Chapter

Interindividual Variability of Cytochromes P450 2B Mediated Oxidation in Human Liver

Abdulmohsen Alrohaimi, Bader Alrohaimi, Nada Alruwais and Kholoud Aldmasi

Abstract

The cytochromes P450 (CYPs) are a group of enzymes that are primarily responsible for oxidative drug biotransformation in people. CYP2B6, which metabolizes numerous drugs including bupropion, propofol and other drug shows great variability in rates of drug oxidation between individuals. In this chapter we discuss the contribution of selected genetic and environmental factors to this variability. Several studies identified and quantified the most common CYP2B6 mRNA splice such as deletion of exons 4 to 6 and of exon 4 which were significantly and negatively correlated with CYP2B6 protein and enzyme activity. CYP2B6 gene expression is highly inducible by phenobarbital. Alcohol ingestion has been associated with increased CYP2B6 levels this involves the constitutive androstane receptor (CAR) and/or the pregnane X receptor (PXR). CYP2B7 is considered a pseudogene because of the presence of a single premature stop codon (TGA) in exon 7. In 10 out of 24 African-Americans (but none out of 48 European-Americans) there is a single nucleotide polymorphism that results in an arginine codon instead of a stop codon (X378R). The results of these studies identify certain CYP2B6 genetic polymorphisms, mRNA splicing variants, and alcohol ingestion as significant factors that determine interindividual variability of CYP2B-mediated oxidation of drugs in people.

Keywords: Cytochromes, cytochromes P450, CYP2B6, CYP2B6 activity, drug oxidation

1. Introduction

Large interindividual variability in drug response is more of a rule than an exception. In fact, among patients treated with the same dose of drug, the response varies widely from no response at all to severe side effects. Many factors contribute to this variability, and apart from the role of non-pharmacological aspects, such as psychological and social issues, it mainly results from the interaction of genetic, pathophysiological and environmental factors that lead to interindividual differences in drug pharmacokinetics and pharmocodynamics. Since the discovery of the debrisoquine/sparteine hydroxylation polymorphism in 1970, research has expanded in the study of the interaction between environmental and genetic factors controlling the rate of drug metabolism. Currently, it is well known that cytochrome P450

(CYP) mediated drug metabolism shows large variability, leading to large differences in steady-state plasma concentrations of drugs. This variability is due to interaction of genetics and environmental factors. In addition, concomitant drug administration influences the variabilty of drug response. The main objective of the work described in this thesis dissertation was to study the role of genetic and environmental factors in determining interindividual variability of the CYP2B subfamily.

1.1 CYP enzymes

The cytochrome P450 (CYP) superfamily of enzymes plays a predominant role in the phase I metabolism of xenobiotics, environmental chemicals and endogenous compounds. An overview of the the most common reaction catalyzed by CYP is as follows:

$$R-H+O2+NADPH+H+ \rightarrow R-OH+NADP++H2O \quad (1)$$

The CYP enzymes are classified into families and subfamilies based on their amino acid sequence similarity. The CYP nomenclature is as follows: "P" stands for "pigment", "cyto" means "hollow vesicle", and "chrome" means "color". "450" is part of the name since the reduced enzyme absorbs light at 450 nm when bound to carbon monoxide. 'CYP' represents the CYP family. Members of the same family represented by a number (e.g. CYP2) share at least 40% identical with respect to their amino acid sequences. If the sequences are 40–55% identical, the enzymes belong to the same subfamily, indicated by an additional letter (e.g. CYP2B). Finally, each individual enzyme is represented by an Arabic numeral (e.g. CYP2B6). Three CYP gene families are mainly responsible for drug metabolism in humans and most other mammalian species i.e. CYP1, CYP2, and CYP3. CYP1 have two subfamilies CYP1A (i.e. CYP1A1 and 1A2) and CYP1B. CYP1A1 is mainly extrahepatic while CYP1A2 is a hepatic enzyme. Both are induced by polycyclic aromatic hydrocarbons (PAR), found for example in cigarette smoke and charbroiled meat. CYP1A2 is involved in metabolism of several. CYP2 is the largest family of human CYPs identified to date. In addition, there is 92% nucleotide sequence similarity of CYP2B7P with CYP2B6.

1.2 *In vitro* models used to study drug biotransformation

Various methods are used to study the metabolic activity of a CYP enzyme in vitro including human liver microsomes, recombinant expressed CYPs, cytosol, S9 fraction, cell lines, transgenic cell lines, primary hepatocytes, liver slices, and perfused liver.

1.2.1 Human liver microsomes

Human liver microsomes are prepared from fresh or frozen liver tissue, and contain different proportions of all CYPs for each donor. Human liver microsomes contain membrane bound Phase I enzymes and Phase II enzymes such as CYPs and UDP glucuronosyltransferases.

1.2.2 Recombinant CYPs

Individual CYPs (including 1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, 3A4, and 3A5) expressed in either lymphoblastoid cells or insect cells are available commercially from BD-Gentest Corporation [1].

1.2.3 Primary hepatocytes

Hepatocytes represent a model for studying biotransformation and drug–drug interactions, such as inhibition and induction [2]. One disadvantage of using hepatocytes is that availability is limited. Once a fresh liver is obtained, the hepatocytes must immediately be plated, used for suspension studies, or cryopreserved for future studies [2].

1.2.4 Liver slices

Liver slices represent one of the earliest in vitro models for metabolism studies, dating back to the earlier part of the 20th century [3].

1.2.5 S9 fraction

The S9 fraction is the fraction of the liver cellscontaining both microsomes and cytosol. It is obtained by centrigation of whole-liver homogenate at 9000 X g [4].

1.2.6 Liver cell lines

Liver cell lines are less popular compared to other models. This is mainly due to their dedifferentiated cellular characteristics and incomplete expression of all families of metabolic enzymes [5].

1.2.7 Bupropion hydroxylation as a CYP2B6 index reaction

If a substrate is biotransformed to one of its metabolites via only one CYP, the pathway is called an index reaction. In this thesis work, conversion of bupropion to hydroxybupropion was used as an index reaction for CYP2B6 based on the evidence as follows. Bupropion is biotransformed to three main metabolites in vivo, including hydroxybupropion, threohydrobupropion, and erythrohydrobupropion. Bupropion is biotransformed to the active metabolite hydroxybupropion mainly via CYP2B6 in vitro [6].

1.3 Factors influencing interindividual variability in CYP2B6 function

On average, CYP2B6 accounts for approximately 1 to 6% of total CYP450 [7, 8]. It was estimated that interindividual variability accounts for a 50-fold difference in CYP2B6 enzyme content [9]. Although CYP2B6 expression is highly variable, it is found at substantial levels in a small percentage of the population [6, 8, 10]. In addition, CYP2B6*6B haplotype and alcohol use history were identified as significant predictors of bupropion hydroxylation. The CYP2B6*6B haplotype was present at an allele frequency of 0.26. These correlations suggest that moderate alcohol consumption (at least 14 drinks of alcohol per week) is associated with enhanced CYP2B6 gene transcription, but the presence of at least one CYP2B6*6B allele reduces this inductive effect.

1.3.1 Genetic polymorphism

Genetic variation in CYPs may affect the biotransformation of drugs metabolized by those CYPs. Variation in a gene could arise from different causes. First, a single nucleotide polymorphism (SNP) is a single nucleotide variation in the genetic sequence. Another type of genetic variation is gene duplication in which a CYP gene is found in multiple copies.

1.3.2 Effect of SNPs on CYP2B6 in vitro

Various laboratories have attempted to correlate CYP2B6 genotype with CYP2B6 phenotype in panels of human liver microsomes. Found novel point mutations in the CYP2B6 coding region: C64T, G516T, C777A, A785G and C1459T, at frequencies of 5.3%, 28.6%, 0.5%, 32.6% and 14.0%, respectively [11]. Furthermore, our laboratory has studied the correlation between several SNPs in the CYP2B6 coding and promoter region (to −3000 base pairs (bp)) verses bupropion hydroxylation activity, CYP2B6protein levels, and CYP2B6 mRNA levels *in vitro* were measured in a bank of 54 human livers.

Initial analysis showed excellent correlation between bupropion hydroxylation activities and CYP2B6 protein content (Rs = 0.88) but relatively poor correlation between CYP2B6 protein levels and CYP2B6 mRNA levels (Rs = 0.44) (**Figure 1A**). We did not find any individual genotypesthat significantly correlated with bupropion hydroxylation activity, CYP2B6 protein or mRNA levels, but found that alcohol use history and the CYP2B6*6B haplotype (−1456 t > c, −750 t > c, G516T, A785G [Q172H, K262R], p = 0.011) were significant predictors of bupropion hydroxylation (**Figure 1B**). The CYP2B6*6B haplotype was present at a frequency of 0.26 [10].

Jinno et al. [12] expressed CYP2B6 mutants (CYP2B6.2,.3,.4,.5,.6, and 7) in COS-1 cells and found that compared to wild type, CYP2B6.6 (G516T, A785G [Q172H, K262R]) was expressed at a lower protein level, but had significantly higher Km and Vmax values for activity of 7-ethoxy-4-trifluoromethylcoumarinO-deethylation (**Figure 1C**). Lang et al. identified four novel CYP2B6 alleles as phenotypic null alleles [13].

1.3.3 Effect of SNPs in vivo

When compared single-dose bupropion pharmacokinetics in 121 healthy male German Caucasian volunteers and found a correlation between the presence of the *4 allele (A785G [K262R]) and a higher (1.7-fold) bupropion clearance, although only a minor fraction of the variability in bupropion and hydroxybupropion kinetics could be explained by this variant [14]. Supporting our *in vitro* results, a clinical study involving efavirenz found that the CYP2B6*6 (G516T, A785G [Q172H, K262R]) genotype correlated with high plasma efavirenz concentrations in HIV-patients treated with standard efavirenz-containing regimens [15].

1.3.4 Ethnic differences in CYP2B6 SNPs

There are ethnic differences in the frequencies of CYP2B6 genotypes that could account for race/ethnic differences in CYP2B6 phenotype. For example, 89 Caucasians and 50 African-Americans who were receiving efavirenz treatment were genotyped for G516T [Q172H] [16].

1.3.5 CYP2B6 inducers

Various CYP2B6 inducers have been identified, including carbamazepine, phenobarbital and related barbiturates, and rifampin [17, 18].

1.4 Mechanisms of CYP induction

Post-transcriptional mechanisms include both mRNA and protein stabilization that may be mediated through transacting regulators or through changes in the phosphorylation status of the enzyme.

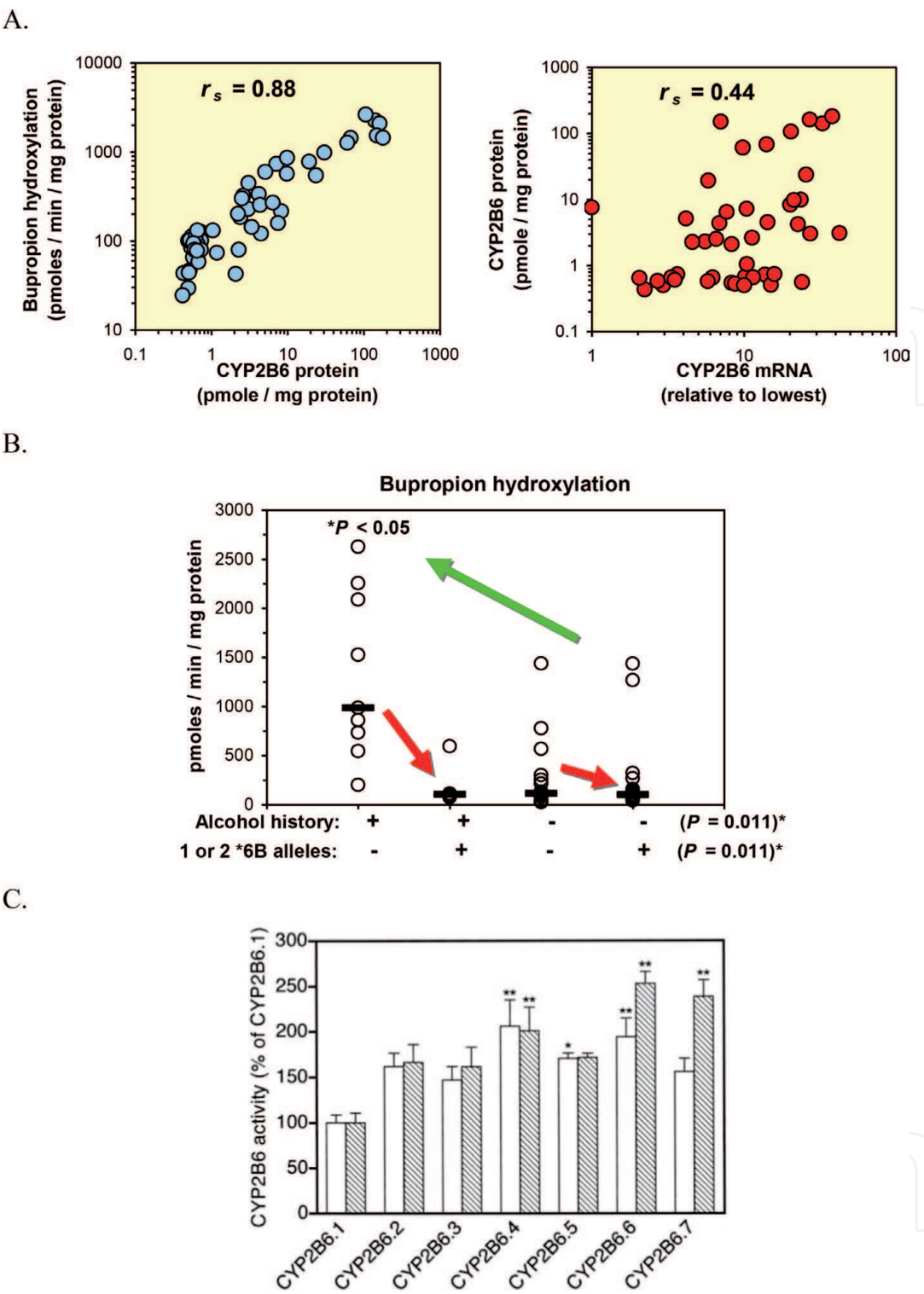


Figure 1.
*Data from our laboratory [10] showing relationships between CYP2B6 activities, protein content and mRNA content measured in a bank of 54 human livers (A); as well as effects of alcohol and *6B allele on CYP2B6 activity (B). A study by [12] showed that some CYP2B6 amino acid coding variants expressed in COS-1 cells are associated with higher activity (C).*

1.4.1 Transcriptional regulation

In addition to the induction of CYP1A genes, at least three other nuclear receptors (NRs) can induce transcription of drug metabolizing enzymes. These are the constitutive androstane receptor (CAR), the pregnane X receptor (PXR), and the peroxisome proliferator-activated receptor (PPAR).

1.4.2 Post-transcriptional regulation

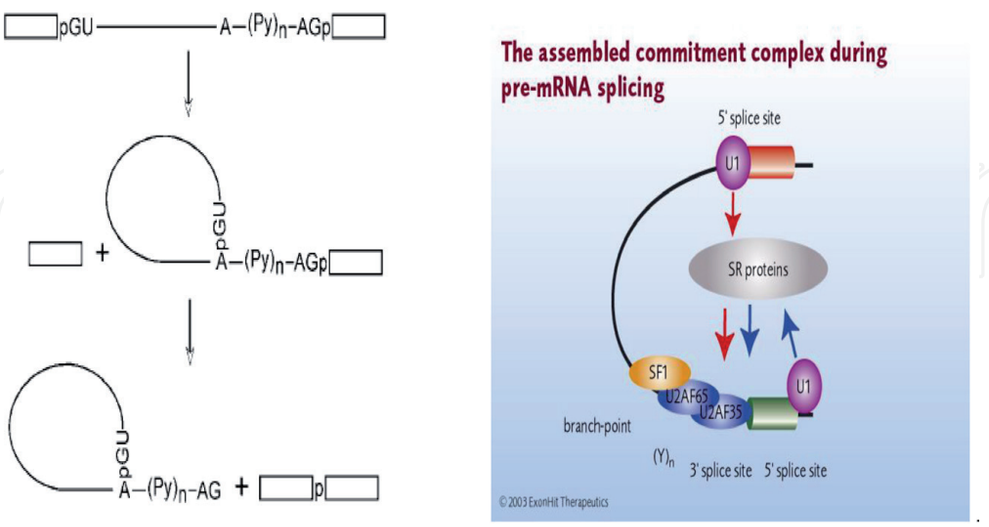
Post-transcriptional regulation mechanisms include both mRNA and protein stabilization as well as stimulated of mRNA and protein degradation. These processes can result in an increase or decrease of enzyme expression, respectively. A good example is CYP2E1 that is regulated by several post-transcriptional mechanisms [19, 20].

1.5 Messenger RNA splicing

As seen in **Figure 2**, splicing is the process that results in excision of the introns from a pre-mRNA and the joining of the resultant exons. The splicing process is directed by special sequences at the intron/exon junctions called splice sites. The 5′ splice site marks the exon/intron junction at the 5′ end of the intron. This includes a GU dinucleotide at the intron end encompassed within a larger, less conserved consensus sequence. At the other end of theintron, the 3′ splice site region has three conserved sequence elements: the branch point, followed by a polypyrimidine tract, followed by a terminal AG at the extreme 3′ end of the intron. Splicing is carried out by the spliceosome, a large macromolecular complex that assembles onto these sequences and catalyzes the two transesterification steps of the splicing reaction. Splicing activators are generally thought to interact with components of the spliceosome to stabilize their binding to adjacent splice sites. SR (splicing regulator) proteins bind to exonic splicing enhancer elements or intronic enhancer elements to stimulate U2AF binding to the upstream 3^{-} splice site, or U1 snRNP binding to the downstream 5′ splice site (**Figure 2B**).

1.5.1 Alternative mRNA splicing

Alternative pre-mRNA splicing is a central mode of genetic regulation in higher eukaryotes (**Figure 3**). Alternative splicingplays an extremely important role in expanding protein diversityand might therefore partially explain the apparent



(A) (B)

Figure 2.
Molecular mechanisms involved in mRNA splicing. (A) Splicing takes place in two transesterification steps. The first step results in two reaction intermediates: the detached 5′- exon and an intron/3′-exon fragment in a lariat structure. The second step ligates the two exons and releases the intron lariat. (B) The complex can be converted into the active spliceosome and involves the recognition of the 5′ splice site by U1 snRNP and the branch-point sequence and 3′ splice site by SF1 and U2AF, respectively with the aid of SR proteins.

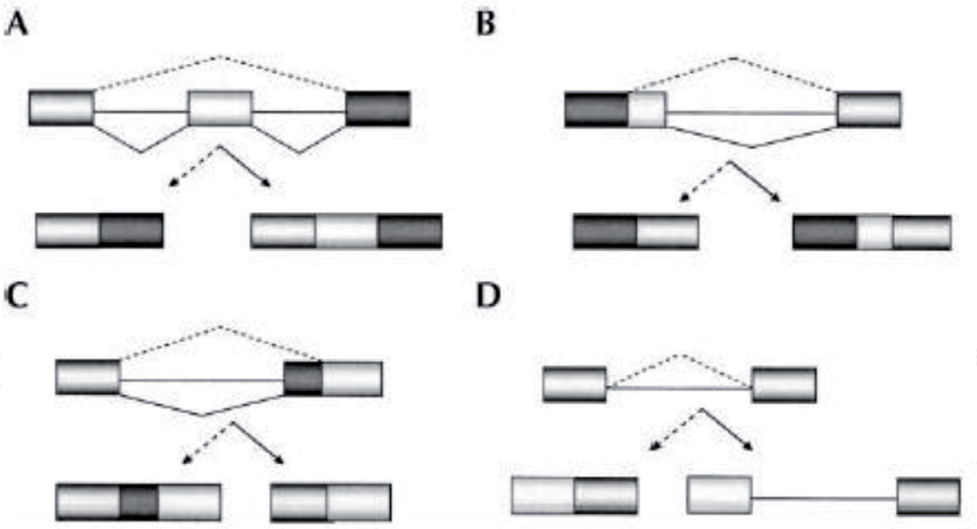


Figure 3.
Patterns of alternative splicing variation. Putative splice variants were classified according to the basic patterns of alternative splicing. (A) Cassette exon: an exon is spliced out with neighboring introns or included in the mature mRNA. (B) Internal donor site: competing donor (5′) splice sites exist for one acceptor site within an intron. (C) Internal acceptor site: competing acceptor (3′) splice sites exist for one donor site within an intron. (D) Retained intron: an intron is spliced out or included in RNA [20].

discrepancybetween gene number and organism complexity [21]. Approximately 40–60% of human genes are estimatedto have distinct splice variants [22]. The regulation of alternative splicing can involve on/off regulationof the products of particular genes and the production of alternativeproducts with clearly separable functions, often in a cell-type-specificmanner [23].

1.5.2 Regulation of splicing: SRproteins and regulatory elements

The SR proteins constitute the best-studied family of splicing regulators. The SR proteins have a common domain structure of one or two RNP-cs RNA binding domains followed by what is called an RS domain containing repeated arginine/serine dipeptides.

The RNA regulatory elements are enhancers or suppressors, diverse in sequence, and often embedded within nucleotides that also code for protein (exonic splice enhancers), but also found in introns. In the intron, the IEs and ISs are often found within a polypyrimidine tract or immediately adjacent to the branch point or 5′ splice site.

However, splicing regulatory elements can also act from a distance, being found hundreds of nucleotides away from the regulated exon.

SR proteins and exonic splicing enhancers (ESEs) play an important role in the regulation of alternative splicing. An SR protein binds to an ESE through its RNA-recognition motifs (RRM) and contacts the components of a spliceosome through its RS domain. Errors in splicing regulation have been implicated in a number of different disease states.

1.6 Effect of alcohol on CYP genes

A Chronic exposure to alcohol produces change in gene expression and alcoholics suffer long-term dysfunction in multiple organ systems, including the liver, immune system and heart [24]. Alcohol is likely to be involved in a significant number of adverse drug reactions.

1.6.1 Inductive effects of ethanol in vitro

Alcohol has been shown to induce hepatic drug metabolism [25].

If administered after a chronic period of alcohol consumption, drugs that are metabolized by enzymes induced by alcohol may have significantly altered biotransformation.

It is found that incubation with ethanol and isopentanol resulted in a synergistic induction of CYP2B1/2 activity and protein levels in cultured rat hepatocytes; and an additive to synergistic induction of CYP2H1/2 activity and protein levels in cultured chick hepatocytes [26].

1.6.2 Inductive effects of ethanol in animals in vivo

It is well established that CYP2E1 is induced by alcohol in humans. In vitro and in vivo human clinical studies have shown that CYP2E1 is induced by ethanol [27–29]. The metabolic ratio of CYP2E1 activity was higher in a group of volunteers that were drinking at least 80 grams of ethanol per day for at least 5 years compared to abstaining alcoholics (for 14 days) and nonalcoholics with liver disease.

In a study subjects drank 40 grams of red wine for a total of four weeks and the metabolic ratio of CYP2E1 activity increased starting after one week of drinking, indicating increased CYP2E1 activity [28]. The ratio continuously increased when measured each week throughout the four-week study.

Brain samples from smoking alcoholics compared to nonsmoking, nondrinking subjects showed increased CYP2B6 protein expression as determined by western blot analysis. In Chapter 3 we use human hepatic and intestinal cell lines to determine whether ethanol can induce CYP2B6 and CYP2B7 mRNA expression; and also explore a role for the nuclear receptor CAR and/or PXR in this induction.

1.6.3 Inhibiting effects of ethanol in vitro

In vitro, concentrations of ethanol (0.1–3%) had inhibitory effects on CYPs 1A1, 1A2, 2A6, 2B6, 2C8, 2C19, 2D6, and 3A4. In human liver microsomes, ethanol inhibits the biotransformation of CYP3A substrates nifedipine, triazolam, and testosterone [30].

1.6.4 Inhibiting effects of ethanol in vivo

There is evidence that an acute dose of alcohol inhibits some human CYPs in vivo. In vitro data may explain the observations that acute ethanol intoxication potentiates the action of barbiturates, while there is increased resistance to the action of some sedatives in sober alcoholics. Ethanol does not appear to inhibit CYP2B6 in vivo in humans since it did not alter pharmacokinetics of bupropion when acutely coadministered with bupropion [31].

1.7 CYP pseudogenes

Pseudogenes are disabled copies of genes that do not produce a functional, full-length copy of a protein. They are of two main types of pseudogenes. Firstly, there are processed pseudogenes. Secondly, there are nonprocessed pseudogenes [32, 33]. With subsequent evolutionary time (generations), these pseudogenes accumulate further coding and noncoding disablements. There are other types of protein-related pseudogenes that are not accounted for in the above classification including semiprocessed pseudogenes.

The extent of the pseudogene population in the human genome is not yet clear. It has been estimated that thereare ~9000 processed and ~ 10,000 nonprocessed pseudogenesin the human genome; [34, 35]. A review of available genomic information indicates that there are many pseudogenes in the CYP family, and it has estimated that there may be more CYP pseudogenes than functional genes within the drug metabolizing enzyme families – CYP 1, 2 and 3 (see **Table 1**). A focus of the studies described in Chapter 4 of this dissertation is CYP2B7P. CYP2B7P has been identified as a pseudogene due to the presence of a single nucleotide change resulting in a premature stop codon in exon 7 (X378) and predicted truncated protein [36].

CYPs	**Function**	**Genes/pseudogenes**
CYP1	drug metabolism	(3 subfamilies 3 genes 1 pseudogene).
CYP2	drug and steroid metabolism	(13 subfamilies 16 genes 16 pseudogenes).
CYP3	drug metabolism	(1 subfamily 4 genes 2 pseudogenes).
CYP4	arachidonic acid or fatty acid metabolism	(5 subfamilies 11 genes 10 pseudogenes).
CYP5	thromboxane A2 synthase	(1 subfamily 1gene).
CYP7A	bile acid biosynthesis 7-alpha hydroxylase of steroid nucleus	(1 subfamily member).
CYP7B	brain specific form of 7-alpha hydroxylase	(1 subfamily member)
CYP8A	prostacyclin synthase	(1 subfamily member).
CYP8B	bile acid biosynthesis	(1 subfamily member).
CYP11	steroid biosynthesis	(2 subfamilies 3 genes).
CYP17	steroid biosynthesis 17-alpha hydroxylase	(1 subfamily 1 gene).
CYP19	steroid biosynthesis aromatase forms estrogen	(1 subfamily 1 gene).
CYP20	Unknown function	(1 subfamily 1 gene).
CYP21	steroid biosynthesis	(1 subfamily 1 gene 1 pseudogene).
CYP24	vitamin D degradation	(1 subfamily 1 gene).
CYP26A	retinoic acid hydroxylase	(1 subfamily member).
CYP26B	probable retinoic acid hydroxylase	(1 subfamily member).
CYP26C	probabvle retinoic acid hydroxylase	(1 subfamily member).
CYP27A	bile acid biosynthesis	(1 subfamily member).
CYP27B	vitamin D3 1-alpha hydroxylase activates vitamin D3 (1 subfamily member).	
CYP27C	unknown function	(1 subfamily member).
CYP39	7 alpha hydroxylation of 24 hydroxy cholesterol	(1 subfamily member).
CYP46	cholesterol 24-hydroxylase	(1 subfamily member).
CYP51	cholesterol biosynthesis lanosterol 14-alpha demethylase	(1 subfamily 1 gene 3 pseudogenes).

Table 1.
Human CYP genes and pseudogenes.

In Chapter 4 we determine whether CYP2B7P is a polymorphic gene in humans, and whether there are genetic variants that code for a full length CYP2B7 protein, lacking the common stop codon (X378). We also evaluate whether the recombinant full-length variant CYP2B7 (R378) is capable of hydroxylating the CYP2B6 substrate bupropion.

2. Conclusion

The main objective of the work described in this thesis dissertation was to study the role of genetic and environmental factors in determining interindividual variability of the CYP2B subfamily.

The results of these studies identify certain CYP2B6 genetic polymorphisms, mRNA splicing variants, and alcohol ingestion as significant factors that determine interindividual variability of CYP2B-mediated oxidation of drugs in people.

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