Original article

Factors influencing efavirenz and nevirapine plasma concentration: effect of ethnicity, weight and co-medication

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Background: The aim of this study was to examine factors influencing plasma concentration of efavirenz and nevirapine.

Methods: Data from the Liverpool Therapeutic Drug Monitoring (TDM) registry were linked with the UK Collaborative HIV Cohort (CHIC) Study. For each patient, the first measurement of efavirenz (600 or 800 mg/day) or nevirapine (400 mg/day) plasma concentration was included. Linear regression was used to evaluate the association of dose, gender, age, weight, ethnicity and concomitant antiretroviral drugs or rifampicin with log-transformed drug concentration, adjusted for time since last intake.

Results: Data from 339 patients on efavirenz (34% black, 17% rifampicin) and 179 on nevirapine (27% black, 6% rifampicin) were included. Multivariable models revealed the following predictors for efavirenz concentration: black ethnicity (59% higher; $P<0.001$), weight (10% lower per additional 10 kg; $P=0.002$), 800 mg/day (52% higher; $P=0.027$), rifampicin (35% lower; $P=0.039$), and zidovudine (25% lower; $P=0.010$). Notably, without adjustment for other factors, patients on rifampicin had 48% higher efavirenz concentration, as these patients were mostly black and on 800 mg/day. For nevirapine the predictors were black ethnicity (39% higher; $P=0.002$), rifampicin (40% lower; $P=0.002$), protease inhibitor (28% higher; $P=0.008$) and tenofovir (22% higher; $P=0.024$).

Conclusions: We observed clear associations between ethnicity and concentrations of nevirapine and efavirenz. Our analyses confirm that concomitant rifampicin substantially decreases concentration of both efavirenz and nevirapine; however, for efavirenz this effect was more than counterbalanced by the effect of ethnicity and increased efavirenz dose. There was also an additional impact of weight, which should be considered when determining optimal dosage. Other associations from our analysis (between tenofovir or protease inhibitor and nevirapine, and zidovudine and efavirenz), require confirmation in formal pharmacokinetic studies.

Introduction

First-line antiretroviral therapy (ART) regimens generally include one of the HIV non-nucleoside reverse transcriptase inhibitors (NNRTIs) nevirapine or efavirenz. Evidence suggests that these two drugs have similar clinical efficacy, at least in a trial setting [1], and both NNRTIs have a low genetic threshold for the development of drug resistance. However, there are also substantial differences between them. Firstly, only nevirapine is licensed for use in pregnant women and very young children. Secondly, the major side effect of efavirenz is central nervous system (CNS) toxicity, whereas nevirapine is mainly associated with hepatotoxicity and drug hypersensitivity.

Considerable interindividual variability has been observed in plasma concentrations of nevirapine and efavirenz after standard dosing [2]. A number of factors have been shown to influence plasma NNRTI exposure including body weight, gender, ethnicity, hepatitis and concomitant medications [2–8], although not consistently across various studies. Efavirenz is mainly metabolised by cytochrome P450 CYP2B6 [9], whereas nevirapine is metabolised by CYP2B6 and CYP3A4 [10]. Although CYP3A4 polymorphisms have not clearly been linked to altered enzyme function, certain haplotypes of CYP2B6 such as *6 and *18 are associated with reduced enzyme function leading to a greater plasma exposure of efavirenz [11–14] and nevirapine [13,15,16]. These alleles are rare in Caucasian populations, but more frequent in Black Africans [11,17,18].

Rifampicin forms an essential part of tuberculosis (TB) treatment regimens and is a potent inducer of CYP enzymes. Decreased serum concentrations of efavirenz [19–21] and nevirapine [7,22–24] have been found when these NNRTIs were given together with rifampicin. Evidence suggests that efavirenz use is less compromised by rifampicin, but controversy remains around whether weight-based dose escalation is required [20,25–29].

In the present study, we used data from the Liverpool HIV Therapeutic Drug Monitoring (TDM) registry to evaluate the association of plasma exposure of nevirapine and efavirenz with ethnicity and other demographic and clinical characteristics.

Methods

Study cohort and participants

The Liverpool TDM Registry contains data from ~18,000 assays performed in HIV-positive patients in whom TDM was requested between 1999 and 2006. For each sample, details of age, gender, weight and medication history (including dose, dosing regimen, time between sampling and last ingestion of HIV medication, and concomitant medications) and reason for asking for drug monitoring were routinely requested. The UK Collaborative HIV Cohort (UK CHIC) study is a collaboration of some of the largest centres for the care of HIV-infected individuals in the UK [30]. The criteria for inclusion of an individual in the study were that a person was HIV-positive, aged over 16 years and had attended one of the collaborating centres for care at any time after 1 January 1996. The dataset used for the present analysis contains information on 25,274 patients seen for care at ten centres (see Additional file). Each centre provided electronic data in a standardised format on demographic characteristics, AIDS diagnoses and mortality, laboratory data ($CD4^+$/$CD8^+$ T-cell counts, viral loads and markers of drug toxicity) and ART. Both the UK CHIC and Liverpool TDM Registry have received ethics approval from Multiregional Research Ethics Committees.

For this cross-sectional study, Liverpool TDM registry records were linked to demographic (ethnicity) and clinical data (antiretroviral drugs) from UK CHIC using hospital identification number and birth date as matching variables. All records were pseudonymised. Linkage was successfully achieved for >90% of records from the TDM Registry. The current analysis is based on the first TDM measurement of efavirenz or nevirapine per patient and includes samples up to the end of 2005. Four inclusion criteria were applied: the availability of a sample taken >4 h after drug intake (to reduce absorption-related variation of drug serum concentrations); patient aged ≥18 years; a once daily 600 mg or 800 mg regimen for efavirenz and a 400 mg once daily or 200 mg twice daily regimen for nevirapine; and white or black African ethnicity. Samples with undetectable dug concentrations were excluded.

Laboratory measurements

Plasma drug concentrations were measured by validated high performance liquid chromatography with UV detection (HPLC-UV), as previously described [27,31].

Nevirapine was extracted from heat-inactivated plasma (200 μl) using dichloromethane after the addition of an internal standard (bromazepam; Sigma Chemical Co, MO, USA). The organic layer was evaporated to dryness and reconstituted in mobile phase (27% ammonium formate buffer (pH 5.0)/ 73% acetonitrile) prior to analysis. Recovery of nevirapine using this method was >95%. Nevirapine and standard were resolved by HPLC (Kontron Instruments Ltd., Hertfordshire, UK) with peak areas quantified using the Chromeleon (Version 6.5) data acquisition system (Dionex Corporation, CA, USA). The lower limit of quantification (LLQ) of nevirapine was taken as the lowest

point on the standard curves (400 ng/ml). Intra-assay and interassay coefficient of varience at 800 ng/ml (low quality control) were both 4.7%.

For efavirenz, internal standard was added to heat-inactivated plasma samples (200 µl) and standards (range 100–8,000 ng/ml) followed by centrifugation with potassium carbonate and ethyl acetate/*n*-hexane. The organic layer was then evaporated to dryness, and reconstituted in mobile phase (150 µl) prior to analysis. Recovery of efavirenz using this method is 98%. Efavirenz and standard were resolved by HPLC (Kontron Instruments Ltd.) with peak areas quantified using the Chromeleon (Version 6.5) data acquisition system (Dionex Corporation). The lower limit of quantification (LLQ) of efavirenz was taken as the lowest point on the standard curves (100 ng/ml). Intra-assay and interassay coefficients of variation at 100 ng/ml were 10.8 and 14.9%, respectively.

The therapeutic range was defined as >3,400 ng/ml for nevirapine and 1,000–4,000 ng/ml for efavirenz [32]. The laboratory participates in an external quality assurance programme (Association for Quality Assessment and Clinical Toxicology [KKGT], The Netherlands).

Statistical analysis

We examined the effect of several factors that could influence the serum concentration of efavirenz/nevirapine using multivariable linear regression: sex, age, ethnicity (white or black), weight, efavirenz/nevirapine dose, time since last efavirenz/nevirapine intake, time since first treatment with efavirenz/nevirapine, time on current ART regimen, concomitant TB drugs (rifampicin or rifabutin), and concomitant antiretroviral drugs. As information on weight was missing in 12% (efavirenz) and 6% (nevirapine) of patients, it was imputed in a linear regression model using the same predictors [33]. Dose was not available for concomitant drugs so they were classified as given versus not given. As protease inhibitors (PIs) were rarely given together with efavirenz/nevirapine, they were initially combined in one variable (on PI yes/no). Year of measurement and centre had only a minor effect on other predictors; therefore, these data were excluded from the final model. Data on hepatitis B and C coinfection as well as alanine transaminase (ALT) values were only available for 57–70% of patients (these measurements have only been performed routinely in relatively recent years); therefore, these data were only considered in sensitivity analyses. The indication for drug monitoring was ignored as a possible predictor because this information was often missing on the request form and was generally regarded as unreliable.

For all models, drug levels were log-transformed to improve the approximation to normality. For continuous variables, presence of a non-linear association with drug concentration was examined using fractional polynomial regression [34]. As the models for nevirapine were sensitive to some influential outliers, robust regression was used for this drug [35]. In brief, gross outliers were eliminated in an initial screening regression if Cook's distance was >1 and, thereafter, regression models were performed iteratively using calculated case weights (Huber and biweight) based on absolute residuals. On the basis of the multivariable models, we then estimated the probability of having a NNRTI concentration beyond the recommended therapeutic range (<1,000 or >4,000 ng/ml for efavirenz; <3,400 ng/ml for nevirapine) for various scenarios.

Results

Efavirenz

Efavirenz concentrations were assessed in 339 patients fulfilling the inclusion criteria (Table 1). The median time from last intake was 14 h (interquartile range [IQR] 12–17 h). Rifampicin was co-administered in 56 (17%) and rifabutin in 11 (3%) patients. Co-administration with rifampicin was strongly associated with efavirenz dose, reflecting UK clinical guidelines: an 800 mg daily dose was used in 48 (86%) patients receiving rifampicin compared with only 8 (3%) patients not receiving this drug. Against UK recommendations, seven patients on rifampicin and with a body weight >50 kg were treated with 600 mg daily dose. TB medication was much more frequent in black patients (56/114, 49%) than in white patients (11/225, 5%).

Factors potentially influencing efavirenz serum concentration are listed in Table 2. Because of strong interrelationships between various patient characteristics, results from the multivariable models are more robust than the unadjusted values from the univariable models. Results from multivariable models with and without consideration of concomitant antiviral drugs were very similar (Table 2, multivariable 1 and 2). Including all potential predictors, efavirenz serum concentration was significantly influenced by ethnicity (59% higher in black than in white patients; $P<0.001$), weight (10% lower per additional 10 kg; $P=0.002$), efavirenz dose (52% higher in 800 mg regimen; $P=0.027$), time post-efavirenz intake (2% lower per additional hour; $P=0.042$), concomitant use of rifampicin (35% lower; $P=0.039$), and co-administration of zidovudine (25% reduction; $P=0.010$). Of note, without adjustment for other factors, the effect for rifampicin was reversed (48% higher efavirenz concentration), owing to its strong association with black ethnicity and higher efavirenz dose. By contrast, there was no demonstrable effect of rifabutin, although due to small numbers the associated confidence interval is wide. Fractional

Table 1. Patient characteristics

	Efavirenz group (*n*=339)	Nevirapine group (*n*=179)
Median age, years (IQR)	40.0 (35.5–46.1)	40.3 (35.2–46.8)
Gender		
Female, *n* (%)	78 (23)	36 (20)
Male, *n* (%)	261 (77)	143 (80)
Ethnicity		
Black, *n* (%)	114 (34)	48 (27)
White, *n* (%)	225 (66)	131 (73)
Median weight, kg (IQR)	70.1 (62.4–80.0)	71.0 (65.0–78.0)
Median time from last NNRTI* intake, h (IQR)	14 (12–17)	12 (12–13)
Median time on NNRTI, weeks (IQR)	42 (14–114)	91 (30–214)
Median time on current regimen, weeks (IQR)	23 (9–73)	40 (14–94)
Dosing schedule		
Twice daily 200 mg, *n* (%)	0	157 (88)
Once daily 400 mg, *n* (%)	0	22 (12)
Once daily 600 mg, *n* (%)	283 (83)	0
Once daily 800 mg, *n* (%)	56 (17)	0
Median NNRTI serum concentration, ng/ml (IQR)	1,918 (1,334–2,896)	4,081 (3,076–5,680)
Concomitant NRTIs		
Zidovudine, *n* (%)	121 (36)	71 (40)
Lamivudine, *n* (%)	219 (65)	128 (72)
Stavudine, *n* (%)	47 (14)	21 (12)
Tenofovir, *n* (%)	115 (34)	55 (31)
Didanosine, *n* (%)	90 (27)	29 (16)
Abacavir, *n* (%)	69 (20)	28 (16)
Concomitant NNRTIs		
Nevirapine, *n* (%)	12 (4)	179 (100)
Efavirenz, *n* (%)	339 (100)	6 (3)
Concomitant protease inhibitors	90 (27)	53 (30)
Concomitant tuberculosis drug		
None, *n* (%)	272 (80)	162 (91)
Rifabutin, *n* (%)	11 (3)	7 (4)
Rifampicin, *n* (%)	56 (17)	10 (6)

*Efavirenz in efavirenz group; nevirapine in nevirapine group. IQR, interquartile range; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor.

polynomial models supported a linear effect of weight, with no evidence of a threshold effect.

Information on hepatitis B surface antigen, hepatitis C antibody and ALT were available in 194 (57%), 214 (63%) and 211 (62%) patients, respectively; these were last measured a median (IQR) of 64 (20–160), 53 (21–127), and 2 (0–9) weeks, respectively, before the measurement of efavirenz concentration. During the course of the study, 17 patients had a positive test result for hepatitis B, 25 patients for hepatitis C and ALT was increased (>2× upper limit of normal) in the last test available in 17 patients. Neither in univariable nor in multivariable models did we find any association between these variables and efavirenz concentration.

The probability of having an efavirenz trough concentration below, within or above the recommended therapeutic range while on concomitant rifampicin is shown in Figure 1, which illustrates the influence of dose, ethnicity and weight. Of interest, the probability of having a trough concentration <1,000 ng/ml when on standard 600 mg dose was about twice as high in white patients than in black patients (for example, at 70 kg, 50% in white and 23% in black patients). Accordingly, an increase in dose to 800 mg considerably improved the probability of having a trough concentration within the 1,000–4,000 ng/ml therapeutic range in white patients (for example, at 70 kg, from 48% to 64%), whereas in black patients this mainly resulted in a lower probability of a trough concentration below and a higher probability of a trough concentration above the therapeutic range.

Nevirapine

Nevirapine concentrations were assessed on 179 patients fulfilling the inclusion criteria (Table 1). Nevirapine was given as 200 mg twice daily in 157 (88%) patients and

Table 2. Factors influencing efavirenz plasma concentration

	Univariable			Multivariable 1			Multivariable 2		
Factor	Effect*	95% CI	*P*-value	Effect*	95% CI	*P*-value	Effect*	95% CI	*P*-value
Age (per 10 years)	-6.2	(-14.7–+3.2)	0.19	+1.2	(-7.6–+10.9)	0.79	-0.1	(-8.7– +9.8)	0.98
Gender (female versus male)	+60.0	(+32.9–+92.5)	<0.001	+3.6	(-17.9–+30.8)	0.76	+7.3	(-15.2–+35.7)	0.56
Ethnicity (black versus white)	+69.2	(+44.0–+98.9)	<0.001	+64.2	(+32.1–+104.0)	<0.001	+58.5	(+27.1–+97.7)	<0.001
Weight (per 10 kg)	-11.9	(-17.0–-6.4)	<0.001	-10.7	(-16.0–-5.0)	<0.001	-9.5	(-15.0–-3.7)	0.002
Hours from last efavirenz intake	-1.4	(-3.4–+0.6)	0.18	-2.0	(-3.9–-0.1)	0.037	-2.0	(-3.9–-0.1)	0.042
Time on efavirenz (per 4 weeks)	-0.5	(-0.9–-0.1)	0.007	0.0	(-0.5–+0.6)	0.86	0.0	(-0.5–+0.6)	0.96
Time on current regimen (per 4 weeks)	-0.8	(-1.4–-0.2)	0.006	-0.6	(-1.4–+0.1)	0.10	-0.4	(-1.2–+0.3)	0.27
Efavirenz dose (800 mg versus 600 mg)	+53.7	(+24.3–+90.1)	<0.001	+49.7	(+4.1–+115.2)	0.030	+51.8	(+5.0–+119.4)	0.027
On rifabutin (yes versus no)	+6.9	(-32.2–+68.6)	0.77	-12.2	(-43.0–+35.3)	0.55	-12.0	(-43.1–+36.2)	0.57
On rifampicin (yes versus no)	+47.9	(+19.5–+83.1)	<0.001	-33.6	(-55.2–-1.5)	0.042	-34.5	(-56.2–-2.1)	0.039
On PI (yes versus no)	-14.6	(-28.8–+2.4)	0.09	–	–	–	-3.1	(-21.2–+19.3)	0.77
On zidovudine (yes versus no)	-9.9	(-23.9–+6.6)	0.22	–	–	–	-24.7	(-39.3–-6.6)	0.010
On lamivudine (yes versus no)	+27.5	(+7.9–+50.6)	0.004	–	–	–	+20.7	(+2.3–+49.2)	0.081
On stavudine (yes versus no)	-16.8	(-34.0–+5.1)	0.12	–	–	–	-15.2	(-33.6–+8.2)	0.18
On tenofovir (yes versus no)	+2.6	(-13.4–+21.7)	0.76	–	–	–	-0.4	(-19.2–+22.7)	0.97
On didanosine (yes versus no)	-9.9	(-24.9–+8.1)	0.26	–	–	–	+2.6	(-15.8–+25.0)	0.80
On abacavir (yes versus no)	+2.8	(-15.9–+25.6)	0.79	–	–	–	0.0	(-17.8–+21.6)	1.0
On emtricitabine (yes versus no)	-18.7	(-40.2–+10.7)	0.19	–	–	–	-12.4	(-38.0–+23.6)	0.45
On nevirapine (yes versus no)	-15.7	(-45.5–+30.4)	0.44	–	–	–	-20.8	(-48.0–+20.5)	0.27

*Change in drug level (% change in ng/ml). Multivariable model 1: concomitant antiretroviral drugs not considered. Multivariable model 2: considering concomitant antiretroviral drugs. CI, confidence interval; PI, protease inhibitor.

as 400 mg once daily in 22 (12%) patients. Rifampicin was co-administered in ten (6%) and rifabutin in seven (4%) patients, despite recommendations to the contrary [29]. All patients with concomitant TB medication (3/17 black ethnicity) were treated with the 200 mg twice daily schedule. In contrast to efavirenz, distribution of time from last intake was much narrower for nevirapine (median [IQR] for once daily regimen 24 [14–25] h and for twice daily regimen 12 [11–13] h).

In multivariable analyses including concomitant antiviral drugs, nevirapine serum concentration was significantly associated with ethnicity (39% higher in black than in white patients; P=0.002), time between drug intake and sampling (6% lower per additional hour; P<0.001), the use of concomitant rifampicin (40% lower plasma drug exposures; P=0.002), and co-administration of tenofovir (23% higher; P=0.024) and a PI (28% higher; P=0.008; Table 3). No effect of nevirapine schedule (once versus twice daily) or concomitant rifabutin was observed. Notably, and in contrast to the finding for efavirenz, weight did not appear to affect nevirapine concentration (P=0.87). We then tried to disentangle the effect of PIs and included all drugs given in at least 5% of the patients in the model (ignoring ritonavir). This revealed an effect of concomitant atazanavir (n=10; 43% higher

nevirapine, *P*=0.027) and saquinavir (*n*=15; 29% higher nevirapine, *P*=0.05), but not lopinavir (*n*=30; *P*=0.33), leaving other predictors unchanged.

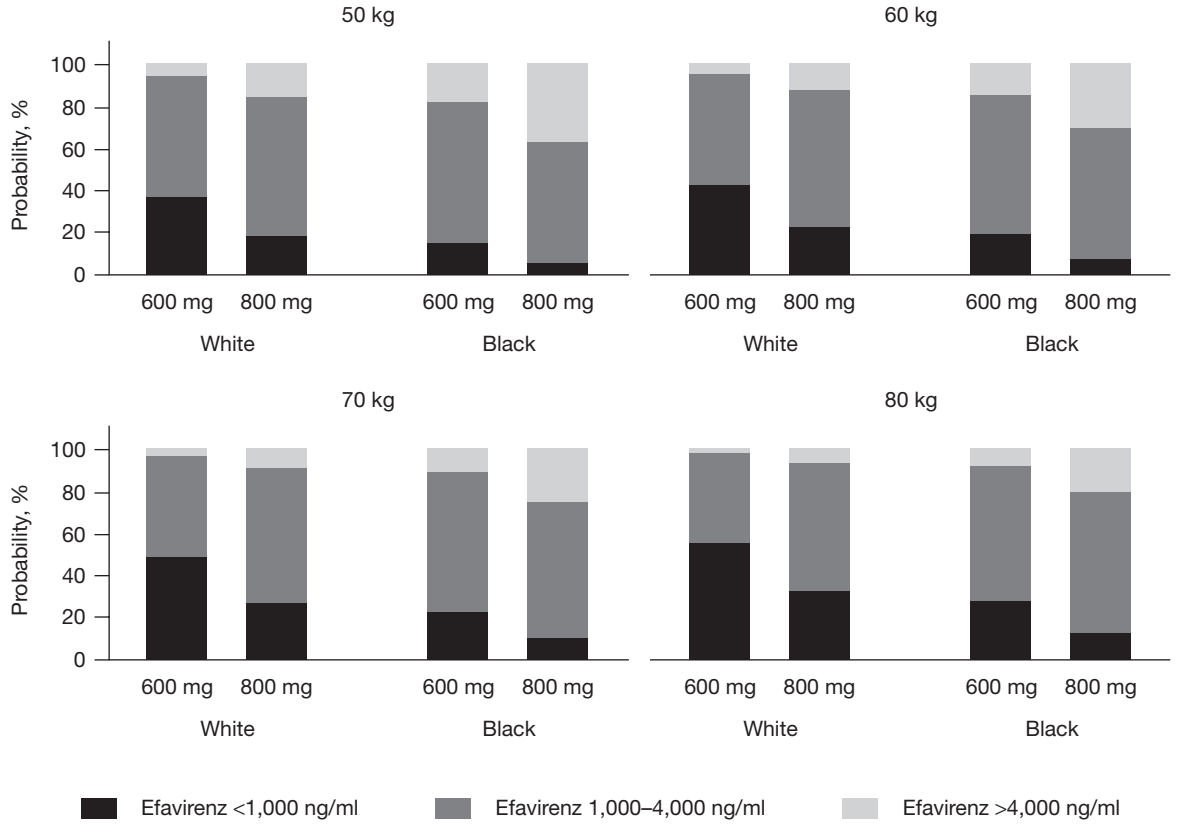
Information on hepatitis B surface antigen, hepatitis C antibody and ALT were available in 111 (62%), 125 (70%) and 125 (70%) patients, respectively; these were last measured a median (IQR) of 10 (0–70), 15 (0–95), and 1 (0–7) weeks before the measurement of nevirapine concentration. During the course of the study, six patients had a positive test result for hepatitis B, 11 patients for hepatitis C and ALT was increased in the last test available in nine patients. In multivariable models, we did not see any association of hepatitis B or ALT with nevirapine concentration, but we found increased drug concentrations (+54%, 95% confidence interval 19–99%; *P*=0.001)in patients who ever tested positive for hepatitis C antibody. Of note, this association did not change the influence of ethnicity, or concomitant rifampicin, tenofovir or PI (results not shown).

The probability of having a nevirapine trough concentration below the recommended therapeutic range while on 200mg twice daily is shown in Figure 2, which illustrates the influence of ethnicity and rifampicin. Of interest, the probability of having a trough concentration <3,400 ng/ml is substantially increased when on concomitant rifampicin, which particularly affects white patients.

Discussion

Conventional drug interaction studies performed during drug development use a crossover or parallel design, usually in healthy volunteers [36]. Whilst the advantages of a controlled approach are obvious, these studies are able only to target suspected drug interactions, and, as a result, many clinically significant drug interactions may be missed. In addition, exposure to all HIV drugs are possibly influenced by body weight,

Figure 1. Probabilities of having a predicted efavirenz concentration below (<1,000 ng/ml), within (1,000–4,000 ng/ml) or above (>4,000 ng/ml) the recommended therapeutic range with rifampicin co-medication for various scenarios



Probabilities were derived from a multivariable regression model. Body weight is given above the charts, and efavirenz dose (600 mg or 800 mg) and ethnicity is shown below. Several assumptions were made: that patients were male (results for women are very similar); age was 41 years; time post-drug intake was 24 h; time since first efavirenz dose 18 months; time on current regimen 12 months (all numbers are means of our study population).

gender, liver function, adherence to therapy, ethnicity or pharmacogenetic variability resulting in considerable interpatient variability. As formal pharmacokinetic studies are usually of small sample size, often with <20 participants [37,38], they are seldom able to examine the effect of these cofactors. In comparison, population studies or TDM registries if sufficiently large are a valuable resource, as they contain 'real world' data incorporating significant numbers of individuals from diverse groups such as ethnic minorities. The linkage of a TDM registry with data from a clinical cohort study allowed us to screen for potential interactions as well as to quantify the effect of demographic and clinical factors on efavirenz and nevirapine exposure in multivariable analyses.

The value of analyses using TDM datasets is a subject of considerable debate, however, not least because these datasets often contain incomplete (or inaccurate) information or samples taken at less than optimal time points. Moreover, TDM datasets are by their very nature selective, as drug monitoring is not universally applied to patients receiving antiretroviral therapy but often for specific indications only. In the UK these include TB and viral hepatitis coinfections, pregnancy, suspected drug interactions or poor adherence, treatment failure and non-licensed use

Table 3. Factors influencing nevirapine plasma concentration

	Univariable			Multivariable 1			Multivariable 2		
Factor	Effect*	95% CI	*P*-value	Effect*	95% CI	*P*-value	Effect*	95% CI	*P*-value
Age (per 10 years)	+3.2	(-4.7–+11.9)	0.43	+6.8	(-0.5–+14.7)	0.07	+3.2	(-4.2–+11.2)	0.40
Gender (female versus male)	+31.8	(+10.6–+57.1)	0.002	+15.7	(-7.6–+44.9)	0.20	+17.3	(-6.6–+47.4)	0.17
Ethnicity (black versus white)	+34.9	(+15.5–+57.5)	<0.001	+34.4	(+9.4–+65.2)	0.005	+38.7	(+12.7–+70.7)	0.002
Weight (per 10 kg)	-0.7	(-6.4–+5.3)	0.81	+0.4	(-4.8–+6.0)	0.87	+0.9	(-4.5–+6.5)	0.75
Hours from last nevirapine intake	-3.6	(-5.1–-2.1)	<0.001	-5.2	(-7.0– -3.4)	<0.001	-5.9	(-7.7–-4.1)	<0.001
Time on nevirapine (per 4 weeks)	+0.2	(-0.1–+0.4)	0.23	+0.2	(0.0–+0.5)	0.07	+0.1	(-0.1–+0.4)	0.35
Time on current regimen (per 4 weeks)	-0.1	(-0.5–+0.3)	0.55	-0.2	(-0.6–+0.2)	0.24	+0.1	(-0.4–+0.5)	0.76
Nevirapine schedule (once versus twice daily)	-20.8	(-36.3– -1.5)	0.036	+16.5	(-10.1–+51.1)	0.25	+12.9	(-14.6–+49.3)	0.39
On rifabutin (yes versus no)	+20.3	(-17.8 –+75.9)	0.34	+3.5	(-31.2–+35.5)	0.84	-5.2	(-32.7–+33.6)	0.76
On rifampicin (yes versus no)	-25.5	(-45.9–+2.4)	0.07	-43.4	(-58.4–-23.1)	<0.001	-39.8	(-56.0–-17.6)	0.002
On PI (yes versus no)	+27.9	(+9.2–+49.8)	0.002	–	–	–	+27.5	(+6.6–+52.5)	0.008
On zidovudine (yes versus no)	-9.0	(-21.7–+5.8)	0.22	–	–	–	-4.3	(-20.7–+15.5)	0.65
On lamivudine (yes versus no)	-12.4	(-25.4–+2.9)	0.11	–	–	–	+10.0	(-8.3–+32.0)	0.30
On stavudine (yes versus no)	-14.3	(-31.7–+7.6)	0.18	–	–	–	-4.6	(-24.2–+20.1)	0.69
On tenofovir (yes versus no)	+7.9	(-8.1–+26.6)	0.35	–	–	–	+22.5	(+2.7–+46.1)	0.024
On didanosine (yes versus no)	+3.6	(-15.0–+26.4)	0.72	–	–	–	+1.3	(-16.6–+23.0)	0.90
On abacavir (yes versus no)	+1.4	(-17.2–+24.0)	0.90	–	–	–	+9.3	(-9.6– 32.2)	0.36
On emtricitabine (yes versus no)	+17.9	(-41.2–+136.7)	0.64	–	–	–	+61.6	(-14.8–+206.7)	0.14
On efavirenz (yes versus no)	+13.1	(-24.7–+70.0)	0.55	–	–	–	-21.6	(-45.4–+12.5)	0.19

*Change in drug level (% change in ng/ml). Multivariable model 1: concomitant antiretroviral drugs not considered. Multivariable model 2: considering concomitant antiretroviral drugs. CI, confidence interval; PI, protease inhibitor.

of doses or regimens of antiretroviral agents [39]. This inherent potential for bias cautions against the uncritical examination of TDM datasets, and may even have led some to doubt the validity of any such analyses even if they are scrupulously undertaken. Nevertheless, providing that the limitations of this approach are understood, we argue that for the purpose of this study – to screen for potential drug interactions and to characterize covariates associated with drug exposure – analysis of TDM datasets may still be valid and add valuable information to the results of formal pharmacokinetic studies.

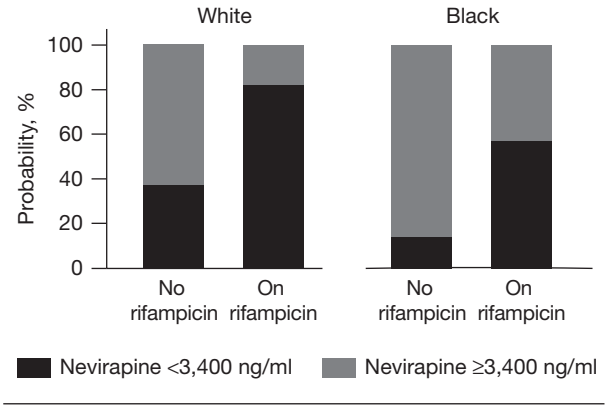
In our study, we saw decreased plasma concentrations for both efavirenz and nevirapine when given with rifampicin, confirming the findings of formal interaction studies. The size of the effect of rifampicin on efavirenz concentrations (decreasing them by 35%) was similar to previous reports (20–32%) [19–21,27,37]. Regarding nevirapine concentrations, small studies of up to 22 participants [24,40,41] have reported a larger effect of concomitant rifampicin (decreasing by 53–68%), whereas two larger trials (n=148 and n=144) in Thai patients, each with half of the patients on concomitant rifampicin [7,22] reported reductions of 46% and 18%, respectively, a magnitude of effect consistent with our observations (decrease by 40%).

In addition, we found that patients on concomitant PI had a 28% higher nevirapine concentration. In previous studies, this has been shown for the (ritonavir boosted) PIs fosamprenavir (trough concentration +22% [42]), darunavir (+47% [43]), and lopinavir (+15% [44]). Whereas the effect of fosamprenavir and darunavir could not be analysed in our study, we did not observe an influence of lopinavir. Instead, we found the effects of PI be driven by concomitant atazanavir and saquinavir (43% and 29% higher nevirapine, respectively); to our knowledge, this has not been shown before. Of note, previous studies have found that nevirapine use was associated with lower concentration of atazanavir [45,46]. Therefore, coadministration of atazanavir and nevirapine might not be without problems.

Furthermore, our study found associations between efavirenz and zidovudine and between nevirapine and tenofovir. Previous drug interaction studies did not provide details of the effect of zidovudine on efavirenz concentrations [37], and we do not have a pharmacological explanation for our finding. It might be a hitherto unrecognised drug interaction, but could also be the result of residual confounding due to unknown patient characteristics. Studies that have explicitly sought an interaction between nevirapine and tenofovir did not observe an effect on nevirapine concentrations [47–49]. Interestingly, a recent paper on efavirenz described a significant statistical interaction between tenofovir and CYP2B6 genotype, with highest efavirenz concentration in those being slow metabolizers and on concomitant tenofovir [50]; however, there was no observed effect of tenofovir when ignoring the genotype, which is consistent with previous results [47]. In our study, we found a significant statistical interaction of ethnicity (a surrogate for genotype) and tenofovir in the analysis of nevirapine (but not efavirenz), with black patients on tenofovir having the highest concentration (P=0.027). The validity of our findings should, however, be confirmed by formal interaction studies, given the potential for bias in TDM datasets.

We observed a strong influence of ethnicity: black patients had around 60% higher efavirenz concentrations, thus confirming previous reports [5,6,8,11]. These differences have been explained by genetic variation, in particular in the polymorphic drug-metabolizing enzyme CYP2B6. For example, in various studies the 516G→T polymorphism – more frequently occurring in black Africans than in white populations – was associated with increased efavirenz concentration [11–13], and in one of these the effect of ethnicity disappeared after statistical adjustment for this polymorphism [11]. The same polymorphism has also been associated with CNS side effects [11,13]. In our study, nevirapine concentration was also higher in black patients compared with white patients, although the effects were less marked than with efavirenz. In the 2NN trial, lower nevirapine clearance was observed in patients from Thailand and South Africa, although ethnicity was not documented [2]. In

Figure 2. Probabilities of having a predicted nevirapine concentration below or above the recommended threshold for efficacy (3,400 ng/ml) for various scenarios in patients on 200 mg nevirapine twice daily



Probabilities were derived from a multivariable regression model. Ethnicity is shown above. Several assumptions were made: age was 42 years, weight was 71 kg, time since first nevirapine dose was 33 months, time on current regimen was 17 months (all numbers are means of our study population), time post-drug intake was 12 h and patients were male (results for women would be similar).

addition, other reports have observed an association between nevirapine concentrations and the CYP2B6 516G→T polymorphism [13,15,16].

Hepatitis B and C has been described as a predictor for high nevirapine concentrations [2,4]. In our study, information on liver disease/function was incomplete. Nevertheless, despite these limitations, we also found a significantly increased nevirapine concentration in patients whose last test for hepatitis C antibody was positive.

Data on associations between NNRTI concentrations and body weight are conflicting. We found that higher body weight was significantly associated with lower efavirenz concentrations, a trend observed in some previous studies [5,21] but not in others [2,6,27]. In keeping with most (but not all [4]) other studies, we did not observe an association between weight and nevirapine concentration. Differences in populations with regard to sample size, ethnicity and other factors are likely to have accounted for these differences and it should be noted that the manufacturer's summary of product characteristics for efavirenz does currently not recommend weight-based dose adjustment for adults [37].

Major controversy remains around whether, in the light of reduced efavirenz exposure with concomitant rifampicin, the dose of efavirenz should be increased in adults. The effect of 800 mg versus 600 mg efavirenz with and without rifampicin was examined in a randomized pharmacokinetic study with 24 HIV-infected individuals [20]. The authors concluded that the decrease caused by rifampicin could be counterbalanced by increasing the efavirenz dose but, given the large interpatient variability in the effect of rifampicin on efavirenz (a finding common to other studies [19,25–28]), increased toxicity might be expected in those patients with high efavirenz concentrations on standard dosing. The authors also pointed out that average efavirenz concentrations in patients with body weight ≥50 kg were half of that of patients <50 kg. Based on these results, recent British and American guidelines recommend a standard dose of 600mg/day in patients weighing <50kg and that a dose increase of 800mg/day should be considered in patients weighing >50 kg [29] or >60 kg [51]. In contrast, current World Health Organization guidelines recommend only the use of 600 mg [52].

Our findings are a useful contribution to this debate. Although efavirenz concentrations are reduced (35%) in the presence of rifampicin, in black African patients (who comprise the majority of TB–HIV-coinfected patients in the UK) this is more than offset by the strong effect of ethnicity (59% higher exposure), which questions the validity of a dose increment of efavirenz in this ethnic group when treating TB coinfection. Supporting evidence for this view comes from a recent report describing a high rate of efavirenz toxicity in black patients on rifampicin and 800 mg efavirenz [25], and from studies reporting a favourable outcome for both TB and HIV when using standard doses of efavirenz with rifampicin in black Africans [27] and Thais (who had a low average body weight of 50 kg) [26]. In addition, we observed a significant association between weight and efavirenz concentrations, and the decrease in exposure of about 10% per additional 10kg may lead to subtherapeutic efavirenz concentrations in patients who are overweight independent of any co-medication, particularly in white patients.

In summary, the linkage of our TDM Registry with a characterized clinical cohort has allowed us to evaluate the important effects of weight and ethnicity on plasma NNRTI exposure and enabled us to study the superimposition of ethnic influences on the key drug interaction between NNRTIs and rifampicin. Several novel suspected drug interactions were identified, but these can only be confirmed in formal prospective studies.

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Additional file

An additional file containing full acknowledgements (list of participating centres and contributors) can be accessed via the Volume 13 Issue 5 contents page for *Antiviral Therapy*, which can be found at www.intmedpress.com (by clicking on 'Antiviral Therapy' then 'Journal PDFs').

Disclosure statement

The costs of TDM of some HIV protease inhibitors (but not non-nucleoside reverse transcriptase inhibitors) were covered by the pharmaceutical industry. The University of Liverpool has spun its TDM service into Delphic Diagnostics Ltd. and SK and DB are non-executive directors of Delphic.

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