UNIVERSITI TEKNOLOGI MARA

DESIGN AND DEVELOPMENT OF A PCR METHOD FOR DETECTION OF SNP OF CYP2B6

TENGKU ALIAA DAYANA BT TUAN MOHD HASHIM

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ABSTRACT

Cytochrome P450 2B6 is an enzyme that involved in the biotransformation of many clinically important drugs. Genetic polymorphisms of the CYP2B6 gene are an important factor that contributes to the inter-individual and inter-ethnic variability in the systemic exposure, therapeutic and toxic responses to CYP2B6 substrate drugs. Therefore, it is important to develop a method to identify and determine all of the genetic variants in a CYP2B6 gene that responsible for altered CYP2B6 expression. The aim of this study was to design and develop rapid and simple method for the detection of SNP in the CYP2B6 gene. The multiplex, allele-specific PCR method was used. This method involves two-stage procedures, which were first and second PCR. First PCR was used to amplify 3 exons, which were exon 4, exon 5+6, and exon 9. After that, the products of the first PCR were used as a DNA template and genotyped by allele-specific PCR. The assay detected the following published single nucleotide polymorphisms such as G516T (Gln172His), A785G (Lys262Arg) and C1459T (Arg487Cys). DNA sequencing analysis was performed to further confirmed genotype results obtained from current method. Multiplex PCR with allele-specific genotyping method is simple, reliable, rapid and maybe more economical compared to other methods to perform. Further studies are required in order to investigate the impact of the SNP of CYP2B6 gene on the clinical response to drugs that are substrates for this gene.

Chapter 1

Introduction

Polymorphisms in all the main cytochrome P450 not only result to interethnic variability in the systemic exposure to a variety of drugs but it also occurs to interindividual. In a study by Nelson *et al.* (1996), the cytochrome P450 are defined as a multigene family of heme-thiolate monooxygenases that catalyse the oxidative biotransformation of a wide variety of xenobiotics and endobiotics. The metabolism in human body is a major factor regulating the therapeutic effect and toxicity of a drug. They are classified into families, subfamilies, and individual isoenzymes based on similarities in their amino acid sequence (Elbekai *et al.,* 2006). Pascussi *et al.* (2003) has proved human beings have 17 known CYP gene families, among which only the first three, CYP1, CYP2 and CYP3, being involved in the metabolism of drugs and xenobiotics.

According to Wang *et al.* (2006) study, cytochrome P450 2B6 (CYP2B6) was first identified in human liver microsomes and later found to be expressed in a variety of extrahepatic tissues, including nasal mucosa, trachea, lung and brain. CYP2B6 genetic polymorphisms can influence the enzyme expression and catalytic activity, probably leading to variation in systemic exposure, therapeutic and toxic responses to CYP2B6