Factors Influencing the Magnitude and Clinical Significance of Drug Interactions Between Azole Antifungals and Select Immunosuppressants

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The magnitude of drug interactions between azole antifungals and immunosuppressants is drug and patient specific and depends on the potency of the azole inhibitor involved, the resulting plasma concentrations of each drug, the drug formulation, and interpatient variability. Many factors contribute to variability in the magnitude and clinical significance of drug interactions between an immunosuppressant such as cyclosporine, tacrolimus, or sirolimus and an antifungal agent such as ketoconazole, fluconazole, itraconazole, voriconazole, or posaconazole. By bringing similarities and differences among these agents and their potential interactions to clinicians' attention, they can appreciate and apply these findings in a individualized patient approach rather than follow only the one-size-fits-all dosing recommendations suggested in many tertiary references. Differences in metabolism and in the inhibitory potency of cytochrome P450 3A4 and P-glycoprotein influence the onset, magnitude, and resolution of drug interactions and their potential effect on clinical outcomes. Important issues are the route of administration and the decision to preemptively adjust dosages versus intensive monitoring with subsequent dosage adjustments. We provide recommendations for the concomitant use of these agents, including suggestions regarding contraindicated combinations, those best avoided, and those requiring close monitoring of drug dosages and plasma concentrations.

Key Words: drug interactions, azole antifungals, immunosuppressants, ketoconazole, itraconazole, fluconazole, voriconazole, cyclosporine, tacrolimus, sirolimus.

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The frequency of infections and mortality due to mycotic disease in the United States has increased dramatically over the past 2 decades. Among infectious disease–related deaths, those due to mycoses increased from being tenth most common in 1980 to seventh most common in 1997.[1] Invasive fungal infections particularly affect immunocompromised patients, including those with cancer, acquired immunodeficiency syndrome, or solid organ or bone marrow transplants, as well as certain high-risk patients with burns or critical conditions and neonates born prematurely.[2, 3]

Fungal infections occur in 11–28% of transplant recipients. Although the highest frequency is reported in liver transplant recipients, 10–15% of patients undergoing bone marrow transplantation become infected, with a mortality rate of 60–75%.[4] The interaction of three factors largely determine the high frequency of fungal infections in transplant recipients: technical and/or anatomic abnormalities, intensity of environmental exposure, and the patient's net state of immunosuppression.[3]

Fungal infections associated with immunosuppression-related, T cell–mediated defects include mucosal candidiasis, cryptococcosis, and mucormycosis, whereas neutropenia-related fungal infections include aspergillosis and disseminated candidiasis.[4] These infections tend to occur early after transplantation, and their frequency decreases over time.[5] In patients undergoing solid organ transplantation, the 1–6-month period after the procedure is highlighted by the emergence of opportunistic infections, such as histoplasmosis, coccidioidomycosis, blastomycosis and *Pneumocystis carinii* (*Pneumocystis jiroveci*) pneumonia.[6] Patients receiving bone marrow transplantation are at highest risk for fungal infections during the preengraftment period, which lasts from the day bone marrow or stem cells are infused (day 0) to approximately day 30 after transplantation.[4]

Given the high mortality rate associated with invasive mycoses in solid organ or bone marrow transplantation, effective prophylaxis and treatment of such infections are worthy goals. Although appropriate antifungal agents and their dosages for treating opportunistic mycoses have been defined, decisions regarding the modes of drug delivery, the patients who should receive antifungal prophylaxis, the duration of treatment or prophylaxis, and the potential for the emergence of resistant fungi remain subjects of uncertainty and controversy.[7]

Investigators have described the need to design a therapeutic prescription for transplant recipients that prevents and treats the two major barriers to successful transplantation: rejection and infection. The therapeutic prescription consists of an immunosuppressive program and an antimicrobial program; changes in the intensity and nature of one program obligate changes in the other. The technical and/or anatomic consequences of transplantation, the time course of infection after transplantation, and the possibility of drug interactions with the immunosuppressive program greatly influence the use of antimicrobials in transplant recipients.[8]

Drug interactions between immunosuppressants and azoles are discussed elsewhere.[9–12] Our objective was to describe certain studies to illustrate similarities and differences among these interactions so that clinicians can appreciate and apply these findings in an individualized patient approach rather than follow only the one-size-fits-all dosing recommendations suggested in many tertiary references. Clinicians must be aware of the combined influence of drug transport and metabolic routes on the likelihood and magnitude of drug interactions. Because data are not available for all potential drug-drug combinations, a thorough understanding of the principles underlying drug interactions is crucial to anticipate potential interactions with new or existing agents used in the clinical setting.

Mechanisms of Drug Interactions Mediated by Cytochrome P450 Enzymes and Transport Proteins

Metabolism Involving the Cytochrome P450 Enzyme System

A thorough understanding of the mechanisms of drug interactions provides clinicians the ability to predict such interactions and to devise strategies to minimize or avoid those of greatest clinical significance.[13] Drug interactions can be categorized as pharmacodynamic or pharmacokinetic. Pharmacodynamic interactions include additive, synergistic, or antagonistic interactions that can affect efficacy or toxicity. Pharmacokinetic interactions result when one drug alters the absorption, distribution, metabolism, or excretion of another drug.

Inhibition or induction of hepatic and extrahepatic cytochrome P450 (CYP) enzymes is the most common mechanism of drug interactions.[13] The major site of drug metabolism is the liver, where two types of reactions occur. In phase I reactions catalyzed by CYP enzymes, oxidation of a parent drug yields a more polar, hydrophilic moiety that may be pharmacologically active or inactive. In phase II reactions, which CYP enzymes do not mediate, conjugation of the parent drug or previously oxidized drug yields a hydrophilic moiety that is excreted in the feces or urine more readily than the parent drug.[14]

Cytochrome P450 enzymes are heme proteins that catalyze phase I metabolism of many endogenous substrates, including steroids, fatty acids, prostaglandins, bile acids, and xenobiotics (including drugs, carcinogens, environmental pollutants, and many synthetic chemicals).[14] These enzymes are found in the smooth endoplasmic reticulum of liver hepatocytes and in the villous columnar epithelium of the jejunum, lungs, kidney, and brain. More than 40 CYP enzymes have been identified in humans. Of these, CYP3A4, CYP2D6, CYP1A2, CYP2C9, CYP2C19, and CYP2E1 are responsible for metabolizing nearly all clinically useful drugs.[15]

The observation that CYP3A4 metabolizes nearly 50% of all clinically used drugs and endogenous steroids may explain why CYP3A4, among the CYP enzymes, is most often involved in drug metabolism and drug interactions.[16] This enzyme accounts for 29% and 70% of the total human hepatic and gastrointestinal tract CYP enzymes, respectively, affecting both presystemic and systemic drug distribution.[14, 17] This first-pass elimination results in variable (often low) oral bioavailability of CYP3A4-metabolized drugs.

An important characteristic of CYP3A is its large interindividual variability in activity, which reflects a genetic effect combined with modulation by environmental factors. The inhibition and induction of enzymes often substantially increases this variability. Hepatic CYP3A4 varies at least 20-fold, and enteric CYP3A4 varies 10-fold among individuals. However, the CYP3A4 content of each site appears to be regulated independently. Thus, one patient might have high CYP3A4 activity in the liver and low CYP3A4 activity in the intestine, whereas another patient has the opposite activities; the liver is the major site of drug metabolism in the first patient, whereas the intestine is the major site in the second.[18] Relative contributions of hepatic and enteric metabolism can be estimated separately by determining differences in drug clearance after oral and intravenous administration of a CYP3A4-metabolized drug.[17]

The CYP group of enzymes can be highly substrate specific and therefore capable of metabolizing only a few substrates, or they may be poorly specific and capable of metabolizing a broad range of substrates. The degree of enzyme specificity greatly affects the outcome of its inhibition. The broader the enzyme specificity, the more drug interactions its inhibition is likely to elicit. To predict, evaluate, and manage such drug interactions, clinicians must first identify the enzymes responsible for each agent's metabolism. Then, they must consider the dosages and timing of administration of the drugs in question, the duration of therapy, the baseline and steady state concentrations, the therapeutic index of each agent, and the potential for interindividual variability in pharmacokinetic variables, including absorption, distribution, metabolism, and elimination.[13]

Substrates, Inducers, and Inhibitors

Drugs can be substrates, inducers, and inhibitors of CYP enzymes. Substrates are moieties that undergo metabolism by one or more CYP enzymes. Inducers increase the amount or activity of a CYP enzyme. By contrast, inhibitors decrease the amount or activity of the enzyme. Enzyme inducers and inhibitors produce their effects in a dose- and concentration-dependent manner; within a range, high doses or concentrations of drugs generally increase induction or

inhibition. Inducers and inhibitors differ in the onset and duration of their effects on CYP enzymes. Although maximum enzyme induction is generally achieved over approximately 2 weeks, enzyme inhibition can be observed immediately after the administration of the first dose of an inhibitor drug.[14–16] Limited data are available regarding the duration of enzyme inhibition or induction after inducers or inhibitors are discontinued; however, the half-lives and protein binding of inducers and inhibitors are factors.

Enzyme induction refers to an increase in enzymatic activity due to increased production of the enzyme (by means of enhanced transcription and translation) or due to a reduction in the natural rate of its breakdown. The CYP enzymes that are known to be inducible are CYP1A2, CYP2C9, CYP2E1, and CYP3A4.[19] Unlike CYP inhibitors, which have an immediate effect, enzyme induction is gradual. Once an enzyme is induced, removal of the inducing agent eventually results in normalization of enzyme activity. Common inducers are phenytoin, carbamazepine, rifampin, and ethanol. As can be predicted, these agents often increase the metabolism of the other CYP substrates, potentially resulting in therapeutic failures.[20, 21]

In general, for CYP3A4 substrates (e.g., immunosuppressants) with low oral bioavailability (high presystemic or first-pass elimination), administration of a single dose of a CYP3A4 inhibitor substantially increases the area under the plasma concentration–time curve (AUC) for the substrate. By contrast, concomitant administration of a single dose of a CYP3A4 inhibitor with a CYP3A4 substrate with high oral bioavailability does not produce a large effect. However, repeat administration of both may cumulatively increase plasma substrate concentrations, and a clinically significant interaction may occur only during steady-state conditions.[17]

An inhibitor can be classified as competitive or noncompetitive. By mimicking the substrate, a competitive inhibitor competes for binding to the active site of an enzyme. For a competitive inhibitor to fully inhibit a site, high concentrations are needed. The higher the concentration, the stronger the inhibition. The clinical significance of drug interactions resulting from competitive inhibition depends on the concentration of the inhibitor achieved at the site of inhibition, the relative doses of the inhibitor and the substrate, the relative bioavailability, the relative affinity constants of the inhibitor and substrate (i.e., how tightly they bind to the site), the interindividual variability, and the therapeutic indices of the drugs.[9] At equimolar concentrations, ketoconazole is the most potent CYP3A4 inhibitor among the azoles, followed by itraconazole, voriconazole, and fluconazole.

A noncompetitive inhibitor binds to a location on the enzyme other than the active site, altering its conformation so that the active site is no longer fully functional. Only minimal amounts of a noncompetitive inhibitor are needed for inhibition to be effective. Table 1 summarizes the metabolism of azoles, the CYP enzymes involved, and the type and relative potency of inhibition.[22–25]

Genetic Polymorphism

A major cause of drug metabolism and subsequent drug effects is genetic polymorphism, in particular CYP2D6, CYP2C9, and CYP2C19. The distribution of these enzymes in the population is polymodal, as determined by genetic polymorphism. Although CYP3A4 drug-metabolizing activity varies widely among individuals, notable polymorphisms have not been identified.[17]

Active Transport of Azoles and Immunosuppressants

In addition to CYP enzymes, active transporters (e.g., P-glycoprotein [P-gp]), and the organic anion–transporting polypeptides (e.g., hepatic canalicular efflux transporter MRP2) play an important role in drug interactions. Active drug transporters play a key role in regulating access of drugs to the drug-metabolizing enzymes and controlling drug concentrations in enterocytes and hepatocytes.

A plasma membrane–associated glycoprotein, P-gp is a member of the adenosine 5′- triphosphate (ATP)–binding cassette transporter superfamily and a product of the multidrug resistance 1 (*MDR1*) gene. Although variably expressed in the population, P-gp is present at high levels in the liver, kidney, pancreas, small intestine, colon, and adrenal gland, as well as in the capillary endothelium of the brain and testes. This glycoprotein functions as an ATP-dependent efflux pump that excretes xenobiotics into bile, gastrointestinal tract, and urine and that prevents access to the central nervous system by limiting transport across the blood-brain barrier. These actions lower plasma and cerebrospinal fluid concentrations of xenobiotics, suggesting that P-

Table 1. Metabolism of Azoles and Immunosuppressants[22–25]

	CYP[a]		Inhibition of CYP3A4		P-glycoprotein	
Agent	Substrate	Inhibitor	Potency	Type	Substrate	Inhibitor
Ketoconazole	3A4	3A4, 2C19	++++	Competitive, noncompetitive	Yes	Yes
Fluconazole	3A4	2C9, 2C19	+	Noncompetitive, mixed	Yes	No
Itraconazole	3A4	3A4	+++	Competitive	Yes	Yes
Hydroxyitraconazole	3A4?	3A4	ND	Competitive	ND	ND
Ketoitraconazole	3A4	3A4	ND	ND	ND	ND
N-desalkylitraconazole	3A4	3A4	ND	ND	ND	ND
Voriconazole	3A4	3A4, 2C9, 2C19	++ to +++	Competitive, noncompetitive	ND	ND
Posaconazole	ND[b]	3A4	+++[c]	ND	Yes	Yes
Cyclosporine	3A4	3A4	ND	Competitive	Yes	Yes
Tacrolimus	3A4	ND	ND	ND	Yes	Yes
13-*O*-desmethyltacrolimus	ND	ND	ND	ND	Yes	Yes
Sirolimus	3A4	ND	ND	Competitive	Yes	Yes
Everolimus	3A4	ND	ND	ND	Yes	Yes

CYP = cytochrome P450; ND = no data.
[a]CYP enzyme involved in the metabolism of the azole.
[b]To date, none have been identified; does not inhibit CYP3A4.
[c]Based on limited data.

gp has a role in defense against xenobiotics.[15] In the gastrointestinal tract, P-gp is located in the brush border on the apical (luminal) surface of mature enterocytes. The colon has the highest P-gp expression, and the stomach and jejunum-ileum, the lowest.[26]

Enzyme-Transporter Cooperativity

Both P-gp and CYP3A4 share some substrate overlap and are colocalized in the small intestine, where they form a cooperative barrier that limits the oral bioavailability of xenobiotics and drugs, such as immunosuppressants. Substrates (e.g., immunosuppressants) for P-gp and CYP3A4 entering an enterocyte may be absorbed directly into the systemic circulation, metabolized by CYP3A4 in the enterocyte, or secreted back into the intestinal lumen by P-gp. Drug pumped back into the lumen may be reabsorbed at a distal site and exposed again to any of the three fates just described.[18]

For dual-substrate drugs, repeated pumping out of the enterocyte by P-gp limits and regulates their access to CYP3A4 metabolism and prevents high drug concentrations in the enterocyte from overwhelming the enzyme. Therefore, the two proteins function in concert to reduce the intracellular concentration of xenobiotics.[22, 27]

Coordinate upregulation of CYP3A was demonstrated in a cell culture model in response to some xenobiotics. However, other drugs have caused selective upregulation of P-gp expression.[12] Despite this shared functionality, no correlation was found between intrasubject enterocyte P-gp levels and CYP3A4 in the small intestine and/or liver. Moreover, CYP3A4 and P-gp do not appear to be coordinately regulated.[28]

The uptake of P-gp substrates can become saturated. When this occurs or when P-gp is inhibited, passive diffusion becomes the rate-limiting step in absorption. Therefore, increased doses of cyclosporine can increase the rate and extent of drug absorption. Drugs with high partition coefficients (i.e., highly lipophilic agents) can diffuse rapidly. The azoles, which are P-gp substrates, differ in their lipophilicity. Inhibition or saturation of P-gp has greatest effect on the oral bioavailability of water-soluble agents (e.g., fluconazole) because relatively lipophilic agents (e.g., itraconazole) can rapidly diffuse across the enterocyte.

Inhibition Constant

Clinical observations suggest that azoles have various potencies with respect to their ability to inhibit CYP3A4 and P-gp.[29] Determining the in vitro inhibition constant, K_i, which quantifies the molecular interaction and inhibitory potency of an agent, assists in predicting the magnitude of in vivo drug interactions. For a specific enzyme, K_i is a constant that is largely independent of substrate identity. This constant can be used to

predict enzyme behavior over a wide range of substrate and enzyme concentrations and, theoretically, to compare the inhibition potency of different drugs.[30] As K_i decreases, the potency of the inhibitor increases exponentially.

The most potent reversible CYP3A inhibitors, which include azole antifungal agents and human immunodeficiency virus protease inhibitors, have K_i values below 1 µmol. Clinically significant inhibition is uncommon for compounds with values above 75–100 µmol because sufficiently high concentrations are not clinically achieved.[31, 32] For example, ketoconazole and fluconazole are inhibitors of human hepatic and intestinal CYP3A4. However, ketoconazole is a more potent inhibitor than fluconazole, with mean ± SD K_i values of 14.9 ± 6.7 and 17.0 ± 7.9 nmol for hepatic and intestinal microsomes, respectively; by contrast, K_i values for fluconazole are 10.7 ± 4.2 and 10.4 ± 2.9 µmol, respectively. These data reflect a 1000-fold difference in the magnitude of CYP3A4 inhibition.[33] An extensive review on the use of inhibitory constants to evaluate the potential for drug-drug interactions has been published.[34]

The practice of using in vitro–in vivo scaling procedures to predict the effect of the coadministration of CYP inhibitory agents on in vivo drug interactions has many limitations. First, because in vitro studies are performed in human or animal preparations of liver microsomes, they do not reflect the possible contributions of extrahepatic CYPs and P-gp.[17] Second, concentrations of the inhibitor at the active site of the enzyme in vitro may not mirror concentrations in vivo.[35] Third, in vitro experiments are usually conducted in media that are devoid of plasma proteins and that do not mimic the in vivo setting with respect to pH, ionic strength, or protein binding, all of which can affect enzyme activity.[30] Finally, metabolites of the inhibitor may contribute to overall inhibitory effects on the enzyme. In general, the overall limitation is that a given concentration of an inhibitor is more potent in vivo than it is in vitro.[35] As a result, human data should be obtained whenever possible.[17] However, in vitro drug interactions can be used as screening tools that provide the rationale for further, in-depth examination of the interaction in human clinical trials.

Metabolism of Azoles

Currently available azoles inhibit the fungal CYP3A4-dependent enzyme lanosterol 14-α-demethylase. They hinder the synthesis of ergosterol, the major sterol component of fungal plasma membranes, resulting in altered membrane fluidity and inhibition of fungal growth and replication.[36] Early azoles, such as ketoconazole, are poorly selective in the inhibition of fungal versus mammalian CYP3A4. As a result, they can cross-inhibit mammalian CYP3A4, leading to drug interactions with other CYP3A4-metabolized drugs and to adverse effects (e.g., gynecomastia, adrenocortical insufficiency) due to the inhibition of CYP3A4-mediated metabolism of human steroid hormones.[36, 37] New azoles have enhanced selectivity for fungal versus mammalian CYP3A4. As a consequence, they have improved toxicity and drug-interaction profiles.[36]

The lipophilicity and lack of aqueous solubility of azoles influence the likelihood of their interacting with immunosuppressants. Ketoconazole, itraconazole, voriconazole, and posaconazole are more lipophilic than fluconazole and require extensive oxidative CYP metabolism to hydrophilic metabolites in order to be eliminated from the body.[36, 38, 39] Itraconazole, the most lipophilic and water-insoluble azole, is prone to enzymatic and transporter-mediated interactions in the intestine, liver, and kidney that interfere with the absorption and elimination of it and other drugs.

More than 30 metabolites have been proposed for itraconazole, only one of which, hydroxyitraconazole, has been studied in humans. The biotransformation of itraconazole is complex and incompletely understood; however, CYP3A4 catalyzes most, if not all, of its metabolism. In addition to hydroxyitraconazole, which is formed primarily in the intestine by CYP3A4, itraconazole and hydroxyitraconazole are converted to ketoitraconazole. In addition, CYP3A4 catalyzes the conversion of ketoitraconazole, and possibly itraconazole and hydroxyitraconazole, to *N*-desalkylitraconazole. Hydroxyitraconazole possesses antifungal activity similar to that of itraconazole, but it circulates at increased plasma concentrations. Itraconazole is a relatively potent inhibitor of CYP3A4 in vitro. Recent in vitro studies demonstrated that hydroxyitraconazole, ketoitraconazole, and *N*-desalkylitraconazole are CYP3A4 inhibitors at least as potent as itraconazole; therefore, they may contribute substantially to the inhibition of CYP3A4 observed clinically. The long half-life of itraconazole and its pharmacologically active

metabolites prolong the inhibitory effects on CYP3A4 metabolism of immunosuppressants.[38, 39]

Voriconazole, which is lipophilic and which has limited water solubility, is well absorbed orally and less prone than other drugs to presystemic interactions in the intestine. Voriconazole drug interactions are dose dependent. Because it has unpredictable nonlinear pharmacokinetics, its drug interactions are difficult to predict and manage. The hepatic biotransformation of voriconazole is fairly complex and involves CYP2C19, CYP3A4, and CYP2C9. The principle *N*-oxide metabolite of voriconazole is formed by CYP2C19 and CYP3A4 and, to some extent, CYP2C9.[38, 39] Two CYPs involved in voriconazole metabolism, CYP2C19 and CYP2C9, exhibit genetic polymorphism. Variability in the CYP2C19 genotype accounts for approximately 30% of the overall between-subject variability in voriconazole pharmacokinetics.[40]

Homozygous poor metabolizers have the highest plasma concentrations of voriconazole, followed by heterozygous extensive metabolizers, then homozygous extensive metabolizers. After oral and intravenous dosing with 200 mg and 3 mg/kg every 12 hours, respectively, mean AUCs in poor metabolizers and heterozygous extensive metabolizers were approximately 4- and 2-fold higher, respectively, than those of extensive metabolizers. The high blood concentrations of voriconazole observed in poor metabolizers may increase the magnitude of their drug interactions; close monitoring of plasma concentrations may be required in these individuals.[41] Furthermore, CYP2C9 expresses polymorphism, which, if expressed, is associated with reduced enzymatic activity. The polymorphism is most prevalent among Caucasians, less frequent among African-Americans, and absent in Asians. The magnitude of interactions with CYP3A4 substrates varies, ranging from no interaction (indinavir) to large increases in exposure (sirolimus).

By contrast, fluconazole is only slightly lipophilic and highly water soluble. It is well absorbed orally and less prone to drug interactions than other drugs, particularly in the intestine.[38, 39] Drugs that interact with fluconazole or voriconazole are substrates of CYP2C9, CYP2C19, or CYP3A4. The CYP-mediated interactions with fluconazole are often dose dependent. Because fluconazole has linear and predictable pharmacokinetics, these interactions may be avoided or minimized by using the lowest effective dose.

Posaconazole, an azole recently approved by the U.S. Food and Drug Administration, is active against a broad spectrum of fungi, including *Aspergillus* and *Candida* species and Zygomycetes. In vitro studies demonstrated that posaconazole is an inhibitor but not a substrate of hepatic (but not total) CYP3A4[25] and that it is both a substrate and an inhibitor of P-gp. This observation suggests that it may have a drug-interaction profile similar to that of other azoles. In addition, posaconazole undergoes glucuronidation by uridine 5′-diphosphate–glucuronosyltransferase enzymes.[42]

All five azoles inhibit CYP3A4 but with various potencies. Ketoconazole is the most potent inhibitor, followed by itraconazole and voriconazole (roughly equipotent), and then fluconazole (Table 1).[38, 41, 43, 44] The relative inhibitory potency of posaconazole is not well described at this time. Its in vitro K_i values have not been published, and the few clinical trials of CYP3A4 substrates have been reported mainly in abstract form.[24, 25, 45] Differences in relative potency translate into various degrees of interaction when each of the azoles is combined with a CYP3A4 substrate (as discussed later).

In clinical practice, fluconazole is the most commonly used agent followed by voriconazole, itraconazole, and, finally, ketoconazole. Although new azoles have widely replaced ketoconazole, its drug interactions with immunosuppressants are important because it is the most potent inhibitor and because it represents an important model for drug-drug interactions.

Metabolism of Cyclosporine, Tacrolimus, and Sirolimus

Both intestinal and hepatic CYP3A4 metabolize all immunosuppressants. Cyclosporine undergoes substantial presystemic metabolism; its oral bioavailability ranges from 30–70%. Tacrolimus is available as oral and parenteral formulations. Its oral absorption is incomplete and variable, with an oral bioavailability of 10–30%.[46] Sirolimus is available as an oral solution and tablets, both of which have wide interpatient and intrapatient variability in oral absorption. Although the oral bioavailability of sirolimus is not known precisely, it has been estimated to be about 15%.[47]

Cyclosporine, tacrolimus, and sirolimus are substrates of CYP3A4 and both substrates and inhibitors of P-gp. As a consequence, they have an immense potential for clinically significant drug-drug interactions that result in increased

plasma concentrations of immunosuppressants, excessive immunosuppression, and toxicity. Because more than 50% of the hepatic metabolism of cyclosporine, tacrolimus, or sirolimus involves CYP3A4, drug interactions resulting in complete induction or inhibition of CYP3A4 are expected to lead to clinically significant pharmacokinetic drug interactions.[28]

Because the correlation between dose and plasma concentrations is poor, with wide variability in interindividual and intraindividual pharmacokinetics, and because many of the adverse effects of immunosuppressants (especially nephrotoxicity) are dose and plasma concentration related, close monitoring of plasma concentrations is necessary to guide dosage adjustments, to minimize dose-related toxicity, and to maximize efficacy.[17, 28, 46–48]

In clinically administered dosages, cyclosporine appears to exert an inhibitory effect on P-gp more potent than that of tacrolimus or sirolimus.[26] Although nephrotoxicity is a well-recognized, concentration-dependent adverse effect of cyclosporine and tacrolimus, the relationship between plasma concentrations and neurotoxicity is hypothetical. Some have proposed that cyclosporine- or tacrolimus-induced inhibition of P-gp in the brain facilitates the distribution of immunosuppressants in the central nervous system, increasing concentrations of immunosuppressants in brain tissue.[28] However, plasma concentrations of sirolimus have not been correlated with any of its documented adverse events.[47]

Drug Interactions Between Azoles and Cyclosporine

Key steps in the metabolism of immunosuppressants are accomplished by CYP3A4. Azoles inhibit this enzyme, decreasing metabolism, increasing serum concentrations of immunosuppressants, and raising the potential for immunosuppressant-induced toxicity (particularly renal), overimmunosuppression, and opportunistic infections.[8] Yet, the degree of inhibition of CYP3A4 by individual azoles varies, and drug interactions with immunosuppressants are best described as an agent-specific rather than class effect for all agents in the class.

Ketoconazole

Among drug interactions between immunosuppressants and azoles, the interaction between cyclosporine and ketoconazole is the best studied. As the most potent CYP3A4 inhibitor of the azoles and an inhibitor of P-gp, ketoconazole and its concomitant administration with cyclosporine increases the AUC of cyclosporine almost 3-fold, allowing for cyclosporine dosage reductions of 70–80%.[49] The magnitude of this interaction becomes fully apparent days to weeks after the addition of ketoconazole to cyclosporine. The interaction resolves 7–10 days after ketoconazole is discontinued.[50]

Fluconazole

Fluconazole is a less potent inhibitor of CYP3A4 than ketoconazole, itraconazole, or voriconazole, and it is not an inhibitor of P-gp.[12] Fluconazole inhibition of CYP3A4 appears to be dose dependent, and interactions with cyclosporine are generally important only with doses of at least 200 mg/day. Although early reports of fluconazole and cyclosporine coadministration suggested a lack of interaction, later reports noted that administration of oral fluconazole 200 mg/day for 14 days doubled the AUC for cyclosporine.[51] Whether fluconazole 800–1200 mg/day, which is often used to treat invasive candidal infections, further increases the AUC is unknown.

Itraconazole

Itraconazole inhibits intestinal and hepatic CYP3A4-mediated metabolism of cyclosporine. Its effect on cyclosporine pharmacokinetics varies. Some, but not all, patients had elevated cyclosporine concentrations during concomitant administration of itraconazole.[50] Itraconazole generally doubles cyclosporine trough concentrations. Inhibitory effects of itraconazole on CYP3A4 metabolism of immunosuppressants persist for several weeks after itraconazole is discontinued because of its long half-life and its pharmacologically active metabolites.[38, 39]

Voriconazole

Data regarding the interaction between voriconazole and cyclosporine are somewhat limited. In kidney transplant recipients whose condition was stabilized for at least 4 weeks with individualized regimens of twice-daily cyclosporine, the addition of oral voriconazole 200 mg twice/day to oral cyclosporine 150–375 mg/day increased the mean AUC for cyclosporine 1.7-fold. Because of these findings, the authors suggested that the daily dose of oral cyclosporine

be decreased 50% when voriconazole therapy is started.[40, 52] However, in patients who withdrew from the study, trough concentrations of cyclosporine increased as much as 3-fold.[52]

Posaconazole

In four heart transplant recipients, the dose of cyclosporine was reduced 0–29% after the addition of oral posaconazole 200 mg twice/day for 10 days. This outcome suggested that low doses of posaconazole, similar to fluconazole, did not substantially inhibit CYP3A4.[25]

Drug Interactions Between Azoles and Tacrolimus

Drug interactions involving tacrolimus are less well characterized in terms of their mechanism, onset, potency, and clinical relevance than those with cyclosporine. The in vitro data are derived from studies performed in various species (rat, human, pig) and tissues (liver, small intestine, gastrointestinal mucosa). All demonstrated differing magnitudes of azole inhibition of CYP3A4-mediated metabolism of tacrolimus in the liver and gastrointestinal tract.[50] Available in vitro data are poorly predictive of clinical results in humans, and few controlled drug trials have addressed this issue.[28]

Ketoconazole

The addition of oral ketoconazole 200 mg/day to the regimen of a patient whose condition was stabilized with tacrolimus prompted a reduction in tacrolimus doses by as much as 80%.[53] The interaction was observed within 1 day after the oral ketoconazole was started and persisted for about 7 days after it was discontinued. Despite a preemptive decrease in the tacrolimus dosage by 45% when ketoconazole was begun, tacrolimus plasma concentrations became supratherapeutic.

Fluconazole

Researchers investigated the interaction between low-dose oral fluconazole 100 mg/day for 7 days and oral tacrolimus in a prospective randomized study of 19 renal allograft recipients.[54] Within 5 days of the start of fluconazole, doses of tacrolimus were decreased substantially. The investigators concluded that a tacrolimus dose equivalent to 60% of the initial dose (i.e., a 40% reduction in the dose of tacrolimus) was appropriate during coadministration with fluconazole. Further dose reductions of tacrolimus are presumably needed with doses of fluconazole higher than this; however, this possibility has not been evaluated. Other researchers reported that, after fluconazole was discontinued, 9 days were required for evidence of the drug interaction to resolve.[55]

Itraconazole

Specific data regarding the interaction between itraconazole and tacrolimus are limited to retrospective studies and case reports. Several investigators demonstrated that, with the addition of oral itraconazole 200–400 mg/day to tacrolimus, a 50–66% decrease in the dose of tacrolimus was needed.[56–58] The long half-life of itraconazole prolonged the inhibition of tacrolimus metabolism. A report of a kidney transplant recipient described an interaction between oral itraconazole 100 mg twice/day and tacrolimus.[56] The onset of the interaction was apparent less than 2 days after the start of itraconazole. Despite the discontinuation of itraconazole after 5 days of therapy, the interaction persisted for an additional 7 days, and a 50% reduction in the dose of tacrolimus was required. Itraconazole 600 mg given orally twice/day increased tacrolimus trough concentrations nearly 5-fold, highlighting the dose-dependent inhibition and nonlinear pharmacokinetics observed with this agent.[57]

Voriconazole

Among healthy volunteers who had received oral voriconazole 400 mg twice/day for 1 day followed by 200 mg twice/day for 6 days, a single oral dose of tacrolimus 0.1 mg/kg tripled the AUC for tacrolimus.[40, 59] Given these findings, the manufacturer of voriconazole recommends reducing the daily dose of tacrolimus to 33% of the initial dose (i.e., 66% reduction) when it is used in combination.[40] However, in a clinical study of liver transplant recipients, trough concentrations of tacrolimus during concomitant oral administration of voriconazole 200 mg twice/day increased nearly 10-fold in the first subject, whose condition had been previously stabilized with tacrolimus 2 mg/day.

The investigators had previously performed an in vitro study in human liver microsomes in which the concentration of voriconazole required to inhibit tacrolimus metabolism by 50% was 10.4 ± 4.3 μg/ml. The inhibition was concentration dependent. At concentrations of voriconazole 5-, 10-, and 50-fold higher than

those of tacrolimus, tacrolimus metabolism was inhibited by 20%, 53%, and 76%, respectively. Because typical maximum and trough serum concentrations of voriconazole clinically achieved after oral dosages of 200 mg twice/day are 2.12–4.8 and 1.4–1.78 μg/ml, respectively, the investigators expected an in vivo interaction of 50% or less. The magnitude of the in vivo interaction appeared to be greater than that predicted by using the in vitro data. They hypothesized that high voriconazole concentrations in the gastrointestinal tract inhibited intestinal CYP3A4 metabolism of tacrolimus, similar to the interaction observed with ketoconazole and tacrolimus.[23]

Posaconazole

Tacrolimus AUCs increased 4.5-fold with the addition of oral posaconazole 400 mg twice/day for 8 days.[24] Of note, the interaction of posaconazole with tacrolimus was greater than that observed with cyclosporine, perhaps because a relatively high dose of posaconazole was used.[25]

Drug Interactions Between Azoles and Sirolimus

Ketoconazole

In healthy subjects, the addition of oral ketoconazole 200 mg/day for 10 days to oral sirolimus 5 mg/day decreased the oral clearance of sirolimus by 90%, similar to its effect on tacrolimus.[60] These findings were confirmed in a prospective study in six kidney transplant recipients.[61] However, careful monitoring and dosage adjustment allowed investigators to maintain appropriate plasma concentrations of sirolimus.

Fluconazole

To our knowledge, no randomized studies have been performed to assess the interaction between fluconazole and sirolimus, and interactions have largely been implied from observations between azoles and other CYP3A4 or P-gp substrate drugs, such as cyclosporine and tacrolimus. However, the management of drug interactions involving sirolimus may be complicated, even during the coadministration of weak CYP3A4 inhibitors such as fluconazole. One report describes a kidney transplant recipient who began sirolimus therapy on day 5 after transplantation and oral fluconazole 200 mg/day on day 25 after transplantation.[62] Despite a preemptive reduction in the dosage of sirolimus from 4 to 3 mg/day on day 26 and an additional reduction to 2 mg/day on day 30, the trough concentration of sirolimus almost doubled by day 29 and tripled by day 32.

Voriconazole

The effect of voriconazole on the pharmacokinetics of sirolimus is even more pronounced than its effect on tacrolimus. In a single-blinded, randomized, placebo-controlled, two-period crossover study of healthy subjects, oral voriconazole 400 mg twice/day for day 1 followed by 200 mg twice/day for 8 days increased the AUC of sirolimus (administered as a 2-mg single oral dose on day 4) by a mean of 11-fold.[40] Based on these findings, the coadministration of voriconazole and sirolimus is contraindicated. However, in a recent case series in eight patients whose sirolimus dose was initially reduced by 90%, concomitant administration of voriconazole and sirolimus for a median of 33 days (range 3–100 days) resulted in trough sirolimus levels similar to those obtained before the administration of voriconazole.[63] No obvious, clinically significant toxicity from either drug was observed. Serious adverse events were observed in two patients in whom sirolimus dosage was not adjusted during voriconazole administration.

Other Azoles

To date, no data are available regarding drug-drug interactions between itraconazole or posaconazole and sirolimus.

Effect of Intravenous versus Oral Administration on Drug Interactions Between Immunosuppressants and Azoles

Because CYP3A4 is expressed in the gastrointestinal tract wall and in the liver, inhibition of CYP3A4 by azoles can occur at both sites. Therefore, inhibition of CYP3A-mediated metabolism of immunosuppressants is more pronounced when the immunosuppressant is administered orally than when it is given intravenously. Dual cooperativity of CYP enzymes and P-gp in the gastrointestinal tract contributes to remarkable differences in the oral bioavailability of immunosuppressants observed with ketoconazole versus fluconazole. Because immunosuppressants are substrates of P-gp and because ketoconazole (but not fluconazole)

inhibits P-gp, ketoconazole inhibition of P-gp–mediated immunosuppressant efflux increases the oral bioavailability of cyclosporine more than fluconazole. For example, the administration of oral ketoconazole 200 mg/day to five healthy subjects increased the oral and hepatic bioavailabilities of cyclosporine 2.6- and 1.2-fold, respectively.[64] Intravenous administration of fluconazole 400 mg/day, a less potent CYP3A4 inhibitor than ketoconazole, reduced the clearance of intravenously administered cyclosporine by only 21%.[65] Similar effects were observed with oral tacrolimus.[65–68] The administration of oral ketoconazole doubled the oral bioavailability of tacrolimus, whereas the addition of intravenous fluconazole reduced tacrolimus clearance by only 16–20%.

Table 2. Recommended Percentage Dose Reductions of Immunosuppressants During Concomitant Azole Therapy[24, 25, 40, 69–71]

Azole	Cyclosporine	Tacrolimus	Sirolimus
Ketoconazole	70–80	50–60	80–90
Fluconazole (≥ 200 mg/day)	21–50[a]	40[b]	50–70[c]
Itraconazole	50–60	50–60	No data
Voriconazole	50	66[d]	90[e]
Posaconazole	0–30[f]	75–80[f, g]	No data[g]

[a]Extent of interaction depends on the route of administration of cyclosporine (see text).
[b]Based on studies of low-dose fluconazole 100 mg/day.
[c]Based on limited data (see text).
[d]Variable (see text).
[e]Used in clinical practice. Coadministration is contraindicated according to the manufacturer (see text).
[f]Based on limited data (see text).
[g]At the time of writing.

Recommendations for the Clinical Management of Drug Interactions Between Immunosuppressants and Azoles

Transplant recipients generally receive numerous drugs, many of which induce, inhibit, or are metabolized by CYP3A4 or utilize the P-gp transport system. Therefore, assessment and management of drug interactions in this population are complex, and the application of generalized recommendations or guidelines in these patients is difficult. Reliable data are lacking, and clinicians should use caution when applying recommendations.

First, the practice of using in vitro and in vivo scaling procedures to predict in vivo effects of coadministered CYP inhibitors on drug interactions is limited. Although in vitro studies provide useful screening tools and a rationale for in vivo human investigations, human data should be used whenever possible.[17]

Second, dosages of immunosuppressants may need to be adjusted. Table 2 provides recommended dosage reductions of immunosuppressants during concomitant therapy with immunosuppressants and azoles.[24, 25, 40, 69–71] Although the purpose of Table 2 is to suggest dosage adjustments, interpatient variability can substantially affect the occurrence, magnitude, and clinical significance of these drug interactions.

Third, although enzyme inhibition is observed immediately after the first dose of an inhibitor is administered, minimal data are available regarding the duration of inhibition after its discontinuation. The half-life of the inhibitor and its protein binding can affect the duration of inhibition. In general, after an azole is discontinued, a mean of 7–10 days is required for concentrations of immunosuppressants to return to baseline values.

Fourth, plasma concentrations of immunosuppressants should be monitored at the start of therapy, throughout combined use, and, most important, after concomitant azole therapy is discontinued.

Fifth, recommendations in the literature suggest preemptive dosage reductions of immunosuppressants when an azole is added. However, most lack validity. Therefore, they are not the standard of practice among transplant clinicians who are concerned about an increased risk of rejection in patients prescribed preemptive dosage reductions of immunosuppressants during concomitant therapy with azoles. Additional studies are needed to assess the validity and clinical outcomes of preemptive dosage reduction.

Additional Factors Affecting Drug Interactions

Both drug- and patient-related factors help determine the susceptibility to potential drug interactions.[28] Factors such as genomic variability, sex, ethnicity, and disease states that alter plasma drug concentrations can affect the magnitude and clinical significance of drug interactions.[17] The effect of wide interpatient variability in the absorption, distribution, metabolism, and clearance of immunosuppressants on the magnitude and clinical significance of drug interactions cannot be overemphasized.[28, 47]

Sex-related differences can influence the

magnitude of drug interactions between azoles and immunosuppressants.[28] Tacrolimus pharmacokinetics were compared in 11 male and eight female kidney transplant recipients with and without the concomitant use of ketoconazole. The coadministration of oral ketoconazole and intravenous tacrolimus decreased tacrolimus clearance significantly more in the female patients than in the male patients. When both drugs were administered orally, a significantly greater increase in absolute bioavailability was observed in the female subjects.[68] Researchers theorize that these sex-related differences could result from lower metabolic capacity of the intestinal microsomes in male subjects than in premenopausal female subjects or from high P-gp activity in female subjects that increases the efficiency of CYP-mediated metabolism.[28]

Ethnicity affects cyclosporine pharmacokinetics because hepatic CYP3A4 activity is increased in some groups.[28] The oral bioavailability of tacrolimus was significantly lower in African-American healthy volunteers and kidney transplant recipients than other subjects perhaps because of differences in intestinal P-gp and CYP3A4 metabolism rather than differences in hepatic metabolism.[28, 72] Differences in the oral bioavailability of tacrolimus result in differences in the dose response. Among kidney transplant recipients, African-Americans require daily doses of tacrolimus 37% higher than those needed by Caucasians to achieve similar plasma concentrations.[73]

Finally, in addition to factors just discussed, inflammatory small-bowel disease, cirrhosis, stress, infections, poor nutritional status, and increased age decrease the amount and activity of CYP3A4 present in tissues.

Advantages of Drug Interactions

Although drug-drug interactions are usually considered undesirable events, they can be beneficial. Therapy with immunosuppressants can be cost-prohibitive. Reduction of daily doses of immunosuppressants due to ketoconazole-induced inhibition of their clearance can reduce therapeutic doses of the immunosuppressants by 70–85%. This drug combination has proved to be well tolerated and effective, and it is considered less costly than cyclosporine monotherapy.[74]

Investigators who reviewed the clinical and cost-saving potential of cyclosporine drug interactions concluded that ketoconazole appeared to be the best candidate for reducing the financial burden of long-term immunosuppressive therapy without sacrificing patients' well-being.[74] A 5-year study of patients receiving combined ketoconazole and cyclosporine showed no clinical difference in outcomes, including renal function, hepatic function, blood pressure, use of antihypertensive drugs, or patient or graft survival.[73] The combined use of ketoconazole and cyclosporine could decrease the yearly per-patient cost of cyclosporine by approximately $3750, which could translate to a national annual savings of more than $100 million in patients undergoing solid organ transplantation. Other reported advantages were decreased frequencies of infections and episodes of acute rejection in patients who received ketoconazole and cyclosporine versus placebo and cyclosporine.[74]

A 10-year follow-up study of transplant patients receiving cyclosporine alone or combined with ketoconazole confirmed the continuity of cyclosporine dosage reduction and cost savings.[75] Clinical benefits associated with combination therapy included similar frequencies of acute-rejection episodes in both groups, although the frequency and rates of unfavorable responses to therapy were higher in the control group. The frequency of chronic allograft nephropathy was also significantly lower in the ketoconazole group than in the control group; this difference was sustained after 10 years. However, data have suggested that the use of ketoconazole to reduce the dosage of cyclosporine may compromise long-term graft survival and increase the risk of late acute or chronic rejection. A potentially detrimental effect of ketoconazole on graft survival might occur because of the delayed and decreased maximum concentrations and because of the flattened or unpredictable absorption of cyclosporine observed during concomitant therapy.[76] In the absence of definitive answers and given the conflicting information, the benefits of combination therapy in terms of infections and graft survival must be investigated further.

Few clinical trials have been conducted to evaluate the economic or clinical outcomes associated with the combined use of fluconazole and cyclosporine. A 3-month course of cyclosporine combined with fluconazole versus clotrimazole resulted in an estimated drug savings of $900/patient, with a significantly decreased rate of fungal infections in the group receiving fluconazole.[77]

Itraconazole coadministration with cyclosporine

decreased the mean dose requirement for cyclosporine by 50% and resulted in an mean cost savings of $233/patient/month.[74] Similar cost savings were reported with the combined use of ketoconazole and sirolimus.[61] The cost of sirolimus 2 mg/day was $625/month compared with $86 for the combination of sirolimus 0.25 mg/day and ketoconazole 200 mg/day.

The cost of tacrolimus was also reduced by 57% when tacrolimus was used concomitantly with ketoconazole. Additional benefits included a decrease in the total days of hospitalization, fungal skin infections, and rate of acute rejection, and a substantial improvement in graft function.[78]

Reports suggest potential economic benefits of fluconazole or itraconazole combined with immunosuppressants. However, ketoconazole remains the drug of choice for this indication because it is the most extensively studied azole and the least expensive and most potent inhibitor. Because only low doses of ketoconazole are needed to achieve major reductions in dosages of immunosuppressants, fewer adverse effects and similar or improved economic benefits can be achieved with ketoconazole than with other azoles.

Drug Interactions Between Azoles and Investigational Immunosuppressants

Everolimus is an investigational immunosuppressant that is structurally similar to sirolimus and that is intended to be given in combination with cyclosporine after solid organ transplantation. Because everolimus is a substrate of P-gp and CYP3A4, CYP3A5, and CYP2C8, a number of drug-drug interactions with azoles can occur. Although the administration of fluconazole has no substantial effect on the pharmacokinetics of everolimus, administration of itraconazole decreases its clearance by 74%.[79]

In subjects administered oral ketoconazole 200 mg twice/day for 8 days, a single 1-mg dose of everolimus coadministered with ketoconazole on day 4 increased the AUC 15-fold. The interaction was evident 24 hours after the administration of everolimus, and plasma concentrations of everolimus were detectable until day 24. The authors concluded that the concomitant use of ketoconazole and everolimus should be avoided if possible.[80] This result emphasizes the need to monitor drug concentrations and to further evaluate drug interactions with everolimus.

Conclusion

Drug interactions between azoles and immunosuppressants are agent specific and depend on the potency of the azole inhibition of CYP and P-gp, the plasma concentrations of each agent, the drug formulation, and interpatient variability. Trough concentrations of cyclosporine, tacrolimus, and sirolimus must be monitored, and their dosages must be adjusted accordingly when an azole is added or discontinued. Clinicians should monitor patients for toxicity and loss of efficacy a few days to a week after starting and 7–10 days after discontinuing combination therapy.

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