Pharmacokinetic and pharmacogenomic modelling of the CYP3A activity marker 4β-hydroxycholesterol during efavirenz treatment and efavirenz/rifampicin co-treatment

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Objectives: To assess the effect of the major efavirenz metabolizing enzyme (CYP2B6) genotype and the effects of rifampicin co-treatment on induction of CYP3A by efavirenz.

Patients and methods: Two study arms (arm 1, n = 41 and arm 2, n = 21) were recruited into this study. In arm 1, cholesterol and 4β-hydroxycholesterol were measured in HIV treatment-naive patients at baseline and then at 4 and 16 weeks after initiation of efavirenz-based antiretroviral therapy. In arm 2, cholesterol and 4β-hydroxycholesterol were measured among patients taking efavirenz during rifampicin-based tuberculosis (TB) treatment (efavirenz/rifampicin) just before completion of TB treatment and then serially following completion of TB treatment (efavirenz alone). Non-linear mixed-effect modelling was performed.

Results: A one-compartment, enzyme turnover model described 4β-hydroxycholesterol kinetics adequately. Efavirenz treatment in arm 1 resulted in 1.74 (relative standard error = 15%), 3.3 (relative standard error = 33.1%) and 4.0 (relative standard error = 37.1%) average fold induction of CYP3A for extensive (CYP2B6*1/*1), intermediate (CYP2B6*1/*6) and slow (CYP2B6*6/*6) efavirenz metabolizers, respectively. The rate constant of 4β-hydroxycholesterol formation [mean (95% CI)] just before completion of TB treatment [efavirenz/rifampicin co-treatment, 7.40 × 10⁻⁷ h⁻¹ (5.50 × 10⁻⁷ – 1.00 × 10⁻⁶)] was significantly higher than that calculated 8 weeks after completion [efavirenz alone, 4.50 × 10⁻⁷ h⁻¹ (4.40 × 10⁻⁷ – 4.52 × 10⁻⁷)]. The CYP3A induction dropped to 62% of its maximum by week 8 of completion.

Conclusions: Our results indicate that efavirenz induction of CYP3A is influenced by CYP2B6 genetic polymorphisms and that efavirenz/rifampicin co-treatment results in higher induction than efavirenz alone.

Keywords: induction, pharmacogenetics, HIV

Introduction

Drug–drug interaction is one of the major concerns of co-treatment of HIV comorbidities because of the potential induction or inhibition of drug metabolizing enzymes and transporters by antiretroviral drugs. Efavirenz is of importance in this regard as both in vitro and in vivo studies have demonstrated its duration-dependent induction of drug metabolizing enzymes.1–6 The magnitude of CYP3A enzyme induction by efavirenz and its implications for outcomes of treated HIV co-morbidities have not been extensively studied. Knowledge of factors influencing the magnitude of efavirenz-based induction of this enzyme system could be useful in aiding appropriate dosage adjustments of concomitant medications.

Determination of the plasma concentration of 4β-hydroxycholesterol and the 4β-hydroxycholesterol/cholesterol ratio was proposed by Diczfalusy et al.7–9 as an alternative
measure of CYP3A4/5 induction or inhibition. It was recently shown that 4β-hydroxycholesterol is comparable to the probe drug midazolam as a marker of induction of CYP3A by rifampicin.\textsuperscript{10} It is therefore a useful alternative to probe drugs since it avoids subjecting patients to pill burden and drug interactions. However, studies employing this marker have reported different magnitudes of CYP3A4/5 induction, probably because of the differences in sampling schedules, study populations or genetic polymorphisms.\textsuperscript{11–13}

Various factors influence the magnitude of induction, including genetic polymorphisms and plasma concentration of the inducer. CYP3A4 and CYP3A5 enzymes are encoded by polymorphic genes, some alleles of which result in enzyme isoforms with low/absent catalytic activity.\textsuperscript{14,15} Such polymorphisms may therefore be responsible for variability in the extent of induction.\textsuperscript{2,16,17}

CYP2B6 is a polymorphic enzyme responsible for efavirenz hydroxylation, a major efavirenz metabolic pathway.\textsuperscript{18,19} Its dysfunctional allele $C_{P Y P 2 B 6}^* 6,20$ resulting in three genotypes ($C_{P Y P 2 B 6}^* 1/1$, $C_{P Y P 2 B 6}^* 1/6$ and $C_{P Y P 2 B 6}^* 6/6$) with differing efavirenz metabolizing capacities, is responsible for the wide inter-individual variation in efavirenz plasma concentration.\textsuperscript{2,5,21} It is expected, therefore, that the $C_{P Y P 2 B 6}^* 6$ genetic polymorphism, influencing CYP2B6 auto-inducibility and hence efavirenz plasma concentrations, will also determine the magnitude of induction of CYP3A4/5.

In this study we aimed to study induction of CYP3A during efavirenz-based antiretroviral therapy (ART) with or without rifampicin co-treatment. The specific aims were to assess the effect of CYP2B6*6 genetic polymorphisms on CYP3A induction and to determine whether rifampicin adds to efavirenz’s induction effect when the two drugs are co-administered.

Patients and methods

Study design, participants and data collection

This study involves patients from a main study on the optimization of HIV/tuberculosis (TB) co-treatment. The design, inclusion criteria, drug regimens and clinical/laboratory follow-up of patients in the main study have been reported previously.\textsuperscript{22} Briefly, HIV treatment-naïve patients were recruited into arm 1 of the study and initiated on efavirenz-based ART immediately, while HIV/TB coinfected, treatment-naïve patients were recruited into arm 2 of the study and initiated on rifampicin-based anti-TB therapy followed by efavirenz-based ART 4 weeks after initiation of anti-TB therapy.

In the current study, blood for quantification of plasma concentration of cholesterol and 4β-hydroxycholesterol was collected (in 4.5 mL EDTA vacutainers) from arm 1 patients ($n=41$) at day 0 and at weeks 4 and 16 after initiation of ART. Blood was also collected from arm 2 patients ($n=21$) just before completion of TB treatment (after ~5 months of efavirenz/rifampicin co-treatment) and then up to about 2 months after discontinuation of TB treatment (with continuation of efavirenz), specifically at days 0, 1, 3, 7, 14, 21, 42 and 56 after completion of TB treatment. This sampling scheme was chosen because of the previously reported long elimination half-life for 4β-hydroxycholesterol.\textsuperscript{7} The collected blood was immediately centrifuged to obtain plasma, which was stored at $-80^\circ$C until analysis. Ethical approval to conduct the study was obtained from the Institutional Review Board of the Muhimbili University of Health and Allied Sciences in Dar es Salaam, Tanzania. Prior written informed consent was obtained from all study participants.

Demographic, laboratory values and genotype data were obtained from the main study database. Procedures and protocols for genomic DNA isolation and genotyping for $C_{P Y P 2 B 6}^* 6$, CYP3A5*3, *5 and *7, UGT2B7*2, ABCB1 c.3435C>T and SLC01B1*1b and *5 were as reported previously,\textsuperscript{5,22}

Quantification of plasma cholesterol and 4β-hydroxycholesterol concentration

Cholesterol was determined by a commercial enzymatic method (Cholesterol CHOD-PAP, Roche Diagnostics GmbH, Mannheim, Germany) run on a Roche/Hitachi Modular instrument. The between-day variation was 1.3% (at 5 mmol/l). Plasma 4β-hydroxycholesterol was quantified by gas chromatography–mass spectrometry with the instrument settings and sample preparation procedure reported by Bodin et al.\textsuperscript{23} and Diczfalusy et al.,\textsuperscript{24} respectively. The within-day variation was 4.5% and the between-day variation was 8.2%. The lower limit of quantification was 4.8 ng/mL. The method was linear up to 600 ng/mL.

Quantification of efavirenz plasma concentrations

Blood sampling and quantification of plasma efavirenz by liquid chromatography–mass spectrometry (LC-MS) were carried out as described previously.\textsuperscript{3} Briefly, plasma obtained from blood sampled 16 h post-dose at week 16 of efavirenz-based ART was analysed using an LC-MS method developed and validated at the Department of Clinical Pharmacology and Pharmacoepidemiology, University of Heidelberg. The lower limit of quantification was 10.0 ng/mL and the calibration range was between 10 and 10000 ng/mL. The method was validated according to the FDA validation guidelines and fulfilled all criteria concerning accuracy, precision, recovery, linearity and stability.

Statistics

Demographic, laboratory, genotype, 4β-hydroxycholesterol and cholesterol plasma concentration data were imported into R statistical software version 2.15 for statistical analyses and preparation of the data for NONMEM-based non-linear mixed-effect modelling. Analysis of variance and Tukey’s honest significant difference (HSD) post-hoc test were used to compare week 16 efavirenz plasma concentrations among the $C_{P Y P 2 B 6}^* 6$ genotypes. A linear regression was used to assess association between the estimated magnitudes of induction and the week 16 efavirenz plasma concentrations.

Non-linear mixed-effect modelling and simulations

The plasma concentrations of 4β-hydroxycholesterol and cholesterol were transformed into molar concentrations for modelling. NONMEM version 7.2 (ICON Development Solutions, Ellicott City, MD, USA) was used for non-linear mixed-effect modelling. Analysis of variance and Tukey’s honest significant difference (HSD) post-hoc test were used to compare week 16 efavirenz plasma concentrations among the $C_{P Y P 2 B 6}^* 6$ genotypes. A linear regression was used to assess association between the estimated magnitudes of induction and the week 16 efavirenz plasma concentrations.

Ngaimisi et al.
model parameters for each dataset. Precisions of estimation of the model parameters (relative standard errors) obtained through the bootstrap approach were compared with those obtained through the NONMEM covariance step. Performance of the final model was tested by simulation-based diagnosis (visual predictive check) using PsN and xpose4 package.

**Rate constants of 4β-hydroxycholesterol formation**

Parameter estimates for induction and baseline rate constants of 4β-hydroxycholesterol formation were used to calculate rate constants of 4β-hydroxycholesterol formation at maximum induction in arm 1 and at maximum decay of induction in arm 2 (for details see the Supplementary data available at JAC Online).

**Determination of the minimum duration of treatment that achieves the maximum increase in 4β-hydroxycholesterol plasma concentrations**

A function describing a one-compartment enzyme turnover model for the induction and fall of induction during treatment and after stopping the inducer, respectively, was written and evaluated using R package deSolve version 1.10–4. The parameters of the model were $K$, $KO$, $IF1$, $γ$ and duration of induction ($T_{\text{max}}$). Monte Carlo simulations of 4β-hydroxycholesterol concentrations were implemented using an FME package version 1.3. The simulated 4β-hydroxycholesterol concentrations were obtained by fixing $K$, $KO$, $IF1$ and $γ$ to estimated parameter values (see Table 2) and $T$ to a preferred value of magnitude fold of induction (2, 5 or 7.5), while $T_{\text{max}}$ was increased from 0 to 100 days. For simulation purposes a unit value for initial enzyme amount was assumed, while baseline concentrations for cholesterol and 4β-hydroxycholesterol were assumed to be equal to the mean baseline values obtained in this study (1353.28 and 48.2 ng/mL, respectively). The percentage increases in 4β-hydroxycholesterol concentration at each $T_{\text{max}}$ were then calculated and plotted versus $T_{\text{max}}$ using an R package ggplot2.

**Results**

**Baseline characteristics**

Baseline characteristics for arm 1 patients (assessed before initiation of ART) were comparable to those for arm 2 patients (assessed just before completion of TB therapy) (Table 1). Among 39 arm 1 patients with genotype data, 14 were fast metabolizers, 18 were intermediate and the rest were slow metabolizers. In arm 2, only 16 patients had genotype data and 9 were fast, 7 were intermediate and the rest were slow metabolizers.

**Changes in 4β-hydroxycholesterol/cholesterol ratio in arms 1 and 2**

A total of 123 and 171 plasma samples for determination of cholesterol and 4β-hydroxycholesterol concentrations were obtained from arm 1 and arm 2 patients, respectively. As shown in Figure 1, efavirenz-based ART resulted in an increase in 4β-hydroxycholesterol/cholesterol ratio from the median (IQR) of $3.07 \times 10^{-5}$ (2.12 $\times 10^{-5}$ to $3.80 \times 10^{-5}$) to $9.90 \times 10^{-5}$ (6.90 $\times 10^{-5}$ to $1.4 \times 10^{-4}$) at week 4 and to 1.03 $\times 10^{-4}$ (8.16 $\times 10^{-5}$ to $1.4 \times 10^{-4}$) at week 16. In patients completing TB treatment but continuing on efavirenz-based ART, the 4β-hydroxycholesterol/cholesterol ratio dropped from a median (IQR) of $1.23 \times 10^{-4}$ (1.03 $\times 10^{-4}$ to $1.78 \times 10^{-4}$) to $8.48 \times 10^{-5}$ (6.7 $\times 10^{-5}$ to $9.67 \times 10^{-5}$) 56 days after completing rifampicin treatment.

**Table 1.** Demographic, genetic and laboratory characteristics of study participants; for arm 1, these are values before initiation of ART, while for arm 2 these are values at completion of TB therapy

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Arm 1 (n = 41)</th>
<th>Arm 2 (n = 23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years, mean (SD)</td>
<td>39.7 (12)</td>
<td>40.2 (7)</td>
</tr>
<tr>
<td>Proportion of female patients (SE)</td>
<td>0.63 (0.075)</td>
<td>0.57 (0.103)</td>
</tr>
<tr>
<td>Body mass index, mean (SD)</td>
<td>19.46 (2.9)</td>
<td>22.47 (4.6)</td>
</tr>
<tr>
<td>Serum creatinine (μmol/L), mean (SD)</td>
<td>78.6 (17)</td>
<td>77.3 (49)</td>
</tr>
<tr>
<td>Proportion of patients who smoke (SE)</td>
<td>0.05 (0.033)</td>
<td>0.13 (0.070)</td>
</tr>
<tr>
<td>Proportion of CYP2B6*6 alleles (SE)</td>
<td>4.9 (0.05)</td>
<td>21.8 (0.07)</td>
</tr>
<tr>
<td>Proportion of UGT2B7*2 alleles (SE)</td>
<td>70.5 (0.05)</td>
<td>43.7 (0.09)</td>
</tr>
<tr>
<td>Proportion of CYP3A5*0 alleles (SE)</td>
<td>50.0 (0.06)</td>
<td>43.0 (0.09)</td>
</tr>
<tr>
<td>Proportion of ABCB1 3435C&gt;T; T SNP (SE)</td>
<td>21.7 (0.05)</td>
<td>9.3 (0.05)</td>
</tr>
<tr>
<td>Proportion of ABCB1 4036AG; G SNP (SE)</td>
<td>20.5 (0.05)</td>
<td>34.3 (0.08)</td>
</tr>
<tr>
<td>Proportion of SLC10A1+b1 alleles (SE)</td>
<td>84.6 (0.04)</td>
<td>81.2 (0.07)</td>
</tr>
</tbody>
</table>

*6CYP3A5 alleles *3, *7 and *7 are non-synonymous and therefore have been replaced by “0” notation.

*6SLCO1B1 allele *1b is due a non-synonymous SNP at c.388A>G and a normal nucleotide at c.521T>C.

**The final 4β-hydroxycholesterol kinetic model**

A one-compartment enzyme turnover model described the plasma kinetics of 4β-hydroxycholesterol. Table S2 (available as Supplementary data at JAC Online) summarizes results from structural, stochastic and covariate model-building approaches. Collinearity assessment of the structural model parameters (see the Supplementary data, Table S1 and Figure S2 available at JAC Online) determined four parameters to be identifiable (collinearity $= 14$). The identifiable parameters were $K$ = 4β-hydroxycholesterol elimination rate constant, $I$ = maximum fold induction in arm 1, $K_{F2}$ = rate constant of 4β-hydroxycholesterol formation just before completion of TB treatment and $D$ = maximum decay of induction. Unidentifiable parameters were fixed to values reported in the literature [enzyme turnover constant ($K_{O}$ = 0.0099)24,25 and rate constant of 4β-hydroxycholesterol formation just before initiation of ART in arm 1 ($K_{F1}$ = 1.785 $\times 10^{-7}$ h$^{-1}$26]. Neither the doubling nor the halving of such fixed parameters resulted in any significant decrease in objective function (see Table S2).

Parameter versus covariate plots did not reveal relationships between stochastic model parameters and any of the continuous covariates (age, weight, body mass index, serum creatinine, viral load, CD4 cell count), but indicated relationships with some categorical covariates (genotypes for CYP2B6*6, UGT2B7*2 and smoking status). CYP3A5 genetic polymorphism did not influence any of the model parameters. After stepwise covariate model building, the final model had CYP2B6*6 genotype and smoking status as covariates for maximum fold induction ($I$) and 4β-hydroxycholesterol elimination rate constant ($K$), respectively. Therefore $I$ and $K$ parameters of the final model can be represented by the following equations:

\[
I = 1.74 \times [1 + 0.91 \times (\text{CYP2B6}^{*1} / \text{6}) + 1.30 \times (\text{CYP2B6}^{*6} / \text{6})]
\]

\[
K = 0.0059 \times (1 - 0.25 \times \text{SMOKER})
\]
Table 2 is a summary of values for median, 2.5th and 97.5th percentiles of final model parameters obtained after fitting 500 non-parametric bootstrap datasets to the model. The precision of the parameter estimates calculated by the NONMEM covariance step was comparable to those generated by the non-parametric bootstrap approach (see Table S2).

Figure 2 shows goodness-of-fit plots for the final model, indicating that the model adequately fitted the data. A visual predictive check (Figure S3, available as Supplementary data at JAC Online) indicates that the final model could simulate 4β-hydroxycholesterol plasma concentration–time profiles very well both for arm 1 and arm 2 patients.

The fraction of the initial enzyme amount remaining after maximum decay of induction in arm 2 patients was calculated to be 0.62 (about 1.56-fold decline in enzyme amount).
Efavirenz plasma concentration is dependent on CYP2B6 genotype and is correlated to magnitude of CYP3A induction

The week 16 efavirenz plasma concentration was significantly higher among individuals with CYP2B6*6/*6 (mean ± SE = 3769 ± 705 ng/mL) and CYP2B6*1/*6 (mean ± SE = 2724 ± 500 ng/mL) genotypes compared with those with CYP2B6*1/*1 (mean ± SE = 1295 ± 164 ng/mL) genotype (P = 0.011 and P = 0.053, respectively). The maximum CYP3A induction at week 16 was in turn positively correlated to week 16 efavirenz plasma concentration [adjusted $R^2 = 70.1$, $P < 0.001$, regression coefficient (relative standard error) = 0.000882 (10%)].

Rate constant of 4β-hydroxycholesterol formation

The rate constant for 4β-hydroxycholesterol formation among treatment-naive arm 1 patients (KF1) was fixed to the reported typical value of $1.785 \times 10^{-7}$ h$^{-1}$.$^{36}$ During structural model building, neither doubling nor halving this value resulted in any significant change in objective function (see Table S2). The estimated population-typical value for rate constants of 4β-hydroxycholesterol formation among treatment-experienced arm 2 patients on ART and anti-TB (KF2) is given in Table 2. The mean (95% CI) for calculated rate constants of 4β-hydroxycholesterol formation among arm 1 patients after maximum induction (see the Supplementary data available at JAC Online for details) was $4.4 \times 10^{-7}$ h$^{-1}$ ($4.20 \times 10^{-7}$ to $4.60 \times 10^{-7}$) and it was $4.50 \times 10^{-7}$ h$^{-1}$ ($4.40 \times 10^{-7}$ to $4.52 \times 10^{-7}$) after maximum decay of induction among arm 2 patients.

Duration of treatment to achieve maximum increase in 4β-hydroxycholesterol plasma concentrations

The simulations using our model parameters showed that, for a given fold induction by an inducer, the maximum increase in 4β-hydroxycholesterol plasma concentrations is only achieved when the duration of exposure to an inducer is >43 days (Figure S4, available as Supplementary data at JAC Online).

Discussion

The identification and use of endogenous markers for enzyme activity, particularly CYP3A4/5, are subjects of active research because of the advantages of such compounds and methods in preclinical and clinical drug development as well as therapeutic drug monitoring and dosage individualization. Our study utilized the plasma concentration of 4β-hydroxycholesterol and cholesterol to assess CYP3A4/5 induction. We show that efavirenz-based ART causes elevation of the 4β-hydroxycholesterol/cholesterol ratio and combined efavirenz/rifampicin treatment causes much higher elevations that decline after stopping rifampicin treatment. Results from mathematical modelling of our data indicate that the induction of CYP3A4/5 production follows a time-dependent e-max model (i.e. early rapid increase, which slows down with time to a constant value).

Our estimate of population maximum fold induction among arm 1 patients agrees with values reported from an in vitro induction assay where an efavirenz concentration of 5 μM (1.6 μg/mL)
caused a 3- to 4-fold induction. We further show that the magnitude of induction is dependent on the CYP2B6*6 genotype and is significantly correlated to efavirenz plasma concentration. The CYP2B6*6 polymorphism results in fast, intermediate and poor metabolizers of efavirenz and therefore causes gene–dose-dependent variation in the efavirenz plasma concentration, which in turn results in induction variation. This is in line with the previous finding in an Ethiopian population where the magnitude of change in 4β-hydroxycholesterol/cholesterol ratio from baseline after efavirenz-based ART was influenced by CYP2B6 genotype and there was significant correlation between 4β-hydroxycholesterol/cholesterol ratio and efavirenz plasma concentration. However, in comparison with the magnitude of increase in median 4β-hydroxycholesterol/cholesterol ratio observed at week 16 in the Ethiopian cohort, the magnitude of induction observed in our cohort is relatively smaller. The concentration-dependent induction of CYP3A by efavirenz was initially reported by Hariparsad et al., who showed that higher efavirenz concentrations were associated with greater mobilization of nuclear receptors (hCAR, hPXR). This implies that, in comparison with efavirenz/rifampicin co-treatment, efavirenz treatment alone might also result in higher induction at higher exposures.

The influence of the CYP2B6*6 polymorphisms on the magnitude of CYP3A4/5 induction is a good example of how genetic polymorphism influences drug–drug interaction. Drug interactions between efavirenz and CYP3A4/5 substrates during the treatment of HIV comorbid conditions would be modified by CYP2B6*6 genotypes with individuals having a CYP2B6*6/*6 genotype, resulting in lower plasma concentrations of the CYP3A4/5 substrates compared with other genotypes. Although CYP3A4/5 metabolizes efavirenz, our previous studies did not show any significant influence of CYP3A5 polymorphisms on efavirenz plasma concentrations, which would otherwise counteract the influence of the CYP2B6 polymorphism.

Figure 2. Goodness-of-fit plots for the final model. (a) Population prediction of the dependent variable (PRED) and individual prediction of the dependent variable (IPRED) versus observed dependent variable (DV) are plotted on the same graph with IPRED versus DV shown by black points. In this panel the PRED and IPRED are very well correlated to DV. (b and c) Individual weighted residuals (IWRES) are plotted versus IPRED and PRED, respectively. In these panels the IWRES is <2.5 and there is no significant trend over the predictions. (d) WRES is plotted versus time and there is no significant trend over time.
Our results show that the CYP3A5 polymorphism did not influence the magnitude of CYP3A induction either in arm 1 or in arm 2. This suggests that a major proportion of 4β-hydroxycholesterol formation might be due to CYP3A4; therefore, under potent PXR and hCAR activators such as rifampicin and efavirenz, induction of CYP3A4 obscures less potent differential induction of CYP3A5 isoforms. It also implies that potent inducers like rifampicin and efavirenz are able to induce CYP3A4 equally in both CYP3A5*1 carriers and non-carriers, as observed previously by Roberts et al.

The utility of 4β-hydroxycholesterol/cholesterol ratio as a marker of rapid changes in enzyme activity is limited by its long elimination half-life, determined in a few European volunteers to be 17 days. By simulating 4β-hydroxycholesterol time profiles using the 17 day half-life and enzyme turnover half-life fixed to 70 h, Yang and Rodrigues were able to support this finding. Our mathematical modelling has estimated a population-typical value for the elimination half-life of 4β-hydroxycholesterol to be about 4.9 days when enzyme turnover half-life is fixed to 70 h. Our analysis is based on data from 64 individuals and involves both an induction phase in arm 1 and the fall of induction in arm 2. Through sensitivity analyses we determined that our data can only support the estimation of 4β-hydroxycholesterol elimination half-life when enzyme turnover half-life is fixed to a certain value. There is lack of consensus on the CYP3A turnover half-life to be used for estimation of induction or inhibition due to a wide variation in reported CYP3A turnover half-lives. The value used in our modelling is therefore based on similar results obtained from two different in vivo studies, one of which assessed the time profile of 6β-hydroxycortic/1 control during both the induction and the fall of induction phases and the other assessed CYP3A induction and fall of induction using midazolam as a probe drug.

In the developed model, the zero-order rate constant of enzyme production is modelled as a function of time. The determined typical value of time constant of induction of 157 h implies that the increase in enzyme production reaches a maximum after about 33 days (five half-lives). This together with the long elimination half-life for 4β-hydroxycholesterol also implies that long treatment duration by an inducer is required for a sensitive measurement of its maximum induction capacity. In fact, our Monte Carlo simulations indicate that, to be able to observe the maximum increase in 4β-hydroxycholesterol plasma concentration, subjects should be treated with an inducer for at least 43 days. This drawback has been pointed out in earlier studies, however, during treatment of chronic conditions, changes in the 4β-hydroxycholesterol/cholesterol ratio compared with baseline values can be used to assess the maximum CYP3A induction capacity of the inducers and therefore aid in dosage adjustments. The size of the cholesterol central pool has been determined to be about 24.2 ± 0.8 g. Assuming a plasma cholesterol concentration of 4.5 mmol/L and a rapid exchange between plasma and the central pool, the pool volume can be calculated to be about 14 L. It is therefore reasonable to suggest that the population-typical value for clearance of 4β-hydroxycholesterol is about 0.0826 L/h. The low clearance value for 4β-hydroxycholesterol is probably due to slow metabolism rather than poor urinary excretion. Bodin et al. were able to show that the compound undergoes very slow conversion into acidic products and is 7α-hydroxylated at a slower rate than cholesterol by recombinant human CYP7A1. The observed effect of smoking on the 4β-hydroxycholesterol elimination rate constant may be attributed to compounds in tobacco smoke that might inhibit its metabolism. Inhaled tobacco smoke has been reported to inhibit the human monoamine oxidase inhibitor and crystalline enzymes.

On the other hand, CYP3A enzymes are promiscuous, having active sites that can accommodate substrates of different sizes and shapes. CYP3A4 exhibits complex in vitro kinetics due to homotropic and heterotropic cooperativity, i.e. similar or different substrates are able to occupy the active site and influence each other’s metabolism. Since our patients were taking other concomitant medications during ART alone and during HIV/TB co-treatment, it is possible that cooperativity might have influenced the formation of 4β-hydroxycholesterol in our study subjects. This phenomenon was not accounted for in our model.

Considering the reported influence of cytokines on enzyme activities in vitro, viral load and opportunistic infections as indicators of cytokine production may be hypothesized to influence induction, decay of induction and even enzyme turnover. In this study viral load did not influence any of the model parameters. In fact, different cytokines have different influences on different human tissues; while some cytokines down-regulate CYP450 enzyme activity in hepatocyte culture they up-regulate the enzymes in PBMC culture. The overall effect of cytokines in HIV patients on enzyme activities in vivo is still unknown. Therefore, at this juncture it may be justified to attribute to drug treatment the observed temporal changes in 4β-hydroxycholesterol concentrations.

During the design and execution of this study, there was no information to guide the calculation of the appropriate sample size for this study. Therefore, while we acknowledge the limitation of the smaller sample size, our results could be useful in the estimation of appropriate sample size and design for future studies with similar objectives.

In conclusion, we have developed a one-compartment enzyme turnover model that describes plasma 4β-hydroxycholesterol kinetics during induction and fall of induction very well. Our results indicate that efavirenz treatment results in appreciable gene-dose-dependent induction of CYP3A4/5 activity during ART; HIV/TB co-treatment results in even higher induction, which declines by <2-fold to a new steady-state level after completion of TB therapy due to the presence of efavirenz in a continuing ART regimen. The magnitude of induction during efavirenz-based ART and HIV/TB co-treatment, particularly in individuals with the CYP2B6*6/*6 genotype, may lead to sub-therapeutic plasma concentrations of concomitant CYP3A4/5 drug substrates and hence treatment failure and resistance, especially for drugs with a narrow therapeutic window. It may be necessary to increase doses of concomitant CYP3A substrates among patients with the CYP2B6*6/*6 genotype taking efavirenz or efavirenz/rifampicin co-treatment. For patients completing HIV/TB co-treatment, dosage adjustments may need to be considered.

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Supplementary data

Supplementary data (including Tables S1 and S2 and Figures S1–S4) are available at JAC (http://jac.oxfordjournals.org/).

References


