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Bioavailability of nevirapine in rats after oral and subcutaneous administration, *in vivo* absorption from gastrointestinal segments and effect of bile on its absorption from duodenum

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ABSTRACT

Nevirapine is a non-nucleoside reverse transcriptase inhibitor of human immunodeficiency virus type-1. The usual dosing regimen is 200 mg twice/day. Reducing the dosing frequency would significantly improve treatment adherence and quality of life of patients. To study new forms of administration, it is necessary to do pre-clinical studies and know the absorption characteristics of nevirapine in laboratory animals. However, there are no studies about its bioavailability in rats and hardly any about its pharmacokinetic. The objectives of this study were to describe the pharmacokinetics of nevirapine in rats after intravenous, oral and subcutaneous administration, to assess its absorption by different regions of the gastrointestinal tract and to evaluate the effect of bile on its intestinal absorption. Nevirapine was well absorbed after oral and subcutaneous administration and the bioavailability estimated in rats (91% for both administration routes) was practically equal to that reported in humans (91–93%) after oral administration of therapeutic doses. Nevirapine was absorbed from the duodenum, ileum and colon, while absorption from the stomach was very low. The rate of absorption was in the order: duodenum > ileum > colon > stomach. The presence of bile in the duodenum increased the absorption rate of nevirapine.

1. Introduction

Nevirapine (NVP) is a non-nucleoside reverse transcriptase inhibitor (NNRTI) of human immunodeficiency virus type-1 (HIV-1) and it is used in combination with other antiretroviral agents for the treatment of HIV-1 infection. Highly active antiretroviral therapy (HAART) is a combination of at least three active drugs of multiple drug classes, among which the drug nevirapine may be one. In general, initial treatment involves combining two nucleos(t)ides reverse transcriptase inhibitors (NRTIs) with a protease inhibitor (PI) (preferably boosted with ritonavir) or an NNRTI, although other combinations are possible (Bartlett et al., 2006). Combination antiretroviral therapy with NVP is frequently used since it has demonstrated a potent and sustained activity in HIV-infected patients (Cheeseman et al., 1995; Montaner et al., 1998; Raffi et al., 2000; Skowron et al., 2004).

NVP is readily absorbed in humans (>90%) after oral administration (Lamson et al., 1999). Effective dosing regimen is 200 mg of NVP once daily for 14 days, followed by 200 mg twice daily. Maximum concentration (C_{max}) is reached approximately 3–4 h after administration of a single dose of 200 mg, and estimated to be approx 2 μg/ml. Following multiple doses, nevirapine peak concentrations appear to increase linearly in the dose range of 200–400 mg/day (Riska et al., 1999a). Data reported in the literature from 20 HIV-infected patients show steady-state C_{max} and C_{min} of 5.74 μg/ml and 3.73 μg/ml, respectively (van Heeswijk et al., 2000). NVP undergoes hepatic biotransformation to several hydroxylated metabolites by cytochrome P450, and is also an autoinducer of isoenzymes 3A (CYP3A) and 2B6 (CYP2B6), being eliminated mainly as glucuronide metabolites in urine. Less than 3% of an administered dose is excreted in urine as the parent compound (Riska et al., 1999a).

Although the metabolism of NVP in different animal species has been studied (Riska et al., 1999b), pharmacokinetic studies in experimental animals are scarce, and there is no information available about the bioavailability of this drug in laboratory animals, e.g. in rats. Knowledge on the absorption characteristics of NVP in laboratory animals is crucial for pre-clinical studies on new forms of administration designed to reduce the dosing frequency. Since HIV-infected patients require long-term therapy with antiretroviral drugs, such as NVP, an improvement in the treatment regimen will improve their quality of life. This objective could be reached through extended-release dosage, i.e. changing the administration regimen from twice to once a day or even to a lower frequency.

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Table 1
Groups of unanesthetized animals ($n = 6$).

Group	Administration route	Dose (mg)
1	i.v.	4
2	p.o.	8
3	s.c.	4

i.v., intravenous; p.o., oral; s.c., subcutaneous.

However, it is essential for NVP to be absorbed by the entire or the greatest part of the gastrointestinal tract, i.e. the small and large intestine.

The objectives of this study were to characterize the pharmacokinetics of NVP in rats after intravenous, oral and subcutaneous administration, to assess its absorption by different regions of the gastrointestinal tract and to evaluate the effect of bile on its intestinal absorption.

2. Materials and methods

2.1. Chemicals

NVP (Viramune®) was obtained from Boehringer Ingelheim (Barcelona, Spain). Carboxymethylcellulose (CMC) and dimethylsulfoxide (DMSO) were purchased from Sigma (Madrid, Spain), and propylene glycol (PG) from Fluka Biochemika (Madrid, Spain). All the other reagents and solvents used in the study were of HPLC or analytical grade.

2.2. Animals

Male Wistar rats (280–310 g) were used in this study. All animals were housed under standard conditions and had "ad libitum" access to water and a standard laboratory diet. The day before administration, rats were cannulated in the jugular vein to facilitate blood sample collection and intravenous dose administration (Torres-Molina et al., 1992). Animals were submitted to overnight fasting, while water was supplied "ad libitum".

2.3. Drug administration and sampling

Rats were divided into ten groups ($n = 6$) depending on the administration route of NVP and the conscious or anesthetized state of animals during blood sampling. Diethyl ether was used as anesthetic. Tables 1 and 2 show the administration route and the NVP dose corresponding to each animal group.

A solution of NVP (8 mg/ml) in DMSO/PG (1/4, v/v) was used for intravenous (through the jugular cannula) and subcutaneous (injection in the back of the rats) administration (groups 1, 3 and 4). Oral dosing in unanesthetized rats (group 2) was performed by gastric gavage with an NVP suspension (8 mg/ml) in 0.5% CMC.

Table 2
Groups of anesthetized animals ($n = 6$). All animals, except those in groups 4 and 10, were subjected to bile duct ligation.

Group	Administration route	Dose (mg)	Drug absorption
4	i.v.	4	–
5	p.o.	8	Stomach
6	i.d.	8	Duodenum
7	i.i.	8	Ileum
8	i.c.	8	Colon
9	i.d.w.	8	Duodenum
10	i.d.w.n.	8	Duodenum

i.v., intravenous; p.o., oral; i.d., intraduodeal; i.i., intraileal; i.c., intracolonic; i.d.w., intraduodenal, washed duodenum; i.d.w.n., intraduodenal, washed duodenum, non-ligated bile duct.

To study the absorption of NVP by different segments of the gastrointestinal tract of anesthetized rats (groups 5–10), segments were conveniently ligated to hold the drug suspension (1 ml of 8 mg/ml) during the study period. In group 5, the pylorus was ligated and the NVP suspension was administered by gastric gavage. In group 6, the pylorus and the small intestine at a distance of 15 cm from the former were ligated, and the NVP suspension was administered through a cannula inserted in the intestine, near to the pylorus ligature. In group 7, an ileal segment of 15 cm was delimited by two ligatures and the NVP suspension was administered in that segment through a cannula. In group 8, the ileocecal valve was ligated and NVP was administered through a cannula inserted into the colon.

Animals in groups 4–8 were subjected to bile duct ligation prior to drug administration. To study the influence of bile on the absorption of NVP, two additional animal groups were included. In group 9, rats were also subjected to bile duct ligation, but before NVP administration in a duodenal segment, remaining bile was washed out with saline. In group 10, the bile duct of rats was not ligated prior to NVP administration in a duodenal segment.

Serial blood samples were collected at different times depending on the route of administration. 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) tempered at 37 °C was perfused. Blood was collected in Eppendorff tubes, which were then centrifuged at 1500 × *g* for 5 min, and the supernatant plasma was used for the analytical determination of NVP.

At the end of the blood sampling period in groups 5–10, rats were sacrificed and the stomach (group 5), or the intestinal segment where NVP was administered (groups 6–10), was excised to determine the remaining amount of the drug.

2.4. Analytical method

NVP was quantified in rat plasma and gastrointestinal tissues using a high performance liquid chromatographic assay (HPLC) with ultraviolet (UV) detection at 254 nm. The HPLC equipment consisted of a quaternary pump SpectraSYSTEM P4000, an autosampler SpectraSYSTEM AS3000 and a spectrophotometric detector SpectraSYSTEM UV 6000LP. Data were processed through "Chromquest Chromatography Workstation Software Version 1.63". The column was a Waters "Nova-Pack" C-18, and the mobile phase was a mixture of acetonitrile and 50 mM NaH_2PO_4 (25:75, v/v). The injection volume was 25 μl, and the flow rate was 1 ml/min.

Plasma samples (100 μl) were subjected to protein precipitation with acetonitrile (100 μl) and, after centrifuging this mixture at 2000 × *g* for 5 min, the supernatant was injected into the HPLC system. To determine the amount of NVP remaining in the gastrointestinal segments at the end of the assays, the gastrointestinal segments were extracted with methanol and, after mixing 1 ml of this extract with 1 ml of acetonitrile/water (50/50, v/v), an aliquot of 25 μl was injected into the chromatograph.

The quantification limit of the analytical method was 0.5 μg/ml and the variation coefficients for accuracy and precision were <15%.

2.5. 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 intravenous NVP administration: half-life ($t_{1/2}$), area under the plasma concentration–time curve from zero to infinity (AUC), clearance (Cl), mean residence time (MRT), and volume of distribution (V_d). In the case of extravasal NVP administration to unanesthetized rats, the estimated parameters were: $t_{1/2}$, AUC, MRT, maximum plasma

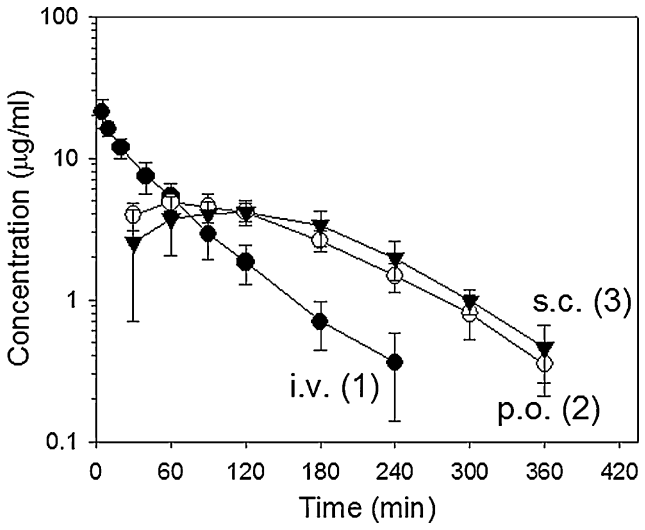

Fig. 1. Plasma NVP concentrations following intravenous, oral and subcutaneous administration in unanesthetized rats. Plasma concentrations corresponding to i.v. and s.c. administration have been normalized for an 8-mg dose. Values in brackets indicate the animal group.

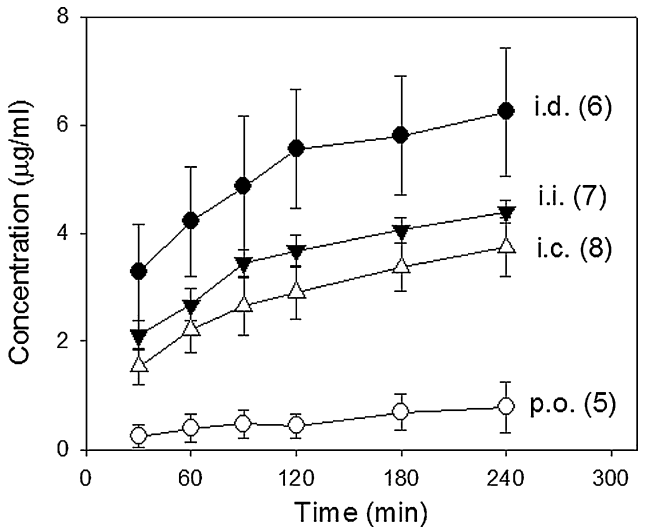

Fig. 2. Plasma NVP concentrations (mean ± S.D.) following intraintestinal and oral administration in anesthetized rats. Values in brackets indicate the animal group.

concentration (C_{max}), time to C_{max} (t_{max}) and mean absorption time (MAT). When NVP was extravasally administered to anesthetized rats, the estimated parameters were: t_{max}, C_{max}, and the area under the plasma concentration–time curve from the time of dosing to the last measurable concentration (AUC_{last}).

2.6. Statistical analysis

Statistical comparisons were performed by means of one-way ANOVA, followed by Tukey's test when more than two groups were compared. A P value of <0.05 was considered statistically significant.

3. Results

3.1. Nevirapine pharmacokinetics and bioavailability

NVP plasma concentration–time profiles obtained after intravenous, oral and subcutaneous dosing in unanesthetized rats are shown in Fig. 1. In that figure, plasma concentrations corresponding to intravenous and subcutaneous administrations have been normalized for an 8-mg dose. NVP plasma concentrations corresponding to oral administration were similar to those obtained after subcutaneous dosing. In fact, the mean AUC value was practically the same for either administration (Table 3), and slightly lower than the mean AUC value obtained after intravenous administration. By comparing the mean AUC values obtained after either way of extravasal administration with the mean AUC value corresponding to the intravenous route, an NVP bioavailability of 91% can be estimated for either extravasal administration route. The C_{max} value was also similar for both extravasal administrations.

Although the mean value of t_{max} corresponding to oral administration was lower than that obtained after subcutaneous administration, the difference was not statistically significant, and the difference between mean MRT values was not statistically significant either. As expected, the MRT value corresponding to intravenous administration was lower than that obtained with either way of extravasal administration, due to the absence of an absorption process. In fact, the difference between MRT values makes it possible to obtain an estimation of MAT for oral and subcutaneous administrations (Table 3).

Other pharmacokinetic parameters of NVP, calculated after intravenous administration, were: Cl = 8.15 ± 1.70 ml/min and V_d = 550 ± 115 ml.

3.2. NVP absorption from gastrointestinal segments

Plasma concentration–time profiles of NVP administered in specific sites of the gastrointestinal tract (groups 5–8) are provided in Fig. 2. All animals in these groups were anesthetized at the time of drug administration and during blood sampling, and their bile ducts were ligated. The plasma concentration versus time curves illustrate the differences in the NVP absorption rate depending on the gastrointestinal segment where the drug was administered. Absorption from the duodenum was faster than absorption from the ileum or from the colon, whereas absorption was slowest when NVP was administered in the stomach. The statistical comparisons

Table 3
Pharmacokinetic parameters after a single intravenous, oral and subcutaneous NVP dose in unanesthetized rats (n = 6).

Parameter	Administration route (group)			Stat. sig.
	i.v. (1)	p.o. (2)	s.c. (3)	
AUC (μg min/ml)	1012 ± 178[a]	918 ± 129	920 ± 116[a]	NS
$t_{1/2}$ (min)	47.5 ± 9.7	56.6 ± 8.7	59.2 ± 6.3	NS
C_{max} (μg/ml)	–	5.05 ± 0.73	4.88 ± 0.94[a]	NS
t_{max} (min)	–	80 ± 24	105 ± 46	NS
MRT (min)	54.4 ± 8.5[b]	138.2 ± 8.9[c]	158.1 ± 23.7[c]	P < 0.001
MAT (min)	–	83.8	103.7	–

NS, not significant.
[b and c]Values with different superscripts are statistically different.
[a] Parameter value normalized for an 8-mg dose.

Table 4
Pharmacokinetic parameters following a single NVP dose in anesthetized rats ($n = 6$).

Parameter	Administration route (group)						Stat. sig.
	p.o. (5)	i.d. (6)	i.i. (7)	i.c. (8)	i.d.w. (9)	i.d.w.n. (10)	
t_{max} (min)	196 ± 63	220 ± 49	228 ± 27	225 ± 30	210 ± 60	228 ± 27	NS
C_{max} (μg/ml)	0.86 ± 0.35[a]	6.31 ± 1.18[b]	4.41 ± 0.20[c]	3.77 ± 0.54[c]	4.55 ± 0.62[c]	7.98 ± 0.38[d]	$P < 0.001$
AUC_{last} (μg min/ml)	96 ± 50[a]	1156 ± 237[b]	774 ± 41[c]	636 ± 99[c]	876 ± 141[c]	1410 ± 107[d]	$P < 0.001$

NS, not significant.
AUC_{last}, area under the plasma-level curve from time of administration to the last measurable concentration.
[a–d]Values with different superscripts are statistically different.

of the C_{max} and AUC_{last} values (Table 4) show no significant differences for these parameters in the case of intraileal (group 7) and intracolonic (group 8) NVP administrations, whereas oral (group 5) and intraduodenal (group 6) administrations provided the lowest and the highest value for these parameters, respectively.

The remaining amount of NVP in each gastrointestinal segment at the end of the study period (Fig. 3) confirms the results drawn from the plasma curves. In fact, the amount of NVP remaining in the stomach was above 80% of the administered dose (group 5), while it was approximately 40% in the case of the duodenum (group 6).

The higher NVP plasma levels obtained in intraduodenally dosed rats (group 6) could be related to the presence of bile in that intestinal segment. Although the bile duct was ligated immediately prior to the administration of NVP, some bile is present in the duodenum at the moment of administration. To evaluate the influence of bile on the absorption of NVP from the duodenum, two additional groups (9 and 10) were included. As can be seen in Fig. 4, when the duodenum was washed out of bile prior to NVP administration (group 9), plasma levels were lower than those obtained in the group with an unwashed duodenum (group 6). However, the highest plasma levels were obtained in rats with continuous bile secretion into the duodenum (group 10) where the highest. On the other hand, the highest NVP plasma concentration obtained in each group was inversely related with the amount of drug remaining in the duodenum at the end of the experimental period (Fig. 5). These results indicate that rat bile increases the absorption rate of NVP.

When NVP was orally dosed to unanesthetized rats (group 2), the maximum in the plasma concentration–time curves reached approximately 80 min after administration. However, a clear maximum in NVP plasma concentrations was not observed in anesthetized rats before the end of the experimental period (240 min). This indicates a slower NVP absorption in anesthetized rats as compared with unanesthetized ones, and it can be attributed to the effect of anesthesia itself and, also, to the surgical manipulation of the gastrointestinal tract prior to drug administration. Nevertheless, the delay in the maximum of the plasma levels could also be

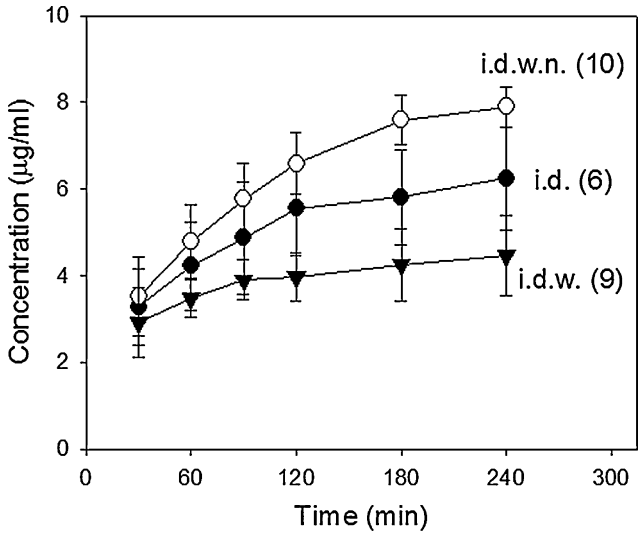

Fig. 4. Plasma NVP concentration–time courses after intraduodenal administration in anesthetized rats with ligated (i.d. and i.d.w.) and non-ligated (i.d.w.n.) bile duct. The duodenum was washed with serum prior to drug administration in groups i.d.w. and i.d.w.n. Values in brackets indicate the animal group.

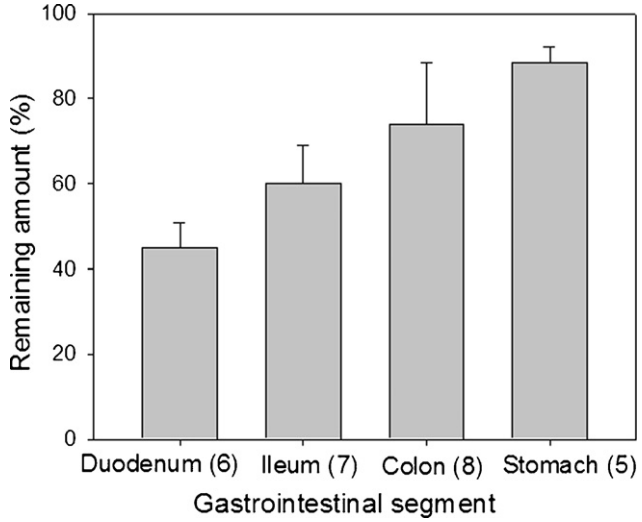

Fig. 3. Percentage (mean ± S.D.) of the NVP administered dose remaining in different segments of the gastrointestinal tract of rats, 4 h after drug administration. Values in brackets indicate the animal group.

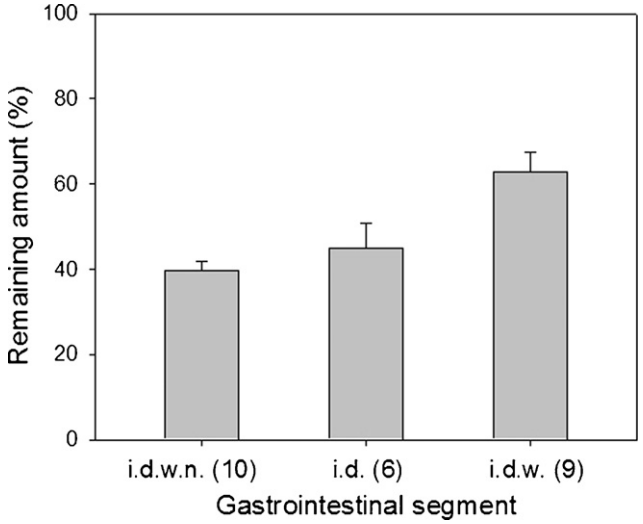

Fig. 5. Percentage (mean ± S.D.) of the NVP administered dose remaining in the duodenum of anesthetized rats with ligated (i.d. and i.d.w.) and non-ligated (i.d.w.n.) bile duct after 4 h of drug administration. The duodenum was washed with serum prior to drug administration in groups i.d.w. and i.d.w.n. Values in brackets indicate the animal group.

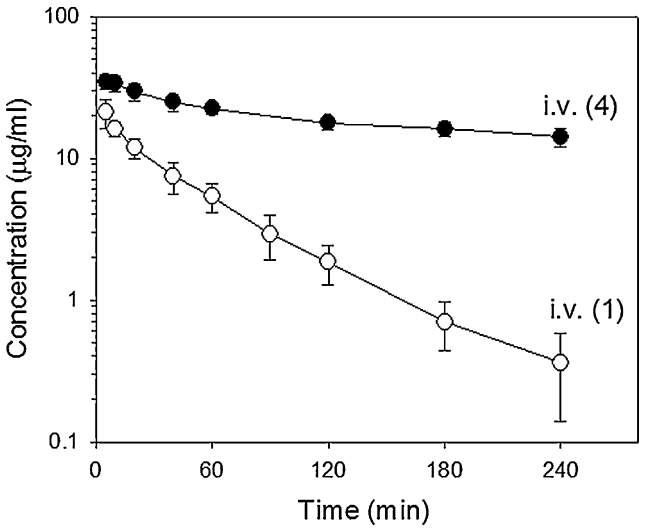

Fig. 6. Mean plasma concentration–time profiles of NVP in rats after intravenous administration. Data are normalized for an 8 mg dose. Values in brackets indicate the animal group.

due, at least in part, to a slower elimination of NVP in anesthetized rats. To evaluate this effect, NVP was intravenously administered to anesthetized rats (group 4), and, as can be seen in Fig. 6, a slower decline of NVP plasma concentrations was observed, as compared with unanesthetized rats.

4. Discussion

The NVP starting dose for adults is 200 mg/day; however, after 14 days the dose is increased to 200 mg/12 h. Although some studies have been carried out to evaluate the feasibility of administering 400 mg once/day instead of 200 mg twice/day (Kappelhoff et al., 2005; van Heeswijk et al., 2000), the highest oscillation in plasma levels obtained for the 400 mg dose, makes the regimen of 200 mg NVP dosed twice/day preferable over the 400 mg dose once/day (Kappelhoff et al., 2005). In fact, some researchers have made efforts in developing sustained release forms of NVP in recent years in order to use a once/day dosage regimen with a small oscillation in plasma levels (Macha et al., 2009a; Parienti and Peytavin, 2011; Ramana et al., 2010; Vedha et al., 2010). In the case of some NVP formulations, such as those containing microparticles, the use of laboratory animals during pre-clinical studies would be helpful for evaluating their sustained release features. Nevertheless, the usefulness of such laboratory animals depends on their similarities with humans with regard to NVP absorption features.

NVP was well absorbed when administered orally or subcutaneously to unanesthetized rats, the extent of bioavailability being similar for both routes. The oral dose was chosen with the aim of obtaining a plasma concentration similar to that described in humans when therapeutic doses of NVP are used. In fact, the mean C_{max} value obtained in rats (5.05 μg/ml) was close to the steady-state C_{max} reported in humans for 200 mg twice/day (5.74 μg/ml) (van Heeswijk et al., 2000). In the case of intravenous and subcutaneous administration, NVP had to be administered at a dose of 4 mg, instead of 8 mg as in the case of oral administration, due to the limited solubility of NVP in the vehicle. The oral bioavailability estimated in rats (91%) is practically equal to that reported in humans (91–93%) (Lamson et al., 1999).

The absorption of NVP from different regions of the gastrointestinal tract of anesthetized rats showed a very low absorption from the stomach, when compared with the rest of the studied regions. Although the absorption from the duodenum was faster than from the ileum or from the colon, this faster absorption can be attributed to the presence of bile in the duodenum. Since NVP is a lipophilic drug with low solubility in water (0.1 mg/ml) (Pereira et al., 2007), its absorption is expected to increase in the presence of bile owing to the emulsifying properties of this.

The absorption of NVP from targeted sites of the human gastrointestinal tract has been studied using remotely activated capsules and gamma scintigraphy (Macha et al., 2009b). In that study it was observed that NVP was absorbed from all four sites of the gastrointestinal tract assayed (jejunum, ileum, ascending colon and descending colon), and that the rate of absorption decreased from the jejunum to the descending colon. This tendency in the absorption of NVP along the gastrointestinal tract has also been observed in the present work, with a decrease in the NVP absorption rate from the duodenum to the colon of rats.

5. Conclusions

NVP bioavailability in rats is higher than 90% and it is absorbed along the small intestine and colon following a pattern similar to that observed in humans, which makes the rat a useful animal model for carrying out studies on the absorption of this drug, and, particularly, for assaying sustained release forms of small size.

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