Pharmacogenomics In Drug Therapy And Interaction: The Role Of Cytochrome P450

Chin-Eng Ong, Yan Pan, Kai-Hung Tiong, Beow-Chin Yiap, Eng-Lai Tan, Peter Pook, Joon-Wah Mak

Abstract: Pharmacogenomics (or pharmacogenetics), the study of the effects of genetic differences on a person's response to drugs, can help in optimizing drug efficacy and minimizing adverse drug reactions. Interperson difference in drug metabolism is one of the important consequences of such genetic variation. This variation is determined in part by mutations in cytochrome P450 enzymes (CYPs). IMU is part of a major collaborative research project in the area of phamacogenetics and drug metabolism. Working together with USM and UiTM, our group has, since 2000, generated useful population database on genetic polymorphism of various CYP isoforms. We have successfully genotyped three major ethnic groups, Malay, Indian and Chinese for their allelic frequency of important isoforms. These include CYP2D6, CYP2C9, CYP2C8 and CYP2A6. Data generated so far collectively have contributed to our effort in mapping and constructing genomic database for Malaysian population.

Since early 2002, our research has been focusing on developing in vitro methods in studying the functional consequences of genetic polymorphism of CYP enzymes. Using site-directed mutagenesis, CYP mutants, carrying nucleotide changes as reported in known alleles in human populations, were generated and expressed in *E. coli* system, and the expressed recombinant proteins were characterized using enzyme assays to determine the functional consequences of mutations. We have established a series of HPLC (high performance liquid chromatography)-based and fluorescence-based assays to investigate CYP activities. Assays that have been developed include tolbutamide methylhydroxylase, paclitaxel 6α-hydroxylase, dextromethorphan O-demethylation, testosterone 6β-hydroxylation and coumarin 7-hydroxylase assays. These assays serve as activity markers allowing comparison of catalytic activities of mutant proteins generated. Another focus of our work is to use the developed assays as a screening tool to investigate drug-herb interactions. This was achieved by co-incubation of herbal extracts and active constituents with the probe substrates in the assays followed by characterization of the kinetic behaviors of the enzymes involved using various pharmacokinetic parameters such as K_m, V_{max}, IC_{50} and K_i. This work is currently carried out with collaboration from the Institute for Medical Research (IMR) and is supported by MOSTI's eScienceFund under RM9. It is envisaged that this screening work will give us insights on the potential of the commonly used herbs to cause pharmacokinetic interactions with other drug substrates, and allow us to elucidate the mechanisms involved in the interactions.

IeJSME 2008: 2 (Suppl 1): S6-S10

Key Words: Pharmacogenomics, Pharmacogenetics, Drug Interaction, Drug Therapy, Cytochromes P450

Introduction

Many factors, such as dietary intake, age, and concurrent drug therapies, affect a person's response to medications. Importantly, genetic makeup determines inherent pharmacokinetics, giving rise to interperson differences in drug absorption, distribution, metabolism, and excretion. Some of these differences can be explained by genetic variations in transport proteins (eg P-glycoprotein and organic anion transporting polypeptide), in drug targets (eg -adrenergic receptors), and in the function of phase 1 or phase 2 drug-metabolising enzymes. Pharmacogenomics (or pharmacogenetics), the study of the effects of genetic differences on a person's response to drugs, can help in optimizing drug efficacy and minimizing adverse drug reactions. Interperson difference in drug metabolism is one of the important areas intensively researched in recent decades. This variation is determined in part by mutations in cytochrome P450 enzymes (CYPs).

Human Cytochrome P450 enzymes

Cytochrome P450 constitutes a ubiquitous superfamily of hemoprotein enzymes. CYP enzymes play crucial roles in the metabolism and biosynthesis of endogenous compounds such as bile acids, biogenic amines, eicosanoids, fatty acids, retinoids, and steroids. To date, we know 57 CYP family members in man, of

Faculty of Medicine and Health, International Medical University, Bukit Jalil, 57000, Kuala Lumpur, MALAYSIA

For Correspondence:
Dr Chin-Eng Ong, School of Pharmacy and Health Sciences, International Medical University, Bukit Jalil 57000, Kuala Lumpur, MALAYSIA
Email: ceong98@hotmail.com

these, isoforms in families 1-3 mediate 70 – 80% of all phase 1-dependent metabolism of clinically used drugs[1] and participate in the metabolism of a huge number of xenobiotic chemicals. The polymorphic forms of CYPs are responsible for the development of a significant number of adverse drug reactions (ADRs) and problems associated with drug interactions. According to Phillips et al[2], 56% of drugs that are cited in ADR studies are metabolized by polymorphic phase 1 enzymes, of which 86% are CYPs. Only 20% of drugs that are substrates for non-polymorphic enzymes are in the ADR reports. It has been estimated that: (i) ADRs cost the US society, US$100 billion; (ii) ADRs cause 100,000 deaths annually in the USA; and (iii) up to 7% of all hospital admissions in the UK and Sweden are due to ADRs[3]. In addition, the costs of treating patients who possess polymorphic forms of CYPs are much higher than those required to treat patients who possess non-polymorphic alleles. Furthermore, the number of non-responders to drug therapy is high and represents 30 – 60% of subjects treated with drugs[4]. Thus, knowledge about the CYP system is fundamental both for drug therapy and for drug development.

Genetic Polymorphism of CYP enzymes

All genes encoding CYP enzymes in families 1-3 are polymorphic. The functional importance of the variant alleles, however, differs and the frequencies of their distribution in different ethnic groups also differ. Updated information can be found on the Human CYP Allele Nomenclature Website (http://www.imm.ki.se/cypalleles). Polymorphic enzymes (in particular CYP2C9, CYP2C19 and CYP2D6) mediate 40% of CYP-mediated drug metabolism, which makes drug dosing problematic. In general, four phenotypes can be identified: poor metabolisers (PMs), who lack the functional enzyme; intermediary metabolisers (IMs), who are heterozygous for one deficient allele or carry two alleles that cause reduced activity; extensive metabolisers (EMs), who have two normal alleles; and ultrarapid metabolisers (UMs), who have multiple gene copies. These various metaboliser statuses may result in wide variability in drug response ranging from lack of drug efficacy, unexpected drug level, to drug toxic or serious side effects.

Population Genotyping of CYP Alleles – Malaysian efforts

Due to the important role that CYPs have in the drug metabolism and emergence of reports on existence of different alleles in different ethnicity, it is of interest to investigate the occurrence of defective CYP alleles in Malaysian population. The identification of polymorphisms in different racial and ethnic populations is vital to understanding differences in clinical responses to drugs. Up to mid-nineties, research efforts in this area are scarce and little work has been carried out on occurrence and comparison of allelic frequency in three major ethnic populations – Malay, Chinese and Indian in Malaysia. Concerted effort to this end however started to emerge at the beginning of the Eighth Malaysian Plan (RM8). IMU is privileged to be part of this endeavour, working together with USM (Assoc Prof Rusli Ismail) and UiTM (Dr Teh Lay Kek and Prof Mohd Zaki Salleh), our group has, since 2000, generated useful population database on CYP genetic polymorphism. We have successfully genotyped Malay, Indian and Chinese for their allelic frequency of important isoforms. These include CYP2D6, CYP2C9, CYP2C8 and CYP2A6 (Table I).

A number of genotyping techniques have been used in our screening with most of these polymerase chain reaction(PCR)-based, including multiplex PCR. Data generated so far collectively have contributed to our effort in mapping and constructing genomic database for Malaysian population.

Development of In Vitro Screening Tools for Pharmcogenetic Study – An IMU effort

In vitro molecular biology techniques serve as convenient tools to study genetic polymorphism of CYP isoforms. A wide set of methodologies such as cDNA cloning, sequence alignments, mutagenesis approaches, heterologous expression systems together with spectroscopic and chromatographic enzyme assays allowed us to investigate many key residues that were

mutated in reported CYP alleles in terms of their roles in ligand binding, substrate or inhibitor orientation within active site, and overall protein folding. The combination of these investigations has aided cooperatively in our understanding of such enzyme-substrate interactions which lead to better insights into the molecular basis of CYP polymorphism. For example, cDNA cloning allows isolation of various isoforms of interest, including defective alleles, from tissue samples. Sequence alignments would reveal the identity of the isolated clones. Following site-directed mutagenesis of specific amino acid residues in a particular isoform, the subsequent effect upon substrate binding and enzyme kinetics can be explored using established enzyme assays (often spectroscopic or high-performance chromatographic based) *via* experimental determinations of K_m, V_{max} or other kinetics parameters. Consequently, it is possible to build up an increasingly clearer picture of the molecular recognition or interaction events operating in CYP-substrate interactions, and thus help to define the structural basis for different substrate selectivity observed in various alleles.

Since early 2002, our research has been focusing on developing in vitro methods in studying the functional consequences of genetic polymorphism of CYP enzymes. Using site-directed mutagenesis, CYP mutants, carrying nucleotide changes as reported in known alleles in human populations, were generated and expressed in *E. coli* system, and the expressed recombinant proteins were characterized using enzyme assays. We have established a series of HPLC (high performance liquid chromatography)-based and fluorescence-based assays to investigate CYP activities. Assays that have been developed include coumarin 7-hydroxylase, paclitaxel 6α-hydroxylase, tolbutamide methylhydroxylase, S-mephenytoin 4'-hydroxylase, dextromethorphan O-demethylation, and testosterone 6β-hydroxylase assays. These assays serve as activity markers allowing comparison of catalytic activities of mutant proteins generated. Our most recent work, published only last month, described the functional consequence of Ile264Met substitution reported in CYP2C8*4[11]. CYP2C8 wild-type cDNA ws first mutated at Ile264 site to different amino acids – methionine, arginine and aspartic acid. These mutated cDNAs were later expressed in *E. coli* DH5α cell. The expressed mutants were examined for protein stability in proteinase K and CYP spectral spectroscopic assays, and for enzymatic activity in paclitaxel 6α-hydroxylase and tolbutamide methylhydroxylase assays. Our results indicated that the presence of isoleucine at position 264 in CYP2C8 was important for proper haem insertion and protein folding; whereas mutated residues were highly disruptive resulting in inactive proteins with minimum spectral and catalytic activities. This was evidenced from the low levels of Soret peak at 450 nm and negligible levels of tolbutamide methylhydroxylase activity. Kinetic study using paclitaxel indicated that all three mutants exhibited only 9.7 to 35.4% of the activity level observed in the wild-type. In addition, the mutants were more sensitive to proteinase K digestion, indicating a possible alteration of conformation. The combined effects of protein instability and compromised catalytic activity resulted in defective CYP2C8 protein which may have clinical implications in carriers of CYP2C8*4, particularly in terms of their capacity to clear potent drugs and their susceptibility to adverse drug reactions. Our current works, still on-going, focus on characterization of alleles from other CYP isoforms including CYP2A6 and CYP2D6.

Another focus of our work is to use the developed assays as a screening tool to investigate drug-herb interactions. This was achieved by co-incubation of herbal extracts and active constituents with the probe substrates in the assays followed by characterization of the kinetic behaviors of the enzymes involved using parameters such as IC_{50} and K_i values. This work is currently carried out in collaboration with the Institute for Medical Research (Dr Badrul Amini Abd-Rashid and Dr Zakiah Ismail) and is supported by MOSTI's eScienceFund under RM9. It is envisaged that this screening work will give us insights on the potential of the commonly used herbs to cause pharmacokinetic

interactions with other drug substrates, and allow us to elucidate the mechanisms involved in the interactions.

Summary and Future Directions

The key word here is translational research. The challenge we face is how to move from bench to bedside, how the research findings, currently largely laboratory-based, could be translated into clinical utility for optimising drug therapy. Although laboratory evaluation for genetic variations in CYP activity can be performed, this testing is infrequent and may be cost prohibitive in clinical settings. The large number of alleles to be screened and the laborious genotyping procedures (using current PCR-based techniques) represent the main obstacles to routine and efficient genotype screening in clinical practice. Recently, a microarray chip technology has been developed that can assess for multiple CYP polymorphisms from a sample of blood[12]. This product is called AmpliChip™ CYP450 Test Kit and is built on the Affymetrix platform and therefore will likely see widespread application in the very near future. In this platform, a light-directed combinatorial chemistry enabling the synthesis of over 15,000 oligonucleotide sequences in precise locations on a glass microarray was used; the platform is then screened by a laser fluorescence scanning system permitting identification of molecular interactions in some areas of the array and excluding inactive areas. This has made possible for the system to simultaneous test hundreds of DNA sequences in a relatively simple, inexpensive way by placing specific oligonucleotide sequences in different specific areas of a glass microarray. The AmpliChip™ CYP450 Test currently classifies individuals on two CYP2C19 phenotypes, EMs and PMs, and four CYP2D6 phenotypes: UMs, EMs, IMs, and PMs allowing individualised dosing for important drugs used in treatment of psychological and gastric acid-related disorders. As this technology continues to evolve and disseminate, it is anticipated that this tool is only the first of a new wave of pharmacogenetic tools that may reach the clinic in the next 10 years or so.

Another area for future effort is the utility of in vitro screening methods in the process of drug development in local industry. The measurement of the effect of new chemical entities on human CYP marker activities using in vitro experimentation represents an important experimental approach in drug development. In vitro drug interaction data can be used in guiding the design of clinical drug interaction studies, or, when no effect is observed in vitro, the data can be used in place of an in vivo study to claim that no interaction will occur in vivo. In recent years, a number of methods have also been proposed to predict the human clearance of drug substances from data obtained from in vitro CYP-mediated reaction assays. Because of their predictive utility and ease of use, these assays using liver enzymes are widely employed in drug discovery and selection process to prospectively identify compounds that will have desirable pharmacokinetics in humans[13,14,15]. Although this in vitro approach is now used widely in developed countries, its utility in local pharmaceutical industry is very little. Greater effort is needed to enhance their awareness and appreciation of availability and utility of these methods in their drug R & D endeavour.

In summary, although pharmacogenetics and related genomic technologies hold the potential to improve drug efficacy and safety as well as to improve the process of drug design and development in the industry, there remains a gap between researchers in the field and the clinicians and industry players. Greater efforts are needed in bridging the gap so that pharmacogenetic testing can be widely applied and have a true impact on society at large. Importantly, scientists in pharmacogenetics should focus on development of a simple, rapid, accurate, and low-cost testing so as to promote wide utility of the testing.

REFERENCES

1. Evans WE, Relling MV. Pharmacogenomics: translating functional genomics into rational therapeutics. Science 1999; 286: 487-91.
2. Phillips KA, Veenstra DL, Oren E, Lee JK, Sadee W. Potential role of pharmacogenomics in reducing adverse drug reactions: a systematic review. J Am Med Assoc 2001; 286: 2270-9.
3. Ingelman-Sundberg M. Pharmacogenetics: an opportunity for a safer and more efficient pharmacotherapy. J Intern Med 2001; 250: 186-200.
4. Spear BB, Heath-Chiozzi M, Huff J. Clinical application of pharmacogenetics. Trends Mol Med 2001; 7: 201-204.

5. Nurfadhlina M, Foong K, Teh LK, Tan SC, Mohd Zaki S, Ismail R. CYP2A6 polymorphisms in Malays, Chinese and Indians. Xenobiotica 2006; 36: 684-92.
6. Muthiah YD, Lee WL, Teh LK, Ong CE, Salleh MZ, Ismail R. A simple multiplex PCR method for the concurrent detection of three CYP2C8 variants. Clin Chim Acta 2004; 349: 191-8.
7. Muthiah YD, Lee WL, Teh LK, Ong CE, Ismail R. Genetic polymorphism of CYP2C8 in three Malaysian ethnics: CYP2C8*2 and CYP2C8*3 are found in Malaysian Indians. J Clin Pharm Ther 2005; 30: 487-90.
8. Zainuddin Z, Teh LK, Suhaimi AWM, Ismail R. Malaysian Indians are genetically similar to Caucasians: CYP2C9 polymorphism. J Clin Pharm Ther 2006; 31: 187-91.
9. Ismail R, Teh LK. Genetic polymorphism of CYP2D6: Malaysian Indians have the highest frequency for CYP2D6*4 in Asia. Eur J Clin Pharmacol 2001; 57: 617-8.
10. Ismail R, Teh LK, Amir J, Alwi Z, Lopez CG. Genetic polymorphism of CYP2D6 in Chinese subjects in Malaysia. J Clin Pharm Ther 2003; 28: 279-84.
11. Singh R, Ting JG, Pan Y, Teh LK, Ismail R, Ong CE. Functional role of Ile264 in CYP2C8: mutations affect haem incorporation and catalytic activity. Drug Metab Pharmacokinet 2008; 23: 165-74.
12. Leon J, Susce MT, Murry-Carmichael E. The AmpliChip™ CYP450 Genotyping Test - integrating new clinical tool. Mol Diag Ther 2006; 10: 135-51.
13. Lave T, Coassolo P, Reigner B. Prediction of hepatic metabolic clearance based on interspecies allometric scaling techniques and in vitro-in vivo correlations. Clin Pharmacokinet 1999; 36: 211-31.
14. Obach RS. Prediction of human clearance of twenty-nine drugs from hepatic microsomal intrinsic clearance data: an examination of in vitro half-life approach and nonspecific binding to microsomes. Drug Metab Dispos 1999; 27: 1350-9.
15. Kohl C, Steinkellner M. Prediction of pharmacokinetic drug/drug interactions from in vitro data: interactions of the nonsteroidal anti-inflammatory drug lornoxicam with oral anticoagulants. Drug Metab Dispos 2000; 28: 161-8.

Table I: Allele Frequencies of Common CYP Polymorphisms in Various Ethnic Groups of Malaysian Population

CYP	ALLELE FREQUENCY FOR EACH ETHNIC GROUP			REFERENCE
	MALAY	CHINESE	INDIAN	
CYP2A6				5
allele*1A	27.0	34.3	52.0	
allele*1B	46.7	44.5	39.4	
allele*2	0	0	0.3	
allele*3	0	0	1.2	
allele*4	7.0	4.9	1.4	
allele*5	0.9	1.2	0.9	
allele*7	4.3	7.0	0	
allele*8	5.0	1.5	0.9	
allele*10	4.3	1.7	0	
CYP2C8				6, 7
allele*1	100	100	91.2	
allele*2	0	0	3.5	
allele*3	0	0	5.3	
allele*4	0	0	0	
CYP2C9				8
allele*1	95.7	97.3	88.2	
allele*2	1.9	0	2.1	
allele*3	2.4	3.3	9.7	
allele*4	0	0	0	
allele*5	0	0	0	
CYP2D6				9, 10
allele*1	36.0	39.0	69.0	
allele*3	0	0	0	
allele*4	3.0	0.2	8.0	
allele*5	5.0	3.0	1.0	
allele*9	4.0	1.0	1.0	
allele*10	49.0	57.0	15.0	
allele*17	0	0.2	1.0	