Cytochrome P450 Part 3: Impact of Drug–Drug Interactions

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ABSTRACT

The role of individual hepatic cytochrome P450 (CYP) enzymes in drug metabolism and the factors that modulate CYP activity are becoming increasingly well understood. These advances have resulted in a better understanding of drug–drug and drug–food interactions and an enhanced capacity to predict drug interactions that may occur with new drugs. This final article in the series describes the issues and principles that are important in identifying and assessing drug interactions that involve CYP enzymes.

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INTRODUCTION

Many drug–drug interactions that involve the hepatic cytochrome P450 (CYP) drug metabolising enzymes can be predicted and potentially avoided.[1] To be able to predict these drug interactions, we need to know about the drugs that act as substrates, inhibitors and inducers of the major CYP drug metabolising enzymes. Although there are approximately 50 human CYP enzymes, only a small number of these are involved in drug metabolism.[2,3] The CYP isoforms of interest in drug metabolism are CYP3A4, CYP2D6, CYP1A2, CYP2C9 and CYP2C19. This information can be found in most pharmaceutical references, such as the detailed and regularly updated table available from <medicine.iupui.edu/Flockhart/table.htm>. A good understanding of basic principles is needed to predict these drug interactions, the most important of which will be covered in this article.

CYP REACTION CYCLE

To comprehend how the CYP enzymes catalyse drug metabolism, we need to understand the CYP reaction cycle. The CYP reaction cycle starts when the substrate molecule binds to the active site of the enzyme (Figure 1). This triggers a series of events involving the transfer of electrons from an electron donor and the binding of oxygen. Ultimately, this results in the generation of a reactive intermediate that attacks the substrate molecule. An oxygen atom is inserted into the substrate and the metabolite (oxidised substrate) dissociates from the enzyme and the cycle can begin again. There are three main steps in this process that are most readily inhibited by drugs – binding of the substrate molecule, binding of oxygen and dissociation of the metabolite. The differences in which step of the cycle is inhibited, partly explains the differences in the potential of drugs to cause clinically significant drug–drug interactions.

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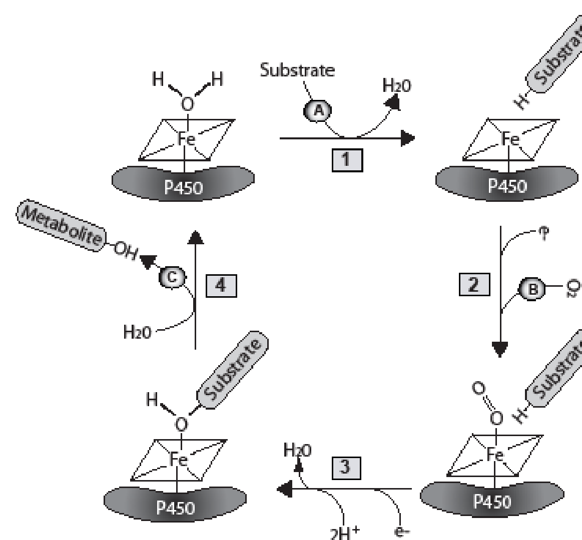

Figure 1. Cytochrome P450 (CYP) reaction cycle. Substrate binds (1) and results in the transfer of an electron to the haem iron (Fe) at the centre of the CYP enzyme, enabling oxygen to bind (2). A second electron is transferred to generate a highly reactive intermediate that attacks the substrate resulting in the insertion of an oxygen atom (3). The oxidised substrate (metabolite) dissociates from the enzyme (4). Inhibitors act by preventing substrate binding (A), oxygen binding (B) or metabolite dissociation (C).

VULNERABLE DRUGS

As a general rule, drugs that are affected by CYP-mediated drug interactions are those that rely extensively on metabolism and specifically CYP metabolism, for clearance from the body. Moreover, when a drug is metabolised predominantly by one CYP enzyme, inhibition of that one enzyme can cause a substantial reduction in the clearance of the drug's metabolite. For example, the conversion of terfenadine (withdrawn because of CYP-mediated drug interactions and associated adverse events) to its caboxylic acid metabolite, fexofenadine, is catalysed almost exclusively by CYP3A4. Inhibition of CYP3A4 by drugs, such as the azole antifungals or macrolides, results in a significant elevation in the blood concentration of the cardiotoxic parent compound.[4]

Another factor influencing the impact of a drug interaction is the therapeutic index of the involved drugs. As a relatively small change in plasma concentration of a drug with a low therapeutic index can lead to serious adverse events. For example, warfarin has a complex metabolic pathway that involves a number of CYP isoforms, most importantly CYP2C9, which metabolises the active S-enantiomer of warfarin.

The clinical status of the patient receiving the drug combination can also influence the impact of the drug interaction.

INHIBITION OF CYP-MEDIATED DRUG METABOLISM

Inhibition of drug metabolism is a common cause of clinically important pharmacokinetic drug interactions. Inhibition of drug metabolism can lead to a dramatic increase in the plasma concentration of the affected drug, which in turn increases the risk of drug toxicity. Inhibition generally occurs because the CYP enzymes that are involved in drug metabolism are capable of interacting with a wide variety of chemicals. Therefore, even when two drugs are structurally distinct they may compete for binding to the catalytic site of a CYP enzyme.

Metabolic Inhibitors

Relatively few drugs are capable of significantly inhibiting drug metabolism. Metabolic inhibitors involved in clinically significant drug interactions include the macrolides (e.g. erythromycin, clarithromycin), azole antifungals (ketoconazole, miconazole and to a lesser extent itraconazole), selective serotonin reuptake inhibitors (e.g. paroxetine, fluoxetine, fluvoxamine), protease inhibitors (e.g. ritonavir), cimetidine and amiodarone. Caution and careful monitoring of clinical signs and symptoms should be exercised whenever one of these metabolic inhibitors is added to or withdrawn from the medication regimen of a patient also taking other drugs primarily eliminated by metabolism.

Competitive Inhibitors

Competitive inhibition occurs when a drug prevents the binding of another drug at the first step of the CYP reaction cycle (Figure 1), e.g. competitive inhibition of fluoxetine by CYP2D6. During competitive inhibition, two drugs share the same space within the CYP enzyme catalytic site where only one drug can be accommodated. Therefore, the drug that binds to the enzyme with the highest affinity will prevent the binding of the other drug. Competitive inhibitors are often metabolised by the same enzyme. For example, although quinidine inhibits CYP2D6 by occupying the space within the CYP enzyme that is required for other substrates, quinidine is not metabolised as it binds at too greater a distance from the site of reactivity.[5]

In contrast, a drug that is a substrate for a particular CYP enzyme will not necessarily be capable of inhibiting the metabolism of other drugs that are also substrates for the same enzyme. To appreciate this, it is necessary to define Ki, which is the concentration of the inhibitor that will cause a twofold reduction in the rate of metabolism of another drug at a given concentration. Ki values are mostly obtained *in vitro* by studying the effects of the inhibitor on the metabolism of a substrate. If in the clinical setting, the unbound concentration of the inhibitor in the vicinity of the enzyme is much less than its Ki value then inhibition will not be discernible.

Mixed Inhibitors

Mixed inhibitors prevent CYP enzymatic function in many ways. Examples of mixed inhibitors include drugs with structures that contain nitrogen heterocycles, such as the azole antifungals. In addition to a competitive mechanism of inhibition, these structures are capable of preventing oxygen from binding to the haem iron and preventing the CYP reaction cycle from proceeding (Figure 1). In human liver microsomes, the Ki value for ketoconazole inhibition of CYP3A4 was reported to be 0.015 mM, while the antifungal effect of ketoconazole occurs at a plasma concentration of around 10 mM.[6] Even allowing for the extensive *in vivo* plasma protein binding of ketoconazole (99%), the unbound concentration of ketoconazole in plasma and presumably within the cell cytosol is approximately 0.1 mM, which is much greater than its *in vitro* Ki value. Therefore, it is entirely predictable that ketoconazole would be a strong *in vivo* inhibitor of CYP3A4-mediated drug metabolism.

Irreversible/Quasi-Irreversible Inhibitors

These drugs are also referred to as mechanism-based inhibitors and are the most clinically significant enzyme inhibitors. Drugs causing inhibition via this mechanism contain a number of different structural features that on CYP catalysed metabolism generate a reactive intermediate that does not dissociate from the enzyme resulting in permanent enzyme inactivation (Figure 1). Enzyme activity is only restored when new enzyme is synthesised. Well known examples of this type of inhibitors include ritonavir, paroxetine and erythromycin (but not azithromycin). In the case of erythromycin and azithromycin, both molecular structures contain the desosamine sugar moiety that has the potential to cause quasi-irreversible inhibition. However, the macrolide ring structure of azithromycin contains an extra member, which sterically prevents the desosamine sugar from being metabolised to the reactive intermediate that produces the inhibition of the enzyme.

Time Course of Inhibitory Drug Interactions

The time taken for the full impact of an inhibitory drug interaction to be elicited depends on the pharmacokinetic properties of the inhibitor and the affected drug. As a general rule, the full impact of a drug interaction is achieved when the drugs reach steady-state *in vivo*. This corresponds to around four half-lives for either drug (inhibitor or affected substrate), whichever is longer. If both the inhibitor and the affected substrate have short half-lives (e.g. cimetidine as an inhibitor of theophylline metabolism), the drug interaction will probably peak in two to four days and clearance of the affected substrate is expected to return to normal within a similar timeframe after the inhibitor is ceased.[7]

In contrast, if both the inhibitor and the affected substrate have long half-lives the full impact of a drug interaction is achieved when the drug with the longer half-life reaches steady-state. For example, warfarin has a half-life of approximately two days and its inhibitor amiodarone (CYP3A4 and CYP2C9 inhibitor) has a half-life of up to a month. Since amiodarone has the longer half-life, it may take months for the full impact of the drug interaction to be observed. In this clinical scenario, prolonged monitoring of blood clotting status is warranted.[8]

In some instances, it may be necessary to consider the half-lives of the metabolites as they too may be responsible for inhibition. For example, fluoxetine has a half-life of three days and its metabolite, norfluoxetine (also inhibits CYP2D6), has a half-life of 16 days.

For mechanism-based inhibitors, the duration of inhibition will persist following drug cessation and is often determined by the time required for the body to synthesise new CYP enzyme. For example, paroxetine has a half-life of approximately 24 hours but the CYP2D6 enzyme has a half-life of approximately 50 hours.[9]

Inhibition of Drug Activation

In some instances, CYP-mediated metabolism represents the drug's mechanism of pharmacological or toxicological activation. For example, the conversion of codeine to its active moiety morphine relies on CYP2D6-mediated o-demethylation. In contrast to the other examples of the effect of CYP inhibition on drug action, inhibition of CYP2D6, the enzyme responsible for codeine's activation, decreases codeine's clinical effect.[10] On this basis, the co-administration of codeine and celecoxib, an inhibitor of CYP2D6, seems irrational. Interestingly, the decrease in codeine's effects has been shown to be greater in Caucasians compared to the Chinese, presumably due to the differences in CYP2D6 genotypes between the two populations. This is an excellent example of how the clinical impact of a drug interaction is dependent on pharmacogenetics.[10] As our understanding of CYP expression develops it may be possible to identify subgroups of patients that are most susceptible to certain drug interactions.

Therapeutic Applications of CYP Inhibition

Although the inhibition of CYP-mediated metabolism is a problem in clinical practice, there are circumstances where this phenomenon may be favourably exploited. In humans, CYP3A4 is expressed in the liver and in various extrahepatic tissues, most notably the intestine. For drugs, such as cyclosporin, saquinavir and midazolam, a significant fraction of an oral dose can be metabolised by CYP3A4 within the intestinal mucosa prior to reaching the mesenteric blood supply. This intestinal metabolism can be reduced significantly by CYP3A4 inhibitors. Considering the intestinal concentration of these inhibitors during the absorption process will be much higher than the corresponding plasma concentration, it is apparent that intestinal inhibition may in some circumstances take on great importance.

In clinical practice, diltiazem (CYP3A4 inhibitor) is coadministered with cyclosporin (CYP3A4 substrate) to reduce the dosage requirements of cyclosporin as it is a costly drug.[11] Similarly, low-dose ritonavir is used as a 'pharmacokinetic modulator' of protease inhibitor pharmacokinetics to enhance bioavailability, as exemplified by the commercial co-formulation of lopinavir and ritonavir (Kaletra). The use of low-dose ritonavir in this manner is frequently referred to as ritonavir-boosted or pharmaco-enhanced protease inhibitor therapy.[12] The development of pharmacologically inactive substrates that inhibit metabolism and reduce the dose and/or dose frequency of another drug without adding to the risk of toxicity would be a more acceptable strategy.

INDUCTION OF CYP-MEDIATED DRUG METABOLISM

Metabolism induction occurs when a drug either induces or stimulates the synthesis of another drug or reduces the natural degradation of enzymes involved in the metabolism of another drug. CYP3A4 is susceptible to induction by a wide range of drugs. Drugs induce CYP3A4 by binding to the pregnane-x-receptor, which in turn binds to the regulatory regions of the *CYP3A4* gene resulting in increased protein levels. The pregnane-x-receptor also induces CYP2C enzymes. Rifampicin a CYP enzyme inducer, increases the hepatic levels of the CYP3A4 and CYP2C enzymes resulting in an increase in the intrinsic metabolic clearance of numerous drugs.[13] These include drugs that are highly reliant on the affected enzymes, such as cyclosporin. Other drugs that induce CYP-mediated drug metabolism include phenytoin, phenobarbitone and carbamazepine.

CYP-mediated drug metabolism can also be induced by a diverse range of non-drug chemicals, such as dietary substances and environmental contaminants. Cigarette smoke is a potent inducer of CYP1A2 and can reduce the plasma concentration of drugs that are substrates for CYP1A2, such as caffeine, clozapine and theophylline. Constituents of St John's Wort have also been identified as strong inducers of some CYP enzymes (via effects on the pregnane-x-receptor) demonstrating the importance of considering the use of complementary medicines in potential drug interactions.

The introduction of an enzyme inducer into a medication regimen leads to a reduction in the plasma concentration of a drug that is a substrate for the inducible CYP enzyme and will most likely lead to a reduction in the clinical efficacy of the drug. However, when an enzyme inducer is withdrawn from the medication regimen there is a possibility that the plasma concentration of the other drugs may increase. For example, cigarette smoke induces CYP1A2 and increases caffeine metabolism.[14] Therefore, smoking cessation can be associated with a reduction in hepatic CYP1A2 and a resultant increase in the plasma concentration of caffeine. This may be the reason for the perceived symptoms of headache and agitation attributed to tobacco withdrawal.

The time course of metabolic induction interactions has implications for how these interactions should be managed in the clinical setting. Drugs that are inducers of drug metabolism result in a change in enzyme expression over a period of weeks. Once the metabolic inducer is ceased the offset of the interaction is determined by the turnover of the enzyme (governed by its half-life) and the half-life of the inducer drug (or its metabolites).

FOOD–DRUG INTERACTIONS

Although dietary changes can also alter the expression and activity of CYP enzymes and lead to changes in drug metabolism, the magnitude of the change in systemic clearance is small.[15] Classic examples include the increased intake of cruciferous vegetables, such as brussel sprouts, or charcoal-broiled beef inducing the oxidative metabolism of drugs such as theophylline.

In the 1990s there was much publicity surrounding the 'grapefruit juice interaction'. Interest in this interaction stemmed from a study in 1991, which demonstrated that a single glass of grapefruit juice caused a twofold to threefold increase in the plasma concentration of felodipine.[16] A similar amount of orange juice did not affect the pharmacokinetics of felodipine.[16] It is postulated that a component of grapefruit juice, possibly a flavonoid or furanocoumarin, inhibits the intestinal but not the hepatic CYP3A4-mediated metabolism of felodipine and at least 25 other similarly metabolised drugs. The growing list of drugs affected by grapefruit juice include calcium channel blockers, cyclosporin, midazolam, terfenadine, ethinyloestradiol, simvastatin, lovastatin and buspirone.[17]

Discovery of the grapefruit juice interaction has created an increased awareness of the potential for dietary substances to alter drug metabolism. As our knowledge of how food–drug interactions contribute to variability

in drug response increases, we will be able to advise patients taking particular drugs on what specific foods to avoid. Alternatively, foods such as grapefruit juice could be used to reduce dosage requirements but the variability in the content of the constituents in grapefruit juice means that this alternative should be considered very carefully.

DRUG DEVELOPMENT

Drug interactions can have a negative impact on drug use and result in the withdrawal of a drug from the market. For example, mibefradil was withdrawn on the basis of its propensity for inhibiting the CYP-mediated metabolism of other drugs.[1]

The drug regulatory authorities have recognised the clinical importance of drug interactions arising from the inhibition and induction of CYP enzymes. As a result, candidate drugs are now screened to establish their potential for CYP metabolism and inhibition at an early stage of drug development. This typically involves *in vitro* experiments and animal studies to determine drug metabolism.

Mathematical models have been developed that assist in deciding whether an *in vitro* drug interaction will be of concern in clinical practice.[18] In general, predictions based on *in vitro* data have been limited to estimation of an average *in vivo* value, i.e. the pharmacokinetics of the 'average person' in a population. However, the latest generation of models are more mechanistic and allow for improved simulation of the pharmacokinetic variability within and between populations. This is an important step forward as the extremes in the population rather than the 'average' are more likely to be at a greater risk of harm from a drug interaction and thus a better estimation of clinically significant drug interactions may be made with this additional information. Similarly, the ability to estimate the inter-ethnic differences in pharmacokinetics and drug interaction risk is also important as most of the early clinical drug studies were undertaken predominantly in a single ethnic group.

In summary, as our understanding of CYP enzymes has increased, so too has our ability to predict drug interactions. While it may be difficult to precisely predict the impact of a drug interaction on an individual basis, it is possible to flag drug interactions that need monitoring and/or consideration when selecting drugs and their dose.

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