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Comparative pharmacokinetics and distribution kinetics in brain of phencynonate enantiomers in rats

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Abstract

To investigate the pharmacokinetics in blood and the distribution kinetics in brain of enantiomers of novel anticholinergic agent phencynonate (*N*-methyl-9α-(3-azabicyclo[3,3,1]nonanyl)-2′-cyclopentyl-2′-hydroxyl-2′-phenylacetate), we collected blood and implanted microdialysis probes in the cerebral frontal cortex of rats. Phencynonate enantiomers (0.35 mg/kg, i.m.) were then cross administered, and the microdialysates were collected using *in situ* microdialysis sampling in the brain of freely moving rats, and the concentration of phencynonate enantiomers was determined by the validated method of liquid chromatography–mass spectrometry. Pharmacokinetic parameters were calculated from the blood and the brain dialysate concentrations of phencynonate enantiomers versus time data. The disposition profiles of the phencynonate enantiomers were best fitted to a first order absorption, two-compartment open model in rats. In general, there were some differences when comparing the mean kinetic parameters of *S*- and *R*-phencynonate in the blood and brain, but the distinct diversity between individual animals made the statistical difference not obvious. Therefore, stereoselective disposition of phencynonate isomers was not obviously observed in rat.

Keywords: Phencynonate; Enantiomer; Pharmacokinetics; Distribution kinetics; Liquid chromatography–mass spectrometry; Microdialysis

1. Introduction

Drug molecular handedness is a crucial structural feature of biologically active compounds, since opposite configurations at pharmacophoric groups frequently influence the biological response, mainly in terms of receptor affinity, receptor subtype selectivity, toxicity, drug metabolism and pharmacokinetics (Ariens, 1984, 1986; Ariens et al., 1988; Baker and Prior, 2002). Therefore, the stereoisomeric composition of drugs is currently receiving considerable attention, owing to its pharmacological, as well as industrial and regulatory implications (Birkett, 1989; Barraud et al., 2004). Phencynonate (*N*-methyl-9α-(3-azabicyclo[3,3,1]nonanyl)-2′-cyclopentyl-2′-hydroxyl-2′-phenylacetate) is a novel anticholinergic drug synthesized and developed by Beijing Institute of Pharmacology and Toxicology, China. Phencynonate has been evaluated in a multi-center clinical trail as a novel anti-motion sickness and anticonvulsant drug. It has been shown to be effective for the prevention of acute motion sickness, and has good effect in Meniere's disease, Parkinson's disease and falling sickness (Xu et al., 1993; Yuan et al., 1995; Dai et al., 1997; Deng and Zhang, 2001; Wang et al., 2005a,b; L.Y. Wang et al., 2005). Previous studies have revealed that phencynonate prevents motion sickness with higher efficacy and lower central inhibitory side effects compared to other motion sickness drugs, such as scopolamine and dimenhydrinate (Dai et al., 1997).

There is one chiral carbonic atom in the molecular structure of phencynonate, thus, there are two optical isomers of phencynonate with different *R*- and *S*-configurations (in Fig. 1). Investigations have indicated that phencynonate enantiomers show a distinct difference in pharmacological activity, in that the *S*-enantiomer has the highest affinity for central muscarinic receptors; among *S*-enantiomer, *R*-enantiomer and racemate,

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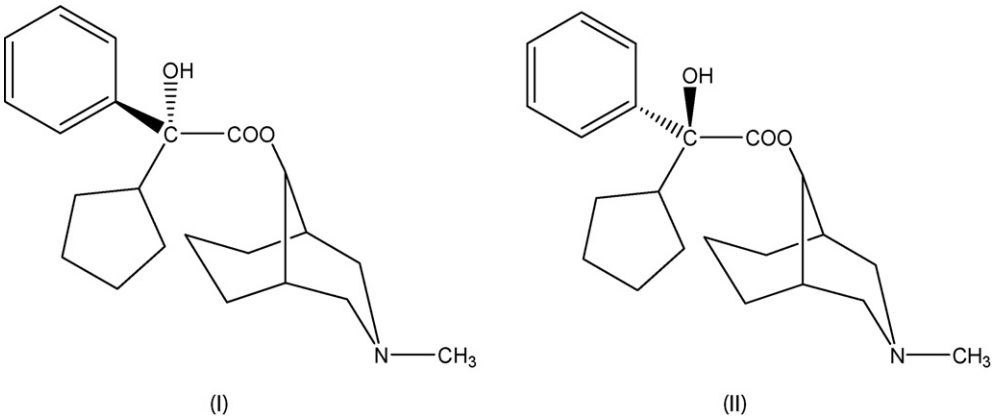

Fig. 1. Chemical structure of phencynonate enantiomers (I) *R*-phencynonate; (II) *S*-phencynonate.

S-enantiomer acts as an eutomer in racemate, and as a competitive acetylcholine muscarinic receptor antagonist (Wang et al., 2005a,b; L.Y. Wang et al., 2005). The nature of the pharmacokinetics and brain distribution of these isomers remains unknown. However, investigations of the enantiomers are typically required for the pharmacokinetics of a racemic drug, because the enantiomer concentration in the blood, and especially in the brain, did necessarily reflect the concentration at the cellular level. This is particular to the case for central nervous system-acting drugs which must negotiate the formidable blood–brain barrier before they can exert their effects, and monitoring drug concentration in the interstitial space is crucial to understanding the time course of the anticholinergic activity of the enantiomers. In addition, correlation between the pharmacokinetics and pharmacodynamics will help with the determination of dosage regimens (Ariens, 1984; Smith, 1989; Baker and Prior, 2002; Waldeck, 2003). In order to further understand the pharmacological differences between the two stereoisomers and their mechanism of action, and to develop a safe eutomer drug, we investigated the pharmacokinetics and brain distribution of the chiral antagonist *in vivo*.

Although reports concerning the analysis and the pharmacokinetics of phencynonate racemate have been published (Yuan et al., 1995; Wang and Gao, 1999), no report dealing with the pharmacokinetics and brain distribution of phencynonate enantiomers has been found. The ordinary method can hardly reach the testing requirement because of the low therapeutic dose during clinical application. We have developed a liquid chromatography–mass spectrometric method equipped with electrospray ionization (LC/ESI/MS), which can markedly improve the limit of detection (Kou et al., 2005; Y. Xu et al., 2006; Y.X. Xu et al., 2006). In the present study, we used an LC/ESI/MS method combined with *in vivo* microdialysis to investigate the pharmacokinetics and distribution kinetics of *R*- and *S*-isomers of phencynonate from the blood into the brain, through simultaneous sampling in the cerebral cortex. We also analyzed the elimination of phencynonate isomers in the systemic and cerebral circulation. Phencynonate and its enantiomers have been shown to display different affinity for the muscarinic receptors in the cerebral cortex (Wang et al., 2005a,b; L.Y. Wang et al., 2005). Therefore, we selected the cerebral cortex as the site for microdialysis sampling to study the stereoselective disposition kinetics of phencynonate enantiomers in the brain. The present study is believed to represent the first attempt at using microdialysis to study the pharmacokinetics of phencynonate and its optical isomers in rat brain. The results in this paper provide important information for developing a novel chiral drug, and for better use of phencynonate isomers in clinic practice.

2. Experimental

2.1. Drug and reagents

Phencynonate racemate, *R*-isomer and *S*-isomer, and D-thiencynonate (*N*-methyl-9α-(3-azabicyclo[3,3,1]nonanyl)-2′-cyclopentyl-2′-hydroxyl-2′-thienylacetate) as the internal standard (IS), were synthesized and provided by the Beijing Institute of Pharmacology and Toxicology, China. The purity of phencynonate enantiomers and D-thiencynonate was >99%, the optical rotation of the enantiomers in chloroform: *S*-enantiomer is $[\alpha]^{20}$ −11.8, and *R*-enantiomer is $[\alpha]^{20}$ +11.8 (Gao et al., 1990; Han et al., 2005; H. Liu et al., 2005; Y.Q. Liu et al., 2005). Methanol was of HPLC grade and was purchased from Fisher Scientific (Fair Lawn, NJ, USA). Formic acid was of HPLC grade and purchased from Dikma Reagent Company (Beijing, China). Distilled water was prepared in our laboratory. The above reagent solutions were filtered through a 0.45 μm organic film. All other reagents and chemicals were of analytical grade.

The artificial cerebrospinal fluid (ACSF) buffer (124 mM NaCl, 5 mM KCl, 1.25 mM NaH_2PO_4, 2 mM MgSO4, 2 mM $CaCl_2$, 26 mM $NaHCO_3$, 10 mM D-glucose) was prepared weekly, filtered, degassed to obtain a pH of 7.1–7.4, and used as the perfusate. All reagents used in the preparation of buffer solutions were of analytical reagent grade.

2.2. Apparatus and conditions

An Agilent HPLC 1100 system (Palo Alto, CA, USA) was used for analysis, which included an HP 1100 G1312A

binary pump, G1379A vacuum degasser, G1313A autosampler and G1315B diode-array detector. The chromatography was performed on a BetaBasic-18 column (150 mm × 2.1 mm i.d., 3 μm; Thermo Electron, CA, USA) at ambient temperature. A C_{18} guard column (13 mm × 4.6 mm i.d., Upchurch Scientific) was used to protect the analytical column. The mobile phase was composed of methanol and water (85:15, v/v), containing 0.5‰ formic acid at pH 6, which was pumped at a flow-rate of 0.2 ml/min. The sample injection volume was 10 μl and the run time of samples was 7 min. The effluent was on-line transferred to the ESI/MS system without splitting.

Mass spectrometric measurements were performed on an LCQ Deca XP ion-trap mass spectrometer (Thermo Finnigan, San Jose, CA, USA), equipped with an electrospray ion source working in positive ion mode. The instrument was connected to the LC system outlet. Nitrogen was used as a sheath gas and an aux/sweep gas in the ion trap. MS detection of phencynonate and the IS was performed at *m/z* 358.40 (phencynonate) and *m/z* 364 (IS), and their reaction ion monitoring (SRM) was both at *m/z* 156, which was used for quantification. ESI was operated at the sheath flow-rate of 35 psi, capillary temperature of 320 °C, capillary voltage of 18 V and skimmer voltage of 70 V. The collision-induced dissociation energy for the two compounds was 36%. The transitions of *m/z* 358.4 → 156 for the analyte and 364 → 156 for the IS were monitored using an isolation width of 1.0 Da. The product ion *m/z* 156 was monitored because it was the most abundant and stable ion for both analyte and internal standard. The divert valve was programmed to waste the first 1 min and the last 0.5 min. The LC system and mass spectrometer were controlled using the Thermo Finnigan Chemstation software (Version 1.3). Data were processed using the IS method, plotting peak area ratios versus relative analyte/IS concentration with a weighting factor 1.

The brain microdialysis systems consisted of a CMA/102 microinjection pump (CMA, Stockholm, Sweden) and microdialysis probes with dialyzing membranes. A dialyzing membrane with a length of 4 mm and an outer diameter of 0.5 mm (CMA/12, CMA, Stockholm, Sweden) was used for brain sampling for freely moving animals.

2.3. Animals and blood samples collection

Adult male Sprague–Dawley rats (250–280 g) were obtained from the Laboratory Animals Center of Capital Medical University (Beijing, China). The animals were pathogen-free and allowed to acclimate in our environmentally controlled quarters (22 ± 1 °C) for at least 5 days before being used for experiments. The rats were fasted for 12 h before administration, but had free access to water. The experiments were carried out in accordance with current guidelines for the care of laboratory animals and ethical guidelines for investigations of experiments in conscious animals (Zimmermann, 1983). In addition, the protocols employed had been approved by the Animal Care and Use Committee of Capital Medical University.

Six rats were randomly divided into two groups (groups A and B), the two groups of rats were used themselves for comparison and administrated alternatively after a washing time. The blank blood was collected from the orbital vein of the rats before administration. The rats in group A were administered phencynonate *R*-isomer 0.35 mg/kg i.m., and group B was administered *S*-isomer in the first experiment. Blood (0.1 ml) was collected at 1, 2, 5, 10, 30, 60, 120, 240 and 480 min after administration. After 2 weeks drug elimination, the second experiment was performed. The rats in group A were administered *S*-isomer 0.35 mg/kg and the rats in group B were administered *R*-isomer 0.35 mg/kg, followed by blood collection as above. All blood samples were sealed and stored at −20 °C until analysis.

2.4. Microdialysis experiments and brain sample collection

Another six rats were randomly assigned into two groups (groups A and B) and cross administered as above. The rats were initially anesthetized with chloral hydrate (10%, 0.4 ml/100 g, i.p.), and remained anesthetized throughout the experimental period. Rats were mounted on a stereotaxic frame (Anhui, China) for brain microdialysis and body temperature was maintained at 37 °C with a heating light. The brain microdialysis probe was perfused with Ringer's solution (147 mM Na^+, 2.2 mM Ca^{2+}, 4 mM K^+, pH 7.0) at a flow-rate of 1.0 μl/min. After an incision made in the scalp, the skull was exposed and a small hole was drilled according to demand. A CMA/12 guide cannula was implanted into the cortex and secured permanently in position with dental cement. After washing with Ringer's solution, the microdialysis probe was implanted via the guide cannula into the frontal cortex (coordinates: AP, −1.2 mm; LAT, −2 mm; VERT, 4 mm), according to the atlas of Paxinos and Watson (1982). The positions of the probes were verified by standard histological procedures at the end of the experiments.

The microdialysis probe was perfused with ACSF via a peep tube connected to a CMA/100 pump, at a flow-rate of 1.0 μl/min (Qiao et al., 2004). Outflows from brain microdialysis probes were connected to a microfraction collector (CMA/140) and collected every 20 min. After dialysate levels were stabilized, the drug-free samples were collected and the *R*- and *S*-isomers of phencynonate (0.35 mg/kg) were administered intramuscularly. The collection time 20, 40, 60, 100, 140 and 180 min, and 5, 10, 20 and 36 h, 20 μl of each sample microdialysate was collected for 36 h and stored at −20 °C until analysis.

2.5. Extraction of samples

Blood blank samples (0.1 ml) were spiked with 100 μl of each phencynonate isomer and 100 μl of IS stock solution (D-thiencynonate, 50 ng/ml) (Xue et al., 2002). Then, 100 μl of 0.2 mol/l NaOH and 2 ml mixed solvent (ethyl ether: dichloromethane, 2:1, v/v) were added. The combined samples were adjusted to pH 10, vortex-mixed for 2 min, and centrifuged at 3000 × *g* for 10 min. The upper organic phase was extracted twice and combined. Then 0.2 ml 0.1 mol/l HCl was added to the combined organic portions and the pH was adjusted to 2–3. The mixed system was vortex-mixed and centrifuged as above, and the upper organic layer was discarded. Then, 0.2 ml 0.2 mol/l NaOH and 2 ml mixed solvent were added to the lower aqueous

phase and the pH was adjusted to 10, which was vortex-mixed, centrifuged and separated twice. The upper organic potions were combined and evaporated to dryness at 40 °C, the residue was dissolved in 100 μl methanol and transferred to HPLC auto-sampler vials, and aliquots (10 μl) were injected into the LC/MS/MS system.

Brain blank samples (20 μl) from microdialysis were spiked with 20 μl of each phencynonate isomer and the IS stock solution. The procedure was the same as that described above. The residue was dissolved in 20 μl the methanol and transferred to HPLC auto-sampler vials, and aliquots (10 μl) were injected into the LC/MS/MS system.

2.6. Assay validation

Calibration samples of blood were prepared by taking 0.1 ml pooled rat blank blood with the appropriate amount of phencynonate (100 μl) and IS (100 μl) solution, so that the final concentration of phencynonate was 1, 2, 5, 10, 25, 50 and 100 ng/ml, and IS was 50 ng/ml. The accuracy and precision of the method were evaluated, based on the data from quality control blood samples at three concentrations in five validation runs. The intra- and inter-day precision and accuracy of the assay were obtained by comparing the experimental to the theoretical concentration of phencynonate spiked in blank samples. The recovery of extracted phencynonate was tested by using the LC/MS assay described to quantify the amount of phencynonate in rat blood, and comparing that to the assay value for a known amount of phencynonate prepared and assayed in methanol.

Microdialysis probe *in vivo* relative recovery was calibrated by measurements of *in vitro* recovery and *in vitro* loss and *in vivo* loss (Scheller and Kolb, 1991; Van Belle et al., 1993). *In vitro* relative recovery was determined by placing the microdialysis membrane in a 100 ng/ml phencynonate solution in ACSF, and perfusing with ACSF at 1 μl/min. Samples of the dialysate were collected at 20 min intervals and phencynonate concentrations were analyzed by LC/MS to give the relative recovery *in vitro*. *In vitro* relative loss was determined by perfusing 100 ng/ml phencynonate solution in ACSF into a microdialysis probe, whose dialysis membrane was placed in a vial containing ACSF at 37 °C. Relative loss *in vitro* was calculated using the different phencynonate concentrations in the perfusate and microdialysate. In a similar manner to the determination of the *in vitro* loss, the *in vivo* loss was calculated by perfusing 100 ng/ml phencynonate solution in ASCF into a microdialysis probe, whose dialysis membrane was inserted into rat brain. The probe relative recovery (RR) and relative loss (RL) are defined by the following Eq. (1), in which C_d is the concentration in the microdialysate, C_s is the concentration of the sample, and C_p is the concentration in the perfusate. The probe *in vivo* relative recovery is calculated by Eq. (2).

$$\mathrm{RR}\,(\%) = \left(\frac{C_d}{C_s}\right) \times 100; \qquad \mathrm{RL}\,(\%) = \frac{C_p - C_d}{C_p} \times 100 \quad (1)$$

$$\frac{\mathrm{RR}_{\text{in vivo}}}{\mathrm{RL}_{\text{in vivo}}} = \frac{\mathrm{RR}_{\text{in vitro}}}{\mathrm{RL}_{\text{in vitro}}} \quad (2)$$

2.7. Pharmacokinetic and statistical analysis

The concentration–time data and pharmacokinetic parameters for phencynonate enantiomers in rats were calculated by 3P87 software (edited by Chinese Mathematic Pharmacological Committee, Chinese Pharmacologic Society). The concentration data for phencynonate enantiomers were obtained by correcting the microdialysis data for *in vivo* recovery of the respective microdialysis probes. The determination of models was judged by the sum of square, the determination coefficient (r^2) and the Akaike's information criterion.

Statistical analysis was performed using SPSS software on an IBM microcomputer. A paired *t*-test was used for comparison of pharmacokinetic parameters between the *R*- and the *S*-isomer of phencynonate. Data are expressed as mean ± S.D. and statistical significance was determined at the $P < 0.05$ level.

3. Results and discussion

Of the several techniques that have been employed to study drug transport to the brain (Pardridge, 1998), one of the more traditional is the brain tissue homogenate method. However, with that technique, only one point of the entire brain drug concentration–time profile can be obtained from each animal, as each determination requires the sacrifice of more experimental animals. Many animals must, therefore, be used, and the inter-animal variability often compromises the results. Microdialysis, an *in vivo* sampling technique, provides a unique tool for evaluating extracellular tissue drug concentration, offering significant advantages over tissue sampling techniques. The main advantage for the present purpose is related to the opportunity to continuously and simultaneously monitor drug concentrations in the same animals when coupled to an adequate analytic technique. These methods can be used to determine the drug concentrations unbounded with protein and the extracellular drug in most tissues (Hurd et al., 1988; Hsiao et al., 1990; Sato et al., 1994, 1996; Paez and Hernandez, 1996). Depending on the sensitivity of the analytical technique, finite volumes of dialysates are required, and the measured drug concentrations, therefore, actually represent the total drug concentrations for a given time period. Microdialysis, in combination with LC/MS/MS, is a better technique for determining lower concentrations of the chiral drug. We studied the concentration–time course of phencynonate enantiomers in rat brain with this technique. The samples were obtained simultaneously and continuously for 36 h from awake animals, with no fluid loss. The full time period for the appearance and disappearance of phencynonate enantiomers in the brain could be acquired from a single animal. The microdialysis sample volumes were quite small and the concentration levels of analytes are often low, therefore, ideal results can be obtained with direct injection with the dialysate samples.

3.1. Quantitative basis

The chromatograms for the same concentration of phencynonate and its two optical isomers in an LC/ESI/MS working in positive ion mode were all similar when the collision-induced

dissociation energy for the two isomers was 36%. The corresponding values of phencynonate enantiomers were both identical when the same concentrations (5, 25, 100 ng/ml) of *R*- and *S*-isomers were prepared. The precision of the same concentration of phencynonate and its two optical isomers was <8%. This is the basis of quantitative analysis for phencynonate enantiomers according to the calibration curve.

3.2. Assay validation

The LC/MS/MS method showed good sensitivity, specificity, precision, accuracy, recovery and linearity for quantification of phencynonate enantiomers. The standard curve was linear over the range 1–100 ng/ml in the blood or brain. Typical equations for the calibration curves for phencynonate were $y = -0.0174 + 0.0164x$ ($r^2 > 0.998$) in rat blood, and $y = 0.0136766 + 0.0523011x$ ($r^2 > 0.997$) in the brain. The inter- and intra-day precision was <10% and the accuracy percentage error was <7%. The limit of detection (LOD) was 0.1 ng/ml and the limit of quantification (LOQ) was 1 ng/ml. The recovery of extracted phencynonate enantiomers in blood was >70%.

For microdialysis, the relative *in vitro* recovery, *in vitro* loss and *in vivo* loss was 27.80, 30.13 and 12.31%, respectively. The *in vivo* recovery, therefore, was calculated according to Eq. (1) to be 11.35%. The concentration of phencynonate enantiomers in rat CSF was calculated using the *in vivo* recovery, by multiplying the concentration determined in the microdialysate by a factor of 100/11.35.

3.3. Pharmacokinetic comparison of phencynonate enantiomers in blood

The data were calculated and treated by program 3P87. The concentration–time curves of phencynonate enantiomers in rat blood were fitted to a first order absorption, two-compartment open model after i.m. administration of single-dose phencynonate (Fig. 2). The main pharmacokinetic parameters are shown in Table 1. The effect of individual animal diversity was decreased further when rats were randomly divided into two groups, the two groups of rats themselves were used as the comparison, and they were administrated alternatively. Comparison of the main pharmacokinetic parameters between the *S*- and the *R*-isomers of phencynonate using the *t*-test gave the following results.

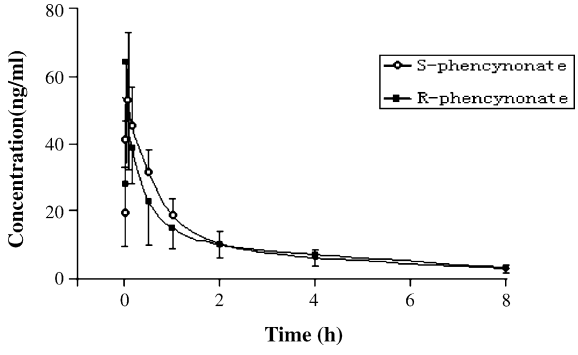

Fig. 2. Blood concentration–time curve of the two optical isomers of phencynonate after a single intramuscular (0.35 mg/kg) administration to rats ($n = 6$, mean ± S.D.).

Table 1
The main pharmacokinetic parameters of the two enantiomers of phencynonate in blood after administration of single i.m. (0.35 mg/kg) doses in rats ($n = 6$, mean ± S.D.)

Parameters (unit)	*S*-Phencynonate	*R*-Phencynonate
V/*F* (L)	0.006 ± 0.002	0.006 ± 0.003
$t_{1/2\alpha}$ (h)	0.350 ± 0.107	0.205 ± 0.146
$t_{1/2\beta}$ (h)	4.68 ± 2.43	3.48 ± 0.64
$t_{1/2Ka}$ (h)	0.021 ± 0.021	0.007 ± 0.005
K_{21} (h^{-1})	0.580 ± 0.242	1.32 ± 0.83
K_{10} (h^{-1})	0.661 ± 0.140	0.880 ± 0.562
K_{12} (h^{-1})	1.13 ± 0.72	2.93 ± 2.04
AUC ((ng/mL) h)	94.33 ± 17.25	89.02 ± 38.09
Cls (L/h)	0.004 ± 0.001	0.005 ± 0.002
t_{max} (h)	0.088 ± 0.067	0.042 ± 0.018
C_{max} (ng/mL)	51.91 ± 9.33	57.21 ± 14.70

Mean values of $t_{1/2\alpha}$, $t_{1/2\beta}$, $t_{1/2ka}$ and mean peak time (t_{max}) for *S*-phencynonate were all greater than that for *R*-phencynonate, but the difference was not significantly different ($P > 0.05$). From these mean half-life values, the absorption, distribution and elimination of *S*-phencynonate were slower than those for *R*-phencynonate, but there were no statistically significant differences in these parameters, because of the distinct difference in S.D. between the two groups. The values of the peak concentration (C_{max}) of the phencynonate enantiomers indicated that there was also no statistically significant difference ($P > 0.05$). There was no statistically significant difference in AUC between the *R*- and the *S*-isomers of phencynonate ($P > 0.05$).

The results showed that the main pharmacokinetic parameters obtained from *S*- and *R*-phencynonate in rat blood were not significantly different. These findings showed that there was no obvious stereoselectivity for the disposition of phencynonate enantiomers in rats.

3.4. Distribution kinetics of phencynonate enantiomers in rat brain

Distribution kinetic parameters of *S*- and *R*-phencynonate in rat brain were estimated by numeral integration with the statistical moment method using program 3P87, and the main kinetic parameters are shown in Table 2. The concentration–time curves of phencynonate enantiomers in rat brain are shown in Fig. 3. Mean AUC of *S*-phencynonate was greater than that of

Table 2
The main pharmacokinetic parameters of the enantiomers of phencynonate in brain after administration of single i.m. (0.35 mg/kg) dose in rats ($n = 6$, mean ± S.D.)

Parameters (unit)	*R*-Phencynonate	*S*-Phencynonate
AUC ((ng/mL) h)	93.36 ± 65.08	147.8 ± 65.42
AUMC ((ng/mL) h^2)	799.31 ± 615.7	909.2 ± 493.2
MRT (h)	8.07 ± 1.55	9.068 ± 1.830
K (h^{-1})	0.059 ± 0.028	0.047 ± 0.035
$t_{1/2}$ (h)	13.94 ± 6.15	22.97 ± 15.94

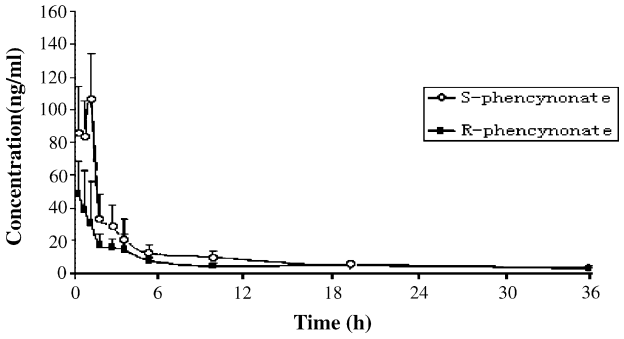

Fig. 3. Brain concentration–time curve of two isomers of phencynonate after a single intramuscular (0.35 mg/kg) administration to rats ($n = 6$, means ± S.D.).

R-phencynonate, which shows that the level of *S*-phencynonate in the brain was higher than that of *R*-phencynonate, but there was no statistically significant difference in AUC between the *R*- and the *S*-isomer ($P > 0.05$). Mean elimination half-life ($t_{1/2}$) of *S*-phencynonate was greater than that of *R*-phencynonate, which shows that elimination of *S*-phencynonate was slower than that of *R*-phencynonate, but because of the distinct difference in S.D. between the two groups, there was no statistically significant difference between *S*- and *R*-phencynonate ($P > 0.05$).

In conclusion, the present study represents an effective attempt to investigate the *in vivo* pharmacokinetics of phencynonate isomers in the blood and brain of rats by LC/MS/MS in combination with microdialysis. Compared with other *in vivo* methods for kinetic study in the brain, microdialysis offers the advantage of being able to continuously monitor drug concentrations in the extracellular compartment in the same animals, causing less biological fluid loss and, therefore, minimal influence on hemodynamics. The disposition profiles of the phencynonate enantiomers were best fitted to a first order absorption, two-compartment open model in rats. In general, there were some differences between the mean kinetic parameters of *S*- and *R*-phencynonate in the blood and brain, animals made the statistical difference not obvious. Thus, the stereoselective deposition of phencynonate isomers was not obviously observed in rats. The results suggest that the pharmacokinetics and the distribution kinetics in the brain of phencynonate isomers in rats had no obvious stereoselectivity. Perhaps stereoselective processes are, nevertheless, involved in relationships between the pharmacokinetics and pharmacodynamics of phencynonate enantiomers in the central nervous system.

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