Inhibition of Efavirenz Metabolism by Sertraline and Nortriptyline and Their Effect on Efavirenz Plasma Concentrations

Virginia Melis, Iris Usach, Patricia Gandía, José-Esteban Peris

Department of Pharmacy and Pharmaceutical Technology, Faculty of Pharmacy, University of Valencia, Valencia, Spain

Between 22 and 45% of HIV-positive subjects are likely to report symptoms of depression. Considering this background, a potential pharmacokinetic interaction between the nonnucleoside reverse transcriptase inhibitor efavirenz (EFV) and two antidepressants, sertraline (SRT) and nortriptyline (NT), was studied. Rats were administered EFV alone or together with the antidepressants, and changes in the plasma levels and pharmacokinetic parameters of EFV were analyzed. Additional *in vitro* experiments with rat and human hepatic microsomes were carried out to evaluate the inhibitory effect of SRT and NT on EFV metabolism by determining the formation rate of the major EFV metabolite (8-OH-EFV). *In vivo* studies showed similar increases in the plasma levels of EFV when it was coadministered with SRT or NT. However, the studies using rat hepatic microsomes showed a more potent inhibitory effect of NT than of SRT on the metabolism of EFV, with values for the 50% inhibition constant (IC_{50}) and inhibitory constant (K_i) for NT about 9-fold lower than those for SRT. An equation was deduced that explains the similar *in vivo* effects of SRT and NT in spite of the different *in vitro* performance data. Using human hepatic microsomes, the strongest inhibitory effect was observed with SRT. In summary, pharmacokinetic interactions between EFV, SRT, and NT, associated with the inhibition of hepatic metabolism of EFV, have been detected in rats. Both antidepressants also inhibit EFV metabolism in human hepatic microsomes, but additional *in vivo* studies in humans are required to evaluate the clinical implication of this interaction.

Human immunodeficiency virus infection/AIDS (HIV/AIDS) is, at present, an incurable disease, and the use of antiretroviral therapy is essential to obtain durable viral suppression, improved immune function, and a good quality of life in infected individuals (1).

Current treatment guidelines recommend the use of a drug combination consisting of at least three antiretroviral drugs of multiple classes, known as highly active antiretroviral therapy (HAART). Combining different anti-HIV agents has proven to be beneficial in terms of sustained efficacy and long-term safety, provided there are no significant negative pharmacokinetic drug-drug interactions. At present, preferred regimens of HAART consist of two nucleos(t)ide reverse transcriptase inhibitors (NRTIs) with either a protease inhibitor (PI) or a nonnucleoside reverse transcriptase inhibitor (NNRTI) although other combinations are possible (2, 3).

Efavirenz (EFV) is an NNRTI of human immunodeficiency virus type 1 (HIV-1) and is one of the preferred components of first-line antiretroviral regimens. It is widely used as a component of initial HAART (except in infected pregnant women) since it has demonstrated potent and sustained activity in HIV-infected patients as it induces a rapid suppression of the HIV-1 viral load and increases the CD4 cell count (1).

In vitro studies with human hepatic microsomes, as well as *in vivo* studies, have demonstrated that EFV undergoes significant metabolism by the cytochrome P450 isoenzymes to give hydroxylated metabolites. The major metabolite is 8-hydroxy-efavirenz (8-OH-EFV), whereas 7-hydroxy-efavirenz (7-OH-EFV) and 8,14-dihydroxy-efavirenz (8,14-diOH-EFV) are minor ones (4, 5). Cytochromes P450 2B6 (CYP2B6) and 2A6 (CYP2A6) have been identified as the isoenzymes involved in the formation of 8-OH-EFV and 8,14-diOH-EFV and of 7-OH-EFV, respectively (4–6). In rats, the major metabolite of EFV is also 8-OH-EFV, whereas 7-OH-EFV and 8,14-diOH-EFV are not detected (7).

EFV has been shown to inhibit CYP2C9, CYP2C19, and CYP3A4 and to induce CYP3A4 and CYP2B6 isoenzymes with the induction of its own metabolism (8). Drugs that are substrates for these isoenzymes induced by EFV may be susceptible to a plasma concentration reduction in individuals receiving EFV. For example, concomitant use of the antidepressant sertraline (SRT) and EFV results in a significant reduction in the area under the plasma concentration-time curve (AUC), maximum plasma concentration (C_{max}), and minimum plasma concentration (C_{min}) of SRT due to the induction of SRT metabolism by EFV, making it necessary to increase the dose of the antidepressant in some patients receiving both drugs (9).

Major depression and substance abuse are the two most prevalent psychosocial comorbidities in patients with HIV/AIDS (10–12), and these problems can interfere with antiretroviral therapy adherence (13–18). In fact, a significant relationship between adherence to HIV treatment and depression has been reported (19).

Selective serotonin-reuptake inhibitors (SSRIs) have become the agents of first choice in the treatment of depression based on side effect profiles that are more favorable than those of tricyclic antidepressants (TCAs). Nevertheless, the effectiveness levels of both groups are similar, with no clinically significant differences,

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Address correspondence to José-Esteban Peris, jose.e.peris@uv.es.

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in the treatment of depressive disorders in general (20–22) as well as depressive symptoms in patients with HIV infection (23).

SRT is a powerful SSRI used in the treatment of depression (24), distress with or without agoraphobia, obsessive-compulsive disorder (25), posttraumatic stress disorder (PTSD) (26), panic attacks, social phobia (27–29), and premenstrual dysphoric disorder (PMDD) (30). SRT is a moderate inhibitor of the isoenzymes CYP2B6 and CYP2D6 (31), and it can, therefore, give rise to an increase in the plasma drug levels of drugs that are substrates of these isoenzymes (32–35).

Nortriptyline (NT) is a TCA which displays superior pharmacological properties to all other TCAs as a psychotropic but has a lower toxicity (36). NT is a weak CYP2D6 inhibitor, and it is one of the least problematic TCAs in terms of drug interactions. Nevertheless, some clinically significant interactions between NT and other drugs have been described, as in the case of chlorpromazine, which shows decreased clearance and increased plasma concentrations in the presence of NT (37).

Considering the lifetime prevalence of depression in patients with HIV (22 to 45%) (38), it was decided to study a potential pharmacokinetic interaction between EFV and the antidepressants SRT and NT. For this purpose, *in vivo* and *in vitro* studies were performed using rats as experimental animals. The studies were carried out *in vivo* to evaluate the effect of the potential interaction on the plasma levels of EFV and *in vitro* with rat hepatic microsomes to confirm the inhibition of EFV metabolism by the antidepressants. Finally, the inhibitory effect of the antidepressants was checked with human hepatic microsomes.

MATERIALS AND METHODS

Chemicals. EFV (Sustiva) was obtained from Bristol-Myers Squibb (Madrid, Spain). SRT (Aremis) was obtained from Esteve Laboratories (Barcelona, Spain). 8-OH-EFV was obtained from Toronto Research Chemicals (North York, Canada). NT (hydrochloride salt), β -NADP (β -NADP⁺), glucose-6-phosphate, glucose-6-phosphate dehydrogenase, and magnesium chloride (MgCl₂) were purchased from Sigma-Aldrich (Madrid, Spain). All the other reagents and solvents used in the study were of high-performance liquid chromatography (HPLC) or analytical grade.

***In vivo* studies.** (i) **Animals.** Protocols for the animal studies were approved by the Animal Care Committee of the Faculty of Pharmacy at the University of Valencia, Spain. Male Wistar rats, 2 to 3 months old and weighing 280 to 310 g, were used in this study. All animals were obtained from the animal facilities of Faculty of Pharmacy, University of Valencia, and were kept in a clean room at a temperature of 23 $\pm$ 1°C, a relative humidity of 60%, and a light-dark cycle of 12 h light/12 h darkness. Rats were fed a standard laboratory diet obtained from Harlan Laboratories, Inc. (Barcelona, Spain), and had *ad libitum* access to water.

(ii) **Drug administration and sampling.** The day before drug administration, rats were cannulated in the jugular vein and the duodenum to facilitate blood sample collection and intraduodenal (i.d.) dose administration using previously reported procedures (39, 40). All rats were anesthetized with ketamine (80 mg/kg) and xylazine (10 mg/kg). Moreover, animals were submitted to overnight fasting, but water was supplied *ad libitum*.

Rats were divided into six groups ($n = 6$ animals per group) to study the *in vivo* interaction between EFV and SRT or NT (Table 1). Drugs were intraduodenally administered in the form of suspension (EFV, 10 mg/ml; SRT, 5 mg/ml) or solution (NT, 5 mg/ml) in distilled water.

In the case of rats receiving both drugs, SRT and EFV or NT and EFV, administrations were separated by a 15-min interval. Animals of groups 1 and 4 (control groups) were intraduodenally administered 1 ml of water and, 15 min later, 1 ml of the EFV suspension (dose, 10 mg). Animals of groups 2 and 3 were administered 1 ml and 2 ml of the SRT suspension,

TABLE 1 Animal groups used for the *in vivo* studies

EFV-SRT interaction			EFV-NT interaction		
Group ($n = 6$)	EFV dose (mg)	SRT dose (mg)	Group ($n = 6$)	EFV dose (mg)	NT dose (mg)
1	10		4	10	
2	10	5	5	10	5
3	10	10	6	10	10

respectively (doses, 5 mg and 10 mg, respectively), and 15 min later, 1 ml of the EFV suspension (dose, 10 mg) was introduced through the i.d. cannula. In the case of groups 5 and 6, the administration schedule was the same as that for groups 2 and 3, but the SRT suspension was replaced by the NT solution (Table 1).

In all rats, a total of eight blood samples were collected at the following times after EFV administration: 15, 30, 60, 120, 180, 240, 360, and 465 min. Sampling was performed through the jugular cannula with previously heparinized syringes to avoid blood coagulation. After each blood collection (0.2 ml), the same volume of heparinized serum (20 IU/ml) kept at 37°C was perfused. Blood samples were collected in Eppendorf tubes, which were then centrifuged at 2,000 $\times g$ for 5 min, and the supernatant plasma was used for the analytical determination of EFV after protein precipitation with acetonitrile (1:1, vol/vol) as indicated below.

***In vitro* studies.** (i) **EFV metabolism and its inhibition by SRT and NT in rat hepatic microsomes.** Wistar rat livers were processed as previously described (41) to obtain hepatic microsomes that were used for the study of the EFV metabolism. The rate of 8-OH-EFV formation by hepatic microsomes was determined using different concentrations of EFV and glass vials as follows: a given volume of EFV in methanol (10 μ g/ml) was evaporated, and hepatic microsomes (equivalents to 0.1 mg of protein) were added to the residue together with 5 μ l of a solution of glucose-6-phosphate in water (20 mg/ml), 5 μ l of a solution of β -NADP⁺ in water (20 mg/ml), 10 μ l of a solution of glucose-6-phosphate dehydrogenase in water (10 U/ml), and 5 μ l of a solution of MgCl₂ in water (13.4 mg/ml). The final volume was adjusted to 0.1 ml by the addition of 0.1 M phosphate buffer (pH 7.4), and the following concentrations of EFV in the final mixture were obtained: 1, 2.5, 5, 10, 25, 50, and 100 μ g/ml (3.2, 7.9, 15.8, 31.7, 79.2, 158.4, and 316.8 μ M). The samples were incubated in a bath at 37°C for 30 min. At the end of the incubation period, 0.1 ml of acetonitrile was added, and the samples were cooled in an ice bath. The deproteinized samples were centrifuged, and the supernatant was utilized for the analytical determination of 8-OH-EFV.

The same protocols were used for the study of EFV metabolism inhibition by SRT and NT, with the following modifications: the EFV concentration in the final mixture was 5 μ g/ml (15.8 μ M), and different amounts of SRT and NT were added to the reaction mixture to obtain final concentrations in the range of 0.33 to 165 μ M.

(ii) **Inhibition of the EFV metabolism by SRT and NT in human hepatic microsomes.** The inhibition of 8-OH-EFV formation by SRT and NT was studied in human hepatic microsomes obtained from Invitrogen (Barcelona, Spain). The procedure was similar to that described in the case of rat hepatic microsomes although the concentration of microsomal protein was doubled (2 mg/ml) to increase the amount of 8-OH-EFV in the final solution since EFV metabolism by human microsomes is slower than that by rat microsomes. The final concentration of EFV in the incubation medium was 5 μ g/ml (15.8 μ M), and the final concentration of SRT and NT was 16.0 μ M.

Analytical method. The analytical determination of EFV concentrations in rat plasma, as well as 8-OH-EFV concentrations in microsome samples, was carried out using an HPLC assay. The HPLC equipment consisted of a quaternary programmable pump SpectraSystem P4000, an autosampler SpectraSystem AS3000, and a spectrophotometric detector SpectraSystem UV6000LP fit at 254 nm. Chromatographic separation was performed on a Waters Nova-Pack C₁₈ (150 mm by 3.9 mm; 4- μ m par-

FIG 1 EFV plasma concentrations (means + standard deviations) in rats following i.d. administration of EFV with and without SRT coadministration (a) and with and without NT coadministration (b). Numbers in parentheses indicate the animal groups.

ticle size). The injection volume of supernatants of deproteinized samples was 20 µl, and the flow rate of the mobile phase was 1 ml/min. The mobile phase consisted of a mixture of acetonitrile and an aqueous solution of dihydrate sodium phosphate (0.78% mass/vol) in proportions of 55/45 (vol/vol) for the analysis of EFV in plasma samples and 50/50 (vol/vol) for the analysis of 8-OH-EFV in microsome samples.

Calibration curves were linear over the concentration range of 0.1 to 20 µg/ml of EFV, and the lower limit of quantification (LLOQ) was 0.03 µg/ml for both analytes, EFV and 8-OH-EFV.

Pharmacokinetic and metabolic analysis. WinNonlin (version 5.1; Pharsight Corp., Mountain View, CA) was used to estimate the following noncompartmental pharmacokinetic parameters of EFV after i.d. administrations: maximum plasma concentration (C_{max}) and time to C_{max} (t_{max}), half-life ($t_{1/2}$), area under the plasma concentration-time curve from the time of dosing to the last measurable concentration (AUC_{last}) and to infinity (AUC_{inf}).

The parameters V_{max} (maximum velocity) and K_m (Michaelis-Menten constant) corresponding to the formation of 8-OH-EFV by rat hepatic microsomes were estimated by nonlinear regression fitting of the Michaelis-Menten equation to the experimental data (rate of 8-OH-EFV formation as a function of the initial concentration of EFV) using the GraphPad Prism program (version 6.01; GraphPad Software, Inc., San Diego, CA). This program was also used to determine the concentration of inhibitor (SRT or NT) required for 50% inhibition (IC_{50}) of EFV metabolism *in vitro*. The Cheng-Prusoff equation (42) was used to estimate the inhibitory constants (K_i s) corresponding to SRT and NT:

$$K_i = \frac{IC_{50}}{1 + \frac{[S]}{K_m}} \quad (1)$$

where [S] is the concentration of EFV (15.8 µM) used in the *in vitro* inhibition studies.

Statistical analysis. Statistical comparisons of EFV pharmacokinetic parameters were performed by means of a one-way analysis of variance (ANOVA) test, and when statistically significant differences were found, Tukey's test was employed to determine which groups were statistically different (IBM SPSS Statistics 19, SPSS, Inc., Chicago, IL, USA). The $\log(IC_{50})$ values obtained for the inhibitory drugs (SRT and NT) by nonlinear regression were statistically compared using a *t* test. A *P* value of <0.05 was considered statistically significant.

RESULTS

***In vivo* studies. (i) Pharmacokinetics of EFV coadministered with SRT.** The mean plasma concentration-time profiles for EFV in the absence or presence of SRT are shown in Fig. 1a, and the values of pharmacokinetic parameters are listed in Table 2. Coadministration of SRT resulted in higher plasma concentrations of EFV, directly related to the administered dose of SRT. The EFV C_{max} , AUC_{last} and AUC_{inf} values increased by 26%, 23%, and 32%, respectively, in the case of the 5-mg dose of SRT and by 75%, 80%, and 87%, respectively, in the case of the 10-mg dose. Statistically significant differences were detected only in the group of animals administered 10 mg of SRT (group 3) since the reduced number of rats used in the experiments and the interindividual variability hampered the increases in the pharmacokinetic parameters observed in the group administered 5 mg of SRT (about 20 to 30%) to be considered statistically significant by the ANOVA test. The increase in the EFV plasma concentrations as a consequence of the coadministration of SRT suggests an inhibitory effect of this drug on the metabolism of EFV, which was confirmed by the *in vitro* studies with rat hepatic microsomes.

TABLE 2 Pharmacokinetic parameters in rats after administration of EFV with and without SRT

EFV parameter	Value for the group (mean ± SD) ^a		
	Group 1	Group 2 (+5 mg of SRT)	Group 3 (+10 mg of SRT)
t_{max} (min)	80.0 ± 45.2	85.0 ± 39.9	90.0 ± 32.9
C_{max} (µg/ml)	1.81 ± 0.53 A	2.29 ± 0.65 A	3.16 ± 0.55 B
$t_{1/2}$ (min)	145 ± 39	165 ± 68	160 ± 4
AUC_{last} (µg · min/ml)	467 ± 104 A	576 ± 139 A	843 ± 178 B
AUC_{inf} (µg · min/ml)	540 ± 100 A	715 ± 197 A	1010 ± 185 B
AUC_{inf} increase (%) ^b		32	87

^a For all groups, 10 mg of EFV was administered i.d. Parameter values with different letters are statistically different (*P* < 0.05).

^b AUC_{inf} increase compared with the value for group 1.

TABLE 3 Pharmacokinetic parameters in rats after administration of EFV with and without NT

EFV parameter	Value for the group (mean $\pm$ SD) ^a		
	Group 4	Group 5 (+5 mg of NT)	Group 6 (+10 mg of NT)
$t_{\max}$ (min)	92.5 $\pm$ 79.6	80.0 $\pm$ 31.0	110.0 $\pm$ 24.5
$C_{\max}$ (μ g/ml)	2.09 $\pm$ 0.53 A	2.30 $\pm$ 0.58 AB	3.20 $\pm$ 0.91 B
$t_{1/2}$ (min)	160 $\pm$ 36	186 $\pm$ 43	193 $\pm$ 36
AUC_{last} (μ g $\cdot$ min/ml)	478 $\pm$ 31 A	653 $\pm$ 249 AB	822 $\pm$ 209 B
AUC_{inf} (μ g $\cdot$ min/ml)	584 $\pm$ 85 A	822 $\pm$ 307 AB	1057 $\pm$ 248 B
AUC_{inf} increase (%) ^b		41	81

^a For all groups, 10 mg of EFV was administered i.d. Parameter values with different letters are statistically different ($P < 0.05$).

^b AUC_{inf} increase compared with the value for group 4.

(ii) **Pharmacokinetics of EFV coadministered with NT.** Co-administration of NT also gave rise to EFV plasma concentrations higher than those obtained in the group of rats administered EFV only (Fig. 1b). The pharmacokinetic parameters of EFV obtained in the absence and presence of NT and their statistical comparison are shown in Table 3. EFV $C_{\max}$, AUC_{last} , and AUC_{inf} increased by 10%, 36%, and 41%, respectively, for the 5-mg dose of NT, and the increases were high and statistically significant in comparison to those in rats administered EFV only when a dose of 10 mg of NT was administered (increases of 53%, 72%, and 81% for $C_{\max}$, AUC_{last} , and AUC_{inf} respectively). These results suggest, as in the case of SRT coadministration, that NT inhibits the metabolism of EFV.

In vitro studies. Using rat hepatic microsomes, the formation rate of the metabolite 8-OH-EFV showed a classic Michaelis-Menten kinetic profile (Fig. 2). The $V_{\max}$ and K_m values ($\pm$ standard error) obtained by nonlinear regression were 79.1 ± 4.3 pmol/min/mg of protein and 38.1 ± 6.7 μ M. The inhibition curves of 8-OH-EFV formation from EFV obtained with different concentrations of SRT and NT (Fig. 3) showed concentration-dependent inhibition of EFV metabolism for both antidepressants. As can be seen from the data shown in Fig. 3, NT was more

FIG 2 Kinetics for the formation rate of 8-OH-EFV from EFV in rat liver microsomes (means $\pm$ standard deviations; $n = 4$). **FIG 3** Concentration-dependent inhibition of 8-OH-EFV formation from EFV (15.8 μ M) by SRT and NT in rat liver microsomes (means $\pm$ standard deviations; $n = 4$).

effective in the inhibition of EFV metabolism than SRT. In fact, the IC_{50} and K_i values were approximately 9-fold lower for NT than for SRT (Table 4). These *in vitro* results confirmed the inhibitory effect of both drugs on EFV metabolism, which had been suggested by the results of the *in vivo* studies. However, the *in vivo* studies did not show the difference in the inhibitory potencies of SRT and NT detected in the *in vitro* experiments.

When human instead of rat hepatic microsomes were used, an inhibitory effect of SRT and NT on the formation rate of 8-OH-EFV was also detected (Fig. 4), with the strongest inhibitory effect corresponding to SRT.

DISCUSSION

Oral administration of EFV to rats and monkeys has been associated with a prolonged absorption phase due to delayed gastric emptying, as demonstrated by a greater than 10-fold slower transit of [14 C]polyethylene glycol through the stomach of EFV-pretreated animals (43). In order to avoid this delayed gastric emptying of EFV, which may alter the absorption profile and hamper the detection of changes in the EFV plasma concentration curves when EFV is coadministered with potential inhibitory drugs, it was decided to administer EFV intraduodenally using a cannula directly connected to the duodenum, which was also used for the administration of the other drugs, SRT and NT.

The usual dose of EFV in humans is 600 mg/daily (approximately 8.6 mg/kg), and the equivalent dose in rats on a direct

TABLE 4 Inhibitory parameters of SRT and NT for 8-OH-EFV formation from EFV in rat liver microsomes

Inhibitory parameter	Value for the drug ^a	
	SRT	NT
$\log(IC_{50})$ ^b	1.99 $\pm$ 0.07 A	1.04 $\pm$ 0.05 B
IC_{50} (μ M)	99.8	11.0
K_i (μ M)	70.5	7.77

^a Parameter values with different letters are statistically different ($P < 0.001$).

^b $\log(IC_{50})$ values were obtained by nonlinear regression and are expressed as estimated value $\pm$ standard error.

FIG 4 Inhibition of 8-OH-EFV formation from EFV (15.8 μM) by SRT (16.0 μM) and NT (16.0 μM) in human liver microsomes (means + standard deviations; $n = 4$).

mg/kg body weight-basis is 2.5 mg, considering 295 g as the average weight of the animals used in the study (280 to 310 g). However, the dose of EFV administered to the rats in this study was four times greater (10 mg) in order to obtain a maximum plasma concentration of EFV similar to that obtained in humans (0.50 to 2.87 $\mu\text{g/ml}$) (44). The same criterion was used for the selection of the lowest NT dose since in a previous study it was observed that an i.d. dose of 5 mg gave rise to a mean maximum plasma concentration of 122 ng/ml in rats (41), which is within the optimal range of 50 to 150 ng/ml described for humans (45). The same lowest dose (5 mg) was selected for SRT, taking into account that, in humans, the usual doses for both antidepressants are similar (75 to 150 mg for NT and 50 to 200 mg for SRT) (45, 46). Furthermore, a higher dose (10 mg) was tested in both cases in order to emphasize a possible pharmacokinetic interaction between these antidepressants and EFV.

Coadministration of SRT or NT with EFV to rats provoked increases in the plasma levels of EFV related to the administered dose of SRT and NT, and the observed increases were similar for both antidepressants. However, the *in vitro* studies using rat hepatic microsomes showed a more potent inhibitory effect of NT than of SRT on the metabolism of EFV, with the IC_{50} and K_i values for NT about nine times lower than those for SRT. Different equations have been proposed to predict *in vivo* drug-drug interactions using *in vitro* data (47, 48), the simpler equation being the one relating the AUC_{inf} ratio (AR) in the presence and absence of the inhibitor [$\text{AUC}_{\text{inf}(+I)}/\text{AUC}_{\text{inf}(\text{control})}$], the concentration of inhibitor *in vivo*, and the K_i value (49, 50):

$$\text{AR} = \frac{\text{AUC}_{\text{inf}(+I)}}{\text{AUC}_{\text{inf}(\text{control})}} = 1 + \frac{[I]}{K_i} \quad (2)$$

In this equation, $[I]$ represents the concentration of inhibitor at the enzyme site *in vivo*, which cannot be determined experimentally, and it is usually replaced by the total (or unbound) peak (or steady-state) concentration of the inhibitor in plasma. Nevertheless, false-negative and -positive predictions are frequently obtained using this equation (51). In order to check if equation 2 could be used to explain the *in vivo* results, an additional group of rats were intraduodenally dosed with 5 mg of SRT, and the follow-

ing pharmacokinetic parameters were obtained: C_{max} of 0.93 μM and AUC_{inf} of 522 $\mu\text{M} \cdot \text{min}$. With regard to NT, previously published data (41) were used (C_{max} of 0.41 μM and AUC_{inf} of 57 $\mu\text{M} \cdot \text{min}$). Using these C_{max} values to replace $[I]$ in equation 2, the predicted AUC_{inf} ratios were clearly lower than those observed *in vivo*: 1.01 versus 1.32 (SRT) and 1.05 versus 1.41 (NT). As indicated before, $[I]$ represents the concentration of inhibitor at the enzyme site *in vivo*, which can be very different from the systemic plasma concentration since it is more closely related to the concentration of drug in the plasma or blood entering the liver (portal vein) than to the concentration in the systemic circulation, especially when drug is absorbed from the gastrointestinal tract (51). According to equation 2, the fractional increase in AUC_{inf} (FIA) of inhibited drug is given by the ratio $[I]/K_i$, and it can be used to compare two inhibitors using the following equation:

$$\text{RAI} = \frac{\text{FIA}_{(1)}}{\text{FIA}_{(2)}} = \frac{[I_{(1)}] \cdot K_{i(2)}}{[I_{(2)}] \cdot K_{i(1)}} \quad (3)$$

where RAI is the ratio of the AUC_{inf} increase for the inhibited drug as a consequence of its coadministration with inhibitors 1 and 2. As before, $[I_{(1)}]$ and $[I_{(2)}]$ represent the concentrations of inhibitors at the enzyme site, but assuming similar absorption and distribution features for both inhibitors, the ratio $[I_{(1)}]/[I_{(2)}]$ could be approximated by the ratio of corresponding plasma concentrations. Furthermore, $[I]$ represents a steady-state concentration, whereas C_{max} is a concentration determined at a specific time (t_{max}), so the ratio $[I_{(1)}]/[I_{(2)}]$ could be better approximated by the corresponding AUC_{inf} ratio $\text{AUC}_{\text{inf}(1)}/\text{AUC}_{\text{inf}(2)}$, than by the C_{max} ratio. When equation 3 is applied using the AUC_{inf} and K_i values corresponding to SRT (as inhibitor 1) and NT (as inhibitor 2), the predicted *in vivo* RAI is 1.01[(522/57)(7.77/70.5)]. Therefore, the approximately 9-fold higher potency of NT as inhibitor is counteracted by the approximately 9-fold higher AUC_{inf} of SRT. The observed RAI was 0.78 for the dose of 5 mg (0.32/0.41) and 1.07 for the dose of 10 mg (0.87/0.81) (Tables 2 and 3), and these values are close to the predicted value of 1.01.

An inhibitory effect of SRT and NT on the metabolism of EFV was also detected using human hepatic microsomes. However, the inhibition of 8-OH-EFV formation was more pronounced in the case of SRT than in the case of NT, which is the opposite of the result observed with rat microsomes. In humans, an increase in the EFV C_{max} has been reported when this drug is coadministered with SRT, which agrees with the inhibition of EFV metabolism by SRT described in the present work. However, the EFV AUC_{inf} was not modified by the administration of SRT (9), which suggests a low clinical significance for this interaction *in vivo*. With regard to the coadministration of EFV and NT in humans, no pharmacokinetic interaction has been described so far.

In summary, SRT and NT inhibited EFV metabolism in rat and human liver microsomes. An increase in the plasma concentrations of EFV was observed in rats as a consequence of such inhibition. Although NT was more potent than SRT in inhibiting EFV metabolism *in vitro*, the increase in the EFV plasma concentrations caused by the coadministration of NT was similar to that obtained with the coadministration of SRT. These results can be explained if the plasma concentrations of the inhibitors, represented by the corresponding AUC_{inf} values, are considered in addition to the K_i values. With regard to the inhibition of EFV metabolism in human liver microsomes, it was more pronounced in

the case of SRT than in the case of NT, which is the opposite of the result observed with rat microsomes.

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