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Update Information on Drug Metabolism Systems—2009, Part I

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Background

The field of drug metabolism has changed dramatically in the past generation. Two of the driving forces are the advances in analytical chemistry and our understanding of the biological systems. With regard to the former, the advances in liquid chromatography-mass spectrometry (LC-MS) are extremely impressive, and the speed of analysis has been increased even more with the recent developments in ultraperformance LC (UPLC). NMR analysis on a sub-microgram scale is not unusual, and the 2-dimensional methods are also very impressive. The biology underlying gene regulation is highly developed, and the recombinant methods have greatly facilitated progress in the field. Today it is common to design discovery and development efforts focused on critical *human* phenomena from the very start, with animal studies supporting the efficacy and safety efforts.

The previous version of this compilation was published in 2002 [1]. Since then the information has continued to accumulate. Major changes since the last version include the addition of information about transporters and some other enzymes, as well as more cytochrome P450 (P450) data. The standardized P450 nomenclature (i.e., 1A1, 1A2, 1B1, ...) is used, and similar nomenclature systems have been developed for the transporters and other systems [2–5]. *Current Drug Metabolism* uses a different reference format for reviews (and is used in this section), and the Editor has also allowed the use of separate numbers in the tables for clarity and inclusion of PMID numbers to facilitate the ease of finding references.

P450s: Significance and Issues

By several unbiased estimates, ~ 75% of drug transformation reactions involve catalysis by P450s [6, 7]. The next most common reactions are glucuronidations and ester hydrolysis. In the P450 reactions with drugs, 90–95% are catalyzed by five of the 57 human P450s: 1A2, 2C9, 2C19, 2D6, and 3A4, with the latter enzyme (3A4) accounting for ~ ½ of the total [6, 7]. Although there are certainly many reports of the involvement of some other (human) P450s in drug metabolism (e.g. 2A6, 2B6, 2C8), it is not clear that the pattern is changing much. Two trends may be predicted for the future: (i) the contributions of the highly polymorphic P450s 2C19 and 2D6 may decrease, in that most pharmaceutical companies try to avoid developing drugs for which these P450s will have a large contribution. (ii) Drugs

(even small molecule drugs) tend to become larger in order to achieve higher selectivity and thus P450 3A4, with its large active site [8] (and possibly P450 2C8), might be expected to have a growing fraction of the contribution.

The *in vitro* approaches to predicting roles of individual P450s to drug metabolism have been discussed elsewhere [9, 10], including the last Update in this series [1]. Major approaches include (i) comparison of rates measured with recombinant P450s (normalized to P450 content), (ii) correlation of the catalytic activity of interest with a marker activities of individual P450s (or immunochemically determined levels) in multiple human tissue samples, and (iii) analysis of the extent of inhibition of the catalytic activity in liver (or other tissue) microsomes with selective chemical inhibitors and/or P450-specific antibodies. *In vivo* methods include non-invasive pharmacokinetic measurements and their relationship with (i) genetics, (ii) treatment with an established P450 inducer, or (iii) treatment with a selective P450 inducer.

Although the field is highly developed and the fraction of drug candidate attrition due to problems with human pharmacokinetics has decreased [11], *in vitro/in vivo* discrepancies are still an issue [12, 13]. What are some of the reasons? At least five issues can be involved: (i) The substrate concentration used may not be comparable to the *in vivo* concentrations associated with a clinically relevant dose of the drug. (ii) Some of the P450s may be co-regulated, e.g. an *in vitro* correlation study may reflect induction of multiple P450s by an inducer, e.g. barbiturates induce 2B, 2C, and 3A subfamily P450s. (iii) Blood flow may limit *in vivo* metabolism. (iv) First pass effects with drugs can result in metabolism prior to entry to the liver. (v) Transporters may distort the cellular concentrations of drug available for metabolism. Finally, the point should be made that a finite, even if slow reaction by other P450s is often seen and thus, even if are P450 is missing, another might account for a low rate of metabolism. For instance, even in homozygous individuals devoid of P450 2D6, there is a low *in vivo* rate of benzylic hydroxylation of the classic P450 2D6 substrate debrisoquine [14].

Variations in P450 Enzymes

One way of categorizing the human P450 enzymes is in terms their substrates. Roughly one-half of the P450s appear to have endogenous substrates (e.g. steroids, eicosanoids, vitamins) (Guengerich, 2005). The levels of these P450s do not vary considerably, without biological problems. However, the levels of the P450s involved in the metabolism of xenobiotics (including drugs) can vary dramatically. In the cases of most of the P450s, variation of at least an order of magnitude is normal [10] and a 10^4 fold *in vivo* variation in the level of debrisoquine benzylic (4') hydroxylation has already been mentioned [14].

One reason for variations in activity is genetic polymorphism, primarily at the level of single nucleotide polymorphisms (SNPs). The consequences of many of the P450 SNPs have been reported (and are compiled on a web site, <http://www.imm.ki.se/CYPalleles/>), although the effects of the majority of these has not been ascertained. Moreover, the effect of a coding region SNP may vary from one substrate (or reaction) to another. Non-coding SNPs can determine the level of expression of a P450. Although potential of SNP analysis has been

discussed for more than a decade, the application in “personalized medicine” is still not developed. However, the knowledge about P450 SNPs has been applied in considerations of drug development in some cases.

Another basis for variations in P450 activity is enzyme induction. The point should be made that human P450 induction may or may not be predicted by animal studies, but the latter may be critical in drug efficacy and safety studies. *In vitro* methods for predicting induction include actual analysis of levels of mRNA or (preferably) protein in cryopreserved human hepatocytes or mammalian cell culture systems with reporter-based constructs. With the latter it is critical that the relevant accessory proteins be present in the cells. *In vivo* induction by a drug (in humans) involves administration of the drug and measurement of characteristic biomarkers (e.g. caffeine *N*³-demethylation for P450 1A2, midazolam oxidation for P450 3A4). A consequence of P450 induction is decreasing plasma concentration with prolonged drug administration or possibly the increased clearance of drugs concurrently.

Inhibition of P450s by drugs has the opposite effect of induction in pharmacokinetics. A drug may inhibit its own metabolism or, more characteristically, the oxidation of others. Inhibition can have serious consequences, including adverse events and even death. A classic example is the inhibition of terfenadine oxidation (P450 3A4) by azoles and erythromycin, which caused the parent drug to accumulate and block the hERG channel, causing abnormal QT intervals [15]. Inhibition analyses can be done *in vitro* using microsomes or recombinant P450s, using competitive methods with LC-MS or fluorescence methods [16]. The procedures for analyses of mechanism-based inactivation are similar and involve the inclusion of pre-incubation modes [17]. A knowledge base of P450 inhibitors is useful for prediction of drug-drug interactions. Different pharmaceutical companies have different views of what parameters of *in vitro* inhibition will translate into *in vivo* problems. Literature in this area is available [18]. Ultimately the question of the importance of inhibition must be addressed *in vivo* in each case.

Transporters

The majority of drugs are hydrophobic, and historically the assumption had been made that drugs diffused into cells through the membranes, were transformed into more polar derivatives, and then left the cells as such. The Multiple Drug Resistance (MDR) transporter was first observed because of its role in inferring resistance to drugs in cancer cells [19] by pumping them out. As knowledge about this system and P450 3A(4) developed in the late 1980s, the overlap in substrate specificity was recognized [20]. Subsequently the expression of the MDR1 protein in liver and small intestine was detected [21]. The role of the MDR1 protein (also called P-170 or P-glycoprotein) as a drug export protein was realized in the 1990s [22,23].

Following the development of the MDR1 system as a drug exporter, scientists in the field characterized other drug exporters and also drug importers. The number of proteins involved in these processes has grown. The overall view today is that passive diffusion of drugs in

and out of cells does occur in many cases but transporters can have important roles in many cases and can major effects on tissue selectivity of drugs [4, 5].

The transporters can be considered in the context of their own activities and also their interactions with P450s. First of all, the regulation of some transporters involves the same systems as P450s, e.g. P-glycoprotein and some of the P450 3A and 2C subfamily enzymes are regulated by PXR [23]. Another situation is that P-glycoprotein can decrease the level of a drug and thereby decrease the induction of a P450 [24, 25]. An export transporter can also lower the level of a drug in a cell and accordingly slow the rate of its metabolism.

Today we have many examples of the roles of transporters in drug disposition. One of the most dramatic is with many of the statins. All statins have the same mechanistic target, 3-hydroxymethylglutaryl (HMG) CoA reductase. Some of the more hydrophilic statins are imported into liver cells by transporters, e.g. OATP2 [26, 27]. Export transporters (e.g. P-glycoprotein) also work on some of the statins. Inhibition of HMGCoA reductase affects not only cholesterol synthesis but also the synthesis of compounds in the mevalonate pathways such as geranylgeranyl. The overall goal with statins is to achieve selective transport into hepatocytes in the liver, the site of cholesterol synthesis, and avoid buildup of the statins in the plasma and extrahepatic tissue such as muscle, where HMGCoA reductase inhibition can lead to rhabdomyolysis [27]. Thus the correct balance of transporters can be important in determining the safety and efficacy of individual statins. The role of transporter polymorphisms in statin toxicity is beginning to be studied [28].

Other Enzymes Involved in Drug Metabolism

Some of the other enzymes involved in drug metabolism are also included in this Update. In most cases the specific roles of individual enzyme family members have not been established, although some of the UGTs and FMOs are now being studied more. We anticipate that more information will be available by the next Update in this series.

Other Resources

Finally, we refer the reader to some websites that contain useful information about drug metabolism and disposition. The David Nelson website contains current information about P450 genes: <http://drnelson.utmem.edu/CytochromeP450.html>. A website managed at the Karolinska Institute has summaries of information about the human P450 gene allelic variants and, when available, the effects on catalytic function (*in vitro* and *in vivo*), plus original literature references <http://www.imm.ki.se/CYPalleles/>. A site maintained by David Flockhart (Indiana University School of Medicine) has information on clinical aspects of drug metabolism and interactions: <http://medicine.iupui.edu/clinpharm/ddis/>.

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