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Effect of mid-dose efavirenz concentrations and *CYP2B6* genotype on viral suppression in patients on first-line antiretroviral therapy

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ABSTRACT

The therapeutic range for efavirenz plasma concentrations is unclear and some studies found no correlation with viral non-suppression. Efavirenz concentrations are variable, driven in part by polymorphisms in *CYP2B6*. We hypothesised that efavirenz mid-dosing concentrations, together with *CYP2B6* metaboliser genotype, could predict viral non-suppression. Participants starting first-line efavirenz-based antiretroviral therapy were monitored for 48 weeks. HIV-RNA and efavirenz mid-dose interval concentrations were determined at Weeks 16 and 48. *CYP2B6* metaboliser genotype status was determined by 516G→T and 983T→C polymorphisms. Cox proportional hazards modelling was used to predict viral non-suppression and to determine the most predictive efavirenz mid-dosing concentration threshold. In total, 180 participants were included. Median efavirenz concentrations were 2.3 mg/L (IQR 1.6–4.6 mg/L) and 2.2 mg/L (IQR 1.5–3.9 mg/L) at Weeks 16 and 48, respectively. Moreover, 49 (27.2%), 84 (46.7%) and 39 (21.7%) participants had extensive, intermediate or slow *CYP2B6* metaboliser genotype, respectively. Log_2 efavirenz concentrations [adjusted hazard ratio (aHR) = 0.77, 95% CI 0.67–0.89] and baseline CD4 cell count (aHR = 0.994, 95% CI 0.989–0.998), but not *CYP2B6* genotype, were predictive of viral non-suppression. For every doubling of efavirenz concentration there was a 23% decrease in the hazard of non-suppression. A threshold of 0.7 mg/L was found to be the efavirenz mid-dosing concentration that was most predictive of non-suppression. Mid-dosing efavirenz concentrations are predictive of viral non-suppression, but the currently recommended lower therapeutic limit (1 mg/L) is higher than our finding. Knowledge of *CYP2B6* metaboliser genotype is not required for prediction of virological outcomes.

1. Introduction

Efavirenz is a good candidate for therapeutic drug monitoring (TDM) because there are reliable assays, its plasma concentrations are characterised by high inter-individual variability, and low concentrations have been linked with viral non-suppression and high concentrations with toxicity [1–3]. However, the relationship between efavirenz concentrations and viral suppression has not always been consistent in studies, perhaps due to the rapid development of high-level resistance to non-nucleoside reverse transcriptase inhibitors (NNRTIs), thus efavirenz TDM is not routinely recommended [1–6].

The high inter-individual variability of efavirenz concentrations is explained in part by polymorphisms in *CYP2B6*, the gene that encodes the cytochrome P450 (CYP) enzyme CYP2B6 [7–9]. The prevalence of genetic slow metabolisers is high in sub-Saharan African populations [7]. Metaboliser genotype status (ultraslow, slow, intermediate or extensive) did not impact virological outcomes in a recent analysis of pooled studies conducted by the AIDS Clinical Trials Group (ACTG), but the impact of genotype status on virological failure has not been fully explored in a South African population [10].

The lower limit of the currently recommended therapeutic range (1–4 mg/L) for efavirenz is controversial [6,11,12]. Marzolini et al reported that mid-dose efavirenz drug concentrations of <1 mg/L were associated with increased rates of virological failure [11]. Whilst pharmacokinetic data from the 2NN (double nonnucleoside) study suggested an increase in virological failure with trough concentrations of <1.1 mg/L, the authors did not recommend using this

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cut-off value to predict virological outcomes as the sensitivity was low [12]. Recently published data from the ENCORE1 study noted that only a small proportion of those failing treatment had mid-dosing efavirenz concentrations of <1.0 mg/L [6].

We hypothesised that mid-dosing interval efavirenz drug concentrations, together with knowledge of *CYP2B6* metaboliser genotype, would be predictive of virological outcome in a sub-Saharan African population starting first-line antiretroviral therapy (ART). We also examined the lower threshold concentration of efavirenz for therapeutic benefit.

2. Methods

2.1. Participants, setting and standard of care

Participants were recruited at the Hannan Crusaid Treatment Centre (HCTC), a large outpatient ART centre in Cape Town, South Africa. The cohort included ART-naïve adults and adolescents who were eligible if they had their own mobile phone and were willing to sign a written informed consent form.

All those entering the treatment programme at HCTC received three group counsellor-driven treatment literacy sessions prior to commencing NNRTI-based ART [13,14]. They were also visited at home by a community care worker to confirm their address and home circumstances. Those with a raised viral load or low adherence based on a count of tablet returns (<90%) received a stepped-up adherence package, including tailored counselling, monthly drug dispensing and further home visits. Participants were traced by phone call and home visit if they were >4 weeks late for a clinic visit.

2.2. Sub-study design

The parent study was a randomised controlled trial over 48 weeks investigating adherence to ART and has been described in detail elsewhere [15]. Participants also had the option of joining a non-randomised voluntary pharmacokinetic and pharmacogenetic sub-study, which required additional blood sampling.

2.3. Visits and sampling

Sub-study visits included screen, baseline, and Weeks 16 and 48. Visits were timed to coincide with booked clinic visits to minimise inconvenience. Participants were reimbursed for local travel (R20 or ca. US$2) at each visit and were offered a gift of a T-shirt, bag or mug valued at R80 (ca. US$8) or less for each on-study visit.

Demographic and psychosocial details were collected at screen. Prescribed ART was recorded at baseline (Week 0). Weight and current ART were confirmed at all visits. Blood was drawn for CD4 cell count (BD FACSCount™; Becton Dickinson, Franklin Lakes, NJ) and HIV-1 viral load (HIV-1 RNA 3.0 assay®; Bayer Healthcare, Leverkusen, Germany) at screen and at Weeks 16 and 48. At Weeks 16 and 48, for those who gave additional consent, blood was drawn in a lithium heparin tube for mid-dosing interval efavirenz concentrations, in the window between 9 h and 16 h after self-reported efavirenz intake time, and in an ethylene diamine tetra-acetic acid (EDTA) tube for *CYP2B6* pharmacogenetic analysis.

At Weeks 16 and 48, most blood samples for efavirenz concentration and viral load were drawn on the same date. However, in a number of participants, viral load measurements were obtained up to 4 weeks before the scheduled pharmacokinetic visit (as part of standard of care) or afterwards (when the measurement had to be repeated due to issues with the measuring procedure). Samples were kept cold (4 °C) until transfer to the laboratory within 2–3 h of blood draw.

2.4. Pharmacokinetic analyses

Samples were centrifuged at 3500 rpm for 10 min and plasma was pipetted into cryovials that were labelled and frozen at –80 °C. Samples were analysed for efavirenz concentrations using a validated liquid chromatography–tandem mass spectrometry (LC-MS/MS) method.

2.5. Pharmacogenetic analyses

Samples were centrifuged at 3000 rpm for 30 min. The white blood cell layer (buffy coat) was transferred to a labelled cryovial and was frozen at –80 °C. Three *CYP2B6* single nucleotide polymorphisms (SNPs) previously associated with efavirenz concentrations were chosen and analysed: rs3745274 (516G→T); rs28399499 (983T→C); and rs4803419 (15582C→T). Genomic DNA was extracted from 100 μL of stored buffy coat, was re-suspended in a total volume of 300 μL of lysis buffer and 30 μL of proteinase K from the Maxwell®16 LEV Blood DNA Kit (Promega, Southampton, UK) and was incubated at 57 °C for 30 min at 1000 rpm. DNA was extracted according to the manufacturer's instructions on a Maxwell® Automated Extraction Platform (Promega) and was eluted in 100 μL of elution buffer.

The quantity and quality of extracted DNA were determined using a Qubit® DNA BR Assay Kit (Molecular Probes; Life Technologies, Carlsbad, CA) and a Qubit® 2.0 Fluorometer (Invitrogen; Life Technologies) according to the manufacturer's instructions. Once the quantity of DNA was determined, it was diluted to 20 ng/μL using sterile nuclease-free water and then 1 μL was aliquoted into one well per sample in a 96-well plate (Life Technologies, Beijing, China). DNA samples were left at room temperature for 12 h to lyophilise.

Amplification and genotyping of each participant for the presence of SNPs in their *CYP2B6* gene were performed using fluorescent-labelled minor groove binding (MGB) allele-specific probes (Applied Biosystems, Foster City, CA). Participants were genotyped for *CYP2B6* (516G→T, rs3745274; 983T→C, rs28399499; and 15582C→T, rs4803419) using 1 μL of lyophilised DNA and 1× *Taq*Man® Universal PCR Master Mix, No AmpErase® UNG (Applied Biosystems) to a total volume of 12.5 μL. Cycling conditions consisted of an initial enzyme activation step of 95 °C for 10 min, followed by a denaturation step of 95 °C for 15 s and a combined annealing and extension step of 60 °C for 1 min. All amplification reactions were performed on a ViiA™ 7 Real-Time PCR System (Applied Biosystems).

A simplified version of Holzinger et al's metaboliser status classifications was used, as used by Dooley et al [8,16]. Each individual was classified as an ultraslow, slow, intermediate or extensive metaboliser. The effect of 516GT|983TC SNP vector was tested as four metabolic subcategories: extensive metabolisers, 516GG|983TT; intermediate metabolisers, 516GT|983TT or 516GG|983TC; slow metabolisers, 516TT|983TT or 516GT|983TC; and ultraslow metabolisers, all participants 983CC irrelevant of 516G→T genotype [8,16,17].

2.6. Study outcome

The outcome of interest was viral load at Week 16 or Week 48.

2.7. Statistical analysis

Descriptive statistics were used to summarise the baseline characteristics of the participant group and the mid-dosing interval efavirenz concentrations using Stata v.13.0 (Stata Corp., College Station, TX).

2.7.1. Prediction of virological outcome

The change in the relative risk of viraemia was estimated using a Cox proportional hazards regression model (Andersen–Gill