Plasma concentrations of efavirenz are associated with body weight in HIV-positive individuals

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Background: Efavirenz is among the most widely used antiretroviral drugs. Increased efavirenz exposure has been associated with CNS side effects and also with the chance of emergence of resistance upon treatment interruptions. The objective of this study was to evaluate factors associated with efavirenz plasma concentrations in a cohort of HIV-infected individuals.

Methods: From July 2009 to March 2010, HIV-infected patients with efavirenz as part of antiretroviral therapy (600 mg at night), undetectable viral load for at least 1 year and CD4 cell count ≥200 cells/mm³ were consecutively enrolled at the HIV/AIDS ambulatory care unit in southern Brazil. Plasma samples were taken 18–23 h after efavirenz last dose and analysed by validated ultra-performance liquid chromatography.

Results: Forty-one subjects were included (21 females). Mean age and weight were 45.4 years and 70.9 kg, respectively. Mean efavirenz plasma concentration was 2.20 ± 2.17 mg/L. Most plasma concentrations (73%) were within the therapeutic window (1–4 mg/L); 17% were below and 10% above the limits. There were no significant associations between efavirenz concentration and age, CD4 cell count, time on antiretroviral treatment and gender. There was significant and inverse correlation between efavirenz concentrations and body weight (P = 0.013) and body mass index (P = 0.001).

Conclusions: In this cohort of well-controlled HIV-positive individuals, patients with lower weight or body mass index had a higher chance of presenting elevated plasma concentrations of efavirenz. Therapeutic drug monitoring to adjust dose might be a helpful tool to decrease efavirenz dose in order to minimize costs and adverse effects.

Keywords: AIDS, antiretroviral therapy, therapeutic drug monitoring

Introduction

The introduction of highly active antiretroviral therapy (HAART) in the treatment of HIV infection has dramatically changed the life expectancy of HIV-infected individuals.1 However, HAART is still associated with the development of important adverse effects such as dyslipidaemia, body changes and CNS toxicity. These effects may limit drug efficacy and further compromise treatment success.2

Efavirenz, a non-nucleoside reverse transcriptase inhibitor (NNRTI), is one of the most prescribed antiretrovirals worldwide. It is relatively well tolerated and has a long plasma half-life that allows one pill to be taken per day.2 Nevertheless, its use may be associated with the development of adverse effects such as dyslipidaemia, CNS toxicity, and the chance of emergence of drug resistance upon HAART interruptions.3–5 Although there is considerable inter-patient variability in efavirenz concentrations (from drug interactions, genetic differences, ethnicity and adherence to treatment), some studies have already related efavirenz plasma concentrations with toxicity and a chance of drug resistance when treatment is discontinued.3,5,6

In this scenario, in order to guarantee plasma concentrations within the therapeutic window, therapeutic drug monitoring (TDM) has been shown to be of some value in controlling drug concentrations. So far, TDM has been of utility in HAART regimens containing protease inhibitors and/or NNRTIs.6 The objective of
this study was to investigate the factors associated with efavirenz plasma concentrations in a cohort of well-controlled (undetectable viral load, <50 copies/mL) HIV-infected individuals on HAART in southern Brazil.

Patients and methods

Study population

From July 2009 to March 2010, HIV-infected patients were consecutively enrolled during routine visits to the HIV/AIDS ambulatory care unit of Hospital de Clínicas de Porto Alegre, a national reference centre for HIV management. Inclusion criteria were the following: an efavirenz-containing HAART regimen (at the usual dose—600 mg at night); age >18 years; undetectable viral load (<50 copies/mL) for at least 12 months; CD4 cell count >200 cells/mm³; on the same HAART for ≥12 months; and no acute disease present. Subjects were excluded from the study if they met any of the following criteria: pregnancy; use of any other medication known to have a pharmacokinetic interaction with efavirenz; the presence of any neurological disorder; a current diagnosis of hepatitis B or C; and patient-reported non-adherence to HAART. Informed consent was obtained from all study participants. The protocol was approved by the Hospital de Clínicas de Porto Alegre Ethics Committee.

Data collection and sample preparation

A questionnaire was used to obtain additional information, such as co-morbidities, concomitant medication and the date and time of last efavirenz dose (the questionnaire is available as Supplementary data at JAC Online). Body weight and height were measured to calculate body mass index (BMI). Plasma samples were taken 18–23 h after efavirenz last dose. This interval was chosen because drug concentration variation is considered minimal on stable HAART regimens containing efavirenz.7,8 Blood samples were collected by venipuncture into tubes containing EDTA as anticoagulant. Tubes were centrifuged at 2500 rpm and 4°C for 10 min and the resulting plasma was aliquoted into numbered Eppendorf tubes and immediately frozen at −70°C until analysis to determine efavirenz concentrations. Investigators who measured plasma drug concentrations remained blinded with respect to patient self-report, medication timing and adherence data.

Determination of efavirenz concentrations

Efavirenz plasma concentrations were analysed using a validated ultra-performance liquid chromatography (UPLC) method, with a lower limit of quantification of 0.1 μg/mL. Detection was performed using a photodiode array detector, acquiring spectra from 210 to 380 nm and with chromatographic monitoring at 240 nm. After liquid–liquid extraction of 0.5 mL of plasma, mixed with 50 μL of 50 μg/mL clomipramine (internal standard solution), the analytes were separated on an AQUITY UPLC BEH 126 C column (Waters Corp., Milford, MA, USA) (2.1×150 mm, p.d. 1.7 μm) eluted with a gradient of acetonitrile and triethylammonium phosphate buffer (5 mM, pH 3.0). The total run time was 9.5 min. Calibration curves were linear in the range 0.1–10.0 μg/mL. Accuracy ranged from 94.9% to 103.5%. Both inter-day and intra-day coefficients of variation were less than 7.7% for all analytes. The extraction yields were greater than 88.2%. Efavirenz plasma concentration classification was based on current recommendations: subtherapeutic, <1 mg/L; therapeutic, 1.0–4.0 mg/L; and toxic, >4.0 mg/L.9-10

Table 1. Demographic data of participants and the factors associated with efavirenz plasma concentrations (N=41)

<table>
<thead>
<tr>
<th>Demographic characteristics</th>
<th>n, % or mean± SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>21 (51.2)</td>
<td>0.211</td>
</tr>
<tr>
<td>European descendants</td>
<td>30 (73.1)</td>
<td>0.585</td>
</tr>
<tr>
<td>African descendants</td>
<td>11 (26.9)</td>
<td>—</td>
</tr>
<tr>
<td>Age (years)</td>
<td>45.4 ±11.1</td>
<td>0.137</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>70.9 ±14.1</td>
<td>0.015a</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.6 ±4.8</td>
<td>0.001a</td>
</tr>
<tr>
<td>Time on antiretroviral therapy (years)</td>
<td>8.8 ±4.7</td>
<td>0.331</td>
</tr>
<tr>
<td>CD4 cell count (cells/mm³)</td>
<td>529 ±224</td>
<td>0.949</td>
</tr>
<tr>
<td>Efavirenz plasma concentration (mg/L)</td>
<td>2.20 ±2.17</td>
<td>—</td>
</tr>
</tbody>
</table>

9Correlation with efavirenz plasma concentrations is significant at P<0.05.

Statistical analyses

Statistical analysis was performed using SPSS version 18.0. Spearman’s rank correlation was used to investigate the relationship between efavirenz plasma concentrations and age, CD4 cell count, lipid profile, weight, BMI, period on ART and time of last dose taken. The association between gender and efavirenz plasma concentrations was determined using the Kruskal–Wallis test. Multiple linear regression was also used to assess the independent association of body weight and efavirenz plasma concentrations. P<0.05 was considered statistically significant for all tests.

Results

A total of 105 patients were initially screened. Unfortunately there were higher than expected losses, as HAART had to be changed in 43 individuals due to difficulties in maintenance of antiretroviral drug supply from the national programme; in addition, 11 patients missed the date of their blood sampling, 7 patients had blood samples inadequately stored and 3 individuals withdrew their informed consent. Table 1 shows the demographic data and the relation to efavirenz plasma concentrations of the remaining 41 individuals (21 females; 11 of African descent).

Most of the efavirenz plasma concentrations were within the therapeutic range (73%). The mean efavirenz plasma concentration was 2.20 ±2.17 mg/L; one patient (a woman of European descent) had a plasma concentration of 13.37 mg/L. We detected 17% (n=7) of individuals with plasma concentrations below the lower therapeutic limit (<1 mg/L) and 10% (n=4) with concentrations above the limit (>4 mg/L). Nervous system symptoms were reported by 49% of subjects, with headache and dizziness being the most common. All patients with efavirenz concentrations above the limit (>4 mg/L) had CNS adverse effects. In the multivariate analysis, only BMI and body weight were still significantly and inversely associated with efavirenz plasma concentrations (Table 1 and Figure 1).

Discussion

As anticipated, we found a high inter-patient variability in efavirenz plasma concentrations.3-6 According to UPLC analysis, most of samples (73%) were within the therapeutic range
Plasma concentrations of efavirenz

Our study has several limitations that should be considered when interpreting the results. First, due to higher than expected losses, our sample size might not be sufficient to draw conclusions about the potential factors associated with efavirenz plasma concentrations. Second, HAART adherence relied on patient self-reporting, which could lead to errors regarding the last dose. Nonetheless, it is important to note that most clinics performing TDM also rely on patient self-reports to obtain blood samples. Lastly, we could have genotyped CYP2B6, as it seems to be a promising approach towards the prediction of efavirenz toxicity and chance of resistance.

In conclusion, we found considerable variations in efavirenz plasma concentrations. Larger and prospectively designed clinical trials are still needed to further investigate efavirenz concentrations and clinical endpoints. We were able to find a significant and inverse relation of body weight and BMI with efavirenz plasma concentrations in that cohort of well-controlled HIV infection and undetectable viral load. Patients with lower body weight might benefit from a lower dose, possibly decreasing adverse events and treatment costs.

Figure 1. Relation between efavirenz (EFV) plasma concentrations (mg/L) and body weight (kg).

We did not find any difference between efavirenz plasma concentrations in relation to the last dose taken (data not shown). It is known that the ideal would be to collect samples close to the trough concentrations. However, due to its prolonged plasma half-life, it is believed that the variability in efavirenz concentrations during a dose interval is minimal and that samples can be taken without regard to the time elapsed between intake and sampling. Therefore the variability in efavirenz plasma concentration for patients whose samples were collected near the trough concentrations would not consistently differ from those whose samples were collected near the peak or midpoint of the dosing interval.

(1.0–4.0 mg/L). However, 17% of individuals had plasma concentrations below the limit. This finding would further support the use of TDM, as efavirenz’s long plasma half-life and its lower genetic barrier to HIV mutations might lead to the emergence of drug resistance. On the other hand, all patients (10%) with efavirenz concentrations above the therapeutic limit presented some CNS toxicity. This could be an opportunity to reduce efavirenz dosage to an acceptable plasma range to minimize toxicity, improve adherence and minimize treatment costs.

Body weight and BMI were significantly and inversely correlated with efavirenz concentrations. Individuals with lower body weight (or BMI) had a higher chance of having increased concentrations of the drug. To our knowledge, this is the first prospective study that found this association. There is another study that found this association, but it was not prospective, and unlike our study, individuals did not need to have undetectable viremia. That study, with individuals of the UK Collaborative HIV Cohort (UK CHIC), also found that ethnicity and zidovudine use interfered with efavirenz concentrations. Ethnicity was not associated with drug concentrations in our study, and the findings regarding zidovudine were no longer confirmed.

Other studies, including clinical trials and exploring the pharmacokinetics of the drug, found that Asian origin and baseline total bilirubin were associated with efavirenz concentrations, but not body weight. In our study, we had neither individuals with Asian ancestry nor baseline bilirubin evaluations. Other factors, such as sex and age, were not associated with efavirenz concentrations. This is in accordance with the other studies that found no association.

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Transparency declarations
None to declare.

Supplementary data
The questionnaire is available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

References

