

The Effect of Single- and Multiple-Dose Etravirine on a Drug Cocktail of Representative Cytochrome P450 Probes and Digoxin in Healthy Subjects

The Journal of Clinical Pharmacology
54(4) 422–431
© 2013, The American College of
Clinical Pharmacology
DOI: 10.1002/jcph.214

Thomas N. Kakuda, PharmD¹, Rodica M. Van Solingen-Ristea, MD¹,
Joelle Onkelinx, MSc^{2,a}, Tanja Stevens, RN^{2,b}, Fatima Aharchi, MSc²,
Goedele De Smedt, MD², Monika Peeters, MSc², Lorant Leopold, MD¹,
Richard M.W. Hoetelmans, PhD²

Abstract

The effect of etravirine on cytochrome P450 (CYP) enzymes and P-glycoprotein were evaluated in two randomized, crossover trials in healthy subjects. A modified Cooperstown 5 + 1 cocktail was utilized to determine the effects of etravirine on single-dose pharmacokinetics of model CYP probes. The cocktail was administered alone, then, after a 14-day washout, etravirine 200 mg twice daily (bid) was given for 14 days with cocktail on days 1 and 14. In a separate study, digoxin (0.5 mg) was administered alone, then, after a 14-day washout, etravirine 200 mg bid was administered for 12 days with digoxin on day 8. In the cocktail study, the AUC_{last} least squares mean (LSM) ratios (90% confidence intervals [CIs]) for cocktail + etravirine versus cocktail were 0.93 (0.88, 0.99; paraxanthine), 0.58 (0.44, 0.75; 7-OH-S-warfarin), 0.43 (0.20, 0.96; 5-OH-omeprazole), 0.85 (0.78, 0.94; dextrophan), and 0.69 (0.64, 0.74; midazolam). Digoxin AUC_{0–8h} was slightly increased with etravirine coadministration (LSM ratio 1.18 [0.90, 1.56]). These data suggest that etravirine is a weak CYP3A isozyme inducer and minimally inhibits CYP2C9, 2C19, and P-glycoprotein activity.

Keywords

etravirine, cytochrome P450, P-glycoprotein, drug–drug interactions, HIV

The nonnucleoside reverse transcriptase inhibitor (NNRTI) etravirine 200 mg twice daily (bid), when given in combination with darunavir/ritonavir and background antiretrovirals (ARVs) has demonstrated durable efficacy with a similar tolerability profile to placebo in the phase III DUET (TMC125 to Demonstrate Undetectable viral load in patients Experienced with ARV Therapy) trials in treatment-experienced, human immunodeficiency virus type-1 (HIV-1)-infected patients.¹ Currently, etravirine combined with other ARVs is indicated for treatment of HIV-1 infection in ARV-experienced adults,^{2,3} and treatment-experienced pediatric patients.⁴

Since ARVs, such as etravirine, are often coadministered with other medications, knowledge of the interaction potential of drugs within a combination regimen is essential. In vitro studies demonstrated that cytochrome P450 (CYP) 2C9 was the most potently inhibited by etravirine ($K_i = 0.58 \mu\text{M}$), whereas K_i values for other CYP enzymes were at least 10-fold higher: $7.0 \mu\text{M}$ (CYP1A2), $83 \mu\text{M}$ (CYP2B6), $22 \mu\text{M}$ (CYP2C19), $15 \mu\text{M}$ (CYP2D6), and $6.7 \mu\text{M}$ (CYP3A) (Janssen, data on file). Investigations with primary hepatocyte cultures indicated that etravirine induced CYP3A expression.⁵ P-glycoprotein was inhibited ($\text{IC}_{50} = 24.2 \mu\text{M}$) by etravirine in vitro at higher concentrations than

expected in vivo⁶ (Janssen, data on file); although etravirine did not inhibit this transporter in another in vitro report.⁷ Many drug–drug interaction trials have been conducted with etravirine and various antiretroviral and nonantiretroviral drugs.^{6,8,9}

To extrapolate in vitro findings with etravirine on CYP enzymes and P-glycoprotein to in vivo, the present studies were conducted in healthy subjects. The “cocktail”

¹Janssen Research & Development, LLC, Titusville, NJ

²Janssen Infectious Diseases BVBA, Beerse, Belgium

³NV Novartis Pharma SA Medialaan 40, Vilvoorde, Belgium.

⁴ClinOps Consulting BVBA, Herfststraat 27, 2590 Berlaar, Belgium.

Submitted for publication 5 July 2013; accepted 17 October 2013.

Corresponding Author:

Thomas N. Kakuda, Janssen Research & Development, LLC, 1125
Trenton-Harbourton Road, Titusville, NJ, USA.

Email: tkakuda@its.jnj.com

Data contained in this article were presented at: the 9th International Workshop on Clinical Pharmacology of HIV Therapy, April 7–9, 2008; New Orleans, LA, USA; Abstract P22 (digoxin study), and at the 48th International Conference on Antimicrobial Agents and Chemotherapy/ 46th Infectious Diseases Society of America meeting, October 25–28, 2008; Washington, DC, USA; Abstract A-955 (cocktail study).

approach, whereby several probe substrates are administered simultaneously, allows the characterization of the activity of multiple CYP enzymes within a single clinical trial.¹⁰ Several cocktail methods exist, among them the Cooperstown 5 + 1 cocktail is validated using bioequivalence criteria,¹¹ and has been shown to minimize intra-individual variability.¹⁰ In addition, since the cardiac glycoside digoxin has no effect on the CYP system, but is a commonly used substrate for P-glycoprotein, it may be considered a suitable candidate for evaluating the effects of etravirine on P-glycoprotein.¹²

The potential inhibitory/inducing effects of etravirine on the single-dose pharmacokinetics of a drug cocktail of representative probes of CYP enzymes (CYP1A2 [caffeine], CYP3A isozymes [midazolam], CYP2C9 [warfarin], CYP2C19 [omeprazole], and CYP2D6 [dextromethorphan]) were investigated using a modified Cooperstown 5 + 1 cocktail approach in healthy subjects. Modifications included use of plasma (instead of urine) caffeine and dextromethorphan concentrations, different sample collection times for some probes, and evaluation of different pharmacokinetic parameters, including parent/metabolite ratios. The latter endpoints have been previously used as a measure of various CYP activities.^{11,13–17} In addition, the effect of steady-state etravirine on the pharmacokinetics of single-dose digoxin in healthy subjects was investigated in a separate study. In both studies, short-term safety and tolerability were also assessed.

Methods

The drug cocktail study was conducted at a single center in Germany and the digoxin study was conducted at a single center in Belgium. Independent ethics committees approved the final protocols and amendments, and all subjects provided written informed consent prior to any study-related procedure. The studies were carried out in accordance with the principles of Good Clinical Practice, the Declaration of Helsinki, and the European Union Clinical Trials Directive.

Participants

Healthy subjects (based on physical examination, medical history, electrocardiograms, vital signs, and clinical laboratory parameters), aged 18–55 years, with a body mass index between 18 and 30 kg/m² were eligible (both studies). For ≥ 3 months prior to selection, subjects were to be nonsmokers (both studies) or light smokers (≤ 10 cigarettes or 2 cigars or 2 pipes per day; digoxin study). Subjects testing positive for HIV-1, HIV-2, or hepatitis A, B, or C infection at screening were excluded. Females were only eligible for enrolment if post-menopausal for >2 years, post-hysterectomy, or post-tubal ligation without reversal operation. Subjects were withdrawn from the studies if they experienced a grade ≥ 3 adverse

event (AE), grade ≥ 2 rash or persistent grade ≥ 2 nausea. For the digoxin study, subjects with a family history of sudden cardiac death, or Wolff–Parkinson–White syndrome or any clinically relevant electrocardiogram abnormality (on day -1 of the first session) were excluded.

In the drug study, at screening, genomic DNA was extracted from blood and analyses of specific alleles of CYP2D6, CYP2C9, and CYP2C19 were performed using TaqMan PCR (Cogenics, Bienred, Germany). Subjects with poor metabolizer genotype (homozygote) for CYP2D6 (*3, *4, *5, and *6), CYP2C9 (*2 and *3), and CYP2C19 (*2, *3, *4, and *8) were excluded from the drug cocktail study. Subjects were not excluded based on P-glycoprotein genotype (digoxin study).

For both studies, over-the-counter (including dietary supplements and herbal medications) and prescribed medication had to be discontinued at least 7 and 14 days, respectively, prior to first intake of study medication. For the cocktail study, any drug or substance known to induce drug-metabolizing enzymes had to be discontinued at least 1 month prior to study start. Alcohol was not permitted in either study from 24 hours before the first intake of trial medication until after collection of the last pharmacokinetic blood sample. Intake of (methyl) xanthines (e.g., coffee, tea, coke, chocolate) was not allowed from 48 hours prior to until 24 hours after each drug cocktail administration. Caffeine intake was restricted to ≤ 3 beverages on the day of digoxin administration.

Study Designs and Treatments

Both studies were phase I, open-label, 2-period crossover studies, with at least a 14-day washout period between treatments (Figure 1). The sampling schemes, drug doses used and metabolites measured were based on those previously used in other drug cocktail studies.^{11,18}

In the drug cocktail study, 14 healthy subjects were randomized 1:1 to AB or BA (Figure 1a). Treatment A was a single dose of drug cocktail on day 1, given within 10 minutes of breakfast, consisting of midazolam 0.025 mg/kg (intravenously over 1 minute), and oral dextromethorphan 30 mg, caffeine 150 mg, omeprazole 40 mg, and warfarin 10 mg (supplemented with 10 mg vitamin K to counteract the pharmacodynamic effects of warfarin). Treatment B consisted of oral etravirine 200 mg bid (within 10 minutes after a meal) for 14 days plus a single dose of the drug cocktail (within 5 minutes of etravirine intake) on days 1 and 14.

In the digoxin study, 16 healthy subjects were randomized 1:1 to receive AB or BA (Figure 1b). Treatment A consisted of a single oral dose of digoxin 0.5 mg (Lanoxin®; GlaxoSmithKline, Research Triangle Park, NC), whereas in Treatment B etravirine 200 mg bid was administered orally for 12 days with a single oral dose

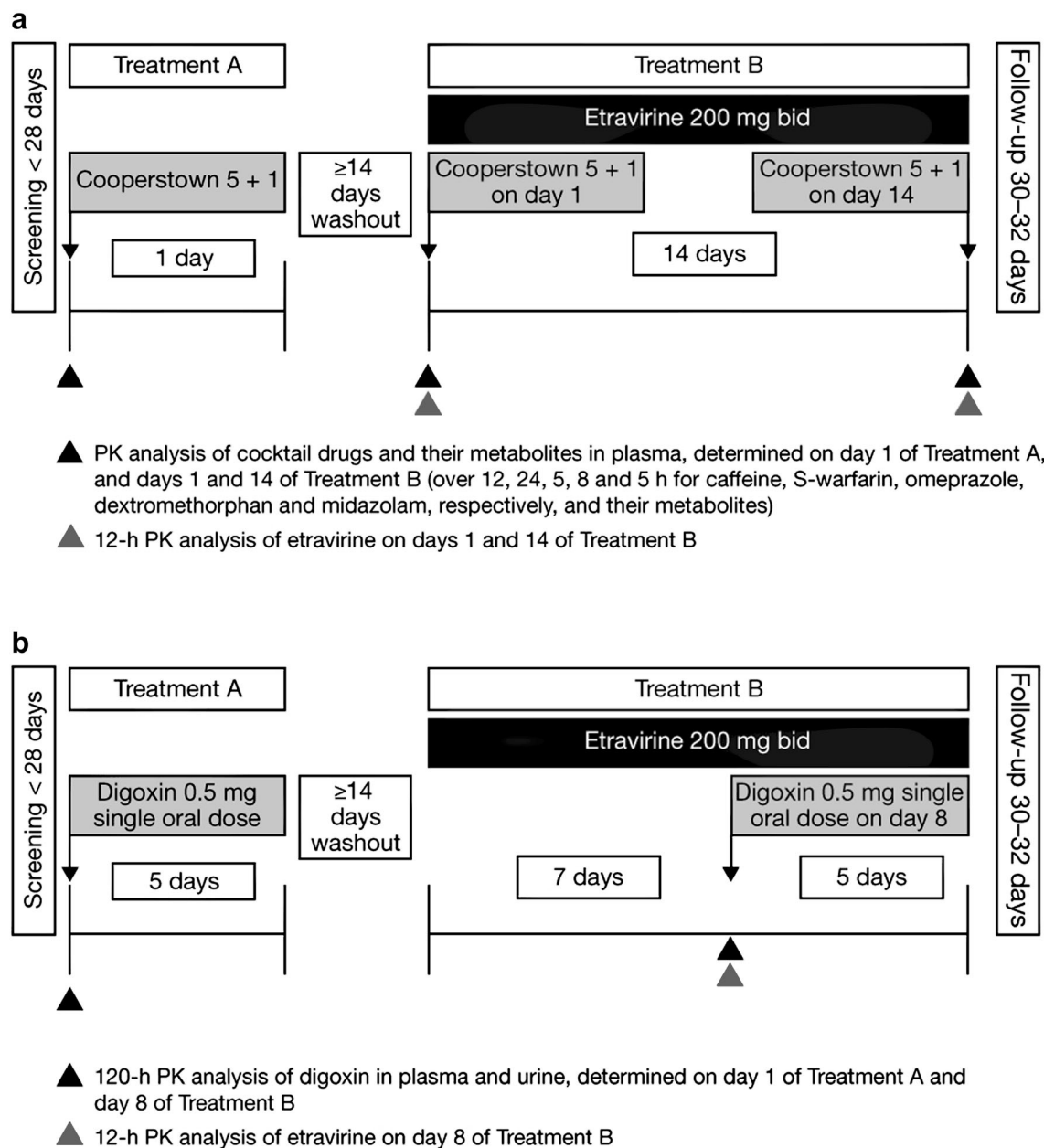


Figure 1. Study design. (a) Drug cocktail study; (b) digoxin study. bid, twice daily; PK, pharmacokinetic.

of digoxin 0.5 mg on day 8. All treatments were administered under fed conditions.

Blood Sampling for Pharmacokinetic Analyses of Probes

In the drug cocktail study, blood samples were collected on days 1 (drug cocktail alone; etravirine and drug cocktail combined) and 14 (etravirine and drug cocktail combined) for the analyses of plasma concentrations of each probe and its metabolite (Figure 1a). The blood sampling times were: caffeine + paraxanthine (CYP1A2): 0 (predose), 0.5, 1, 1.5, 3, 6, and 12 hours; midazolam + 1-OH-midazolam (CYP3A isozymes): 0, 5, and 15 minutes, 0.5, 1, 2, and 5 hours; S-warfarin + 7-OH-S-warfarin

(CYP2C9): 0, 0.5, 1, 2, 4, 8, and 24 hours; omeprazole + 5-OH-omeprazole (CYP2C19): 0, 0.5, 1, 1.5, 2, 3, and 5 hours; dextromethorphan + dextrorphan (CYP2D6): 0, 0.5, 1, 2, 3, 5, and 8 hours post-dosing.

In the digoxin study, blood samples collected on day 1 (digoxin alone), and day 8 (etravirine plus digoxin) (Figure 1b) at 0 (predose), 0.5, 1, 1.5, 2, 3, 4, 6, 9, 12, 24, 48, 72, 96, and 120 hours post-dosing.

Analyses of Analytes

Liquid chromatographic-mass spectrometry/mass spectrometry methods (validated for accuracy, precision, sensitivity, stability, recovery, matrix effects, and calibration

range) were developed for the cocktail probes (parent drugs and metabolites) and digoxin. The accuracy and precision of quality control samples were within the pre-specified criteria at all concentrations (accuracy: overall bias $\leq 20\%$ for the lower limits of quantification [LLOQ] and $\leq 15\%$ for all other concentrations; precision: total coefficient of variation $\leq 20\%$ for the LLOQs and $\leq 15\%$ for all other concentrations).

The LLOQs were: 0.05 ng/mL for dextromethorphan; 0.1 ng/mL for midazolam, 1-OH-midazolam and digoxin; 0.8 ng/mL for dextropropranolol; 1 ng/mL for omeprazole; 2 ng/mL for 5-OH-omeprazole; 5 ng/mL for S-warfarin and 7-OH-S-warfarin; and 25 ng/mL for caffeine and paraxanthine (Janssen data on file).

Data Analyses

No formal sample size calculation was performed. A minimum of 10 and 12 subjects completing all sessions was considered sufficient to allow for relevant conclusions in the drug cocktail and digoxin studies, respectively. An additional 4 subjects were randomized in each study to allow for any dropouts.

Plasma concentration–time curves were plotted for all parent drugs and their metabolites, and digoxin, and descriptive statistics were calculated using a noncompartmental model with extravascular input (WinNonLin Professional version 4.0 or 4.1, Pharsight, Mountain View, CA). Key pharmacokinetic parameters were maximum plasma concentrations (C_{\max}) and the area under the plasma concentration–time curve (AUC; determined using the linear–linear trapezoidal rule).

Statistical analyses of key pharmacokinetic parameters were performed using SAS version 8.2 (SAS Institute, Inc., Cary, NC). In the drug cocktail study, C_{\max} and AUC_{last} for each parent drug, its metabolite, and the parent:metabolite ratios were used to assess the effects of etravirine on CYP isoenzyme activities. For both day 1 and day 14 data, these parameters were compared for etravirine plus drug cocktail (test) with drug cocktail alone (reference). Statistical analyses were performed for the digoxin study for etravirine plus digoxin (test) versus digoxin alone (reference). In both studies, least square means (LSM) ratios and 90% confidence intervals (CIs) for C_{\max} and AUC_{last} were estimated with a linear mixed-effects model controlling for treatment, session and sequence as fixed effects, and subject as a random effect. The predefined limits of the 90% CIs of the LSM ratios to establish no effect were 0.80–1.25.

In the digoxin study, for subjects providing informed consent for pharmacogenetic assessments, an exploratory analysis of C_{\max} and AUC_{last} data was conducted to evaluate the effects of P-glycoprotein genotype, that is, exons 21 (position 2677) and 26 (position 3435), which are reported to affect P-glycoprotein expression.¹⁹

Safety Evaluations

In both studies, safety and tolerability data were collected throughout the study until 30–32 days after the last administration of study drug. AEs were coded using the Medical Dictionary for Regulatory Activities (MedDRA version 8.1 [drug cocktail study] or version 10.0 [digoxin study]). Safety parameters were evaluated by descriptive statistics and frequency tabulations.

Results

Participants

In the drug cocktail study, 47 healthy subjects were screened; 33 were screening failures (mainly due to not meeting the inclusion/exclusion criteria). Two subjects discontinued (day 6 and day 9) due to AEs during treatment with etravirine/drug cocktail. Day 1 pharmacokinetic data for both treatments were available for these 2 subjects. Of the 14 randomized subjects, 12 completed the drug cocktail study. In the digoxin study, 21 healthy subjects were screened; 5 were screening failures. One subject discontinued treatment due to an AE (day 11 of treatment with etravirine/digoxin), all pharmacokinetic data were available for this subject. All 16 randomized subjects completed the digoxin study. Baseline demographics are shown in Table 1.

During the drug cocktail study, 4 (29%) subjects used at least 1 concomitant therapy (dexamethasone, $n = 2$; aceponate, $n = 1$; cefaclor, $n = 1$). Eight subjects used concomitant therapy in the digoxin study (paracetamol, $n = 3$; heparinoid, seretide, sinutab, ibuprofen, loperamide hydrochloride, metoclopramide, and tobramycin, $n = 1$ for each drug). None of these therapies were considered to have any relevant pharmacokinetic effects.

Assessment of CYP1A2 Activity: Caffeine and Paraxanthine Pharmacokinetics

Mean plasma concentration–time profiles of caffeine were similar with drug cocktail given alone and with a single dose of etravirine (Figure 2a). With multiple doses of etravirine, plasma concentrations of caffeine were slightly lower compared with drug cocktail alone (Figure 2a).

Coadministration of a single etravirine dose with the drug cocktail had no effect on C_{\max} and AUC_{0-12h} of either

Table 1. Baseline Demographics

	Drug cocktail study (n = 14)	Digoxin study (n = 16)
Male, n (%)	14 (100)	16 (100)
Caucasian race, n (%)	14 (100)	16 (100)
Age, median (range), years	34.0 (21–49)	31.5 (18–46)
Body mass index, median (range), kg/m ²	23.6 (19–30)	24.0 (18–29)

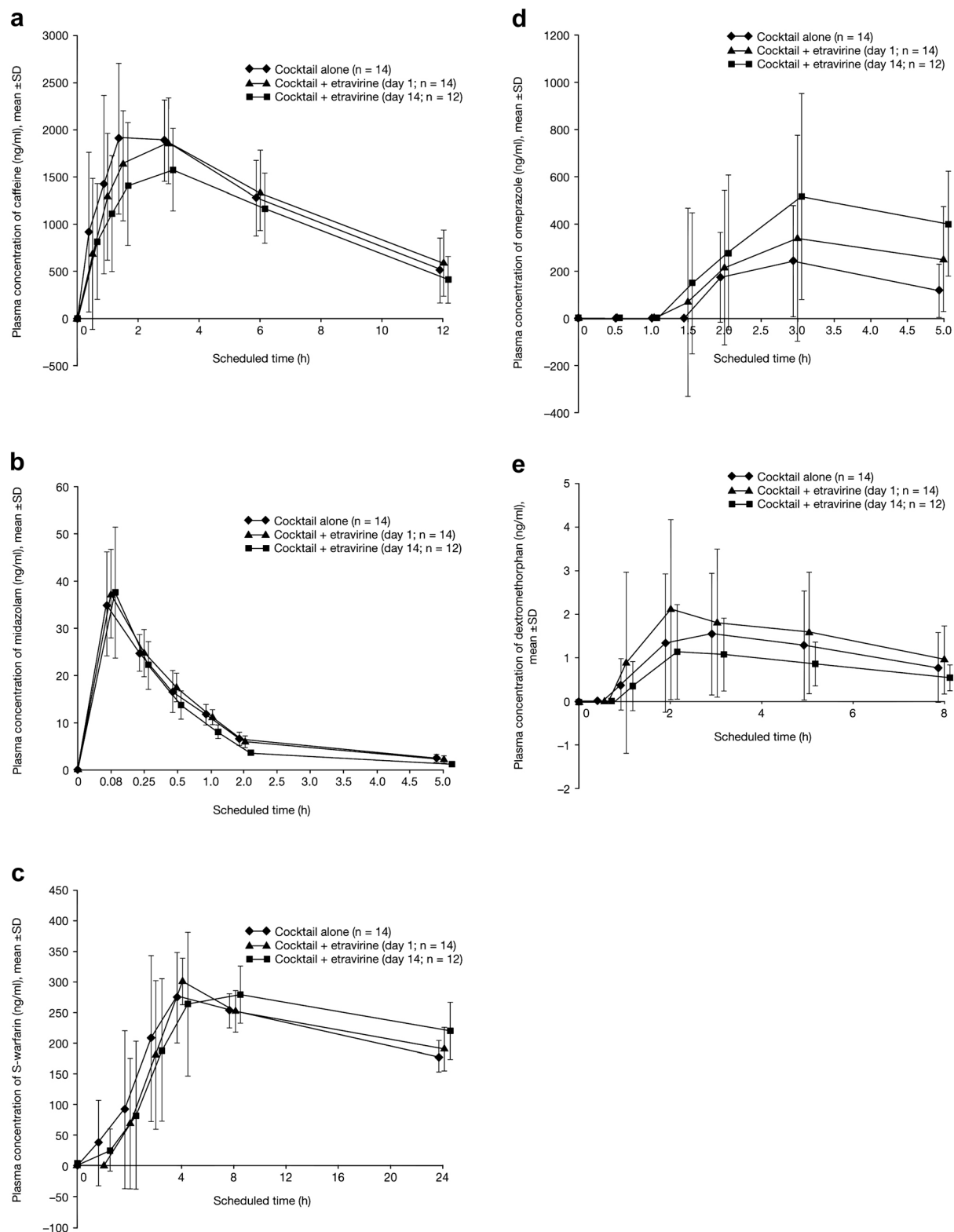


Figure 2. Mean (\pm SD) plasma concentration–time curves following administration of drug cocktail alone and in combination with etravirine. (a) Caffeine (day 1 and day 14); (b) midazolam (day 1 and day 14); (c) S-warfarin (day 1 and day 14); (d) omeprazole (day 1 and day 14); (e) dextromethorphan (day 1 and day 14). SD, standard deviation.

caffeine or paraxanthine, or the parent:metabolite ratio for these parameters, as the 90% CIs of the LSM ratios were all within the limits of 0.80 and 1.25 (Table 2). Overall, steady-state etravirine did not affect CYP1A2 activity as the 90% CIs of the LSM ratios for paraxanthine C_{\max} and AUC_{0-12h} , and the parent:metabolite ratios were within 0.80 and 1.25. The lower 90% CIs for caffeine C_{\max} and AUC_{0-12h} were only slightly below 0.80 (Table 2).

Assessment of CYP3A Isozyme Activity: Midazolam and 1-OH-Midazolam Pharmacokinetics

Mean plasma concentration–time profiles of midazolam were similar with drug cocktail given alone and with a single etravirine dose, and slightly decreased after coadministration of drug cocktail with multiple doses of etravirine (Figure 2b).

With coadministration of the drug cocktail with a single etravirine dose, the 90% CIs of the LSM ratios of midazolam and 1-OH-midazolam C_{\max} and AUC_{0-5h} and for the parent:metabolite ratios were generally within 0.80 and 1.25; except for the upper 90% CI for 1-OH-midazolam C_{\max} (1.40) and the lower 90% CI for C_{\max} P/M (0.68; Table 2). Compared with drug cocktail alone, steady-state etravirine affected midazolam metabolism. The 90% CIs of the LSM ratios for midazolam C_{\max} were outside the 0.80 and 1.25 limits, and the midazolam AUC_{0-5h} was decreased by 31% (90% CI of LSM ratio: 0.64–0.74) (Table 2). Although the AUC_{0-5h} of 1-OH-midazolam was unaffected by steady-state etravirine, C_{\max} was increased by 57% (90% CI of LSM ratio: 1.30–1.89) (Table 2). Consequently, the LSM ratios for the midazolam:1-OH-midazolam ratio for C_{\max} and AUC_{0-5h} were decreased by approximately 36%, and the 90% CIs were outside the no effect limits (except for the upper 90% CI for C_{\max} P/M; Table 2).

Assessment of CYP2C9 Activity: S-Warfarin and 7-OH-S-Warfarin Pharmacokinetics

Mean plasma concentration–time profiles of S-warfarin after drug cocktail with and without a single etravirine dose were similar, and the time to C_{\max} was delayed with steady-state etravirine (Figure 2c). The mean plasma concentration–time profiles of the metabolite, 7-OH-S-warfarin, were still increasing at the end of the 24-hour sampling period.

A single dose of etravirine had no effect on CYP2C9 activity as the 90% CIs of the LSM ratios were all within 0.80–1.25 for S-warfarin and 7-OH-S-warfarin C_{\max} and AUC_{0-24h} , and for C_{\max} P/M and AUC_{0-24h} P/M (Table 2). With steady-state etravirine, the 90% CIs of the LSM ratios for S-warfarin C_{\max} and AUC_{0-24h} were within the no effect limits versus drug cocktail alone. However, these parameters for 7-OH-S-warfarin were decreased by 27% and 42%, respectively, at steady-state etravirine (90% CIs 0.60, 0.88 and 0.44, 0.75, respectively)

Table 2. Effect of Etravirine on the Single-Dose Pharmacokinetics of the Drug Cocktail Components (n = 14, Day 1 and n = 12, Day 14)

Parameter	Day 1, LSM ratio (90% CI) ^a	Day 14, LSM ratio (90% CI) ^b
Caffeine (CYP1A2)		
C_{\max}	0.98 (0.86, 1.11)	0.84 (0.75, 0.94)
AUC_{0-12h}	1.02 (0.96, 1.08)	0.85 (0.78, 0.91)
Paraxanthine		
C_{\max}	0.97 (0.91, 1.03)	0.93 (0.88, 0.99)
AUC_{0-12h}	1.00 (0.94, 1.05)	0.93 (0.88, 0.99)
P/M ratio		
C_{\max} P/M	1.01 (0.90, 1.14)	0.90 (0.81, 1.00)
AUC_{0-12h} P/M	1.02 (0.97, 1.07)	0.91 (0.85, 0.96)
Midazolam (CYP3A isozyme)		
C_{\max}	1.00 (0.89, 1.12)	1.00 (0.78, 1.27)
AUC_{0-5h}	0.96 (0.90, 1.03)	0.69 (0.64, 0.74)
1-OH-midazolam		
C_{\max}	1.16 (0.96, 1.40)	1.57 (1.30, 1.89)
AUC_{0-5h}	1.07 (1.02, 1.13)	1.09 (1.00, 1.18)
P/M ratio		
C_{\max} P/M	0.86 (0.68, 1.09)	0.64 (0.49, 0.83)
AUC_{0-5h} P/M	0.90 (0.81, 1.00)	0.63 (0.57, 0.70)
S-warfarin (CYP2C9)		
C_{\max}	1.01 (0.94, 1.08)	0.99 (0.88, 1.12)
AUC_{0-24h}	1.01 (0.98, 1.04)	1.05 (0.93, 1.18)
7-OH-S-warfarin		
C_{\max}	1.05 (0.94, 1.17)	0.73 (0.60, 0.88)
AUC_{0-24h}	1.05 (0.92, 1.21)	0.58 (0.44, 0.75)
P/M ratio		
C_{\max} P/M	0.96 (0.83, 1.10)	1.38 (1.17, 1.63)
AUC_{0-24h} P/M	0.96 (0.85, 1.08)	1.82 (1.51, 2.19)
Omeprazole (CYP2C19)		
C_{\max}	1.33 (0.90, 1.97)	1.79 (0.92, 3.50)
AUC_{0-5h}	1.62 (1.00, 2.63)	1.83 (0.78, 4.29)
5-OH-omeprazole		
C_{\max}	1.07 (0.78, 1.47)	0.46 (0.26, 0.81)
AUC_{0-5h}	1.25 (0.76, 2.05)	0.43 (0.20, 0.96)
P/M ratio		
C_{\max} P/M	1.24 (1.04, 1.47)	4.04 (3.40, 4.81)
AUC_{0-5h} P/M	1.30 (1.12, 1.51)	4.32 (3.74, 5.00)
Dextromethorphan (CYP2D6)		
C_{\max}	1.16 (0.91, 1.47)	0.85 (0.59, 1.23)
AUC_{0-8h}	1.27 (1.03, 1.57)	0.94 (0.72, 1.23)
Dextrorphan		
C_{\max}	0.90 (0.82, 0.99)	0.81 (0.69, 0.94)
AUC_{0-8h}	0.99 (0.93, 1.04)	0.85 (0.78, 0.94)
P/M ratio		
C_{\max} P/M	1.28 (1.06, 1.56)	1.11 (0.86, 1.44)
AUC_{0-8h} P/M	1.28 (1.07, 1.55)	1.12 (0.90, 1.38)

AUC, area under the plasma concentration–time curve for the time interval indicated; CI, confidence interval; C_{\max} , maximum plasma concentration; CYP, cytochrome P450; LSM, least square means; P/M, parent/metabolite ratio.

^aTest = etravirine (single-dose, day 1) + cocktail versus reference = single-dose cocktail alone.

^bTest = etravirine (at steady state, day 14) + cocktail versus reference = single-dose cocktail alone.

(Table 2). Consequently, the parent:metabolite ratios were significantly increased by 38% (C_{\max} P/M) and 82% (AUC_{0-24h} P/M).

Assessment of CYP2C19 Activity: Omeprazole and 5-OH-Omeprazole Pharmacokinetics

Mean plasma concentration–time profiles of omeprazole were increased with a single dose of etravirine, and with steady-state etravirine compared with drug cocktail alone (Figure 2d).

The LSM ratios of omeprazole C_{\max} and AUC_{0-5h} were increased by 33% and 62%, respectively, with a single etravirine dose, and by 79% and 83%, respectively, with steady-state etravirine, compared with drug cocktail alone; the upper 90% CIs of the LSM ratios were above 1.25 (Table 2). For 5-OH-omeprazole, C_{\max} and AUC_{0-5h} were significantly increased (7% and 25%, respectively) by a single dose of etravirine, and significantly decreased (54% and 57%, respectively) by steady-state etravirine, as the 90% CIs of the LSM ratios were all outside the 0.80–1.25 range (Table 2). Etravirine significantly increased the parent/metabolite ratios of omeprazole and 5-OH-omeprazole by 24% and 30%, respectively (single-dose etravirine), and 304% and 332%, respectively (steady-state etravirine); most of the 90% CIs were outside the no effect range (Table 2).

Assessment of CYP2D6 Activity: Dextromethorphan and Dextrorphan Pharmacokinetics

Relative to drug cocktail alone, the mean plasma concentration–time profiles of dextromethorphan were increased with a single etravirine dose and decreased with multiple doses of etravirine (Figure 2e).

A single dose of etravirine slightly increased the LSM ratio for dextromethorphan C_{\max} and AUC_{0-8h} , and the parent/metabolite ratios for both pharmacokinetic parameters; the upper 90% CIs of the LSM ratios were above 1.25. The C_{\max} and AUC_{0-8h} of dextrorphan were not affected by a single etravirine dose (Table 2). Compared with drug cocktail alone, coadministration of multiple etravirine doses with drug cocktail slightly decreased the LSM ratios of C_{\max} and AUC_{0-8h} of dextromethorphan and dextrorphan (lower 90% CIs were below 0.85), and slightly increased the parent/metabolite ratios by 11% and 12%, respectively (upper 90% CIs were above 1.25).

Assessment of P-Glycoprotein Activity: Digoxin Pharmacokinetics

Mean plasma concentration–time profiles of digoxin were slightly higher for up to 2 hours post-dosing with etravirine plus digoxin versus digoxin alone (Figure 3). From 2 hours post-dose onwards, similar plasma concentration–time profiles were observed (Figure 3).

In the presence of steady-state etravirine, the LSM ratio (90% CIs) for digoxin C_{\max} and AUC_{last} were 1.19 (0.96,

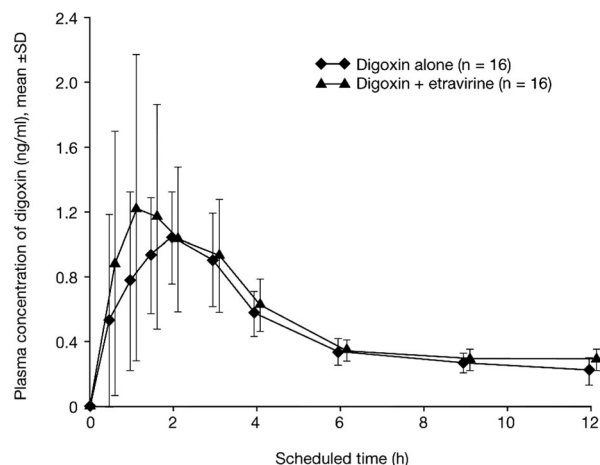


Figure 3. Mean (\pm SD) plasma concentration–time curves following administration of digoxin alone and in combination with etravirine. SD, standard deviation.

1.49) and 1.18 (0.90, 1.56), respectively, relative to digoxin alone.

The number of subjects with G2677T polymorphisms (exon 21) were 7, 7, and 2 for G/T or A/G combined, G/G and T/T, respectively. Individual digoxin values for C_{\max} and AUC_{last} were generally in the same range, with larger interindividual variability in the G/T or A/G combined group for C_{\max} , and in the G/G genotype group for AUC_{last} (data not shown). For C3435T polymorphisms (exon 26), 5, 7, and 4 subjects had C/C, C/T, and T/T genotypes, respectively. Digoxin C_{\max} and AUC_{0-12h} values were generally comparable across these groups (data not shown).

Safety and Tolerability

Two subjects discontinued treatment in the drug cocktail study ($n=1$ grade 2 rash; $n=1$ grade 2 pharyngolaryngeal pain; both during combined treatment). One subject in the digoxin study discontinued during the coadministration phase due to grade 2 viral bronchitis. All 14 subjects in the drug cocktail study, and 12 of 16 subjects in the digoxin study, experienced at least 1 treatment-emergent AE, all of which were grade 1 or 2.

In the cocktail study, the most frequent AEs were fatigue and hypoesthesia occurring in 9 subjects each, whereas headache was the most common AE in the digoxin study (4 subjects during etravirine administration alone; 5 subjects during coadministration of etravirine and digoxin). In the drug cocktail study, AEs considered at least possibly related to etravirine or drug cocktail were reported by 12 and 13 subjects, respectively. In the digoxin study, seven subjects reported an AE considered at least possibly related to etravirine and 1 subject an AE considered at least possibly related to digoxin.

Two subjects in the drug cocktail study ($n=1$, hyponatremia during combined treatment; $n=1$ hypophosphatemia during the follow-up phase), and 1 in the

digoxin study (hypophosphatemia during digoxin and etravirine coadministration) developed a grade 3 treatment-emergent laboratory abnormality. None of the laboratory abnormalities were reported as an AE. No consistent or clinically relevant changes in cardiovascular safety parameters were observed in either study.

Discussion

Based on 90% CIs of the LSM ratios (no effect limits 0.80 and 1.25) for AUC_{last} of the parent compounds, key metabolites and parent:metabolite ratios (summarized in Figure 4), using the well-established cocktail approach,^{10,11} the drug cocktail study demonstrated that etravirine is an inhibitor of CYP2C9 and CYP2C19 activity, is a CYP3A inducer, and has no effect on CYP1A2 or CYP2D6 activity in vivo. The digoxin study indicated that etravirine inhibits P-glycoprotein in vivo. Results are consistent with in vitro findings (Janssen, data on file),^{5,6} and drug–drug interaction studies with etravirine and known substrates for these isoenzymes.^{6,8,9}

The finding that etravirine had no effect on CYP1A2 or CYP2D6 activities in vivo, confirms in vitro findings in which etravirine K_i values for these isoenzymes were high (7.0 and 15 μ M, respectively, Janssen, data on file). No

specific drug–drug interaction studies have been conducted for CYP1A2 substrates. Coadministration of etravirine had no effect on the AUC or C_{max} of the CYP2D6 substrate paroxetine.⁸

A single dose of etravirine had no effect on CYP3A activity, whereas this enzyme was induced by steady-state etravirine. As midazolam AUC_{last} was decreased by 31% (LSM ratio 0.69, 90% CIs 0.64, 0.74), etravirine can be considered a weak (i.e., 20–50% AUC decrease) CYP3A inducer. This finding confirms the etravirine-induced CYP3A expression observed in vitro,⁵ so caution should be exercised when coadministering etravirine with agents that affect the CYP3A pathway. Therefore, numerous drug–drug interaction studies with etravirine and CYP3A substrates including other antiretrovirals (HIV protease inhibitors, maraviroc, elvitegravir)^{6,9} and other drugs (anti-infectives, certain statins, sildenafil)^{6,8} have been conducted. Etravirine should not be used with unboosted protease inhibitors.^{6,9} Etravirine can be coadministered with some ritonavir-boosted protease inhibitors such as darunavir, lopinavir, or saquinavir.⁹ No clinically relevant effects on the pharmacokinetics of ritonavir-boosted darunavir, lopinavir (soft gel or meltrex formulations), or saquinavir were observed when coadministered with etravirine.^{9,20,21} Coadministration of etravirine significantly decreased maraviroc AUC_{0-12h} by 53% compared with maraviroc alone,^{8,22} but had no significant effect on elvitegravir pharmacokinetic parameters.⁹ Rifabutin, clarithromycin, sildenafil, and atorvastatin AUCs have also been shown to significantly decrease in the presence of etravirine, that is, the LSM ratios (90% CIs) 0.83 (0.75, 0.94), 0.61 (0.53, 0.69), 0.43 (0.36, 0.51), and 0.63 (0.58, 0.68), respectively.⁹

In vitro etravirine inhibits CYP2C9 ($K_i = 0.58 \mu$ M; Janssen, data on file). This finding was confirmed in vivo in the drug cocktail study as the LSM ratio (90% CIs) of 7-OH-S-warfarin was 0.58 (0.44, 0.75), although interpretation of the pharmacokinetic findings is limited by too short a sampling period; warfarin should have been sampled at least over 72 hours, although truncated sampling times have been explored.²³

CYP2C19 enzyme activity was inhibited by etravirine, confirming in vitro data (Janssen data on file), although consistent with previous findings^{24–26} interindividual variability in the data was high. Omeprazole metabolism was inhibited by etravirine (LSM ratio 0.43, 90% CI 0.20, 0.96 for 5-OH-omeprazole at steady-state etravirine). However, etravirine had no clinically relevant effects on the pharmacokinetics of CYP2C19 substrates fluconazole, voriconazole, and ethinylestradiol^{8,27,28} possibly because alternative metabolic pathways other than CYP2C19 may be involved.

In vitro data with P-glycoprotein are conflicting as etravirine has been reported to inhibit (Janssen, data on file) and have no effect on this transporter.⁷ Digoxin

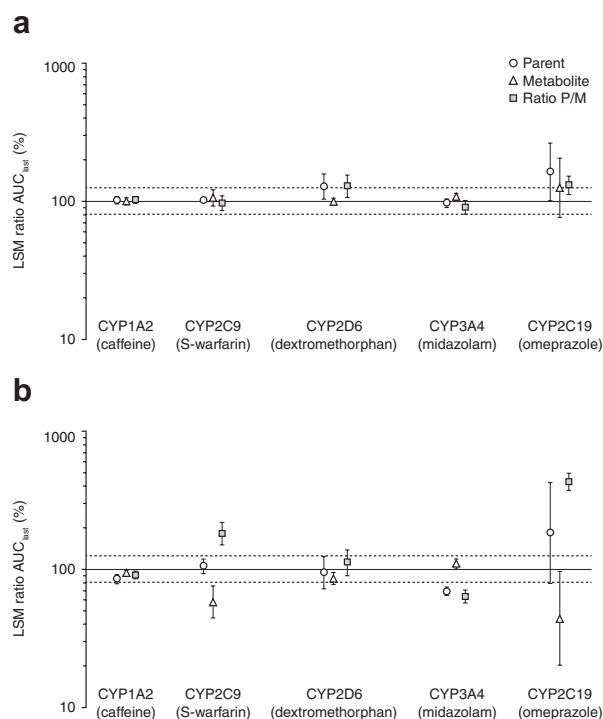


Figure 4. Summary of the effect of etravirine on the single-dose pharmacokinetics of CYP probes in the drug cocktail on (a) day 1 (single-dose etravirine) and (b) day 14 (multiple doses of etravirine). Values are LSM ratios and 90% CIs, the dotted lines represent the 80% and 125% limits. AUC_{last} , area under the concentration–time curve from time 0 to the time of the last quantifiable concentration; CI, confidence interval; LSM, least square means.

AUC_{0–8h} was slightly increased in the presence of etravirine (LSM ratio 1.18, 90% CI 0.90, 1.56), indicating inhibition of P-glycoprotein activity in vivo. However, given this small effect, the inhibition of P-glycoprotein by etravirine is not expected to lead to clinically relevant interactions. For example, the AUC of tenofovir disoproxil fumarate, a P-glycoprotein substrate, was not significantly affected by etravirine coadministration (LSM ratio 1.15; 90% CI 1.09, 1.21).⁹ Therefore, no dose adjustments are necessary; although if etravirine is coadministered with digoxin, plasma concentrations of the latter should be monitored. In retrospect, digoxin could have been combined with the Cooperstown cocktail, as no interaction is observed between digoxin and midazolam²⁹ however, this “6 + 1” approach has not been fully validated.

The two studies have some limitations. Firstly, the analyses are reliant on the probes being selective. The Cooperstown 5 + 1 cocktail is a validated method to assess CYP1A2, 3A, 2C9, 2C19, and 2D6 using caffeine, midazolam, warfarin, omeprazole, and dextromethorphan as respective probes.¹¹ The approach has also been used to assess the effects of other drugs such as the HIV entry inhibitor apilavir, the HIV protease inhibitor lopinavir/ritonavir,³¹ oral contraceptives,³² and panopazib³³ on CYP enzymes. However, as detailed above, we recognize that several of our analyses used probes that slightly deviated from the validated phenotypic indexes (e.g., S-warfarin/7-OH-S-warfarin ratio was used as a biomarker for CYP2C9). Nevertheless, S-warfarin 7-hydroxylation has been suggested as a possible reaction to probe CYP2C9-based drug interactions.¹³

Furthermore, there are no validated probes available for P-glycoprotein. Although digoxin is a commonly used probe for this transporter,^{34,35} there are limitations in its use, such as lack of specificity (digoxin is also a substrate for OATP2), no optimal single oral dose for phenotyping or partial AUC time to use, influence of formulation on digoxin pharmacokinetics, and the contribution of hepatic P-glycoprotein activity.³⁶

Another possible limitation of the analyses is the relatively small sample size (14–16 patients). Although no formal a priori sample size calculation was performed, the study design took into consideration the variability, and the expected magnitude of effects. In addition, our studies were of a similar sample size to similar previous studies.^{37,38} We also acknowledge that we used AUC_{last} rather than AUC_∞ to calculate the LSM ratios. While this is not as robust as full characterization of the AUC_∞, we believe that using this parameter did not change the overall findings. Finally, since the statistical analyses use pharmacokinetic parameters that include drug concentrations measured during the absorption phase, it is theoretically possible that drug interactions at the absorption stage could affect the results. However, our

findings show consistency with levels of both the drug and the metabolite profiles from previous cocktail probe studies.^{11,18,24,37,39,40} This, coupled with the use of validated probes which are selective, suggests this would have only a negligible effect.

In conclusion, these studies have shown that etravirine is a weak inducer of CYP3A isozymes and an inhibitor of CYP2C9, CYP2C19, and P-glycoprotein.

Acknowledgments

Both trials were sponsored by Janssen. The authors wish to thank the study subjects, study center staff, and Janssen study personnel. The authors are also grateful to Monika Schöller-Gyüre, MD, for her input into the design and conduct of the study. Medical writing support was provided by Karen Pilgram (all drafts and incorporating comments up to submission) and Jackie Phillipson (response to reviewers' comments) of Gardiner-Caldwell Communications, Macclesfield, UK, and funded by Janssen.

Financial Disclosures

TNK, RMVS-R, FA, GDS, MP, LL, and RMWH are full-time employees of Janssen. JO was a full-time employee of Janssen at the time the research was carried out and is now an employee of NV Novartis Pharma SA. TS was a full-time employee of Janssen at the time the research was performed and is now owner and consultant at ClinOps Consulting BVBA.

References

1. Katlama C, Clotet B, Mills A, et al. Efficacy and safety of etravirine at week 96 in treatment-experienced HIV-1-infected patients in the DUET-1 and DUET-2 trials. *Antivir Ther*. 2010;15:1045–1052.
2. DHSS. Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. Department of Health and Human Services (updated 7 May 2013). <http://www.aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf>. Accessed May 19, 2013.
3. European AIDS Clinical Society (EACS). Guidelines: clinical management of treatment of HIV infected adults in Europe. Version 6.1. November 2012. http://www.europeanaidsclinicalsociety.org/index.php?option=com_content&view=article&id=59&Itemid=41. Accessed April 25, 2013.
4. DHHS. Guidelines for the use of antiretroviral agents in pediatric HIV infection. November 2012. <http://aidsinfo.nih.gov/contentfiles/lvguidelines/pediatricguidelines.pdf>. Accessed June 11, 2013.
5. Yanakakis LJ, Bumpus NN. Biotransformation of the antiretroviral drug etravirine: metabolite identification, reaction phenotyping, and characterization of autoinduction of cytochrome P450-dependent metabolism. *Drug Metab Dispos*. 2012;40:803–814.
6. Schöller-Gyüre M, Kakuda TN, Raoof A, De Smedt G, Hoetelmans RM. Clinical pharmacokinetics and pharmacodynamics of etravirine. *Clin Pharmacokinet*. 2009;48:561–574.
7. Zembruski LCL, Haefeli WE, Weiss J. Interaction potential of etravirine with drug transporters assessed in vitro. *Antimicrob Agents Chemother*. 2011;55:1282–1284.
8. Kakuda TN, Schöller-Gyüre M, Hoetelmans RMW. Pharmacokinetic interactions between etravirine and non-antiretroviral drugs. *Clin Pharmacokinet*. 2011;50:25–39.

9. Kakuda TN, Schöller-Gyüre M, Hoetelmans RM. Clinical perspectives on antiretroviral drug-drug interactions with the non-nucleoside reverse transcriptase inhibitor etravirine. *Antivir Ther*. 2010;15:817–829.
10. Zhou H, Tong Z, McLeod JF. Cocktail approaches and strategies in drug development: valuable tool or flawed science? *J Clin Pharmacol*. 2004;44:120–134.
11. Chainuvati S, Nafziger AN, Leeder JS, et al. Combined phenotypic assessment of cytochrome P450 1A2, 2C9, 2C19, 2D6 and 3A, N-acetyltransferase-2, and xanthine oxidase activities with the ‘Cooperstown 5+1 cocktail’. *Clin Pharmacol Ther*. 2003;74:437–447.
12. Fenner KS, Troutman MD, Kempshall S, et al. Drug–drug interactions mediated through P-glycoprotein: clinical relevance and in vitro–in vivo correlation using digoxin as a probe drug. *Clin Pharmacol Ther*. 2009;85:173–181.
13. Yuan R, Madani S, Wei XX, Reynolds K, Huang SM. Evaluation of cytochrome P450 probe substrates commonly used by the pharmaceutical industry to study in vitro drug interactions [Office of Clinical Pharmacology and Biopharmaceutics, Center for Drug Evaluation and Research, US FDA]. *Drug Metab Dispos*. 2002;30:1311–1319.
14. Kim M, Nafziger AN, Kirchheiner J, Bauer S, Gaedigk A, Bertino JS. Effect of fluvastatin on CYP2C9 activity using warfarin as a probe. *Clin Pharmacol Ther*. 2004;75:P28. doi: 10.1016/j.clpt.2003.11.106
15. Hakooz NM. Caffeine metabolic ratios for the in vivo evaluation of CYP1A2, N-acetyltransferase 2, xanthine oxidase and CYP2A6 enzymatic activities. *Curr Drug Metab*. 2009;10:329–338.
16. Quinney SK, Malireddy SR, Vuppalandhi R, et al. Rate of onset of inhibition of gut-wall and hepatic CYP3A by clarithromycin. *Eur J Clin Pharmacol*. 2013;69:439–448.
17. Jurica J, Barteczek R, Zourkova A, et al. Serum dextromethorphan/dextrorphan metabolic ratio for CYP2D6 phenotyping in clinical practice. *J Clin Pharm Ther*. 2012;37:486–490.
18. Streetman DS, Bleakley JF, Kim JS, et al. Combined phenotypic assessment of CYP1A2, CYP2C19, CYP2D6, CYP3A, N-acetyltransferase-2, and xanthine oxidase with the ‘Cooperstown cocktail’. *Clin Pharmacol Ther*. 2000;68:375–383.
19. Cascorbi I, Gerloff T, John A, et al. Frequency of single nucleotide polymorphisms in the P-glycoprotein drug transporter MDR1 gene in white subjects. *Clin Pharmacol Ther*. 2001;69:169–174.
20. Schöller-Gyüre M, Kakuda TN, Sekar V, et al. Pharmacokinetics of darunavir/ritonavir and TMC125 alone and coadministered in HIV-negative volunteers. *Antiviral Ther*. 2007;12:789–796.
21. Schöller-Gyüre M, Kakuda TN, Witek J, et al. Steady-state pharmacokinetics of etravirine and lopinavir/ritonavir melt extrusion formulation, alone and in combination, in healthy HIV-negative volunteers. *J Clin Pharmacol*. 2013;53:202–210.
22. Kakuda TN, Abel S, Davis J, et al. Pharmacokinetic interactions of maraviroc with darunavir-ritonavir, etravirine, and etravirine-darunavir-ritonavir in healthy volunteers: results of two drug interaction trials. *Antimicrob Agents Chemother*. 2011;55:2290–2296.
23. Ma JD, Nafziger AN, Kashuba AD, et al. Limited sampling strategy of S-warfarin concentrations, but not warfarin S/R ratios, accurately predicts S-warfarin AUC during baseline and inhibition in CYP2C9 extensive metabolizers. *J Clin Pharmacol*. 2004;44:570–576.
24. Zhang W, Han F, Guo P, et al. Simultaneous determination of tolbutamide, omeprazole, midazolam and dextromethorphan in human plasma by LC-MS/MS—a high throughput approach to evaluate drug-drug interactions. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2010;878:1169–1177.
25. Andersson T, Andren K, Cederberg C, Lagerstrom PO, Lundborg P, Skanberg I. Pharmacokinetics and bioavailability of omeprazole after single and repeated oral-administration in healthy-subjects. *Br J Clin Pharmacol*. 1990;29:557–563.
26. Andersson T. Pharmacokinetics, metabolism and interactions of acid pump inhibitors—focus on omeprazole, lansoprazole and pantoprazole. *Clin Pharmacokinet*. 1996;31:9–28.
27. Kakuda TN, Van Solingen-Ristea R, Aharchi F, et al. Pharmacokinetics and short-term safety of etravirine in combination with fluconazole or voriconazole in HIV-negative volunteers. *J Clin Pharmacol*. 2013;53:41–50.
28. Schöller-Gyüre M, Kakuda TN, Woodfall B, et al. Effect of steady-state etravirine on the pharmacokinetics and pharmacodynamics of ethinylestradiol and norethindrone. *Contraception*. 2009;80:44–52.
29. Kirby B, Kharasch ED, Thummel KT, Narang VS, Hoffer CJ, Unadkat JD. Simultaneous measurement of in vivo P-glycoprotein and cytochrome P450 3A activities. *J Clin Pharmacol*. 2006;46:1313–1319.
30. Johnson BM, Song IH, Adkison KK, et al. Evaluation of the drug interaction potential of aplaviroc, a novel human immunodeficiency virus entry inhibitor, using a modified Cooperstown 5 + 1 cocktail. *Clin Pharmacol*. 2006;46:577–587.
31. Yeh RF, Gaver VE, Patterson KB, et al. Lopinavir/ritonavir induces the hepatic activity of cytochrome P450 enzymes CYP2C9, CYP2C19, and CYP1A2 but inhibits the hepatic and intestinal activity of CYP3A as measured by a phenotyping drug cocktail in healthy volunteers. *J Acquir Immune Defic Syndr*. 2006;42:52–60.
32. Shelepova T, Nafziger AN, Victory J, et al. Effect of a triphasic oral contraceptive on drug-metabolizing enzyme activity as measured by the validated Cooperstown 5+1 cocktail. *J Clin Pharmacol*. 2005;45:1413–1421.
33. Goh BC, Reddy NJ, Dandamudi UB, et al. An evaluation of the drug interaction potential of pazopanib, an oral vascular endothelial growth factor receptor tyrosine kinase inhibitor, using a modified Cooperstown 5 + 1 cocktail in patients with advanced solid tumors. *Clin Pharmacol Ther*. 2010;88:652–659.
34. Hirabayashi H, Sugimoto H, Matsumoto S, Amano N, Moriwaki T. Development of a quantification method for digoxin, a typical P-glycoprotein probe in clinical and non-clinical studies, using high performance liquid chromatography-tandem mass spectrometry: the usefulness of negative ionization mode to avoid competitive adduction formation. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2011;879:3837–3844.
35. Keogh JP, Kunta JR. Development, validation and utility of an in vitro technique for assessment of potential clinical drug-drug interactions involving P-glycoprotein. *Eur J Pharm Sci*. 2006;27:543–554.
36. Ma JD, Tsunoda SM, Bertino JS, et al. Evaluation of in vivo P-glycoprotein phenotyping probes: a need for validation. *Clin Pharmacokinet*. 2010;49:223–237.
37. Blakey GE, Lockton JA, Perrett J, et al. Pharmacokinetic and pharmacodynamic assessment of a five-probe metabolic cocktail for CYPs 1A2, 3A4, 2C9, 2D6 and 2E1. *Br J Clin Pharmacol*. 2004;57:162–169.
38. Ieiri I, Tsunemitsu S, Maeda K, et al. Mechanisms of pharmacokinetic enhancement between ritonavir and saquinavir; micro/small dosing tests using midazolam (CYP3A4), fexofenadine (p-glycoprotein), and pravastatin (OATP1B1) as probe drugs *J Clin Pharmacol*. 2013;53:654–661.
39. Frye R, Matzke G, Adedoyin A, Porter JA, Branch RA. Validation of the five-drug ‘Pittsburgh cocktail’ approach for assessment of selective regulation of drug-metabolizing enzymes. *Clin Pharmacol Ther*. 1997;62:365–376.
40. Turpault S, Brian W, Van Horn R, et al. Pharmacokinetic assessment of a five-probe cocktail for CYPs 1A2, 2C9, 2C19, 2D6 and 3A. *Br J Clin Pharmacol*. 2009;68:928–935.