

XEN and the Art of Pharmacology: New Learning from an Old Science

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THE FUTURE DEVELOPMENT OF EFFECTIVE AND SAFE ANTIRETROVIRAL agents—and the continued study of ways to maximize the utility of currently available therapies—are highly dependent on a scientific field that has evolved in recent years at an incredible rate: pharmacology. While *The PRN Notebook* has published numerous articles focusing on the issues of pharmacokinetics (PK) and pharmacodynamics (PD) as they apply to antiretroviral therapy, very little has been published in these pages regarding the actual pharmacologic mechanisms responsible for host- and drug-related PK and PD variability. To help explain the science of drug metabolism and drug interactions—and how it is translating into new treatment strategies—PRN turned to Dr. Charles Flexner, a longtime supporter and friend, to discuss the latest developments and future directions of pharmacology in the much larger arena of HIV treatment research.

The XEN of Drug Metabolism and Disposition

“UNTIL VERY RECENTLY,” DR. FLEXNER BEGAN, “PHARMACOLOGISTS TYPICALLY viewed the body as a complex organism designed to eliminate and respond to drugs. Our view of the world has been so idiosyncratic that we actually started out, a couple of decades ago, simply naming the various enzymatic pathways involved in drug elimination after the drugs that were first used to make the pathways known to us. For example, we had chloramphenicol reductase and debrisoquine hydroxylase, which worked for quite some time until we began running into questions that we as pharmacologists could not easily answer.”

One example cited by Dr. Flexner was hepatic enzyme induction, a process that involves the upregulation of enzymes involved in drug metabolism. It wasn't clear how a single drug could turn on multiple elimination pathways. Nor was it clear why inducers of drug-metabolizing enzymes—such as cytochrome P450 3A4 (CYP3A4), the most common enzyme involved in drug metabolism—were also upregulating expression of drug-transport proteins, such as P-glycoprotein.

Over the past decade, a great deal has been learned about the regulation of CYP3A4 expression. For many years, all that was known about enzyme-inducing substrate drugs—such as phenobarbital, rifampin, efavirenz (Sustiva), and ritonavir—was that they were increasing the transcription of an elusive gene encoding CYP3A4. It wasn't until seven or eight years ago that pharmacologists discovered two proteins in the nucleus of cells associated with increased transcription of the CYP3A4 gene: the pregnane X receptor (PXR) and the retinoid X receptor (RXR).

Simply put, PXR is an “orphan” nuclear receptor that upregulates transcription of downstream genes encoding drug-metabolizing enzymes, after it binds to substrate drugs. PXR then meshes with RXR to

form a heterodimer that binds to an enhancer sequence in DNA, resulting in increased transcription, increased enzyme production, and increased enzyme activity.

More recently, pharmacologists identified yet another piece of the puzzle: the xenobiotic response element (XRE), a genetic locus on the 5'-flanking region of the CYP3A4 gene. And it is this enhancer—triggered by the PXR/RXR heterodimer—that upregulates the transcription of the CYP3A4 gene, resulting in increased production and activity of the CYP3A4 isoenzyme (see Figure 1). Most interestingly, it turns out that XRE is involved in a lot more than hepatic induction of CYP3A4.

“We know that phenobarbital, rifampin, efavirenz, and ritonavir don't just turn on CYP3A4,” Dr. Flexner said. “They can turn on a number of drug-metabolizing enzymes. They also turn on transport proteins

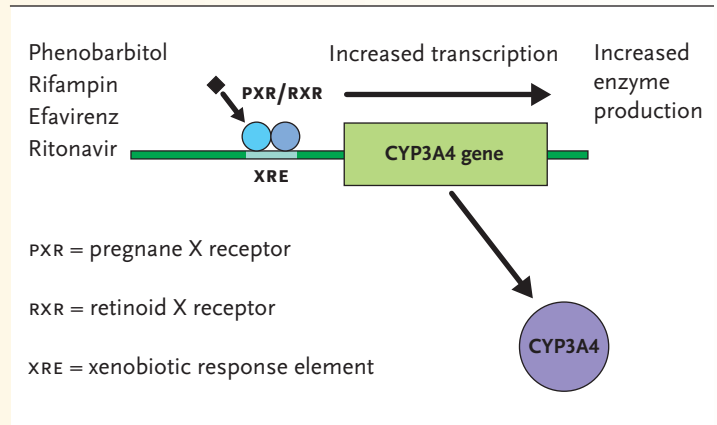


FIGURE 1. Molecular Mechanism of Hepatic Enzyme Induction

Hepatic enzyme induction, a significant component of drug metabolism, involves the upregulation of enzymes involved in drug metabolism. Over the past decade, a great deal has been learned about the regulation of cytochrome P450 3A4 (CYP3A4) expression. Depicted here are two proteins in the nucleus of cells associated with increased transcription of the CYP3A4 gene: the pregnane X receptor (PXR) and the retinoid X receptor (RXR). PXR is an “orphan” nuclear receptor that upregulates transcription of downstream genes encoding drug-metabolizing enzymes, after it binds to substrate drugs. PXR then meshes with RXR to form a heterodimer that binds to an enhancer sequence in DNA, resulting in increased transcription, increased enzyme production, and increased enzyme activity. Also depicted here is the xenobiotic response element (XRE), a genetic locus on the 5'-flanking region of the CYP3A4 gene. It is this enhancer—triggered by the PXR/RXR heterodimer—that upregulates the transcription of the CYP3A4 gene, resulting in increased production and activity of the CYP3A4 isoenzyme.

Source: Charles Flexner, MD

that affect the absorption and elimination of drugs and the intracellular concentrations of drugs. We've come to realize that XRE is responsible for much of this."

Aside from its role in increasing transcription of the CYP3A4 gene, XRE is also responsible for upregulating the activity of the CYP2D6 gene. XRE also increases transcription of the uridine diphosphate glucuronosyl-transferase (UGT) gene, responsible for the glucuronidation of zidovudine (Retrovir) and abacavir (Ziagen). It also increases activity of P-glycoprotein—formerly known as the multi-drug resistance-1 (MDR-1) protein—responsible for pumping a variety of drugs out of the interior of cells. Studies have also concluded that XRE upregulates organic anion transporting polypeptide (OATP) genes, thus tying together an entire family of enzymes and transporters involved in the intestinal, hepatocellular, and renal uptake and elimination of exogenous compounds with a variety of divergent chemical structures. These compounds are all xenobiotics, from the Greek words *xenos*, meaning foreign, and *biotiko*, meaning organic or living.

"Now we know that one drug can turn on multiple enzymes and transport proteins, via its effect on XRE," Dr. Flexner commented. "Interestingly, these regulatory networks are not unique to humans. They're highly conserved in mammals and, even in insects, similar systems can be found. Metabolizing enzymes, drug excretion, and transport genes are really part of an ancient network designed to detoxify and eliminate ingested substances." And this, Dr. Flexner explained, is the xenobiotic elimination network (XEN).

Obviously, these biochemical systems did not evolve simply to eliminate drugs from the body. XEN is characterized by coordinated induction of enzymes and transporters, along with redundancy of elimination pathways. This system, Dr. Flexner pointed out, evolved to prevent vertebrates and invertebrates from being poisoned by organic and inorganic toxic matter. "The fact is, our bodies can't tell the difference between ritonavir, saccharin, strychnine, or a mushroom toxin. This is important for us to remember when we develop new antiretrovirals: our bodies work very hard to get rid of them before they can do what we pharmacologists would like them to do."

The effects of XRE on metabolism and drug transport are variable. CYP3A4 is the most heavily influenced gene, more so than CYP2D6 and UGT, and significantly more so than P-glycoprotein and OATP. As an example, rifampin induces a tenfold increase in CYP3A4, compared to a twofold increase in P-glycoprotein or OATP. As explained by Dr. Flexner, CYP3A4 is one of the most promiscuous enzymes in biochemistry. It affects more than 10,000 different substrates and is the workhorse of detoxification in humans. And it's not just the classic P450 isoenzymes and transporters that are involved in the elimination of xenobiotics. Certain vitamins, bile acids, and other substances play a role in activating a variety of possible drug-elimination pathways.

XEN and Drug-Drug Interactions

IT IS WITH HOPE THAT AN ENHANCED UNDERSTANDING OF XEN WILL translate into much more precise clinical management of HIV, particularly when it comes to drug-drug interactions. As a case in point, Dr. Flexner discussed the interactions documented in studies involving multiple hepatic-inducing agents, which include both non-nucleoside reverse transcriptase inhibitors and protease inhibitors.

There are new *in vitro* data demonstrating that one hepatic enzyme-inducing agent can be used to antagonize the effects of other hepatic enzyme-inducing agents. There has also been some historical

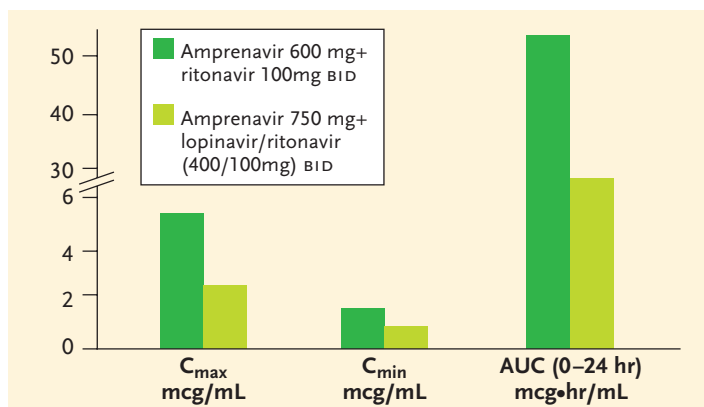


FIGURE 2. Comparison of Amprenavir Concentrations Following Administration of Amprenavir/ Ritonavir or Amprenavir/Lopinavir/ Ritonavir

The coadministration of multiple protease inhibitors can make for complex and problematic interactions. This is the case with amprenavir (Agenerase) and lopinavir/ritonavir (Kaletra). Amprenavir is a moderate inhibitor and inducer of CYP3A4, whereas ritonavir is a major inhibitor of CYP3A4 but also a moderate inducer of the enzyme. Lopinavir may be a CYP3A4 inhibitor and inducer as well. A regimen consisting of 750 mg amprenavir, 400 mg lopinavir, and 100 mg ritonavir results in amprenavir concentrations— C_{max} , C_{min} , and AUC—that are approximately 50% lower compared to a regimen consisting of 600 mg amprenavir and 100 mg ritonavir.

Source: Hsu, 2000; Wood, 2000

data confirming this *in vivo*. For example, efavirenz decreases the indinavir (Crixivan) AUC by approximately 30% when added to a regimen consisting of indinavir (800 mg TID) and ritonavir (100 mg BID). "If you increase the ritonavir dose from 100 mg to 200 mg BID, you completely reverse the effect of efavirenz, the inducer, on indinavir, the substrate," Dr. Flexner said. "One might argue that this occurs simply because more ritonavir is being taken to inhibit CYP3A4. However, in other studies, increasing the ritonavir dose by 100 mg had little effect on raising concentrations of other protease inhibitors, such as saquinavir. So, one possible explanation is competition for enzyme induction."

Another example involves combining efavirenz with lopinavir/ritonavir (Kaletra). If efavirenz is added to lopinavir/ritonavir, the lopinavir AUC decreases by approximately 20% and the C_{trough} decreases by roughly a third. "But if you add one more Kaletra capsule, which is only 33 extra milligrams of ritonavir, the effect is reversed. It was initially believed that this dose of ritonavir led to stronger inhibition of lopinavir clearance. However, we've since discovered that such a small increase in the ritonavir dose is unlikely to explain this large change in the lopinavir concentrations. This may be another example of competition for PXR binding leading to reduced enzyme induction, rather than increased ritonavir inhibition of CYP3A4."

A more recent example discussed by Dr. Flexner involves the coadministration of multiple PIs—an increasingly popular treatment option for antiretroviral-experienced patients—specifically amprenavir (Agenerase) and lopinavir/ritonavir. Amprenavir is a moderate inhibitor and inducer of CYP3A4, whereas ritonavir is a major inhibitor of CYP3A4 but also a moderate inducer of the enzyme. Lopinavir may be a CYP3A4 inhibitor and inducer as well.

What happens when these three drugs are coadministered results in

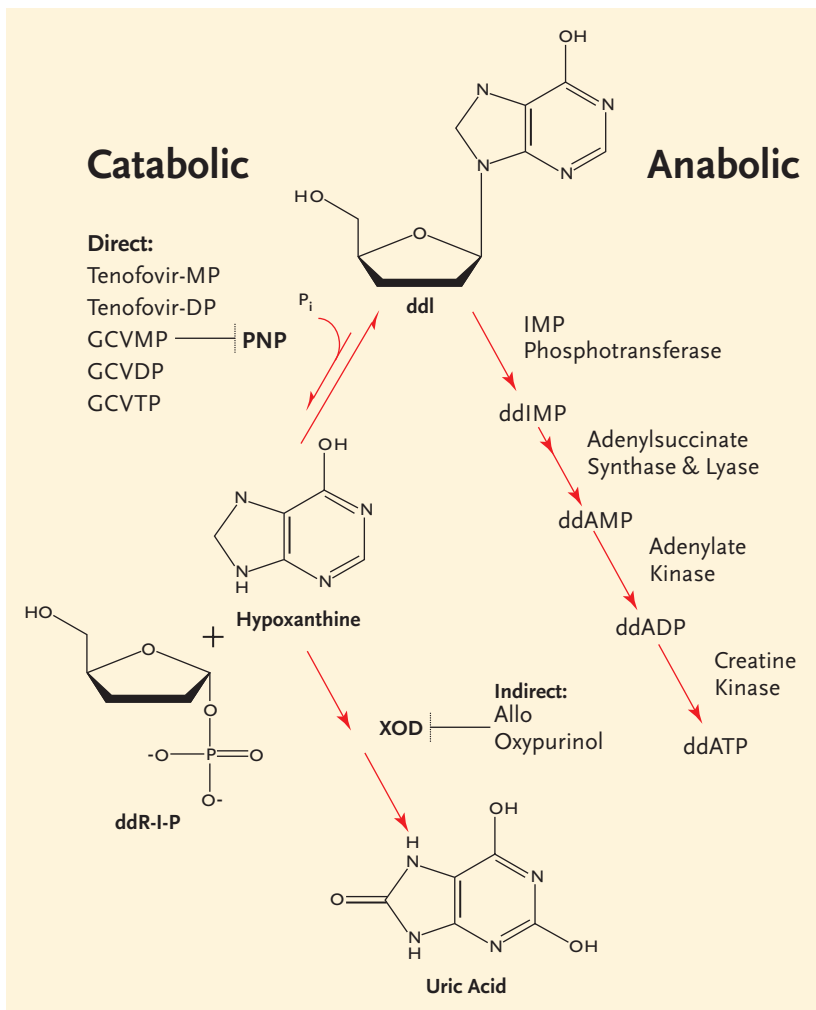


FIGURE 3. Tenofvir and PNP

New data may help explain the drug-drug interactions associated with tenofvir (Viread), most notably its profound effect on didanosine (Videx) concentrations (a 44% to 60% increase in didanosine's AUC). Building on the observation that one metabolic route for didanosine clearance is its breakdown of the enzyme purine nucleoside phosphorylase (PNP), researchers at Gilead Sciences have demonstrated that the monophosphate and diphosphate forms of tenofvir (as well as the monophosphate, diphosphate, and triphosphate forms of ganciclovir) are inhibitors of PNP-dependent degradation, resulting in increased didanosine concentrations. Inhibition of PNP is also associated with increased intracellular concentrations of dGTP and dATP. The phosphorylated anabolites of abacavir (Ziagen), a guanosine analog, and didanosine, an adenosine analog, must compete with dGTP and dATP to inhibit HIV reverse transcriptase. If intracellular levels of dGTP and dATP are substantially increased as a result of PNP inhibition, the chain-terminating nucleotides of abacavir and didanosine will be outnumbered—and out-competed—by the increased levels of natural dGTP and dATP. This, in turn, may explain the poor results seen in studies combining tenofvir with abacavir and tenofvir with didanosine, particularly as components of triple-NRTI regimens.

Source: Ray, 2004. Reprinted with permission of *Antimicrobial Agents and Chemotherapy* and the American Society for Microbiology

what Dr. Flexner bluntly called “a big mess.” A regimen consisting of 750 mg amprenavir, 400 mg lopinavir, and 100 mg ritonavir results in amprenavir concentrations— C_{max} , C_{min} , and AUC—that are approximately 50% lower compared to a regimen consisting of 600 mg amprenavir and 100 mg ritonavir (see Figure 2). “Increasing the amprenavir dose from

600 mg BID to 750 mg BID may increase amprenavir-mediated induction, but I think this is unlikely given that it's really a very minor increase in the amprenavir dose,” Dr. Flexner suggested. “The more likely explanation is that lopinavir is a CYP3A4 inducer.”

In discussing a possible dosing scheme to circumvent this negative drug-drug interaction, Dr. Flexner shared some preliminary data from a study evaluating the effects of a modest increase in the lopinavir/ritonavir dose on amprenavir pharmacokinetics. In this study, one group of 13 patients received lopinavir/ritonavir (533 mg/133 mg BID) coadministered with 750 mg amprenavir BID. Another group, consisting of seven patients, received the same regimen plus efavirenz (600 mg QD).

Increasing the lopinavir/ritonavir dose to 533/133 mg BID resulted in an approximate doubling of the amprenavir geometric mean trough (to 1.18 $\mu\text{g/ml}$) as compared with historical controls receiving amprenavir plus lopinavir/ritonavir (400 mg/100 mg BID). Interestingly, the addition of a fourth CYP3A4 inducer, efavirenz, did not further alter the pharmacokinetic parameters.

Tenofvir and PNP

SWITCHING GEARS, DR. FLEXNER DISCUSSED SOME INTRIGUING new data that might help explain some of the unexpected drug-drug interactions associated with tenofvir (Viread), most notably its profound effect on didanosine (Videx) concentrations (a 44% to 60% increase in didanosine's AUC). Like didanosine and some other nucleoside reverse transcriptase inhibitors, tenofvir is cleared by the kidneys and thus wasn't expected to result in any significant drug-drug interactions.

One theory was that tenofvir alters intracellular didanosine phosphorylation. However, in a study published last year in *Pharmacotherapy*, no significant interaction between tenofvir and didanosine in human PBMCs was documented, as determined by the extent of formation of the phosphorylated anabolites (Robbins, 2003).

In another study, researchers at Gilead Sciences followed up on the observation that one metabolic route for didanosine clearance is its breakdown by the enzyme purine nucleoside phosphorylase (PNP) (Ray, 2004) (see Figure 3). This study demonstrated that the monophosphate and diphosphate forms of tenofvir were inhibitors of PNP-dependent degradation, resulting in increased didanosine concentrations. “PNP is involved in the breakdown of purines, like inosine, which is the parent compound for didanosine,” Dr. Flexner explained. “If you inhibit PNP, you wind up with more of the parent compound. PNP is responsible for turning didanosine into hypoxanthine, which is eventually converted to uric acid. Didanosine is the only current antiretroviral known to be cleared by PNP.”

One location where PNP could affect didanosine is in the gastrointestinal tract. “We used to think that didanosine was poorly absorbed simply because it is acid labile and gets broken down in the stomach,” Dr. Flexner said. “We now think that part of the poor oral bioavailability of didanosine may result from the action of PNP. How much does PNP reduce the bioavailability of didanosine? Possibly by as much as 40% to 60%, based on coadministration data involving tenofvir.

Also of interest are data from a team of oncologists looking to use a PNP inhibitor to inhibit growth of leukemia cells, based on the observation that PNP deficiency in humans produces a selective depletion of T-lymphocytes (Bantia, 2003). Using a powerful PNP inhibitor dubbed BCX-1777, the study team found that it increased intracellular concentrations of dATP by eightfold, and dGTP by 154-fold, a dramatic effect on intracellular nucleotide pools.

With respect to the treatment of HIV infection, this could have significant implications and may explain the poor results of two recent clinical trials: one evaluating the efficacy of a regimen consisting of tenofovir, didanosine, and lamivudine, and another evaluating the efficacy of a regimen consisting of tenofovir, abacavir (Ziagen), and lamivudine.

In the latter study (E53300009), 345 patients were randomized to receive either tenofovir or efavirenz, combined with a fixed-dose combination tablet containing 600 mg abacavir and 300 mg lamivudine, all to be taken once a day (Gallant, 2003). Patients were naive to antiretroviral therapy prior to starting the study and had an average baseline viral load of 4.63 log₁₀ copies/mL and a baseline CD4+ cell count of 260 cells/mm³.

Because of a high number of premature treatment failures in the triple-NRTI group, the study investigators conducted an unplanned analysis involving the first 194 patients to complete eight weeks of follow-up. Approximately 49% of patients in the triple-NRTI group met the definition of virologic failure, compared to only 5.4% patients in the efavirenz-based arm. Among patients who had viral loads that were high enough to test for drug resistance, 64% of the triple-NRTI failures had both the K65R and M184V mutation and 36% had only the M184V mutation. Based on these preliminary results, this arm of the study was immediately halted and closed to enrollment.

The reason for the differences in response to therapy is still being debated. One possibility was the low genetic barrier for selection of resistance for the regimen. Tenofovir, abacavir, and lamivudine can all select for the K65R mutation and both abacavir and lamivudine can select for the M184V mutation, which can potentially yield rapid and high-level resistance to a regimen consisting of these three drugs.

Another possible explanation may be a pharmacological mechanism. One possibility is an interaction at the phosphorylation level, perhaps resulting in decreased carbovir triphosphate and/or tenofovir diphosphate with the coadministration of these NRTIs. However, two studies reported at the 5th International Workshop on Clinical Pharmacology of HIV Therapy, held in early April in Rome, indicate that such an interaction does not occur (Ray, 2004a; Hawkins, 2004). But when data is considered from the oncology study employing BCX-1777, another pharmacological mechanism comes into play. The phosphorylated anabolites of abacavir, a guanosine analog, and didanosine, an adenosine analog, must compete with dGTP and dATP to inhibit HIV reverse transcriptase. If intracellular levels of dGTP and dATP are substantially increased as a result of PNP inhibition, the chain-terminating nucleotides of abacavir and didanosine will be outnumbered—and out-competed—by the increased levels of natural dGTP and dATP. This, in turn, could help explain why tenofovir—an inhibitor of PNP—combined with abacavir and didanosine in the once-daily therapy studies discussed above, may have contributed to premature virologic failure and drug resistance.

“These emerging data provide one possible pharmacologic explanation for the unexpected antagonism between abacavir and didanosine in the once-daily tenofovir-based triple-NRTI regimens,” Dr. Flexner said. “There is no direct evidence for this at present, and tenofovir does not antagonize the anti-HIV effects of abacavir or didanosine *in vitro*. This interaction would only be expected to affect didanosine and abacavir,

given that we don't have any other guanosine or adenosine analogues that would have to compete with these two endogenous nucleosides. These recent study results will change what we do in the clinic and I think many are already being cautious about giving tenofovir with once-daily abacavir or once-daily didanosine.”

Ritonavir Redux

PREVIOUS PHARMACOKINETIC STUDIES DETERMINED THAT RITONAVIR has a plasma half-life of approximately three hours, and that a single dose of ritonavir produces detectable plasma levels for only 12 to 18 hours. If ritonavir is a competitive, reversible inhibitor of CYP3A4, it would need to be dosed twice-daily if used to boost the pharmacokinetic properties of other drugs. However, new data indicate that ritonavir may be, in part, an irreversible inhibitor of CYP3A4, suggesting that its inhibitory effects on CYP3A4 last significantly longer than its plasma pharmacokinetic properties. The implications here couldn't be more optimistic: the possibility of using ritonavir, less frequently, while maintaining its favorable pharmacokinetic effect on coadministered drugs.

To help determine whether ritonavir has a reversible or irreversible inhibitory effect on CYP3A4, the AIDS Clinical Trials Group (ACTG) conducted a study evaluating staggered versus simultaneous administration of 750 mg nelfinavir (Viracept), 400 mg saquinavir, and 400 mg ritonavir (Washington, 2003). ACTG 378 enrolled 18 healthy volunteers and had a complex design involving six treatment periods for each person.

When saquinavir was given four hours before ritonavir, saquinavir's AUC was reduced by 63%, compared with saquinavir's AUC when the ritonavir/saquinavir were given simultaneously. When saquinavir was given four hours before nelfinavir, saquinavir's AUC was reduced by 68% compared with saquinavir's AUC when saquinavir/nelfinavir were given simultaneously. When nelfinavir was given four hours before saquinavir, saquinavir's AUC was increased by 76% compared with when the PIs were given simultaneously.

Of particular interest to Dr. Flexner has been the finding that, when given 48 hours before a second PI, ritonavir boosted concentrations of a later PI—a 20-fold increase in the case of saquinavir—without any lingering concentrations of ritonavir detected.

“What does this mean?” Dr. Flexner asked. “If there's irreversible inhibition of CYP3A4, which has been our observation, then the concentration of ritonavir in the plasma doesn't matter as much as we previously thought. Ritonavir can be used less frequently as a booster. It's still not clear if similar effects would be seen using lower doses of ritonavir.”

Herbal Medicines and XRE

WHILE THERE HAS ALWAYS BEEN SOME DEGREE OF SPECULATION AMONG clinicians regarding the safety of herbal/complementary medicines in the broader context of HIV/AIDS therapy, it was a study published by Dr. Stephen Piscitelli and his colleagues in a February 2000 issue of *The Lancet* involving an interaction between indinavir (Crixivan) and St. John's wort that solidified the need for caution (Piscitelli, 2000). St. John's wort reduced the AUC of indinavir by a mean of 57% and decreased the indinavir trough by 81% in healthy volunteers. The authors concluded that a reduction in indinavir exposure of this magnitude could lead to the development of premature drug resistance and treatment failure.


St. John's wort contains at least one ingredient, hyperforin, that binds to and activates PXR. This, Dr. Flexner surmised, may explain why

it is a CYP3A4 inducer. “P450 and P-glycoprotein inducers are likely to be common in herbal products, given the multitude of chemical ingredients and the evolutionary design of the XEN,” he pointed out. “These have very important implications for clinicians. It is important to know what patients are taking besides prescription drugs.”

Earlier this year, long-awaited *in vivo* data involving the effects of Echinacea on cytochrome P450 isoenzymes were published (Gorski, 2004). The effects of *Echinacea purpurea* root on CYP activity was assessed using the CYP probe drugs caffeine (CYP1A2), tolbutamide (CYP2C9), dextromethorphan (CYP2D6), and midazolam (hepatic and intestinal CYP3A4). Six male and six female healthy study volunteers completed the two-period, open-label, fixed-schedule study. Caffeine, tolbutamide, dextromethorphan, and oral and intravenous midazolam were administered before and after a short course of echinacea (400 mg four times a day for eight days) to determine *in vivo* CYP activities.

Echinacea administration increased the systemic clearance of intravenously administered midazolam by 34% and reduced the midazolam AUC by 23%, indicating induction of hepatic CYP3A4. Conversely, Echinacea did not affect the clearance of orally administered midazolam, although the oral bioavailability of midazolam after Echinacea dosing was significantly increased.

Conclusion

WHILE DR. FLEXNER DID NOT DISCUSS RECENT TRENDS IN PHARMACOGENETICS and pharmacogenomics—reviewed, in some detail, in the June 2004 issue of the *Notebook*—he did point out that the future of this area of research is very exciting. “However,” he stated, “I think there’s an awful lot out there besides genetics that we’re neglecting. In part, maybe it’s because all the other stuff doesn’t have a sexy name like pharmacogenomics. So I’m going to propose that we consider a new name for the old science of external influences on human pharmacology: *pharmacoeecology*. The fact is, there are many things in the environment that have a dramatic effect on drug metabolism. Until very recently we did not have the tools to understand these interactions on a molecular level. But now we do. Here’s hoping that pharmacologists may be able to use XEN to find inner peace.” 

References

- Bantia S, Ananth SL, Parker CD, et al. Mechanism of inhibition of T-acute lymphoblastic leukemia cells by PXR inhibitor—BCX-1777. *Int Immunopharmacol* 3(6):879-87, 2003.
- Gallant JE, Rodriguez AE, Weinberg W, et al. Early Non-response to tenofovir DF (TDF) + abacavir (ABC) and lamivudine (3TC) in a randomized trial compared to efavirenz (EFV) + ABC and 3TC: ESS30009 [abstract 1722a]. 43rd Interscience Conference on Antimicrobial Agents and Chemotherapy, September 2003.
- Gorski JC, Huang SM, Pinto A, et al. The effect of echinacea (*Echinacea purpurea* root) on cytochrome P450 activity *in vivo*. *Clin Pharmacol Ther* 75(1):89-100, 2004.
- Hawkins T, Veikley W, St. Claire R, et al. Intracellular pharmacokinetics of tenofovir-DF and carbociclovir in patients receiving triple nucleoside regimens [abstract 2.4]. 5th International Workshop on Clinical Pharmacology of HIV Therapy, Rome, 2004.
- Hsu A, Bertz R, Ashbrenner E, et al. Interaction of ABC-378/ritonavir with protease inhibitors in healthy volunteers [Abstract 2.4]. First International Workshop on Clinical Pharmacology of HIV therapy, Noordwijk, 2000.
- Piscitelli SC, Burstein AH, Chait D, et al. Indinavir concentrations and St John's wort. *Lancet* 355(9203):547-8, 2000.
- Ray AS, Olson L, Fridland A. Role of purine nucleoside phosphorylase in interactions between 2',3'-dideoxynosine and allopurinol, ganciclovir, or tenofovir. *Antimicrob Agents Chemother* 48(4):1089-95, 2004.
- Ray AS, Vela JE, Olson L, et al. Lack of negative interaction between tenofovir and abacavir in human cells [abstract 2.3]. 5th International Workshop on Clinical Pharmacology of HIV Therapy, Rome, 2004a.
- Robbins BL, Wilcox CK, Fridland A, et al. Metabolism of tenofovir and didanosine in quiescent or stimulated human peripheral blood mononuclear cells. *Pharmacotherapy* 23(6):695-701, 2003.
- Washington CB, Flexner C, Sheiner LB, et al. Effect of simultaneous versus staggered dosing on pharmacokinetic interactions of protease inhibitors. *Clin Pharmacol Ther* 73(5):406-16, 2003.
- Wood R, Trepco C, Livrozet JM, et al. Enhancement of pharmacokinetic parameters of amprenavir when combined with low dose ritonavir (APV 600 mg/rtv 100 mg bid) and preliminary efficacy results [Abstract P283]. Fifth International Congress on Drug Therapy in HIV Infection, Glasgow, 2000.