between beneficial and harmful cytokines or whether some cytokines are beneficial in low amounts but toxic at high levels. Studies addressing these types of questions will provide new insights into the liver injury and clinical manifestations of alcoholic hepatitis.

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PREDICTING DRUG INTERACTIONS USING CULTURED HUMAN HEPATOCYTES

Pichard L, Fabre I, Fabre G, Domergue J, Saint Aubert B, Mourad G, Maurel P. Cyclosporin A drug interactions: screening for inducers and inhibitors of cytochrome P-450 (cyclosporin A oxidase) in primary cultures of human hepatocytes and in liver microsomes. Drug Metab Dispos 1990;18:595-606.

ABSTRACT

In previous papers we demonstrated that cyclosporin A (CsA) was specifically oxidized in rabbit and human liver by cytochrome P-450IIIA. We therefore anticipated that any drug that is an inducer or an inhibitor of this cytochrome should lead to interaction with CsA when given in association with it. In order to confirm this hypothesis, primary cultures of human hepatocytes and human liver microsomes were used to "reproduce" *in vitro* clinically significant interactions observed between CsA and drugs known either as specific inducers (i.e., rifampicin) or as specific inhibitors (i.e., erythromycin) of P-450IIIA. Our results were in close agreement with the clinical reports. Human hepatocytes maintained in primary cultures for 72 hr in the presence of 50 μM rifampicin exhibited increased levels of P-450IIIA, determined by Western blot using specific antibodies, and concomitant increase in CsA oxidase activity, determined by HPLC analysis of extra and intracellular media. Conversely, these cultures exhibited erythromycin concentration-dependent decreases in CsA oxidase activity when incubated in the presence of 5, 20, and 100 μM erythromycin. In addition, a Lineweaver-Burk analysis of the erythromycin-mediated inhibition of CsA oxidase activity in human liver microsomes revealed competitive inhibition (with K_i of 75 μM) as expected, this macrolide being a specific substrate of P-450IIIA. Using this experimental approach, 59 molecules representative of 17 different therapeutic classes were screened for inducers and inhibitors of CsA oxidase activity. Our results allowed us to elucidate the molecular mechanism of previously observed, but unexplained, drug interactions involving CsA, and to detect drugs that should interfere with CsA metabolism as inducers or inhibitors. Drugs detected as potential inducers of CsA oxidase included: rifampicin, sulfadimidine, phenobarbital, phenytoin, phenylbutazone, dexamethasone, sulfinpyrazone, and carbamazepine. Drugs detected as potential competitive inhibitors included: triacetyloleandomycin, erythromycin, josamycin, midecamycin, ketoconazole, miconazole, midazolam, nifedipin, diltiazem, verapamil, nicardipine, ergotamine, dihydroergotamine, glibenclamide, bromocryptine, ethynylestradiol, progesterone, cortisol, prednisone, prednisolone, and methylprednisolone. Finally cefoperazone, cefotaxime, ceftazidime, isoniazide, doxycycline, spiramycin, sulfamethoxazole, norfloxacin, pefloxacin, vancocin, trimethoprime, amphotericine B, valproic acid, quinidine, cimetidine, ranitidine, omeprazole, diclofenac, aspirin, paracetamol, debrisoquine, guanoxan, captopril, furosemide, acetazolamide, sparteine, gliclazide, and imipramine were found not to interfere with the hepatic metabolism of CsA.

COMMENTS

More than 100 different cytochromes P450 (P450s) have been identified in laboratory animals, and at least 20 have been identified in man (1). The P450s that appear to be most involved in drug metabolism belong to three distinct families (designated as I, II and III), which are further divided into subfamilies (designated by capital letters). A variety of techniques have been used to determine which P450s metabolize which drugs. The newest technique is the insertion of specific P450 cDNAs into cell lines that are deficient in drug-metabolizing capability. Such cell lines, some of which are now commercially available, then produce functional enzymes and can therefore be used to characterize the catalytic properties of individual P450s. This technique has been used to show that two closely related enzymes within the P450IIIA subfamily are capable of generating the major metabolites of cyclosporin A (CsA) produced in patients *in vivo* (2). P450IIIA catalytic activity varies at least 10-fold and appears to have a unimodal distribution among patients (i.e., discrete "rapid" and "poor" metabolizer populations have not been identified) (1).

Enzymes outside the P450IIIA subfamily probably contribute little to CsA metabolism because antibodies that selectively recognize P450IIIA enzymes inhibit most of the CsA metabolism that occurs in human liver microsomes (3-5).

In a recent study (6) where P450IIIA activity was noninvasively measured in 32 patients before they began treatment with CsA, patients with higher P450IIIA activity had lower dose-adjusted blood levels of CsA, and patients with low P450IIIA activity had higher dose-adjusted blood levels. This inverse correlation was highly significant and suggests that the catalytic activity of P450IIIA is rate limiting in the metabolism of CsA. It logically follows that any drug that influences the catalytic activity of P450IIIA could produce an interaction with CsA. If a drug is normally metabolized by P450IIIA and therefore competes with CsA for binding to the substrate binding (active) site of the enzyme, the rate of CsA metabolism should fall. Conversely, treatment with some drugs could result in the induction of P450IIIA catalytic activity, which could result in accelerated metabolism of CsA.

Pichard et al. directly investigated the role of P450IIIA in drug interactions by measuring CsA metabolism in cultured human hepatocytes exposed to a battery of medications. When drugs previously reported to cause clinically significant elevations in CsA blood levels were added to the culture medium, the result was a diminished rate of CsA metabolism. The investigators then characterized the interactions between these drugs and P450IIIA in human liver microsomes. In most cases, they found that the inhibition of CsA metabolism in the hepatocytes resulted from competitive inhibition of P450IIIA catalytic activity, most likely because the inhibiting drugs were also metabolized by P450IIIA. When drugs known to produce clinically significant decreases in CsA blood levels were added to the culture medium, accelerated metabolism of CsA was observed. This induction was accompanied by increases in the hepatocyte concentration of P450IIIA protein. These data strongly support the idea that most drug interactions involving CsA result from competitive inhibition or induction of P450IIIA.

It should be pointed out that the data presented are not entirely consistent with a primary role of liver P450IIIA in drug interactions. For example, the K_i determined for erythromycin inhibition of CsA metabolism in human liver microsomes was 75 μ-mol/L, a concentration that exceeds by at least an order of magnitude the peak blood levels observed in patients treated with erythromycin. Indeed, intravenous administration of erythromycin has been reported to have relatively small effects on the pharmacokinetics of CsA (7). It may therefore be important that P450IIIA enzymes are also abundant in jejunal enterocytes where they may significantly contribute to "first pass" metabolism of CsA (8). After oral dosing, the enterocyte concentrations of erythromycin could well exceed the determined K_i, resulting in significant inhibition in gut metabolism of CsA.

The data presented by Pichard et al. add to the growing body of literature supporting cultured human hepatocytes as a valid model for studying the regulation of human liver P450s. In this regard, human hepatocytes appear to be "hardier" than rat hepatocytes in that the latter tend to rapidly lose responsiveness to many inducers in culture. The importance of the work by Pichard et al. is that it provides the best evidence to date that *in vitro* models of P450 regulation are capable of yielding new and important information for clinicians. For example, the investigators were able to identify some drugs that are potent inhibitors of P450IIIA (such as miconazole) or inducers of P450IIIA (sulfinpyrazone and phenylbutazone) that have not been previously reported to interact with CsA. It seems reasonable to alert physicians to these likely interactions. Pichard et al. were also able to identify many drugs that appeared to have no effect on P450IIIA activity and that may therefore be safe alternatives to drugs that interact with CsA. For example, they suggested that the macrolide antibiotic spiramycin, which had no effect on CsA metabolism, would be a suitable substitute for erythromycin in patients receiving CsA. Finally, although the investigators focused on CsA, their data provide potential explanations for some well-known interactions involving other drugs. Any drug shown to be a substrate for P450IIIA should be metabolized at a reduced rate in the presence of a P450IIIA inhibitor and at an increased rate in the presence of a P450IIIA inducer. For example, prednisone and troleandomycin (TAO) were shown to inhibit CsA metabolism and to bind to P450IIIA. It therefore seems likely that the "steroid sparing" effect of TAO in patients with chronic obstructive pulmonary disease is caused by reduced metabolism of prednisone by P450IIIA resulting from the inhibition of the enzyme by TAO. Conversely, because at least some estrogens are metabolized by P450IIIA (9), induction of P450IIIA by rifampicin may explain why women treated with this drug may experience failure of oral contraceptives (1).

It remains to be determined how often the activity of a P450 is rate limiting in the metabolism and elimination of drugs. Nonetheless, the concepts raised by this article have not escaped the notice of drug companies who would obviously like to be alerted to potential drug interactions before they are experienced in clinical trials. Many companies are setting up human hepatocyte culture facilities, although it is likely that difficulty obtaining human liver tissue will limit some of the potential applications. Fortunately, Pichard et al. found that the inhibition of CsA metabolism in hepatocytes could be reproduced in microsomes in most cases. It should therefore be possible to use recombinant P450s and/or human liver microsomes to identify most drugs likely to produce interactions by inhibiting P450s. Induction studies, on the other hand, currently require living cells. Unfortunately, animal-model results often cannot be extrapolated to humans because interspecies

differences exist in the regulation of at least some of the P450s (10). As the molecular mechanisms that underlie induction of human liver P450s are characterized, it is likely that cell-free assays will be developed to screen drugs for induction properties.

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IDENTIFICATION OF GENETIC DIFFERENCES IN DRUG METABOLISM: PREDICTION OF INDIVIDUAL RISK OF TOXICITY OR CANCER

Gough AC, Miles JS, Spurr NK, Moss JE, Gaedigk A, Eichelbaum M, Wolf CR. Identification of the primary gene defect at the cytochrome P_{450} *CYP2D* locus. Nature 1990;347:773-776.

Heim M, Meyer UA. Genotyping of poor metabolisers of debrisoquine by allele-specific PCR amplification. Lancet 1990;336:529-532.

EDITOR'S ABSTRACT

These two reports describe recombinant DNA tests that can identify individuals having a defect in the cytochrome P450IID6 *(CYP2D6)*–mediated oxidative metabolism of debrisoquine and more than two dozen other drugs that are commonly prescribed. The poor metabolizer (PM), representing 5% to 10% of the northern European white population is homozygous for an autosomal recessive trait. Compared with the extensive metabolizer (EM) phenotype, the PM individual is more prone to toxicity caused by some of these drugs. Curiously, the PM phenotype appears to be associated with a lower risk of lung and bladder cancer.

Gough and coworkers propose that *the* primary gene defect associated with the *CYP2D6* polymorphism appears to be a G to A transition mutation affecting the splice junction of intron 3/exon 4 of the *CYP2D6* gene. They offer a polymerase chain reaction (PCR) amplification method for diagnosing three variant alleles and claim to have predicted the phenotype of all normal and 34 of 42 (81%) PM individuals. Based on the known frequencies of those variant alleles detectable by this method, however, the assay would be expected to predict less than 60% of PM patients. Heim and Meyer, on the other hand, have combined allele-specific PCR with restriction fragment length polymorphism (RFLP) patterns to identify more than 95% of all mutant alleles, suggesting that their assay would accurately predict more than 90% of all PM individuals. Using this latter assay or a similar test with further improvements, especially in combination with family studies, it should soon be possible for the physician to determine the *CYP2D6* phenotype of the patient, thereby avoiding toxic drug overdoses.

COMMENTS

To appreciate these two recent papers, this commentary briefly reviews our current understanding of pharmacogenetics and drug metabolism, what the debrisoquine polymorphism represents and how pharmacogenetic disorders could lead to differences in individual risk of toxicity. Also, it is interesting to consider why striking ethnic differences in drug metabolism might exist in the first place.

Pharmacogenetics is defined as the study of inherited traits that are responsible for unusual drug responses (1, 2). These responses can be immediate (e.g., hypotension, rash), subacute (e.g., peripheral neuropathy, lupoid hepatitis) or delayed (e.g., cancer). Many idiosyncratic responses reflect underlying differences, ranging from 10-fold to more than 200-fold, in the expression of genes-encoding, drug-metabolizing enzymes. In the medical field it is important to recognize that subpopulations exist with substantially increased risk of cancer or toxicity caused by prescribed medications, exposures to chemicals in the workplace and in the diet and other pollutants in the environment. Prediction of individual risk will require the development of inexpensive, relatively simple and unequivocal tests. As has already been found with many "inborn errors of metabolism," the development of a predictive test for detecting genetic variants is not always straightforward because of the