IMPLICATIONS OF POLYMORPHIC CYTOCHROME P450-DEPENDENT DRUG METABOLISM FOR DRUG DEVELOPMENT

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ABSTRACT:
The main part of human cytochrome P450-dependent drug metabolism is carried out by polymorphic enzymes that can cause abolished, quantitatively or qualitatively altered, or enhanced drug metabolism. Ultrarapid metabolism is due to stable duplication, multiduplication, or amplification of active genes. Several examples exist where subjects carrying certain alleles suffer from a lack of drug efficacy due to ultrarapid metabolism or, alternatively, adverse effects from the drug treatment due to the presence of defective alleles. The polymorphic enzymes create a problem for the drug industry because of the extensive interindividual variability in the metabolism of candidate drugs that are substrates for such enzymes. The new area for lead generation has a more preclinical emphasis and involves combinatorial chemistry in conjunction with high-throughput-based analysis of thousands of substances with respect to their absorption, metabolism, and excretion characteristics. The outcome is that companies drop substrates for polymorphic enzymes at an early stage in development, which will of course create fewer problems with polymorphic enzymes in the future. The risk is that very valuable candidates, which cannot be replaced easily, never come out on the market. The alternative, however, of using the patient’s genotype as a basis for individualized drug treatment constitutes, in light of rapid methodological developments, a very feasible approach to safer and more efficient drug therapies.

Pharmacokinetics and drug metabolism have been shown to be of greater importance during drug development today. It is evident that drugs that are too rapidly metabolized by drug-metabolizing enzymes mainly localized in the liver and the intestine are nonoptimal therapeutic candidates. In published overviews, it has been concluded that as many as 30 to 40% of the drugs undergoing clinical trials were withdrawn from further development due to unfavorable pharmacokinetic properties (Prentis et al., 1988; Monro, 1996). The recent and very rapid progress in the field of drug metabolism has allowed for the identification of the major phase I and phase II enzymes responsible for the metabolic conversions. This knowledge, in combination with combinatorial chemistry techniques, opens up the possibility for pharmaceutical industries to rapidly screen drug candidates with respect to their pharmacological and pharmacokinetic properties at an early stage in drug development, which prevents unnecessary and costly clinical trials. First, human liver microsomes as well as heterologously expressed enzymes can be used for in vitro primary screening of the metabolic stability and enzyme selectivity of the compound. In support of this approach, the intrinsic microsomal in vitro clearance of drugs largely predicts their in vivo clearance. The absorption can then be measured in vitro using Caco-2 or Madin-Derby canine kidney cell containing membranes. Finally, in vivo absorption and pharmacokinetic properties are determined using cassette protocols with simultaneous administration of up to 10 compounds and concomitant mass spectrometry analysis (Tarbit and Beman, 1998).

A major obstacle for the drug industry today is, however, the extensive interindividual variation in human drug metabolism. This variation can lead to a variety of outcomes that are often difficult to foresee, which include therapeutic failure, adverse effects, and toxicity in selected subpopulations undergoing treatment. Indeed, the incidence of serious and fatal adverse drug reactions might be extremely high among hospitalized patients and may result in over 100,000 deaths per year in the U.S., making it between the fourth and sixth leading cause of death in 1994 (Lazarou et al., 1998). It is plausible that a substantial amount of these deaths could be prevented by predictive genotyping preceding the drug treatment in question. Over- and underdosage of drugs has been estimated to cost more than 100 billion dollars per year in the U.S. because of prolonged hospitalization, decreased productivity, and premature death.

The main causes for the variation observed in drug metabolism are 1) genetic polymorphisms, 2) induction or inhibition due to concomitant drug therapies or environmental factors, 3) physiological status, and 4) disease states. Of these, the first two appear to be of major importance for the occurrence of adverse effects or lack of therapeutic efficacy in many cases. Recently, knowledge concerning the molecular basis for P4501 induction caused by several drugs, including rifampicin, nifedipine, and phenobarbital, has made great progress. This has occurred through the identification of the function of two orphan receptors, namely the constitutive androstane receptor and the pregnane X receptor, as mediators for drug-induced increases in P450 gene transcription (Honkasoski et al., 1998; Kliewer et al., 1998). In particular the pregnane X receptor, with its ability to sense cellular levels of steroids and xenobiotics and control the rate of their degradation through the induction of P450 enzymes, appears to be a very interesting target for further studies regarding its role in drug-medi-

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1 Abbreviations used are: P450, cytochrome P450; PM, poor metabolizer.
ated P450 induction. It remains to be established the extent to which polymorphism in these genes can explain interindividual differences in induction and constitutive expression of drug-metabolizing enzymes.

With respect to the genetic polymorphism of drug-metabolizing enzymes, the drug industry has now started to drop drug candidates that are primarily selective substrates for the polymorphic enzymes. The similar structure of the active site of CYP2D6 and receptors of importance for psychoactive drugs is striking; however, the difficulty in developing drugs that indeed do interact with the same receptors but which are not substrates for CYP2D6 is apparent. An alternative to this approach would be to individualize the drug dose based on the genotype of the specific patient and take both pharmacokinetic and pharmacodynamic aspects into consideration. This method could be more advantageous and avoid the elimination of candidate drugs that would otherwise be the most suitable. Novel high-throughput methods for genotyping, which include the oligonucleotide chips array technology and automated single nucleotide polymorphism detection techniques whereby multiple mutations can be screened rapidly, make this a feasible approach that can be easily applied to clinical situations.

P450 Enzymes in Drug Metabolism

An evaluation of the mechanism for the metabolic clearance of 315 different drugs revealed that 56% of them were primarily cleared through the action of the cytochrome P450 enzymes, with CYP3A4 being by far the most important (50%) followed by CYP2D6 (20%), CYP2C9/19 (15%), and the remaining metabolism carried out by CYP2E1, CYP2A6, CYP1A2, and unidentified P450s (Bertz and Granneman, 1997). Of these enzymes, all are inducible except for CYP2D6. Here induction in response, most likely to dietary stress, has instead been accomplished by a selection for alleles with duplicated, multiduplicated, and amplified active CYP2D6 genes (Johansson et al., 1993; Ingelman-Sundberg et al., 1999). A very clear relationship between the CYP2D6 catalytic activity and the number of active genes has been seen. All these CYP enzymes have been shown to be polymorphic, most recently also CYP3A4 (Sata et al., 2000). A summary of the most important functionally variant alleles is shown in Table 1. Also, the phase II enzymes are to the main extent polymorphic (see Evans and Relling, 1999).

### Polymorphic P450 Genes

In general, alleles causing defective, qualitatively altered, diminished, or enhanced rates of drug metabolism have been identified for many of the P450 enzymes. Gene deletions, gene conversions with related pseudogenes, and point mutations causing frameshifts, premature termination of translation, or aberrant splicing are all causes of inactive alleles. The number of known defective alleles is growing and at present at least 30 different defective CYP2D6 alleles and about 55 CYP2D6 gene variants in total have been identified (Marez et al., 1997). In addition, at least four defective CYP2D6 alleles (Oscarson et al., 1999) and six defective CYP2C19 alleles are known (Ibeanu et al., 1999). Concerning CYP2D6, however, it appears that genotyping for only the six most common defective alleles will predict the phenotype with about 95 to 99% certainty. A continuously updated database where all old and new polymorphic human P450 alleles encoding enzymes participating in the metabolism of xenobiotics has been established. Here, the location of the single nucleotide polymorphism, the functional consequences, as well as direct links to the relevant literature references are given. The site, which is found at http://www.imm.ki.se/CYPalleles/, has Mikael Oscarson as webmaster; Magnus Ingelman-Sundberg, Ann K. Daly, and Daniel W. Nebert as editors, and an International Advisory Committee with initially Jürgen Brockmöller, Michel Eichelbaum, Seymour Garte, Joyce A. Goldstein, Frank J. Gonzalez, Fred F. Kadlubar, Tetsuya Kamataki, Urs A. Meyer, David R. Nelson, Michael R. Waterman, and Ulrich M. Zanger. The web site contains the latest updated knowledge about the allelic variants of genes in the human CYP1, CYP2, and CYP3 families. Ultimately, a listing of the common alleles of all 49 human CYP genes that are presently known is planned, and the page will in due time be continuously updated as new allelic variants of each CYP gene are described.

The principal function of this web homepage is to encourage scientists worldwide to speak the same language and to avoid “home-made” allelic designations that would only confuse the nomenclature system and the scientific literature. An important additional function of this web site is to rapidly update everyone about the progress in this rapidly moving field, thereby avoiding unnecessary work to characterize alleles that have been already described. It is anticipated that several new alleles will be published only on the web site.
Impact of P450 Polymorphism on in Vivo Metabolism of Drugs

The P450 alleles carried by a patient will influence the success of some drug treatments. Subjects with multiple gene copies will metabolize drugs more rapidly, and therapeutic plasma levels will not be achieved at ordinary drug dosages. For example, subjects with 13 CYP2D6 gene copies on one allele treated with nortriptyline form a substantially higher amount of the metabolite (10-hydroxynortriptyline) than subjects carrying lower numbers of active CYP2D6 genes. If such subjects are taking the prodrug codeine, extensive formation of morphine occurs in a reaction catalyzed by CYP2D6 (Hedenmalm et al., 1997). Severe abdominal pain, a typical adverse effect of morphine, has been observed in an ultrarapid metabolizer treated with codeine (Dalén et al., 1997).

Individuals lacking functional CYP2D6 genes have been shown to metabolize selective CYP2D6 substrates at a lower rate, particularly antidepressants and neuroleptics. The CYP2D6 genotype has been successfully shown to predict the clearances of, for example, the antidepressants desipramine, fluvoxamine, fluoxetine, mexitetin, and citalopram as well as the clearance of the neuroleptics perphenazine and zuclopenthixol and the competitive muscarinic receptor antagonist tolterodine (Ingelman-Sundberg et al., 1999). Adverse effects due to elevated drug plasma levels would be expected to occur more frequently in cases wherein the drug clearance is dependent on CYP2D6.

A lack of CYP2D6 enzyme would be expected to result in reduced effectiveness in drug therapy in instances where prodrugs requiring activation by CYP2D6 are used. For example, the antiarrhythmic effect of the prodrug encainide and the decreased analgesic effect of the CYP2D6-catalyzed 6- and 7-hydroxylation of S-warfarin, as well as many other substrates such as losartan, phenytoin, and tolbutamide. The corresponding enzyme variants, in particular CYP2D9*3, are much less effective in the in vitro conversion of warfarin. Thus, the clearance of S-warfarin among subjects homozygous for the CYP2D9*3 allele has been shown to be reduced by 90% compared with subjects homozygous for the wild-type allele. Cases have been described wherein a dose of only 0.5 mg of racemic warfarin per day was necessary for homozygous carriers of the CYP2D9*3 allele as opposed to the 5- to 8-mg daily dose required for subjects homozygous for wild-type CYP2C9 alleles (Steward et al., 1997). Patients in low-dose warfarin groups displayed a substantial overrepresentation of variant CYP2C9 alleles and exhibited a much higher risk of experiencing side effects at the beginning of therapy as well as an increased risk of major bleeding complications, when compared with random clinical controls (Aithal et al., 1999).

Subjects of the CYP2C19 PM phenotype have an area under the curve of omeprazole more than 12-fold higher than efficient metabolizers, and the drug has a severely prolonged half-life in PM individuals (Andersson et al., 1992). A similar relationship is seen for other proton pump inhibitors (Andersson et al., 1998). To reach similar plasma levels, PMs of CYP2C19 would take about 1 to 2 mg of omeprazole instead of the recommended dose of 20 mg. In fact, there have been reports wherein treatment of omeprazole in combination with amoxicillin treatment has a much higher cure rate on peptic ulcer and Helicobacter pylori infection in CYP2C19 PMs as compared with efficient metabolizers (Furuta et al., 1998). The antimalarial prodrug proguanil is another selective CYP2C19 substrate, and its oxidation to cycloguanil is dependent on CYP2C19.

CYP2A6 is responsible for the C-oxidation of nicotine, which is the major pathway in nicotine metabolism (Benowitz and Jacob, 1997). CYP2A6 is also active in the metabolism of several clinically used drugs; among them, Zendraand is able to metabolically activate some precarcinogens. At present four different defective alleles of CYP2A6 have been found. Pianezza and coworkers (1998) have proposed that individuals with at least one defective CYP2A6 allele are underrepresented in a group of smokers compared with a never-tobacco-dependent control group. Furthermore, smokers carrying a variant CYP2A6 allele, as determined by the authors, smoked significantly fewer cigarettes. This is an interesting finding, but the method used for determination of both the CYP2A6*2 and CYP2A6*3 alleles has been inaccurate and the correct genotyping has therefore not been achieved. In fact, results by Oscarson et al. (1998, 1999) show that the allele frequency of the CYP2A6*2 allele is only 1 to 3% and that the CYP2A6*3 allele does not exist in a large Spanish and Chinese population examined. The basis for this erroneous genotyping is the common distribution of the CYP2A6*1B allele in the populations, which has a gene conversion event with a small piece of the pseudogene CYP2A7 just 3′ of exon 9, resulting in the absence of CYP2A6 gene amplification in such subjects, using the genotyping method originally described by Fernandez-Salguero et al. (1995) and used by Pianezza et al. (1998).

CYP2E1 is relatively well conserved between individuals, and only three rare alleles yielding amino acid substitutions have been described (Hu et al., 1997; Fairbrother et al., 1998). Only one of those (CYP2E1*2) causes any measurable effect on function, yielding a smaller amount of catalytically active enzyme expressed. A relatively high extent of conservation is also seen in the CYP3A4 gene. Full sequencing of the open reading frame from almost 100 individuals revealed that only two alleles creating amino acid substitutions were seen (Sata et al., 2000). One of those, CYP3A4*2, is causing a S222P amino acid change and an enzyme with altered substrate affinity; the other, found in one Chinese subject, is CYP3A4*3, having M445T with unknown consequences.

In conclusion, we believe that the most important polymorphisms causing genetic differences in phase I drug metabolism are known and therapeutic failures or adverse drug reactions caused by polymorphic genes can to a great extent be foreseen. This information is currently being used by the drug industry during drug development. Many drug industries regularly genotype the patients involved in their clinical trials to obtain more information regarding pharmacokinetic properties and observed side effects. Furthermore, candidate drugs that are selectively metabolized by polymorphic enzymes are often dropped early in drug screening; therefore, fewer problems with polymorphic enzymes during drug therapy will apparently be the case in the future. This might result in the failure to develop efficient drugs where the target receptors have structural similarities to the polymorphic drug-metabolizing enzymes. However, due to the rapid development of efficient and inexpensive methods for genotyping and the need to genotype a patient only once in a lifetime, it would be more advisable to include the genotypes in the patient’s medical record, providing the doctor with valuable information to individualize the drug treatment. Due to the rapid development in the field of genotypes affecting the pharmacodynamic effects of drugs, this could also include genotypes of drug receptors, which taken together would provide the doctor with highly predictive genetic information concerning the likelihood of successful drug therapy.

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References


