Pharmacokinetic interaction between nevirapine and nortriptyline in rats: inhibition of nevirapine metabolism by nortriptyline

Running title: Nevirapine metabolism inhibition by nortriptyline

Authors: Iris Usach, Virginia Melis, Patricia Gandía, José-Esteban Peris*

Department of Pharmacy and Pharmaceutical Technology
Faculty of Pharmacy, University of Valencia.
Avda. V. Andrés Estellés, s/n
46100-Burjassot, Valencia, Spain.

* Department of Pharmacy and Pharmaceutical Technology
Faculty of Pharmacy, University of Valencia.
Avda. V. Andrés Estellés, s/n
46100-Burjassot, Valencia, Spain.
Tel: +34 963543353
Fax: +34 963544911
e-mail: jose.e.peris@uv.es
ABSTRACT

One of the most frequent comorbidities of HIV infection is depression, with a lifetime prevalence of 22-45%. It was, therefore, decided to study a potential pharmacokinetic interaction between the non-nucleoside 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 the plasma levels and pharmacokinetic parameters were analysed. Experiments with rat and human hepatic microsomes were carried out to evaluate the inhibitory effect of NT on NVP metabolism. NVP plasma concentrations were significantly higher when this drug was coadministered with NT. The maximum plasma concentration of NVP increased 2-5 times and the total plasma clearance decreased 7-fold in the presence of NT. However, statistically significant differences were not found between the pharmacokinetic parameters of NT obtained in absence and presence of NVP. The in vitro studies with rat and human hepatic microsomes confirmed the inhibition of NVP hepatic metabolism by NT in a concentration-dependent way, the inhibition being more intense in the case of rat microsomes. In conclusion, a pharmacokinetic interaction between NVP and NT has been 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 effect of this interaction on the pharmacokinetics of NVP before it can be taken into account in patients receiving NVP.
ABBREVIATIONS

AUC<sub>inf</sub>: area under the concentration-time curve from the time of dosing to infinity

AUC<sub>last</sub>: area under the plasma concentration–time curve from the time of dosing to the last measurable concentration

Cl: total plasma clearance

C<sub>max</sub>: maximum plasma concentration

IC<sub>50</sub>: concentration of inhibitor required to achieve half maximal inhibition of substrate metabolism

i.d.: intraduodenal

i.v.: intravenous

MRT<sub>inf</sub>: mean residence time estimated using areas extrapolated to infinity

MRT<sub>last</sub>: mean residence time estimated using areas from the time of dosing to the last measurable concentration

NT: nortriptyline

NVP: nevirapine

2-OH-NVP: 2-hydroxynevirapine

3-OH-NVP: 3-hydroxynevirapine

8-OH-NVP: 8-hydroxynevirapine

12-OH-NVP: 12-hydroxynevirapine

p.o.: oral

t<sub>1/2</sub>: half-life

t<sub>max</sub>: time to C<sub>max</sub>

V<sub>d</sub>: volume of distribution
INTRODUCTION

Human immunodeficiency virus infection / acquired immunodeficiency syndrome (HIV/AIDS) is, at present, an incurable disease. However, the use of an adequate antiretroviral therapy (ART) has resulted in a dramatic reduction of AIDS-related morbidity and mortality. 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 (1).

In general, the initial treatment of HIV infected individuals involves drug combinations consisting of at least three antiretroviral drugs of multiple classes, which is known as highly active antiretroviral therapy (HAART). Currently, preferred regimens of HAART use combinations of two nucleos(t)ide reverse transcriptase inhibitors (NRTIs) with a protease inhibitor (PI) (preferably boosted with ritonavir) or with a non-nucleoside reverse transcriptase inhibitor (NNRTI), although other combinations are possible (2).

Nevirapine (NVP) is an NNRTI of human immunodeficiency virus type-1 (HIV-1) widely used as a component of HAART since it has demonstrated a potent and sustained activity in HIV-infected patients because it induces rapid suppression of the HIV-1 viral load and rises in the CD4 cell count (3-6). NVP efficacy is comparable to efavirenz (another first-generation NNRTI) and ritonavir-boosted PIs, the other antiretroviral drugs currently used in the initial HAART in addition to the two NRTIs (7). Moreover, NVP-based regimens are commonly prescribed to HIV infected pregnant women as one of its most relevant benefits is its efficacy in the prevention of mother-to-child transmission of the HIV-1 infection (8).
The effective dosing regimen of 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 show steady-state $C_{\text{max}}$ and $C_{\text{min}}$ of 5.74 µg/ml and 3.73 µg/ml, respectively (9). In humans as well as in mice, rats, rabbits, dogs, monkeys and chimpanzees, NVP undergoes a significant hepatic metabolism mainly by 2-, 3-, 8-, and 12-hydroxylation (2-OH-NVP, 3-OH-NVP, 8-OH-NVP, 12-OH-NVP) followed by glucuronidation of these hydroxyl metabolites (10, 11). In humans, cytochrome P450 3A4 (CYP3A4) was identified as the major enzyme involved in formation of 2-OH-NVP and 12-OH-NVP, whereas cytochrome P450 2B6 (CYP2B6) was the predominant enzyme forming 3-OH-NVP and 8-OH-NVP. NVP has been shown to be a substrate and inducer of these 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 (11). For example, several published drug interaction studies have shown that NVP reduced the steady-state ketoconazole AUC and $C_{\text{max}}$ by 63 % and 40 %, respectively (12). Similarly, methadone and oral contraceptive steroids such as ethinylestradiol/norethindrone suffer a reduction of their plasmatic levels when they are administered with NVP (13), (14). Nevertheless, drugs and substances that induce these isoenzymes may reduce NVP plasma concentrations. For example, rifampicin and rifabutin, two potent CYP3A inducers, cause significant reductions of NVP plasma concentrations (11). The herbal remedy St. John's Wort is another CYP3A4 inducer that decreases NVP levels in blood when they are administered concomitantly (15). By contrast, there are some inhibitors of cytochrome P450 (CYP450) isoenzymes, such as fluconazole, that increase NVP plasma concentrations (16).
Variations in NVP plasma levels could be associated with a loss of efficacy and viral resistance, or drug toxicity (17-20).

The two most prevalent and interfering psychosocial comorbidities of HIV infection are clinical depression and substance use (21-23). These problems, besides causing distress and functional impairment, can interfere with the antiretroviral therapy adherence (24-29). In fact, a meta-analysis carried out by Gonzalez et al. (30) 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, based on their more favorable side-effect profiles than tricyclic antidepressants (TCAs). However, there is no clinically significant difference in the effectiveness between SSRIs and TCAs in the treatment of depressive disorders (31-33) and both groups of drugs appear to be effective in treating depressive symptoms in patients with HIV infection without affecting their immune status (34).

Nortriptyline (NT) is a TCA that shows superior pharmacological properties to all other TCAs as a psychotropic, with a lower toxicity (35). 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 (36). The main routes of NT metabolism are E-10-hydroxylation (the major) and N-demethylation (the minor) (37, 38). E-10-hydroxylation is mediated mainly by cytochrome P450 2D6 (CYP2D6), although in vitro studies have shown that CYP3A4 is involved too (39). 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. Concomitant therapy with drugs that inhibit CYP2D6, such as terbinafine, fluoxetine, norfluoxetine, sertraline, and paroxetine, result in a major increase in the plasma NT concentrations caused by a decrease in NT clearance,
whereas the volume of distribution remains unchanged (40-42). On the other hand, the plasma concentration 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 (43).

The involvement of CYP3A4 in the NT metabolism seems likely, when considering either case reports of increased NT metabolism by concomitant use of CYP3A4 inducers, such as pentobarbital, carbamazepine and rifampicin (44-47), or reported cases about the inhibition of NT metabolism by CYP3A4 inhibitors, such as fluconazole and cimetidine (48, 49).

Considering the lifetime prevalence of depression in patients with HIV (22-45 %) (50), a study of a possible pharmacokinetic interaction 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 (10).
MATERIALS AND METHODS

Chemicals

NVP (Viramune®) was obtained from Boehringer Ingelheim (Barcelona, Spain). 2-, 3- and 12-OH-NVP were from Toronto Research Chemicals (North York, Canada). NT (hydrochloride salt), carboxymethylcellulose (CMC), dimethylsulfoxide (DMSO), β-nicotinamide adenine dinucleotide phosphate (β-NADP+), glucose-6-phosphate, glucose-6-phosphate dehydrogenase and magnesium chloride (MgCl2) were purchased from Sigma-Aldrich Corporation (Madrid, Spain). Propylene glycol (PG) and 9H-fluoren-9-ylmethyl chloroformate (Fmoc-Cl) were supplied by Fluka Biochemika (Buchs, SG, Switzerland). All the other reagents and solvents used in the study were of HPLC or analytical grade.

In vivo studies

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, 2-3 months old and weighing 280–310 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 ± 1 °C, a relative humidity of 60 % and a light-dark cycle of 12 hours light:12 hours 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 dose administration using the procedure reported previously (51). All rats were anaesthetized with ketamine (80 mg/kg) and xylazine (10 mg/kg). Moreover, animals were submitted to overnight fasting, but water was supplied *ad libitum*.

*Drug administration and sampling*

Rats were divided into 9 groups (n = 6) depending on the administration route of 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, v/v) 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 a gastric gavage, and intravenous administrations were performed through the jugular cannula.

In the case of rats receiving both drugs, NT was administered first in groups 2, 4 and 7, whereas NVP was the first administered drug in group 5. Both administrations were separated by a 15-min time period.

Serial blood samples were collected at different times depending on the route of administration of both drugs. Sampling was performed through the jugular cannula with previously heparinized syringes. Sample volume was 0.2 ml and after each blood
collection, the same volume of heparinized serum (20 UI/ml) kept at 37 °C was perfused. Blood was collected in Eppendorff tubes, which were then centrifuged at 1500 x g for 5 min, and the supernatant plasma was used for the analytical determination of the drugs.

**In vitro studies**

**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 a 50 mM sodium-potassium phosphate buffer (pH 7.4) solution containing 1.15 % (w/v) of potassium chloride (KCl), 2 mM ethylenediaminetetraacetic acid (EDTA) and 0.25 M sucrose. The homogenate was centrifuged at 9000 x g for 10 min and the supernatant fraction was further centrifuged at 150000 x g for 1 h. The microsomal pellet was resuspended in 0.1 M potassium phosphate buffer (pH 7.4). Microsomal protein concentration was determined by the method of Lowry et al. (52).

The components added to test tubes in the inhibition studies were: hepatic microsomes (equivalent to 0.1 mg of protein); 10 µl of a solution of NVP in a mixture of water, methanol and DMSO (final concentrations: 5 µg/ml of NVP, 0.12 % of methanol and 0.08 % of DMSO); 10 µl of NT solutions in water (final NT concentrations: 100, 200, 300, 500, 1000, 2000, 3000 and 10000 ng/ml); 5 µl of a 20 mg/ml glucose-6-phosphate solution; 5 µl of a 20 mg/ml β-NADP⁺ solution; 10 µl of a glucose-6-phosphate dehydrogenase solution (10 UI/ml) and 5 µl of MgCl₂ solution (13.4 mg/ml). The volume was adjusted to 0.1 ml by adding 0.1 M phosphate buffer (pH 7.4) and the components were incubated at 37 °C for 30 minutes. At the end of the incubation time,
the reaction was terminated by addition of 0.1 ml of acetonitrile and the remaining amount of NVP was determined. Controls with no inhibitor were performed by replacing NT solution by the same volume of water.

Inhibition of NVP metabolites 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 in order to increase the concentration 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 (0.2 ml total assay volume, 5 µg/ml NVP final concentration). After incubation at 37 °C for 30 min, 25 µl 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 µl of the mobile phase described in the analytical method. The assayed NT concentrations in the final incubation medium were 100, 1000 and 10000 ng/ml.

Analytical method

NVP was quantified in rat plasma using a high performance liquid chromatographic assay (HPLC) with ultraviolet (UV) detection at 254 nm as previously described (53). A Waters Nova-Pack C18 (150×3.9 mm) analytical column was used and the mobile phase consisted of a mixture of acetonitrile and 50 mM sodium phosphate monobasic buffer (25:75, v/v). The injection volume was 25 µl 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 µg/ml, the lower limit of quantification (LLOQ) was 0.1 µg/ml, the accuracy (as ratio of found concentration to spiked concentration) was higher than 90 % and the precision (as percent coefficient of variation) was lower than 15 %. Concentrations of NVP and its hydroxylated metabolites in microsome samples were determined using a slightly modified mobile phase, which was comprised of acetonitrile and sodium phosphate monobasic buffer (0.05 M, pH 4.6) containing 0.1 % (v/v) triethylamine (12:88, v/v), and the detection was performed at 240 nm.

For the chromatographic analysis of NT in plasma, a previously developed method based on HPLC and fluorescence detection was employed (54). The fluorescence detector was set at excitation and emission wavelengths of 260 nm and 310 nm, respectively. Chromatographic separation was performed on a Waters Spherisorb S5 ODS2 (4.6×250 mm) column at room temperature. The mobile phase consisted of a mixture of acetonitrile/water (85:15, v/v), 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 10-5000 ng/ml, the LLOQ was 10 ng/ml, the accuracy was higher than 90 % and the precision was lower than 15 %.

Pharmacokinetic analysis

WinNonlin (version 5.1, Pharsight Corp., Mountain View, CA) was used to estimate non-compartmental pharmacokinetic parameters. The following parameters were estimated after extravascular administrations: maximum plasma concentration (C_max) and time to C_max (t_max), half-life (t½), area under the plasma concentration–time curve from the time of dosing to the last measurable concentration (AUC_{last}) and to infinity.
(AUC_{inf}), and mean residence time estimated using areas from the time of dosing to the last measurable concentration (MRT_{last}) and to infinity (MRT_{inf}).

In the case of intravenous administrations, the estimated parameters were: t_{1/2}, AUC_{last}, AUC_{inf}, MRT_{last}, MRT_{inf}, volume of distribution (Vd) and total plasma clearance (Cl).

**Statistical analysis**

Data are presented as mean ± SD. Student’s t-test was used for comparing differences between two groups, except when variances of compared groups were not homogeneous, in which case the Mann-Whitney U test was employed. A one-way analysis of variance (ANOVA) test was used for comparison of more than two groups, and when statistically significant differences were found, Tukey’s test was employed to determine which groups were statistically different. A P value of < 0.05 was considered statistically significant. All calculations were performed with IBM SPSS Statistics 19 (SPSS Inc., Chicago, IL, USA).

The concentration of NT which is required for 50 % inhibition of NVP metabolism *in vitro* (IC50) was determined by non-linear 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

Pharmacokinetics of NVP after p.o. administration (groups 1 and 2)

The mean concentration-time profiles of 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 \( C_{\text{max}} \) and \( AUC_{\text{last}} \) values being approximately about 2-3 times greater. The values of \( t_{\text{max}} \) and \( \text{MRT}_{\text{last}} \) of 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 both drugs, NVP and NT, are coadministered orally. Due to the scarce number of data in the terminal phase of the plasma curve corresponding to animals of group 2, it was not possible to determine \( t_{1/2} \), \( AUC_{\text{inf}} \) or \( \text{MRT}_{\text{inf}} \) for this group, and the values of these parameters are reported in Table 2 for rats of group 1 only (solely dosed with NVP).

The plasma concentrations of NT could not be quantified reliably in animals of group 2, since they were, for some animals, very low and near the quantification limit of the analytical method. For this reason, the effect of the pharmacokinetic interaction on the pharmacokinetic parameters of NT after p.o. administration was not evaluated (including an additional group only dosed with NT). In order to confirm the effect of NT on NVP pharmacokinetics, and also to evaluate the effect of NVP on NT pharmacokinetics, the following groups of animals were administered intraduodenally instead of orally, since previous studies had shown good duodenal absorption for NVP (53) and NT (data not shown).
Pharmacokinetics of NVP after i.d. administration (groups 3-5)

NVP plasma concentrations obtained 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 both drugs were coadministered intraduodenally, the increase in the plasma levels of NVP was more pronounced than that obtained after p.o. administration, with 5-fold increments in $C_{\text{max}}$ and AUC$_{\text{last}}$. In the case of $t_{\text{max}}$ and MRT$_{\text{last}}$, the increases were similar to those observed when both drugs were administered orally (groups 1 and 2). In order to check that the interaction did not occur at the level of absorption, NVP and NT were administered by different routes (NVP i.d. and NT i.v. (group 5), NVP i.v. and NT i.d. (group 7)). Animals of group 5 (NVP i.d. and NT i.v.) showed higher NVP plasma concentrations than those obtained in group 3 (only NVP i.d.), although not as high as those obtained in group 4 (NVP i.d. and NT i.d.).

Pharmacokinetics of NVP after i.v. administration (groups 6 and 7)

The mean concentration-time profiles of NVP achieved after its i.v. administration to rats of groups 6 (only NVP i.v.) and 7 (NVP i.v. and NT i.d.) showed a slower decline of NVP plasma concentrations during the post-distribution phase (which starts between 20 and 40 min after NVP dosing) in the group 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 MRT$_{\text{inf}}$ of NVP as a consequence of the i.d. administration of NT. These results confirm that the coadministration of NT gives rise to a slower elimination rate of NVP, regardless of the administration routes used for both drugs. The decreased elimination rate of NVP could be attributed to an inhibition of its hepatic metabolism caused by NT. In order to check
this, additional studies were carried out in vitro with rat hepatic microsomes (shown below).

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 or presence of NVP are shown in Fig. 2. NT concentrations obtained after its i.d. administration (groups 4, 7 and 8) were higher when it was coadministered with NVP. However, statistically significant differences were not found between the pharmacokinetic parameters of NT obtained in absence and presence of NVP, due to the wide interindividual variability of the parameters (groups 4 and 8, Table 3). Except t_{1/2}, AUC_{inf} and MRT_{inf}, the other pharmacokinetics parameters of NT obtained in rats coadministered intravenously with NVP (group 7) were not statistically compared with 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).

In vitro studies

To investigate the inhibitory effect 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 a concentration-dependent decrease of the NVP metabolism in the range of 200 to 1200 ng/ml of NT, although a complete inhibition of the NVP metabolism could not be reached. An IC50 value of 864 ng/ml (2.88 μM) of NT was
obtained after fitting the equation corresponding to a competitive inhibition model to
the experimental data.

The inhibitory effect of NT on the metabolism of NVP was also observed in human
hepatic microsomes (Fig. 3b). NT decreased the formation rate of the three assayed
metabolites in a concentration-dependent way, the metabolite 3-OH-NVP being the
most inhibited with all the assayed NT concentrations. Although the measured analytes
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 effect of NT on the NVP metabolism is more intense in rat
microsomes than in human microsomes. In fact, for a concentration of 1000 ng/ml of
NT, the NVP metabolism in rat microsomes was about 50 % of the control, whereas in
human microsomes the formation rate of metabolites was inhibited by less than 50 %.
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 described in humans when therapeutic doses of these drugs are used. Thus, the mean NVP $C_{\text{max}}$ value obtained in rats dosed orally with 8 mg (4.10 µg/ml) was close to the steady-state $C_{\text{max}}$ value reported in humans for 200 mg twice/day (5.74 µg/ml) (9). The i.v. dose of NVP was reduced to 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 the obtention of high plasma concentrations. In fact, NVP plasma concentrations achieved in the post-distribution phase (which starts between 20 and 40 min after i.v. NVP dosing) were lower than 10 µg/ml (Fig. 1c). In the case of the i.d. administration of NT, the mean $C_{\text{max}}$ value obtained with a dose of 5 mg (122 ng/ml) was within the optimum range of 50 to 150 ng/ml described in humans (36). In order to maintain the same relationship between i.v. and i.d. doses as in the case of NVP administrations, half of the i.d. dose was intravenously administered (2.5 mg).

The absorption of NVP was faster when it was administered orally than after its i.d. administration. This fact 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 (53), p.o. administration gives rise to a higher dissolved fraction when it reaches the duodenum after passing through the stomach than when it is directly injected into the duodenum. As a consequence of this higher drug dissolved fraction, a faster absorption is obtained after p.o. administration than after i.d. administration. However, the absorbed amount is similar since the $\text{AUC}_{\text{inf}}$
value is practically the same for both administration routes (873 µg·min/ml and 877 µg·min/ml, Table 2).

In all groups of animals coadministered with NVP and NT, NVP plasma concentrations increased significantly as compared to the plasma concentrations obtained with NVP administered alone. The effect of NT on the NVP plasma levels was a consequence of a slower elimination rate of NVP. In fact, the Cl of NVP was reduced by 7-fold when it was coadministered with NT. The slower elimination of NVP in presence of NT gave rise to increased C\textsubscript{max} and t\textsubscript{max} values of NVP administered orally or intraduodenally. The decreased elimination of NVP in presence of NT was attributed to an inhibition of the NVP metabolism. In fact, \textit{in vitro} studies with rat liver microsomes confirmed such a pharmacokinetic interaction.

The \textit{in vitro} results with rat hepatic microsomes showed that NT did not cause the complete inhibition of NVP (20 % metabolism was not inhibited), which suggests that some metabolic routes of NVP remain unaffected by NT.

The effect of NT on the metabolism of NVP was studied in rat microsomes determining the changes in the rate of NVP disappearance. However, this could not be done in the case of human microsomes 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 formation rate 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 observed in rat microsomes. NVP is a substrate of CYP3A4 (2- and 12-OH-NVP metabolites), and it has been shown that
this isoenzyme is also involved in the NT metabolism. On the other hand, the metabolism of NT is mediated mainly by CYP2D6 and the implication of this isoenzyme in the NVP metabolism (12-OH-NVP metabolite) has also been described (11). Therefore, these two isoenzymes are likely to be involved in the inhibition of the 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 with the metabolism of NT. In fact, this was the most inhibited of the three assayed metabolites.

In contrast to the marked effect of NT on NVP pharmacokinetics, significant differences were not observed in NT parameters when coadministered with NVP. Although the mean plasma concentrations of NT in rats administered intraduodenally were higher when both 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 a marked inhibition of the NVP metabolism by NT. Nevertheless, so far, it is not possible to extrapolate these results directly to humans receiving both drugs concomitantly and pharmacokinetic studies conducted in humans are required to assess the clinical relevance of this interaction.
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REFERENCES


cannulation of the jugular vein on pharmacokinetics of amoxycillin and antipyrine in the rat. Pharm. Res. 9:1587-1591.


Legend to Figures

FIG 1. NVP plasma concentrations (mean ± SD or mean - SD) following p.o. (a), i.d. (b) and i.v. (c) administration in rats with and without NT coadministration. Numbers in brackets indicate the animal group.

FIG 2. NT plasma concentrations (mean ± SD or mean - SD) after i.d. (a) and i.v. (b) administration in rats in the presence or absence of NVP.

FIG 3. Concentration-dependent inhibition of NVP (5 µg/ml) metabolism by NT (mean ± SD, n = 4) in rat (a) and human (b) liver microsomes. See text for details.
TABLE 1. Groups of animals (n = 6).

<table>
<thead>
<tr>
<th>Group</th>
<th>NVP dose and administration route</th>
<th>NT dose and administration route</th>
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<tbody>
<tr>
<td>1</td>
<td>8 mg p.o.</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>8 mg p.o.</td>
<td>5 mg p.o.</td>
</tr>
<tr>
<td>3</td>
<td>8 mg i.d.</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>8 mg i.d.</td>
<td>5 mg i.d.</td>
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<tr>
<td>5</td>
<td>8 mg i.d.</td>
<td>2.5 mg i.v.</td>
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<tr>
<td>6</td>
<td>4 mg i.v.</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>4 mg i.v.</td>
<td>5 mg i.d.</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>5 mg i.d.</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>2.5 mg i.v.</td>
</tr>
</tbody>
</table>

p.o., oral; i.d., intraduodenal; i.v., intravenous
### TABLE 2. Pharmacokinetic parameters of NVP (mean ± SD) after p.o., i.d. and i.v. administration in rats with and without NT. Numbers in brackets indicate the animal group.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NVP p.o.</th>
<th>NVP i.d.</th>
<th>NVP i.v.</th>
<th>Parameter</th>
<th>NVP i.v.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without NT</td>
<td>With NT p.o.</td>
<td>With NT i.d.</td>
<td>With NT l.v.</td>
<td>Without NT</td>
</tr>
<tr>
<td>$t_{\text{max}}$ (min)</td>
<td>110 ± 24</td>
<td>300 ± 75***</td>
<td>95.0 ± 85.2$^{a}$</td>
<td>260 ± 90$^{b}$</td>
<td>205 ± 4$^{a,b}$</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (ng/ml)</td>
<td>4.10 ± 1.06</td>
<td>8.92 ± 4.26*</td>
<td>2.64 ± 1.13$^{a}$</td>
<td>11.9 ± 3.4$^{b}$</td>
<td>7.24 ± 1.46$^{c}$</td>
</tr>
<tr>
<td>$t_{\frac{1}{2}}$ (min)</td>
<td>63.2 ± 22.9</td>
<td>-</td>
<td>164 ± 63</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>AUC_{\text{inf}} (µg·min/ml)</td>
<td>819 ± 224</td>
<td>2470 ± 1000**</td>
<td>650 ± 333$^{a}$</td>
<td>3360 ± 530$^{b}$</td>
<td>1260 ± 231</td>
</tr>
<tr>
<td>AUC_{\text{inf}} (µg·min/ml)</td>
<td>873 ± 291</td>
<td>-</td>
<td>877 ± 462</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MRT_{\text{inf}} (min)</td>
<td>137 ± 12</td>
<td>242 ± 14***</td>
<td>162 ± 24$^{a}$</td>
<td>222 ± 25$^{b}$</td>
<td>156 ± 5</td>
</tr>
<tr>
<td>MRT_{\text{inf}} (min)</td>
<td>152 ± 24</td>
<td>-</td>
<td>285 ± 77</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Significantly different from without NT, *P < 0.05, **P < 0.01, ***P < 0.001.

Parameter values with different superscripts are statistically different. AUC_{\text{inf}} and MRT_{\text{inf}} values obtained after i.v. NT administration (group 5) were not statistically compared with the other two groups (see text).

Significantly different from without NT, **P < 0.01, ***P < 0.001.
TABLE 3. Pharmacokinetic parameters of NT (mean ± SD) after i.d. and i.v. administration in rats with and without NVP.

Numbers in brackets indicate the animal group.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NT i.d.</th>
<th>NT i.v.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without NVP (8)</td>
<td>With NVP i.d. (4)</td>
</tr>
<tr>
<td>( t_{\text{max}} ) (min)</td>
<td>35.0 ± 7.7</td>
<td>37.5 ± 8.2</td>
</tr>
<tr>
<td>( C_{\text{max}} ) (ng/ml)</td>
<td>122 ± 48</td>
<td>163 ± 80</td>
</tr>
<tr>
<td>( t_{\frac{1}{2}} ) (min)</td>
<td>86.0 ± 14.4</td>
<td>70.3 ± 36.6</td>
</tr>
<tr>
<td>( \text{AUC}_{\text{last}} ) (µg·min/ml)</td>
<td>15.0 ± 2.9</td>
<td>22.9 ± 8.7</td>
</tr>
<tr>
<td>( \text{AUC}_{\text{inf}} ) (µg·min/ml)</td>
<td>17.0 ± 3.4</td>
<td>26.7 ± 10.9</td>
</tr>
<tr>
<td>( \text{MRT}_{\text{last}} ) (min)</td>
<td>110 ± 15.0</td>
<td>113 ± 38</td>
</tr>
<tr>
<td>( \text{MRT}_{\text{inf}} ) (min)</td>
<td>149 ± 19</td>
<td>135 ± 50</td>
</tr>
</tbody>
</table>

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

In the case of group 7, only \( t_{\frac{1}{2}}, \text{AUC}_{\text{inf}} \) and \( \text{MRT}_{\text{inf}} \) were compared with the corresponding values obtained in the other two groups (see text).

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