

# Development of a competitive ELISA and genotype technique to determine caffeine pharmacokinetics and CYP1A2 status in humans

C4ME SUPPLEMENT

## AUTHOR INFORMATION

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### **Background**

The cytochrome P-450 1A2 (CYP1A2) enzyme accounts for approximately 13% of the CYP family of enzymes. (1) It metabolises drugs and is important for drug-drug interactions. Interindividual differences in CYP1A2 phenotype and genotype result in variable enzyme expression and functional activity, thus can alter drug plasma concentration. Caffeine is largely metabolised by CYP1A2 and is often used to investigate an individual's CYP1A2 metabolic activity, (2) which may help in drug prescription and dose adjustment to avoid toxicity or inefficacy. (3)

The aim of this project is to develop two protocols:

- A) To evaluate CYP1A2 phenotype through determining salivary caffeine pharmacokinetics using a competitive ELISA.
- B) To identify the most widely studied single nucleotide polymorphism (SNP) CYP1A2\*1F (SNP rs762551 A/C), with polymerase chain reaction-restriction enzyme fragment length polymorphism (PCR-RFLP) assay.

Once protocols have been developed, we would determine if they are appropriate for undergraduate laboratory teaching of pharmacogenomic concepts, i.e. how interindividual differences in CYP1A2 activity can alter the pharmacokinetic parameters of caffeine.

## Methods

Ethical approval was sought but not required, as confirmed by the Chair of the School of Medicine Research Ethics Committees.

### *Development, optimization and validation of ELISA*

Competitive ELISA to measure caffeine in saliva is based on a previously described protocol. (4) The concentrations of commercially available antibodies, caffeine conjugated to horse-radish peroxidase and unlabelled caffeine were optimised for the assay. The optimised ELISA was then validated with saliva collected at various time intervals from ten volunteers, after ingestion of 100 mg caffeine in tablet form.

The ELISA standard curve was generated on myassays.com using four-parameter logistic curve fit. Pharmacokinetic parameters of caffeine were determined; coefficient of variation (CV) was calculated to evaluate precision and repeatability of the immunoassay. Data were analysed using GraphPad Prism 7.04 and expressed as mean  $\pm$  SD.

### *Development of PCR-RFLP assay to determine a SNP in the CYP1A2 gene*

DNA was isolated from buccal cells of three volunteers using two commercially available DNA extraction kits. It was used for identification of CYP1A2\*1F through PCR-RFLP, and its association with the fast metaboliser phenotype using two published protocols. (5-6) Two pairs of forward and reverse primers were evaluated to identify and amplify gene segment containing the rs762551 SNP. PCR products were digested using two restriction enzymes specific for the C-allele. Fragments were analysed by agarose gel electrophoresis.

## Results

### *Development, optimization and validation of caffeine competitive ELISA*

The optimised ELISA allowed detection of caffeine ranging from 0.169 ng/ml - 20.0 ng/ml in saliva. CV between days was <15%, and <5% between replicates, indicating the ELISA had acceptable precision and repeatability. Saliva was collected before taking the caffeine tablet, then at 0.5, 1, 2, 3, 5, 7, 9, 12 and 24 hours. Levels of caffeine from 1 to 12 hours was statistically different from baseline at 0 hour ( $p = <0.05$ ) and peaked at  $1.56 \pm 0.98$  hours. Large interindividual variations were observed in  $C_{max}$  ( $1215 \pm 2927$  ng/ml).

### *PCR-RFLP assay to determine a SNP in the CYP1A2 gene*

The PCR-RFLP did not yield any restriction fragments, suggesting all three subjects had two copies of the most common A-allele. Use of a control restriction enzyme, which cuts outside the SNP, yielded fragments within the expected base pair range, demonstrating validity of the assay. Further development of the PCR-RFLP protocol is required to determine CYP1A2\*1F genotype.

## Discussion

### *ELISA and CYP1A2 phenotype*

Competitive ELISA, optimized and validated, is sensitive enough to quantify salivary caffeine; it is comparable to Carvalho's immunoassay (7) and a commercial ELISA kit using similar antibodies. (8) Large interindividual variations in  $C_{max}$  and  $T_{max}$  may represent differences in CYP1A2 activity or that caffeine's pharmacokinetic may not accurately represent CYP1A2 metabolic activity. Further study with a larger sample size is required.

### *PCR-RFLP and CYP1A2 genotype*

The PCR-RFLP assay requires further optimization and a larger sample size to identify CYP1A2\*1F. In addition to the enzymes specific for C-allele, those specific for A-allele could be used.

### *Are the protocols appropriate for teaching the concepts of pharmacogenomics to undergraduate students?*

The competitive ELISA offers a simple and inexpensive way to quantify salivary caffeine in a standard laboratory, making it suitable for undergraduate laboratory teaching. During the experiments, students can practise laboratory skills, learn the principles of ELISA, understand pharmacokinetics and drug metabolism. Fast metabolisers metabolise drugs quickly, resulting in drug inefficacy; whereas drugs may accumulate in slow metabolisers, leading to toxicity. Through understanding the metaboliser phenotypes, drug dose can be adjusted accordingly for maximum efficacy and to avoid side effect. Many factors affect CYP1A2 phenotype, but their mechanisms and impact are unclear. The significance of different genotypes on its phenotype also has to be confirmed. The current PCR-RFLP protocol is not appropriate for undergraduate laboratory and requires further optimization.

## Lessons Learnt

Constant reflection on the experimental process was required to produce an optimised immunoassay for the accurate quantification of salivary caffeine level.

As it was the first time carrying out a laboratory project by myself, I felt quite uncertain initially. However, under the guidance of my supervisors and reading the literature, I quickly understood and became more interested in laboratory research.

I was surprised by the viscosity of saliva and it was hard to pipette. In later experiments, to minimize error in volume of saliva used, a larger volume was pipetted and vortexed after each dilution. In addition, large interindividual differences were observed in caffeine pharmacokinetic. This may be due to saliva collection not being monitored; the duration of saliva collection could be standardised. Moreover, it may be accounted for by differences in gastric emptying. Large volume and high density food are emptied slower, (9) which may affect caffeine absorption rate. Some volunteers fasted before taking caffeine tablets, whereas others did not. This increased my understanding on the interaction between drug pharmacokinetics and consumption of food or liquid. Mealtime and saliva collection times should be standardized in order to minimize interference of food in future experiments. Moreover, pharmacokinetics of caffeine could be affected by differences in body mass, smoking and other drugs, which should be considered in the future.

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