Current progress toward a better understanding of drug disposition within the lungs: summary proceedings of the 1st Workshop on Drug Transporters in the Lungs

Carsten Ehrhardt[1,*], Per Bäckman[2], William Couet[3], Chris Edwards[4], Ben Forbes[5], Markus Fridén[6], Mark Gumbleton[7], Ken-ichi Hosoya[8], Yukio Kato[9], Takeo Nakanishi[10], Mikihisa Takano[11], Tetsuya Terasaki[12], Ryoko Yumoto[11]

[1]School of Pharmacy and Pharmaceutical Sciences, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland

[2]Mylan Global Respiratory Group, Sandwich, UK

[3]Inserm U1070, Université de Poitiers, UFR Médecine-Pharmacie, Poitiers, France CHU Poitiers, Poitiers, France

[4]Refractory Respiratory Inflammation DPU, GlaxoSmithKline Medicines Research Centre, Stevenage, Hertfordshire, United Kingdom

[5]Institute of Pharmaceutical Science, King's College London, London, United Kingdom

[6]Translational PKPD Group, Department of Pharmaceutical Biosciences, Uppsala University, Uppsala, Sweden; Respiratory, Inflammation and Autoimmunity Innovative Medicines, AstraZeneca R&D Gothenburg, Mölndal, Sweden.

[7]Experimental Therapeutics, School of Pharmacy and Pharmaceutical Sciences, Cardiff University Cardiff, Wales, United Kingdom

[8]Department of Pharmaceutics, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Toyama, Japan

[9]Department of Molecular Pharmacotherapeutics, Faculty of Pharmaceutical Sciences, Kanazawa University, Kanazawa, Japan

[10]Department of Membrane Transport and Biopharmaceutics, Faculty of Pharmaceutical Sciences, Kanazawa University, Kanazawa, Japan

[11]Graduate School of Biomedical & Health Sciences, Hiroshima University, Hiroshima, Japan

[12]Graduate School of Pharmaceutical Sciences, Tohoku University, Miyagi, Japan

Corresponding author

Dr. Carsten Ehrhardt, School of Pharmacy and Pharmaceutical Sciences, Trinity College Dublin, Panoz Institute, Dublin 2, Ireland, tel.: +353-1-896-2441, email: ehrhardc@tcd.ie

Formatted: French (France)

ABSTRACT

The School of Pharmacy and Pharmaceutical Sciences at Trinity College Dublin hosted the *1st Workshop on Drug Transporters in the Lungs* in September 2016 to discuss the impact of transporters on pulmonary drug disposition and their roles as drug targets in lung disease. The workshop brought together about 30 scientists from academia and pharmaceutical industry from Europe and Japan. The primary questions addressed were: What do we know today, and what do we need to know tomorrow about transporters in the lung? The three themes of the workshop were: (1) model systems for drug transporter studies in the lungs; (2) drug transporter effects on pulmonary pharmacokinetics (PK) – case studies; and (3) transporters as drug targets in lung disease.

Some of the conclusions of the workshop included the following: ~~suitable~~ experimental *in vitro*, *ex vixo*, *in vivo* and *in silico* models that allow studies of transporter effects are available; data from these models convincingly show a contribution of both uptake and efflux transporters on pulmonary drug disposition; the effects of uptake and efflux transporters on drug lung PK is now better conceptualised; transporters are associated with several of lung diseases. More studies are therefore required to better understand these phenomena, particularly to establish which of the available models best translate to the clinical situation.

KEYWORDS: Absorption; Computational ADME; Efflux pumps; In vitro models; Organic cation transporters; P-glycoprotein; Peptide Transporters; Pulmonary delivery/absorption; ABC Transporters; Organic anion-transporting polypeptide transporters

LIST OF ABBREVIATIONS

ASP^+, 4-(4-(dimethylamino) styryl)-N-methylpyridinium iodide; BAL, broncho-alveolar lavage; BCRP, breast cancer resistance protein; CDF, carboxy-dichlorofluorescein; COX, cyclooxygenase; ECF, extra-cellular fluid; ELF, epithelial lining fluid; HPAEpiC, human primary alveolar epithelial cells; HTEpiC, human tracheal epithelial cells; IPRL, isolated and perfused rat lung; LC-MS/MS, liquid chromatography–linked tandem mass spectrometry; MDR, multidrug resistance; MRP, multidrug resistance-associated protein; OAT, organic anion transporter; OATP, organic anion transporting polypeptide; OCT, organic cation transporter; NHBE, normal human bronchial epithelial cells; PEPT; peptide transporter; PG, prostaglandin; P-gp, P-glycoprotein; PBPK, physiologically based pharmacokinetics; PK, pharmacokinetics; PKC, protein kinase C; SLC; solute carrier; SV, simian virus; $V_{u,lung}$, volume of distribution in the lung

INTRODUCTION

In pre-clinical drug development, drug transporter interactions are routinely studied in epithelia of the intestine, liver and kidney as well as the endothelium of the blood–brain barrier. Transporter effects in pulmonary drug disposition have been hypothesised for several years (reviewed, e.g. in 14, 15, 31 and 32). The vast majority of data that are available were generated using organotypic *in vitro* or *ex vivo* models and are consistent with the idea that drug absorption from the lungs is not exclusively mediated by passive diffusion. These initial studies confirmed the expression of drug transporters in lung tissues *in situ* and/or demonstrated transporter-related effects in cultured cells, but the translation of these data into clinical practice is still missing.

It was the aim of this workshop to bring together a group of international experts who shall summarise and discuss the available information on lung drug transporters and to find a consensus on what steps to take next, in order to advance the field. The workshop had three main themes, i.e. (1) techniques to study drug transporter expression and actions in the lungs~~model systems for drug transporter studies in the lungs~~; (2) drug transporter effects on pulmonary pharmacokinetics (PK) – case studies; and (3) Transporters as drug targets in lung disease.

~~In~~ This report briefly summarises the lectures given at the workshop. In the ~~the~~ first part, existing methods and techniques to study the expression and action of pulmonary transporters will be discussed, ranging from targeted proteomics to cell-based *in vitro* systems to complex *ex vivo* models. In the second part, case studies will highlight the impact of specific ~~drug~~ transporters on the distribution of pulmonary administered drugs~~. And~~ and

in the ~~finally~~final section, the evidence on drug transporter contribution to lung disease and/or progression will be summarised ~~in the third section~~.

Characterisation of lung transporters in lung tissues and primary cultured and immortalised lung cells based on quantitative targeted absolute proteomics

~~Quantitative targeted absolute proteomics (QTAP) for transporter protein analysis of lung tissues and human *in vitro* models~~

The lung is a very important organ for local drug targeting and systemic delivery. QTAP studies have revealed transporter protein expression in human lung tissues,[4] primary cultured cells[4,7] and immortalised cell lines.[8] Nineteen transporters (i.e. MDR1, MRP1, MRP3, MRP4, MRP5, MRP6, MRP8, BCRP, OCT1, OCT2, OCTN1, OAT2, OAT3, OAT4, PEPT2, OATP1A2, OATP1B3, OATP2B1 and PGT/OATP2A1) in human lung tissue has been confirmed by QTAP LC-MS/MS analysis.[4] The most abundantly expressed protein was OCTN1.[4] High expression of MRP1, BCRP and PEPT2 protein was also revealed.[4] Interestingly, MRP8 protein expression in female lung was 8-fold higher than that in male specimens, demonstrating a significant gender difference of transporter protein expression.[4]

When commercially available human pulmonary epithelial cells (i.e. tracheal (HTEpiCs), bronchial (NHBEs) and alveolar (HPAEpiCs)) in primary culture were studie~~s~~d, 9 transporters (i.e. MRP1, MRP4, MRP5, MRP6, OCT1, OCT2, OCTN1, OATP1B3 and OATP2B1), 14 transporters (i.e. MRP1, MRP2, MRP3, MRP4, MRP5, MRP6, BCRP, OCT1, OCT2, OCTN1, OAT3, PEPT2, OATP1B3 and OATP2B1) and 8 transporters (i.e. MDR1, MRP1, MRP4, MRP5, MRP6, OCT2, OCTN1 and OATP2B1) were found in detectable quantities in HTEpiCs, NHBEs

and HPAEpiCs, respectively.[4] OATP2B1 was detected in all cell types and therefore, might have important physiological roles and could be a useful target transporter for pulmonary drug delivery. MRP1 expressed abundantly in bronchi and alveoli, exhibited an 18-fold maximal inter-individual difference in the bronchial region among 5 donors.[4] Inter-individual differences in apparent efflux activities evaluated by the steady state cell-to-medium ratio of carboxy-dichlorofluorescein (CDF), a model substrate of MRPs in HTEpiCs, NHBEs and HPAEpiCs correlated well with MRP1 protein expression levels in the respective cells examined.[7] OCTN1 expression in primary cultured cells of all three different regions was similar to that of lung tissue. The maximum uptake rate of the organic cation, 4-(4-(dimethylamino) styryl)-N-methylpyridinium iodide (ASP^+) into HTEpiCs, NHBEs and HPAEpiCs correlated well with OCTN1 transporter protein levels in the plasma membrane fraction of cells from 5 different donors.[7]

QTAP studies were also been performed for 5 commonly used respiratory epithelial cell lines.[8] Interestingly, OCTN1 protein expression in NCI-H441 was shown to be most closely resembling that in primary cells. Similarities and differences of transporter protein expression were shown between the immortalised cell lines and the primary cultured cells, showing limitations of the use of immortalised cell lines.[8] In conclusion, quantitative targeted absolute proteomics is a useful tool to characterise drug transporter protein expression in lung tissues and to ~~evaluate~~ compare primary cultured cells and immortalised cell lines in the context of inhalation biopharmaceutics.

Cell culture models of the air-blood barrier

Cell culture models are a relatively quick and simple means to study mechanisms of drug disposition at the molecular level. Free of the limitations of complex organ systems or indeed living subjects, uptake, transport, metabolism~~, irritation~~ and toxicity can hence be studied *in vitro*.[9,10] On the other hand, these models have obvious shortcomings regarding, for example their lack of clearance mechanisms or reduction to mostly a singular cell type, which needs to be considered when interpreting data generated in *in vitro* assays.

A number of questions need to be addressed when deciding which *in vitro* model to use:

- ~~s~~Should the model be of human or animal origin? Animal-derived (primary) cells are easier to obtain, but due to species differences they should mainly be used to support *in vivo* or *ex vivo* data generated in the same species.
- Should freshly isolated cells in primary culture be used or a continuously growing cell line? Primary cells have arguably the closest resemblance to the *in vivo* situation, but they are associated with considerably higher cost and effort and eventually will dedifferentiate. Continuously growing cell line, either of cancerous origin or immortalised are generally easier to obtain and can be kept in culture for several passage numbers, but they might differ significantly from their original cell types.
- Also important is the choice of culture conditions. Can the cells be grown at an air-liquid interface or do they require submersed culture conditions?[11,12] Which medium and medium supplements should be used and what type of extracellular matrix? For how long can the cells be kept in culture? What is the impact of oxygen pressure? In any case, cell lines that have not been used in a biopharmaceutical context require a thorough characterisation.

The ideal *in vitro* model would have a cellular phenotype similar to the cell *in situ*, which in the case of respiratory epithelium, implies the ability to grow to confluent, polarised cell layer(s), a mixed population of cell types, functional cilia (trachea/bronchi/bronchioles), mucus secretion (trachea/bronchi/bronchioles) and/or surfactant production (bronchioles/alveoli) and the expression of drug transporters and metabolic enzymes at the same level and activity as the corresponding barrier. Many of the widely used cancer-derived cell lines, i.e. Calu-3, A549, NCI-H441 and NCI-H292 have significant shortcomings in many of the above mentioned aspects. Similarly, the first generation of simian virus (SV)40 large T antigen-immortalised cell lines, e.g. 16HBE14o- and BEAS-2B often present phenotypes different from the original cell type. More recently generated immortalised cell lines such as NuLi-1, UNCN1T - 3T, VA10, BCi-NS1.1 and hAELVi appear to better resemble the native cells, but most of them have not yet been sufficiently characterised in term of biopharmaceutical applications.[13, 106-109]

In vitro models of lung epithelium have been extensively used for uptake and transport studies. P-glycoprotein and organic cation transporters are probably the most studied transporters, but also MRPs, peptide transporters and several others have been investigated.[14,15] Absorption across cell monolayers has been compared to drugs' physicochemical parameters (e.g. log *P*, polar surface area), to other cell model of lung origin or from other organs (e.g. Caco-2), and with absorption kinetics in isolated lungs or experimental rodent models.[16-18] The results obtained, however, were frequently contradictory, which points to a certain heterogeneity in culture conditions, study protocols and marker selection.

Some of the open questions remaining are: do we need organotypic cell culture models? The changing cellular phenotype along the respiratory tree makes it plausible to have different models available representing the large airways, small airways and alveoli, respectively, but are different models for different lung sections really required? Do complex co-culture models offer benefits over mono-cultures? Is the predictive power of the currently available models sufficient to allow IVIVC? Do we need a "Caco-2-like" gold standard for the lungs? Do transport study conditions matter?

Precision-cut lung slices for profiling of inhaled compounds

Development of locally acting inhaled drugs for the treatment of respiratory disease relies on the optimisation of compound and/or formulation properties to achieve retention in the lung that provides a sufficient level and duration of local exposure.[19] Whilst most small drug molecules are rapidly absorbed across the pulmonary epithelium, molecular properties such as lipophilicity and basicity which are normally associated with increase tissue binding,[20] have been found to be also associated with enhanced lung retention.[19] This observation triggered the investigation of how the extent of lung tissue binding, determined in precision-cut lung slices, relates to lung retention and the effect duration of inhaled bronchodilators. Using a framework originally developed for studying brain tissue[21] lung tissue binding described as the unbound drug volume of distribution in the lung ($V_{u,lung}$) was found to be lower for salbutamol (2.2 ml/g) than for longer acting β_2-adrenergic bronchodilators including the AZD3199 (2970 ml/g), suggesting that binding is a drug property that may be relevant to profile and modulate in the optimisation of inhaled drugs. Further insight to the mechanisms of lung tissue binding was provided by modulation of lysosomal pH using

monensin: a substantial proportion of basic bronchodilators was found to reside in these compartments and to slow down the rate of drug release from tissue as studied in the slices.[22]

Moreover it was shown that ipratropium, a quaternary amine and an anti-muscarinic bronchodilator, had an accumulation in lung slices that was 8-fold higher than what would be expected from lung tissue binding determined in homogenate of lung tissue. This points to the existence of a carrier mechanism in its cellular accumulation. Interestingly, ipratropium is a substrate for OCT and OCTN transporters[23] and the intracellular accumulation of the prototypic OCT substrate MPP^+ was 100-fold higher then binding in lung homogenate.[24] Whilst both beta-agonist[25] and beta-antagonists[26] are also substrates for OCT transporter the contribution to cellular accumulation is likely lower.

Besides providing an integrated experimental system to predict lung retention for certain classes of drugs, the specific information of lung tissue binding obtained from slices is can be integrated with other measured drug or formulation properties through physiologically based pharmacokinetic (PBPK) modelling by which the complex interplay between drug properties and lung physiology can be explored and understood. PBPK modelling also bears promise for scaling inhalation pharmacokinetics from animals to man.[27,28]

Isolated and perfused lung models

Absorptive clearance of drug from the lungs is important for the efficacy and safety of inhaled medicines.[29,30] Transporters have the potential to significantly influence drug absorption, but to date most of the experimental evidence for the presence and impact of

drug transporters in the lungs comes from immunohistochemical identification of transporters in lung tissue and *in vitro* studies using respiratory epithelial cell cultures, respectively[14,15,31,32] Such techniques cannot show the influence of transporters on pulmonary absorption – for this, air to blood solute transfer in intact lungs must be measured.

Isolated perfused lungs offer unique opportunities to investigate the effect of drug transporters on lung permeability.[33] In this technique, the lungs are isolated from the systemic circulation, perfused via the pulmonary circulation and ventilated via the trachea. Using isolated perfused lungs, it is possible to deliver precise concentrations of transporter substrates/inhibitors, measure bi-directional drug transfer (from lung to perfusate and perfusate to lung), pre-administer transporter inhibitors and use inhibitors in concentrations and combinations that are not possible *in vivo*. Whilst rat lungs are the most commonly used, it is possible to use the lungs of genetic knockout mice[34] and lobes of human lungs.[35]

Intrinsic and formulation-driven pharmacokinetics have been investigated following pulmonary administration of drugs to isolated perfused lungs. The rate and extent of drug transfer from airway to perfusate has been used to establish relationships with molecular properties,[16,36] permeability in epithelial cell models[16,18] and drug absorption *in vivo*.[16] The effectiveness of a variety of absorption-modifying drug delivery strategies on absorptive clearance from the lungs has been investigated, including polymer microparticles,[37] liposomes,[38] sequence-specific phage display-derived peptide conjugated dendrimers,[39] and drug-ester polymer conjugates.[40]

Isolated perfused lungs have been used less extensively to study the impact of drug transporters on absorptive clearance. Airway to perfusate transfer of losartan, a P-

glycoprotein substrate, was not retarded in absorptive permeability in isolated rat lungs.[16] This finding was consistent with rapid the pulmonary absorption of losartan *in vivo*;[41] similarly, no effect on the absorption of another P-gp substrate, digoxin, has been reported in MDR1a-deficienct mice.[42] However, subsequent detailed investigations have demonstrated convincingly using isolated perfused lungs with inhibitors and genetic knockout that the impact of P-gp on absorptive pulmonary permeability is substrate specific (*see below*).[41] The OCT/OCTN transporter substrates ipratropium and L-carnitine have been shown to transfer into the pulmonary circulation of isolated rat lungs by passive processes rather than active uptake.[43] However, the effect of methacholine on transport of β-agonists through competition for organic cation/carnitine transporters has been demonstrated in human lungs and linked to lung mechanics.[35] In other studies, active transport in isolated rat lungs has also been demonstrated for IgG transported by neonatal constant region fragment receptor[44] and the absorptive permeability transport of polyhydroxyethylaspartamide.[45] To fully exploit the potential of isolated perfused lungs to study the influence of drug transporters, it would be useful to establish best practice in performing such studies, e.g. demonstration of saturation / concentration-dependency, use of inhibitors, controls and genetic knockouts, confirmation of findings using complimentary techniques and the demonstration that drug kinetics link to effect on lung mechanics.

A novel Quantitative Structure-Activity Relationship (QSAR) model to accurately predict pulmonary absorption

A literature~~s~~ analysis of the physicochemical properties of respiratory drugs concluded that inhaled respiratory drugs have a higher hydrogen bonding capacity, polar surface area and

molecular weight and lower lipophilicity compared to oral respiratory drugs.[46] The authors also concluded that inhaled drugs with different pharmacological action occupy distinct property space which may limit the application of this information to the design of compounds for novel targets of most diverse respiratory disease.

To facilitate inhaled drug design for more novel respiratory targets a ~~A~~ novel *in silico* model was constructed using the largest, diverse and relevant pulmonary absorption data set available to date, combining both marketed inhaled drugs and novel inhaled compounds.[36] A pulmonary absorption dataset generated using the isolated and perfused rat lung (IPRL) *ex vivo* model, for 82 drug discovery compounds and 17 marketed drugs, was used to build a novel Quantitative Structure-Activity Relationship (QSAR) model based on calculated physicochemical properties. The model predicted the percentage of the solubilised fraction of the dose, crossing the lungs into the perfusate over a 20 minute timeframe, following intra-tracheal instillation as an aqueous solution/suspension. A further 9 compounds were used to test the model's predictive capability, with the QSAR model performing well on this "test set" with a predicted versus observed correlation of $R^2 = 0.85$, and >65% of compounds correctly categorised. Calculated descriptors associated with permeability and hydrophobicity positively correlated with pulmonary absorption, whereas those associated with charge, ionisation and size negatively correlated. These findings were in keeping with literature describing physicochemical drivers of pulmonary absorption for a variety of compounds.[41,47] The novel QSAR model described can replace routine generation of IPRL model data for ranking and classifying compounds prior to synthesis therefore facilitating compound design through improved prediction of pulmonary absorption.[36] It will also provide scientists working in the field of inhaled drug discovery with a deeper understanding

of the physicochemical drivers of pulmonary absorption based on a relevant respiratory compound dataset.

Computer based mechanistic models as a means to understand the impact epithelial permeability/transport on local and systemic drug exposure after inhalation

Effective drug design requires a means to predict the impact of changes to product, material and molecular properties on the clinical performance of the medicine. Lately, computer based mechanistic models have shown promise. For instance, it was demonstrated that the clinically observed variation in rate and extent of absorption into the systemic circulation of a poorly soluble inhaled drug could be rather accurately predicted based on deposition pattern, dissolution rate and molecular physiochemical properties for a range of formulations and devices.[48] Using the same mechanistic model (Gastroplus™ 9.0, Simulations Plus Inc., Lancaster, CA), extent and rate of systemic absorption was simulated as a function of: Deposition region; Solubility and Permeability (Figure 1).

Results show that the extent and rate of absorption follow a pattern with respect to the impact of solubility and permeability which is akin to that of an oral drug. A rough comparison with the oral biopharmaceutical classification system[49] revealed that:

- BCS1 like drugs (high permeability and high solubility) are generally rapidly and completely absorbed from peripheral lung regions. It is likely that these drugs would show limited lung targeting
- BCS4 like drugs (low permeability and low solubility) are generally expected to be very poorly absorbed from conducting airways regions as result of mucociliary clearance. It

is likely that these drugs may demonstrate a very poor effect in conducting airway regions.

- BCS2/3 like drugs show some promise in terms of having some conducting airway bioavailability and a delayed absorption rate. This is likely the classes of compounds where we would expect to find most successful inhaled drugs. Nevertheless, the predicted low uptake (F) in large (0.1 – 0.4%) and small airways (3.4 – 11.6%) would put emphasis on highly potent drugs, especially given the low doses of standard inhalation formulations (cf. Figure 1)~~Nevertheless, the low bioavailability would put emphasis on highly potent drugs, especially given the low doses of standard inhalation formulations~~.

Overall, simulation results indicated that the clinical property of an inhaled medicine would (as could be expected) be very dependent on deposition pattern, but also on the interplay between solubility, permeability (and obviously target affinity).

The heterogeneity of the lung (ranging from large, low permeable, mucociliary cleared conducting airways to the large-surface, highly permeable, highly perfused, alveolar gas exchange region),[50] could be expected to generate regional differences in active drug concentration time profiles.[48] For instance, selective local targeting of fluticasone propionate to conducting airway tissue was suggested to explain its observed local clinical efficacy in absence of total lung targeting (as measured by receptor occupancy).[27]

Although computer-based mechanistic modelling thus show significant promise in bringing about a better understanding of local drug tissue levels in relation to drug and product properties, access to good quality input parameters is vital. Today, airway permeability and the impact (if any) of active transport in airways is among the least understood of these

parameters. Especially, in conducting airways, a region of significant clinical importance, airway permeability could ~~to~~ govern both extent and rate of absorption and should therefore be further investigated.

Drug transporter effects on pulmonary PK – case studies

P-glycoprotein transport in the lungs

P-glycoprotein (P-gp), encoded by the *MDR1* gene in humans and the *mdr1a* and *mdr1b* genes in rodents, is amongst the most widely studied pharmaceutically relevant transport. P-gp substrates cover a broad range of drug classes and physicochemical properties although they tend to be lipophilic or amphipathic.[51]

Evidence for P-gp expression within whole lung includes both mRNA and protein data in both humans and rodents[52-58] (*reviewed in*[14,15,31,59]), although indications are that the lung displays lower levels of P-gp than in other tissues normally associated with pharmaceutical barrier functions. However, it is the spatial microanatomic localisation of P-gp to lung epithelium that needs to be considered: P-gp expression is recognised at the luminal surface of bronchial/bronchiolar epithelium[54-59] and within alveolar epithelium.[54,55] Nevertheless, the impact of P-gp upon pulmonary PK of inhaled drugs is poorly understood, and requires experiments performed in the intact lung where anatomically accurate tissue architecture is maintained and appropriate parallel processes of clearance from the airways are preserved, including studies performed in isolated perfused organ models.

Using an IPRL model Kuhlmann *et al.* in 2003 reported P-gp to limit the transport of a P-gp substrate, idarubicin, from the pulmonary circulation into lung tissue,[60] indicative of P-gp

expression within pulmonary capillary endothelial cells. In 2004, Roerig *et al.* using a rabbit model made a similar report for rhodamine 6G. However, these findings have not been corroborated.[61]

Functional studies that directly or indirectly address airway to blood absorption and the impact of P-gp include: Tronde *et al.* where as part of a broader study of pulmonary absorption and drug structural properties the extent of absorption for the P-gp substrate losartan was reported to be >90% from the airways.[41] In 2008, Manford *et al.* reported the pulmonary absorption of the P-gp substrate digoxin to remain unchanged in CF-1 mice, which display spontaneous *mdr1a* knockout, although retaining *mdr1b* expression.[42] In an IPRL model the same group reported that co-administration into the airways of a P-gp inhibitor had no effect upon the pulmonary absorption profile of digoxin.[62] Contrary to this, a similar study in the IPRL showed a significant increase in the absorption of the P-gp substrate rhodamine 123 when co-dosed with the P-gp inhibitor, GF120918.[63]

Subsequently, a study was undertaken in both IPRL and isolated perfused mouse (IPML) lung models that began to explore the above divergent findings.[34] Using a comparatively small panel of five P-gp substrates the impact of P-gp upon airway instilled P-gp substrates (including the archetype digoxin and rhodamine 123 molecules) resulted in disparate outcomes with some substrates affected by P-gp and others not. From here the same group used genetic knockout P-gp mice in an IPML model and examining a much broader panel (i.e. 18 molecules) of P-gp substrates instilled into the airways (Price *et al.* unpublished observation). The outcome for this larger panel again showed divergent outcomes between the substrates separating into two distinct sub-populations; those whose pulmonary absorption was affected by P-gp and those unaffected. These polarised groupings could be

entirely distinguished by their differing computed physicochemical properties and not by more global biological properties such as P-gp binding affinity (K_m) or turnover (V_{max}). In general the more polar the P-gp substrate the less its pulmonary absorption was impacted by P-gp. To put this in context, the same panel was tested in intestinal absorption studies in the P-gp knockout and control mice. Here no distinct sub-populations could be identified. The only study on P-gp effect on pulmonary absorption in man found that oral verapamil increased the area under the curve (AUC) of inhaled umeclidinium bromide and vilanterol by approximately 40%.[64] In that study, however, the concentrations of inhalants were a multiple of the approved clinical dose and the timing between administration of the oral inhibitor and the begin of the inhalation manoeuvre was suboptimal.

In summary, work is beginning to identify specific physicochemical properties for P-gp substrates that predict if their absorption from the lung may be affected by P-gp; these same physicochemical signatures do not predict the effect of P-gp upon intestinal absorption.

Interaction of organic cation transporters with bronchodilators *in vitro*, *ex vivo* and *in vivo*

Organic cation transporters (i.e. OCT1-3 and OCTN1, 2) belong to the *SLC22* gene-family.[65] The majority of inhaled drugs, e.g. bronchodilative β_2-adrenergic agonists and anti-muscarinics are either permanent cations or bases, whilst several inhaled corticosteroids have been reported to be OCT inhibitors (Table 1). Therefore, this family of transporters has received considerable attention in the past 10 to 15 years.[66] The vast majority of data have been generated in cell-based *in vitro* models, often reporting conflicting findings.[15] This might be explained by different culture conditions of primary cells and cell lines which can

result in changes in transporter expression and activity. Organic cation accumulation and absorption in lung tissues, however, was also studied in lung slices and perfused organ models, respectively.[24,35] An involvement of OCT/N transporters in the pulmonary absorption of salbutamol was proposed already in 2005,[67] and although many publications were published demonstrating OCT/N expression and activity in lung tissues and cell cultures,[68-70] the molecular identity of the transporters responsible was not clarified until recently, when functional studies using OCT over-expressing expression systems showed that OCT1 and OCT3 exhibit high affinities for β_2-adrenergic agonists.[25] Direct evidence, using either radiolabelled probes, RNAi or overexpression studies demonstrating evidence on the contribution of OCTN1, 2 in β_2-adrenergic agonists transport is still outstanding. OCTN contribution to the uptake of ipratropium, on the other hand, has been shown *in vitro* and *in vivo*,[23,71] but the role of the transporters in pulmonary transport and lung PK of anti-cholinergics is still controversially discussed.[43]

Functional expression of PEPT2 and its regulation in alveolar epithelial cells

The di- and tri-peptide transporters, PEPT1 (SLC15A1) and PEPT2 (SLC15A2) transport various peptidomimetic drugs such as β-lactam antibiotics and antivirals, and have an important role, for example in the absorption of these drugs from the intestine. PEPTs are secondary active transporters, and transport their substrates coupled with an electrochemical proton gradient.[72]

PEPT2 is functionally expressed in alveolar epithelial type 2 cells, but not in type 1 cells.[73] However, the widely used cell lines with alveolar epithelial type 2-like phenotype such as A549 (a cell line derived from human lung adenocarcinoma) and RLE-6TN (a cell line derived

from rat normal lung) do not have substantial PEPT2 activity. The human distal lung epithelial cell line, NCI-H441 has recently been reported to have PEPT2 activity.[74] In NCI-H441 cells, PEPT2 protein is expressed on the apical membrane and facilitates the uptake of PEPT2 substrates into the cells. At this moment, the pharmacological role of alveolar PEPT2 in the pulmonary absorption of peptidomimetic drugs after inhalation is not entirely clear. Physiologically, PEPT2 might be involved in the innate immune response via a nucleotide-binding oligomerisation domain 1 (NOD1)-dependent mmechanism,[75] as it has been shown that the bacterial dipeptide, γ-D-glutamyl-meso-diaminopimelic acid (γ-iE-DAP), an initiator of this response, can be taken up by PEPT2. It is very interesting and important to further clarify the pharmacological and physiological roles of PEPT2 in the human lung.

Implications of the carrier-mediated transport of nicotine in lung and other tissues

Although nicotine is rapidly absorbed from the lung and distributed to the brain after tobacco smoking,[76] our knowledge of the transport mechanism by which nicotine crosses the alveolar epithelial barrier and blood-brain barrier is incomplete. Nicotine uptake by A549 human carcinoma-derived cells with an alveolar epithelial type 2 cell-like phenotype was found to be time-, temperature-, and concentration-dependent with a Michalis-Menten constant of 50 μM, suggesting that a carrier-mediated process is involved in nicotine transport in alveolar epithelial cells. Nicotine absorption was reduced by hydrophobic cationic drugs such as verapamil and pyrilamine, whereas typical substrates and inhibitors of organic cation transporters did not show inhibitory effect.[77] The transport mechanism of nicotine in alveolar epithelial cells shows great similarity to that in a brain capillary endothelial cell line *in vitro*.[78] This evidence suggests that the newly identified organic cation

transport system is involved in nicotine transport process in lung and the blood-brain barrier. ~~T~~Once taken up by alveolar epithelial and brain endothelial cells, nicotine could further be efflux to the blood from the lung and to the brain from the blood, respectively. Currently, however, little is known regarding the mechanisms underlying the efflux of nicotine at the basolateral membrane of alveolar epithelial and brain endothelial cells. Nevertheless, this transport might offer the opportunity of delivering cationic drugs to the brain after inhalation.

Pulmonary transporters in the drug disposition of antibiotics

Pulmonary PK is relatively difficult to investigate. Drug assays in whole tissue homogenates do not differentiate between intra- and extracellular concentrations and are therefore of little value. Unbound concentrations in tissue are more difficult to assess but should be much more informative, since with only few exceptions, in the absence of active transport, unbound concentrations in plasma and tissues should be equal at steady-state. Following the same reasoning, unbound AUC in plasma and tissue extra-cellular fluid (ECF) should be identical, if tissue distribution is only governed by passive diffusion, whereas unbound AUC should be lower in tissue ECF than in plasma in the presence of active efflux transport. This implies that unbound drug concentrations should be measured in lung ECF and then compared with unbound plasma concentrations to properly assess the effect of active efflux transporters on pulmonary PK. Drug concentrations can be measured within lung epithelial lining fluid (ELF) after broncho-alveolar lavage (BAL), in laboratory animals or humans, and correction for dilution can obtained by measuring urea in plasma and BAL. As in healthy

subjects lung ELF contains virtually no proteins, one can assume that estimated ELF concentrations correspond to unbound drug concentrations.

A series of experiments has recently been initiated using well controlled experimental conditions, in order to develop a biopharmaceutical classification of nebulised antimicrobial agents, based on their water solubility and membrane permeability.[79-83] Healthy rats were used for these experiments. Antibiotics were administered intravenously or nebulised using a Penn-Century MicroSprayer®. This system allows most of the dose to reach bronchial alveoli where the drug can be systemically absorbed. This ensures proper experimental control but does not correspond to the clinical situation where, depending on the inhaler, only a small fraction of the dose (in the order of 10 to 20%) is eventually absorbed. Simultaneous BAL and plasma samplings were conducted at various times post-dosing for assessment of drug concentrations. The route of administration had a major effect on lung PK for antibiotics presenting low membrane permeability (and therefore low and even virtually negligible oral bioavailability allowing only parenteral administration in clinical practice), such as colistin,[80] tobramycin[81] or aztreonam,[82] with much higher ELF concentrations after nebulisation when compared to intravenous administration at the same dose, and much higher ELF concentrations than unbound plasma concentrations after nebulisation. By contrast, the route of administration had no detectable effect on ELF and unbound plasma concentrations for antibiotics with relatively high membrane permeability (and therefore high oral bioavailability allowing oral administration in patients), such as ciprofloxacin or moxifloxacin.[79] These results were obtained in experimental conditions precluding direct extrapolation to the clinical situation, but provide important information before selecting the route of administration and drug formulation in case of nebulisation. Yet the spectacular differences observed between compounds were related to differences

in membrane permeability, essentially controlled by passive diffusion and irrespective of active transport phenomenon.

However, the higher ELF than unbound plasma concentrations observed with moxifloxacin suggested or at least were consistent with an active efflux transport (Figure 2). Moxifloxacin is highly permeable and therefore, its distribution equilibrium occurs rapidly after both intravenous administration and nebulisation. Nonetheless, moxifloxacin is also a P-glycoprotein substrate,[84] which could explain why ELF concentrations were higher than unbound plasma concentrations. Although unlikely, one cannot totally exclude that ELF concentrations may have been over-estimated after moxifloxacin present within cells (e.g. macrophages) was released to ELF during BAL sampling. In fact such differences between ELF and unbound plasma concentrations were not observed with ciprofloxacin, also a known P-gp substrate. Therefore, the *in vivo* effect of P-gp on moxifloxacin lung PK should be further confirmed using appropriate knock-out animals. Alternatively, interaction studies with potent, specific and non-toxic competing P-gp substrates or inhibitors could be conducted in whole animals or/and using an isolated and perfused lung model. For low-permeability drugs it will take time to reach equilibrium distribution and therefore, the effect of an efflux transport system on the lung PK would probably be observable after multiple dosing at steady-state, but not after single dose administration, which was confirmed by PK studies.

Transporters as drug targets

Organic cation transporter OCTN1 as possible target for lung pathology

The *SLC22A4* gene has been identified to be associated with several diseases such as rheumatoid arthritis,[85] Crohn's disease,[86] autoimmune thyroid disease[87] and recessive non-syndromic hearing loss DFNB60 in humans.[88] This implies that the OCTN1 transporter could play a role in onset and/or deterioration of these diseases although little information is available on the molecular mechanisms. Since OCTN1 was originally identified as a xenobiotic transporter, which accepts various types of organic cations as substrates *in vitro*, identification of its substrate(s) *in vivo* may help understanding of how this transporter is associated with those diseases. Thus *octn1* gene knockout (*octn1*$^{-/-}$) mice were generated and metabolome analysis of blood and several organs in both wild-type and *octn1*$^{-/-}$ mice was carried out, leading to the identification of the food-derived antioxidant, ergothioneine (ERGO) as the physiological substrate of OCTN1.[89] This finding was in agreement with metabolome analysis using *SLC22A4* gene transfected cell lines, which originally identified ERGO as OCTN1 substrate.[90] ERGO is furthermore demonstrated to be possible biomarker substance in both rheumatoid arthritis[91] and Crohn's disease.[92]

Since oxidative stress is associated with various types of inflammatory diseases, speculating about a protective role for OCTN1 by transporting ERGO into cells to reduce oxidative stress is warranted. This protective role of OCTN1 was recently studied in tobacco smoke-induced chronic obstructive pulmonary disease (COPD).[93] Exposure to tobacco smoke leads to oxidative stress, which contributes to alveolar wall destruction, mucus hyper secretion, inflammation and defective tissue repair. Semi-quantitative PCR and immunoblot revealed elevated expression levels of catalase, thioredoxin and sulfiredoxin-1 following treatment with ERGO in NCI-H441 cells. Moreover, lower levels of oxidative stress were observed in cells, which were cultured in the presence of ERGO prior to the exposure to cigarette smoke extract. When exposed to room air, *octn1*$^{-/-}$ mice showed little differences compared to

wild-type mice. However, numbers of total cells and PNMs in BAL fluid as well as increased alveolar damage and increased inflammatory markers were observed in *octn1*$^{-/-}$ mice compared to wild-type mice, when exposed to second-hand smoke. These data suggest that ERGO can protect lung epithelial cells from oxidative damage and consequently, variants of OCTN1 might play a role in the pathogenesis of tobacco smoke-induced COPD by regulating ERGO transport.

Pathophysiological role of prostaglandin transporter OATP2A1/SLCO2A1 in pulmonary fibrosis

Prostaglandin (PG) E_2 is a bioactive lipid produced from arachidonic acid via the cyclooxygenase (COX)-PGE synthase (PGES) pathway. The prostaglandin transporter, OATP2A1 (aka PGT) encoded by the *SLCO2A1* gene has been characterised as an importer with high affinity to PGE_2, $PGF_{2\alpha}$ and PGD_2 at plasma membranes. [94,95] Recent genome-wide association studies indicate a link of loss-of-function mutations in *SLCO2A1* with primary hypertrophic osteoarthropathy[96] and chronic non-specific multiple ulcers of the small intestine.[97] Since PGE_2 is anti-fibrotic to lung stromal cells[98] and OATP2A1 is expressed functionally in mouse lungs[99] and the human bronchial epithelial BEAS-2B cells,[100] the effect of the absence of *Slco2a1* on pulmonary fibrosis in intra-tracheally (i.t.) bleomycin-injected mice was studies.[101] Immunohistochemistry showed that abundant expression of Oatp2a1 in mouse type 1 alveolar epithelial cells (AT1), and PGE_2 uptake was almost diminished in *Slco2a1*$^{-/-}$mice-derived AT1-like cells. Bleomycin-induced interstitial pneumonia and fibrosis became more severe in *Slco2a1*$^{-/-}$ mice, manifesting greater airway infiltration of inflammatory cells, collagen deposition and TGF-β1 signalling-related gene expressions (e.g.

Tgf-b1 and *Pai-1*). Two weeks after the i.t. injection, Western blot analysis demonstrated a significant activation of protein kinase C (PKC)-δ in the lungs of *Slco2a1*$^{-/-}$ mice, which contributes to an excessive production of extracellular matrix. Moreover, PGE_2 levels approximately 5-fold elevated significantly in BAL fluid of bleomycin-injected *Slco2a1*$^{-/-}$ mice, compared to that of wild type (WT) counterparts, although no other eicosanoids were found to increase in the BAL fluid by means of eicosanoid targeting metabolomics analysis. On the other hand, PGE_2 concentration in lung homogenates tended to decrease in *Slco2a1*$^{-/-}$ mice. Accordingly, altered PGE_2 disposition in the lung of *Slco2a1*$^{-/-}$ mice may contribute to aggravation of pulmonary fibrosis induced by bleomycin. It is hypothesised that PGE_2 released into alveolar lumen from epithelium and inflammatory cells infiltrated airway could transverse across AT1 cells into the lung interstitium via luminal OATP2A1, and then, inhibits fibroblast activation. This hypothesis is also supported that fact that PKC-δ was negatively regulated by c-AMP produced by PGE_2 signals in human lung fibroblasts.[102,103] Moreover, it was recently found that intracellular OATP2A1 mediates exocytosis of PGE_2 from murine macrophages;[104] therefore, PGE_2 autocrine signal may be altered in infiltrated monocytes and involved in severe inflammation. Revealing the precise role of OATP2A1 in tissue remodelling, which has been unappreciated to date, may provide a new rationale for mechanism of action of PGE_2 and possibly for pulmonary fibrosis.

Summary and conclusions

Lung transporter research is conducted in a small number of academic and industry laboratories with wider interest limited pending the first case studies to illustrate that drug-transporter interactions in the lungs have clinical impact. Although some studies have

shown effects of transporters on drug absorption from the lungs, there are no scientific reports to the authors' knowledge that provide evidence of a significant impact on target engagement or drug activity in the lungs. However, the potential impact that transporters may have in determining local free drug concentrations in target lung compartments (and thus drug action in the lungs) may be missed by insensitive measures such as net lungs-to-blood absorptive clearance. The potential for inhaled medicines to affect normal physiological processes, i.e. those mediated via the endogenous substrates of transporters, provides a further reason to understand better the way in which inhaled drugs interact with transporters during their residence in the lungs.

A barrier to progress in studying drug transporters in the lungs is that techniques required for evaluating impacts on local drug disposition are not routinely available or relatively recently developed. This includes methods to deliver appropriate doses and distributions of aerosolised drug accurately into the lungs of small animals or isolated perfused lungs, methods to study receptor occupancy as a surrogate for drug action following administration of drugs to the lungs and mechanistic modelling approaches, which can discern the impact of transporter kinetics in the context of competing pathways for drug clearance. Such models can be used to generate hypotheses for experimental testing and develop deeper understanding of inhaled drug biopharmaceutics. Priorities for further research in the field have been laid out previously by Gumbleton and colleagues in 2011 and it is interesting to revisit these to see what progress has been made (Table 2).[14]

The advantages and limitations of the different systems in which lung transporters are studied should also be considered (Table 3), so that these can be used in complementary study designs to investigate transporter interactions using triangulation to confirm

hypotheses and observations. Cell cultures provide convenient systems in which transporters are expressed and can be characterised for their substrate specificity, drug-interactions and kinetics. *Ex vivo* lungs enable drug transport to be studied in a model that possesses the architecture of the lungs and can be combined with delivery by inhalation and measurement of lung function. For example, the IPL has been used to examine the link between PK and lung mechanics.[35] *In vivo* studies permit less precise control of experimental conditions, but are the ultimate proof of impact of transporter actions.

In conclusion, it is well established that transporters for which licensed inhaled molecules are substrates are present in the lungs. New drug candidates for treating respiratory disease may include different chemical classes, with different transporter affinities. Furthermore, the molecularly diverse drugs that may be delivered via the lungs for systemic action may also bring different transporters into play. The physiological role for these transporters may provide clues regarding their likely impact on local disposition of drugs in the lungs. The influence of disease on these transporters may also be important. For drugs that have targets in the lungs, the effect of transporters on inhaled drug biopharmaceutics is not adequately investigated if only systemic exposure is measured and carefully designed studies are needed to measure effects in the lungs themselves.

ACKNOWLEDGEMENTS

This work has been financially supported by an International Strategic Collaboration Award from Science Foundation Ireland and co-sponsored by the Japanese Society for the Study of

Xenobiotics (JSSX). The authors would like to thank Joe Moore (NUI Galway) for logistical support and acknowledge the contribution of the COST Actions BM1201 and MP1404.

1. Kamiie J, Ohtsuki S, Iwase R, Ohmine K, Katsukura Y, Yanai K, Sekine Y, Uchida Y, Ito S, Terasaki T~~, 2008~~. Quantitative atlas of membrane transporter proteins: Development and application of a highly sensitive simultaneous LC/MS/MS method combined with novel in-silico peptide selection criteria. Pharm Res 2008;25(6):1469-1483.

2. Ohtsuki S, Uchida Y, Kubo Y. Terasaki T. 2011. Quantitative targeted absolute proteomics-ADME research as a new path to drug discovery and development: Methodology, Advantages, Strategy and Prospects. J Pharm Sci 2011; 100(9):3547-3559.

3. Uchida Y, Ohtsuki S, Terasaki T. 2014. Pharmacoproteomics-based reconstruction of in vivo P-glycoprotein function at blood-brain barrier and brain distribution of substrate verapamil in pentylenetetrazole-kindled epilepsy, spontaneous epilepsy and phenytoin treatment models. Drug Metab Dispos 2014;42(10):1719-1726.

4. Sakamoto A, Matsumaru T, Yamamura N, Uchida Y, Tachikawa M, Ohtsuki S, Terasaki T. 2013. Quantitative expression of human drug transporter proteins in lung tissues: analysis of regional, gender and inter-individual differences by liquid chromatography-tandem mass spectrometry. J Pharm Sci 2013;102(9):3395-3406.

5. Ohtsuki S, Kawakami H, Inoue T, Nakamura K, Tateno C, Katsukura Y, Obuchi W, Uchida Y, Kamiie J, Horie T, Terasaki T. 2014. Validation of uPA/SCID mouse with humanized liver as a human liver model: protein quantification of transporters, cytochromes P450, and UDP-glucuronosyltransferases by LC-MS/MS. Drug Metab Dispos 2014;42(6),1039-1043.

6. Akazawa T, Uchida Y, Tachikawa M, Ohtsuki S, Terasaki T. 2016. Quantitative targeted absolute proteomics of transporters and pharmacoproteomics-based reconstruction of P-glycoprotein function in mouse small intestine. Mol Pharm 2016;13(7):2443-2456.

7. Sakamoto A, Suzuki S, Matsumaru T, Yamamura N, Uchida Y, Tachikawa M, Terasaki T. 2016. Correlation of OCTN1 and MRP1 transport activities with protein expression levels in primary cultured human tracheal, bronchial and alveolar epithelial cells, J Pharm Sci 2016;105(2):876-883.

8. Sakamoto A, Matsumaru T, Yamamura N, Suzuki S, Uchida Y, Tachikawa M, Terasaki T. 2015. Drug transporter protein quantification of immortalized human lung cell lines derived from tracheo-bronchial epithelial cells (Calu-3 and BEAS2-B), bronchiolar-alveolar cells (NCI-H292 and NCI-H441) and alveolar type II-like cells (A549) by liquid chromatography-tandem mass spectrometry. J Pharm Sci 2015;104(9):3029-3038.

9. Forbes B, Ehrhardt C. 2005. Human respiratory epithelial cell culture for drug delivery application. Eur J Pharm Biopharm 2005;60(2):193-205.

10. Sporty JL, Horálková L, Ehrhardt C. 2008. In vitro cell culture models for the assessment of pulmonary drug disposition. Expert Opin Drug Metab Toxicol 2008;4(4):333-345.

11. Ehrhardt C, Kneuer C, Fiegel J, Hanes J, Schaefer UF, Kim KJ, Lehr CM 2002. Influence of apical fluid volume on the development of functional intercellular junctions in the human epithelial cell line 16HBE14o-: implications for the use of this cell line as an in vitro model for bronchial drug absorption studies. Cell Tissue Res 2002;308(3):391-400.

12. Lin H, Li H, Cho HJ, Bian S, Roh HJ, Lee MK, Kim JS, Chung SJ, Shim CK, Kim DD 2007. Air-liquid interface (ALI) culture of human bronchial epithelial cell monolayers as an in vitro model for airway drug transport studies. J Pharm Sci 2007;96(2):341-350.

13. Benediktsdóttir BE, Arason AJ, Halldórsson S, Gudjónsson T, Másson M, Baldursson O 2010. Drug delivery characteristics of the progenitor bronchial epithelial cell line VA10. Pharm Res 2010;30(3):781-791.

14. Gumbleton M, Al-Jayyoussi G, Crandon-Lewis A, Francombe D, Kreitmeyr K, Morris CJ, Smith MW 2011. Spatial expression and functionality of drug transporters in the intact lung: Objectives for further research. Adv Drug Deliv Rev 2011;63(1–2):110–118.

15. Nickel S, Clerkin CG, Selo MA, Ehrhardt C 2016. Transport mechanisms at the pulmonary mucosa: implications for drug delivery. Expert Opin Drug Deliv 2016;13(5):667–690.

16. Tronde A, Nordén B, Jeppsson A-B, Brunmark P, Nilsson E, Lennernäs H, Bengtsson UH 2003. Drug Absorption from the Isolated Perfused Rat Lung–Correlations with Drug Physicochemical Properties and Epithelial Permeability. J Drug Target 2003; 11(1):61–74.

17. Mathia[s] NR, Timoszyk J, Stetsko PI, Megill JR, Smith RL, Wall DA 2002. Permeability characteristics of calu-3 human bronchial epithelial cells: in vitro-in vivo correlation to predict lung absorption in rats. J Drug Target 2002;10(1):31-40.

18. Manford F, Tronde A, Jeppsson AB, Patel N, Johansson F, Forbes B 2005. Drug permeability in 16HBE14o- airway cell layers correlates with absorption from the isolated perfused rat lung. Eur J Pharm Sci 2005;26(5):414-420.

19. Cooper AE, Ferguson D, Grime K 2012. Optimisation of DMPK by the inhaled route: challenges and approaches. Curr Drug Metabol 2012;13(4):457-473.

20. Rodgers T, Leahy D, Rowland M 2005. Physiologically based pharmacokinetic modeling 1: predicting the tissue distribution of moderate-to-strong bases. J Pharm Sci 2005;94(6): 1259-1276.

21. Kakee A, Terasaki T, Sugiyama Y 1996. Brain efflux index as a novel method of analyzing efflux transport at the blood-brain barrier. J Pharm Exp Ther 1996;277(3): 1550-1559.

22. Bäckström E, Boger E, Lundqvist A, Hammarlund-Udenaes M, Fridén M 2016. Lung Retention by Lysosomal Trapping of Inhaled Drugs Can Be Predicted In Vitro With Lung Slices. J Pharm Sci 2016;105(11): 3432-3439.

23. Nakamura T, Nakanishi T, Haruta T, Shirasaka Y, Keogh JP, Tamai I ~~2010~~. Transport of ipratropium, an anti-chronic obstructive pulmonary disease drug, is mediated by organic cation/carnitine transporters in human bronchial epithelial cells: implications for carrier-mediated pulmonary absorption. Mol Pharm 2010;7(1):187-195.

24. Bäckström E, Lundqvist A, Boger E, Svanberg P, Ewing P, Hammarlund-Udenaes M, Fridén M ~~2016~~. Development of a Novel Lung Slice Methodology for Profiling of Inhaled Compounds. J Pharm Sci 2016;105(2): 838-845.

25. Salomon JJ, Hagos Y, Petzke S, Kühne A, Gausterer JC, Hosoya K, Ehrhardt C ~~2015~~. Beta-2 Adrenergic Agonists Are Substrates and Inhibitors of Human Organic Cation Transporter 1. Mol Pharm 2015;12(8):2633-2641.

26. Dudley AJ, Bleasby K, Brown CD ~~2000~~. The organic cation transporter OCT2 mediates the uptake of beta-adrenoceptor antagonists across the apical membrane of renal LLC-PK(1) cell monolayers. Br J Pharmacol 2000;131(1): 717-719.

27. Boger E, Evans N, Chappell M, Lundqvist A, Ewing P, Wigenborg A, Fridén M ~~2016~~. Systems Pharmacology Approach for Prediction of Pulmonary and Systemic Pharmacokinetics and Receptor Occupancy of Inhaled Drugs. CPT Pharmacometrics Syst Pharmacol 2016;5(4):201-210.

28. Caniga M, Cabal A, Mehta K, Ross DS, Gil MA, Woodhouse JD, Eckman J, Naber JR, Callahan MK, Goncalves L, Hill SE, Mcleod RL, McIntosh F, Freke MC, Visser SA, Johnson N, Salmon M, Cicmil M ~~2016~~. Preclinical Experimental and Mathematical Approaches for Assessing Effective Doses of Inhaled Drugs, Using Mometasone to Support Human Dose Predictions. J Aerosol Med Pulm Drug Deliv 2016;29(4):362-377.

29. Hastedt JE, Bäckman P, Clark AR, Doub W, Hickey A, Hochhaus G, Kuehl PJ, Lehr CM, Mauser P, McConville J, Niven R, Sakagimi M, Weers JG ~~2016~~. Scope and relevance of a pulmonary biopharmaceutical classification system AAPS/FDA/USP Workshop March 16-17th, 2015 in Baltimore, MD. AAPS Open 2016;~~2~~(1):1.

30. Forbes B, Asgharian B, Dailey LA, Ferguson D, Gerde P, Gumbleton M, Gustavsson L, Hardy C, Hassall D, Jones R, Lock R, Maas J, McGovern T, Pitcairn GR, Somers G, Wolff RK ~~2011~~. Challenges in inhaled product development and opportunities for open innovation. Adv Drug Deliv Rev 2011;63(1–2):69–87.

31. Bosquillon C ~~2010~~. Drug transporters in the lung—do they play a role in the biopharmaceutics of inhaled drugs? J Pharm Sci 2010;99(5):2240–2255.

32. Gustavsson L, Bosquillon C, Gumbleton M, Hegelund-Myrbaeck T, Nakanishi T, Price D, Tamai I, Zhou XH ~~2016~~. Drug transporters in the lung: expression and potential impact on pulmonary drug disposition. In: Nicholls G, Youdim K, ed~~itor~~s. *Drug transporters: role and importance in ADME and drug development*~~.~~ London: RSC; 2016 ~~p.~~:184–227.

33. Tronde A, Bosquillon C, Forbes B ~~2008~~. The isolated perfused lung for drug absorption In: Ehrhardt C, Kim KJ, ed~~itor~~s. *Drug absorption studies – In situ, in vitro and in silico models*~~.~~ New York: Springer; 2008 ~~p.~~:135–163.

34. Al-Jayyoussi G, Price DF, Francombe D, Taylor G, Smith MW, Morris C, Edwards CD, Eddershaw P, Gumbleton M ~~2013~~. Selectivity in the impact of P-glycoprotein upon pulmonary absorption of airway-dosed substrates: A study in *ex vivo* lung models using chemical inhibition and genetic knockout. J Pharm Sci 2013;102(9):3382–3394.

35. Gnadt M, Trammer B, Freiwald M, Kardziev B, Bayliss MK, Edwards CD, Schmidt M, Friedel G, Högger P ~~2012~~. Methacholine delays pulmonary absorption of inhaled β_2-agonists due to competition for organic cation/carnitine transporters. Pulm Pharmacol Ther 2012;25(1):124–134.

36. Edwards CD, Luscombe C, Eddershaw P, Hessel EM ~~2016~~. Development of a Novel Quantitative Structure-Activity Relationship Model to Accurately Predict Pulmonary Absorption and Replace Routine Use of the Isolated Perfused Respiring Rat Lung Model. Pharm Res 2016;33(11):2604–2616.

37. Beck-Broichsitter M, Stoisiek K, Bohr A, Aragão-Santiago L, Gessler T, Seeger W, Kissel T ~~2016~~. Potential of the isolated lung technique for the examination of sildenafil absorption from lung-delivered poly(lactide-co-glycolide) microparticles. J Control Release 2016;226:15–20.

38. Ong HX, Benaouda F, Traini D, Cipolla D, Gonda I, Bebawy M, Forbes B, Young PM ~~2014~~. *In vitro* and *ex vivo* methods predict the enhanced lung residence time of liposomal ciprofloxacin formulations for nebulisation. Eur J Pharm Biopharm 2014;~~-~~86(1):83–89.

39. Morris CJ, Smith MW, Griffiths PC, McKeown NB, Gumbleton M ~~2011~~. Enhanced pulmonary absorption of a macromolecule through coupling to a sequence-specific phage display-derived peptide. J Control Release 2011;151(1):83–94.

40. Bayard FJC, Thielemans W, Pritchard DI, Paine SW, Young SS, Bäckman P, Ewing P, Bosquillon C ~~2013~~. Polyethylene glycol-drug ester conjugates for prolonged retention of small inhaled drugs in the lung. J Control Release 2013;171(2):234–240.

41. Tronde A, Nordén B, Marchner H, Wendel A, Lennernäs H, Bengtsson UH ~~2003~~. Absorption Rate and Bioavailability of Drugs in Vivo in Rats: Structure–Absorption Relationships and Physicochemical Profiling of Inhaled Drugs. J Pharm Sci 2003;92(6):1216–1233.

42. Manford F, Riffo-Vasquez Y, Spina D, Page CP, Hutt AJ, Moore V, Johansson F, Forbes B ~~2008~~. Lack of difference in pulmonary absorption of digoxin, a P-glycoprotein substrate, in mdr1a-deficient and mdr1a-competent mice. J Pharm Pharmacol 2003;60(10):1305–1310.

43. Al-Jayyoussi G, Price DF, Kreitmeyr K, Keogh JP, Smith MW, Gumbleton M, Morris CJ ~~2015~~. Absorption of ipratropium and L-carnitine into the pulmonary circulation of the ex-vivo rat lung is driven by passive processes rather than active uptake by OCT/OCTN transporters. Int J Pharm 2015;496(2):834–841.

44. Sakagami M, Omidi Y, Campbell L, Kandalaft LE, Morris CJ, Barar J, Gumbleton M ~~2006~~. Expression and Transport Functionality of FcRn within Rat Alveolar Epithelium: A Study in Primary Cell Culture and in the Isolated Perfused Lung. Pharm Res 2006;23(2):270–279.

45. Sakagami M, Byron PR, Rypacek F ~~2002~~. Biochemical evidence for transcytotic absorption of polyaspartamide from the rat lung: Effects of temperature and metabolic inhibitors. J Pharm Sci 2002;91(9):1958–1968.

46. Ritchie TJ, Luscombe CN, Macdonald SJ ~~2009~~. Analysis of the calculated physicochemical properties of respiratory drugs: can we design for inhaled drugs yet? J Chem Inf Model 2009;49(4):1025-1032.

47. Enna SJ, Schanker LS ~~1972~~. Absorption of saccharides and urea from the rat lung. Am J Physiol 1979;222(2):409-414.

48. Bäckman P, Tehler U, Olsson B 2016. Predicting Exposure After Oral Inhalation of the Glucocorticoid Receptor Modulator, AZD5423, Based on Dose, Deposition Pattern, and Mechanistic Modeling of Pulmonary Disposition. J Aerosol Med Pulm Drug Deliv [Epub ahead of print] DOI:10.1089/jamp.2016.1306

49. Waiver of In Vivo Bioavailability and Bioequivalence Studies for Immediate-Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System. Available at: http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm070246.pdf. [aAccessed 16 FebApril 1, 2017]

50. Patton JS, Byron PR 2007. Inhaling medicines: delivering drugs to the body through the lungs. Nat Rev Drug Discov 2007;6(1):67-74.

51. Stouch TR, Gudmundsson O 2002. Progress in understanding the structure-activity relationships of P-glycoprotein. Adv Drug Deliv Rev 2002;54:315–328.

52. Bleasby K, Castle JC, Roberts CJ, Cheng C, Bailey WJ, Sina JF, Kulkarni AV, Hafey MJ, Evers R, Johnson JM, Ulrich RG, Slatter JG 2006. Expression profiles of 50 xenobiotic transporter genes in humans and pre-clinical species: a resource for investigations into drug disposition. Xenobiotica 2006;36:963-988.

53. Brady JM, Cherrington NJ, Hartley DP, Buist SC, Li N, Klaassen CD 2002. Tissue distribution and chemical induction of multiple drug resistance genes in rats. Drug Metab Dispos 2002;30:838-844.

54. Campbell L, Abulrob AN, Kandalaft LE, Plummer S, Hollins AJ, Gibbs A, Gumbleton M 2003. Constitutive expression of p-glycoprotein in normal lung alveolar epithelium and functionality in primary alveolar epithelial cultures. J Pharmacol Exp Ther 2003;304:441-452.

55. Endter S, Becker U, Daum N, Huwer H, Lehr CM, Gumbleton M, Ehrhardt C 2007. P-glycoprotein (MDR1) functional activity in human alveolar epithelial cell monolayers. Cell Tissue Res 2007;328(1):77-84.

56. Cordon-Cardo C, O'Brien JP, Boccia J, Casals D, Bertino JR, Melamed MR 1990. Expression of the multidrug resistance gene product (P-glycoprotein) in human normal and tumor tissues. J Histochem Cytochem 1990;38:1277-128

57. Valk PVD, Kalken CKV, Ketelaars H, Broxterman HJ, Scheffer G, Kuiper CM, Tsuruo T, Lankelma J, Meijer CJLM, Pinedo HM, Scheper RJ 1990. Distribution of multi-drug resistance-associated P-glycoprotein in normal and neoplastic human tissues Analysis with 3 monoclonal antibodies recognizing different epitopes of the P-glycoprotein molecule. Ann Oncol 1990;1(1):56-64.

58. Lechapt-Zalcman E, Hurbain I, Lacave R, Commo F, Urban T, Antoine M, Milleron B, Bernaudin JF 1997. MDR1-Pgp 170 expression in human bronchus. Eur Respir J 1997;10:1837-1843.

59. Scheffer GL, Pijnenborg AC, Smit EF, Muller M, Postma DS, Timens W, van der Valk P, de Vries EG, Scheper RJ 2002. Multidrug resistance related molecules in human and murine lung. J Clin Pathol 2002;55(5):332-339.

60. Kuhlmann O, Hofmann HS, Muller SP, Weiss M 2003. Pharmacokinetics of idarubicin in the isolated perfused rat lung: effect of cinchonine and rutin. Anticancer Drugs 2003;14:411–416.

61. Roerig DL, Audi SH, Ahlf SB 2004. Kinetic characterization of P-glycoprotein-mediated efflux of rhodamine 6G in the intact rabbit lung. Drug Metab Dispos 2004;32:953–958.

62. Madlova M, Bosquillon C, Asker D, Dolezal P, Forbes B ~~2009~~. In-vitro respiratory drug absorption models possess nominal functional P-glycoprotein activity. J Pharm Pharmacol 2009;61:293-301.

63. Francombe D, Taylor G, Somers G, Edwards CE, Gumbleton M ~~2008~~. Functional role of P-gp efflux in limiting pulmonary drug absorption within an intact lung: application of an isolated perfused rat lung model. In: Dalby RN, Byron PR, Peart J, Suman JD, Farr SJ, Young PM, ed~~itor~~s., *Respiratory Drug Delivery 2008 Volume II*, ~~-~~ River Grove: Davis Healthcare International Publishing; 2008 ~~p. :~~461–464.

64. Mehta R, Kelleher D, Preece A, Hughes S, Crater G ~~2013~~. Effect of verapamil on systemic exposure and safety of umeclidinium and vilanterol: a randomized and open-label study. Int J Chron Obstruct Pulmon Dis 2013;8:159-167.

65. Koepsell H ~~2013~~. The SLC22 family with transporters of organic cations, anions and zwitterions. Mol Aspects Med 2013;34(2–3):413-435.

66. Salomon JJ, Ehrhardt C ~~2012~~. Organic cation transporters in the blood-air barrier: expression and implications for pulmonary drug delivery. Ther Deliv 2012;3(6):735-747.

67. Ehrhardt C, Kneuer C, Bies C, Lehr CM, Kim KJ, Bakowsky U ~~2005~~. Salbutamol is actively absorbed across human bronchial epithelial cell layers. Pulm Pharmacol Ther 2005;18(3):165-170.

68. Lips KS, Volk C, Schmitt BM, Pfeil U, Arndt P, Miska D, Ermert L, Kummer W, Koepsell H ~~2005~~. Polyspecific cation transporters mediate luminal release of acetylcholine from bronchial epithelium. Am J Respir Cell Mol Biol 2005;33(1):79-88.

69. Endter S, Francombe D, Ehrhardt C, Gumbleton M 2009. RT-PCR analysis of ABC, SLC and SLCO drug transporters in human lung epithelial cell models. J Pharm Pharmacol 2009;61(5):583-591.

70. Salomon JJ, Endter S, Tachon G, Falson F, Buckley ST, Ehrhardt C 2012. Transport of the fluorescent organic cation 4-(4-(dimethylamino)styryl)-N-methylpyridinium iodide (ASP+) in human respiratory epithelial cells. Eur J Pharm Biopharm 2012;81(2):351-359.

71. Nakanishi T, Hasegawa Y, Haruta T, Wakayama T, Tamai I 2013. In vivo evidence of organic cation transporter-mediated tracheal accumulation of the anticholinergic agent ipratropium in mice. J Pharm Sci 2013;102(9):3373-3381.

72. Daniel H, Kottra G 2004. The proton oligopeptide cotransporter family SLC15 in physiology and pharmacology. Pflugers Arch 2004;447:610-618.

73. Takano M, Horiuchi T, Sasaki Y, Kato Y, Nagai J, Yumoto R 2013. Expression and function of PEPT2 during transdifferentiation of alveolar epithelial cells. Life Sciences 2013;93:630-636.

74. Takano M, Sugimoto N, Ehrhardt C, Yumoto R 2015. Functional expression of PEPT2 in the human distal lung epithelial cell line NCl-H441. Pharm Res 2015;32:3916-3926.

75. Swaan PW, Bensman T, Bahadduri PM, Hall MW, Sarkar A, Bao S, Khantwal CM, Ekins S, Knoell DL 2008. Bacterial peptide recognition and immune activation facilitated by human peptide transporter PEPT2. Am J Respir Cell Mol Biol 2008;39:536-542.

76. Rose JE, Mukhin AG, Lokitz SJ, Turkington TG, Herskovic J, Behm FM, Garg S, Garg PK, 2010. Kinetics of brain nicotine accumulation in dependent and nondependent smokers

assessed with PET and cigarettes containing ^{11}C-nicotine. Proc Natl Acad Sci USA 2010;107:5190-5195.

77. Tega Y, Yuzurihara C, Kubo Y, Akanuma S, Ehrhardt C, Hosoya K 2016. Functional expression of nicotine influx transporter in A549 human alveolar epithelial cells. Drug Metab Pharmacokinet 2016;31:99-101.

78. Tega Y, Akanuma S, Kubo Y, Terasaki T, Hosoya K 2013. Blood-to-brain influx transport of nicotine at the rat blood-brain barrier: involvement of a pyrilamine-sensitive organic cation transport process. Neurochem Int 2013;62:173-181.

79. Gontijo AV, Brillault J, Grégoire N, Lamarche I, Gobin P, Couet W, Marchand S 2014. Biopharmaceutical characterization of nebulized antimicrobial agents in rats: 1. Ciprofloxacin, moxifloxacin, and grepafloxacin. Antimicrob Agents Chemother 2014;58(7):3942-3949.

80. Colistin. Gontijo AV, Grégoire N, Lamarche I, Gobin P, Couet W, Marchand S 2014. Biopharmaceutical characterization of nebulized antimicrobial agents in rats: 2. Antimicrob Agents Chemother 2014;58(7):3950-3956.

81. Marchand S, Grégoire N, Brillault J, Lamarche I, Gobin P, Couet W 2015. Biopharmaceutical Characterization of Nebulized Antimicrobial Agents in Rats: 3. Tobramycin. Antimicrob Agents Chemother 2015;59(10):6646-6647.

82. Marchand S, Grégoire N, Brillault J, Lamarche I, Gobin P, Couet W 2016. Biopharmaceutical Characterization of Nebulized Antimicrobial Agents in Rats. 4. Aztreonam. Antimicrob Agents Chemother 2016;60(5):3196-3198.

83. Galindo Bedor DC, Marchand S, Lamarche I, Laroche J, Pereira de Santana D, Couet W ~~2016~~. Biopharmaceutical Characterization of Nebulized Antimicrobial Agents in Rats: 5. Oseltamivir Carboxylate. Antimicrob Agents Chemother 2016;60(8):5085-5087.

84. Brillault J, De Castro WV, Harnois T, Kitzis A, Olivier JC, Couet W ~~2009~~. P-glycoprotein-mediated transport of moxifloxacin in a Calu-3 lung epithelial cell model. Antimicrob Agents Chemother 2009;53(4):1457-14621

85. Tokuhiro S, Yamada R, Chang X, Suzuki A, Kochi Y, Sawada T, Suzuki M, Nagasaki M, Ohtsuki M, Ono M, Furukawa H, Nagashima M, Yoshino S, Mabuchi A, Sekine A, Saito S, Takahashi A, Tsunoda T, Nakamura Y, Yamamoto K ~~2003~~. An intronic SNP in a RUNX1 binding site of SLC22A4, encoding an organic cation transporter, is associated with rheumatoid arthritis. Nat Genet 2003;35(4):341-348.

86. Peltekova VD, Wintle RF, Rubin LA, Amos CI, Huang Q, Gu X, Newman B, Van Oene M, Cescon D, Greenberg G, Griffiths AM, St George-Hyslop PH, Siminovitch KA ~~2004~~. Functional variants of OCTN cation transporter genes are associated with Crohn disease. Nat Genet 2004; 36(5):471-475.

87. Hou X, Mao J, Li Y, Li J, Wang W, Fan C, Wang H, Zhang H, Shan Z, Teng W ~~2015~~. Association of single nucleotide polymorphism rs3792876 in SLC22A4 gene with autoimmune thyroid disease in a Chinese Han population. BMC Medical Genetics 2015;16:76.

88. Ben Said M, Grati M, Ishimoto T, Zou B, Chakchouk I, Ma Q, Yao Q, Hammami B, Yan D, Mittal R, Nakamichi N, Ghorbel A, Neng L, Tekin M, Shi XR, Kato Y, Masmoudi S, Lu Z, Hmani M, Liu X ~~2016~~. A mutation in SLC22A4 encoding an organic cation transporter expressed in

the cochlea strial endothelium causes human recessive non-syndromic hearing loss DFNB60. Hum Genet 2016;135(5):513-524.

89. Kato Y, Kubo Y, Iwata D, Kato S, Sudo T, Sugiura T, Kagaya T, Wakayama T, Hirayama A, Sugimoto M, Sugihara K, Kaneko S, Soga T, Asano M, Tomita M, Matsui T, Wada M, Tsuji A ~~2010~~. Gene knockout and metabolome analysis of carnitine/organic cation transporter OCTN1. Pharm Res 2010;27(5):832-840.

90. Gründemann D, Harlfinger S, Golz S, Geerts A, Lazar A, Berkels R, Jung N, Rubbert A, Schömig E ~~2005~~. Discovery of the ergothioneine transporter. Proc Natl Acad Sci USA 2005;102(14):5256-5261.

91. Taubert D, Grimberg G, Jung N, Rubbert A, Schömig E ~~2005~~. Functional role of the 503F variant of the organic cation transporter OCTN1 in Crohn's disease. Gut 2005;54(10):1505-0506.

92. Taubert D, Lazar A, Grimberg G, Jung N, Rubbert A, Delank KS, Perniok A, Erdmann E, Schömig E ~~2006~~. Association of rheumatoid arthritis with ergothioneine levels in red blood cells: a case control study. J Rheumatol 2006;33(11):2139-2145.

93. Nickel S, Selo MA, Clerkin CG, Talbot BN, Walsh JJ, Lewis JB, Reynolds PR, Kato Y, Nakamichi N, Ehrhardt C ~~2016~~. Ergothioneine protects lung epithelial cells from tobacco smoke-induced oxidative damage *in vitro* and *in vivo*. Am J Respir Crit Care Med 2016;193:A7514.

94. Kanai N, Lu R, Satriano JA, Bao Y, Wolkoff AW, Schuster VL ~~1995~~. Identification and characterization of a prostaglandin transporter. Science 1995;268:866-869.

95. Lu R, Kanai N, Bao Y, Schuster VL 1996. Cloning, in vitro expression, and tissue distribution of a human prostaglandin transporter cDNA(hPGT). J Clin Invest 1996;98:1142-1149.

96. Seifert W, Kuhnisch J, Tuysuz B, Specker C, Brouwers A, Horn D 2012. Mutations in the prostaglandin transporter encoding gene SLCO2A1 cause primary hypertrophic osteoarthropathy and isolated digital clubbing. Hum Mutat 2012;33:660-664.

97. Umeno J, Hisamatsu T, Esaki M, Hirano A, Kubokura N, Asano K, Kochi S, Yanai S, Fuyuno Y, Shimamura K, Hosoe N, Ogata H, Watanabe T, Aoyagi K, Ooi H, Watanabe K, Yasukawa S, Hirai F, Matsui T, Iida M, Yao T, Hibi T, Kosaki K, Kanai T, Kitazono T, Matsumoto T 2015. A Hereditary Enteropathy Caused by Mutations in the SLCO2A1 Gene, Encoding a Prostaglandin Transporter. PLoS Genet 2015;11:e1005581.

98. Vancheri C, Mastruzzo C, Sortino MA, Crimi N 2004. The lung as a privileged site for the beneficial actions of PGE2. Trends Immunol 2004;25:40-46.

99. Chang HY, Locker J, Lu R, Schuster VL 2010. Failure of postnatal ductus arteriosus closure prostaglandin transporter-deficient mice. Circulation 2010;121:529-536.

100. Shirasaka Y, Shichiri M, Kasai T, Ohno Y, Nakanishi T, Hayashi K, Nishiura A, Tamai I 2013. A role of prostaglandin transporter in regulating PGE2 release from human bronchial epithelial BEAS-2B cells in response to LPS. J Endocrinol 2013;217:265-274.

101. Nakanishi T, Hasegawa Y, Mimura R, Wakayama T, Uetoko Y, Komori H, Akanuma S, Hosoya K, Tamai I 2015. Prostaglandin Transporter (PGT/SLCO2A1) Protects the Lung from Bleomycin-Induced Fibrosis. PLoS One 2015;10:e0123895.

102. Huang S, Wettlaufer SH, Hogaboam C, Aronoff DM, Peters-Golden M ~~2007~~. Prostaglandin E(2) inhibits collagen expression and proliferation in patient-derived normal lung fibroblasts via E prostanoid 2 receptor and cAMP signaling. Am J Physiol Lung Cell Mol Physiol 2007; ~~-~~292:L405-L413.

103. Huang SK, Wettlaufer SH, Chung J, Peters-Golden M ~~2008~~. Prostaglandin E2 inhibits specific lung fibroblast functions via selective actions of PKA and Epac-1. Am J Respir Cell Mol Biol 2008;39:482-489.

104. Shimada H, Nakamura Y, Nakanishi T, Tamai I ~~2015~~. OATP2A1/SLCO2A1-mediated prostaglandin E loading into intracellular acidic compartments of macrophages contributes to exocytotic secretion. Biochem Pharmacol 2015;98:629-638.

105. Dinis-Oliveira RJ, Duarte JA, Sánchez-Navarro A, Remião F, Bastos ML, Carvalho F ~~2008~~. Paraquat poisonings: mechanisms of lung toxicity, clinical features, and treatment. Crit Rev Toxicol 2008;38(1):13-71.

106. Zabner J, Karp P, Seiler M, Phillips SL, Mitchell CJ, Saavedra M, Welsh M, Klingelhutz AJ. Development of cystic fibrosis and noncystic fibrosis airway cell lines. Am J Physiol Lung Cell Mol Physiol 2003;284(5):L844-854.

107. Fulcher ML, Gabriel SE, Olsen JC, Tatreau JR, Gentzsch M, Livanos E, Saavedra MT, Salmon P, Randell SH. Novel human bronchial epithelial cell lines for cystic fibrosis research. Am J Physiol Lung Cell Mol Physiol 2009;296(1):L82-91.

108. Walters MS, Gomi K, Ashbridge B, Moore MA, Arbelaez V, Heldrich J, Ding BS, Rafii S, Staudt MR, Crystal RG. Generation of a human airway epithelium derived basal cell line with multipotent differentiation capacity. Respir Res 2013;14:135.

109. Kuehn A, Kletting S, de Souza Carvalho-Wodarz C, Repnik U, Griffiths G, Fischer U, Meese E, Huwer H, Wirth D, May T, Schneider-Daum N, Lehr CM. Human alveolar epithelial cells expressing tight junctions to model the air-blood barrier. ALTEX 2016;33(3):251-260.

110. Hendrickx R, Johansson JG, Lohmann C, Jenvert RM, Blomgren A, Börjesson L, Gustavsson L. Identification of novel substrates and structure-activity relationship of cellular uptake mediated by human organic cation transporters 1 and 2. J Med Chem 2013;56(18):7232-7242.

absorption.

Deposition (region)	Solubility (uM)	Peff (cm/s)	F (%)	Cmax (pg/ml)	Tmax (h)
BB	0.1	Hi	0.1	0.04	19.9
BB	0.1	Lo	0.003	0.004	8.6
BB	10	Hi	4.3	3.2	9.4
BB	10	Lo	0.03	0.02	10.7
BB	1000	Hi	39	37	3
BB	1000	Lo	0.4	0.3	4.1
bb	0.1	Hi	3.4	1.5	24
bb	0.1	Lo	0.02	0.009	24
bb	10	Hi	82	77	5.5
bb	10	Lo	2.1	0.9	24
bb	1000	Hi	97	112	1.5
bb	1000	Lo	11.6	5.7	9.2
Al	0.1	Hi	100	127	1.2
Al	0.1	Lo	100	83	8.6
Al	10	Hi	100	140	0.08
Al	10	Lo	100	137	0.24
Al	1000	Hi	100	140	0.08
Al	1000	Lo	100	137	0.16

Figure 2. Concentration-time profiles of moxifloxacin following i.v. administration and administration of the nebulised form in plasma (red line, closed symbols) and in ELF (blue

line, open symbols), predicted from simultaneous PK modelling of plasma and ELF data.

Symbols represent means ± SD concentrations measured in plasma and ELF. Taken from[79]

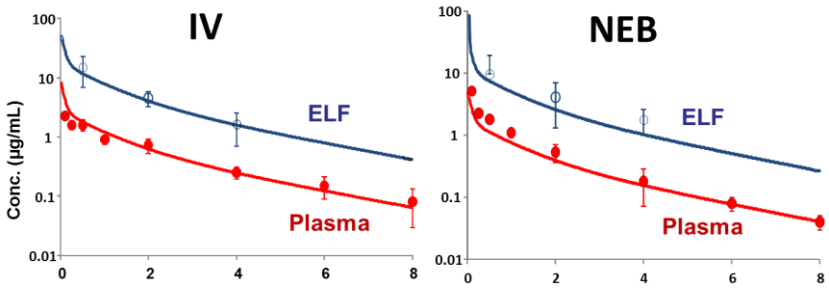

Table 1. List of FDA-approved drugs for inhalation with reported organic cation transporter interactions (adapted from[15, 110])

Compound	Drug Class	Transporters
Salbutamol	β_2-Adrenergic agonist	OCT1, OCT3, OCTN1(?), OCTN2(?)
Beclomethasone diproprionate	Corticosteroid	OCT1, OCT2
Budesonide	Corticosteroid	OCT1-3
Ciprofloxacin	Antibiotic	OCT-3, OCTN2*
Fenoterol	β_2-Adrenergic agonist	OCT1, OCT2
Fluticasone propionate	Corticosteroid	OCT2, OCT3
Formoterol	β_2-Adrenergic agonist	OCT1, OCT3, OCTN2
Indacaterol	β_2-Adrenergic agonist	OCT1, OCT2
Ipratropium bromide	Anticholinergic	OCT2, OCTN2*
Levofloxacin	Antibiotic	OCT2*
Olodaterol	β_2-Adrenergic agonist	OCT1*
Pentamidine	Antiprotozoal	OCT1-3*
Salmeterol xinafoate	β_2-Adrenergic agonist	OCT1, OCT3
Terbutaline	β_2-Adrenergic agonist	Oct1*, Oct2 *
Tiotropium bromide	Anticholinergic	OCT2, OCTN2*

Bold face indicates drugs which act as inhibitors

*, Interactions were found in non-lung tissues or cells

?, Contradictory information

Formatted: Font: Bold

Formatted: Font: Bold

Formatted: Font: Bold

Formatted: Font: Not Bold

Formatted: Font: Not Bold

Formatted: Font: Not Bold

Research priorities	Examples of ~~P~~progress
Identify transporters in human lungs and other species used in preclinical evaluation of inhaled medicines	Summarised by Nickel *et al.* 2016[15] and Gustavsson et al. 2016[32]
Map levels and locations of transporter expression within the lungs	Summarised by Nickel *et al.* 2016[15] and Gustavsson et al. 2016[32]
Demonstrate the impact of transporters on drug retention or disposition in the lungs after inhalation	Data on P-gp effects from IPL and *in vivo* animal work, e.g.[34,63,71]
Study the effect of transporters on accumulation of drug in the lungs from the systemic circulation	Forbes has investigated uptake via the polyamine transporter (unpublished data); Paraquat accumulation in lung tissue[105]
Do inhaled drugs alter transporter expression or function (thereby affecting normal physiology)	No evidence yet
Clinical impact of transporters, e.g. drug-drug interactions, effect of disease, inter-individual variability	Methacholine caused decrease of β_2 agonist absorption in human IPL;[35] Oral verapamil increased AUC of inhaled umeclidinium and vilanterol in human volunteers[64]

Formatted: Font: 11 pt

Formatted: Font: 11 pt, Superscript

Formatted: Font: 11 pt

Table 3. Experimental models for measuring effects of transporters on drug disposition in the lungs

Experimental system	Advantages	Limitations
Respiratory cell cultures	• Primary cell cultures and many cell lines available • High capacity, readily available • Measurement of transport or cell uptake • Measurement of metabolism • Molecular identification of pathways possible	• Usually only single cell type • Many clearance mechanisms missing • Relevance to lung effects unclear?
Isolated perfused lungs	• Human lung lobes can be used • Can be linked to lung function measurement • Can study air to blood and blood to lung transport • Can use inhibitors in high concentrations, controlled concentrations • Use of genetically modified animals possible	• Species differences unclear • Access to human lung is limited • Short study duration • Not trivial to set up
In vivo experiments	• Lung function can be measured • Disease models can be used • Use of genetically modified animals possible	• Use of inhibitors and concentrations limited • Studies in humans are limited