No Evidence for Induction of ABC Transporters in Peripheral Blood Mononuclear Cells in Humans after 14 Days of Efavirenz Treatment\textsuperscript{\textdagger}

Jürgen Burhenne,* Anne-Kathrin Matthée, Ivana Pasáková,† Claudia Röder, Tilman Heinrich, Walter Emil Haefeli, Gerd Mikus, and Johanna Weiss

Department of Clinical Pharmacology and Pharmacoepidemiology, University of Heidelberg, Im Neuenheimer Feld 410, D-69120 Heidelberg, Germany

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Intracellular concentrations of antiretroviral drugs in peripheral blood mononuclear cells (PBMCs) are an important determinant of therapeutic success. In vitro data indicate that efavirenz induces several ATP-binding cassette (ABC) transporters, and pharmacogenetic studies found an association between \textit{ABCB1(C3435T)} and efavirenz exposure and between this polymorphism and improved virological outcomes. We therefore aimed to clarify whether efavirenz also induces ABC transporters in vivo in PBMCs and whether intracellular concentrations might be altered after induction. Twelve healthy individuals received multiple oral doses of efavirenz over 14 days (400 mg once daily). Blood samples were drawn on study days 1 (single dose) and 14 (multiple dose), and efavirenz concentrations were analyzed by liquid chromatography-tandem mass spectrometry. Expression of P glycoprotein (P-gp) and of the multidrug resistance-associated proteins 1 and 2 as well as P-gp activity was analyzed in PBMCs on day 1 and day 14 using real-time reverse transcription-PCR (RT-PCR) and rhodamine 123 efflux. Although a clear autoinduction could be confirmed by a significant decrease of efavirenz exposure from day 1 to day 14, efavirenz did not change expression of the ABC transporters or P-gp activity in PBMCs. Moreover, intracellular concentrations of efavirenz were 1.3- to 1.8-fold higher than the corresponding plasma concentrations, and the intracellular/plasma concentration ratio remained constant during the treatment and did not correlate with ABC transporter expression or function. In conclusion, our study confirmed that intracellular concentrations of efavirenz are independent from these efflux transporters and demonstrated for the first time that the transporters are not induced in PBMCs in vivo after 2 weeks of treatment with efavirenz.

Even though highly active antiretroviral therapy (HAART) has markedly improved the clinical outcome of HIV-infected patients, virological treatment failure often occurs as early as within the first year of therapy (1, 37). There is growing evidence that suboptimal drug concentrations at the site of virus replication (e.g., within CD4\textsuperscript{+} cells) confer selective pressure to the virus that ultimately results in the emergence of mutant virus isolates with reduced susceptibility to antiviral drugs (1, 29). Reduced plasma and intracellular drug concentrations may result from drug-drug interactions by induction of liver P450 cytochromes (e.g., CYP3A4 and CYP2B6) and also from induction of ATP-binding cassette (ABC) efflux transporters located in the cell membrane, e.g., P glycoprotein (P-gp, MDR1/\textit{ABCB1}) and multidrug resistance-associated protein 1 (MRP1/\textit{ABCC1}) and MRP2/\textit{ABCC2}. These transporters have been demonstrated to transport many antiretroviral drugs, especially HIV protease inhibitors (PIs) (20, 22, 23, 34). Thus, ABC transporters limit not only the access of antiretroviral drugs to the systemic circulation but also their access to the infected cells and contribute to sanctuary sites for virus replication. Moreover, recent evidence suggests that interactions with anti-HIV drugs also evolve at the level of the ABC transporters (5, 11, 12, 31). Indeed, induction and inhibition of this system may profoundly affect the pharmacokinetics of concomitantly administered drugs and thus impair the effectiveness of antiretroviral therapy (28).

At least for PIs, a close correlation between intracellular drug concentration and antiviral activity has been demonstrated (7, 27), leading to failure of antiretroviral therapy when insufficient intracellular drug concentrations are achieved. Therefore, monitoring of HIV drug concentrations in plasma and at the site of action is a valuable tool to improve efficacy and consider factors limiting optimum therapeutic response (37). Because the membrane of peripheral blood mononuclear cells (PBMCs) is an actively regulated barrier between plasma and the target site, plasma concentration monitoring may not closely reflect drug concentrations reaching the site of action. Hence, quantification of anti-HIV drug within PBMCs or even their subfractions (CD4/CD8) might have distinct advantages over the determination of plasma concentrations (17).

The nonnucleoside reverse transcriptase inhibitor (NNRTI) efavirenz is commonly used in HAART, which usually consists of two nucleoside reverse transcriptase inhibitors (NRTIs) combined with one NNRTI or one or two protease inhibitors.
Efavirenz is known to induce its own metabolism via CYP2B6 (41), resulting in a reduced half-life (t1/2) and exposure to the drug. Additionally, efavirenz induces CYP3A4 in vivo (2, 28), and we have recently demonstrated that this NNRTI induces several ABC transporters in vitro (39). However, it is so far unclear whether efavirenz also induces these transporters in PBMCs in vivo. Moreover, although efavirenz is no P-gp substrate (9, 35, 39), there are studies demonstrating an association between ABCB1 (C3435T) and efavirenz exposure (15) and between this polymorphism and improved virological outcomes (18). We therefore aimed to clarify whether efavirenz induces ABC transporters in vivo in PBMCs, being the main target cells of HIV infection, and whether possible changes in PBMC transporter expression also translate into altered intracellular concentrations of efavirenz. We developed analytical methods to quantify efavirenz in plasma, plasma ultrafiltrate (unbound drug concentration), and PBMCs and investigated in a clinical study the influence of efavirenz on PBMC transporter expression. In addition, efavirenz potentially induced ABC transporters in PBMCs, being the main target cells of HIV infection, and whether possible changes in PBMC transporter expression also translate into altered intracellular concentrations of efavirenz. We developed analytical methods to quantify efavirenz in plasma, plasma ultrafiltrate (unbound drug concentration), and PBMCs and investigated in a clinical study the influence of efavirenz on P-gp expression and activity as well as on the expression of other ABC transporters and compared these results to plasma and intracellular PBMC efavirenz concentrations.

Materials and Methods

Materials. The efavirenz reference standard was purchased from Sequoia Research Products (Pangbourne, United Kingdom). Efavirenz (Sustiva) was purchased from Bristol-Myers Squibb, flecainide reference substance from Sigma-Aldrich (Steinheim, Germany), rhodamine 123 from Calbiochem (San Diego, CA), and zosudar (LY-335979) from Eli Lilly Company (Bad Homburg, Germany). Media, medium supplements, and buffers were purchased from Invitro (Karlsruhe, Germany), Casy ton and Casy clean from Schärfe System (Reutlingen, Germany), and Biocol from Biochrom (Berlin, Germany). Water was deionized and filtered by an HP6/UV/UF TKA system (TKA, Niederelbert, Germany). All other reagents and solvents were of analytical quality.

Clinical study. The study protocol was approved by the Ethics Committee of the Medical Faculty of Heidelberg, the competent authority (BfArM, Germany), and was conducted at the Department of Clinical Pharmacology and Pharmacoeconomics in accordance with good clinical practice guidelines and the Declaration of Helsinki (EudraCT 2006-004388-74). None of the healthy participants (n = 12, 4 females, mean age ± standard deviation [SD], 26.4 ± 6.1 years) had taken any medications for 2 months prior to the study, nor did they take any nonstudy medication during the study. All selected female participants were nonpregnant and nonlactating, and they were asked to use appropriate barrier contraception throughout the study. The participants received two capsules of efavirenz (400 mg) on study day 1 (single dose), and after a washout of at least 8 days, efavirenz (400 mg per day) was orally administered again over a period of 14 days. Blood samples were drawn on study day 1 and after the last administration on study day 14.

Plasma sampling. Blood samples for quantification of mRNA expression and function were taken before the first dosing of efavirenz and on study day 14.

Whole-blood sampling. Blood samples for quantification of mRNA expression and function were taken before the first dosing of efavirenz and on study day 14.

Plasma sampling. Blood samples (4.5 ml) for efavirenz quantification were drawn before and 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12, and 24 h after dosing and centrifuged immediately (15 min, 3,000 × g, 4°C). On study day 1, additional plasma samples were drawn after 48, 72, 120, and 168 h. Plasma samples were stored at −20°C until analysis. The isolation of the unbound efavirenz fraction from plasma was performed 5 h after dosing by ultrafiltration with Centricon YM-30 (Millipore, Bedford, MA) (14). Then, plasma (2.0 ml) was centrifuged (20 min, 1,800 × g, 37°C) and the protein-free plasma (400 μl) was used for efavirenz quantification.

PBMC sampling. For efavirenz quantification in PBMCs, whole-blood samples (three 8-ml samples) were taken 5 and 24 h after efavirenz administration in a Vacutainer CPT (Becton Dickinson, Heidelberg, Germany) on study days 1 and 14 and centrifuged (20 min, 1,700 × g, room temperature) (14). In brief, the supernatant containing plasma, PBMCs, and residual platelets was transferred into reaction tubes and gently centrifuged (10 min, 180 × g, room temperature) to separate PBMCs from platelets. Subsequently, plasma and platelets were decanted, and the PBMC pellet was resuspended in ice-cold phosphate-buffered saline (PBS) and centrifuged again (5 min, 180 × g, 4°C). Efavirenz potentially adsorbed to the PBMC surface was removed by three washes with ice-cold PBS, which is widely used (10, 14, 30) and has been validated using radioactively labeled drugs (21). During the last of the three PBS washes, 60 μl was taken for cell counting and cell size/volume determination. One aliquot (50 μl) was used for cell counting of PBMCs and remaining platelets (Beckman Coulter, Krefeld, Germany), and 10 μl was used for PBMC size determination (Casy cell counter; Schärfe System, Reutlingen, Germany). For quality control (QC) and calibration samples, human blank PBMCs were isolated from the buffy coat (local blood bank, Heidelberg, Germany).

Quantification of efavirenz in plasma, ultrafiltrate, and PBMCs. Appropriate stock solutions for the internal standard flecainide, efavirenz calibration, and efavirenz QC were prepared. Blank plasma (200 μl), blank ultrafiltrate (400 μl), and blank PBMCs (3 × 10^6 cells) were spiked with internal standard solution and respective calibration solutions, yielding concentrations of 5 to 10,000 ng/ml plasma, 0.5 to 500 ng/ml plasma ultrafiltrate, and 0.2 to 40 ng/3 × 10^6 PBMCs. QC samples at three concentrations were prepared as described for calibration samples. For protein precipitation, acetonitrile was added to plasma (800 μl), ultrafiltrate (400 μl), and PBMC pellets (400 μl). After centrifugation (15 min, 16,100 × g, 6°C), liquid phases were separated, evaporated to dryness (nitrogen, 40°C), reconstituted with 200 μl water, and transferred to autosampler vials, and injected (25 μl) into a liquid chromatography tandem mass spectrometry (LC-MS-MS) system. The LC-MS-MS system (Thermo, San Jose, CA) consisted of an LC system (Surveyor Plus) and a triple-stage quadrupole mass spectrometer (TSQ 7000). For chromatographic separation, a Synergi Fusion RP column (4 μm, 80 Å, 150 mm by 2 mm; Phenomenex, Aschaffenburg, Germany) with an integrated guard column was used at 40°C. The LC eluent consisted of 0.1% (vol) aqueous and 99.9% (vol) acetonitrile, containing 5 mM ammonium acetate (A), acetonitrile (B), and methanol (C). The gradient started with 74% A/12% B/14% C, was changed within 1 min to 42% A/26% B/32% C, and kept until 13 min. Within the next minute, the ratio was changed to 2% A/49% B/49% C and was kept until 15 min. The eluent (flow rate of 0.45 ml/min) was introduced directly without splitting into the electrospray ion (ESI) source. Selected reaction monitoring was performed in the negative ion mode. MS transitions monitored were m/z 318 → m/z 180 for efavirenz and m/z 413 → m/z 270 at 28 V for flecainide. Analytical method validation for plasma and PBMCs was performed according to the recommendations of the U.S. Food and Drug Administration (FDA) regarding selectivity, accuracy and precision, linearity, recovery, and stability (16).

Quantification of mRNA expression of ABC transporters. RNA was isolated using an RNasefree minikit (Qiagen, Hilden, Germany). Quality and concentration were measured spectrophotometrically, and isolated RNA was stored at −80°C until analysis. cDNA was synthesized with a Transcripter first-strand cDNA synthesis kit (Roche Applied Science, Mannheim, Germany) according to the manufacturer’s instructions. Expression of mRNA was quantified by real-time reverse transcription-PCR (RT-PCR) with a LightCycler instrument (Roche Applied Science, Mannheim, Germany), as described previously (39). The most suitable housekeeping genes tested in PBMCs were 18S (Center for Medical Genetics, Ghent, Belgium), GAPDH (Thermo, San Jose, CA), and glyceraldehyde-3-phosphate dehydrogenase (G6PDH), GAPDH, and hypoxanthine-guanine phosphoribosyltransferase 1 gene (HPRT1), ribosomal protein L13 gene (RPL13), and 60S [human] acidic ribosomal protein P1 gene (HPLO), GU was the most stable and therefore used for normalization. Data were evaluated by calibrator-norm-}

P-gp activity assay (rhodamine 123 efflux). Flow cytometry assessment of P-gp activity in PBMCs was performed as earlier reported (3, 39), with minor modifications. In brief, one sample of 10^6 PBMCs was suspended in incubation medium (500 μl RPMI medium-2% fetal calf serum [FCS]) containing rhodamine 123. Another sample was suspended in incubation medium containing rhodamine 123 and the specific P-gp inhibitor zosuquidar. These samples were incubated (30 min, 37°C) on a rotary shaker. Cells were washed with ice-cold incubation medium (1 ml). The first sample was resuspended in incubation medium (500 μl) and the second in incubation medium containing zosuquidar. After incubation (50 min, 37°C) on a rotary shaker, cells were washed, resuspended in ice-cold PBS-2% FCS, and kept on ice until flow cytometry. Intracellular fluorescence was analyzed using a Becton Dickinson LSR II flow cytometer (Heidelberg, Germany) with a solid-state coherent sapphire blue laser and...
TABLE 1. Plasma pharmacokinetic data for efavirenz (400 mg once daily) on study days 1 (single dose) and 14 (multiple dose)
in 12 healthy individuals.

<table>
<thead>
<tr>
<th>Parameter*</th>
<th>Value (mean ± SD) on study day:</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_{max} (ng/ml)</td>
<td>2,735 ± 691</td>
<td>1</td>
</tr>
<tr>
<td>t_{1/2} (h)</td>
<td>78.7 ± 27.0</td>
<td>0.0005</td>
</tr>
<tr>
<td>T_{max} (h)</td>
<td>2.3 ± 0.6</td>
<td>0.6523</td>
</tr>
<tr>
<td>AUC_{c-o} (mg · h/ml)</td>
<td>97.46 ± 20.33</td>
<td>0.0005</td>
</tr>
<tr>
<td>AUC_{C-24} (mg · h/ml)</td>
<td>47.72 ± 13.71</td>
<td>0.0005</td>
</tr>
<tr>
<td>V (liters)</td>
<td>462.8 ± 119.1</td>
<td>0.0342</td>
</tr>
<tr>
<td>CL_F (ml/min)</td>
<td>70.67 ± 12.94</td>
<td>0.0005</td>
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* C_{max}, maximum concentration of drug in plasma; t_{1/2}, half-life in plasma; T_{max}, time to maximum concentration of drug in plasma; AUC_{c-o}, area under the plasma concentration-time curve from 0 h to infinity; AUC_{C-24}, area under the plasma concentration-time curve from 0 to 24 h; V, volume of distribution; CL_F, apparent oral clearance.

** Values in parentheses were calculated from data collected within a 24-h dosing interval.

a 530 band-pass filter. Cell debris was eliminated by gating the living cells in forward versus side scatter. To quantify possible inductive effects of efavirenz on P-gp, the ratio between the cells incubated with and without the specific P-gp inhibitor zosuquidar during the efflux period was calculated.

** Performance of the analytical methods.** The PBMC isolation and protein precipitation procedures combined with LC–MS–MS quantitation entirely met U.S. FDA requirements for bioanalytical method validation (16). Drug concentrations in samples from clinical trials were measured within the calibration range of 5 to 10,000 ng/ml (plasma), 0.5 to 500 ng/ml (ultrafiltrate), and 0.2 to 40 ng/3 × 10^6 cells (PBMCs). The lower limit of quantitation (accuracy/precision) for efavirenz in plasma was 5 ng/ml (~0.5%/5.2%), that in ultrafiltrate was 0.5 ng/ml (~1.3%/14.0%), and that in PBMCs was 0.2 ng/3 × 10^6 cells (~10.9%/7.3%). Generally, within-batch accuracies (precision) ranged from ~13.5% to +8.8% (1.0% to 7.7%), and batch-to-batch accuracies (precision) ranged from ~6.8% to +3.9% (4.1% to 10.4%).

** Quantification of efavirenz.** Table 1 shows the pharmacokinetic data for efavirenz in 12 healthy individuals on study days 1 (single dose) and 14 (multiple dose). After 14 days of treatment, the efavirenz area under the concentration–time curve (AUC) was reduced to 50% and apparent oral clearance roughly doubled. The efavirenz plasma concentration–time profiles, intracellular PBMC concentrations, and corresponding free plasma concentrations at study days 1 and 14 are shown in Fig. 1. Even after a single dose of 400 mg efavirenz, therapeutic plasma concentrations were reached and the intracellular efavirenz concentrations always exceeded concurrent plasma concentrations (P = 0.015 on day 1 and P = 0.004 on day 14). Intracellular concentrations on study day 1 (and day 14) were 1,469 ± 496 ng/ml (3,597 ± 1,172 ng/ml) after 5 h and 974 ± 427 ng/ml (2,029 ± 733 ng/ml) after 24 h. Individual mean (±SD) intracellular/plasma concentration ratios remained constant after 5 h on study days 1 and 14 and were 1.63 ± 0.87 and 1.76 ± 1.00, respectively (Fig. 2A). After 24 h, this result was not changed significantly, and the intracellular/plasma concentration ratios were 1.53 ± 0.55 and 1.36 ± 0.38 on study days 1 and 14, respectively (Fig. 2B). Individual intracellular efavirenz concentrations were correlated with concurrent plasma concentrations after 24 h (trough levels). The slopes were considerably above 1 and reached a correlation coefficient (r^2) of 0.1331 (Fig. 3A) on study day 1 and r^2 of 0.5077 (Fig. 3B) on study day 14. The mean (±SD) free efavirenz plasma concentrations were 4.67 ± 0.91 ng/ml (0.47% ± 0.10% free fraction) on study day 1 and 11.3 ± 2.7 ng/ml (0.52% ± 0.13% free fraction) on study day 14, resulting in accumulation factors of intracellular efavirenz concentration compared to free plasma concentration of 325 ± 131 (study day 1) and 330 ± 115 (study day 14).

** Quantification of ABC transporter mRNA expression and P-gp activity.** After 14 days of efavirenz treatment, ABCB1 mRNA expression was significantly reduced compared to baseline on study day 1 (mean ± SD of 2.0 ± 1.4 on study day 1 and 1.2 ± 0.4 on study day 14; P = 0.034 by Wilcoxon signed-rank test) (Fig. 4B), whereas ABCCl and ABCC2 expression was unchanged (for ABCCl, mean ± SD of 0.8 ± 0.4 on study day 1 and 0.8 ± 0.3 on study day 14; for ABCC2, mean ± SD of 0.9 ± 0.3 on study day 1 and 0.7 ± 0.2 on study day 14) (Fig. 4C and D). In contrast, P-gp activity in PBMCs was unaffected by efavirenz treatment (mean ± SD of 1.1 ± 0.2 on study day 1 and 1.0 ± 0.3 on study day 14; P > 0.05 by Wilcoxon signed-rank test) (Fig. 4A). There was no correlation between PBMC/plasma ratio of efavirenz and P-gp activity or expression (Fig. 4A).
5A and B) or expression of MRP1 (Fig. 5C) and MRP2 (Fig. 5D) in PBMCs.

**DISCUSSION**

**Analytical methods.** Selective and sensitive LC–MS-MS quantification of efavirenz was achieved by reversed-phase chromatography using a Synergi Fusion RP column in combination with a fast gradient from aqueous to organic eluent, direct connection to the ESI source, and analysis in the selected reaction monitoring mode. Extraction of efavirenz and flecainide from blood and PBMCs by protein precipitation allowed fast and convenient analysis as well as PBMC collection and isolation using a Vacutainer CPT, which was an accurate and fast isolation procedure with a low risk of infection. When this method was applied to clinical study samples, the extracted PBMC pellets contained a mean (±SD) of $4.1 \times 10^6 \pm 1.8 \times 10^6$ PBMCs/sample, resulting in a mean (±SD) cell pellet volume of $1.4 \times 10^9 \pm 0.6 \times 10^9$ fl, which corresponds to a volume of 0.34 ± 0.15 pl of a single PBMC. This is in agreement with published data (32). The total numbers of PBMCs were always sufficient to quantify drug concentrations for efavirenz at the trough level.

**Influence of efavirenz on ABC transporter mRNA expression and P-gp activity.** The study participants were treated with a rather low efavirenz dose under fasting conditions, two factors that may result in lower concentrations and less central nervous system (CNS) toxicity than those observed in HIV-positive patients (33). Nevertheless, efavirenz plasma concentrations achieved were in a range that is expected to be therapeutic (1,000 to 4,000 ng/ml). Due to the significantly decreased AUC and increased apparent oral clearance between study days 1 and 14, a clear autoinduction of the efavirenz metabolizing system CYP2B6 was observed, and this is in accordance with published results (2, 28, 41). This finding is supported by the $t_{1/2}$, which decreased from 78.7 h (day 1) to about 33.0 h (day 14) significantly, although the $t_{1/2}$ on day 14 was calculated within a 24-h dosing interval. The mean free efavirenz plasma concentration and resulting free fraction were quite similar to results from earlier reports (2, 33, 36). In one previous report, the intracellular PBMC concentration correlated better with free efavirenz plasma concentration than with total plasma concentration (36), but this cannot be supported by our results and may depend on the timing of the samples. Intracellular efavirenz concentrations in our study always exceeded concurrent plasma concentrations, confirming accumulation in PBMCs and suggesting distinct mechanisms controlling the access of efavirenz to PBMCs, in accordance with published data (4, 36). Due to the nonsignificant difference in slopes at study days 1 and 14 (Fig. 3), the slopes do not indicate a change in intracellular efavirenz accumulation. Moreover, there was no correlation between intracellular/plasma ratio and expression or function of ABC transporters, validating previous *in vitro* findings which have demonstrated that intracellular efavirenz levels are independent from P-gp activity (19). This, however, appears to be in
contrast to earlier studies demonstrating an association between \(ABCB1(C3435T)\) and efavirenz exposure and between this polymorphism and improved virological outcomes (15, 18). Thus, how this polymorphism can influence the pharmacokinetics of a drug not transported by P-gp remains unsolved.

Whereas it is unequivocal that efavirenz can induce ABC transporter expression and function \textit{in vitro} in LS180 cells (35, 39, 40), data for PBMCs are contradictory (8, 9), and there are two studies (one in humans and one in rats) indicating that efavirenz does not provoke changes in intestinal P-gp expression \textit{in vivo} (6, 26). Although our data indicate a slight decrease in P-gp expression in PBMCs, these changes did not translate to changes in P-gp function. This is in agreement with

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**FIG. 4.** Quantification of P-gp activity by means of rhodamine 123 efflux (A) and mRNA quantification of \(ABCB1\) (B), \(ABCC1\) (C), and \(ABCC2\) (D) expression (normalized to the level for the glucuronidase-\(\beta\) housekeeping gene [GU]) in PBMCs of study participants on study day 1 (before efavirenz application) and on study day 14 of repeated efavirenz treatment. Individual/mean data are represented as black circles/gray squares, respectively. Statistical significance was evaluated using the Wilcoxon signed-rank test.

**FIG. 5.** Correlation between P-gp activity (A), \(ABCB1\) expression (B), \(ABCC1\) expression (C), or \(ABCC2\) expression (D) and PBMC/plasma concentration ratio of efavirenz (combined data from study days 1 and 14). The correlation was assessed by the Spearman rank correlation (\(\rho\) always not significant).
previous studies demonstrating that P-gp expression is not always related to P-gp function (3, 13, 25). Moreover, the detected decrease in ABCB1 expression (Fig. 4A) is no longer significant when the two individuals with very high ABCB1 expression on study day 1 are excluded from the analysis. We thus conclude that efavirenz treatment had no influence on ABC transporter expression in our study.

In vitro and in vivo data indicate that induction of CYPs and P-gp by efavirenz continues beyond the second week of treatment (39, 41; E. Ngaimisi, S. Mugusi, O. Minzi, P. Sasi, K.-D. Riedel, A. Suda, N. Ueda, M. Janabi, F. Mugusi, W. E. Haefeli, J. Burhennes, and E. Akilu, submitted for publication). Thus, we cannot completely rule out the possibility that long-term treatment with efavirenz could provoke different effects on ABC transporter expression. This, however, appears unlikely, because in this case an effect should already have been observed after 2 weeks of treatment.

Taken together, our results have demonstrated for the first time that efavirenz does not induce P-gp, MRP1, and MRP2 in PBMCs in vitro. The reasons for the discrepancies between in vitro and in vivo data are not completely clear. One underlying reason may be different equipment of the PBMCs with nuclear factors mediating transporter induction compared to the in vitro cell models used. Recently published data demonstrate that even the prototype inducer rifampin does not induce P-gp in PBMCs, which express only very low levels of the pregnane X receptor (PXR) and the constitutive androstane receptor (CAR) (24).

Moreover, intracellular concentrations reached in PBMCs might not be high enough to trigger induction. Peak total plasma concentrations in our study reached ~10 \(\mu\)M, closely matching the concentration used in induction assays in vitro (9, 39), and intracellular concentrations were even higher. However, efavirenz is highly protein bound, and intracellular concentrations in vitro are possibly much higher than those in vivo because the free concentration of efavirenz in cell culture medium or buffer is much higher. Interestingly, even in the intestine, where local concentrations after oral intake of drugs are much higher, a clinical study did not find an induction of P-gp (26). However, the authors used Western blotting for the quantification of protein expression, a method less sensitive than quantification of mRNA by PCR, and might therefore have missed small changes in the expression level.

Conclusion. Our in vivo study for the first time demonstrates that treatment with efavirenz over 2 weeks does not induce the ABC transporters P-gp, MRPI, and MRP2 in PBMCs in vivo and that the intracellular/plasma concentration ratio of efavirenz does not change during this treatment. Moreover, we confirmed that intracellular levels of efavirenz are independent from these efflux transporters.

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