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COMMENTARY

In Vitro Approaches to Predicting Drug Interactions *In Vivo*

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ABSTRACT. *In vitro* metabolic models using human liver microsomes can be applied to quantitative prediction of *in vivo* drug interactions caused by reversible inhibition of metabolism. One approach utilizes *in vitro* K_i values together with *in vivo* values of inhibitor concentration to forecast *in vivo* decrements of clearance caused by coadministration of inhibitor. A critical limitation is the lack of a general scheme for assigning intrahepatic exposure of enzyme to inhibitor or substrate based only on plasma concentration; however, the assumption that plasma protein binding necessarily restricts hepatic uptake is not tenable. Other potential limitations include: flow-dependent hepatic clearance, "mechanism-based" chemical inhibition, concurrent induction, or a major contribution of gastrointestinal P450-3A isoforms to presystemic extraction. Nonetheless, the model to date has provided reasonably accurate forecasts of *in vivo* inhibition of clearance of several substrates (desipramine, terfenadine, triazolam, alprazolam, midazolam) by coadministration of selective serotonin reuptake-inhibitor antidepressants and azole antifungal agents. Such predictive models deserve further evaluation, since they may ultimately yield more cost-effective and expeditious screening for drug interactions, with reduced human drug exposure and risk. BIOCHEM PHARMACOL **55**;2:113–122, 1998.

KEY WORDS. cytochromes P450; chemical inhibition; scaling; *in vitro* metabolism; drug interactions

Cytochrome P450 enzymes have long been recognized as the primary enzymes responsible for human drug metabolism. Although the number of individual isoforms that have been identified and characterized is increasing continuously, the metabolism of xenobiotics in humans is handled mainly by enzymes from three families: CYP1†, CYP2, and CYP3. The increasing amount of biochemical information available for individual enzymes, as accumulated primarily over the last decade, has allowed improved insight into the mechanisms of biotransformation and identification of major metabolic routes in humans [1–11]. It has also allowed for prediction of metabolic pathways that may be required to handle novel pharmaceutical compounds. The development and refinement of *in vitro* methods have allowed a wealth of information to be obtained without the expense and potential risks involved in conducting human *in vivo* studies. Systems for *in vitro* experimentation include microsomes, liver slices, hepatocyte culture or suspension, cell lines, and expressed enzyme. Many excellent reviews on the advantages and disadvantages of each system currently exist in the literature [1–11].

MEDICAL AND ECONOMIC NEED FOR PREDICTIVE MODELS

Impairment of drug metabolism as a consequence of drug interactions has obvious clinical implications including toxicity as a result of increased bioavailability and decreased clearance. Kinetic variability can result if metabolic inhibition leads to nonlinearity. Toxicity can also occur if a previously minor pathway becomes subsequently favored, and that pathway produces a toxic or reactive metabolite. Inhibition can reduce clinical efficacy if the substrate is a prodrug requiring metabolic activation, and this step is blocked.

Thus, understanding and anticipating pharmacokinetic drug interactions are important components of rational therapeutics. Undertaking of controlled pharmacokinetic studies of drug interactions in humans has become a routine part of pharmaceutical drug development, and such studies continue to be performed after drugs are approved for clinical use. Human studies provide the most definitive data on the probability and magnitude of pharmacokinetic drug interactions during medication coadministration in clinical therapeutics. However, such studies have important limi-

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† *Abbreviations:* CYP, cytochrome P450; CL_o, *in vivo* clearance in control condition without inhibitor; CL_I, *in vivo* clearance with inhibitor coadministered; *FDCL*, fraction decrement of clearance *in vivo*; *FDV*, fractional decrement in reaction velocity *in vitro*; SSRI, selective serotonin reuptake inhibitor; V_o, reaction velocity *in vitro* in control condition with no inhibitor; and V_I, reaction velocity *in vitro* with inhibitor present.

tations and drawbacks. The issue of risk to human subjects is always of concern, even if the actual risk is small and acceptable. The increasingly stringent regulatory requirements for control and monitoring of premarketing studies, as well as the scientific needs for appropriate design and adequate sample size, generally cause these studies to be costly and time-consuming. As a consequence, there is a realistic limit to the number and scope of clinical drug interaction studies that can be performed, whether in the premarketing phases of drug development or after approval for clinical use. Inevitably some important drug interactions will be overlooked simply because they were not among those that were studied; the often-cited case of the terfenadine–ketoconazole interaction is an important example [12]. On the other hand, many clinical interaction studies are negative.

These dilemmas have stimulated the search for alternative approaches to studying drug interactions. The possibility of predicting *in vivo* metabolic events from *in vitro* models has been discussed for decades and some of the fundamental principles and assumptions involved in the *in vitro–in vivo* predictions have been described [13–15]. Identifying the cytochromes involved in biotransformation of a specific drug can identify sources of variation in clearance secondary to population demographics, genetic polymorphisms, extrahepatic enzyme distribution, and known chemical inducers or inhibitors. The ability to accurately predict drug interactions secondary to inhibition of cytochrome metabolism has been greatly enhanced by the knowledge of which cytochrome mediates a specific reaction.

MODEL COMPONENTS AND ASSUMPTIONS

Predictive models for *in vitro–in vivo* scaling of pharmacokinetic drug interaction can be constructed from a combination of laboratory and theoretical components. Many of the pieces are based on well-established principles, while others represent hypotheses and assumptions. The following describes the steps in development of a representative model.

Biotransformation In Vitro

The objective of the laboratory component is to replicate *in vitro* the principal metabolic pathways of the index substrate as they occur *in vivo*, and to characterize the mechanism and quantitative inhibiting potency of the model inhibitor.

Human liver microsomes appear to be the most applicable *in vitro* system for purposes of scaling, since the various cytochromes are present in proportion to their *in vivo* representation. This is particularly important when more than one specific cytochrome contributes to the biotransformation of the index substrate. Microsomal preparations from various animal species have been used for predictive studies of drug interactions, but no species has been generally established as a substitute for human tissue.

Mathematical analysis of *in vitro* reaction kinetic data in the absence of inhibitors usually includes application of the Michaelis–Menten (MM) equation, with or without linearizing transformation of the data [16]. However, with increasing numbers of data points and/or range of substrate concentrations, it may become apparent that the simplest form of the MM equation is not applicable. Examples of complicating features requiring modification of modeling approaches include: simultaneous contribution of two or more enzymes with distinct K_m values [17, 18]; cooperative binding resulting in apparent substrate activation [19–23]; concentration-dependent inhibition by substrate and/or product [21, 22, 24–26]; sequential metabolism of the primary metabolite to yield secondary downstream metabolic products. Goodness-of-fit criteria from mathematical modeling techniques may tentatively identify which one (or more) of these features is applicable to a given data set, but definitive confirmation of a biochemical mechanism requires supplemental information [21]. In any case, complex reaction kinetic mechanisms may compromise the conceptual validity of absolute or relative intrinsic clearance values as calculated from the V_{max}/K_m ratio [27].

It is nonetheless important to recognize that the accuracy of potency calculations for specific chemical inhibitors is not necessarily compromised importantly when simplifying approximations are used to handle complex reaction kinetics. When two distinct enzyme components contribute to a specific biotransformation, the component with the lowest K_m (the "high-affinity" site) may account for a large percentage of net intrinsic clearance. In such cases, the high-K_m enzyme may be approximated by a simple linear function, and inhibition of the low-K_m enzyme accounts for most of the clinically important activity of chemical inhibitors [17].

Chemical Inhibitors In Vitro

Coincubation of a candidate chemical inhibitor with the target substrate using the same *in vitro* system can yield an estimate of the intrinsic inhibitory potency of the candidate compound. The most straightforward approach utilizes a fixed concentration of substrate [*S*] coincubated with various concentrations of inhibitor (Fig. 1). Analysis of the relation between inhibitor concentration [*I*] and reaction velocity decrement (i.e. reaction velocity at that concentration of inhibitor divided by reaction velocity with no inhibitor present) may yield an estimated concentration of inhibitor corresponding to a 50% decrease in reaction velocity (IC_{50}). This approach has the advantage of being model independent. That is, IC_{50} can be calculated without knowledge of the biochemical mechanism of inhibition. The IC_{50} values are quite useful when comparing the relative inhibitory potency of different candidate inhibitors of the same chemical class, such as the SSRI antidepressants [17] or azole antifungal agents [28]. On the other

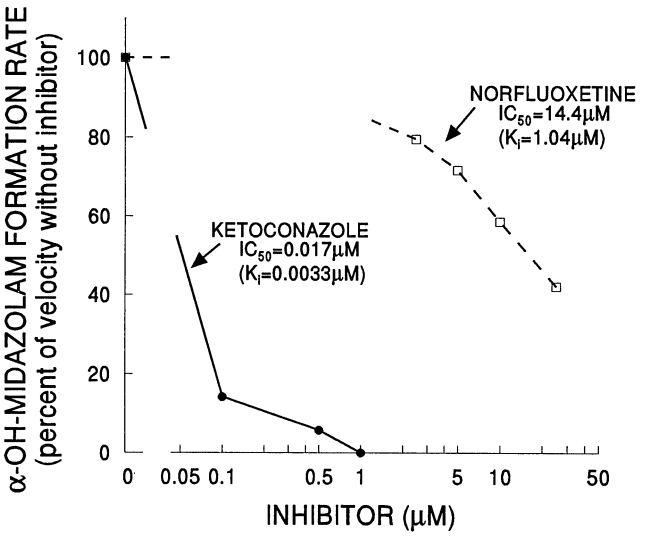

FIG. 1. Effect of two inhibitors, ketoconazole and norfluoxetine, on the *in vitro* biotransformation of midazolam to α-OH-midazolam by a representative human liver microsomal preparation. A fixed concentration of midazolam (= 5 μM, higher than the K_m value of 2.5 μM as determined previously [24]), was incubated with various concentrations of inhibitor. Reaction velocities are expressed as values with inhibitor present divided by control values without inhibitor. The IC_{50} values, determined by nonlinear regression analysis, in both cases overestimate the *in vitro* competitive K_i values as determined previously [24], but both methods provide similar estimates of the relative inhibitory potency of the two compounds.

hand, IC_{50} values have important limitations in the context of *in vitro–in vivo* scaling. In principle, the IC_{50} value obtained from an *in vitro* model is applicable only to the specific substrate concentration at which the IC_{50} was generated. If it can be demonstrated that the biochemical mechanism of inhibition is noncompetitive, then IC_{50} is equal to the inhibition constant (K_i), and both are independent of substrate concentration. If the mechanism of inhibition is competitive, then IC_{50} equals K_i only when the substrate concentration is much less than K_m. If the substrate concentration approaches or exceeds K_m, then IC_{50} overestimates the competitive K_i. This limitation can be overcome by actual calculation of *in vitro* K_i values, based on methodology involving coincubation of varying concentrations of substrate with varying concentrations of a candidate chemical inhibitor. Resulting data are typically analyzed by nonlinear regression, with or without linearizing transformation, yielding parameter estimates of K_i. Since the theoretical relationships among V, $[S]$, K_m, $[I]$, and K_i are different depending on the specific biochemical mechanism of inhibition, estimated values of K_i will accordingly differ, even for the same data set, depending on the underlying model of inhibition (i.e. competitive, noncompetitive, or "mixed"). Ironically, independent biochemical or molecular validation of the mechanism of inhibition is seldom available. Rather, a mechanism is assigned based on the data itself, using either visual inspection of linearizing reciprocal plots (such as a Lineweaver–Burk plot), statistical goodness-of-fit criteria from nonlinear regression, or a combination of these.

Procedural and methodologic variables potentially add further uncertainty. Estimates of K_i may well be influenced by the number of data points analyzed, as well as the range of substrate and inhibitor concentrations. Distortions may be imparted by reciprocal transformation, or by outlying data points [29, 30]. When the underlying reaction velocity versus substrate concentration relationship is "sigmoidal," as may occur with substrate for P450-3A isoforms [19–23], a mathematical model for superimposed competitive inhibition is not established, and K_i values are calculated using simplifying assumptions. Apparent K_i values can also be calculated even when inhibition is "mechanism-based" [31, 32], although the interpretation and *in vivo* implications of K_i under these circumstances are unclear. Finally, it is also evident that K_i values apply only to the specific substrate–inhibitor pair under study. Even if two substrates are biotransformed by the same cytochrome, it cannot be assumed that a given inhibitor will have the same K_i value for *in vitro* metabolism of those two substrates. Likewise, if a substrate can be transformed to more than one metabolite by the same cytochrome, it cannot be assumed that a given inhibitor will have the same K_i value for each of the metabolic pathways [24].

In Vivo *Application of* In Vitro *Data*

The principal hypothesis of *in vitro–in vivo* scaling of pharmacokinetic interactions involving metabolic inhibition is that the *in vivo* decrement in clearance of a substrate drug caused by coadministration of a metabolic inhibitor, present at concentration $[I]$, can be predicted by the decrement in reaction velocity for the same substrate *in vitro* when the inhibitor is present in the same concentration [13–15]. In procedural terms, the clearance of the substrate for a specific human subject is measured in the control condition with no inhibitor present (CL_o) and again during coadministration of the inhibitor (CL_I). The fractional decrement in clearance ($FDCL$) is:

$$FDCL = \frac{CL_o - CL_I}{CL_o} \tag{1}$$

In vitro, the fractional decrement in reaction velocity (FDV) is related to the velocity without inhibitor (V_o) and the velocity with inhibitor present (V_I) as follows:

$$FDV = \frac{V_o - V_I}{V_o} \tag{2}$$

In mathematical terms, the core hypothesis is:

$$FDCL = FDV \tag{3}$$

FDV can be calculated using an *in vitro* K_i value along with an appropriate value of $[I]$. If the mechanism of

inhibition is competitive, it can be shown that Equation 2 becomes:

$$FDV = \frac{[I]}{[I] + K_i\left(1 + \frac{[S]}{K_m}\right)} \quad (4)$$

If [*S*] is much less than K_m, or if the mechanism of inhibition is noncompetitive, then:

$$FDV = \frac{[I]}{[I] + K_i} \quad (5)$$

If the substrate is biotransformed in parallel to two or more metabolic products, the net value of *FDV* is calculated using the K_i and K_m for the separate pathways, followed by weighing of inhibition based on the relative contribution of intrinsic clearance for the separate pathways. When more than one inhibitor is present simultaneously, as happens when a parent drug is converted to a metabolite (i.e. fluoxetine and norfluoxetine), the net degree of inhibition is calculated under the assumption of exclusivity. These procedures have been described in detail previously [33].

Constraints, Limitations, and Assumptions

The testability of this hypothesis is immediately constrained by a number of pharmacokinetic and biochemical factors. The model is applicable when *in vivo* clearance is dependent on cytochrome activity, and chemical inhibition is reversible and concentration dependent. Applicability diminishes or disappears when hepatic clearance has a flow-dependent component, as occurs after intravenous administration of drugs with high hepatic clearance [34, 35]. Predictability of *in vivo* inhibition is also reduced when inhibition is mechanism-based [31, 32]. Impairment of *in vivo* clearance by mechanism-based inhibitors such as erythromycin is substantially greater than predicted from *in vitro* studies, which indicate only weak inhibitory potential (i.e. high K_i values) [36–38]. Concurrent *in vivo* induction because of chronic exposure to a competitive inhibitor would not be reflected by *in vitro* K_i values, and would therefore reduce the predictive accuracy of the model.

Even when biochemical and pharmacokinetic circumstances suggest that the hypothesis is testable, a major limitation remains. Calculation of *FDV* in Equations 4 or 5 is critically dependent on the availability of an accurate value of [*I*] that reflects the amount or concentration of inhibitor to which the enzyme is exposed *in vivo*. Similar considerations may hold for [*S*], but only if the value of [*S*] available to the enzyme approaches K_m (Equation 4). Plasma concentration of inhibitor can be measured in human pharmacokinetic studies, but no adequately validated general model exists for extrapolation of the measurable plasma concentration to the hypothetical concentration to which the enzyme is exposed at the site of metabolic activity. One cogent hypothesis proposes that only the unbound concentration in plasma is available for diffusion to intrahepatic sites of metabolic activity; therefore, the value of [*I*] to be used for calculation of *FDV* in Equations 4 or 5 is the unbound plasma concentration [39–43]. However, this hypothesis is not supported by considerable experimental data indicating that the hepatic uptake of many lipophilic compounds is not necessarily restricted by protein binding [44, 45]. *In vitro* and cell culture models demonstrate extensive partitioning of lipophilic compounds into liver tissue homogenates or intact cells in culture [46–53]. *In vivo* experimental data also demonstrate extensive hepatic uptake of drugs such as benzodiazepines, neuroleptics, antidepressants, and other lipophilic compounds [24, 50–56]; liver concentrations greatly exceed *total* plasma concentrations, let alone unbound plasma concentrations (Figs. 2 and 3). Results from human autopsy studies [57–65] yield similar results (Fig. 4). Although drug distribution may be modified by postmortem conditions [66–69], these human data show high liver to total plasma concentration ratios for lipophilic drugs despite extensive plasma protein binding (Fig. 4). Pharmacokinetic data on presystemic extraction provide further evidence that plasma binding does not necessarily restrict hepatic uptake [70, 71]. Many extensively protein-bound drugs have extraction ratios after oral dosage that greatly exceed the free fraction (Table 1). Biotransformation in the gastrointestinal mucosa may contribute to presystemic extraction of some substrates of P450-3A [72, 73], such as midazolam [74, 75] and cyclosporin [76, 77]. Even accounting for this contribution, it remains evident that the hepatic component of biotransformation is not limited by plasma binding.

The available data therefore indicate that complete restriction of hepatic uptake by plasma protein binding

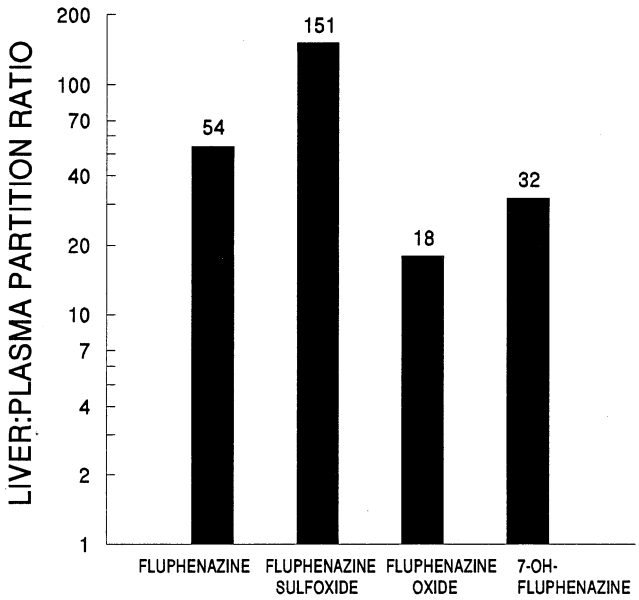

FIG. 2. Mean liver:plasma partition ratios *in vivo* in rats for fluphenazine and its metabolites. Adapted, in part, from Ref. 54.

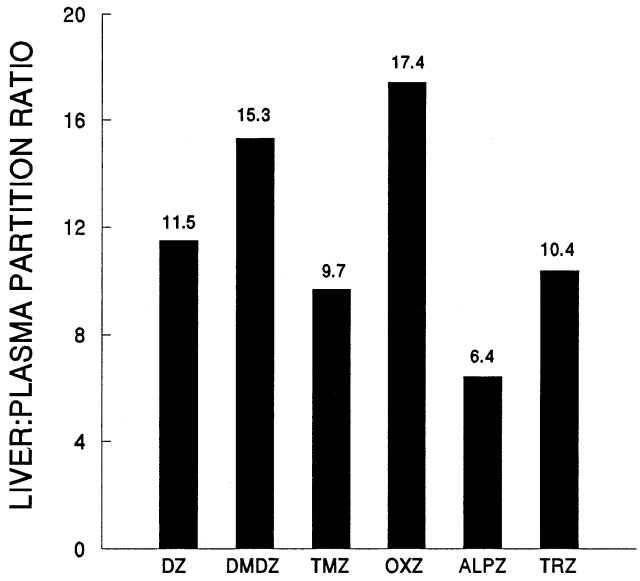

FIG. 3. Mean liver:plasma partition ratios *in vivo* in rats for several benzodiazepine derivatives. Abbreviations are: DZ, diazepam; DMDZ, desmethyldiazepam; TMZ, temazepam; OXZ, oxazepam; ALPZ, alprazolam; and TRZ, triazolam. Adapted, in part, from Ref. 55.

cannot be assumed *a priori*. Construction of models for prediction of intrahepatic substrate or inhibitor concentration based on measured plasma levels should incorporate the possibilities that plasma binding might completely restrict, partially restrict, have no effect upon, or actually facilitate drug transfer between plasma and intrahepatic sites of cytochrome activity. Given consistent evidence for extensive hepatic uptake of lipophilic drugs despite high degrees of plasma binding, projected intrahepatic concentrations are a plausible reference point for model development. Nonetheless, it should be emphasized that "whole liver" drug concentrations still do not necessarily reflect "enzyme-available" concentrations.

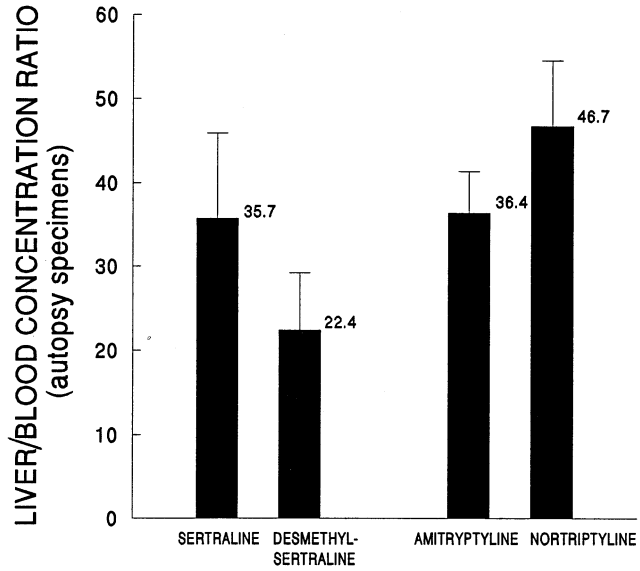

FIG. 4. Mean (± SEM) postmortem liver:blood concentration ratios determined in human autopsy specimens. Left: Sertraline (n = 5) and its metabolite desmethylsertraline (n = 5). Right: Amitriptyline (n = 30) and its metabolite nortriptyline (n = 23). Adapted, in part, from Refs. 62 and 63.

TABLE 1. Representative drugs for which the extraction ratio is reported to exceed the free fraction in plasma

Alprenolol	Midazolam
Amiodarone	Nifedipine
Amitriptyline	Nortriptyline
Chlorpromazine	Prazosin
Cyclosporine	Propanolol
Desipramine	Triazolam
Imipramine	Trimipramine
Lidocaine	Verapamil

OUTCOME OF HYPOTHESIS TESTING

Data available in the literature have allowed testing of the principal hypothesis in a preliminary way. The components necessary for application of the model are: (a) *in vitro* data on the inhibitory capacity of the specific inhibitor for the specific substrate; (b) clinical data on *in vivo* clearance of the substrate, with and without inhibitor coadministration; and (c) plasma concentrations of inhibitor, and an estimate of liver-plasma partitioning.

Azole Antifungal Agents and P450-3A *Substrates*

The clinically important inhibitory capacity of azole antifungal agents (ketoconazole, itraconazole, and, to a lesser degree, fluconazole) versus substrates of human cytochrome P450-3A isoforms has been documented extensively. Application of the predictive model in the case of P450-3A substrates is particularly complex because of the significant contribution of gastrointestinal 3A isoforms to biotransformation of some 3A substrates after oral administration [72–77]. If the inhibitor and the substrate are administered orally, concentrations of both at the absorptive sites are likely to be very high transiently just after dosage, and to fluctuate between doses to a much greater degree than the concentrations in plasma. Model testing nonetheless proceeds with the simplifying assumption that the overall change in substrate clearance due to inhibitor coadministration reflects the net contribution of gastrointestinal and hepatic P450-3A isoforms, and that the same K_i and inhibitor concentration values apply to both sites.

Ketoconazole is estimated to be 99% bound to blood/plasma components in humans [78], whereas liver versus total plasma concentration ratios in experimental *in vitro* and *in vivo* studies consistently exceed 0.5 [79–82]. This strongly suggests that binding incompletely restricts hepatic uptake (Table 2). In two *in vitro* studies, the K_i value for ketoconazole versus α-OH midazolam formation was 0.1 μM (noncompetitive) [36] and 0.004 μM (competitive) [24]; in a clinical study [83], coadministration of ketocon-

TABLE 2. Partitioning of Ketoconazole Between Plasma and Liver Tissue

Experimental system	Liver:plasma partition ratio	Ref.
Rat, *in vivo*	5	79
Rat, *in vivo*	0.5–1.0	80
Rat, *in vivo*	≥3.2*	81
Mouse, *in vivo*	2.03	82
In vitro: human plasma vs liver homogenate	1.12	82

* At plasma concentrations <3.8 μg/mL, liver:plasma partition ratios exceeded 3.2.

azole (total plasma concentration ≥1 μg/mL) reduced oral midazolam clearance by an average of 94%. By applying these K_i values along with the total plasma ketoconazole concentrations for [*I*] in the predictive model, the predicted degrees of oral midazolam clearance inhibition are 95% [36] and >95% (assuming [*S*] ≪ K_m) [24], respectively. However, use of the projected unbound plasma ketoconazole concentration (0.01 × total plasma concentration) yields underestimations of the degree of clearance inhibition (16 and 82%, respectively). Reasonable predictions have also been derived, using total plasma ketoconazole concentrations and *in vitro* K_i values, for the clinical interactions of ketoconazole with triazolam [82, 84], terfenadine [12, 85], and alprazolam [86, 87]. It is of interest that coadministration of ketoconazole reduces clearance of oral alprazolam by an average of 69% [87], although absolute bioavailability of oral alprazolam is greater than 80% [88, 89].

Itraconazole, like ketoconazole, is 99% bound to blood/plasma components [78], whereas liver versus total plasma concentration ratios in experimental models range from 11:1 to 20:1 [79]. The competitive K_i for itraconazole versus α-OH-midazolam formation was 0.28 μM [24], and the impairment of midazolam clearance by coadministration of itraconazole (total plasma concentrations generally ≥0.1 μM) in three clinical studies was 90, 83, and 85% [83, 90, 91]. These are well predicted by the scaling model if a liver:plasma ratio in the range of 10:1 to 20:1 is assumed for itraconazole, but poorly predicted based on total or unbound plasma itraconazole levels. It is of interest that itraconazole coadministration also impairs clearance of intravenous midazolam [91].

SSRI Antidepressants and P450-2D6 *Substrates*

In vitro inhibition of desipramine hydroxylation, a reaction mediated largely if not entirely by human cytochrome P450-2D6 [92, 93], has been evaluated for SSRI antidepressants and their pertinent metabolites [94, 95]. Based on *in vitro* competitive K_i values, fluoxetine, norfluoxetine, and paroxetine are potent inhibitors, while sertraline, desmethylsertraline, and fluvoxamine are substantially weaker [96]. Clinical studies have evaluated the impairment of desipramine clearance due to coadministration of a number of

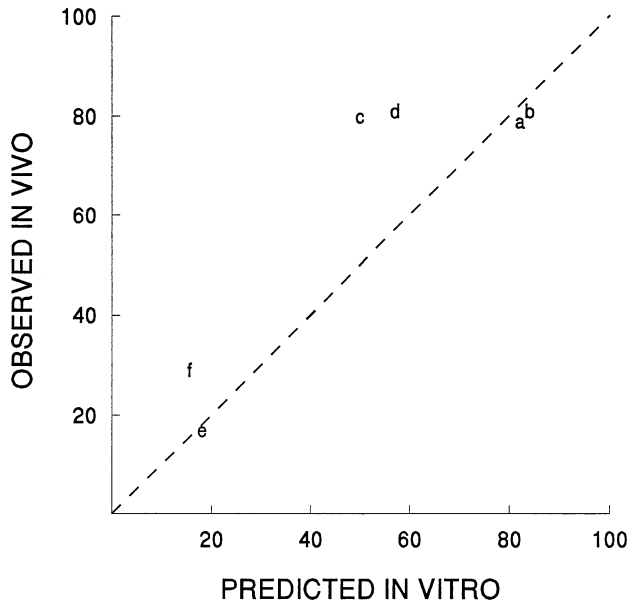

FIG. 5. Outcome of six studies evaluating the actual inhibition of oral desipramine clearance in humans as compared with that predicted for the *in vitro* model. *In vitro* competitive K_i values for six SSRIs versus desipramine hydroxylation in human liver microsomes were determined as described previously [94–96]. Predicted inhibition (x-axis) was determined using the model described in the text, based on K_i values and plasma SSRI concentrations which were then corrected for anticipated hepatic uptake. Actual inhibition of desipramine clearance *in vivo* (y-axis) was determined in clinical pharmacokinetic studies. Dashed line is the line of identity (y = x). Symbols *a* and *b* are studies of fluoxetine/norfluoxetine [97, 98]; *c* and *d* are studies of paroxetine [100, 101]; and *e* and *f* are studies of sertraline/desmethylsertraline [98, 99].

these SSRIs [97–100]. Application of the predictive model yields reasonably accurate forecasting of *in vivo* desipramine clearance decrements based on *in vitro* data, provided that values of [*I*] determined from plasma SSRI concentrations are adjusted for anticipated liver partitioning, consistent with experimental and human autopsy data (Fig. 5). Use of unbound or even total SSRI plasma concentrations in the predictive model leads to substantial underestimation of observed clearance decrements.

SSRI Antidepressants and P450-3A *Substrates*

In vivo decrements in clearance of alprazolam, a substrate for P450-3A isoforms, due to coadministration of fluoxetine [101, 102] or fluvoxamine [103] were reasonably well predicted from *in vitro* data [86, 95]. Minimal clinical interaction of terfenadine with paroxetine was predicted *in vitro* [104] and confirmed *in vivo* [105]. However, the predictive model forecasted a significant decrement in triazolam clearance due to coadministration of fluoxetine [82], whereas a clinical study demonstrated only a minimal *in vivo* pharmacokinetic interaction [106]. The reasons for these discrepancies are not established. Gastrointestinal P450-3A isoforms are likely to account for an important

component of presystemic extraction after oral administration of drugs such as triazolam. Inhibition of P450-3A isoforms by fluoxetine is largely attributable to the metabolite norfluoxetine [53, 107], which may have incomplete access to gastrointestinal mucosal cells following administration of the parent compound. It is also possible that fluoxetine and other SSRIs may have inducing effects that offset competitive inhibition under some circumstances.

COMMENT

Major advances in the use of *in vitro* systems to understand human drug metabolism have logically led to exploration of the application of such systems to focus, supplement, or even replace, human pharmacokinetic study programs. *In vitro* models to predict drug interactions based on *in vitro* data have been proposed. The models are preliminary, and the drawbacks, limitations, weaknesses, and assumptions are numerous. The models can, in principle, forecast the magnitude of an interaction, but not its potential clinical consequences [96]. Nonetheless, the concept is promising, and the potential long-range benefit is a drug-development process that is more rapid and cost-effective, with reduced risk to human subjects.

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