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WRL68-CYP3A4 AND WRL68-CYP2C9 MUTAGENESIS IN VITRO DRUG METABOLISM MODELS FOR THE ASSESSMENT DRUG-HERB INTERACTIONS

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ABSTRACT

In vitro screening of drug interactions is one of the most important steps in early drug discovery. Inhibition and stimulation of the phase 1 metabolism enzymes known as CYP450 isozymes is the most prominent form of drug interactions. More reliable and high specificity *in vitro* models are needed to improve the predictions of clinical metabolism-based drug interactions related to CYP450 and its variants. The use of herbal remedies in complementary and alternative medicine has been a popular practice all around the world with little information regarding its interaction with common drugs. Potential drug-herb interaction due to co-treatment of herb and conventional drug has become an issue of concern in healthcare practice. Therefore, *in vitro* screening of medicinal plant for its potential CYP450 related drug-herb interaction using a reliable system is an important step in the evaluation of safety of use. This study aimed to develop a metabolism-based cell model for *in vitro* assessment of drug interactions related to two major CYP450 isozymes, which are CYP3A4 and CYP2C9. A foetal liver cell line, WRL 68, was evaluated for its hepatic marker genes, basal CYP450 and the redox partners expression, and was found suitable as a cell host for the models. Using an optimized polyethylenimine (PEI) mediated transient transfection protocol, WRL 68 cells were incorporated with CYP3A4 and CYP2C9 genes carrying plasmids to overexpress the isozymes. Without alteration of basal redox partners expression, optimum CYP3A4 and CYP2C9 overexpression were achieved at 48 hours post-transfection which were confirmed by qRT-PCR and western blot analyses. Here, two HPLC-DAD methods were developed and validated for quantification of 6β-hydroxytestosterone and 4'-hydroxydiclofenac, the metabolite from enzymatic reactions of CY3A4 and CYP2C9, respectively. WRL68-CYP3A4 and WRL68-CYP2C9 models were successfully developed and evaluated for kinetic parameters which were found in good agreement with data reported in the literature. These models were also developed for SNPs variants of the isozymes. WRL68-*CYP3A4*4*, WRL68-*CYP3A4*18*, WRL68-*CYP2C9*2* and WRL68-*CYP2C9*3* systems were successfully developed as *in vitro* models and can be used to study metabolic activities and drug interactions on different enzyme variants. Finally, the WRL68-CYP2C9 and its variants models were utilized to investigate inhibitory drug-herb interactions by *Clinacanthus nutans* towards CYP2C9. In this study, ethanolic extract of *C. nutans* leaves was prepared. Five common flavonoids of *C. nutans* which are schaftoside, isoorientin, orientin, vitexin and quercetin were identified from HPLC-UV phytochemical profiling of the extract. *C. nutans* extract and quercetin exhibit varied potency in inhibiting different CYP2C9 variants. From the evaluation of kinetic parameters as well as Lineweaver-Burk plots constructed, both *C.nutans* extract and quercetin inhibited wild-type CYP2C9 in an uncompetitive manner. Inhibition level of both inhibitors were varied on CYP2C9*2 and CYP2C9*3 in comparison to wild type CYP2C9. In conclusion, the metabolism-based cell models for *in vitro* assessment of drug interactions related to CYP3A4 and CYP2C9 variants has been successfully developed.

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CHAPTER ONE
INTRODUCTION

1.1 Research Background

In drug discovery, it is worth to consider the drug safety in the early stage by conducting *in vitro* screening of potential drug-drug interaction (DDI) in which the drug could pose clinically. Development of a reliable model for an *in vitro* screening of DDI such as CYP450 inhibition is important to reduce the number of animals used in preclinical studies (Costa et al., 2014). Vast number of CYP450 isoenzymes are species-dependant in terms of substrate and inhibitor specificities, making the extrapolation from animal to human quite chancy (Martignoni et al., 2006). Furthermore, CYP450 enzymes are polymorphic and single nucleotide polymorphism (SNP) is the most common type of allelic variants in this enzyme family (Li et al., 2015).

Single nucleotide polymorphism (SNP) is a form of genetic variation that is most commonly found in CYP450 genes. A SNP is a variation of a single nucleotide located at a specific location in the genome, either at exonic or intronic region within a gene, or at inter-gene regions, that may contribute to gene alterations (Ahmad et al., 2018). The single nucleotide changes may involve substitution, insertion, deletion or duplication of nucleotide that results in alteration of amino acid sequence, occurrence of premature stop codon of splicing defect which eventually affects the substrate specificity (Rosdi et al., 2017).

It is important to study the role of genetic polymorphisms in genes encoding enzymes involved in drug metabolism, such as CYP450 family, for better insights on the interindividual variability of drug pharmacokinetics (Hodel et al., 2013). Polymorphism in the major CYP450 isozyme, CYP3A4, is known to be ethnicity related (Guttman et al., 2019). It was reported previously that up to 40-fold interindividual variations were observed in the human liver and about 10-fold variation involving *in vivo* metabolism of CYP3A4 substrates (Dai et al., 2001). *CYP3A4*4*, *CYP3A4*5* and *CYP3A4*18* are the allelic variations in CYP3A4 gene that are known to affect the enzyme's catalytic activity (Ruzilawati et al., 2007).

Many cases demonstrate the clinical relevance of pharmacogenetics involving variations in phase I metabolism and one of it includes the influence of CYP2C9