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A Phenotype–Genotype Approach to Predicting CYP450 and P-Glycoprotein Drug Interactions With the Mixed Inhibitor/Inducer Tipranavir/Ritonavir

JB Dumond[1], **M Vourvahis**[1], **NL Rezk**[1], **KB Patterson**[2], **H-C Tien**[3], **N White**[1], **SH Jennings**[1], **SO Choi**[1], **J Li**[1], **MJ Wagner**[4], **NM La-Beck**[1], **M Drulak**[5], **JP Sabo**[5], **MA Castles**[5], **TR MacGregor**[5], and **ADM Kashuba**[1]

[1] Division of Pharmacotherapy and Experimental Therapeutics, School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

[2] School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

[3] FPG Institute, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

[4] Institute for Pharmacogenomics and Individualized Therapy, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

[5] Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, Connecticut, USA

Abstract

The effects of tipranavir/ritonavir (TPV/r) on hepatic and intestinal P-glycoprotein (P-gp) and cytochrome P450 (CYP) enzyme activity were evaluated in 23 volunteers. The subjects received oral (p.o.) caffeine, warfarin + vitamin K, omeprazole, dextromethorphan, and midazolam and digoxin (p.o. and intravenous (i.v.)) at baseline, during the first three doses of TPV/r (500 mg/200 mg b.i.d.), and at steady state. Plasma area under the curve $(AUC)_{0-\infty}$ and urinary metabolite ratios were used for quantification of protein activities. A single dose of TPV/r had no effect on the activity of CYP1A2 and CYP2C9; it weakly inhibited CYP2C19 and P-gp; and it potently inhibited CYP2D6 and CYP3A. Multiple dosing produced weak induction of CYP1A2, moderate induction of CYP2C19, potent induction of intestinal P-gp, and potent inhibition of CYP2D6 and CYP3A, with no significant effects on CYP2C9 and hepatic P-gp. Several P450/transporter single-nucleotide polymorphisms correlated with the baseline phenotype but not with the extent of inhibition or induction. Although mixed induction and inhibition are present, this approach offers an understanding of drug interaction mechanisms and ultimately assists in optimizing the clinical use of TPV/r.

Cocktail phenotyping involves simultaneous, single-dose administration of marketed drugs (also known as probes) to measure the activity (or phenotype) of multiple hepatic and intestinal drug-metabolizing enzymes and transporters for rapid and efficient assessment of the drug interaction potential of a new compound.[1–5] The "Cooperstown 5 + 1 cocktail" consists of caffeine, warfarin, omeprazole, dextromethorphan, intravenous (i.v.) midazolam, and vitamin K (to negate warfarin's anticoagulant effect).[6] We have modified this approach to elucidate the specific influences of drugs on hepatic and intestinal proteins by evaluating enzyme activity

Correspondence: ADM Kashuba (akashuba@unc.edu).

SUPPLEMENTARY MATERIAL is linked to the online version of the paper at http://www.nature.com/cpt

CONFLICT OF INTEREST

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under baseline conditions in addition to conditions of both acute and chronic drug exposure. Oral (p.o.) midazolam and i.v. and p.o. digoxin were also added as probe substrates in order to measure hepatic + intestinal CYP3A activity, hepatic P-glycoprotein (P-gp) activity, and hepatic + intestinal P-gp activity, respectively.[7,8] The genotyping of cytochrome P450 (CYP) genes corresponding to enzymes investigated with phenotype probes (CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5/7/43) and P-gp (ABCB1) also allows for investigating genotype–phenotype correlations and determining genetic influences on drug interactions.

We used this modified cocktail approach to investigate the drug interaction potential of tipranavir (TPV) administered along with low-dose ritonavir (RTV). TPV is a nonpeptidic protease inhibitor (PI) with potent activity against HIV-1-resistant virus. Because TPV is a potent inducer of CYP3A,[9] coadministration of RTV is required in order to inhibit CYP3A and ensure therapeutic concentrations of TPV. However, both TPV and RTV have coinciding induction and inhibition effects on metabolic enzymes and transporters, which preclude making accurate drug–drug interaction predictions from *in vitro* data.

In vitro data suggest that tipranavir/ritonavir (TPV/r) likely inhibits CYP3A4, CYP1A2, 2C9, 2C19, and 2D6.[9] However, when TPV/r was combined with other CYP3A4 substrates in HIV-1-infected patients (saquinavir, amprenavir, or lopinavir (LPV)), coadministered PI exposures did not increase; rather, they unexpectedly decreased by 40–80%.[10] The aim of this study was to gain an understanding of these unexpected interactions by simultaneously determining the effects of TPV/r on various CYP enzymes and P-gp through a phenotype–genotype approach evaluating both acute and chronic TPV/r exposure.

RESULTS

Baseline characteristics

A total of 33 subjects were enrolled in the study, and data from 23 subjects were available for analyses, including those from 16 subjects who were receiving the TPV self-emulsifying drug delivery system (SEDDS) formulation and 7 who were receiving the p.o. solution. Although equal numbers in each group were planned, the study was modified in order to prioritize completion of the SEDDS arm because this was the US Food and Drug Administration–approved dosage form. Two subjects were withdrawn from the study prior to the administration of TPV/r: one of the subjects developed caffeine-withdrawal migraine headaches; in the other subject, there was inadequate venous access. An additional five subjects (four of whom were receiving TPV p.o. solution) prematurely discontinued the study because of nausea and vomiting. One more subject, who was receiving the TPV SEDDS formulation, discontinued prematurely because there was inadequate venous access.

Two subjects prematurely discontinued participation in the study (investigator's choice) after Boehringer Ingelheim issued a letter to health-care professionals describing 14 events of intracranial hemorrhage in 13 HIV-1-infected subjects receiving TPV/r. None of the study subjects experienced clinically significant changes in prothrombin time, activated partial thromboplastin time, or international normalized ratio after receiving warfarin (+ vitamin K). However, this warning caused an unfavorable shift in the risk:benefit ratio for volunteer subjects receiving TPV/r with warfarin, despite the coadministration of vitamin K. After discussion with the institutional review board, enrollment was closed.

Of the 23 subjects evaluated (16 men and 7 women), 16 were Caucasians and 7 were African Americans. The mean (±SD) values of the demographic parameters of the subjects were as follows: age, 27.0 years (±7.1); height, 174.3 cm (±8.3); weight, 76.0 kg (±14.4); and body mass index, 24.8 (±3.2).

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In all the subjects, there was >90% adherence to the study medications, and TPV/r concentrations were consistent with those in previous reports.[9] TPV concentrations (mean (±SD)) in plasma at the end of the dosing interval ($Cp_{12\ h}$) after the first dose and at steady state were 65 μmol/l (±22) and 41 μmol/l (±26), respectively, for the SEDDS formulation and 44 μmol/l (±18) and 36 μmol/l (±23), respectively, for the p.o. solution. For RTV, the mean (±SD) $Cp_{12\ h}$, was 1.5 μg/ml (±1.4) after the first dose and 0.6 μg/ml (±1.1) at steady state for subjects receiving the SEDDS formulation and 1.1 (±0.6) μg/ml at first dose and 0.4 μg/ml (±0.3) at steady state for subjects receiving the p.o. solution. No significant differences in TPV or RTV pharmacokinetics were observed between TPV dosage forms. All study medications were generally well tolerated and found to be consistent with TPV and RTV safety profiles.[9,11]

Pharmacokinetic/pharmacogenomic analysis

A summary of the phenotyping measurements for P-gp and for each CYP studied and the geometric mean ratios (GMRs) (phase II/phase I and phase III/phase I) are shown in Table 1. Figure 1 illustrates the mean (±SE) pharmacokinetic profiles for each probe drug for phases I–III. It was noted that the influence of TPV/r on enzyme/transporter activity was not different between the SEDDS or liquid formulation (data not shown), and therefore the results for these two types of formulations were combined. The data that follow are reported as median percentage changes (phase II/phase I and phase III/phase I) for each biomarker.

CYP1A2, NAT-2, and XO activity—A slight inhibition of CYP1A2 occurred after the initial TPV/r dosing: although plasma caffeine area under the curve (AUC) and peak plasma concentration (C_{max}) increased by 9.5 and 4.6%, respectively, the 90% confidence interval (CI) for the GMR demonstrated equivalence. Urinary metabolite ratios decreased by 16%, and the GMR 90% CI did not show bioequivalence. At steady-state dosing of TPV/r, plasma AUC and C_{max} decreased by 47 and 16%, respectively, with the nonbioequivalent GMR 90% CI demonstrating weak CYP1A2 induction (Figure 1a). The urinary ratio increased by 5%, although the urinary GMR 90% CI was bioequivalent. Under first-dose and steady-state conditions, changes in NAT-2 and XO caffeine urinary ratios were not clinically significant (Table 1). CYP1A2, NAT-2, and XO genetic polymorphisms showed no effects on caffeine pharmacokinetics.

CYP2C9 activity—First-dose TPV/r increased S-warfarin AUC and C_{max} by 24 and 8%, respectively. At steady-state dosing of TPV/r, S-warfarin AUC and C_{max} declined by 15 and 3%, respectively, suggesting very minimal CYP2C9 inhibition and induction after the first dose and at steady state with TPV/r (Figure 1b).

Of the 23 subjects evaluated, 4 were heterozygotes for the A→ C variant at the rs1057910 single-nucleotide polymorphism, which corresponds to the CYP2C9*3 polymorphism, a nearly null allele. Of these four subjects, three had two- to fourfold higher plasma S-warfarin concentrations at 72 h during phase I (baseline) than those in other subjects. One subject had an S-warfarin half-life of 124 h as compared to the geometric mean S-warfarin half-life of 30 h for the whole study group. The *P* value for the equality-of-populations test for this allele, based on warfarin AUC at baseline, was statistically significant after correcting for the number of CYP2C9 alleles tested ($P = 0.047$). One of the four subjects was also heterozygous for CYP2C9*11 and had the highest baseline warfarin AUC of all the subjects, as well as higher TPV exposures, both after the first dose and at steady state (1.5 times the geometric mean AUC). The other three subjects in this group of heterozygotes showed no differences relative to the total study group with respect to pharmacokinetic parameters of TPV.

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CYP2C19 activity—First-dose TPV/r increased omeprazole concentration in the majority of the subjects (21/23). AUC and C_{max} increased by 57 and 18%, respectively, indicating CYP2C19 inhibition. At steady state, AUC and C_{max} decreased by 70 and 68%, respectively, suggesting CYP2C19 induction (Figure 1c). The GMR 90% CI lacked bioequivalence with baseline for both single and multiple doses. The 2-h 5-hydroxyomeprazole/omeprazole ratios were consistent with these results (data not shown).

Of the 23 subjects evaluated, 7 were heterozygous for the G→ A variant at the rs4244285 single-nucleotide polymorphism, which corresponds to the CYP 2C19*2 allele. These subjects with genotypes of CYP2C19*1/*2 are considered heterozygous extensive metabolizers, but they had higher (geometric mean C_{max} = 1.95 μmol/l) systemic omeprazole concentrations at baseline than the other subjects did (geometric mean C_{max} = 0.92 μmol/l). In the equality-of-population testing, a significant result was obtained for the ratio of omeprazole to its 5-hydroxy metabolite at baseline (P = 0.0026). This may indicate reduced CYP2C19 activity, given that the 5-hydroxy metabolite is formed through the CYP2C19 pathway. The mean 2-h omeprazole/5-hydroxyomeprazole ratio in subjects identified as CYP2C19*2 heterozygotes was 2.6-fold higher at baseline, 2.9-fold higher after the first dose, and 4.4-fold higher at steady state, when compared with the value of this ratio in the other subjects. Although the metabolite ratios in the CYP2C19*2 heterozygotes decreased with the enzyme induction seen in phase III, the decrease was less dramatic than that seen in the other subjects. For heterozygotes, the mean phase I ratio was 1.78, which decreased to 0.99 at phase III, as compared to ratios of 0.67 and 0.22 at the same phases for the other subjects. Although the differences in the ratios were not statistically significant, these results are consistent with the effects on CYP2C19 activity after acute and chronic TPV/r dosing.

CYP2D6 activity—First-dose TPV/r substantially increased dextromethorphan AUC and C_{max} by 188 and 164%, respectively. A similar increase in the dextromethorphan urinary ratio (178%) was noted, demonstrating CYP2D6 inhibition. One subject was phenotyped as a CYP2D6 poor metabolizer (PM; urinary ratio >0.3) at baseline and remained a PM throughout the TPV/r dosing. Of the 22 individuals phenotyped as CYP2D6 extensive metabolizers or intermediate metabolizers at baseline, 4 became PMs (PM phenocopies) by phenotype at first dose. At steady state after TPV/r dosing, further CYP2D6 inhibition was noted, with dextromethorphan AUC increasing by 490%, C_{max} increasing by 363%, and the urinary ratio increasing by 193% (Figure 1d). Under these conditions, two additional subjects became PM phenocopies. For both single and multiple doses, the GMR 90% CI lacked bioequivalence with baseline. Dextrorphan/dextromethorphan AUC ratios in plasma demonstrated similar results (data not shown).

One subject demonstrated a PM phenotype at all phases and was classified genetically as a PM (CYP2D6*4/*4). Six subjects, each of whom was heterozygous for one wild-type allele and one low-activity or null allele (three subjects were heterozygous for the *4 allele and one each for the *10, *17, and *41 alleles), demonstrated the intermediate metabolizer phenotype (urinary ratios of 0.01–0.3) at baseline, along with nine wild-type subjects. Of the six subjects who demonstrated PM phenocopies after TPV dosing, four displayed wild-type genotypes. Equality-of-population rank tests comparing heterozygous subjects against wild-type subjects showed differences that were marginally significant for dextromethorphan AUC at baseline (P = 0.06) and significant for change from baseline in dextromethorphan urinary ratio after steady-state dosing (P = 0.02), with heterozygotes demonstrating lower percentage changes than wild-type subjects.

Hepatic and intestinal CYP3A enzyme activity—Intravenous midazolam administration was used to measure hepatic CYP3A activity. First-dose TPV/r increased i.v midazolam AUC and C_{max} by 400 and 14%, respectively. These increases were less

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pronounced at the TPV/r steady state, suggesting auto-induction of CYP3A activity; as compared to baseline, AUC increased by 203%, and C_{max} decreased by 11% (Figure 1e). For both single and multiple doses, the GMR 90% CI lacked bioequivalence with baseline.

Oral midazolam measured combined CYP3A activity in the intestine and liver. First-dose TPV/r increased p.o. midazolam AUC and C_{max} by 3,549 and 333%, respectively. These increases were greater than those seen with i.v midazolam, confirming that TPV/r has a greater effect on intestinal enzyme activity than on hepatic enzyme activity. When TPV/r was dosed to steady state, these increases were less pronounced (AUC and C_{max} were increased by 944 and 200%, respectively), suggesting additional intestinal CYP3A induction (Figure 1f). No effects of CYP3A genetic polymorphisms on midazolam pharmacokinetics were noted.

Hepatic and intestinal P-gp activity—Intravenous administration of digoxin measured the hepatic P-gp activity, and p.o. digoxin measured the P-gp activity in both the intestine and the liver. With i.v digoxin, first-dose TPV/r resulted in an increase in AUC of 9% and a decrease in C_{max} of 10% (n = 10 subjects), suggesting very minor P-gp inhibition after a single dose. Under steady-state conditions, digoxin AUC and C_{max} decreased 9 and 16%, respectively, suggesting very minor induction of hepatic P-gp activity over time (Figure 1g), and the GMR 90% CI demonstrated bioequivalence with baseline.

When p.o. digoxin was administered, first-dose TPV/r increased the AUC and the C_{max} by 87 and 87%, respectively (n = 13 subjects), suggesting pronounced inhibition of intestinal P-gp activity. Under steady-state conditions, digoxin AUC and C_{max} declined by 2 and 32%, respectively, relative to baseline values. These data suggest induction of P-gp activity in the intestine after prolonged TPV/r exposure (Figure 1h). This level of induction overcame the inhibition observed after the first dose, as demonstrated by the GMR 90% CI, but did not induce P-gp activity beyond baseline conditions. No effects of ABCB1 genetic polymorphisms were noted with respect to the pharmacokinetics of digoxin.

DISCUSSION

This study demonstrates that utilizing a phenotype approach is clinically useful for predicting complex drug–drug interactions, and it also further validates the use of biomarker probes. Although interaction studies with commonly coadministered drugs are useful, extrapolating the results to other drugs with mixed inhibition/induction potential is a challenging task. The additional information obtained by genotyping relevant drug-metabolism enzymes and transporters generated useful genotype–phenotype correlations for biomarker probes at baseline, but these were not apparent after the compound of interest had affected the metabolism of the probe.

In this study, the strength of enzyme/transporter inhibition was defined as weak if the AUC increased 1.25- to less than 2-fold, moderate if the AUC increased 2- to less than 5-fold, and potent if the AUC increased =5-fold.[11] Induction was classified as weak if the AUC decreased by 25–<50%, moderate if the AUC decreased by 50–<75%, and potent if the AUC decreased by ≥75%. Overall, after the first dose, TPV/r had no effect on CYP1A2 and CYP2C9, weakly inhibited CYP2C19 and P-gp, and potently inhibited CYP2D6 and hepatic and intestinal CYP3A. At steady state, TPV/r weakly induced CYP1A2, moderately induced CYP2C19, potently induced intestinal P-gp, and potently inhibited CYP2D6 and hepatic and intestinal CYP3A, whereas no effect was observed with CYP2C9 and hepatic P-gp. Although healthy subjects were studied here, these results are applicable to HIV-1-infected patients, given that the steady-state exposures of TPV and RTV were similar in volunteers and patients.[9] Ethical concerns preclude treating HIV-infected subjects with monotherapy.

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Both plasma and urinary measures of caffeine concentration indicated induction of CYP1A2 at steady-state dosing with TPV/r, although the magnitude in the change from baseline differed (47% decline for plasma AUC vs. 5% increase in urinary ratio). The complex metabolism of caffeine[12] and the presence of other factors that influence the renal excretion of caffeine's metabolites[13] may render the urinary ratio less accurate as a reffection of CYP1A2 activity than plasma exposure of caffeine. Alternatively (not done in this study), the plasma ratio of paraxanthine:caffeine may have more closely mimicked the caffeine urinary ratio.[14] Nonetheless, these data suggest that concentrations of CYP1A2 substrates may be significantly decreased when coadministered with TPV/r, potentially necessitating dose adjustment.

After multiple dosing with TPV/r, no clinically significant effect on CYP2C9 activity was noted, as evidenced by a 15% decrease in S-warfarin AUC and a bioequivalent GMR 90% CI as compared to baseline. This change is less than the 29% decrease in S-warfarin exposure seen with lopinavir/ritonavir (LPV/r).[5] Subtherapeutic international normalized ratios have been reported in patients receiving warfarin in combination with LPV/r[15] and also in those receiving RTV 400 mg twice daily.[16,17] However, no changes in international normalized ratios have been seen with the administration of TPV/r.[9] These results support and explain the clinical findings.

When omeprazole was used as a CYP2C19 probe, activity decreased by 57% after the first dose and increased by 70% at steady state. Little information is available on the influence of PI therapy on CYP2C19 activity, although previous investigations with LPV/r have found a 53% decrease in omeprazole exposure[5] and a 31% decrease in phenytoin exposure.[3] Conversely, fosamprenavir (without RTV) increases the exposure of esomeprazole by 55%.[18]

Consistent with *in vitro* data,[9] TPV/r was found to be a potent CYP2D6 inhibitor *in vivo*. With TPV/r dosing, 27% (6/22) of subjects phenotyped as extensive metabolizers at baseline became PMs. The genotype showed correlation with baseline plasma parameters and urinary phenotype and also predicted the degree of CYP2D6 inhibition by TPV, using the urinary ratio. Therefore, CYP2D6 substrates should be used with caution when they are to be administered in combination with TPV/r.

The differential results between i.v and p.o. administration of midazolam (203% inhibition vs. 944% inhibition) after 10 days of TPV/r therapy demonstrate a larger inhibitory effect on intestinal CYP3A enzyme activity than on hepatic activity. This observation is consistent with the large amount of CYP3A located in the intestine[19] and the high local concentration of RTV present after TPV/r dosing. This phenomenon has also been seen after administration of LPV/r.[5] As this study evaluated first-dose effects, the attenuation of CYP3A inhibition from first dose to steady state for both i.v and p.o. administration of midazolam suggests that there is induction of the enzyme at both sites over time.

As seen in the results after the administration of midazolam, TPV/r also inhibited hepatic and intestinal P-gp activity after the first dose. Under steady-state conditions, TPV/r did not appear to alter P-gp activity relative to baseline conditions. This is an important finding because if the evaluation of this interaction had been carried out only under steady-state conditions, one would have concluded that TPV/r has no effect on P-gp activity. The attenuation at steady state after the observed first -dose inhibition demonstrates that, *in vivo*, TPV/r is an inducer of P-gp activity.

We believe these data help to explain the mechanism through which TPV/r decreased the exposure (AUC and C_{min}) of the CYP3A and P-gp substrates[11,18,20] saquinavir, amprenavir, LPV, and atazanavir by 44–76%—a finding that was unexpected at the time.[10,21] Although the decrease in CYP3A activity after the administration of TPV/r is similar to the effect seen with other PIs, the increase in P-gp activity associated with TPV/r is not seen in the case of

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other inhibitors. Other investigations have shown that coadministration of RTV, LPV/r, or saquinavir/ritonavir with digoxin increases the AUC of digoxin by 86, 81, and 49%, respectively, suggesting P-gp inhibition.[20,22,23] Given that RTV-boosted PIs rely on both CYP3A and P-gp inhibition for maximal exposure, PI exposure decreases in the absence of P-gp inhibition. The use of other PIs in combination with RTV will be an important area of investigation of drug interactions.

The small sample size of this study and the low allele frequencies for some of the polymorphisms that were tested limited the power to detect genetic effects on the metabolism of the probe drugs or on changes in metabolism after TPV/r administration. However, significant genotype–phenotype relationships were observed for several of the alleles present in the study subjects. Both CYP2C9*3 and CYP2C19*2 had significant effects on warfarin AUC and hydroxyomeprazole/omeprazole ratios. Notably, these effects between genotype and phenotype were seen only at baseline, suggesting that genotypic information may not predict drug metabolism in the face of interactions. In this scenario, phenotyping is likely to provide the most clinically useful information. Vormfelde and colleagues recently demonstrated the presence of differential metabolism of tolbutamide in subjects with CYP2C9 polymorphisms, but no discernible differences between subjects were found when rifampin, a potent CYP450 inducer, was administered concomitantly.[24] Some race-related effects were noted for biomarkers, both at baseline and after TPV dosing, but, given the small sample size, it is difficult to determine the clinical relevance of these effects (results presented in Supplementary Materials and Methods online). Larger studies involving more diverse populations should be done to further explore the role of racial differences in biomarker phenotyping and pharmacogenomic interactions. Alternatively, the availability of a diverse pool of genotyped volunteers would permit investigators to “mix and match” populations for the exploration of specific phenotype interactions predicted from *in vitro* experiments. Combined with *in silico* hypotheses and *in vitro* experiments, this genotype–phenotype method of screening drug interactions affords a clinically realistic, yet time-efficient, method for developing accurate prescribing information.

In summary, this study offers a novel, comprehensive approach to understanding and predicting the interactions between drugs already in use and compounds in development. We believe that the incorporation of this phenotyping approach in early-phase drug development can provide important data for managing complex drug interactions in phase II/III studies, in addition to providing useful information for clinicians in the prescribing materials. The results of this study offer a better understanding of the mechanisms of drug interactions with TPV/r and will ultimately assist in optimizing the administration of TPV/r in clinical practice. Given the results of this study, clinicians should closely monitor CYP1A2 and CYP2C19 substrates for reduced efficacy in patients receiving TPV/r and cautiously initiate CYP2D6 and CYP3A4 substrates at the lowest possible doses.

METHODS

Subjects

Thirty-three healthy volunteers were investigated to determine whether they met the required criteria for enrollment. The inclusion criteria were age 18–45 years, weight ≥50 kg, willingness to complete all study-related activities, and seronegative for HIV-1, hepatitis B, and hepatitis C. In addition, women volunteers who were sexually active were required to test negative for pregnancy (serum or urine pregnancy test) and use a barrier form of contraception for the duration of the study. The exclusion criteria were significant medical conditions or abnormalities in laboratory test results. The institutional review board of the University of North Carolina at Chapel Hill approved the study protocol, and all subjects provided written informed consent before any study procedures were performed.

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Study design

This investigation was a single-center, open-label, pharmacokinetic study consisting of three in-patient visits—a 72-h baseline visit (phase I), a 48-h TPV/r first-dose visit (phase II), and a 72-h TPV/r steady-state visit (phase III)—and six outpatient visits at the Verne S. Caviness General Clinical Research Center in Chapel Hill, NC. The study duration was 22 days, with a safety follow-up visit at day 35.

Caffeine, warfarin, omeprazole, dextromethorphan, midazolam, and digoxin were administered in order to assess the activity of CYP1A2, 2C9, 2C19, 2D6, 3A, and P-gp, respectively (see Supplementary Materials and Methods online for materials used). At the time of the study, caffeine, warfarin, omeprazole, dextromethorphan, and midazolam were validated for simultaneous use[2,5,6] and were considered acceptable probes for assessing CYP enzyme activity in accordance with Food and Drug Administration guidelines.[25] Digoxin was considered appropriate for P-gp phenotyping.[25] Because the interaction potential of digoxin and the components of the phenotyping cocktail had not been established, this study separated digoxin dosing from phenotyping cocktail dosing. At phase II, subjects were randomized to receive either p.o. or i.v. digoxin in order to capture first-dose effects.

Figure 2 depicts the results from the three study visits and the phenotyping activities at each visit. From phase II to phase III, subjects self-administered TPV/r 500 mg/200 mg with food at ~8:00 AM and 8:00 PM. The subjects were randomized to receive either the SEDDS formulation or the p.o. solution of TPV; all subjects received the capsule formulation of RTV (see Supplementary Materials and Methods online for additional details).

Safety was monitored at each visit via laboratory blood testing, adverse-event reporting, changes observed during physical examination, alterations in vital signs, or 12-lead electrocardiogram. Adverse events were assessed using the Boehringer Ingelheim modified Division of AIDS severity grading scale,[26] which included minor revisions to the grading criteria pertaining to hemoglobin, platelets, potassium, creatinine, and alanine transaminase.

Analytical methods

Blood samples were collected in K_2 EDTA-containing Vacutainers (BD Diagnostics, Franklin Lakes, NJ). Each sample was processed within 30 min of collection and kept on wet ice until centrifugation (15 min at 2,800 rpm at 4 °C). Plasma was aliquoted into labeled cryovials and stored at −80 °C until analysis (within 7 months of collection). During urine collection, containers with 5 g of ascorbic acid (to maintain urine pH <4) were kept refrigerated at 4 °C. After collection, the urine was mixed thoroughly, the total volume was measured, and 15-ml aliquots were stored at −80 °C until analysis. Validated bioanalytical assays[27] were used to determine plasma and/or urine analyte concentrations (see Supplementary Materials and Methods online for additional details).

DNA was extracted from whole blood using QIAamp DNA Blood Mini Kits (Qiagen, Valencia, CA) and genotyped for multiple CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5/7 polymorphisms and P-gp single-nucleotide polymorphisms, using the DMET panel chip at Affymetrix (Santa Clara, CA).[28]

Data and statistical analysis

Sampling strategies to determine each measure of activity are outlined in Table 2. Pharmacokinetic parameters were calculated using noncompartmental methods in WinNonlin Pro, version 4.0.1 (Pharsight, Mountain View, CA). The AUC was calculated using the linear/log trapezoidal rule.

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The primary end points were the $\log_e$-transformed intraindividual differences and their associated arithmetic means. For these arithmetic means, 90% CIs were calculated assuming a normal distribution of the log-transformed data. Exponentiation of these arithmetic means and CIs resulted in estimates for GMRs with 90% CIs, to compare intrasubject changes in pharmacokinetic parameters (relative to baseline values) after the first dose and at steady-state dosing of TPV/r (phase II/phase I and phase III/phase I), applying the typical regulatory bounds for bioequivalence (0.8–1.25). The median percentage changes from baseline to study period were also calculated for these parameters.

Genotype–phenotype analysis was performed using the Kruskal–Wallis equality-of-populations rank test after the data had been tested for normality and found not to follow a normal distribution for biomarker AUCs and metabolite ratios (Stata, version 10.0; StataCorp, College Station, TX). Differences in results between racial groups were also tested in this manner. A P value of 0.05 was considered significant and was adjusted for multiple comparisons resulting from testing multiple polymorphisms in a gene for an effect on phenotypes related to that gene. Analysis was confined to genes relevant to the measured phenotypes, and monomorphic polymorphisms were excluded.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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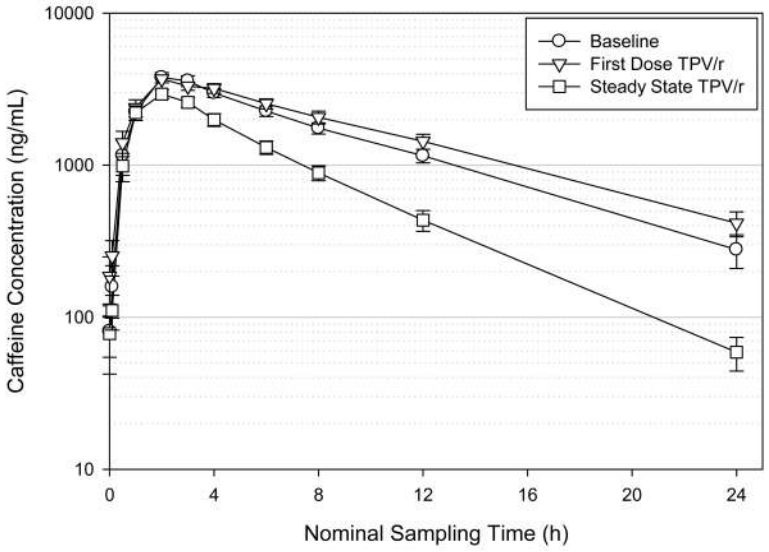
Figure 1a.



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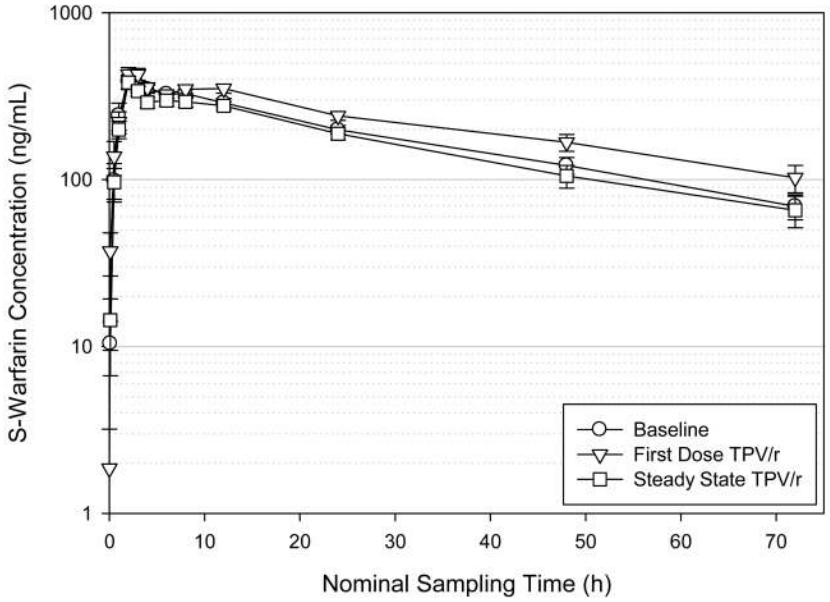

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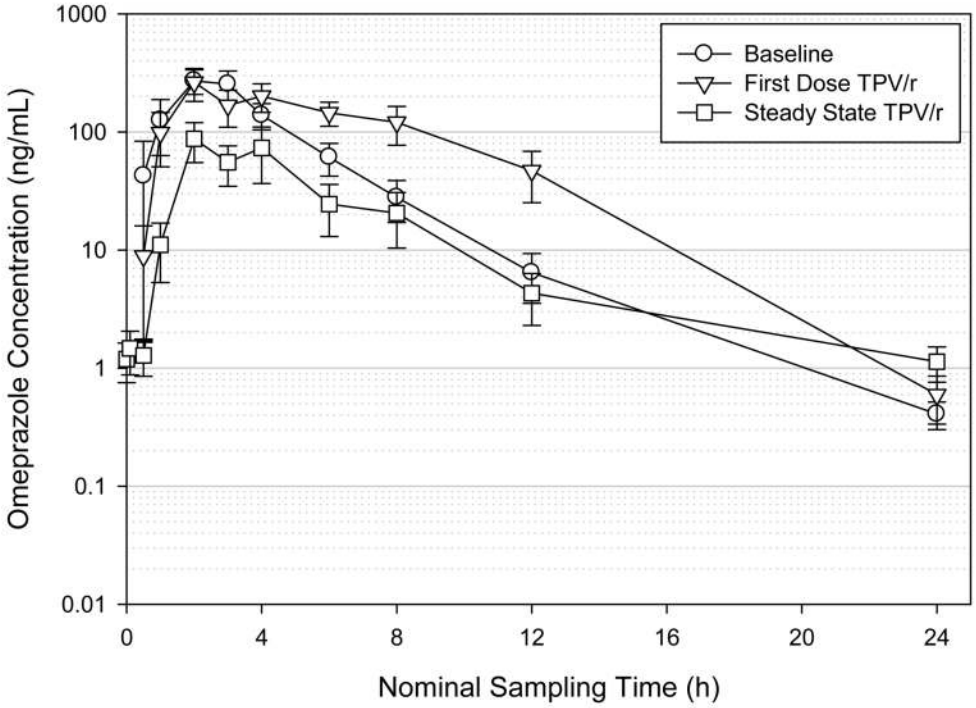

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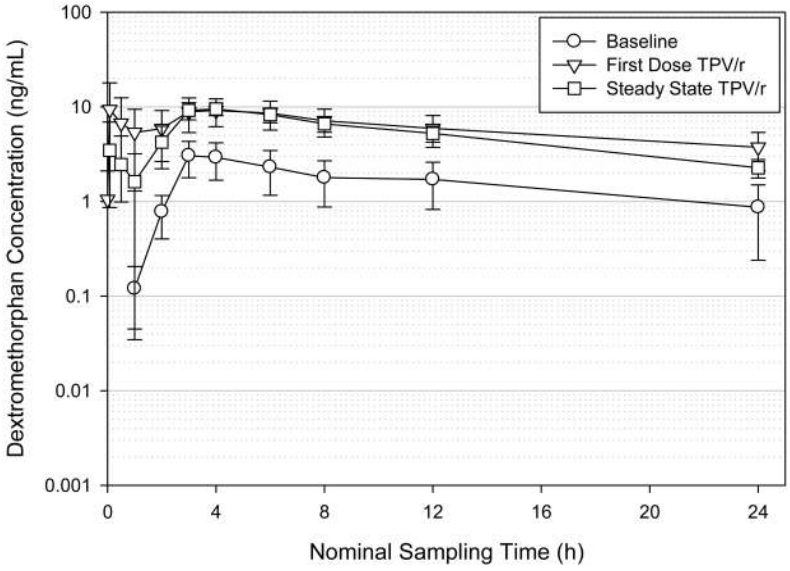

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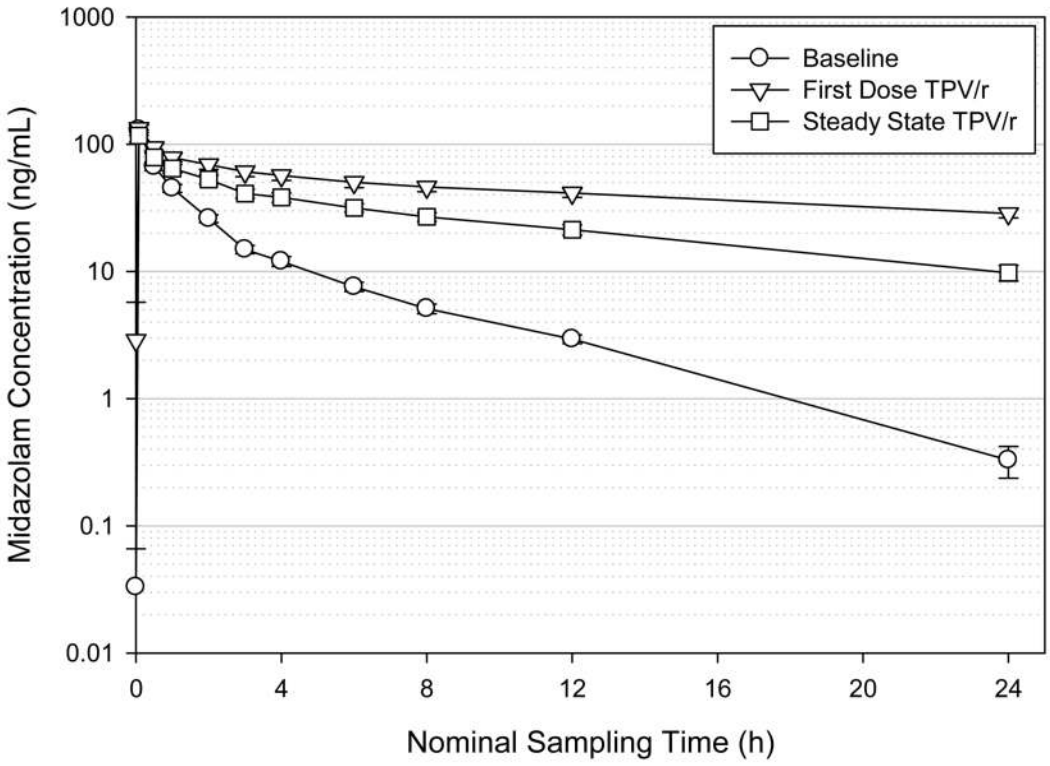

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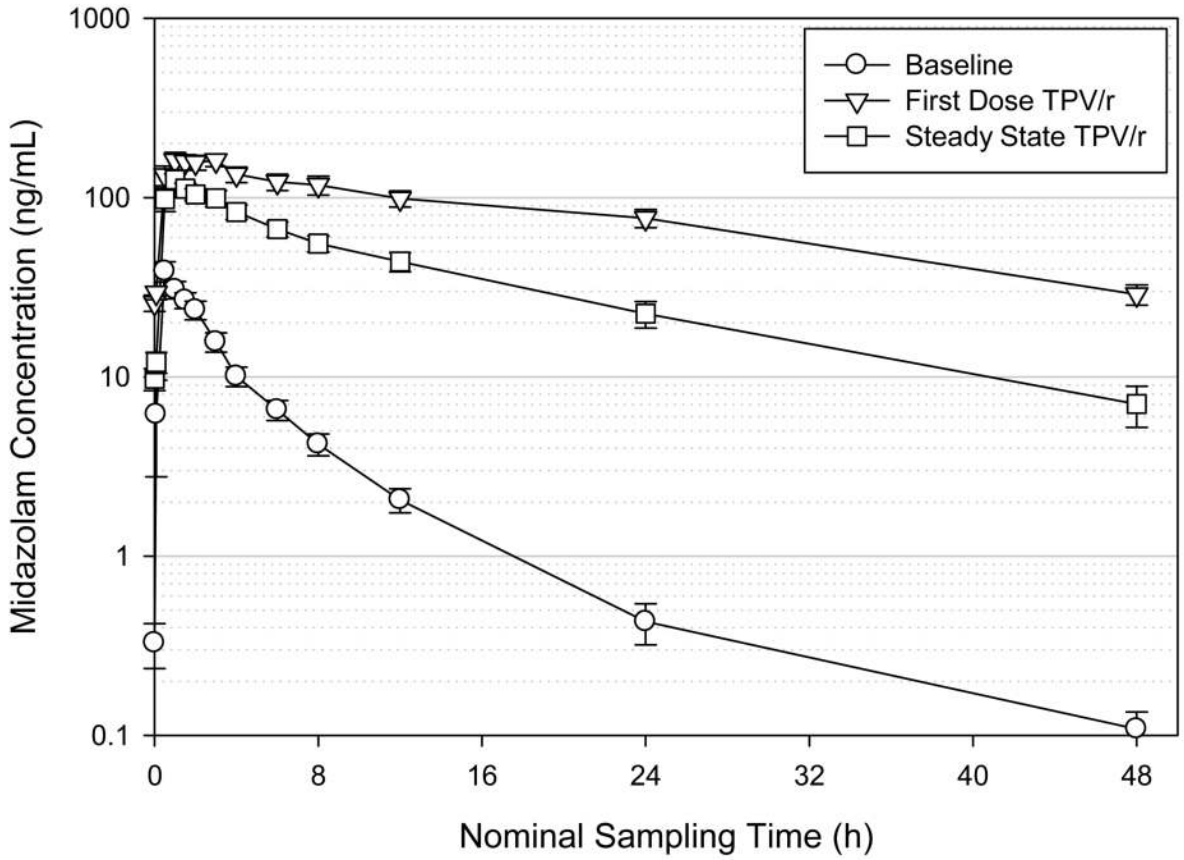

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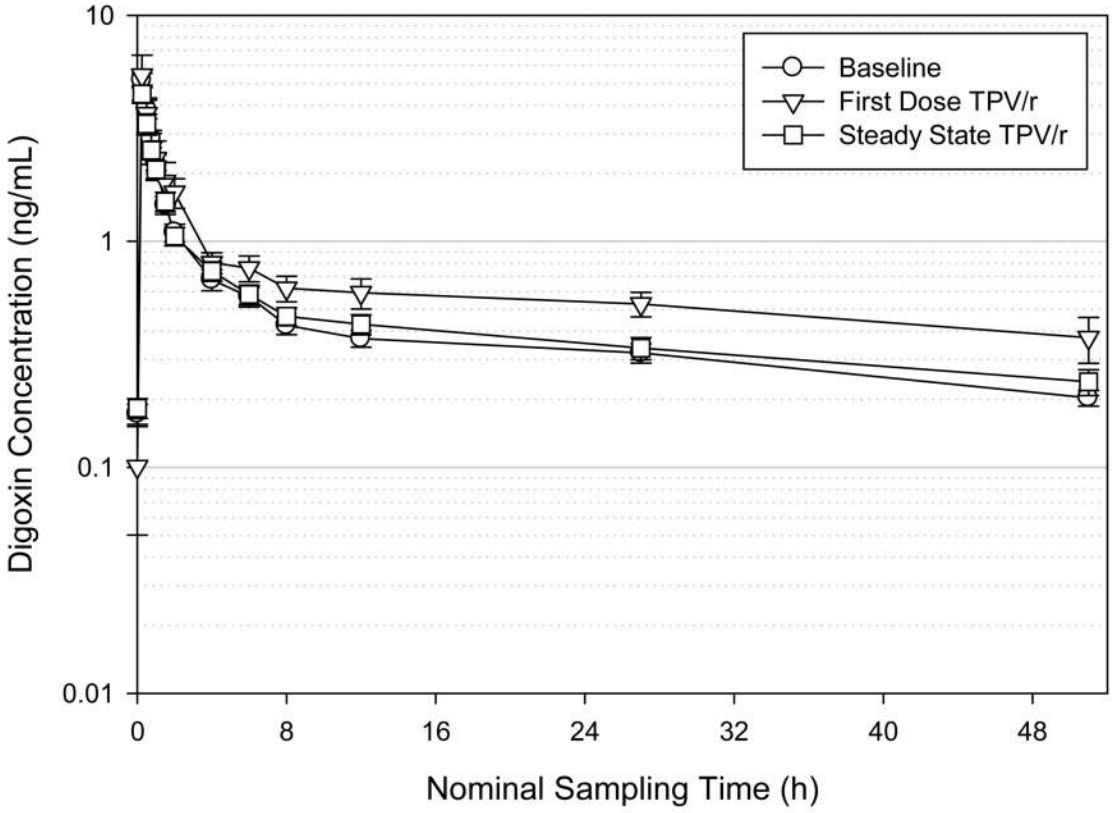

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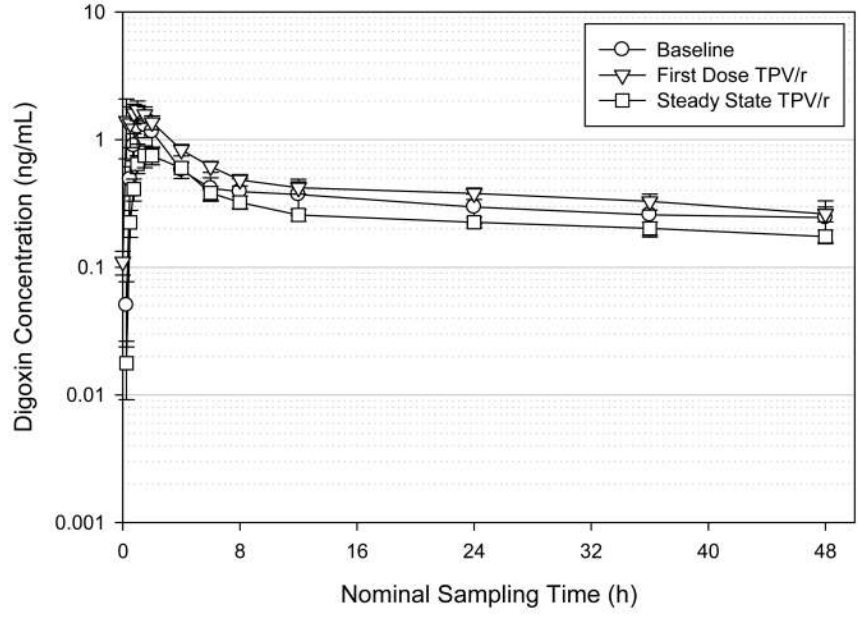

Figure 1.

Concentration–time curve at baseline, after first dose, and at steady state of (**a**) caffeine (CYP1A2), (**b**) S-warfarin (CYP2C9), (**c**) omeprazole (CYP2C19), (**d**) dextromethorphan

(CYP2D6), (**e**) i.v. midazolam (hepatic CYP3A), (**f**) p.o. midazolam (hepatic + intestinal CYP3A), (**g**) i.v. digoxin (hepatic P-gp), and (**h**) p.o. digoxin (hepatic + intestinal P-gp). i.v., intravenous; P-gp, P-glycoprotein; p.o., oral; TPV/r, tipranavir/ritonavir.

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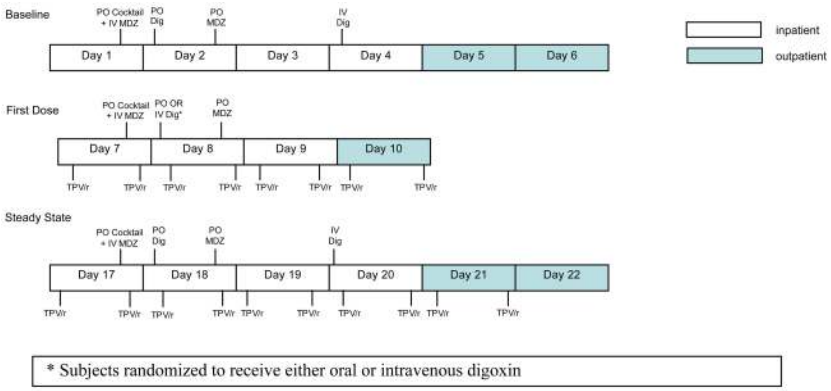

Figure 2.

Study schematic. Dig, digoxin; i.v., intravenous; MDZ, midazolam; p.o., oral; TPV/r, tipranavir/ritonavir. *Subjects randomized to receive either oral or intravenous digoxin.

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Table 1

P-gp and CYP phenotyping results at baseline (PK1), after first dose (PK2), and at steady state (PK3)

		Results				
Enzyme/transporter	**Phenotyping measure**	**Baseline**	**First-dose TPV/r**	**GMR (PK2/PK1)**	**Steady-state TPV/r**	**GMR (PK3/PK1)**
Blood plasma						
CYP1A2	Caffeine AUC (h·μg/ml)	31.40 (27.51, 35.30)	32.31 (27.92, 37.39)	1.10 (1.01, 1.19)	16.77 (14.40, 19.52)	0.57 (0.50, 0.65)
CYP2C9	S-warfarin AUC (h·μg/ml)	12.02 (10.86, 13.31)	14.75 (13.22, 16.46)	1.22 (1.16, 1.28)	10.64 (9.36, 12.13)	0.89 (0.82, 0.96)
CYP2C19	Omeprazole AUC (h·ng/ml)	652.34 (462.85, 919.41)	1,148.80 (879.63, 1,500.36)	1.76 (1.47, 2.10)	194.79 (136.41, 278.16)	0.30 (0.22, 0.41)
CYP2D6	Dextromethorphan AUC (h·ng/ml)	17.33 (8.98, 33.46)	48.52 (28.67, 82.12)	3.95 (3.04, 5.13)	80.29 (57.49, 112.15)	5.46 (3.58, 8.34)
CYP3A4/5 (hepatic)	Midazolam AUC (h·ng/ml)	199.47 (177.43, 224.24)	1,022.97 (896.91, 1,166.75)	5.13 (4.76, 5.53)	581.96 (500.74, 676.35)	2.92 (2.64, 3.22)
CYP3A4/5 (intestinal and hepatic)	Midazolam AUC (h·ng/ml)	128.04 (107.65, 152.28)	3,445.45 (2,941.78, 4,035.35)	26.91 (22.46, 32.25)	1,314.22 (1,054.50, 1,637.91)	10.26 (8.23, 12.80)
P-gp (hepatic)	Digoxin AUC (ng/ml)	21.80 (19.01, 25.00)	31.83 (24.48, 41.38)	1.20 (1.01, 1.41)	21.04 (18.14, 24.42)	0.97 (0.85, 1.10)
P-gp (intestinal and hepatic)	Digoxin AUC (h·ng/ml)	12.00 (10.70, 13.47)	19.82 (16.73, 23.49)	1.75 (1.55, 1.98)	11.44 (9.60, 13.62)	0.95 (0.82, 1.11)
Urine						
CYP1A2	(1X+1U+AFMU)/17U	6.52 (5.84, 7.27)	5.50 (4.85, 6.23)	0.84 (0.78, 0.91)	6.70 (5.87, 7.6)	1.05 (0.94, 1.17)
CYP2D6	DM/DX	0.02 (0.01, 0.05)	0.05 (0.02, 0.13)	3.12 (1.61, 6.06)	0.11 (0.05, 0.24)	6.26 (2.72, 14.42)
NAT-2	AFMU/(1X+1U+AFMU)	0.08 (0.06, 0.12)	0.09 (0.06, 0.12)	0.96 (0.82, 1.12)	0.08 (0.06, 0.12)	0.84 (0.67, 1.05)
XO	1U/(1X+1U)	0.56 (0.54, 0.58)	0.55 (0.53, 0.57)	0.99 (0.94, 1.04)	0.56 (0.54, 0.57)	0.99 (0.95, 1.03)

The results are presented as geometric means (90% confidence interval).

1U, 1-methylurate; 17U, 1,7-dimethylurate; 1X, 1-methylxanthine; AFMU, 5-acetylamino-6-formylamino-3-methyluracil; AUC, area under the curve; CYP, cytochrome P450; DM, dextromethorphan; DX, dextrorphan; GMR, geometric mean ratio; NAT-2, N-acetyltransferase-2; P-gp, P-glycoprotein; TPV/r, tipranavir/ritonavir; XO, xanthine oxidase.

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Table 2

Phenotyping probe and sampling scheme

Enzyme/transporter		Pharmacologic probe	Phenotypic index	Sampling
CYP1A2		Caffeine 200 mg orally	Plasma caffeine $AUC_{0–24\ h}$	T = 0, 5 min, 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 h
			Urinary molar ratio of (1X+1U+AFMU)/17U	12-h Urine
CYP2C9		Warfarin 10 mg + vitamin K 10 mg orally	Plasma S-warfarin $AUC_{0–72\ h}$	T = 0, 3, 6, 8, 12, 24, 48, and 72 h
CYP2C19		Omeprazole 40 mg orally	Plasma omeprazole $AUC_{0–24\ h}$	T = 0, 5 min, 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 h
CYP2D6		DM 30 mg orally	Plasma dextromethorphan $AUC_{0–24\ h}$	T = 0, 5 min, 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 h
			Urinary molar ratio of dextromethorphan/ dextrorphan	12-h Urine
CYP3A4/5	H	Midazolam 2 mg intravenously	Intravenous midazolam plasma $AUC_{0–24\ h}$	T = 0, 5 min, 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 h
	H and I	Midazolam 5 mg orally	Oral midazolam plasma $AUC_{0–48\ h}$	T = 0, 5 min, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, and 48 h
P-gp	H	Digoxin 0.25 mg intravenously	Plasma digoxin $AUC_{0–51\ h}$	T = 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 12, 27, and 51 h
	H and I	Digoxin 0.25 mg orally	Plasma digoxin $AUC_{0–48\ h}$	T = 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 12, 24, 36, and 48 h

1U, 1-methylurate; 17U, 1,7-dimethylurate; 1X, 1-methylxanthine; AFMU, 5-acetylamino-6-formylamino-3-methyluracil; AUC, area under the curve; CYP, cytochrome P450; DM, dextromethorphan; H, hepatic; I, intestinal; P-gp, P-glycoprotein; *T*, sampling time.