Complex Drug Interactions of the HIV Protease Inhibitors 3: Effect of Simultaneous or Staggered Dosing of Digoxin and Ritonavir, Nelfinavir, Rifampin or Bupropion

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Nonstandard Abbreviations**:**

RTV, ritonavir; RIF, rifampin; NFV, nelfinavir; PI, anti-HIV Protease inhibitors; P-gp, P-glycoprotein; AUC, area under the plasma concentration-time curve

ABSTRACT

As part of a larger clinical drug-drug interaction (DDI) study aimed at *in-vitro* to *in-vivo* prediction of HIV protease inhibitor metabolic and transporter based DDIs, we measured the inductive (staggered administration) and inductive plus inhibitory (simultaneously administered) effect of multiple dose ritonavir (RTV), nelfinavir (NFV) or rifampin (RIF) on the pharmacokinetics of the P-glycoprotein probe, digoxin (DIG), when administered simultaneously or staggered with the protease inhibitors or RIF. In both cases, NFV did not significantly affect DIG disposition. RTV decreased DIG Cl_{renal} when administered simultaneously or staggered, but significantly increased DIG AUC_{0-24hr} only when administered simultaneously. RIF decreased DIG AUC_{0-24hr} only when RIF and DIG administration was staggered. When RIF and DIG were administered simultaneously, DIG C_{max} and AUC_{0-4hr} were significantly increased and DIG Cl_{renal} was decreased. An unexpected and potentially clinically significant DDI was observed between DIG and the CYP2B6 probe, bupropion, which decreased DIG AUC_{0-24hr} 1.6-fold and increased Cl_{renal} 1.8-fold. Because this was an unexpected DDI and our studies were not specifically designed to quantify this interaction, further studies are required to confirm the interaction as well as understand the mechanistic basis of the DDI. In summary, RTV or NFV do not induce P-glycoprotein activity measured with digoxin and RIF does so only under staggered administration.

INTRODUCTION

Clinical use of the HIV protease inhibitors (PIs) is complicated by the profound, paradoxical and unpredictable nature of drug-drug interactions (DDIs) with the PIs (Unadkat and Wang, 2000). Many of these DDIs arise from potent inhibition or inactivation of cytochrome P450 3A (CYP3A) by the PIs (Josephson, 2010). In addition, the PIs are known to be *in-vitro* inducers or inhibitors of many CYP enzymes including CYP3A and the drug efflux pump P-glycoprotein (P-gp) (Dixit et al., 2007, Hsiao et al., 2008). These multiple modes of interaction are likely the cause of the unpredictable and paradoxical nature of DDIs with the PIs. For example, many of the PIs are believed to be predominantly cleared *in-vivo* by CYP3A and/or P-gp, but they are capable of inducing their own clearance (ritonavir [RTV] and nelfinavir [NFV]) or the clearance of other PIs. In addition, multiple dose RTV has no effect on the clearance of the CYP3A probe drug alprazolam where on acute dosing of RTV the clearance of alprazolam is decreased. These DDIs have been hypothesized to be the result of net induction of CYP3A *in-vivo*, but may also be the result of induction of other CYP enzymes or drug transporters significantly contributing to the clearance of the PIs or alprazolam. In an effort to understand these paradoxical DDIs with the PIs, we designed two DDI studies in healthy volunteers to determine if RTV or NFV and the induction positive control rifampin (RIF) are net inducers of CYP3A, inducers of other CYP enzymes and/or inducers of P-gp. In our first manuscript from these studies, we showed that multiple dose treatment with RTV or NFV do not result in net induction of CYP3A, rather CYP3A activity is substantially decreased (Kirby et al., 2011b). In our second manuscript we showed that RTV or NFV do in fact induce CYP1A2, 2B6 and 2C9, but the magnitude of induction is not substantial enough to explain the induced clearance of the

PIs or alprazolam (Kirby et al., 2011a). Therefore, in this manuscript we determine if induction

of P-gp by RTV or NFV would provide explanations for these paradoxical DDIs.

P-gp is highly expressed in the intestine and is thought to play a role in the absorption of

P-gp substrates such as DIG, the PIs, as well as other drugs (Endres et al., 2006). After oral

administration of P-gp inhibitors, the inhibitor concentrations in the intestinal lumen and portal

vein are expected to be high and therefore can potentially produce profound inhibition and/or

induction of intestinal and/or hepatic P-gp and CYP3A activity. Because of this potential for

simultaneous inhibition and induction of P-gp or CYP enzymes, the design of clinical DDI

induction studies is critical for accurate interpretation of study outcomes from a mechanistic

perspective (e.g. induction of P-gp). Therefore in our study we administered the P-gp probe drug

DIG in a staggered and simultaneous manner with RTV, NFV or the induction positive control

rifampin (RIF).

Here in, we describe the effect of multiple dose treatment of RTV, NFV or RIF

administered in a staggered or simultaneous fashion on the pharmacokinetics of DIG as a marker

for P-gp activity. In addition, we describe an unexpected DDI between DIG and bupropion

(BUP, a CYP2B6 probe) that may be clinically significant.

METHODS

Study Design

The general study design, subject selection criteria, and subject safety monitoring have been described in detail in our previous manuscript (Kirby et al., 2011b) (Figure 1). The design of the study with respect to the P-gp mediated DDIs is described herein. Briefly, digoxin (0.5 mg PO) was used to probe P-gp activity in the intestine, liver and kidney as part of a larger DDI study to determine the inductive effect of ~14 day treatment with RTV, NFV or RIF in healthy volunteers. In Study 1, we administered the probe drug cocktails staggered by ~12 hrs following the dose of the inducers, RTV, NFV or RIF. This staggered design allowed a more accurate assessment of induction because the likelihood of reversible inhibition by RTV, NFV or RIF was minimized. In Study 2, we measured the combined effect (induction and inhibition) by simultaneous administration of RTV, NFV or RIF with the probe drugs. In Study 2, to measure the effect of RTV, NFV or RIF on CYP2B6 activity, we also administered BUP in a staggered fashion similar to administration of CYP probe drugs in Study 1 (the results are presented in Kirby et al. (Kirby et al., 2011a). BUP was not included in Study 1 as a validated phenotyping cocktail containing BUP was not available. BUP was administered on the first of two consecutive study days and DIG and midazolam (P-gp and CYP3A probes) were administered on the second day (~24 hrs after BUP) (Kirby et al., 2006). Blood and urine samples were collected prior to and up to 48 hours after probe drug administration. Although desirable, we were unable to sample blood and urine for longer periods due to difficulty in recruiting subjects willing to collect over longer periods. Plasma and urine samples were stored at -20°C until analysis.

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Study Drugs, Chemicals and Reagents

All study drugs were supplied via the University of Washington Investigational Drug Services. Study drugs were purchased from the following suppliers: DIG (Lanoxin, 0.25 mg tablets), GlaxoSmithKline (Philadelphia, PA), nelfinavir (625 mg tablets, Agouron Pharmaceuticals, La Jolla, CA), ritonavir (100 mg tablets, Abbott Labs, Abbott Park, IL) and rifampin (300 mg capsules, Sandoz, Broomfield, CO).

Digoxin Analysis

Reference standards of DIG and digitoxin (internal standard for DIG analysis) were purchased from ICN Biomedicals Inc (Aurora, OH). Optima grade water, methanol and methyl t–butyl ether (MTBE) were purchased from Fisher (St. Louis, MO). All other chemicals used were reagent grade or higher. Plasma and urine samples were assayed for DIG concentration following a previously published method utilizing a liquid/liquid extraction and LC/MS detection (Kirby et al., 2008).

Pharmacokinetic Analysis

Noncompartmental analysis of the plasma concentration-time profiles of DIG was performed using WinNonlin Professional v 5.0 (Pharsight Corp, Mountain View, CA). Parameters estimated included area under the plasma concentration-time profile (AUC_{0-t}) with t=4 and 24 hours, maximum plasma concentration (C_{max}), time of maximum plasma concentration (T_{max}). Renal clearance (Cl_{renal}) of DIG was estimated by the ratio of total amount

of DIG excreted in the urine over 24 hours ($A_{e,0-24hr}$) and AUC_{0-24hr}. DIG $t_{1/2\beta}$, and oral clearance were not estimated because of the limited sampling time (24hr) after DIG administration.

Statistical analyses

Statistical analysis was conducted on log-transformed pharmacokinetic parameters. This was done by calculating the geometric mean ratio (GMR) by exponentiation of the average difference of log transformed pharmacokinetic parameters. If the 90% confidence interval of this GMR included the value of unity, the treatment was considered to not have significantly altered the pharmacokinetic parameter. Because of an unexpected DDI between DIG and bupropion (administered 24 hours prior to DIG in Study 2), we compared the pharmacokinetic parameters of DIG prior to and after treatment with RTV, NFV or RIF in both studies using an unpaired students t-test assuming equal variance. A p-value of ≤ 0.05 was considered to be statistically significant.

Using historical data of DIG pharmacokinetics in healthy volunteers, we conducted a priori power analysis using plasma AUC as the primary outcome measure. Assuming equal variance between control and treatment groups, our analysis indicated that n=7 would provide 80% power ($\alpha < 0.05$) to discern a 30% change in plasma AUC of DIG.

RESULTS

Subject demographics, treatment periods for RTV, NFV and RIF and cocktail administration were previously described (Kirby et al., 2011b). Briefly, 16 healthy volunteers (33 ± 9 yr, 78 ± 14 kg, 5 males and 11 females) completed Study 1 (staggered administration) with n=16, 7, 8 and 16 completing the control, NFV, RTV, RIF treatment respectively (one subject did not complete the NFV treatment period). Nine subjects (29 ± 9 yr, 79 ± 14 kg, 3 males and 6 females) completed Study 2 (simultaneous administration) (Figure 1).

Effect of RTV, NFV or RIF on P-gp activity (Digoxin)

The average plasma concentration-time profiles of DIG before and after NFV, RTV or RIF treatment in Studies 1 and 2 are shown in Figure 2. Compared with pretreatment, RIF (staggered dosing) significantly but modestly decreased DIG $AUC_{0-24\ hr}$ (0.81, 90%CI 0.69-0.96) which is a composite of intestinal, hepatic and renal P-gp activity. Staggered RIF dosing did not affect Cl_{renal} of DIG. No change was observed in DIG AUC_{0-4hr} (0.79, 0.62-1.02) or C_{max} (0.78, 0.57-1.06), indicators of intestinal P-gp activity. In contrast, simultaneous administration of RIF (Study 2) significantly increased DIG C_{max} (1.55, 1.20-1.99), AUC_{0-4hr} (1.37, 1.05-1.80) and decreased Cl_{renal} (0.87, 0.78-0.97) (Table 1 and Figure 3A). When compared with staggered RIF administration, simultaneous administration of RIF significantly increased DIG C_{max}, $AUC_{0-4\ hr}$ and $AUC_{0-24\ hr}$.

Multiple doses of NFV had no significant effect on any measured pharmacokinetic parameters of DIG in either Study 1 or 2 (Table 1). Multiple doses of RTV significantly decreased DIG Cl_{renal} when administered in a staggered (0.79, 0.67-0.93) or simultaneous manner

(0.64, 0.55-0.75) (Table 1 and Figure 3B). RTV did not alter other DIG pharmacokinetic parameters following staggered administration of RTV. In the simultaneous administration study, RTV significantly increased DIG T_{max} (1.54, 1.22-1.94) and AUC_{0-24hr} (1.37, 1.11-1.70). A comparison of staggered vs. simultaneous RTV administration showed no significant differences in any of the DIG parameters.

Unexpected Interaction Between Digoxin and Bupropion

An unexpected DDI was observed when the pharmacokinetics of DIG prior to any treatment (control) were compared between staggered (Study 1) vs. simultaneous (Study 2) administration (Figure 4A). In the control phase of Study 2 where DIG was given ~24 hours after bupropion (extended release, 150 mg), the DIG AUC_{0-24hr} was decreased 1.6-fold and Cl_{renal} was increased 1.8-fold compared to Study 1 (Table 1 and Figure 5). This interaction was also observed during RTV, NFV or RIF treatment (Figure 4B-D). DIG C_{max}, T_{max} and AUC_{0-4hr} were not significantly different between Studies 1 and 2. Bupropion and 4-OH-BUP plasma concentration profiles during digoxin administration are shown in Figure 4 E and F respectively to show the level of exposure to these drugs over the interval during which the DDI was observed..

DISCUSSION

RIF induces intestinal and hepatic P-gp by pregnane X receptor (PXR)-mediated transcription, thereby decreasing bioavailability and increasing non-renal clearance of DIG (Drescher et al., 2003, Greiner et al., 1999). Consistent with these reports, we observed a decrease in the DIG AUC_{0-24hr} and slight, but not statistically significant decreases in AUC_{0-4hr} and C_{max} when RIF and DIG administration was staggered. In contrast, when RIF and DIG were administered simultaneously, DIG AUC_{0-24hr} was unchanged, but AUC_{0-4hr} and C_{max} were increased (Figure 3A). The different effect of RIF on DIG AUC_{0-4hr} and C_{max} between staggered and simultaneous administration indicates the presence of an interaction mechanism other than induction of intestinal/hepatic P-gp. RIF is an inhibitor and substrate of the hepatic transporters, organic anion transporting polypeptides (OATPs) and P-gp (Lau et al., 2007, Reitman et al., 2011, Tirona et al., 2003). Simultaneously administered RIF could inhibit intestinal P-gp and/or hepatic P-gp/OATPs during hepatic first-pass, increasing DIG C_{max} and bioavailability. Such an effect on hepatic OATPs has been shown in rats using IV DIG and RIF (Lam et al., 2006). Recently, Reitman et al. (Reitman et al., 2011) verified the findings of Lam et al. in the rat and our results that simultaneous administration of RIF and DIG increased DIG C_{max} and AUC_{0-3hr} , masking P-gp induction. The magnitude of increase in DIG C_{max} and AUC_{0-3hr} observed by Reitman et al. is comparable to our observations, implying that the underlying DIG-BUP interaction (described below) did not substantially alter the effect of simultaneous RIF administration on DIG pharmacokinetics. Reitman et al. attributed this interaction to inhibition of intestinal P-gp whereas Lam et al. showed inhibition of hepatic OATPs. Recently, DIG was shown to not be a substrate of the human OATPs 1A2, 1B1, 1B3 or 2B1, but is a substrate of an

as yet unidentified transporter that might be the sodium dependent uptake transporter expressed in HEK293 cells (Kimoto et al., 2011, Taub et al., 2011).

Mixed inhibition/induction interactions have significant implications for induction DDI study design. The purpose of induction studies may be two-fold. First, to characterize the effect of an inducer on the object drugs pharmacokinetics. Second, to assess induction of specific enzymes or transporters. To address the first purpose, co-administration of the inducer and the object drug is logical provided the two drugs are usually dosed simultaneously. However, to address the second and mechanistic purpose, our data demonstrate the need for staggered administration of the two drugs to avoid confounding inhibitory interactions from simultaneous administration of the two drugs which may mask induction of transporters or enzymes. Unfortunately, the current FDA draft guidance for industry on the conduct of DDI studies (www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM0 72101.pdf) does not specify such design considerations. We believe it should.

NFV and RTV are ligands of PXR (Dussault et al., 2001) and possibly Aryl Hydrocarbon Receptor AHR (Frotschl et al., 1998) thereby inducing transcription of many CYP enzymes (3A, 1A2, 2B6, 2C9 and 2C19) as well as P-gp (Dixit et al., 2007, Gupta et al., 2008). In our study, NFV (1250 mg bid, 14 days) had no effect on intestinal, hepatic or renal P-gp activity, in agreement with a previous study using fexofenadine to measure intestinal P-gp (Kharasch et al., 2009). Likewise, staggered or simultaneous administration of RTV (400 mg bid, 14 days) did not result in net induction of P-gp activity, in contrast to a previous study using fexofenadine which indicated slight induction of P-gp by RTV (Kharasch et al., 2008). Upon simultaneous administration of RTV and DIG, DIG T_{max} was prolonged, but AUC_{0-4hr} was unchanged

suggesting a slower rate but not extent of absorption, or decreased DIG oral clearance. The latter is supported by the increased DIG AUC_{0-24hr} and decreased Cl_{renal}, suggesting inhibition of hepatic and/or renal P-gp. However, inhibition of intestinal or hepatic uptake transport cannot be ruled out. In fact digoxin is actively taken up into human hepatocytes by a saturable process other than OATP1B1, 1B3 or 2B1(Kimoto et al., 2011).

Previously, 200 mg bid RTV simultaneously administered with DIG increased DIG AUC_{0-72hr} by inhibiting hepatic but not renal P-gp (Penzak et al., 2004). Therefore, in our staggered administration study DIG AUC_{0-24hr} would be expected to increase when renal P-pg was inhibited by RTV (decreased Cl_{renal}). The reason for this discrepancy is unclear, but may include competing inhibition/induction of hepatic P-gp. Collectively, our data suggest that P-gp is not induced by NFV, and that 400 mg bid RTV may induce hepatic P-gp slightly, but the net effect, irrespective of staggered or simultaneous administration, is inhibition of P-gp or no effect respectively. We don't believe these data are confounded by the underlying BUP-DIG interaction because the observed inhibition of renal P-gp by simultaneous RTV administration in the presences of the BUP-DIG interaction is comparable to that observed with staggered administration when BUP was not present.

When designing our studies, we assumed BUP given 24 hours before DIG would have no effect on the pharmacokinetics of either drug. We were surprised by a substantial interaction between these drugs because BUP is extensively metabolized (Lai and Schroeder, 1983), whereas DIG is minimally metabolized and its excretion is mediated by filtration and net secretion (via transporters). The metabolites of BUP are extensively excreted in the urine (Laizure et al., 1985), but whether they are secreted and/or filtered is unknown. The effect of

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DIG on BUP and its metabolite 4-hydroxy bupropion was described previously (Kirby et al.,

2011a). The effect of BUP and/or its metabolites (BUP/Met) on DIG pharmacokinetics is

evident when comparing DIG pharmacokinetics in the absence of BUP (Study 1) and in the

presence of BUP (Study 2) prior to treatment (control phase). In the presence of BUP/Met, we

observed a statistically significant decrease ($p<0.05$, with an unpaired t-test since subjects were

not paired between the studies) in DIG AUC_{0-24hr} (1.6-fold) and increase in DIG Cl_{renal} (1.8-fold)

(Figure 5). Clinically, steady-state digoxin plasma concentrations 6 hr post-dose are maintained

between 0.5-1.0 ng/ml. Clearly, the 60% decrease in DIG AUC_{0-24hr} in the presence of BUP/Met

is clinically significant because it would result in sub-therapeutic DIG plasma concentrations.

There are multiple possible mechanisms of this DDI. First, BUP/Met may have increased DIG

free fraction in plasma resulting in increased DIG renal and non-renal clearances. Since DIG is

only 25% bound in plasma (Evered, 1972), complete protein binding displacement cannot

explain the increased DIG Cl_{renal}. Secondly, DIG is actively secreted in the renal proximal

tubules by basal uptake by OATPs (likely 4C1) and apical efflux by P-gp. For BUP/Met to

increase secretion of DIG, activation of the rate limiting step of these processes would be

necessary. Activation of P-gp has been shown *in-vitro* (Soldner et al., 1999), but to date has not

been demonstrated *in-vivo*. Since BUP was administered 24 hours before DIG, induction of P-gp

or OATP4C1 by BUP/Met is unlikely. Therefore it is more likely that BUP/Met increased DIG

renal secretion possibly by inhibiting reabsorption. Currently it is not known if DIG is actively

reabsorbed in the kidney. Least likely is the possibility that BUP/Met increased glomerular

filtration by increasing renal blood flow. There are no reports that BUP/Met can alter renal

blood flow. BUP/Met did not affect DIG AUC_{0-4hr} which is used as a measure of intestinal P-gp

activity, implying that BUP/met did not affect DIG intestinal bioavailability. Assessing the

effect of BUP/Met on hepatic clearance of DIG wasn't possible from our data because of insufficient plasma sampling to estimate non-renal clearance.

Irrespective of the underlying mechanism(s) of this DDI, an important question to address is whether this interaction would be greater upon co-administration or multiple dosing. Initiating BUP treatment for a patient stabilized on DIG could result in substantially decreased DIG concentrations necessitating increasing DIG dose to avoid therapeutic failure. Further dose adjustments may be needed if the DDI is greater following multiple doses of BUP. On the other hand, when BUP therapy is terminated, DIG plasma concentrations could dramatically increase causing clinically significant toxicity. Because our study was not designed to confirm or quantify this unexpected interaction, a study where DIG and BUP are co-administered to steady-state is warranted. To gain insight into the site (hepatic/intestinal vs. renal) and mechanisms of this interaction, the study design should include IV and oral administration of DIG.

In summary, we have shown that the PIs, NFV or RTV, do not substantially induce hepatic or intestinal P-gp activity measured with DIG. These finding do not provide an explanation for the paradoxical DDIs with the PIs such as autoinduction of the PIs clearance. Hence, other mechanism such as induction of enzymes other than CYP3A, 1A2, 2B6, or 2C9 or other drug transporters may explain these paradoxical DDIs. Our contrasting results of RIF induction of P-gp dependant on DIG and RIF dosing exemplify the need for careful clinical DDI study design and attention to both influx and efflux transporters. Surprisingly, we discovered evidence of an unexpected, novel DDI between the CYP2B6 probe drug BUP and DIG that has clinical relevance. The mechanistic basis of this DDI is not clear and warrants further study.

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AUTHORSHIP CONTRIBUTIONS

Participated in research design: Collier, Kharasch, Kirby, Thummel and Unadkat

Conducted experiments: Kirby and Whittington

Contributed new reagents or analytical tools: Whittington

Performed data analysis: Kirby and Whittington

Wrote or contributed to the writing of the manuscript: Collier, Kharasch, Kirby, Thummel, Unadkat and Whittington

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FOOTNOTES

FIGURE LEGENDS

Figure 1: Study design showing administration of probe drug cocktails prior to (control) and after RTV, NFV or RIF treatment. In Study 1 (staggered), the probe drug cocktails were staggered ~12 hrs after the last dose of RTV, NFV or RIF. In Study 2 (simultaneous), a dose of RTV, NFV or RIF was simultaneously administered with MDZ and digoxin.

Figure 2: Average ($\pm$ SD) plasma concentration-time profiles for DIG prior to treatment (control) and after RTV, NFV or RIF treatment for Study 1 (Staggered administration) (A and B) and Study 2 (Simultaneous administration) (C and D). Panels A and C show the full profiles whereas Panels B and D show only the first 8 hours after DIG dosing.

Figure 3: Comparison of staggered vs simultaneous administration of RIF (A) or RTV (B) after ~14 day treatment on the pharmacokinetics of DIG. Staggered administration of RIF and DIG did not significantly alter DIG C_{max}, T_{max}, AUC_{0-4hr} or Cl_{renal}, but significantly decreased AUC_{0-24hr} of DIG compared to pretreatment. Upon simultaneous administration of RIF and DIG these effects were reversed, showing only a statistically significant increase in DIG C_{max} and AUC_{0-4hr} compared to pretreatment. Staggered vs. simultaneous administration of RIF and DIG significantly altered the C_{max}, AUC_{0-4hr} and AUC_{0-24hr}, but not the T_{max}, or Cl_{renal} of DIG. Simultaneous administration of RIF and DIG masked the apparent induction of intestinal and/or hepatic P-gp by RIF and resulted in an apparent increase in DIG bioavailability. No statistically significant difference in DIG pharmacokinetic parameters was observed between staggered and simultaneous administration for RTV. * 90%CI does not include unity, therefore the treatment significantly altered the parameter relative to control. † $p<0.05$ unpaired T-test comparison of treatment/control between Studies 1 and 2.

Figure 4: Comparison of average ($\pm$ SD) plasma concentration-time profiles for DIG in Study 1 (simultaneous administration) and Study 2 (staggered administration) prior to (Control, A) and after nelfinavir (B), ritonavir (C) or rifampin (D) treatment. Average ($\pm$ SD) plasma concentrations of racemic bupropion (E) and racemic 4-OH-bupropion (F) during DIG administration (24-48 hrs after BUP administration) prior to (CON) and after ritonavir, nelfinavir or rifampin treatment are shown for reference.

Figure 5: Comparison of pharmacokinetics of DIG during the pretreatment (Control) phase of Study 1 (n=16) and Study 2 (n=9, 24 hours after bupropion administration). Administration of bupropion 24 hours prior to DIG did not significantly affect DIG C_{max} (A), T_{max} (B) or AUC_{0-4hr} (C), but significantly decreased AUC_{0-24hr} (D), and significantly increased DIG Cl_{renal} (E).

Table 1: Pharmacokinetic parameters of digoxin prior to (Control) and after nelfinavir, ritonavir or rifampin treatment.

		Control Study 1 n=16 Study 2 n=9	Nelfinavir Study 1 n=7 Study 2 n=9		Ritonavir Study 1 n=8 Study 2 n=9		Rifampin Study 1 n=16 Study 2 n=9	
		Ave $\pm$ SD	Ave $\pm$ SD	GMR (90%CI)	Ave $\pm$ SD	GMR (90%CI)	Ave $\pm$ SD	GMR (90%CI)
Study 1 Staggered Admin.	C_{max} (ng/ml)	1.34 $\pm$ 0.53	1.44 $\pm$ 0.96	1.05 (0.63-1.74)	1.59 $\pm$ 0.80	0.98 (0.61-1.57)	1.02 $\pm$ 0.38	0.78 (0.57-1.06)
	T_{max} (hr)	1.66 $\pm$ 0.77	2.56 $\pm$ 2.47	1.32 (0.66-2.64)	2.13 $\pm$ 1.03	1.10 (0.75-1.62)	1.82 $\pm$ 0.85	1.04 (0.78-1.38)
	AUC_{0-4hr} (hr*ng/ml)	2.96 $\pm$ 0.97	3.08 $\pm$ 1.64	1.05 (0.63-1.74)	4.13 $\pm$ 2.15	1.10 (0.71-1.73)	2.26 $\pm$ 0.67	0.79 (0.62-1.02)
	$AUC_{0-24\ hr}$ (hr*ng/ml)	8.66 $\pm$ 2.57	9.46 $\pm$ 2.93	1.23 (1.00-1.48)	12.7 $\pm$ 6.22	1.16 (0.81-1.66)	6.83 $\pm$ 1.68	**0.81 (0.69-0.96)**
	Cl_{renal} (ml/min)	146 $\pm$ 43.3	141 $\pm$ 65.4	0.87 (0.65-1.16)	115 $\pm$ 39.1	**0.79 (0.67-0.93)**	162 $\pm$ 51.4	1.06 (0.95-1.19)
Study 2 Simultaneous Admin. (24 hrs after BUP)	C_{max} (ng/ml)	1.24 $\pm$ 0.35	1.66 $\pm$ 0.81	1.23 (0.91-1.68)	1.55 $\pm$ 0.71	1.16 (0.86-1.57)	2.12 $\pm$ 1.04	**1.55* (1.20-1.99)**
	T_{max} (hr)	1.56 $\pm$ 0.64	2.21 $\pm$ 1.04	1.38 (0.93-2.05)	2.30 $\pm$ 0.89	**1.54 (1.22-1.94)**	1.56 $\pm$ 0.89	0.93 (0.68-1.26)
	AUC_{0-4hr} (hr*ng/ml)	2.47 $\pm$ 0.49	3.14 $\pm$ 1.68	1.02 (0.59-1.74)	3.06 $\pm$ 1.41	1.13 (0.85-1.49)	3.71 $\pm$ 1.72	**1.37* (1.05-1.80)**
	$AUC_{0-24\ hr}$ (hr*ng/ml)	5.45 $\pm$ 1.99*	7.35 $\pm$ 3.50	1.20 (0.90-1.60)	7.59 $\pm$ 3.14	**1.37 (1.11-1.70)**	7.08 $\pm$ 3.25	1.25* (0.93-1.70)
	Cl_{renal} (ml/min)	258 $\pm$ 64.9*	236 $\pm$ 83.0	0.92 (0.75-1.13)	168 $\pm$ 46.3	**0.64 (0.55-0.75)**	225 $\pm$ 59.8	**0.87 (0.78-0.97)**

Bold values are statistically significant (90%CI does not include 1.00)
*Values are significantly different between Studies 1 and 2 (unpaired T-test p<0.05)

Figure 1
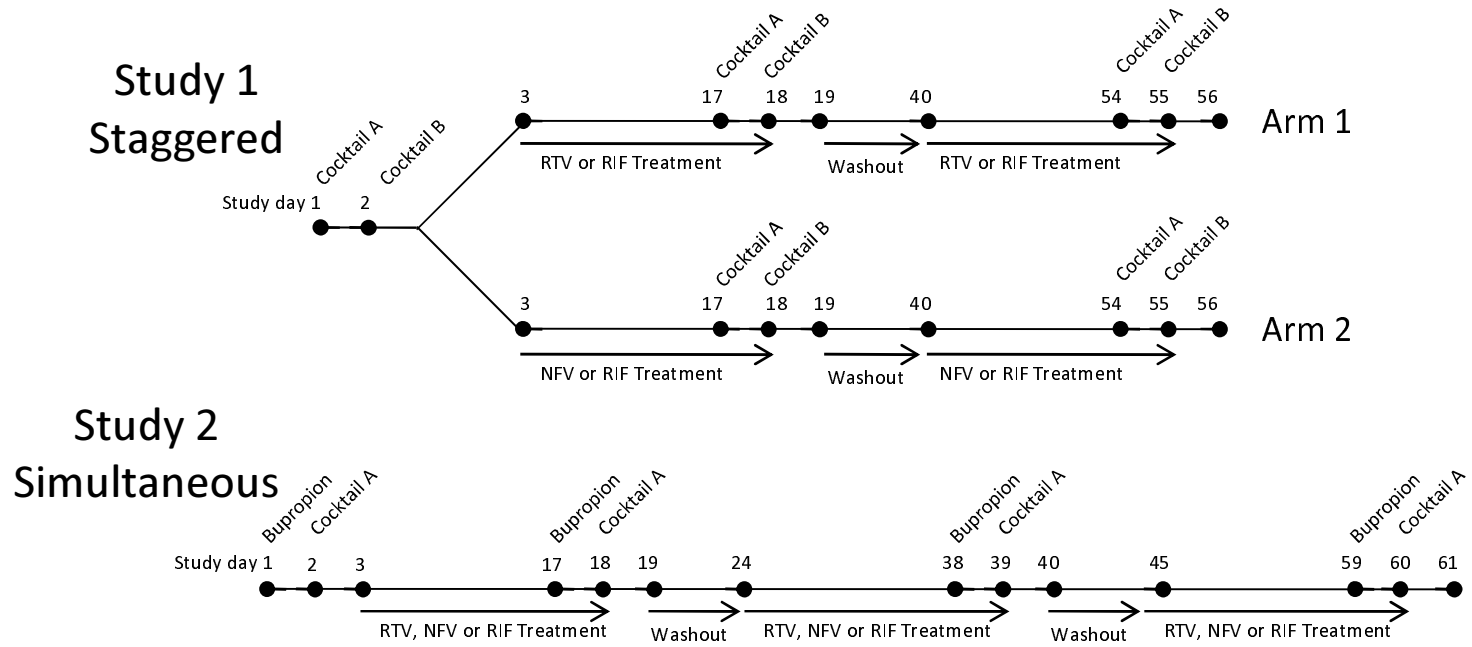
Cocktail A: midazolam (2 mg PO), digoxin (0.5 mg PO)
Cocktail B: midazolam (1 mg IV), caffeine (200 mg PO), tolbutamide (500 mg PO), dextromethorphan (30 mg PO)
Bupropion: bupropion ER (150 mg PO)
Treatment ~14 days: ritonavir (RTV, escalating dose to 400 mg bid), nelfinavir (NFV, 1250 mg bid), rifampin (RIF, 600 mg qd)
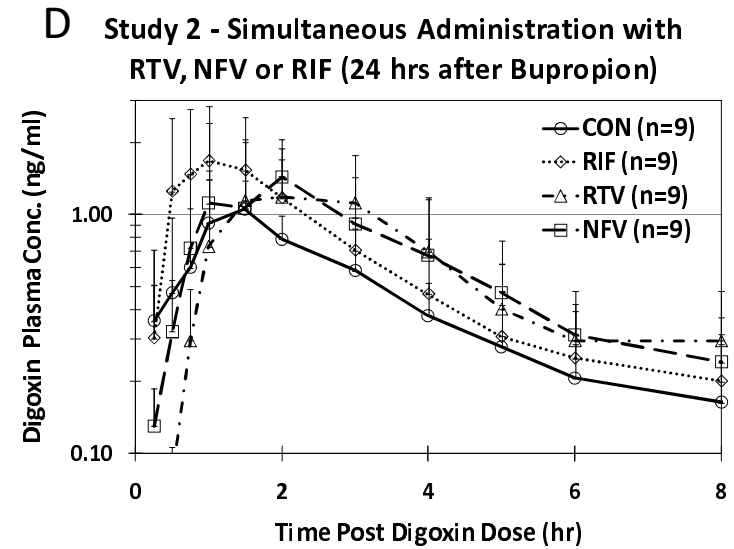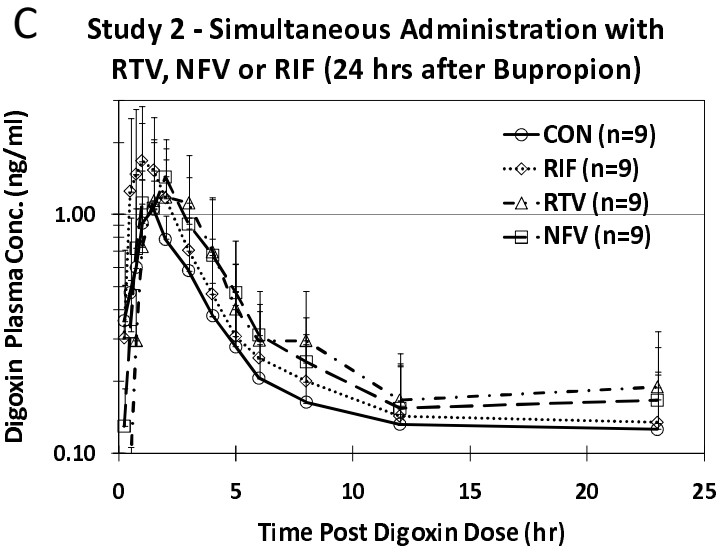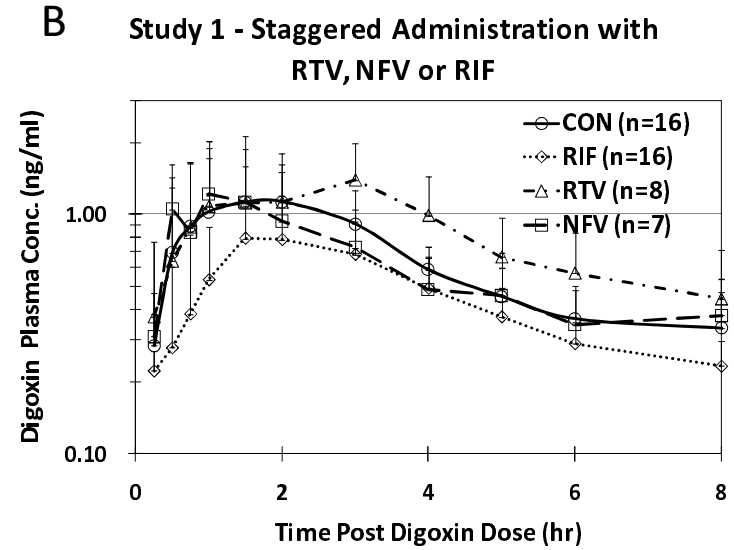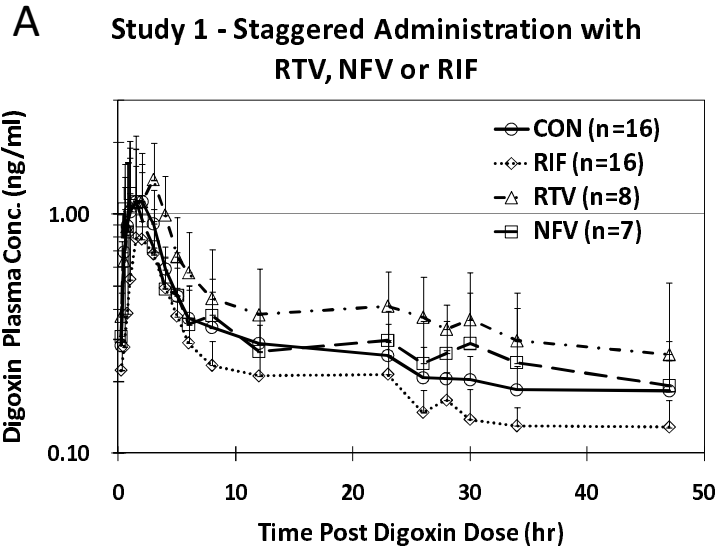
Figure 2

A **Study 1 - Staggered Administration with RTV, NFV or RIF**

Legend:
- CON (n=16)
- RIF (n=16)
- RTV (n=8)
- NFV (n=7)

Y-axis: Digoxin Plasma Conc. (ng/ml), 0.10 to 1.00
X-axis: Time Post Digoxin Dose (hr), 0 to 50

B **Study 1 - Staggered Administration with RTV, NFV or RIF**

Legend:
- CON (n=16)
- RIF (n=16)
- RTV (n=8)
- NFV (n=7)

Y-axis: Digoxin Plasma Conc. (ng/ml), 0.10 to 1.00
X-axis: Time Post Digoxin Dose (hr), 0 to 8

C **Study 2 - Simultaneous Administration with RTV, NFV or RIF (24 hrs after Bupropion)**

Legend:
- CON (n=9)
- RIF (n=9)
- RTV (n=9)
- NFV (n=9)

Y-axis: Digoxin Plasma Conc. (ng/ml), 0.10 to 1.00
X-axis: Time Post Digoxin Dose (hr), 0 to 25

D **Study 2 - Simultaneous Administration with RTV, NFV or RIF (24 hrs after Bupropion)**

Legend:
- CON (n=9)
- RIF (n=9)
- RTV (n=9)
- NFV (n=9)

Y-axis: Digoxin Plasma Conc. (ng/ml), 0.10 to 1.00
X-axis: Time Post Digoxin Dose (hr), 0 to 8

Figure 3

A

Rifampin Treatment

GMR (90%CI) of Digoxin Parameter (RIF/CON)

Study 1 Staggered Admin. (n=8)
Study 2 Simultaneous Admin. (n=9)

Cmax   Tmax   AUC 0-4hr   AUC 0-24hr   Clrenal

B

Ritonavir Treatment

GMR (90%CI) of Digoxin Parameter (RTV/CON)

Study 1 Staggered Admin. (n=8)
Study 2 Simultaneous Admin. (n=9)

Cmax   Tmax   AUC 0-4hr   AUC 0-24hr   Clrenal

Figure 4

A **Control**

B **Ritonavir Treatment**

C **Nelfinavir Treatment**

D **Rifampin Treatment**

E **Average Bupropion Plasma Concentration**

F **Average 4-OH-Bupropion Plasma Concentration**

Figure 5: