DRUG–DRUG INTERACTIONS: SCIENTIFIC AND REGULATORY PERSPECTIVES

Edited by

Albert P. Li

In Vitro Technologies Inc.
University of Maryland Technology Center
Baltimore, Maryland

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F. Peter Guengerich

Department of Biochemistry and Center in Molecular Toxicology
Vanderbilt University School of Medicine
Nashville, Tennessee 37232–0146

Role of Cytochrome P450 Enzymes in Drug–Drug Interactions

I. Introduction

Cytochrome P450 (P450) enzymes were discovered in independent studies on the metabolism of drugs, carcinogens, and steroids (Guengerich, 1993). These enzymes are components of mixed-function oxidase systems that catalyze reactions of the overall stoichiometry

$$NAD(P)H + H^+ + O_2 + R \rightarrow NAD(P)^+ + H_2O + RO,$$

where R is an organic substrate (e.g., drug in our discussion here). At the present time the conventional wisdom is that there are ~40 different P450 enzymes expressed in each mammalian species, including humans (Nelson *et al.*, 1993; Guengerich, 1995). Many of these P450 enzymes have specific roles in the anabolism of steroids. The P450 enzymes that oxidize drugs are localized in the liver, although some of this group are also found in sites

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such as lung and small intestine, where an appreciable contribution to overall metabolism of a drug can occur depending on the route of administration.

Fortunately, it appears that the metabolism of most drugs can be accounted for by a relatively small subset of the P450s. One estimate is that ≥90% of human drug oxidation can be attributed to six enzymes: P450s 1A2, 2C9/10, 2C19, 2D6, 2E1, and 3A4 (Guengerich, 1995; Wrighton and Stevens, 1992). Further, most could probably be attributed to P450s 1A2, 2C9/10, 2D6, and 3A4 and, by some estimates, half can be attributed to P450 3A4 (Guengerich, 1995; Guengerich *et al.*, 1994a). This view is based primarily on (*in vitro*) microsomal studies done with drugs studied to date and may change somewhat with time. For instance, the fraction of drugs oxidized by P450 2D6 may be too high in current estimates because of the ease of identifying these and the attention that has been given to this particular enzyme. Nevertheless, the concept that most drug oxidations are catalyzed primarily by a small number of P450 enzymes is important in that the approaches to identifying drug–drug interactions are feasible, both *in vitro* and *in vivo*.

This chapter operates from the premise that many significant drug–drug interactions can be understood in terms of P450s. However, drug–drug interactions are more complex for at least two reasons. First, some drug–drug interactions can be attributed to pharmacokinetic differences due to other enzymes such as monoamine oxidases, flavin-containing monooxygenases, UDP-glucuronosyl transferases, and sulfotransferases. These and other so-called "drug-metabolizing" (or "xenobiotic-metabolizing") enzymes also show the characteristics of induction and inhibition by drugs that are associated with P450s, although most have not yet been studied as extensively. The other aspect of drug–drug interactions is that some of these are probably pharmacodynamic instead of pharmacokinetic. For instance, drugs can compete for binding to a receptor directly related to the pharmacological response.

II. Potential Consequences of Drug–Drug Interactions

From a pharmacokinetic standpoint, the major effects of drug–drug interactions can be understood in terms of causing the disposition of a durg to be unusually slow or fast. The major consequence is a high or low plasma and tissue level of the drug.

If the metabolism of a drug is impeded due to enzyme inhibition, then a high plasma level may follow (Fig. 1). One of the major effects will be increased pharmacological activity, and this may or may not be a problem, depending on the therapeutic window. Of course, not only the desired effect may be increased but also any undesirable side effects. If activation of a pro-drug is inhibited, then a lower level of therapeutic effectiveness might

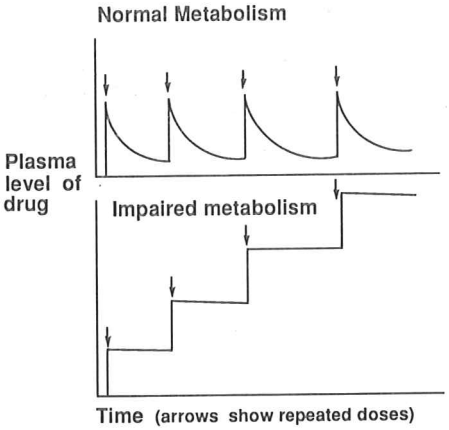

FIGURE 1 Effect of enzyme inhibition on drug metabolism and plasma drug levels.

be anticipated. Another possibility is that when the major pathway of metabolism of a drug is blocked, secondary pathways may become more favorable. This can be a problem if the secondary pathway leads to a toxic product. An example of this is seen with the analgesic phenacetin (no longer on the U.S. market). If O-deethylation (P450 1A2) is slow, then other pathways are favored that lead to quinoneimine formation and methemoglobinemia (Fischbach and Lenk, 1985; Klehr *et al.,* 1987). Another possibility is that the increased level of a drug due to inhibition of the P450 involved in its oxidation may lead to inhibition of another P450. Although direct evidence for such a situation has not been presented, one could postulate that accumulation of quinidine due to P450 3A4 inhibition might lead to inhibition of P450 2D6, an enzyme for which quinidine is an inhibitor but not a substrate (Guengerich *et al.,* 1986b; Otton *et al.,* 1984).

When levels of P450 (or, for that matter, another enzyme) are induced, the major consequence is a lack of therapeutic effectiveness. Although this might seem to be a common event, the number of real clinical situations in which this has been a problem are rather limited. Two of the best documented examples are cyclosporin and 17α-ethynylestradiol (*vide infra*). Another possibility with a pro-drug is that activation may be too rapid and a seriously high level of active drug could result. This could be a problem, as one of the primary reasons for developing pro-drugs is to avoid a transiently high level of the active drug. However, no good examples of clinical problems resulting from a phenomenon of this type are known yet.

There are two other possibilities that can be considered in regard to issues of drug–drug interactions. One involves metabolism of chemical carcinogens. Most of the P450s that transform drugs can also oxidize chemical carcinogens (Guengerich and Shimada, 1991; Guengerich, 1995). The possibility exists that a P450 induced by a drug could lead to enhanced levels

of DNA–carcinogen adducts due to increased carcinogen activation. The induction of human P450s 1A1 and 1A2 by omeprazole was postulated to present a risk due to such considerations (Diaz *et al.*, 1990). Whether or not this is a serious issue is unknown, as levels of P450 1A2 are only one of many factors linked to cancer risk from known carcinogenic substrates for the enzyme (Lang *et al.*, 1994). Nevertheless, most pharmaceutical companies and the Food and Drug Administration (FDA) would rather avoid drugs that induce P450 1A subfamily enzymes, which have been suggested to be related to cancer development (Ioannides and Parke, 1990, 1993). Again, it should be emphasized that increased cancer risk due to P450 (1A or other) induction is still a hypothesis. Another matter to consider is that some P450s are involved in the detoxication of potential carcinogens and that induction or inhibition might have an impact on this process, as well as the activation (Guengerich and Shimada, 1991; Richardson *et al.*, 1952; Nebert, 1989).

The other possibility involves the influence of drugs on P450s involved in the transformation of endogenous compounds, i.e., those normally found in the body. This matter has not been extensively investigated, but some possibilities exist. Depending on the tissue, 17β-estradiol is oxidized by P450 3A4 (Brian *et al.*, 1990; Guengerich *et al.*, 1986a), P450 1A2 (Guo *et al.*, 1994), or P450 1B1 (Liehr *et al.*, 1995). It is not known what the impact of changes in these enzymes is on total body levels. Testosterone is a substrate for P450 3A4 (Guengerich *et al.*, 1986a; Waxman *et al.*, 1988). Another case to consider is animals devoid of bilirubin UDP-glucuronosyl transferase activity, who can be administered P450 1A inducers to lower their levels of bilirubin to nontoxic levels (Kapitulnik and Gonzalez, 1993; Kapitulnik and Ostrow, 1978).

A final point to consider is that some drugs may affect the disposition of chemicals in foods and beverages through P450 interactions. For instance, the drug disulfiram (Antabuse) inhibits P450 2E1. This would affect ethanol oxidation by P450 2E1, although the more serious effect is on aldehyde dehydrogenase (Guengerich *et al.*, 1991). The P450 1A2 inhibitor furafylline blocks caffeine N^3-demethylation to the point where severe insomnia is associated with drinking coffee (Sesardic *et al.*, 1990; Kunze and Trager, 1993).

III. Use of Information about Human P450s

Much of the information abot drug metabolism by human P450s has been acquired in the past decade. Induction was first seen in clinical settings in the 1950s (Remmer, 1959) and there were many *in vitro* studies with human tissue samples (Distlerath and Guengerich, 1987). By 1980 some purification work had commenced and several of what are now recognized

as the major P450 2C and 3A subfamily proteins were isolated (Kitada and Kamataki, 1979; Beaune *et al.,* 1979; Wang *et al.,* 1980). Further work led to the isolation of more of the major human P450s (Guengerich, 1989), and cDNA cloning methods were used to obtain DNA sequences for the human P450s (Gonzalez, 1989).

In recent years the access to human tissue samples in the United States and Europe has facilitated characterization of P450 reactions catalyzed by human P450s. The availability of the recombinant human P450s expressed in various systems has also facilitated studies on their catalytic selectivity (Gonzalez *et al.,* 1991a,b; Guengerich *et al.,* 1996). Thus, it is now relatively straightforward to use *in vitro* studies to determine which P450s oxidize a particular drug and which drugs can inhibit oxidations catalyzed by this P450. The *in vitro* determination of inducibility is not as easily done, but a number of possibilities exist with cultures of human hepatocytes (Guillouzo *et al.,* 1993; Loretz *et al.,* 1989) (also see chapter by A. P. Li).

It is also possible to do logical *in vivo* studies to test the relevance of *in vitro* findings. For instance, individuals known to be high or low in a particular P450 from the use of other noninvasive assays can be examined with regard to the pharmacokinetics of the new drug to see if there is a match. In some cases, inducers or inhibitors of a specific P450 can be given safely to people to verify that a P450 is involved in the oxidation of a drug. Also, the drug under consideration can be given to people to determine if it affects the pharmacokinetics of other drugs through enzyme induction.

The acquisition of the *in vitro* information about a new drug can be extremely useful. In many cases, the FDA now expects *in vitro* information on the P450s involved in the oxidation of a drug early in the registration process. The *in vitro* information can be used to guide the more expensive and time-consuming *in vivo* studies. In particular, potential adverse drug interactions due to pharmacokinetics can be predicted and the number of *in vivo* interaction studies can be restricted, as some of those historically done with all new drugs may be found irrelevant. The *in vitro* procedures, if used early in the drug development process, may be used to select from a series of potential candidates, in terms of which will be least likely to cause problems with drug–drug interactions. Another point is that the *in vitro* studies can be used as a guide in predicting bioavailability, simply by screening candidate drugs for resistance to oxidation by the major known human P450s.

IV. Mechanisms of Drug Interactions Attributable to P450s

A. Induction

This is a phenomenon first identified a half century ago in *in vivo* studies with humans, primarily in the laboratories of Remmer and Brodie (Remmer,

1959; Brodie *et al.,* 1958). Individuals who were administered certain drugs developed a certain "tolerance," in that increasing doses were needed to produce the same effect. Work with experimental animals demonstrated that the effect could be reproduced. For instance, animals treated with barbiturates decreased their "sleeping time," a parameter indicating how long a certain dose of a barbiturate would keep animals sedated (Burke, 1981). Other studies on chemical carcinogenesis reinforced the concept of enzyme induction (Conney *et al.,* 1956; Conney, 1967), particularly with what are now termed the P450 1A subfamily genes.

The mechanism of P450 1A induction is perhaps the most well characterized in this field (for reviews, see Hankinson, 1993; Denison and Whitlock, 1995). Although barbiturate induction was also discovered early, mechanistic studies on this phenomenon are not as well developed and there is not general agreement regarding observations in different laboratories (Rangarajan and Padmanaban, 1989; Liang *et al.,* 1995; Ramsden *et al.,* 1993). Nevertheless, there seems to be a rather general agreement that most human P450 2C and 3A subfamily proteins are induced by barbiturates (Zilly *et al.,* 1975; Morel *et al.,* 1990). Studies with experimental animals indicate that subfamily 2B proteins are induced by barbiturates (Burke, 1981; Guengerich, 1987), but direct information on inducibility in humans is not available. Evidence indicates that human P450 2E1 is inducible by ethanol and isoniazid, although the mechanism of the process is complex (Perrot *et al.,* 1989; Kim *et al.,* 1994). Compounds (including drugs) that cause peroxisomal proliferation induce P450s in the 4A subfamily in experimental animals (Muerhoff *et al.,* 1992; Rao and Reddy, 1991; Gibson, 1993); presumably this can also happen in humans, although the system is suspected to be less responsive (Bell *et al.,* 1993). The mechanism involves the interactions of ligand-bound peroxisomal proliferation activation receptor (PPAR): retinoid X receptor (RXR) heterodimers with upstream recognition sequences (Lee *et al.,* 1993), and compounds such as fatty acids and retinoids may be involved in this response.

The effect of induction is simply to increase the amount of the P450 present and make oxidation and clearance of a drug faster. As mentioned earlier, details of mechanisms remain to be understood. The overall situation is complicated because even in situations where a response element can be identified, there are probably interactions with other response systems that must be considered. However, knowledge of such phenomena will be useful in the further development of *in vitro* systems that can be used to screen new drug candidates for their potential as inducers of P450s and other enzymes.

B. Inhibition

Inhibition is decreased enzyme activity due to direct interaction with a drug (or other chemical). In a sense, this can be considered more serious

than enzyme induction because inhibition happens rather immediately and does not take time to develop, in the manner that induction does. Further, there seem to be more reported incidences of drug–drug interaction problems that can be attributed to inhibition rather than induction. There are different types of enzyme inhibition, and the clinical effects are influenced by the basic mechanisms.

The first type of inhibition is competitive, where the inhibitor and substrate compete for the same binding site on an enzyme. In the situations under consideration here, the inhibitor and the substrate would be drugs, competing for the binding site of a P450. Insofar as is currently known, P450s are thought to have a single substrate-binding site (aside from some possible allosteric situations, *vide infra*) (Raag and Poulos, 1991; Cupp-Vickery and Poulos, 1995), although the sizes and flexibility of the microsomal P450s we are concerned with here are not known. The inhibitor may be a substrate itself. For examples, see the section on P450 2D6 (*vide infra*). This type of inhibition is easily identified by the classic intersecting plots seen in *in vitro* studies (Kuby, 1991).

Another type of inhibition has precedent in the classical studies of enzymology. The two situations are called *noncompetitive* inhibition, where the inhibitor binds at a site on the enzyme distinct from the substrate, and *uncompetitive* inhibition, where the inhibitor binds *only* to the enzyme–substrate complex (Kuby, 1991). Actually, neither of these have many clear examples in the literature of drug–drug interactions or in drug metabolism in general. (An example of a noncompetitive inhibitor would be a reagent that modifies sulfhydryl groups remote from the substrate-binding site to attenuate the activity of an enzyme.)

A fairly common mechanism of inhibition related to drug–drug interactions is *mechanism-based,* or suicide, inhibition (Silverman, 1988, 1995; Ortiz de Montellano and Correia, 1983; Ortiz de Montellano and Reich, 1986; Halpert and Guengerich, 1997). In the strict definition of the mechanism, a substrate (the inhibitor) is transformed by the enzyme in the normal course used for other substrates and an intermediate is formed, which usually has a fleeting but finite half-life. This intermediate can partition between reaction with the enzyme (at the active site), to inactivate the enzyme, or undergo a different transformation (e.g., reaction with water or proton loss) to yield a stable product. The ratio of the two processes (latter : former) is termed the *partition ratio* and is used to compare the efficiencies of different mechanism-based inactivators. Mechanism-based inactivators are characterized *in vitro* by a number of properties, including time-dependent loss of enzyme activity, requirement for normal enzyme cofactors, blockage by noninhibitory substrates, saturation kinetics, and (usually) single irreversible modification of the protein or prosthetic group (Silverman, 1988). Examples of this type are seen in the P450 drug metabolism literature with compounds such as secobarbital (Levin *et al.,* 1973), gestodene (Guengerich, 1990a),

furafylline (Kunze and Trager, 1993), and disulfiram (Guengerich *et al.*, 1991; Brady *et al.*, 1991), and many clinical interactions may be understood in these terms. The inhibition of specific enzymes by mechanism-based inactivators is an approach used in the design of new drugs. In principle, a substrate can be designed as a mechanism-based inactivator of a single enzyme. This approach has been used to attenuate monoamine oxidase (Thull and Testa, 1994). The only good examples of development of drugs to specifically inhibit P450s deal with P450 19, the steroid aromatase, which is a target in breast and ovarian tumors because of its role in estrogen production (Brodie, 1994). Nevertheless, there are many examples of experimental compounds that are selective inactivators of individual P450 enzymes *in vitro* and in experimental animals (Ortiz de Montellano and Reich, 1986). These can be used in a diagnostic manner (to help identify P450s involved in various reactions) (Guengerich and Shimada, 1991) to label enzyme active sites (Roberts *et al.*, 1994; Yun *et al.*, 1992) and to identify a drug target in a complex mixture of proteins (Rando, 1984).

Several other types of irreversible enzyme inhibition are related to mechanism-based inactivation but can be distinguished. In one case, there is time-dependent inhibition at the active site by reaction of a substrate (or analog) with the protein, unrelated to the normal enzyme mechanism. A good example is not available for P450, except perhaps a substrate such as acrylonitrile that reacts rather nonselectively with all protein sulfhydryls but is oxidized by P450 2E1. A slow-binding inhibitor of testosterone 5α-reductase is the prostate growth inhibitor finasteride (Proscar) in which the enzyme bonds with the drugs at a slow rate, competitive with normal steroids, and irreversibly inactivates the enzyme (Tian *et al.*, 1995).

Another case involves the conversion of a substrate to a product that is reactive enough to modify the protein. An example of this latter case is chloramphenicol, which is oxidized by P450 to an acyl chloride (Halpert *et al.*, 1985). The acyl chloride is not an enzyme intermediate in the strict sense. The product can be readily hydrolyzed by water. It would also leave the protein and modify other proteins; however, the similarity of the molecule to the substrate seems to keep it in the active site so that it will label groups there. Distinguishing inhibitors of this type from true mechanism-based inactivators may not be easy; one test is to determine if a scavenger such as glutathione (which would not enter the active site of the enzyme) can block inhibition. Another test is to find a certain P450 enzyme ("P450 1") that is not inactivated when incubated with the drug (plus normal cofactors). This P450 ("P450 1") can be mixed with the drug, cofactors, and another P450 known to be inactivated ("P450 2"). If P450 1 is now inactivated, then the most direct explanation is that a reactive product has migrated from P450 2 to P450 1. Although the mechanistic distinction may seem subtle, these properties influence the selectivity of inhibitors of P450s. An

example of this type of inhibition involves 4-alkyl dihydropyridines oxidized by P450 3A4 that inhibit P450 2C9 (Böcker and Guengerich, 1986).

Sometimes the product of a P450 reaction may inhibit by liganding to the heme iron instead of covalent modification of amino acid residues. For instance, many amines are oxidized to nitroso compounds that form spectral complexes with absorbance maxima at 455 nm (Jönsson and Lindeke, 1992; Mansuy *et al.*, 1983). A classical case in pesticide biochemistry is the synergist piperonyl butoxide, which is oxidized to a carbene that binds the heme (Ortiz de Montellano and Reich, 1986). Evidence shows that mechanisms of this type may be important in inhibition under physiological conditions (Bensoussan *et al.*, 1995).

C. Stimulation

Enzyme stimulation refers to the process by which direct addition of one compound to an enzyme enhances the rate of reaction of the substrate. This phenomenon has been observed in a number of cases with P450s (Halpert and Guengerich, 1997; Huang *et al.*, 1981).

Distinguishing enzyme induction and stimulation *in vivo* is not easy because some of the compounds that seem most effective in P450 stimulation are also enzyme inducers, e.g., flavonoids. One approach used was the treatment of rats with a substrate in which product formation was accompanied by the release of tritiated water, for a short period of time (15 min), in the absence or presence of flavone (Lasker *et al.*, 1982). The increase in product formation observed (in total body radioactive water) in the presence of flavone provides evidence that stimulation occurred in a time frame before significant enzyme induction could have occurred.

In our laboratory we have been studying the effect of α-naphthoflavone on the oxidation of the carcinogen aflatoxin B_1 by P450 3A4 (Raney *et al.*, 1992; Ueng *et al.*, 1995). α-Naphthoflavone has the interesting effect of inhibiting the 3α-hydroxylation of aflatoxin B_1 but stimulating the 8,9-epoxidation, and our current working hypothesis is that an allosteric mechanism is involved (Ueng *et al.*, 1995; Guengerich *et al.*, 1994b). In line with this view, plots of rates of these reactions versus substrate concentration are sigmoidal in the absence of α-naphthoflavone but hyperbolic in the presence of α-naphthoflavone (Ueng *et al.*, 1995). There is also evidence in the literature that sigmoidal kinetics are observed in the *in vitro* oxidation of drugs [e.g., carbamazepine (Kerr *et al.*, 1994) and possibly acetaminophen (Lee *et al.*, 1991)] and steroids [e.g., progesterone and 17β-estradiol (Schwab *et al.*, 1988)], usually with P450 3A subfamily enzymes.

The *in vivo* relevance of these phenomena to drug metabolism remains to be established, as does the mechanism(s).

V. Examples of P450-Based Interactions

A. Cimetidine

Cimetidine (Tagemet, Fig. 2) is a drug that inhibits antihistamine H_2 receptor binding and is used in the treatment of gastric ulcers. There is considerable literature on the inhibition of drug metabolism by cimetidine in both animal and human models (Gerber *et al.*, 1985). A similar H_2 receptor antagonist, ratinidine (Zantac), was developed by another company and was devoid of the inhibitory properties, a point that was exploited in marketing.

Analysis of the scientific literature indicates that cimetidine is a relatively weak P450 inhibitor (Knodell *et al.*, 1991). No serious acute episodes of adverse health have been attributed to cimetidine despite long use in many patients, many of whom are undoubtedly using other drugs.

The mechanism of inhibition appears to involve the imidazole ring of cimetidine, which is not present in ranitidine. Cimetidine shows selectivity for inhibiting reactions catalyzed by P450s 2D6 and 3A4 (Knodell *et al.*, 1991). The inhibition has generally been regarded as due to competitive binding of cimetidine, possibly through interaction of the imidazole with the P450 heme. However, some evidence for mechanism-based inactivation of P450 has also been published (Coleman *et al.*, 1991), although a chemical basis has not been established.

B. P450 2D6

P450 2D6 inhibitors and substrates have attracted considerable concern. In the early 1970s Smith personally experienced an adverse response in a clinical trial of the antihypertensive agent debrisoquine. This episode led him to study the basis in more detail, and the work led to the identification of a subset of the population (~7% Caucasians) as "poor metabolizers," who hydroxylated the drug at a much slower rate than the rest of the population (Mahgoub *et al.*, 1977).

Subsequent work led to characterization of this enzyme, P450 2D6, by purification, cDNA cloning, and genetic analysis (Gonzalez *et al.*, 1988; Gonzalez and Meyer, 1991). P450 2D6 is now recognized to be involved in the oxidation of >30 drugs. Some of these show relatively narrow thera-

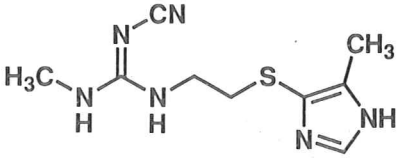

FIGURE 2 Structure of cimetidine.

peutic windows and adverse side effects have been reported. The case of debrisoquine itself has already been mentioned. The accumulation of perhexiline in poor metabolizers has been reported to cause peripheral neuropathy (Shah *et al.*, 1982). However, P450 2D6-deficient individuals do not convert the pro-drug encainide to its active form as effectively as the rest of the population (Woosley *et al.*, 1981). A number of drugs are also potent inhibitors of P450 2D6 (Fig. 3) (Strobl *et al.*, 1993). Prominent among these are alkaloids such as quinidine and the ajmalicine derivatives (Strobl *et al.*, 1993; Fonnę-Pfister and Meyer, 1988).

It is now relatively easy to identify P450 2D6 substrates and inhibitors *in vitro* early in the development process. Strong P450 2D6 inhibitors are generally avoided. An issue can be raised, though, as to how serious a P450 2D6 inhibitor really is. Because ~5% of the population (depending on the country) is already deficient in P450 2D6, the effect of the inhibitor is to extend this group of individuals. The problem would be slow metabolism of P450 2D6 substrates, but this may not be a serious issue.

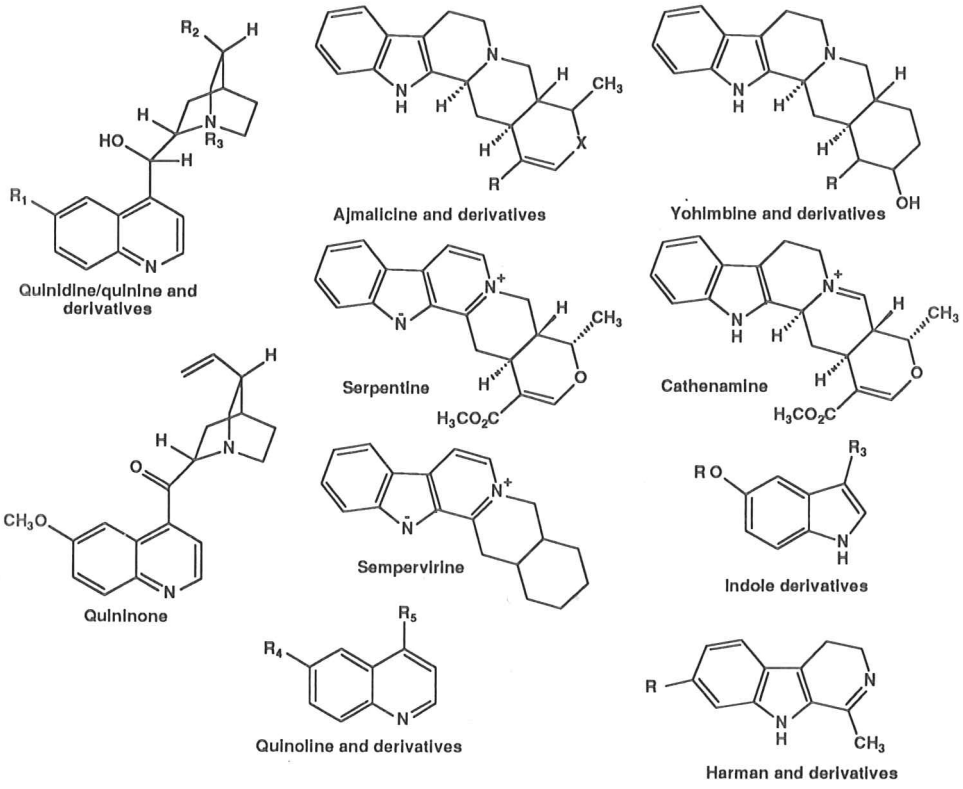

FIGURE 3 Some inhibitors of P450 2D6 (Strobl *et al.*, 1993).

The issue of development of P450 2D6 substrates has been a more serious matter, and some pharmaceutical companies had developed policies of dropping these from development. A realistic way of addressing the issue is to test candidate drugs *in vitro* to determine if they are substrates and then proceed to examine them *in vivo* to establish the pharmacokinetics and the therapeutic window. The majority of P450 2D6 substrates can probably be tolerated reasonably well even by P450 2D6-deficient individuals.

The molecular basis of the P450 2D6 polymorphism has been described in detail, and there are a number of alleles that contribute to cause both unusually slow and also unusually rapid oxidation (Broly *et al.*, 1991; Johansson *et al.*, 1993). The P450 2D6 substrates and inhibitors all seem to share a basic nitrogen group, which is positioned 5–7 Å away from the site of hydroxylation. The carboxylate anionic moiety of Asp 301 has been suggested to interact with the basic nitrogen of the substrate, on the basis of modeling and site-directed mutagenesis work (Ellis *et al.*, 1995). Substrates and inhibitors have been used to develop pharmacophore models of P450 2D6 (Strobl *et al.*, 1993; Islam *et al.*, 1991; Koymans *et al.*, 1992). The strong inhibitors of P450 2D6 (e.g., quinidine) are not readily oxidized (Strobl *et al.*, 1993; Guengerich *et al.*, 1986b), and the conclusion has been reached that the basic nitrogen in these binds to the same protein anion as the substrates (Asp 301) but no atoms that can be oxidized are accessible to the FeO complex (Islam *et al.*, 1991). The model does not explain the oxidation of deprenyl by P450 2D6 (Grace *et al.*, 1994). A modification involves the transient deprotonation of the amine and electron transfer (Grace *et al.*, 1994; Guengerich, 1995).

C. 17α-Ethynylestradiol

This is a classic example of a drug–drug interaction and one of the few attributed to induction, instead of inhibition. In the early 1970s several German reports indicated that women who were using oral contraceptives began spotting or became pregnant after using rifampicin or barbiturates (Reimers and Jezek, 1971; Nocke-Finck *et al.*, 1973; Janz and Schmidt, 1974). The major estrogen in oral contraceptives is 17α-ethynylestradiol (Fig. 4), which is metabolized via 2-hydroxylation, plus other pathways (Bolt *et al.*, 1973; Guengerich, 1990b). Administration of rifampicin resulted in the faster elimination of 17α-ethynylestradiol in volunteers (Bolt *et al.*, 1977).

Subsequently, P450 3A4 was shown to be a major enzyme involved in the (2-)hydroxylation of 17α-ethynylestradiol (Guengerich, 1988). P450 3A4 can also be induced by rifampicin or barbiturates in cultured human hepatocytes (Morel *et al.*, 1990), and *in vivo* induction has also been reported (Watkins *et al.*, 1985).

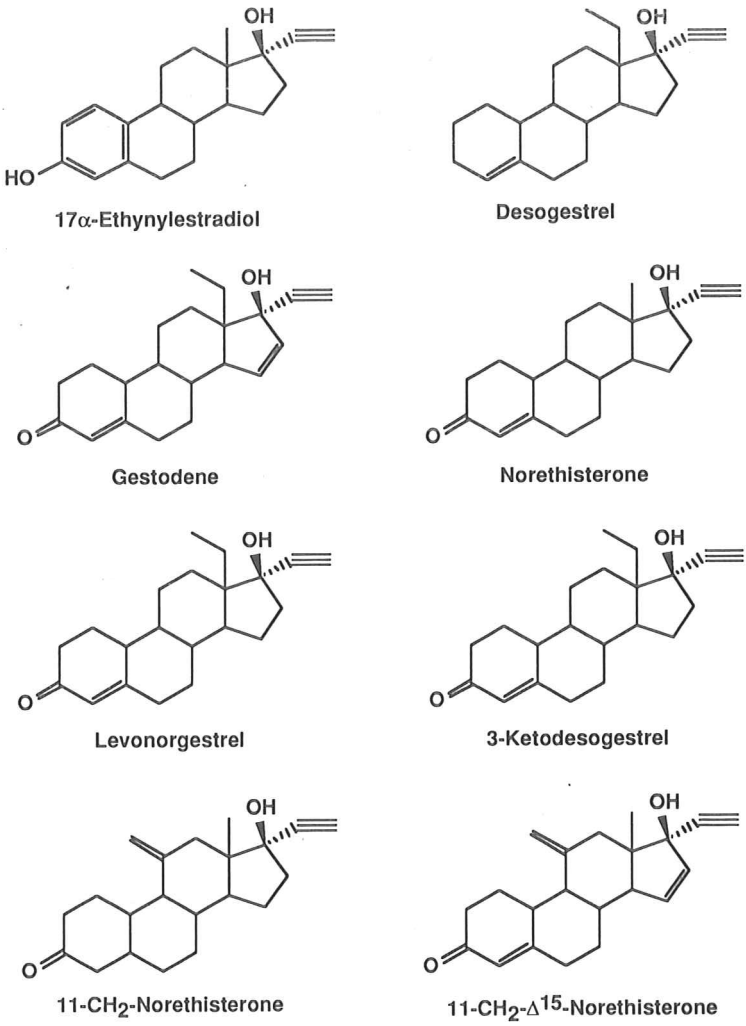

FIGURE 4 Structures of 17α-ethynylestradiol and several progestins used in oral contraceptives (Guengerich, 1990a,b).

The ineffectiveness of oral contraceptives due to P450 3A4 induction can be explained in these terms. There could also be contributions of induced conjugating enzymes (e.g., UDP-glucuronosyltransferases), but these have not been documented. This phenomenon of lack of efficacy of oral contraceptives is still a problem because of the low doses of 17α-ethynylestradiol used (to prevent unwanted effects of estrogens) and the sensitivity to changes due to variations in P450 3A4.

In the course of work with 17α-ethynylestradiol and oral contraceptives, the progestin gestodene (Fig. 4) was found to be a relatively effective and

selective mechanism-based inactivator of P450 3A4 in *in vitro* experiments (Guengerich, 1990a). This inactivation is due in part to the presence of an ethynyl moiety, which is also a part of many P450 inactivators (Ortiz de Montellano *et al.*, 1979; Gan *et al.*, 1984). However, most of the progestins used in oral contraceptives have 17α-ethynyl groups (Fig. 4), and other features of gestodene are apparently responsible for the inactivation (Guengerich, 1990a). This inactivation phenomenon has been postulated to account for the increased levels of estradiol and cortisol in women using oral contraceptives (Jung-Hoffmann and Kuhl, 1990), although it is not clear that the dose of gestodene is sufficient to inhibit a large fraction of hepatic or intestinal P450 3A4 (Guengerich, 1990a).

D. Terfenadine

Terfenadine is a component of the antihistamine formulation Seldane. It is rapidly oxidized by P450 3A4 to two products, acyclinol and an alcohol derived from oxidation of a *t*-butyl methyl group (Fig. 5) (Yun *et al.*, 1993). Acyclinol is inactive. The alcohol is further oxidized to a carboxylic acid by either P450 3A4 (Rodrigues *et al.*, 1995) or by dehydrogenases; the relative contributions of the two enzyme systems are not known (Fig. 6). This carboxylic acid, like terfenadine itself, binds to the H_1 histamine receptor and should produce relief of allergy symptoms. However, the acid is a zwitterion and does not readily cross the blood–brain barrier and does not cause

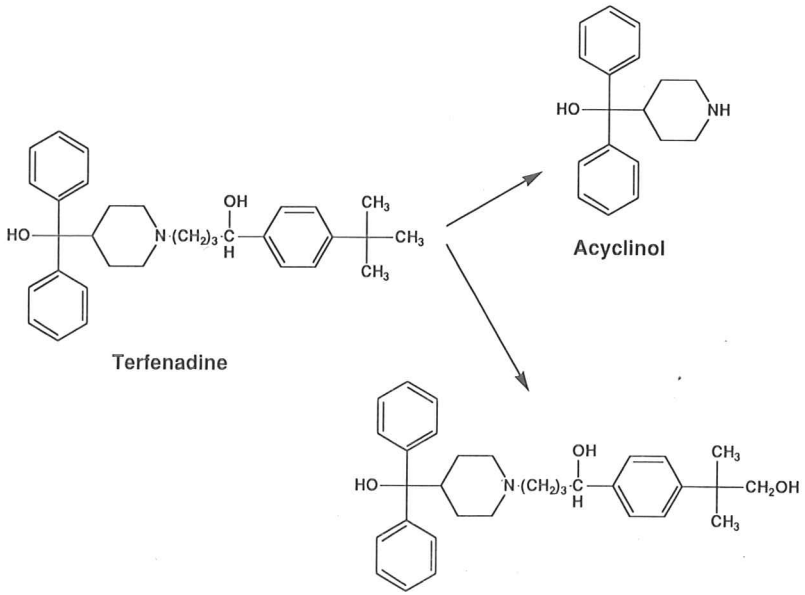

FIGURE 5 Major routes of oxidation of terfenadine.

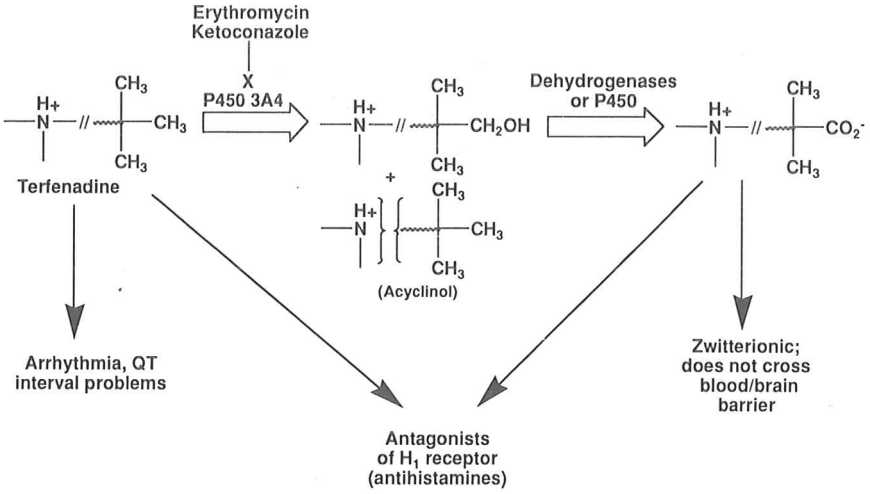

FIGURE 6 Scheme depicting role of terfenadine as a pro-drug and effects of influences on P450 3A4 (Yun *et al.*, 1993).

drowsiness. Terfenadine is metabolized very rapidly in most individuals and the levels in the plasma account for <1% of the administered dose (Yun *et al.*, 1993; Kivistö *et al.*, 1994). Thus, terfenadine fits the description of a pro-drug and the carboxylic acid is the effective agent (Kivistö *et al.*, 1994).

The oxidation of terfenadine by P450 3A4 can be inhibited by agents such as ketoconazole and erythromycin (Kivistö *et al.*,.1994; Woosley *et al.*, 1993). High plasma levels of terfenadine have been associated with cardiac problems, including arrhythmias, torsade de pointes, and abnormal heart ventricular rhythms (Woosley *et al.*, 1993; Kivistö *et al.*, 1994). For this reason, the package insert for Seldane warns against the concomitant use of erythromycin or ketoconazole. Ingestion of grapefruit juice (*vide infra*) has also been reported to increase the plasma level of terfenadine, although apparently not to a point considered serious (D. G. Bailey, personal communication).

E. Grapefruit Juice

The inhibitory effect of grapefruit juice was discovered rather serendipitously in an interaction study with ethanol and felodipine (Bailey *et al.*, 1991). The *in vivo* effect can be quite dramatic, with a single large glass of grapefruit juice producing fivefold increases in the plasma C_{max} and AUC parameters for dihydropyridines (Bailey *et al.*, 1991, 1993, 1994). Effects have also been demonstrated for other P450 oxidations, e.g., terfenadine (*vide supra*) and cyclosporin. Several lines of investigation indicate that the effect is on the oxidation itself, probably by intestinal P450 3A4 (Bailey, 1995).

The identity of the components(s) responsible for the inhibition is not known. One problem is that not all individuals seem to respond, and the incidence of response is not directly linked to rates of oxidation (Bailey *et al.*, 1993). One clue to the active component is that orange juice does not show this inhibitory effect. Presumably the inhibitor is grapefruit specific. The major grapefruit-specific flavonoid is naringin, which can account for up to 10% of the dry weight. Naringin did not inhibit P450 3A4 *in vitro* but its aglycone naringenin did (Guengerich and Kim, 1990). The effect may not be strong enough to explain the *in vivo* results. Naringin administered *in vivo* did not show the inhibitory effect (Bailey *et al.*, 1993), but there are questions about the bioavailability of an aqueous formation and also the stereochemistry of the naringin relative to that in the grapefruit. To date there have been no reports of attempts to use grapefruit juice extracts in *in vitro* assays.

F. Erythromycin

Erythromycin is known to inhibit a number of drug oxidations, essentially all of which are catalyzed by P450 3A4. For instance, the oxidations of terfanidine (Woosley *et al.*, 1993; Kivistö *et al.*, 1994), cyclosporin (Godin *et al.*, 1986), and numerous other drugs show inhibition by erythromycin in both *in vitro* and *in vivo* settings, and erythromycin is often regarded as a diagnostic inhibitor of P450 3A4 (Guengerich, 1995), as well as the 3A family enzymes of experimental animals. Erythromycin N-demethylation is a reaction rather specifically catalyzed by P450s 3A4 and 3A5 (Brian *et al.*, 1990; Gillam *et al.*, 1995). However, not all P450 3A4 reactions are inhibited by erythromycin. For instance, lovastatin oxidations were not inhibited by erythromycin either *in vitro* (even at concentrations of 1 m*M*) or *in vivo* (Wang *et al.*, 1991). The reasons are not clear at this time. These observations suggest that (i) lack of inhibition of a reaction by erythromycin may not always be a reliable guide that the reaction is not catalyzed by P450 3A4 and (ii) that not all P450 3A4-catalyzed reactions may be prone to erythromycin interactions.

G. Cyclosporin

Cyclosporin A (Sandimmune) has been the most popular immunosuppressant used in organ transplantation. Plasma levels of this drug are critical because high levels lead to renal toxicity but low levels can lead to organ rejection. In contrast to chronic diseases where there is an opportunity to adjust the drug dosage, the first few days after organ transplant are usually critical and there is often not much opportunity for dose adjustment in this period. The major pathway of cyclosporin metabolism is via P450 3A4 (Kronbach *et al.*, 1988; Combalbert *et al.*, 1989; Aoyama *et al.*, 1989), with

three major metabolites formed (Aoyama *et al.*, 1989). Another complication in a liver transplant is that the level of P450 3A4 in the donor liver must be considered as well as in the recipient's liver, small intestine, and other tissues.

The effect of modulations on cyclosporin levels was apparently first noted by Godin *et al.* (1986). Rifampicin treatment led to lower plasma levels of cyclosporin and erythromycin treatment led to higher levels. Subsequently the involvement of P450 3A4 in the oxidation was clearly demonstrated (Combalbert *et al.*, 1989; Kronbach *et al.*, 1988; Aoyama *et al.*, 1989). Similar cases were seen by Watkins and associates (Lucey *et al.*, 1990), where plasma levels rose and fell. This group was also able to demonstrate that humans are oxidizing cyclosporin during the period when no liver is present in the body, arguing for a significant role of intestinal (or other extrahepatic) P450 3A4 in the process (Kolars *et al.*, 1991). Because of the serious nature and high cost of organ transplants, efforts are being made to use noninvasive assays for hepatic and intestinal P450 3A4 to estimate what doses of cyclosporin will be most appropriate for individuals about to undergo transplantation surgery (Watkins *et al.*, 1990).

Other immunosuppressants have been introduced and two of these, tacrolimus (FK506) and rapamycin, have been studied. Both are metabolized primarily by P450 3A4 (Sattler *et al.*, 1992; Vincent *et al.*, 1992). Tacrolimus is a more potent immunosuppressant but is not without toxic side effects. Evidence shows that the metabolites may retain more of the biological activity of the parent drug than in the case of cyclosporin.

H. Omeprazole

Omeprazole is an acid pump inhibitor used in the treatment of gastric ulcers. In general, it is considered to be a relatively safe drug and little in the way of adverse episodes have been reported. There have been two concerns about omeprazole, induction and inhibition.

Maurel and associates reported that omeprazole could induce P450s 1A1 and 1A2 in cultured human hepatocyte cultures (Diaz *et al.*, 1990). This observation was considered of interest because many procarcinogens are known to be activated to genotoxic forms by P450s 1A1 and 1A2 (Ioannides and Parke, 1987; Guengerich and Shimada, 1991), although it should be emphasized that there is a 40-fold variation in P450 1A2 among humans and there is not strong evidence to date that differences have a major influence in the risk of any cancers (Butler *et al.*, 1989; Lang *et al.*, 1994). Further studies with noninvasive assays of P450 1A2 showed that induction by omeprazole could be demonstrated, but only in individuals deficient in P450 2C19 (Rost *et al.*, 1992). This phenomenon is due to the involvement of P450 2C19 [along with P450 3A4 (Andersson *et al.*, 1990, 1993)] in the metabolism of omeprazole.

The induction of P450 1A family enzymes by omeprazole was unusual in the sense that such induction of the orthogous P450s was not seen in mice or rats, *in vitro* or *in vivo* (Diaz *et al.*, 1990). Omeprazole did not displace known radiolabeled ligands bound to the Ah receptor, and a non-Ah receptor mechanism has been proposed (Lesca *et al.*, 1995; Diaz *et al.*, 1990). However, Tukey and associates have shown that a sensitive construct containing an Ah receptor enhancer element connected to a reporter gene can be stimulated in cell culture in the presence of very high doses of omeprazole (Quattrochi and Tukey, 1993). Thus, the exact mechanism of P450 1A induction by omeprazole has not been definitively established. However, we can conclude that (i) very high doses of omeprazole itself are needed for induction and (ii) the general significance of a small amount of P450 1A induction in humans has yet to be established.

Omeprazole also appears to be a competitive inhibitor of P450 2C19, an enzyme involved in its metabolism (Unge *et al.*, 1992). Such competition may be a reflection of the low amount of P450 2C19 present in the body (de Morais *et al.*, 1994), especially those individuals showing the deficient genetic polymorphism (Guengerich, 1995).

VI. Other Issues

Two other issues will be mentioned with regard to interactions. Neither can be considered a direct problem in the sense of human drug–drug interactions, but both bear on studies in this area and need to be considered.

A. Barbiturate and Peroxisome Proliferation Inducers

Chemicals in these two categories are of interest in the pharmaceutical industry. We have already considered how barbiturates can induce human P450 enzymes such as P450 3A4 and 2C9 and lead to more rapid elimination of drugs (e.g., 17α-ethynylestradiol, *vide supra*). Induction of enzymes by the peroxisome proliferation agents does not seem to affect the clearance of drugs, as the only P450s that seem to be induced are in the 4A subfamily and these are apparently not extensively involved in the metabolism of drugs.

A major concern with both of these groups of compounds is the correlation between enzyme induction and tumor promotion (Lubet *et al.*, 1989; Rao and Reddy, 1991). There is a concern that a compound in either of these groups (i.e., that shows, e.g., P450 2B or 4A induction in animals) might also be a tumor promoter and be positive in a long-term animal cancer bioassay. The barbiturate group includes a variety of barbiturates, hydantoins, and miscellaneous compounds (Diwan *et al.*, 1988). The peroxisome proliferation group includes plasticizers, phthalates, and many phar-

maceutical groups such as leukotriene receptor antagonists (Rao and Reddy, 1991; Bars *et al.*, 1993).

The correlation between tumor promotion and induction is certainly not very strict in either group of compounds. Further, the relevance to humans and human health is still a matter of speculation. A considerable amount of epidemiology evidence is available from people who have used barbiturates for epilepsy on a long-term basis, and no real increase in tumors was seen (Olsen *et al.*, 1989). It would appear that even the doses administered to humans in such settings are not enough to show the hepatocyte proliferation observed when animals are treated with high doses.

Good evidence has been obtained that humans have a functional PPAR system, but actual *in vivo* induction has not been demonstrated in humans, and even those individuals using clofibrate, a known inducer of this response in animals, have not shown any evidence of liver tumors. It is possible that rodents contain genes relevant to cancer that are activated by the PPAR. Of interest are recent observations that the PPAR system is a mixture of different receptors that interact with individual members of the RXR receptor family to activate PPAR-associated genes. Also, certain fatty acids have actually proven to be better inducers than any of the prototypic synthetic ligands; the possibility exists for highly active metabolites of fatty acids.

B. *In Vitro*/*in Vivo* Comparisons

One general problem involves approaches to searching for possible drug–drug interactions. Ideally this should be done first in *in vitro* settings to expedite the process, and some approaches looking for enzyme inhibition and induction have been mentioned (*vide supra*). However, there is the matter of validation of *in vivo* results in *in vivo* settings.

In principle, one can readily look for the inhibition and induction if an appropriate noninvasive assay is available. The compound under investigation can be administered to humans, and the alteration of pharmacokinetics of another drug can be observed. This process seems to work reasonably well in some cases. For instance, the induction and inhibition of human P450 1A2 can be inferred from changes in the N^3-demethylation of caffeine (Butler *et al.*, 1992; Kalow and Tang, 1993). Other noninvasive assays in which a generally acceptable degree of validation has been obtained include P450 2A6 and coumarin 7-hydroxylation (Cholerton *et al.*, 1992), P450 2C9 and tolbutamide hydroxylation and warfarin 7-hydroxylation (Breimer *et al.*, 1978; Rettie *et al.*, 1992, 1994; Knodell *et al.*, 1987), P450 2D6 and debrisoquine 4-hydroxylation (Mahgoub *et al.*, 1977) and several other assays (Evans *et al.*, 1989; Eichelbaum *et al.*, 1979), and now P450 2E1 and chlorzoxazone 6-hydroxylation (Kim *et al.*, 1995). However, there seems to still be a problem with P450 3A4, which is unfortunate as this appears to be the main human P450 involved in the oxidation of drugs (Kinirons *et*

al., 1993). The differences among individuals are not genetic, and temporal intraindividual changes in *in vivo* parameters are observed. A number of reactions (of different substrates) that clearly seem to be associated with P450 3A4 *in vitro* do not show good *in vivo* corrections (Kinirons *et al.,* 1993). There are several possible reasons, including the balance between hepatic and small intestine oxidation with individual compounds, the presence of inhibitors in the diet (Bailey *et al.,* 1994), and possibly the contribution of the MDR1 and other pump proteins in influencing cellular concentrations (Schuetz *et al.,* 1995).

The problem has been choosing the most appropriate *in vivo* assay for P450 3A4, and the merits and disadvantages of several probe drugs have been discussed (Kinirons *et al.,* 1993; Lown *et al.,* 1992; Ged *et al.,* 1989; Thummel *et al.,* 1993). Currently the list of possibilities includes nifedipine and felodipine oxidation, erythromycin conversion to CO_2, 6β-hydroxycortisol production, midozolam 4-hydroxylation, lidocaine N-deethylation, and dapsone N-hydroxylation.

VII. Summary and Conclusions

Many adverse drug–drug interactions are attributable to pharmacokinetic problems and can be understood in terms of alterations of P450-catalyzed reactions. Much is now known about the human P450 enzymes and what they do, and it has been possible to apply this information to issues related to practical problems. A relatively small subset of the total number of human P450s appears to be responsible for a large fraction of the oxidation of drugs. The three major reasons for drug–drug interactions involving the P450s are induction, inhibition, and possibly stimulation, with inhibition appearing to be the most important in terms of known clinical problems.

With the available knowledge of human P450s and reagents, it is possible to do *in vitro* experiments with drugs and make useful predictions. The results can be tested *in vivo,* again using assays based on our knowledge of human P450s. This approach has the capability of not only improving predictions about which drugs might show serious interaction problems, but also decreasing the number of *in vivo* interaction studies that must be performed. These approaches should improve with further refinement and technical advances.

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