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Cytochrome P450 Enzymes and Psychopharmacology

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INTRODUCTION

The study of cytochrome P450 (P450) enzymes has implications for psychopharmacology at many levels as follows:

> *At the most practical level, it has dramatically increased our ability to understand, anticipate, and avoid clinically important, pharmacokinetically mediated drug-drug interactions.*
>
> *It has become as much an integral part of the modern drug development process as screening for effects on neural targets of interests (e.g., a drug's receptor binding profile).*
>
> *It serves as a prime example of the role genetic and environmental influences can play in determining drug response.*
>
> *It can serve as a model for understanding the relationship between a drug's in-vitro* potency (with respect to its mechanism of action), the drug's plasma concentration, and the resultant clinical effects.

CONCEPTUAL BACKGROUND

Any drug effect is a function of three variables as expressed in the equation 1 below:

Equation 1

Effect = Potency at the site(s) of action x Drug concentration at the site of action x Biological variance

This equation expresses the relationship between pharmacodynamics (i.e., *response* in the dose-response curve), pharmacokinetics (i.e., *dose* in the dose-response curve), and biological variance (i.e., *shift* in the dose-response curve). Pharmacodynamics deals with drug potency at one or more sites of action. Pharmacokinetics deals with the qualities of the drug that determine what the drug concentration is at its site of action. Biological variance deals with patient-specific factors that individualize expression of the dose-response relationship.

As expressed in equation 1, the concentration of interest is the concentration at the site of action. Such sites of relevance to psychopharmacology include specific enzymes, neuroreceptors, and uptake pumps. In both research and clinical practice, plasma drug concentration is often used as a surrogate for drug concentration at

the site of action. Plasma concentration is far more easily measured, and it generally correlates well with drug concentration in deeper compartments (e.g., brain) [80] and even at specific sites of action (e.g., dopamine receptors) [30, 31, 55, 74, 85].

The concentration achieved after administration of a given dose is determined by the pharmacokinetics of the drug: its absorption, distribution, metabolism, and elimination (65). Most drugs are converted into polar metabolites as a necessary step in their elimination (i.e., phase I metabolism). These metabolites then undergo phase II metabolism (i.e., conjugation reactions, including glucuronidation and sulfation) prior to elimination via the kidneys. The rate-limiting step in this process is phase I metabolism, the bulk of which is mediated by cytochrome P450 enxymes(P450s).

P450s

Our knowledge of these enzymes has expanded dramatically over the last decade as a result of advances in molecular biology. P450s are heme-containing monooxygenases and are divided into two primary groups: steroidogenic and xenobiotic (37, 38, 39, 66, 67, 68, 69). Steroidogenic P450s are found both in prokaryotes and in the mitochondria and smooth endoplasmic reticulum of eukaryotes. They synthesize steroids and other substances necessary for the maintenance of cell wall integrity and cellular differentiation. Xenobiotic P450s are found in the smooth endoplasmic reticulum of eukaryotes and metabolize foreign (i.e., *xeno-*) biological substances. This latter group of P450s appears to have evolved from the steroidogenic P450s during the era of plant-animal differentiation. These enzymes conveyed a survival advantage by allowing animals to detoxify the substances that they consumed. These P450s are also responsible for most oxidative drug metabolism.

Over the last decade, the genes coding for these enzymes have been identified, and the amino acid sequences of the individual P450s have been deduced. A classification system has been developed in which each P450 is assigned a family, subfamily and number (66, 69). For example, CYP2D6 stands for family 2, subfamily D, and gene 6. Within a family, P450s usually share at least 40% identity in amino acid sequence. Mammalian sequences within the same subfamily are >55% identical and (thus far) represent clusters of genes on a given chromosome.

Table 1 lists the human steroidogenic and xenobiotic P450s (77). The latter group comprises families 1, 2, and 3. Known drug metabolism is largely mediated by CYP3A (approximately 50%) and CYP2D6 (approximately 30%). CYP1A2, 2C9/10, 2C19, and 2E1 account for approximately equal percentages of the remaining 20%. However, these numbers must be viewed cautiously, since the relevant P450s have been established for only perhaps 20% of marketed medications. Hence, these numbers and the relative importance of specific P450s may change as more research is done to establish the metabolic pathways for other commonly used medications.

The importance of the P450s to psychopharmacology is based in part on the fact that they mediate most of phase I drug metabolism (i.e., biotransformation into polar metabolites). Phase I metabolism can occur during drug absorption, either in the gut wall or in the liver , before the drug reaches the systemic circulation. This presystemic clearance (or first-pass metabolism) determines the fraction of the oral dose that will reach the systemic circulation (i.e., the fraction of the drug that is bioavailable). Drug that reaches the systemic circulation is redistributed to the liver via hepatic arterial blood. The unmodified term "clearance" refers to the rate at which the drug is cleared from the systemic circulation. Thus, presystemic and systemic clearance determine the concentration that is achieved after a given dose of a given drug in a given patient. Recall that

concentration is the second variable in equation 1 that determines the clinical effect observed in the patient.

The importance of P450 enzymes extends beyond determining the concentration of the parent drug. The P450s also determine the concentration of drug metabolites, which may or may not be pharmacologically active. Active metabolites can have a pharmacological profile quite different from the parent compound. For example, nefazodone is an antidepressant with anxiolytic effects. But its active metabolite, meta-chlorophenylpiperazine (mCPP), is anxiogenic (26, 54, 103). The concentration of mCPP is generally only a fraction of that of the parent drug, but mCPP is dependent on CYP2D6 for its biotransformation prior to elimination. Thus, the concentration of this metabolite can be appreciably higher than usual in individuals who are either genetically or phenotypically deficient in CYP2D6. In sum, the activity of P450 enzymes can be an important determinant of atypical responses to medications if the usual ratio of parent drug to active metabolite is altered.

The biotransformation of a drug may be mediated by one or more P450 enzymes. The role of a specific enzyme is determined by several factors: the affinity of the drug for the enzyme, the speed with which the enzyme metabolizes the drug, and the abundance of the enzyme in the body. A drug may be almost exclusively dependent on a specific P450 enzyme for its elimination (e.g., terfenadine and CYP3A). Some drugs may be principally dependent on a specific P450 enzyme (e.g., desipramine and CYP2D6) [10, 22, 24]. Other drugs may utilize several relatively equal routes of elimination (e.g., mirtazapine and CYP1A2, CYP2D6, and CYP3A). The principal P450 enzyme is the one that is normally responsible for most of the biotransformation of a drug. The more a drug is dependent on a principal P450 enzyme, the greater the impact of changes in that enzyme's functional activity.

THE ROLE OF GENETICS IN DETERMINING DRUG RESPONSE

CYP2C19 and CYP2D6 are two P450s known to be genetically polymorphic (36, 59). Certain mutations in the genes that code for these enzymes produce nonfunctional variants. The genetic polymorphisms vary depending on the population in question. For example, Caucasian populations contain a large percentage of individuals who are genetically deficient in CYP2D6— perhaps 5–10% of the population (63, 64). Conversely, perhaps 20% of Orientals are genetically deficient in CYP2C19 (63).

Such polymorphisms are one example of the third factor in equation 1, in this case a genetically determined biological variance in elimination rates that shifts the dose-response curve. Such variance is one reason why specific patients may respond in an unusual way to the usual dose of a drug. Or, to state this in another way, well established, concentration-dependent toxic effects of a drug may develop at an unexpectedly low dose due to substantially reduced clearance. Slowed clearance resulting in accumulation of what is usually a minor metabolite can also lead to development of unexpected effects, whether beneficial or adverse.

Whereas genetic polymorphisms are traits and thus enduring characteristics, environmental factors such as medications and dietary constituents can alter the characteristic activity of P450 enzymes. Phenocopies of genetically "poor metabolizers" or of "unusually extensive metabolizers" can be produced, depending on whether the environmental factor inhibits or induces the enzyme, respectively. In terms of diet, P450s can be induced or inhibited by foodstuffs such as alcohol, caffeine, constituents of tobacco, charcoal-broiled foods, cruciferous vegetables and grapefruit juice (64). Some P450s are also affected by endogenous substances such as steroid hormones. Steroid induction of CYP3A, for example, may provide an explanation of some of the pharmacokinetic differences between men and women and within individuals over their life cycle from the prepubertal years to puberty and on through menopause. P450s can also be induced or inhibited by a variety of medications, including antibiotics (e.g., erythromycin), anticonvulsants (e.g., carbamazepine),

antidepressants (e.g., fluoxetine), antifungals (e.g., ketoconazole), antipsychotics (e.g., thioridazine), H_2 blockers (e.g., cimetidine), and herbal medicines (16, 19, 41, 46, 48, 56, 70, 93, 96, 100).

These environmental factors are obviously state rather than trait phenomena, since the person must have them in their system to affect the activity level of the enzyme. For this reason, an individual may behave at some times as if they are an "extensive metabolizer" with regard to a specific P450 enzyme, but at other times as if they are a "poor metabolizer", and at still other times as if they are an "unusually extensive" metabolizer—all as a function of their internal environment. This situation can be confusing to the physician and potentially dangerous for the patient, as the response to a medication can change from "typical" to "sensitive" (due to slowed clearance) to "resistant" (due to enhanced clearance). This situation provides the impetus for efforts in the drug development process to screen out new agents that either dramatically affect P450s or are themselves dependent on a single P450 enzyme for their elimination.

RELEVANCE OF P450S TO PHARMACOKINETIC DRUG-DRUG INTERACTIONS

The relationship between psychiatric medications and P450s is a two-way street. P450s can act on (i.e., biotransform) psychiatric medications and psychiatric medications can act on (i.e., inhibit, induce, enhance) P450s. Hence, psychiatric medications can be the target and/or the cause of pharmacokinetic drug-drug interactions. This issue is particularly germane to psychopharmacology for several reasons. First, this therapeutic area may have a larger number of commonly used drugs with effects on P450s than any other therapeutic area. The medications include: 1) inducers of one or more P450s (e.g., some anticonvulsants) and 2) inhibitors of more than one P450 enzyme (e.g., some antidepressants and antipsychotics). Second, psychopharmacologic medications are often taken for years and thus have the potential to alter the activity level of these enzymes for an extended period of time. The long-term consequences, if any, of such alterations are not known. Third, medications in this therapeutic area are commonly co-prescribed with other medications, increasing the probability of drug-drug interactions (25, 47, 78).

As an example of the last point, 30–80% of patients taking antidepressants also take two or more other medications (78). The higher percentages occur in older, more medically ill populations, patients who generally have more refractory or more complicated forms of major depression. The same factors which cause these patients to require more medications also increase their susceptibility to the adverse consequences of drug-drug interactions. By understanding the role of P450s in drug metabolism and the effects of drugs on P450s, physicians can minimize the likelihood of adverse pharmacokinetically mediated drug-drug interactions. They can either avoid combinations that are likely to result in significant alterations in clearance or they can make appropriate dosage adjustments to compensate for changes in clearance. This approach is analogous to understanding the pharmacodynamics of drugs in order to make informed treatment decisions that minimize the risk of a serious pharmacodynamically mediated drug-drug interaction.

Two sets of information are being developed currently, as illustrated in Fig. 1 a) to what extent will a given drug induce or inhibit a specific P450 enzyme, and b) to what extent is a specific P450 enzyme responsible for the metabolism of specific drugs. With these two data sets, one can deduce whether the addition of a second drug to an ongoing regimen is likely to cause a clinically meaningful change in the clearance of the first drug. If so, the physician can do one of several things. He can adjust the dose of the affected drug to compensate for its altered clearance. Alternatively, he can choose not to prescribe the inducer/inhibitor but rather to use a drug with the same therapeutic benefit that lacks significant effects on the metabolism of the initial drug. The latter course would clearly be necessary if a dosage adjustment would not adequately address the problem, such as when the interaction led to an altered ratio of the parent drug to an active metabolite. A comparable situation arises when a patient already being given a P450 inhibitor or inducer is

prescribed a second drug whose clearance depends on the affected P450.

Pharmacokinetic interactions can manifest themselves in myriad ways. Most often, they present as a "sensitivity" or "resistance" problem that may be attributed. wrongly, to the patient rather than to a drug interaction. In such cases, the interaction affects the level of drug such that the patient achieves either subtherapeutic or toxic levels with a dose that is usually effective. Thus, the patient appears to be either "resistant" or sensitive" to the affected drug. The interaction may appear either when a medication is added or when it is stopped, depending upon the sequence of prescribing. If the patient is already on an inhibitor or inducer and a second drug is added, the physician may titrate the dose of the second drug and unwittingly compensate for its altered clearance. If the inhibitor or inducer is later stopped but the second drug is continued, its levels will increase or decrease, respectively, in the absence of a compensatory dose adjustment. Without such an adjustment, discontinuation of an inducer can lead to an increase in levels of the second drug and the development of toxicity or tolerability problems. Discontinuation of an inhibitor can lead to reduced levels of the second drug, loss of efficacy, or symptoms of withdrawal (from the continued drug) if a state of tolerance has developed previously (e.g., benzodiazepines, narcotic analgesics).

In all of the above ways, the pharmacokinetic interaction produces a quantitative rather a qualitative change in the response. In other words, the response is a known dose-dependent effect of the affected drug but is occurring at an unusual dose due to the alteration in clearance. The fact that it is an expected effect of the drug may paradoxically increase the likelihood that it will likely to be missed or misattributed to a problem with the patient.

Pharmacokinetic interactions can also present as a qualitative change in response (i.e., an unexpected effect). This typically occurs when the interaction leads to unusual accumulation of a minor moiety with a substantially different pharmacologic effect than the normal moiety. The minor moiety may be the parent drug, as in the case of terfenadine, or it may be a metabolite, as with mCPP. Terfenadine is virtually completely biotransformed by CYP3A to the pharmacologically active metabolite prior to reaching the systemic circulation (102). That presystemic biotransformation is substantially blocked by CYP3A inhibitors such as ketoconazole, leading to unusually high levels of the parent drug in the systemic circulation (33). While the metabolite does not slow intracardiac conduction, the parent drug does (49, 104). The interaction between terfenadine and ketoconazole results in the unexpected occurrence of serious and even fatal disturbances in intracardiac conduction. The mirror image of this situation applies to the altered ratio of nefazodone and its active metabolite, mCPP, which can occur as a result of inhibition of CYP2D6, the enzyme that normally clears mCPP.

ROLE OF *IN-VITRO* AND *IN-VIVO* STUDIES IN ESTABLISHING THE HUMAN METABOLISM OF A DRUG

Research in this area is an excellent example of the complimentary nature of *in-vivo* and *in-vitro* studies. *In-vitro* studies permit rapid screening for interactions which are likely to be clinically meaningful; *in-vivo* studies confirm or reject the *in-vitro* prediction. This research can also serve as a model for understanding the relationships among *in-vitro* potency with respect to mechanism of action, drug concentration, and clinical effects.

In-vitro studies can be used to determine the ability of a P450 enzyme to metabolize a drug and the ability of a drug to inhibit a specific P450 enzyme. Both are based on conventional enzymology approaches. A drug

can be incubated with different human P450s to determine the affinity of the drug for the enzyme, the capacity of the enzyme to biotransform the drug, and the nature of the biotransformation it mediates (e.g., demethylation, hydroxylation). Different experimental approaches use different sources of P450s: human liver microsomal preparations, purified forms of the enzyme, or cells (e.g., bacteria or yeast transfected with human cDNA coding for a specific P450 enzyme).

A full discussion of the advantages and disadvantages of these different approaches is beyond the scope of this chapter. However, a few comments are warranted, since these different approaches can be quite complimentary. Panels of microsomes have been used to relate enzymatic activities of interest to either the amounts of specific P450s present in the microsomal sample or to its efficiency in metabolizing model substrates (46, 95, 98). Microsomes are particularly helpful in revealing a polymodal distribution of metabolism across the population. That is because microsomes from individuals with a full complement of P450s will differ in activity from those of individuals genetically deficient in a specific P450 enzyme (4, 14, 91). With microsomes, one can also compare results before and after the addition of specific P450 inhibitors, enhancers, or antibodies (91). The advantages (and disadvantages) of human microsomes arise because metabolism occurs in the presence of a naturally occurring complement of human P450s. This approach gives one a sense of the extent of variation in metabolism that may occur in the population. However, it also means that multiple P450s are available to mediate metabolism. Such is not the case when using an isolated P450 enzyme or cells transfected with cDNA for a single P450 enzyme. These systems are used primarily to establish the ability of a particular enzyme to oxidize a substrate and to evaluate the kinetics of that enzyme in isolation.. The relative importance of each P450 enzyme in mediating the clearance of the drug in man can be predicted using kinetic information and taking into consideration the usual abundance of the enzyme in the liver and bowel wall and the usual concentration of the drug achieved under clinically relevant dosing conditions

These approaches are now being used in the drug development process to guide the selection of candidate drugs as follows. Early in drug development, several compounds will be synthesized which have the desired structure-activity characteristics for clinical testing (e.g., an investigational antipsychotic capable of selectively blocking the dopamine-4 receptor). Those compounds will then be screened further to chose a promising compound for clinical testing. Part of that process involves determining how that drug is metabolized, using the *in-vitro* approaches outlined above. The goal is to find the one compound out of the group of candidates that most closely meets the following three criteria. First, the drug should be metabolized equally well by more than one P450 enzyme. That characteristic reduces the likelihood that the compound will be the victim of a clinically significant pharmacokinetic interaction with concomitantly prescribed drugs or other environmental agents (e.g., dietary factors). Second, metabolism should not be dependent to any appreciable extent on a P450 enzyme with a significant genetic polymorphism (i.e., CYP2C19 and CYP2D6). That feature will simplify dosing, because it will avoid the prospect of a significant proportion of the population being "slow" metabolizers of the drug and hence likely to need a dose other than the "usually effective" dose. Third, the drug should not have significant inhibitory or inductive effects on P450s, particularly those mediating most known oxidative pathways of drug metabolism—CYP3A3/4, CYP2D6, CYP1A2, CYP2C19, and CYP2C9/10.

At the same time, *in-vitro* testing of drug metabolism has appreciably improved the ability to select target doses for Phase I and subsequent phases of clinical research. In the past, complicated formulas were used to try to predict these doses by extrapolation from *in-vivo* data obtained with different animal species. The problem with that older approach is that animals often metabolize drugs quite differently than man, both in terms of specific enzymes and enzyme activity. Modern *in-vitro* approaches utilize human P450s to permit a

better estimation of the likely *in-vivo* human metabolic rates and pathways.

Now, two sets of *in-vitro* data can be used to predict the clinically relevant dose: the *in-vitro* data for the presumed site of action and the *in-vitro* data for human metabolism. The underlying strategy is reflected in equation 1 as follows: The affinity for the presumed site of action (i.e., the first variable in equation 1) is coupled with knowledge of the distribution characteristics of the drug between plasma and the compartment containing the site of action. This can be used to estimate the plasma concentration needed for clinical efficacy (i.e., the second variable in equation 1). Based on the *in-vitro* human metabolism work, the dosing strategy that will be needed to achieve that plasma drug concentration can also be predicted. Together, these pieces of information can significantly increase the efficiency and yield of early Phase I studies in man. Similarly, work that has been done on the effect of different antidepressants on CYP2D6 can be used as a model for understanding the difference between *in-vitro* potency for a site of action and *in vivo* effect which, of necessity, must take the concentration of the drug into consideration. That issue is further discussed later in this chapter.

Predictions based on *in-vitro* studies are then confirmed or revised based on focused *in-vivo* studies. As with *in-vitro* studies, several complimentary approaches can be taken with the *in-vivo* studies. The fact that genetic polymorphism of CYP2C19 and CYP2D6 exists can be used to determine the role these P450s play by comparing the rate and type of metabolism in individuals with and without functional forms of these enzymes (11, 21, 24). The role of both polymorphic and non-polymorphic P450s can be assessed in several other ways, including: a) the degree of correlation between the rate of metabolism of the new drug via a specific pathway versus the rate for a known model substrate for the enzyme (7, 10, 89), and b) the effects of known selective P450 enzyme inhibitors (18, 90). This is an excellent example of the use of complementary *in-vitro* and *in-vivo* approaches as a means of acquiring clinically relevant knowledge of psychopharmacologic drugs.

ROLE OF *IN-VITRO* AND *IN-VIVO* STUDIES IN PREDICTING PHARMACOKINETIC DRUG-DRUG INTERACTIONS

The approach outlined above can also be used to predict the likelihood of a pharmacokinetic drug-drug interaction mediated via an effect on a specific P450 enzyme. This approach has revolutionized research in this area.

In the past, pharmacokinetic drug-drug interaction studies were pursued because two drugs were likely to be used together or because drugs in a particular "class" were known to interact with certain other drugs. The term ,"class", was used loosely, as in therapeutic "class" (e.g., antidepressant) rather than pharmacologic "class" (e.g., beta blocker). Moreover, there was little theoretical basis for extrapolating the results beyond the specific drugs tested in the study. For example, one could not confidently state that because a drug increased plasma levels or augmented the effects of one benzodiazepine that it would necessarily do the same with another. In fact, we know that a drug might affect one group of benzodiazepines but not another, based on which P450s mediate metabolism (42). Before, interactions had to be memorized by rote, but now they can be deduced and organized based on the P450 enzyme that mediates the particular interaction.

Figure 1 illustrates the two sets of data that are being developed and the way that they are used to organize and predict P450 enzyme-mediated pharmacokinetic drug-drug interactions (78). The first data set concerns whether a drug affects a specific P450 enzyme (i.e., inhibition or induction). The second data set concerns which drugs are metabolized by specific P450 enzymes and to what degree they are metabolized. Using these two sets of information, we can predict whether one drug will alter the metabolism of another drug. In addition, the results of a specific pharmacokinetic study can be extrapolated well beyond the specific drugs

studied. The altered pharmacokinetics of the affected drug in such a study actually reflects the effect of the causative agent on the P450 enzyme responsible for the biotransformation of the affected drug.

Research can move in a cooperative fashion from *in-vitro* to *in-vivo* studies and back (28). *In-vitro* studies use a drug as a substrate for the enzyme and test the effect of the drug on the enzyme. Studies of induction are more difficult than those of inhibition, since they require DNA with all its transcriptional factors. Also, enzyme induction may require a period of weeks to occur. In the rest of this chapter, we will focus on inhibition studies.

In-vitro studies to determine the ability of a drug to inhibit a specific P450 enzyme follow the same pattern as those to determine which specific P450 enzyme is responsible for the biotransformation of a drug (46, 95). These studies can be performed with purified P450 enzymes, cells transfected with cDNA, or with hepatic microsomal preparations. The goal is to determine the potency of a drug for inhibiting a specific P450 enzyme by using a model substrate which is known to be principally and (ideally) exclusively dependent on a single P450 enzyme for its biotransformation. The potency of the causative drug is expressed as an inhibition constant, K_i (46, 78, 95).

Data acquired by these means can then be used to determine whether the causative drug is likely to produce a potentially meaningful inhibition of the P450 enzyme under clinically relevant dosing conditions; that is, will the interaction alter the plasma level of a concomitantly prescribed medication (86). That prediction is based on the *in vitro* potency of the drug and the concentration of the drug that should be achieved at the enzyme under clinically relevant dosing conditions. The latter is generally assumed to be the hepatic concentration of the drug, which is estimated based on the expected plasma concentration of the drug under clinically relevant dosing conditions and the plasma:liver partition coefficient under steady state conditions (45, 95).

The *in-vitro* approach permits rapid screening of drugs for their potential to cause clinically meaningful pharmacokinetic drug-drug interactions via effects on P450s. *In-vivo* studies can be limited to those which are likely to confirm a potentially clinically meaningful interaction. Since the *in-vivo* studies involve human exposure, the *in-vitro* prediction can also be used to guide dose and substrate selection to reduce risk to the subjects. In addition, this approach increases the yield of *in-vivo* studies, which are considerably more expensive and time-consuming than *in-vitro* studies. This area then is another example of the complementary nature of *in-vitro* and *in-vivo* studies.

The sequence of events can also go in the opposite direction. For example, a case report (i.e., an in vivo observation) may suggest an interaction that had not previously been suspected. *In-vitro* work can then be done to explore the mechanism underlying the interaction. Subsequently, formal *in-vivo* pharmacokinetic studies can then be done to confirm the interaction.

A case in point is the work that has been done on the effects of selective serotonin reuptake inhibitors (SSRIs) on P450s. Interest in this area began with case reports of appreciable increases in plasma levels of tricyclic antidepressants when fluoxetine was added to the treatment regimen (6, 79, 94). A considerable amount of *in-vitro* and *in-vivo* work has now been done to characterize the differential effects of these drugs on various P450s (46, 78). The work with SSRIs will be reviewed later in this chapter as an example of research in this area.

The relationship between the *in-vitro* and *in-vivo* approaches again harkens back to equation 1. The *in-vitro* work determines the potency of the drug's effect on the enzyme. *In-vivo* work determines the magnitude of the effect under clinically relevant dosing conditions (i.e., potency in the context of the concentration of drug

achieved at the enzyme [the second variable in equation 1). Such work can serve as model for research where the site of action is a neural mechanism, such as an uptake pump or neuroreceptor. In the case of metabolism studies the consequence of the *in-vivo* effect of the drug on the site of action (is the change in the clearance of an exogenously administered substrate [such as a drug] for the enzyme). That is a much simpler end point to study and quantitate than the effect on a neural circuiti.

In-vivo studies are important for several reasons. First, they take into account the effects of metabolites of the drug as well as the parent drug. *In-vitro* studies are often limited to studying only the effect of the parent drug and perhaps its primary metabolite. Second, *in-vivo* studies can test for induction as well as inhibition, whereas *in-vitro* studies usually assess only inhibition. A drug that both induces and inhibits a specific P450 enzyme may have offsetting effects *in vivo* which would not be apparent in many *in-vitro* studies. Third, *in-vivo* studies can incorporate pharmacodynamic measures to assess the potential clinical significance of a change in the levels of the affected drug. For example, *in-vivo* studies testing the effect of a drug on the clearance of warfarin could include measures of clotting time to measure the consequence of a change in the levels of substrate. However, most often clinical significance is inferred rather than directly measured, for a variety of reasons, including cost and limited application. Since a specific P450 enzyme can metabolize a wide variety of drugs with different pharmacodynamics, an appropriate physiological measure for one substrate may have no relevance to another substrate for the same P450 enzyme. For example, CYP2D6 metabolizes antiarrythmics, analgesics, and antidepressants—which have quite different pharmacodynamic properties (17).

Instead, it is probably more useful to infer the potential clinical significance based on the magnitude of the change in clearance and a knowledge of the pharmacology of the affected drug, in terms of its concentration- or dose-dependent toxicity, tolerability and efficacy. A clinician can ask what the level of concern would be if the dose of the affected drug were changed to the same degree as the plasma drug level. They can ask the question: What would be my level of concern if the patient were taking a 25% or 400% higher dose than the dose I prescribed? Obviously, the answer to that question involves several considerations, including the nature of the prescribed dose (e.g., a benzodiazepine vs. a tricyclic antidepressant) and the dose that was initially prescribed (e.g., a quite low dose or a dose that was already near the upper end of the drug's therapeutic range).

EFFECTS OF SSRI ON P450

This chapter is not meant to be an exhaustive review of the effects of psychiatric medications on P450 enzymes nor the converse. Instead, the SSRIs will be used merely as an example. SSRIs were chosen for several reasons. They are arguably the best studied class of psychiatric medications, in terms of their effects on P450s (78). Also, these effects are the ones that differentiate the SSRIs from each other, as opposed to their more conventional psychopharmacological effects (46, 86).

Five SSRIs are marketed world-wide. The effects of these drugs on five P450s (CYP1A2, CYP2C9/10, CYP2C19, CYP2D6, and CYP3A3/4) have been studied using both *in-vitro* and *in-vivo* approaches.

The *in-vitro* studies and supporting references are summarized in Table 2 (23, 27, 57, 71, 72, 82, 87, 96, 97, 98, 99). Fluvoxamine is the most potent SSRI with regard to the *in-vitro* inhibition of CYP1A2. It is one to two orders of magnitude more potent than the next most potent SSRI. Norfluoxetine is the most potent SSRI inhibitor of CYP2C19, but the difference between norfluoxetine and the other SSRIs or their metabolites is

less than an order of magnitude. While citalopram is the least potent of the SSRIs in terms of CYP2C19 inhibition, its metabolite (didemethylcitalopram) is in the same range as the other SSRIs., Paroxetine is the most potent SSRI with regard to CYP2D6, but it is only 2–3-fold more potent than its metabolite (M2), which is equipotent with fluoxetine and its metabolite, norfluoxetine. However, paroxetine and fluoxetine are one or more orders of magnitude more potent than citalopram, fluvoxamine, and sertraline. With regard to CYP3A3/4, fluvoxamine and norfluoxetine are the most potent of the SSRIs and are approximately equipotent. However, the *in-vitro* potency of fluvoxamine and norfluoxetine on CYP3A3/4 is more than an order of magnitude less than their effects on CYP1A2 (fluvoxamine) and CYP2D6 (norfluoxetine) and more than two orders of magnitude less potent than antifungal agents such as ketoconazole. The other SSRIs, with the possible exception of citalopram, are within an order of magnitude of the potency of fluvoxamine and norfluoxetine in terms of CYP3A3/4 inhibition.

As can be seen in Table 2, discrepancies in findings can occur from one *in-vitro* study to another; thus, it is helpful to replicate a finding with several independent studies. For example, an early study by Crewe and colleagues (27) suggested that sertraline was virtually equipotent to fluoxetine in terms of the inhibition of CYP2D6. Later studies, however, consistently found sertraline to be an order of magnitude less potent. Similarly, some discrepancy exists among the three published *in-vitro* studies of the effects of the SSRIs on CYP1A2. While the rank order is generally the same, the study by von Moltke and colleagues (99) found only an order of magnitude difference between fluvoxamine and the other SSRIs, whereas two other studies found more than two orders of magnitude difference. (23, 82)

The *in-vitro* studies establish the first variable in equation 1. That information can be coupled with the plasma concentration of the SSRIs achieved under clinically relevant dosing conditions and their plasma:liver partition coefficients. The result can be used to predict which SSRIs, if any, are likely to cause a potentially clinically meaningful inhibition of a specific P450 enzyme during clinical use. Table 3 (45) shows the usual plasma levels of the SSRIs at their usually effective antidepressant doses, along with their plasma:liver partition coefficients (45, 78). The *in-vivo* effects of fluoxetine on P450 enzymes would be predicted to be magnified, relative to other SSRIs. That is because the combined plasma level of fluoxetine and its active metabolite is up to an order of magnitude higher than that of any other SSRI (Table 3). Similarly, the effects of fluvoxamine and paroxetine would be predicted to be magnified relative to both their *in-vitro* potency and their plasma levels, because these two drugs have the highest plasma:liver partition coefficients—approximately twice as high as fluoxetine or sertraline (Table 3).

Taking all of these factors into account, the following prediction could be made. Fluvoxamine would be the SSRI most likely to affect CYP1A2. Fluoxetine and fluvoxamine would be the SSRIs most likely to affect CYP2C19. Paroxetine and fluoxetine would be the SSRIs most likely to affect CYP2D6. Fluoxetine (due to norfluoxetine) and fluvoxamine would be the SSRIs most likely to affect CYP3A3/4, although their effects would likely be substantially less than those of other CYP3A3/4 inhibitors such as ketoconazole.

In addition to these *in-vitro* studies, a number of *in-vivo* studies have been done with the SSRIs, and these generally have confirmed the *in-vitro* predictions. Two formal studies have been done with regard to CYP1A2. Coadministration of fluvoxamine (100 mg/day for 8 days) reduced the clearance of caffeine by 80% (from 107 mL/min to 21 mL/min) and increased its half-life from 5 to 31 hours (52). In another study by Jeppensen and colleagues, fluvoxamine was the only one of four SSRIs to substantially inhibit the clearance of caffeine after a single dose (51). In separate studies, warfarin plasma levels were appreciably increased by the coadministration of fluvoxamine but not by fluoxetine, paroxetine, or sertraline (5, 8, 84, 101). The findings with warfarin are consistent with the notion that fluvoxamine inhibits CYP1A2. Interpretation of studies with warfarin are limited by the fact that it is a racemic compound, with the active enantiomer being

S-warfarin (which is metabolized by CYP2C9/10) and the inactive enantiomer being R-warfarin (which is metabolized by CYP1A2) [46, 58, 83]. Any increase in R-warfarin is particularly problematic because it can cause an increase in S-warfarin levels by competitively inhibiting CYP2C9/10 (58). Unless the study used an assay that can distinguish between the two enantiomers, the results could reflect either an effect on CYP1A2 or an effect on CYP2C9/10. Nevertheless, the results of individual studies of SSRIs on the pharmacokinetics of racemic warfarin are consistent with both the *in-vitro* and *in-vivo* studies. Finally, several case reports have reported that coadministration of fluvoxamine increased plasma levels of other coadministered drugs which are known CYP1A2 substrates, including clozapine, tertiary amine tricyclic antidepressants, and theophylline (12, 13, 44, 53, 88, 92).

The study by Jeppenson and colleagues is important because it tested the effects of four SSRIs on three different P450s in the same study (51). The four SSRIs were citalopram, fluvoxamine, fluoxetine, and paroxetine. The enzymes (and model substrates) were: CYP1A2 (caffeine), CYP2C19 (S-mephenytoin), and CYP2D6 (sparteine). Nevertheless, the interpretation of the results was seriously compromised by the fact that only single, albeit ascending, doses of the SSRIs were tested. As discussed above, the inhibition of P450s by SSRIs is concentration-dependent (45, 46). Hence, a single dose approach underestimates the effect of these drugs to the extent that it underestimates the steady-state concentration of the SSRI and any relevant metabolites. The limitation of this study is particularly problematic when interpreting the effects of fluoxetine for two reasons. First, norfluoxetine is more potent than fluoxetine in terms of the inhibition of these three P450s, and the metabolite requires time to accumulate. In addition, the half-lives of fluoxetine and norfluoxetine are such that single dose studies—even using loading dose strategies—will significantly underestimate the concentration that will be achieved on 20 mg/day under steady-state conditions. These caveats must be kept in mind when interpreting the results of this study to avoid underestimating the effects that will occur during the usual clinical use of fluoxetine.

The results of four *in-vivo* studies examined the effects of different SSRIs on CYP2C19 substrates (35, 51, 62, 73). The results are consistent with the *in-vitro* predictions. The effects of fluoxetine, fluvoxamine, and sertraline on diazepam pharmacokinetics were examined in three separate studies. Fluvoxamine at a mean dose of 112 mg/day for 16 days produced a 300% increase in diazepam levels (73). Fluoxetine,using at a loading dose of 60 mg/day to adieve plasma fluoxetine and norfluoxetine levels near those expected understeadly-state conditions at 20mg/ day for 8 days produced a 50% increase (62). Sertraline at 200 mg/day for 16 days caused a 13% increase (35). In the single dose study by Jeppensen and colleagues, fluvoxamine and fluoxetine increased the mephenytoin S/R ratio whereas citalopram and paroxetine did not (51).

CYP2D6 has been the most extensively studied P450 enzyme with regard to inhibition by the different SSRIs Table 4. As predicted by *in-vitro* modeling, paroxetine and fluoxetine produced substantial inhibition of this P450 enzyme, while the other three SSRIs did not. The average magnitude of the effect on plasma levels of CYP2D6 substrates is as follows: paroxetine, a 327–421% increase (1, 2, 20); fluoxetine, a 380–640% increase (9, 75); citalopram, a 47% increase (40); sertraline, a 0–37% increase (2, 50, 75); and fluvoxamine, a 14% increase (90). The single-dose study by Jeppensen and colleagues is also consistent with these results, although the underestimation of the effect of fluoxetine is obvious when comparing the result of this study to those of the multiple dose studies that have been done with fluoxetine.

Sertraline has been the only SSRI studied at higher doses. The results of these studies are generally consistent with the concentration-dependent nature of such inhibition. Sertraline at 100mg/day in two separate studies produced a 0-5% increase in plasma levels of a CYP206 substrate and at 150mg/day in two other studies produced a 54-70% increase Table 4.

The effect of the various SSRIs on CYP2D6 has also been assessed in terms of the change in dextromethrophan to dexorphan ratio or the conversion of CYP2D6 extensive metabolizers (Ems) to a phenocopy of CYP2D6 "poor" metabolism (PM) status Table 4. Both of these approaches have also demonstrated an appreciable different between fluoxetine and paroxetine on one hand and fluvoxamine and sertraline on the other. For example, over 60% of CYP2D6 extensive metabolizers (EMs) are converted to PM status under dosing conditions which approximate 20mg/day of fluoxetine or paroxetine given to steady-state versus 0% for fluvoxamine or sertraline dosed at 100mg/day to steady-state conditions.

Several studies have also been done testing the effects of the different SSRIs on CYP3A3/4 substrates. The results with this enzyme are consistent in several ways with those predicted based on the *in-vitro* data. First, fluvoxamine and fluoxetine produced the greatest effect, but the magnitude was quite modest, both in comparison to the effect of these SSRIs on other P450s and in comparison to the effects of inhibitors such as ketoconazole on CYP3A3/4 (93). The CYP3A3/4 substrates that have been used in these studies include alprazolam, carbamazepine, and terfenadine. In studies using alprazolam as a substrate, fluvoxamine produced a 100% increase in alprazolam plasma levels (32), fluoxetine a 26–33% increase(42,61), and sertraline had no effect (76). In studies that used carbamazepine as a substrate, there was a reported 30–70% increase with fluvoxamine (15, 34), while paroxetine and sertraline had no effect (3, 81). Fluoxetine produced a 27% increase, which is an underestimatim since the dosing strategy used produced plasma levels of fluoxetine and norfluoxetine below those that would be expected at steady-state with a dose of 20 mg/day (43). Paroxetine and sertraline also had no effect on terfenadine plasma levels in two separate studies (76).

Thus, the *in-vivo* work with SSRIs demonstrates the value of the *in-vitro* modeling approach. The fact that there is also consistency between studies in terms of the effects of different SSRIs on different substrates for the same P450 enzyme further confirms the generalizability of the studies. This is important, since time and expense prohibit individually testing the effect of a drug on every substrate for a P450 enzyme. Since the results generalize as expected, the clinician can use the result with one P450 enzyme substrate as a general indication of whether and to what extent a dose adjustment is needed when he prescribes a specific P450 enzyme inhibitor for a patient who is already on a drug that is principally dependent on that P450 enzyme for its clearance.

In summary, the research examining the effects of P450s on psychiatric medications and vice versa has multiple implications for psychopharmacology, from the drug development process to the safe and effective use of the medications in clinical practice.

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