Effect of widely used combinations of antiretroviral therapy on liver CYP3A4 activity in HIV-infected patients

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Aims

To evaluate the effects of combined antiretroviral drugs (HAART) on liver CYP3A4 activity using the [¹⁴C-N-methyl]-erythromycin breath test (ERMBT).

Methods

HIV-infected patients (31 women, 30 men) with mean ($\pm$ SD) age of 38 ± 9 years were enrolled and underwent complete clinical and laboratory evaluation. Patients were divided into five groups and were treated with two nucleoside analogues (NAs) and one of the following: nelfinavir alone ($n = 13$), any ritonavir-boosted protease inhibitor with ($n = 8$) or without ($n = 13$) nevirapine, nevirapine alone ($n = 15$), or a third NA ($n = 12$). Three or four ERMBTs were performed 7 days prior to (D-7) and at the beginning of treatment (D0), D14 (only for patients taking nevirapine) and on D28.

Results

Mean baseline liver CYP3A4 activity displayed high interindividual variability (47%) but low intraindividual variability (15%). Women had 30% higher ERMBT values than men [2.7 ± 1.3 vs. 1.9 ± 0.7 ; 95% confidence interval (CI) 20.5, 49.5; $P = 0.003$]. The ERMBT data correlated with body weight, α - and β -globulins and alanin aminotransferases ($0.10 < r_s < 0.20$; $P < 0.01$). Whereas nevirapine had no effect on liver CYP3A4 activity, nelfinavir-based and ritonavir-boosted drug regimens inhibited it by 69% (95% CI 64.7, 72.9; $P = 0.005$) and by 95% (95% CI 93.3, 96.7; $P = 0.001$), respectively.

Conclusion

Evaluation of the effect of HAART on liver CYP3A4 activity may aid in preventing inappropriate treatment regimens in HIV-infected patients.

Introduction

Several diseases, including infection with the human immunodeficiency virus (HIV), can alter hepatic metabolic capacity [1–5]. In these conditions, multiple mechanisms involving infectious, inflammatory or nutritional factors can modulate the activities of cytochromes P450 (CYPs), the major enzymes involved in the metabolism of drugs and other xenobiotics [6–10]. Of the CYPs

expressed in human liver, CYP3A4 accounts for up to 35% of total CYP content and is believed to metabolize up to 60% of currently marketed drugs, including a number of HIV-1 protease inhibitors (PIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) [11, 12]. HIV-infected patients often require multiple drug therapy and are thus considered at high risk for drug–drug interactions, which in turn pose challenges to

clinicians treating HIV-infected individuals [12]. However, the effect of combination therapy on hepatic CYP3A4 activity is not known, making it difficult for the clinician to predict the likelihood of drug–drug interactions.

One method often used to predict hepatic metabolic capacity *in vivo* in humans involves highly selective, reproducible and non-invasive drug probes. The [^{14}C -N-methyl]-erythromycin breath test (ERMBT) is one of the most reliable and reproducible indices of CYP3A4 activity [13–15]. This test requires injection of a trace amount of ^{14}C -labelled erythromycin, which is N-demethylated exclusively by CYP3A4 in the liver. Because the N-demethylated ^{14}C -labelled group ultimately appears in breath as ^{14}C -labelled carbon dioxide, CYP3A4 activity can be determined from changes in the rate of production of $^{14}\text{CO}_2$ from a single breath sample. The ERMBT has been used in healthy subjects [16, 17], cancer patients [18] and transplant recipients to evaluate both inter- and intraindividual variability in liver CYP3A4 activity [19–22].

Of the drugs used to treat HIV infection, PIs and NNRTIs have proved effective, given either alone or in combination, as part of highly active antiretroviral therapy (HAART). In addition to undergoing extensive metabolism by hepatic (and intestinal) CYP3A4 [12, 23–26], these agents are well-known CYP3A4 modulators and have been implicated in drug–drug and drug–food interactions [12, 23–29]. Limited data are available regarding hepatic CYP3A4 activity in HIV-infected patients, as well as the effect of HAART on hepatic CYP3A4 activity. This lack of information, coupled with the known large interindividual variability in efficacy and tolerability of HAART, frequently leads to difficulties in anticipating the extent of drug–drug interactions and in the optimization of therapeutic regimens.

In the current study, we have characterized, in 61 HIV-infected patients starting HAART, the effect of several drug combinations on liver CYP3A4 activity using the ERMBT. Based on previous reports, we hypothesized that a nelfinavir-based drug regimen would inhibit liver CYP3A4 activity by at least 50% [26], that any drug regimen containing ritonavir would inhibit activity by at least 90% [26–28], that a nevirapine-based drug regimen would induce activity by at least 50% [12, 26, 29] and that a combination of three nucleoside analogues (NAs) would have no effect [12, 26]. Furthermore, as the ERMBT may not comply with the radiation safety guidelines in all countries [30], we have attempted to identify independent surrogate markers to estimate liver CYP3A4 activity.

Methods

Subjects and study design

The study was approved by the ethics committee of Paris-Saint Louis and all patients gave their written informed consent prior to enrolment. All patients attending the Internal Medicine Department at Lariboisière Hospital (Paris, France) with documented HIV infection and requiring HAART (based on CD4 count and plasma viral load [31]) and who had never been treated prior to the study, or who had been on drug holiday awaiting new antiretroviral regimen at the time of enrolment for at least 1 month, were eligible to participate. Drug combinations were chosen by the referring physician. Patients were excluded if they had a history of allergy to erythromycin. Women of childbearing age were required to have a negative pregnancy test before enrolment.

Patients were asked to fast overnight and to return to the Internal Medicine Department on three or four separate occasions during the course of the study. Seven days prior to the beginning of treatment (D–7), patients underwent clinical and laboratory evaluations and an ERMBT. A medical history was taken, and height, weight, and body mass index (BMI) were documented. Laboratory evaluations included nutritional (albumin, prealbumin, transferrin serum levels), inflammatory [haptoglobin, C-reactive protein (CRP), α_1 -acid glycoprotein (AAG), α - and β -globulins] and immunological (CD4⁺ and CD8⁺ count as measured by flow cytometry) testing, assessment of hepatic function [alanine aminotransferase (ALT) and prothrombin time] and HIV-1 RNA measurement (by the polymerase chain reaction; Quantiplex Bayer[®] bDNA method, Paris-La Défense, France). Current medications were also recorded.

On day D0, immediately before the beginning of treatment, patients underwent a second ERMBT. Patients then received two NAs and one of the following: nelfinavir alone, a ritonavir-boosted PI with or without nevirapine, nevirapine alone, or a third NA. Nelfinavir and ritonavir dosage regimens were 1250 mg bid and 100 mg bid, respectively. The ritonavir-boosted PI was indinavir (400 mg bid), lopinavir (400 mg bid) or amprenavir (400 mg bid). Nevirapine dosage was 200 mg day^{–1} for the first 2 weeks, which was increased to 200 mg bid thereafter.

On D28, patients underwent a third ERMBT and the trough concentration of the PI and/or nevirapine was determined. Patients were also asked to return their empty pill boxes back to one of the study investigators (S.M.) to assess compliance with treatment. Patients taking nevirapine underwent an additional ERMBT on D14 to evaluate whether the increase in the daily dosing

regimen induced hepatic CYP3A4 activity, as previously described with efavirenz, another NNRTI [32]. At the end of the study, patients were made appointments with their respective referring physicians for subsequent follow-up.

Erythromycin breath test procedure

Erythromycin breath test kits were purchased from Metabolic Solutions, Inc. (Nashua, NH, USA) and were administered as described previously [13, 14]. To decrease interindividual variation in CO₂ production, the ERMBT was always performed on patients who had rested. The percentage of administered ¹⁴C exhaled per minute was determined at 20 min after erythromycin administration, assuming that at least 98% of ¹⁴C-erythromycin was injected into the patient and an endogenous carbon dioxide production of 5 mmol min⁻¹ m⁻² body surface area [33]. Liver CYP3A4 activity was expressed as the percent of erythromycin metabolized per hour, as described previously [33].

Determination of steady-state trough concentrations

To assess compliance with treatment, trough concentrations were determined on the morning of D28 for all of the PIs (except ritonavir) and nevirapine using previously validated high-performance liquid chromatography (HPLC) methods [34]. For the PIs, the HPLC system (Thermoquest, Fremont, CA, USA) consisted of an isocratic pump, an AS3000 autosample injector, a PC-1000 intergrator, and a UV-1000 variable-wavelength ultraviolet detector. Analytes were separated at 37 °C using a column heater (Temperature Control System; Waters, Milford, MA, USA) and a Symmetry 5 µm C₁₈ column (250 × 4.6 mm i.d.) protected by a Guard-Pak µBondapack C₁₈ precolumn (Waters). For nevirapine, the HPLC system consisted of three 114M pumps (Beckman, Berkeley, CA, USA), a Wisp 717 plus autosample injector (Waters), two switch valves (Lea Switch I&T, Labmetrics Technology, Roissy, France), a 481 variable-wavelength ultraviolet detector model (Waters) and a System Gold 2 integrator (Beckman). Analytes were separated at 30 °C with a column heater (Croco-Cil, Cil Cluzeau, Paris la Défense, France) and a Symmetry Shield 5 µm C₁₈ column (250 × 4.6 mm i.d.) (Waters) protected by a 2 µm Upchurch filter (Oak Harbor, WA, USA). For all drugs analysed, the calibration curves were linear up to 40 000 ng ml⁻¹. Within-run coefficients of variation ranged from 1% (amprenavir) to 3% (nevirapine) at high concentrations, from 1.5% (nelfinavir) to 3.5% (nevirapine) at medium concentrations and from 2.6% (nelfinavir) to 4.6% (indinavir) at low concentrations. Between-run coefficients of varia-

tion ranged from 3.4% (indinavir) to 5.9% (nevirapine) at high concentrations, from 1.4% (nelfinavir) to 6% (nevirapine) at medium concentrations and from 5.8% (amprenavir) to 10% (indinavir) at low concentrations. The lower limits of quantification were 25 ng ml⁻¹ (amprenavir), 50 ng ml⁻¹ (indinavir, nelfinavir, lopinavir) and 100 ng ml⁻¹ (nevirapine).

Statistical analysis

The primary aim of the study was to determine the interindividual variability in hepatic CYP3A4 activity and the effect of HAART on this parameter. A sample size of at least 10 patients for each treatment group was estimated to be required assuming an intraindividual variability of 20% [i.e. the mean D-7/D0 ERMBT ratio was assumed to be 20% with a 95% distribution-free confidence interval (CI) of 0.8, 1.25] and an interindividual variability of 50%, with a significance level (α) of 0.05 and a power of 90%. Likewise, a sample size of 10 patients for each treatment group was estimated to be required with a power of 80% to detect a treatment effect on liver CYP3A4 activity, as determined by an effect size (Δ/σ_d) of 1, where Δ corresponds to the difference in the ERMBT values between D0 and D28 and σ_d to the SD of the observed effect. Considering that 20% of the patients could be lost to follow-up, or may not undergo an ERMBT on D28, 61 patients were enrolled.

All continuous variables were expressed as means $\pm$ SD. Intraindividual variability in liver CYP3A4 activity was determined by paired comparisons using the non-parametric Spearman rank correlation coefficient (r_s) between ERMBT values obtained at D-7 and D0. Interindividual variability in liver CYP3A4 activity was expressed as the coefficient of variation of the D-7:D0 ERMBT ratio. Comparisons with respect to gender and ethnicity were made using a two-way ANOVA, followed by Scheffe's test (allowing corrections for multiple comparisons) when an overall statistical difference was found. The ERMBT data were then incorporated into single and multiple regression (for continuous variables) or logistic regression analyses (for nominal variables), where appropriate, to determine whether liver CYP3A4 activity could be predicted from routine clinical or biological markers. The ERMBT data obtained at D28 were compared with the mean baseline results (combined D-7 and D0 values) using two-way MANOVA, followed by Scheffe's test when an overall statistical difference was found. The percentage change in the ERMBT results was expressed as mean $\pm$ SD with 95% CI.

Based on previous reports, we hypothesized that a nelfinavir-based drug regimen would inhibit liver

CYP3A4 activity by at least 50% [26, 35], that any drug regimen containing ritonavir would inhibit activity by at least 90% [26–28, 36] and that a nevirapine-based drug regimen would induce liver activity by at least 50% [12, 26, 29]. In addition, we expected that any HAART consisting of three NAs would have no effect [12, 26]. Finally, the Spearman rank correlation coefficient was used to evaluate whether a relationship existed between baseline liver CYP3A4 activity and trough concentrations of the PI and NNRTI. All statistical analyses were performed using SAS software v8.2 (SAS Institute Inc., Cary, NC, USA). A *P*-value of ≤ 0.05 was considered significant for all comparisons.

Results

Between September 2002 and May 2003, 30 men and 31 women with documented HIV infection (mean $\pm$ SD duration 44 ± 60 months) were enrolled in the study. The mean ($\pm$ SD) age, weight, height and BMI were 38 ± 9 years, 61 ± 11 kg, 170 ± 8 cm and 21.3 ± 3.5 kg m⁻², respectively. Forty patients (65%) were identified as black African and 21 as caucasian. Nine African (23%) and 11 caucasian (57%) patients had received at least one combination therapy prior to enrolment. In 30 patients, at least one medication known to alter CYP3A4 activity *in vitro* and/or *in vivo* [11, 12] was taken at the time of the study. Such drugs included benzodiazepines ($n = 15$), calcium channel blockers ($n = 2$), rifampicin ($n = 3$), oral contraceptives ($n = 3$), steroids ($n = 2$), fluconazole ($n = 14$), clarithromycin ($n = 3$) and morphine or methadone ($n = 5$). In five patients, at least one medication was added after starting HAART. These included benzodiazepines ($n = 3$), steroids ($n = 1$), fluconazole ($n = 1$) and morphine ($n = 2$). Overall, 31 patients were treated with at least one additional drug, which was continued throughout the study. These patients were distributed homogeneously among the five treatment groups, except for the three patients receiving rifampicin, who were also being treated with three NAs.

All but five patients completed the study. Within 14 days of starting treatment, three died, one from severe wasting syndrome and two from multiorgan failure due to *Streptococcus pneumoniae* infection. Two patients withdrew from the study, one due to an extended skin rash 7 days after starting HAART and the other for personal reasons. Nine patients also had chronic hepatitis B ($n = 6$) or hepatitis C infection ($n = 3$). Of the 61 patients enrolled, 13 received nelfinavir, six received ritonavir-boosted indinavir, 13 received boosted lopinavir (seven of whom also received nevirapine), one received boosted amprenavir in associ-

ation with nevirapine, 11 received nevirapine and 12 received a third NA.

Baseline (D-7) immunovirological, nutritional, inflammatory and hepatic function characteristics of the 61 patients are presented in Table 1. The five treatment groups were generally comparable regarding any clinical and biological parameter. CD4 and CD8 counts were lower in patients taking ritonavir-boosted PI with nevirapine compared with the remaining groups. α -Globulins were lower in patients taking three NAs compared with those treated with a nelfinavir-based drug regimen. ALT was lower in patients taking three NAs compared with the remaining groups (Table 1).

ERMBT values ranged between 0.1% and 6.4% on both D-7 and D0 (mean $\pm$ SD, $2.5 \pm 1.1\%$ and $2.3 \pm 1.1\%$, respectively; median, 2.4 and 2.2, respectively). Interindividual variability expressed as a coefficient of variation (CV) in liver CYP3A4 activity on D-7 and D0 was 45.6% and 48.8%, respectively. When the three patients taking rifampicin (ERMBT values $> 5\%$) were excluded, interindividual variability in liver CYP3A4 activity remained unchanged. Intraindividual variability was low ($15 \pm 13\%$) and D-7 ERMBT results were well correlated with those from D0 ($r_s = 0.7$, $P = 0.0001$, Figure 1). Accordingly, D-7 and D0 ERMBT values were averaged and used as baseline liver CYP3A4 activity in subsequent analyses. Inter- and intraindividual variability in liver CYP3A4 activity was similar in all patient subgroups. A trend toward lower baseline activity was noted in patients taking ritonavir-boosted PI with nevirapine (Figure 2).

The interindividual variation in CYP3A4 activity observed at baseline was further characterized using stepwise forward and backward logistic regression and multiple regression analyses. An independent and significant influence of gender was observed on baseline liver CYP3A4 activity. Women had a 30% higher mean ERMBT value than men ($2.7 \pm 1.3\%$ vs. $1.9 \pm 0.7\%$; 95% CI for the difference 20.5, 49.5; $P = 0.003$). Further multiple or logistic regression analyses accounted for gender bias. Caucasian patients had a 20% lower CYP3A4 activity than Africans ($2.0 \pm 0.7\%$ vs. $2.5 \pm 1.3\%$), but the difference did not reach statistical significance ($P = 0.2$). A trend towards lower CYP3A4 activity was observed in the nine patients with coinfection compared with the remaining patients ($1.9 \pm 0.7\%$ vs. $2.5 \pm 1.2\%$; 95% CI for the difference -6.5, 58.5; $P = 0.12$). No influence of concomitant medications was noted when this variable was incorporated into the model ($P = 0.33$).

Of the 18 clinical (age, height, weight, BMI, mean duration of HIV infection at the time of the study)

Table 1

Mean ($\pm$ SD) baseline (D-7) immunological (CD4, CD8, plasma viral load), nutritional (albumin, prealbumin, transferrin), inflammatory [α_1 -acid glycoprotein (AAG), haptoglobin, α - and β -globulins, C-reactive protein (CRP)] status and liver function [albumin, alanine-aminotransferase (ALT), prothrombin time (PT)] in the 61 patients studied

D-7 variables (normal range)	Nelfinavir (N = 13)	Boosted PI (N = 13)	Nevirapine (N = 15)	Boosted PI + nevirapine (N = 8)	NA (N = 12)	All (N = 61)
CD4 (mm^{-3}) (400–1600)	202 $\pm$ 173	145 $\pm$ 117	131 $\pm$ 101	80 $\pm$ 84*	198 $\pm$ 142	155 $\pm$ 132
CD8 (mm^{-3}) (200–1100)	892 $\pm$ 742	1193 $\pm$ 913	772 $\pm$ 424	399 $\pm$ 337*	1042 $\pm$ 504	891 $\pm$ 662
Plasma viral load ($\times 10^3$ copies ml^{-1}) (<50)	138 $\pm$ 171	265 $\pm$ 266	257 $\pm$ 237	239 $\pm$ 270	184 $\pm$ 211	217 $\pm$ 229
Albumin (g l^{-1}) (35–48)	35 $\pm$ 6	36 $\pm$ 4	33 $\pm$ 7	40 $\pm$ 4	37 $\pm$ 4	36 $\pm$ 6
Prealbumin (g l^{-1}) (0.22–0.29)	0.19 $\pm$ 0.05	0.19 $\pm$ 0.07	0.22 $\pm$ 0.06	0.27 $\pm$ 0.07	0.22 $\pm$ 0.05	0.22 $\pm$ 0.06
Transferrin (g l^{-1}) (1.9–2.9)	2 $\pm$ 0.3	2.4 $\pm$ 0.5	2.2 $\pm$ 0.5	2.1 $\pm$ 0.4	2.2 $\pm$ 0.6	2.2 $\pm$ 0.5
AAG (g l^{-1}) (0.5–1.2)	1.3 $\pm$ 0.6	1.1 $\pm$ 0.6	1.2 $\pm$ 0.7	1.2 $\pm$ 0.2	0.8 $\pm$ 0.3	1.1 $\pm$ 0.6
Haptoglobin (g l^{-1}) (0.7–1.9)	1.8 $\pm$ 0.6	1.5 $\pm$ 0.9	1.2 $\pm$ 0.7	1.7 $\pm$ 0.6	1.3 $\pm$ 0.8	1.5 $\pm$ 0.8
CRP (mg l^{-1}) (<4)	17 $\pm$ 16	9 $\pm$ 13	7 $\pm$ 7	12 $\pm$ 17	6 $\pm$ 3	10 $\pm$ 12
α -Globulins (g l^{-1}) (1.2–3.2)	2.4 $\pm$ 0.7	2.2 $\pm$ 0.6	2.1 $\pm$ 0.7	2.1 $\pm$ 0.5	1.9 $\pm$ 0.3**	2.1 $\pm$ 0.6
β -Globulins (g l^{-1}) (6.2–12.1)	12.1 $\pm$ 3.3	10.8 $\pm$ 1.1	10.3 $\pm$ 1.5	10.8 $\pm$ 2.1	10.8 $\pm$ 2.1	11.1 $\pm$ 2.2
ALT (IU l^{-1}) (<50)	32.2 $\pm$ 24.1	30.8 $\pm$ 25.2	26.1 $\pm$ 16.1	35.1 $\pm$ 28.4	20.4 $\pm$ 11.3*	28.4 $\pm$ 21.2
PT (%) (75–105)	90 $\pm$ 16	93 $\pm$ 16	94 $\pm$ 14	99 $\pm$ 15	94 $\pm$ 11	94 $\pm$ 14

* $P = 0.04$ vs. other patients groups (ANOVA, followed by Scheffe's test). ** $P = 0.04$ vs. the nelfinavir group (ANOVA, followed by Scheffe's test). PI, Protease inhibitor; NA, nucleoside analogue.

Figure 1

Intraindividual variability in baseline liver CYP3A4 activity, as measured by the erythromycin breath test (ERMBT), in the 61 HIV-infected patients enrolled

and biological continuous variables tested (Table 1), only body weight ($R^2 = 0.12$, $P = 0.01$), α -globulins ($R^2 = 0.12$, $P = 0.05$), β -globulins ($R^2 = 0.11$, $P = 0.01$) and ALT ($R^2 = 0.12$, $P = 0.005$) were significantly and

independently related to baseline liver CYP3A4 activity. No effect of gender on these measurements was observed. When these four independent continuous variables were combined, baseline ERMBT was described by the following equation: $\text{ERMBT}_{\text{baseline}} = 6.25 - 0.02 \times \text{ALT} - 0.21 \times \beta\text{-globulins} - 0.03 \times \text{body weight} + 0.4 \times \alpha\text{-globulins}$ (adjusted $R^2 = 0.32$).

As shown in Figure 2, relative to baseline, three of the five treatments altered liver CYP3A4 activity by the end of the 28-day treatment period. A drug regimen including nelfinavir decreased mean activity by $53.2 \pm 25.8\%$ (95% CI for percent change 39.2, 67.2; $P = 0.01$). In four patients, CYP3A4 activity was unchanged on D28. These patients had undetectable nelfinavir serum concentrations and, when excluded from the analysis, the extent of nelfinavir-mediated hepatic CYP3A4 inhibition increased to $68.8 \pm 7.6\%$ (95% CI 64.7, 72.9; $P = 0.005$). A positive correlation between baseline liver CYP3A4 activity and the extent of inhibition by nelfinavir was observed in those patients with detectable nelfinavir serum concentrations ($r_s = 0.53$, $P = 0.04$). However, when the patient with the highest baseline activity (ERMBT of 5%) was excluded, the observed correlation was no longer significant ($r_s = 0.2$, $P = 0.44$).

Figure 2

Baseline (mean of D-7 and D0) erythromycin breath test (ERMBT) and the effect of highly active antiretroviral therapy after 1 month of treatment. Bars and error bars represent means and SDs, respectively. * $P < 0.01$ vs. baseline (MANOVA, followed by Scheffe's test). #PI, Protease inhibitor; NA, nucleoside analogue

Table 2

D28 trough concentrations (ng ml⁻¹) of the protease inhibitors (Pis) and nevirapine during the current study

	Nelfinavir (1500–5500)* (N = 13)	Indinavir (150–550) (N = 6)	Lopinavir (2500–7000) (N = 13)	Nevirapine (3000–5500) (N = 19)
Mean ± SD	1375 ± 1192	837 ± 595	4482 ± 2225	5398 ± 5434
Median	1163	799	4073	4565
Range	0–4037	158–1771	1587–7998	326–23 625
95% CI	727, 2023	361, 1313	3273, 5691	2648, 8148
$r_s^\dagger$	0.23	0.90	0.003	0.003
P -value †	0.29	0.03	0.65	0.65

*Therapeutic range. † Spearman rank correlation for baseline erythromycin breath test vs. trough drug concentration.

Four of the 15 patients in the nevirapine group withdrew before the end of the study. Of the 11 patients remaining, no change was observed in liver CYP3A4 activity at D14 and D28 (Figure 2) (mean percent change from baseline, $18.6 \pm 30.3\%$; 95% CI -0.2 , 37.4 ; $P = 0.60$). The mean serum nevirapine concentration was within the therapeutic range (5398 ± 5434 ng ml⁻¹, range 3000–5500, Table 2). In patients treated with lopinavir, indinavir or amprenavir boosted with ritonavir, CYP3A4 activity decreased by $95 \pm 2.9\%$ (95% CI for the difference 93.3, 96.7; $P < 10^{-4}$) (Figure 2). When nevirapine was associated with the boosted PI regimen, CYP3A4 activity decreased by $85.7 \pm 5.4\%$ (95% CI for the difference 81.9, 89.5; $P = 0.0005$). CYP3A4 activity was unchanged in patients treated with three NAs (mean percent change $11 \pm 24.1\%$; 95% CI for the difference -2.6 , 23.6 ; $P = 0.6$) (Figure 2).

In patients taking nelfinavir (four women, nine men), as well as those taking any boosted PI regimen (six women, six men), the sex difference observed at baseline was no longer present. Conversely, in patients taking nevirapine with two or three NAs (16 women, eight men), women had a 32% higher baseline activity compared with men ($3.1 \pm 1.8\%$ vs. $2.1 \pm 1.0\%$; 95% CI for the difference 17.8, 80.9; $P = 0.003$). In patients taking nevirapine in association with any boosted PI, a sex difference could not be assessed at D28 due to the small sample size (two women, six men).

Trough concentrations of nelfinavir, indinavir, lopinavir and nevirapine were measured on D28 (Table 2). Mean values for all of these drugs were highly variable. Plasma trough concentrations were below the therapeutic range in eight patients receiving nelfinavir, two receiving lopinavir and five receiving nevirapine. Conversely, three patients receiving indinavir, two receiving

lopinavir and two receiving nevirapine had plasma trough concentrations above the therapeutic range. Indinavir trough concentrations correlated positively with baseline liver CYP3A4 activity ($r_s = 0.9$, $P = 0.03$, Table 2), and nelfinavir trough concentrations correlated negatively with liver CYP3A4 activity on D28 ($r_s = -0.70$, $P = 0.02$). Significant correlations were not observed for lopinavir or nevirapine.

Discussion

The current study provides the first description, in a large cohort of HIV-infected patients, of the effect of the most widely prescribed antiretroviral drug combinations on liver CYP3A4 activity. We believe that the effects of HAART on liver CYP3A4 are consistent among the various treatment groups, as patients receiving concomitant medication known to affect CYP3A4 activity were uniformly distributed. Moreover, concomitant medication was not predictive of interindividual variability in baseline CYP3A4 activity.

We observed up to 69% inhibition of liver CYP3A4 activity in patients taking nelfinavir, which is consistent with the increase (twofold) in systemic exposure to rifabutin, saquinavir and indinavir following single doses of nelfinavir in healthy subjects [12, 26]. Moreover, the current study demonstrated an inverse correlation between nelfinavir trough concentrations and the D28 ERMBT values, which is consistent with concentration-dependent inhibition of hepatic CYP3A4 [11, 12, 23, 24, 26]. Nelfinavir is metabolized predominantly by CYP2C19, which is polymorphically expressed [37]. However, CYP2C19 genotype was not determined in the current study since, to our knowledge, no relevant drug–drug interactions between nelfinavir and other CYP2C19 substrates have been described. Moreover, based on a recent meta-analysis, only 3.9% of unrelated black Africans (who represented two-thirds of our patient group) are CYP2C19 poor metabolizers [38]. Hence, whether genetic polymorphisms of CYP2C19 influence the extent of CYP3A4 inhibition by nelfinavir requires further investigation.

We also observed at least a 95% inhibition of liver CYP3A4 activity during the boosted PI regimens, which is consistent with previous findings in healthy subjects [36]. In contrast, in patients taking nevirapine alone, CYP3A4 activity was unchanged. This drug is a potent inducer of CYP3A4 in humans, causing a 20–60% decrease in the systemic exposure of the CYP3A4 substrates, indinavir, nelfinavir, saquinavir and methadone [12, 26, 29, 39]. Dose-dependent induction of liver CYP3A4 activity, as measured on days 14 and 28, was also not evident in the current study. This was unlikely

to have been due to an inability of the ERMBT to detect CYP3A4 induction by nevirapine, as the test has been used to characterize liver CYP3A4 induction by several well-established inducers, including efavirenz [32], dexamethasone [40] and rifampicin [41]. Nevirapine is also a substrate and modulator of CYP2B6 *in vitro* and *in vivo* [42]. Hence, some drug–drug interactions observed with nevirapine may involve CYP2B6 rather than CYP3A4 induction, especially that with methadone, another CYP2B6 substrate [42, 43]. Further studies, including the identification of CYP2B6 genetic variants, are needed to elucidate the mechanisms involved in drug–drug interactions involving nevirapine.

As in the current study, large interindividual variability in liver CYP3A4 activity in HIV-infected patients has been reported by others [44]. Slain *et al.* [44] also found that the ERMBT did not correlate with any of the immunological, inflammatory or nutritional parameters tested. Because most of the current patients receiving other drugs were being simultaneously treated with inducers and inhibitors of liver CYP3A4, its baseline activity was not correlated with the concomitant medication using logistic regression analysis. However, further analysis detected independent correlations between the ERMBT data and routine clinical and biological variables, namely, body weight, α -globulins, α -globulins and ALT. Although such correlations were statistically significant, all coefficients has values of <0.20 . Combining all of these variables into a single equation improved the correlation coefficient to 0.32. However, this model is probably not clinically useful, since it would predict only 32% of the observed interindividual variability in liver CYP3A4 activity. Hence, the lack of strong correlations (≥ 0.70) indicates further studies are needed to identify more reliable surrogate markers of CYP modulation in HIV-infected patients. The trend towards lower CYP3A4 activity observed in patients with chronic hepatitis B or C coinfection compared with non-infected patients supports the potential role of cytokines in the variation in liver CYP3A4 activity [7–9]. Most of the current patients had no active inflammatory condition at the time of enrolment, which may explain why baseline liver CYP3A4 activity in the non-infected patients was similar to that observed in healthy subjects [16, 17].

The single ERMBT performed in the Slain *et al.* study [44] did not allow the full characterization of the effect of concomitant medication on liver CYP3A4 activity, as patients receiving therapy known to affect liver CYP3A4 activity modulators were compared with others who did not. In addition, almost 25% of patients were already being treated with HAART at the time of enrolment. Hence, the variability observed by Slain

et al. [44] may be partly attributed to the effect of HAART rather than the disease itself. In a study of 75 HIV-infected caucasians (>70% male), the authors used oral midazolam to evaluate liver and intestinal CYP3A activity before and during HAART [45, 46]. They found significantly lower baseline CYP3A activity in their patients compared with unmatched controls (>70% female) [45]. The discrepancy in the gender distribution between these two groups may partly explain the data from the study. In the present work, women had a 30% higher baseline ERMBT value than men, with the highest ERMBT being observed in the African females, which is consistent with previous work [47, 48]. In the current study, the ERMBT was performed in patients who had rested for a period to decrease interindividual variability in CO₂ output, which may cause fluctuations in data from repeat ERMBT administrations [49]. Although men and women are unlikely to have similar resting CO₂ production [49], the current results are consistent with the recent observations by Wolbold *et al.* [50], who analysed a large collection ($n = 94$) of well-characterized surgical liver specimens. European women were shown to have a 29% higher median CYP3A4 protein content compared with men, which was independent of age or concomitant medication [50]. Furthermore, CYP3A4 content was normally distributed and varied 55-fold, which is in agreement with the current observations.

Using oral midazolam as a probe for combined liver and intestinal CYP3A activity in 21 patients, Fellay *et al.* observed similar effects of several antiretroviral drug combinations (including nevirapine, efavirenz and/or ritonavir) on CYP3A activity [46]. Whereas nelfinavir decreased the midazolam metabolic ratio by up to 95%, nelfinavir decreased the ERMBT (current study) by at most 69%. This apparent discrepancy might be explained by the additional effect of nelfinavir on intestinal CYP3A4 or CYP3A5, which is not measured by the ERMBT, the latter being only a selective probe for liver, but not intestinal CYP3A4 activity [13–15]. The potential role of intestinal CYP3A4 or CYP3A5 and enterocyte P-glycoprotein in the oral bioavailability of PIs and, to a lesser extent, NNRTIs [32], may also explain the lack of correlation between baseline liver CYP3A4 activity and antiretroviral trough concentrations (excluding indinavir). Other explanations may include the small sample size per treatment in the current study or the use of ritonavir as a potent liver and intestinal CYP3A4 inhibitor. The significant correlation between indinavir trough concentrations and baseline ERMBT involved only six patients in the current study. Because our results are in disagreement with those pub-

lished by Slain *et al.* [44], further studies are needed for a better understanding of this unexpected positive correlation between concentrations of indinavir, a CYP3A4 substrate, and liver CYP3A4 activity.

In summary, the ERMBT disclosed wide interindividual variability in liver CYP3A4 activity in HIV-infected patients and provides the first description of the effect of various HAART on the activity of this CYP form under routine treatment and follow-up conditions. Whether a single ERMBT can predict antiretroviral drug concentrations or the extent of drug–drug interactions that may occur in HIV-infected individuals is uncertain and may require a more sensitive probe that measures aggregate presystemic first-pass metabolism at various stages of HIV infection. Since poor treatment adherence to antiretroviral therapy and drug–drug interaction-related toxicity may account for as much as 75% of treatment failure during the course of HIV infection, the current study supports the potential usefulness of this approach in clinical practice.

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References

- Williams ML, Bhargava P, Cherrouk I, Marshall JL, Flockhart DA, Wainer IW. A discordance of the cytochrome P450 2C19 genotype and phenotype in patients with advanced cancer. *Br J Clin Pharmacol* 2000; 49: 485–8.
- Piscitelli SC, Vogel S, Figg WD, Rajé S, Forrest A, Metcalf JA, Baseler M, Falloon J. Alteration in indinavir clearance during interleukin-2 infusions in patients infected with the human immunodeficiency virus. *Pharmacotherapy* 1998; 18: 1212–6.
- Kaufmann GR, Wenk M, Taeschner W, Peterli B, Gyr K, Meyer UA, Haefeli WE. N-Acetyltransferase 2 polymorphism in patients

- infected with human immunodeficiency virus. *Clin Pharmacol Ther* 1996; 60: 62–7.
- 4 Lee BL, Wong D, Benowitz NL, Sullam PM. Altered patterns of drug metabolism in patients with acquired immunodeficiency syndrome. *Clin Pharmacol Ther* 1993; 53: 529–35.
 - 5 O’Neil WM, Pezzullo JC, Di Girolamo A, Tsoukas CM, Wainer IW. Glucuronidation and sulphatation of paracetamol in HIV-positive patients and patients with AIDS. *Br J Clin Pharmacol* 1999; 48: 811–8.
 - 6 Armstrong SG, Renton KW. Factors involved in the down-regulation of cytochrome P450 during *Listeria monocytogenes* infection. *Int J Immunopharmacol* 1994; 16: 747–54.
 - 7 Craig PI, Tapner M, Farrell GC. Interferon suppresses erythromycin metabolism in rats and human subjects. *Hepatology* 1993; 17: 230–5.
 - 8 Lescut D, Fouin-Fortunet H, Moore N, Petit J, Hecketsweiler B, Lemeland JF, Denis P, Colin R. Liver glutathione and cytochrome P450 activity in experimental infection: study of the relative effects of infectious stress and malnutrition. *Crit Care Med* 1991; 19: 1183–7.
 - 9 Ansher S, Thompson W, Watson R. Alterations of hepatic drug metabolism in mice following infection with the murine retrovirus LP-BM5. *Immunopharmacology* 1994; 27: 215–23.
 - 10 Topfer F, Lenton LM, Bygrave FL, Behm CA. Importance of T-cell-dependent inflammatory reactions in the decline of microsomal cytochrome P450 concentration in the livers of rats infected with *Fasciola hepatica*. *Int J Parasitol* 1995; 25: 1259–62.
 - 11 Von Moltke LL, Greenblatt DJ, Schmider J, Harmatz JS, Shader RI. Metabolism of drugs by cytochrome P4503A isoforms. *Clin Pharmacokinet* 1995; 29 (Suppl.): 33–44.
 - 12 Piscitelli SC, Gallicano KD. Interactions among drugs for HIV and opportunistic infections. *N Engl J Med* 2001; 344: 984–96.
 - 13 Watkins PB, Murray SA, Winkelman LG, Heuman DM, Wrighton SA, Guzelian PS. Erythromycin breath test as an assay of glucocorticoid-inducible liver cytochrome P-450. Studies in rat and patients. *J Clin Invest* 1989; 83: 688–97.
 - 14 Watkins PB. Non-invasive tests of CYP3A enzymes. *Pharmacogenetics* 1994; 4: 171–84.
 - 15 Streetman DS, Bertino JS, Nafziger AN. Phenotyping of drug-metabolizing enzymes in adults: a review of in-vivo cytochrome P450 phenotyping probes. *Pharmacogenetics* 2000; 10: 187–216.
 - 16 Harris RZ, Tsunoda SM, Mroczkowski P, Wong H, Benet LZ. The effects of menopause and hormone replacement therapies on prednisolone and erythromycin pharmacokinetics. *Clin Pharmacol Ther* 1996; 59: 429–35.
 - 17 Kinirons MT, O’Shea D, Downing TE, Fitzwilliam AT, Joellenbeck L, Groopman JD, Wilkinson GR, Wood AJJ. Absence of correlations among three putative in vivo probes of human cytochrome P450 3A activity in young healthy men. *Clin Pharmacol Ther* 1993; 54: 621–8.
 - 18 Hirth JA, Watkins P, Strauwerderman M, Schott A, Bruno R, Baker L. The effect of an individual’s cytochrome CYP3A4 activity on docetaxel clearance. *Clin Cancer Res* 2000; 6: 1203–12.
 - 19 Cakaloglu Y, Tredger JM, Delvin J, Williams R. Importance of cytochrome P-450IIIa activity in determining dosage and blood levels of FK 506 and cyclosporine in liver transplant recipients. *Hepatology* 1994; 20: 309–15.
 - 20 Turgeon DK, Leichtman AB, Blake DS, Schmouder RL, Lown KS, Annesley TM, Watkins PB. Prediction of interpatient and inpatient variation in OG 37-325 dosing requirements by the erythromycin breath test. A prospective study in renal transplant recipients. *Transplantation* 1994; 57: 1736–41.
 - 21 Turgeon DK, Leichtman AB, Lown K, Normolle DP, Deeb GM, Merison RM, Watkins PB. Cytochrome P450 3A activity and cyclosporine dosing in kidney and heart transplant recipients. *Clin Pharmacol Ther* 1994; 56: 253–60.
 - 22 Turgeon DK, Normolle DP, Leichtman AB, Annesley TM, Smith DE, Watkins PB. Erythromycin breath test predicts oral clearance of cyclosporine in kidney transplant recipients. *Clin Pharmacol Ther* 1992; 52: 471–8.
 - 23 Barry M, Gibbons S, Back D, Mulcahy F. Protease inhibitors in patient with HIV disease. Clinically important pharmacokinetic considerations. *Clin Pharmacokinet* 1997; 32: 194–209.
 - 24 Hoetelmans RMW, Meenhorst PL, Mulder JW, Burger DM, Koks CHW, Beijnen JH. Clinical pharmacology of HIV protease inhibitors: focus on saquinavir, indinavir, and ritonavir. *Pharm World Sci* 1997; 19: 159–74.
 - 25 Koudriakova T, Iatsimirskaya E, Utkin I, Gangl E, Vouros P, Storozhuk E, Orza D, Marinina J, Gerber N. Metabolism of the human immunodeficiency virus protease inhibitors indinavir and ritonavir by human intestinal microsomes and expressed cytochrome P4503A4/3A5. Mechanism-based inactivation of cytochrome P4503A by ritonavir. *Drug Metab Dispos* 1998; 26: 552–61.
 - 26 Barry M, Mulcahy F, Merry C, Gibbons S, Back D. Pharmacokinetics and potential interactions amongst anti-retroviral agents used to treat patients with HIV infection. *Clin Pharmacokinet* 1999; 36: 289–304.
 - 27 Eagling VA, Back DJ, Barry MG. Differential inhibition of cytochrome P450 isoforms by the protease inhibitors, ritonavir, saquinavir and indinavir. *Br J Clin Pharmacol* 1997; 44: 190–4.
 - 28 Kempf DJ, Marsh KC, Kumar G, Rodrigues AD, Denissen JF, McDonald E, Kukulka MJ, Hsu A, Granneman GR, Baroldi PA, Sun E, Pizzuti D, Plattner JJ, Norbeck DW, Leonard JM. Pharmacokinetic enhancement of inhibitors of the human immunodeficiency virus protease by coadministration with ritonavir. *Antimicrob Agents Chemother* 1997; 41: 654–60.
 - 29 Altice FL, Friedland GH, Cooney EL. Nevirapine induced opiate withdrawal among injection drug users with HIV infection receiving methadone. *AIDS* 1999; 13: 957–62.
 - 30 Salvat C, Mouly S, Rizzo-Padoin N, Knellwolf AL, Simoneau G, Duet M, Nataf V, Bailliat O, Bergmann JF. The [¹⁴C-N-methyl]-erythromycin breath test dosimetry complies with the French regulations for radiation safety. *Fund Clin Pharmacol* 2003; 17: 349–53.
 - 31 Hoen B, Raffi F. Quand et comment débuter un traitement antiretroviral? In: *Prise en Charge Thérapeutique des Personnes*

- Infectées par le VIH, ed. Delfraissy JF. Paris: Médecine-Sciences Flammarion 2004; 43–58.
- 32 Mouly S, Lown KS, Kornhauser D, Joseph JL, Fiske WD, Benedek IH, Watkins PB. Hepatic but not intestinal CYP3A4 displays dose-dependent induction by efavirenz in humans. *Clin Pharmacol Ther* 2002; 72: 1–9.
 - 33 Wagner D. CYP3A4 and the erythromycin breath test. *Clin Pharmacol Ther* 1998; 64: 129.
 - 34 Aymard G, Legrand M, Trichereau N, Diquet B. Determination of twelve antiretroviral agents in human plasma sample using reversed-phase high-performance liquid chromatography. *J Chromatogr B* 2000; 744: 227–40.
 - 35 Merry C, Barry MG, Mulcahy F, Halifax KL, Back DJ. Saquinavir pharmacokinetics alone and in combination with nelfinavir in HIV-infected patients. *AIDS* 1997; 11: F117–F120.
 - 36 Khaliq Y, Gallicano K, Tisdale C, Carignan G, Cooper C, McCarthy A. Pharmacokinetic interaction between mefloquine and ritonavir in healthy volunteers. *Br J Clin Pharmacol* 2001; 51: 591–600.
 - 37 Khaliq Y, Gallicano K, Seguin I, Fyke K, Carignan G, Bulman D, Badley A, Cameron DW. Single and multiple dose pharmacokinetics of nelfinavir and CYP2C19 activity in human immunodeficiency virus-infected patients with chronic liver disease. *Br J Clin Pharmacol* 2000; 50: 108–15.
 - 38 Xie HG, Kim RB, Stein CM, Wilkinson GR, Wood AJJ. Genetic polymorphism of (S)-mephenytoin 4'-hydroxylation in populations of African descent. *Br J Clin Pharmacol* 1999; 48: 402–8.
 - 39 Merry C, Barry MG, Mulcahy F, Ryan M, Tjia JF, Halifax KL, Breckenridge AM, Back DJ. The pharmacokinetics of combination therapy with nelfinavir plus nevirapine. *AIDS* 1998; 12: 1163–7.
 - 40 McCune JS, Hawke RL, LeCluyse EL, Gillenwater HH, Hamilton G, Ritchie J, Lindley C. In vivo and in vitro induction of human cytochrome P4503A4 by dexamethasone. *Clin Pharmacol Ther* 2000; 68: 356–66.
 - 41 Gharaibeh MN, Gillen LP, Osborne B, Schwartz JI, Waldman SA. Effect of multiple doses of rifampin on the [^{14}C N-methyl] erythromycin breath test in healthy male volunteers. *J Clin Pharmacol* 1998; 38: 492–5.
 - 42 Erickson DA, Mather G, Trager WF, Levy RH, Keirns JJ. Characterization of the in vitro biotransformation of the HIV-1 reverse transcriptase inhibitor nevirapine by human hepatic cytochrome P-450. *Drug Metab Dispos* 1999; 27: 1488–95.
 - 43 Haas DW, Ribaud HJ, Kim RB, Tierney C, Wilkinson GR, Gulik RM, Clifford DB, Hulgand T, Marzolini C, Acosta EP. Pharmacogenetics of efavirenz and central nervous system side effects: an adult AIDS Clinical Trials Group study. *AIDS* 2004; 18: 2391–400.
 - 44 Slain D, Pakiz A, Israel S, Monroe S, Polk RE. Variability in activity of hepatic CYP in patients infected with HIV. *Pharmacotherapy* 2000; 20: 898–907.
 - 45 Eap CB, Fellay J, Buclin T, Bleiber G, Golay KP, Brocard M, Baumann P, Telenti A. CYP3A activity measured by the midazolam test is not related to 3435 C>T polymorphism in the multiple drug resistance transporter gene. *Pharmacogenetics* 2004; 14: 255–60.
 - 46 Fellay J, Marzolini C, Decosterd L, Powell Golay K, Baumann P, Buclin T, Telenti A, Eap CB. Variation of CYP3A4 activity induced by antiretroviral treatment in HIV-1 infected patients. *Eur J Clin Pharmacol* 2005; 60: 865–73.
 - 47 Min DI, Lee M, Ku YM, Flanagan M. Gender-dependent racial difference in disposition of cyclosporine among healthy African-American and white volunteers. *Clin Pharmacol Ther* 2000; 68: 478–86.
 - 48 Zhu B, Liu ZQ, Chen GL, Chen XP, Ou-Lang DS, Wang LS, Huang SL, Tan ZR, Zhou HH. The distribution and gender difference of CYP3A activity in Chinese subjects. *Br J Clin Pharmacol* 2003; 55: 264–9.
 - 49 Rivory LP, Slaviero KA, Hoskins JM, Clarke SJ. The erythromycin breath test for the prediction of drug clearance. *Clin Pharmacokinet* 2001; 40: 151–8.
 - 50 Wolbold R, Klein K, Burk O, Nussler AK, Neuhaus P, Eichelbaum M, Schwab M, Zanger UM. Sex is major determinant of CYP3A4 expression in human liver. *Hepatology* 2003; 38: 978–88.