

Pharmacokinetic Interaction between Nevirapine and Nortriptyline in Rats: Inhibition of Nevirapine Metabolism by Nortriptyline

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One of the most frequent comorbidities of HIV infection is depression, with a lifetime prevalence of 22 to 45%. Therefore, it was decided to study a potential pharmacokinetic interaction between the nonnucleoside reverse transcriptase inhibitor nevirapine (NVP) and the tricyclic antidepressant nortriptyline (NT). NVP and NT were administered to rats either orally, intraduodenally, or intravenously, and the changes in plasma levels and pharmacokinetic parameters were analyzed. Experiments with rat and human hepatic microsomes were carried out to evaluate the inhibitory effects of NT on NVP metabolism. NVP plasma concentrations were significantly higher when this drug was coadministered with NT. The maximum plasma concentrations of NVP were increased 2 to 5 times and the total plasma clearance was decreased 7-fold in the presence of NT. However, statistically significant differences in the pharmacokinetic parameters of NT in the absence and presence of NVP were not found. *In vitro* studies with rat and human hepatic microsomes confirmed the inhibition of NVP hepatic metabolism by NT in a concentration-dependent way, with the inhibition being more intense in the case of rat microsomes. In conclusion, a pharmacokinetic interaction between NVP and NT was detected. This interaction was a consequence of the inhibition of hepatic metabolism of NVP by NT. *In vivo* human studies are required to evaluate the effects of this interaction on the pharmacokinetics of NVP before it can be taken into account for patients receiving NVP.

Human immunodeficiency virus infection/AIDS is, at present, an incurable disease. However, the use of adequate antiretroviral therapy (ART) has resulted in dramatic reductions in AIDS-related morbidity and mortality rates. ART decreases HIV RNA levels (<50 copies/ml) at 48 weeks and increases CD4⁺ cells in the vast majority of patients. Durable viral suppression improves immune function and quality of life, lowers the risk of both AIDS-defining and non-AIDS-defining complications, and prolongs life (53).

In general, the initial treatment of HIV-infected individuals involves drug combinations consisting of at least three antiretroviral drugs of multiple classes, known as highly active antiretroviral therapy (HAART). Currently, preferred HAART regimens use combinations of two nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) with a protease inhibitor (PI) (preferably boosted with ritonavir) or with a nonnucleoside reverse transcriptase inhibitor (NNRTI), although other combinations are possible (1).

Nevirapine (NVP) is an NNRTI of HIV-1 that is widely used as a component of HAART since it has demonstrated potent sustained activity in HIV-infected patients, because it induces rapid suppression of the HIV-1 viral load and increases in CD4⁺ cell counts (2–5). The efficacy of NVP is comparable to that of efavirenz (another commonly used NNRTI) and ritonavir-boosted PIs, the other antiretroviral drugs currently used in addition to the two NRTIs in initial HAART regimens (6). Moreover, NVP-based regimens are commonly prescribed for HIV-infected pregnant women, because one of the most relevant benefits of NVP is its efficacy in the prevention of mother-to-child transmission of HIV-1 infection (7).

The effective dosing regimen for NVP is 200 mg once daily for 14 days, followed by 200 mg twice daily. Data reported in the literature from 20 HIV-infected patients showed steady-state maximum plasma concentration ($C_{\max}$) and minimum plasma concentration ($C_{\min}$) values of 5.74 $\mu\text{g/ml}$ and 3.73 $\mu\text{g/ml}$, respectively (8). In humans as well as in mice, rats, rabbits, dogs, mon-

keys, and chimpanzees, NVP undergoes significant hepatic metabolism, mainly through 2-, 3-, 8-, and 12-hydroxylation (yielding 2-hydroxynevirapine [2-OH-NVP], 3-OH-NVP, 8-OH-NVP, and 12-OH-NVP, respectively) followed by glucuronidation of the hydroxyl metabolites (9, 10). In humans, cytochrome P450 3A4 (CYP3A4) was identified as the major enzyme involved in the formation of 2-OH-NVP and 12-OH-NVP, whereas cytochrome P450 2B6 (CYP2B6) was the predominant enzyme involved in the formation of 3-OH-NVP and 8-OH-NVP. NVP has been shown to be a substrate and inducer of these cytochrome P450 enzymes, resulting in the induction of its own metabolism and multiple drug-drug interactions. Plasma concentrations of drugs that are substrates for CYP2B6 and CYP3A4 may be susceptible to reduction in individuals receiving NVP (10). For example, several published drug interaction studies showed that NVP reduced the steady-state ketoconazole area under the curve (AUC) and $C_{\max}$ by 63% and 40%, respectively (11). Similarly, methadone and oral (p.o.) contraceptive steroids such as ethinylestradiol/norethindrone show reductions in their plasma levels when they are administered with NVP (12, 13). Drugs and substances that induce these isoenzymes may reduce NVP plasma concentrations. For example, rifampin and rifabutin, two potent CYP3A4 inducers, cause significant reductions in NVP plasma concentrations (10). The herbal remedy St. John's wort is another CYP3A4 inducer that decreases NVP levels in blood when they are administered concomitantly (14). In contrast, there are some inhibitors

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of cytochrome P450 isoenzymes, such as fluconazole, that increase NVP plasma concentrations (15). Variations in NVP plasma levels could be associated with loss of efficacy and viral resistance or with drug toxicity (16–19).

The two most prevalent and interfering psychosocial comorbidities of HIV infection are clinical depression and substance use (20–22). These problems, besides causing distress and functional impairment, can interfere with antiretroviral therapy adherence (23–28). In fact, a meta-analysis carried out by Gonzalez et al. (29) revealed a significant relationship between depression and adherence to HIV medications. In general, selective serotonin reuptake inhibitors (SSRIs) are considered first-line therapy for treatment of depressive disorders because of their more-favorable side effect profiles, compared with those of tricyclic antidepressants (TCAs). However, there is no clinically significant difference in the effectiveness of SSRIs versus TCAs for the treatment of depressive disorders (30–32), and both groups of drugs appear to be effective in treating depressive symptoms in patients with HIV infections, without affecting their immune status (33).

Nortriptyline (NT) is a TCA that shows superior pharmacological properties, compared with all other TCAs, as a psychotropic agent, with lower toxicity (34). It is administered orally, and doses of 25 mg 3 or 4 times daily are recommended to achieve plasma NT levels within the optimum range of 50 to 150 ng/ml (54). The main routes of NT metabolism are *E*-10-hydroxylation (the major route) and *N*-demethylation (a minor route) (35, 36). *E*-10-Hydroxylation is mediated mainly by cytochrome P450 2D6 (CYP2D6), although *in vitro* studies have shown that CYP3A4 is also involved (37). NT is a weak CYP2D6 inhibitor, and it is one of the least problematic TCAs in terms of drug interactions. However, some clinically significant interactions between NT and other drugs have been described. Concomitant therapy with drugs that inhibit CYP2D6, such as terbinafine, fluoxetine, norfluoxetine, sertraline, and paroxetine, results in major increases in plasma NT concentrations, caused by decreased NT clearance (CL), whereas the volume of distribution (*V*) remains unchanged (38–40). On the other hand, the plasma concentrations of some drugs are affected by the coadministration of NT, as occurs in the case of chlorpromazine, which shows decreased clearance and increased plasma concentrations in the presence of NT (41). The involvement of CYP3A4 in NT metabolism seems likely, considering either case reports of increased NT metabolism with concomitant use of CYP3A4 inducers, such as pentobarbital, carbamazepine, and rifampin (42–45), or reported cases of inhibition of NT metabolism by CYP3A4 inhibitors, such as fluconazole and cimetidine (46, 47).

Considering the lifetime prevalence of depression in patients with HIV (22 to 45%) (48), a study of possible pharmacokinetic interactions between NVP and NT was carried out. For this purpose, *in vivo* and *in vitro* studies were performed using rats as experimental animals, since the same metabolites are formed in humans and in rats (9).

MATERIALS AND METHODS

Chemicals. NVP (Viramune) was obtained from Boehringer Ingelheim (Barcelona, Spain). 2-, 3-, and 12-OH-NVP were obtained from Toronto Research Chemicals (North York, Canada). NT (hydrochloride salt), carboxymethyl cellulose (CMC), dimethyl sulfoxide (DMSO), β -NADP, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, and $MgCl_2$ were purchased from Sigma-Aldrich (Madrid, Spain). Propylene glycol

TABLE 1 Animal groups ($n = 6$)

Group	Dose (mg) and administration route of ^a :	
	NVP	NT
1	8, p.o.	
2	8, p.o.	5, p.o.
3	8, i.d.	
4	8, i.d.	5, i.d.
5	8, i.d.	2.5, i.v.
6	4, i.v.	
7	4, i.v.	5, i.d.
8		5, i.d.
9		2.5, i.v.

^a p.o., oral; i.d., intraduodenal; i.v., intravenous.

(PG) and 9H-fluoren-9-ylmethyl chloroformate (Fmoc-Cl) were supplied by Fluka BioChemika (Buchs, Switzerland). All 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 (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 the Faculty of Pharmacy, University of Valencia, and were kept in a clean room with a temperature of $23 \pm 1^\circ\text{C}$, a relative humidity of 60%, and a light-dark cycle of 12 h of light and 12 h of darkness. Rats were fed a standard laboratory diet obtained from Harlan Laboratories Inc. (Barcelona, Spain) and had *ad libitum* access to water.

The day before drug administration, rats were cannulated in the jugular vein to facilitate blood sample collection and intravenous (i.v.) dose administration, using a procedure reported previously (49). All rats were anesthetized with ketamine (80 mg/kg) and xylazine (10 mg/kg). Animals were subjected to overnight fasting, but water was available *ad libitum*.

(ii) **Drug administration and sampling.** Rats were divided into 9 groups ($n = 6$) depending on the administration routes for NVP and NT (Table 1). A suspension of NVP (8 mg/ml) in 0.5% CMC was used for intraduodenal (i.d.) and oral (p.o.) administrations. A solution of NVP (8 mg/ml) in DMSO-PG (1:4 [vol/vol]) was used for intravenous (i.v.) administration. NT was administered as a solution (5 mg/ml) in water.

To administer the drugs intraduodenally, a cannula was inserted in the intestine the day before the administration, immediately after cannulation of the jugular vein. Oral dosing was performed by gastric gavage, and intravenous administration was performed through the jugular cannula. In the case of rats receiving both drugs, NT was administered first for groups 2, 4, and 7, whereas NVP was administered first for group 5. The two administrations were separated by a 15-min interval.

Serial blood samples were collected at different times, depending on the routes of administration of the two drugs. Sampling was performed through the jugular cannula, with previously heparinized syringes. The sample volume was 0.2 ml; after each blood collection, the same volume of heparinized serum (20 IU/ml) maintained at 37°C was perfused. Blood was collected in Eppendorf tubes, which were then centrifuged at $1,500 \times g$ for 5 min, and the supernatant plasma was used for the analytical determination of drug levels.

***In vitro* studies.** (i) **Inhibition of NVP metabolism by NT in rat hepatic microsomes.** The disappearance of NVP in the absence and presence of NT was studied in microsomes prepared from Wistar rat livers. The livers were homogenized (4°C) in 50 mM sodium-potassium phosphate buffer (pH 7.4) containing 1.15% (wt/vol) KCl, 2 mM EDTA, and 0.25 M sucrose. The homogenate was centrifuged at $9,000 \times g$ for 10 min, and the supernatant fraction was further centrifuged at $150,000 \times g$ for 1 h. The microsomal pellet was resuspended in 0.1 M potassium phosphate

buffer (pH 7.4). Microsomal protein concentrations were determined by the method of Lowry et al. (50).

The components added to test tubes in the inhibition studies were hepatic microsomes (equivalent to 0.1 mg of protein); 10 μ l of a solution of NVP in a mixture of water, methanol, and DMSO (final concentrations of 5 μ g/ml NVP, 0.12% methanol, and 0.08% DMSO); 10 μ l of NT solutions in water (final NT concentrations of 100, 200, 300, 500, 1,000, 2,000, 3,000, and 10,000 ng/ml); 5 μ l of a 20-mg/ml glucose-6-phosphate solution; 5 μ l of a 20-mg/ml β -NADP solution; 10 μ l of a glucose-6-phosphate dehydrogenase solution (10 IU/ml); and 5 μ l of a $MgCl_2$ solution (13.4 mg/ml). The volume was adjusted to 0.1 ml with the addition of 0.1 M phosphate buffer (pH 7.4), and the components were incubated for 30 min at 37°C. At the end of the incubation time, the reaction was terminated by the addition of 0.1 ml of acetonitrile, and the amount of NVP remaining was determined. Controls with no inhibitor were prepared by replacing NT solution with the same volume of water.

(ii) Inhibition of NVP metabolite formation by NT in human hepatic microsomes. The inhibition of 2-, 3- and 12-OH-NVP formation by 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, with the following changes to increase the concentrations of metabolites in the final solution to be used for the analysis: 0.2 mg of microsomal protein were used in each assay, and the amounts of all other solutions were doubled (total assay volume of 0.2 ml and final NVP concentration of 5 μ g/ml). After incubation at 37°C for 30 min, 25 μ l of a 2 N sodium hydroxide solution and 5 ml of ethyl acetate were added to the reaction tube to extract NVP and its metabolites. The extraction was repeated with 3 ml of ethyl acetate, and the combined extracts were evaporated at 40°C. The dried residue was dissolved into 100 μ l of the mobile phase described for the analytical methods. The assayed NT concentrations in the final incubation medium were 100, 1,000, and 10,000 ng/ml.

Analytical methods. NVP was quantified in rat plasma using a high-performance liquid chromatographic (HPLC) assay with UV detection at 254 nm, as described previously (51). A Waters Nova-Pack C_{18} analytical column (150 by 3.9 mm) was used, and the mobile phase consisted of a mixture of acetonitrile and 50 mM sodium phosphate monobasic buffer (25:75 [vol/vol]). The injection volume was 25 μ l, and the flow rate was 1 ml/min. Plasma samples containing NVP were subjected to protein precipitation with acetonitrile. The analytical method was linear in the range of 0.1 to 50 μ g/ml, the lower limit of quantification (LLOQ) was 0.1 μ g/ml, the accuracy (determined as the ratio of the measured concentration to the spiked concentration) was >90%, and the precision (determined as the percent coefficient of variation) was <15%. Concentrations of NVP and its hydroxylated metabolites in microsomal samples were determined using a slightly modified mobile phase composed of acetonitrile and sodium phosphate monobasic buffer (0.05 M [pH 4.6]) containing 0.1% (vol/vol) triethylamine (12:88 [vol/vol]), and detection was performed at 240 nm.

For the chromatographic analysis of NT in plasma, a previously developed method based on HPLC with fluorescence detection was employed (52). The fluorescence detector was set to excitation and emission wavelengths of 260 nm and 310 nm, respectively. Chromatographic separation was performed on a Waters Spherisorb S5 ODS2 column (4.6 by 250 mm) at room temperature. The mobile phase consisted of a mixture of acetonitrile and water (85:15 [vol/vol]), delivered at a flow rate of 1 ml/min. The assay involved derivatization with Fmoc-Cl. Calibration curves were linear over the concentration range of 10 to 5,000 ng/ml, the LLOQ was 10 ng/ml, the accuracy was >90%, and the precision was <15%.

Pharmacokinetic analysis. WinNonlin (version 5.1; Pharsight Corp., Mountain View, CA) was used to estimate noncompartmental pharmacokinetic parameters. The following parameters were estimated after extravascular administration: maximum plasma concentration (C_{max}), time to C_{max} (T_{max}), half-life ($t_{1/2}$), area under the plasma concentration-time

curve (AUC) from the time of dosing to the last measurable concentration (AUC_{last}), AUC from the time of dosing to infinity (AUC_{inf}), mean residence time (MRT) from the time of dosing to the last measurable concentration (MRT_{last}), and MRT from the time of dosing to infinity (MRT_{inf}). In the case of intravenous administration, the estimated parameters were $t_{1/2}$, AUC_{last} , AUC_{inf} , MRT_{last} , MRT_{inf} , volume of distribution (V), and total plasma clearance (CL).

Statistical analysis. Data are presented as mean $\pm$ standard deviation (SD). Student's t test was used for comparisons of two groups, except when the variances of the compared groups were not homogeneous, in which case the Mann-Whitney U test was employed. One-way analysis of variance (ANOVA) was used for comparisons of more than two groups; when statistically significant differences were found, Tukey's test was employed to determine which groups were statistically different. P values of <0.05 were considered statistically significant. All calculations were performed with IBM SPSS Statistics 19 (SPSS Inc., Chicago, IL). The concentration of NT required for 50% inhibition of NVP metabolism *in vitro* (i.e., 50% inhibitory concentration [IC_{50}]) was determined by nonlinear regression fitting of a standard competitive inhibition model to data obtained in the assay with rat hepatic microsomes (GraphPad Prism, version 4.00; GraphPad Software Inc., San Diego, CA).

RESULTS

In vivo studies. (i) Pharmacokinetics of NVP after p.o. administration (groups 1 and 2). The mean concentration-time profiles for NVP in the absence or presence of NT are shown in Fig. 1, and the values of pharmacokinetic parameters are listed in Table 2. Coadministration of NVP p.o. and NT p.o. resulted in higher plasma concentrations of NVP, with C_{max} and AUC_{last} values being approximately 2 to 3 times greater. The T_{max} and MRT_{last} values for this drug were also higher when it was coadministered with NT. All of these results suggest that a pharmacokinetic interaction, causing important changes in the pharmacokinetic parameters of NVP, takes place when NVP and NT are coadministered orally. Due to the scarcity of data in the terminal phase of the plasma curve for animals in group 2, it was not possible to determine $t_{1/2}$, AUC_{inf} , or MRT_{inf} for this group, and the values of these parameters are reported in Table 2 only for rats in group 1 (dosed with NVP alone).

The plasma concentrations of NT could not be quantified reliably for animals in group 2, since concentrations were, for some animals, very low and near the quantification limit of the analytical method. For this reason, the effects of the pharmacokinetic interaction on the pharmacokinetic parameters of NT after p.o. administration were not evaluated (including an additional group dosed only with NT). In order to confirm the effects of NT on NVP pharmacokinetics and also to evaluate the effects of NVP on NT pharmacokinetics, the subsequent groups of animals were treated intraduodenally instead of orally, since previous studies showed good duodenal absorption for NVP (51) and NT (data not shown).

(ii) Pharmacokinetics of NVP after i.d. administration (groups 3 to 5). NVP plasma concentrations measured after i.d. NVP administration were higher in the presence of NT (groups 4 and 5) than in its absence (group 3). In fact, when the two drugs were coadministered intraduodenally, the increase in the plasma levels of NVP was more pronounced than that observed after p.o. administration, with 5-fold increases in C_{max} and AUC_{last} . In the case of T_{max} and MRT_{last} , the increases were similar to those observed when the two drugs were administered orally (groups 1 and 2). In order to determine that the interaction did not occur at the level of absorption, NVP and NT were administered by different

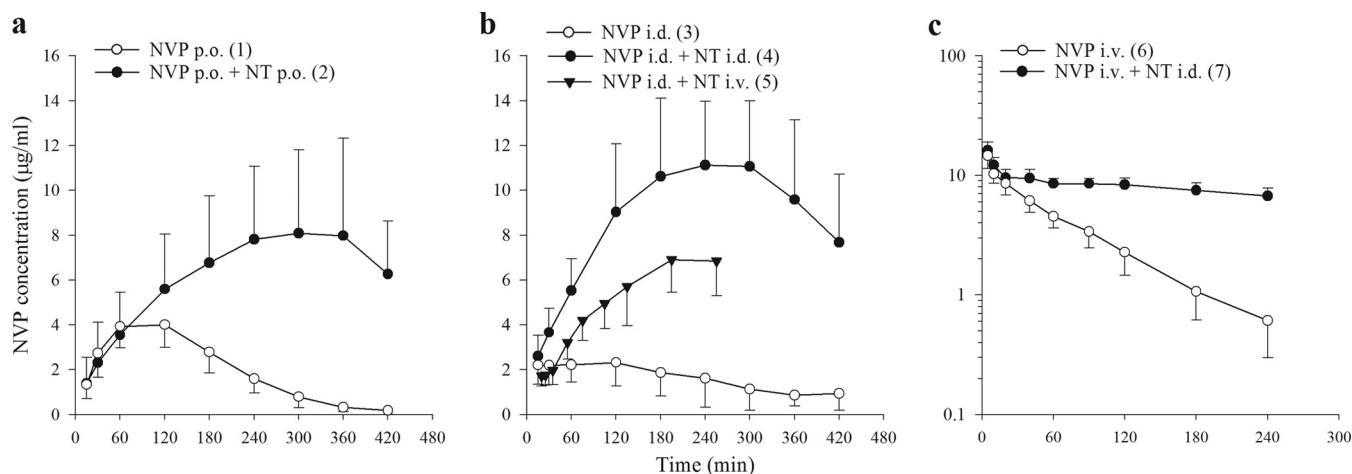


FIG 1 NVP plasma concentrations (mean $\pm$ SD) in rats following p.o. (a), i.d. (b), and i.v. (c) administration, with and without NT coadministration. Numbers in parentheses indicate the animal groups.

routes (NVP i.d. and NT i.v. [group 5] or NVP i.v. and NT i.d. [group 7]). Animals in group 5 (NVP i.d. and NT i.v.) showed higher NVP plasma concentrations than did those in group 3 (only NVP i.d.), although the levels were not as high as those observed for group 4 (NVP i.d. and NT i.d.).

(iii) Pharmacokinetics of NVP after i.v. administration (groups 6 and 7). The mean concentration-time profiles for NVP achieved after i.v. administration to rats in group 6 (only NVP i.v.) and group 7 (NVP i.v. and NT i.d.) showed slower decreases of NVP plasma concentrations during the postdistribution phase (which starts between 20 and 40 min after NVP dosing) in the group with NVP coadministered with NT. The NVP pharmacokinetic parameters summarized in Table 2 show a 7-fold decrease in clearance, a 7-fold increase in $t_{1/2}$, and an 8-fold increase in the MRT_{inf} of NVP as a consequence of the i.d. administration of NT. These results confirm that the coadministration of NT gives rise to slower elimination of NVP regardless of the administration routes used for the two drugs. The decreased NVP elimination rate could be attributed to inhibition of NVP hepatic metabolism caused by

NT. In order to check this, additional studies were carried out *in vitro* with rat hepatic microsomes (shown below).

(iv) Pharmacokinetics of NT after i.d. and i.v. administration (groups 4, 5, 7, 8, and 9). The NT plasma concentration-time profiles obtained in the absence and presence of NVP are shown in Fig. 2. NT concentrations obtained after i.d. administration (groups 4, 7, and 8) were higher when NT was coadministered with NVP. However, statistically significant differences between the pharmacokinetic parameters of NT obtained in the absence and presence of NVP (groups 4 and 8) were not found, due to the wide interindividual variability of the parameters (Table 3). Except for $t_{1/2}$, AUC_{inf} , and MRT_{inf} , the pharmacokinetic parameters for NT obtained with rats treated with NT coadministered with NVP i.v. (group 7) were not statistically different from the corresponding values obtained in the other two groups, because of the different sampling times. NT plasma concentrations obtained after i.v. administration (groups 5 and 9) were similar in the absence and presence of NVP, and no significant differences in pharmacokinetic parameters were detected (Fig. 2b and Table 3).

TABLE 2 Pharmacokinetic parameters for NVP (mean $\pm$ SD) in rats after p.o., i.d., and i.v. administration with and without NT

Parameter	NVP p.o.		NVP i.d.			NVP i.v.	
	Without NT (group 1)	With NT p.o. (group 2)	Without NT (group 3)	With NT i.d. (group 4)	With NT i.v. (group 5)	Without NT (group 6)	With NT i.d. (group 7)
T_{max} (min)	110 $\pm$ 24	300 $\pm$ 75 ^a	95.0 $\pm$ 85.2 ^b	260 $\pm$ 90 ^c	205 $\pm$ 45 ^{b,c}		
V (ml)						492 $\pm$ 140	454 $\pm$ 134
C_{max} (μ g/ml)	4.10 $\pm$ 1.06	8.92 $\pm$ 4.26 ^c	2.64 $\pm$ 1.13 ^b	11.9 $\pm$ 3.4 ^c	7.24 $\pm$ 1.46 ^d		
CL (ml/min)						6.40 $\pm$ 2.18	0.955 $\pm$ 0.314 ^f
$t_{1/2}$ (min)	63.2 $\pm$ 22.9		164 $\pm$ 63			54.4 $\pm$ 7.7	370 $\pm$ 189 ^f
AUC_{last} (μ g $\cdot$ min/ml)	819 $\pm$ 224	2,470 $\pm$ 1,000 ^f	650 $\pm$ 333 ^b	3,360 $\pm$ 530 ^c	1,260 $\pm$ 231 ^g	643 $\pm$ 223	1,770 $\pm$ 403 ^a
AUC_{inf} (μ g $\cdot$ min/ml)	873 $\pm$ 291		877 $\pm$ 462			683 $\pm$ 212	4,840 $\pm$ 2,530 ^f
MRT_{last} (min)	137 $\pm$ 12	242 $\pm$ 14 ^a	162 $\pm$ 24 ^b	222 $\pm$ 25 ^c	156 $\pm$ 5 ^g	51.5 $\pm$ 11.6	105 $\pm$ 5 ^a
MRT_{inf} (min)	152 $\pm$ 24		285 $\pm$ 77			65.2 $\pm$ 8.2	528 $\pm$ 272 ^f

^a Significantly different from without NT, $P < 0.001$.

^{b,c,d} Parameter values with different superscript letters are statistically different.

^e Significantly different from without NT, $P < 0.05$.

^f Significantly different from without NT, $P < 0.01$.

^g AUC_{last} and MRT_{last} values obtained after i.v. NT administration (group 5) were not statistically compared with the other two groups (see the text).

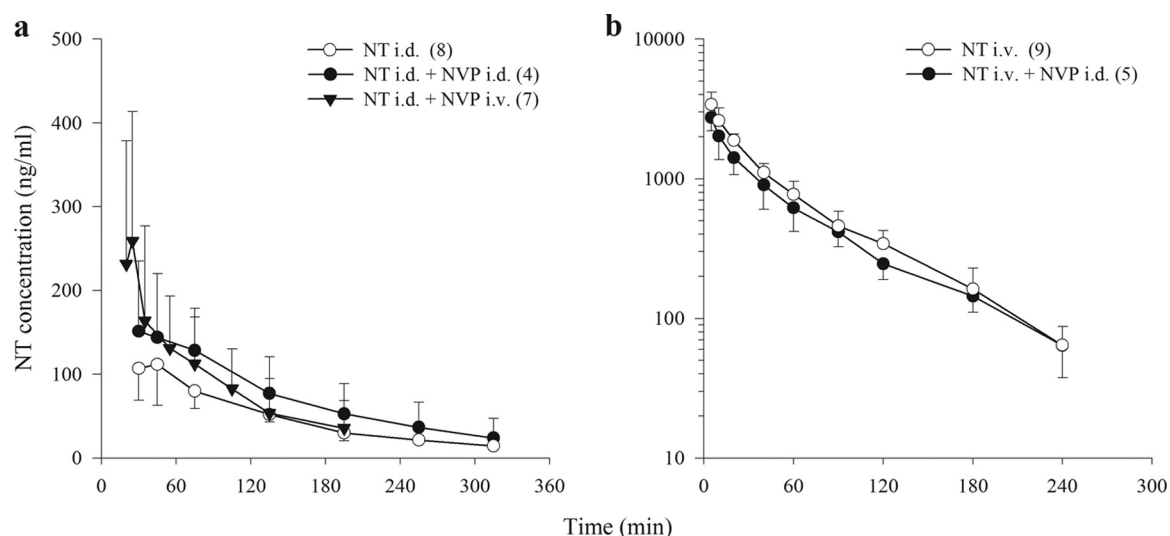


FIG 2 NT plasma concentrations (mean $\pm$ SD) in rats after i.d. (a) and i.v. (b) administration in the presence and absence of NVP. Numbers in parentheses indicate the animal groups.

In vitro studies. To investigate the inhibitory effects of NT on the metabolism of NVP, different concentrations of NT were tested *in vitro* using rat hepatic microsomes. As can be seen in Fig. 3a, NT provoked concentration-dependent decreases of NVP metabolism in the range of 200 to 1,200 ng/ml NT, although complete inhibition of NVP metabolism could not be reached. An IC_{50} value of 864 ng/ml (2.88 μ M) was obtained for NT after fitting the equation corresponding to a competitive inhibition model to the experimental data.

The inhibitory effects of NT on the metabolism of NVP were also observed in human hepatic microsomes (Fig. 3b). NT decreased the rate of formation of the three assayed metabolites in a concentration-dependent way, with the metabolite 3-OH-NVP being the most inhibited at all of the assayed NT concentrations. Although the measured parameters were different in the experiments with rat hepatic microsomes (rate of NVP disappearance) and human hepatic microsomes (rate of metabolite formation), it seems that the inhibitory effects of NT on NVP metabolism were more intense in rat microsomes than in human microsomes. In fact, with a concentration of 1,000 ng/ml NT, the NVP metabolism in rat microsomes was about 50% of the control level,

whereas the rate of formation of metabolites was inhibited by less than 50% in human microsomes.

DISCUSSION

The doses of NVP and NT used in this study were selected with the aim of obtaining plasma concentrations in rats similar to those observed in humans when therapeutic doses of these drugs are used. Thus, the mean NVP C_{max} value obtained in rats dosed orally with 8 mg (4.10 μ g/ml) was close to the steady-state C_{max} value reported for humans receiving 200 mg twice per day (5.74 μ g/ml) (8). The i.v. dose of NVP was reduced to one-half of that used extravascularly (4 mg per rat instead of 8 mg per rat) due to the limited solubility of NVP in the vehicle. Furthermore, this reduced dose avoided high plasma concentrations. In fact, NVP plasma concentrations achieved in the postdistribution phase (which starts between 20 and 40 min after i.v. NVP dosing) were lower than 10 μ g/ml (Fig. 1c). In the case of i.d. administration of NT, the mean C_{max} value obtained with a dose of 5 mg (122 ng/ml) was within the optimal range of 50 to 150 ng/ml described for humans (54). In order to maintain the same relationship between

TABLE 3 Pharmacokinetic parameters for NT (mean $\pm$ SD) in rats after i.d. and i.v. administration with and without NVP

Parameter	NT i.d. ^a			NT i.v. ^a	
	Without NVP (group 8)	With NVP i.d. (group 4)	With NVP i.v. (group 7) ^b	Without NVP (group 9)	With NVP i.d. (group 5)
T_{max} (min)	35.0 $\pm$ 7.7	37.5 $\pm$ 8.2	23.3 $\pm$ 2.6		
V (ml)				1,080 $\pm$ 162	1,660 $\pm$ 595
C_{max} (ng/ml)	122 $\pm$ 48	163 $\pm$ 80	273 $\pm$ 148		
CL (ml/min)				15.8 $\pm$ 3.5	19.2 $\pm$ 3.1
$t_{1/2}$ (min)	86.0 $\pm$ 14.4	70.3 $\pm$ 36.6	76.4 $\pm$ 42.2	48.5 $\pm$ 8.3	58.8 $\pm$ 16.5
AUC_{last} (μ g $\cdot$ min/ml)	15.0 $\pm$ 2.9	22.9 $\pm$ 8.7	17.9 $\pm$ 8.8	159 $\pm$ 27	127 $\pm$ 24
AUC_{inf} (μ g $\cdot$ min/ml)	17.0 $\pm$ 3.4	26.7 $\pm$ 10.9	23.9 $\pm$ 13.7	164 $\pm$ 29	133 $\pm$ 22
MRT_{last} (min)	110 $\pm$ 15.0	113 $\pm$ 38	68.5 $\pm$ 16.1	51.3 $\pm$ 6.7	53.5 $\pm$ 4.1
MRT_{inf} (min)	149 $\pm$ 19	135 $\pm$ 50	122 $\pm$ 54	58.5 $\pm$ 10.1	66.8 $\pm$ 15.0

^a No statistically significant differences in the values of the parameters were found.

^b In the case of group 7, only $t_{1/2}$, AUC_{inf} , and MRT_{inf} were compared with the corresponding values obtained for the other two groups (see the text).

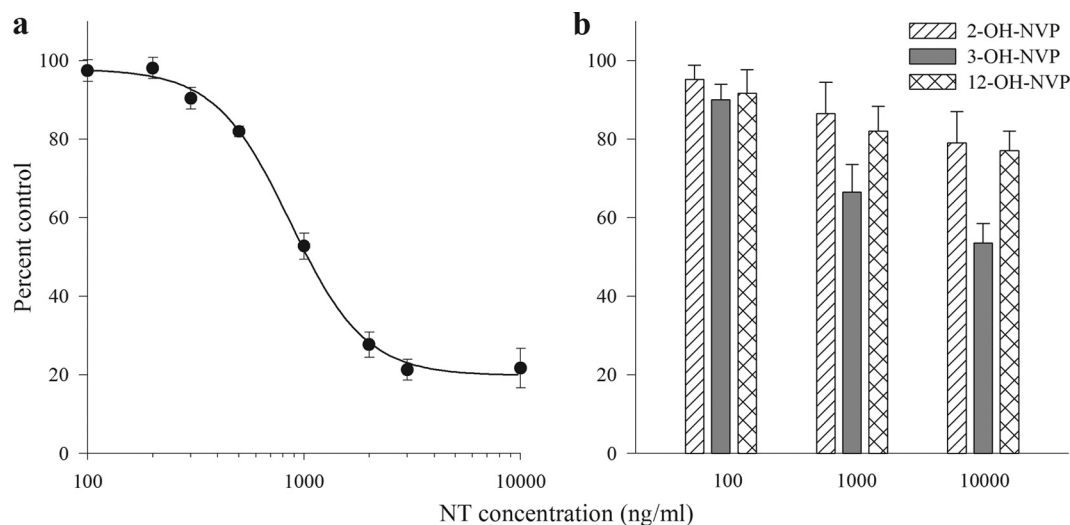


FIG 3 Concentration-dependent inhibition of NVP (5 µg/ml) metabolism by NT (mean $\pm$ SD, $n = 4$) in rat (a) and human (b) liver microsomes. See the text for details.

i.v. and i.d. doses as in the case of NVP administrations, one-half of the i.d. dose was administered intravenously (2.5 mg).

The absorption of NVP was faster after oral administration than after i.d. administration. This finding can be attributed to the favorable solubility of the drug in the acidic medium of the stomach, since NVP is a weak base and its solubility is strongly pH dependent, with increased solubility at acidic pH. Although NVP is better absorbed from the duodenum than from the stomach (51), p.o. administration gives rise to a greater dissolved fraction when the drug reaches the duodenum after passing through the stomach than when it is injected directly into the duodenum. As a consequence of this greater dissolved fraction of drug, faster absorption is obtained after p.o. administration than after i.d. administration. However, the absorbed amounts are similar, since the AUC_{inf} values are practically the same for the two administration routes (873 µg · min/ml and 877 µg · min/ml) (Table 2).

In all groups of animals treated with NVP and NT, NVP plasma concentrations were increased significantly, compared to the plasma concentrations obtained with NVP administered alone. The effect of NT on NVP plasma levels was a consequence of slower elimination of NVP. In fact, the CL of NVP was reduced 7-fold when NVP was coadministered with NT. The slower elimination of NVP in the presence of NT gave rise to increased C_{max} and T_{max} values for NVP administered orally or intraduodenally. The decreased elimination of NVP in the presence of NT was attributed to inhibition of NVP metabolism. In fact, *in vitro* studies with rat liver microsomes confirmed such a pharmacokinetic interaction.

The *in vitro* results with rat hepatic microsomes showed that NT did not cause complete inhibition of NVP metabolism (20% of metabolism was not inhibited), which suggests that some metabolic routes for NVP remain unaffected by NT. The effect of NT on the metabolism of NVP was studied in rat microsomes by determining the changes in the rate of NVP disappearance. This could not be done in the case of human microsomes, however, as the rate of NVP metabolism was very slow and very small changes in the concentration of NVP were detected at the end of the incubation period. Therefore, it was decided to study the rate of for-

mation of NVP metabolites, which is a more sensitive assay for detecting changes in metabolism than measuring the rate of NVP disappearance (changes in the parent drug concentration).

In the case of human hepatic microsomes, inhibition was detected for the three assayed metabolites but the inhibition was less intense than that observed in rat microsomes. NVP is a substrate of CYP3A4 (yielding 2- and 12-OH-NVP metabolites), and it has been shown that this isoenzyme is also involved in NT metabolism. On the other hand, the metabolism of NT is mediated mainly by CYP2D6, and the implication of this isoenzyme in NVP metabolism (yielding 12-OH-NVP) has also been described (10). Therefore, these two isoenzymes are likely to be involved in the inhibition of NVP metabolism by NT, probably through a competitive mechanism. However, an unexpected result obtained in the human microsome assays was the inhibition of 3-OH-NVP formation, which is mediated exclusively by CYP2B6, an isoenzyme not related to the metabolism of NT. In fact, this was the most inhibited of the three assayed metabolites.

In contrast to the marked effects of NT on NVP pharmacokinetics, significant differences in NT parameters were not observed when NT was coadministered with NVP. Although the mean plasma concentrations in rats for NT administered intraduodenally were higher when the two drugs were coadministered, statistically significant differences were not found because of the large interindividual variability of NT pharmacokinetic parameters.

The results obtained in the present study show marked inhibition of NVP metabolism by NT. To date, however, it is not possible to extrapolate these results directly to humans receiving the two drugs concomitantly, and pharmacokinetic studies conducted in humans are required to assess the clinical relevance of this interaction.

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