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Research Article

Thiencynonate *S*-Isomer as the Novel Eutomer of Muscarinic Receptor Antagonist: A Comparison of Pharmacodynamics, Pharmacokinetics and Brain Distribution Kinetics

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Abstract

The aim of this study was to investigate whether thiencynonate S-isomer can act as the eutomer of muscarinic receptor antagonist via the competitions with binding of [^{3}H]-QNB, *in vivo* pharmacokinetics and brain distribution kinetics of its enantiomers. The muscarinic acetylcholine receptors were produced from rat cerebral cortex. The samples from brain implanted micro dialysis probes in the cerebral cortex of rats were collected. Thiencynonate enantiomers were cross administered to rats, the microdialysates were collected using *in situ* microdialysis sampling while the rats were freely moving. The concentrations of the two enantiomers were determined by a validated method using a liquid chromatography–mass spectrometry. The results suggested that the affinity of S-isomer to the muscarinic receptor was more potent than that of *R*-isomer, and there was statistically significant difference. The disposition profiles of both the enantiomers exhibited a best fit with a first order absorption, two-compartment open model. There were some marked differences when comparing the mean values of the main kinetic parameters of *S*- and *R*-isomer. From the data of the main kinetic parameters and M receptor affinity activities, thiencynonate *S*-isomer is better for acting as the eutomer and novel competitive chiral candidate for the acetylcholine muscarinic receptor antagonist.

Keywords: Thiencynonate; *S*-isomer; Eutomer; Receptor affinity; Pharmacokinetics; Brain distribution

Introduction

Stereochemistry is a crucial structural characteristic for biological active compounds, since various configurations at the pharmacophore influence the results of the pharmacological response, mainly in terms of receptor affinity, toxicity, drug metabolism and pharmacokinetics [1,2]. Therefore, the importance of stereo chemistry in drug action is gaining greater attention owing to its pharmacological, industrial, and regulatory implications [3]. Thiencynonate {N-methyl-9α-(3-azabicyclo [3,3,1] nonanyl-2'-cyclopentyl-2'-hydroxyl-2'-thienylacetate} is a novel anticholinergic lead compound synthesized by the Beijing Institute of Pharmacology and Toxicology in China [4-7]. The structure of thiencynonate is similar to that of phencynonate, which is a very effective anticholinergic drug and has been widely used in clinic practice for many years [8-14]. Thiencynonate has been also shown to be effective for the prevention of motion sickness, and has especially beneficial effects in Parkinson's disease; this compound has also potent sedative and hypnotic effects when given in combination with a sub-threshold dose of pentobarbital, inhibiting tracheobronchial contractile responses of guinea pig and salivation-induced oxotremorine [7,15,16]. Therefore, this chiral compound is a very valuable drug candidate for anticholinergic agent.

There is a single chiral carbon in the molecular structure of thiencynonate, yielding its two optical isomers, each with an *R* or *S* configuration (Figure 1), thus, the evaluation of the dynamic and kinetic stereo selective properties of this chiral drug is of great relevance. Up to date, the affinity for muscarinic receptors, pharmacokinetics and brain distribution kinetics of these two isomers remains unknown; however, the investigations of the enantiomers are typically required to understand the pharmacodynamics and pharmacokinetics of a racemic drug, because the enantiomer concentrations in the blood, especially in the target tissue brain and the receptor affinity necessarily reflect the drug concentration at cellular level and drug action. 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. In this regard, for thiencynonate, monitoring drug concentration in the interstitial space is crucial to understanding the

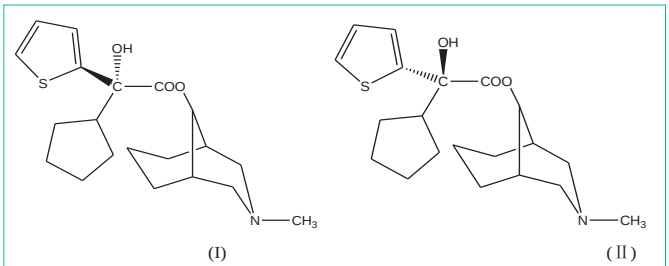

Figure 1: Chemical structure of thiencynonate enantiomers, (I) *S*-thiencynonate; (II) *R*-thiencynonate.

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time course of the anticholinergic activity of its enantiomers [17]. To further understand the pharmacological differences between these two isomers, and to develop a safe and effective eutomer, we investigated the pharmacodynamics, pharmacokinetics and brain distribution of thiencynonate.

Binding sites with a high affinity and specificity for [^{3}H]-Quinuclidinyl Benzilate (QNB) were present in homogenates of rat brain [18]. The characteristics of the binding sites resemble those of central muscarinic cholinergic receptors. Muscarinic antagonists displaced the specific [^{3}H]-QNB binding sites, while nicotinic and non-cholinergic drugs possess little affinity for [^{3}H]-QNB-binding sites [19], thus, we used the [^{3}H]-QNB to study thiencynonate isomers for the selective affinity of muscarinic cholinergic receptors. Only one analysis of the pharmacokinetics of racemic thiencynonate using Liquid Chromatography–Mass Spectrometry with Electrospray Ionization (LC/ESI/MS) has been reported by our laboratory [20]. No report of the receptor affinity, pharmacokinetics and brain distribution of the two isomers has been published to date. In the present study, we used the methods of competitions with the binding of [^{3}H]-QNB, LC/ESI/MS combined with *in vivo* microdialysis to investigate the pharmacokinetics and distribution kinetics of the *R*- and *S*-isomers of thiencynonate, via sampling of the membrane receptor, blood and the extracellular fluid in the cerebral cortex. As the present study represents the first attempt to investigate the pharmacodynamics and pharmacokinetics of the thiencynonate enantiomers, the results provide the very valuable information for discovering a novel chiral eutomer and drug candidate.

Materials and Methods

Chemicals and materials

Racemic thiencynonate, *R*-thiencynonate, *S*-thiencynonate, and *S*-phencynonate as the Internal Standard (IS) were synthesized by the Beijing Institute of Pharmacology and Toxicology, China. The purities of the two thiencynonate enantiomers and *S*-phencynonate were all more than 99% [5, 6]. [^{3}H]-Quinuclidinyl Benzilate (QNB) was purchased from Amersham Pharmaceutical Company (Amersham, UK). Atropine was purchased from Sigma (St Louis, MO, USA). 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 the highest quality or analytical grade available.

The Artificial Cerebrospinal Fluid (ACSF) buffer (124 mM NaCl, 5mM KCl, 1.25mM NaH_2PO_4, 2mM MgSO4, 2mM $CaCl_2$, 26mM $NaHCO_3$, 10mM 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.

Apparatus and conditions

An Agilent HPLC 1100 system (Palo Alto, CA, USA) was used for the analysis, which included an HP 1100 G1312A binary pump, a G1379A vacuum degasser, a G1313A autosampler, and a G1315B diode-array detector. The chromatography was performed on a BetaBasic-18 column (150×2.1 mm i.d., 3 μm; Thermo Electron, CA, USA) at ambient temperature. A C_{18} guard column (13×4.6 mm i.d., Upchurch Scientific) was used to protect the analytical column. The mobile phase was composed of methanol and water (70:30, v/v), containing 0.75% 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 the samples was 6 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 thiencynonate and the IS was performed at *m/z* 364 (thiencynonate) and *m/z* 358 (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 was 36% for each of the two compounds. The transitions of *m/z* 364→156 for the analyte and 358→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 analytes and the internal standard. The divert valve was programmed to go to waste for the first minute and to last 0.5 min. The LC system and mass spectrometer were controlled using Thermo Finnigan Chemstation software (version 1.3). Data were processed using the internal standard method, plotting the peak area ratios versus the relative analyte/IS concentrations.

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

Animals and blood samples collection

Adult male Sprague–Dawley rats (250–300 g) were obtained from the Laboratory Animal Center of Capital Medical University (Beijing, China). The animals were pathogen-free and allowed to acclimate in our environmentally controlled colony (22±1°C) for at least 5 days before being used for experiments. The rats were fasted for 12 hour before drug administration, but had free access to water. The experiments were carried out in accordance with the current guidelines for the care of laboratory animals and ethical guidelines for investigations of experiments in conscious animals [21]. In addition, the protocols employed were approved by the Animal Care and Use Committee of Capital Medical University.

Rats were randomly divided into two groups (group A and group B); the two groups of rats provided their own controls. Each group received both isomers of thiencynonate in an alternating design, with an adequate washout time for drug elimination provided between each administration. Blood was collected from the intubation vein of the rats before and after drug administration. The rats in group A were intramuscularly administered *R*-thiencynonate at 0.35 mg/kg. The rats in group B were administered *S*-thiencynonate with the same dose in the first experiment. Blood (0.1 ml) was collected at 1, 2, 5, 10, 30, 60, 120, 240, 480 and 640 min after drug administration. To allow for drug elimination, 2 weeks later the second experiment was

performed. The rats in group A were administered *S*-thiencynonate at 0.35 mg/kg, and the rats in group B were administered *R*-thiencynonate at 0.35 mg/kg, followed by blood collection as described above. All blood samples were sealed and stored at –20°C until analysis.

Microdialysis experiments

Another six rats were randomly assigned into two groups (group A and group B). The rats were initially anesthetized with chloral hydrate (10 %, 0.4 ml/100 g, i.p.), and remained anesthetized throughout the experimental period. The 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. 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). The positions of the probes were verified by standard histological procedures at the end of the experiments [22,23].

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. Outflows from brain microdialysis probes were connected to a micro fraction collector (CMA/140) and collected every 20 min. After dialysate levels stabilized, the drug-free samples were collected and the *R*- and *S*-isomers of thiencynonate (0.35 mg/kg) were administered intramuscularly. The collection times were 20, 40, 60, 100, 140 min, and 3, 5, 8, 15 and 21 h. At each time point, 20 μl of the sample microdialysates was collected and stored at –20°C until analysis.

Extraction of samples

Blood samples (0.1 ml) were spiked with 100 μl of each thiencynonate isomer and 100 μl of IS stock solution (*S*-phencynonate, 50 ng/ml). Then, 100 μl of 0.2 mol/l NaOH and 2 ml of 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 of 0.1 mol/l HCl was added to the combined organic portions and the pH was adjusted to 2–3. The system was vortex-mixed and centrifuged as above, and the upper organic layer was discarded. Then, 0.2 ml of 0.2 mol/l NaOH and 2 ml of 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 portions were combined and evaporated 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 microdialysis samples (20 μl) were spiked with 20 μl of each thiencynonate 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.

Assay validation

Calibration blood samples were prepared by taking 0.1 ml of rat blood pooled with the appropriate amount of thiencynonate (100 μl) and the IS (100 μl) solution, so that the final concentration of thiencynonate was 1, 2, 5, 10, 25, 50 and 100 ng/ml, and IS was 50 ng/ml. Both 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. Both the intra- and inter-day precision and accuracy of the assay were obtained by comparing the experimental to the theoretical concentration of spiked thiencynonate in blank samples. The recovery of extracted thiencynonate was tested using the same LC/MS assay as that used to quantify the amount of thiencynonate in rat blood, and comparing that to the assay value for a known amount of thiencynonate 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 [24]. *In vitro* relative recovery was determined by placing the microdialysis membrane in a 100 ng/ml thiencynonate solution in ACSF, and perfusing ACSF at 1 μl/min. Samples of the dialysate were collected at 20-min intervals and thiencynonate concentrations were analyzed by LC/MS to give the relative recovery *in vitro*. *In vitro* relative loss was determined by perfusing 100 ng/ml thiencynonate 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 thiencynonate concentrations in the perfusate and microdialysate. Similarly, *in vivo* loss was calculated by perfusing 100 ng/ml thiencynonate solution in ASCF into a microdialysis probe, whose dialysis membrane was inserted into rat brain. The probe Relative Recovery (*RR*) was the ratio of the drug concentrations in the dialysate and out of the dialysis tube. The Relative Loss (*RL)* was the drug concentration ratio of the concentration difference obtained from the input and output perfusate, and the incipient drug concentration in the perfusate [24]. The *in vivo RR* and *RL* were defined by the following equations [a], in which C_d is the drug concentration in the microdialysate, C_s is the drug concentration in the sample, and C_p is the incipient drug concentration in the perfusate. The probe *in vivo* relative recovery was calculated with equation [b]

$$RR = (C_d/C_s)\times 100\%;\ RL = (C_p - C_d)/C_p \times 100\% \quad [a]$$

$$RR_{in\ vivo}/RL_{in\ vivo} = RR_{in\ vitro}/RL_{in\ vitro} \quad [b]$$

Pharmacokinetics and brain distribution

The concentration over time data and pharmacokinetic parameters for the thiencynonate enantiomers in rats were calculated by 3P97 software (edited by Chinese Mathematic Pharmacological Committee, Chinese Pharmacologic Society). The concentration data for the thiencynonate 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.

Measurement of competitive inhibition form receptor

To explore and compare the anticholinergic activities of thiencynonate enantiomers, the affinity of these compounds were tested by the radioligand binding assay with muscarinic acetylcholine receptors from rat cerebral cortex. According the methods of Yamamura and Snyder [18], the male Sprague-Dawley rats (100-150 g) were decapitated and their brains were rapidly removed.

After excision of the cerebella, each rat brain was homogenized in 10 volumes of ice-cold 0.32 M sucrose in a Polytron homogenizer (15000 rpm, 1 min, at 4 °C). The whole homogenate was centrifuged for 10 min at 1000 g and 4 °C. The pellet (crude nuclear fraction) was discarded and the resultant supernatant fluid was centrifuged again for 30 min at 20,000 g. After the supernatant fluid was discarded, the precipitate was suspended with 5 volumes of 50 mM Na^+/K^+ PBS buffer (pH 7.4). The membrane protein was determined by the method of Lowry using bovine-serum albumin as the standard [26], and stored at –80 °C for the [^{3}H]-QNB-binding studies.

The assays were conducted in 0.5 ml total reaction volume and 100 µl of the receptor membrane protein. 120 µl of [^{3}H]-QNB was added to each test tube and formed the final concentrations, and for the inhibition experiments, 6 nM [^{3}H]-QNB was used. Nonspecific binding was assayed in the presence of 10 µM atropine sulfate. The test tube was added by the different concentrations of test drugs. All test tubes were filled to 0.5 ml of final volumes with Na^+/K^+ PBS buffer, mixed and incubated for 30 min at 37 °C. The reaction was terminated in ice-water bath. The contents were passed through a Glass Fiber filter (GF/C) over a vacuum. The filters were washed three times under vacuum with 3 ml of ice-cold buffer. Every determination of binding was performed in triplicate, together with triplicate samples containing unlabeled QNB to determine nonspecific [^{3}H]-QNB binding. The filters were placed in vials containing 1 ml of scintillation liquid, maintained for 12 hours. The radioactivity was then assayed by liquid scintillation spectrometry (Beckman LS6500).

Data calculation and statistical analysis

Data were analyzed by curvilinear regression using the program Graph Pad (ISI, Philadelphia, PA). All data points were determined in duplicate. The points were plotted as the mean of at least three independent experiments. In the [^{3}H]-QNB equilibrium binding assay, the data from the direct binding experiments were fitted to the equation $B = B_{max} \times F/(K_d + F)$, where K_d is the dissociation constants, B_{max} is the maximum binding capacity, and F is the free concentration of the radioligand [^{3}H]-QNB. In inhibition binding assay, the curves were fitted by computer according to a logisteric four parameter function by using the program GraphPad. The IC_{50} values were obtained from at least three separate experiments performed in triplicate with 6–8 concentrations of drugs. The inhibition constants (K_i) were calculated using the Cheng-Prusoff equation, $K_i = IC_{50}/(1 + L/K_d)$, where L and K_d are the concentration and the equilibrium dissociation constant of [^{3}H]-QNB, respectively.

A paired *t* test was used for comparison of pharmacokinetic parameters between the *R*- and the *S*-isomers of thiencynonate. The data are expressed as the mean ± S.D. Variance (ANOVA) was used for statistical analysis of the data. $p<0.05$ was considered statistically significant.

Results and Discussion

Microdialysis is an *in vivo* sampling technique that provides a unique tool for evaluating extracellular tissue drug concentrations, 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 unbound drug concentrations and the extracellular drug concentration in most tissues [23,24]. Depending on the sensitivity of the analytical technique, finite volumes of dialysates are required, and the measured drug concentrations actually represent the total drug concentrations for a given time period. Microdialysis, in combination with LC/MS/MS, is a much better technique for determining lower concentrations of the chiral drug. We can use the technique to study the concentration–time course of thiencynonate enantiomers in rat brain. The samples were obtained continuously for 24 h from the awake animals, with no fluid loss. The full time course for the distribution and elimination of thiencynonate enantiomers in the brain could be acquired from a single animal. The microdialysis sample volumes were quite small and the concentration levels of analytes were often low. Therefore, better results could be obtained with direct injection with the dialysate samples (Figure 2).

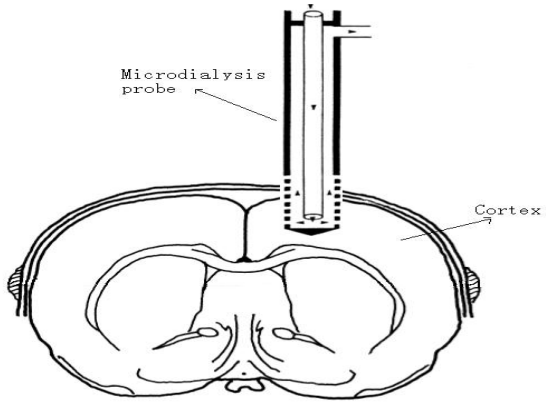

Figure 2: The location of the micro dialysis probe in rat brain.

Quantitative basis

The chromatograms for the same concentration of thiencynonate 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 retention time was 2.84 min for thiencynonate and its two optical isomers, and 2.94 min for the IS. The corresponding values of thiencynonate enantiomers were both identical when the same concentrations (5, 25 and 100 ng/ml) of *R*- and *S*-isomers were prepared. The precision of the same concentration of thiencynonate and its two optical isomers was <15 %. This was the basis of the quantitative analysis for the thiencynonate enantiomers according to the calibration curve.

Assay validation

The LC/MS/MS method showed good sensitivity, specificity, precision, accuracy, recovery and linearity for the quantification of thiencynonate enantiomers. The standard curve was linear over the range 1–100 ng/ml in the blood or brain. The typical equations for the calibration curves for thiencynonate were y= –0.1298+0.0328x (r^2>0.9973) in rat blood, and y= 0.02395+0.01477x (r^2>0.9955) in rat brain. The inter- and intra-day precision was less than 10 % and the accuracy percent error was less than 10 %. The Limit Of Detection (LOD) was 0.3 ng/ml and the Limit Of Quantification (LOQ) was 1 ng/ml. The recovery of extracted thiencynonate enantiomers in blood was more than 65 %.

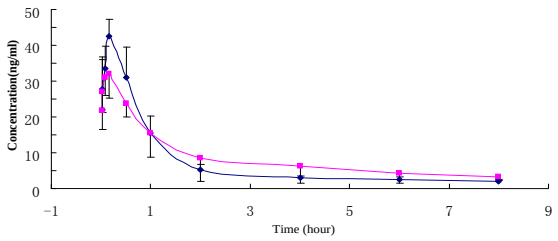

Figure 3: Blood concentration-time curve of the two optical isomers of thiencynonate after a single intramuscular (0.35mg/kg) administration to rats (n=6, mean ± S.D.). ♦ indicates *R*-thiencynonate, ■ indicates *S*-thiencynonate.

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

Parameters (Unit)	*R*-thiencynonate	*S*-thiencynonate
plasma		
$t_{1/2\alpha}$ (h)	0.630±0.609	0.691±0.593
$t_{1/2\beta}$ (h)	6.89±4.62	11.14±6.57
$t_{1/2Ka}$ (h)	0.606±1.49	0.587±1.51
K_{21} (h^{-1})	0.968±1.78	1.02±1.77
K_{10} (h^{-1})	1.51±2.00	1.22±2.11
K_{12} (h^{-1})	1.73±2.33	1.76±2.33
AUC ((ng/mL)h)	68.73±31.87	94.79±64.20
t_{max} (h)	1.54±3.73	1.47±2.76
C_{max} (ng/mL)	38.83±13.62	31.08±9.51
brain tissue		
$t_{1/2}$ (h)	19.80±9.63	41.11±21.41
AUC ((ng/ml)h)	78.99±12.86	100.91±43.02

For microdialysis, the relative *in vitro* recovery, *in vitro* loss, and *in vivo* loss were 13.81, 96.53, and 87.33%, respectively. The *in vivo* recovery, therefore, was calculated according to equation [a] to be 12.49%. The concentration of thiencynonate enantiomers in rat CSF was calculated using the *in vivo* recovery, by multiplying the concentration determined in the microdialysates by the factor of 100/12.49.

Pharmacokinetic differences of thiencynonate enantiomers

The concentration over time data were calculated and analyzed. The concentration-time curves for both thiencynonate enantiomers in rat blood exhibited excellent fits to a first order absorption and two-compartment open model after i.m. administration of a bolus dose (Figure 3). The main pharmacokinetic parameters of the enantiomers in rats are shown in Table 1. The effects of individual animal differences decreased when the rats were randomly divided into the two groups and the two groups of rats were used as their own controls. Comparison of the main pharmacokinetic parameters between the *S*- and the *R*-isomers of thiencynonate using the *t* test gave the following results.

The mean values of $t_{1/2\alpha}$, and especially, $t_{1/2\beta}$ for *S*-thiencynonate were longer than those for *R*-thiencynonate, and the mean values of the $t_{1/2\beta}$ for *S*-thiencynonate were 38.2% longer than those for *R*-thiencynonate. From these mean half-life values, the distribution

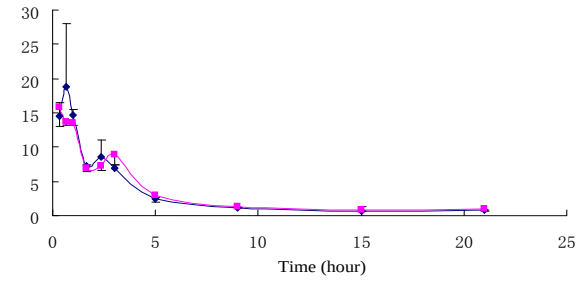

Figure 4: Brain concentration-time curve of the two isomers of thiencynonate after a single intramuscular (0.35mg/kg) administration to rats (n=6, means ± S.D.). ♦ indicates *R*-thiencynonate, ■ indicates *S*- thiencynonate.

as well as elimination of *S*-thiencynonate was slower than *R*-thiencynonate, indicating that the elimination of the S-isomer was slower and favorable for the clinic treatment. The time to reach the peak concentration (t_{max}) was similar for these two isomers. Although there was no statistically significant difference in the AUC between the *R*- and the *S*-isomer, the mean AUC was 27.5% higher for the *S*-isomer than the *R*-isomer, indicating that the *S*-isomer had higher content and was more favorable for the clinic treatment. Although the main pharmacokinetic parameters obtained from *S*- and *R*-isomer in rat blood were not significantly different, which the individual differences among the animals (the standard deviation values were much vicariance) clouded the pharmacokinetic parameter differences, the mean values of the main pharmacokinetic parameters such as AUC and $t_{1/2\beta}$ of the *S*-isomer were still favorable than that of the *R*-isomer; these data indicated that there was relative dominant in the disposition of S-thiencynonate *in vivo*.

Distribution kinetics of thiencynonate enantiomers in rat brain

The distribution kinetic parameters of *S*- and *R*-isomer in rat brain were estimated and shown in Table 1. The concentration-time curves of both the enantiomers in rat brain are shown in Figure 4. Although there was no statistically significant difference in the main kinetic parameters between the *R*- and the *S*-isomer in rat brain distribution ($p>0.05$), the mean value of AUC of *S*-isomer was greater than that of *R*-thiencynonate, and the mean AUC for the S-isomer were higher by 21.7%, indicating that the distribution of *S*-isomer was greater than that of *R*-isomer. The mean elimination half-life of the *S*-isomer was greater by 51.8 %. These data indicated that the *in vivo* disposition of *S*-isomer was better and beneficial for the clinical use.

Competitive inhibition for m receptor

The *S*/*R* isomers of thiencynonate competitions with the binding

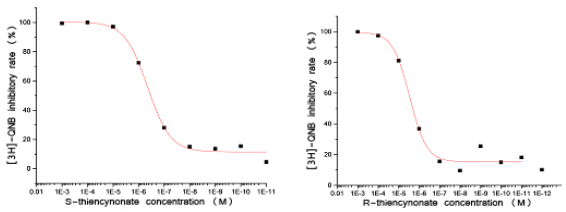

Figure 5: Effects of *S*-thiencynonate and *R*-thiencynonate on the binding of [^{3}H]-QNB to rat central muscarinic acetylcholine receptors. Rat cerebral cortex homogenate was incubated with 6 nM [^{3}H]-QNB for 30 min at 37 °C in the absence and presence of increasing concentrations of different drugs.

of [^{3}H]-QNB to the acetylcholine muscarinic receptors in rat brain were assayed individually (Figure 5). The corresponding IC_{50} (M) values of the *S*- and *R*- isomers of thiencynonate were $4.06\times10^{-7}\pm1.14\times10^{-8}$ and $2.90\times10^{-6}\pm8.72\times10^{-7}$; and the K_i (nM) mean values of the *S*- and *R*-isomers were 216.76 and 1548.29, respectively. The hill coefficients (nH) of the M receptors were 0.89 ± 0.18 and 1.05 ± 0.24, respectively, suggesting that the affinity of *S*-isomer to the M receptor was more potent than that of *R*-isomer. The nH values of *S*-isomer was less than 1, indicating that there were more possible allosteric interactions between the *S*-isomer and muscarinic receptors [25]. The investigations indicated that thiencynonate enantiomers showed distinct differences in pharmacological activities to the central muscarinic receptors, i.e. the *S*-enantiomer had the higher affinity for the central M receptors.

Approximately 50% of the marketed drugs are chiral compounds and of these approximately 50% are used as a racemate, a mixture of equal proportions of the two enantiomers. According to the Food and Drug Administration of the United States, among the new molecular entities approved, 47% are optically active and only 8% are race mates. It shows that in the last decade there was a tendency for the development of stereo chemically pure substances with the aim to reduce the total dose of the administered drug, to simplify dose-response relationships and to minimize toxicity due to the inactive isomer [26]. Thus, the evaluation of the physicochemical, dynamic and kinetic stereo selective properties of chiral drugs is much great important. There is a chiral carbon in thiencynonate structure, yielding two optical isomers with the *S* or *R* configuration. Our investigations have showed that thiencynonate enantiomers show distinct differences in pharmacological activity, in that the *S*-enantiomer has the highest affinity for the central muscarinic receptors and lower toxicity. The elimination of *S*-thiencynonate was slower than that of *R*-thiencynonate and the mean AUC of *S*-isomer was greater than that of *R*-isomer in rat blood and brain tissue, indicating that the *in vivo* disposition of *S*-isomer was better and beneficial for the clinical use. Indeed, as the CNS-acting drug, the *S*-isomer can act as a novel eutomer and as a potent and competitive candidate of acetylcholine muscarinic receptor antagonist.

Conclusion

In summary, the present study represents an effective attempt to investigate the muscarinic receptor affinity, *in vivo* pharmacokinetics of the thiencynonate isomers in rat blood and brain tissue by competitions with the binding of [^{3}H]-QNB, LC/MS/MS in combination with the microdialysis techniques. The results suggested that the affinity of *S*-isomer to the muscarinic receptor was more potent than that of *R*-isomer, and there was statistically significant difference. The disposition profiles of the two isomers were all best fitted by a first order absorption, two-compartment open model in rats. In general, there were some differences between the mean values of the main kinetic parameters of *S*- and *R*-isomer in the blood and brain. Thus, thiencynonate *S*-isomer could act as the novel eutomer and competitive chiral candidate for the acetylcholine muscarinic receptor antagonist.

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