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In Vitro Systems for Prediction of Rates of Drug Clearance and Drug Interactions

Interindividual variation in the metabolism of drugs is a significant problem in clinical pharmacology. This variability is particularly important with drugs having narrow therapeutic indexes. Rapid metabolism and clearance of a drug can result in little sustained effectiveness, and slow metabolism can cause hyperresponse and, in some cases, toxicities.

Another problem of immense clinical importance is drug interactions. In many pathophysiologic states, patients receive several drugs simultaneously. If two or more compounds are metabolized or inactivated by the same enzyme, problems can arise because of a slower clearance of one of the agents. Again, these interactions are particularly important with drugs having narrow ranges of serum concentrations for their therapeutic effectiveness.

Complications due to interindividual variabilities in response to drugs and drug-drug interactions have usually been recognized during clinical trials or after a period of time on the market. It is now possible by use of *in vitro* analyses to predict whether problems might arise with a particular drug. These procedures have been used and validated by a number of studies on drugs currently in use and having known compromising determinants. In this issue of ANESTHESIOLOGY, Yun *et al.*¹ determine the enzyme responsible for metabolic activation of the synthetic opioid alfentanil. Use of this drug is complicated by a high degree of interindividual variability in the plasma concentration, requiring customized, individually dosed infusion.² Metabolism was postulated to be the pri-

mary determinant in this variability,³ but the polymorphically expressed cytochrome P450 CYP2D6, debrisoquine 4-hydroxylase, was found not to be a factor.⁴ However, a number of other human P450s are known to exist. To date, 17 P450s known to metabolize foreign compounds have been identified.⁵⁻⁷ Almost all of these enzymes exhibit a high degree of interindividual variability in their levels of expression. Some are also subject to induction or inhibition by drugs and even dietary substances. Thus, it is necessary to determine which P450 is responsible for metabolism of a particular drug in order to understand fully and, more importantly, predict whether it will present problems.

Yun *et al.*¹ use two independent methods to determine which P450 form is responsible for metabolism of alfentanil. Using a panel of human liver specimens obtained from accident victims and kidney donors in which levels of individual P450 forms have been well characterized, they measured metabolism of the drug. The rate of metabolism was highly variable among livers and correlated quite well with known levels of the P450 designated CYP3A4. These results were confirmed by immunoinhibition of activity using an antibody directed against CYP3A4-type P450s. In addition, the authors directly demonstrated that CYP3A4, produced by complementary DNA-directed expression in yeast, was also able to metabolize alfentanil. Another important aspect of this study, as the authors note, is that a number of drugs are known to be metabolized by CYP3A4, allowing predictions of drug-drug interactions with alfentanil. The concentrations of CYP3A4 can also be induced by steroids, and the enzyme is inhibited by a number of agents including na-

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ringenin, found in grapefruit juice, and certain antibiotics.

To date, most studies on human drug metabolism *in vitro* have been retrospective in nature: the molecular basis of known problems in the clinical use of drugs has been investigated using procedures as described by Yun and coworkers. Historically, during drug development, animal models have been the primary means of assessing drug metabolism and safety. In many cases, however, rodents can metabolize a compound quite differently from humans, and this difference can create problems in the prediction of future side effects during clinical use.

It would be preferable to use human-based systems to test the chemicals to which we will be exposed. Indeed, production of recombinant human drug-metabolizing enzymes with systems such as the yeast used in the work by Yun *et al.* is becoming quite common.⁸ Cell lines containing individual human P450s are now available to academic and industrial laboratories by purchase from the Gentest Corporation (Woburn, MA). Cells have also been modified to contain a number of P450s and other foreign compound-metabolizing enzymes.⁹ These can be used to predict whether a compound will be activated to a toxic or mutagenic metabolite by human P450s and how a particular chemical will be metabolized in humans. Once the enzymes responsible for metabolism in humans are identified, one has only to refer to the literature to determine other drugs metabolized by the same P450 and whether this P450 is subject to wide interindividual variability in expression. In addition, the extent of extrahepatic expression of a particular P450 might also bear some clinical relevance to the use of certain drugs.

The possibility of phenotyping or genotyping for multiple P450 expression in humans may soon become a reality. A polymerase chain reaction (PCR)-based genotyping assay for the presence of normal and mutant CYP2D6 genes is now available for use in research and could be developed for the clinic.¹⁰ Allelic mutants and enzymatic variants of other P450 forms undoubtedly will be found, and these could be detected by PCR. Multiple pharmacogenetic defects in drug metabolizing enzymes such as the N-acetyltransferases,^{11,12} butyrylcholinesterases¹³ and glutathione S-transferases may lend themselves to simultaneous PCR analysis using nonradioactive allele-specific probes.¹⁴

Chemical probes are currently being used to determine levels of P450 expression by analysis of P450-derived urine metabolites. For example, caffeine can be used to evaluate levels of CYP1A2¹⁵ and perhaps even CYP2E1¹⁶ activity in humans. Chlorzoxazone, coumarin, and warfarin are also potential probes for levels of CYP2E1¹⁷ CYP2A6¹⁸ and CYP2C9,¹⁹ respectively. Erythromycin has been used for determining the expression of the CYP3A P450s.²⁰ Thus, within a few years, with combinations of

PCR and chemical probes, we may be able to determine the foreign compound metabolic potential of individuals to aid in drug therapy and in molecular epidemiology studies to determine if P450 expression is associated with increased risk to environmentally based disease.

FRANK J. GONZALEZ, PH.D.

Head, Nucleic Acids Section
Laboratory of Molecular Carcinogenesis
National Cancer Institute
National Institutes of Health
Building 37, Room 3E24
Bethesda, Maryland 20892

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