Intracellular and plasma efavirenz concentrations in HIV-infected patients switching from successful protease inhibitor-based highly active antiretroviral therapy (HAART) to efavirenz-based HAART (SUSTIPHAR Study)

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Objectives: To assess intracellular and plasma efavirenz concentrations in HIV-infected patients who switched to efavirenz-based highly active antiretroviral therapy (HAART) from successful protease inhibitor-based HAART.

Patients and methods: A total of 49 patients were included in this observational cohort study. At inclusion, all patients had plasma HIV-RNA levels <50 copies/mL and switched to efavirenz combined with two nucleoside reverse transcriptase inhibitors. Intracellular and plasma concentrations were measured 12 h post-dose, 1 month (M1) and 6 months (M6) after starting efavirenz. Virological success (VS) was defined as plasma HIV-RNA level <50 copies/mL within the first 12 months and remaining undetectable at the end of the follow-up.

Results: VS was documented in 48 patients for at least 12 months (range 12–78 months). High inter-patient variabilities of intracellular and plasma efavirenz concentrations were observed (coefficients of variation >40%). At M1 and M6, respectively, median [Q1; Q3] intracellular efavirenz concentrations were 5300 [2830; 11 530] and 6790 [3870; 8790] ng/mL, median plasma efavirenz concentrations were 2050 [1600; 3100] and 2100 [1410; 2500] ng/mL. No correlation was found between intracellular and plasma concentrations. Plasma efavirenz levels exceeded the proposed threshold of 1000 ng/mL in 96% of patients from M1.

Conclusions: For moderately pre-treated HIV-infected patients with few mutations who switched to efavirenz from previous successful HAART, the proposed plasma efficacy-threshold was reached without any dosage adaptation. VS was maintained beyond 12 months, despite high inter-patient and intra-patient variabilities of intracellular and plasma efavirenz concentrations.

Keywords: pharmacokinetics, therapeutic drug monitoring, drug efflux, drug metabolism

Introduction

Clinical studies have demonstrated that antiretroviral therapies based on efavirenz combined with two nucleoside reverse transcriptase inhibitors (NRTI) were as potent as protease inhibitor (PI)-based HAART.1 Several authors have reported that this efficacy was correlated with plasma efavirenz levels.2,3 Thus, they have recommended efavirenz-dose adaptation to achieve 12 h post-dose plasma concentrations ranging from 1000 to 4000 ng/mL, which were associated with a higher
Intracellular and plasma efavirenz concentrations in HAART switch patients

probability of virological success (VS) at study endpoint.\textsuperscript{2} Relatively few data are available on intracellular efavirenz pharmacokinetics. Thus, our objective was to assess, at months 1 (M1) and 6 (M6) of treatment, intracellular and plasma efavirenz concentrations in HIV-infected patients who switched to efavirenz-based HAART from successful PI-based HAART.

Patients and methods

Study design and patients

A total of 49 HIV-infected patients were enrolled in this observational cohort study. At inclusion, all patients had plasma HIV-1-RNA levels <50 copies/mL and switched from 1 PI to efavirenz 600 mg daily in association with the same NRTI that they had been taking previously to limit gastrointestinal side effects and attenuate lipid abnormalities. VS was defined as a plasma HIV-1-RNA level remaining consistently <50 copies/mL for at least 12 months or until the end of the follow-up. All subjects gave written informed consent. The protocol was approved by our local Ethics Committee.

Drug sampling and data collection

All patients had follow-up visits at inclusion, M1, M6 and every 3 months thereafter. Collected data included plasma HIV-1-RNA levels, determined with the Cobas/Taqman HIV assay (Roche Diagnostics, Basel, Switzerland) and flow cytometric determination of CD4 cell counts. Adherence was measured using a patient-completed questionnaire. For drug monitoring, blood samples were drawn at the pharmacokinetic steady-state 12 h post-dose at M1 and M6. Blood (−8 mL/tube) was drawn into Vacutainer CPT\textsuperscript{TM} tubes (Becton Dickinson) containing sodium citrate as the anticoagulant, a polyester-gel layer and a Ficoll solution enabling isolation of peripheral blood mononuclear cells (PBMCs) and plasma by density-gradient centrifugation (1650 g, 20 min, 20°C). The cell layer was collected and washed twice with 2 mL of ice-cold phosphate-buffered saline and centrifuged at 4°C to limit drug efflux. Cells were put on KOVAT\textsuperscript{TM} slides for cell counting. An aliquot of PBMCs (3 × 10\textsuperscript{6} cells) was removed for intracellular analysis and resuspended in 200 µL of a solution of α-L glycoprotein acid (1 mg/mL) in sodium azide (0.1%, v/v) prior to extraction.\textsuperscript{4} Plasma and PBMCs were frozen at −80°C until analysis.

Efavirenz pharmacokinetic data

Pharmacokinetic parameters were intracellular and plasma efavirenz concentrations 12 h post-dose, and intracellular to plasma concentration ratios determined at M1 and M6. Overall, 98 cell and plasma samples were assayed. Plasma and cell samples were mixed with 200 µL of sodium carbonate (0.05 M) and 10 µL of internal standard (A-86093, 1 mg/mL, Abbott Laboratories). Two consecutive liquid–liquid extractions of efavirenz were performed with an n-pentane/ethyl acetate solvent mixture (50/50, v/v). Efavirenz was quantified using a validated HPLC assay coupled with mass spectrometry.\textsuperscript{4} Mean extraction recoveries were 88.6% and 91% in PBMCs and plasma, respectively.

Statistical analyses

Quantitative variables are expressed as medians and interquartile ranges [Q1; Q3]. A Wilcoxon test for paired data was used to assess the significance of the longitudinal changes of CD4 cell levels. Correlations between efavirenz concentrations were evaluated with

### Table 1. Baseline characteristics of the patients (n = 49)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>46 [41; 51]\textsuperscript{a}</td>
</tr>
<tr>
<td>Number of males, n (%)</td>
<td>39 (80)</td>
</tr>
<tr>
<td>Baseline CD4 cells count (cells/mm\textsuperscript{3})</td>
<td>505 [269; 673]\textsuperscript{a}</td>
</tr>
<tr>
<td>Previous antiretroviral treatment lines</td>
<td>1 [1; 2]\textsuperscript{a}</td>
</tr>
<tr>
<td>NNRTI experienced, n (%)</td>
<td>4 (8)</td>
</tr>
<tr>
<td>Genotype characteristics at baseline (n = 24)</td>
<td></td>
</tr>
<tr>
<td>NRTI resistance-related mutations</td>
<td>0 [0; 1]\textsuperscript{a}</td>
</tr>
<tr>
<td>PI resistance-related mutations major</td>
<td>0 [0; 0]\textsuperscript{a}</td>
</tr>
<tr>
<td>PI resistance-related mutations minor</td>
<td>1 [1; 2]\textsuperscript{a}</td>
</tr>
<tr>
<td>NNRTI resistance-related mutations, n (%)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Antiretroviral therapy before study entry, n</td>
<td></td>
</tr>
<tr>
<td>nelfinavir</td>
<td>22</td>
</tr>
<tr>
<td>indinavir</td>
<td>11</td>
</tr>
<tr>
<td>saquinavir (plus ritonavir)</td>
<td>4 (1)</td>
</tr>
<tr>
<td>lopinavir</td>
<td>8</td>
</tr>
<tr>
<td>nelfinavir plus saquinavir</td>
<td>1</td>
</tr>
<tr>
<td>didanosine plus lamivudine plus abacavir</td>
<td>2</td>
</tr>
<tr>
<td>Duration of RNA &lt;50 copies/mL before study entry (months)</td>
<td>14 [11; 24]\textsuperscript{a}</td>
</tr>
</tbody>
</table>

NRTI, non-nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; EFV, efavirenz. Major and minor protease mutations were defined according to the IAS USA panel.\textsuperscript{a}Values are expressed as medians [Q1; Q3].

the non-parametric Spearman correlation coefficient. Inter-patient coefficient of variation (CV\%) was determined for PBMCs and plasma using the formula [standard deviation/mean of concentrations] × 100. The Fisher’s exact test was used to compare CV\% at M1 and M6. Ratios were compared using the Mann–Whitney U-test. \textit{P} < 0.05 was considered statistically significant.

Results

Baseline characteristics of the patients

Baseline characteristics of the 49 patients are summarized in Table 1.

Immunological and virological outcomes

VS was maintained in 48 subjects for at least 12 months, with a median of 31 (range 12–78) months of follow-up. Median CD4 cell counts/mm\textsuperscript{3} were 548 [310; 677.5], 552 [306; 723] and 529 [339; 728], at M1, M6 and M12, respectively. Self-reported adherence was above 95% in all patients during follow-up.
Pharmacokinetic results of patients achieving VS

At M1 and M6, respectively, median [Q1; Q3] intracellular efavirenz concentrations were 5300 [2830; 11 530] and 6790 [3870; 8790] ng/mL, median plasma efavirenz concentrations were 2050 [1600; 3100] and 2100 [1410; 2500] ng/mL, and median intracellular concentration/plasma concentration ratios were 2.82 [2.18; 5.36] and 1.13 [0.54; 1.83] (Figure 1).

M1 and M6 intracellular or plasma concentrations were not correlated (\( r = 0.24, P = 0.14 \) and \( r = 0.27, P = 0.30 \), respectively). Inter-patient CV% were >40% (Figure 1), with significantly higher variabilities at M1 than M6 (\( P = 0.004 \) and 0.005, in PBMCs and plasma, respectively). No correlation was observed between intracellular and plasma concentrations either at M1 (\( r = 0.40, P = 0.10 \)) or M6 (\( r = 0.18, P = 0.44 \)). M1 and M6 ratios differed significantly (\( P = 0.0038 \)).

Pharmacokinetic and virological characteristics of the patient failing on efavirenz

The only patient who experienced virological failure after 6 months of efavirenz treatment had switched from a successful 14 month lopinavir/ritonavir regimen, with 428 CD4 cells/mm\(^3\) at baseline. He had also failed on a previous nevirapine-based regimen, which was associated with the selection of the K103N mutation, archived in PBMCs and re-selected under efavirenz pressure. At M1 and M6, his intracellular and plasma efavirenz concentrations were 6000 and 7500 ng/mL, and 4000 and 4900 ng/mL, respectively.

Discussion

In our study, switching to efavirenz-based HAART was an effective option for patients with long-term VS on HAART. All but one patient who switched to efavirenz maintained adequate viral suppression for a median of 31 months.

According to our results, inter-patient variabilities of intracellular and plasma efavirenz concentrations are high. The absence of correlation between M1 and M6 concentrations suggested intra-patient variability of efavirenz concentrations over time. Nor did we find a correlation between concentrations in the two compartments, indicating that plasma concentrations could not predict intracellular concentrations, as previously described by Rotger et al.\(^5\). However, those authors noted only a moderate correlation (\( r = 0.49 \)).

That intracellular and plasma concentrations were not correlated suggested that cellular mechanisms control intracellular efavirenz accumulation.\(^5\) A major mechanism described in the literature is active transport by P-glycoprotein (P-gp) and multidrug-resistance protein-1 (MRP1) expressed on PBMCs. Several PIs have been shown to be in vitro substrates and inducers of P-gp-regulated efflux from PBMCs.\(^6\) Unfortunately, relatively few data are available on the active transport of efavirenz by the P-gp efflux pump. Although efavirenz is not thought to be a P-gp substrate, Fellay et al.\(^7\) reported a relationship between allelic variants of MDR1, the gene encoding P-gp, and plasma efavirenz concentrations. However, controversy persists and other mechanisms have been proposed. Intracellular efavirenz accumulation has been linked to its affinity for binding to intracellular proteins.\(^8\) Intracellular drug metabolism\(^9\) and affinity for transporters, whose expression is correlated to that of P-gp\(^7\) (MRP1 and other ATP-dependent drug export proteins), are likely to be determinants.

Drug efflux and cytochrome P450 (CYP450) metabolizing enzymes are compensatory mechanisms. Overlapping of substrate specificity by intestinal mechanisms CYP3A and P-gp raises the possibility that the two proteins cooperate to limit absorption.\(^7\)
In the liver, efavirenz is metabolized by CYP2B6, CYP2C19 and CYP3A.\textsuperscript{10} Thus, intracellular efavirenz availability is likely to be co-regulated by cellular mechanisms (drug efflux, binding) and metabolizing enzymes. Efavirenz induces the expression of P-gp \textit{in vitro}\textsuperscript{6} and CYP3A4, thereby modulating its own metabolism. The concentration variabilities that we observed probably reflected variabilities of the expression of these mechanisms. We noted significantly higher variability of observed concentrations at M1 than M6 and significantly decreased intracellular penetration ratio at M6. Those findings raised a hypothetical efavirenz induction of P-gp, CYP450 metabolism enzymes and indirectly other transporters, which reached between M1 and M6 with intracellular binding (ratios reached ~1 at M6). Determination of P-gp, CYP3A4, CYP2C19 and CYP2B6 expression at M1 and M6 and their association with efavirenz concentrations would allow verification of this hypothesis.

Allelic variants of CYP2B6, CYP2C19 or CYP3A genes could co-modulate efavirenz concentrations and enhance inter-patient variability of pharmacokinetics.\textsuperscript{10} Inter-patient variability of pharmacokinetic parameters is thought to dictate virological response. Thus, efavirenz-dose adaptation has been recommended to achieve a plasma efficacy-threshold of 1000 ng/mL.\textsuperscript{2} For our response. Thus, efavirenz-dose adaptation has been recommended to achieve a plasma efficacy-threshold of 1000 ng/mL.\textsuperscript{2} For our response.

Allelic variants of CYP2B6, CYP2C19 or CYP3A genes could co-modulate efavirenz concentrations and enhance inter-patient variability of pharmacokinetics.\textsuperscript{10} Inter-patient variability of pharmacokinetic parameters is thought to dictate virological response. Thus, efavirenz-dose adaptation has been recommended to achieve a plasma efficacy-threshold of 1000 ng/mL.\textsuperscript{2} For our moderately pre-treated patients with few mutations who switched to efavirenz with virus loads <50 copies/mL, 96% had plasma levels above the proposed efficacy-threshold by M1 and all had reached that threshold by M6 without any dose adaptation. Moreover, 98% of them achieved prolonged VS despite this variability. Virological failure was recorded for only one patient who had therapeutic concentrations but an archived efavirenz-resistance-related mutation.

In conclusion, for our moderately pre-treated patients who had been on successful HAART, switching to efavirenz-based HAART constituted an effective option. Viral suppression was maintained despite high inter- and intra-individual variabilities of intracellular and plasma efavirenz concentrations. Therapeutic plasma levels were obtained without any dose adaptation.

Lastly, our results support the need for further investigations on intracellular and plasma efavirenz concentrations and their ratio in future studies aiming to link efavirenz pharmacokinetics to virological outcome, particularly in patients failing on HAART and receiving efavirenz-based combinations as a salvage therapy.

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Transparency declarations

None to declare.

References