Clinical Pharmacokinetics™
Supplement

WI L629

1995, Vol. 29, Supplement 1 (pp. 1-61)
ISSN: 0312-5963

Drug Interactions and the Cytochrome P450 System: Clinical Implications for Selective Serotonin Reuptake Inhibitors

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Clin. Pharmacokinet. 29 (Suppl. 1): 33-44, 1995
0312-5963/95/0001-0033/$06.00/0

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Metabolism of Drugs by Cytochrome P450 3A Isoforms

Implications for Drug Interactions in Psychopharmacology

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Summary

Members of the P450 3A subfamily are the most abundant of the human hepatic cytochromes. CYP3A isoforms mediate the biotransformation of many drugs, including a number of psychotropic, cardiac, analgesic, hormonal, immunosuppressant, antineoplastic, and antihistaminic agents. Activity of CYP3A in humans is variable among individuals, but there is no evidence of genetic polymorphism. Significant amounts of CYP3A are present in the gastrointestinal tract, and may contribute to presystemic extraction of drugs such as cyclosporin. The azole antifungal agents ketoconazole and itraconazole are potent inhibitors of human CYP3A isoforms. Selective serotonin reuptake inhibitor (SSRI) antidepressants are also CYP3A inhibitors, but much less potent than ketoconazole or itraconazole. *In vitro* models can provide important information on the qualitative and quantitative activity of potential inhibitors of human cytochromes. However, *in vitro* inhibition constant (K_i) values alone do not predict the magnitude of an *in vivo* interaction, nor whether an interaction will be of clinical importance. For example, SSRIs are predicted to impair clearance of the antihistamine terfenadine in humans. However, the magnitude of this effect is much less than would be associated with a pharmacokinetic interaction of clinical importance.

Understanding the role of cytochrome P450 (CYP) 3A isoforms in the metabolism of drugs, and the factors influencing the activity of CYP3A, is of major clinical importance. Studies of the relative content of various cytochromes in large numbers of human liver specimens[1] indicate that CYP3A isoforms on average constitute the largest component (approximately 30%) of identified cytochromes (fig. 1). The CYP3A isoforms are partially or entirely responsible for biotransformation of many drugs in humans, including many psychotropic agents, cardiovascular drugs, antineoplastic and immunosuppressive agents, the nonsedating histamine H_1-antagonists, and various analgesics (table I). A number of endogenous steroid hormones are also metabolised by these isoforms.

1. Features of Metabolism by CYP3A Isoforms

Extensive *in vitro* and biochemical and clinical studies of drug metabolism in humans have established a number of important characteristics of cytochrome CYP3A and its metabolic activity.[2-5] Specifically, 2 isoforms, CYP3A3 and 3A4, are of

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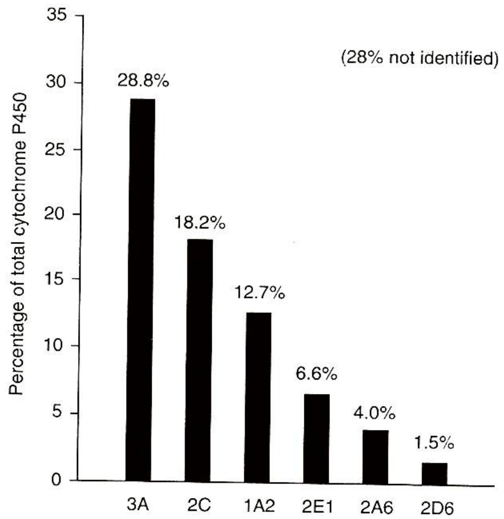

Fig. 1. Mean relative amounts of identifiable human cytochromes determined immunologically in a study of 60 human liver samples (Shimada et al.,[1] with permission).

established clinical importance. These 2 isoforms are structurally distinct but very difficult to distinguish either immunologically or functionally. Recent data suggest that a CYP3A5 isoform may also be of clinical importance. It is possible that varying relative amounts of these 3 specific isoforms may contribute to individual variation in clearance of CYP3A substrates, but this currently remains speculative. For the purposes of this report, CYP3A3, 3A4, and 3A5 are collectively referred to as CYP3A. A CYP3A7 isoform has been identified in fetal liver but appears not to be important in adults.[6-9]

Evidence from *in vitro* studies that immunologically quantitate CYP3A in series of human liver samples or that determine activity functionally based on the capacity of liver samples to biotransform model CYP3A substrates shows that there is large individual variation.[1] The pattern of substrate binding is complex. More than one substrate may bind simultaneously, and one substrate may actually activate the metabolism of another.[10-12] Variation is also evident clinically, if metabolic activity is quantitated by *in vivo* clearance of specific CYP3A substrates. Several studies have evaluated individual variations in clearance within relatively homogeneous groups of volunteers. A study of the clearance of triazolam, a CYP3A substrate, in a series of 54 healthy young male subjects indicated that the coefficient of variation in clearance was 53% of the mean (fig. 2).[13] Findings were similar in a study of the clearance of alprazolam, also a CYP3A substrate.[14,15] Individual variations in clearance can be expected to be even larger in heterogeneous populations.[16] It is of considerable interest that no single substrate has been agreed upon as an index compound to profile *in vivo* activity of CYP3A activity in humans. Compounds such as erythromycin, dapsone, and midazolam have been used in this context, and each has some experimental support.[17-22] However, there is incomplete correlation of clearance for the various substrates within the same individuals.[23,24]

Some sources of individual variation in CYP3A activity have been identified, while others remain unexplained. Subjects who are exposed to medications that are inducers or inhibitors of CYP3A ac-

Table I. List of representative drugs whose metabolism is partially or entirely dependent on cytochrome P450 3A isoforms in humans

Psychotropics	**Hormones/steroids**
Triazolam	Tamoxifen
Alprazolam	Testosterone
Midazolam	Cortisol
Diazepam	Progesterone
Bromazepam	Ethinylestradiol
Imipramine	Paclitaxel
Amitriptyline	**Antihistamines**
Nefazodone	Terfenadine
Antiarrhythmics	Loratadine
Amiodarone	Astemizole
Lidocaine	**Antimicrobial agents**
Quinidine	Erythromycin
Propafenone	Troleandomycin
Disopyramide	Dapsone
Calcium antagonists	**Immunosuppressants**
Diltiazem	Cyclosporin
Verapamil	Tacrolimus
Nifedipine	**Antiulcer agents**
Opioid analgesics	Omeprazole
Alfentanil	**Anticonvulsants**
Dextromethorphan (mainly 2D6)	Carbamazepine

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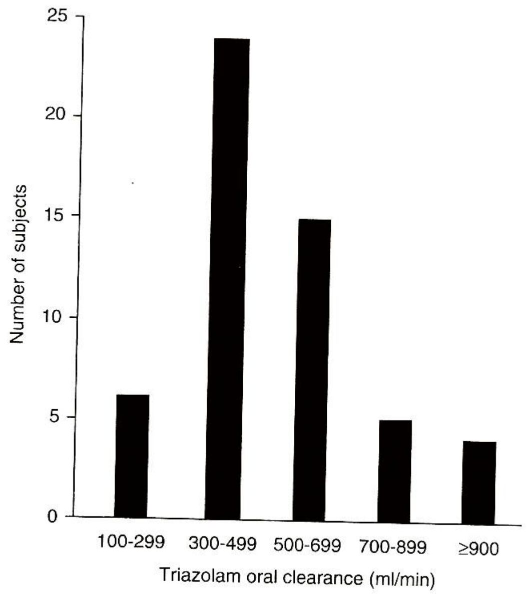

Fig. 2. Distribution of triazolam oral clearance values among 54 healthy male volunteers administered a single 0.5mg dose of triazolam. The mean value of clearance was 526 ml/min, the standard deviation was ± 279, and the coefficient of variation was ± 53% (Friedman et al.,[13] with permission).

tivity will have correspondingly increased or decreased activity of CYP3A. Examples of inducers include rifampicin and barbiturates, while inhibitors include ketoconazole, itraconazole, cimetidine, and macrolide antibiotics. Activity may also be influenced by environmental or dietary factors.[25] For example, CYP3A activity can be inhibited by an as yet unidentified component of grapefruit juice, and may be inhibited or enhanced *in vitro* by certain flavonoids.[11,26,27] CYP3A activity may be nonspecifically depressed by debilitation or disease,[28] particularly cirrhosis or hepatocellular, disease. *In vivo* evidence strongly suggests reduced metabolic clearance of some CYP3A substrates in otherwise healthy elderly humans;[29-31] however, biochemical and *in vitro* studies have not clearly elucidated the mechanism of the age-related change.[1,29,32] There is some suggestion that gender may influence the activity of human CYP3A, but the data are inconsistent.[33] It should be emphasised that studies of the pattern of individual variation in clearance fail to reveal evidence of polymodal distributions consistent with genetic polymorphisms (as observed with CYP2D6 and CYP2C19). Essentially, all data are consistent with a unimodal distribution of variance of CYP3A activity in human populations (fig. 2).[13]

The liver is the most important site of cytochrome CYP3A activity in humans, although significant amounts of this cytochrome are present in the gastrointestinal tract.[34-38] For some CYP3A substrates, gastrointestinal tract metabolism contributes importantly to total metabolic clearance. For example, studies of cyclosporin clearance in humans indicate that presystemic extraction after oral administration is greater than would be expected on the basis of first-pass hepatic extraction

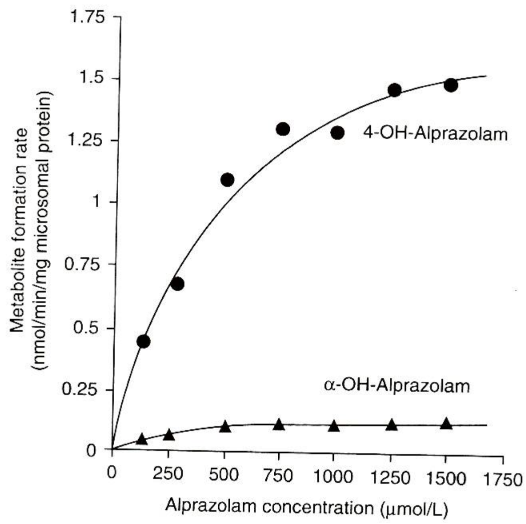

Fig. 3. Rates of formation of 4-OH- and α-OH-alprazolam in relation to the concentration of the substrate, alprazolam, based on an *in vitro* study using microsomes from a representative human liver. The lines represent the Michaelis-Menten function of best fit determined by nonlinear least squares regression. Parameter values for 4-OH-alprazolam formation were as follows: the maximum reaction velocity (V_{max}) = 1.9 nmol/min/mg microsomal protein; the substrate concentration at which the reaction velocity is 50% of maximum (K_m) = 394 μmol. Corresponding values for α-OH-alprazolam were 0.136 and 203. Comparison of intrinsic clearance values (V_{max}/K_m ratios) for the 2 pathways indicated that 88% of clearance was accounted for by 4-OH-alprazolam formation, and 12% by α-OH-alprazolam formation.

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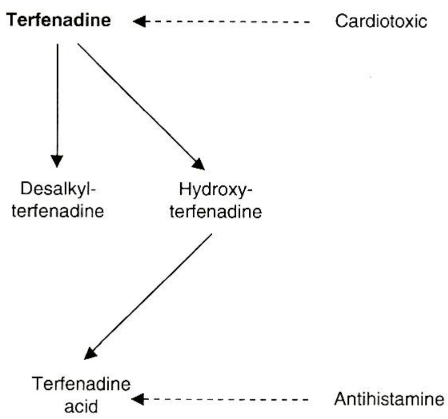

Fig. 4. Schematic representation of the principal pathways of terfenadine biotransformation in humans. The initial metabolic steps, presumed to be mediated by cytochrome P450 3A (CYP3A) isoforms, yield 2 parallel metabolites: desalkyl- and hydroxy-terfenadine. The hydroxy derivative is further metabolised to terfenadine acid, which is presumed to account for antihistaminic effects. Under usual circumstances, presystemic extraction (first-pass metabolism) of oral terfenadine is so extensive that only very low concentrations of intact terfenadine are reached in the systemic circulation. However, coadministration of a potent CYP3A inhibitor such as ketoconazole impairs presystemic extraction, leading to potentially important systemic concentrations of intact terfenadine.

alone.[39] Furthermore, changes in presystemic extraction attributable to coadministration of inducing or inhibiting agents are far greater than can be accounted for on the basis of changes in hepatic clearance alone.[39] Indirect evidence also suggests that the gastrointestinal tract participates in the metabolism of two other CYP3A substrates, triazolam and midazolam,[40-42] but this is not the case for all CYP3A substrates. For example, systemic availability of orally administered diazepam and alprazolam is close to 100%, indicating that presystemic extraction is of minimal clinical importance.[43-45]

2. Methods for Verifying the Role of CYP3A in Human Drug Metabolism

The use of *in vitro* techniques to study pathways and mechanisms of drug metabolism in humans has assumed increasing importance over the last decade. These methods can be used to identify the cytochrome(s) responsible for biotransformation of a specific drug. One of the most commonly applied models involves the use of human liver tissue, from which the drug metabolising cytochromes present in the microsomal fraction can be isolated by ultracentrifugation techniques. If *in vitro* incubation conditions are appropriately adjusted and the necessary cofactors added to a reaction mixture, drug biotransformation will proceed *in vitro* in much the same way as in the intact human (fig. 3).

A common mathematical approach to analysing *in vitro* metabolic data involves evaluating the relationship between the rate of metabolite formation and the concentration of substrate.[46] In many cases, such data can be analysed by classical enzyme kinetic methods (assuming the involvement of a single enzyme with one binding site), yielding 2 typical reaction characteristics: V_{max}, the maximum reaction velocity; and K_m, the substrate concentration at which the reaction velocity is 50% of maximum (fig. 3).

To identify the cytochrome(s) responsible for a specific biotransformation, several techniques may be considered. The chemical inhibition approach uses a series of chemicals known to have relatively high specificity as inhibitors of cytochromes. Studies of metabolite formation rate versus substrate concentration are repeated with coaddition of varying concentrations of specific cytochrome inhibitors.[47-50] A high degree of inhibition by a low concentration of a specific inhibitor implicates the corresponding cytochrome as participating in the reaction. In an appropriately designed experiment, the inhibiting potency of a chemical inhibitor can be quantitatively characterised by a competitive inhibition constant (K_i), which reciprocally reflects inhibiting activity on a molar basis: low values of K_i indicate high inhibiting potency, whereas high K_i values indicate a weak capacity for inhibition.[51] As an example, biotransformation of the nonsedating histamine H_1-antagonist terfenadine to its desalkyl and hydroxy metabolites (fig. 4) is strongly inhibited *in vitro* by low concentrations of the azole antifungal agent ketoconazole.[52-54] Although the mechanism of inhibition is not clearly

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