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FOREWORD

The School of Pharmacy and Pharmaceutical Sciences, Cardiff University, is the only school of pharmacy in Wales and is one of the top schools of pharmacy in the UK. The quality of teaching and learning has been rated "excellent" by the National Teaching Quality Assessment. Our students graduate well-prepared and satisfied, as seen by the consistently high pass rate in the pharmacist registration examination and high ranking in the National Student Survey respectively.

In addition to supporting individual pharmacists in their initial and ongoing education and development, the School is active in research that has been independently judged to be predominantly of international standing, more than half of which is recognised as world-leading or internationally excellent and with many interdisciplinary and external collaborators. Research at the School encompasses medicinal chemistry, drug delivery and microbiology, pharmacology and physiology, and pharmacy practice and clinical pharmacy, and it impacts on healthcare and pharmaceutical sciences throughout the UK and the world. Further information on the School's research activities and degree programmes, along with contact details for academic staff can be found at http://www.cardiff.ac.uk/phrmy.

At the Cardiff School of Pharmacy and Pharmaceutical Sciences, the combination of these strengths allows us to successfully deliver research-led learning and teaching. All of our MPharm students undertake a significant, independent Masters level research project in the final year of the four year degree, and present and defend their research. We also attract large numbers of well-qualified UK, EU and international applicants to our postgraduate diplomas and degrees. This is the 12th year in which we have published the abstracts of student research.

Within this publication the student is the first named author, and collaborators and supervisors follow. An alphabetical list of authors appears in the index. The following may be used to cite an abstract:
Authors' names. Abstract title, *Cardiff School of Pharmacy & Pharmaceutical Sciences Research Abstracts 2012,* (ed. Price-Davies, R) STS Publishing, Cardiff (2012) Page number. ISBN: 978 0 948917 45 5.

I am grateful to my colleagues for their assistance in collating this book, most especially to Dr Dai John and Dr Keith Brain.

Rebecca Price-Davies
July 2012

The Effect of Covalent Modification of 5-HT by Methylglyoxal on the Biological Activity of 5-HT

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Approximately 10% of the general population is affected by irritable bowel syndrome (IBS).[1] The exact aetiological mechanism of IBS remains unknown; this is one of the main reasons why the current treatment of IBS is unsatisfactory, resulting in a loss in quality of life and huge costs to the National Health Service. The bacterial 'toxins' hypothesis explains the involvement of food in the pathogenesis of irritable bowel syndrome.[1] The majority of mammals lose lactase-phlorizin hydrolase (LPH) after weaning. This means that 4000 million people cannot digest lactose properly. This hypothesis proposes that carbohydrates not absorbed in the small intestine reach the bacteria present in the large intestine. Anaerobic digestion of carbohydrates by bacteria in the large intestine produces a range of alcohols, diols, aldehydes, ketones and acids. One of these products is the aldehyde methylglyoxal, which is a metabolic toxin. Methylglyoxal (MG) could potentially modify 5-HT inducing abnormal serotonergic signalling, which has already been implicated in IBS through a number of studies.[2] The aim was to investigate whether methylglyoxal covalently modifies 5 HT, altering the biological activity of 5 HT and its interaction with receptors in the gut.

Methylglyoxal was mixed with 5-HT at 23°C for 24 hours to determine whether MG covalently modified 5-HT. Thin layer chromatography was also used to confirm that methylglyoxal modified 5-HT and to isolate the different compounds present in order to examine their absorbance spectra and biological activity. The reaction mixture and 5-HT was added to the TLC plate, and the TLC plate was then inserted into the solvent. The different components present on the TLC plate were identified under a fluorescent lamp at 366 nm. They were then individually eluted in a fixed amount of ethanol. UV Spectroscopy was likewise used to confirm the presence of two new compounds using absorbance spectra. The last thing was to test the biological activity of 5-HT and the modified compounds on isolated segments of ileum. The cumulative effect of 5-HT (0.1 µM-10 µM), mixture of 5-HT & methylglyoxal (0.1 µM-10 µM), yellow band from TLC (0.1 µM-20 µM), black band from TLC (0.1 µM-20 µM) and 5-HT from TLC (0.1 µM-20 µM) were investigated.

The mixture of MG and 5-HT appeared yellow after 24 hours, indicating that a reaction had occurred.[3] The next step was to isolate the different compounds present in the reaction mixture using TLC. Two new bands appeared on the TLC plate, a yellow fluorescent band and a black band, suggesting the formation of two new compounds. The absorbance spectra for the mixture of MG and 5-HT showed the presence of two new peaks. This suggested the formation of two new compounds, and the loss of 5-HT. Lastly, the biological effect of 5-HT and the modified products was investigated on the ileum. 5-HT caused an increase in twitch height contraction, highlighting the involvement of the 5 HT receptors in gut contraction. However, there was no response to both the purified compounds from the TLC plate and the crude mixture of 5-HT and MG, indicating that the modified 5-HT is biologically inactive.

The results confirm for the first time that methylglyoxal covalently modifies 5-HT and that it inactivates its biological activity. These findings help explain the role of MG in the systemic symptoms of lactose intolerance and IBS.[4] It also shows that the bacterial metabolic 'toxins' hypothesis provides an evidence-based molecular mechanism for the involvement of food in the pathogenesis of irritable bowel syndrome, which in the past was a matter of contention.

1. Campbell AK *et al*. Bacterial metabolic 'toxins': A new mechanism for lactose and food intolerance, and irritable bowel syndrome. *Toxicology*. 2010;**278**(3): 268-276.
2. Yamada K, *et al*. A novel synthesis of 3, 4, 5, 6-tetrahydro-7-hydroxy-1H-azepino[5,4,3-cd] indole derivatives from serotonin heterocycles. *Chem Pharm*. 2011;**83**(11): 2547-2562.
3. Szent-Györgyi A, McLaughlin JA. Interaction of glyoxal and methylglyoxal with biogenic amines. *Proc Natl Acad Sci* 1975;**72**:1610–1611.
4. Campbell AK, Waud JP, Matthews SB. The molecular basis of lactose intolerance. *Science Progress*. 2005;**88**: 157–202.

Micro-patterning of Materials for Medical Application (antimicrobial surfaces)

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Device associated infections (DAI) have been widely taking place in hospitals especially among the urinal catheters patients. Since the urinal catheters are made of biomaterials in the polymer form, this device has always becoming a target for bacteria such as *E. coli* or *Staphylococci* to cause catheter associated urinary tract infections (CAUTI).[1] Bacterial biofilm formation has been identified to be a major cause of the biomaterial-associated infection, and is triggered by bacterial adhesion on the surface of the catheters. Traditional methods such as the release of biocidal compounds such as antibiotic into the aqueous surrounding were less efficient to overcome the bacterial adhesion, due to emergence of antibiotic-resistant pathogenic strains. A novel idea of applying surface patterning on the material surface has been suggested to formulate an adhesion-resistant material. One of the techniques involved is structural topography i.e. physical or mechanical patterning. The aim of this study was to investigate the effect of micro-patterned features of different sizes on the behaviour of bacterial adhesion.

Micro-pattern features stamp templates were mechanically created on silicone master wafer plate using Nano-second Laser Machining to produce an array of pockets with 10mm by 10mm dimension. Medical grade PDMS was selected to become sample model surface to represent commonly used silicone polymer in the construction of the catheters. Spreading of silicone against the stamp templates was conducted and after heat treatment, solidified PDMS samples were created. The PDMS samples copied the imprinted micro-patterned features on the micro-patterned stamp templates. Two different strains of *Escherichia coli* MG1655 (wild type and non-modified strain) and PHL628 (a thick biofilm former strain) were challenged to be exposed on the micro-patterned PDMS sample surface. Simulated urine solutions was used as a fresh media and maintained at pH6.7 to imitate the real condition in urinary environment. The PDMS samples were cultured and incubated in the urine solution containing the bacteria strains for 1 hour, 6 hour and 24 hour exposure time. The behaviour of bacterial adhesion was illustrated in the form of fluorescence images and absorbance reading of the crystal violet stain.

Fluorescence images demonstrated the proportions of adhered *E. coli* strains on the surface and absorbance readings indicated the approximation of the amount of biofilm cells. Both MG1655 and PHL628 showed response when being exposed to micro-patterned surface. All micro-patterned PDMS samples successfully reduced the proportions and amount of biofilm cell of on the surface. Lower proportions of adhered bacteria were obviously spotted on micro-patterned PDMS with features of 25-30 micrometres as compared to PDMS with 20 and 40 micrometres feature size after 1 hour and 24 hour exposure time. The absorbance readings supported these findings by revealing only 25-30 micrometres feature samples gave big reductions for both short (after 1 hour) and long (between 1 and 24 hour) exposure time. The stronger adherent PHL628 strain was markedly reduced by these micro-pattern feature sizes as well.

Topographically modified surface was able to influence the nature of original attachment of *E. coli* strains through the micro-pattern features. This influence took form in the change of the orientation for adhesion via physical mean. Micro-patterned surface has been shown to produce impacts on chemical signalling pathways between and within bacterial cells, introducing interference in the recognition ability of the bacteria to start their adhesion on the surface and also communication between them as well.[2] However, all the micro-pattern features were way bigger than the size of *E. coli* strains used, which indicate that a perfect comparison of sizes between bacteria and features could not be made. Further studies using nanometre size of features can be carried out to investigate this. In summary, this study proved that micro-patterning of material is able to create potential antimicrobial surface.

1. Epstein AK *et al*. Control of Bacterial Biofilm Growth on Surface by Nanostructural Mechanics and Geometry. *Nanotechnology*. 2011; **22**: 1-8.
2. Hochbaum AI and Aizenberg J. Bacteria Pattern Spontaneously on Periodic Nanostructure Arrays. *Nano Letters*. 2010; **10**: 3717-21.

The effect of pulmonary P-glycoprotein upon Rhodamine-123 absorption in an *ex-vivo* lung model

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Pulmonary drug delivery proves to be of great interest among drug discovery and development scientists in recent years, primarily in the treatment of respiratory diseases. The lung, which is a portal of entry of various molecules, may be utilised for delivering drugs either locally or to the systemic circulation. In both instances, the understanding of the impact of pulmonary physiology on the local drug pharmacokinetics is essential. One such physiological factor is drug transporters. P-glycoprotein (P-gp) is a major ABC transporter known to influence the pharmacokinetics of a wide range of drugs. Lung expression of P-gp has previously been confirmed in both human and rodent species where it has been observed to be localised in the apical membranes of the bronchial and alveolar epithelia.[1,2] Nonetheless, the functional importance of P-gp in lung on the pulmonary absorption of inhaled drugs has received only limited attention. The aim of this study is to study the effect of P-gp inhibition on the pulmonary absorption of P-gp substrate rhodamine-123.

Rhodamine-123 (1.32nmole in 25µL) was administered into the airways of an isolated perfused lung using a stoplock Hamilton. This was carried out in the presence and absence of a known P-gp inhibitor, GF120918, and controlled to exclude any possibility that GF120918 affected Rhodamine-123 by a mechanism(s) other than P-gp inhibition. At specified time points, 400µL of sample was taken and rhodamine-123 concentration was determined using HPLC-fluorescence.

There was a 170% increase in bioavailability (F) in both sets of experiments where P-gp function was diminished by either chemical or genetic means. The Student t-test demonstrated that the percentage absorption of rhodamine-123 was significantly different at all data points (in both sets of experiments).

The results indicated that P-gp limits the absorption of rhodamine-123 across the lung epithelia. Using the isolated perfused lung, it was proven that this a good model to study the impact of P-gp on drug handling by the lung. Note that the responses in the model would be consistent and do not require co-administration of chemical modulators.

1. Campbell L *et al*. 2003. Constitutive Expression of P-Glycoprotein in Normal Lung Alveolar Epithelium and Functionality in Primary Alveolar Epithelial Cultures. *The Journal of Pharmacology and Experimental Therapeutics*. **1**:304.
2. Endter S *et al*. 2007. P-glycoprotein (MDR1) functional activity in human alveolar epithelial cell monolayers. *Cell Tissue Research*. **328**:77–84.

Exploratory Study on the Use of Self-management Rescue Packs for Exacerbations of Chronic Obstructive Pulmonary Disease (COPD)

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Chronic Obstructive Pulmonary Disease (COPD) is a progressive condition, characterised by airflow obstruction due to prolonged lung inflammation.[1] Patients suffering from COPD, experience acute episodes known as exacerbations, which interrupt their stable steady state.[1,2] A self-management rescue pack contains a course of both oral antibiotics and corticosteroid tablets and is designed for the patient to keep at home, for the self-treatment of an exacerbation. This study aimed to explore the views of health care professionals (HCPs) and COPD patients on the use of rescue packs. The study objectives were to use the interview results to identify the facilitators and barriers to COPD rescue pack use and to devise potential future improvements to their application.

This study has been designed as a continuation of the MSc project conducted by Green[3], where insufficient numbers of COPD patients were interviewed to fully ascertain their views on rescue pack use. Consequently, qualitative semi-structured interviews were chosen as the most appropriate research instrument to achieve the exploratory aim.[4] Non-probability quota sampling was used to attempt to recruit 20 COPD patients, who had been admitted to the hospital for COPD exacerbations: 10 who had previous rescue pack experience, and 10 who had not. Non-probability purposive sampling was used to recruit seven HCPs, including respiratory consultant, COPD specialist respiratory nurse, frailty team nurse and long-term condition nurse participants. The semi-structured interview consisted of seven main open questions, which were used to identify the facilitators and barriers for patients using rescue packs. The interviews were conducted at the Hospital and were audio-recorded, transcribed verbatim and then thematically analysed.

In total, nine interviews were conducted: two COPD patients and seven HCPs. The main rescue pack facilitators showed the majority of patients preferred home exacerbation treatment and displayed enthusiasm towards rescue packs. Contact from HCPs (direct and 'over the phone') and non-HCPs (family and social workers) was thought to be beneficial for patients, alongside education about rescue pack usage. The current lack of written information provided within the pack, as well as their unsuitable use for some COPD patients, were identified as the two main barriers to rescue packs. Future improvements to be made were identified as: production of a simplified patient information leaflet and inclusion of a sputum colour chart.

This qualitative study, using small numbers of COPD patients and HCPs, was successful in identifying the facilitators, barriers and improvements to rescue pack usage. What may be considered interesting is that HCPs and patients identified similar facilitators and barriers regarding rescue pack use. Overall the participants thought most patients would prefer to stay at home for exacerbation treatment and use a self-management rescue pack, if they had the appropriate support required. This support was thought to be HCP and non-HCP contact, education and written information. The literature explains this is more likely for exacerbations experienced by younger patients, with the required support offered and where anxiety is less prevalent.[1] The major study limitation experienced was the low number of COPD patients interviewed, both due to high refusal rates and low numbers of patients meeting the study criteria. Low participation rates are commonly experienced amongst COPD studies.[1] To conclude, the study findings suggest patients need more simplified written information, HCP support and education, which were also identified by Green.[3] Findings from these two studies, was thought to have enabled data saturation, within both the patient group (Green's study n=4, current study n=2) and HCP group (current study n=7), to be reached, however due to the low patient participation rate, further interviews are still advised.

1. Dowson, C. A., Kuijer, R. G., Mulder, R.T. Anxiety and self-management behaviour in chronic obstructive pulmonary disease: what has been learned? Chronic Respiratory Disease. 2004;**1**: 213-220.
2. Bourbeau, J. Preventing Hospitalization for COPD Exacerbations. Seminars in respiratory and critical care medicine. 2010;**31**(3): 313-320.
3. Green, V. An assessment of reasons why patients are admitted to hospital with a simple exacerbation of chronic obstructive pulmonary disease (COPD) [MSc Dissertation]. Cardiff: Cardiff University; 2011.
4. Oppenheim, A. N. Questionnaire Design, Interviewing and Attitude Measurement. New ed. London and New York: Continuum; 1992.

Caveolin-1 and Molecular Pathways Regulating Renal Cell Carcinoma – *in vitro* Studies in 786-0 Cells

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Renal cell carcinomas (RCC) are the commonest group of heterogeneous malignancies of kidney. Clear cell RCC (ccRCC) is the major histological subtype accounting for about 75-80% of all RCC cases. The incidence of RCC is rising, currently it accounts for about 3% of adult cancers and 2% of all cancer-related deaths. At initial diagnosis two-thirds of patients with clinically localised disease will undergo nephrectomy. Despite this intervention approximately 40% of these patients will later relapse.[1] Lack of appropriate diagnostic tool and effective treatment intervention has lead to extensive research into the tumour biology of renal cell carcinoma. Caveolin-1 (cav-1) is a 24 KDa membrane protein that its overexpression was found to be associated with high-grade tumours, vascular invasion and poor disease-free survival in clinically-confined RCC patients.[1] Further studies by Campbell and collaborators reveal that an association exists between cav-1 over-expression and key signalling pathways involved in RCC. The co-expression of cav-1 with either activated PI3K/Akt, mTOR or ERK pathway were found to be associated with reduced disease-free survival in clinically-confined RCC.[2,3] The aim of this study is to investigate if caveolin-1 is down-stream of PI3K/Akt, mTOR or ERK pathway.

An MTT cell viability assay was carried out to determine whether the inhibitors of PI3K/Akt, mTOR and ERK pathways can affect the cellular viability of 786-0 cells *in vitro*. The 786-0 cell line is a human renal cell carcinoma that is VHL and PTEN (both tumour suppressor genes) deficient. Cells were seeded in a 24-well plate (10,000 cells/cm^2), grown for 24 hours, and then treated for 48 hours with their known inhibitors at various concentrations. Western blot analysis were then carried out to investigate any changes in the expression of caveolin-1 and phospho-proteins.

The Akt inhibitor (LY294002) significantly reduced the proliferation of 786-0 cells in a dose-dependent manner ($P< 0.0001$). The western blots demonstrate no changes in caveolin-1 expression regardless of the LY294002 concentration. pAKT expression were reduced in the 50 µM treatment. 1 nM and 10 nM rapamycin resulted in a dose-dependent reduction in proliferation of 786-0 cells. Interestingly the western blots revealed an over-expression in cav-1. level upon mTOR pathway inhibition. Phospho-S6 expression was completely inhibited. Treatment of 786-0 cells with PD98059 (ERK inhibitor) also significantly reduced its proliferation ($P< 0.0001$). No changes were observed in cav-1 expression. pERK expression was reduced in a dose-dependent manner.

The exact mechanism by which caveolin-1 assists carcinogenesis is still largely unknown. Other pathways in RCC need to be investigated. This study has demonstrated that the PI3K/Akt and ERK pathway are not an upstream regulator of caveolin-1. The presence of dual feedback loop involving mTOR may be the answer to cav-1 overexpression.[4] Further studies are needed to exploit the complex nature of mTOR pathway and establish any association between caveolin-1 and the mTOR pathway.

1. Campbell L *et al*. 2003. Caveolin-1 overexpression predicts poor disease-free survival of patients with clinically confined renal cell carcinoma. *British J Cancer*. **89**:1909-13.
2. Campbell L *et al*. 2008. Combined expression of caveolin-1 and an activated AKT/mTOR pathway predicts reduced disease-free survival in clinically confined renal cell carcinoma. *British J Cancer*. **98**:931-40.
3. Campbell L *et al*. 2009. Activated extracellular signal-regulated kinase is an independent prognostic factor in clinically confined renal cell carcinoma. Cancer. **115**:3457–3467.
4. Carracedo A *et al*. 2008. Inhibition of mTORC1 leads to MAPK pathway activation through a PI3K-dependent feedback loop in human cancer. *J Clin Invest*. **118**:3065–74

Ex-vivo bladder tissue for investigating the urothelial permeation of oxybutynin hydrochloride

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Intravesical drug delivery (IDD) involves the administration of a considered amount of drug solution to the urinary bladder via a catheter.[1] IDD avoids first pass metabolism, minimises systemic side-effects and provides an increased release of active constituents at the site of disease.[2] At present, there is little scientific knowledge and understanding regarding the tissue levels achieved by intravesical dosing in practice. In this study we aim to investigate the permeation and tissue distribution of oxybutynin hydrochloride into the bladder wall using an *ex vivo* model.

The deposition of oxybutynin into the different layers of the bladder wall was performed using Franz-type diffusion cells. Urothelial and muscle tissue layers were separated to study bladder permeability and analysed using high performance liquid chromatography. Histology studies examined urothelial separation procedures establishing its presence and removal. The partition coefficient was determined between the muscle tissue and the oxybutynin (1.1 mg/ml) drug solution.

The drug extraction procedures indicated that approximately 95% of the drug is removed within the first 24 hour cycle. Histology studies illustrated the difficulties in removing the urothelium, though it is possible to selectively remove the urothelium and lamina propria. A clinically relevant concentration of oxybutynin hydrochloride has demonstrated that it is able to permeate through the urothelium and lamina propria. Higher proportions of oxybutynin were seen in the urothelium layer presenting as the rate-limiting step to drug delivery to the bladder. An experimental partition coefficient of 0.143 indicates the lack of partitioning in the muscle tissue. Moreover it has emphasised that drug permeation reaching the muscle tissue to be 30% of the applied dose after 6 hours.

The data obtained from partition coefficient study can be used to estimate clearance from the bladder to predict tissue concentration levels in the *in vivo* setting. In addition the study has assessed the tissue concentrations achieved to investigate the urothelial permeation of oxybutynin hydrochloride and with continued optimization a drug delivery system to deliver a clinically relevant concentration into the bladder wall can be ascertained.

1. GuhaSarkar, S. and Banerjee, R. 2010. *Intravesical drug delivery: Challenges, current status, opportunities and novel strategies*, *Journal of Controlled Release* **148** (2): 147-159.
2. Barthelmes J. *et al* 2011. *Development of a Mucoadhesive nanoparticulate drug delivery system for a targeted drug release in the bladder. International Journal of Pharmaceutics* , **416** (1): 339-345

An Investigation of the Antibacterial Properties of Propolis

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Bee products have been used medicinally since 2000BC. The advent of readily available antibiotics moved the use of natural products from mainstream medicine to more of a health supplement. The poor control over the use of antibacterials when they were first discovered has lead to problems with resistance which is becoming a real problem for modern treatment. Due to this resistance to synthetic antibacterials, interest has turned to back the natural products from which we used to obtain our medicines. Honey is undergoing many trials currently and has been shown to exhibit potent antibacterial properties. This success has lead to the study of other bee products including propolis. Propolis is a "…generic term for certain sticky substances secreted by plants, mainly trees …also used for the material in the hive which is a mixture of (plant) propolis and beeswax." [1] The aim of this study is to investigate the antibacterial properties of propolis against methicillin resistant *Staphylococcus aureus* (MRSA) and *Bacillus subtilis.*

Appropriate concentrations of bacteria were chosen by enumeration of bacterial cell count using turbidity measurements. Curves were plotted to obtain the concentration of bacteria which would be used for each strain. Antibacterial activity was investigated by measuring the zone of clearance in a lawn of bacteria with using the agar well diffusion method. Phenol (1-10%) was used as a control to ensure that each propolis experiment was comparable. There were six propolis samples studied in this experiment which came in either dry solid, liquid or unprocessed form. Ethanolic extractions of all samples were made using a 1:1 ratio of propolis to 70% ethanol. Six types of agar media were investigated to ensure the media which gave the clearest results was chosen for use in further experiments. The effect the concentration of ethanolic extractions had on antibacterial activity was investigated for concentrations of 10-100%. The minimum effective concentration of each propolis sample was then investigated using a liquid broth and drop count method. To investigate the activity of propolis as compared to honey, the effect of the inactivation of hydrogen peroxide and methylglyoxal was investigated.

By using the drop count method on bacterial solutions of various concentrations, concentrations of $0.01OD \approx 10^6 CFU/ml$ and $0.05OD \approx 10^9 CFU/ml$ were chosen for *B. subtilis* and MRSA respectively. Mueller Hilton was chosen as the media for use in further experiments as it gave the clearest zones of inhibition and is the most commonly used for propolis experiments. The propolis extracts showed antibacterial activity at all concentrations. The activity varied from sample to sample even between samples of a similar original form. The minimum effective concentration of the propolis samples showed that the inhibition of bacterial growth was different between MRSA and *B. subtilis*. Neither the inactivation of hydrogen peroxide nor methylglyoxal proved to show any decrease in the antibacterial activity of any propolis sample investigated.

The hypothesis of this study was that propolis could have antibacterial activity methicillin-resistant *Staphylococcus aureus* and *B. subtilis*. If the propolis exhibited antibacterial activity, further experiments could be carried out to isolate the biologically active components and use them as a lead compound for a new antibacterial drug. By using a known source of antibacterials the search for new antibacterial compounds can be continued. This is becoming more important with the rise of resistant bacterial strains and the inability of modern synthetic drugs to treat them.

1. Crane, E. 1973. "Bee Products." *Bee World.* 54: pp71-74

Topical Delivery of Gabapentin for Neuropathic Pain

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Neuropathic pain is a potentially devastating condition that may result in both physical pain and a decreased quality of life. Gabapentin is used currently as an oral medication for neuropathic pain, but is limited by side effects such as dizziness and somnolence. Clinicians propose that a topical formulation of Gabapentin may be an alternative for patients unable to tolerate oral Gabapentin. This theory is currently being tested in patients with peripheral neuropathies such as vulvodynia via clinical trials. However there are no published studies which prove Gabapentin permeates the stratum corneum or give a definitive peripheral site of action for Gabapentin.[1,2] Microneedles will therefore be tested to assess their effect on Gabapentin flux through the skin. Microneedles range in length from 150-800µm and create microchannels in the stratum corneum enabling transdermal delivery of drugs by increasing permeability.[3] The aims of this project were therefore; to create an alternative gel formulation to those currently used, to establish whether Gabapentin permeates the stratum corneum, and to determine whether microneedles can increase the flux of Gabapentin across skin.

A carbomer based 6% Gabapentin gel was produced following the creation of a working formula and method based on existing literature. The release properties of the gel were compared to a currently used Lipoderm based gel using a Franz cell diffusion study with cellulose nitrate membranes. Once Gabapentin release had been established a second Franz cell diffusion study was undertaken with human epidermal heat separated sheets and both carbomer and Lipoderm based 6% Gabapentin gels to determine whether Gabapentin can cross intact stratum corneum. Microneedle studies were then used to ascertain the most appropriate microneedle array to use with Gabapentin gel. These studies included; visualisation of microneedle arrays using environmental scanning electron microscopy (ESEM) and light microscopy, trans-epidermal water loss readings before and after microneedle puncture and heat separation of the epidermis post puncture to enable imaging of puncture pattern and dimensions via ESEM. The most suitable microneedle array was then used to puncture epidermal sheets for a third Franz cell diffusion study; which used both unpunctured and microneedle punctured epidermal sheets with carbomer 6% Gabapentin gel to establish the effect of microneedle puncture on Gabapentin flux. High Performance Liquid Chromatography (HPLC) was used to quantify Gabapentin in all Franz cell diffusion studies.

Carbomer was a suitable gel base for preparing a topical Gabapentin gel preparation. The release of Gabapentin from the carbomer based gel was statistically greater than Lipoderm gel release according to an ANOVA and post-hoc Tukey's test ($p<0.05$). A Franz cell diffusion study showed that Gabapentin did not permeate intact stratum corneum, at least not at a level detectable using HPLC. Microneedle studies indicated that the most appropriate microneedle array for use with Gabapentin was the 500µm length microneedle array due to their durability and consistent puncture pattern and dimension. An ANOVA and post-hoc Tukey's test following the third Franz cell diffusion study concluded that microneedles cause a statistically significant increase in Gabapentin flux across intact skin.

As release of Gabapentin from carbomer based gels exceeds that of current formulations it may be a more efficacious preparation for use in future studies and treatment. The lack of Gabapentin flux across the stratum corneum suggests that Gabapentin is too polar to permeate the stratum corneum, although quantities below the HPLC limit of detection may have permeated but remain undetected. Therefore clinical trials would need to address whether the pain relief observed in neuropathic pain patients using topical Gabapentin is due to a placebo effect. Both microneedle and Franz cell diffusion studies indicated that microneedles are a viable option for use in conjunction with Gabapentin to enhance delivery, via an increased flux of Gabapentin across the stratum corneum. Further studies in addition to clinical trials, using differing membranes and HPLC methods are necessary to definitively address whether topical Gabapentin is a viable alternative treatment for neuropathic pain.

1. Gilron I, *et al*. Neuropathic Pain: a practical guide for the Clinician. *Canadian Medical Association Journal*. 2006; **175** (3): 265-275
2. Boardman LA *et al*. Topical Gabapentin in the treatment of Localised and Generalized Vulvodynia. *Obstetrics & Gynaecology*. 2008 Sept; 112(3): 579-58
3. Tyndall National Institute. Microneedle Technology. 2012 [accessed 10 April 2012] Available from: http://www.tyndall.ie/content/microneedle-technology

A survey to discover the extent and level of teaching dedicated to "Clinical Nutrition" in undergraduate medical and pharmacy schools

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Malnutrition is defined as a nutritional state where an imbalance of energy, protein or other nutrients causes measurable adverse effects on the body. Malnutrition represents a serious health problem within the U.K; in 2008 24% of men and 25% of women in England were categorised as obese.[1] In addition to this, 25% of adults admitted to hospital and care homes in 2007 were found to be at risk of malnutrition, largely as a result of under-nutrition.[2] In 1992 the King's Fund report "A Positive Approach to Nutrition as Treatment" found that very little "Clinical Nutrition" (CN) was taught to medical and pharmacy students, with no attempt made to co-ordinate basic scientific teaching with clinical experience later in the curriculum.[3] CN is the nutritional management of patients with established disease[4], and studying it in depth as part of an undergraduate medical or pharmacy degree would better educate future healthcare professionals in the identification and treatment of malnutrition.[3] This research aims to discover the extent and level of teaching dedicated to CN in undergraduate medical and pharmacy schools in order to discover if changes have occurred since the 1992 King's Fund report.

A structured questionnaire was designed to be completed by members of staff currently teaching CN at undergraduate medical and pharmacy schools. The questions were based upon themes identified following thorough a literature search, and a 5-point Likert scale was created for use within the majority of the questions. Once the questionnaire had been designed it was piloted on a small number of medical and pharmacy students in order to maximise face and content validity. The questionnaire was sent as an e-mail attachment to the head of every medical and pharmacy school with instructions to forward to the relevant member of staff teaching CN. This was considered the most appropriate method due to the time constraints, geographical spread of the study population and the ease with which questionnaire results would be analysed. Completed questionnaires were requested to be returned within three weeks of receipt. An e-mail reminder being sent ten days, after the questionnaires were first e-mailed in an attempt to increase response rate.

8 out of 52 questionnaires were returned giving a response rate of 15.38%. The average time spent teaching CN was between 9 and 11 hours, with little difference between medical and pharmacy schools. 100% of respondents either disagreed or strongly disagreed that CN was not relevant to pharmacy and medical students due to dieticians specialising in this area. Respondents neither agreed nor disagreed that a greater number of hours spent teaching CN would be beneficial to students. The same response was also observed with respect to a greater number of assessments in the area of CN. 100% of respondents either agreed or strongly agreed that students were aware of the impact of malnutrition on health and well-being yet only 25% of respondents agreed that the teaching they currently provide allows students to accurately identify and treat malnutrition.

This research has gained insight into the current teaching of CN in undergraduate medical and pharmacy schools. Respondents believe that CN is a relevant topic for future doctors and pharmacists; however, they are unconvinced whether an increase in the number of hours spent teaching CN or greater levels of assessments in the area will be beneficial to students. Current teaching seems to adequately cover the impact of malnutrition on health and well-being but it appears that students are not able to accurately identify and treat malnutrition. This suggests that a deficiency in the teaching of CN has been identified.

1. British Association for Parenteral and Enteral Nutrition. 2007. *BAPEN study reveals that 1 in 4 of all adults across all age groups admitted to hospital and care homes in the U.K at risk of malnutrition.* [Online] Available at: http://www.bapen.org.uk/pdfs/press_releases/press_nsw_2007_results.pdf [Accessed: 28th November 2011]
2. NHS Information Centre 2010. *Statistics on obesity, physical activity and diet: England 2010.* [Online] Available at: http://www.ic.nhs.uk/webfiles/publications/opad10/Statistics_on_Obesity_Physical_Activity_and_Diet_England_2010.pdf [Accessed 18th February 2012]
3. Lennard Jones *et al.* 1992. *A positive approach to nutrition as treatment.* King's fund report. Available at: http://www.bapen.org.uk/pdfs/bapen_pubs/pub_kings_fund.pdf [Accessed 15th November 2011]
4. Gibney, M. *et al.* 2005. *Clinical Nutrition.* United Kingdom: Blackwell Science.

Does magnesium inhibit bronchoconstriction and can it potentiate salbutamol-induced bronchodilation in isolated lung?

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Asthma is a chronic relapsing and remitting respiratory condition characterised by airway hyperresponsiveness and inflammation of the bronchi. Asthma exacerbations occur in response to a trigger and are of varying severity. Where hospitalization is required treatment can include magnesium sulphate. Magnesium has been shown to reduce bronchoconstriction *in vitro*[1] and many clinical studies show that magnesium in combination with a β_2-agonist improves pulmonary function.[2] However, other clinical studies have shown magnesium has no effect on treatment outcomes.[3] The aims of this study were to determine firstly if magnesium inhibits bronchoconstriction and secondly if magnesium potentiates salbutamol-induced bronchodilation.

Lungs were isolated and separated at the first bifurcation. Baseline pressure was set at approximately 20mmHg by adjusting the flow rate of the perfusate. Bronchoconstriction was induced with bolus doses of methacholine and histamine ($1x10^{-3}$-1mmol). To determine if magnesium inhibits bronchoconstriction the lungs were perfused with Krebs bicarbonate containing increasing magnesium concentrations (10mM-100mM) with Krebs bicarbonate (1.2mM magnesium) acting as the control. To determine if magnesium potentiates salbutamol-induced bronchodilation the perfusates used contained 10µM salbutamol alone, 5mM magnesium alone or 10µM salbutamol +5mM magnesium. Responses were measured in mmHg and converted to a ratio by dividing peak pressure by baseline pressure.

Magnesium decreased methacholine and histamine-induced bronchoconstriction in a concentration-dependent manner with these decreases being statistically significant compared to control with the higher doses of methacholine and histamine administered ($p<0.05$, $n=3$). Magnesium did not potentiate the bronchodilating effects of salbutamol but did appear to have additive effects as the responses produced in the presence of salbutamol alone and magnesium alone were greater than those produced in the presence of both. With a dose of 1mmol methacholine the responses induced in the presence of salbutamol alone and magnesium alone were significantly greater than in the presence of both ($p<0.05$, $n=3$) therefore the combination of magnesium and salbutamol results in significantly reduced bronchodilation.

This study shows that magnesium can act as a bronchodilatory agent which is consistent with the findings of other *in vitro* studies.[1] The enhanced bronchodilation seen with a combination of magnesium and salbutamol is in contrast to the findings of a similar study.[4] However, the actual difference in the responses produced in the presence of salbutamol + magnesium compared to in the presence of either alone are relatively small thus this enhanced bronchodilation may not be clinically significant. The concentrations of magnesium used in this study and other studies like it are much higher than the doses used clinically. This may indicate why *in vitro* studies provide more conclusive evidence of the bronchodilating effects of magnesium compared to clinical studies where conclusions have been conflicting as magnesium has a concentration-dependent effect. This study did not investigate the mechanism of action of magnesium but several have been proposed, this along with the use of higher doses of magnesium in *in vivo* studies warrant further investigation.

1. Hirota, K. *et al*. 1999. Relaxant effect of magnesium and zince on histamine-induced bronchoconstriction in dogs. *Critical Care Medicine* 27(6), pp. 1159-1163.
2. Mohammed, S. and Goodacre, S. 2007. Intravenous and nebulised magnesium sulphate for acute asthma: systematic review and meta-analysis. *Emergency Medicine Journal* 24, pp. 823-830.
3. Rowe, B. *et al*. 2000. Magnesium sulphate for treating exacerbations of acute asthma in the emergency department (Review). *Cochrane Database of Systematic Reviews* 1.
4. Joseph, S. and Ford, W. R. 2011. *The effect of magneisum sulpahte on salbutamol-induced relaxation of isolated trachea*. Cardiff.

Distribution and Permeation of Nanoparticulate and Soluble Model Compounds Following Microneedle Delivery

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The skin presents an ideal target for drug delivery due to its large surface area and ease of access.[1] In the past decade, there has been heightened interest in transdermal drug delivery systems due to their advantages over conventional dosage forms. However, the major barrier that limits the type of therapeutic agent delivered to the skin is the physical barrier of the stratum corneum.[2] Microneedles are a minimally invasive drug delivery method, able to puncture and thereby disrupt the stratum corneum to facilitate drug delivery via the transdermal route.[3] There is currently a lack of information on the distribution and permeation properties of therapeutic agents following microneedle treatment. Therefore, the objectives were to determine the spatial distribution and permeation of nanoparticle suspension and sulforhodamine b solution following microneedle treatment.

To determine the distribution properties of the nanoparticle suspension and sulforhodamine B solution, they were delivered to *ex vivo* human skin using two methods of delivery; coated microneedle delivery and liquid delivery. Coated microneedle delivery involved coating the surface of microneedles with the fluorescent formulations. The microneedles were left to dry before insertion into human skin. Liquid delivery entailed applying a small volume of the fluorescent formulations onto the surface of the skin and applying the microneedle array directly on top of the formulation. The distribution properties of the fluorescent formulations were determined by fluorescent microscopy. Epidermal membranes were used to determine the permeation properties of the nanoparticle suspension and the sulforhodamine B solution. Permeation studies using Franz-type diffusion cells assessed the permeation of nanoparticle suspension and sulforhodamine B solution through untreated and microneedle treated membranes. Fluorescence values were converted to concentrations and the percentage of the formulations permeated was determined at the conclusion of the experiment.

A significant increase in the distribution of the sulforhodamine B solution compared to the nanoparticle suspension was observed with both methods of microneedle delivery. It was observed that the nanoparticle suspension remained localised to the needle tract area whereas the sulforhodamine B solution was shown to distribute outside of this area and into the surrounding dermal layer. The results from the permeation studies indicate that a significant increase in the permeation of sulforhodamine B solution was observed following microneedle treatment. Although there was a small increase in the permeation of the nanoparticle suspension, the difference in permeation between intact and microneedle treated membrane was not significant.

The results have shown that the type of formulation (suspension vs. solution) and the molecular weight of the proposed therapeutic agent are important for distribution and permeation through untreated and microneedle treated skin. The use of nanoparticles and sulforhodamine B in these studies has given a broad idea on the type of formulation that would be suitable for transdermal delivery. The results obtained will provide information to researchers to optimize the development of new therapeutic agents for successful microneedle-mediated drug delivery. Further work is required on the characteristics of therapeutic agents to develop the perfect microneedle-mediated formulation.

1. Ro, BI. and Dawson, T. L. 2005. The role of sebaceous gland activity and scalp microfloral metabolism in the aetiology of seborrheic dermatitis and dandruff. *The Journal of Investigative Dermatology*. **10**(3), pp.194-197.
2. Mitragotri, S. 2004. Breaking the skin barrier. *Advanced Drug Delivery Reviews*. **56**, pp.555-556.
3. Prausnitz, M. R. and Langer, R. 2008. Transdermal Drug Delivery. *Nature Biotechnology*. **26**(11), pp.1261-1268.

Post-Polymerisation Hydrolysis of Molecularly Imprinted Polymers

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Molecular imprinting is the introduction of sites of specific recognition into a homogenous polymer matrix.[1, 2] The technology allows the natural process of molecular recognition to be mimicked using a polymer construct which is robust to harsh environmental conditions such as extremes of temperature and pH. These attributes make molecular imprinting amenable to a variety of applications such as purification, separation and recently *in vivo* technologies.[3] The main draw backs are problems with binding characteristics caused by heterogeneity and difficulty removing the template molecule. In addition re-binding can be slow due to the densely cross linked nature of the polymer matrix. This project aims to test the hypothesis that: The speed of template re-binding can be increased by post-polymerisation hydrolysis of a proportion of the cross-linking monomers. The proposed mechanism of the hydrolytic treatment is that by cleaving some of the matrix cross-links the flexibility of the polymer matrix is increased, which in turn improves the speed of access for the template molecule to internal imprinted sites.

A propranolol molecularly imprinted polymer (MIP) was produced, composed of 20% hydrolysable EGDMA (ethylene glycol dimethacrylate) and 80% non-hydrolysable DVB (divinyl benzene) cross-linking monomers. A control polymer was used composed of 100% DVB as were non-imprinted polymers (NIPs) of both cross-linker compositions. The effects of hydrolysis by potassium hydroxide on the kinetics of rebinding were investigated using non-equilibrium studies. Equilibrium studies were conducted to rule out any possible effects of hydrolysis on re-binding affinity and capacity. Since the hydrolysis process was carried out at 60˚C, heated controls of each polymer composition were also produced.

Binding by non-imprinted polymer controls was significantly less than binding by MIPs. During equilibrium studies the 20% EGDMA heated MIPs bound more propranolol than the control MIPs, but binding by heated and hydrolysed MIPs was similar. During the non-equilibrium binding by the 20% EGDMA MIP was increased post-hydrolysis compared to control and heated MIPs and there was no increase in post-hydrolysis binding by the 0% EGDMA MIP.

It can be concluded that specific binding sites for propranolol were successfully produced using molecular imprinting. There was no observable change in the proportion of propranolol bound by the MIPs post-hydrolysis in equilibrium binding studies, indicating that hydrolysis does not affect the affinity or capacity of MIP binding. The changes which were observed were also seen in the heated MIP and as such can be attributed to the heating process, which is thought to cause the polymer matrix to swell. As increased binding post-hydrolysis was only observable during non-equilibrium biding studies it may be inferred that the kinetics of rebinding were affected. The findings support the initial hypothesis that partial post-polymerisation hydrolysis increases the speed of template rebinding.

1. Turner N *et al*. From 3D to 2D: a review of the molecular imprinting of proteins. *Biotechnology Progress*. 2006;**22**:1474-1489.
2. Bossi A *et al*. Molecularly imprinted polymers for the recognition of proteins: the state of the art. *Biosensors and Bioelectronics*. 2007;**22**:1131-1137.
3. Hoshino Y *et al*. Recognition, neutralisation and clearance of target peptides in the bloodstream of living mice by molecularly imprinted polymer nanoparticles: a plastic antibody. *Journal of the American Chemical Society*. 2010;**132**(19):6644-6645.

Exploring the role of FAK in drug-resistant breast cancer modulation of osteoclast precursor cells

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Considerable advances in the treatment of breast cancer have been made in recent years, particularly through the use of endocrine agents to treat oestrogen receptor positive breast cancers. However, these agents are limited in the clinical setting by the phenomenon of acquired resistance, which often presents as bone metastases.[1] Cell models of acquired endocrine resistance have a more aggressive phenotype, mediated by increased activity of Src and the closely related protein FAK. Src is known to enhance the differentiation of monocyte precursor cells into bone degrading osteoclasts, which leads to further morbidity and mortality in breast cancer sufferers.[2,3] Whilst Src inhibitors have proven to have some use as suppressors of osteoclast differentiation, the role of FAK in this process is currently unknown. Therefore the aim of this project is to determine whether FAK inhibition affects osteoclast precursor (RAW cell) differentiation, signalling and proliferation, and hence whether FAK is a potential therapeutic target in preventing bone metastasis in breast cancer.

The methods used to explore these aims include TRAP staining, western blotting and MTT assays. The cell lines used were RAW cells (mouse monocytes with the potential to differentiate into bone cells), endocrine sensitive (MCF7) and endocrine resistant (TamR) breast cancer cells. Conditioned media was harvested from these breast cancer cells, pre-treated with FAK or Src inhibitor (PF228 or PP2 respectively). RAW cell differentiation in response to this media was then investigated by staining for TRAP (tartrate-resistant-acid-phosphatase), a compound produced by osteoclasts but not their progenitors. Western blotting was used to investigate the effects of breast cancer conditioned media on RAW cell signalling. RAW cell samples were run by gel electrophoresis, and the resultant blots were incubated with primary and secondary antibodies, before being probed with chemiluminescent substrates. Finally, changes in RAW cell proliferation in response to breast cancer conditioned media was assessed by MTT assays. Viable cells converted MTT to purple formazan crystals and so colorimetry allowed RAW cell growth to be quantified.

TRAP staining suggested that MCF-7 cell conditioned media had no effect on RAW cell differentiation; in contrast to TamR conditioned media, which appeared to cause a change in RAW cell morphology, to one representing intermediately differentiated cells. Furthermore this morphological change was prevented by pre-treatment of TamR cells with a FAK inhibitor (PF228). Western blotting highlighted the ability of both MCF-7 and TamR cells to evoke elevated signalling in RAW cells. Pre-treatment of MCF-7 cells with PF228 suppressed the ability of conditioned media to induce Src and MAPK activity in RAW cells. In contrast, conditioned media from breast cancer cells exposed to FAK inhibitor augmented the ability of TamR culture media to stimulate MAPK and GSK3 signalling in RAW cells. Surprisingly, it was observed that Src inhibition in breast cancer cells vastly increased the ability of TamR conditioned media to induce Src and GSK3 activity in RAW cells. MTT assays showed that both MCF-7 and TamR conditioned media induced proliferation in RAW cells; however this was reduced by inhibition of FAK, with the greatest effect seen in TamR cells.

These results suggest that TamR cells secrete ligands (e.g. RANKL) in a FAK dependent manner, capable of forcing RAW cell differentiation towards an osteoclast phenotype. Inhibition of FAK may lead to a decrease in RAW cell signalling, whilst also preventing RAW cell proliferation. Overall it seems inhibition of FAK may be useful in preventing osteoclastogenesis, clinically this may have relevance in metastatic, drug-resistant breast cancer, where FAK inhibition may prevent degradation of the bone micro-environment.

1. Hiscox S *et al*. Src as a therapeutic target in anti-hormone/anti-growth factor-resistant breast cancer. *Endocrine-related cancer*. 2006;**13**(Supplement 1):S53-S9.
2. Mitra SK, Schlaepfer DD. Integrin-regulated FAK–Src signaling in normal and cancer cells. *Current opinion in cell biology*. 2006;**18**(5):516-23.
3. Saad F, Lipton A. SRC kinase inhibition: targeting bone metastases and tumor growth in prostate and breast cancer. *Cancer treatment reviews*. 2010;**36**(2):177-84.

Investigating the relationship between polar surface area and biological activity of anticancer drug molecules and its application in drug design

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Despite rapid advancement in drug design, in the last few years R&D expenditure has increased by 80%, while productivity has decreased by 43%.[1] This has resulted in the increasing use of predictive tools to try to improve the cost effectiveness of drug design. "PSA ($Å^2$) is the sum of the contributions to the molecular (usually van der Waals) surface area of polar atoms such as oxygen, nitrogen and their attached hydrogens".[2] This parameter has shown good correlation with experimental transport data, resulting in it becoming a successful predictive tool for molecular transport properties, especially intestinal absorption and BBB penetration.[3] The overall aims of this project are to develop a strategy to determine whether PSA correlates with anticancer activity and to establish whether this correlation could be used as an accurate predictive tool to aid drug design.

A quantitative meta-analysis approach was developed where hundreds of journals were examined and analysed to find datasets with a wide range of PSA and anticancer activity in whole cell assays. Overall, the datasets attempted to examine a wide range of cell lines, enabling investigation into different types of cancers. Due to the complexity and the need for specialised software in order to calculate PSA, it was decided to use a method proposed by Ertl *et al* instead; topological polar surface area (tPSA). tPSA is based on "the summation of tabulated surface contributions of polar fragments" [3] and has shown to be a faster and less demanding computer method than traditional 3D PSA but with comparable quality. Investigation into three different tPSA calculation software packages was conducted; ChemDraw, Daylight and Molinspiration. This showed that ChemDraw produced very different tPSA values compared to the other two software packages, due to the fact that ChemDraw uses a different mathematical infrastructure. Nevertheless, ChemDraw produced results of a very similar pattern and gradient as Daylight and Molinspiration and its much higher ease of use in drawing structures justified ChemDraw as the chosen software package to calculate the tPSA of the datasets. Additionally, scattergraphs and the squares of correlation coefficients (R^2) were used to explore the linear and exponential relationships of each dataset.

A total of sixteen different datasets were analysed which produced low R^2 values, demonstrating that there is not a good correlation between PSA and anticancer activity. Most of the datasets showed an extremely poor correlation with a mean R^2 value for the linear and exponential relationship being 0.127 and 0.144 respectively. A wide range of R^2 values was produced and it was decided to investigate the triazolopyrimidine dataset further due to its high R^2 value and ease of synthesis compared to the other datasets. An analogue was synthesised and its relationship equation used to predict its anticancer activity.

In conclusion, this research proved that there is not a good correlation between PSA and anticancer activity, with the highest correlation, R^2= 0.759, found in the naphthoquinone amides dataset. The wide range and inconsistent R^2 values produced between different datasets show that a universal predictive tool is not likely. Furthermore, the difficulty in finding datasets with enough structural variety suggests that this method is not suitable for pharmacophore optimization but with the use of less closely related analogues it could be used for pharmacophore discovery.

1. Kwong E, *et al*. Strategies for bringing drug delivery tools into discovery. *International Journal of Pharmaceutics*. 2011;**412**;1–7
2. Prasanna S, Doerksen R J. Topological Polar Surface Area: A Useful Descriptor in 2D-QSAR. *Current Medicinal Chemistry*. 2009;**16;**21-41
3. Ertl P, Roahde B, Selzer P. Fast contribution of molecular polar surface area as a sum of fragment-based contributions and its application to the prediction of drug transport properties. *Journal of Medicinal Chemistry*. 2000;**43**;3714-3717.

Impact of a Consultation Skills Training Programme on Pharmacy Students' Perceived Confidence

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The role of the pharmacist increasingly requires the use of good consultation skills and therefore they need to be confident in their ability to conduct a consultation. The Cardiff School of Pharmacy and Pharmaceutical Science's consultation skills training programme in MPharm 4 utilises the Medication-Related Consultation Framework (MRCF)[1] during one workshop. The aim of the study was to examine the impact of the consultation skills training programme on MPharm 4 students' perceived confidence to conduct a consultation with a patient.

Two Confidence Rating Questionnaires (CRQs) were distributed to each MPharm 4 student who attended the consultation skills training workshop; one was completed before the workshop the other afterwards. The CRQ contained 12 items each describing a different ability related to consultation skills and was adapted from the validated questionnaire used in a study by Otter[2]. The CRQ had a four-part rating scale ranging from "No confidence" to "Very confident". Also a feedback form was given to all MPharm 4 students following receipt of the consultation skills training. It contained three open questions on positive aspects of the workshop, suggestions for improvement and any other comments. A further sample of MPharm 4 students were interviewed on a one-to-one basis. Questions asked were about their views on the consultation skills training programme, plus assessment and focussed on the main topics that emerged from the feedback form.

A total of 100 students attended the workshop. The response rates for the CRQ and feedback form were 93% and 96% respectively. A statistically significant increase in confidence was seen in each of the 12 items in the CRQ; with the greatest increase in confidence seen in the item "Ability to construct a pharmaceutical problem list" ($MD=-0.69$, $t=-8.51$, $p<0.001$). The main topics that appeared from the feedback form were; "wanting more practise scenarios" and "good practise for OSCA". These were also the main themes that appeared in the interviews; reasons behind the themes were explored. A need for extra scenarios in a more accessible and life-like format was identified among the students interviewed. Students also exhibited a considerable focus on assessment throughout interviews.

Findings from both quantitative and qualitative studies support the widespread increase in confidence levels after the consultation skills workshop. These findings are similar to a former study involving medical students[3]. The increase in confidence levels was concomitant with a major interest in assessment among the students interviewed, suggesting an engagement in the consultation skills training process due to the students' awareness of upcoming assessment. The CRQ was an effective tool to measure confidence levels and its validity was strengthened in this study. The feedback forms and interviews were successful in identifying areas where the consultation skills training programme could be developed in the future. Further research would ideally look at the effect the consultation skills training programme has on assessment results, and possibly investigate the long term effects the consultation skills training programme had on the confidence levels in conducting a consultation within different aspects of healthcare professions.

1. Abdel-Tawab R *et al*. 2011. Development and validation of the Medication-Related Consultation Framework (MRCF). *Patient Education and Counseling* 83(3), pp. 451-457.
2. Otter, S *et al*. 2003. An Evaluation of an Interdisciplinary Joint Clinical Placement between Podiatry and Pharmacy Students. *Pharmacy Education* 3(4), pp. 253-260.
3. Papageorgiou, A *et al*. 2011. Cross-sectional evaluation of a longitudinal consultation skills course at a new UK medical school. *BMC Medical Education* 11, pp. 55.

The Isolation and Characterisation of Novel Compounds from the Mediterranean Sponge *Aplysina aerophoba*

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Natural products have been one of the most successful sources of novel and innovative compounds to date. Research into this area is ever increasing, once again making natural products the focal point in the pharmaceutical industry.[1] Marine sponges are known to synthesize an array of diverse and novel compounds, with varying biological activity, as a mechanism of defence which is why research is being directed toward this field in particular.[2] The Mediterranean sponge, *Aplysina aerophoba* is known to synthesize brominated isoxazoline alkaloids, compounds which have shown antibacterial properties.[3] The aim of this research project is to extract, isolate and characterise novel compounds from this sponge to decipher whether *A. aerophoba* produces compounds other than the brominated species that have already been reported in the literature.

A small sample of the *A. aerophoba* sponge species was cut from a larger specimen and ground to a fine powder using a pestle and mortar. The sponge material was then exposed to a three stage solvent system and a hot methanol Soxhlet extraction. The residual crude extracts were subject to preparative thin layer chromatography (TLC) and column chromatography which resulted in the separation and isolation of several fractions. Based on the fractions recovered weights, seven were investigated furthers using ^{1}H NMR, electron impact (EI) mass spectrometry and electrospray (ES) mass spectrometry.

^{1}H NMR successfully characterised fraction 1Aa1 to be cholesterol, which was further confirmed using analytical TLC. This result was expected based on the biology of the marine sponges.[4] ^{1}H NMR also characterised fractions 1Aa2.2 and 3Aa3.4 to be plasticizer contaminants, belonging to a group of compounds known as the dialkyl phthalate esters. Contamination of such compounds can be explained by two theories; either contamination of the sponge habitat prior to collection or contamination by the plastic bag the sponge was being stored in post collection. ^{1}H NMR alone did not provide enough information for full characterisation of the remaining fractions and hence mass spectrometry was carried out. The resultant EI mass spectrometry and ^{1}H NMR spectra for fraction 3Aa3-Z suggests that this fraction is a mixture of two aromatic, non brominated compound with accurate masses of 230.1418 and 244.1786. However complete structure characterisation was not possible for these two compounds. The mass spectrometry data of fraction 1Aa2.1 was strongly predictive of clionasterol acetate, a sterol compound commonly synthesised and utilised by an array of different sponge species.[4] Finally, EI and ES mass spectrometry confirmed the remaining two fractions (3Aa3.1 and 3Aa3.2) to be mixtures of brominated compounds, all of which have molecular weights lower than 500. These compounds did not demonstrate characteristic molecular weights of the published structures of the brominated isoxazoline alkaloids *A. aerophoba* is known to produce, and thus it is assumed they do not belong to this group of compounds.

To conclude, unknown compounds can be successfully extracted and isolated from marine sponges using simple methods such as preparative TLC and column chromatography. Cholesterol, clionasterol acetate and two different dialkyl phthalate esters were extracted, isolated and characterised from the *A. aerophoba* species. A mixture of non-brominated and brominated compounds were also extracted and isolated, all of which had MW below 500. Despite the fact that full structure characterisation was not possible for these compounds, the brominated species were not characteristic of the brominated isoxazoline alkaloids *A. aerophoba* is known to produce, suggesting the extraction and isolation of novel low molecular weight brominated molecules from this particular sponge.

1. Harvey AL. *Natural products in drug discovery*. Drug Discovery Today. 2008; **13**(19-20):894-901.
2. Anand TP, *et al. Antimicrobial activity of marine bacteria associated with sponges in the waters off the coast of South East India*. Microbiological Research. 2006 Jul; **161**(3):252-262.
3. Haefnre B. *Drugs from the deep: marine natural products as drug candidates*. Drug Discovery Today. 2003 Jun; **8**(12):536-544.
4. Valentine F.R Jr, Bergmann W. *Contributions to the study of marine products. VIII. The sterols of sponges: Clionasterol and Poriferasterol*. Journal of Organic Chemistry. 1941. **6**(3):452-461

Determination of Tissue Calcium Concentration in the Isolated Heart Previously Treated with Hydrogen Peroxide and Tetracaine

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When myocardial ischaemia occurs due to occlusion of one of the coronary arteries, reperfusion is necessary to save viable tissue. Reperfusion however has been shown to result in injury additional to that caused by ischaemia alone.[1] It is thought that reperfusion injury is caused by a combination of raised intracellular calcium levels and oxidant stress resulting in cell death.[2] Hydrogen peroxide (H_2O_2) is used to simulate the oxidative stress seen in reperfusion injury.[3] Tetracaine is a known ryanodine receptor blocker and was used to look at the effect seen on tissue calcium levels when this channel is prevented from releasing calcium into the cytosol.[3] The primary objective of this study is to develop a method able to detect cellular levels of calcium using aequorin. The secondary objectives of this study were to determine if there is a rise in calcium levels in cardiac tissue following treatment of isolated hearts with H_2O_2 compared to that found in control hearts and also to determine if treatment with tetracaine along with H_2O_2 reduced the amount of calcium detected in cardiac tissue compared with H_2O_2 only treated hearts.

A novel method was developed to enable tissue calcium content to be determined using wild type aequorin. A luminometer was used to detect photon emission from the calcium-aequorin reaction. All samples were prepared by breaking down the heart tissue in nitric acid 68% and then neutralising with tris acetate 1M and potassium hydroxide 5M to pH 7.4. The resulting sample was filtered using 0.22 micron filters to minimise light absorption by particulate matter. HEPES buffer 25mM / EDTA10µM / gelatin 0.1%w/v and 20µl aequorin 1:1000 were added to the sample tubes to be placed inside the sample chamber of the luminometer. 500µl of sample was injected into each sample tube and counts per second recorded for 2 minutes. All samples were analysed in triplicate. The k sec^{-1} was calculated by plotting counts per second against time and dividing the x value taken from the equation of the line by log e. k sec^{-1} is proportional to $[Ca^{2+}]$ which may be determined by interpolation from a calcium standard curve.

The findings of this study were: Control samples appeared to have higher levels of calcium than H_2O_2 treated samples and H_2O_2 plus tetracaine treated samples appeared to have higher levels of calcium than H_2O_2 treated samples. However, no significant difference was found between any of the groups using a one way ANOVA followed by a Newman-Keuls post hoc test with $p<0.05$ considered significant.

The results recorded were the opposite of what was hypothesised would be seen. Literature references to studies looking at reperfusion injury look at the effects of ischaemia rather than oxidant stress, therefore direct comparison cannot be made. It is possible that simulating conditions of oxidant stress alone is not fully representative of the pathology of reperfusion injury, known to be a complex cellular event. The higher calcium levels seen in the tetracaine treated samples were unexpected. One explanation may be that it is myotoxic due to increased calcium levels.[4]

1. Burley, D.S., Ferdinandy, P., Baxter, G.F., 2007 Cyclic GMP and protein kinase –G in myocardial ischaemia-reperfusion: opportunities and obstacles for survival signalling. *British Journal of Pharmacology*., 152, 855-869.
2. Piper, H.M. *et al.* 2008 Sarcoplasmic reticulum-mitochondrial interaction in the mechanism of acute reperfusion injury. *Cadiovascular Research.,* 77, 234-236.
3. Andreou, D.E., 2011 The role of cardiac ryanodine receptors in hydrogen peroxide induced oxidative stress. MSc Dissertation, Cardiff University.
4. Zink, W., and Graf, B.M. (2004). Local anaesthetic myotoxicity. *Regional Anaesthesia and Pain Medicine*., 29 (4), 333-340.

The effect of storage conditions and Vitlipid® on parenteral nutrition lipids within syringes

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Parenteral Nutrition (PN) provides nutritional support via the intravenous route, where the oral or enteral route of feeding cannot be established.[1] PN admixtures usually consist of glucose, amino acids, lipids, vitamins, electrolytes and trace elements. Lipids within PN have a multifunctional role: they serve as a non-carbohydrate source of energy and as a medium for fat-soluble vitamins, another essential component of the admixture.[2] Usually, the vitamin additives are only added to admixtures shortly before use, as prolonged storage may result in physical instabilities and chemical degradation.[3] Vitlipid is an example of such an additive, containing a concentrated emulsion of lipid-soluble vitamins. Vitlipid contains Vitamin A, which is photosensitive and known to breakdown on exposure to light. It has therefore been used as a model in this study to monitor breakdown in the various storage conditions. The aim of the study is to investigate the effect of different storage conditions and the addition of parenteral nutrition additive, Vitlipid, on the stability of intravenous lipids (Intralipid® and SMOF®) within syringes, to assess the feasibility of standard ready-to-administer injectable products.

Fifteen 60 ml syringes were prepared aseptically in a laminar flow unit on Day 0. One of the following formulations was prepared each week: Intralipid, SMOF, Intralipid + Vitlipid and SMOF + Vitlipid. 10 ml from each syringe was removed daily (excluding weekends) and tested. Samples were placed in each storage condition and removed daily at the same time as required. Five conditions were tested: light, darkness, room temperature (18-25°C), incubation (37°C) and refrigeration (2-8°C). Syringes were monitored over a 7 day period (Day 0-Day 7). Changes in physical and chemical properties were assessed by laser diffraction, microscopy, pH testing, osmolality testing and HPLC. HPLC assays were conducted on Day 0 and Day 7 only, for purpose of comparison. Syringes in the light condition were stored on a window sill and dark condition syringes were covered in foil and placed on the same sill, to exclude temperature as a variable. Syringes for room temperature, incubation and refrigeration were placed on a laboratory bench, in a stability chamber and pharmacy refrigerator respectively.

All experimental conditions showed a similar trend in physical analysis but to differing degrees. Generally the pH of formulations decreased and particle size and number increased, more dramatically when Vitlipid was present. Osmolality testing demonstrated small changes, which were not deemed to be significant. The production of degradation products during storage may be responsible for the drop in pH recorded. This may have led to the loss of zeta potential between lipid globules, causing coalescence hence explaining the increase in lipid globule size. However, changes may be attributable to other factors such as the interaction of the sample with plastic from the syringes. The most significant changes in these parameters were seen in the incubation experiment and in the light experiment. The smallest changes were seen in the refrigeration experiment: the pH stayed relatively stable and there was no significant increase in particle size or number. Chemical analysis showed significant Vitamin A degradation in the light experiment, as Vitamin A is a photosensitive vitamin. It was more stable in Intralipid than in SMOF. Large breakdown of Vitamin A was also recorded in the room temperature and incubation experiments. This may be as a result of the lipid itself being less physically stable in these conditions or human error.

Storage conditions and the PN additive, Vitlipid, had a significant effect on the chemical and physical properties of Intralipid and SMOF kept within syringes. Exposure to light and increased temperatures were the two conditions that caused the greatest change. Refrigeration decreased rate of lipid degradation. Intralipid and SMOF showed similar stability patterns under the different storage environments, however upon addition of Vitlipid, stability of both decreased. The optimum condition to store intravenous lipids and lipids containing additives would be in a light-protected, refrigerated environment.

1. American Society for Parenteral and Enteral Nutririon.2011. *What is Parenteral Nutrition?* [Online]. Available at: http://www.nutritioncare.org/wcontent.aspx?id=270 [Accessed: 14 March 2012].
2. ESPGN. 2005. Vitamins. *Journal of Paediatric Gastroenterology and Nutrition* **41**(2), pp, S47-S53.
3. Allwood MC, Kearney MC.1998.Compatibility and stability of additives in parenteral nutrition admixtures. *Nutrition* **14**(9),pp, 697-7

Synthesis of Fluorinated FLT ProTides for Application in PET Imaging

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Positron emission tomography (PET) is a non-invasive nuclear imaging modality that uses compounds labelled with positron-emitting radionuclides to image tumours.[1] ^{18}Fluorine (^{18}F) is the most widely used radionuclide due to its practical half-life; however the high basicity and poor nucleophilicity of fluorine make its late-stage installation an on-going synthetic challenge.[1] The proliferation radiotracer [^{18}F]-FLT can image tumours with greater specificity than the 'gold standard' metabolic radiotracer [^{18}F]-FDG, but its poor cellular uptake by passive diffusion and rate-limiting monophosphorylation by Thymidine Kinase-1 must be overcome.[1] ProTides (pro-nucleotides) have potential to overcome these problems, but their use in PET is unexplored. Thus, the first novel aim of this study was to synthesise a [^{19}F]-FLT ProTide precursor, using either 2,3'-anhydrothymidine or 3'-*O*-nosyl-thymidine, as proof of concept for overcoming the limitations of [^{18}F]-FLT. In view of the poor nucleophilicity and high basicity of fluorine, the second aim of this study was to investigate the feasibility of fluoridating ProTide precursors, and address the key question of whether the fluoride ion binds to the electrophilic centre at the 3'-carbon to give the desired product, to the electrophilic phosphorous to cause ProTide decomposition, or forms side-products via E2 elimination.

2,3'-anhydrothymidine ProTide synthesis was first attempted using *N*-methyl imidazole as a base, in keeping with Van Boom's procedure.[2] Repeated failure of this method led to attempts at nosylation, in keeping with the wide-spread use of nosylates as FLT precursors.[1] Failure of nosylation led to application of *tert*-butylmagnesium chloride (^{t}BuMgCl) as the base following Uchiyama's method, and successful 2,3'-anhydrothymidine ProTide synthesis.[3] The first fluoridation test reaction was then performed at 120°C for 1-2 h using CsF (3.0 eq), kryptofix (0.5 eq) and anhydrous acetonitrile (15 ml) in keeping with current ^{18}F-radiolabelling trends.[1] Due to inconclusive results the solvent was changed to pyridine/acetonitrile (6ml: 6ml), but fluoridation still failed. 2,3'-anhydrothymidine and phenyl-(isopropoxy-L-alaninyl)-phosphorochloridate were thus fluoridated separately in acetonitrile at 120°C (1-2 h) and room temperature (30 min) respectively to better determine where fluoride binds.

2,3'-anhydrothymidine ProTide synthesis failed with NMI due to the base causing decomposition, but was accomplished with ^{t}BuMgCl due to the bulky butyl group blocking the 3'-carbon to facilitate nucleophilic attack of 5'-O^{-} at the electrophilic phosphorous centre. Results of the first fluoridation test were inconclusive due to the poor solubility of CsF in acetonitrile; moreover multiple ^{31}P NMR peaks showed the phosphorochloridate decomposed which suggests ProTide precursors will not survive ^{18}F-radiolabelling conditions. Unfortunately ^{1}H NMR results of the second fluoridation test only showed the presence of pyridine due to pyridine-mediated P-F bond cleavage and phosphorochloridate hydrolysis resulting in the loss of unexpected aqueously soluble products during pyridine extraction. ^{31}P NMR showed phosphorochloridate fluoridation led to spin-spin coupling between ^{19}F and ^{31}P. Unfortunately, ^{1}H NMR showed that 2,3'-anhydrothymidine fluoridation formed the elimination side-product 3'-deoxy-2',3'-didehydrothymidine – this is disappointing as the addition of K_2CO_3 to the kryptofix reaction medium will compound competitive elimination in [^{18}F]-FLT-PET radiolabelling practise. Molecular modelling with the human histidine triad nucleotide binding protein (hHINT-1) does however suggest that FLT ProTides are reasonable HINT substrates, should they be formed in future work, as two key interactions with His^{112} and Ser^{107} were formed even with un-optimised substituents, and binding could easily be improved by optimising the aryl, alkyl and amino acid ProTide moieties.

In conclusion, novel 2,3'-anhydrothymidine ProTides can be synthesised using ^{t}BuMgCl, however fluoridation results were inconclusive. Further testing and optimisation of fluoridation conditions are needed to determine whether fluoride can be encouraged to bind to 3', and thus whether fluoridating 2,3'-anhydrothymidine ProTides is a feasible option for addressing the physiological and synthetic limitations of [^{18}F]-FLT, and achieving the broader objective of improving PETs application in oncology.

1. Vallabhajosula, S., 2007 ^{18}F-Labeled positron emission tomographic radiopharmaceuticals in oncology: an overview of radiochemistry and mechanisms of tumor localization. *Seminars in Nuclear Medicine* **37**: 400-419.
2. Van Boom, J.H., *et al*. 1975 Phosphorylation of nucleoside derivatives with aryl phosphoramidochloridates *Tetrahedron* **31**: 2953–2959.
3. Uchiyama, M. *et al*. 1993 O-selective phosphorylation of nucleosides without N-protection. *Journal of Organic Chemistry* **58**, 373-379.

Accounting for the skin permeation of each component in a simple formulation casts light on the significance of the co-permeation effect in topical delivery

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The stratum corneum (SC) is generally considered to be the main barrier to delivering drugs across the skin.[1] Because of this, drugs which can be delivered by this route are limited to those with and suitable physico-chemical properties. To increase the permeation, and thus flux, of drug across the SC, permeation enhancers are used. Traditionally, they have been considered to work by causing a transient reduction in the barrier function of the SC following the classical theories of drug permeation. However, recent work has brought to light an alternative mechanism that may explain skin permeation enhancement through a simple comparison of the simultaneous permeation of the drug, vehicle and the enhancer. Drag/pull/co-permeation refers to the concomitant transport of drug and vehicle across the skin barrier, where the movement of the vehicle facilitates the movement of the drug.[2] The aim of this work was to analyse the permeation of all components of a topical formulation, in this case ethanol, caffeine and water, to rigorously investigate efficacy of the classical or co-permeation theories for drug delivery.

Saturated solutions of caffeine were prepared in a binary solvent mixture comprised of EtOH (0, 5, 10, 20 and 30%) and deuterium oxide (D_2O). Franz-type diffusion cells were used with full thickness skin, analysis was carried out over 24 hr with maximal loading. D_2O was used as a model for water to allow for the detection and analysis of water permeation via the use of NMR, EtOH analysis was carried out using head space gas chromatography and caffeine via HPLC.

Caffeine, EtOH and D_2O were all detected in the receptor phases. It was found that increasing the EtOH content of the vehicle from 0 to 10% had little effect on the flux of caffeine and ethanol. However, between 10 and 30% EtOH a linear increase in the flux of both caffeine and EtOH was seen. D_2O was able to permeate skin from 0% EtOH solution, with a substantial increase in permeation found at 10% EtOH. Between 10 and 20% EtOH concentrations, a decrease in D_2O permeation was found. The molar permeation of EtOH is less than that of the molar permeation of caffeine; however the molar permeation of D_2O is much greater than that of caffeine. The total molar permeation of vehicle (EtOH + D_2O) is always greater than the molar permeation of caffeine.

The overall picture that emerged from this work was unclear, as none of the theories relating to skin permeation were supported entirely. Interpretation of the data was hampered by a number of singlicate determinations. A more in-depth analysis of the permeation of caffeine, EtOH and D_2O is required to elucidate how each component is interacting with each other, in solution and during permeation. The variation of EtOH concentration needs to be more specific and the effect of caffeine concentration on the permeation of either solvent needs to be investigated. It is apparent that EtOH concentration greatly effects the permeation of both D_2O and caffeine; at low concentrations (5, 10%) greatly increasing D_2O permeation and at higher concentrations (10, 20 and 30%) greatly increasing caffeine permeation. The 10% concentration is a definitive point which varies the permeation of the drug and solvent, having greater than or lower than this concentration of EtOH has opposing effects on the permeation of caffeine and D_2O. This phenomenon has not been mentioned in the literature and does not follow any conventional or new models for the skin permeation of drugs and or solvents.

1. Williams, A C. 2003. Transdermal and Topical Drug Delivery. London: Pharmaceutical Press.
2. Heard, C M, D Kung, and C P Thomas. 2006. Skin penetration enhancement of mefenamic acid by ethanol and 1,8-cineole can be explained by the 'pull' effect. International Journal of Pharmacy., pp.167-170.

Molecular Modelling Simulations of Coxsackievirus B3 RNA-dependent RNA-polymerase

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Coxsackievirus B serotype 3 (CVB3) is a positive strand RNA virus which belongs to the *Picornaviridae* family. It is one of the most common aetiological causes of acute myocarditis in children and young adults, being prevalent in up to 32% of cases.[1] There are currently no approved treatments for coxsackievirus infections.[2] A novel inhibitory binding site has been discovered on the RNA-dependent RNA polymerase (RdRp) of CVB3. The discovery of compounds that interact with this site may have clinical benefits in the future for the treatment of acute myocarditis and other coxsackievirus related diseases. The aim of this study was to identify potential new inhibitors for CVB3 RdRp, by using molecular modelling and virtual screening.

The crystallised structure of CVB3 RdRp (PDB code: 3CDU [2]) was used as the target structure model in all simulations. This 3D structure allowed a filter query to be built directly in the virtual space of the binding site. Compounds from a compound library that fit this 3D query were then docked into the binding pocket using Glide. 19 compounds with a high docking score, or a good visual fit into the binding site were bought and sent for biological testing. To achieve a more accurate model of how the RdRp would behave *in vivo*, molecular dynamics simulations were ran. Although the general conformation of the protein before and after the simulation remained similar, some of the binding site residues changed their position and so both conformations were used in docking. Homology models for CVB4 and CVA10 were generated and the 19 test compounds docked in them, to predict whether they would present selectivity for CVB3.

The test compounds possess chemical diversity and a range of different docking scores, as some compounds were selected solely for their optimum positioning within the binding site. With the homology models docks, most compounds scores remained within a close range of their score for CVB3, suggesting that they may demonstrate activity across the coxsackieviruses. Some of the 19 test compounds did not dock successfully in the homology models suggesting that these may be more selective for CVB3.

The final 19 compounds selected are currently being biologically tested against CVB3. Particular compounds of interest are compound 1 which had the highest docking score in the original RdRp, compound 16 which scored the highest in the MD5 RdRp, and compound 28 which had the highest normalised score.

1. Kok, C.C., McMinn, P.C. 2009. Picornavirus RNA-dependent RNA polymerase. *The International Journal of Biochemistry and Cell Biology*. **41**, pp: 498-502.
2. Gruez, A. *et al*. 2008. The crystal structure of coxsackievirus B3 RNA-dependent RNA polymerase in complex with its protein primer VPg confirms the existence of a second VPg binding site on *Picornaviridae* polymerases. *Journal of Virology*. **82**(19), pp: 9577-9590.

Influence of Cycled Storage Conditions on the Performance of Salbutamol Sulphate Pressurised Metered-Dose Inhalers

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Asthma is a common condition in which pressurised metered-dose inhalers (pMDI) are used. The first line therapy for the management of asthma is short acting $beta_2$ agonists that are used when required to relieve acute exacerbations of asthma.[1] The most commonly used of these drugs is salbutamol, of which there are a number of brands and formulations including several pMDIs. Due to their use as relievers at unpredictable times, patients often store salbutamol inhalers in convenient locations. As found in previous studies, an inhaler can be exposed to changing conditions outside of its recommended storage limit during its lifetime.[2] Moisture ingress is known to be a major issue and can cause the increase the size of drug particles in the formulation, reducing clinical efficacy. A process known as Ostwald Ripening may cause this, in which smaller particles in a suspension dissolve and crystallise onto larger particles, causing an increase in particle diameter.[3] It is known to happen in aqueous suspensions, however it is unknown whether this process occurs in the non-aqueous suspension of a pMDI. The aim of this study was to investigate the impact of changing storage conditions on the performance of salbutamol pMDIs and to determine whether Ostwald Ripening is responsible.

Three salbutamol sulphate pMDI formulations were manufactured which contained different amounts of 96%v/v ethanol, to produce 2%, 8% and 16% w/w ethanol in HFA134a; to determine whether ethanol/water content would have an effect on the changes caused by storage. To simulate the storage conditions which a salbutamol sulphate pMDI may be subjected, the inhaler canisters were cycled every 2-3 days between 40°C/75% RH (relative humidity) and refrigerated at <5°C over the course of four weeks. The level of moisture ingress over the course of the storage time was monitored. This was carried out by measuring the amount of water contained in the formulations using a Karl Fischer Titration method. The aerodynamic particle size of the emitted aerosol from the inhalers was measured using a Next Generation Pharmaceutical Impactor and High Performance Liquid Chromatography with the Fine Particle Fraction (FPF), Mass Median Aerodynamic Diameter (MMAD) and Geometric Standard Deviation (GSD) being calculated as measures of aerosol performance. The total emitted dose, propellant loss and shot weight were also measured throughout the study period.

The results of this study showed that storage under cycled accelerated stress conditions initiated the ingress of water into all of the salbutamol sulphate pMDI preparations. However this had little effect on the aerosol performance of the pMDI formulations. The presence of the phenomenon Ostwald Ripening was not detected as although the GSD tended to decrease, especially at higher ethanol concentrations, the MMAD and FPF remained constant over the storage time. The influence of 96%v/v ethanol concentration on the aerosol performance was dramatic, e.g. FPF decreased from 65.6% to 38.8 to 23.8 as the ethanol concentration increased from 2% w/w to 8% w/w to 16% w/w respectively. This effect however was likely to be due to ethanol/water causing a decreased rate of propellant evaporation during aerosolisation rather than an effect on the size of salbutamol sulphate particles within the formulation.

From the results in this study it cannot be concluded that moisture ingress under cycled storage conditions does not initiate Ostwald Ripening in pressurised metered-dose inhalers, as the study period was very short compared to typical accelerated stability studies. It can however be concluded that Ostwald Ripening does not happen rapidly in this type of pMDI formulation. Further studies are required using longer storage times under accelerated stress conditions to draw definite conclusions on the role of Ostwald Ripening in suspension pMDI formulations.

1. Joint Formulary Committee. *British National Formulary,* 62nd edition. London: BMJ Group, London: Pharmaceutical Press, 2011.
2. W.L. Hoye, E.M. Mogallan, P.B. Myrdal, Effects of extreme temperatures on drug delivery of albuterol sulfate hydrofluoroalkane inhalation aerosols, *American Journal of Health-System Pharmacy* **62** (2005), 2271-2277.
3. J.H. Yao *et al*. Theory and simulation of Ostwald ripening, *Physical Review B* **47** (1993), 14110-14125.

Evaluation of the clinical and validation aspects of the Dermatitis Family Impact questionnaire (DFI)

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The Dermatitis Family Impact (DFI) questionnaire is a renowned disease-specific measure to assess the impact of atopic dermatitis (AD) on the quality of life (QoL) of parents and family members of affected children.[1] The DFI has been the subject of great interest in recent years, garnering international use and acceptance as a QoL measure, especially considering its casual introduction to practice. The aim of this project was to collect and review all the clinical and psychometric aspects of the Dermatitis Family Impact (DFI) questionnaire from its development in 1998 to 2011. In so doing, assembling all the dispersed information regarding the DFI so that a complete and up-to-date formal validation of the instrument can be made for the first time. This will help to bring together previous validation data to create a one-stop single source of reference to summarise the DFI and aid clinical decision-making.[2]

A detailed literature search was performed using the online bibliographic databases Pubmed and Google Scholar as well as a manual search through the Cardiff University Department of Dermatology library. Inter-library journal requests were made for inaccessible articles. All articles and abstracts published for studies using the DFI from its development in 1998 to the end of 2011 were identified for evaluation. The exclusion criteria included those articles published in a language other than English and without an accessible English translation. Relevant clinical and psychometric data were extracted from the publications for inclusion in the review.

In total, 48 publications were identified referencing the use of the DFI (32 full articles and 16 abstracts/summaries). Only one study demonstrated test-retest reliability for the DFI ($r = 0.95$).[3] Three studies demonstrated the internal consistency of the DFI with Cronbach's alpha values ranging from 0.85-0.90 showing the high correlation amongst the scores of the items. Fourteen studies showed sensitivity to change of the DFI scores with significant differences between baseline and the end of study. As of yet there are currently no validated score banding descriptors set for the DFI, however some studies have adopted their own approach to this issue. The DFI has been correlated to other objective and subjective instruments to demonstrate its construct (convergent) validity in 22 studies. Currently, there are 18 validated translations of the DFI that have been used in 15 different countries (including two multinational studies). The DFI has been used in nine clinical studies assessing the effectiveness of five different topical drugs, one probiotic supplement, and in two studies of the effectiveness of the care of dermatology nurses and dermatologists.

This evaluative review illustrates that the DFI is a robust instrument with proved validity that can accurately and reliably assess changes in the disease-state of AD through a familial construct. Although it does possess some unproved psychometric limitations in differential item functioning, factor structure and dimensionality. This review brings together previously scattered DFI validation data supporting its use in both clinical settings and research; highlighting areas requiring further validation. Interestingly, there appears to be some unapproved use of the DFI in various diseases other than AD despite the availability of more appropriate dermatology-specific measures.[4] More in-depth statistical involvement of the DFI in clinical practice would further consolidate the status of the DFI as a reliable measure of QoL.

1. Lawson, V. *et al*. 1998. The family impact of childhood atopic dermatitis: the Dermatitis Family Impact questionnaire. *British Journal of Dermatology*. **138(1)**, pp.107-113.
2. Beattie, P.E. and Lewis-Jones, M.S. 2006. An audit of the impact of a consultation with a paediatric dermatology team on quality of life in infants with atopic eczema and their families: further validation of the Infants' Dermatitis Quality of Life Index and the Dermatitis Family Impact Score. *British Journal of Dermatology*. **155**, pp. 1249-1255.
3. Lewis-Jones, M.S. *et al*. 2001. The Infants' Dermatitis Quality of Life Index. *British Journal of Dermatology*. **144(1)**, pp. 104-110.
4. Basra, M.K.A. *et al*. 2007. The Family Dermatology Life Quality Index: measuring the secondary impact of skin disease. *British Journal of Dermatology*. **156(3)**, pp. 528-538.

An investigation into the intravesical delivery of tamsulosin to the urinary bladder

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This project's purpose was to improve the data available for intravesical delivery. Intravesical delivery is the direct administration of a drug into the bladder, typically for the purpose of treating a localised urological condition.[1] Intravesical delivery has the potential to raise the efficacy of treatments while limiting systemic side effects. This project will set out to analyse the barrier properties of the urothelium[2] by using tamsulosin as a model drug in a series of permeability studies. Using an *ex vivo* bladder model the concentrations of drug permeating into the tissue will be monitored.

Bladder tissue was placed in Franz diffusion cells. They were then left in a warm water bath, for the various lengths of time used in the different experiments carried out. The Franz diffusion cells were then removed from the bath and the urothelium and lamina propria were then surgically separated from the underlying muscle layer and all tissue sections left in methanol to facilitate drug extraction over 24 hours. All experiments were variations of this basic model and at the end of the project some cell histology was carried out to confirm the method.

The results showed that tamsulosin could permeate across the bladder wall but in much lower amounts than the amount applied as the urothelium successfully stopped the majority of the drug (in one experiment for example 90.06% of the tamsulosin applied failed to cross the urothelium). Through the course of the project the K_p was calculated as 3.98×10^{-6} cm/ second at the steady state period of 1 -2.5 hours. The tamsulosin had a partition coefficient ratio, between muscle tissue and saline solution of 1:1.6 showing that tamsulosin preferred being in solution to tissue. The extraction method was shown to extract approximately 70% of drug in the first 24 hours for the muscle and almost 100% in the urothelium and lamina propria.

This study shows that tamsulosin was able to permeate across the urothelium at detectable levels. Although the majority of the drug tamsulosin by the end of a 90 minute period which is estimated to be the clinical dosing time[2] hadn't yet crossed through the urothelium and lamina propria into the detrusor muscles. Increases in time increased the permeation to up to 8%. The project shows that there is an opportunity to deliver tamsulosin intravesically whilst confirming the barrier properties of the urothelium.

1. GuhaSarkar S and Banerjee R. Intravesical drug delivery: Challenges, Current status, opportunities and novel strategies .*Journal of Controlled Release.*2010,148,pp147-159
2. Lewis S.A. Everything you needed to know about the bladder but were afraid to ask. *American Journal of Physiology Renal Physiology.* 2000,278,ppF867-F874
3. Beiko D.T. *et al.* Second prize: Double-blind randomized controlled trial assessing the safety and efficacy of intravesical agents for ureteral stent symptoms after extracorporeal shockwave lithotripsy. *Journal of endourology.* 2004, 18(8), pp723-730.

A Comparison between Human and Veterinary Nursing Education and Practice related to Wound Management

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It has long been common practice in veterinary wound management to use wound management materials designed for human use.[1] It is unclear whether there is sufficient training for both human veterinary nurses during their pre-registration courses to use these materials for the optimum benefit of their patients. There are 67 UK higher education institutions where "adult nursing" is a listed course. Entry to the register is controlled by the Nursing and Midwifery Council. Veterinary nurses can qualify through a vocational employment route or a degree level education at one of 14 higher education facilities. Entry to the veterinary nurse register is controlled by the Veterinary Nurse Council, a branch of the Royal College of Veterinary Surgeons. Both professional bodies issue learning objectives with regards to wound management which need to be covered throughout the respective courses, but these aims were found to lack the detail needed for this research project. The first objective was to identify what is taught at the respective training centres and observe practices to compare wound management in animal and human patient groups. The ultimate aim of the research was to investigate and evaluate wound management education, with the objective of possibly developing an academic training course suitable for both human and veterinary nurses.

In order to judge more objectively the courses available for undergraduate nurses and veterinary nurses, email contact was made with the registered institutions of education. Contact email addresses were obtained from University websites and an email requesting information on their wound management syllabus sent. This process was carried out for 61 out of 67 human nursing training centres, and all 14 veterinary nursing training centres. Observation at a human outpatient wound clinic was carried out. Data was recorded using a standard form. Animal case studies were sourced online from the veterinary wound library *(www.vetwoundlibrary.com)* following unsuccessful attempts at to observe wound management cases at a number of local veterinary practices, charities and Schools of Veterinary Science. Ethics consent was not required for information requests or veterinary patients, but was observed in the human wound clinic by the obtaining of verbal consent from the patient and anonymising patient data.

The response rates from the education and training centres were disappointingly low; 26 out of 67 (39%) of the human nursing schools replied with relevant data to requests for information in the time available. The results showed that topics covered over the three year of training varied from place to place. Themes common to all nursing schools were management of leg ulcers, dressing materials and wound assessment. There was a trend for the complexity of the topics to increase from years one to three, along with the independence of the nurses in their wound management competence. Reponses from the veterinary nurse training centres were too low for valid conclusions to be drawn, but the two of the 14 who replied showed a range of topics covered. Observation at a human wound clinic showed a variety of wounds and their management amongst seven patients. Having sourced an animal case study, comparisons could be made between the management of human wounds and that of the animal subject.

There were more differences than similarities found between the two types of nurses' approach to wound management education and training practices. Differences were rooted in the respective patient groups. Communication between human nurses and their patients is vastly more effective than that between a veterinary nurse and the patient. Common wounds seen in human patients are chronic, whereas the majority of animal wounds acute. These differences are reflected in the syllabi of each training course. Other differences found were the needs of the patient as a whole, such as psychological care in the human. Similarities included treatment aims for all wound healing and education on the wound healing process. The conclusions drawn from the information received would be more valid had there been a greater response rate. Overall the project highlighted areas where a mutual learning environment for both categories would be appropriate and benefit relevant patients.

1. Turner. T. 1997. Interactive Dressings used in the management of human soft tissue injuries and their potential in veterinary practice. *Veterinary Dermatology* **8**, pp. 235 – 242.

An Evaluation of the Administration of Subcutaneous Insulin at Mealtimes in Acute Inpatients at Betsi Cadwaladr University Health Board and of the Associated Knowledge and Understanding of Nursing Staff

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Insulin as a treatment for diabetes is frequently prescribed within the hospital. However, when used incorrectly, it is also a medicine that can cause harm to patients.[1] With the increasing number of new insulin preparations available today, it has been reported that nurses have insufficient knowledge on insulin therapy.[1] This study aims to evaluate whether in-patients at BCUHB receive their insulin during the correct time frame, and to explore the knowledge and confidence of nursing staff on the subject of insulin administration at BCUHB.

Multiple (triangulated) research methods were used at both Glan Clwyd and Wrexham Maelor Hospital. Non-participant observations of the administration of soluble or short-acting analogue insulins in relation to meal times took place. Data collected was input into SPSS®, were descriptive statistics were calculated. Eighty-two short, fact-based, multiple-choice questionnaires were distributed to nursing staff at both sites. Data collected from questionnaires was input into SPSS® and descriptive statistics obtained. Four one-to-one, semi-structured exploratory interviews were conducted using 'purposive', 'convenience' and 'snowball' sampling methods in combination. The interviews were audio recorded, transcribed and the data analysed using 'Content Analysis'.

Out of 75 observations, 45% of the insulin administrations were given at the incorrect time. When patient administrations were compared with nurse administrations, it was found that out of the 44 correctly administered observations, only 18 were administered by a nurse. Of the 82 questionnaires distributed, 51 were completed, giving a response rate of 62%. The majority of the respondents who completed the questionnaire understood how insulin should be correctly prescribed, however only 51% knew that insulin could be stored at room temperature for up to four weeks. When asked to match correctly five types of insulin with their duration of action only 14% matched correctly all five types, making this question the most poorly answered. When tested on when NovoRapid®, NovoMix® and Humulin M3® should be given in relation to meal times, 71%, 53% and 35% respectively knew the correct administration times. Questionnaire scores were recorded out of a possible score of 11; 6 was the mean, mode and median score. All respondents who had completed the e-learning training package in January 2012 had a score above the overall mean. The interviews strengthen the findings of the questionnaire by supporting the fact that nurses have a lack of knowledge with regards to the different type of insulins available. The interviewees all confirmed that additional training on insulin treatment would be beneficial to nursing staff, with the majority having the opinion that an informal approach would be most suitable, such as an e-learning training package.

It can be concluded that at BCUHB, a substantial number of diabetic patients included in the study received their insulin at the incorrect time. The questionnaire results demonstrate that nurses have a deficient knowledge regarding insulin types and when each type of insulin should be administered, and these results are reinforced by the answers provided from the interviewees. As insulin has a narrow therapeutic window, it is of great importance that nurses are competent in administering all types of insulin, in order to prevent frequent occurrences of hypo- and hyperglycaemic events.[2] The results from this study have exposed gaps in nurses' diabetic knowledge and it is therefore recommended that nurses receive further education, within an informal setting, such as an e-learning package. Additional training would not only benefit nurses, but also their patients, undoubtedly helping to improve their standard of care.

1. Trepp R, *et al. Diabetes-related knowledge among medical and nursing house staff.* Swiss Med Wkly. 2010; **140** (25-26):370-5.
2. Clement S. *Better glycemic control in the hospital: beneficial and feasible.* Cleve Clin J Med. 2007; **74** (2):111-2, 4-20.

Measuring patients' self-medicating beliefs and behaviours in response to symptoms of a cough, cold or flu

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On average, an adult has between two and four colds a year [1] and as a result, the symptoms of a cold are a common presenting complaint in a community pharmacy. It is important that we have an understanding of why a patient self-medicates in the way that they do, yet this is an area which lacks published research. Previously, work has been conducted to develop a questionnaire to measure patients' self-medicating beliefs and behaviours. This self-medication scale (SMS) was designed based on qualitative studies and tested on students' responses to the symptoms of pain [2] and self-medication with analgesics. The aim of this study was to explore patients' self-medicating beliefs and behaviours in response to the symptoms of a cough, cold or flu with view to modifying the SMS for use in this area.

The qualitative method of choice was face-to-face semi-structured interviews as this enabled exploratory research. Participants were chosen based on purposive and snowball sampling to achieve a wide range of demographics. The participants were selected on the basis of the following sampling framework: gender, occupation, ethnicity and whether they had experienced symptoms of a cough, cold or flu in the preceding six months. The interview schedule was designed based on those used in previous qualitative studies.[2] Following ethical approval, the interviews were carried out, audio-taped, transcribed verbatim and then thematically analysed.

Fifteen interviews were conducted and from the analysis of these interviews, eleven broad themes were identified, (with a number of sub-themes to represent each one). These were: 1) Specific symptoms experienced, 2) Response to these symptoms, 3) Length of response, 4) Reason for response, 5) Prevention approaches, 6) Beliefs about medication, 7) Health-seeking behaviour, 8) Self-medication, 9) Influences, 10) Recommendations and 11) First port of call. The ability to identify the various ways patients respond and their reasoning for their particular response helps to explain the self-medication behaviour. Based on these findings, the original SMS was modified to be relevant to the symptoms of a cough, cold or flu. Specific self-medicating beliefs and behaviours matched the original three sub-scales of the SMS, which were 'reluctance', 'don't think twice' and 'run its course' and each sub-scale consisted of three items.

These qualitatively-derived statements about coughs, colds or flu have enabled the original self-medication scale to be modified, so that patients' self-medicating beliefs and behaviour in response to these symptoms can be measured. Further work needs to be carried out to test the psychometric properties of the self-medication scale in the context of coughs, colds and flu.

1. NHS Choices. Common Cold. 2011 [accessed 20 Mar 2012]. Available from: http://www.nhs.uk/conditions/cold-common/pages/introduction.aspx.
2. James DH, French DP. The development of the Self-Medicating Scale (SMS): a scale to measure people's beliefs about self-medication. *Pharmacy World Science*. 2008;**30**:794-800.

An evaluation of shared care arrangements between a Medical Centre and a University Health Board

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A shared care arrangement is an agreement between a hospital consultant and a General Practitioner (GP) to jointly care for a patient with a chronic disease.[1] The regular monitoring of these patients used to take place in a hospital setting. However with the emergence of shared care arrangements, both the monitoring and prescribing for these patients can now take place in primary care. This type of shared care arrangement is called 'Near Patient Testing (NPT)'.[2] Very few studies have evaluated these NPT shared care arrangements. This study evaluates the NPT shared care arrangements between a Medical Centre (MC) and a University Health Board (UHB). The aim of this project was to investigate if MC is adhering to the NPT Shared Care protocols and to determine what the issues are with the current system. The objectives were to explore the views of health care professionals working on each side of the arrangement. To determine the number of patients at MC that are prescribed medication through a NPT shared care arrangement and to determine what proportion of these patients are receiving the care that is outlined in the protocols. Finally to determine the reasons why the NPT shared care protocols are not being adhered to and to make recommendations for improvements.

Two key informant interviews were arranged. After appropriate literature was looked at, a semi- structured 20 minute interview schedule was written and carried out, findings were summarised.[3] Quantitative data collection at MC was performed in which patients' notes were accessed in order to identify whether patients had received the appropriate monitoring outlined in the protocols. Requirements such as the frequency of certain blood tests and action taken on notification of an out of range result were assessed. The data was organised in summary tables and a descriptive analysis was carried out.

From the interviews, it was found that there are still problems with the communication in a shared care arrangement. When patients miss appointments this is a barrier to the efficient operation of these arrangements. From the quantitative data collection it was found there were 57 patients and 7 drugs which were part of the NPT. Fourteen out of the 57 patients eligible for the study were receiving monitoring that was following the protocols completely. The monitoring of 2 of the 7 NPT drugs being prescribed at MC were being carried out well. The monitoring of the other NPT drugs needs greater attention; blood tests weren't being carried out at the correct frequency and in some patients, certain tests weren't carried out at all. The action taken on notification of an out of range result was also sometimes not following the protocol. In addition blood pressure and weight weren't measured often enough in the majority of patients that required these measurements.

In order to improve these arrangements, the researcher puts forward that better communication between the GPs and specialists is required and that patients need to be better educated on how a shared care arrangement operates. The main finding of the quantitative data collection was that the majority of patients were receiving the correct monitoring but not necessarily at the correct frequency. A similar finding was seen in a study by McGhee *et al*.[4] Presentation of the results to the team at MC and informing them of the amendments to the computer warnings required is thought to improve the arrangements. All five objectives of the study were met; the limitations of the study were that the researcher didn't document the individual GP that wasn't engaging in contact with the specialist. Further work would be to interview more GPs at the medical centre to attain what they think are the cause of the issues and any suggestions they have in which improvements could be made. The researcher concludes that if the improvements proposed are implemented then this will increase protocol adherence.

1. Horne, R. *et al*. 2001. Shared care: a qualitative study of GPs' and hospital doctors' views on prescribing specialist medicines. *British Journal of General Practice* **51**: 187-193
2. Bro Taf Localities Drugs and Therapeutics Committee. *Shared Care and Near Patient Testing. General Guidance*. 2012. [Online] Welsh Medicines Information Centre Available at: http://www.wmic.wales.nhs.uk/shared_care.php. [Accessed 18 April 2012]
3. Oppenheim, A. N. 1992. *Questionnaire design, interviewing and attitude measurement*. New Ed. London: Continuum.
4. McGhee *et al* 1994 Coordinating and standardizing long-term care: evaluation of the west of Scotland shared-care scheme for hypertension. *British Journal of General Practice,* **44**: 441-445

The role of caveolae-mediated endocytosis in Alzheimer's Disease

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Alzheimer's Disease (AD) is a form of dementia that results in the progressive loss of memory and decreasing cognitive function. The deposition of β-amyloid (Aβ) is a known causative factor of AD and it is formed by cleavage from amyloid precursor protein (APP) by secretases.[1] Caveolae are a specialised form of lipid raft involved in endocytosis which contain the proteins and machinery required to form the toxic Aβ fragment.[2] Moreover, altered endocytosis has been shown to precede the deposition of Aβ.[3] Finally, Caveolin-1 (Cav-1), a major protein in the normal functioning of caveolae, has been shown to be upregulated in AD brains.[4] Thus the aim of the current research was to examine whether altering levels of caveolins by overexpressing Cav-1, would lead to changes in the processing of APP to Aβ and/or surrounding proteins.

Cells cultured for the present study were human astrocytoma cells, MOG-G-UVW (MOGs). Cav-1 overexpression (O/E) was achieved by the introduction of a plasmid containing the Cav-1 gene. O/E was shown by immunocytochemistry. Western blotting was also used to confirm O/E of Cav-1 and then to examine the levels of Cav-2, Cav-3 and APP. Flotillin-1 is a marker of non-caveolar endocytosis and is found in neurons. Western blotting was used to determine whether Cav-1 O/E affected this separate form of endocytosis. ELISAs were performed to examine levels of APP and $A\beta_{40}$.

Cav-1 O/E was successful as shown by Western blotting. Immunocytochemistry revealed that O/E was only achieved in around 10% of transfected cells. Cav-1 O/E had no effect on the levels of Cav-2, Cav-3 or the non-caveolar protein, Flotillin-1 compared to the media control. Similarly, neither APP nor $A\beta_{40}$ levels were affected by Cav-1 O/E as detected by the methods above.

O/E of Cav-1 did not affect the levels of other caveolins or the non-caveolar protein Flotillin-1. Similarly, neither APP levels nor its processing to $A\beta_{40}$ were altered by overexpressing Cav-1. The literature reports that caveolae may be involved in changes that occur in the brain in AD. The present study has shown that these alterations are unlikely to occur as a result of a sudden increase in the expression of the Cav-1 protein. While previous studies indicate that a certain level of Cav-1 is needed for the processing of APP to Aβ by caveolae, the current study indicates that Cav-1 O/E does not affect this processing.

1. Hardy, J. and Allsop, D. 1991. Amyloid deposition as the central event in the aetiology of Alzheimer's disease. *Trends Pharmacol Sci* **12**: 383-388.
2. Cohen, A. W. *et al.* 2004. Role of caveolae and caveolins in health and disease. *Physiol Rev* **84**: 1341-1379.
3. Cataldo, A. M. *et al.* 2000. Endocytic pathway abnormalities precede amyloid beta deposition in sporadic Alzheimer's disease and Down syndrome: differential effects of APOE genotype and presenilin mutations. *Am J Pathol* **157**: 277-286.
4. Gaudreault, S. B. *et al.* 2004. Increased caveolin-1 expression in Alzheimer's disease brain. *Neurobiol Aging* **25**: 753-759.

Bevacizumab Use in Patients with Advanced Pancreatic Cancer: A Systematic Review

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The prognosis for advanced pancreatic cancer is very poor, most patients capitulate their disease in a year or so. The clinical management of this disease is mainly palliative because most about (80%) of the patients present with a metastatic disease. Gemcitabine is the treatment of choice where systematic chemotherapy is applicable.[1] Chemotherapy modestly improve median survival compared to supportive care alone. The incidence and mortality rates of this disease are nearly identical reflecting the malignancy of pancreatic cancer and how insufficient current therapies are.[2] Targeted therapies have shown promising results in trials[3] and Bevacizumab is one of them. The objective of this study was to assess the effectiveness of Bevacizumab in advanced pancreatic cancer.

Randomised controlled trials of Bevacizumab use in pancreatic cancer were identified in Medline, Embase and Cochrane Central Register of controlled Trials (CENTRAL). Then the titles and abstracts from the electronic search were screened and only randomised controlled trials that fitted our inclusion and exclusion criteria were selected. The information retrieved from data extraction was analysed in Review manager 5.[4] The outcomes of interest included overall survival, progression free survival and adverse events.

Identified studies enrolled a total of 1209 patients, addition of Bevacizumab to standard chemotherapy did not increase overall survival Hazard ratio (HR) 0.97, 95% confidence interval (CI) 0.86-1.10, P =0.63 but it had a statistically significant positive effect on progression free survival HR=0.75, CI=0.66-0.85 and P< 0.00001. The adverse event profile of Bevacizumab and existing treatment regimens was found to be the same.

The combination of Bevacizumab to chemotherapy is an effective therapy for prolonging disease free survival with a similar adverse event/toxicity profile to the current treatment regimens.

1. National Institute for Health and Clinical Excellence (NICE). Guideline on the use of gemcitabine for the treatment of pancreatic cancer. Technology appraisal guidance no. **25**. London: NICE; 2001.
2. American Cancer society. Pancreatic cancer http://www.cancer.org/Cancer/PancreaticCancer/DetailedGuide/index
3. H. Hurwitz, *et al*. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer . *N Engl J Med*, **350** (23) (2004), pp. 35–2342
4. Review Manager 5. Available at: http://ims.cochrane.org/revman

Evaluation of the clinical and validation aspects of the Infant Dermatitis Quality of Life (IDQOL) Index

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The IDQOL index is a questionnaire completed by parents that measures the impact that Atopic Dermatitis (AD) has on the quality of life (QOL) of infants aged between 0-4 years.[1] The aim was to collect and review all clinical and psychometric data on the use of the IDQOL index from its inception in 2001 until 2011, to serve as a single reference source.

A detailed literature search was carried out using the Cardiff University Dermatology Department library and Medline, PubMed and Google Scholar. Articles and abstracts that described the clinical use of the IDQOL index and those that investigated its psychometric properties, were included. Articles not in English were excluded. Data were extracted and tabulated using pre-designed templates.[2]

Thirty-five articles were identified of which 32 fulfilled the inclusion criteria. Five aspects of the IDQOL index were studied: psychometric, descriptive, clinical practice research, drug trials and therapeutic interventions. The IDQOL index has been translated into 15 languages and used in 14 countries including in two multinational studies. Twenty-five of these studies demonstrated psychometric properties such as test-retest reliability, validity, responsiveness to change and interpretability, though there is no valid score description system yet published. No studies investigated the dimensionality and internal consistency of the tool. Eight studies used the IDQOL index to assess the effectiveness of therapeutic interventions such as education programmes, consultations and wet-wrap therapy.[3] No studies focused on its use in clinical practice research. Six studies have used the IDQOL index in drug trials.[4] The IDQOL index has been used simultaneously with other instruments such as the Dermatitis Family Index and a severity assessment tool in most studies.

This review has demonstrated that IDQOL index is a reliable and valid measure that has been used extensively worldwide. This unique review has the potential to serve as a single reference source allowing potential users of the IDQOL index to make an informed decision regarding its use in their clinical studies.

1. Lewis-Jones M, *et al*. 2001. The Infant's Dermatitis Quality of Life Index. *Br J Dermatol* **144:** 104-110
2. Basra M, *et al*. 2008. The Dermatology Life Quality Index 1994-2007: a comprehensive review of validation data and clinical results. *Br J Dermatol* **159**: 997 – 1035
3. Beattie P, Lewis-Jones M. 2006. An audit of the impact of a consultation with a paediatric dermatology team on QOL in infants with AD and their families: further validation of the IDQOL and DFI score. *Br J Dermatol* **155:** 1249-1255
4. Msika P, *et al*. 2008. New emollient with topical corticosteroid-sparing effect in treatment of childhood AD: SCORAD and QOL improved. *Pediatric Dermatology* **25(6**): 606-612

Vasoconstrictor responses of aorta to amphetamine, β-phenylethylamine and 2-(2-chlorophenyl)-ethylamine

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The main trace amines β-phenylethylamine, tyramine and octopamine are endogenous compounds formed in trace amounts as a product in the body during metabolism of amino acids and along the biosynthesis pathway of other biogenic amines.[1] They cause vasoconstriction and increase blood pressure via indirectly sympathomimetic action (ISA).[2] There is also evidence that they may exert their action through a novel receptor system, trace-amine-associated receptors (TAAR).[3] This study examined whether the vasoconstrictor responses of isolated aorta to amphetamine, β-phenylethylamine (β-PEA) and 2-(2-chlorophenyl)-ethylamine (C-PEA) is mediated by ISA or the TAAR system. To meet the aim, experiments were performed in the presence and absence of the α_1-adrenoceptor antagonist, prazosin.

Isolated thoracic aorta was cut into 4-8mm of length to set up in a 50ml organ bath. Two hooks were passed through the lumen on the ring. The fixed hook was secured and mobile hook was connected to a transducer to retrieve the contractions. Concentration response curves (CRCs) were constructed in the absence and presence of prazosin either cumulatively or non-cumulatively depending upon the drug type. The contractions were recorded at the plateau of every peak response. The values were expressed as a percentage of the contraction to the either its own maximum or 1st curve and the mean responses (±SEM) were taken (n= 4, or 5). The EC_{50} values for individual experiments were converted to negative log molar EC_{50} (pD_2) and mean (±SEM) was taken. Paired or unpaired Student's t-test was employed for statistical analysis of pD_2 values.

Vasoconstrictor responses were not significantly affected by the presence of prazosin for β-PEA, C-PEA and amphetamine. Some inhibition was observed in responses for amphetamine and C-PEA but no substantial modification was present. Rate of onset of the vasoconstriction seemed to be slightly slowed by the presence of the antagonist for β-PEA and C-PEA and unaffected for d-amphetamine. The potency order according to pD_2±SEM in the presence of prazosin was D-amphetamine>β-PEA>C-PEA.

The vasoconstrictor effect in aorta rings by d-amphetamine, β-PEA and C-PEA does not appear through indirect sympathomimetic activity. Some inhibition with prazosin seen in the responses of amphetamine and C-PEA indicates that there may be a small component of α_1-adrenoceptors being activated. The major response, however, is mediated via the novel receptor system TAAR because the expected inhibition by prazosin was missing. Prazosin also slowed the rate of onset of action of β-PEA and C-PEA and thus, the efficacy of the two were reduced, whereas, it was unaltered for d-amphetamine. Also, the potency for C-PEA differed from that reported in the cloned TAAR receptors.[4] Thus, these amines do not cause vasoconstriction by an indirect sympathomimetic action.

1. Kim, K. and Zastrow, M. V. (2001). Old Drugs Learn New Tricks: Insights from Mammalian Trace Amine Receptors. *Molecular Pharmacology*. 60 (6), pp.1165-1167 [online]. Available at: http://molpharm.aspetjournals.org/content/60/6/1165.full [Accessed 01/02/2012]
2. Bunzow, J. R. *et al.* (2001). Amphetamine, 3,4-Methylenedioxymethamphetamine, Lysergic Acid Diethylamide, and Metabolites of the Catecholamine Neurotransmitters Are Agonists of a Rat Trace Amine Receptor. *Molecular Pharmacology*. 60 (6), pp.1181-1188 [online]. Available at: http://molpharm.aspetjournals.org/content/60/6/1181.full [Accessed 03/02/2012]
3. Borowsly B. *et al.* (2001). Trace amines: Identification of a family of mammalian G protein-coupled receptors. *PNAS*. 98 (16), pp.8966-8971 [online]. Available at: http://www.pnas.org/content/98/16/8966.full [Accessed 01/02/2012]
4. Wainscott, D. B. *et al.* (2006). Pharmacologic Characterization of the Cloned Human Trace Amine-Associated Receptor1 (TAAR1) and Evidence for Species Differences with the Rat TAAR1. *The Journal of Pharmacology and Experimental Therapeutics*. 320 (1), pp.475-485 [online]. Available at: http://jpet.aspetjournals.org/content/320/1/475.long [Accessed 07/02/2012]

The Role of Hydrogen Peroxide Induced Oxidative Stress on Ryanodine Receptor Mediated Calcium Release and Apoptosis in the Myocardium

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Reperfusion injury is the paradoxical injury caused by reperfusion of ischaemic tissue, as seen when restoring blood flow to previously ischaemic left ventricular myocardium in the clinical treatment of acute myocardial infarction (AMI). There are several types of injury, one of which being lethal reperfusion injury (LRI). LRI is cell death that is specifically caused by reperfusion, rather than the period of ischaemia preceding it. Necrosis is the main form of cell death produced, yet apoptosis has also been found to be present. Apoptosis in ischaemia/reperfusion injury is less well understood and provides a potential target to limit infarct size. Reactive oxygen species (ROS) are generated at reperfusion and have been implicated in both necrotic and apoptotic cell death. Cytosolic calcium overload is important in the induction of apoptosis, and ROS have been implicated in causing this calcium overload. In this study, Langendorff perfusion with hydrogen peroxide (H_2O_2) was used to promote oxidative stress, mimicking reperfusion conditions, in order to investigate induction of apoptosis. Tetracaine, the ryanodine receptor (RyR) inhibitor, was also used to investigate the contribution of calcium release from the endoplasmic/sarcoplasmic reticulum (ER/SR) via the RyR. It was hypothesised that, following perfusion of myocardium with H_2O_2 as a model of oxidative stress, there is an increase in caspase activation and inhibition of the ryanodine receptor with tetracaine will inhibit the rise in intracellular calcium, thus reducing caspase activation. To this end, the activation of both caspase-3 (executioner caspase) and caspase-9 (initiator caspase) was used as a biomarker to determine the effect of oxidant stress, and the role of the RyR, on myocardial apoptosis.

Four groups were studied, including a naïve tissue control (n=7) and three Langendorff heart perfused groups (20 mins stabilisation, 45 mins treatment): Krebs-Henseleit buffer time-matched control (n=6); 75 µM H_2O_2 (n=11); 75 µM H_2O_2 + 100 nM tetracaine (n=8). Following this, the left ventricles were removed and frozen. Caspase-3 and caspase-9 specific activity was then assessed using colorimetric assays; the activated caspases cleave DEVD-pNA and LEHD-pNA respectively, with the released *p*-nitroaniline producing a yellow colour proportional to caspase activation. Data are mean ± SEM.

Caspase activation was measured as pmol pNA liberated.hour^{-1}.µg protein^{-1}. 45 minutes of perfusion with 75 µM H_2O_2 did not result in statistically increase caspase-3 specific activity compared to time-matched control (3.2 ± 0.1 v 4.1 ± 0.1), but a significant increase in caspase-9 specific activity was observed compared to time-matched control (61.6 ± 2.8 v 44.9 ± 3.5, $P<0.01$). Perfusion with tetracaine alongside H_2O_2 produced no statistically significant attenuation of caspase-3 activation compared to H_2O_2 perfusion alone (3.1 ± 0.2 v 3.2 ± 0.1), nor any statistically significant attenuation of caspase-9 activation compared to H_2O_2 perfusion alone (66.7 ± 3.3 v 61.6 ± 2.8).

These results show no direct evidence of oxidant stress-induced apoptosis, due to the lack of caspase-3 activation. However, the significant activation of caspase-9, an upstream initiator of the intrinsic apoptotic pathway, following H_2O_2 perfusion suggests that the intrinsic apoptotic pathway was activated. There was no evidence that inhibition of calcium release from the ER/SR, by using tetracaine to block the ryanodine receptor, attenuated oxidant stress-induced apoptosis.

An Investigation into Alternative Synthetic Routes to Antitumour 2-Arylbenzothiazoles

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The ability of the 2-arylbenzothiazole to interact with a variety of biological receptors designates it a 'privileged pharmacophore'. This encompasses various anti-tumour targets, epitomised by the clinical trial agent *Phortress*, a tumour activated prodrug with selectivity attributable to CYP450 1A1 induction via arylhydrocarbon receptor binding. The widespread application of 2-arylbenzothiazoles makes it unsurprising that a plethora of synthetic routes, using both conventional and microwave heating, exist. Many routes require condensation of carbonyl derivatives with, unstable and unsubstituted, *o*-aminothiophenol.[1] To overcome these limitations, this reagent has been replaced by its substituted dimer, 2-aminophenyl disulphide, to synthesise 2-arylbenzothiazoles on reaction with benzaldehydes, in two synthetic methods.[1,2] The catalogue of 2-arylbenzothiazoles produced is therefore limited to commercially available substituted benzaldehydes. To expand this library, the viability of benzaldehyde replacement with alternative carbonyl derivatives in the method developed by Weekes[2] was investigated. Additionally, as 4-aminobenzaldehyde is not commercially available, 2-(4-aminophenyl)benzothiazole synthesis from 4-acetamidophenyl benzaldehyde and subsequent 'acetamido' group hydrolysis was also considered.

Arylcarbonyl (1 eq.), 2-aminophenyl disulphide (0.86/0.88 eq.) and sodium metabisulphite (1 eq.) were added to DMSO (5ml), stirred at 120°C and reaction monitored for completion by TLC analysis.[2] Product identification was made by ^{1}H-NMR spectroscopy and purification by column chromatography. 2-(4-acetamidophenyl)benzothiazole was synthesised and acetamido group cleaved by addition of c.HCl (0.25ml/1mmol) and ethylene glycol (0.75ml/1mmol) under reflux,[3] to access 2-(4-aminophenyl)benzothiazole. Optimisation of this cleavage was attempted by 3-fold increase in reagents and purification step removal. *In situ* cleavage by replacement of the final water extraction in 2-arylbenzothiazole synthesis, with c.HCl, was also investigated.

2-(4-methoxyphenyl)benzothiazole was synthesised from aldehyde only (42% yield), whilst mixtures of starting materials remained where carboxylic acids, ester and acyl chlorides replaced aldehydes. 2-(4-acetamidophenyl)benzothiazole was synthesised from 4-acetamidobenzaldehyde in 1.5, 3 (34% yield) and 7 hour reactions but 2-aminophenyl disulphide was identifiable in all crude products. Acetamido group cleavage produced 2-(4-aminophenyl)benzothiazole from both standard (9% yield) and optimised methods (10% yield) but was inaccessible from *in situ* cleavage, where starting materials remained. The optimised method was transferable to fluorinated disulphides to produce 6-fluro-2-(4-aminophenyl)benzothiazole (16% yield).

2-arylbenzothiazoles can be synthesised by reaction of 2-aminophenyl disulphide with benzaldehydes, in Weekes' method,[2] but not with alternative carbonyls. It is hypothesised that the α-hydrogen, provided by aldehydes, donates an electron for thioamide bond formation and subsequent disulphide bond cleavage in the 'dimer' intermediate. This step is not required when *o*-aminothiophenol is used, hence the accessibility of 2-arylbenzothiazoles from carboxylic acids on reaction with the monomer. 2-(4-acetamidophenyl)benzothiazole synthesis from 4-acetamido benzaldehyde was suboptimal due to an excess of 2-aminophenyl disulphide, a 25-50% reduction of which could improve efficacy of future synthesis. 2-(4-aminophenyl)benzothiazole synthesis is achievable by acetamido group cleavage, and transferable to fluorinated benzothiazoles, but at low yields. Furthermore, the increased yield expected from method 'optimisation' was not realised therefore longer reaction times, particularly of the cleavage step may be required. Reduction of 2-(4-nitrophenyl)benzothiazole, synthesised by Weekes' method,[2] could result in yields of 2-(4-aminophenyl)benzothiazole superior to those in literature reports, therefore would provide interesting further work.

1. Weekes AA, Westwell AD. 2-Arylbenzothiazoles as a Privileged Scaffold in Drug Discovery. *Curr. Med. Chem.* 2009;**16**:2430-2440.
2. Weekes AA. Synthesis of 2-Arylbenzothiazoles with Known and Potential Therapeutic Activity [PhD thesis]. Cardiff: Cardiff University;2010.
3. Peng Y, *et al.* Highly Efficient *N*-Monomethylation of Primary Aryl Amines. *Chin. J. Chem.* 2009;**27**:1339-1344

The Effects of Local Anaesthetics on the Mechanosensitive Channel of Large Conductance

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Bacterial mechanosensitive (MS) channels are gated by mechanical forces and are essential in bacteria. When a bacterial cell is subject to hypoosmotic shock these channels can detect the increasing turgor pressure and open to release osmoprotectants, which relieves the pressure and prevents cellular damage.[1] The mechanosensitive channel of large conductance (MscL) is one of several subtypes of MS channels. It is a non-selective channel with an open pore diameter of ~40 Å, a unitary conductance of 3 nS and is activated by high negative pressures.[2] Blocking MS channels causes cell lysis while opening MS channels causes cell death through loss of essential osmolytes, therefore MS channels have potential as targets for novel antibiotics. MscL in particular has excellent potential as a selective target for broad spectrum antibiotics as it is highly conserved in all of the 120+ prokaryotic species it has been identified in but no homologues have been found in mammals.[2] Numerous agents have been shown to manipulate MS channels (either through direct channel binding or penetration of the surrounding lipid bilayer) including the local anaesthetics tetracaine and procaine.[3] Their anaesthetic action depends on binding to the sodium channel at positions 1764 (phenylalanine, F) and 1771 (tyrosine, Y).[4] The aims of this study were to determine the effects of 4 local anaesthetics (tetracaine, procaine, lidocaine and the permanently charged QX-314) on MscL and to identify their most likely mechanism of action.

Azolectin liposomes were formed containing purified *E. coli* MscL protein using the conventional dehydration-rehydration method. Channel activity was then studied using the inside-out patch configuration of the patch-clamp technique. Single channel recordings were made in symmetrical solutions (mM: KCl 200, $MgCl_2$ 40, HEPES 5, pH 7.4 KOH). Images of protein structures were constructed using UCSF Chimera. Clustal W was used to identify sequence similarities between *E. coli* MscL and the sodium channel ($Na_{v1.8}$).

Phenylalanine and tyrosine residues were identified in the *M. tuberculosis* MscL protein at distances similar to 1764F and 1771Y (10Å)[4] in the sodium channel. The sequence similarity between *E. coli* MscL and the sodium channel was found to be 11%. Patch-clamp experiments showed that 300 µM tetracaine, 1 mM lidocaine and 1 mM QX-314 significantly decreased the pressure threshold for opening of MscL. 500 µM procaine, 500 µM lidocaine, 50 µM and 150 µM tetracaine also decreased the pressure threshold of MscL. None had any effect on the open channel characteristics; however 300 µM tetracaine induced spontaneous channel activity in one instance after ~20 minutes.

From this study it is clear that local anaesthetics affect MscL by decreasing their pressure threshold for opening. It has been previously reported that the more lipophilic an amphipath the more effective it is at decreasing MS channel pressure threshold.[3] In this work tetracaine was the most lipophilic local anaesthetic used and had the lowest effective concentration, which corresponds with the previous findings. However most of the results did not correspond with the previous findings. 500 µM procaine was more effective than 500 µM of the more lipophilic lidocaine, and the hydrophilic QX-314 was also effective. As QX-314 (a permanently charged hydrophilic molecule) decreased the pressure threshold of MscL, it suggests that direct channel binding is involved in the local anaesthetics mechanism of action in affecting MscL. This is because if the mechanism of action was solely down to interacting with the lipid bilayer surrounding the channel, QX-314 should be ineffective as it would be unable to penetrate into the lipid. Numerous phenylalanine and tyrosine pairs were found in MscL at distances apart similar to their counterparts in the sodium channel (8.779-16.563Å), which could constitute binding sites for local anaesthetics. Although MscL and the sodium channel have a potential similar local anaesthetic binding site, their protein sequence similarity is only 11%, which is advantageous in terms of developing a potential novel antibiotic in the future as it allows for drug selectivity.

1. Booth, I. R., *et al.*, 2007. Mechanosensitive channels in bacteria: signs of closure? *Nature Reviews ,* **5**: 431-440.
2. Kung, C., *et al.*, 2010. Mechanosensitive Channels in Microbes. *Annual Review of Microbiology ,* **64**: 313-329.
3. Martinac, B., *et al.*, 1990. Mechanosensitive ion channels of E.coli activated by amphipaths. *Nature ,* **348**: 261-263.
4. Ragsdale, D. S., *et al.*, 1994. Molecular determinants of state-dependent block of Na+ channels by local anesthetics. *Science,* **265**: 1724-172

Antibacterial Activity of Capped Silver Nano-Particles

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Interest with silver as an antibacterial agent has been renewed since the increasing bacterial resistance to widely used antibiotics. Silver has long been known to have a broad range antibacterial activity and this is utilised in topical preparations such as dressings and creams. Nanotechnology has allowed silver to be created as Nano scale particles that demonstrate superior antimicrobial activity to ionic silver.[1] The techniques used to generate silver nanoparticles (Ag-NP) affect the shape and surface properties of the particles and hence the effect on their activity.[1,2] This study aims to determine the antimicrobial efficacy of Ag-NPs created by a green chemistry method and capped by Polypeptide 'Agent B'. Particles created with different ratios of silver nitrate and Agent B were used in antibacterial testing to observe the effect of Agent B on the antimicrobial activity. The objectives were to measure the minimum antimicrobial concentration (MIC) and the minimum bactericidal concentration (MBC) of *E. coli* and *S. aureus* for the different Ag-NP. These values would then be compared to those found in literature.

The MIC was determined using broth micro dilution method and the results were read by plate reader and visually after 24 hour of incubation. The Ag-NP solutions were tested with 12 different concentrations ranging from 43.2 mg/ml to 0.021mg/ml. Three different Ag-NP solutions were tested named 1:1, 1:5, 5:5. This refers to the ratios of $AgNO_3$ and Agent B (mol:mol) used in synthesis of the particles using the filtrate of *E. coli* suspension as the reducing agent. *S. aureus* was used as example of Gram-positive bacteria and *E. coli* as Gram-negative bacteria in MIC and MBC determination. The bacteria were serially diluted down to 10^5 CFU/ml level after being incubated for 24 hours in BHI at 37℃. MBC values were determined by serial dilution to -3 of the samples used for the MIC assay. Samples from the MIC and dilutions down to -3 of the dilutions were subcultured on an BHI agar plate for 24 hours in 37℃.

Nanoparticles tested showed antibacterial activity against both *S. aureus* and *E. coli.* MIC and MBC were determined to be 21.6mcg/ml and 43.2mcg/ml respectively for both bacteria irrespectively of the ratio $AgNO_3$ to capping agent.

Identical MIC and MBC values indicated Ag-NPs having bactericidal mechanism of action. Aggregation of the particles was noted which also explains the higher than expected MIC and MBC concentrations. Differences in ratios of $AgNO_3$ and polypeptide capping Agent B during synthesis did not influence the antibacterial activity of the Ag-NPs. In contrast to literature, *S. aureus* was more susceptible to Ag-NPs than *E. coli*.[2,3] This could be related to the production method where *E. coli* was employed and Ag-NPs were less effective against *E. coli*. Alternatively the peptide capping Agent B has increased activity against gram-positive cells. This is an exciting notion as Ag-NPs are mainly used topically and MRSA, a gram positive bacterium, is a major threat. Optimising Ag-NPs to kill gram-positive bacteria in low concentrations would have a wide range of topical applications in health care. However, the MIC values recorded were still high compared to results achieved with different Ag-NPs of similar size, so further optimization of activity is needed.

1. Sotiriou GA, Pratsinis SE. Antibacterial Activity of Nanosilver Ions and Particles. *Environmental Science & Technology*. 2010;**44**(14):5649-54.
2. Guzman M, Dille J, Godet S. Synthesis and antibacterial activity of silver nanoparticles against gram-positive and gram-negative bacteria. *Nanomedicine*. 2012;**8**(1):37-45.
3. Taglietti A, Diaz Fernandez YA, Amato E, Cucca L, Dacarro G, Grisoli P, *et al*. Antibacterial Activity of Glutathione-Coated Silver Nanoparticles against Gram Positive and Gram Negative Bacteria. *Langmuir*. 2012.

Patients' views of the written information provided during a Medicine Use Review (MUR) in England and Wales

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Medicine Use Reviews (MURs) have been available as an advanced level service since 2005.[1] It is estimated that the cost of unused or unwanted medicines is around £100million annually.[2] The aim of an MUR is to increase patients' knowledge and understanding of their medicines, to improve adherence and reduce cost to the National Health Service (NHS). Interventions and referrals are documented on the MUR form, where one copy should be given to the patient, and one to their General Practitioner (GP).[3] Current research shows the MUR form is given to less than 50% of patients, and those who received it reported it was difficult to understand.[4] The aim of this study is to investigate patients' views of the written information provided during MURs in England and Wales.

A sample of community pharmacists was asked to distribute a previously designed and tested 'MUR satisfaction questionnaire' to ten consecutive patients receiving an MUR consultation at their pharmacy. The questionnaire consisted of 58 questions, using Yes/No/NA (n=7), and Likert (n=51) response scales. Patients were asked to complete the questionnaire in their own time and return it to the WSP using a freepost envelope. Returned questionnaires were analysed using the Statistical Package for the Social Sciences (SPSS©). Patients who left their contact details within the questionnaire were later invited to take part in a semi-structured telephone interview to further investigate their views of the information provided during MURs. Patients' interview responses were paraphrased and recurring themes were identified and grouped.

Sixteen pharmacists known to the research team agreed to participate and 63 questionnaires were distributed. Twenty-six were returned to the WSP before the study end date (26/63 = 41%). These were added to the existing database and a total of 98 questionnaires were analysed. Of these, 42/98 (43%) patients received written information, of which most (35/42; 83%) were satisfied with its quality, and thought it was appropriate and easy to understand. A further 32/98 (33%) did not receive the MUR form. However 53% (17/32) of these said they prefer verbal to written information. All bar one of the seven sub-scales within the questionnaire had a high internal consistency (Cronbach's alpha >0.7), and positive Spearman's Rho values were found between the scales indicating good construct validity. Four telephone interviews were conducted, where two had received the MUR form whilst the others had not, y*et all* commented that written information could be useful and acknowledged its potential.

The evidence suggests that pharmacists are assessing patients during MURs and are supplying written information to those who they think want it, or would benefit from having it. Most people were satisfied with the way information was provided to them, however some patients expressed that they would like written information although they were not given any. Overall, patients expressed a high level of satisfaction with the service, however the study was limited by a number of different types of bias (e.g. non-response, sampling), and no reliable conclusions could be drawn from the small number of interviews conducted. Further research should use the same questionnaire with a wider range of pharmacists, so that results can be generalised to all pharmacists. Also, a sample should be obtained that is large enough for factor analysis to be carried out in order to reduce the questionnaire size. The techniques used by pharmacists to assess patients during MURs should also be investigated so that suggestions can be made on ways of improving information provision and optimising the service.

1. Department of Health. 2003. *A vision of pharmacy in the new NHS.* [Online] Available at: http://www.dh.gov.uk/prod_consum_dh/groups/dh_digitalassets/@dh/@en/documents/digitalasset/dh_4070099.pdf [Accessed: 23rd March 2012]
2. Department of Health. 2008. *Pharmacy in England: building on strengths – delivering the future.* [Online] Available at: http://www.dh.gov.uk/en/Publicationsandstatistics/Publications/PublicationsPolicyAndGuidance/DH_083815 [Accessed: 3rd March 2012]
3. Department of Health. 2005. *The pharmaceutical services (advanced and enhanced services) (England) Directions 2005 and determination.* [Online] Available at: http://www.dh.gov.uk/prod_consum_dh/groups/dh_digitalassets/@dh/@en/documents/digitalasset/dh_4107597.pdf [Accessed: 2nd March 2012]
4. Royal Pharmaceutical Society of Great Britain. 2010. *MUR patient report* [Online] Available at: http://www.qi4pd.org.uk/index.php/Medicines-Use-Review-Audit.html%20 [Accessed: 23rd March 2012]

Evaluation of a patient's medicines discharge information from hospital: an exploratory study

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When patients transfer between care providers, effective communication between the healthcare professionals responsible for their care is essential. When patients are admitted to hospital, changes are often made to their medication regimen. It is well established that when patients are discharged from hospital, errors in the medication information provided to their GP often occur and can cause harm to patients.[1] Within the University Health Board (UHB), the current process used to provide GPs with discharge information is in the format of a handwritten discharge advice letter that is given to the patient on discharge for them to give to their GP. Handwritten, patient-reliant discharge communication systems are known to have drawbacks such as untimely transfer of poor quality information to GPs.[2,3] The aim of this study was to explore the opinions of hospital staff and GPs on the current medicines discharge process within UHB and to identify any improvements needed.

To meet the aim of this study, qualitative research methods were chosen. Semi-structured interviews took place as they allow open questions to be asked to explore participants' views and experiences[4]. Participants were chosen by purposive then convenience sampling and were sent an invitation letter, participant information sheet and consent form. A follow up e-mail was sent to participants to arrange the interview and a further telephone call was made if no reply was obtained. The interview schedule was developed and contained a mixture of open and closed questions and probes to further explore points mentioned by participants.[4] The interview schedule was piloted and the interviews took place and were tape-recorded, transcribed verbatim and quality assured. The interview transcripts were analysed by thematic analysis.

Seventeen participants were invited to participate in the study and all were interviewed. Sixteen interviews took place in person and one interview took place as a telephone interview. The main themes derived from the data were: problems of the current process, ways to improve the current process, positive aspects of the current process and information a GP wants to receive / others believe a GP wants to receive. The main problems included that the information provided to GPs was often inadequate, inaccurate and illegible and was transferred to GPs in an untimely manner. The most frequently mentioned way to improve the process was to move to an electronic system. The main benefits of this were that more detailed, legible information could be provided to GPs in a more timely way. Other ways to improve included having a dedicated section on the discharge document for changes to medication, improving the appreciation hospital doctors have for the importance of discharge communication, providing better information to patients on discharge and utilising community pharmacists in the discharge process. Positive aspects of the current process included the involvement of hospital pharmacists in clinically checking the discharge document and that the process can be good if used effectively. Additionally, the perceived limitations of an electronic system such as limited availability of computers on wards were considered positives of continuing with the current process. The information that a GP wants to receive included medication changes, reasons for those changes, indications, intended duration of treatment and follow up information.

All participants expressed their concerns relating to problems with the current discharge process and generally perceptions were negative. Some of the problems identified represent a significant threat to the safety of patients. RPS guidance provides core principles for healthcare professionals that, if adhered to, should protect patient safety and encourage continuity of care.[1] These principles encourage the timely transfer of accurate, comprehensive, legible medicines discharge information.[1] Many of the required attributes are considered inadequate in the process within UHB showing a definite need for improvement and for the problems with the process to be addressed.

1. Picton, C., 2011 Keeping patients safe when they transfer between care providers - getting the medicines right: good practice guidance for healthcare professionals. London: Royal Pharmaceutical Society.
2. Motamedi, S. M. *et al*. 2011 The efficacy of computer-enabled discharge communication interventions: a systematic review. *BMJ Quality & Safety* **20**(5): 403-415.
3. Chen, Y. *et al*, 2010 Is email an effective method for hospital discharge communication? A randomized controlled trial to examine delivery of computer-generated discharge summaries by email, fax, post and patient hand delivery. *International Journal of Medical Informatics* **79**(3): 167-172.
4. Oppenheim, A., 1992 Questionnaire design, interviewing and attitude measurement (New Edition). London: Pinters.

Measuring the cytotoxicity effect of disulfiram in MCF 7 and MDA-MB-231 breast cancer cell lines

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The dithiocarbamate drug, disulfiram has been used for decades to support the treatment of alcohol dependence. Studies have indicated that disulfiram is cytotoxic to several cancer cell lines. It has been shown to have metal chelating properties[1], able to block the efflux pump P-glycoprotein[2], inhibits the transcription of nuclear factor-kappaB[3], and inhibits growth of *BCA2*-expressing breast cancer cells.[4] In this study, the effect of disulfiram on estrogen receptor (ER)-positive and ER-negative cell lines; MCF 7 and MDA-MB-231 were examined. The aim of the study was to evaluate if a synergistic cytotoxicity effect could be achieved if disulfiram was used in combination with Doxorubicin (Dox) and Faslodex® which have a different mechanism of action to disulfiram.

The cytotoxicity effect of disulfiram, doxorubicin and Faslodex® were examined by exposing MCF 7 and MDA-MB-231 cell lines to a series of drugs concentrations (0.0001 - 100µM) obtained by 10-fold serial dilutions. The effect of the drug solvent, dimethyl sulfoxide (DMSO) on cell proliferation was also examined to ensure that the inhibition of cell growth is only mediated by the cytotoxic drugs, and the solvent is not responsible in inducing cell death. Cell viability was determined using CellTitre Blue® viability fluorometric assay after 24 hours of exposure to the drugs.

Results of the fluorescence intensity showed that the proliferation of MCF 7 and MDA-MB-231 were inhibited in a dose-dependent manner, with MCF 7 cell line being more sensitive to disulfiram (IC_{50}: 4µM) as compared to MDA-MB-231 (IC_{50}: 50µM). Disulfiram enhanced the cytotoxicity of Dox (IC_{50}, MCF 7: 10µM to 7.5µM; MDA-MB-231: 47.5µM to 25µM, when used alone and in combination respectively), however no synergistic effect was seen with Faslodex®.

In conclusion, our results demonstrated that different types of combination had different effects on the proliferation of MCF 7 and MDA-MB-231 cell lines. A synergistic cytotoxicity effect is seen when both cell lines were treated with DSF/DOX. Therefore disulfiram could be a promising anticancer agent with marked therapeutic benefits and the potency of combination use of disulfiram/DOX should also be tested more in future clinical trials involving cancer patients in order to proof the effectiveness without causing any undesirable adverse effects.

1. Chen, D. *et al.* (2006) Disulfiram, a clinically used anti-alcoholism drug and copper-binding agent, induces apoptotic cell death in breast cancer cultures and xenografts via inhibition of the proteasome activity. *Cancer Research,* 66**,** 10425-10433.
2. Sauna, Z. E.*et al.* (2004) The Molecular Basis of the Action of Disulfiram as a Modulator of the Multidrug Resistance-Linked ATP Binding Cassette Transporters MDR1 (ABCB1) and MRP1 (ABCC1). *Molecular Pharmacology,* 65**,** 675-684.
3. Wang, W. G., H. L. McLeod and J. Cassidy (2003) Disulfiram-mediated inhibition of NF-kappa B activity enhances cytotoxicity of 5-fluorouracil in human colorectal cancer cell lines. *International Journal of Cancer,* 104**,** 504-511.
4. Burger, A. M., Y. Amemiya and A. K. Seth (2006) Disulfiram inhibits the ubiquitin E3 ligase activity of the novel breast cancer associated gene BCA2 and the growth of BCA2 expressing breast cancer cell lines. *AACR Meeting Abstracts,* 1296-b.

Oral Delivery of Vitamin D 3 from a Spray Formulation

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Vitamins are essential nutrients that one requires to sustain normal body functions and growth. They can be obtained from variable and multiple sources. Vitamin D 3 (VD 3) is an important, fat soluble vitamin needed for growth and development of the human systems. It is mainly obtained through a photochemical reaction that occurs via the skin in the presence of UV light. The progression of a westernised culture and poor diet regimes, has led to vitamin supplementation playing a vital role in maintaining the required levels of nutrient uptake. This research has probed the efficacy of sublingual VD 3 supplementation from an oral spray, via the *in vitro* permeation of VD 3 through the excised sublingual membranes; the efficacy of VD 3 across the buccal and soft palate membranes is also included. Comparative studies against the sprays were conducted using two simple oil formulations which contained VD 3 in a 9:1 ratio of olive oil: 1-methyl-2pyrrolidinone with a variant of 5% menthol. There is no scientific evidence as yet to show that this supplement works however, it is known that sublingual sprays offer a faster onset action in comparison to a tablet which would require dissolution.[1] This investigation also takes into account the differences between the different types of non-keratinised membranes in relation to the permeation of VD 3.[2]

Permeation studies were conducted using all glass Franz Diffusion Cells, where each experimental set up was run over a period of 12 h with sampling taking place after every 2 h. The receptor phase used in the cells was Cetrimide at a concentration of 0.03%. The samples obtained were analysed using reverse phase HPL with a mobile phase of methanol, ethanol and phosphoric acid at 1%. Calibration curves for vitamin D have been carried out using Cholecalciferol dissolved in ethanol. Porcine membranes have been used due to their similarities to human membranes.[3] Two membrane extraction techniques were used to excise the porcine membranes; blunt dissection to excise the ventral tongue surface membrane and lower soft palate, and heat separation for the buccal membranes. The in-vitro analysis for each membrane was carried out separately. For each experimental run cells were exposed to 200 µL of either the commercial or the simple oil preparation.

Permeation across the sublingual membranes was compared using varied concentrations of the commercial sprays and the two simple oil formulations. The commercial sprays showed an overall better delivery across all membranes. The permeation profiles for the ventral surface of the tongue showed linearity, whilst the other two membranes (lower soft palate and buccal) showed a non-linear permeation profile of VD 3. Comparative studies of the different formulations showed that the commercial micro-emulsion spray permeated the membranes better than the simple oil formulations. The flux values of the commercial sprays of three different concentrations across the ventral surface of the tongue showed no significant difference showing that permeation was rate limiting. Three application techniques were assessed to estimate VD 3 permeation from a spray plume of 8.55 cm^2, over an available surface area of 214.7 cm^2 ± 12.9 cm^2 [4]; the buccal membrane showed the best permeation profile for all three techniques assessed.

The results confirmed the permeation of VD 3 across oral membranes; however there was a vast difference in the extent of permeation seen with each membrane. The differences in the permeation can be attributed to structural differences and/or location in the oral cavity. However with the formulations being so different it can be assumed that the difference seen is due to the number of excipients used. The buccal region is seen to have the best permeation profile with the commercial spray. The overall conclusion is that the oral commercial spray depending on the type of technique used, buccal permeation is the highest delivering ~20% of the dose administered.

1. Marmor, A., 1990. Comparative evaluation of a new formulation of isosorbide dinitrate oral spray and sublingual nitroglycerin tablets. Am. J. Cardiol. Vol. 65, 43J-45J
2. Lesch, C.A. *et al*. 1989. The permeability of the human oral mucosa and skin to water. Journal of Dental Research. Vol. 68. No. 9 Pp.1345-1349.
3. Squier, C.A., Cox, P., Wertz, P.W. 1991. Lipid content and water permeability of skin and oral mucosa. J. Invest. Dermatol. Vol. 96, 123-126.
4. Collins, L.M.C., Dawes, C., 1987. The surface area of the adult human mouth and thickness of the salivary film covering the teeth and oral mucosa. Journal of Dental Research. Vol. 66(8), pp. 1300-1302.

Exploring the performance of undergraduate pharmacy and medicine entry students' in a diagnostic numeracy tool

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Numeracy skills are required by all and are defined as more than using mathematical skills alone.[1] Healthcare professionals are one group of people who require numeracy competence for reasons including patient safety.[2] However, studies have shown a decline in healthcare professional entry students' numeracy skills, despite being mathematically qualified prior to university.[3,4] The aim of this study was to evaluate the performance of pharmacy and medicine entry students' in a basic numeracy diagnostic tool and to explore the influence of their educational background, such as qualifications and country of education, on their performance.

The study population consisted of first year pharmacy and medicine entry students enrolled either on Cardiff School of Pharmacy and Pharmaceutical Sciences MPharm programme or the Cardiff School of Medicine degree programme. Data was collected using a 25-question contextualised diagnostic numeracy tool and adjoining questionnaire that aimed to gather demographic information and feedback. Numeracy questions consisted of six categories (multiplication, division, fractions, percentage, ratios and unit conversions) all of which are considered key stage 3 level skills. The diagnostic tool was administered to first year students in the second week of the academic year 2011-2012 with students not informed of the test beforehand and not permitted to use calculators. Students were assigned to a numeracy competency category based on their total score; categories were satisfactory (>21/25), borderline (15-21/25) and unsatisfactory (≤14/25). Ethical approval was obtained from Cardiff School of Pharmacy and Pharmaceutical Sciences, prior to the study start date.

The study population consisted of 165 pharmacy and 274 medicine entry students (response rate= 97.1%). Over 60% of participants were categorised as satisfactory. The difference between scores of pharmacy and medicine entry students' was statistically significant (p=0.019 Mann-Whitney U) with higher percentages of pharmacy students' in the borderline and unsatisfactory categories. Students pre-University educated primarily in South East Asia had the highest percentage in the satisfactory category. There were students with A-level mathematics in each of the numeracy competency categories. Generally the number of wrong answers increased as questions progressively increased in difficulty and unit conversion followed by division proved the most challenging domains. Students answering the most challenging questions incorrectly had similar pre-University mathematics qualifications and grades to those answering correctly.

The majority of students performed satisfactorily. However some students' with A-level mathematics qualifications at grades ≥A were classified in borderline and unsatisfactory categories, suggesting that pre-University qualifications are not reliable indicators of basic numeracy. A statistical difference between pharmacy and medicine entry students' performance suggests that student entering pharmacy students are less competent, however a larger study is needed to indicate generalizability. The difference in performance between students' from different countries suggests this should also be considered when identifying future students' who may require numeracy support, however greater 'N' values are required. Finally, the tool successfully diagnosed areas that students found difficult; this could be used to tailor and improve future numeracy teaching strategies for undergraduate healthcare students'.

1. Barwell, R. 2004. What is Numeracy? *For the Learning of Mathematics*. **24:** 20-22.
2. Meyler, L, Ramtoola, Z and Barlow, J. 2011. Evaluation of the ability of pharmacy and medicine students to calculate drug dosage. *Pharmacy Education*. **11:**186-189.
3. Malcolm. R. K and McCoy. C. P. 2007. Evaluation of numeracy skills in first year pharmacy undergraduates 1999-2005. *Pharmacy Education*. **7:** 53-59
4. Sheridan, S. L and Pignone, M. 2002. Numeracy and the medical student's ability to interpret data. *Effective Clinical Practice* **5:** 35-40.

The role of 5-HT in contractions of electrically stimulated ileum

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The aim of the project was to determine the cause of contractions seen in isolated ileum when electrically stimulated. The effect of electrical stimulation in changing the way the ileum tissue responds to 5-HT was also looked at. It is normally regarded that the twitch seen from an electrical impulse is a parasympathetic response involving release of acetylcholine; this project asks the question is 5-HT also involved in generating the contraction seen with the twitch? The role of 5-HT2 and 5-HT3 receptors in causing the twitches seen when stimulated at a frequency of 0.5 HZ was studied. The electrical trains seen when tissue is stimulated at a higher frequency of 15HZ was also looked at in an attempt to determine if 5-HT is involved.

Transmurually electrically stimulated ileum was submerged in a 50ml organ bath with Krebs solution. Cumulative dose response curves were carried out for 5-HT, acetylcholine and tyramine these were then repeated in the presence of the antagonists ondansetron and ritanserin. Increases in baseline contractions to 5-HT, acetylcholine and tyramine were plotted as a percentage of the maximum response seen for each drug as well as the maximum peak contraction seen for each drug. Electrical Trains were generated via electrodes located inside and outside the ileum. These were generated at a frequency of 0.5HZ, a voltage of 20 and a pulse width of 5ms for a period of 2 minutes. Electrical twitches were generated via electrodes located inside and outside the ileum at a frequency of 0.5HZ, a voltage of 20 and a pulse width of 5ms. Contractions were recorded by use of a force transducer coupled to a Power Lab data acquisition system. All experiments were carried out at least three times to ensure their reliably and significance and graphs were plotted with the standard error of the mean included.

The contractile effect of 5-HT was completely inhibited ondansetron (5-HT3 receptor antagonist) suggesting that the contractile effects of 5-HT are completely due to 5-HT3 receptors. Ondansetron did not show inhibition of the effect of Ach in causing contraction suggesting that the contractile effect seen with Ach is not due to 5-HT 3 receptors. The contractile effect of tyramine was also completely inhibited by ondansetron, suggesting that tyramine may release 5-HT to cause contraction via HT3 receptors. In the presence of the 5-HT2 receptor antagonist ritanserin, the 5-HT dose response curve was not affected. This suggests that in the electrically stimulated tissue it is not the 5-HT2 receptors leading to the contraction seen when 5-HT is administered. Interestingly in the non stimulated tissue there was much more of an inhibitory effect, suggesting that when the tissue is not electrically stimulated the 5-HT2 receptors play a far greater role in contractions seen from the administration of 5-HT. With administration of ondansetron there was reduction in the electrical trains suggesting that it is 5-HT3 receptors causing some of the contraction. With 1uM atropine a significant decrease was not recorded. This suggests that it is not the muscarinic receptors responding to a release of acetylcholine which play a major role in causing the contractions seen. Administration of 1uM atropine led to an almost complete abolishment of the electrical twitches seen suggesting that they are almost completely due to the action of acetylcholine on muscarinic receptors.

5-HT exerts its contractile effects completely via 5-HT3 receptors in the electrically stimulated tissue however 5-HT effects appear to be far more reliant on 5-HT2 receptors in non electrically stimulated tissue. The electrical twitches were abolished by administration of atropine suggesting that they are indeed caused by acetylcholine via muscarinic receptors. The electrical trains were in some part caused via the release of 5-HT via 5-HT3 receptors and by another unknown mechanism.

Use of a contextualised diagnostic tool to investigate numeracy skills of pharmacy and medicine undergraduate students

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There has been increasing concern amongst universities that the numeracy skills of new undergraduate students are declining.[1-3] Basic numeracy skills are required to be able to carry out calculations. The ability to perform medicine-based calculations competently is a vital skill for both pharmacists and doctors, as errors in calculations can compromise patient safety. The value of GCSE and A-level mathematics as indicators of students' mathematical ability has been questioned.[2,3] Diagnostic testing can be used to assess the inherent numeracy skills of new undergraduate students to identify the areas of strengths and weakness for individuals or cohorts of students. This can provide 'rapid evaluation' of the students' mathematical knowledge, allowing teaching to be targeted appropriately.[2,4] The aim of this study was to investigate the use of a diagnostic tool to identify areas of weakness in pharmacy and medicine entry student's numeracy skills and identify factors that may influence overall performance.

Ethical approval for the study was provided by the Cardiff School of Pharmacy and Pharmaceutical Sciences Research Ethics committee. A diagnostic numeracy tool, developed in 2010 by Coulman and colleagues, was used for this research study. The numeracy tool consists of twenty-five calculations to be completed in forty-five minutes, without the use of a calculator. Questions in the numeracy tool were contextualised to medicines; however specific training in medicines-based calculations was not required to be able to answer questions on the tool. After each question participants are also asked to indicate their level of confidence in their answer. The test was administered in the second week of the first semester. Entry students included in the study were undergraduate pharmacy students from Cardiff School of Pharmacy and Pharmaceutical Sciences and Taylors University (n=168) and medicine students from Cardiff School of Medicine (n=284). On completion of the test students were asked to complete a short questionnaire to provide feedback on the test and also details of their age, gender, highest math qualifications and country of pre-university education. Data from the diagnostic tests were collated and analysed using SPSS version 18.

The response rate for the study was 97.1%. The mean score for pharmacy students (n=165) was 20.36 and the mean score for medicine students (n=274) was 21.76. Medicine students were also generally more confident that their answers were correct compared to pharmacy students. Unit conversions, multiple step division and multiplication were the least competently answered numeracy domains by both pharmacy and medicine students. A two-step multiplication question was the worst answered by pharmacy and medicine students. Students who scored 60% or less were considered to have the weakest numeracy skills; 12.7% of pharmacy and 6.2% of medicine students were classified in this category. The mean score for students with A-level maths was 21.71 and 20.17 for those without. Pharmacy students from South East Asia outperformed UK pharmacy students in the numeracy tool.

The diagnostic tool was able to identify the areas of strengths and weaknesses in basic numeracy skills amongst students entering pharmacy and medicine degrees in Cardiff University. Studies have suggested that A-level mathematics is no longer a good predictor of a student's mathematical ability[3] and some students with A-level mathematics were classified in the weakest category, thus indicating that A-level is not a good predictor of numeracy ability either. Further work is required to understand how national differences in pre-University education contribute to significant differences in the numeracy ability of students.

1. Barry J G, Colville J A, Donnelly R F. Attitudes of pharmacy students and community pharmacists to numeracy. *Pharmacy Education*. 2007; **7**(2): 123-131.
2. Batchelor H. The Importance of a Mathematics Diagnostic Test for Incoming Pharmacy Undergraduates. *Pharmacy Education*. 2004; **4(**2): 69-74.
3. Tariq V. Numeracy, Mathematical Literacy and the Life Sciences. *MSOR Connections*. 2004; **4:25**-30.[accessed 15 March 2012]. Available from: http://www.heacademy.ac.uk/assets/documents/subjects/bioscience/key-skills-numeracy-math-literacy-life-sciences.pdf.
4. Sharif S. *et al*. Diagnostic Testing of First Year Pharmacy Students: A Tool for Targeted Student Support. *Pharmacy Education* 2007; **7**(3): 215-221.

Novel formulation and manufacturing method validation of 1 mg Glycopyrronium Bromide capsules

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Hard capsules were first patented by Dublanc and Mothes in 1834[1], and are now one of the most common dosage forms within the pharmaceutical industry. Hard capsules can be filled on machines that are operated either manually or automatically. Manual filling methods are used in hospital pharmacy to produce small quantities of special formulations. This project was carried out in conjunction with St. Marys Pharmaceutical Unit (SMPU), Cardiff. SMPU had received a request to manufacture a glycopyrrolate capsule. Glycopyrrlorate tablets were in the top 50 most imported unlicensed medicines in 2010 [2], being used to treat hyperhidrosis.[3] The objective of this study was to develop a novel capsule containing 1 mg of Glycopyrronium Bromide, using their standard stock excipients. The challenge was to produce a powder blend containing <1% of active for filling using a Feton manual machine to produce capsules complying with Pharmacopoeial limits for uniformity of fill weight and content.

Lactose monohydrate and microcrystalline cellulose were selected as the excipients due to their common usage in immediate release oral capsule formulations.[4] Particle size analysis and visual inspection of the excipients and active were used in selecting a suitable excipient to make a trituration with the glycopyrronium bromide to aid the dispersion of the active into the mixture. Four candidate powder blends, of varying excipient proportions, underwent flowability assessment using tapped-density testing and calculation of their compressibility indexes. Two candidate formulations were chosen for the next stage of development: to determine the composition of the mixtures and the possible capsule fill weights. The time to achieve a homogeneous mix in a rotating cuboid bench-top mixer was investigated for each formulation. Samples were taken at set intervals of time from 6 positions in the mixer bowl and tested for content uniformity using an HPLC assay. Formulations were prepared for capsule sizes 2 and 4, to investigate whether the extra dilution of the active would affect the results. Two final formulations were chosen for encapsulation and samples were assayed for uniformity of weight and content.

This study discovered that lactose monohydrate promoted the adhesion of glycopyrronium bromide to the plastic polymer walls of the cuboid mixing bowl, reducing the amount of active in the formulation. This effect was highlighted in formulations that contained a high proportion of lactose; as they produced a homogenous mix with an insufficient concentration of active. The effect of trituration adhesion was confirmed further by the formulation containing equal parts lactose monohydrate and microcrystalline cellulose, which produced a homogenous mix in shorter period of time, with the correct levels of glycopyrronium bromide being attained. The effect of increasing the capsule size increased the homogeneity of glycopyrronium bromide in formulations containing high concentrations of lactose. This was due to the extra quantity of excipients reducing the adhesion of the active to the mixing bowl.

It was shown that a formulation containing equal parts lactose monohydrate and microcrystalline cellulose was the most suitable powder mixture of the potential formulations tested. The final formulation was lactose monohydrate 67.5 mg, microcrystalline cellulose 67.5 mg, glycopyrronium bromide 1 mg for a size 4 capsule. This formula was also recommended for dilution for filling into size 2 capsules.

1. Jones, B.E. 2004. The history of the medicinal capsule. In: Podczeck, F. and Jones, B.E. (eds) *Pharmaceutical Capsules.* 2nd Ed. London: Pharmaceutical Press; pp 1-22.
2. Matthews, G.P. 2011. Export Certificates & Unlicensed Imports: Summary Report for Importation of Unlicensed Medicines. MHRA. [Available online] http://www.mhra.gov.uk/home/groups/is-lic/documents/websiteresources/con140847.pdf [Last accessed: 13th April 2012]
3. Bajaj, V. Langtry, J.A.A. 2007. Use of oral glycopyrronium bromide in hyperhidrosis. *British Journal of Dermatology.* **157** (1) pp 118-121
4. Jones, B.E. 1998. New thoughts on capsule filling. *S.T.P. Pharma sciences.* **8** (5) pp 277 – 283.

Characterisation of a bilateral model of α-synucleinopathy

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Parkinson's disease, PD, is a progressive neurodegenerative disorder characterized primarily by cardinal motor dysfunctions,[2] caused by a loss of dopaminergic neurons in the substantia nigra and a subsequent loss of dopamine in the striatum. Such motor symptoms are vastly improved with the use of dopamine replacement therapies, however these therapies have a lesser effect on the debilitating non-motor symptoms of the disease.[1,3] The non-motor symptoms of PD are thought to arise from a broader neuropathology, involving abnormal aggregation of α-synuclein; a key pathological hallmark in PD. Limited understanding of this pathology pushes the need for better models to be used as tools for studying the neuropathological and neurochemical aspects behind the symptoms, and for the development of future therapeutic interventions. This study aims to evaluate the non-motor symptoms of PD when α-synuclein is overexpressed, developed using an adeno-associated viral vector.

Four rats were injected bilaterally into the SN and the PFCx with an adeno-associated viral vector containing the human wildtype α-synuclein, and 12 were treated with sterile PBS and deemed the control group. The behavioural tests carried out included: the elevated plus maze, open field test, sucrose consumption test, olfactory discrimination tests and a spatial memory test with the aim of studying four key non-motor symptoms of PD, including, anxiety, depression, olfactory dysfunction and spatial memory. Immunohistochemistry was conducted to identify regions of the brain showing α-synuclein overexpression.

The AAV-α-synuclein group displayed different anxiety-like behaviour when subject to the elevated plus maze test and a significant impairment in olfaction when subject to the novel odour test and the social odour test; potentially attributable to the overexpression of α-synuclein observed in the locus coeruleus and prefrontal cortex and the olfactory tubercle and striatum respectively. Overall the AAV-α-syn vector successfully produced a bilateral model of α-synucleinopathy, and induced two key non-motor symptoms associated with early stage PD.

The adeno-associated viral vector provides a successful tool for overexpression of α-synuclein in specific brain regions, and for inducing certain non-motor symptoms of PD when injected bilaterally. A-synuclein is neuron specific, and the AAV vector can be injected into any anatomical location in the brain, allowing α-synuclein induced pathology to be studied in specific neurons. The progressive nature of the AAV-α-syn model allows for bilateral overexpression and produces a pathology which mimics the human form of the disease more accurately than previous neurotoxin or transgenic based models. A-synuclein overexpression presents only one pathological hallmark of PD, and therefore not all of the non-motor symptoms associated with the disease were displayed in this model, however the AAV vector is valuable when studying symptoms associated with early stage PD, before the manifestation of motor symptoms. Further studies are needed to evaluate the pathological effect of α-synuclein on specific neurons; dopaminergic, noradrenergic, serotonergic and cholinergic, to improve our understanding on the neuropathology and neurochemistry behind the non-motor symptoms of PD and aid the development of future therapies.

1. Hely, M.A. *et al.* (2005). Sydney multicentre study of Parkinson's disease: non-L-dopa-responsive problems dominate at 15 years. *Mov disord,* **20**:190-199.
2. Marsden, C.D. (1994). Parkinson's disease. *Journal of Neurology, Neurosurgery and Psychiatry*, **57**:672-681.
3. Schrag, A. (2004). Psychiatric aspects of Parkinson's disease – an update. *J Neurol*, **251**:795-804.

Does the method of Electrical Field Stimulation (EFS) affect the cannabinoid response in isolated ileum?

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The therapeutic potential of cannabinoids in gastrointestinal (GI) diseases has been the subject of much research over the last two decades. An endocannabinoid system is present throughout the gut and known to play a role in many GI functions including gut motility. Cannabinoid agonists can decrease smooth muscle contractility by acting on presynaptic CB_1 receptors to decrease acetylcholine release.[1] This may be of use in diseases where an excess of gut motility is a problem e.g. irritable bowel syndrome (IBS). A common research tool used to identify decreases in smooth muscle contractility is Electrical Field Stimulation (EFS), which delivers electrical pulses to tissues causing acetylcholine release and smooth muscle contraction. There are two main methods of EFS, train pulsing and single pulsing. Train pulsing delivers high frequency bursts of pulses with a period of relaxation in between. Single pulsing delivers regular low frequency pulses leading to isotonic contraction and relaxation of the tissue. The aim of this research project was to determine whether using different methods of EFS affects the cannabinoid response.

Four oxygenated 50ml organ baths were perfused with fresh Krebs and heated to 37°C. For each bath a 2cm section of isolated ileum was threaded onto a stainless steel electrode. Another electrode ran parallel. The ileum was secured to the electrode and to a transducer by cotton threads using loops to avoid direct damage. Initial tension was set at 0.5g. The ileum was left to rest for at least 30mins. Both methods of EFS were tested for each protocol, train pulsing (manually: frequency 5Hz, pulse width 0.5mS, 5s on 10s off) and single pulsing (frequency 0.5Hz, pulse width 5mS). Protocol 1 involved treating the tissue with WIN 55,212-2, a non-selective cannabinoid agonist. Concentrations of $1x10^{-9}$M to $1x10^{-5}$M increasing in log increments were added two minutes apart. As the drug was dissolved in ethanol, an ethanol control was run alongside the experiment. The aim of this protocol was to find the concentration at which a significant decrease in contraction size was seen for each method of EFS. Protocol 2 aimed to block this decrease using the CB_1 receptor antagonist, rimonabant. The tissue was pre-incubated for 15 minutes with $1x10^{-6}$M rimonabant before adding WIN 55,212-2 in the same way as in protocol 1. A WIN 55,212-2 control was run alongside the experiment to ensure that the tissue was responsive. Statistical analysis was carried out on experiments with an $n \geq 3$ using two-tailed Student's paired t test. Significance was defined as a p value <0.05.

Data was split into responders and non-responders where a responder was defined as a tissue that showed more than a 10% decrease in contractility in response to WIN 55,212-2. 50% of experiments were non-responders. This may have been to do with WIN 55,212-2 itself or regional variation of CB_1 receptor density throughout the ileum. For protocol 1 a significant decrease in contractility was seen at $1x10^{-5}$M WIN 55,212 for both train and single pulsing ($p=0.033$ and 0.04 respectively). Due to high tissue variability, an insufficient n number was obtained for experiments in protocol 2. However rimonabant did not appear to be blocking WIN 55,212-2 irrespective of the EFS type.

No difference was seen between the methods of EFS. An unusually high concentration of WIN 55,212-2 was required to see a decrease in contraction for protocol 1. One study noted that a response to WIN 55,212-2 could take up to 15mins [2] so a longer period between doses may be required. Rimonabant was unsuccessful in blocking the response in protocol 2. This suggests that WIN 55,212-2 is not mediating its effect via CB_1 receptors. As these results do not follow what has been found by previous studies,[1-3] further experimentation is required. If this study were to be repeated it should strictly use ileum from the same anatomical location each time, test more than one agonist and antagonist, and use stimulators with a wider range of frequencies and an inbuilt train pulsing mechanism.

1. Coutts AA, Pertwee RG. Inhibition by cannabinoid receptor agonists of acetylcholine release from the guinea-pig myenteric plexus. *Br J Pharmacol.* 1997;**121**(8):1557-66. Epub 1997/08/01.
2. Makwana R, Molleman A, Parsons ME. Evidence for both inverse agonism at the cannabinoid CB1 receptor and the lack of an endogenous cannabinoid tone in the rat and guinea-pig isolated ileum myenteric plexus-longitudinal muscle preparation. *Br J Pharmacol.* 2010;**160**(3):615-26. Epub 2010/07/02.
3. Izzo AA, *et al.* Excitatory transmission to the circular muscle of the guinea-pig ileum: evidence for the involvement of cannabinoid CB1 receptors. *Br J Pharmacol.* 1998;**124**(7):1363-8. Epub 1998/09/02.

Does Bevacizumab Improve Survival in Patients with Glioblastoma Multiforme? : A Systematic Review

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Glioblastoma multiforme (GBM) is a highly malignant, rapidly growing type of brain tumour arising from astrocytic glial cells of the central nervous system. It is the most common and most malignant brain tumour with very poor prognosis despite advances in treatment.[1] Bevacizumab (Avastin), a prominent antiangiogenic agent, has evolved recently as a treatment option for recurrent glioblastoma multiforme. It inhibits the binding of vascular endothelial growth factor (VEGF) to its receptors, reducing vascularisation of tumours thereby inhibiting tumour growth.[1] Bevacizumab is hailed as the wonder drug to tame GBM but there is no consensus on its efficacy in treating GBM. This study therefore aims to evaluate the effectiveness of bevacizumab in prolonging survival in glioblastoma multiforme.

The following databases were searched: the Cochrane Central Register of controlled trials, Medline and EMBASE. Conference proceedings and reference lists of identified studies were searched. Drug manufacturer was contacted regarding any on-going and unpublished trials. Selection criteria included Randomised clinical trials (RCTs) and Interventions using bevacizumab during primary therapy and recurrent disease. Patients included all ages with a proven pathological diagnosis of GBM. Data extraction and quality assessment were undertaken. Outcome measures included overall survival, progression-free survival and adverse events. RevMan 5.1 [2] statistical software was used for analysing the data.

In recurrent disease two RCTs[3] were identified, enrolling a total of 330 patients, that investigated Bevacizumab plus Irinotecan versus Bevacizumab alone treatment regimens. The analysis showed no difference between effects of the treatment regimens in overall survival (Odds Ratio 1.33, 95% CI 0.85 to 2.07, P= 0.2). Both regimens improved overall survival to similar extent. However, there is a significant difference between the effects of the treatment regimens in favour of Bevacizumab plus Irinotecan therapy in 6-month progression-free survival (Odds ratio 0.57 95% CI 0.37 to 0.88; P= 0.01). In newly diagnosed GBM, a single trial[4] enrolling 65 patients in total investigated the treatment regimens Bevacizumab plus Irinotecan (BV+IR) versus Bevacizumab plus Temozolomide (BV+TMZ). The analysis demonstrates that BV+IR regimen is favoured against BV+TMZ at both 6 months and 12 months Overall Survival (Odds ratio 8.42 95% CI 1.13 to 62.74, P=0.04) and (OR 2.97 95% CI 1.09 to 8.07) respectively. For 6-month Progression-Free Survival, it did not demonstrate a statistically significant effect between both regimens. None is favoured in terms of patient survival for 6 months or 12 months (OR 1.07 95% CI 0.41 to 2.83; P=0.89) and (OR 0.75 95% CI 0.16 to 3.58; P=0.72) respectively. Regarding safety, a benefit for BV+IR combination therapy against BV+TMZ treatment is shown.

This review indicates that Bevacizumab plus Irinotecan treatment regimen is effective in improving progression free survival in patients with recurrent GBM but not necessarily their Overall Survival. No reliable evidence was found to advocate the use of BV in treating newly diagnosed GBM. The review did not find any randomised controlled trial that included a bevacizumab-free control arm. Under such circumstance, the effect of bevacizumab cannot be clearly demonstrated. It has therefore contributed to the divided opinion about the drug among professionals. Further trials that have improved methodology to provide the evidence required for the use of bevacizumab in treating glioblastoma are urgently needed. A re-visitation of this review probably in 2 years will proffer a better insight into the role of BV in the treatment of GBM at primary and recurrent disease when the results of the robust phase III trials in progression mature.

1. Brown G.L, Matthew E, and Kurt A.W. (2010) A Review of Glioblastoma Multiforme. *US Pharmacist* **35** (5): 3-10
2. Cochrane Information Management System (2011) RevMan http://ims.cochrane.org/revman
3. Friedman H.S. *et al* (2009) BV Alone and in Combination With Irinotecan in Recurrent Glioblastoma. *Journal of Clinical Oncology*, **27** (28): 4733-4740.
4. Hofland, K.F. *et al* (2011) Randomised phase II study of neoadjuvant Bevacizumab and Irinotecan versus Bevacizumab and Temozolomide followed by concomitant chemotherapy in newly diagnosed primary GBM. *Journal of Clinical Oncology*, **29**S: 2052.

Are the current teaching methods employed to deliver Sterile Formulation teaching on the MPharm course in Cardiff appropriate for today's modern pharmacist?

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Sterile formulation is an umbrella term referring to a number of inter-related topics, namely being aseptic techniques, radiopharmacy and sterilisation.[1] Individual universities employ different teaching methods to teach sterile formulation depending on factors such as time and the facilities available. Current teaching methods include GMP suites, laboratory-based workshops, CAL packages as well as theory-based lectures. The competency and confidence that hospital pre-registration students are equipped with can be partially reflected on the teaching methods that their university employed to teach sterile formulation. The principle aim of this project is to evaluate whether the current teaching methods employed at university level sufficiently prepares students who will enter hospital pre-registration placements.

A questionnaire consisting of three sections was electronically distributed to lecturers teaching sterile formulation at UK schools of pharmacy, as well as being distributed to pre-registration tutors at hospitals who have a sterile manufacturing/dispensing facility of some sort. The questionnaire comprised of open and closed questions allowing for qualitative and quantitative data to be collated, respectively.

The response rate from the lecturers was relatively good, at 64.2%. However, the response rate obtained from the pre-registration tutors was relatively poor, with only 18.3% returning their completed questionnaires. Amongst the findings obtained from the lecturers, a common theme from them was that a range of teaching methods to teach sterile formulation was the most appropriate way of equipping students with knowledge and competency. For the data collated from the pre-registration tutors, most agreed that although pre-registration pharmacists have sufficient knowledge, they can sometimes lack competency when working with the practical aspects of sterile formulations. Expanding from this, a common opinion amongst pre-registration tutors was that more practical teaching methods would better prepare students for work in hospital pharmacies.

The culmination of the findings, coupled with published data, would suggest that although a range of teaching methods were employed at university level in providing students with sufficient knowledge on sterile formulation, in particular, the use of more practical teaching methods to supplement the traditional theory-based teaching methods would increase student competency and confidence in the practical aspects of sterile formulation in a true hospital pharmacy setting. The use of GMP suites and live video demonstrations of a sterile formulation facility in a hospital, or interactive teaching methods, such as CAL[2], are all teaching methods which could be employed at universities to supplement the conventional theory-based lectures.

1. Sharp, J. 2005. *Good Pharmaceutical manufacturing practice- Rationale and compliance*. London: CRC Press
2. Scitteck, M. *et al*. 2001. Computer assisted learning. A review. *European Journal of Dental Education*. **5**, pp.93-99

Determining the forces used by patients to apply a microneedle patch

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Microneedles are a topic of increasing interest in the field of drug delivery and clinical practice. They are designed to overcome the barrier properties of the skin by penetrating through the stratum corneum whilst avoiding blood vessels and nerve endings in the dermis.[1] Therefore, they provide a minimally invasive, bloodless and relatively painless method of intradermal and transdermal drug delivery.[2] The force used to apply a microneedle patch to the skin is paramount in determining the functionality of the patch and its ability to deliver the therapeutic agent to the patient.[3] A study conducted in 2011 (unpublished) explored the forces that patients would intuitively use to apply a microneedle patch to the skin. The results showed large inter- and intra- patient variation in the forces used to apply the patches. The primary aim of this study was to determine whether providing instructions reduced the variability in the forces used to apply a microneedle patch to the skin.

The Cardiff School of Pharmacy and Pharmaceutical Sciences Ethics Committee granted ethical approval. A patient instruction sheet was designed and created to provide the study participants with instructions on how to apply a dummy microneedle patch to the skin. Participants that were naïve to microneedle technology were recruited. After volunteers consented to participate in the study, demographic information was recorded including the participant's age, gender and their medical/injection experience (if any). The participant was then provided with the patient instruction sheet, including specific directions on how to apply a microneedle patch. Each participant then applied a downward force to patches located on their own forearm (N=3) and deltoid (N=3) and also the researchers forearm (N=3) and deltoid (N=3). The force applied to each patch was recorded using a Sauter digital force gauge. Results of this study (information intervention group) were then compared to data collected in the 2011 study (control group).

There was no statistically significant difference between the control group and the information intervention group with regard to the mean application of the forces used to apply the patches at each anatomical site ($p<0.05$). There was also no significant difference in the standard deviations ($p<0.05$). The mean force applied to the researcher's deltoid by male participants was significantly higher than the force applied by females ($p<0.05$). In the information intervention group, participants with medical/injection experience applied significantly less force at all patch sites than those without ($p<0.05$). The SD in the forces applied to each site by participants with medical/injection experience was significantly less than those without at the participant and researcher's deltoid ($p<0.05$).

The introduction of a detailed patient instruction sheet had little impact on the inter-patient variation of the application forces used by members of the public to apply a microneedle patch. In order for patients to be able to apply microneedle patches to themselves, it may be prudent to consider the use of an applicator device to ensure the correct force of insertion into the skin.[3]

1. Kaushik, S. *et al*. 2001. Lack of Pain Associated with Microfabricated Microneedles. *Anesthesia & Analgesia* 92(2), pp. 502-504.
2. Haq, M. *et al*. 2009. Clinical administration of microneedles: skin puncture, pain and sensation. *Biomedical Microdevices* 11(1), pp. 35-47.
3. Yang, M. and Zahn, J. D. 2004. Microneedle Insertion Force Reduction Using Vibratory Actuation. *Biomedical Microdevices* 6(3), pp. 177-182.

The Role of Hydrogen Peroxide Induced Oxidative Stress on Ryanodine Receptor Mediated Calcium Release in the Myocardium

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Ischaemic heart disease is the leading cause of human mortality with most deaths being caused by sudden thrombotic occlusion of a coronary artery. Early Reperfusion is the treatment of choice, with coronary angioplasty the mainstay treatment to limit the extent of irreversible injury caused by ischaemia.[1] Sudden reperfusion of an ischaemic myocardium can cause further injury; this paradoxical phenomenon is known as reperfusion injury. Reperfusion causes damage which is seen as myocardial stunning, irreversible cell death and arrhythmias. Oxidative stress has long been associated with ischaemia reperfusion injury and may contribute to cardiac dysfunction.[2] Too much reactive oxygen species (ROS) causes oxidative stress and can cause cellular injury and death. Hydrogen peroxide (H_2O_2) is formed naturally in the body by enzymes and metabolic processes, under normal conditions forms ROS such as the hydroxyl radical. Under some pathological conditions oxidative stress rises to toxic levels causing damage to cells. Mitochondria are central regulators of cell fate during exposure to stress. The mitochondrial permeability transition pore (mPTP) is a non selective pore, which under certain conditions such as ischaemia/ reperfusion can open to allow molecules smaller than 1.5 kDa through. High concentrations of cytosolic calcium are known to cause mPTP to open, and it has been demonstrated to cause mPTP to become more sensitive to calcium concentrations in the presence of ROS.[3] When mPTP opens it releases apoptotic factors and ROS. ROS causes profound lipid peroxidation, and therefore can be measured by using a thiobarbituric acid reactive substances (TBARS) assay. This measures the amount a decomposition product of lipid peroxides MDA, providing an indication of whether lipid peroxidation and therefore oxidative stress has occurred. Using tetracaine to block the ROS induce calcium release the aim of the study is to see if it has an effect on the lipid peroxidation and oxidative stress levels which occur during ischaemia reperfusion.

Thirty two hearts were perfused with Krebs-Henseleit bicarbonate (KHB) solution for twenty minutes to stabilize the hearts using a Langendorff perfusion.[4] Following the stabilization period the hearts were perfused with KHB, 75 µM hydrogen peroxide or 75 µM hydrogen peroxide plus 100 µM tetracaine depending on which of the three groups they were split into. The fourth group was Naive hearts and they were also used to control against the perfusion protocol, and see if the perfusion process may have had an effect on the hearts. The left ventricle of the heart was removed and placed into microcentrifuge tubes and snap frozen in liquid nitrogen and stored at -80ºC for further biochemical analysis. Tissue samples were then powdered and allowed to lyse in a lysis buffer. Samples were centrifuged and the supernatant was taken for analysis of protein and TBARS using a bicinchonic acid TBARS assay respectively.

It was found that that H_2O_2 + tetracaine significantly increased the amount of MDA present in the sample. This suggests that there was higher lipid peroxidation. A smaller increase in TBARS was caused by H_2O_2 alone suggesting that there was a smaller increase in lipid peroxidation. These results were the opposite of what was hypothesised would happen.

Lipid peroxidation effect of oxidative stress on the myocardium appears to be enhanced by the presence of tetracaine, thus indicates something else maybe occurring. Although further research would be needed based on this study to confirm if tetracaine would not be of benefit to reducing reperfusion injury through reducing lipid peroxidation.

1. Downey JM, Cohen MV (2006). Reducing infarct size in the setting of acute myocardial infarction. *Prog Cardiovasc Dis* **48**(5)**:** 363-371.
2. Bolli R (1988). Oxygen-derived free radicals and postischemic myocardial dysfunction ("stunned myocardium"). *J Am Coll Cardiol* **12**(1)**:** 239-249.
3. Halestrap AP, Pasdois P (2009). The role of the mitochondrial permeability transition pore in heart disease. *Biochim Biophys Acta* **1787**(11)**:** 1402-1415.
4. Langendorff O (1895). Untersuchungen am überlebenden Säugetierherzen. *Pflügers Archiv*(61)**:** 291–332.

Discovering strategies to control ER loss and aggressive behaviour in acquired endocrine resistant breast cancer

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Acquired resistance to anti-hormonal therapies is a significant clinical problem in breast cancer. Up to 20% of such tumours have lost oestrogen receptor alpha (ERα) during adjuvant treatment.[1] Tumours lacking ERα are often more invasive, and lack targeted therapies.[2] Currently, there is a lack of models to represent such acquired resistant tumours, with little known about the biology of ER loss during treatment. The BCMP group, Cardiff University have derived a series of unique *in vitro* acquired resistant models that have developed significant ERα loss during continuous, long term (> 2years) anti-oestrogen treatment of MCF7 breast cancer cells i.e. tamoxifen (TOYA line) or Faslodex (FASR and FLO lines). By using signalling (EGFR/HER2/MAPK/PI3K/Src) and epigenetic inhibitors, this study has used these models to determine mechanisms driving aggressive migratory behaviour and ERα loss. Such studies aimed to reveal potential treatments for tumours that have become ERα negative on anti-hormone relapse to 1) control tumour aggressiveness and/or 2) allow recovery of functional ERα which may consequently restore sensitivity to anti-hormonal agents.

Basal migratory capacity of TOYA, FASR and FLO cell models was assessed using *in vitro* migration assays, evaluating migration before/after 3 day treatment with gefitinib (EGFR), Herceptin (HER2) UO126 (MAPK), LY29004 (PI3K) and AZD0530 (Src) inhibitors through fibronectin coated membranes. Migrating cells were evaluated by counting across 10 fields/treatment; Expression of nuclear ERα/PR and cytoplasmic pS2 was also analysed in the models (vs. MCF7 cells) before/after treatment (Src blockade using AZD0530; epigenetic modulation using the agents TSA and/or 5-AZA) or antihormone withdrawal (w/d) using *in vitro* immunocytochemistry (ICC). Staining was assessed (across n=6 fields/treatment) using HScore analysis.

FLO and FASR cells had lost all nuclear ERα, while TOYA retained partial ERα expression. All three acquired ERα loss models were highly migratory and had marked decreases in the ERα regulated proteins PR and pS2. The Src kinase inhibitor, AZD0530, significantly reduced migration of all these resistant models by~ 80%. Inhibition of EGFR, MAPK AND PI3K signalling (gefitinib, UO126, and LY29004) only partially reduced migration in the models, although HER2 blockade with Herceptin significantly inhibited the substantial migration of TOYA only. AZD0530 treatment fully restored ERα and PR in TOYA but 1 week tamoxifen w/d resulted in only partial restoration. Neither Src inhibition nor Faslodex w/d recovered ERα in FLO and reversal of epigenetic DNA methylation (using 5-AZA) and/or histone decetylation events (using TSA) also had no impact on ER in this model.

As in the clinic, ERα loss in the models was paralleled by a more aggressive phenotype in acquired endocrine resistance.[2] Targeting Src proved superior in controlling migration of all the acquired endocrine resistant models, suggesting a potential role for Src inhibitors in controlling aggressive tumours losing ER. The TOYA findings also indicated some migration dependency on HER2, thus Herceptin may also have some value in controlling acquired tamoxifen resistant tumours, where there is partial ERα loss. In TOYA, while tamoxifen withdrawal was only partially effective, ERα/PR were substantially recovered with Src blockade, indicating Src dependant ERα loss occurs, potentially due to Src enhanced proteolysis or Src suppression of ER gene expression.[3] Src inhibition may thus provide a mechanism to prevent ERα loss and gain in tumour aggressiveness alongside tamoxifen treatment.[4] However, lack of recovery of ERα expression by Faslodex w/d, AZD0530 or epigenetic reversing agents in FLO suggested unidentified mechanisms can drive ERα loss following long-term Faslodex, requiring future investigation in this model to reveal further treatment avenues.

1. Johnston SR, *et al*. Changes in estrogen receptor, progesterone receptor, and pS2 expression in tamoxifen-resistant human breast cancer. *Cancer Res* 1995; **55**: 3331-8.
2. Gee JM, Giles MG, Nicholson RI. Extreme growth factor signalling can promote oestrogen receptor-alpha loss: therapeutic implications in breast cancer. *Breast Cancer Res*. 2004; **6**: 162-3.
3. Chu I, *et al*. Src promotes estrogen-dependent estrogen receptor alpha proteolysis in human breast cancer. *J Clin Invest*. 2007; **117**: 2205-15.
4. Hiscox S, *et al*. Elevated Src activity promotes cellular invasion and motility in tamoxifen resistant breast cancer cells. *Breast Cancer Res Treat*. 2006; **97**: 263-74.

Interaction of drugs with albumin assessed by chemiluminescence

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Albumin is the most abundant protein in the blood and plays an important role in transportation of drugs. Albumin can have vast effects on pharmaceutical agents with high binding affinities and narrow therapeutic indexes, primarily by effecting their distribution. Bioluminescence is an emission of light from a living organism and was first discovered in luminous jellyfish, the luciferin was coelenterazine. Studies have shown that the mono-oxygenase enzymatic activity of albumin can be detected by chemiluminescence in the presence of coelenterazine.[2] Metabolic toxins such as methylglyoxal produced by gut bacteria modify the binding sites of albumin by altering its enzymatic activity; this alters the pharmacokinetics of some drugs.[3] Many pharmaceutical agents used to treat IBS and IBD bind strongly to albumin. Thus, conditions, which affect the binding of these agents to albumin, will affect their pharmacokinetics. This study was conducted to determine whether pharmaceutical agents used to treat IBS and IBD interact with albumin and whether covalent modification of albumin by methylglyoxal has an effect on this interaction.

The two methods used in this study were the measurement of chemiluminescence using a home built chemiluminometer and the use of spectroscopy. 100µl volumes of each solution containing albumin, coelenterazine and drugs were tested in glass vials for chemiluminescence, buffered by 50mM HEPES pH 7.4, and then quantified digitally using the chemiluminometer. Chemiluminescent counts were recorded for 6x10s and the mean calculated. Drugs were dissolved in ethanol, which had no significant effect on the background chemiluminescence. Initially the optimum temperature for using a chemiluminometer and the justification for taking readings for the first 60s was established. Chemiluminescence was demonstrated with coelenterazine and two types of albumin. Then methylglyoxal and drugs were introduced individually and in combinations to albumin to gather their chemiluminescence. A time course of the absorbance of methylglyoxal's reaction with albumin was done, as well as an absorbance in the presence and absence of methylglyoxal.

The major findings of the study are that bovine serum albumin was discovered to exert a 3.5 fold greater chemiluminescence than that for human serum albumin. Warfarin used as a reference in this study inhibited the chemiluminescence of both bovine serum albumin by 80% ($p=0.0004$) and human serum albumin by 40% ($p=0.006$). The warfarin inhibition was decreased in the methylglyoxal-attenuated albumin compared to albumin in its absence. The only other drug, which proved to inhibit the chemiluminescence of albumin with coelenterazine, was 6-mercaptopurine monohydrate. This drug showed a greater inhibition than warfarin with an inhibition of 86.4% ($p=0.001$) compared to the warfarin's inhibition of 40% in human serum albumin. Additionally methylglyoxal proved to affect both warfarin and dexamethasone's binding to albumin, methylglyoxal increased the inhibition of dexamethasone by 12% ($p=0.037$) and decreased the inhibition of warfarin by 6.7% ($p=0.044$).

Consistent with previous studies warfarin was proven to compete for Sudlow binding Site I of albumin, 6-mercaptopurine monohydrate was additionally proven to bind to this site. The clinical consequences are that binding to albumin may have a significant effect on the distribution of these drugs throughout the body. Methylglyoxal does induce structural modifications of albumin as it has been proven to modify the inhibition of the drugs warfarin and dexamethasone. Methylglyoxal is a biologically reactive glucose metabolite; it's implicated in diabetic complications, and therefore may have a substantial effect on the binding of drugs to albumin in these patients. This study has shown that chemiluminescence is an excellent assay to establish the interaction of a drug with Sudlow Site I of human albumin and may be used more widely. Additionally further investigation is needed to establish whether the methylglyoxal levels within the body are enough to modify albumin and attenuate the binding of protein bound drugs.

1. Sudlow, G. *et al*. 1975. The Characterization of Two Specific Drug Binding Sites on Human Serum Albumin. *Molecular Pharmacology* **11** (6), pp. 824-832.
2. Vassel, N. *et al*. 2012. Enzymatic activity of albumin shown by coelenterazine chemiluminescence. *Luminescence*.
3. Kimzey, M.J. *et al*. 2011. New site(s) of methylglyoxal-modified human serum albumin, identified by multiple reaction monitoring, alter warfarin binding and prostaglandin metabolism. *Chemico-Biological Interactions* **192** (1-2), pp. 122-128.

Design and presentation of a computer-assisted learning package on Tuberculosis and treatment

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Tuberculosis is an infectious disease, affecting one third of the world's population. Early recognition and the implementation of effective pharmacological treatment will prevent further development of the disease.[1] The aim of the project was to design and develop a computer-assisted learning package on tuberculosis and its treatment, which could serve as a teaching aid. This was presented to MPharm II students. Computer assisted learning covers a range of computer-based learning packages, offering interactive study within a specific subject area.[2] It is an effective tool in education providing a number of advantages such as its individualized and flexible use.[3]

In order to evaluate the views of the MPharm II cohort, a 5-point Likert Scale questionnaire was designed and developed.[4] It incorporated five potential responses: strongly agree, agree, no opinion, disagree or strongly disagree. Due to the restrictive nature of a Likert scale questionnaire, a facility for free text entry was provided. The questionnaire was filled in by MPharm II students following completion of the tuberculosis CAL package. It aimed to identify their opinion on the package as a whole, its presentation and content, as well as the overall use of CAL.

Thirty five questionnaires were returned producing a response rate of 30.17%. In general, there was a very positive response to the Tuberculosis package. 89% of respondents found the package beneficial and useful to them. Comments received included '…*excellent, appeared to cover all areas of Tuberculosis very well...'* 89% of respondents believed the package was well presented. The main issues addressed were controversy in the use of colour and animation and the need for more written text to back up the visual aids. While 88% believe that CAL is an effective learning method, not one respondent agreed that it should replace the more traditional learning methods. A common opinion amongst MPharm II was that a combination of both would be most beneficial.

It was concluded, from those that responded that CAL packages are an effective learning aid. However, up to now, they are not regarded as a suitable replacement to the more traditional learning methods. Teaching staff of the MPharm degree should consider the development of new packages to be used within the undergraduate course. Furthermore students should have a higher awareness of packages already available through the university. The reason why such a large proportion of students did not participate in the research project needs to be considered. The project should be repeated in an aim to improve response rate and ensure that the views observed are representative of MPharm II as a whole. The package should be made available to all years and possibly postgraduate students to receive a wider range of views. This should be done before considering the use of these learning packages within the undergraduate course.

1. Pratt, R. Grange, J. Williams, V.2005. *Tuberculosis: A foundation for nursing and healthcare practice.* Great Britain: Hodder Education.
2. Schittek, M. *et al.* 2001. Computer assisted learning. A Review. European J Dental Education. 5. p.p. 93-100
3. Rouse DP. 2007. Computer-Assisted Instruction: An effective instructional Method. Teaching and Learning in Nursing (2007) 2, 138-143.[online] available at: http://www.sciencedirect.com/science?_ob=MImg&_imagekey=B82X44PRGK32C7&_cdi=33020&_user=7381840&_pii=S1557308707000650&_origin=search&_zone=rslt_list_item&_coverDate=10%2F31%2F2007&_sk=999979995&wchp=dGLzVtz-zSkWA&md5=3916cec353ae964f06495ea8eae246f3&ie=/sdarticle.pdf [accessed 15/02/11]
4. Boynton, P. M. 2004. Administering, analysing and reporting your questionnaire. British Medical Journal. 328 p.p. 1372-1375. [Online] Available at: http://www.bmj.com/cgi/reprint/328/7452/1372?maxtoshow=&HITS=10&hits=10&RESULTFORMAT=&searchid=1&FIRSTINDEX=0&minscore=5000&resourcetype=HWCIT [Accessed 4/02/09]

Comprehensive review of psychometric aspects of the Children's Dermatology Life Quality Index (CDLQI)

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Traditionally, quality of life (QoL) assessments were not valued as such to biomedical laboratory results. Nonetheless, with more emphasis directed to measuring the QoL impact, health-related quality of life (HRQoL) tools are now readily available in clinics to capture patient's QoL. Skin diseases such as atopic dermatitis can pose a major impact on the QoL of children. Therefore, the development of the CDLQI has been a significant turning point in the dermatology field with its ability to measure QoL impairment in children aged between 4 to 16 years.[1] Since its inception in 1995, the CDLQI is one of the most widely used HRQoL measure with numerous validations in the literature regarding its psychometric aspects. The aim was to collect and evaluate articles describing the psychometric aspects of the CDLQI from 1995 to the end of 2011.

The data collection was carried out using a relatively up-to-date reference list available on the website of the Department of Dermatology of Cardiff University.[2] To supplement this, a comprehensive literature search was performed using Pubmed and Google Scholar. Due to vast quantities of articles collected, inclusion and exclusion criteria were set up to only include the articles that contained sufficient amount of psychometric data. Consequently, any articles published in abstract format were excluded on the basis of limited psychometric information. A standardised psychometric data collection sheet was used to extract relevant data and this process was followed by data formatting stage, which involved insertion of the collected data into tables.

A total of 81 publications fulfilled the inclusion criteria and were reviewed carefully of the psychometric aspects of the CDLQI. Two articles showed exactly same results for responsiveness to change, so the earlier publication was removed which reduced the number of articles reviewed to 80. Internal consistency, performed as part of the validation of translated CDLQI, was determined by Cronbach's alpha with the range of 0.83 to 0.92. This range is above the guideline defined by Streiner and Norman[3] and therefore the CDLQI demonstrated good internal consistency. The number of publications describing the validity studies, responsiveness to change and test-retest reliability were 37, 16 and 6 respectively. For the test-retest reliability, Spearman's correlation was used in 4 trials to determine the statistical significance of the data (range of $r = 0.74\text{-}0.97$, $p<0.01$). Sensitivity to change was mainly demonstrated using paired t-test and the Wilcoxon signed rank test with only 2 out of 16 studies failing to confirm statistical significance. All 37 trials showed construct validation in which 19% showed construct divergent properties. The most readily used statistical tests for validation studies were Spearman's and Pearson's correlation, observed in 18 and 7 studies, respectively. Despite over 40 translated CDLQI versions available, only 5 journal articles reported the procedures of cross-cultural adaptations. Also, only one article described the clinical meaningfulness of the CDLQI for psoriatic patients with the MCID of 2.5. None of the studies tested for DIF or factor structure.

A steady increase in the use of the CDLQI since its inception has demonstrated its robustness in the clinical and research settings. The aim of the project was fulfilled by evaluation of the psychometric aspects of the CDLQI documented in full English articles published between 1995 and 2011. With the possibility of the extracted data being published as a review article, this information can aid potential users to make informed decisions regarding their specific needs. The project has also highlighted the need for further research of the CDLQI's dimensionality, clinical meaningfulness and DIF.

1. Lewis-Jones, MS. and Finlay, AY. 1995. The Children's Dermatology Life Quality Index (CDLQI): Initial validation and practical use. *British Journal of Dermatology* **132**, pp. 942-949.
2. Department of Dermatology. A. *Children's Dermatology Life Quality Index CDLQI: Articles describing the use* [Online]. Available at: http://www.dermatology.org.uk/quality/cdlqi/quality-cdlqi-publications.html [Accessed at: 01/02/2012].
3. Streiner, DL. and Norman, GR. 2003. *Health Measurement Scales: A practical guide to their development and use*, 3rd edition. Oxford: Oxford University Press.

An Investigation into the Pharmacology of Trace Amines in the Isolated Ileum

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Trace amines are biogenic amines present in the human body at very low concentrations, generally in the region of 0.1 to 10nM concentrations.[1] Examples of trace amines include tyramine, tryptamine, octopamine and β-phenylethylamine (BPEA). They are also found in various foods, notably cheeses, red wine and cocoa-based foods, and can be produced by lactic acid-producing bacteria.[2] Their structure, metabolism and cellular localisation are very similar to that of classical biogenic amines, such as catecholamines, histamine and 5-HT.[3] Their exact function in the body has not yet been defined; however they have been implicated in numerous disease states such as irritable bowel syndrome (IBS), migraine, hypertension and schizophrenia.[1] Trace amines were thought to act as neuromodulators for the classical biogenic amines by increasing the release and inhibiting the reuptake of them, hence the effects seen by trace amines were simply due to this.[3] However, in 2001 receptors that could selectively bind to trace amines were first discovered.[4] They have now been named trace-amine-associated-receptors (TAARs) and it is via these receptors that trace amines are now thought to act. Yet, it is still difficult to ascertain the precise pharmacological mechanism by which trace amines elicit a response. Therefore this project aims to identify the pharmacological effects, if any, of tyramine, tryptamine, octopamine and BPEA in the ileum and to determine if these responses are mediated by the interaction with classical biogenic amine receptors.

Isolated ileum (2cm) was suspended in a water jacketed Krebs solution (50ml, 37ºC) and aerated with gas (95% O_2, 5% CO_2). The ileum was attached to a transducer which recorded contractions as an electrical signal. Prior to each experiment sufficient time (approximately 60 minutes) was left in order for the section of ileum to equilibrate with its surroundings. Concentration response curves (CRCs) were constructed to tyramine, tryptamine, octopamine and BPEA to determine their effect, if any, on the ileum. CRCs were then produced in the presence of the antagonists: atropine, ICI118551, ondansetron, ketanserin and ritanserin to see if they affected the responses originally produced by the four trace amines. In each case the antagonists were incubated for 30 minutes.

Tyramine, tryptamine and BPEA produced concentration-dependent contractions in the ileum above nanomolar concentrations. A significant response to octopamine was not seen. The antagonists atropine and ICI118551 had no effect on the responses produced by tyramine, tryptamine, octopamine and BPEA. Similarly, ondansetron, ketanserin and ritanserin had no effect on the contractile response produced by tyramine.

Antagonism of muscarinic and β_2-adrenergic receptors had no effect on the responses produced by tyramine, tryptamine, octopamine and BPEA. Blockage of 5-HT_2 and 5-HT_3 receptors had no significant effect on the contractile-response produced by tyramine. This could suggest that they are eliciting their responses via TAARs. However, since the endogenous concentrations of trace amines failed to produce a significant response in the ileum this could imply that the physiological role of trace amines and TAARs is minimal.

1. Zucchi, R. *et al*. 2006. Trace amine-associated receptors and their ligands. *British Journal of Pharmacology*. **149**(8):967-978.
2. Broadley, K. *et al*. 2009. Effects of dietary amines on the gut and its vasculature. *British Journal of Nutrition*. **101**:1645-1652.
3. Lindemann, L., and Hoener, M. A. 2005. Renaissance in trace amines inspired by a novel GPCR family. *Trends in Pharmacological Sciences*. **26**(5):274-281.
4. Borowsky, B. *et al*. 2001. Trace amines: Identification of a family of mammalian G-protein coupled receptors, *Synaptic Pharmaceutical Corporation*. **98**(16):8966-8971.

Analysing the capacity of the cell penetrating peptide octaarginine to recycle from the endocytic pathway of cells

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Delivery of therapeutic macromolecules into cells first requires penetration of the cell membrane. This can be a problem for many compounds and carrier molecules have been utilised to aid their delivery.[1] Cell penetrating peptides (CPPs) have been widely studied as vectors for delivering molecules into cells. These are small peptides capable of penetrating the cell membrane, but their exact penetration mechanism is poorly understood.[2] Little information is also available on their fate once inside cells and to what extent, if any, they are recycled. This is important to understand because, if CPPs are recycled back to the cell surface, the therapeutic cargo may also leave the cell. The aims of this investigation were to ascertain whether a fluorescently labelled CPP, octaarginine, is recycled out of A431 cells, and to develop a method of quantifying this using a fluorescence microplate reader. Transferrin and dextran were used as comparison endocytic probes as both molecules are known to be internalised by endocytosis and recycled.[3,4] Like octaarginine, these endocytic probes were analysed as fluorescent labelled probes.

Firstly, experiments using confocal microscopy were used to visualise recycling of the compounds. Control cells were incubated with the fluorescent probe for 90 minutes and separate populations of cells were incubated for 90 minutes with the probes then washed and incubated for a further 2 hours in the absence of the probe to investigate recycling. Confocal microscopy was used to compare the fluorescence of these cells. A second set of experiments were then carried out using a fluorescence microplate reader (FLUOstar OPTIMA) to quantify the changes in fluorescence. As in the previous experiment, control cells were incubated for 90 minutes only with the probes and separate populations of cells were incubated with the probes for 90 minutes then washed and incubated for a further 20, 40, 60, 80, 100 or 120 minutes without the probe. The fluorescence of the control cells was then compared with the other groups to determine how much of the probes were recycled.

Both the confocal microscope and fluorescence microplate reader experiments showed recycling of transferrin: a decrease in fluorescence was seen in the microscope images of the recycling cells compared with the control cells and the fluorescence microplate reader experiments show around 60-80% is recycled after 20 minutes. The confocal microscope images for the dextran experiments show little uptake of the compound in 90 minutes and it was therefore difficult to analyse how much was recycled. The fluorescence microplate reader experiments however do show recycling of dextran as a decrease in fluorescence of about 80% is seen after 20 minutes of further incubation. For the octaarginine experiments, the confocal microscope images show an apparent increase in fluorescence when incubated further suggesting that octaarginine remains in the cell after 2 hours. The fluorescence microplate reader experiments, however, show around 50-80% of octaarginine is recycled out of the cells after 20 minutes.

The fluorescence microplate reader experiments were successful in quantitating fluorescence, however there are concerns as to the reliability of the results as loss of cells due to washing cannot be ruled out. This is apparent from the fact that the results did not correlate with the confocal microscope images, except for the transferrin images which show a decrease in fluorescence due to recycling. The octaarginine images show an increase in fluorescence when incubated further, possibly due to concentration of the compound in lysosomes, and the dextran images show little uptake at all which may be due to not using a high enough concentration. The results from the fluorescence microplate reader experiments suggest up to 80% of octaarginine is recycled out of the cells after 20 minutes, however further experiments may be needed to confirm these findings and to determine the suitability of using this method to quantify recycling of these probes.

1. Torchilin V., 2008. Intracellular delivery of protein and peptide therapeutics. *Drug Discovery Today: Technologies*. **5**(2-3): 95-103.
2. Mae M, Langel U., 2006. Cell-penetrating peptides as vectors for peptide, protein and oligonucleotide delivery. *Current Opinion in Pharmacology*. **6**(5): 509-514.
3. Maxfield FR, McGraw TE., 2004. Endocytic Recycling. *Nature Reviews: Molecular Cell Biology*. **5**: 121-132.
4. Seib PF, Jones AT, Duncan R., 2007. Comparison of the endocytic properties of linear and branched PEIs, and cationic PAMAM dendrimers in B16f10 Melanoma cells. *Journal of Controlled Release*. **117**: 291-300.

Sustained Anti-Inflammatory Activity from Topically Applied PRE-Loaded polyNIPAM Nanogels

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Nanogels are colloidal, cross-linked particles with a size range between 100nm and 1µm, are very penetrative and possess dual temperature-sensitive multiphase behaviour.[1] Poly(NIPAM) nanogel undergoes lattice collapse close to the human body temperature, depending on the chemical environment.[2] An acrylic acid co-monomer in the poly (NIPAM) nanogel is thought to increase thermoresponsiveness.[1] It is due to such properties that there has been interest in using nanogels as vehicles for topical drug delivery. Pomegranate rind extract contains phytochemicals such as ellagitannins which possess many pharmacological properties, one of which is reduction of inflammation.[3,4] The aim was to synthesise and purify poly(NIPAM) and poly(NIPAM-*co*-AAc) nanogels and respectively load them with pomegranate rind extract before testing the anti-inflammatory effects of pomegranate rind extract in *ex vivo* skin by monitoring the levels of PGE_2, an inflammatory mediator, over a period of time.

Nanogel synthesis was achieved using a single step, surfactant-free, emulsion polymerisation. Purification of the nanogels involved five successive steps of ultracentrifugation. The nanogels were consequently freeze-dried before undergoing particle size analysis using a Malvern Mastersizer 2000. The "breathing-in" technique was used to load each nanogel with pomegranate rind extract. Fresh *ex vivo* ear skin was used as a model for determining topical anti-inflammatory activity. The dorsal skin was obtained by blunt dissection and cut into 2.5 cm^2 sections. The skin sections were mounted in glass Franz diffusion cells, with Hanks buffer as receptor solution. 30 mg of drug-loaded nanogel was loaded onto each area of skin using a blunt rod, rotated 10 times under very light pressure. After 2, 5, 10 & 15 hours, cells were dismantled and the skin carefully recovered and the areas exposed to the nanogel excised and homogenised following the addition of indomethacin, prior to centrifugation. Extract from the homogenised tissue was subjected to Solid Phase Extraction using Alltech C18 cartridges. Determination of PGE_2 was achieved via the use of enzyme immunoassay. Samples of 100% and 66% were included in assay. Dilution was achieved by addition of assay buffer. Instructions from the package insert were followed. PGE_2 levels were determined using a plate reader at 450 nm and built-in 4PLC software.

For the poly(NIPAM) nanogel, the surface weighted mean was in the range 0.3-0.45µm, indicating that a gel of nanoscale proportions has been synthesized. The PRE-loaded nanogels were both found to suppress PGE_2 levels by 86% 3 hours after application to the skin, and suppression was sustained. Overall, there was no significant difference in anti-inflammatory effect between the two types of nanogel.

The sustained anti-inflammatory effect was a result of more than one mechanism. PRE inhibits COX enzymes as well as inhibiting the synthesis of arachidonic acid.[3] Such results are promising for future work on using polyNIPAM nanogels for topical *in situ* delivery of anti-inflammatory natural products.

1. Gillian, S *et al.* 2010. Enhanced topical delivery and anti-inflammatory activity of methotrexate from an activated nanogel. *European Journal of Pharmaceutics and Biopharmaceutics.* ; 76**(2)**: 275-281.
2. Das *et al.* 2006. Microgels: old materials with new applications. *Annual Review of Materials Research* 36, pp. 117–142.
3. Panichayupakaeanant, P *et al.* 2010. Antibacterial, anti-inflammatory and anti-allergic activities of standardised pomegranate rind extract. Food *Chemistry* 123**(2)**, pp. 400-403.
4. Houston DMJ. 2011. Towards a nanomedicine-based broad-spectrum topical virucidal therapeutic system. PhD thesis.

Development of Computer-Assisted Learning (CAL) on the Clinical Features, Diagnosis and Management of Eating Disorders

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During the last two decades, computer-assisted learning (CAL) has grown to become an effective tool to supplement teaching and learning material, especially in the health care field. In this project, a CAL package, consisting of educationally appropriate material on eating disorders was developed. The aim of this undertaking was to evaluate the potential of the computer package as a supplementary learning aid for third year pharmacy students. Consequently, the learning package was presented through the Cardiff University Virtual Learning Environment (Learning Central) web site and a questionnaire was developed for completion by third year pharmacy students following presentation of the package. The questionnaire was distributed to obtain student views on three attributes: presentation, content and overall impression of the CAL package.

Generation of the learning package content was preceded by a background literature search which was necessary to capture the most up to date information on eating disorders.[1] Literature concerning the evaluation and development of CAL was also simultaneously scrutinized to produce a high quality presentation for the students. The package presented information on the aetiology, signs and symptoms, diagnosis, risk factors, prevalence statistics, complications, treatment aims, strategies and challenges for the most common types of eating disorder. It was divided into six sections which included a background to eating disorders, anorexia nervosa, bulimia nervosa, binge eating disorders, a glossary and summaries. A survey questionnaire composed of 25 closed statements was constructed and divided into three sections. It was assessed by a 5-point centre-weighted Likert scale with a facility for additional free text comments which were employed to collect both qualitative and quantitative data. The questionnaire was arranged randomly with positively and negatively phrased statements to ensure that all the statements were interpreted appropriately by the participants in order to obtain reliable and valid results.[2] A pilot study was conducted whereby two pharmacy students voluntarily responded to the questionnaire upon completion of CAL package and minor questionnaire modifications were accordingly made.

A response rate of 30% was obtained from 105 questionnaires issued to the third year pharmacy students and these were returned electronically via email. The majority of students agreed with the positively worded statements and disagreed with the negatively worded statements. Additionally, more positive comments were obtained from the students than negative comments in the qualitative free text data. A high percentage of students believed that the CAL package was well presented since the majority of them agreed that the use of animation, a glossary, hyperlinks and content layout were helpful. Ninety-one percent (29 out of 32) of students agreed/strongly agreed that the amount of information on anorexia nervosa, the use of summaries and the information on the background of eating disorders were sufficient and that they helped them to understand the topic more clearly. The majority (84%; 27 out of 32) of students agreed/strongly agreed that the information on bulimia nervosa in the package was sufficient. However, the mean response for the question concerning the amount of information on binge eating indicated that the majority of students had no opinion regarding the statement that the amount of information on binge eating was sufficient. All students (100%) agreed/strongly agreed that the information provided in the package would benefit their future practice as pharmacists. A large number (88%; 28 out of 32) of students, agreed/strongly agreed that the package would be of potential use for continuing professional development (CPD).

The overall response rate was reasonable and it did provide a representative view of third year pharmacy students on the package. Arising from the questionnaire evaluation, it can be concluded that the CAL package was well-perceived and genuinely acceptable as a useful tool to supplement the lectures on eating disorders.

1. Wilson, G. (2007). Cavan, J. *et al* (2010). Eating disorder clinical features and diagnosis. Clinical Pharmacist, p. 325. *American Psychological Association*, **62(3),** 188-216.
2. Boynton, P. M. 2004. Administering, analysing and reporting your questionnaire. *British Medical Journal*. **328**, pp. 1372-1375.[Online] Available: http://www.bmj.com/cgi/reprint/328/7452/1372?maxtoshow=&HITS=10&hits=10&RESU LTFORMAT=&se archid=1 &FIRSTINDEX=0&minscore=5000&resourcetype=HWCIT [Accessed: 23 March 2012]

mRNA expression profiling reveals novel Faslodex response genes in breast cancer

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The antihormone Faslodex (fulvestrant), currently approved as a treatment option in ER+ postmenopausal breast cancer patients after Tamoxifen failure[1], is expected to increase in clinical utility after promising efficacy reports for 500mg vs. 250mg Faslodex in recent clinical trials.[2,3] However, resistance to Faslodex is a problem in breast cancer, with only very few patients achieving complete Faslodex response.[4] Predictive markers are needed to distinguish Faslodex responsive from resistant patients so the latter can be given more-appropriate treatment. There is also a need to decipher the mechanisms underlying Faslodex response and failure to develop improved therapeutic strategies to use alongside this antihormone to maximize its impact. This project employed four ER+ breast cancer models (ER+/HER2+: BT474 & MDAMB361; ER+/HER2-: MCF-7 & T47D) to reflect genetic variability of clinical ER+ breast cancer. The BCMP group, Cardiff University previously showed T47D cells achieved a complete Faslodex response contrasting all other cell models which developed resistance, providing an opportunity to determine genes contributing to complete response and thereby possible response markers and new therapeutic strategies. Preliminary Affymetrix microarray studies by HE Francies in the group revealed Faslodex induced three genes in T47D cells only: Decorin (DCN), Thioredoxin Interacting Protein (TXNIP) and Transforming Growth Factor-Beta 2 (TGF-β2) after 10-day treatment. This project aimed to verify the Affymetrix microarray mRNA signatures with RT-PCR and to evaluate potential function for DCN, TXNIP and TGF-β2 induction in complete Faslodex response.

Web-based GeneSifter software was employed to confirm microarray mRNA expression profiles across the 4 models for untreated control versus 10-day Faslodex treatment. Signatures were then verified by semi-quantitative RT-PCR. Interrogation of gene ontology versus key cancer endpoints was performed using online ontological resources. The clinical relationship between intrinsic gene expression versus patient survival was analysed using a virtual dataset from 392 ER+ endocrine (tamoxifen)-treated breast cancer patients (online KMplot algorithm).

Genesifter studies and RT-PCR verification showed DCN, TXNIP and TGF-β2 were significantly and robustly induced in T47D cells only. Gene ontology studies suggested tumor suppressive functions for DCN, TXNIP and TGF-β2 in relation to growth, cell survival and metastasis. KMplot showed high levels of DCN, TXNIP and TGF-β2 significantly related to improved duration of endocrine response.

DCN, TXNIP, TGF-β2 have been reported as growth-inhibitory signaling genes of prognostic significance in breast cancer and other cancers of various histogenetic origins. Additionally, the anti-oncogenic signaling mediated by DCN, TXNIP and TGF-β2 was reported to have potential to yield new anti-cancer modalities. The novel findings from this project reveal DCN, TXNIP and TGF-β2 are induced only in the T47D model showing complete Faslodex response, as well as having relevance to extended clinical response and potential tumor suppressive function. The genes are therefore worthy of future evaluation as potential clinical predictive biomarkers for Faslodex sensitivity and also to yield new therapies to maximize Faslodex response and delay resistance in breast cancer.

1. Peter, F.B. *et al*. 2000. FDA Drug Approval Summaries: Fulvestrant. *The Oncologist* **7**(6), pp. 477-480.
2. Di Leo, A. *et al*. 2010. Results of the CONFIRM phase III trial comparing fulvestrant 250 mg with fulvestrant 500 mg in postmenopausal women with estrogen receptor-positive advanced breast cancer. *Journal of Clinical Oncology* **28**(30), pp. 4594-4600.
3. Kuter, I. *et al*. 2012. Dose-dependent change in biomarkers during neoadjuvant endocrine therapy with fulvestrant: results from NEWEST, a randomized Phase II study. *Breast Cancer Research and Treatment*, pp. 1-12.
4. Cheung, K.L. *et al*. 2006. Endocrine response after prior treatment with fulvestrant in postmenopausal women with advanced breast cancer: experience from a single centre. *Endocrine-Related Cancer* **13**(1), pp. 251-255.

An evaluation of the administration of subcutaneous insulin at mealtimes in acute inpatients at Betsi Cadwaladr University Health Board (Central and East areas) and of the associated knowledge and understanding of nursing staff

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Increasing occurrence of hypoglycaemic cases has raised concerns that they may primarily derive from the incorrect administration of insulin[1]. Short-acting insulin (SAI) is commonly prescribed for patients with diabetes mellitus however insulin treatment has lately become more complex as a result of the vast range of insulin preparations that are available in the UK. Insulin, when given incorrectly can be one of the most harmful drugs.[1] Therefore insufficient knowledge surrounding insulin can lead to errors and may cause adverse patient effects. The aim was to evaluate nurses' knowledge and understanding of appropriate insulin administration as well as evaluating whether SAI is appropriately administered on the wards in order to determine whether more comprehensive training is required.

Ethical approval was obtained from the Welsh School of Pharmacy Research Ethics Committee and the Research and Development Office. A mixed methods approach was used to collect both qualitative and quantitative data and to allow for incorporation of the triangulation design.[2] Non-participant observations were carried out to identify whether patients were receiving their insulin appropriately in relation to meals. Purposive sampling was used to select patients prescribed SAI and patients either self-administered or were given their insulin by nursing staff. A questionnaire intended to evaluate nurses' knowledge of appropriate insulin administration. As well as fact-based questions, the questionnaire asked nurses whether they had completed an insulin e-learning package. Questionnaires were distributed to a variety of nursing grades before answers were coded and entered on SPSS for analysis. Semi-structured interviews were constructed to explore nurses' views on appropriate insulin administration following completion of the questionnaire.

Of the ten wards included in the study, eight patients consented to participate and a total of 75 observations were made. Approximately seven patients had to be ruled out as they were too ill to consent. Only 55% (41/75) of insulin administered was given correctly. Patients who self-administered received theirs most appropriately (24/41) however 47.1% (16/34) of incorrectly administered insulin was given by nurses. A response rate of 62% was obtained for the questionnaires (n=51). For the fact-based questions, scores ranged from 1 to 10 out of 11 and the mean score was 5.9 (± 2.2). Nurses who had completed the e-learning package had a greater mean score of 7.8 (± 0.5) in comparison to 5.8 (± 2.2) for those who did not complete the package. An Independent-Samples T Test was performed and identified that there was a statistically significant difference between nurses who had completed the e-learning package and nurses who had not regarding questions addressing the strength, storage and prescribing of insulin. Four interviews were carried out to explore nurses' views on the appropriate administration of insulin. Nurses admitted that they were unfamiliar with certain brands of insulin and identified a need for further training in the style of online packages.

The evaluation determined that patients frequently received their SAI dose outside the recommended time scale and nurses' knowledge of insulin was limited. Observations posed the risk of the Hawthorne Effect occurring[2] but it did not appear to be problematic in this study. Nurses were associated with the high percentage of incorrectly administered insulin, placing patients at risk of adverse effects. Pharmacists could play a huge part by annotating drug charts with the appropriate administration times. Nurses' knowledge of appropriate insulin administration was greater for those who had completed the e-learning package. It is evident that all nurses should be encouraged to complete the package in order to refresh their knowledge. Interviews identified that nurses prefer the method of online teaching but in reality nurses are unlikely to find time to complete this style of learning. Further work is necessary in order to gain a true reflection of all insulin administered in Wales and to identify further causes of inappropriate insulin administration.

1. Trepp, R. *et al*. 2010.Diabetes-related knowledge among medical and nursing house staff. *Swiss Med Wkly* **140** (25-26), pp. 370-375.
2. Smith, F.2001.*Research methods in pharmacy practice*. London: Pharmaceutical Press.

Study of the time course of rejection of primary cells grafted in models of Parkinson's disease

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Parkinson's disease (PD) is a neurodegenerative disorder characterised by tremor, bradykinesia, postural instability and rigidity.[1] It occurs due to loss of dopamine (DA) neurons in the substantia nigra (SN) which project into the striatum.[1] Due to the limitations of current treatments, intrastriatal transplantation is being investigated as an alternative approach for treating PD. This procedure involves cells from the ventral mesencephalon (VM) of embryos being implanted into the striatum, in order to replace lost dopaminergic innervations.[2] Graft rejection however, has been found to be a significant concern, hence Cyclosporin A (CsA) is typically given. Despite benefits in clinical symptoms, 15% of patients developed post-graft dyskinesias on withdrawing the CsA.[3] The host immune response could be responsible for these dyskinesias[3] and therefore good models of rejection are required for further investigation. The overall aim of the study was to determine the time course of rejection in subjects that had received graft VM cells, and in doing so to assess whether the local or peripheral immune system is implicated in graft rejection.

All rats were unilaterally lesioned in the SN using 6-OHDA to create models of PD, before receiving an intra-striatal injection of VM cells and were split into experiment I and II. Experiment I received CsA for 8, 9 or 10 weeks, followed by perfusion at 10 weeks. Experiment II received CsA for 4 weeks, with perfusion at 8 or 14 weeks. Amphetamine induced rotations were carried out regularly until perfusion, to assess graft function. Immunohistochemistry was performed for tyrosine hydroxylase (TH), CD45 and OX-42. For TH, DA neurons were counted in the SN and ventral tegmental area (VTA) on both the grafted and intact side, and in the grafted side of the striatum. For OX-42 and CD45 optical density was measured on the intact and grafted striatum.

Amphetamine induced rotations did not change within the time course of Experiment I, however both groups in Experiment II showed a considerable increase in rotations to the right the week before perfusion, compared to 4 weeks post-graft. No significant difference was found for TH expression within Experiment I or II, however a noticeable decline in DA neurons was observed in Experiment II compared to Experiment I. Optical density for CD45 and OX-42 found no significant difference between the intact and grafted sides in Experiment I, however a significant increase was noted in Experiment II.

No signs of graft rejection were seen within the time frame of Experiment I. In contrast, Experiment II showed a clear graft rejection with a decline in DA neurons, activation of microglia within the graft and infiltration of leucocytes. This demonstrates the involvement of both the local and peripheral immune system in graft rejection. Due to the length of CsA given, it is unlikely that graft rejection took place before 'the critical time period'[4] and therefore entry of peripheral immune cells through the blood brain barrier during surgery is an unlikely route of rejection. Amphetamine rotations were seen to become more positive the week before perfusion indicating loss of graft function, while a strong correlation with TH and immune cell response was found, indicating that rotations are a good method for determining graft function prior to perfusion. In order to produce a good model of rejection, it can be concluded that CsA should be given for 4 weeks before withdrawing for a minimum of 4 weeks. Both groups within Experiment II presented a comparable rejection indicating that withdrawing CsA for longer than 4 weeks is not necessary. This study has only partially fulfilled the aims since the time course of graft rejection was unable to be fully established due to the findings of the study. To fully determine the time course of rejection, it may be useful to carry out further studies.

1. NHS Clinical Knowledge Summaries. 2011. Parkinson's Disease [Online]. Available at: http://www.cks.nhs.uk/clinical_knowledge/clinical_topics/previous_version/parkinsons_disease.pdf [Accessed 24 March 2012].
2. Brevig, T., Holgersson, J., Widner, H. 2000. Xenotransplantation for CNS repair: immunological barriers and strategies to overcome them. *Trends Neuroscience* **23**(8):337-344.
3. Hagell, P. *et al*. 2002. Dyskinesias following neural transplantation in Parkinson's Disease. *Nature neuroscience* **5**(7): 627-628.
4. Brundin, P., Olanow, C.W. 2006. *Restorative Therapies in Parkinson's Disease*. Springer Science and Business Media, LLC.

Efflux - Mediated Resistance to Chlorhexidine Digluconate in *Salmonella Enterica* Serovar Typhimurium using a 96 Well Plate Fluorescence Assay

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Nowadays, effective use of biocides is essential to prevent bacterial outbreaks such as *Salmonella*-associated infections. These infections are becoming harder to treat as a result of the emerging resistant strains to commonly used antibiotics. However, the presence of biocides in the environment especially at non-inhibitory concentrations can favour bacteria resistance to the biocide which is commonly associated with efflux pump activity (EPA). Therefore, the study aimed to investigate and analyse the response of *Salmonella* bacteria to low concentrations of biocide by observing the transport of efflux pump substrate, ethidium bromide (EthBr) across the cell envelope of *Salmonella enterica* serovar Typhimurium strain SL1344 and their susceptibility towards antimicrobials, before and after chlorhexidine digluconate (CHG) exposure.

In order to evaluate the susceptibility of SL1344 to CHG and some clinically relevant antibiotics, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of CHG were determined which comply BS EN ISO 20776-1:2006 protocol[1] while the antibiotic susceptibility testing was performed using BSAC disk diffusion protocol.[2] The antibiotics tested were ceftriaxone, piperacillin/tazobactam, ampicillin, gentamicin, tetracycline and ciprofloxacin. These were then repeated using SL1344 that has been exposed to low concentration of CHG for 5 min following BS EN 1276:2009: Suspension Testing methods.[3] As for the assessment of the EPA, a 96-well plate assay was utilised. SL1344 were incubated with shaking until mid-log phase for 3h with the presence or absence (control) of ranges of non lethal concentrations of CHG (0.0001% w/v, 0.0002% w/v and 0.0004% w/v). After 3h, the cells were then treated with EthBr and the intracellular accumulation of EthBr was measured with and without the presence of an efflux pump inhibitor (EPI) called carbonyl cyanide 3-chlorophenyl hydrazone (CCCP), using a plate reader FLUOstar optima.

All data sets were subjected to statistical analysis using single factor ANOVA with α=0.05. Without the presence of CCCP, the results indicated that the fluorescence intensity of EthBr decreases significantly with increasing concentrations of CHG exposed to SL1344. Low fluorescence intensity values mean low amount of EthBr accumulated intracellularly, thus indicates high level of EPA. With the presence of CCCP, although there was an increased accumulation of EthBr, no significant difference between data was observed because the activity of the efflux pumps was inhibited. Moreover, exposure to CHG led to a significant increase in MIC and MBC so higher concentration is needed to inhibit and kill the bacterial growth. But, no profound differences in clinical susceptibility of SL1344 to several of antibiotics tested because single exposure of biocide did not compromise with the clinical effectiveness of the antibiotics.

These phenomena demonstrated that enhanced activity of efflux pump can select reduced susceptibility and efflux mediated resistance in *Salmonella* when low concentrations of biocide present in their environment. In fact, fluorescence assay using FLUOstar optima is a reliable and powerful tool, which provides sensitive and specific test to assess the role of overall active efflux, hence demonstrating a concrete evidence of emerging efflux mediated biocide resistance in bacterial strain.

1. British Standard Institution (BSI). 2006. *BS EN ISO 20776-1:2006: Clinical laboratory testing and in vitro diagnostic test systems. Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices. Reference method for testing the in vitro activity of antimicrobial agents against rapidly growing aerobic bacteria involved in infectious diseases.* London, UK.
2. The British Society for Antimicrobial Chemotherapy (BSAC). 2002. *BSAC Disc Diffusion Method for Antimicrobial Susceptibility Testing*. London, UK.
3. British Standard Institution (BSI). 2009. *BS EN 1276:2009: Chemical disinfectants and antiseptics. Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas. Test method and requirements (phase 2, step 1).* London, UK.

Biocide Resistance of *Staphylococcus aureus* Strains Isolated from the Food Industry

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Biocide resistance has been studied over 60 years ago but due to belief that its occurrence was rare it was deemed clinically insignificant. Nowadays, its study has become more common due to increasing fears of antibiotic resistance, the link between this with biocide resistance and the in depth study and evaluation of resistance mechanisms.[1] Recent outbreaks of pathogenic bacteria in food produce have caused detrimental effects to human health and to decrease the likelihood of their reoccurrence, one major aspect is to validate biocide efficacy. The purpose of this study was to test strains of *Staphylococcus aureus* isolated from a conveyor belt from a meat processing plant in France. The isolates were collected after disinfection procedures and were tested against the same compound, in which disinfection was performed. The aims were to compare results from the two isolated strains, *S. aureus EMPF2* and *EMPF85*, against a culture reference strain of *S. aureus NCIMB 9518*, explain the differences in results and propose how these could affect disinfection procedures currently used in the food industry.

A compound comprising glutaraldehyde and the QAC was tested against all strains. Two test methods were carried out to investigate biocide efficacy. These were the Carrier Test and the Sedimentation Biofilm. Both utilised a stainless steel disc to represent bacterial adherence to surfaces in industry. Overnight cultures of the bacteria to be tested were grown and a count of viable bacteria was performed (Miles and Misra) before each test was carried out. The Carrier test demonstrated the changes in biocide efficacy in 'dirty' conditions – in the presence of bovine serum albumin while the Sedimentation Biofilm showed how biocide susceptibility was affected when bacterial cells were contained in a biofilm.

Results from the Carrier Test showed that the presence of BSA decreased efficacy of the biocide. The isolated *F2* strain showed the greatest susceptibility as no counts were obtained at 5 min. The reference *9518* strain survived at 5 min but was killed at 30 min and the isolated *F85* strain survived at 30 min but no counts were obtained at 60 min. The Sedimentation Biofilm showed that the reference strain *9518* was the most susceptible to biocide when contained in a biofilm. No counts were obtained for this strain at 60 min whereas both isolated strains *F2* and *F85* survived at this time point with no counts being obtained at 120 min.

The presence of BSA decreased biocide efficacy significantly as both 9518 and F85 strains were not killed within 5 min. Bacterial cells contained in the biofilm led to a decreased susceptibility to biocide. However from results obtained, it could only be concluded that this was due to increased 'tolerance'. Biofilms act as a physiological barrier against biocide and although they have also been shown to influence acquired resistance mechanisms[2], this could not be proven in this study. Results could not be validated due to time constraints and therefore necessary repeats could not be performed. Further research is required in order to investigate into the resistance mechanisms, if any, that the isolated strains possess. Results did, however, prove the importance of biofilm and organic matter removal in industry. Since proteins can inactivate biocide and bacteria are more difficult to remove when contained in biofilms[3], then manufacturers must take necessary measures to ensure they are not present in processing plants. This could lead to the re-evaluation of many disinfection procedures in industry including biocide choice.

1. Assessment of the Antibiotic Resistance Effects of Biocides. 2009. Scientific Committee on Emerging and Newly Identified Health Risks.
2. McDonnell, G. Russell A,D. 1999. Antiseptics and Disinfectants: Activity, Action, and Resistance. Clinical Microbiology Reviews. American Society for Microbiology. 12. (2) 147 – 179.
3. Carpentier. B, Cerf. O. 2003. Review: Biofilms and their Consequences, with particular reference to hygiene in the food industry. Journal of Applied Bacteriology. 75. 499-511

The sporicidal activity of polyphenols: an examination of the ability of polyphenols to inactivate pathogenic bacteria such as *Clostridium difficile*

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Tea is one of the most popular drinks in the world[1] and is derived from the leaves of the plant *Camellia sinesis.*[1] Tea has been found to have a range of beneficial effects such as antioxidant activity, anticarcinogenic potential, ability to lower plasma cholesterol levels, antiviral and antibacterial activity.[1] Tea's antimicrobial effects are thought to be due to the presence of polyphenols; collectively termed as catechins. There are four major catechins found in tea extracts (epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC) and epigallocatechin gallate (EGCg)), however studies have shown that the EGCg catechin is the most potent antibacterial of the four.[2] The bactericidal effects of EGCg are due to the catechin's ability to disrupt the bacteria's phospholipid membrane, causing leakage of the cell's content.[2] Therefore we sought to see whether there was similar activity against spores. *Clostridium difficile* is Gram-positive, spore-forming anaerobic bacteria and currently one of the most common health-acquired infections in the western hemisphere, causing symptoms of the infection such as diarrhoea and pseudomembranous colitis.[3] The bacterial spore is the etiologic factor of *C. difficile* and is essential for the spread of the infection, often by the faecal-oral route.[4] In order to cause the disease, the *C. difficile* spores must germinate and return to vegetative cell growth, also allowing for the production of toxins.[4] Therefore the aim of the study was to investigate the sporicidal activity of the catechin EGCg using the spore forming bacteria *Bacillus subtilis* and *C. difficile*.

The method described by Miles and Misra[5] was employed in order to assess sporicidal activity following exposure of EGCg. Following exposure, a 20µl aliquot was serially diluted in TSC (180µl) using a 96 well microtitre plate. Three 10µl aliquots per dilution were plated onto the dried surface of the appropriate agar plate. *B. subtilis* was plated onto Tryptone Soya Agar (TSA) plates while *C. difficile* was plated onto degassed Brain-Heart Infusion (BHI) agar plate supplemented with 1% of the bile salt sodium taurocholate. Each count was repeated twice. Plates were incubated for 24 hours under the appropriate atmospheric conditions and the mean colony forming units per ml (CFU/ml) for each plate and dilution were calculated. All experiments were performed in triplicate and negative controls using sterile deionised water (SDW) were also carried out.

After 7 and 4 days incubation of *B. subtilis* and *C. difficile* spores, respectively with EGCg concentrations of 0, 800, 1600, 3200 and 6400µg/ml, there was no evidence of sporicidal activity. However, after 24 hours of exposure of vegetative *B. subtilis* to the same concentrations of EGCg, there was a marked decrease in viable growth. EGCg concentrations of 800, 1600 and 3200µg/ml gave a 2 log CFU/ml reduction, with an EGCg concentration of 6400µg/ml giving a 3 log CFU/ml reduction in viable growth.

In conclusion, EGCg did not have any sporicidal activity on *B. subtilis* and *C. difficile* spores after 7 and 4 days respectively, at concentrations up to 6400µg/ml, however activity could potentially be observed if the spores were incubated with the EGCg for a longer period of time. EGCg did show bactericidal activity against vegetative *B. subtilis* after 24 hours exposure, possibly suggesting that there would be similar activity against other spore-forming bacteria such as *C. difficile*.

1. J.M.T. Hamilton-Miller, Antimicrobial properties of tea (*Camellia sinensis L.*), Antimicrobial Agents and Chemotherapy 39 (1995), 2375-2377.
2. H. Ikigai, *et al.* Bactericidal catechins damage the lipid bilayer, Biochimica et Biophysica Acta - Biomembranes 1147 (1993), 132-136.
3. V.K. Viswanathan, M.J. Mallozzi, G. Vedantam, *Clostridium difficile* infection: An overview of the disease and its pathogenesis, epidemiology and interventions, Gut Microbes 1 (2010), 234-242.
4. D.D. Hedge, *et al.* New advances in the treatment of *Clostridium difficile* infection (CDI), Ther Clin Risk Manag 4 (2008), 949-964.
5. A.A. Miles, S.S. Misra, J.O. Irwin, The estimation of the bactericidal power of the blood, J Hyg (Lond) 38 (1938), 732-749

Association of ZIP6 and STAT3 in Breast Cancer Cells

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Breast cancer is a disease with a complex aetiology, for which new treatments are required. Zinc has been identified as an element of interest in breast cancer as it is crucial for a number of signalling pathways involved in cell growth, division and apoptosis. For this reason it is important that intracellular zinc levels are tightly controlled, this is achieved through zinc transporters, including ZIP6. ZIP6 is a member of the LIV-1 subfamily of zinc influx transporters, which control zinc entry into cells.[1] It is known to be present in increasing amounts in ER positive breast cancer, and through the regulation of intracellular zinc, is thought to be involved in the cell cycle. Furthermore, recent evidence has shown ZIP6 to be a downstream target of the transcription factor STAT3,[2] a protein that has also been significantly linked with cancer development and has been shown to enhance cell proliferation and prevent apoptosis in a variety of tumorgenic cells.[3]

In light of this, the aim of this study was to assess whether ZIP6 and STAT3 are present in higher levels in tamoxifen resistant breast cancer cells compared to wild-type MCF-7 cells, and to characterise their relationship. In order to fulfil this aim two techniques were performed on tamoxifen resistant (TamR) breast cancer cells. The first technique was immuno-fluorescent microscopy imaging, for which cells were probed with ZIP6 and STAT3 antibodies, followed by nuclear staining with DAPI to identify cell nuclei. Secondly, proximity ligation assays were performed to assess the binding of ZIP6 and STAT3 in cells. This relatively new technique ultimately results in the production of red immuno-fluorescent dots (proximity ligation assay signal) at each site of protein-protein interaction, when the cells are viewed on a Leica RPE automatic microscope. The numbers of red immuno-fluorescent dots per cell were determined using ImageTool software.

A combination of immuno-fluorescent microscopy imaging and proximity ligation assays was used to investigate the role of ZIP6 and STAT3 in Tamoxifen-resistant cells.

Previous research suggests that ZIP6 is activated by STAT3 in cells, resulting in an increase in intracellular zinc levels.[2] This increase in zinc is known to activate a signalling pathway that ultimately leads to reduced E-cadherin expression and cell rounding.[4] Existing evidence has been developed further by the novel findings of this investigation, as proximity ligation assays demonstrate binding of ZIP6 and STAT3 in cells. This leads to the conclusion that the binding of these two proteins causes loss of E-cadherin and cell roundings. As both STAT3 and ZIP6 have been found in high levels in a number of ER positive breast cancer specimens, this new phenomenon may be highly significant in the development of future treatments for tamoxifen resistant breast cancer.

1. Taylor KM *et al*. The emerging role of the LIV-1 subfamily of zinc transporters in breast cancer. *Molecular Medicine*. 2007;**13**:396-406.
2. Yamashita S, *et al*. Zinc transporter LIV-1 controls epithelial-mesenchymal transition in zebrafish gastrula organizer. *Nature*. 2004;**429**:298-302.
3. Buettner R, Mora LB, Jove R. Activated STAT3 signalling in human tumors provides novel molecular targets for therapeutic intervention. *Clin Cancer Res*. 2002;**8**:945-954.
4. Shen H, Qui H, Guo J. Concordant correlation of LIV-1 and E-cadherin expression in human breast cancer cell MCF-7. *Mol Biol Rep*. 2009;**36**:653-659.

Investigating the Effects of Changing pMDI Alcohol Concentration on Particle Size Distribution of Beclomethasone Inhalers

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Changing particle size of aerosols produced by pressurised metered dose inhalers (pMDI) may have a large influence on their therapeutic potency[1] and thus gives the potential of lowering doses of drugs with problematic side effects such as the steroid, beclomethasone. This study aimed to investigate the effects of altering the co-solvent concentration, ethanol, on the particle size distribution of beclomethasone inhalers. The commercial inhalers QVAR (Teva) and Clenil Modulite (Chiesi) are available in different dose strengths with QVAR having a potency around 2 to 2.5-fold greater than that of Clenil, largely due to differences in aerosol particle size between the formulations. Experimental solution formulations of beclomethasone in an attempt to modify the aerosol particle size distribution.

Particle size distribution was analysed with the use of a Next Generation Impactor (NGI) using a flow rate of 30L/min. Samples from all collection surfaces were recovered with a methanol using a reverse phase HPLC assay. Inhalers with a range of ethanol concentrations were produced and the aerosol particle size distribution was assessed by mass median aerodynamic diameter (MMAD), geometric standard deviation (GSD), fine particle fraction (FPF) and extra fine particle fraction (EFPF). The FPF was defined as the percentage of particles less than 5µm in diameter. The EFPF was defined as the percentage of particles less than 1.4µm in diameter; this latter is a reflection of the percentage of particles deposited from plate 6 of the impactor to the filter.

The results from analysis of QVAR and Clenil Modulite showed that QVAR produces aerosol particles with a MMAD of 0.82µm in comparison to the 3.3µm of Clenil. The FPF and EFPF of QVAR were also found to be approximately 3-10 fold-higher than with Clenil. For the experimental inhalers, there were no statistically significant differences in terms of MMAD. However, higher alcohol concentrations were significantly associated with reduced FPF, from 73% with 3%w/w ethanol to 36% with the 15%w/w ethanol formulations with concomitant decreases in EFPF. The deposition of drug in the induction port of the NGI was also correlated with ethanol content, from 24% deposition with the 3%w/w ethanol to 62% with the 15%w/w ethanol formulation. In addition, the GSD increased with higher ethanol content in the formulations.

The results indicate that the increased potency of QVAR is most likely due to the smaller aerosol particle size, higher FPF and a decreased oropharyngeal deposition (as predicted by NGI throat deposition) in comparison to Clenil. It was found that changing ethanol concentrations solely did not have a profound effect on the MMAD, thus particle size within the range measured by the NGI is not profoundly affected by alcohol content. However, the GSD trends showed increased variation in particle size with a 15%w/w ethanol concentration in the experimental formulations. Furthermore, reduced ethanol concentrations were associated with decreased drug deposition in the NGI induction port, thus potentially decreasing the risk of oral candidiasis that is a particular problem with steroidal inhalers.[2] Therefore increasing the ethanol concentration of inhalers decreases the therapeutic efficacy of inhalers. From the four experimental formulations tested, 3%w/w ethanol inhalers produced the most optimum inhaler with greater the highest FPF. Currently, there aren't any licensed CFC-free beclomethasone pMDI's with alcohol content of less than the 8% of QVAR.[3] Therefore the results from this investigation prove the possibility of enhancing inhaler performance and ultimately patient care through decreasing co-solvent concentration.

1. C. Leach, G.L. Colice, A. Luskin. Particle size of inhaled corticosteroids: Does it matter? *Journal of Allergy and Clinical Immunology*. **125** (2009), Pp88-93.
2. N. Godara R. Godara, M. Khullar. Impact of inhalation therapy on oral health. *Lung India*.**28** (2011), Pp.272-5.
3. J.P. Mitchell, *et al.* The Abbreviated Impactor Measurement (AIM) Concept: Part II—Influence of Evaporation of a Volatile Component—Evaluation with a "Droplet-Producing" Pressurized Metered Dose Inhaler (pMDI)-Based Formulation Containing Ethanol as Cosolvent. *American Association of Pharmaceutical Scientists.* 2009.

Domiciliary Visits to Elderly Patients by a Pharmacy Technician: The Patients' Perspective

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The ageing population is often associated with greater burden of disease. The elderly population often taking multiple and complex drug regimens are some of the most vulnerable to problems with their medications.[1,2] It is widely recognised that the risk of miscommunication and unintended changes to medications is significantly higher when patients are transferred from one care setting to another.[1] This may consequently lead to decreasing patients' adherence to their medications as patients are confused on which treatment plan to follow after discharge from hospital (NICE).[3] In an attempt to reduce these issues, a new service has been introduced at a hospital whereby the Pharmacy Technician (PT) conducts domiciliary visits to elderly patients' homes after they have been discharged from hospital. The aims of the study were to identify the number of patients suitable to be included into the new domiciliary visit project and to explore patients' views of a domiciliary visit by a PT post discharge from hospital.

Ethical approval for the study was obtained. The study was divided into two parts (Study 1 and Study 2). For Study 1, a data collection form was designed to cater a cross sectional descriptive survey method using a representative sample approach.[4] The form allowed the researcher to gather data on the number of patients who would meet the essential criteria to be included into the domiciliary visit project. 59 discharged prescriptions were analysed between the months of October 2011 to January 2012. Study 2 involved the use of a semi-structured qualitative interview method to obtained patients' views on the domiciliary visit by the PT after discharge from hospital. The convenience sampling method was used in this study. The interview schedule took an iterative approach whereby constant changes were made to the interview schedule if valuable information was generated from previous interviews. Eleven patients were recruited into the study. These interviews were conducted at the patients' home. All interviews were tape recorded, transcribed and analysed. Thematic analysis was conducted for this interview whereby the transcripts were repeatedly analysed to identify common themes that emerged from the interviews.

Study 1 showed a high number of patients (n=35/59; 59%), both male and female who fitted into the three main essential criteria, which gave a good indication of the number of patients who were eligible for the domiciliary visit by the PT. In Study 2, thematic analysis allowed the identification of common themes from the transcripts. One of the main themes identified was the benefit of the service to patients. Majority of the patients found the service very beneficial. The service had also improved patients' knowledge about their medicine and therefore improved patient compliance. However, patients who were not responsible for their own medicines and were reliant on family and care packages did not benefit from this service compared to those who were managing their own medicines. Another major finding was the use of the Medicine Reminder Chart (MRC). Majority of the patients found the MRC very useful as the chart provided information on when to take their medicines as well as information about their medicines. However, some patients who were on medicines for a long period of time did not use the chart often as they were familiar with their medicines and therefore had a routine in place.

The aims and objectives of the study were achieved. The data collection tools used in both studies was valuable to give very satisfactory and reliable results. The baseline data presented a good indication of the need of this service to be implemented. The positive feedback from patients about the domiciliary visit demonstrated that meeting patients in their home environment can provide useful information. The majority of patients appreciated the PT's visit indicating that the time and effort devoted to a home visit is likely to be perceived favourably by the patient and increase cooperation in managing the medication regimen. In this sense, domiciliary pharmacy should be implemented in the National Health Service for the benefit of elderly patients.

1. Royal Pharmaceutical Society [RPS] (2011) *Keeping patients safe when they transfer between care provider – getting the medicines right*: *Good Practice Guide for Healthcare Professionals*. London, UK: RPS Publications.
2. Welsh Assembly Government (2006) Medicines & older people. In: National Service Framework for Older People in Wales
3. National Institute for Health and Clinical Excellence [NICE] (2009) *Medicines Adherence: Involving Patients in Decision about Prescribed Medicines and Supporting Adherence*. London, UK: NHS Publications
4. Babbie E. (2007) *'The practice of Social Research'*. Belmont, USA: Thomson Wadsworth

Improving Self-Administration of Microneedle Devices

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Microneedles are tiny micron-scale needles that can increase skin permeability by disrupting the stratum corneum whilst avoiding the nerve endings and blood vessels of the skin. Microneedles are considered more suitable for self-administration than hypodermic. This could prove extremely useful in developing countries where healthcare professionals are in short supply, in the event of a pandemic outbreak or for conditions such as diabetes.[1,2] It is important however that the correct force is applied in order for the needles to successfully puncture the stratum corneum.[3] An application method or applicator will therefore likely be required if wide scale microneedle self-administration is to become a reality.[3] The aims of this study are to develop a simple, testable microneedle applicator in order to standardise the force at which individuals apply a microneedle patch and to develop appropriate methods to test its effectiveness at causing skin puncture.

After initial testing, it was decided that an adapted sports cap from a drinking bottle was the most feasible and effective applicator. Further tests were carried out between different types of sports cap with the Powerade® cap eventually being chosen. A standard protocol was then developed for testing the devices ability to standardise force on 50 volunteers. Once the data had been collected it was collated and analysed. Further tests were then carried out in order to determine the applicators ability to puncture *ex vivo* porcine and human skin samples (both full thickness skin and heat separated epidermal sheets). These tests included methylene blue staining, trans-epidermal water loss and Franz-type cell diffusion studies. Any microneedles that were used were also examined using an Environmental Electron Scanning Microscope both before and after testing in order to analyse physical damage.

From testing the applicator on 50 volunteers and comparing the data to a previous study[3] it was shown that the range of forces individuals used to apply a dummy microneedle patch were significantly reduced. The range was reduced from 42.71N to 28.33N. Inter-individual standard deviation was decreased from 11.16 to 5.14 as was intra-individual standard deviation from 5.63 to 1.63. The applicator was also shown to be capable of puncturing full thickness human skin as well as the epidermal layers of both human and pig skin, however punctures were inconsistent.

The applicator was shown to significantly reduce variation in application force. Any remaining notable variation was likely due to differences in the three applicators used, the gradual seizing up of each applicator as well as incorrect use of the applicator by a number of volunteers. Improvements could be made to the applicator/study in order to reduce variation further. The inconsistencies in skin puncture can likely be explained by the variations in speed at which the applicator was pressed down for each attempted puncture[3] along with other factors.

1. M.R. Prausnitz, R. Langer, Transdermal drug delivery, *Nature Biotechnology*, **26** (2008), 1261-1268.
2. M.R. Prausnitz, *et al.* Microneedle-based vaccines, *Current Topics in Microbiology and Immunology*, **333** (2009), 369-393.
3. F. Arikat. Evaluating the potential for patient administration of a microneedle device (Jul-Aug 2011)

Comparison of the Antibacterial Activity of Polar and Non-Polar Components in Black Tea against *Staphylococcus aureus*

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Antibiotic drug resistance is a major problem worldwide; the most prevalent of which is methicillin resistant *Staphylococcus aureus* (MRSA). MRSA infection can result in minor skin disease to the more severe respiratory tract infections, such as pneumonia.[1] Tea (*Camellia sinensis*) is a natural product of particular interest in recent years due to its numerous pharmacological effects, for instance as an antimicrobial agent.[2] The majority of research in this area has focused on the pharmacology of polar compounds in tea infusions; however there has been little investigation of the non-polar compounds which are also present in tea leaves. The aim of this study was to compare the antimicrobial activity of non-polar and polar pigments of black tea against pathogenic isolates of *S aureus* and MRSA.

Two black teas were investigated in this study, the first from India (A) and the second from Sri Lanka (B). A 25g sample of each tea underwent a three phase solvent system extraction, first with petroleum ether then with ethyl acetate and lastly with methanol, allowing compounds to be categorised in terms of polarity. After each extraction the mixtures were filtered and samples of the liquid supernatant were analysed using thin layer chromatography (TLC). Preparatory TLC allowed for isolation of various tea pigments as well as comparative analysis between TLC profiles of the two teas. A microbial suspension test, using non-resistant *S. aureus,* was first carried out in order to screen the pigments isolated as well as the crude extracts. Six of the pigments were then chosen to undergo further testing in a contact time suspension test in which two concentrations of each pigment were tested; 0.1mg/ml and 0.01mg/ml. Minimum inhibitory concentration testing was additionally carried out on the crude methanol fractions against *S. aureus*.

The TLC plates showed significant differences between the tea samples. The petroleum ether extract originating from tea A gave rise to just three different compounds compared to tea B which gave rise to five compounds. The ethyl acetate fractions from both teas were however very similar, both showing an additional grey compound not present in the petroleum ether fractions. The screening test indicated potent bactericidal activity of all compounds tested at a concentration of approximately 1mg/ml, however there was no reduction in bacterial growth when pigments were tested at lower concentrations of 0.1mg/ml and 0.01mg/ml.

The different TLC profiles of the two teas suggest that environmental factors such as temperature, humidity and sunlight affect the non-polar composition of tea leaves. However, there are other factors which could influence the relative compounds in tea, for example the manufacture process.[3] Neither of the concentrations (0.1 and 0.01mg/ml) showed any significant antimicrobial activity over the 18 hour time scale. This suggests that the activity lies between 0.1 and 1mg/ml. One possible explanation for the apparent loss of activity was that the pigments precipitated out of the DMSO solution upon dilution during the test. In terms of drug delivery a large MIC concentration of 1mg/ml could present problems. However, there may be scope for drug development in an attempt to increase potency of some of the compounds isolated.

1. Deleo, F. R. *et al.* 2010. Community-associated meticillin-resistant Staphylococcus aureus. *Lancet* **375**(9725), pp. 1557-1568.
2. Hamilton-Miller, J. M. 1995. Antimicrobial properties of tea (Camellia sinensis L.). *Antimicrob Agents Chemother* **39**(11), pp. 2375-2377.
3. Astill, C. *et al.* 2001. Factors affecting the caffeine and polyphenol contents of black and green tea infusions. *J Agric Food Chem* **49**(11), pp. 5340-5347.

Design and Synthesis of Novel Potential HCV Helicase Inhibitors

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Approximately 170 million people worldwide are infected with hepatitis C virus (HCV) and the majority will proceed to develop chronic HCV. HCV is curable, but current therapy is mostly non-specific; poor efficacy and tolerability renders the treatment insufficient for many patients.[1] There is a move towards the development of 'specifically targeted antiviral therapy for HCV' (STAT-C) agents. There are a number of STAT-C compounds in clinical development. Recently, boceprevir and telaprevir became the first two to be approved. However, resistance appears to be an issue when these agents are used alone.[2] It is therefore believed that a combination of STAT-C agents targeting different aspects of the HCV life-cycle could be used to increase treatment success whilst overcoming the problem of resistance. HCV helicase offers a possible target for such a therapeutic strategy. Helicase has been shown to be essential for HCV replication whilst structural analysis has revealed unique motifs and ligand-binding clefts which could accommodate small molecule inhibitors.[3] The aim of this project was to design and synthesise novel potential inhibitors of the RNA binding site of HCV helicase using rational modification of a lead compound.

The structure of the lead compound was compared with that of known inhibitors targeting the RNA binding site to identify what chemical features might be required for antiviral activity. It was subsequently decided to retain the aromatic sulfonamido groups of the lead compound but vary the diamide linker connecting the two groups. The chlorine in the aromatic sulfonamido group was varied to lower molecular weight. The inhibitor compounds along with a number of known inhibitors were then docked into a crystal structure of HCV helicase to visualise how they might interact with key residues in the RNA binding site and indicate which compounds were worth pursuing.

Each of the potential inhibitors formed three-four ligand interactions with key HCV helicase residues, often in addition to other interactions, which measured up to the interactions formed when known helicase inhibitors were also docked. Sulfonyl and carbonyl oxygens of the designed compounds tended to form hydrogen bonds with key residues which mimics the interactions formed with phosphoryl oxygens of the RNA backbone. Based on the interactions formed between the designed compounds and HCV helicase, it seems all of the compounds could potentially inhibit the RNA binding site and so were subsequently synthesised. Ten compounds were successfully synthesised and purified in respectable yields.

The synthesised compounds are due to be tested in cell-based HCV replicon and isolated helicase enzymatic assays. The results will determine future investigations. If any of the compounds are found to be more active than the lead compound, further modifications will be undertaken to optimise activity, this may involve using molecular docking studies to identify alterations. There are currently no HCV helicase inhibitors in clinical development; however the move towards STAT-C therapy and the rapid resistance encountered with other STAT-C agents make HCV helicase an attractive target for further drug design.

1. Dvory-Sobol H, Pang PS, Glenn JS. 2006 The future of HCV therapy: NS4B as an antiviral target. *Viruses* **2**: 2481-2492.
2. Yang PL, *et al.* 2011 Anti-HCV drugs in the pipeline. *Current Opinion in Virology* **1**: 607-616.
3. Belon CA, Frick DN. 2009 Helicase inhibitors as specifically targeted antiviral therapy for hepatitis C. *Future Virology*. **4**: 277-293.

Evaluation of the Training and Preparedness for Medicines-Based Calculations in Health Care Professionals and Undergraduate Students in Betsi Cadwaladr University Health Board (West)

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The ability to perform medicines-based calculations (MBCs) is an essential skill required by health care professionals and undergraduate students in pharmacy, nursing and medicine. However, weaknesses when performing MBCs have been identified by several research studies[1-2] and have been highlighted by patient safety incidents.[3] Such weaknesses may be due to underlying factors such as insufficient education, lack of confidence and/or mathematics anxiety. Greater understanding of the training received by healthcare professionals and undergraduate students and their preparedness for MBCs in practice may be a beneficial way to identify weaknesses and suggest improvements. This study aimed to explore how doctors, nurses and pharmacists and undergraduate medicine and nursing students are trained and prepared for their role in performing MBCs in one hospital.

Ethical approval was obtained from Cardiff School of Pharmacy and Pharmaceutical Sciences Ethics Committee. This study was also registered with the Research & Development Office in BCUHBW as a service evaluation. Participants were recruited from the pharmacy, nursing and medical professions using three non-probability sampling methods; purposive sampling, snowball sampling and convenience sampling. Semi-structured interviewing was chosen as the qualitative methodology. Following the development of an interview schedule, semi-structured interviews were conducted with forty-three participants (13 medicine, 17 nursing and 13 pharmacy professionals) in order to gain insight into their experiences and opinions on their training and preparedness for MBCs. Written informed consent was obtained prior to an interview. The interviews were face to face, audio-recorded and transcribed ad verbatim. All data were analysed thematically.

Nurses and pharmacists acknowledged they had dedicated pre-qualification and post-qualification education on MBCs. Doctors indicated they had dedicated post-qualification education but this was not the same for their pre-qualification education on MBCs. Doctors had either integrated education on MBCs or none at all before qualification. Many participants, including doctors, reported there should be more dedicated education on MBCs before and after qualification. A number pharmacists spoke of frequent reassessment during pre-registration training and nurses mentioned reassessment once qualified. Participants reported being confident in performing those MBCs required in their current roles. Some participants admitted to being less confident in less frequently used MBCs. More pharmacists were confident in performing most types of MBCs. Some doctors and pharmacists felt that nurses lacked confidence when performing MBCs. This was confirmed by some nurses, who indicated that they relied on other health care professionals. Furthermore, some participants identified specific MBCs, involving infusion rates, that they found challenging. Participants believed any MBC errors could potentially compromise patient safety.

This study successfully explored the training received and preparedness for MBCs in three health care professional groups at BCUHBW. The results support evidence in the literature that some students and health care professionals have weaknesses in relation to performing at least some types of MBCs.[1,2] The data will be used to inform BCUHBW of these issues. Further research involving larger numbers, different cohorts and in a greater number of settings will be valuable, and may address some limitations of the present study.

1. Wheeler D. W, *et al*. 2004. Doctors' confusion over ratios and percentages in drug solutions: the case for standard labeling. *Journal of the Royal Society of Medicine* **97**: 380-383
2. Harne-Britner, S. *et al*. 2006. Improving medication calculation skills of practicing nurses and senior nursing students. *Journal of nurses in staff development* **22**(4). pp 190-195.
3. National Patient Safety Agency 2007. *Fourth report from the patient safety observatory. Safety in doses: medication safety incidents in the NHS 2007-2008*. London: The National Patient Safety Agency.

Design and Synthesis of Novel Inhibitors of Chikungunya Virus

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Chikungunya virus (CHIKV), an RNA alphavirus from the *togaviridae* family, is transmitted to humans by the *aedes* genus of mosquito vector.[1] It causes the infectious disease: Chikungunya Fever[1], which is typically characterised by fever, rash, and athralgia[1], and is often highly debilitating to sufferers. Due to a recent mutation in the E1 glycoprotein of CHIKV, and a change in vector distribution patterns, CHIKV has been responsible for an epidemic outbreak of unprecedented magnitude, and has established itself as a worldwide threat.[2] To date, no treatment or vaccine is available.[2] Due to its essential role to the viral replication cycle, and the recent publication of its crystal structure (PDB ID: 3TRK), nsP2 protease has been identified as an attractive anti-viral target.[3] Low μM activity demonstrated by lead compounds against nsP2 protease *in vitro*, has provided the foundations for the development of a novel series of nsP2 protease inhibitors. This report proposes the computer-aided design and synthesis of novel structures with an enhanced potency for nsP2 protease.

Over 80 compounds structurally related to the initial leads were docked into the crystal structure of nsP2 protease using PLANTS[4] software, and analysed by visual inspection. The docking criteria required for ligands to achieve, was devised as a result of analysing key binding characteristics of active compounds. The most potent compound was used as a docking reference. Substituent variation studies were conducted in a logical manner to assess the effects of different groups on ligand-protein interactions. The most promising compounds identified from the docking studies were selected to be synthesised to create two novel series of anti-CHIKV compounds with a proposed increased selectivity for nsP2 protease.

Ligand binding characteristics determined from docking lead compounds into the crystal structure of CHIKV nsP2 protease were found to be mostly hydrophobic, with the presence of key interactions between the carbonyl nitrogen and residue Tyr1047, and the acidic carbonyl oxygen with Trp1084. The ligands also exhibited a preferred docking orientation within the pocket which was incorporated in the docking criteria for subsequently docked ligands to meet. Substituent variation studies have enabled the characterisation of specific structural requirements at three different positions.

In conclusion, two series of novel nsP2 protease inhibitors with proposed enhanced potency for CHIKV nsP2 protease were designed, based on the key findings from docking studies. Of the structures designed, eight compounds were selected to be synthesised. Overall the chosen compounds were synthesised with ease and obtained in moderate yields. The study is currently awaiting the results of a cell-based assay testing these compounds for their inhibitory activity against CHIKV-induced CPE.

1. Powers, A. M. *et al*. Re-emergence of chikungunya and o'nyong-nyong viruses:evidence for distinct geographical lineages and distant evolutionary relationships. *J. Gen. Virol.* 2000, **81**, 471–479.
2. Sourisseau, M. *et al*. Characterization of re-emerging chikungunya virus. *PLoS Pathog.* 2007, **3**(6): e89.
3. Strauss, J. H.; Strauss, E. G. The Alphaviruses: Gene Expression, Replication, and Evolution. *Microbiol. Rev.* 1994, **58**(3), 491-562.

Study of clinical effectiveness for clozapine augmentation strategies

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Schizophrenia is a severe long term Mental Health Illness affecting 1% of the population.[1] The main treatment for schizophrenia is the use of antipsychotic drugs. Approximately 30% of patients do not benefit from antipsychotics and are known as treatment resistant. Treatment resistance is known to be increasing. Clozapine is an atypical antipsychotic used in treatment resistant schizophrenia. Due to clozapine unresponsiveness in a minority of patients, there is need for alternative treatment. Therefore, the use of clozapine in combination with another drug is initiated as last line treatment. This combination is known as clozapine augmentation. The aim of the study was to assess the effectiveness of clozapine augmentation strategies within a male low secure forensic psychiatric service. Effectiveness of treatment was evaluated using drug discontinuation and clinical monitoring as outcome measures.

To establish the effectiveness of various clozapine augmentation strategies in clinical practice, a retrospective study within a male low secure forensic psychiatric service at a South Wales hospital was conducted. A retrospective study was the preferred methodology as it involved review of existing data. Information that was documented in patient's case notes and medical records was analysed to obtain the relevant details. Data for 30 male patients, diagnosed with treatment resistant schizophrenia were involved in the study. This included 5 in-patients and 25 out-patients. A literature search, revealed that a CATIE study[2] was found to have reformed the methods of measuring clinical effectiveness of antipsychotics. Based on their conclusion, the same methodological rationale was applied, whereby clinical monitoring and discontinuation were used as the study outcome measures. A data collection form was created to aid an efficient data collecting process.

A total of 30 male patients were included. Of these 30 patients, 14 were termed 'Non-augmenters' and 16 termed 'Augmenters'. A total of 25 augmentation strategies were included of which 19 (76%) were continued and 6 (24%) discontinued due to reasons such as ineffectiveness and clozapine discontinuation. Through analysis, the breakdown of patient numbers receiving each strategy was, aripiprazole (n=10), lamotrigine (n=6), amisulpride (n=4), haloperidol (n=2), haloperidol decanoate (n=1), olanzapine (n=1) and lithium (n=1). This study reported no significant difference in age, ethnicity and in-patient/out-patient status between augmentation continuers and discontinuers. The duration of all augmentation treatment was above a 6-8 weeks trial[3] (as recommended by NICE). Analysis of serum clozapine concentration highlighted a decrease in plasma levels of smoking patients receiving clozapine (n=10) compared with non-smoking patients (n=20). When comparing plasma levels between smokers and non-smokers receiving clozapine augmentation, there was no difference, but the higher dose range prescribed for smokers was of some interest.

This small patient study reported augmentation discontinuation as 24% (6) and continuation as 76% (19). The main reason for discontinuation was ineffectiveness of augmentation strategy or discontinuation of clozapine, whereby augmentation was no longer needed. It is apparent that few people discontinue clozapine as it is a last line treatment for this patient group. Therefore few patients discontinue augmentation for the same reason. Aripiprazole augmentation was found to be the most popular strategy (n=9) of which 3 patients discontinued. Overall, the study outlined that clozapine augmentation is of modest benefit in resistant schizophrenia, and continuation of this treatment is a marker of its effectiveness.

1. D Bhugra. *Schizophrenia: Core Interventions in the Treatment and Management of Schizophrenia in Adults in Primary and Secondary Care. London: The British Psychological Society and The Royal College of Psychiatrists*; 201
2. JA Lieberman. *et al. Effectiveness of antipsychotic drugs in patients with chronic schizophrenia*. N Eng J Med. 2005 Sep 22;353(**12**):1209-23
3. Schizophrenia: Core interventions in the treatment and management of schizophrenia in adults in primary and secondary care, NICE clinical guidelines 82. London: National Collaborating Centre for Mental Health; 2009 [Accessed 2 March2012] Available from: http://www.nice.org.uk/nicemedia/pdf/CG82NICEGuideline.pdf
4. DM Taylor, *et al.* Augmentation of clozapine with a second antipsychotic-meta analysis. Acta Psychiatr Scand. 2011:1-10

Intravenous Filtration of Lipids for Pre-term Infants

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Parenteral Nutrition (PN) is commonly required in pre-term infants due to them having immature organ systems such as the gastro-intestinal tract. As such they are unable to achieve adequate nutrition via the oral or enteral route to meet their nutritional requirements for growth and maintenance of body tissues.[1] Lipids are an important part of PN for these patients as an energy source and also to provide essential fatty acids (EFA) in order to a avoid EFA deficiency and associated neurodevelopmental issues.[2,3] Filtration during the administration of PN serves to protect the patient from the infusion of potentially damaging contaminants; however there have been an increasing number of reports of filter blockage occurring during the administration of lipid-containing PN. This research aimed to determine whether there was a loss of physical stability of lipid emulsions when stored in combination with injectable vitamins that may contribute to or cause filter blockage. It also investigated the filtration of lipid-containing solutions to determine whether filter blockage is occurring as a result of an increase in mean lipid globule size and loss of lipid emulsion stability.

Two testing protocols were used to study the physical stability of Intralipid® 20% and SMOFlipid® 20% lipid emulsions when stored in combination with the injectable Solivito N® and Vitlipid N® Infant. Four different formulations were stored for either three days at room temperature (RT) or six days under refrigeration followed by a further two days at RT. The samples were analysed by visual inspection, microscopy, measurement of pH and laser diffraction to determine the mean lipid globule size. Further samples were then manufactured and passed through a PALL Biomedical Total Nutrient Admixture 1.2 micron filter using an Alaris® CC syringe pump. The samples were analysed using microscopy and laser diffraction at a series of time points during filtration.

All samples were observed to be yellow, homogenous emulsions for the duration of both storage testing protocols. No significant changes in globule number or arrangement were noted with microscopy and laser diffraction data showing no significant changes. All formulations produced a normal bell shaped globule size distribution curve, at all points of analysis with no tailing. A decrease in pH of all formulations was noted, this being greater in the formulations manufactured in glass bottles, however no corresponding change in the lipid globule size distribution profile, measured by microscopy or laser diffraction, occurred.

Correspondence with hospital pharmacists in practice has brought to light issues with filtration during the administration of lipid-containing PN[3]. Several factors could contribute to filter blockage occurring in practice including loss of stability of the lipid emulsion. The data obtained during storage testing indicated that there was no loss of lipid emulsion stability or change in the lipid globule size distribution profile under the conditions used in this study. The decrease in pH observed could be attributed to the presence of dissolved oxygen in the formulations causing oxidation of lipid and degradation of vitamins into acidic products. Data obtained during filtration also did not indicate a loss of stability and again no change in lipid globule size distribution profile occurred. Also, no significant difference was seen in the stability of Intralipid® in comparison to SMOFlipid®, supporting previous research[4]. Future research is needed to address all possible variables including different flow rates and pressure settings, different PN formulations and different storage conditions. However, it can be concluded that this research cannot support the claim that issues with filter blockage experienced in practice can be attributed to an increase in mean lipid globule size and loss of lipid emulsion stability.

1. Milla P. Paediatric Nutrition Requirements. In: Payne-James J, Grimble G, Silk D, editors. *Artificial Nutrition Support in Clinical Practice.* 1st ed. London: Edward Arnold; 1995. :167-174.
2. Krohn K, Koletzko B. Parenteral lipid emulsions in paediatrics. *Curr. Opin. Clin. Nutr. Metab. Care.* 2006; **9**: 319–323.
3. Personal communication between NHS staff and Dr AG. Cosslett.
4. Tomsits E, *et al*. Safety and Efficacy of a Lipid Emulsion Containing a Mixture of Soybean Oil, Medium-chain Triglycerides, Olive Oil and Fish Oil: A Randomised, Double-blind Clinical Trial in Premature Infants Requiring Parenteral Nutrition. *JPGN*. 2010; **51**: 514–521.

Is blocking the RyR and activating the SK channel neuroprotective in a hypoxia cell model?

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Neurodegeneration is the progressive loss of nerve cells in the brain and has been linked to a disruption in Ca^{2+} homeostasis. The RyR (ryanodine receptor) is a relatively non-specific cation channel, which can be activated by intracellular Ca^{2+}.[1] Blocking the receptor with the antagonist dantrolene prevents Ca^{2+} release from the ER (endoplasmic reticulum) into the cytoplasm. The SK channel is a small conductance calcium-activated K^+ channel, primarily activated by Ca^{2+}.[2] Activation of this channel allows K^+ ions to leave the cell, hence preventing Ca^{2+} influx through voltage-gated channels into the cytosol. The primary aim of this study was to elucidate whether SK channel activation and RyR antagonism is neuroprotective against a cobalt insult in two cell lines, MOG-G-UVW cells (human astrocytoma) and undifferentiatedSH-SY5Y cells (human neuroblastoma).

RT-PCR analysis was carried out to identify the SK subunits in each cell line. RNA was extracted with Trizol from undifferentiated SH-SY5Y cells and MOG-G-UVW cells. Equal amounts of RNA were used in each reverse transcription to obtain cDNA. PCR was carried out to compare the SK messages. Experiments studied the effect of dantrolene (0.1 µM-1 mM) in MOG-G-UVW cells and (0.1 µM-100 µM) in SH-SY5Y cells and dose-response curves were constructed. $CoCl_2$ was used as a model for hypoxia. The effects of dantrolene against a $CoCl_2$ insult (EC_{50}=32.2 µM for MOG-G-UVW, EC_{50}=40 µM for SH-SY5Y) were investigated using the same concentrations as with dantrolene alone. The effect of NS309 (30nM - 30µM) was tested in both cell lines. The effect of NS309 in the presence of $CoCl_2$ was also investigated over the same concentration ranges. Cell number was measured with the MTS assay. Cells were seeded at a density of 15000cells/well, left overnight, challenged and left for 24 hours. MTS reagent was then applied. After 3 hours, the plates were read at 490nm using a plate reader.

The major findings of this study are as follows: PCR illustrated the presence of the message for the SK1, SK2, SK3 (weaker message) and IK (stronger message) channels in MOG-G-UVW cells. However, only the message for SK1 and SK3 channels was present in SH-SY5Y cells. The exact proportions of these could not be ascertained. Dantrolene was without effect in both cell lines up to 10 µM. At 100µM, it causes a significant reduction in cell number. The effect of dantrolene against the $CoCl_2$ insult suggests that it affords significant protection up 10 µM in both cell lines. NS309 alone is without effect over 30 nM-30 µM. When used in conjunction with $CoCl_2$, NS309 offers a significant protective effect in MOG—G-UVW cells up to 300nM. However, in SH-SY5Y cells, NS309 does not offer protection. On the contrary, it causes reduction in relative cell number and ca. 2 µM NS309, there is a significant reduction in cell number.

From this study it is clear that dantrolene affords a significant protective effect against a cobalt insult up to 10 µM in both cell lines. However, NS309 offers a significant protective effect only in MOG-G-UVW cells up 300nM. In SH-SY5Y cells, concentrations ca. 2 µM begin to significantly reduce cell viability. This is contrary to previous studies where NS309 reduced ischaemic brain damage after experimental middle cerebral artery occlusion.[3] However, it can be argued that NS309 has higher binding affinity to IK channels than SK channels.[2] The fact that SH-SY5Y cells lack the IK channel means that only SK channels can be activated. It is possible that the conductance change was too small to cause neuroprotection. NS309's binding site has been proposed to be functionally and possibly physically different from the SK blocker apamin.[2] qPCR and further experiments on the SH-SY5Y cells are needed to confirm the findings.

1. Stutzmann, G. E. and M. P. Mattson (2011). "Endoplasmic reticulum $Ca^{(2+)}$ handling in excitable cells in health and disease." *Pharmacol Rev* **63**(3): 700-727.
2. Strøbaek, D. *et al.* (2004). "Activation of human IK and SK Ca^{2+} -activated K^+ channels by NS309 (6,7-dichloro-1H-indole-2,3-dione 3-oxime)." *Biochim Biophys Acta* **1665**(1-2): 1-5.
3. Dolga, A. M. *et al.* (2011). "$K_{Ca}2$ channels activation prevents $[Ca^{2+}]_i$ deregulation and reduces neuronal death following glutamate toxicity and cerebral ischemia." *Cell Death Dis* **2**: e147.

Pharmacy and Medicine Undergraduates Learning Together - Evaluating an Interprofessional Education Pilot at Cardiff University

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Interprofessional learning (IPL) is becoming more important in healthcare professions to encourage students to develop an understanding of the roles and values of other health professionals.[1] The General Pharmaceutical Council (GPhC) states that learning in the MPharm degree should be a result of providing education through interprofessional practice and procedures.[2] The General Medical Council (GMC) also recognises this and states that medical schools must ensure students work alongside other health professionals to recognise the importance of teamwork. The aim of this study was to evaluate pharmacy and medical students' views on an IPL undertaken session at Cardiff University.

Medical and pharmacy undergraduate students attended one of three sessions where they were required to work through role-play scenarios in interprofessional pairs. After participating in the session students were asked to complete a questionnaire. The questionnaire consisted of both quantitative and qualitative items. Responses to quantitative items were analysed using SPSS software and qualitative results were analysed using thematic analysis. A literature search was undertaken as part of this study to identify evaluation tools used in previous IPL studies. Relevant items from the evaluation tools identified were adapted to improve their appropriateness. These items then formed the basis of a new evaluation tool. Cardiff School of Pharmacy and Pharmaceutical Sciences ethics committee was obtained.

In total 431 questionnaires were collected resulting in a response rate of 96%. Eighty-five percent of all respondents agreed or strongly agreed that more IPL should be introduced into the undergraduate degrees. There were significant differences between medical students working interprofessionally and pharmacy students working interprofessionally, where medical students showed a higher level of disagreement that they felt well prepared for the session, in terms of both therapeutics and drug history taking. The most commonly-cited 'best' feature of the session was working with another healthcare professional student and the organisation was the most frequent component students thought could be improved. Review of the literature resulted in the identification of an alternate evaluation method, that is, the use of a pre-post test questionnaire approach. As a result of the literature review and findings in the study, draft versions of pre- and post-questionnaires were developed (largely based on the Readiness for Interprofessional Learning Scale[3]). This pre-questionnaire contains 8 items and the post-test questionnaire contains the same 8 items plus an additional 5 items specific to the session itself and 3 open ended items.

The feedback from the IPL session was largely positive from both student cohorts. Students found the session both useful and enjoyable and are in favour of introducing more IPL into the curriculum with many recognising its benefits from just a single session. Evaluation of future IPL using a pre-post test questionnaire will identify changes in attitudes and knowledge that have occurred as a direct result of the IPL session. Overall, the findings support the development and introduction of IPL between pharmacy and medicine undergraduate students at Cardiff University.

1. Quynh, L. *et al*. 2008. . Development of a tool to evaluate health science students' experiences of an interprofessional education (IPE) programme. *Annals Academy of Medicine* **37**(12), pp. 1027-1033
2. General Pharmaceutical Council. 2011. *Future Pharmacists: Standards for the initial education and training of pharmacists* [Online]. Available at: http://www.pharmacyregulation.org/sites/default/files/GPhC_Future_Pharmacists.pdf [Accessed: 23 April 2012].
3. Parsell, G and Bligh, J. 1999. The development of a questionnaire to assess the readiness of health care students for interprofessional learning (RIPLS). *Medical Education* **33**(2), pp. 95-100.

The Kinetics of Responses of Aorta to Octopamine in the Absence and Presence of Prazosin

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Octopamine and β-phenylethylamine (β-PEA) are naturally occurring trace amines. These are produced in the body from amino acids or absorbed from the diet or herbal supplements.[1] Trace amines act by causing noradrenaline release, or by acting directly on receptors e.g. octopamine acts on α1-adrenoreceptors and on the recently discovered trace-amine associated receptor (TAAR) on aorta to cause vasoconstriction.[1,2,3] The main aim of this study was to see if initial onset rate of contraction is different between α1 and TAAR stimulation. To do this the α1 antagonist prazosin was used and the Lineweaver-Burk plot from enzyme kinetics was utilised. This is the plot of 1/rate vs. 1/concentration which gives enzyme constants Km, equivalent to drug-receptor affinity, and Vmax, equivalent to the maximum onset rate.[4] It was hypothesised that once all α1 vasoconstriction was blocked by prazosin a different rate of onset would be seen as a different receptor was responsible.

To test this, a thoracic aortic ring was set up in an organ bath. The endothelium was removed to eliminate nitric oxide release. The tissue was incubated with cocaine, pargyline and ICI 118,551, to block noradrenaline release, monoamine oxidase breakdown and β2 adrenoreceptor stimulation, respectively. Cumulative dose-response curves were constructed for octopamine alone and with 1, 10 and 30uM prazosin and 0.3ml DMSO. Non-cumulative curves were obtained for octopamine alone and with 1 and 10uM prazosin and for β-PEA alone and with 1uM prazosin. The initial rates of onset of these non-cumulative contractions were measured over the first 1-2 minutes, by plotting the line of best fit. These were then used to construct the Lineweaver-Burk plot and the Km was found from the gradient multiplied by the Vmax and the Vmax from 1/y-intercept. The initial offset rate of contraction after washout was also found.

1uM prazosin shifted the dose response curve for octopamine to the right, increasing the –logEC50, whereas increasing the concentration to 10 and 30uM did not shift the curve any further. Therefore, responses seen with 1uM prazosin are due to a different receptor e.g. TAAR as all α1 stimulation is blocked. The initial rate of onset of contraction and Vmax were slower with 1 and 10uM prazosin, further proving that a different receptor is responsible. The –logKm with prazosin was smaller, meaning the affinity for the receptor causing this response, e.g. TAAR, is less than when it acts on both α1 and TAAR. The offset rate of contraction after washout was not different with prazosin. Thus, changes in onset rate and affinity are not down to a different diffusion barrier but due to a different receptor or a different post-receptor event. To determine if TAAR is the cause, other receptors must be eliminated e.g. DA, 5-HT, α2, angiotensin and prostaglandins, as a TAAR antagonist does not yet exist. There was no difference in onset rate, Vmax or –logKm between β-PEA with and without prazosin, proving that β-PEA does not act on α1-adrenoreceptors.[2] The onset rates and Vmax between β-PEA and octopamine both with 1uM prazosin were not significantly different indicating they both act on the same receptor e.g. TAAR. However, -logKm and affinity was higher for β-PEA, following the known potency order; tryptamine > β-PEA > octopamine > D-amphetamine > tyramine.[2]

In conclusion, there is a slower onset rate of contraction to octopamine when α1 vasoconstriction is blocked with prazosin. The likely receptor is $TAAR_1$, although definitive proof will only come once a TAAR antagonist is available.[2] The Lineweaver-Burk plot seems like a viable way of determining drug-receptor affinities as those affinities found follow the known potency order. Further studies will be needed to see if other trace amines follow this order. However, because the transformation leads to increased chance of error in Kms and Vmaxs found, a more accurate method for finding these constants such as the non-linear regression of the Michaelis-Menten equation may be more useful.[4]

1. Broadley, K.J. 2010. The vascular effects of trace amines and amphetamines. *Pharmacol Therapeut* **125**: 363–375.
2. Fehler, M. *et al*. 2010. Identification of trace-amine-associated receptors (TAAR) in the rat aorta and their role in vasoconstriction by β-phenylethylamine. *Naunyn-Schmiedebergs Archive of Pharmacology* **382**: 385–398.
3. Brown, C.M. *et al*. 1988. Activities of octopamine and synephrine stereoisomers on α-adrenoceptors. *Br J Pharmacol*. **93**: 417-429.
4. Ranaldi, F. *et al*. 1999. What students must know about the determination of enzyme kinetic parameters. *Biochem Educ* **27**: 87-91.

How Does the MPharm Degree in Cardiff Facilitate and Impede the Effective Learning of Pharmacy Undergraduate Students?

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The MPharm (Masters in Pharmacy) degree must cover a vast amount of information in order to produce responsible graduates, who are capable of providing a wide range of care services.[1] The pharmacy career is evolving to meet growing demands and the curriculum of the Cardiff MPharm must adapt in order to keep up with the career.[2] There is a new curriculum in place currently for first and second year MPharm. There has been movement of the weighting between types of assessment and integration between subject areas to make the course more realistic and give context to the pharmacy career. Students have a variety of learning styles which should be considered in planning for teaching; however, there is no agreement over a single model. Categorisation of learning styles tends to be based on the students' preference of environment, personality types, sensory modalities or cognitive styles.[3] Students and academic staff can have opposing views on the most effective aids for students learning. To improve this, teachers must understand the techniques which students use, and supply a broad media of delivery.[4] The aim of this research project was to evaluate how the MPharm degree facilitates and impedes the effective learning of first and second year MPharm students.

Participants were recruited via email and lecture presentations. The interviews were arranged according to the participants, and conducted either one-to-one or in small groups. The first and second year students' and lecturers' opinions on the new curriculum were explored and the recordings were transcribed and analysed by thematic analysis method, to determine areas of the degree which facilitate and impede effective learning and to compare the lecturers' and students' views to aid further improvement to the MPharm degree at Cardiff.

Personal interest was found to be the main reason for enjoyment in a module which also correlated with the module the students felt they learnt the most in. Students suggested formative assessments after each topic, to maintain motivation, and to highlight the areas they don't understand. Lecturers were also aware that exams increase motivation; however they thought that there are currently too many assessments, which are leading to surface learning by the students. Students found workshops were the best teaching activity for learning; lecturers agreed that it enabled active work on examples, and that if time constraints allow it, there should be more workshop teaching.

A variety of teaching activities is needed with constant modification of teaching and course structure based on student feedback and career progress, in order to maintain Cardiff as a leading school of pharmacy. Computer assisted leaning (CAL) would be a good way of delivering more formative assessments and exam question examples to fuel students' revision. Workshop learning was highly regarded by all participants, and there should be more regular use of this method; however, staff time and facilities are limited. Placements are useful but nevertheless, success relies on the suitability of the pharmacy and staff to enable students learning. Students would benefit from more feedback, however, there must be a balance of expectations and students must also make the most of what is given to them.

1. Smith, L. *et al*. 2010. Pharmacy Students' Approaches to Learning in Undergraduate and Graduate Entry Programs. *American Journal of Pharmaceutical Education*. **74** (6) Article 106.
2. Smith, A. and Darracott, R. 2011. Modernising Pharmacy Careers Programme: Review of pharmacist undergraduate education and pre-registration training and proposals for reform. Medical Education England. [Online]. Available at: http://www.mee.nhs.uk/pdf/MPC_Discussion_Paper.pdf [Accessed: 8th April 2012].
3. Romanelli, F. *et al*. 2009. Learning Styles: A Review of Theory, Application, and Best Practices. *American Journal of Pharmaceutical Education*. **73** (1) Article 9.
4. Suda, K. *et al*. 2011. Faculty and Student Perceptions of Effective Study Strategies and Materials. *American Journal of Pharmaceutical Education*. **75** (10) Article 201.

Ser^{276} is essential for zinc release through ZIP7 channel in MCF7 breast cancer cells from the endoplasmic reticulum

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Zinc is a metal ion, essential to all forms of life and has been linked to various signalling pathways, including those which contribute to cell proliferation.[1] Abnormal expression of zinc transporter ZIP7 and elevated concentrations of intracellular zinc levels are prevalent in tamoxifen-resistant breast cancer cells.[2] Increased intracellular concentrations of free zinc cause inhibition of several phosphatases activating tyrosine kinases and other cell signalling pathways which promotes cell growth and migration.[3] An exogenous stimulus such as zinc treatment causes zinc release from the intracellular store, ER to the cell cytoplasm. Two neighbouring serine residues located on ZIP7 transporter, Ser^{275} and Ser^{276} have been revealed[4] to be essential for ZIP7-mediated zinc release from intracellular stores, such as the ER. These residues on ZIP7 are phosphorylated by protein kinase CK2 (casein kinase 2) before zIP7-mediated zinc release. Previously, Taylor *et al*. discovered that a S275AS276A mutant of ZIP7 failed to release zinc from the endoplasmic reticulum (ER) when compared to wild type ZIP7.[4] We now investigated whether there was a requirement for both residues to be phosphorylated by CK2 or whether one alone was sufficient. Here, we investigated the role of individual residues, Ser^{275} and Ser^{276} on ZIP7 channel and/or their involvement in zinc transport in the MCF7 breast cancer cell line transfected with recombinant constructs.

Zinc release through ZIP7 transfected into MCF7 breast cancer cell lines in mutants S275A S276A, S275A, S276A and wild type ZIP7 was determined. Cells were loaded with cell membrane permeable zinc specific dyes, Fluozin-3 and Zinquin (UV) following treatment with 20μM zinc and ionophore for 20 minutes. The localization of ZIP7 in cells was also examined. Cells were viewed on Leica fluorescent microscopy at x63 magnification. Pictures of cells were quickly captured before the dyes would deteriorate. Statistics were also applied to determine the level of ZIP7 expression in all assays.

ZIP7 was confirmed to be localized in the endoplasmic reticulum membrane. In wild type and S275A transfected cells, an increase in cytosolic zinc was seen after 10 minutes of zinc treatment with both dyes. In contrast, S275AS276A and S276A mutants, no fluorescence of both dyes were observed. Mutation of serine 276 in breast cancer cells prevented ZIP7-dependent zinc induced zinc release to the cell.

ZIP7 channel acts as a gatekeeper of cytosolic zinc release from ER[3] and highlights the involvement of zinc in downstream signalling pathways that promote cell proliferation. ZIP7-CK2 association allows intracellular zinc to be transported into the cell cytoplasm.[4] This mechanism subsequently leads to phosphatase inhibition which prevent deactivation of phosphorylated of tyrosine kinases. In our results, we have successfully demonstrated that functional mutation of Ser^{276} on ZIP7 interferes with zinc transport and completely inhibits downstream signalling events that cause cell division and migration. In conclusion, phosphorylation of Ser^{276} residue on zinc transporter ZIP7 in endoplasmic reticulum by CK2 is important in zinc release and holds potential as a therapeutic target in breast cancer. This novel finding yields new insights into the areas of zinc signalling and its role in mediating transcriptional responses.

1. Taylor, K. M. 2008. A distinct role in breast cancer for two LIV-1 family zinc transporters. *Biochemical Society Transactions* **36**, pp. 1247-1251.
2. Taylor, K. M. *et al*. 2008. ZIP7-mediated intracellular zinc transport contributes to aberrant growth factor signalling in antihormone-resistant breast cancer cells. *Endocrinology* **149**, pp. 4912-4920.
3. Hogstrand, C. *et al*. 2009. Zinc transporters and cancer: a potential role for ZIP7 as a hub for tyrosine kinase activation. *Trends in Molecular Medicine* **15**, pp. 101-111.
4. Taylor, K. M. *et al*. 2012. Protein Kinase CK2 triggers cytosolic zinc signalling pathways by phosphorylation of zinc channel ZIP7. *Science Signalling* **5**(210), pp. 1-10.

Protocol development for confocal imaging of pulpal tooth infection and its control

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Streptococcus anginosus are implicated in early pulpal infections[1] resulting in eventual necrosis of the pulp and ultimately a patient requiring a root canal, a painful and expensive procedure. The potential use of triclosan as an early treatment is promising as triclosan has both antibacterial and antiinflammatory properties[2], both required for healing of the pulp. A recent study investigating the growth of *S. anginosus* on pulpal cells has found the bacteria colonised in clusters within the dental pulp in 2D histological section, rather than growing as a lawn.[3] Results were obtained using a co-culture system (combining DMEM with 10% BHI) permitting the growth of both bacterial and mammalian cells over 24 h. The aims of this project were to use the *ex vivo* co-culture model to produce a pulpal infection using *S. anginosus* and to develop an confocal microscopy imaging protocol to visualise pulp section in 3D allowing to visualise the antibacterial properties of triclosan against *S. anginosus* bacteria.

S. anginosus (10^5 cfu) were stained with 1% w/v FDA in acetone for 30 min, captured in a 0.22µm filter and re-suspended with co-culture media. Two mL of this filtrate was used to inoculate tooth slices and incubated overnight anaerobically (6% CO_2) at 37ºC. Samples were viewed under a Leica, confocal laser scanning microscope at X20 magnification. A further 2 mL of this filtrate was inoculated with 125 µg/mL of triclosan to test its antimicrobial activity. A number of technical approaches were tried and tested; the most successful involved removing of the pulp from the harder dentinal and enamel layers, fixing in paraformaldehyde, staining with 0.1 µL of rhodamine phalloidin and viewing transverse section. The MIC and MBC of triclosan (dissolved in 100% DMSO; concentration in the range of 0.25 - 125 µg/ml) was determined following microbial challenge with 10^5 cfu and overnight incubation anaerobically (6% $CO_{2)}$ at 37ºC.

The selected protocol produced images depicting *S. anginosus* growth in clustered of colonies scattered throughout pulpal tissue. Furthermore co-localisation between pulpal and bacterial cells was observed indicating bacterial attachment after a period of 12 h, as well as pulpal cell death after 24 h depicted through blackened areas surrounding *S. anginosus* colonies. Pulpal cell staining was observed in areas where no *S. anginosus* had colonised, indicating viable pulpal cells. Triclosan MBC for *S. anginosus* was > 125µg/mL, thus when teeth were inoculated with bacteria and 125 µg/mL of triclosan growth was still observed in pulpal tissue.

Co-culture media is sufficient to support the growth of pulpal and *S. anginosus* cells. The refined selected protocols have produced the most successful confocal images, however a further protocol able to obtain a true z-stack image of the growth of *S. anginosus* in pulpal tissue outlined in the further works section will need to be implemented in order to image the presence of a possible cone shaped growth in pulpal tissue. Higher concentrations of triclosan were not tested against *S. anginosus* to obtain its bactericidal concentration as there is the concern that 1% DMSO cannot hold more than 16 µg/mL of triclosan and that higher concentrations of triclosan used may not accurately represent the actual amount of triclosan in solution, thus further solubility testing needs to be conducted. There were initial concerns that 125 µg/mL may have toxic effects upon pulpal cells, however triclosan is used at concentrations of 0.2 – 0.3% in oral hygiene products thus 0.125% used in these experiments may possibly be used to treat pulpal infections in the future.

1. Love, R.M. and Jenkinson, H.F. 2002. Invasion of Dentinal Tubules by Oral Bacteria. *Crit.Rev Oral Biol Med.* **13**(2) pp. 171-183.
2. Kjaerheim, V. *et al.* 2007. Antiplaque, antibacterial, and anti-inflammatory properties of triclosan mouthrinses in combination with zinc citrate or polyvinylmethylether maleic acid (PVM-MA) copolymer. *European Journal of Oral Science.***104**(5-6), pp 529-534
3. Roberts,J, L. 2010. Development of an *Ex vivo* Co-Culture System to Model Pulpal Infection by Streptococcus Anginosus Group Bacteria. PhD thesis, Cardiff University.

A biological approach to fragment based drug discovery, using the marine sponge *Agelas oroides*

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The use of fragments as a starting point for drug discovery has proven to be an effective alternative to traditional methods in recent years.[1-2] Another trend in the pharmaceutical industry has been a shift of focus back to natural products as a source of new chemical entities (NCEs) for drug development. This has included many marine organisms such as sponges.[3] Hymenialdisine is a bromopyrrole metabolite produced in *Agelas oroides* sponges, which has been identified as having potent anti-cancer and neuro-protective activity. The strong binding interaction is partly due to three crucial hydrogen bonds formed with Leu83 and Glu81 in CDK2, a kinase important for cell division.[4] 2-(Trichloroacetyl)pyrrole is an electrophilic fragment containing the substructure responsible for this binding. The project aim was to react this fragment with material extracted from *Agelas oroides* in order to synthesize and isolate NCEs with potentially strong affinity for clinically relevant kinases.

2-(Trichloroacetyl)pyrrole was initially reacted with a range of nucleophiles in order to determine the optimal reaction conditions. 1-Hydroxybenzotriazole (HOBt) was found to be an effective catalyst but the reaction mixture required heating at reflux in order to advance. Following this, sponge extract from *Agelas oroides* was reacted with 2-(trichloroacetyl)pyrrole and HOBt in acetone and heated under reflux. TLC was used to separate fractions, which were then analysed using NMR spectroscopy.

5-Pentylamine was found to be the most reactive nucleophile as it produced the largest yield, however none of the proof of concept reactions were fully quantitative. No novel compounds were identified following isolation of the sponge-based reaction products, signifying that either the starting material did not react, or that the products were synthesized in quantities too small to be detected.

The preliminary reactions provided evidence to support the use of 2-(trichloroacetyl)pyrrole in the biological fragment based assay. The proposed technique was not effective using this particular sponge and fragment, but this does not rule out the possibility of further biological fragment-based drug discovery screens, potentially using a different source of natural products. A stronger electrophile, such as a pyrrole-2-carbonyl chloride may be necessary for sufficient reactivity for this particular target.

1. Carr, R. A. et al. Fragment-based lead discovery: leads by design. *Drug Discovery Today* 2005, **10**, 987-992.
2. Congreve, M. et al. Recent developments in drug discovery. *J. Med. Chem.* 2008, **51**, 3661-3680.
3. Baker, D. D. The value of natural products to future pharmaceutical discovery. *Nat. Prod. Rep.* 2007, **24**, 1225-1244.
4. Meijer, L. et al. Inhibition of cyclin-dependent kinases, GSK-3β and CK1 by hymenialdisine, a marine sponge constituent. *Chem Biol.* **2000**, *7*, 51-63.

Investigating the Synergistic Activity of Hops with Antibiotics for the Treatment of Tuberculosis

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The activity of anti-tuberculosis drugs are steadily decreasing due to the development of resistant strains of the bacterium. Hops – a natural product has known anti microbial activity[1], this project aims to determine whether any synergistic action exists between the current tuberculosis treatments and hops.

An agar plating method was used in which the responses of overlapping inhibition rings were measured and compared. The responses observed provided insight into whether synergy exists or not.

Results showed that for those hops tested, no effect was observed on the activity of the traditional treatments. In addition to this no effect was observed between the traditional treatments and myrcene – an activity enhancing hop constituent.[2]

This suggests that the hops tested have limited use for increasing the activity of the traditional antibiotics. No assumptions can be made regarding other hop types that weren't tested. Potential further study appears to revolve around the use of myrcene for its activity enhancing effect on hops.

1. Srinivasan V at el. Contributions to the antimicrobial spectrum of hop constituents. *Economic Botany*. Vol 58. Pp S230-S238. 2004
2. Onawunmi GO *et al*. Antibacterial constituents in the essential oil of *Cymbopogon citrates*. *Journal of Ethnopharmacology*. 1984 Dec;12(3):279-86

The effect of PICALM siRNA on the expression of amyloid precursor protein in an Alzheimer's disease cell model

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Alzheimer's disease (AD) is a neurodegenerative disorder which is characterised by progressive neuronal degeneration and the formation of beta-amyloid (Aβ) plaques and neurofibrillary tangles. The formation of Aβ from the transmembrane amyloid precursor protein (APP) is considered central to the pathology of AD. Non-amyloidogenic processing of APP is believed to occur at the cell surface and involve the sequential action of the enzymes α- and γ-secretase. This prevents Aβ formation as cleavage occurs within the Aβ domain.[1] Amyloidogenic processing is believed to involve the enzymes β- and γ-secretase and occur in the endosome after APP has been internalised into a clathrin-coated vesicle in the process of clathrin-mediated endocytosis (CME).[2] PICALM is a protein which exists in a number of different isoforms and is considered essential in the formation of the clathrin vesicle in endocytosis.[3] PICALM has been implicated in the pathology of AD since any change in endocytosis can affect the degree of amyloidogenic processing and Aβ formation.[4] This study aimed to compare the effects of knocking down the expression of isoform 1, isoform 2 and total PICALM on the expression of APP and other endocytic proteins.

siRNA targeting either isoform 1 (TV1), isoform 2 (TV2) or total PICALM expression (PIA and PIB) were transfected into cultured H4 neuroglioma cells. Cells transfected with siRNA targeting non-mammalian green fluorescent protein (GFP) or with the transfection reagent oligofectamine alone or incubated with media alone were used as negative controls. Western blotting was used to detect any changes in protein expression following transfection. Immunocytochemisty was performed on fixed cells to identify any changes in the cellular expression and localisation of PICALM following transfection. A sandwich ELISA was performed for quantification of APP expression. An MTS assay was performed to ensure the siRNA were not adversely affecting cell viability.

Western blotting revealed PICALM expression to be significantly reduced by TV2, PIA and PIB siRNA. TV1 siRNA, targeting isoform 1, had no significant effect on PICALM expression. Immunocytochemisty revealed a distinct cellular localisation of the isoforms of PICALM. Isoform 1 appeared as diffuse peri-nuclear staining while isoform 2 appeared as larger aggregations near the plasma membrane. Interestingly PIB siRNA, although designed to target both isoforms, appeared to reduce mainly the diffuse peri-nuclear expression of isoform 1 of PICALM. Western blotting showed PICALM siRNA to have no significant effect on the expression of APP, clathrin heavy chain or dynamin II. However, the ELISA results showed a statistically significant reduction in APP levels with TV2 and PIA siRNA.

The high expression and membrane localisation of isoform 2 suggests this is the dominant isoform in this cell line. Furthermore, a reduction in the expression of isoform 2 was able to reduce APP levels significantly. It is possible to hypothesise that when isoform 2 is reduced, there is a reduction in CME and therefore, in the internalisation and amyloidogenic processing of APP to Aβ. As a consequence, this may increase the amount of APP at the cell surface which can be processed in the non-amyloidogenic pathway, again reducing Aβ formation. The lack of effect of PICALM on the expression of clathrin heavy chain and dynamin II suggests that PICALM is not essential for the expression of these endocytic proteins. In conclusion, the results suggest for the first time a dominant role of isoform 2 of PICALM in CME in neuroglioma cells. Further studies are needed to understand the impactions on Aβ formation and AD pathology.

1. Blennow, K, Leon, M.J.D. and Zetterberg, H. 2006. Alzheimer's disease. *The Lancet* **368**: 387-403.
2. Koo, E.H and Squazzo S.L. 1994. Evidence that the production and release of amyloid B-protein involves the endocytic pathway. *The Journal of Biological Chemistry* **269**: 17386-17389.
3. McMahon, H. T. and Boucrot, E. 2011. Molecular mechanism and physiological function of clathrin mediated endocytosis. *Molecular Cell Biology* **12:** 517-533.
4. Harold, D. *et al.* 2009. Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. *Nature Genetics* **41**: 1088-1093.

Investigating the numeracy skills of students entering Pharmacy and Medicine at Cardiff University

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Diversity in the educational background of students continues to increase. Faced with differences in mathematics education, larger class sizes and recent reports suggesting a steady decline in numeracy skills, many employers and admissions tutors are starting to feel they can no longer rely on student's pre-University mathematics grades as a sole indicator of numerical competency.[1] For future healthcare professionals, aptitude in areas of basic numeracy such as multiplication and unit conversions will be essential for maintaining both patient safety and patient confidence in their future profession. Consequently, numerous studies over the last decade have called into question the ability of healthcare students to adequately demonstrate competence in this area.[2,3] Diagnostic numeracy tests can be used to inform staff of student competency in basic numeracy, influence future teaching strategies, and inform individual students of any gaps in their knowledge. The study aimed to use a contextualised diagnostic tool, to evaluate both numeracy competence and confidence of students entering the first year of pharmacy and medicine at Cardiff University.

Ethical Approval for this study was obtained from the Cardiff School of Pharmacy and Pharmaceutical Sciences ethics committee. A contextualised pilot diagnostic numeracy tool was designed by the study team in 2010, following semi-structured interviews, focus groups and a small piloting exercise.[4] This tool was administered to first year healthcare students in week two of the academic year 2011-2012. Pharmacy students at Cardiff School of Pharmacy (n=118), and Taylor's University Malaysia (n=47), and medical students at Cardiff University (n=274) all sat the paper based diagnostic tool. It contained 25 medicines-based questions covering six numeracy domains (multiplication, division, fractions, percentages, ratios and unit conversion). Students were given 45 minutes to complete the diagnostic tool. The use of calculators was not permitted. After completing each question, candidates were asked to indicate their level of confidence in the correctness of their answer. An accompanying semi-structured questionnaire was attached to obtain demographic data and collect feedback on the numeracy tool. All raw data was entered into SPSS version 18.0.3, data was re-coded for further analysis and group comparisons were made using the Mann Whitney U test.

The tool was completed by 97% (n=439/452) of eligible participants and 23% (n=99/439) gave feedback. The mean score was 20.93 (out of 25), and marks ranged from 4-25. Overall, 14% of students that sat the numeracy tool achieved full marks (n=63/439). Unit conversion was found to be the weakest calculation domain. Students also had difficulty with questions that involved a method consisting of two or more steps. Individuals with a pre-University A-level mathematics qualification outperformed those with only a GCSE ($p < 0.05$). Students whose highest qualification in mathematics was a B grade at GCSE had a mean score of 13.67 (out of 25), performing significantly worse than those with an A or A*.

The use of a diagnostic numeracy tool proved to be a useful method for highlighting weaknesses in individual students numeracy skills. Entry medical students were found to be more competent and confident in their basic numeracy skills than entry pharmacy students. Males were no more competent than females, however they were more confident in their ability. Results suggest that students who study mathematics to A-level were more confident and competent in basic numeracy skills. These results may inform future teaching strategies in both degree programmes. Future work could aim to pilot the tool in other Schools of Pharmacy and Medicine.

1. Tariq, V. 2002. A decline in numeracy skills among bioscience undergraduates. *Journal of Biological Education*. **36**(2): 76-83.
2. Barry, J.G. *et al*. 2007. Attitudes of pharmacy students and community pharmacists to numeracy. *Pharmacy Education* **7**(2): 123-131.
3. Batchelor, H. 2004. The importance of a mathematics diagnostic test for incoming pharmacy undergraduates. *Pharmacy Education* **4**: 69-74.
4. Jarman, S and Coulman, SA (2010). The development of a pilot diagnostic numeracy tool for first year pharmacy students. *Welsh School of Pharmacy MPharm Research Abstracts 2010,* (ed. John DN) STS Publishing, Cardiff p48 ISBN 97809489173.

Antihormone-resistant breast cancer cells modulate bone cell function *in vitro*

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Breast cancer is the second most common cause of cancer related death amongst women. Despite the benefits of antihormone agents for breast cancer, their clinical effectiveness is limited by acquired resistance. Acquired resistance is associated with deregulation of secreted ligands which potentially regulate other cell types *in vitro* and metastases *in vivo*. Breast cancer in particular is known to preferentially metastasise to bone tissue with the bone microenvironment being hypothesised to potentiate this metastatic progression of the disease. The most prevalent form of bone metastases present as osteolytic bone lesions where uncoupled functioning of osteoclasts and osteoblasts are observed. Osteoclasts specifically have been shown through previous research to have increased functioning during osteolytic metastasis, resulting in the associated symptoms such as severe pain and nerve compression.[1]

This study sought to investigate the ability of antihormone-sensitive and antihormone-resistant breast cancer cells to modulate the function of RAW cells, an osteoclast progenitor cell line. Conditioned media (CM) was collected from antihormone-sensitive MCF-7 cells and their tamoxifen resistant (TamR) and faslodex resistant (FasR) counterparts. CM was then applied to RAW cells and the effects on proliferation, differentiation to osteoclasts and intracellular signalling determined by MTT assay, TRAP staining and Western Blotting respectively.

Antihormone-resistant CM was found to be able to promote RAW cell proliferation whilst this was not observed upon stimulation with CM from antihormone-sensitive cells. TamR and FasR conditioned media was also found to have a potential to induce RAW cell differentiation into osteoclast-like cells. Investigations into cellular signalling in RAW cells highlighted an up regulation of FAK and Src following treatment with TamR and FasR media but not MCF-7 CM. Cellular signalling investigations also illustrated treatment with TamR and FasR CM reduced RAW cell expression of the Wnt signalling mediators' β-catenin S33/37 and GSK3β.

This data suggests antihormone-resistant breast cancer has a potentially higher capacity to modulate bone cell function than antihormone-sensitive breast cancer, clinically paralleling the more aggressive nature observed with the resistant phenotype. Treatment with the antihormone-resistant CM resulted in promotion of RAW cell proliferation and differentiation into osteoclasts corresponding with the up regulation of FAK, known to regulate cellular processes such as proliferation and differentiation[2], and up regulation of Src, known to activate pro-survival pathways further promoting proliferation.[3] FAK has previously been linked with induction of AKT signalling and conserved induction patterns in signalling data suggests up regulation of FAK Y397 could result in induction of RAW cell AKT signalling, known to activate further pro-survival pathways.[2] The observed down regulation of Wnt signalling mediators could further contribute to the promotion of RAW cell differentiation into osteoclasts with Wnt signalling down regulation promoting osteolysis *in vivo*.[4] Overall, this data potentially provides early evidence of antihormone-resistant mechanisms of increased osteoclastogenesis, suggesting antihormone-resistant breast cancer cells may possess the ability to modulate their bone microenvironment to favour osteolytic metastasis.

1. Akhtari, M. *et al*. Biology of breast cancer bone metastasis. *Cancer biology and therapy*. 2008;**7**(1):3-9.
2. Luo, M. and Guan, J. Focal adhesion kinase: A prominent determinant in breast cancer initiation, progression and metastasis. *Cancer Letters*. 2010;**289**(2):127-139.
3. Saad, F. and Lipton, A. SRC kinase inhibition: Targeting bone metastases and tumour growth in prostate and breast cancer. *Cancer Treatment Reviews*. 2010;**36**(2):177-184.
4. Pinzone, JJ. *et al*. The role of Dickkopf-1 in bone development, homeostasis and disease. *Blood*. 2009;**113**(3):517-25.

Transplantation for the treatment of Parkinson's disease: The effect of L-DOPA

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A chronic, progressive neurodegenerative movement disorder, Parkinson's disease (PD) is characterised by resting tremor, bradykinesia and rigidity. L-DOPA remains a cornerstone of PD motor symptom control, however chronic treatment is associated with response fluctuations and the development of involuntary movements. With limited treatment options available in late stage PD and the complications associated with current therapies, it's evident that a novel treatment strategy is essential. Transplantation is just that, and has remained under development over the past 30 years. Since L-DOPA remains the gold standard therapy and grafting takes a long time to reach effect, patients undergoing transplantation are likely to be taking L-DOPA before and after surgery. There is evidence to suggest that L-DOPA can affect graft survival, however results are ambiguous with some studies demonstrating toxic effects and others demonstrating neuroprotective effects.[1] Further studies have also shown L-DOPA's ability to alter the microvascular environment within the basal ganglia,[2] however it is unknown whether this may influence graft survival. The aim of this study is to investigate L-DOPA's effects on grafted DAergic neurons and graft vascularisation in order to elucidate the ambiguity surrounding these issues.

Fifty-one 6-OHDA lesioned rats were divided into five experimental groups. Four out of the five groups received VM (E12) grafts, while the remaining group received sham surgery. Each group received varying treatment schedules of L-DOPA and/ or saline before and after grafting. Amphetamine-induced rotation was assessed pre (post-lesion) and post-grafting. The rats were then perfused transcardially and standard peroxidase based immuno-histochemical experiments were performed for TH and RECA-1.

A successful PD model was used in the study, apparent by a reduction in the number of TH+ cells in the lesioned SN for all experimental groups. Grafts were found to be functional by a reduction in the number of ipsilateral rotations following grafting. The immunohistochemical experiments demonstrated that L-DOPA treatment had no effect on graft DAergic cell body count, graft area or graft density; although the L-DOPA treated groups were associated with an increased number of striatal TH+ cells and larger grafts. L-DOPA plus grafting was also found to increase the number of RECA-1 stained blood vessels within the medial region of the striatum.

This study demonstrates that L-DOPA does not negatively affect any aspect of graft survival and may actually exert a beneficial effect on DAergic neurons. This is good news for the PD patient as it means they can continue taking L-DOPA before and after grafting knowing it will not have any detrimental effects on graft survival. It is also the first study to show that L-DOPA increases the microvasculature within the medial region of the striatum, in the area where the grafts are placed. The implications of this increased microvasculature are unknown, thus further studies looking at the microvascular effects of L-DOPA are warranted.

1. Agid, Y. 1998. Levodopa: is toxicity a myth? *Neurology* **50**(4), pp. 858-863.
2. Westin, J. *et al*. 2006. Endothelial Proliferation and Increased Blood–Brain Barrier Permeability in the Basal Ganglia in a Rat Model of 3,4-Dihydroxyphenyl-L-Alanine-Induced Dyskinesia. *The Journal of Neuroscience* **26**(37), pp. 9448-9461.

An Evaluation of the Training and Preparedness for Medicines-based Calculations in Health Care Professionals and Undergraduate Students

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It has been reported that some medicine, pharmacy and nursing health care professionals (HCPs) lack confidence in performing medicines-based calculations (MBCs).[1,2] MBCs are calculations producing numerical data involving patient care or regarding the patient themselves.[3] Errors in MBCs may affect patient safety. The aims of this study were to identify the training and preparedness of HCPs to perform MBCs.

Ethical approval was obtained from an ethics committee within Cardiff University and approval as a service evaluation was obtained from BCUHB before the study commenced. A semi-structured interview was chosen as this would capture valid responses in relation to the study objectives. A small pilot study confirmed the interview validity. Purposive sampling was the main sampling technique employed. However, snowball and convenience sampling were also used to identify participants. A mixture of different career levels (undergraduates, pre-registration and qualified participants) and specialties (medical, surgical and information provision) were sampled in order to gather varied opinions. The interviews took place in Ysbyty Gwynedd, a district hospital part of the Betsi Cadwaladr University Health Board (BCUHB) in North Wales, during a five week period; 6th February to 8th March 2012. The interviews lasted fifteen minutes and captured individuals' personal viewpoints via audio recorder. Each interview was subsequently transcribed ad verbatim. The thematic analysis guide by Braun and Clarke was employed to analyse the interviews,[4] allowing for the generation of main themes, sub-themes and sub-node names.

Forty-three participants were interviewed (13 pharmacy, 17 nursing and 13 medicine). Twelve main themes were identified which include: "level of confidence in MBCs", "initial thoughts on MBCs", "training and assessment", "MBCs performed", "calculator use", "double checking MBCs", "challenging MBCs", "implications of calculation error", "supervise or train others in MBCs", "suggested changes to training and assessment", "using other resources to assist MBCs" and "inter- and intra-professional comparison". Any data which did not fit in to these main themes were coded as "other" data.

Pharmacy participants are specifically assessed by the General Pharmaceutical Council (GPhC) as part of the registration exam and this might explain why they were more aware of the importance of MBCs. Each of the professions (pharmacy, nursing and medicine) were more confident in MBCs they perform frequently. This is similar to recently-reported findings[1] whereby participants rated their calculation skill higher with increased frequency of performing calculations. Nursing and medicine participants recognised there was a need for teaching and training in MBCs, but the style of teaching needed careful consideration in order to suit the individual profession and the learners. The present study was only conducted in one district hospital within one health board over a five-week period; this is a limitation of the study. Further research involving qualitative and quantitative research could provide more data. An important finding was the increased confidence in those calculations that were performed routinely. With other studies finding a correlation between improved numeracy ability and confidence, perhaps a combination of both aspects would enhance MBC confidence further.

1. Meyler L, Ramtoola Z, Barlow J. Evaluation of the ability of pharmacy and medicine students to calculate drug dosage. *Pharmacy Education*. 2011; **11**(1): 186-9.
2. Elliott M, Joyce J. Mapping drug calculations skills in an undergraduate nursing curriculum. *Nurse Education in Practice. 2005*; **5**(4): 225-9.
3. McQueen DS, Begg MJ, Maxwell SR. eDrug Calc: an online self-assessment package to enhance medical student' drug dose calculation skills. *British Journal of Clinical Pharmacology*. 2010; **70**(4): 492-9.
4. Braun V, Clarke V. Using thematic analysis in psychology. *Qualitative Research in Psychology*. 2006; **3**(2): 77-101.

Does FAK represent a therapeutic target in RAW cells to prevent RANKL induced differentiation and signalling?

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Breast cancer is the most common type of cancer in the UK and the prevalence of bone metastasis in those with advanced disease is as much as 85%[2], a trend that is largely due to cross-talk between breast cancer and bone cells. RANKL, an integral part of this cross talk, induces differentiation of osteoclast progenitors into osteoclasts which in turn sustains breast cancer cells in the bone microenvironment and ultimately results in bone-related morbidities including hypercalcaemia, bone fragility and overwhelming pain.[3] Focal adhesion kinase (FAK) is known to play a key role in breast cancer cells where it promotes pro-metastatic cell behaviour and is also implicated in the release of growth factors and chemokines which may include RANKL. The role of FAK in bone cells is currently unknown. The aim of this study was thus to explore the role of the intracellular kinase, FAK, in RANKL-induced osteoclast progenitor differentiation.

Osteoclast progenitor cells (RAW cells) were treated with RANKL in the presence or absence of a FAK inhibitor (PF228) after which RAW cell differentiation was assessed using TRAP staining. The role of FAK in RANKL-induced RAW cell signalling was determined using Western blotting of samples treated with RANKL +/- PF228 whilst RANKL-induced growth following FAK-inhibition was assessed with MTT assays.

RANKL increased FAK signalling and promoted RAW cell differentiation. PF228 treatment suppressed RANKL-induced signalling. FAK and Src signalling were particularly decreased following treatment. In all the results were promising, showing that PF228 can inhibit RANKL-induced signalling in RAW cells.

TRAP staining demonstrated that RANKL caused differentiation in 93.5% of RAW cells; inclusion of the FAK inhibitor decreased this to 85.34%. MTT assays showed that RANKL caused an initial increase in proliferation (a phase that lasts around four days) followed by a decrease in proliferation by day 6. Whilst the initial growth phase appeared unaffected by the addition of a FAK inhibitor, proliferation increased with the addition of PF228 over the longer time point. These data suggest a role for FAK in RANKL-induction of RAW cell differentiation and associated signalling. Given that FAK also plays a role in breast cancer itself, the use of FAK inhibitors may represent an interesting future therapeutic option to suppress breast cancer metastasis and its associated bone morbidity.

1. Bendre, M. *et al*. 2003 Breast cancer metastasis to bone: it is not all about PTHrP *Clin Orthop Relat Res*. **415:** S39-45.
2. Chen, Y. C. *et al*. 2010 Breast cancer metastasis to the bone: mechanisms of bone loss *Breast Cancer Res* **12:** 215.
3. Nicolin, V. *et al*. 2008 Breast adenocarcinoma MCF-7 cell line induces spontaneous osteoclastogenesis via a RANK-ligand dependent pathway *Acta histochemica* **110**: (5) 388-96.

Immunohistochemical Characterisation of Novel Models for Acquired Anti-Hormone Resistance in Breast Cancer

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Anti-hormone (AH) therapies, for example tamoxifen and Faslodex (fulvestrant), are used to treat ER-positive breast cancer. Although initially responsive to AHs, ~30% of ER-positive early breast cancer patients relapse with acquired resistance to adjuvant AH which can mean poorer prognosis.[1] To determine the pathways underlying endocrine resistance and reveal new therapeutic targets, development of preclinical models for acquired AH resistance is vital. Human breast cancer cell lines (e.g. MCF-7) have been widely used *in vitro* for research into AH response. MCF-7-derived cell lines are also established to decipher acquired AH resistance and to screen drugs that target implicated mechanisms. Loss or retention of functional ER and increased erbB receptors EGFR and HER2 can play an important role in acquired resistance to AHs in these simple *in vitro* models.[2] However, tumour microenvironment is known to contribute substantially to cancer biology, and so there is a need to maximise similarities between research models and clinical disease through more complex acquired AH resistant model development.

In this study, a novel panel of acquired tamoxifen (TAMR) and faslodex resistant (FASR) models (vs. MCF-7), produced by the Garvan Institute and Biosciences Dept. Cardiff University to encompass tumour microenvironment, were characterised for expression of ER, HER2 and EGFR using immuno-histochemistry (IHC) to begin to establish if these signalling proteins can also contribute to more complex acquired resistant model systems. This study determined if local environment used to generate the resistant cells influenced the signalling proteins. Impact of duration of tumour cell growth and oestrogen exposure (E2+) on ER, HER2 and EGFR were examined in the new FASR model and capacity for cell metastasis was monitored, using IHC staining for ER, HER2 and EGFR to establish whether these signalling proteins are potential contributors to any AH progression.

Although the new TAMR model retained ER positivity, contrary to results observed in simpler *in vitro* models, EGFR expression was shown to be equivalent in TAMR vs. MCF-7 indicating local environment can influence EGFR expression in tamoxifen resistant cells. The new FASR model recovered significant ER positivity; this was somewhat augmented by modifying the local environment including by E2+ exposure. ER recovery was paralleled by decline in HER2 and EGFR. Recovery of ER in the FASR model vs. the reported ER- status of simpler *in vitro* models indicated an impact of tumour environment in promoting outgrowth of ER positive cells with lower levels of EGFR and HER2. A subsequent decline in ER and some rebound in EGFR was seen after prolonged growth of the FASR model, which also showed evidence of cell metastasis with both ER+ and ER- cells and EGFR and HER2 staining in the spread cells.

IHC characterisation of the new TAMR and FASR (and MCF-7) model panel has revealed significant impact of tumour environment (modified by E2+ exposure and duration of cell growth) on ER, EGFR and HER2 levels in acquired resistant disease which may lead to important clinical implications in the future. Further characterisation of the mechanisms holding EGFR levels in check in this TAMR model could help determine why breast cancers are invariably poorly responsive to EGFR inhibitors.[3] If the mechanisms behind the FASR model's restoration of ER can be determined, it may also yield ways of restoring ER in patients to improve endocrine response. With further characterisation, these novel TAMR and FASR models may help identify further mechanisms and thereby new therapeutic targets to overcome resistance and metastatic progression during AH treatment.

1. Early Breast Cancer Trialists' Collaborative Group. Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. *Lancet*. 2005; **365**: 1687-1717
2. Knowlden JM *et al*. Elevated levels of growth factor receptor/ c-erbB2 heterodimers mediate an autocrine growth regulatory pathway in tamoxifen-resistant MCF-7 cells. *Endocrinology*. 2002; **144**: 1032-1044.
3. Agrawal A *et al*. Overview of tyrosine kinase inhibitors in clinical breast cancer. *Endocrine-related Cancer*. 2005; **12**: S135–S144.

Synthesis and Evaluation of a Series of Oxadiazolyl Indoles with Potential Anti-tumour Activity

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Worldwide cancer is the leading cause of death and is defined as cells that undergo uncontrolled proliferation. A key hallmark of cancer cells is their ability to evade apoptosis.[1] Apoptosis can be induced via two main pathways known as the intrinsic and extrinsic pathways. The Bcl-2 family of proteins play a key role in the intrinsic apoptosis pathway. The Bcl-2 family of proteins is divided into pro and anti-apoptotic proteins. Following an apoptotic stimuli the pro-apoptotic Bcl-2 proteins cause the release of cytochrome c from the mitochondria. This leads to the formation of the apoptosome, activation of effector caspase 3 and induction of apoptosis in the cell. However, anti-apoptotic Bcl-2 proteins can form heterodimers with pro-apoptotic Bcl-2 proteins and prevent them from carrying out their normal function. In normal healthy cells there is a balance between the pro and anti-apoptotic Bcl-2 proteins. However, a common feature of cancer cells is the overexpression of these anti-apoptotic Bcl-2 proteins.[2] The aim of this research is to synthesise a series of oxadiazolyl indoles with potential anti-tumour activity that will be achieved by inhibiting anti-apoptotic Bcl-2 proteins.

The compounds' ability to bind in the hydrophobic BH3 domain of anti-apoptotic Bcl-2 proteins were assessed by performing docking experiments using "Molecular Operating Environment" software. A series of oxadiazolyl indoles were synthesised via two different synthetic routes. The first method used to synthesise these compounds involved refluxing an equimolar suspension of indole-3-carboxylic acid hydrazide and different carboxylic acid derivatives in phosphorous oxychloride.[3] The second synthetic route was a two step process. Step one involved refluxing an equimolar solution of indole-3-carboxylic acid hydrazide and different substituted phenyl isothiocyanate derivatives in ethanol (95%). The resulting compounds were then refluxed in a solution of ethanol (95%), sodium hydroxide (4M) and potassium iodide solution (5%). The purification technique used was recrystallisation from absolute ethanol. The activity of one compound was tested using the MTT biological assay. The assay was performed on Hela cell lines and the cells were exposed to 0.001-25µM of compound 2b for 72hours.

The docking results indicated that the compounds were capable of binding in the hydrophobic BH3 domain. The results also indicated the compounds were capable of forming a number of ligand interactions with key amino acid residues such as Asn 102. The oxadiazolyl indoles synthesised were analysed by a range of different techniques to ensure that they had been successfully synthesised including H'NMR, ^{13}C'NMR, mass spectrometry and elemental analysis. The MTT biological assay performed on compound 2b produced an IC_{50} value of 9µM indicating that the compound possessed anti-tumour activity in Hela cell lines.

In conclusion, this research resulted in the successful synthesis of a series of oxadiazolyl indoles via two different synthetic routes. The synthesis achieved good yields ranging from 40-64% apart from compound 5b which was unsuccessful. The docking results indicated that the compounds should bind in the hydrophobic BH3 domain in the anti-apoptotic Bcl-2 proteins. All the compounds synthesised complied with the Lipinski rule of five. Finally, the MTT biological assay performed on compound 2b indicated that the compound had an IC_{50} value of 9µM and therefore possessed anti-tumour activity.

1. Hanahan, D. Weinberg, R. Hallmarks of Cancer: The Next Generation *Cell*. 2011, **144**(5), 646-67
2. Westwell, A. Zieden, N. Kadri, H. The development of pro-apoptotic cancer therapeutics *Mini-reviews in Medicinal Chemistry*. 2008, **8**, 711-718
3. Sun, J. *et al.* Oxadiazole derivatives containing 1,4-benzodioxan as potential immunosuppressive agents against RAW264.7 cells *Bioorganic and Medicinal Chemistry*. 2011, **19**, 4895-4902

How does the Cardiff MPharm Degree Prepare Students for Pre-registration Training?

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The MPharm (Master of Pharmacy) is a four year, full time degree course accredited by the General Pharmaceutical Council (GPhC). Cardiff is one of the 24 fully accredited Universities that offer the course. Together with a pre-registration year, GPhC registration exam and the following of the fitness to practice standards, the MPharm prepares students to become pharmacists.[1] A pharmacist is a healthcare professional involved in manufacturing, dispensing and reviewing medicines. The pre-registration year is a 52 week, paid placement of 'satisfactory and assessed training'[2] completed within a chosen hospital or community pharmacy or in industry or other approved establishments but at least 26 weeks of the placement must involve patient contact[2]. Throughout the year the students work under the supervision of a tutor and collect a portfolio of evidence, signed off by a tutor, demonstrating their competency at carrying out tasks.[2] In the past few years there have been lots of changes within the pharmacy profession. Do these changes mean changes are needed within the MPharm to better prepare students for pre-registration? The aim of the study is to assess how well the Cardiff MPharm degree and the School prepare students for pre-registration training.

A semi-structured interview plan for both students and teacher practitioners was drafted and sent to the Cardiff School of Pharmacy and Pharmaceutical Sciences Research Ethics Committee for approval. Once obtained, invitation emails were sent out to all MPharm IV students and teacher practitioners at the School. Participation information sheets and consent forms were sent out to those who showed interest in taking part. A mock interview was undertaken for practice and interviews were then conducted and audio recorded. Eighteen participants were interviewed with a range of backgrounds, experience and research areas. Interviews were transcribed and named A to R to anonymise the data. Thematic analysis was applied to the data. Data was coded and arranged into initial themes. Themes were reviewed, renamed and used to explore the objectives.

Eight themes emerged from the data and most participants agree that the degree prepares students well for the pre-registration year however there were parts of it they would like to have altered to better prepare students. Role models, especially teacher practitioners were useful to give examples of real life patients and job roles. Therefore more access to real patients and pharmacist role models within the degree would be useful. Also more posting of job opportunities on learning central and placements, especially hospital ones, so knowledge can be put practice earlier is needed. Furthermore there were arguments for and against the proposed 5 year integrated degree. Calculations and Practice of Pharmacy (POP) were thought to be useful to prepare students for pre-registration but some students would like more of them throughout the whole MPharm not just around the assessment. OSCAs were described as fake and not useful to prepare students for pre-registration but despite this altering the assessment was regarded. Clinical modules were thought best to prepare students for pre-registration but the amalgamation of the non clinical areas with the clinical would allow students to better transfer and develop their knowledge. The School spends a lot of time developing inter professional and spiral learning but no participants mentioned them so greater reinforcement should be used. Introduction of workshops on interview skills and application filling is also thought as beneficial.

The study allowed the researcher to obtain the information set out in the aims. However only 13 MPharm IV students took part in the study and not all teacher practitioners had been pre-registration tutors so results may not be representative. If a greater time frame was available more students could have been interviewed and questionnaires could also have been developed to gain quantitative results as well as qualitative. More Schools of Pharmacy could be involved to make results more generalisable.

1. GPhC 2011. *Education* [Online]. Available at: http://www.pharmacyregulation.org/education [Accessed 3rd November 2011].
2. GPhC 2011.*Pharmacists pre-registration training* [Online]. Available at: http://www.pharmacyregulation.org/education/pharamcists-pre-registration-training [Accessed 3rd November 2011].

Age-dependent Changes in Endocytic-related Proteins in the Brain

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Endocytosis is a crucial cellular process which allows the internalisation of nutrients, fluids and components from the plasma membrane. In particular, endocytosis is key to brain function due to its role in protein trafficking and recycling of synaptic vesicles.[1] Clathrin-mediated endocytosis (CME), the most prevalent endocytic mechanism, relies upon a specific group of proteins, particularly clathrin, to internalise cargo which can then be organised by the endosomal pathway.[2] Normal ageing is inevitable in all organisms, yet its effect on the crucial cellular process of endocytosis is under-researched at present. Hence, the present study aimed to investigate how the expression of proteins involved in endocytosis is altered by increasing age in the brain.

Optimised immunohistochemistry assays were performed using 3, 9 and 18 month old brain tissue sections. 20x microscopy images were taken of the cortex, thalamus and CA1, CA2 and dentate gyrus regions of the hippocampus. By counting the number of labelled cells in each regional image, age-dependent changes in protein expression could be analysed by one-way ANOVA and Dunnett's statistical tests. Since tissue from both male and female subjects was used, the effect of gender on protein expression with age was analysed by two-way ANOVA and Bonferroni's test. The significant gender difference seen with EEA1 meant that male and female age data were analysed separately for EEA1, whilst the age data for clathrin and APP were pooled.

In the cortex and thalamus, there was a significant increase in clathrin-labelled cells with age, whilst a significant decrease was seen in the CA1 region. A significant increase in EEA1-labelled cells was observed in the male cortex with age, but a significant decrease was seen in the thalamus and CA1 region of both genders. A significant age-dependent increase in APP-labelled cells was seen in the CA1 region. It was not possible to achieve good cellular staining for either the caveolin-1 or flotillin proteins and therefore no age-dependent analysis could be performed for clathrin-independent endocytic mechanisms.[2]

The effect of increasing age on clathrin, involved in vesicle internalisation, and EEA1, a marker for early endosomes, are region-dependent. A rise in these proteins in a particular brain region suggests an age-dependent increase in CME, and vice versa. However, a correlating pattern with both clathrin and EEA1 was not always observed. Since APP is internalised by CME[3], the general increase infers an age-related increase in APP due to increased CME. The disparity between brain regions and apparent uncoupling of different stages of the same endocytic pathway proposes an imbalance in endocytosis in normal ageing, which could be implicated in reduced learning and memory.

1. Slepnev VI and De Camilli P, 2000. Accessory factors in clathrin-dependent synaptic vesicle endocytosis. *Nature Reviews Neuroscience* **1**(3) pp. 161-172
2. Doherty GJ and McMahon HT, 2009. Mechanisms of Endocytosis. *Annual Review of Biochemistry* **78** pp. 857-902
3. Coughlan CM and Breen KC, 2000. Factors influencing the processing and function of the amyloid β precursor protein – a potential therapeutic target in Alzheimer's disease? *Pharmacology and Therapeutics* **86** pp. 111-144

Feedback on Assessed Work: The Views of Pharmacy Undergraduate Students

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National Student Survey (NSS) data from 2011 revealed that despite giving their School an overall satisfaction rating of 94%, MPharm students awarded the feedback and assessment element of the course just 51%.[1] This data is worrying, as effective feedback is essential at university level and has been described as, *"…more strongly and consistently related to achievement than any other teaching behaviour."* [2] Additionally the Higher Education Funding Council bases the amount of funding the university receives on feedback scores, with lower scores equalling less funding. For these reasons, the aim of this study was to explore the views of students at the School with regard to feedback on assessed work in order to hopefully improve the quality of feedback they receive.

Semi-structured interviews were chosen as the initial qualitative method for research into the views of students and any issues raised from the interviews were used to develop a quantitative questionnaire to be distributed to all four MPharm years. Non-probability convenience sampling was used to recruit MPharm students (n=5) for interviews, which were conducted in the Redwood building. The interviews were recorded and transcribed verbatim and data was thematically analysed by using both inductive and deductive approaches before being used to develop a questionnaire.[3] This questionnaire was distributed to MPharm I-III (n=248) during lectures and to MPharm IV (n=66) in laboratories or via email. Completion of the questionnaire gave complied consent and due to its anonymous nature, there were no follow-ups. Quantitative data from the questionnaire was entered into an SPSS database and qualitative data was entered into a Word® 2007 document to be coded and thematically analysed.

The questionnaire response rate was 73%, which is comparable to studies of a similar nature. It was found that students viewed feedback in a positive way with 100% agreeing that they wanted feedback and 94% agreeing that getting feedback is always beneficial. Ninety six percent of students agreed that getting feedback boosted their confidence although interestingly, significantly fewer ($p=0.009$) males (91%) than females (98%) felt this way. Results showed that 60% of students wanted feedback within two weeks although 55% understood if feedback was delayed due to a tutor's busy schedule. It is evident from the answers given that students care about the form of feedback. The most popular format of receiving feedback is work annotated with comments (90% like) followed by reviewing a marked test paper (82% like). Worryingly the most disliked form of feedback is the commonly used method of giving generalised feedback on Learning Central (51% dislike). To determine whether students were using feedback to its maximum potential, they were asked to identify different forms of feedback. Those most recognised by students were meeting with a tutor (92%) and receiving written criticism (90%). Those least recognised included being marked by a peer (37%) and doing a formative exercise in class (30%). Improvements that students most wanted to see on the course included more individualised as opposed to generalised feedback and for feedback to be quicker and more detailed.

The positive way in which students view the concept of feedback at the School was encouraging as it's important to establish that students want this type of support before making any changes to it. A balance of both positive and negative feedback appears to be optimum, however it appears that the nature of feedback is less important to males that females. Generalised feedback is the most commonly given and the most disliked form of feedback on the course. This is an issue that needs to be addressed by introducing more individualised feedback whenever possible. This study has generated new data in the field of undergraduate student views on feedback. It is hoped that the results can help to improve the feedback given on the course. Simple changes could be made that don't compromising practicality, such as labelling feedback so that students can recognise it. Hopefully these findings will go some way to improving NSS scores and enhancing the quality of feedback on the course for current and future students.

1. Directgov 2011. *Unistats* [Online]. Available at: http://unistats.direct.gov.uk/nStudentSurvey.do [Accessed 10th March 2012].
2. Bellon, J. 1991. *Teaching from a Research Knowledge Base: a Development and Renewal Process.* Facsimile edition. Prentice Hall, New Jersey, USA.
3. Smith, F. 2010. *Conducting your Pharmacy Practice Research Project*. Pharmaceutical Press.

Caveolin-1: Is it downstream signalling molecule in renal cell carcinoma cell line A498

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Renal cell carcinoma represents 3% of all cancers and 2% of all cancer related deaths. Two-thirds of all RCC present with localised disease initially and undergo surgery but up to 40% will develop metastasis later.[1] Survival rates for metastatic RCC rarely exceed 5% due to resistance of tumours to chemotherapy, radiotherapy and hormonal therapy.[2] Caveolin-1 expression in combination with activated components of PI3K/AKT/mTOR pathway[1] and Raf/Ras/ERK pathway[2] is correlated with metastasis, poor prognosis and poor disease-free survival in renal cell carcinoma. Caveolin-1, a 21-24kDa scaffold protein, is the essential constituent of caveolae, flask=shaped (50-100nm) invaginations that can occupy up to 20% of the plasma membrane.[3] This study aimed to address if caveolin-1 is a downstream signalling molecule in respect to other pathways correlated to poor prognosis in renal cell carcinoma therefore objectives were to examine the effect upon cell growth and caveolin-1 expression of inhibition of PI3K/AKT (inhibitor LY294002), inhibition of mTOR (inhibitor rapamycin) and inhibition of MEK/ERK-1/-2 (inhibitor PD98059) in renal cell carcinoma A498 cell line.

Cells were treated with inhibitors at various concentrations and cell viability was determined by MTT proliferation assay. This assay is based on the conversion of the yellow tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to purple formazan crystals by metabolically active cells and provides a quantitative estimate of viable cells.[4] The expression profiles of caveolin-1, phosphorylated-AKT, total-AKT, phosphorylated-S6, total-S6, phosphorylated-ERK, total-ERK and β-actin were examined by Western blot. Western blot is an analytical technique that uses electrophoresis to separate proteins.

Reduction in cell growth by 60% and 80% was present in cells treated with concentrations of 10μM and 50μM LY294002. Reduction in cell growth by 30% and 50% was present in cells treated with concentrations of 1nM and 10nM rapamycin. Reduction of cell growth by 25% and 50% was present in cells treated with concentrations of 10μM and 50μM PD98059. Caveolin-1 expression remained unchanged as did total-AKT, total-S6 and total-ERK levels when levels of p-AKT, p-S6 and p-ERK decreased.

Caveolin-1 is not a downstream effector of AKT, mTOR or ERK. Correlation between poor prognosis and caveolin-1 expression and activated Akt, mTOR and ERK has been revealed by other studies. This combined with our data suggests that caveolin-1 and PI3K/AKT, mTOR and Raf/Ras/MAPK signalling pathways may have a common upstream activator. This may afford Caveolin-1 to represent a good independent target for new therapies.

1. Campbell, L. *et al* 2008. Combined expression of caveolin-1 and an activated AKT/mTOR pathway predicts reduced disease-free survival in clinically confined renal cell carcinoma. *British Journal of Cancer* **98**, pp. 931-940.
2. Campbell, L. *et al* 2009. Activated extracellular signal-regulated kinase is an independent prognostic factor in clinically confined renal cell carcinoma. *Cancer,* pp.3457-3467.
3. Burgermeister, E. *et al*. 2008.Caveats of calveolin-1 in cancer progression. *Cancer Letters* **268**, pp.187-201.
4. Sourbier, C. *et al* 2006. The Phosphoinositide 3-kinase/Akt pathway: a new target in human renal cell carcinoma therapy. *Cancer Research* **66**(10), pp. 5130-5142.

The following abstracts have been withheld as they have been or will be published elsewhere, and/or due to intellectual property or confidentiality issues:

Determining the Effect of Stem Cell Populations on Oral Cancer Cell Proliferation

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Design, Synthesis, and Antitumour Evaluation of Novel 5-fluoro-2'-deoxyuridine (FUDR) Prodrugs

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Synthesis and Optimisation of the Diaryldiamines as Inhibitors of *C. difficile* MetRS

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Probing the potentiated antibacterial effect of Pomegranate Rind Extract (PRE) alone and in Combination with Zinc

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Design and synthesis of novel MetRS inhibitors as potential *Clostridium difficile* therapeutics

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The design and synthesis of novel inhibitors of *Mycobacterium tuberculosis* CYP121

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Does magnesium sulphate play a role in the delivery of salbutamol via an air jet nebulizer?

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Synthesis and evaluation of pro-drug motifs on 2C-thiophenyl-6O-methyl-2'C-methylguanosine nucleoside analogues for hepatitis C virus

Heather L Weerdenburg, K Madela and C McGuigan
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MSc

The Use of Electronic Injection Devices to Monitor and Improve Adherence to Growth Hormone Therapy

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The aim is to demonstrate that improved patients outcome could be achieved through better adherence and support. The objective of this review is to assess the level of adherence of subjects receiving growth hormone therapy via the electronic injectable device easypod and the impact of adherence on clinical outcomes.

I will report and summarize the study design as well as analyse the results, published in 2011, of the first study of adherence to treatment as recorded by the auto-injector easypod. The primary objective of this survey study was to evaluate adherence to r-hGH therapy over 3 months of use. I will then compare the results and the author's conclusion to leading publication having similar objectives. Descriptive analysis was used on the data as reported in the respective publications using tables and figures and where possible, graphical analysis.

The survey reported a significantly increased adherence in treatment-naïve children (89.7%) compared with treatment-experienced children (81.7%; p=0.0062). There was an increased trend in missing injections over the course of the three-month treatment period. Rates of reported adherence were higher than those of recorded adherence at each time point as well as overall (90.2% *vs* 87.5%). Additionally, aspects of ease of device operation, device use and features and an assessment of the pain intensity experienced by patients confirmed reports of previous findings.

The findings concur with previous studies, that poor adherence to recombinant human growth hormone (r-hGH) leads to reduced efficacy outcomes and that the electronic injector device easypod™ is the only GH injection device that can accurately record patients' dose and injection history, allowing healthcare professionals the ability to monitor adherence.

Renal Function Estimation Equations to Guide Enoxaparin Dosing

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Two of the most commonly used estimation equations for renal function (eRF) are the Cockcroft and Gault (C&G) and the Modification of Diet in Renal Disease (MDRD). There is no definitive guidance as to which equation to use for calculating eRF and for dosing drugs. This pilot study recruited patients with Non ST Segment Elevation Myocardial Infarction (NSTEMI) and a calculated renal function of CKD 3 (≥30mL/min) with one equation and CKD 4 (<30mL/min) with the other. Empirical treatment for NSTEMI includes enoxaparin dosed according to an eRF. An anti factor Xa assay was used to detect an accumulation of enoxaparin.

The qualitative element involved semi-structured telephone interviews with renal, medical admissions and cardiology pharmacists. The participants all stated that they would calculate a C&G, particularly if the drug had a narrow therapeutic window or if the patient were of an extreme of age or weight. Very few had experience with the anti factor Xa assay due to lack of laboratory facilities and short duration of enoxaparin therapy.

The quantitative part randomly allocated patients with a discrepancy in eRF above and below 30mL/min to receive a dose of enoxaparin according to MDRD or C&G. An anti factor Xa test was taken after the third enoxaparin dose, and this along with patient demographics, adverse events and readmission rates were analysed. Out of 5 potentially eligible patients, two consented to participate in the study, one in each arm. There was not enough data to carry out statistical analysis. Both patients were elderly and overweight; neither had a major adverse event although both suffered minor bleeds.

Limitations included a short data collection period during summer months and single centre recruitment. Future work could include a longer study period with multicentre recruitment. Medical staff could also be interviewed to gain their opinion on the issue of eRF.

Development and application of a measurement tool for the training of commercial clinical researchers – the SPITFIRE

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Clinical trials are essential for the development of medicines and treatments for patients. Effective training is fundamental for ensuring the safety of trial subjects, obtaining accurate and credible data and reducing errors in clinical research. The aims of this study was to comprehensively evaluate a standardised approach to protocol training for clinical research staff working on multiple, and often complex clinical trials.

The attitudes and opinions towards this training approach will be explored in three areas: i) General feedback from all staff ii) Study Coordinator feedback iii) Feedback from staff who were closely involved in a complex study using this approach. In the absence of a measurement tool to pursue the aims of this study, an electronic questionnaire (SPITFIRE) was developed to obtain feedback on a variety of characteristics of this approach, specifically, the perceived improvement in the following five key areas: Safety; Quality; Knowledge; Skills; Confidence.

The findings demonstrate very strong positive opinions towards the standardised approach to protocol training, with over two thirds of study participants (n=42) rating Strongly Agree in all of the five key areas. The most compelling feedback was with regard to the preparation and delivery of the training by the Study Coordinators in which 75% strongly agreed this approach helped them gain a greater understanding of the protocol and disease area/drug being studied. The most important aspects of dry run training on the complex trial by were performing the task 'hands on' (94%) and being able to educate and train subjects (88%). Ongoing support, training and communication were the highest rated features in the lengthy and complex trial for maintaining safety, quality and consistently high standards of clinical conduct throughout.

The implementation of this questionnaire has successfully obtained valuable feedback which clearly highlights the importance of a standardised approach to protocol training for helping to improve the safety and quality of clinical trials and the knowledge, skills and confidence of research staff.

An *in vitro* evaluation of immunosuppressant and antifungal drugs in combination against *Aspergillus fumigates*

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Invasive fungal infections (IFIs) caused by *Aspergillus spp.* continue to be responsible for unacceptably high mortality rates. The major risk factor for developing IFIs is immunosuppression, however, a number of these agents have antifungal activity themselves. Understanding this activity and any interactions that occur when taken in combination with antifungal agents could potentially improve treatment for IFIs. Optimal combinations and dose adjustments could be made to harness synergy or manage antagonism. To address this, an *in vitro* microdilution susceptibility study was performed to detect interactions these drugs (cyclosporine A (CsA), tacrolimus (FK-506), hydrocortisone acetate (HCA), cyclophosphamide (CPM) and mycophenoate mofetil (MMF)) have in combination with antifungal agents. Susceptibility of five isolates of *A. fumigatus* was investigated against eight antifungals and five immunosuppressive agents, both as single agents and in combination. Results for amphotericin B and the triazoles were easy to read macroscopically but the echinocandin class of drugs needed to be interpreted microscopically. Of the immunosuppressive agents tested only CsA and FK-506 showed activity on their own. When used in combination FK-506 was shown to be synergistic with the triazoles and echinocandins. CsA was also shown to be synergistic with the echinocandins however antagonism was detected when in combination with posaconazole and itraconazole.

MSc

A review of pharmacists' knowledge, attitudes and barriers towards pharmacovigilance and the reporting of adverse drug reactions through the yellow card scheme

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The aim of this dissertation is to evaluate the knowledge and attitudes of pharmacists in regards to the reporting of ADRs through the Yellow Card Scheme (YCS). It is also intended to investigate the possible pressures or factors that may affect the reporting of such ADRs.

An online questionnaire survey of 100 pharmacists from the Nottinghamshire and Derbyshire area was conducted. The survey included a mixture of demographic questions, questions related to the Yellow Card Scheme reporting criteria and various attitudinal questions regarding the reporting habits.

The response rate to the survey was 45% (n=45). The vast majority of the pharmacists surveyed considered that reporting ADRs was a professional obligation and felt positive towards the reporting of ADRs, however only 40% of those responding had reported an ADR in their career. More than half of the pharmacists were unclear as to what should be reported. The time available in practice and time to complete reports were deemed to be major deterrents to reporting as well. More time spent in educating pharmacist and publicizing the scheme were seen as factors that could help improve the community pharmacist population reporting habits.

Pharmacists are supportive of the Yellow Card spontaneous reporting scheme and have a reasonable knowledge in general. However, the lack of time and misconceptions on what should be reported may be contributing to the low reporting figures for this health professional group. It has been seen that education and training and the development of a smarter reporting system embedded in the dispensing activities could contribute to maintaining and increasing ADR reports from pharmacists in the UK.

An Investigation of the Knowledge, Perceptions and Practices of General Practitioners and Pharmacists Concerning Generic Substitution in Bristol, England

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This research sought to investigate the views of pharmacists and general practitioners about generic substitution, their knowledge of the science behind generic substitution and their willingness in practice to substitute or allow the substitution of generic medicines for branded ones. A descriptive cross-sectional postal survey was conducted in Bristol using an anonymous self-administered structured questionnaire.

The overall response rate for the questionnaire was 58 %. Most respondents had a positive attitude towards generic drugs overall. The majority of respondents thought generic drugs were not inferior to branded drugs in quality (81%), efficacy (84%) and safety (78%). A large majority (76%) did not know the bioequivalence criteria used to evaluate generic drugs, and 67% thought clinical data to demonstrate efficacy and safety were required for generic drug approval. Most could define bioavailability (74%), bioequivalence (74%), and 84% could distinguish between generic and therapeutic substitution. Respondents who believed generic drugs were manufactured to the same GMP standards as branded drugs were more likely to support generic substitution.

Although generic substitution remains controversial among some doctors and pharmacists in primary care, and despite the lack of proper understanding of the bioequivalence criteria applied to generic drugs, the large majority of pharmacists and general practitioners have a positive view of generic drugs.

MSc

The management of chemotherapy induced nausea and vomiting (CINV) in gynaecology oncology patients

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Velindre Cancer Centre is a tertiary centre for the non-surgical treatment of solid tumour oncology patients. Gynaecology oncology patients commonly receive carboplatin alone or in combination with paclitaxel, and all patients are pre-assessed within an outpatient clinic prior to their next cycle of chemotherapy. Medical and non-medical prescribers (NMPs) within the team commonly prescribe chemotherapy and supportive medicines i.e. anti-emetics.

The study aimed:
To investigate the management of CINV in gynaecological oncology patients receiving carboplatin alone or in combination with paclitaxel chemotherapy
To establish the patients views of their CINV management by the team and the pharmacist NMP within this team

The study was divided into two sections. Study one used data collection forms to retrospectively collect data on the grade of CINV and the corresponding anti-emetics prescribed on each cycle. Study two involved explorative interviews which enabled production of a patient questionnaire, which was used to obtain patients' views of their CINV management and the pharmacist prescriber.

Out of 39 patients, 18 experienced nausea and 11 experienced vomiting with or without nausea. Generally, as the cycles of chemotherapy progressed, the grade of CINV decreased. A wide range of anti-emetics were prescribed for patients from cycle two onwards for differing grades of CINV experienced, but standard anti-emetics were prescribed on the first cycle. By cycle three, 30 patients had their CINV controlled to grade zero. Out of 32 patients, 31 felt their CINV management by the team was at least satisfactory, and out of 19 patients, 100% were at least "satisfied" with the pharmacist NMPs care.

CINV was controlled for the majority of patients, but there is limited continuity with the anti-emetics prescribed. The majority of patients felt their CINV was managed well by the team overall, and were satisfied with their care from the pharmacist NMP.

Medicines Management in Elderly Care Home Residents: Impact of a Pharmacist Clinical Medication Review

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The Department of Health's Care Home Alert was issued in January 2010 following the publication of the Care Homes Use of Medicines (CHUMS) report in November 2009[1]. The CHUMS study revealed a high prevalence of medication errors; on any one day 7 out of 10 patients experienced at least one medication error (mean of nearly 2 errors per resident).[2]

To evaluate the impact of a pharmacist clinical medication review (CMR) on the medicines management of elderly care home residents within Cwm Taf Health Board (HB).

Prospective CMRs of all (n=40) patients (residential and nursing) within the purposively sampled care home took place between April to July 2011.The impact that the research pharmacist had on the medicines management of patients was measured by quantitatively recording the number and nature of interventions made following the CMR and the rate of acceptance and implementation of interventions by the General Practitioner (GP). Cost implications were calculated by comparing the cost differences of monthly repeat medication pre- and post- CMRs and the pharmacist resource requirement. Qualitative data was collected by assigning the interventions a consequence severity score based on the potential for patient harm had the intervention not been made.

The forty care home residents were prescribed a total of 326 medicines, averaging 8 items per patient. A total of 147 interventions were identified, averaging 3.68 interventions per patient (range 0-7). Of all the prescribed medicines, 45.1% (147/326) required an intervention. The most frequent types of interventions were stopping medication 23.8% (35/147) where the duration was inappropriate or indication no longer valid and technical interventions 20.4% (30/147), including amending quantities and archiving medicines. Two-thirds, 67% (99/147) of interventions were accepted by GPs and implemented. A pre-agreed pharmacist enabling policy allowed a fifth (30/147) of interventions to be made without referral to the GP. The highest number of recommendations were for medicines in the cardiovascular, endocrine and nutrition and blood chapters of the BNF.

The annual reduction in cost of the repeat medication resulting from the interventions for the 40 patients was £14,576 (average £365 per patient or £100 per intervention). On average 3 interventions, saving £300 were made per hour at a gross staff cost of £20 per hour. Therefore for one hour of pharmacist time, a net saving of £280 per patient, equivalent to a total saving to Cwm Taf HB of £11,200 per annum was achieved.

Pharmacist-led CMRs in care home patients is highly cost-effective and improves the quality of prescribing. A fourteen fold return on investment in medicines expenditure was demonstrated. The majority of interventions had a negligible consequence severity score, however, whilst the average score of potential harm was relatively low, results from the literature indicate opportunity for more serious harm.[2] A robust system aiming to improving medication safety and cost effectiveness of prescribing in care home patients should be seen as a high priority for Cwm Taf HB. Further potential work includes domiciliary medication reviews in housebound patients and targeted 'Medication-Use-Reviews' in community.

1. Department of Health (DoH). 2010. Central Alerting System. The use of medicines in care homes for older people.
2. Barber ND, Alldred DP, Raynor DK, Dickinson R, Jesson B, Lim R *et al.* 2009. Care homes' use of medicines study (CHUMS): prevalence, causes and potential harm of medication errors in care homes for older people. QualSaf Health Care 18:341-346.

MSc

Joints manifestations in Anderson-Fabry disease

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The research project is an investigation of joints manifestations in Anderson-Fabry Disease (AFD). AFD is an X-linked lysosomal storage disorder caused by the lysosomal accumulation of neutral glycosphingolipids due to deficiency of the α-galactosidase A. Progressive accumulation of globotriaosylceramide is associated with a wide range of disease symptoms, including renal failure, cardiovascular dysfunction, neuropathy, stroke and dermatological manifestations. There is a clinical impression of an increase in joint problems in Fabry patients. However, only single instances were reported in publications.

This study aims to determine whether patients with AFD have joints symptoms. This is a prevalence study and thus aimed to invite to participate all eligible patients known to the Lysosomal Storage Disease Unit (LSDU) at the Royal Free Hospital, London. We have developed a Joint pain questionnaire ǁ as a combination of the well known and validated questionnaires, including: Euro Quality of Life 5D questionnaire (EQ5D), Brief Pain Inventory (BPI), Patient‘s assessment of physical function (HAQ-DI), SF-36 Health Survey and McGregor‘s questionnaire.

We aimed to determine prevalence of joint symptoms in patients with AFD, its impact on quality of life, pain and ability to look after themselves for patients suffering from AFD, to compare the prevalence of joint manifestations in male and female population of patients with AFD and to correlate it with patients‘ age.

We found a significant prevalence of joint problems in AFD patients especially in the <40 group compared to prevalence estimates of the normal population. This has clinical impact for diagnostic assessments and management of AFD patients.

An Investigation of a New Compound for the Treatment of Asthma; the Potential for a Shift in Metabolism

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This thesis reports the findings from a clinical trial which was conducted to assess whether C000459, a novel drug for the treatment of asthma, has the potential to influence one of the most important enzymes involved in drug metabolism, CYP3A4. The ultimate objective of the study was to deduce whether OC000459 could influence or be influenced by co-administered concomitant therapy and therefore, later be implicated in drug-drug interactions when prescribed to asthma patients.

The CYP3A4 substrate, midazolam served as a probe drug to help define any changes evident in CYP3A4 enzyme activity following administration of OC000459. The pharmacokinetics of midazolam (and its major metabolite, 1-hydroxymidazolam) was studied both before (Day 1) and after multiple administrations of OC000459 (Day 9) and the results compared.

Twenty healthy volunteers underwent a ten day treatment period, during which two single doses of 5 mg oral midazolam were administered (each separated by a period of eight days), interrupted by thirteen doses of 100 mg OC000459 tablets (each separated by a period of twelve hours). Various safety assessments were performed at pre-determined time-points throughout the treatment period and blood samples obtained for pharmacokinetic assessment.

The mean Cmax and AUC (including AUC0-t and AUC0-!) values for midazolam demonstrated a clinically significant decrease from Day 1 to Day 9, such that bioequivalence could not be concluded for the two administrations. However, this was not accompanied by the increase in 1-hydroxymidazolam mean Cmax and AUC values expected on Day 9, which instead also decreased. Although it seems that OC000459 is not a potent inducer of CYP3A4 and therefore is unlikely to have major DDI implications in the clinic, the results from this study are inconclusive and may benefit from further investigation in patient populations before the inducing possibility of OC000459 is altogether eradicated.

Evaluation of the National Institute for Health Research Clinical Research Network's Capacity to Improve the Delivery of Commercial Research in the NHS

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In recent years the government has sought to address the issues associated with conducting commercial research in the UK. Problems such as long approval times, unsuccessful recruitment and expense have been detrimental to the industry conducting research in the UK. Best Research for Best Health targeted NHS collaborations with the industry and has attempted to improve the ability and capacity of the NHS to meet the needs of commercial sponsors. The implementation of this strategy was rolled out by the National Institute of Health Research (NIHR) who were charged with addressing the requirements of the industry, and via the newly established Clinical Research Networks (CRN), set about enhancing the overall delivery of commercial trials in the NHS. The aim of this study was therefore to evaluate if the NIHR CRNs have been successful in improving the delivery of commercial research in the UK.

To reveal if NIHR adopted portfolio studies were delivered more successfully than non adopted studies, the five Acute Trusts in the Trent CLRN area were approached to provide data on non adopted commercial studies that were approved between November 2008 and November 2010. Study set up metrics were complied in a database for both adopted studies and non adopted studies within the specified time frame, and the time taken to gain NHS permissions at each Trust were compared. Key performance indicators were measured for adopted studies across all of the CRNs in correlation with the NW Exemplar Programme. The following assessments were made: relationship to the NW Exemplar Programme, and contrast in commercial research activity/performance between Networks where full coverage was not present in the Trent area. To evaluate how the CRNs support the industry via methods which are not captured in key performance indicators, data available locally was analysed to demonstrate the provision of infrastructure, and to show how commercial sponsors engage and use the resource available.

The NHS permissions for adopted studies are not achieved faster than approval times for non adopted studies. Inconsistency in approval processes and the start point of local governance checks affects the measurements; however the NIHR CRNs do have the potential to make improvements as demonstrated by the results of the NW Exemplar Programme. Commercial trial activity tends to be greater where there is coverage and support of a CRNs, and the successful placement of clinical studies could be maximised by the industry if they used to the full potential local intelligence and knowledge of research capacity within the CLRNs.

These findings show that while the study set up metrics does not reveal adopted studies to be at an advantage over non adopted studies, there is great potential to improve these times across all commercial trials if the systems and tools introduced by the NIHR to make efficiencies were fully embraced and implemented consistently across all NHS organisations. The underpinning infrastructure provided by the NIHR CRNs is key to delivering commercial research in the UK, and the industry must fully engage with the CRNs to maximise on the potential to deliver studies in the UK successfully via the NHS. Collaborative approaches are essential; as disjointed and isolated improvements by individual bodies will do little to advance the UK nationally towards the aspired status of achieving excellence.

The Use of Monitored Dosage Systems (MDSs) in Patients with Learning Disabilities

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Monitored Dosage Systems (MDSs) are issued to people with learning disabilities in an attempt to improve the management of their medication. Their use may not be appropriate as there is no evidence base to support this. The aim was to explore this group's pharmaceutical needs and identify barriers and facilitators to meeting them. The study was designed to identify issues this group may have with MDSs and to ascertain patient expectation and satisfaction of the service.

This qualitative study was carried out at Lloydspharmacy©, Porth, where twenty six patients with learning disabilities received their medication in MDSs. The patients were under the care of social services or residents in a care home and had never received a Disability Discrimination Act (DDA) assessment. Twenty six patients were sent a DDA assessment form. Patients who met the eligibility criteria were sampled purposively and semi-structured interviews were conducted. Face to face interviews took place in the patient's house, with a carer present. The interviews were transcribed verbatim, a coding framework was developed and transcripts were thematically analysed.

Nine patients with learning difficulties were interviewed. Six main themes were identified and these were: 1) knowledge of medication, 2) attitude to medication, 3) problems using MDSs, 4) positive opinions on MDSs, 5) expectations of the service and 6) recommendations and suggestions. Feedback from these MDS service users, indicate that people with learning disabilities are a heterogeneous group with differing abilities, knowledge of their medications, opinions and pharmaceutical needs.

This study successfully engaged people with learning disabilities in research and many methodological challenges in researching this population were overcome. Several issues were identified leading to the conclusion that a patient-centred approach is needed to identify and address the pharmaceutical needs of the individual, rather than providing an MDS without this assessment. These are preliminary findings based on a small study and further work is needed to establish if these can be generalised to all patients with learning disabilities who take medication.

MSc

Antidepressants as a short-term treatment for selfinjurious behaviour in young adults in the UK and their effectiveness in the long-term management of self-harm

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Deliberate Self-Injury (DSI) is a serious behavioural problem that can affect any age group, but appears to be more prevalent in young adult and adolescent women. The reasons for the behaviour are complex and poorly understood by health professionals and the general public. Rates of DSI in the UK are among the highest in Europe and rising. Various psychological and psychosocial treatments are offered by GPs. Pharmacological treatments are yet to be recommended by NICE. Antidepressants are the most common pharmacological therapy available.

To investigate the efficacy of antidepressants in treating self-injuring young adults in the UK, both in the short and long-term. To explore the effect of antidepressants on the common co-disorders: 'anxiety', 'Obsessive Compulsive Disorder (OCD)' and 'anger'.

A short confidential web-based survey was answered by 44 out of an anticipated 50 young adult self-injuring men and women. Participants had taken antidepressants and had not received any other treatment (e.g. Cognitive Behavioural Therapy). The primary endpoint was: the proportion of participants for whom antidepressants was an effective short-term treatment for DSI. Secondary objectives were: the proportion of participants for whom antidepressants were effective in the longer-term (up to 3 months or more); the proportion of participants for whom antidepressants had a positive effect on the co-morbidities: anxiety, OCD and anger.

All evaluable participants (n=38) returned to self-injuring after treatment with antidepressants, 57.9% of them (n=22) within just a week. There were 5 participants (13.2%) who refrained from self-injuring for up to 3 or more months. However, only 1 of them was still free from the disorder for >6 months. The majority of participants (83.8%, n=31) for whom anxiety was a co-disorder saw an improvement in their condition. About half of the participants with OCD and/or anger symptoms experienced some improvement; but half experienced no improvement at all.

There is no evidence that antidepressants are effective as a short or long-term treatment for DSI. However, there is some evidence that antidepressants could be useful for certain people for up to 3 months or more. Antidepressants may be useful for treating anxiety in patients who self-injure. There is insufficient evidence that OCD and anger can be alleviated by taking antidepressants.

The Impact of the European Union Clinical Trial Directive on Multinational Non-Commercial Clinical Trials – A UK Perspective

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The European Union Clinical Trial Directive (2001/20/EC) was aimed at harmonising the many divergent national regulations that governed the conduct of clinical trials however, after its entry into force in May 2004, it was apparent that differences in interpretation had led to further disparity in national requirements. The aim of this research project was to indentify key differences implementation of the Directive and to assess its impact on the conduct of multinational trials carried out by non-commercial sponsors; the group believed to have been most affected by the Directive.

Following an extensive literature review, a web-based questionnaire was distributed to 48 UK Clinical Research Collaboration Registered Clinical Trials Units to establish their experience of conducting multinational clinical trials and their opinions on the success of the Clinical Trials Directive. 20 questionnaires were returned and from this it was derived that 16% of all the trials being conducted by respondents were being carried out in the EU, across 14 of the 27 Member States. Respondents reported that the time, cost and number of employees required to conduct a clinical trial had increased since the introduction of the Directive and that overall the number of multinational non-commercial clinical trials conducted had fallen. 67% of respondents gave a verdict of an overall negative impact of the Directive on multinational, non-commercial trials.

This project has identified some of the key areas where the aims of the Clinical Trial Directive have been unsuccessful. The perceived negative impact of the Directive on the non-commercial research community is also supported by the results of this research. In order to facilitate the conduct of non-commercial research, a key factor for continuing medical advancement, it will be essential for the EU to address the barriers and divergence of national legislation associated with the Clinical Trial Directive.

MSc

Barriers to recruitment and retention in adults' respiratory clinical trials: the patients' perspective

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To assess the attitudes of patients about their willingness to participate in clinical trials.

This is a self-administered questionnaire survey of patients who attended an adult respiratory outpatients department in a local DGH during a two month study period in 2011. It measured patient knowledge of access to clinical trials, their attitudes toward participation, their recruitment preferences, and their beliefs about research safety and integrity.

Of 600 randomly selected patients, 228 (38%) completed the questionnaire. 214 (94%) stated ethnicity as white, compared to 14 (6%) non-white. Previous participation in clinical trials was reported by 47 (20.6%) patients. The majority of patients were unaware of online information about clinical trials (192 [84%), were satisfied with their current knowledge (117 [51.3%]), expected their treating physician to inform them about current trials (171 [75%]), showed an almost equal amount of interest in participating in conventional or complementary intervention trials (78 [34.3%] vs. 68 [29.9%]). Of the 228 respondents, 154 (67.5%) found it appropriate to be contacted by mail compared to 109 (47.8%) by telephone regarding study participation. The majority of respondents (197 [86.4%]) wanted to be informed about research findings or else would not take part in future clinical trials (145 [63.6%]). The preferred compensation was free parking (151 [66.2%]). An equal proportion of respondents (198 [89.9]) thought their privacy and safety would be maintained in a trial conducted within the NHS.

Patients are interested in taking part in clinical trials, but lack the necessary information. If patients received more information from Consultants and the research teams, recruitment might improve. This single-site dose has limited generalisation. Further studies across multiple sites, utilising a more naturalistic methodology, and involve in a more diverse population of patients from a broader geographic distribution are needed to provide more conclusive results.

Post-Marketing Surveillance Strategy: Approach To Modified Risk Tobacco Products

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Although no tobacco product is safe, it has been proposed that smokers who cannot or will not stop smoking are offered alternative reduced risk tobacco products in an attempt to reduce the overall incidence of cigarette smoking-related diseases. Tobacco companies are interested in launching such products. The Institute of Medicine (IOM) recommended to undertake post-marketing surveillance (PMS) and epidemiological studies as necessary to determine the short-term behavioural and long-term consequences of using tobacco products marketed with claims of reduced risk of tobacco-related disease.

To suggest a model of PMS strategy and tools to be adopted by a tobacco company to monitor modified risk tobacco products (MRTPs) for safety, use and reduced risk or harm to the consumer population over a long period of time compared with conventional tobacco products currently on the market. A literature review on tobacco product surveillance was carried out. The pharmaceutical and food industry's PMS systems were also reviewed to inform any post-market activity for MRTPs.

The potential role of a MRTP should be evaluated by appropriate monitoring to gather reliable data with respect to its safety profile and efficacy as compared with a conventional tobacco product. Early indicators, such as consumer perception of risk reduction, impact on smoking behaviour, biomarkers of exposure and biological effect, should be assessed together with long-term health outcomes and mortality. Active and passive PMS methods for pharmaceutical and food products can be adopted to monitor MRTPs.

A PMS strategy for MRTPs may be divided in three main areas: a passive monitoring of unexpected adverse reactions to evaluate safety; an active tracking of MRTP usage patterns, consumer behaviours and perceptions; and evaluation of population health effects under natural conditions of use to verify lower incidence of morbidity and mortality from tobacco-related diseases compared to conventional products.

Factors Affecting Patient Adherence to Eye Drop Medication in a Rural Setting

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Non-adherence to medicines is a well recognised multi-factorial problem. Living in a rural area poses additional barriers but it is not clear if this influences adherence levels. The aim of the study was to identify factors that influence adherence in a rural setting and to determine the level of adherence to eye medication.

To explore the barriers, ten stakeholder interviews, with healthcare professionals and patients, were undertaken. The themes identified were used to develop a questionnaire to quantify the extent patients experience these issues when attending an eye clinic in rural Mid-West Wales. Patients were invited to complete the questionnaire while waiting for their appointment. The questionnaire was divided into five sections, covering a range of issues, including: demographics, adherence (7 items, adapted from MARS-5, low score = high adherence), information (8), education (5) physical administration (6), access (4) and the doctor: patient relationship (1) using a five point Likert scale.

Of the 53 patients approached to take part in the study, 51 questionnaires were completed. Approximately 80% of the patients demonstrated a good level of adherence, not found to be affected by the distance from the clinic. Patients who had not been assessed for ability to administer their eye drops were statistically significantly more likely to be non-adherent ($rho=-0.324$, $n=51$, $p<0.02$). The study identified that only 29% (15/51) of patients responded they would mention their eye drops when asked about their medicines. The shape of the bottle (size and thickness) was an additional factor identified by patients.

Non-adherence to eye medication is multi-factorial with 50% of patients indicating problems with administration. Improvement of the assessment process of patients' ability to administer their drops is essential, with potential for the pharmacists' role, through medicines use review and education to be strengthened and extended.

History, Current Standard and Future of Clinical Research in Egypt

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Egypt with its central geographical location and huge treatment naïve population make it ideal for clinical research. Egypt has a huge history in medicine dating back to the pharaonic period. Historically CTs are mainly done in the triparate of USA Japan and EU i.e. ICH regions. However clinical research is spreading globally and new markets are emerging. The aim of my thesis is to gain an understanding on the history and standard of CTs in Egypt and to demonstrate Egypt as a country of investment for the pharmaceutical industry and future growth in this emerging market for clinical research

To conduct a survey and meta-analysis of RCTs for self analysis and interpretation to determine Egypt's status in Clinical Research, past present and future in the region.

Survey distributed to medical professionals in Egypt with the aim for 300 surveys to be returned. Returned surveys will be counted. The answers will be collated and coded for general observations and analysis. Data from RCTs in Egypt and the region will extracted from the ICTRP and imported to Excel spreadsheet for categorization, a tally and visual graphical representation of the data will be created in order for trends and patterns to be observed. No formal statistical analysis will be done on the data gathered. Ethics Committees were consulted, approval was not needed for this survey. Ministry of Health (MOHP) in Egypt requested submission for review by security services.

ICTRP: A total of 314 CTs were registered in Egypt until 06 June 2011. Phase 1 trials were seen despite being disallowed by the MOHP, 24.4% of trials were conducted in females only compared to 2.9% conducted in males In the EMR a total of 5670 RCTs. Israel and Iran rank number 01 and 02 respectively for total RCTs conducted. Egypt ranks number 03 for total trials conducted. Afghanistan and Sudan conducted the greatest proportion of pediatric CTs. (over 60%). Growth was seen in the region, 2004 – 2005 EMR growth of over 600%.

After review of the history, current standard I believe that Egypt is one of many growing countries in the industry of clinical research for the future. Although the revolution and political change may delay growth I expect it to continue.

Remission in Rheumatoid Arthritis: a perspective from clinicians in daily practice in the UK, USA and France

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Remission in Rheumatoid Arthritis: a perspective from clinicians in daily practice in the UK, USA and France. To investigate the use of the different composite indices such as the ACR (American College of Rheumatology), DAS (Disease Activity Score) and others in clinical practice amongst experienced rheumatologists (UK, USA, France) how these indices are used and perceived, how useful they are and how rheumatologists would like them to be refined

Before the advent of drugs capable of modifying the course of RA, remission was elusive. However recent years have witnessed a flurry of new way to assess remission. Starting from the historical ACR definition, indices were developed and extrapolated from ACR such as DAS and DAS28.

A survey was designed in which 45 rheumatologists from UK, USA and France indicated the index they use, the index they prefer, explain these choices, why they are satisfied or not with the available indices and give their opinion on the next step for these indices. Fifteen of the forty five rheumatologists participated in the survey. Few indices are used. DAS 28 was overwhelmingly the criteria the most used for a variety of reasons. Its place of true reflexion of remission was challenged by the ACR criteria. Only half of the rheumatologists are happy with available criteria. Future ways to assess remission might be necessary and should include other aspects of the disease such as the absence of synovitis.

Use and perception of remission criteria shows differences amongst the rheumatologists. Does this mean remission indices can help understanding remission but only some rheumatologists do appreciate their limitation? Could it be that the tools of the past are obsolete now? This could have an implication on additional intervention for achieve “true remission” which needs to be defined.

Investigation of possible acceleration processes from project nomination to first dose in human

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Recent benchmark data show that within MerckSerono's clinical project development the timelines at early stage from "First Pivotal Toxicology Dose" (MS0) to "First Dose in Human" (MS1) are longer compared to industry benchmark data (CMR benchmark). The data demonstrate that MerckSerono spends more time than other companies to reach MS1 once a preclinical developed compound went into clinical development. This master thesis aims to investigate the obstacles the company faces that may hinder to reach MS1 in a similar time as the benchmark shows. The value of an accelerated approach for MerckSerono within the organisation and the Global Product Teams (GPTs) will be evaluated; and conclusive assumptions on the major impacts on timelines are made.

Project work will be discussed in general. This includes the therapeutic areas MerckSerono has develop programs and compound classes (NCE, NBE). Approaches that have been initiated to shorten development timelines to MS1 at MerckSerono, like the use of fast track medications for NCE's or standards in clone selection for NBE's and exploratory clinical trials (ECT) will be introduced.

The method used for data acquisition was a questionnaire had been designed and distributed to representative employees of MerckSerono GPTs such as regulators, preclinical, non-clinical and clinical colleagues. The analysed feedback of this survey leads the discussions of this work. The introductory part and the discussion part are stressed by references from the literature.

The evaluation of the survey demonstrated where the company faces challenges with regard to reduce timelines from MS0 to MS1. Key factors that prolong the overall development timelines, such as resource constraints, have been identified. Secondly, the evaluation of the survey exposed several options for further steps that might lead to a significant time reduction.

MSc

Primary Care research in Poland: Challenges and Opportunities

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Physicians who practice in primary care area are caring out for people general health condition and welfare. As first line of contact for patients, they must be able to recognise a potential health issues. Their help is measured by how much early they can see if something wrong is going on with the patients. Clinicians who practice in Primary care area are unique in ability of finding and recognising people with potential health problems and according to their knowledge are dedicated to serve with appropriate intervention. Clinical research conducted in area of primary care may include:

- The aspects of natural history combined with epidemiology issues together with the most often seen clinical cases diagnosis;
- The management of the most often seen clinical cases;
- Clinical trials which may be conducted in primary care area with patients recommended from family doctors
- Studies of patients health results and symptoms

The aim of this thesis is to show and discuss opportunities and challenges in primary care research in Poland, focusing mostly on clinical research, conducted in Poland and the current situation of clinical trials conducted in the primary care area.

This study presents the methodological approach, and the methods employed in the collection and analysis of data:

- Introduction of ClinicalTrials.gov database and requirements for registration
- All “Industry Sponsored” trials in Poland conducted between 2005 and 2010 in primary care area were captured in the ClinicalTrials.gov database.
- Data set analysis

Presents the results and study analysis, including a description of the statistical from the set of data grabbed in the form of graphs and charts. Data from key themes are analysed. Concludes the study by summarising the significant points and proposes recommendations for the future.

Systematic Review and Meta-Analyses of Adverse Events of Special Interest in Ankylosing Spondylitis Patients Treated with Tumor Necrosis Factor (TNF) Antagonists

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TNF antagonists provide an important treatment option for chronic inflammatory disorders such as ankylosing spondylitis (AS), however adverse events of special interest (AESI) such as serious infections, neurologic disorders and malignancies have been demonstrated to be a risk in rheumatoid arthritis (RA) treated with TNF antagonists. Few studies have studied incidence or prevalence of AESI in AS patients treated with TNF antagonists. The objective of this study was to perform a systematic review and meta-analyses to assess the risk of AESI (serious and opportunistic infections, malignancies, neurologic disorders such as demyelinating disease and progressive multifocal leukoencephalopathy) in AS patients treated with TNF antagonists compared to control conditions (e.g. NSAIDs, nbDMARDs).

A study protocol and data eligibility forms were prospectively created and a systematic literature search using DataStar, PubMed, The Cochrane library and Web of Science was performed between November 2010 to January 2011. All randomized controlled trials (RCTs), open-label extensions (OLEs) and controlled observational studies monitoring AESI in AS patients diagnosed by the modified New York criteria and treated with TNF antagonists were assessed. A second person (VI) independently extracted and assessed the methodological quality of 20 % of the RCT data.

Twelve RCTs and ten OLEs were retrieved (2821 patients). The methodological quality of the RCTs were measured using the Downs and Black Checklist and Jadad Scale. Meta-analyses of risk difference (RD) were performed on the RCT data of SAEs [RD=0.01 95 % CI: -0.01,0.02] and serious infections [RD=0.01, 95 % CI: -0.00,0.02] but not on other AESI because the overall number of patients retrieved was too small to make robust conclusions.

There was no evidence that the risk of serious adverse events (SAEs) and serious infections in AS patients treated with TNF antagonists was different from placebo, however, the overall number of patients was low and the calculations may have lacked power, therefore continued monitoring is necessary.

Nanogels-based carriers for topical delivery

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Temperature-responsive *N*-isopropylacrylamide (NIPAM) was co-polymerised with butyl acrylate monomer to yield poly(NIPAM-*co*-BA), which was loaded with a model permeant, methotrexate. *In vitro* study of the loaded nanogel showed that it was capable of delivering methotrexate across the epidermis in levels that significantly reduce the biosynthesis of prostaglandin E_2 (PGE_2), a key inflammation mediator. Moreover, reduced lag time and enhanced delivery by the addition of sodium carbonate to the nanogel were observed. However, Western blotting for cyclooxygenase-2 (COX-2) in *ex vivo* skin, found the nanogel to be pro-inflammatory. Next, a temperature- and pH-responsive nanogel based on polyNIPAM copolymerised with acrylic acid, or poly(NIPAM-*co*-AAc)(5%), was synthesised. Studies demonstrated that poly(NIPAM-*co*-AAc)(5%) nanoparticles were capable of penetrating the porcine epidermis and migrating across the skin, as shown by the presence of the particulates in the diffusion cell receptor phase. Furthermore, the nanogels were shown to enhance the delivery of loaded drugs across the epidermis in comparison to saturated solutions of the corresponding drugs. Western blotting for COX-2 demonstrated that the nanogel did not induce significant inflammatory reactions post-topical application, suggesting its compatibility with skin. A preliminary investigation examined a single-compartment system comprised of poly(NIPAM-*co*-AAc)(5%) nanogel and pH modulator-containing liposome, designed to remain stable until its application onto the skin. However, the composite system proved unsuccessful, primarily due to liposome instability. Overall, this novel smart topical drug delivery system is within reach, provided the pH modulator-loaded liposome can be adequately stabilised.

siRNA Depletion of Endocytic Proteins and Pathways for Analysing the Cellular Uptake of Cell Penetrating Peptides as Vectors for Drug Delivery

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Cell-penetrating peptides (CPPs) have the potential to deliver a host of macromolecular therapeutics into cells including peptides, proteins, and nucleotides. The mechanism by which they are internalised has been hotly-disputed but is important if improvements are to be made in their delivering capacities. Endocytosis is thought to be of significant importance but identifying the exact uptake mechanism has been difficult due predominantly to a lack of specific tools. Multiple pathways have been reported to contribute to uptake, including macropinocytosis and those regulated by clathrin and cavaeolin-1.

The aim of this thesis was to utilise siRNA-depletion to develop cell models with defects in specific endocytic proteins and pathways that could then be utilised to study the uptake of drug delivery vectors such as CPPs. Targeted pathways were those regulated by clathrin heavy chain, dynamin-2, caveolin-1, flotillin-1 and P21-activated kinase (PAK-1). Significant variation between cell lines emerged in the expression of these proteins and the ease with which they could be depleted. Single siRNA sequences were, however, discovered that effectively depleted these proteins and using a variety of endocytic probes the effects of depletion could be determined. Eventually, model cell lines were generated that were measurably defective in at least one of the five different endocytic pathways and these were tested to determine routes utilised by two well characterised CPPs, HIV-Tat and octaarginine. Only cells depleted of pak-1 protein and thus macropinocytosis were defective in CPP uptake. Further analysis revealed defective actin organisation in these cells that could have caused the effects and support data presented here and elsewhere on actin disruption with cytochalasin D.

With comparative studies using pharmacological inhibitors of endocytic pathways these methods provide new tools to study drug delivery systems as shown here for CPPs and also for polyplexes through a collaboration with the University of Ghent, Belgium.

PhD

Measuring the influence of chronic diseases on health-related major life changing decisions and development of a patient-based novel instrument for its measurement

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The permanent nature of chronic disease may impair patients' psycho-social and physical well being, may change their attitude towards life goals and influence major life changing decisions (MLCDs) over time. Very little information is available in the literature about the long term impact of chronic diseases, particularly their influence on MLCDs. The aim of this study was to investigate the influence of chronic diseases on MLCDs and to develop a standardised tool for use across all chronic conditions to assess the impact of disease on MLCDs.

675 patients (100 from 6 specialties: cardiovascular, rheumatoid, diabetes, cystic fibrosis, chronic obstructive pulmonary disease, nephrology and 75 from dermatology) from the University Hospital of Wales, Cardiff and University Hospital Llandough, Llandough were invited to take part. The data was obtained through surveys, interviews and focus group discussions and this was reduced to core items through "content analysis". Themes and statements generated from evaluable responses were used for the development of the MLCD Profile. Standard techniques in terms of language, reading age and item length were applied for the developmental version. The panel of judges rated each item for its language clarity, completeness, relevance and scaling. Agreement among the panel members was examined using interclass correlation and kappa coefficient. The suggested changes by the panel were implemented to produce the revised version of the new tool MLCDP (version 1a). In a further study, 225 patients from the seven specialties were asked to complete the MLCDP (version 1a) and, factor analysis was applied to confirm the breadth and depth of the allocated domains and to determine construct validity of the MLCDP.

385 (57%) patients of 675 took part in the first phase of the study. Themes and statements generated from 316 (82%) evaluable responses (postal survey=258, individual interviews=50, focus group=8; female=140 (44.3%), male=176 (55.7%); mean age=51.7 yrs, range=17-92 yrs; mean disease duration=19.1 yrs, range=2-61 yrs; no influences on MLCDs: n=56, 18%, reported influences on MLCDs: n=260, 82%) were used for the development of the MLCD Profile. The most frequently reported MLCDs concerned early retirement, having children, job, career choice, relationships, housing, moving abroad and education. The correlation between the patients' age and the total number of reported MLCDs was significant (r_s = -0.46, p=<0.001, n=308) showing negative relationship. In total, 41 affected MLCD themes were identified and grouped into 15 core MLCD categories. The working definition of health-related "Major Life Changing Decision" was also developed. The 45-item draft profile was grouped into six MLCD domains. 19 clinicians took part in the "content validation" stage and there was good agreement among the panel members for their ratings of language clarity, relevance, completeness and scaling. (Interclass correlation coefficient=0.71, p=<0.0001, CI=0.61-0.78, kappa coefficient=0.81, p=<0.0001, CI=0.69-0.93). This led to a new 41-item version of the MLCDP (version 1a), covering five MLCD domains: education, job/career, family/relationships, social and physical. 210 patients (30 from each of the seven specialties) recruited in the second phase (female=108 (51.4%), male=102 (48.6%); mean age=50.8 yrs, range=16-89 yrs; mean disease duration=19 yrs, range=2-74 yrs) completed the MLCDP and data were analysed using factor analysis. The Cronbach's alpha value of 0.8 indicated good reliability. Several items were made redundant as a result of factor analysis; this analysis supported the evidence of construct validity. Item prevalence ranking helped to retain conceptually important items at this stage. This profile was easy to complete for most patients (n=131, 97%) and mean completion time was 5.7 minutes. A 32-item version of the tool MLCDP (version 2) was finally developed, which requires future examination of its other psychometric properties.

The findings of this study provide a new insight into the long term impact of disease on important life decisions and identified MLCD as a new domain in patient reported outcome assessment. The MLCDP is potentially of benefit in alerting clinicians to the long term impact of a chronic disease on patients, and as a tool to assess the true burden of chronic diseases on individuals' long term quality of life. Clinicians' knowledge about the influence of chronic diseases on MLCDs is important to provide better and timely guidance to patients, to support better treatment decisions and eventually to lead to better health outcomes. Strategies were developed to support and advise patients when taking MLCDs. Such support might result in more appropriate decision-taking and improved health outcomes.

Cardioprotection afforded by targeting guanylyl cyclase during early reperfusion

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Guanylyl cyclase - cyclic guanosine monophosphate (cGMP) signalling has been demonstrated to play an important role in the endogenous cardioprotective signalling of the myocardium during early reperfusion. It is proposed that infarct limitation is afforded by elevating cGMP and activating protein kinase G and its distal targets.

It was hypothesised that increasing the activity of soluble guanylyl cyclase (sGC) would limit myocardial ischaemia-reperfusion injury. Primarily using the rat isolated perfused heart method, the experiments reported in this thesis investigate the role of exogenous targeting of sGC during early reperfusion, specifically exploring targeting different redox states of the enzyme and their effects on myocardial infarct size. The novel sGC stimulator BAY 41-2272 and activator BAY 60-2770 were selected to investigate this hypothesis.

Both administration of BAY 41-2272 and BAY 60-2770 during early reperfusion significantly limited infarct size compared to controls. This was associated with elevated total tissue cGMP levels. Inhibition of nitric oxide could not completely abrogate this protection, but exogenous perfusion of nitric oxide along with BAY 41-2272 showed synergistic action. Oxidation of the prosthetic haem group by ODQ abrogated the protection afforded by BAY 41-2272 but potentiated the protection afforded by BAY 60-2770. Targeting both the reduced and oxidised forms of sGC together did not afford additive protection, in fact it reduced the protection afforded compared to the individual treatments. Preliminary data also suggest that targeting the particulate form of guanylyl cyclase increases activity of Akt signalling during early reperfusion suggesting common signalling between soluble and particulate guanylyl cyclase.

These data suggest that targeting sGC during early reperfusion can afford cardioprotection by limiting infarct size. The relationship between cGMP elevation and infarct size needs to be investigated further. Nevertheless, these studies suggest that sGC may be a tractable target for the therapeutic management of acute myocardial infarction.

Detection of Lipopolysaccharide Pyrogens by Molecularly Imprinted Polymers

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Sepsis is a complex and life-threatening condition that arises when the body's response to an infection injures its own tissues and organs.[1] It is estimated that 1.8 million people annually worldwide are affected by sepsis and with mortality rates of up to 50 % it is the leading cause of death in non-cardiac intensive care units. In the UK it is responsible for more deaths than breast and bowel cancer combined and the economic burden is significant.[2] With the emergence of multi-drug resistant pathogens, in an ageing population with an increased number of immuno-compromised patients, it is not surprising that infective diseases such as sepsis are more prevalent. Lipopolysaccharide (LPS) is a bacterial endotoxin located in the cell wall of Gram-negative organisms that is directly implicated in the development and rapid progression of sepsis, even when the causative pathogen is of Gram positive or fungal origin. Its presence in the blood is associated with elevated mortality rates[3,4], however it is not routinely tested for in the clinical setting. The over-arching aim of this project was therefore to develop a synthetic recognition system capable of efficiently detecting and binding LPS in a variety of biologically relevant environments. It was hypothesised that target selective peptides could be used as high affinity 'functional monomers' in a molecular imprinting approach to give rise to such a system.

Polymyxin B, a small, conformationally constrained cyclic peptide that possesses high affinity for lipopolysaccharide (LPS) was used to provide proof-of-principle. To reduce the concept to practice, a bi-functionalised Merrifield resin was prepared so as to allow the use of two independent surface attachment strategies. Alternative functionality was introduced by reacting the resin with sodium azide to generate an azidomethyl polystyrene intermediate, with subsequent reduction to aminomethyl resin using the Staudinger method. The native chloromethyl groups of the resin were used to introduce iniferter groups from which controlled polymer growth could be initiated, whilst the attachment of polymyxin was achieved through amine-amine imidoester linkages or via azide-alkyne "click" chemistry.

Solid supports with pre-defined densities of azide/amine and chlorine groups can be produced by varying the molar ratio of sodium azide to the loading of chlorine on the Merrifield resin. Polymyxin resins were able to efficiently bind LPS from aqueous solutions with an apparent K_d of ~ 0.2 μM. Growth of a polymer in the presence of polymyxin immobilised via amine-amine linkage demonstrated an imprinting effect, however those systems produced via the immobilisation of alkyne derivitised polymyxin B on the surface of azidomethyl polystyrene via "click" chemistry suggested an overall reduction in binding efficiency. Whether the observed reduction in binding is due to changes in the B_{max} or the K_d of the resin remains to be elucidated.

The results from these studies suggest that the hypothesised approach is feasible but that optimisation of a number of variables, including relative loading of peptide to polymerisation initiator species and experimental conditions used in the immobilisation chemistries, is needed before definitive results can be obtained.

1. The Global Sepsis Alliance. Stamping Out Sepsis A Global Goal [Internet]. 2011 Feb 15 [cited 2011 Apr 25]; Available from: http://www.infectioncontroltoday.com/articles/2011/02/stamping-out-sepsis-a-global-goal.aspx
2. Daniels R. NHS Evidence - Emergency and Urgent Care [Internet]. 2009 [cited 2010 Nov 1]; Available from: http://www.library.nhs.uk/emergency/Page.aspx?pagename=SEPSIS
3. Marshall JC, Foster D, Vincent J-L, Cook DJ, Cohen J, Dellinger RP, *et al*. Diagnostic and Prognostic Implications of Endotoxemia in Critical Illness: Results of the MEDIC Study. *Journal of Infectious Diseases*. 2004; **190(3)**: 527 -534.
4. Opal SM, Scannon PJ, Vincent J-L, White M, Carroll SF, Palardy JE, *et al*. Relationship between Plasma Levels of Lipopolysaccharide (LPS) and LPS-Binding Protein in Patients with Severe Sepsis and Septic Shock. *Journal of Infectious Diseases*. 1999; **180(5)**: 1584 -1589.

Examining the functional role of transporters in modulating drug absorption across lung epithelium

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The aims of this project were to establish the relative mRNA expression of several ABC, SLC and SLCO drug transporters within the rat lung through use of RT-PCR; expression suggesting the potential to serve as targets for pulmonary drug delivery. Further, validation of an Isolated Perfused Rat Lung preparation for use in assessment of drug transport across the lung was conducted. In order to assess the functional significance of the ABC drug transporter P-glycoprotein on drugs intra-tracheally instilled to the IPRL set-up, se of the P-gp substrates; Rhodamine 123, Digoxin, and Flunisolide and the P-gp inhibitor, GF120918 was employed. Further, use of kinetic modelling was employed to establish pharmacokinetic parameters involved.

Using the IPRL, the P-gp dependent pulmonary absorption of the P-gp substrate, Rh123, was demonstrated. Dose-dependent absorption, consistent with a saturable component in the molecule's pulmonary absorption, was demonstrated. Further, the absorption of low dose Rh123 was promoted by the presence of the highly selective P-gp inhibitor GF120918, consistent with a functional role of P-gp mediated efflux within an intact lung; an efflux process which may limit the pulmonary absorption of a lung administered molecule. Further studies using this system and extending the range of molecules studied will provide greater understanding of the quantitative significance of P-gp in limiting pulmonary absorption across lung epithelium.

PhD

Pain, Sensation and Biological Responses following Human Puncture with Microneedles

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Injections using hypodermic needles cause pain, discomfort, localised trauma and apprehension. As an alternative, microneedles facilitate drug delivery without significantly impacting on pain receptors or blood vessels that reside within the dermis. In this study we aimed to investigate, for the first time, whether two silicon microneedle arrays (36 equally spaced 180μm or 280μm length microneedles) elicit pain and sensory response when applied to human volunteers. In addition to *in vivo* clinical testing of silicon microneedles, alternative polymer microneedle designs were characterised and tested *ex-vivo*.

Prior to applying silicon microneedles clinically, ethically approved testing of applicator devices determined that inverted-syringe plungers caused minimum discomfort when applied to human volunteers. Microneedle arrays mounted onto inverted-syringe plungers reproducibly created microconduits through the stratum corneum of *ex-vivo* skin. Following ethical approval, 12 subjects received single-blinded insertions of a 25G hypodermic needle and both microneedle arrays. A visual analogue scale (VAS), perception questionnaire and audio-recording collected descriptions of the pain intensity and sensory perception following each application. The creation and temporal retention of skin microchannels was assessed over 24 hours by external dye staining and measurement of transepidermal water loss (TEWL). Characterisation of wound healing markers, including keratin K16, was carried out by immunohistochemistry.

Mean VAS scores, verbal descriptions and questionnaire responses showed that the 180μm and 280μm silicon microneedles caused significantly less pain and discomfort than the hypodermic needle. Dye staining and TEWL analysis confirmed that microchannels were formed in the skin following microneedle application with repair and resealing apparent at 8-24 hrs post-application. A spring-loaded applicator device was developed to reproducibly accelerate polymer microneedles into the skin along a trajectory perpendicular to the skin surface.

Microneedles provide a less discomforting method of skin penetration than hypodermic needles. Future work should optimise the design of microneedle devices for clinical delivery of active molecules.

Identification of Lyn kinase as a therapeutic target for tamoxifen resistant breast cancer

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Tamoxifen has made a significant contribution in decreasing breast cancer related deaths for over 30 years and until recently was the gold standard for treatment of ER positive breast cancer. Resistance to tamoxifen is however a considerable issue with cells utilising a number of molecular mechanisms to bypass the growth inhibition caused by blocking ER activity. This move towards an anti-hormone resistant state from an anti-hormone responsive state is associated with the transition to a much more aggressive phenotype including increased proliferation and also invasiveness. Thus unfortunately, acquisition of tamoxifen resistance is not only associated with a recurrence of breast cancer, but this cancer is also much more aggressive in nature with fewer treatment options available than the initial cancer.

This study has identified Lyn kinase as increased in tamoxifen resistant breast cancer cells compared to oestrogen-responsive breast cancer cells. Subsequent removal of Lyn kinase from tamoxifen resistant breast cancer cell lines using RNAi technology led to a significant decrease in cell proliferation, increased apoptosis and also a decrease in migration and invasion. A mechanism has been suggested whereby Lyn kinase is involved in a calcium dependent zinc wave which ultimately leads to the activation of tyrosine kinases.

Metastasis to other sites in the body is ultimately responsible for fatalities due to breast cancer and so being able to block its action is key to treating breast cancer in the clinic. Therefore identifying Lyn kinase as a gene target that leads to the advancement of breast cancer to a more aggressive state provides a powerful tool for treating breast cancer in the clinic.

PhD

Synthesis of Potential Cancer Therapeutic and Diagnostic Agents Based on Stilbenes

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The need to find novel anticancer agents with better potency, efficacy and safety are highly demanded. Therefore, the first part of the study was aimed to synthesis new compounds based on stilbenes, indole-based isoxazoles and structure based design compounds as potential antitumour agents, which later will be evaluated for their anticancer properties.

The syntheses of substituted stilbenes were achieved via catalyzed or uncatalyzed methods, yielding stilbene analogues in moderate to good yields. Preliminary antiproliferative study on four cancer cell lines (prostate, non-small lung, colon and breast) demonstrated their antiproliferative potential in the micromolar range. Unfortunately, the stilbenes were unable to inhibit the downstream level of the Wnt-signaling pathway on colon cancer stem cells.

Next, the synthesis of indole-based isoxazole analogues were gained via two different methods; affording the compounds in low to moderate yields. The compounds will later be tested for their anticancer property.

The synthesis of 3289-8625 analogues, compounds which showed potent inhibitory activities on the PDZ domain of Dishevelled (PDZ-Dvl) as an important component in the Wnt signaling pathway was also described. The synthesis was achieved via various methods which gave rise to the formation of two analogues, which showed better binding affinities towards the PDZ-Dvl compared to the parent compounds.

Finally, the therapeutic potential of the stilbenes was expended to the synthesis of stilbene-based analogues as novel positron emission tomography (PET) imaging probes especially for the detection of β-amyloid plaques in brain, which is the hallmark of Alzheimer's disease. The syntheses of stilbenes were sought using fluorine-19, which later will be expended to ^{18}F-PET radiochemistry. The syntheses of stilbenes attached to ^{19}F-linker were afforded in good yields. Stilbenes directly attached to potassium trifluoroborate was synthesised in moderate yield. Nevertheless, the attempt to synthesis stilbene derivatives attached to potassium trifluoroborate linker using novel procedure was failed.

Evaluation of the pharmaceutical regulatory review process in Iran and its impact on patient access to medicines

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The regulatory authorities and pharmaceutical companies have invested considerable time and resources into improving their performance by timely approval and efficient practices. The aim of this research was to evaluate Iran's review processes and milestones by monitoring its key performance indicators, measuring the satisfaction by pharmaceutical companies with the review system, comparing Iran's regulatory process and quality measures with those in a number of emerging markets and evaluating the relative efficiency of the regulatory departments of the Iranian pharmaceutical companies.

Differences in the regulatory practices of Iran's Drug Selecting Committee (IDSC) (responsible for reviewing safety, efficacy and cost effectiveness) and the Registration Department (responsible for reviewing quality of the medicines) were identified. The results showed that there are significant differences between the two departments, as well a degree of overlap.

The number of products and the regulatory approval times by the Iranian regulatory authority were measured and the results showed a significant upward trend ($p=0.001$) in the number of products approved during the period of study (2004-2009). In addition, there was a significant increase in the review times ($p \leq 0.05$) and this was due to a number of factors including changing management teams and the sequential review by the regulatory authority.

In order to measure the satisfaction of the Iranian companies with the country's regulatory review system, a questionnaire (Company Satisfaction Index) was developed and this showed that the main points of concern for the authority are to improve its weaknesses reflected in the limited number of reviewers and the current model of the review process; while for companies it is related to regulatory structure and resources.

PhD

Evaluation of a community pharmacy cardiovascular risk assessment service

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The aim of the study was to evaluate a community pharmacy-based cardiovascular risk assessment (VRA) service introduced into two pharmacies in south Wales.

A longitudinal methodology was adopted where participants had an initial assessment with a follow-up after 12 months. Body mass index, waist circumference, blood pressure and total/HDL cholesterol levels were measured and the Framingham 10-year cardiovascular risk was estimated and communicated to patients. Demographic details and lifestyle information (smoking, alcohol, diet and exercise) were obtained via self-complete questionnaires at each consultation.

A total of 172 individuals accessed the service and had either a brief assessment (n=26) without the calculation of the Framingham score or a full VRA (n=146). Mean age was 60 years (±10.3), 59% were female and 25% (37/146) were at high risk (>20%) of developing cardiovascular disease. High satisfaction with the VRA was obtained via an anonymous questionnaire provided immediately after the initial consultation (74% response rate). The short-term outcomes of the service (including recall of advice, lifestyle improvement and/or making the visit to their GP if they were referred) were reported through a semi-structured telephone interview two weeks after the initial assessment. In total 105/172 (61%) who attended the twelve-month follow-up had results of the two assessments compared using paired Student's t-test. There was a statistically significant increase in mean HDL 0.08 mmol/L (95% CI 0.02 to 0.14) and a statically significant reduction in mean systolic BP -8.5 mmHg (95% CI -11.0 to -5.9), diastolic BP -7.7 mmHg (95% CI -10.4 to -5.0) and Framingham score -1.07 (95% CI -1.9 to -0.2). A comparison between Framingham and QRISK2 algorithms showed the importance of using the most accurate tool available in estimating cardiovascular risk.

This is the first study to investigate short- and longer-term outcomes of a community pharmacy-based VRA service in Wales and provides a basis for future research.

The following abstracts have been withheld as they have been or will be published elsewhere, and/or due to intellectual property or confidentiality issues:

Dendrimer biopharmaceutics: toward active dendrimer-cannabinoid drugs

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Controlled delivery of bacterial viruses for the eradication of bacterial infection

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Towards a nanomedicine-based broad-spectrum topical virucidal therapeutic system

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Cannabinoids as potential new therapeutics of gastrointestinal motility and inflammatory disorders

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