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Analysis of the catalytic activity of cytochrome P450 2C8

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Cytochrome P450(CYP) is a diverse group of haem-containing enzymes, which catalyze the biotransformation of endogenous and foreign substances. The human CYP2C subfamily is of particular interest because it encodes a constitutively expressed group of CYPs, *CYP2C8*, 2C9 and *2C19*, which collectively metabolize at least 20% of the commonly used drugs. *CYP2C8* is the focus of current study as it has been less well characterized in terms of substrate and inhibitor specificity and population genetic polymorphism. The objective of this project is to develop and optimize a simple variant specific PCR techniques to detect and determine the frequencies of *CYP2C8* mutant alleles (*CYP2C8*2, CYP2C8*3* and *CYP2C8*4*) in Malaysian population and to clone and express NADPH P450-reductase and the *CYP2C8* and its variants protein in bacterial expression system for the use of in vitro kinetic studies. For genotyping, blood samples were obtained from healthy unrelated volunteers of three major races in Malaysia. Genomic DNA were extracted and subjected to variant -specific PCR newly developed to examine *CYP2C8* genotypes. *CYP2C8* variants would be generated by PCR - site directed mutagenesis. They would be expressed as recombinant proteins and analyzed for kinetic studies in comparison with the wild type protein. The variant specific PCR method was validated and DNA sequencing further confirmed genotype results. Data were compiled according to the genotype and allele frequencies with the 95% confidence intervals. Expected genotype frequencies were calculated using the Hardy-Weinberg equation from the allele frequencies ($p^2+2pq+q^2=1$). The Fisher- Exact test was used to compare allele frequencies between the subpopulations. A p value of 0.05 or less was regarded as significant. All statistics were done using SPSS for Windows Version 11 on an IBM-PC® compatible computer. Only the Indians showed *CYP2C8* polymorphism with allele frequency of 98 % for *CYP2C8*1*, 0.8 % for *CYP2C8*2* and 1.2 % for *CYP2C8*3*. *CYP2C8*4* was not detected in any of the ethnic groups. E.coli cells were successfully transformed with the *CYP2C8* (wild type) and reductase plasmids. Protein expression was optimized at 30ºC with culture time of 48 hours and 24 hours for *CYP2C8* and reductase respectively. The immunoblot step confirmed that the correct proteins were expressed of sizes about 50kDa and 80kDa for *CYP2C8* and reductase respectively. We have successfully developed and optimized a multiplex PCR method suitable for use in population studies of *CYP2C8* polymorphism. To the best of our knowledge, for the first time polymorphisms of *CYP2C8* in Malaysia Indian was reported. The cytochrome P450 2C8 and reductase were successfully cloned and expressed in this study.