Original Research Article

Pharmacokinetics of enantiomers of oxiracetam in rats

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Abstract

Purpose: To investigate the differences in pharmacokinetics of S-oxiracetam (S-ORT) and R-oxiracetam (R-ORT) in rats.
Methods: Sprague-Dawley rats (20) were randomly divided into two groups (ten rats per group), viz, S-ORT and R-ORT groups. Rats in S-ORT group received 200 mg S-ORT/kg while rats in R-ORT group were given 200 mg R-ORT/kg. Both treatments were given orally, and blood samples were collected at fixed time intervals for analysis. Ultra performance liquid chromatography-electrospray ionization-tandem mass spectrometry (UPLC–ESI-MS/MS) was used for pharmacokinetic analysis. Portions of the rat plasma were also subjected to configurational transformation analysis using normal phase-high performance liquid chromatographic (NP-HPLC) fitted with a Chiral OC column. Blank blood samples from five rats were used for plasma protein binding rate studying.
Results: The two enantiomers did not transform into each other after oral administration, and the concentrations of S-ORT at 1, 1.5 and 2 h were significantly higher than those of R-ORT ($p < 0.05$). The area under the curve ($AUC_{0-\infty}$) and maximum concentration (C_{max}) of S-ORT were also significantly larger than those of R-ORT ($p < 0.05$). There were no stereoselective differences between the two enantiomers.
Conclusion: There are significant differences in absorption between two ORT enantiomers, and this may result in different pharmacological effects.

Keywords: S-oxiracetam, R-oxiracetam, Configuration, Pharmacokinetics, Rats

INTRODUCTION

Chiral compounds constitute more than 50 % of pharmaceuticals in use currently, and biomolecules such as proteins, amino acids, fatty acids, nucleic acids, and monosaccharides exhibit chirality. Enantiomers have the same physicochemical properties, but may exhibit differences in pharmacokinetics, pharmacodynamics, and toxicity [1].

Oxiracetam (ORT) belongs to the cyclic γ-aminobutyric acid class of drugs and as a nootropic agent, it is used to treat various cognitive disorders due to its ability to promote both learning and memory processes [2-5]. In clinical practice, it is used as a racemic mixture of S-ORT) and R-ORT (Figure 1).
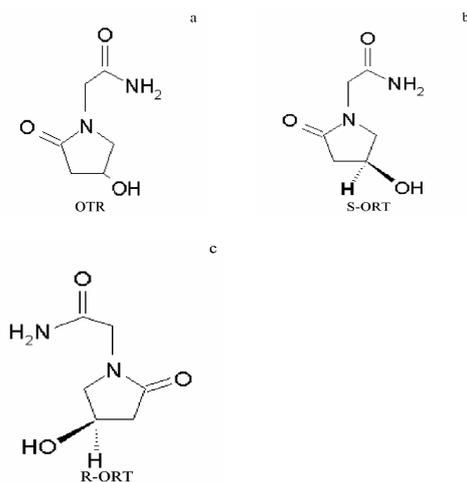
Figure 1: Chemical structures of ORT and its enantiomers

The S enantiomer of ORT is more biologically active than the R form, probably due to differences in their pharmacokinetics [6,7]. The aim of the present study was to investigate the differences in pharmacokinetics of S-ORT and R-ORT in rats.

EXPERIMENTAL

Chemicals and reagents

All chemicals and solvents used in this study were of analytical grade. The enantiomers of ORT were products of Nanjing Yoko Biomedical Research Co. Ltd., while piracetam (internal standard) was obtained from the National Institute for Food and Drug Control, Beijing, China. High-performance liquid chromatography (HPLC) grade n-hexane was bought from Tedia (USA).

Experimental rats

A total of 25 rats of Sprague-Dawley strain were used in this study. They were purchased from the Shanghai Institute of Xingang Experimental Animal Center, and housed in iron cages with free access to food and water. Twenty of the rats were randomly divided into two equal groups of ten rats each: S-ORT and R-ORT, while the remaining five rats were used to obtain blood samples which served as blank in the analysis of plasma protein binding. Rats in S-ORT group received 200 mg S-ORT/kg, while rats in R-ORT group were given 200 mg R-ORT/kg BW. Both treatments were administered orally.

After drug administration, 0.2 mL of blood sample was collected from the orbital venous plexus of each rat at 0, 0.08, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 12, and 24 h in anti-coagulated tubes. The

blood samples were centrifuged at 4000 rpm for 5 min and the plasma obtained stored at -80 °C until analysis. Plasma samples at different time interval were used for pharmacokinetic studies, while portions of the plasma at 0, 1, 1.5, and 2 h were used for configurational transformation analysis. Ethical clearance for the animal experiments was obtained from the Ethics Committee of the Yijishan Hospital (approval no. 20170116) and international guidelines for animal studies were followed [8].

Chromatographic conditions

Enantiomer transformation

Enantiomers of ORT were separated using a Waters HPLC system equipped with a binary pump, a thermostated column oven and a UV detector. Chromatographic separation was carried out on a Chiral OC Daicel (4.6 × 250 mm) column. The column temperature and detection wavelenght were set at 30 °C and 210 nm, resprctively, while the injected volume was 50 μL. The isocratic mobile phase which consist of a mixture of hexane and ethanol (75:25, v/v), was delivered at 1.0 ml/min and the run time for each sample was 18 min.

Pharmacokinetics and plasma protein binding rate studies

Sensitive UPLC–ESI-MS/MS was used to measure plasma S-ORT and R-ORT concentrations. A Shimadzu LC system equipped with a binary pump, vacuum degasser, and a thermostated column oven. Temperatures of the column and autosampler were maintained at 40 and 4 °C, respectively, and the chromatographic separation was performed on an HP amide LC-MS/MS column (100 mm × 3.00 mm, 5 μm). The isocratic mobile phase which consisted of a mixture of methanol and water (85:15, v/v), was delivered at 0.2 ml/min and the run time for each sample was 5 min. Positive ion mode fitted with multiple reaction monitors was used for detection (mass transition (*m/z*) 159.0 →114.1 and 143.0 →126.1 for ORT and piracetam, respectively).

Samples and standards

A portion of the plasma (200 μL) and 40 μL acetonitrile-water solution (50:50, v/v) were added to an Eppendorf tube spiked with 20 μL of piracetam, (internal working standard solution), and vortexed for 2 min. This was followed by the addition of 0.6 mL of acetonitrile, and vortexing again for another 2 min. The resultant mixture was centrifuged at 12,000 rpm at 4 °C for 5 min, and 2 mL of the supernatant was collected, and

dried with liquid nitrogen in a thermostated water bath at 38 °C. After drying, 300 µL of mobile phase solution was added, and the tube was again centrifuged at 12,000 rpm at 4 °C for 5 min. A portion of the resultant supernatant (100 µL) was pipetted into an auto-sampler vial, out of which 50 µL was injected into the column for analysis.

Pharmacokinetic analysis

Plasma sample spiked with piracetam was extracted with methanol and centrifuged at 12,000 rpm for 5 min at 4 °C. The resultant supernatant was pipetted into an auto-sampler vial for analysis. Quality control (QC) samples of three different concentrations (0.1, 1, and 40 µg/mL) in five replicates were used to assess the assay performance. Both enantiomers analyses were performed within a concentration range of 0.05 - 50 µg/mL . Inter-assay precision and accuracy were determined by comparing with values obtained for the QC samples.

Plasma protein binding studies

A dialysis membrane bag was refluxed first with ethanol for 2 h, and then with 0.01 M $NaHCO_3$ solution for 30 min, followed by multiple rinses with water to equilibrate it before the dialysis proper. Glass tubes (40 mL) were prepared for placement of the dialysis bag to create two chambers in it. Dialysates (30 mL) mixed with different concentrations of S-ORT (0.5, 2, 10 and 40 µg/mL) and 1.0 mL of blank plasma was injected into the dialysis bag. The tubes were stoppered and the bag was tied and carefully immersed in Hank's balanced salt solution (pH 7.4).Dialysis took place while the bag was rotated at 10 rpm at 37 °C in a temperature-controlled water bath. After 24 h of dialysis, 50 µL of plasma and dialysate samples were withdrawn from the two chambers and stored at -20 °C until drug concentrations analysis. An aliquot (50 µl) of the plasma or buffer dialysate, spiked with 10 µL of piracetam was vortex-mixed for 30 secs and extracted with 1 mL of methanol using a vortex mixer for 1 min. This was followed by centrifugation at 12,000 rpm at 4 °C for 3 min. A portion of the resultant supernatant (100 µL) was pipetted into an auto-sampler vial, while 50 µL of it was injected into the column for analysis. The percentage drug-protein binding was calculated as in Eq 1.

Binding (%) = (1-C_{out}/C_{in}) × 100

where C_{out} is the concentration of drug outside the bag, and C_{in} is the drug concentration inside the bag.

Statistical analysis

Data are expressed as mean ± standard deviation (SD). Groups were compared using *t*-test and the statistical analysis was performed with Drug Supply Modelling Software (1.0). Values of $p < 0.05$ were considered statistically significant.

RESULTS

Stereo-chemical stabilities of enantiomers of ORT

The retention times of R-ORT and S-ORT were 10.7 and 15.9 min, respectively, and they did not transform into each other after oral administration to rats (Figure 2).
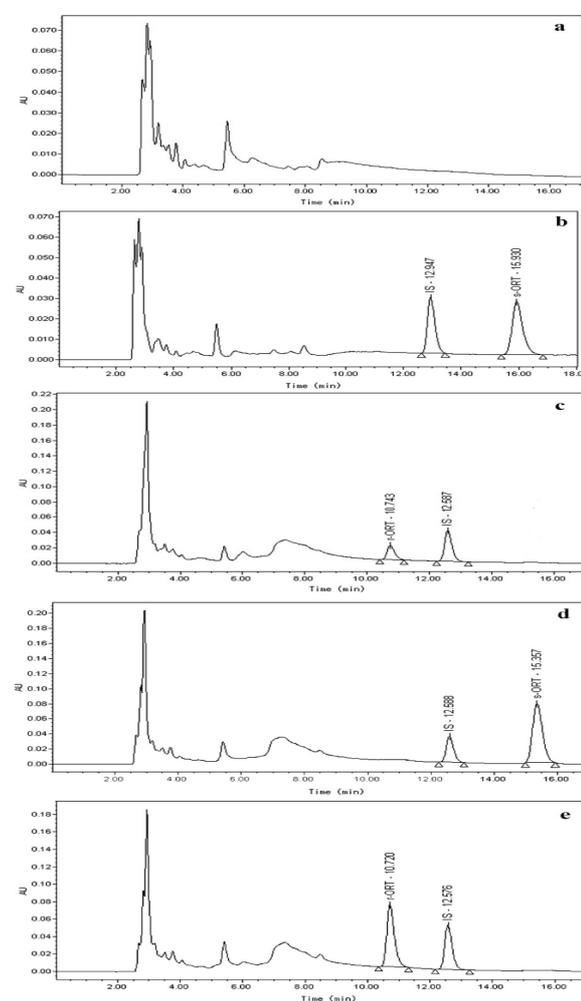
Figure 2: Normal phase-HPLC chromatograms of ORT samples. *(a) blank rat plasma, (b) blank rat plasma spiked with 10 µg/mL of S-ORT, (c) blank rat plasma spiked with 10 µg/mL of R-ORT, (d) plasma sample 1.5 h after oral administration of 200 mg S-ORT/kgand (e) plasma sample 1.5 h after oral administration of 200 mg R-ORT/kg*

Pharmacokinetics of S-ORT and R-ORT in rats

The time-concentration curves of S-ORT and R-ORT after oral administration to rats were best fitted as two-compartment open models, and the concentrations of S-ORT at 1, 1.5 and 2 h were higher ($p < 0.05$) than those of R-ORT at corresponding periods (Figure 3). The area under the curve ($AUC_{0-\infty}$) and maximum concentration (C_{max}) of S-ORT after oral administration were also larger ($p < 0.05$) than those of R-ORT. The absorption half-life ($t_{1/2ka}$), peak time (t_{max}), volume of distribution (V_d), elimination half-life ($t_{1/2\beta}$), systemic clearance (Cl), and mean resident time (MRT) of S-ORT were similar to those of R-ORT (Table 1).

* Significantly different from R-ORT
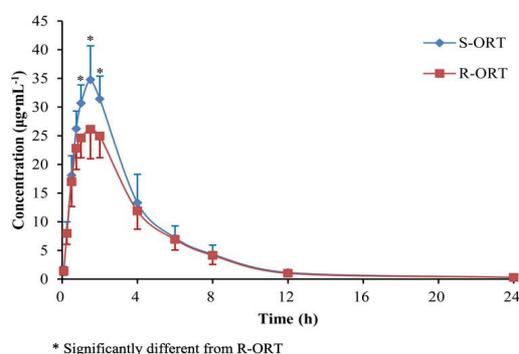
Figure 3: Mean plasma concentration *vs* time profiles after oral administration of S-ORT and R-ORT, each at 200 mg/kg

Table 1: Pharmacokinetic parameters for S-ORT and R-ORT

Parameter	S-ORT	R-ORT
$t_{1/2\alpha}$ (h)	2.13 ± 0.97	1.99 ± 0.71
$t_{1/2\beta}$ (h)	6.10 ± 2.72	6.06 ± 3.13
$t_{1/2ka}$ (h)	0.33 ± 0.16	0.31 ± 0.25
V_d (L/kg)	8.79 ± 3.77	9.15 ± 5.17
Cl/F (L/h/kg)	2.74 ± 0.76	3.03 ± 0.73
$AUC_{0-\infty}$ (mg/L/h)	153.13 ± 30.52[b]	114.15 ± 17.47
$MRT_{0-\infty}$ (h)	4.39 ± 0.83	4.67 ± 0.72
t_{max} (h)	1.68 ± 0.17	1.79 ± 0.25
c_{max} (mg/L)	37.13 ± 6.01[b]	27.14 ± 4.67

[b] $P < 0.05$, when compared to R-ORT

Plasma protein binding capacity of enantiomers of ORT

The degrees of plasma protein binding of four different concentrations of S-ORT and R-ORT ranged from 5.12 to 5.59 %, and from 5.13 to 5.62 %, respectively (Table 2). There were no evidence of stereoselective differences between the two enantiomers.

Table 2: Percentage protein binding capacities of ORT enantiomers after 24 h of dialysis

Initial concentration (µg/mL)	0.5	2	10	40
S-ORT	5.12 ± 0.50	5.43 ± 0.52	5.27 ± 0.54	5.59 ± 0.76
R-ORT	5.13 ± 0.51	5.43 ± 0.72	5.35 ± 0.65	5.62 ± 0.57

DISCUSSION

In the present study, there was no interconversion of S-ORT and R-ORT after oral administration, and the plasma concentrations of S-ORT at 1, 1.5 and 2 h were significantly higher than those of R-ORT. The C_{max} and $AUC_{0-\infty}$ of S-ORT were also larger than those of R-ORT. While these results are in agreement with those reported in beagle dogs [8], they are however not consistent with results obtained in some previous studies [9,10]. In the present study, there was no significant differences in Cl values of both enantiomers, but the C_{max} of S-ORT was significantly higher than that of R-ORT. This result appears to suggest that the differences in the pharmacokinetics of the two enantiomers may be partly due to their different rates of absorption. Results from previous studies on beagle dogs indicated significant differences in the rates of absorption of the two enantiomers after oral administration [11-14]. In the classical theory of pharmacology, the higher the concentration and rate of absorption of a drug, the better its effect. Other parameters of S-ORT were not significantly different from those of R-ORT after oral administration. This seems to suggest that there is probably no difference in the metabolism and excretion of both enantiomers. In this study, there were no evidence of stereoselective differences between the two enantiomers, an indication that the differences in their pharmacokinetics may not be related to their plasma protein binding capacities.

CONCLUSION

There are significant absorption differences between the two enantiomers of ORT. Thus, the enantiomers may differ in their pharmacological effects.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work

Contribution of authors

This work was done by the authors named in this article and the authors accept all liability resulting from claims which relate to this article and its contents. The study was conceived and designed by Yu-Feng Wen; Wu-San Wang, Yu-Feng Wen collected and analysed the data; Wu-San Wang wrote the text and all authors have read and approved the text prior to publication.

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