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INTERINDIVIDUAL VARIABILITY IN INHIBITION AND INDUCTION OF CYTOCHROME P450 ENZYMES

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■ **Abstract** Drug interactions have always been a major concern in medicine for clinicians and patients. Inhibition and induction of cytochrome P450 (CYP) enzymes are probably the most common causes for documented drug interactions. Today, many pharmaceutical companies are predicting potential interactions of new drug candidates. Can in vivo drug interactions be predicted accurately from in vitro metabolic studies? Should the prediction be qualitative or quantitative? Although some scientists believe that quantitative prediction of drug interactions is possible, others are less optimistic and believe that quantitative prediction would be very difficult. There are many factors that contribute to our inability to quantitatively predict drug interactions. One of the major complicating factors is the large interindividual variability in response to enzyme inhibition and induction. This review examines the sources that are responsible for the interindividual variability in inhibition and induction of cytochrome P450 enzymes.

INTRODUCTION

Drug interactions have always been a major concern in medicine for clinicians and patients. When two or more drugs are administered over similar or overlapping time periods, the possibility of drug interactions exists. Drug interaction can be pharmacokinetic or pharmacodynamic in nature. However, in many cases, the interactions have a pharmacokinetic rather than a pharmacodynamic basis. Inhibition and induction of cytochrome P450 (CYP) enzymes are probably the most common causes for documented drug interactions (1). Inhibition of drug metabolism by competition for the same enzyme may result in undesirable elevations in plasma concentrations of drugs, which can lead to serious adverse effects and toxicity. Fatal drug interactions have been reported (2, 3), and several prominent drugs have been withdrawn from the market because of serious adverse events related to drug interactions (4, 5). On the other hand, enzyme induction, which is defined as an

increase in the amount and activity of a drug-metabolizing enzyme, may increase the drug's elimination and attenuate its pharmacological effect as a result of decreases in plasma concentrations (6, 7). Therefore, drug interactions represent not only a medical problem for clinicians and patients, but also an economic loss for pharmaceutical companies. Today, many pharmaceutical companies are predicting potential interactions of new drug candidates, and thus these companies are minimizing such losses, as well as more effectively safeguarding the welfare of patients.

With major advances in molecular biology and biochemical technology, our knowledge of the structures and regulatory mechanisms of CYP enzymes has grown exponentially. To date, both inhibition and induction of CYP enzymes have been better understood in terms of mechanistic details. However, in spite of our better understanding, it is still very difficult to accurately predict the magnitude of drug interactions (8). There are many factors that contribute to our inability to quantitatively predict drug interactions. The large interindividual variability in drug metabolism is one of the major reasons. Sometimes, the interindividual differences in the extent of changes in plasma area under the concentration-time curve (AUC) are $\leq$100-fold. For example, the oral bioavailability of felodipine increased with a range from 5% to 500%, when normal subjects were pretreated with grapefruit juice (9–11).

The sources of such variability in drug interactions are many, including genetics, disease, diet, cigarette smoking, age, and a variety of environmental factors. This review examines the sources that are responsible for the interindividual variability in the extent of drug interactions. Theoretical considerations are briefly reviewed and used to explain why the magnitude of variability in drug interactions depends on the kinetic properties of drugs (high or low hepatic extraction ratio), route of drug administration (oral or intravenous), and genetic makeup of individuals. In addition, representative examples are drawn from literature to illustrate the interindividual variability in drug interactions involving enzyme inhibition and induction.

HUMAN CYTOCHROME P450 ENZYMES

The most important enzyme system for drug metabolism is the CYP system. To date, at least 17 *CYP* gene families have been identified in mammals (12, 13). The mammalian CYP families can be functionally subdivided into two major classes: those that involve the biosynthesis of steroids, fatty acids, and bile acids and those that primarily metabolize xenobiotics. Three main *CYP* gene subfamilies, *CYP*1, *CYP*2, and *CYP*3, are responsible for most drug metabolism. The liver, which constitutes $\sim$2.5% of human adult body weight and contains an abundance of CYP enzymes, has long been recognized as the major organ for drug metabolism. Therefore, the liver often serves as the locus of metabolic drug interactions. The CYP enzymes are found primarily in the endoplasmic reticulum of the liver, where

the enzymes are synthesized on membrane-bound polyribosomes and inserted directly into the lipid bilayer via the signal sequence recognition system (14, 15).

The distribution of CYP enzymes in the liver has been studied by several investigators. Although Ratanasavanh et al (16) suggested that CYP3A enzymes are expressed in a heterogenous fashion throughout the hepatic lobule, mainly in the centrolobular and midzonal hepatocytes in adult human livers, the data by Watkins et al (17), who investigated the CYP3A content in 10 different locations in a human liver including the zones most commonly biopsied either by precutaneous needles or by surgery during laparotomy, indicated that the CYP3A is homogeneously distributed in the human liver. Studies in rats by Debri et al (18) also suggested that the CYP enzymes in the liver are evenly distributed. The composition of hepatic CYP enzymes has been studied in 30 Japanese and 30 Caucasians (19). CYP3A (specifically CYP3A4 and CYP3A5) and CYP2C (specifically CYP2C8, CYP2C9, CYP2C18, and CYP2C19) are the most abundant subfamilies, accounting for 30% and 20% of the total amount of hepatic CYP enzymes, respectively. Other isoforms are minor contributors to the total CYP enzymes: CYP1A2 at 13%, CYP2E1 at 7%, CYP2A6 at 4%, CYP2D6 at 2%, and CYP2B6 at 0.4%. However, it should be noted that the importance of the isoforms in drug metabolism depends not only on their abundance but also on the affinity of substrate to the enzyme.

Although the liver plays a major role in drug metabolism, drug-metabolizing enzymes are also present in other tissues, such as the mucosa of the intestine, kidney, lung, brain, or skin (20). Among these, enzymes in the intestinal mucosa are probably the most important contributors to the extrahepatic metabolism of drugs. Using immunoblotting techniques, a comparative study of hepatic and intestinal distribution of CYP enzymes in humans has been investigated in detail by de Waziers et al (21). The levels of CYP3A4 were estimated to be 350 pmol/mg of microsomal protein in the liver and 160, 120, and 70 pmol/mg of microsomal protein in the duodenum, jejunum, and ileum, respectively. CYP3A4 present in human small intestine appears to be functionally and structurally identical to CYP3A4 in human liver. Although the level of CYP3A4 in human intestine is only slightly lower than that of the liver, the estimated total mass of CYP3A4 in the whole small intestine is roughly 30-fold lower than in the whole liver (21). Similarly, a 20-fold difference between the total mass of hepatic and intestinal CYP3A4 was reported by Back & Rogers (22). Other CYP enzymes detected in human intestine are CYP2C and CYP2D6, whereas CYP1A2 and CYP2E1 are not detected. The estimated total mass of CYP2C and CYP2D6 in the whole intestine is 100- to 200-fold lower than that in the whole liver (21). The low total mass of intestinal CYP enzymes is due to the very low yield of microsomal protein for intestine as compared with the liver, because the CYP enzymes are expressed only in the enterocytes of the tip epithelial cells of villi, which account for only a very small fraction of the total intestinal cell population (23). Additionally, the human liver ($\sim$1.5 kg) by weight is about twice as large as the small intestine ($\sim$0.7 kg).

The distribution of these enzymes is not uniform along the length of small intestine. In a recent study of 20 human donor intestines (24), it has been shown that

CYP3A4 expression varies along the length of the small intestine. Median values of 31, 23, and 17 pmol/mg of protein were found in duodenum, distal jejunum, and distal ileum, respectively. It should be noted that the values of the content of intestinal CYP3A4 reported by Paine et al (24) were significantly lower than those observed by de Waziers et al (21). Although the reason for the differences in the content of intestinal CYP3A4 between these two laboratories is not clear, they might be caused by the segment of small intestine and the method used for enterocyte isolation. Depending on the segment of small intestine and the method used for enterocyte isolation, various populations of enterocytes, in which CYP enzymes are located, were obtained. It is also possible that the differences reflect interindividual variability in intestinal CYP3A4. Furthermore, the distribution of CYP enzymes is also not uniform along the villi within a cross-section of mucosa. The localization and distribution of CYP3A4 along the villi in human small intestine has been studied using a monoclonal antibody (25). The columnar absorptive epithelial cells of the villi exhibited the strongest immunoreactivity, whereas no immunostaining was detectable in the goblet cells or the epithelial cells in the crypts.

Consistent with the levels of CYP enzyme protein, the catalytic activities of CYP enzymes also were higher in the liver than the small intestine. The CYP3A4 catalytic activity, measured by erythromycin demethylation, was estimated to be 2.8, 1.6, 1.1, and 0.15 nmol/min/mg of protein in the liver, duodenum, jejunum, and ileum, respectively (21). Similar results also were observed by other investigators (26, 27), who reported that CYP3A4 enzyme activity was higher in the liver than the small intestine. In addition to CYP3A enzymes, higher hepatic catalytic activity was also reported for other CYP enzymes. The metabolism of (+) bufuralol, a substrate of CYP2D6, was studied in human hepatic and intestinal microsomes (28). Although the K_m values (5–10 μM) were similar in both hepatic and intestinal microsomes, the V_{max} values were much higher in human hepatic microsomes (383 pmol/min/mg of protein) than in intestinal microsomes (3–9 pmol/min/mg of protein).

Anatomically, the small intestine has a serial relationship with the liver relative to the absorption of drugs. The amount of an orally administered drug that reaches the systemic circulation can be reduced by both intestinal and hepatic metabolism. The metabolism of drugs before entering the systemic circulation is referred to as first-pass metabolism. Although it is widely believed that the liver is the major site of such first-pass metabolism because of its size and its high mass of CYP enzymes, some recent studies have suggested that the contribution of intestinal metabolism to the overall first-pass metabolism of drugs is quantitatively more important than that of hepatic metabolism (29–31). From the above data, it is evident that both the total mass of individual CYP enzymes and their catalytic activity in the whole small intestine are much lower than those in the whole liver (20- to 300-fold less), when the yield of microsomal protein is taken into consideration. With the limited amount of CYP enzymes in the small intestine, it is expected that the contribution of intestinal metabolism to the overall first-pass metabolism of drugs would be less

likely to be quantitatively as important as that of the liver, unless the oral dose is very small or the rate of absorption is very slow (32). Therefore, the contribution of small intestine in drug interactions becomes quantitatively important only when a very small dose is given.

The point that the size of oral dose is an important factor in determining whether the small intestine plays a significant role in the first-pass metabolism of orally administered drugs is best exemplified by the case of midazolam. Based on the organ size and CYP3A content, the total hepatic intrinsic clearance (15.8 ml/min) of midazolam is 75-fold that of the small intestine (0.21 ml/min) (27). From these values, it is expected that the small intestine would contribute very little to the first-pass metabolism of midazolam in vivo. However, studies in patients (26, 27) provided convincing evidence that both liver and small intestine can contribute equally to the first-pass metabolism of the drug, when a very small oral dose of midazolam (2 mg for human adults) is given. Therefore, it is clear that at this small dose even a low content of intestinal CYP3A enzymes is sufficient to metabolize a large fraction of midazolam during its first passage through the small intestine. The dose-dependent intestinal first-pass metabolism of midazolam has also been demonstrated in rats (33). The intestinal first-pass metabolism of midazolam in untreated and dexamethasone-pretreated rats, estimated by the mesenteric blood-collecting method in situ, was 25% and 49%, respectively, when a small dose of 16 μg was administered into the jejunal loop. The intestinal first-pass metabolism of midazolam was reduced substantially to 16% and 21%, respectively, in untreated and pretreated rats when a dose of 160 μg was given. Dose-dependent intestinal first-pass metabolism was also observed for indinavir in rats (34). The intestinal first-pass metabolism of indinavir was 23% when a small dose of 30 μg was administered into the jejunal loop, whereas the intestinal first-pass metabolism decreased to 6% when a high dose of 3000 μg was given. From these results, it is clear that unless the oral dose is very small, the small intestine does not serve as an important locus for drug interactions.

Variability in the Content of Cytochrome P450 Enzymes

A major characteristic of CYP enzymes is the large range of interindividual variability in the expression of enzyme protein. In a comprehensive study of human liver microsomes of 30 Japanese and 30 Caucasians, Shimada et al (19) have found that the content of individual CYP enzyme is highly variable. The interindividual differences in the enzyme content are approximately 5-fold for CYP2C and CYP3A4, 12-fold for CYP2E1, 20-fold for CYP1A2, and $>$50-fold for CYP2A6, CYP2B6, and CYP2D6. Large interindividual variability in hepatic CYP 3A4 and CYP 3A5 also has been reported by other investigators. There was a 37-fold variation in the level of CYP3A4 protein in biopsy tissues from 21 donor livers (35). The content of CYP3A4 enzyme ranged from 1.1 to 40.8 pmol/mg of protein. On the other hand, CYP3A5, which is known to be polymorphically expressed in adult human liver, was detected in only 4 of the 21 donor livers, ranging from 1.3

to 3.5 pmol/mg of protein. It is interesting that, although absolute CYP3A5 levels were low in the four positive livers, the relative contribution of CYP3A5 to the total hepatic CYP3A protein reached a high of 53% in one donor liver. In another study of 20 donor livers, a 64-fold variation in the CYP3A content was observed, ranging from 4.1 to 262 pmol/mg of protein (24). Interindividual variability in the content of CYP enzymes has also been observed in the small intestine. In the same study, Paine et al (24) found that the levels of intestinal CYP3A4 in duodenum, jejunum, and ileum were extremely variable among 20 human intestine donors. The content of CYP3A protein ranged from <3.0 to 90.8, 2.1 to 98, and <1.9 to 59.5 pmol/mg of protein for duodenum, jejunum, and ileum, respectively.

Because CYP3A enzymes are expressed exclusively in mature enterocytes in the villous tip of intestinal mucosa, differences in the percentage of mature enterocytes in individual biopsies may contribute to variability. For example, a deep mucosal biopsy may contain a relatively low proportion of enterocytes, while a more superficial biopsy may have a relatively high proportion of enterocytes. To correct these factors, Lown et al (36) used villin, a constitutively expressed protein in enterocytes, as an internal standard. There was still a significant variability (>11-fold) in the intestinal CYP3A4 protein after the correction with villin. The CYP3A4 protein level of duodenal biopsies from 20 patients ranged from 0.63 to 7.16 fmol/mg of protein/U of villin.

For a given substrate, the higher the enzyme content, the greater is the catalytic activity. Thus, a large variability in catalytic activities is expected for both hepatic and intestinal CYP enzymes, because the contents of enzymes are highly variable, as indicated above. Paine et al (24) found a large degree of interindividual variability in both V_{max} and intrinsic clearance (V_{max}/K_m) in microsomes prepared from 20 human liver samples. The intrinsic clearance of midazolam varied >29-fold. As expected, there were significant correlations between hepatic microsomal CYP3A content and both the V_{max} and intrinsic clearance ($r = 0.80$, $P < 0.001$; $r = 0.86$, $P < 0.001$). In another in vitro study with 21 liver biopsy samples (35), the formation rate of 1′-hydroxy-midazolam ranged from 1.2 to 482.5 pmol/min/mg of protein when 4 μM of midazolam was incubated. Again, there was a strong correlation between the formation rate of midazolam 1′-hydroxylation and CYP3A content. Good correlation between the individual CYP content and their catalytic activity was also found for all CYP enzymes examined in the liver microsomes from 30 Japanese and 30 Caucasians by Shimada et al (19).

Highly variable catalytic activities of CYP enzymes were also observed in the small intestine among individuals. In an in vitro study with intestinal microsomes prepared from four donors, kinetic parameters K_m and V_{max} of midazolam were determined (26). The estimated K_m values for midazolam 1′-hydroxylation were relatively constant, ~3.5 μM. However, a fourfold variation was noted in the V_{max} values, ranging from 222 to 842 pmol/min/mg of microsomal protein. Lown et al (36) found that there was a 48-fold variation in the rate of 1′-hydroxy-midazolam formation in S9 fractions prepared from 20 human duodenal biopsies, ranging from 5.8 to 277.6 pg/min/U of villin. In these 20 duodenal samples, there was a

strong correlation between the content of CYP3A4 and midazolam 1′-hydroxylase activity. In another study by Paine et al (24), 15 intestines were examined for the metabolism of midazolam in the duodenal, jejunal, and ileal regions. There was a high degree of interindividual variability in the intrinsic clearance of midazolam for duodenal, jejunal, and ileal regions, which respectively had 29-, 22-, and 18-fold variability. Again, good correlations between the CYP3A content and both intrinsic clearance and V_{max} of midazolam were observed for duodenum and jejunum. In addition to midazolam, highly variable intestinal metabolism was reported for other drugs. In an in vitro study with intestinal microsomes prepared from 14 donors, the formation rate of 13-*O*-demethyl tacrolimus varied from 24 to 110 pmol/min/mg of microsomal protein (37).

An obvious question is the extent to which the variations seen in the content of CYP enzymes and their catalytic activity in the liver and intestine are due to technical conditions and/or to "genuine" biological reasons. Undoubtedly, the conditions of organ procurement, storage, and transfer determine the quality of tissues, which in turn affects the variability of CYP content and catalytic activity. Furthermore, it is almost impossible to obtain uniform biopsy tissues from different patients with respect to the location of the samples within organs. Uneven distribution of CYP content in organs, particularly in the small intestine, contributes to the variability in CYP content and their catalytic activity when the tissue samples are obtained by biopsy. However, as discussed earlier, after correction with villin, which serves as an internal standard for enterocytes, a large variation in the CYP3A4 content (11-fold) and its catalytic activity (48-fold) was still observed in S9 fractions prepared from 20 human duodenal biopsies (36), suggesting that biological factors contribute significantly to the variability. To further test whether the large interindividual variability seen in the enzyme content and catalytic activity is due mainly to experimental conditions and the quality of tissue samples, an in vitro-in vivo correlation study was conducted by Thummel et al (38), using midazolam as a model drug. There was a strong correlation between the in vivo clearance of midazolam determined from 10 patients and the hepatic CYP3A content in liver biopsies obtained from these patients ($r = 0.93$, $P < 0.001$). In this study, the clearance of midazolam in the 10 patients was quite variable and ranged from 1.3 to 11.2 ml/min/kg. The formation rate of midazolam 1′-hydroxylation and the amount of CYP3A protein detected in the liver samples also varied considerably, from 17 to 380 pmol/min/mg of protein and 1.6 to 27.3 pmol/mg of protein, respectively. In this study, predicted hepatic clearance of midazolam from in vitro K_m and V_{max} correlated well with in vivo observed clearance. These results strongly suggest that the large interindividual variability in the in vivo clearance was largely reflected by the individual variations in the hepatic enzyme content. Thus, biological factors, a mixture of genetic and environmental factors, are probably the major sources for the variability. Recently, Ozdemir et al (39) have analyzed a total of 16 clinical studies ($n = 161$ subjects) with 10 different CYP3A4 substrates and concluded that ~90% of variation in hepatic CYP3A4 activity is under genetic control.

Inhibition and Induction of Cytochrome P450 Enzymes

The mechanisms of CYP inhibition can be grossly categorized as (*a*) reversible inhibition, (*b*) quasi-irreversible inhibition, and (*c*) irreversible inhibition (1). Of these, reversible inhibition is probably the most common mechanism responsible for documented drug interactions. Both quasi-irreversible and irreversible inhibitions are caused by the formation of reactive metabolites. Quasi-irreversible inhibition involves the formation of a noncovalent, but tight metabolic intermediate (MI) P450 complex, whereas irreversible inhibition is caused by enzyme inactivation (destruction) resulting from the covalent binding of reactive intermediates to the heme and/or protein of CYP. Although MI complex does not destroy the enzyme, the MI complex is so stable in vivo that the enzyme involved in the complex is unavailable for drug metabolism, and biosynthesis of new enzymes is the only means by which enzyme activity can be restored. In a practical sense, MI complex can be considered as irreversible inhibition in vivo. Depending on whether enzyme inhibition is a reversible or irreversible process, in vivo pharmacokinetic consequences caused by inhibition can be quite different (40). Reversible enzyme inhibition is transient; the normal function of CYPs continues after the inhibitor has been eliminated from the body. In contrast, the loss of enzyme activity by irreversible inactivation persists even after elimination of the inhibitor, and de novo biosynthesis of enzymes is the only means by which functional activity is restored. Kinetically, reversible inhibitors show only dose-dependent inhibition, whereas irreversible inhibitors exhibit both dose-dependent and time-dependent inhibitory effects on the disposition of substrates (40–42). It is, therefore, important to understand the mechanisms of inhibition and to differentiate between reversible inhibition and irreversible inhibition. Reversible inhibition can be further classified as competitive, noncompetitive, or uncompetitive (43). In competitive inhibition, the binding of inhibitor prevents binding of a substrate to the active site of the enzyme, whereas in noncompetitive inhibition, the inhibitor binds to a site other than the active site of the enzyme and has no effect on binding of substrate. The enzyme-substrate-inhibitor complex is nonproductive. In uncompetitive inhibition, the inhibitor, instead of binding the free enzyme, binds to the enzyme-substrate complex, which results in the formation of a nonproductive enzyme-substrate-inhibitor complex.

One of the intriguing aspects of the CYP enzymes is that most of these enzymes are inducible. Human CYP1A1/2, CYP2A6, CYP2C9, CYP2C19, CYP2E1, and CYP3A4 are known to be inducible (7). Unlike enzyme inhibition, which is an almost immediate response, induction is a slow regulatory process that increases the intracellular enzyme concentration. The increase in enzyme protein is normally, but not always, caused by an increase in transcription of the associated gene. The molecular mechanisms involved in CYP induction have been extensively studied in recent years. The transcriptional processes of CYP1A induction include a sequence of events: binding of inducing agent to the Aryl hydrocarbon (Ah) receptor, ligand-dependent heterodimerization between Ah receptor and an Ah receptor nuclear translocator, interaction of the heterodimer with a

xenobiotic-responsive enhancer, transmission of the induction signal from the enhancer to the CYP1A promoter, and alteration of the chromatin structure (7, 44). Recent studies have established that an orphan nuclear receptor, PXR, is involved in CYP3A4 induction, whereas a different receptor, CAR, is involved in CYP2B6 induction (45–47).

THEORETICAL CONSIDERATIONS

The metabolism of many drugs follows Michaelis-Menten kinetics as illustrated in the following scheme:

$$[\mathrm{E}] + [\mathrm{S}] \underset{\mathrm{K2}}{\overset{\mathrm{K1}}{\longleftrightarrow}} [\mathrm{ES}] \xrightarrow{\mathrm{k3}} [\mathrm{E}] + [\mathrm{P}]. \qquad \text{(Scheme 1)}$$

Kinetically, the velocity (ν) of a reaction can be expressed as in Equation 1, where $V_{\max}$ is the maximum velocity of metabolism and K_{m} is the Michaelis-Menten constant of the substrate (43), so that

$$\mathrm{v} = \frac{V_{\max} \cdot [S]}{K_{\mathrm{m}} + [S]} \qquad 1.$$

The $V_{\max}$ and K_{m} are described by Equations 2 and 3, respectively:

$$V_{\max} = K_3 \times [E_{\mathrm{t}}] \qquad 2.$$

and

$$K_{\mathrm{m}} = (K_2 + K_3)/K_1 \qquad 3.$$

where K_1 and K_2 are the association and dissociation rate constants, respectively, while K_3 is the catalytic rate constant. $[E_{\mathrm{t}}]$ is the concentration of total enzyme (free and occupied enzyme; $[E] + [ES]$). As shown in Equation 2, for a given substrate, the higher the enzyme content is, the greater the catalytic activity. Thus, wide variability in the $V_{\max}$ is expected for both hepatic and intestinal CYP enzymes, because of the large variability in the content of enzymes. On the other hand, the K_{m} value of a substrate is expected to be constant for a given enzyme. However, the K_{m} value of a substrate can be quite different for allelic variants. For example, the Cys-144-containing CYP2C9 had a markedly lower K_{m} value for *S*-warfarin hydroxylation and 4′-hydroxylation of (*R/S*)-flurbiprofen than the Arg-144-containing enzyme (48, 49).

To extrapolate the in vitro kinetic data to metabolic activity in vivo, the concept of intrinsic clearance (CL_{int}) is very important and useful. In fact, the intrinsic clearance can be considered the cornerstone for in vitro/in vivo extrapolation of metabolic interactions. The intrinsic clearance is defined as the velocity (v) of the enzymatic reaction divided by the substrate concentration [*S*]. In the absence of inhibitor, the intrinsic clearance ($CL_{\mathrm{int,o}}$) can be described by

$$CL_{\mathrm{int,o}} = \frac{V_{\max}}{K_{\mathrm{m}} + [S]} \qquad 4.$$

whereas in the presence of competitive inhibitor, the intrinsic clearance ($CL_{int,i}$) can be expressed by

$$CL_{int,i} = \frac{V_{max}}{K_m\left(1 + \frac{[I]}{K_i}\right) + [S]} \quad 5.$$

where K_i is the inhibition constant of the inhibitor, and $[I]$ is the inhibitor concentration. As indicated by Equation 5, a competitive inhibitor will increase the apparent K_m value of a substrate and has no effect on the V_{max}. In contrast to inhibitor, during induction an inducer will increase the V_{max} value of a substrate, but it will have little effect on the apparent K_m value. Consequently, enzyme inhibition causes a decrease in the intrinsic clearance, whereas enzyme induction results in an increase in the intrinsic clearance. Intuitively, because the V_{max} value is directly related to the enzyme content, which is known to be quite variable among individuals, one might think that large interindividual variability is seen only in drug interactions caused by enzyme induction, but not by enzyme inhibition. However, it is evident from the literature on this subject that drug interactions caused by CYP inhibition are subject to a degree of interindividual variability similar to that caused by CYP induction. In addition to the variations in enzyme content, the interindividual variability in CYP inhibition and induction can be partly explained by the following theoretical considerations.

If a drug is metabolized exclusively by the liver, the total clearance (CL_{total}) of the drug is equal to the hepatic clearance (CL_H), which can be expressed by Equation 6 or 7, according to the well-stirred model or parallel-tube model, respectively (50):

$$CL_{total} = CL_H = Q_H \cdot E = (Q_H \cdot fu \cdot CL_{int})/(Q_H + fu \cdot CL_{int}) \quad 6.$$

and

$$CL_{total} + CL_H = Q_H \cdot E = Q_H \cdot (1 - e^{-fu \cdot CLint/Q_H}) \quad 7.$$

where Q_H is the hepatic blood flow, E is the hepatic extraction ratio, and fu is the unbound fraction of drug in the blood. Drugs can be further classified as low- or high-clearance compounds, depending on whether their clearance is enzyme limited or flow limited (50).

Because the hepatic first-pass metabolism reflects the hepatic intrinsic clearance (CL_{int}), hepatic bioavailability (F_H) can be expressed as in Equation 8 (the well-stirred model),

$$F_H = 1 - E = Q_H/(Q_H + fu \cdot CL_{int}) \quad 8.$$

or Equation 9 (the parallel-tube model),

$$F_H = 1 - E = e^{-fu,Clint/Q_H} \quad 9.$$

As shown in Equations 8 and 9, a decrease in the CL_{int} caused by enzyme inhibition will result in a decrease in first-pass metabolism leading to an increase

in bioavailability of drugs, regardless of the hepatic model. On the other hand, an increase in the CL_{int} as a result of induction will cause an increase in first-pass metabolism resulting in a decrease in bioavailability.

It is important to note that the pharmacokinetic consequences of enzyme inhibition should always be an increase in plasma concentrations, because CYP inhibition will cause a decrease in the hepatic metabolism and an increase in bioavailability. Conversely, a decrease in plasma concentrations is expected during enzyme induction, because of increased hepatic metabolism and decreased bioavailability. A simple and direct way of assessing the effect of enzyme inhibition and induction in vivo is to compare the plasma AUC of a substrate before and after administration of an inhibitor or inducer. The AUC after oral administration can be expressed by Equation 10 or 11; depending on whether the well-stirred model,

$$\mathrm{AUC_{po}} = \frac{F_H \cdot fa \cdot \mathrm{dose}}{CL_H} = \frac{fa \cdot \mathrm{dose}}{fu \cdot CL_{int}} \qquad 10.$$

or parallel-tube model,

$$\mathrm{AUC_{po}} = \frac{F_H \cdot fa \cdot \mathrm{dose}}{CL_H} = fa \cdot \mathrm{dose}(e^{-fu \cdot CLint}/Q_H)/Q_H(1 - e^{-fu \cdot CLint}/Q_H) \qquad 11.$$

is used, where *fa* is the fraction of dose absorbed from the gastrointestinal lumen.

On the other hand, the AUC after intravenous administration can be expressed by Equation 12 or 13; that is:

$$\mathrm{AUC_{iv}} = \frac{\mathrm{dose}}{CL_H} = \frac{\mathrm{dose}}{\left[\frac{Q_H \cdot fu.CL_{int}}{Q_H + fu \cdot CL_{int}}\right]} \qquad 12.$$

or

$$\mathrm{AUC_{iv}} = \mathrm{dose}/CL_H = \mathrm{dose}/\,Q_H(1 - e^{-fu \cdot CLint}/Q_H) \qquad 13.$$

Computer simulations have been carried out to illustrate the effect of enzyme inhibition and induction on the AUCs after oral and intravenous administration for high- and low-clearance drugs, by using Equations 10–13. For low-clearance drugs ($E < 0.5$), a decrease in the CL_{int} caused by enzyme inhibition yields an almost proportional increase in the AUC, regardless of the route of administration or the choice of hepatic models (Figures 1 and 2). However, for high-clearance drugs ($E > 0.9$), changes in the AUC are more profound after oral administration than after intravenous dosing. A reduction of the CL_{int} has little effect on the $\mathrm{AUC_{iv}}$ for high-clearance drugs after intravenous administration. A significant increase in the $\mathrm{AUC_{iv}}$ is observed only when >70% of the CL_{int} is inhibited, namely when the ratio of CL_{int} (inhibited)/CL_{int} (control) is <0.3 (Figure 2). It is interesting that the parallel-tube model appears to be more sensitive to enzyme inhibition for high-clearance drugs, as compared with the well-stirred model. This is particularly true after oral administration. It should be noted that the parallel-tube model describes kinetics more accurately than the well-stirred model for high-clearance drugs (50).

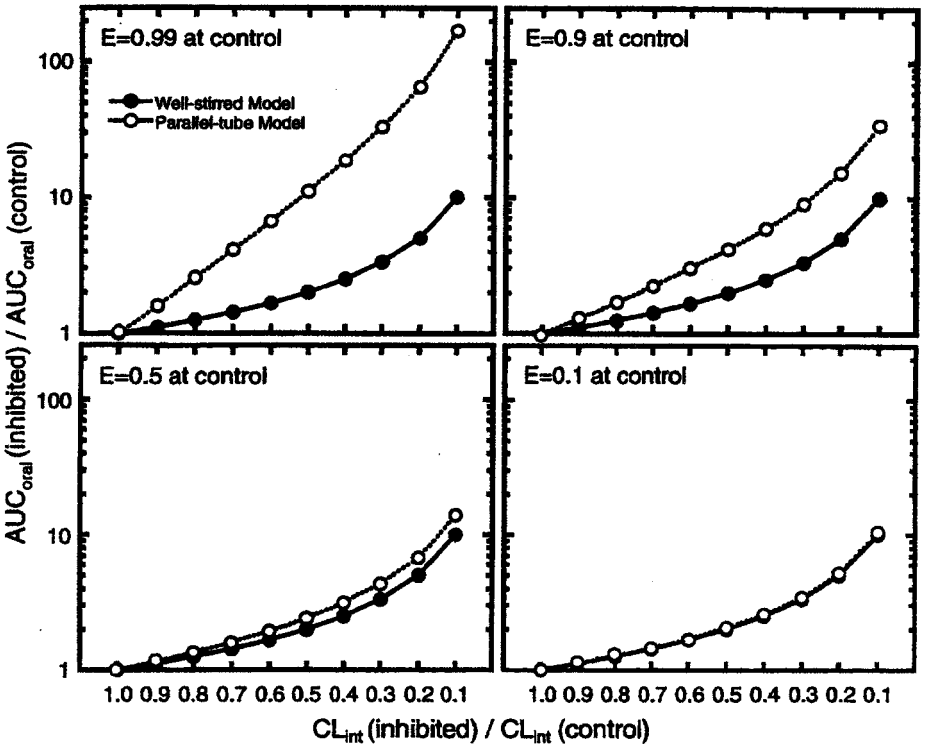

Figure 1 Simulated effects of enzyme inhibition on the area under the concentration-time curve (AUC) after oral administration of a high-clearance ($E = 0.99$ and $E = 0.9$), intermediate-clearance ($E = 0.5$), and low-clearance ($E = 0.1$) drug, using Equations 10–13 (for details, see text). For simplicity, the fraction of absorption of drug from the gastrointestinal tract and the fraction of drug not bound to plasma proteins are assumed to be unity. The hepatic blood flow used in the calculation is 20 ml/min/kg, and the dose is 10,000 nmol/kg.

Similarly, pharmacokinetic consequences of enzyme induction are dependent on the route of substrate administration and kinetic properties of the substrate. For low-clearance drugs ($E < 0.1$)), an increase in the CL_{int} caused by enzyme induction yields an almost proportional decrease in the AUC, regardless of the route of administration or the choice of hepatic models (Figures 3 and 4). However, for high- and intermediate-clearance drugs ($E > 0.5$), changes in the AUC are more profound after oral administration than after intravenous dosing. Induction has little effect on the AUC after intravenous administration for high-clearance drugs. Again, the parallel-tube model appears to be more sensitive to enzyme inhibition for high- and intermediate-clearance drugs ($E > 0.5$) after oral administration. Collectively, these computer simulations show that pharmacokinetic consequences of enzyme inhibition and enzyme induction are dependent on the route of substrate administration and kinetic properties of the substrate (high or low clearance).

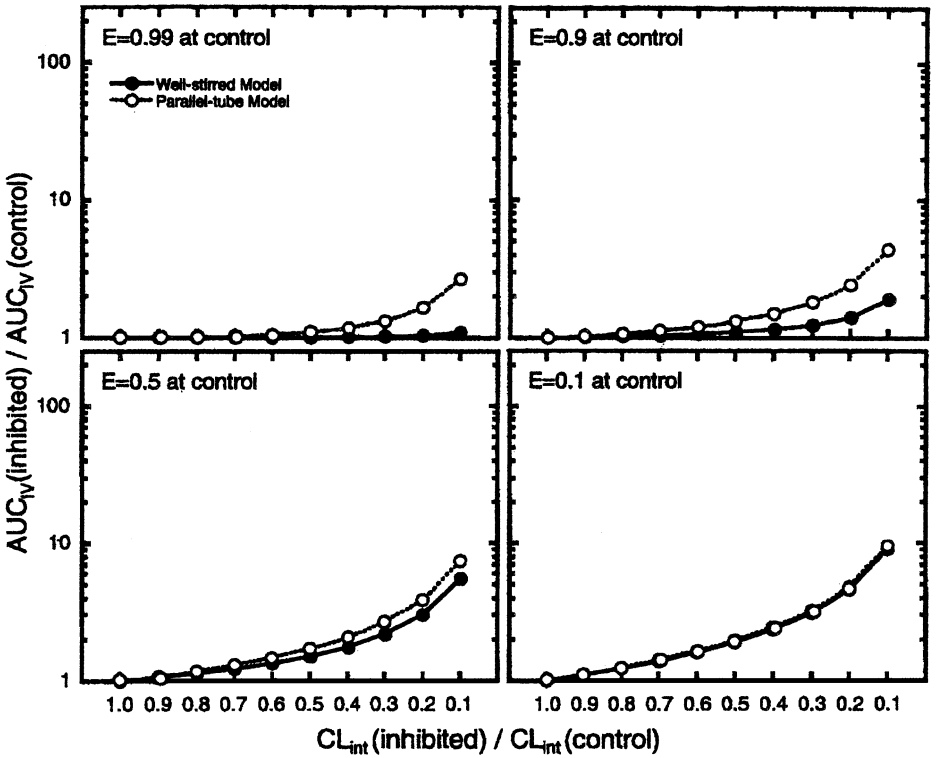

Figure 2 Simulated effects of enzyme inhibition on the area under the concentration-time curve (AUC) after intravenous administration of a high-clearance ($E = 0.99$ and $E = 0.9$), intermediate-clearance ($E = 0.5$), and low-clearance ($E = 0.1$) drug, using Equations 10–13 (for details, see text). For simplicity, the fraction of absorption of drug from the gastrointestinal tract and the fraction of drug not bound to plasma proteins are assumed to be unity. The hepatic blood flow used in the calculation is 20 ml/min/kg, and the dose is 10,000 nmol/kg.

INTERINDIVIDUAL VARIABILITY IN CYTOCHROME P450 INHIBITION

Large interindividual variations in response to cytochrome P450 inhibition have been observed in vivo. A significant interindividual variability was reported for terfenadine-ketoconazole interactions in humans, and there was a fivefold variation in the response to ketoconazole inhibition. The interaction with ketoconazole resulted in a 1500% to 7200% increase in the antihistamine's oral AUC (51). In an encainide-quinidine interaction study, the differences in the extent of increase in oral AUC and bioavailability of encainide during quinidine administration were >10-fold in seven extensive metabolizers (EMs) of CYP2D6 (52). A 20-fold variation in the extent of increase in oral AUCs of venlafaxine was also observed during quinidine administration, ranging from a 50% to 1000% increase

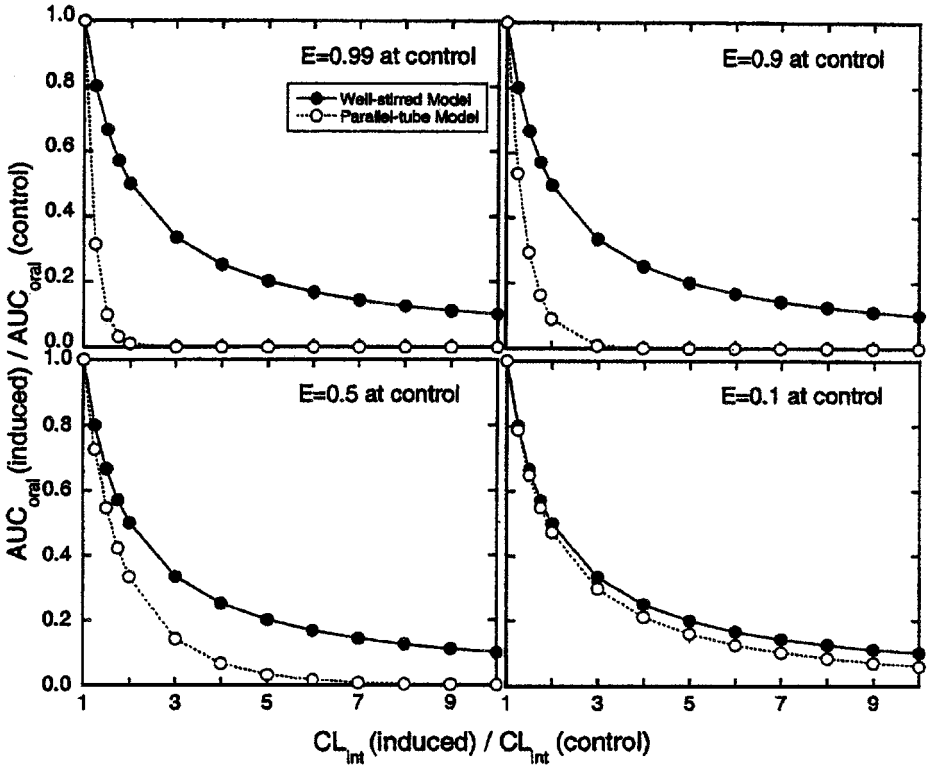

Figure 3 Simulated effects of enzyme induction on the area under the concentration-time curve (AUC) after oral administration of a high-clearance ($E = 0.99$ and $E = 0.9$), intermediate-clearance ($E = 0.5$), and low-clearance ($E = 0.1$) drug, using Equations 10–13 (for details, see text). For simplicity, the fraction of absorption of drug from the gastrointestinal tract and the fraction of drug not bound to plasma proteins are assumed to be unity. The hepatic blood flow used in the calculation is 20 ml/min/kg, and the dose is 10,000 nmol/kg.

in eight EMs of CYP2D6 (53). Similarly, considerable variations in response to CYP inhibition in healthy volunteers have also been reported for cyclosporine-ketoconazole interaction (54), triazolam-itraconazole interaction (55), and alprazolam-ketoconazole interaction (56).

In addition to the variability in drug-drug interactions, large variations in the extent of food-drug interactions have also been reported. There have been a number of reports showing that the oral intake of grapefruit juice results in increased AUCs of several drugs administered orally, and pronounced variability was found in the magnitude of increase in oral AUCs of drugs after ingestion of grapefruit juice. For example, the increase in oral AUCs by grapefruit juice inhibition ranged from −10% to 480% for felodipine (57), −16% to 200% for cyclosporine (58), and 26% to 100% for midazolam (59). The common denominator for these grapefruit

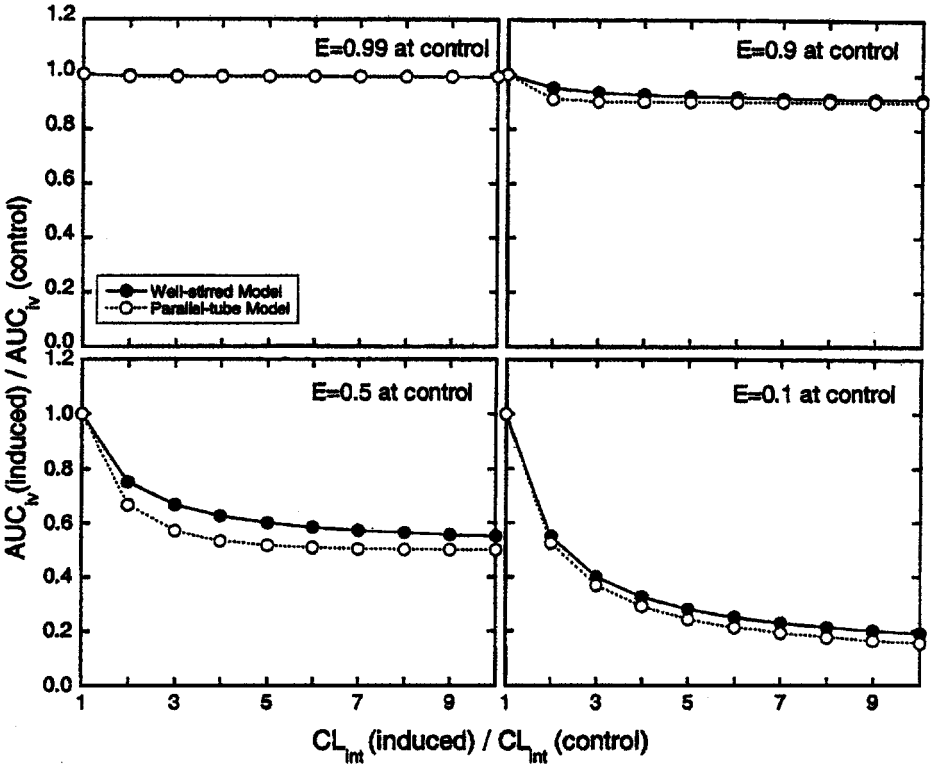

Figure 4 Simulated effects of enzyme induction on the area under the concentration-time curve (AUC) after intravenous administration of a high-clearance ($E = 0.99$ and $E = 0.9$), intermediate-clearance ($E = 0.5$), and low-clearance ($E = 0.1$) drug, using Equations 10–13 (for details, see text). For simplicity, the fraction of absorption of drug from the gastrointestinal tract and the fraction of drug not bound to plasma proteins are assumed to be unity. The hepatic blood flow used in the calculation is 20 ml/min/kg, and the dose is 10,000 nmol/kg.

juice-drug interactions is that most of the drugs involved in grapefruit juice interactions are CYP3A4 substrates, and their clinical dose is relatively small. Thus, it is very possible that the small intestine plays an important role in grapefruit juice-drug interactions. Recently, using duodenal biopsies, it has been shown that grapefruit juice causes a nontranscriptionally mediated reduction in CYP3A4 protein levels in the small intestine but not the liver (60). This finding supports the possible involvement of intestinal metabolism in grapefruit juice-mediated interactions. Although the underlying mechanism(s) of the selective downregulation of CYP3A4 in the small intestine is not fully understood, it is speculated that naringin, 6′,7′-dihydroxybergamottin, and other ingredients of grapefruit juice may contribute to the downregulation (57). As discussed earlier, there is a considerable interindividual variation in the expression of CYP3A4 in the small intestine.

Therefore, a greater inhibitory effect of grapefruit juice would be expected to occur in those individuals with high protein levels of CYP3A4, compared with those in whom no enzyme was present and available for interaction.

Many factors contribute to the large variability observed in CYP inhibition-mediated drug interactions. As shown in Equation 5, for competitive inhibition both the concentration of inhibitor [*I*] and inhibition constant K_i value are important in determining the degree of enzyme inhibition. One major contributing factor in the variability in enzyme inhibition is the individual variability in the [I] after oral absorption of inhibitors. Both the rate and extent of drug absorption can vary considerably among individuals and even within the same individual during chronic or multiple dosing. Interindividual and intraindividual variations in oral absorption are best exemplified by studies on verapamil (61, 62). By using a stable isotope-labeling technique, the kinetics of verapamil were studied in six healthy volunteers on two separate occasions (10 days apart). The interindividual differences in the oral AUCs of verapamil were more than fivefold. In addition to large interindividual variations in oral AUCs, profound day-to-day intraindividual variations in verapamil AUCs were also observed after oral administration. In one subject, the AUCs deviated from one study to another study by as much as threefold. Considering the large variability in drug absorption, it is not surprising to see large variations in drug-drug interactions. If a drug interaction involves mechanism-based enzyme inactivation, timing of administration between substrate and inhibitor, and route of administration become important. Variations in the timing of administration may affect the variability in the degree of inhibition (41, 42, 63).

Although variability in the inhibitor concentrations after administration of CYP inhibitors is one of the major sources of interindividual variability in response to enzyme inhibition, variability in the K_i values among individuals also contributes significantly to interindividual variability in enzyme inhibition. The variation in K_i values may result from differences in the differential expression of CYP enzymes, when multiple enzymes with different K_i values are involved in the metabolism of drugs. For example, both CYP3A4 and CYP3A5 are capable of metabolizing midazolam, and the metabolism of midazolam by these two isoforms can be inhibited by ketoconazole. In a recent in vitro study, Gibbs et al (64) have found that there is a significant difference in the affinity of CYP3A4 and CYP3A5 for ketoconazole. The ketoconazole K_i values on midazolam metabolism were estimated to be 27 and 109 nM for cDNA-expressed CYP3A4 and CYP3A5 enzymes, respectively. Given that the relative amounts of CYP3A4 and CYP3A5 frequently differ among individuals, it is expected that the extent of inhibition on the metabolism of midazolam caused by ketoconazole also varies among individuals. As expected, in the same study, the investigators demonstrated that the ketoconazole K_i values for liver microsomes containing CYP3A4 alone were significantly lower than the K_i for liver microsomes containing both CYP3A4 and CYP3A5 (14.9 nM vs 53 nM). Clearly, the 3.5-fold difference in the K_i values can be explained by the significant difference in the affinity of these two isoforms for ketoconazole. However, it should be noted that, even in the liver microsomes

containing only CYP3A4, there was a large coefficient of variation (45%) for ketoconazole K_i values (14.9 $\pm$ 6.7 nM; mean $\pm$ SD), suggesting that other factors may also contribute to the large variability in the K_i values.

In another in vitro study, the inhibitory effects of eight antipsychotic drugs (perphenazine, thioridazine, chlorpromazine, haloperidol, fluphenazine, risperidone, clozapine, and *cis*-thiothixene) on CYP2D6-mediated dextromethorphan *O*-demethylation were examined (65). The K_i values of these drugs were determined from human liver microsomes obtained from 11 donors. All of the antipsychotic drugs competitively inhibited dextromethorphan *O*-demethylation in a concentration-dependent manner. Perphenazine and thioridazine were the most potent inhibitors, with K_i values of 0.8 and 1.4 μM. However, these K_i values were quite variable, with a large coefficient of variation of 50–60%. Since CYP2D6 is a polymorphic enzyme, genetic factors may contribute to the large degree of variability. Unfortunately, no information was given on the genotype or phenotype of these 11 livers. Genetic factors can affect the K_i values for CYP inhibitors. Three naturally occurring allelic variants of CYP2C9 have been identified: the wild-type Arg-144 Leu-359 (CYP2C9*1), Cys-144 Leu-359 (CYP2C9*2), and Arg-144 Ile-359 (CYP2C9*3) (66–69). In vitro studies with cDNA-expressed variants have shown that the CYP2C9*3 variant exhibited lower intrinsic clearance (V_{max} /K_m) of tolbutamide, *S*-warfarin, phenytoin, piroxicam, and torsemide than CYP2C9*1, while the intrinsic clearances of these drugs were similar between CYP2C9*1 and CYP2C9*2 variants (66, 70, 71). Depending on the substrate, the lower intrinsic clearance is caused either by an increase in the K_m value or a decrease in the V_{max} value or a combination of both. Therefore, it is anticipated that individuals who are CYP2C9*3 homozygotes or heterozygotes will have higher K_i values for CYP inhibitors compared with those who are CYP2C9*1 homozygotes.

In addition to the variability in the inhibitor concentrations and K_i values, susceptibility of drugs to CYP inhibition in vivo is highly dependent on their kinetic properties (high or low clearance). This is because a high-clearance drug is subject to extensive first-pass metabolism and is more sensitive to CYP inhibition than a low-clearance drug, resulting in a greater magnitude of increases in oral AUCs (Figure 1). Coadministration of a high-clearance drug with a potent inhibitor produces a synergistic effect on the oral AUC of the drug by increasing bioavailability through inhibition of first-pass metabolism and by decreasing the hepatic clearance, whereas, for a low-clearance drug, the first-pass metabolism is minor and the inhibitory effect is only on hepatic clearance (Equations 10 and 11). In a recent clinical study, Greenblatt et al (56) demonstrated that ketoconazole causes a more profound increase in the oral AUC of triazolam than in that of alprazolam. Although both triazolam and alprazolam are anxiolytic agents that are predominantly metabolized by CYP3A4, the human pharmacokinetics of the two drugs differ substantially. Triazolam has a clearance (10 ml/min/kg) in the intermediate range relative to hepatic blood flow (20 ml/min/kg) and bioavailability of $\sim$50%, probably due, in large part, to its first-pass metabolism. In contrast, alprazolam has a low clearance of $<$5% of hepatic blood flow and bioavailability exceeding

90%. Coadministration of ketoconazole resulted in a 14-fold increase in the oral AUC of triazolam, but only a 4-fold increase in the alprazolam oral AUC. The extent of changes in AUC caused by enzyme inhibition also depends on the route of substrate administration. Route-dependent enzyme inhibition of drug metabolism has recently been demonstrated by Tsunoda et al (72), who showed that ketoconazole caused an 11-fold increase in the oral AUC of midazolam, but only a 4-fold increase in the intravenous AUC of midazolam. Taken together, these findings are consistent with theoretical simulations (Figures 1and 2), which clearly show that the extent of changes in plasma AUCs is route and substrate dependent.

Because of interindividual variability in the basal level of enzymes, a drug can be defined as a high-clearance compound in some individuals and a low-clearance compound in others. For example, there is an 11-fold interindividual variability in the systemic clearance of alfentanil in patients, ranging from 1.5 to 16.7 ml/min/kg (73). Alfentanil, a short-acting narcotic widely used in minor surgical procedures, is metabolized mainly by CYP3A4 in humans. The 11-fold variation in clearance is most likely due to individual variability in the CYP3A4 protein levels. Conceivably, if the drug were given orally, the subject with high clearance of 16.7 ml/min/kg would be expected to be more sensitive in response to CYP3A4 inhibition than the subject with the low clearance of 1.5 ml/min/kg. The following examples are used to illustrate the point that sensitivity in response to enzyme inhibition is dependent on the basal clearance of drugs for each individual. There is a 40-fold difference in oral clearance of encainide, an antiarrhythmic agent metabolized mainly by CYP2D6, ranging from 6.3 to 251 ml/min/kg in normal volunteers. When coadministered with quinidine, the extent of the changes in oral AUCs and bioavailability of encainide was greater in subjects with higher basal clearance (before inhibition) than those with lower initial clearance (52). A wide interindividual variability in the extent of quinidine inhibition was also seen with venlafaxine. Again, the magnitude of changes in oral clearance and AUC of venlafaxine during quinidine coadministration correlated well with the basal clearance of venlafaxine before administration of the inhibitor (53). It is interesting that the results of these examples are consistent with the observations by Hellerieгel et al (74), who conducted a meta-analysis of 143 suitable publications and concluded that "the lower a drug's bioavailability (probably due to extensive first-pass metabolism), the greater the interindividual variability in bioavailability." Collectively, these results strongly suggest that variability in basal enzyme levels is one of the major sources of interindividual variability in response to enzyme inhibition.

Genetic polymorphism also contributes to interindividual variability in response to enzyme inhibition. Quinidine exhibited significant inhibitory effects on the CYP2D6-mediated encainide metabolism in subjects with the extensive metabolism phenotype for debrisoquine (EMs), but quinidine had little effect on the metabolism of encainide in subjects with poor metabolism phenotype (PMs) (52). Quinidine decreased encainide systemic clearance from 935 to 190 ml/min in EMs but from 128 to 119 ml/min in PMs. On the other hand, quinidine increased oral bioavailability from 50% to 70% in EMs and 76% to 82% in PMs. This is

not surprising because in PMs there is no active enzyme for which quinidine and encainide can compete. Similarly, coadministration of quinidine did not alter the plasma concentration of venlafaxine in the PMs of debrisoquine, whereas quinidine increased the AUCs of venlafaxine by more than eightfold in the EMs (53). Genetic variability in drug interactions also was observed with the CYP2C19 polymorphism. Both diazepam and omeprazole are mainly metabolized by CYP2C19. Coadministration of omeprazole resulted in a significant increase in the AUCs of diazepam in EMs of *S*-mephenytoin, but this coadministration had no effect on the diazepam AUCs in PMs (75). These results suggest that EMs are more susceptible to enzyme inhibition than PMs. In contrast to these examples that PMs are less sensitive to CYP inhibitors, coadministration of ketoconazole significantly increased oral AUCs of omeprazole in PMs of CYP2C19, but not in EMs (76). In EMs, omeprazole is metabolized predominantly by CYP2C19 to form the major hydroxylated metabolite, whereas sulfoxidation by CYP3A4 is the major metabolic pathway of omeprazole in PMs. Therefore, the differential effect of ketoconazole on the metabolism of omeprazole between EMs and PMs is due mainly to the switch of major metabolic pathways.

INTERINDIVIDUAL VARIABILITY IN CYTOCHROME P450 INDUCTION

In drug metabolism research, enzyme induction has been used as a generic term, describing an increase in the amount and activity of an enzyme as a result of exposure to an inducing agent, regardless of the underlying mechanisms. The basic tenet is that induction leads to an increased amount of existing enzymes and not to qualitatively different enzymes. Unlike enzyme inhibition, which is an almost immediate response, enzyme induction is a slow regulatory process, and it takes time to increase the amount of enzymes, either as a result of increased transcription and translation or as a result of stabilization of enzymes, leading to a new steady-state level between biosynthesis and degradation (77). In animal studies, enzyme induction can be evidenced by direct measurement of the amount and catalytic activity of enzyme in the liver. However, owing to ethical considerations and practical limitations, only a few reports have provided direct evidence of induction CYP enzymes in humans. In these limited reports, there is a large interindividual variability in the levels of enzyme protein (or mRNA) and catalytic activity.

Ged et al (78) reported that there was a large interindividual variation in the changes in protein level of CYP3A4 in human liver before and after rifampicin treatment. Fourteen patients were included in the study, in which liver biopsies were collected before and after rifampicin treatment (600 mg/day for 4 days). After rifampicin treatment, there was an 18-fold difference in the induction of CYP3A4 protein. The extent of increase ranged from 160% to 2900% among these patients. Similar to the hepatic enzyme induction, considerable interindividual variability in human CYP3A4 induction in small intestine by rifampicin was reported by Kolars et al (79), using endoscopic biopsies in five healthy volunteers. The extent

of increase in mRNA of CYP3A4 in enterocytes during rifampicin treatment (600 mg/day for 7 days) ranged from no change in one volunteer to 12-fold in another volunteer. Like the variability in CYP3A4 induction, a large variation was also seen in intestinal CYP1A induction by omeprazole (80). Endoscopic tissue specimens from 6 healthy volunteers were analyzed for mRNA and enzymatic activity measured by deethylation of ethoxyresorufin before and after omeprazole treatment (20 mg/day for 1 week). The extent of increases in both mRNA and enzymatic activity of CYP1A was quite variable among individuals and ranged from 0% to 600%. The individual who did not initially respond (20 mg/day) had a marked increase in both mRNA and enzymatic activity after receiving 60 mg of omeprazole daily for 1 week, suggesting that enzyme induction is dose dependent. Similarly, dose-dependent induction of CYP1A by omeprazole has been demonstrated by Rost et al (81), who observed a more than fivefold interindividual difference in the magnitude of CYP1A induction, as measured by a ^{13}C-[*N*-3-methyl]-caffeine breath test. These results clearly demonstrated that there is a large interindividual difference in enzyme induction in response to inducing agents.

Although direct measurements of enzyme amount and catalytic activity by induction are limited in humans, the reduction in plasma AUCs of drugs has commonly been used as an indirect index for enzyme induction when concomitantly administered with inducers. As mentioned earlier, the basic tenet is that induction leads to an increase in the amount of existing enzymes but not to qualitatively different enzymes. Therefore, enzyme induction always causes an increase in the intrinsic clearance (V_{max}/K_m), as a result of increased V_{max}, and the concept of intrinsic clearance is the cornerstone for relating the changes in plasma AUC to enzyme induction. Using the indirect approach, large individual variations in the decreases in plasma AUCs have been reported for many drugs during enzyme induction, for example for the verapamil-rifampicin interaction. The extent of decrease in oral AUC of *S*-verapamil caused by rifampicin induction ranged from 5- to 60-fold with a mean value of 30-fold in eight healthy volunteers (82). In another study involving 10 subjects, rifampicin treatment decreased oral AUCs of midazolam by 11.6- to 55-fold (83). Similarly, a large interindividual variability was observed for the inductive effect of rifampicin on triazolam in 10 healthy volunteers (84). A significant interindividual variability was also observed for cyclosporin. Daily administration of rifampicin (600 mg/day) for 11 days resulted in a 2.5- to 6.6-fold increase in oral clearance of cyclosporin (85). Rifampicin also induces theophylline metabolism. In a clinical study involving six healthy subjects, pretreatment with rifampicin resulted in a significant decrease in oral AUC of theophylline, ranging from 23% to 150% (86). A large interindividual variability was also observed for the inductive effect of pentobarbital on nortriptyline metabolism (87). The extent of decrease in oral AUCs of nortriptyline during pentobarbital treatment ranged from 1.6- to 4.1-fold. Collectively, these examples show that there is a large interindividual variability in the changes in oral AUCs of drugs in response to enzyme induction, and many factors may contribute. Among these, environmental and genetic factors are probably the major sources for the variability.

Many environmental and physiological factors, such as diet, tobacco smoking, and age, can contribute to the interindividual variability in response to inducing agents. Unlike that of other animal species, the human diet is extremely variable among individuals. The compositions of diets vary according to availability, religion, and the method of preparation (88). It has been determined, by using a "breath test" as an indirect measure of $^{14}CO_2$, that a high-protein–low carbohydrate diet for 2 weeks increases the metabolism of caffeine and aminopyrine (89). Similarly, the metabolic clearance of antipyrine and theophylline was enhanced by a high-protein–low-carbohydrate diet for 2 weeks, whereas fat and total calories had little effect on drug metabolism (90). These results demonstrated the potential for dietary composition to modulate enzyme level and activity. The method of food preparation also has been shown to have impact on the metabolism of drugs. Individuals who ate a charcoal-broiled 8-ounce hamburger at lunch and a 6-ounce steak for dinner each day for 4 days had >80% lower plasma concentrations of phenacetin compared with those who ate their usual home diet (91). Similar observations were reported for theophylline and antipyrine (92). The lower drug concentrations after the consumption of charcoal-broiled meat are believed to result from the induction of CYP enzymes through the contamination of polycyclic hydrocarbons, which results from the incomplete combustion of meat drippings (93). Additionally, certain vegetables including brussel sprouts, cabbage, broccoli, and cauliflower contain chemicals that induce drug-metabolizing enzymes (94, 95).

Like charcoal-broiled meat, tobacco smoke contains a variety of polycyclic hydrocarbons. It is believed that the polycyclic hydrocarbons in cigarette smoke induce the metabolism of CYP1A substrates as a result of CYP1A induction (96). The average content of CYP1A in the liver biopsies from smokers (16.3 pmol/mg of protein) was significantly higher than that from nonsmokers (4.7 pmol/mg of protein). Consistent with the increase in enzyme protein, the hepatic activity of CYP1A, measured by phenacetin *O*-deethylation, was increased from 54 pmol/min/mg of protein in nonsmokers to 230 pmol/min/mg of protein in smokers (97, 98). Similarly, CYP1A activity in human duodenal mucosa was induced by cigarette smoking (97–99). Cigarette smoke also enhances metabolic clearance of CYP1A substrates in vivo. Theophylline clearance is significantly increased in smokers compared with nonsmokers (100, 101). Similarly, metabolic clearance of caffeine, fluvoxamine, clozapine, and olanzapine was found to be increased by cigarette smoke (102–105). The inductive effect of cigarette smoke appears to be CYP1A specific. Smoking does not induce CYP3A4 enzyme in humans, as determined by the pharmacokinetics of triazolam, a CYP3A4 substrate (106).

Although the increase in enzyme levels caused by enzyme induction is highly variable among individuals, there appears to be a limit to which the enzyme can be maximally induced. In other words, the maximally induced enzyme levels tend to be quantitatively similar among individuals. In a study with human hepatocytes, the maximally induced values of CYP3A were quantitatively similar in six hepatocyte cultures, probably representing the maximal extent to which CYP3A can be induced. Similar observations were reported by Madan et al (107), who

showed a maximal induction for CYP1A, CYP2B, and CYP3A in hepatocytes from different rats. It is interesting that the increase in testosterone 6β-hydroxylase activity by rifampicin and taxol in human hepatocyte cultures was inversely related to the basal levels of CYP3A in untreated hepatocytes (108). Similarly, the induction of oxazaphosphorine 4-hydroxylation activity in human hepatocytes was also inversely related to the basal activity in untreated cells (109). These results strongly suggest that a greater degree of enzyme induction will be observed in individuals with lower basal enzyme levels. Therefore, the degree of induction caused by inducing agents will be dependent on an individual's dietary habits and cigarette smoking.

The aging process involves many complex changes in physiology. Although it is believed that the hepatic activity of CYP enzymes is generally lower in old rats compared with young animals (110–112), the age-related impairment of enzyme activity in humans is highly controversial. In a study with a total of 54 liver samples from donors ranging in age from 9 to 89 years, Schmucker et al (113) failed to detect an age-related decline in the content of CYP2C and CYP3A enzymes. Studies with an erythromycin breath test in humans have also suggested no age-related decrease in the activity of CYP3A4 in vitro and in vivo (114, 115). However, Sotaniemi et al (116) concluded that age decreases drug metabolism in humans, as evidenced by the content of total CYP enzymes in liver biopsies obtained from 226 subjects with similar histopathologic conditions. The mean total CYP enzymes and antipyrine clearances decreased from 7.2 nmol/g of liver and 46.4 ml/min in the young subjects (20 to 29 years) to 4.8 nmol/g of liver and 32.5 ml/min in the elderly (>70 years), respectively. Similarly, the clearance of omeprazole (a substrate of CYP2C19) was decreased in the elderly (250 ml/min) compared with that in the young (594 ml/min) individuals (117). Similar to the controversy over the age-related decline in enzyme activity, there are conflicting reports regarding enzyme induction in the elderly. Using rifampicin as an inducing agent, Twum-Barima et al (118) found that the inducing effect of rifampicin on antipyrine metabolism was greater in young than in old subjects. Similarly, impaired enzyme induction in the elderly was reported by Salem et al (119). However, other investigators found similar degrees of induction in old and young subjects. Treatment with rifampicin resulted in the same degree of induction, and a 30-fold decrease in oral AUCs of verapamil in both old and young volunteers (82). The induction of theophylline metabolism by phenytoin has also been found to be unaffected by age (120). The reason for these discrepancies is unknown. However, given the conflicting data from different pharmacokinetic studies, it is not possible to generalize about an effect of aging on the induction response.

Genetic polymorphism can also influence enzyme induction. Depending on whether the individual has or does not have the functioning enzyme, there are interindividual differences in response to inducing agents. For example, induction of polymorphic 4-hydroxylation of *S*-mephenytoin by rifampicin occurred only with EMs of CYP2C19, but not with PMs (121). Mephenytoin is a 1:1 racemic mixture of *R*- and *S*-enantiomers. The *S*-mephenytoin is completely and rapidly

metabolized by CYP2C19 to form 4-OH-mephenytoin, whereas *R*-mephenytoin is metabolized very slowly to form 5-phenyl-5-ethylhydantoin. The stereoselective differences in the excretion of unchanged enantiomers in urine (urinary *R/S* ratio) have been used as an index of metabolic capacity. Daily dosing with rifampicin for 20 days resulted in a three- to eightfold increase in the 0- to 8-h urinary *R/S* ratio of mephenytoin after oral administration of racemic drugs to EMs of CYP2C19, although the urinary ratio was unaffected in PMs after rifampicin treatment. In another clinical study, rifampicin treatment caused a twofold increase in systemic clearance of propafenone in PMs of CYP2D6, but it had little effect on the clearance in EMs (122). CYP2D6-mediated polymorphic hydroxylation is the major pathway of propafenone metabolism in EMs, whereas CYP3A4-CYP1A2-mediated *N*-dealkylation is the main metabolic pathway in PMs. Thus, induction of propafenone *N*-dealkylation (CYP3A4-CYP1A2) by rifampicin resulted in more pronounced increases in the clearance of propafenone in PMs, but not in EMs. Omeprazole is known to induce CYP1A enzymes. A clinical study was conducted to evaluate the effect of genetic factors on enzyme induction by omeprazole. This study included 18 volunteers, 12 EMs, 5 PMs, and one intermediate metabolizer (IM) of *S*-mephenytoin. The CYP1A enzyme activity was measured by a ^{13}C-[*N*-3-methyl]-caffeine breath test. Omeprazole treatment caused a significant increase in CYP1A activity in all PMs and the IM, whereas omeprazole had little inductive effect in EMs (123). Data analysis of all 18 subjects revealed that there was a good correlation between the percent increase in enzyme activity (measured by cumulative $^{13}CO_2$ exhalation) and the plasma AUC of omeprazole. It is believed that the lack of omeprazole-inductive effect in EMs was due to less systemic exposure of omeprazole, as a result of rapid metabolism of omeprazole in EMs.

CYP3A5, a member of CYP3A subfamily, demonstrates 84% amino acid sequence similarity with CYP3A4. Because of their similarity, there is a large overlap in substrate specificity for both CYP isoforms. However, unlike CYP3A4, CYP3A5 is polymorphically expressed in human liver, appearing in only 25% of the human population, and CYP3A5 is reportedly not inducible by rifampicin (124–126). It is conceivable that the response of CYP3A substrates to rifampicin induction will be highly dependent on the molar ratio of CYP3A4 to CYP3A5. A greater increase in the metabolism of CYP3A substrates by rifampicin induction is expected for individuals with CYP3A4 enzyme only, compared with those with CYP3A4 and CYP3A5.

In addition to the environmental and genetic factors discussed above, kinetic properties of substrates can also influence the interpretation of enzyme induction when the changes in plasma AUCs of substrates are used. As shown in Figure 3 (parallel-tube model), the magnitude of changes in oral AUCs by enzyme induction is highly dependent on the kinetic properties of drugs; high-clearance drugs are more sensitive to the changes in the CL_{int}. Although an increase in the CL_{int} results in an almost proportional decrease in the AUCs for low-clearance drugs, for high- and intermediate-clearance drugs, the changes in the AUCs are more than proportional to the changes in the CL_{int}. Therefore, it is expected that enzyme induction

has less effect on low-clearance drugs than on high-clearance drugs. O'Reilly (127) reported that treatment with rifampicin caused a significant reduction of warfarin plasma concentration in patients. As expected, the extent of decreases in the AUCs of warfarin [a low-clearance drug (clearance = 0.05 ml/min/kg)] in response to rifampicin induction was twofold after oral administration of warfarin. Similarly, a twofold decrease in warfarin AUCs caused by induction was observed during phenobarbital treatment (128). In contrast to warfarin, verapamil [a high-clearance drug (clearance = 12 ml/min/kg)] shows profound inductive response to rifampicin treatment. Rifampicin increased the oral AUCs of *S*-verapamil by 32-fold (31). In another study, after 12 days of rifampicin treatment a marked reduction (12-fold) in the oral AUCs of verapamil was observed (129). Similar observations were obtained after barbiturate treatment. The extent of decrease in the oral AUC for alprenolol (clearance = 15 ml/min/kg) was more dramatic than the extent for warfarin after barbiturate induction (130–132). For drugs with intermediate clearance, the extent of decrease in oral AUC caused by enzyme induction would be greater than that for low-clearance drugs, but less than that for high-clearance drugs. Quinidine is an intermediate-clearance drug with a systemic clearance of 5 ml/min/kg. The oral AUC of quinidine was reduced by four- to six-fold with concurrent oral administration of rifampicin (133). From these examples, it is clear that the extent of decrease in oral AUCs of drugs highly depends on their clearance. Because of interindividual variability in the basal level of enzymes, a drug could be defined as a high-clearance compound in some individuals and a low-clearance compound in others. Thus, the decrease in the oral AUC of a given drug is expected to be greater for individuals with higher basal clearance than for those with lower basal clearance.

The magnitude of decrease in plasma AUCs of drugs by enzyme induction is also dependent on the route of drug administration. Although for low-clearance drugs, an increase in the CL_{int} owing to enzyme induction causes a proportional decrease in AUC regardless of route of drug administration, for high- and intermediate-clearance drugs, the change in AUC is much greater after oral administration than after intravenous administration (Figures 3and 4). Enzyme induction has little effect on the AUCs of high-clearance drugs after intravenous administration because the hepatic clearance of high-clearance drugs is limited by hepatic blood flow (Equations 10 and 11). Therefore, an increase in the intrinsic clearance caused by enzyme induction has little effect on the hepatic clearance of high-clearance drugs, unless inducers also alter hepatic blood flow. As shown in the examples cited above, the extent of reduction in the AUC of warfarin (a low-clearance drug) by rifampicin was almost identical, approximately twofold, after either oral or intravenous administration of warfarin (127). On the other hand, treatment of rifampicin caused a 15-fold decrease in the AUC of verapamil (a high-clearance drug) after a single oral dose, whereas rifampicin treatment had little effect on the intravenous AUC of verapamil (129). Similar results were reported by Fromm et al (31), who found that rifampicin decreased the

AUC of *S*-verapamil by 32-fold after oral administration of racemic verapamil, but it decreased the AUC of *S*-verapamil by only 1.3-fold after intravenous administration.

CONCLUSIONS

Undoubtedly, drug interactions involving enzyme inhibition and induction will continue to be a major medical concern for clinicians and patients, and pharmaceutical companies will continue to put their efforts into predicting the potential of drug interactions of new drug candidates during drug development. When predicting drug-drug interactions, the following questions are often addressed. Can in vivo drug-drug interactions be predicted accurately from in vitro studies? Should such predictions be qualitative or quantitative? Some scientists believe that a quantitative prediction of drug interaction is possible (134–136), whereas others are less optimistic and believe that quantitative predictions would be extremely difficult, if not impossible (8).

There are many factors that contribute to our inability to quantitatively predict drug interactions. One of the major contributing factors is the large interindividual variability in response in enzyme inhibition and induction. In addition, the difficulty in predicting drug interactions caused by enzyme inhibition stems from the inaccuracy of K_i estimation from in vitro studies and insufficient methods for direct measurement of inhibitor concentration at the sites of metabolism (8). It is even more difficult to quantitatively predict in vivo interactions involving enzyme induction from in vitro induction studies, because many underlying mechanisms for enzyme induction remain largely unknown. Until optimal experimental conditions are established for accurate estimation of K_i values and inhibitor concentrations and the underlying mechanisms of enzyme induction and the sources of interindividual variability in enzyme inhibition and induction are completely understood, information obtained from in vitro metabolic studies will go only so far in predicting whether there will be a lack of an interaction or a probability of one.

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