Semisimultaneous Midazolam Administration to Evaluate the Time Course of CYP3A Activation by a Single Oral Dose of Efavirenz

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Abstract

This study aimed to assess whether a single oral dose of the nonnucleoside reverse transcriptase inhibitor efavirenz can alter CYP3A in vivo. In 12 healthy participants individual CYP3A activity was quantified using a semisimultaneous methodology (midazolam orally and 6 hours later intravenously) both alone and during a period of 22 days after a single oral dose of 400 mg efavirenz. Twelve hours after efavirenz administration, midazolam apparent oral clearance was significantly increased by 70%, and midazolam systemic clearance after intravenous administration was significantly increased by 27%. Similar effects were still present on day 6, after which midazolam clearances slowly returned to baseline on day 22. At least on day 1, the midazolam clearance increase is consistent with the in vitro observed CYP3A activation. The onset of an efavirenz treatment will almost immediately result in enhanced elimination of CYP3A substrates.

Keywords

CYP3A, midazolam, efavirenz, enzyme activation

Efavirenz is a clinically effective nonnucleoside reverse transcriptase inhibitor used in the first-line treatment of human immunodeficiency virus infection.[1,2] Efavirenz-based regimens have performed favorably compared with protease inhibitor–based therapies both in treatment-naive patients and as simplification strategies in pretreated patients.[2] It has a long elimination half-life (>48 hours), and elimination is almost exclusively by metabolism.[2] Efavirenz is primarily metabolized by the cytochrome P450 (CYP) isozymes CYP2B6 and CYP3A4 to form the (inactive) major metabolite 8-hydroxyefavirenz, which is further metabolized to 8,14-dihydroxyefavirenz, also by CYP2B6.[3] In vitro and in vivo studies consistently revealed that efavirenz can act both as an inducer (eg, CYP3A4, CYP2B6) and inhibitor (CYP3A4) of CYP isozymes.[4–8] In contrast to most known CYP inducers, which are activators of the human nuclear pregnane X receptor, induction by efavirenz is mediated preferentially by the constitutive human androstane receptor.[4,9]

Several methods to assess efavirenz-induced changes in CYP3A activity in vivo have been used. In 1 study in healthy participants, the erythromycin breath test revealed a mean induction of 55% after 400 mg/d for 10 days.[5] Hepatic P-glycoprotein is a potentially confounding factor for the interpretation of CYP3A activity measured by the erythromycin breath test.[10] The use of midazolam is much more reliable to assess CYP3A activity.[11] In human immunodeficiency virus–infected patients on 600 mg/d efavirenz, the mean plasma ratio for 1-OH-midazolam/midazolam 30 minutes after oral administration of 0.075 mg midazolam performed after a median treatment period of 29 days was increased 3-fold, but this change was highly variable.[7] In a previous study we observed significantly increased CYP3A activity 1 day after efavirenz administration with a midazolam area under the concentration-time curve (AUC) that was 35% lower compared to baseline.[12] However, the study participants all had received efavirenz beforehand to study the pharmacokinetics of efavirenz.

Due to the complex study design and previous efavirenz administration, it was unclear whether this

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apparent acute induction (activation) was actually related to efavirenz. Therefore, we decided to study the effect of a single oral dose of efavirenz on CYP3A activity to prove an acute activation of CYP3A. We studied the time profile of CYP3A activity using midazolam every fifth day over 3 weeks. It has been shown that a semisimultaneous administration of midazolam (first orally followed at 6 hours by intravenous infusion) is a suitable approach to determine hepatic and intestinal CYP3A activity at baseline and with enzyme inhibition or induction.[13,14]

Methods

The study (EudraCT: 2007-004356-36) was approved by the responsible Ethics Committee of the Medical Faculty of the Heidelberg University and the competent authority (BfArM) responsible for Germany. It was conducted between November 2007 and January 2008 in the ISO9001-certified clinical research center of the Department of Clinical Pharmacology and Pharmacoepidemiology, in accordance with the standards of good clinical practice (as defined in the ICH E6 Guideline for Good Clinical Practice), in agreement with the Declaration of Helsinki and all specific legal requirements in Germany. Written informed consent was obtained from each participant.

Study Population

Twelve healthy participants (6 females) were enrolled and completed the trial. They were nonsmokers, between 21 and 34 years old, and mentally and physically healthy as defined by medical history, physical examination, electrocardiogram, and routine laboratory analyses that included hematology, blood chemistry, urinalysis, and a urine drug screening. None of the participants had taken any medication for at least 1 month prior to or during the study except for oral contraceptives. Exclusion criteria included inability to communicate with the investigator, a history of allergic reactions, pregnancy or lactation, blood donation or participation in a clinical trial within the last 2 months, or excessive alcohol drinking (more than approximately 20 g alcohol per day).

Study Design

The study was designed as an open-label single-center trial (Figure 1). On the first day the baseline investigation of CYP3A activity was carried out by administering a 4-mg oral dose of midazolam (Dormicum® V 5 mg/5 mL solution for injection; Roche, Grenzach-Wyhlen, Germany) followed by a 30-minute infusion of 2 mg midazolam 6 hours later (semisimultaneous method) to determine hepatic and intestinal CYP3A activity.[13,14] A week later all participants received a single oral dose of 400 mg efavirenz (Sustiva®; BMS,
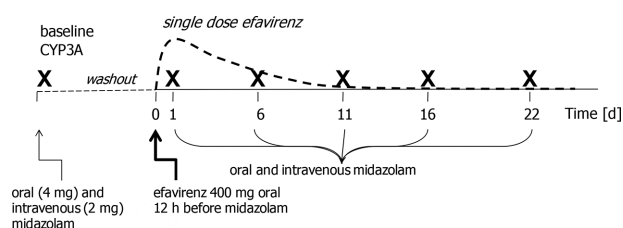

Figure 1. Study design.

Uxbridge, UK) in the evening (12 hours before midazolam administration) to avoid central nervous system stimulation and dizziness and to reach maximum concentration, which is reached approximately 5 hours after administration.[15] On days 1, 6, 11, 16, and 22 after efavirenz administration, the midazolam testing was repeated. Blood samples were collected via an indwelling catheter from a vein contralateral to the midazolam infusion arm immediately before and at 10, 20, 30, and 45 minutes and 1, 1.5, 2, 3, 4, 5, and 6 hours after administration of oral midazolam and at 15, 30, 40, and 50 minutes and 1, 1.25, 1.5, 2, 3, 4, 6, and 8 hours after the start of midazolam infusion. Additional blood samples for the analysis of efavirenz were taken before efavirenz administration and 12, 18, 24, 132, 252, 372, and 516 hours thereafter. Blood samples were immediately centrifuged at 4°C, and the separated plasma was stored at −20°C until analysis. Immediately before the administration of midazolam, the participants emptied their bladders, and urine was collected for the subsequent 24 hours (6-hour interval after oral administration; then 6-14 hours and 14-24 hours). Urine volumes were measured, and a 10-mL aliquot of each was kept frozen at −20°C until analysis.

Quantification of Midazolam, 1-OH-Midazolam, and Efavirenz

Midazolam and 1-OH-midazolam concentrations in plasma and urine were determined by high-performance liquid chromatography coupled to tandem mass spectrometry using a modified, previously published method.[14] The bioanalytical method was validated according to the US Food and Drug Administration guidance for industry.[16] For plasma and urine, the within-batch accuracies varied between −14.3% and +12.3%, and the batch-to-batch accuracies ranged from −13.3% to +10.3%. The within-batch and batch-to-batch precision for urine and plasma were not higher than 8.16%. The lower limits of quantification (lowest calibration points) for midazolam and 1-OH-midazolam in plasma were 0.2 ng/mL (with corresponding accuracy/precision of −4.2%/+5.0%, +11.6%/+4.6%) and in urine 0.5 ng/mL for midazolam and 25 ng/mL for 1-OH-midazolam

(accuracy/precision of +8.0%/+7.9%, +3.8%/+7.1%). Recovery rates of midazolam and 1-OH-midazolam from plasma and urine varied between –13.3% and +15.5%.

Efavirenz plasma concentrations were determined by high-performance liquid chromatography coupled to tandem mass spectrometry using a validated and previously published method.[17] The lower limit of quantification (accuracy/precision) for efavirenz in plasma was 5 ng/mL (–0.5%/5.2%). Within-batch accuracies (precision) ranged from –13.5% to +8.8% (1.0% to 7.7%), and batch-to-batch accuracies (precision) from –6.8% to +3.9% (4.1% to 10.4%).

Pharmacokinetic and Statistical Analysis

Noncompartmental analysis using Kinetica 5.0 (Thermo, Waltham, Massachusetts) was performed to determine the pharmacokinetic parameters of midazolam and 1-OH-midazolam. The terminal elimination half-life of midazolam and 1-OH-midazolam after oral and intravenous administration was calculated as $\ln2/\lambda$ using the plasma concentration-time data from 0 to 6 hours after oral administration and from 6 to 14 hours after intravenous administration (which is 6 hours after oral administration). The terminal slope, λ, is calculated by linear regression of the log concentration vs time data. The AUC from time 0 to infinity ($AUC_{0-\infty}$) after oral administration was calculated using the mixed log-linear method and the λ value. $AUC_{0-\infty}$ after intravenous administration was calculated as AUC from the start of intravenous administration minus the extrapolated AUC attributable to the oral dose ($AUC_{6-\infty}$). Bioavailability (F) was calculated on the basis of $AUC_{0-\infty}$ after oral midazolam and $AUC_{0-\infty}$ after intravenous midazolam minus oral $AUC_{6-\infty}$. The apparent oral clearance (CL_o) and the systemic clearance (CL) were calculated as midazolam dose divided by the corresponding $AUC_{0-\infty}$. Hepatic extraction (E_H) was calculated as the ratio of CL and estimated hepatic plasma flow (Q_H),[13] with the assumption that extrahepatic elimination after intravenous midazolam is negligible. Q_H was estimated as $25.4\,\text{mL} \times \text{min}^{-1} \times \text{kg}^{-1} \times$ body weight in kilograms $\times$ (1 − hematocrit). Intestinal extraction (E_G) was calculated as $(1 − F/[1 − E_H])$.[13] AUC of the metabolic ratio of plasma 1-OH-midazolam divided by plasma midazolam was calculated separately from 0 to 6 hours (oral administration) and 6 to 14 hours (intravenous administration). Total excretion of midazolam and 1-OH-midazolam was determined in urine from 0 to 24 hours.

Statistics

From the CYP3A induction data with St John's wort[14] a sample size of 8 participants will be sufficient to detect a
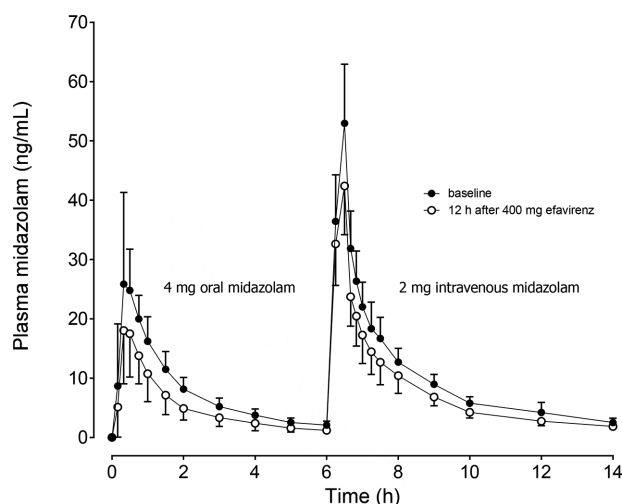

Figure 2. Mean ($\pm$SD) midazolam plasma concentrations after oral administration of 4 mg and, 6 hours later, intravenous administration of 2 mg midazolam at baseline (closed symbols) and 12 hours after a single oral dose of 400 mg efavirenz (open symbols).

reduction of AUC after oral midazolam administration to 63% of baseline with a power of 0.80 and $\alpha = 0.05$ (http://biomath.info/power/prt.htm). After log transformation, the primary (AUC) and secondary variables (CL, F, MR, E_H, and E_G) of this study were analyzed by standard repeated-measures analysis of variance with post hoc analysis (Dunnett multiple-comparison test; baseline vs days 1, 6, 11, 16, and 22) using Prism 6.0 (GraphPad Software, San Diego, California). A *P*-value < .05 was considered significant.

Results

At baseline, midazolam plasma concentrations after administration of 4 mg orally as a solution were lower than those after 2 mg intravenous administration 6 hours later, resulting in an absolute bioavailability of 29.9 % (Figure 2; Table 1). At baseline, midazolam apparent oral clearance was 1313 mL/min, and systemic clearance 393 mL/min. Twelve hours after a single oral dose of efavirenz, plasma midazolam concentrations were significantly decreased after both oral and intravenous administration (Figure 2). Midazolam CL_o was significantly increased by 70%, and CL after intravenous administration of midazolam was increased by 27% (*P* < .05) (Table 1, Figure 3). Hepatic extraction was increased by 30%, whereas intestinal extraction did not change at all (Table 2), and midazolam bioavailability decreased by 24% (Table 1). No significant correlation was observed between E_H and E_G (Pearson correlation coefficient r = 0.23; *P* = .48). On day 6, the efavirenz effects on oral midazolam were still present in the same magnitude, but thereafter (day 11 to day 22) they gradually vanished, and on day 22 the pharmacokinetic parameters had returned
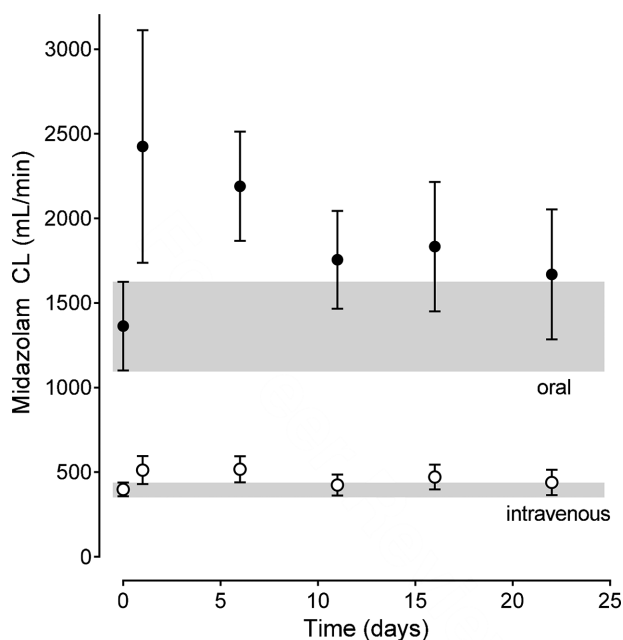
Figure 3. Mean (± 95%CI) midazolam clearance (total after intravenous; apparent oral clearance after oral administration) over time after a single oral dose of 400 mg efavirenz. Baseline is shown using open symbols. The gray bands represent the 95%CI of the baseline clearances.

In 10 of the 12 participants, efavirenz plasma concentrations after a single oral dose of 400 mg could be quantified until 22 days after administration with a terminal elimination half-life of 104 hours (geometric mean; 95%CI 85-128 hours) (Figure 4). Apparent oral clearance was estimated at 58 mL/min (95%CI 47-70 mL/min). There was a relationship between midazolam CL_o and plasma efavirenz concentrations with the Pearson correlation coefficient $r = 0.87$ ($P = .025$).

Within 1 hour after efavirenz intake, 5 of 12 participants reported dizziness, which disappeared within the next hour. Two participants had a euphoric episode during the night following efavirenz intake. One participant was very tired after efavirenz intake. No other adverse effects were reported after efavirenz. Due to the low doses, almost no adverse effects were reported after midazolam: only 1 participant developed transient headaches on days 16 and 22, but no intervention using medication was necessary.

Discussion

The most important finding of this study is the immediate influence of efavirenz on CYP3A activity. Following the first complex study reported in abstract form[12] in which we observed a significantly increased CYP3A activity the day after efavirenz administration, we performed a series of in vitro experiments[18] and planned and carried out a study of the time dependency of CYP3A activity after a single oral dose of efavirenz in vivo. Indeed, 12 hours after administration, a single oral dose of 400 mg efavirenz increased total CYP3A4 activity by 70%. This is in agreement with the distinct acute activation of midazolam metabolism observed in vitro, which is likely caused by binding of efavirenz

to baseline. For the metabolic ratios of the AUCs of plasma 1-OH-midazolam and plasma midazolam, a significant increase was observed only on day 1 after oral midazolam administration (Table 2). The urinary excretion over a 24-hour interval of midazolam and its conjugated metabolite 1-OH-midazolam was unchanged over the 22-day observation period (Table 1).

Table 1. Pharmacokinetic Parameters of Oral and Intravenous Midazolam Before and After a Single Oral Dose of 400 mg Efavirenz

	4 mg Oral Midazolam			2 mg Intravenous Midazolam			
	AUC (ng·h/mL)	CL_o (mL/min)	F (%)	AUC_c (ng·h/mL)	% AUC Extrapol	CL (mL/min)	Total Excretion (Midazolam + 1-OH-Midazolam (%)
Baseline	50.8	1313	29.9	84.8	6.9	393	63.7
	42.5–60.6	1100–1568	25.4–35.3	76.3–94.4	5.4–8.4	353–437	59.0–68.8
Day 1	30.0*	2231*	22.9*	66.6*	4.1	500*	66.3
	22.9–39.0	1708–2915	18.3–28.5	57.2–77.6	2.8–6.3	430–583	62.2–70.7
Day 6	31.2*	2138*	23.8	65.9*	3.8	506*	66.5
	27.0–36.0	1852–2468	20.5–27.7	56.5–77.0	2.8–5.9	433–590	62.3–71.1
Day 11	39.1*	1703*	27.2	79.5	5.3	420	64.9
	33.3–46.0	1449–2002	24.5–30.3	69.2–91.3	4.3–8.1	365–482	60.1–70.0
Day 16	38.0*	1755*	25.4	72.4	5.0	461	58.6
	31.4–45.9	1452–2123	21.7–29.8	62.6–83.6	2.3–7.6	399–532	52.4–65.5
Day 22	42.6	1564	27.6	78.2	5.9	426	59.9
	33.3–54.5	1223–2001	23.2–32.9	65.8–93.0	3.5–8.0	359–507	55.4–64.6

Data are shown as geometric means and 95% confidence intervals (n = 12). AUC, area under the concentration-time curve; AUC_c, AUC after intravenous administration corrected for contribution of preceding oral administration; % AUC extrapol, percentage of AUC after intravenous administration resulting from preceding oral administration; CL, clearance; CL_o, oral clearance.

*$P < .05$ in comparison to baseline.

Table 2. Metabolic Ratio of the AUC of 1-OH-Midazolam and Midazolam and Hepatic and Intestinal Extraction Ratios of Midazolam After Oral (0-6 hours) and Intravenous (6-14 hours) Midazolam Administration Before and After a Single Oral Dose of 400 mg Efavirenz

	MR Oral Midazolam	MR Intravenous Midazolam	E_H	E_G
Baseline	1.91	1.03	0.199	0.606
	1.38–2.64	0.72–1.48	0.173–0.229	0.545–0.674
Day 1	3.14*	1.12	0.258*	0.671
	2.17–4.53	0.57–2.20	0.208–0.319	0.621–0.725
Day 6	1.80	0.76	0.261*	0.662
	1.26–2.58	0.41–1.40	0.221–0.308	0.615–0.712
Day 11	1.76	0.47	0.210	0.651
	1.08–2.87	0.17–1.28	0.180–0.245	0.619–0.684
Day 16	1.64	0.66	0.235	0.654
	0.88–3.05	0.27–1.63	0.204–0.271	0.605–0.707
Day 22	2.37	0.84	0.213	0.631
	1.79–3.14	0.49–1.45	0.174–0.259	0.578–0.689

Data are shown as geometric means and 95% confidence intervals (n = 12). AUC, area under the concentration-time curve; E_G, intestinal extraction ratio; E_H, hepatic extraction ratio; MR, metabolic ratio.
*$P < .05$ in comparison to baseline.
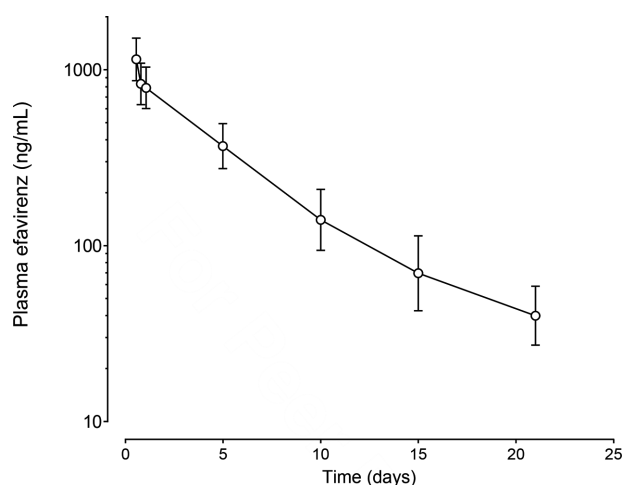
Figure 4. Mean plasma concentration-time profile of efavirenz after a single oral dose of 400 mg (n = 12; at the last time point n = 10).

(and not its metabolite 8-OH-efavirenz) to a peripheral site of the enzyme, resulting in enhanced turnover at the active site (allosteric activation).[18] A similar effect has been proposed for the picornavirus inhibitor pleconaril.[19,20]

Twelve hours after efavirenz administration (day 1), both total (after oral administration) and systemic (after intravenous administration) midazolam clearances were significantly increased. Total excretion of midazolam and its metabolites over a 24-hour period was unchanged, suggesting that renal clearance was unaffected by efavirenz. Oral midazolam bioavailability was reduced by efavirenz because the clearance increase after oral administration was larger than that after intravenous administration. It would be obvious to consider activation of intestinal and hepatic CYP3A responsible. However, because intestinal extraction is high and unchanged, the increase of low hepatic extraction must be the cause of the reduced bioavailability. After oral administration of midazolam, the concentrations reaching the liver by the portal vein are much higher than after intravenous administration, and the increased hepatic extraction caused the increase of clearance. The fact that the increase of oral clearance was higher indicates a predominant effect of efavirenz on hepatic first-pass metabolism. This is consistent with the increase of the midazolam metabolic ratio only after oral administration, which can be explained by the enhanced metabolism in the liver during the first-pass metabolism because of high midazolam concentrations in the portal vein. Because efavirenz absorption is complete 12 hours after its administration, it is no longer present in the intestine, and therefore, the high intestinal extraction of midazolam was unchanged. If there were activation of CYP3A in the enterocytes, intestinal extraction would increase, resulting in even higher first-pass metabolism and further reduced bioavailability.

The most relevant difference between activation and induction is the time of onset: whereas induction takes some days to be of any relevance (enhanced clearance with reduced drug effect), activation is much more rapid (within hours). This can be explained by the different mechanisms because induction initiates gene transcription and ultimately increases the amount of enzyme, a time-consuming process. This was demonstrated for the potent inducer rifampin, for which an in vivo induction half-life of 24 hours was observed using verapamil as a substrate.[21] In contrast, activation results from a conformation change of the already expressed enzyme resulting in a rapid (in vitro within minutes)[18] increase of metabolic turnover. Therefore, the effects of efavirenz on CYP3A metabolism will be observed almost instantaneously and as quickly as

enzyme inhibition. The clinical consequence of enzyme activation will be an almost immediate requirement of dose increase of a CYP3A substrate. If no dose adjustment is performed, a reduction of the therapeutic effects might result.

In our study just a single oral dose of 400 mg efavirenz was administered, and 12 hours later we observed a 32% increase of hepatic extraction that lasted 6 days, but there was no significant change of intestinal extraction. These data can be explained by the observed in vitro activating mechanism.[18] Due to the long elimination half-life of efavirenz observed (104 hours), CYP3A activation was still present 6 days after dosing. Enzyme induction after 6 days is also possible, and a mixture of activation and induction 6 days after a single oral dose of efavirenz seems likely.

Both mechanisms (activation and induction) may be present after multiple dosing. CYP2C19 and CYP3A4 are reported to be induced after 17 days of 600 mg efavirenz daily.[22] Michaud et al compared omeprazole 5-hydroxylation and sulfoxidation between single dose and multiple doses of efavirenz and reported a difference, which was attributed to enzyme induction.[22]

In addition, efavirenz is able to induce multiple drug transporters such as P-glycoprotein,[23] BCRP, MRP1-3, and OATP2B1.[24]

Baseline midazolam bioavailability in our study was 29.9%, which is lower than the 34% reported in the original publication of the semisimultaneous method by Lee et al.[13] This is probably due to the use of an oral solution in our study and consistent with an earlier study.[14] The hepatic extraction ratio of midazolam, which is regarded as a low-extraction drug, is below 0.3. This is in agreement with the low extraction observed by Lee et al (0.267)[13] but not with our own previous data, where midazolam was found to be a moderate-extraction drug ($E_H = 0.37$).[14] Earlier studies reported hepatic extraction of midazolam ranging from 0.32 to 0.96, and it was questioned whether midazolam can serve as a valid biomarker for CYP3A activity in the liver, especially when a high extraction is observed.[25]

We observed a much longer half-life of efavirenz than originally reported, which was 52 to 76 hours after a single oral dose.[26] Our long half-life is consistent with the long elimination half-life (121 hours) after a single 400-mg dose reported recently[27] but different from others who reported half-lives around 50 hours after a single oral dose.[28,29] This is probably the result of insufficient sampling times (up to 72 hours).

Limitations

In this study we have not addressed the CYP3A polymorphisms because they were not the topic of this investigation. However, it is known that systemic clearance of midazolam is about 30% lower in the CYP3A4*B1 polymorphic variant.[30] Because it is not known if the degree of CYP3A activation can be influenced by genetics (CYP3A polymorphisms), this matter remains to be investigated.

Conclusions

Within hours after administration of a single oral efavirenz dose, CYP3A is moderately activated, thereby enhancing the elimination of drugs that are major CYP3A substrates.

Acknowledgments

Declaration of Conflicting Interests

None to declare.

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