Journal of Pharmaceutical and Biomedical Analysis 111 (2015) 153–158

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Short communication

Comparative pharmacokinetic studies of racemic oxiracetam and its pure enantiomers after oral administration in rats by a stereoselective HPLC method

Qiuyang Zhang, Wei Yang, Yue Yang, Han Xing, Qing Zhang, Junxiu Li, Yang Lu, Jiake He, Shuoye Yang, Di Zhao, Xijing Chen*

Center of Drug Metabolism and Pharmacokinetics, China Pharmaceutical University, Jiangsu, China

ARTICLE INFO

Article history:
Received 20 December 2014
Received in revised form 3 March 2015
Accepted 28 March 2015
Available online 4 April 2015

Keywords:
Oxiracetam
Stereoselective HPLC
Pharmacokinetics
Enantiomer
Racemate

ABSTRACT

Oxiracetam (ORC), a nootropic drug used for improving the cognition and memory, has an asymmetric carbon in its structure and exists as (S)- and (R)-ORC. The pharmacokinetic profiles of racemic oxiracetam and its pure enantiomers in rats were evaluated and compared by enantioselective high-performance liquid chromatography, which was performed on a Chiralpak ID column with a mobile phase of hexane–ethanol–trifluoroacetic acid (78:22:0.1, v/v/v). The method was validated with respect to selectivity, linearity, accuracy and precision, stability and the limit of quantification. The validation acceptance criteria were met in all cases. A saturating phenomenon of (S)-ORC was observed when the dosage ranged from 200 mg/kg to 800 mg/kg. The two enantiomers showed similar profiles in the absorb phase, and reached the maximum concentration at 2 h after oral administration. However, compared with the racemate group, the AUC/dose and C_{max}/dose ratios of (S)-ORC were higher and Cl/f was lower in enanpure (S)-ORC group. The C_{max} of (S)-ORC decreased from 21.3 ± 5.0 μg/ml to 13.2 ± 4.2 when (R)-ORC was co-administrated at the dose of 200 mg/kg. AUC_{0-t} values of (S)-ORC were different after oral administration of 200 mg/kg (S)-ORC and 400 mg/kg racemic ORC (96.7 ± 15.5 and 50.1 ± 16.3 μg h/ml). The higher absorption and slower elimination suggest that enantiopure (S)-ORC could be a promising drug that efficiently reduces clinical dosage, improves therapeutic indices, decreases toxicology risks, and results in increased therapeutic ration.

1. Introduction

Oxiracetam (ORC), 4-hydroxy-2-oxo-1-pyrrolidine acetamide (Fig. 1), is a nootropic drug used clinically to improve cognition and memory and also has protective effect on ischemic stroke [1–4]. ORC is a chiral drug with an asymmetric carbon at position 4 of the ring and exists as (S)- and (R)-ORC (Fig. 1). Clinically it is used in the form of racemic mixture. (S)-ORC is mainly responsible for the pharmacological activity of racemic ORC, and is more active than (R)-ORC in inducing long-term potentiation in rat hippocampal slices, potentiating glutamate stimulated Ca^{2+} uptake in cultured cerebellar granule cells and reverting the scopolamine including amnesia in rats [5].

Nowadays, nonstereoselective methods (such as HPLC-UV, HPLC-FLD and LC–MS/MS) have been developed and are applied to determine racemic oxiracetam in biological samples [6–9]. However, drug enantiomers may have different pharmacokinetic, toxicological and pharmacodynamic properties due to biological stereoselectivity and potential inversion. Pharmacokinetic evaluations without chiral assays could be misleading when the disposition of enantiomers is different. Camilleri et al. reported a chiral HPLC method to separate ORC enantiomers directly. However, the analysis time of that method to achieve the baseline separation of ORC enantiomers in purified samples was very long (40–50 min) and endogenous interference from biological samples was not considered in the method [10–12]. Therefore, the aim of this study is to develop and validate a simultaneous and specific stereoselective HPLC method to determine the concentration of ORC enantiomers in rat plasma.

In this study, the stereoselective pharmacokinetic profiles of racemic ORC and its pure enantiomers were investigated and compared using a specific and accurate chiral HPLC method.

Abbreviations: ORC, oxiracetam; HPLC, high performance liquid chromatography.

* Corresponding author at: Mailbox 210, #24 Tongjiaxiang, Nanjing 210009, Jiangsu, China. Tel.: +86 25 86185379; fax: +86 25 86185379.
E-mail address: chenxj-lab@hotmail.com (X. Chen).

http://dx.doi.org/10.1016/j.jpba.2015.03.039
0731-7085/

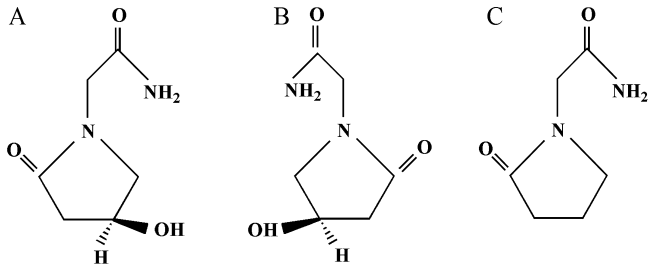

Fig. 1. Structures of (S)-ORC (A), (R)-ORC (B), and piracetam (C, internal standard).

2. Materials and methods

2.1. Chemicals and reagents

ORC and its enantiomers (purity >98%) were obtained from Luoxin Pharmaceutical Co., Ltd. (Shandong, China). Piracetam (used as internal standard, IS, purity >99.0%) was obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). HPLC-grade n-hexane and ethanol were purchased from Tedia (Cincinnati, OH, USA).

2.2. Chromatographic conditions

The analysis was performed on a rapid resolution HPLC system with a thermostated-column device and a UV detector. The chromatographic separation was achieved on a Daicel Chiralpak ID column (250 mm × 4.6 mm, 5 μm, Daicel Chemical Industries, Tokyo, Japan) maintained at 35 °C with an injection volume of 20 μl. The stationary phase is amylose tris (3-chlorophenylcarbamate). The mobile phase is hexane–ethanol–trifluoroacetic acid (78:22:0.1, v/v/v) and the flow rate was 1.0 ml/min. The determination was carried out with UV detector at 214 nm.

2.3. Sample preparation

After being thawed and equilibrated to room temperature, an aliquot of 80 μl sample was spiked with 350 μl methanol containing 3 μg/ml IS. The samples were vortexed for 5 min, followed by centrifugation at 16,000 rpm for 10 min at 4 °C. Supernatant of 350 μl was separated and evaporated to dryness. The dried samples were reconstituted with 80 μl mobile phase and centrifuged again before analysis.

2.4. Preparation of stock and standard solutions, calibration standards, and quality control (QC) samples

Stock solution containing 1 mg/ml of each ORC enantiomer was prepared in methanol. A series of standard solutions ranging from 2 μg/ml to 100 μg/ml were prepared by serial dilution of the stock solution with methanol. Piracetam was prepared at 2 mg/ml in methanol as stock solution and diluted to working solution of 3 μg/ml with methanol. All solutions were stored at 4 °C until use.

Calibration standards were prepared by evaporating a series of 80 μl standard solutions and spiking them with 80 μl blank plasma. The final concentrations of calibration were 2, 5, 10, 20, 50 and 100 μg/ml of each enantiomer in plasma. The QC samples were prepared in the same way at three concentrations: 5, 20 and 80 μg/ml of each enantiomer in plasma. The calibration standards and QC samples underwent the preparation procedure described above and were reconstituted before use.

2.5. Method validation

Validation procedures of the method were carried out according to US FDA guidelines as follows [13].

The specificity of the method was evaluated by analyzing six batches of blank plasma. The linearity was assessed by plotting the peak area of the ORC/IS ration against the concentration of ORC (2–100 μg/ml) on three consecutive days with least-squares linear regression analysis.

The lower limit of quantification (LLOQ) was established as the lowest concentration (2 μg/ml), and determined at five replicates. Intra- and inter-day precision and accuracy were evaluated by analyzing five QC samples at each concentration (5, 20, and 80 μg/ml) on the same day and mean values of five replicates within 3 consequent days. The criteria for acceptability of the data included accuracy within ±15% relative error (R.E.) from the nominal values and a precision within 15% relative standard deviation (R.S.D.) except for LLOQ (LLOQ not exceed ±20%).

The extract recovery of each enantiomer was evaluated at three concentrations (5, 20, and 80 μg/ml) in five replicates by comparing the peak area of spiked samples with untreated QC solutions. The recovery of IS was also evaluated in the same way.

The stability was evaluated at three concentrations (5, 20, and 80 μg/ml) in five replicates by analyzing samples that were kept at room temperature before preparation procedure for 4 h, kept at −20 °C after preparation procedure for 24 h, stored at −20 °C for 30 days, and after three freeze and thaw cycles. Analytes were considered stable when accuracy variance was within ±15%.

2.6. Pharmacokinetic studies

Healthy Sprague-Dawley rats were obtained from animal center of Jiangsu University. They were fed with standard rat chow and water in temperature and humidity controlled rooms. Animal studies were approved by the Animal Ethics Committee of China Pharmaceutical University.

Sprague-Dawley rats were randomly divided into six groups (six animals per group). Rats were fasted for 12 h before drug administration. Three groups of rats received 200, 400, and 800 mg/kg (S)-ORC by gavage administration, respectively. Another three groups were given racemic ORC at 400 mg/kg, 800 mg/kg and (R)-ORC at 400 mg/kg by gavage administration, respectively. Blood samples (0.2 ml) were collected *via* orbital venous plexus from rats into heparinized Eppendorf tubes at 0.5, 1, 2, 3, 4, 6, 8, 10, and 12 h after administration. Samples were centrifuged at 8000 rpm for 5 min. Plasma was collected and stored at −80 °C until analysis.

2.7. Data analysis

Pharmacokinetic analysis was performed by noncompartmental approach using Drug and Statistics (DAS 2.1, Mathematical Pharmacology Professional Committee of China, Shanghai, China). The maximum plasma concentration (C_{max}) and the time to reach maximal plasma concentration (T_{max}) were obtained directly from the concentration–time curve. Other parameters were obtained from the DAS output. Data were expressed as mean ± SD. Statistical analysis was performed using SPSS Statistics 19 (SPSS Inc., Chicago, IL, USA). Bivariate correlations analysis was used to evaluate the correlation and a p value < 0.01 indicated significant correlations. Student's t test was used to analyze the significance and a p value < 0.05 indicated significant differences.