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Stereoselective Pharmacokinetics of Ornidazole After Intravenous Administration of Individual Enantiomers and the Racemate

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ABSTRACT The pharmacokinetics of ornidazole (ONZ) were investigated following i.v. administration of racemic mixture and individual enantiomers in beagle dogs. Plasma concentrations of ONZ enantiomers were analyzed by chiral high-performance liquid chromatography (HPLC) on a Chiralcel OB-H column with quantification by UV at 310 nm. Notably, the mean plasma levels of (−)-ONZ were higher in the elimination phase than those of (+)-ONZ. (−)-ONZ also exhibited greater $t_{1/2}$, MRT, $AUC_{0\text{-}t}$ and smaller CL, than those of its antipode. The area under the plasma concentration-time curve ($AUC_{0\text{-}t}$) of (−)-ONZ was about 1.2 times as high as that of (+)-ONZ. (+)-ONZ total body clearance (CL) was 1.4 times than its optical antipode. When given separately, there were significant differences in the values of $AUC_{0\text{-}\infty}$ and CL between ONZ enantiomers ($P < 0.05$), indicating that elimination of (+)-ONZ was more rapid than that of (−)-ONZ. No significant differences were found between the estimates of the pharmacokinetic parameters of (+)-ONZ or (−)-ONZ, obtained following administration as the individual and as a racemic mixture. This study demonstrates that the elimination of ONZ enantiomers is stereoselective and chiral inversion and enantiomer/enantiomer interaction do not occur when the enantiomers are given separately and as racemic mixture. *Chirality 18:799–802, 2006.*

KEY WORDS: stereoselective pharmacokinetics; chiral high-performance liquid chromatography analysis; *rac*-ONZ; (+)-ONZ; (−)-ONZ

INTRODUCTION

Certain derivatives of nitroimidazole are known to possess antibacterial, antiprotozoan, and anticancer activity. Ornidazole, 1-(3-chloro-2-hydroxy) propyl-2-methyl-5-nitroimidazole (ONZ), acts selectively against anaerobic and microaerophilic bacteria and protozoa,[1] with a half-life longer than that of metronidazole, that is most widely used therapeutically. The mechanism of action of nitroimidazoles, as ornidazole, is thought to involve interference with DNA by a metabolite in which the nitro group has been reduced.[2] ONZ exhibits an excellent tolerance in humans when administered during pregnancy, but reproductive studies in rats showed that the main side-effect detected being spermatotoxicity.[3–6] ONZ has a chiral center, which gives rise to two optical isomers (Fig. 1) and are often used as the racemate, clinically. However, its enantiomers may differ qualitatively and/or quantitatively in their pharmacological effects due to stereoselective difference with optically active biological molecules.[7,8]

Stereoselectivity in the pharmacokinetics of a chiral drug may be determined by estimating the differences between pharmacokinetic parameters of the individual enantiomers. It is important to determine the pharmacokinetics of the drug enantiomers not only following the administration of the racemic mixture, but also after the separate administration of the individual enantiomers, in order to avoid misinterpretation of data relating to drug disposition.[9,10] Previous studies had demonstrated the pharmacokinetics of ONZ as a mixture form.[11] However, there were no reports on stereoselective pharmacokinetics of ONZ enantiomers.

In this experiment, plasma concentrations of ONZ in dogs following intravenous (i.v.) administration were determined using a novel plasma extraction procedure followed by a chiral high-performance liquid chromatography (HPLC) assay. The drug was given as individual enantiomers in order to estimate stereoselective differences in the pharmacokinetic parameters and uni- or bidirectional chiral inversion, and as a racemic mixture to see

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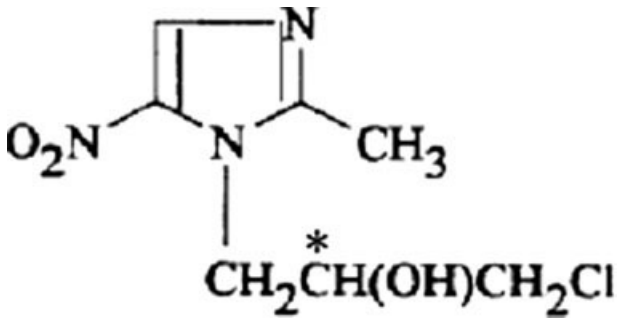

Fig. 1. Chemical structure of *rac*-ornidazole (*chiral center).

whether these parameters are affected in the presence of the optical antipode.

MATERIALS AND METHODS

Chemicals

Rac-ONZ, (+)-ONZ, (−)-ONZ (optical purity >99.5%), (+)- and (−)-ONZ injections were provided by Sanhome Pharmaceutical. (Nanjing, China). Hexane, isopropyl alcohol, and methyl *t*-butyl ether (HPLC-grade) were purchased from Tedia, (Fairfield, USA), and all other chemicals and reagents were of the analytical grade.

Animals and Drug Administration

Four beagle dogs weighing 9–11.5 kg (Center of Xingang Experimental Animals, Shanghai, China) were housed under controlled conditions (20°C ± 2°C, 50% ± 20% relative humidity) with a natural light–dark cycle. They were fasted for 12 h before the experiment, with free access to water. The studies were approved by the Animal Ethics Committee of China Pharmaceutical University.

Four dogs received (+)-ONZ (15 mg/kg) or (−)-ONZ (15 mg/kg) or *rac*-ONZ (15 mg/kg) via the foreleg vein. Blood samples (1.5–2 ml) were collected immediately before drug administration and postdose at 0.083, 1, 2, 4, 6, 8, 12, and 16 h. Plasma was separated by centrifugation (4000 rpm, 10 min), and stored at −20°C until analysis.

HPLC Assay

Concentrations of ONZ enantiomers in plasma were determined by a chiral high-performance liquid chromatography (HPLC) method. The plasma samples (0.2 ml) were extracted with 0.4 ml of a mixture of methanol and isopropyl alcohol (1:1, v/v), after centrifugation (15,000 rpm) for 10 min; 10 μl of the supernatant was taken and injected directly.

Shimadzu HPLC-2010C (Shimadzu Corporation, Tokyo, Japan) chromatographic system, including a standard vacuum degasser, column oven, and a multiwavelength detector, was used to conduct the analysis. Stereoselective separation was achieved on a Chiralcel :OB-H column (250 × 4.6 mm i.d., Daicel, Tokyo, Japan). The mobile phase consisted of hexane-isopropyl alcohol-methyl *t*-butyl ether-glacial acetic acid (90:2:8:0.5 v/v) and was delivered at a flow-rate of 1.0 ml/min. The column temperature was maintained at 40°C with the UV detector set at 310 nm. Under these chromatographic conditions, retention times for (+)- and (−)-ONZ were about 57 and 62 min. Excellent linearity was obtained over the concentration range of 0.313–20 μg/ml for each enantiomer in dog plasma (R^2 > 0.998). The extraction recoveries of ONZ from plasma were >87% (RSD <6%), The quantification limit was 0.313 μg/ml for (+)- and (−)-ornidazole with a signal-to-noise ratio of about 10:1. The inter- and intraday precision of the method ranged from 1.6 to 8.4% (RSD) and from 3.5 to 6.8% (RSD), respectively.

Pharmacokinetics Analysis

The pharmacokinetic parameters of ONZ enantiomers were calculated by noncompartmental analysis. The terminal elimination rate constant (k) was determined by linear least squares regression of the terminal portion of the plasma concentration-time curve and the corresponding elimination half-life ($t_{1/2}$) was calculated as $0.693/k$. The area under the plasma concentration-time curve (AUC) and the area under the first-moment time curve (AUMC) were calculated by the trapezoidal method and extrapolated to infinity, using the last measurable plasma concentration and the terminal elimination rate constant. Mean residence time (MRT) was calculated using the equation MRT = AUMC/AUC. Clearance (CL) was derived from the ratio dose/AUC, and distribution volume (V) was estimated by the ratio CL/k.

Statistical Analysis

Data are expressed as mean ± standard deviation. Differences between pharmacokinetic parameters of (+)- and (−)-ONZ were evaluated by two independent samples analysis of nonparametric statistical test, and two enantiomers in *rac*-ONZ were evaluated by two related samples analysis of nonparametric statistical test. All statistical analyses were performed using SPSS 11.0. A value of $P < 0.05$ was considered statistically significant.

RESULTS

Plasma concentration-time profiles for ONZ enantiomers following i.v. administration of 15 mg/kg *rac*-ONZ to dogs are shown in Figure 2. In plasma, both enantiomers declined rapidly with slight difference in elimination phase. Visual inspection of the concentration versus time courses showed that (−)-ONZ was higher than that of (+)-ONZ during the elimination phase. Pharmacokinetic parameters determined by noncompartmental analysis are listed in Table 1. The mean AUC_{0-t} value of (−)-ONZ was significantly higher ($P < 0.05$) than that of (+)-ONZ (40.87 vs.30.60 μg h/ml). The mean MRT and V of (−)-ONZ was 8.97 h, 10.64 l, respectively, which were not statistically different from those of (+)-ONZ. No significant differences between enantiomers were observed for half-life, although in each dog (+)-ONZ gave rise to values for $t_{1/2}$ that were shorter than the corresponding values for (−)-ONZ. The *P*-value of clearance did approach significance ($0.068 > P > 0.05$), the use of a

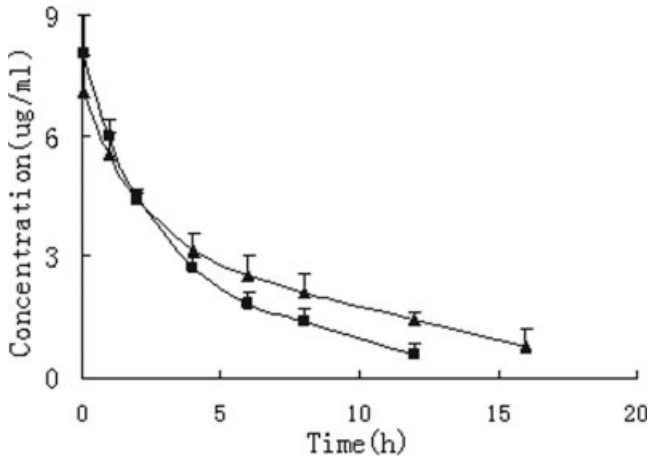

Fig. 2. Plasma concentration-time profiles for ONZ enantiomers in dogs following i.v. administration of *rac*-ONZ (15 mg/kg); (▲) (−)-ONZ; (■) (+)-ONZ. Each data point represents the mean values ± SD, from four dogs.

larger number of dogs ($n > 5$) might have resulted in a significant difference.

The time course of (−)-ONZ and (+)-ONZ concentrations in the dog plasma after i.v. administration of 15 mg/kg individual enantiomers are shown in Figure 3. The concentrations of ONZ enantiomers were found to be distinctively different in the elimination phase. It turned out that the elimination of the (+)-ONZ was much faster than that of (−)-ONZ in the beagle dog. Pharmacokinetic parameters for both enantiomers are presented in Table 1. Pharmacokinetic analysis indicated that (−)-ONZ had a greater $t_{1/2}$, MRT, AUC_{0-t}, and smaller CL, than the corresponding values of (+)-ONZ. The (−)/(+)-isomer ratios for $t_{1/2}$, MRT, and AUC_{0-t} range from 1.24–1.70, while the ratio for CL was about 0.71. The total body clearance of (+)-ONZ was significantly larger (1.95 ± 0.25, 1.39 ± 0.18 l/h, $P < 0.05$) and the areas under the curve ($AUC_{0-\infty}$) was significantly smaller (61.53 ± 11.40, 85.68 ± 13.21, $P < 0.05$) than those of (−)-ONZ. The result suggested stereoselectivity in the pharmacokinetics of ONZ enantiomers in dogs.

The $t_{1/2}$ and CL of (−)-ONZ were slightly lower, but not statistically different from that of (−)-ONZ when dosed with *rac*-ONZ. Similarly, no significant differences were found between the estimates of the pharmacokinetic parameters of (+)-ONZ obtained following administration as a racemic mixture and as the individual enantiomer.

DISCUSSION

In the present study, enantiomers were considered as separate chemical entities and the presence or absence of any interaction had been investigated. Stereoselective pharmacokinetics of the separated enantiomers were observed after intravenous administration to dogs. We found higher concentrations of (−)-ONZ, when compared with its optical antipode in the elimination phase. These concentration profiles resulted in a 39% increase of the AUC for the (−)-ONZ enantiomer. In addition, total body clearance of (−)-ONZ was significantly lower (29%) than that of (+)-ONZ, indicating that the elimination of (+)-ONZ was faster than its antipode. The findings from our laboratory showed that the binding of (+)-ONZ to dog plasma protein (28%) was not significantly greater than that of (−)-ONZ (24%), which strongly supported the stereoselective elimination of ONZ enantiomers. Stereoselectivity in the pharmacokinetics may be contributed to the difference in the hepatic extraction and the metabolism of the two enantiomers in the intestine and liver, which need further investigation. Moreover, after administration of an individual enantiomer, its optical antipode was not detected at any time point under our experimental conditions. These results suggest that stereochemical inversion does not occur in the beagle dogs.

The statistical significance between pharmacokinetic parameters of ONZ enantiomers would tend to suggest the stereoselective difference when given separately. However, the enantiomers which were administered as mixture form tend to blur the picture somewhat, and the presence of statistically significant difference between enantiomers could easily be masked by the small number of animals. The values of AUC_{0-t} parameter were statistically significant, and the *P*-value of clearance did approach significance ($0.068 > P > 0.05$). No significant differences were observed for some parameters such as $t_{1/2}$, MRT, and V after administration of the racemate. These parameters showed the same trend with the administra-

TABLE 1. The pharmacokinetic parameters estimated following i.v. administration of racemate (15 mg/kg) and individual enantiomers (15 mg/kg) in beagle dogs (mean ± SD, n = 4)

	Administration of pure enantiomers		Administration of racemic mixture	
Parameters	(−)-ONZ	(+)-ONZ	(−)-ONZ	(+)-ONZ
$T_{1/2}$ (h)	6.37 ± 1.06	3.73 ± 1.31	6.22 ± 3.73	4.93 ± 2.02
MRT (h)	9.19 ± 1.67	5.38 ± 1.89	8.97 ± 5.39	7.11 ± 2.91
Cl (L/h)	1.39 ± 0.18[a]	1.95 ± 0.25	1.25 ± 0.31	1.67 ± 0.34
V (L)	12.60 ± 1.10	10.14 ± 2.13	10.46 ± 4.34	10.72 ± 2.09
AUC(0–*t*) (μg h/ml)	72.33 ± 7.06	57.99 ± 8.85	40.87 ± 4.75[a]	30.60 ± 4.39
AUC(0–∞) (μg h/ml)	85.68 ± 13.21[a]	61.53 ± 11.40	49.14 ± 12.66	37.82 ± 7.72

[a]Significantly different from (+)-ONZ at $P < 0.05$.

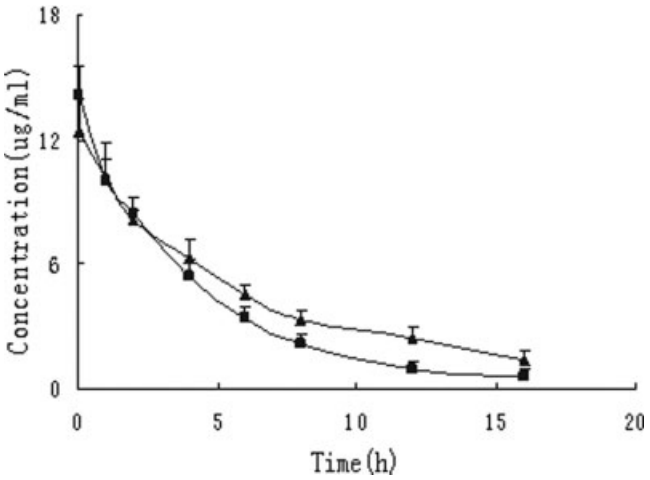

Fig. 3. Plasma concentration-time profiles for ONZ enantiomers in dogs following i.v. administration of single (+)- and (−)-ONZ (15 mg/kg); (▲) (−)-ONZ; (■) (+)-ONZ. Each data point represents the mean values ± SD, from four dogs.

tion of individual enantiomers. The results for clearance and half-life confirmed the slightly slower elimination rate of (−)-ONZ compared with (+)-ONZ. The half-life for (−)-ONZ was slightly longer than that of (+)-ONZ; conversely, the clearance of (−)-ONZ was smaller than that of (+)-ONZ. The longer half-life and smaller clearance of (−)-ONZ that were also observed, following separate administration of ONZ enantiomers, indicate that the preferential faster elimination of (+)-ONZ is not dependent upon the presence of the (−)-ONZ. No evidence of an interaction between ONZ enantiomers was found in the current study, on the basis of the half-life or the clearance values.

This study demonstrates that the elimination of ONZ enantiomers is stereoselective and that chiral inversion does not occur when the enantiomers are given as separated enantiomers or racemic mixture in dogs. Moreover, the presence of a potential enantiomer/enantiomer interaction has not been detected when ONZ is administered in racemic form.

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