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Study of stereoselective pharmacokinetics of anisodamine enantiomers in rabbits by capillary electrophoresis

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Abstract

The purpose of this study was to determine the pharmacokinetics of anisodamine enantiomers in plasma after oral and intravenous administration of racemic anisodamine in rabbits. A capillary electrophoresis method for the simultaneous separation of two pairs of enantiomers in plasma has been firstly developed and validated. Using a 75 mM phosphate buffer containing 25 mM carboxymethylated-γ-cyclodextrin at pH 2.5, good resolution was achieved on a 45-cm uncoated fused-silica capillary at the voltage of 20 kV and 25 °C. The pharmacokinetics of individual anisodamine enantiomers were characterized using the CE assay, the sole method of enantiomeric separation for anisodamine. Pharmacokinetic analysis of results indicated that anisodamine enantiomers showed non-stereoselective disposition or stereoselective disposition in different rabbits. For the rabbits with non-stereoselective disposition, similar pharmacokinetic characteristics were observed between (6*S*, 2′*S*)- and (6*R*, 2′*R*)-, or (6*S*, 2′*R*)- and (6*R*, 2′*S*)-anisodamine. For the rabbits with stereoselective disposition, (6*S*, 2′*S*)- and (6*R*, 2′*S*)-anisodamine were below the established LOD, while the two remaining enantiomers also had similar pharmacokinetic profiles. Further investigations remain necessary to find out the underlying mechanism about the stereoselective disposition of (6*S*, 2′*S*)- and (6*R*, 2′*S*)-anisodamine.

Keywords: Enantiomer separation; Anisodamine

1. Introduction

Anisodamine (6-[*s*]hydroxyhyoscyamine), an alkaloid first extracted from the Chinese herb *Scopolia tangutica* Maxim, is very similar to atropine in structure and is considered as a kind of antagonist of the M-choline acceptor but with a weaker effect on the central nervous system [1]. Anisodamine is extensively used in clinics, especially in cases of toxic shock and organophosphorus intoxication. In China, anisodamine has been used as a vasoactive drug for decades to improve microcirculation [2–4]. In addition, anisodamine has also been used to protect against arrthythmias, myocardial ischemic reperfusion injury and cardiopulmonary bypass surgery-induced hypertension [5–7].

The chemical structure of anisodamine is shown in Fig. 1. The molecule has two chiral centers, 6- and 2′-position. Natural anisodamine is (6*S*, 2′*S*)-enantiomer. Anisodamine is used clinically in its synthetics (trade name 654-2). Synthetic anisodamine is a racemic mixture of two pairs of optical enantiomers, that is (6*S*, 2′*S*)- and (6*R*, 2′*R*)-, or (6*S*, 2′*R*)- and (6*R*, 2′*S*)-anisodamine. Pharmacological studies have demonstrated that anisodamine enantiomers have slightly different interactions with M-choline acceptor [8].

No report dealing with pharmacokinetics of individual enantiomers was found due to the lack of an enantioseparation method. However, enantiomers should be considered as separate chemical entities and individual pharmacokinetic properties should thus be investigated.

In recent years, capillary electrophoresis (CE) has been widely developed for the separation and quantification of drugs in biological fluids (plasma, serum, urine and saliva), due to its many attractive features such as high peak

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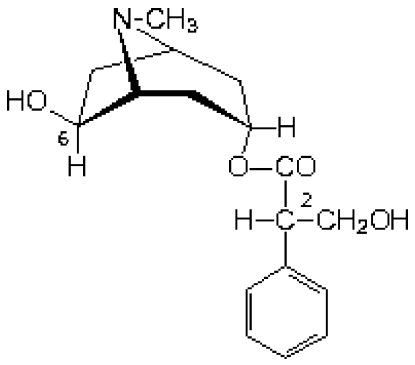

Fig. 1. Chemical structure of anisodamine.

efficiency, high resolution, small sample volume and reduced analysis time [9,10]. CE is also well established as one of the major techniques in the field of chiral separations of pharmaceutical drugs [11–13]. The most common approach for enantiomeric CE separation involves the addition of cyclodextrins (CDs) to the running buffer, instead of expensive HPLC and GC chiral columns.

Therefore, the present study was performed using a CE enantioseparation method to determine pharmacokinetics of individual anisodamine enantiomers. Method development and validation are discussed. The results from pharmacokinetic studies with oral and intravenous administration of racemic anisodamine are presented and analyzed.

2. Experimental

2.1. Chemicals and apparatus

Methanol, ethyl acetate, triethylamine, sodium hydroxide, sodium dihydrogen phosphate monohydrate and sodium chloride, of analytical reagent grade, were purchased from Shanghai Reagents Company (Shanghai, China); racemic anisodamine synthetics were provided by Hangzhou Minsheng Pharmaceuticals (Hangzhou, China), and scopolamine hydrobromide was provided by Department of Clinical Pharmacology, Shanghai Changhai Hospital (Shanghai, China). Carboxymethyl (CM)-α-CD, CM-γ-CD and CM-β-CD were provided by Dr. Zhu Ming-de from Bio-Rad Company, USA. Double-distilled water was used for the preparation of all solutions and 0.45 μm pore size filters (Millipore, MA) was used to filter the solutions.

Separations were performed on a BioFocus 3000 capillary electrophoresis system (Bio-Rad, USA) using an uncoated fused-silica capillary (45 cm × 50 μm i.d., effective length 40.4 cm). The capillary was purchased from Hebei Yongnian Optical Fiber Factory (Hebei, China). A new uncoated capillary was conditioned by rinsing with 1 M NaOH, 0.1 M NaOH, H_2O and 0.1 M HCl (30 min each) sequentially. Everyday before starting a series, the capillary was flushed with 0.1 M NaOH for 6 min, and the running buffer for 4 min. The capillary was rinsed before each sample injection, according to the scheme shown in Table 1 to achieve high reproducibility of migration time and electroosmotic flow and to avoid plasma proteins adsorption to the capillary wall.

2.2. Animals

Specific pathogen-free rabbits (2.06 ± 0.32 kg, female and male) were obtained from the Laboratory Animal Center of the Chinese Pharmaceutical University, Nanjing, China. Animals were housed under normal conditions and allowed to acclimatize for at least 1 week before initiation of studies. Water and standard laboratory food were given until 12 h before the experiments.

2.3. Blood sampling

This study was performed on two groups of five rabbits, using different administration routes.

Five rabbits were orally administrated with anisodamine hydrobromide (140 mg/kg, i.g.) dissolved in water. Blood samples (each 2.5 ml) were collected before drug administration and post-dose at 15, 30, 45, 60, 90, 120, 150, 180, 240 and 300 min. Each collected blood sample was transferred to a heparinized microcentrifuge tube and plasma was separated out by centrifugation at 3000 rpm for 5 min. All plasma samples were stored at −20 °C until analysis.

Another five rabbits were injected with anisodamine hydrobromide (50 mg/kg, i.v.) dissolved in water. Blood samples (each 2.5 ml) were collected before drug administration and post-dose at 5, 15, 30, 45, 60, 90, 120, 150, 180 and 240 min. Each collected blood sample was transferred to a heparinized microcentrifuge tube and plasma was separated out by centrifugation at 3000 rpm for 5 min. All plasma samples were stored at −20 °C until analysis.

Table 1
Automatic wash program of capillary electrophoresis

Circle	Solution	Pressure process (psi × s)	Description
1	0.5 ml water	0	Washing for injection end of capillary and positive electrode
2	0.5 ml water	120	Removing plasma proteins adsorbed to inner wall of capillary
3	0.5 ml HCl (0.1 M)	120	Keeping uniform physical state of inner wall of capillary
4	0.5 ml running buffer	150	Filling capillary with running buffer
5	0.2 ml water	0	Field-amplified stacking for on-column concentration

2.4. Treatment of plasma samples

To 1.0 ml of the resulting plasma in a 10-ml stoppered centrifuge tube, 0.1 g NaCl and 0.1 ml of triethylamine were added and the mixture was shaken vigorously for 2 min. The mixture was extracted with 5.0 ml ethyl acetate during 2.5 min of vortexing, followed by centrifugation at 3500 rpm for 5 min. An accurately measured 4.0 ml of the supernatant ethyl acetate layer was evaporated to dryness in a stream of nitrogen on a 45 °C water bath. The residue was reconstituted in 60-μl methanol and 60-μl IS solution (9.0 μg/ml scopolamine hydrobromide in 1 mM HCl), followed by centrifugation at 15,000 rpm for 5 min. An 80-μl aliquot of the supernatant was directly injected into CE system. The same sample handling process was used for recovery, precision and accuracy determinations.

2.5. Calibration curves

Blank plasma samples were spiked with synthetic anisodamine hydrobromide at concentrations of 0.2–50 μg/ml. The calibration samples were prepared and spiked with IS as described above. The calibration curves were generated by the ratios of the peak area of each enantiomer to the peak area of the internal standard versus the concentration of the enantiomer spiked in the samples.

2.6. Pharmacokinetic analysis

The pharmacokinetic parameters were determined based on the non-compartment model and calculated with an in-house validated computer program. 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 were extrapolated to infinity using the last detectable plasma concentration and the terminal elimination rate constant. Mean residence time (MRT), total plasma clearance (CL), and apparent volume of distribution at steady state (Vd_{ss}) were calculated using the equations MRT = AUMC/AUC, CL = Dose/AUC and Vd_{ss} = CL·MRT, respectively. Peak plasma concentration (C_{max}) and the time to achieve maximal plasma concentration (t_{max}) were determined directly by visual inspection of the concentration–time curves. The terminal elimination half-life ($t_{1/2}$) was derived by linear regression analysis of the terminal phase of the plasma concentration–time curve. Pharmacokinetic calculations were performed on each individual set of data.

2.7. Statistical analysis

All values were reported as mean ± standard deviation. Differences between pharmacokinetic parameters of the two enantiomers were evaluated by Pair *t*-test with the prior level of significance set at $\alpha = 0.05$.

3. Results and discussion

3.1. Method optimization

Electrophoretic conditions were optimized, in particular with regard to choice of chiral selectors, concentration of the cyclodextrin used, buffer pH value, applied voltage, temperature and the length of the capillary.

Different cyclodextrins, such as native α-, β- and γ-cyclodextrin and their carboxymethylated derivatives, have been investigated with regard to the simultaneous enantioseparation of all two enantiomeric pairs. Carboxymethylated-γ-cyclodextrin (CM-γ-CD) was found to be the most efficient chiral selector for this purpose. The effect of CM-γ-CD concentration on the migration time and resolution was further examined in the range of 10–35 mM. The suitable CM-γ-CD concentration was found to be 25 mM.

The pH of buffer has an important effect on surface characteristics of the capillary, the effective electric charge of the ion and electric characteristics of CM-γ-CD. The use of a 75 mM phosphate buffer containing 25 mM CM-γ-CD at pH 6.5, 4.5 or 2.5 was investigated. At pH 6.5, CM-γ-CD was negatively charged and has a "countercurrent" flow with respect to electroosmotic flow (EOF). The two enantiomeric pairs could be separated while the baseline and peak shape were not good enough for the quantification. With a pH of 4.5, which was close to the pK_a of CM-γ-CD, no enantioseparation was observed. At pH 2.5, CM-γ-CD acted as neutral chiral selector and best enantioseparation was obtained.

The applied voltages ranging from 15 to 25 kV were then examined. In order to obtain short migration times, the applied voltage was chosen as high as possible, but limited by the heating of the capillary. In our experiment, the applied voltage was set at 20 kV due to the high resolution and the acceptable migration time.

The inside diameter of the capillary was usually 50 or 75 μm, and in this study, capillaries of 50 μm i.d. × 45 cm (effective length 40.4 cm) and 75 μm i.d. × 50 cm (effective length 45.4 cm) gave similar migration times, but the peak resolution of the former capillary was better than that of the latter, and thus was used for further experiments.

Capillary temperatures of 25 and 30 °C were also investigated. At 30 °C the peak resolution declined significantly, so a temperature of 25 °C was finally selected.

3.2. Electrophoretic conditions and electropherograms

The final optimized conditions were as follows. A short zone of purified water was hydrodynamically injected with 0.5 psi (1 psi = 6894.76 Pa) for 2 s, followed by electrokinetic sample injection at 16 kV for 8 s. Since purified water had low conductivity, the analytes injected electrokinetically at high velocity could thus be stacked at the interface between the water zone and the running buffer. The capillary temperature was kept at 25 °C. During separation, the voltage was set at 20 kV in normal polarity mode and the detection

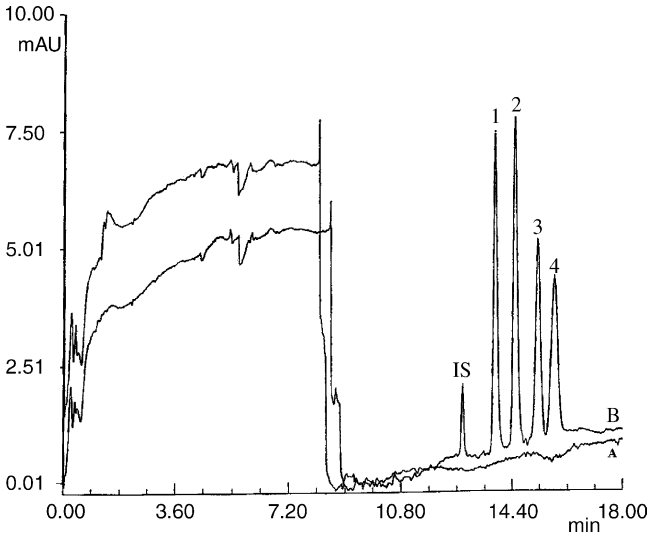

Fig. 2. Electropherograms of (A) a blank plasma and (B) plasma spiked with anisodamine (10 μg/ml). Electrophoretic conditions: uncoated fused-silica capillary (45 cm × 50 μm i.d., effective length 40.4 cm), the background electrolyte consisted of 75 mM phosphate buffer, pH 2.5, containing 25 mM carboxymethylated-γ-cyclodextrin, applied voltage, +20 kV; temperature, 25 °C; injection, 16 kV × 8 s. (1) (6*R*, 2′*S*)-, (2) (6*S*, 2′*R*)-, (3) (6*R*, 2′*R*)-, (4) (6*S*, 2′*S*)-anisodamine.

wavelength was 200 nm. The background electrolyte consisted of 75 mM phosphate buffer (pH 2.5), containing 25 mM carboxymethylated-γ-cyclodextrin.

Under the conditions described, the migration times of the analytes were 13.84 and 15.77 min for (6*R*, 2′*S*)- and (6*S*, 2′*S*)-anisodamine, 14.49 and 15.24 min for (6*S*, 2′*R*)- and (6*R*, 2′*R*)-anisodamine. The migration time of scopolamine hydrobromide (IS) was 12.78 min. Representative electropherograms of blank rabbit plasma and spiked plasma sample containing all analytes of interest were shown in Fig. 2. Comparison of the spiked plasma sample with the blank plasma showed the high selectivity, since no matrix peaks interfered with the determination. A baseline separation of all peaks indicated excellent separation efficiencies.

3.3. Assay validation

The CE method was validated by evaluating linearity, limit of quantification (LOQ) and limit of detection (LOD), method precision and accuracy. Coefficients of variation values and relative errors of less than 15% were considered acceptable, except for the quantification limit which was extended to 20%, as recommended by Bressole et al. [14] and Shah et al. [15] for analyses of biosamples for pharmacokinetic studies.

The concentration ratio of four enantiomers (6*S*, 2′*S*)-/(6*S*, 2′*R*)-/(6*R*, 2′*R*)-/(6*R*, 2′*S*)-anisodamine in synthetic product was 2/3/2/3 (w/w). The peak area ratios (each enantiomer/scopolamine hydrobromide) were linearly related to the concentrations of enantiomers, with r^2 consistently greater than 0.9990. Calibration functions for all enantiomers with r^2 were given in Table 2. The LOQs for (6*S*, 2′*S*)- and (6*R*, 2′*R*)-anisodamine, (6*S*, 2′*R*)- and (6*R*, 2′*S*)-anisodamine were found to be 0.04 and 0.06 μg/ml, respectively. The LODs for (6*S*, 2′*S*)- and (6*R*, 2′*R*)-anisodamine, (6*S*, 2′*R*)- and (6*R*, 2′*S*)-anisodamine were found to be 0.01 μg/ml and 0.015 μg/ml at a signal-to-noise ratio of 3:1, respectively.

The results of intra- and inter-day reproducibility experiments were presented in Table 3. Precision (relative standard deviation) and accuracy values (percentage deviation of the found concentration from the nominal concentration) were consistently less than 10%. Therefore, the method meets the requirements for bioassays.

Table 2
The calibration functions and r^2 values

Enantiomer	Range (μg/ml)	Calibration function*	r^2	Number of data points (*n*)
(6*S*, 2′*S*)-	0.04–10.0	$y = 0.009345 + 0.7418C$	0.9996	8
(6*S*, 2′*R*)-	0.06–15.0	$y = 0.009664 + 0.7421C$	0.9990	8
(6*R*, 2′*R*)-	0.04–10.0	$y = 0.007810 + 0.7399C$	0.9994	8
(6*R*, 2′*S*)-	0.06–15.0	$y = 0.01445 + 0.7409C$	0.9992	8

* *C*: concentration of individual enantiomer; *y*: peak area ratio of enantiomer/IS.

Table 3
Intra- and inter-day accuracy and precision data for the determination of anisodamine enantiomers (*n* = 5)

Nominal concentrations (μg/ml) of (6*S*, 2′*S*)-/(6*S*, 2′*R*)-/(6*R*, 2′*R*)-/(6*R*, 2′*S*)-anisodamine			
	0.10/0.15/0.10/0.15	0.40/0.60/0.40/0.60	4.0/6.0/4.0/6.0
Intra-day			
Concentration found	0.0971/0.151/0.0992/0.145	0.387/0.589/0.393/0.582	3.98/5.94/3.97/5.99
Precision (R.S.D. %)	5.90/5.86/5.93/6.33	5.21/4.81/4.95/5.44	4.06/4.14/4.18/4.17
Accuracy (%)	97.1/100.7/99.2/96.7	96.8/98.2/98.2/97.0	99.6/99.0/99.2/99.8
Inter-day			
Concentration found	0.0986/0.154/0.101/0.147	0.394/0.597/0.397/0.590	3.97/5.95/3.98/5.96
Precision (R.S.D. %)	7.78/7.44/7.50/8.40	7.14/6.85/6.71/6.83	6.84/6.90/6.15/6.37
Accuracy (%)	98.6/102.7/101.0/98.0	98.5/99.5/99.2/98.3	99.2/99.2/99.5/99.3

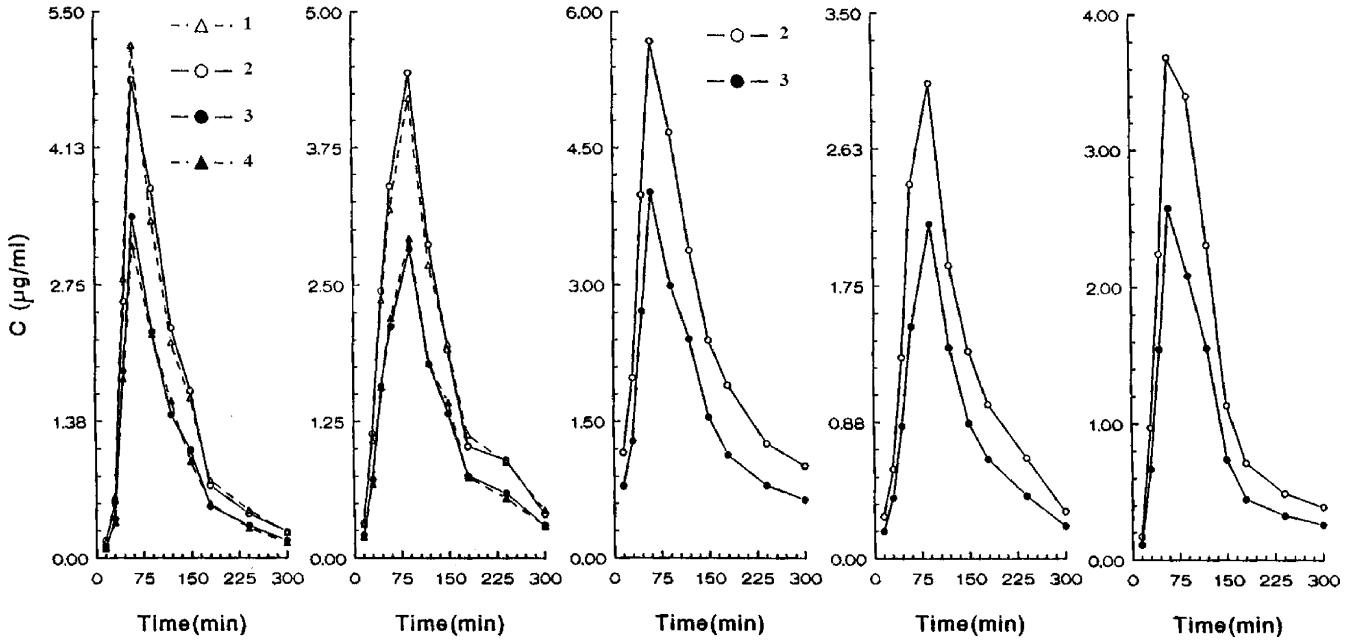

Fig. 3. Individual plasma concentration–time curves of anisodamine enantiomers after an i.g. administration of 140 mg/kg in five rabbits. (1) (6*R*, 2′*S*)-, (2) (6*S*, 2′*R*)-, (3) (6*R*, 2′*R*)-, (4) (6*S*, 2′*S*)-anisodamine.

3.4. Pharmacokinetic analysis of synthetic anisodamine hydrobromide after oral administration

Plasma concentration–time profiles for anisodamine enantiomers after an i.g. administration of 140 mg/kg dose synthetic anisodamine hydrobromide to five conscious rabbits were presented in Fig. 3. As shown in Fig. 3, following i.g. administration of racemic anisodamine, four enantiomers of anisodamine quickly appeared in the plasma samples of two rabbits, reached maximum concentrations at approximately 1–1.5 h, and then declined biexponentially with time. Similar plasma concentration–time profiles of (6*S*, 2′*S*)- and (6*R*, 2′*R*)-, or (6*S*, 2′*R*)- and (6*R*, 2′*S*)-anisodamine were observed. In contrast, only (6*R*, 2′*R*)- and (6*S*, 2′*R*)-anisodamine were detectable in the plasma samples of another three rabbits and after reaching peaks, plasma concentrations also declined biexponentially. It suggested that anisodamine enantiomers showed non-stereoselective disposition or stereoselective disposition in different rabbits. Fig. 4 presented the typical electropherograms of plasma samples from the rabbits with non-stereoselective disposition or stereoselective disposition. Pharmacokinetic parameters determined by non-compartment analysis method were summarized in Table 4. For the rabbits with non-stereoselective disposition, no statistically significant differences ($P > 0.05$) were detected between the values of C_{max}, T_{max}, $AUC_{0\sim\tau}$, $AUC_{0\sim\infty}$, $t_{1/2}$ and MRT of (6*S*, 2′*S*)- and those of (6*R*, 2′*R*)-anisodamine, and so was those of (6*S*, 2′*R*)- and (6*R*, 2′*S*)-anisodamine. The four enantiomers showed similar pharmacokinetic characteristics. For the rabbits with stereoselective disposition, the C_{max}, $AUC_{0\sim\tau}$ and $AUC_{0\sim\infty}$ ratios of (6*S*, 2′*R*)- and (6*R*, 2′*R*)-anisodamine were 1.425 ± 0.009112, 1.477 ± 0.01431 and 1.494 ± 0.0377, respectively, which were similar to their concentration ratio (≈1.5). No significant differences ($P > 0.05$) were detected between the values of $t_{1/2}$ and MRT of (6*S*, 2′*R*)- and (6*R*, 2′*R*)-anisodamine, suggesting similar elimination courses. Except the absence of (6*S*, 2′*S*)- and

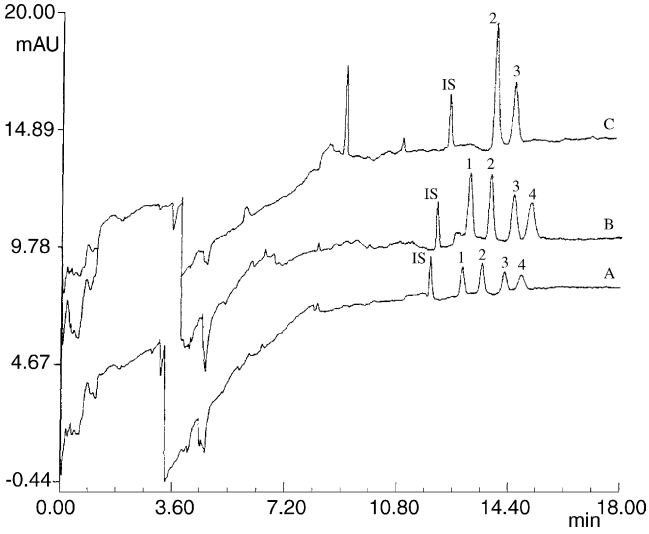

Fig. 4. Electropherograms of (A) plasma spiked with anisodamine, (B) plasma sample from the rabbit with non-stereoselective disposition and (C) plasma sample from the rabbit with stereoselective disposition, after an i.g. administration of anisodamine (140 mg/kg) in five rabbits. Electrophoretic conditions: uncoated fused-silica capillary (45 cm × 50 μm i.d., effective length 40.4 cm), the background electrolyte consisted of 75 mM phosphate buffer, pH 2.5, containing 25 mM carboxymethylated-γ-cyclodextrin, applied voltage, +20 kV; temperature, 25 °C; injection, 16 kV × 8 s. (1) (6*R*, 2′*S*)-, (2) (6*S*, 2′*R*)-, (3) (6*R*, 2′*R*)-, (4) (6*S*, 2′*S*)-anisodamine.

Table 4
Pharmacokinetic parameters of anisodamine enantiomers following oral administration of racemic anisodamine in rabbits (dose: 140 mg/kg, five rabbits)

Pharmacokinetic parameters	Non-stereoselective disposition				Stereoselective disposition	
	(6*S*, 2′*S*)-	(6*R*, 2′*R*)-	(6*S*, 2′*R*)-	(6*R*, 2′*S*)-	(6*R*, 2′*R*)-	(6*S*, 2′*R*)-
C_{max} (μg/ml)	3.030±0.1534	3.127 ± 0.4271	4.636 ± 0.2652	4.689 ± 0.6739	2.910 ± 0.9796	4.142 ± 1.372
T_{max} (min)	75.00±21.21	75.00 ± 21.21	75.00 ± 21.21	75.00 ± 21.21	70.00 ± 17.32	70.00 ± 17.32
$AUC_{0\sim\tau}$ (μg min/ml)	344.2±36.13	347.4 ± 26.02	517.0 ± 48.79	509.7 ± 38.47	356.8 ± 133.5	528.1 ± 201.7
$AUC_{0\sim\infty}$ (μg min/ml)	367.7±47.02	372.2 ± 38.61	555.2 ± 57.49	549.1 ± 52.82	412.4 ± 184.7	617.2 ± 278.6
$t_{1/2}$ (min)	66.34±9.037	69.65 ± 10.98	68.58 ± 10.25	69.36 ± 10.70	102.0 ± 24.23	98.69 ± 24.24
MRT (min)	127.1±15.49	128.4 ± 18.67	131.1 ± 10.75	132.3 ± 15.63	150.4 ± 27.10	157.7 ± 24.33

(6*R*, 2′*S*)-anisodamine, the two remaining enantiomers also had similar pharmacokinetic characteristics.

3.5. Pharmacokinetic analysis of synthetic anisodamine hydrobromide after intravenous administration

Plasma concentration–time profiles for anisodamine enantiomers after an i.v. administration of 50 mg/kg dose synthetic anisodamine hydrobromide to five conscious rabbits were presented in Fig. 5. Like the oral administration, anisodamine enantiomers also showed non-stereoselective disposition or stereoselective disposition in different rabbits after i.v. administration. As shown in Fig. 5, four enantiomers of anisodamine can be detected in the plasma samples of three rabbits 5 min after administration, and then declined biexponentially with time. Maximum plasma concentrations (C_{max}) were in the range of 6.007–9.952 and 6.119–9.666 μg/ml for (6*S*, 2′*S*)- and (6*R*, 2′*R*)-anisodamine, 9.389–15.63 and 9.137–14.53 μg/ml for (6*S*, 2′*R*)- and (6*R*, 2′*S*)-anisodamine, respectively. Similar plasma concentration–time profiles of (6*S*, 2′*S*)- and (6*R*, 2′*R*)-, or (6*S*, 2′*R*)- and (6*R*, 2′*S*)-anisodamine were also observed. Interestingly, (6*S*, 2′*S*)- and (6*R*, 2′*S*)-anisodamine still remained undetectable in the plasma samples of another two rabbits, even at 5 min after dosing, which implied that stereoselective disposition of anisodamine enantiomers may happen during the distribution or elimination phase, but not during the adsorption phase. Pharmacokinetic parameters determined by non-compartment analysis method were summarized in Table 5. For the rabbits with non-stereoselective disposition, no statistically significant differences ($P > 0.05$) were detected between the values of $AUC_{0\sim\tau}$, $AUC_{0\sim\infty}$, $t_{1/2}$ and MRT of (6*S*, 2′*S*)- and those of (6*R*, 2′*R*)-anisodamine, and so was those of (6*S*, 2′*R*)- and (6*R*, 2′*S*)-anisodamine. The four enantiomers had similar pharmacokinetic characteristics. For the rabbits with stereoselective disposition, the $AUC_{0\sim\tau}$ and $AUC_{0\sim\infty}$ ratios of (6*S*, 2′*R*)- and (6*R*, 2′*R*)-anisodamine were similar to their concentration ratio (≈1.5). No significant differences ($P > 0.05$) were detected between the values of $t_{1/2}$, MRT, CL and Vd_{ss} of (6*S*, 2′*R*)- and (6*R*, 2′*R*)-anisodamine. Except

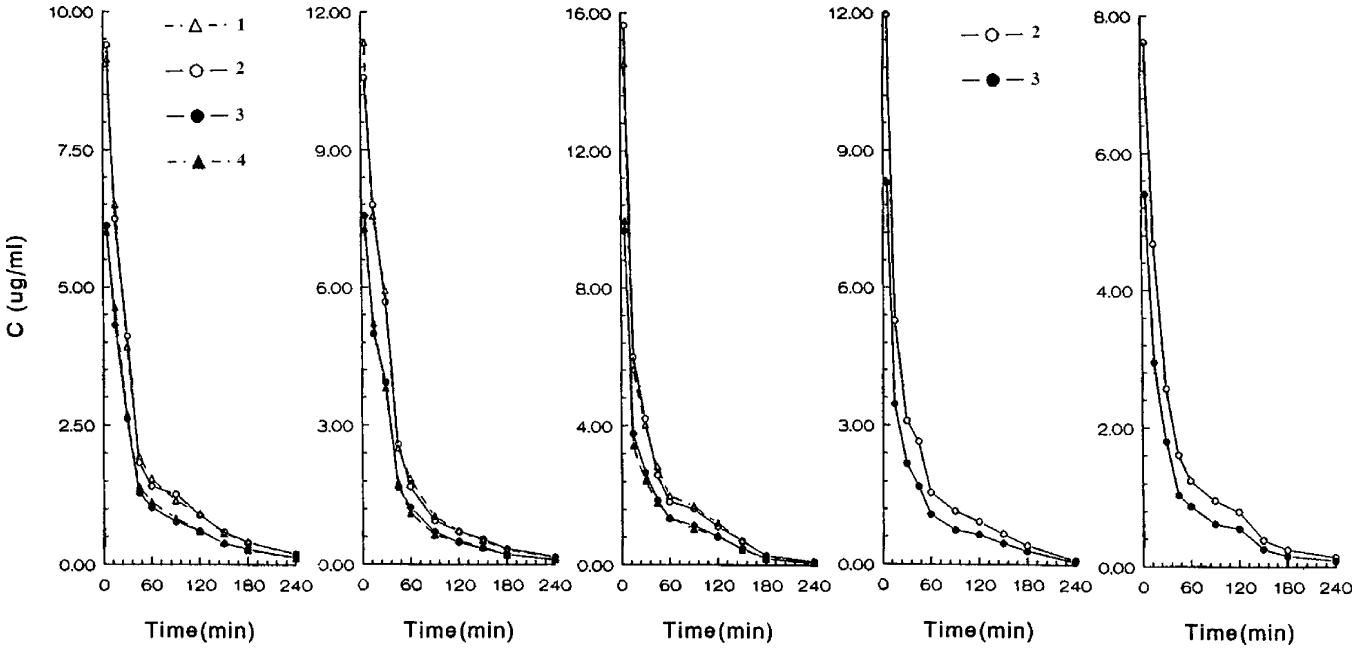

Fig. 5. Individual plasma concentration–time curves of anisodamine enantiomers after an i.v. administration of 50 mg/kg in five rabbits. (1) (6*R*, 2′*S*)-, (2) (6*S*, 2′*R*)-, (3) (6*R*, 2′*R*)-, (4) (6*S*, 2′*S*)-anisodamine.

Table 5
Pharmacokinetic parameters of anisodamine enantiomers following intravenous administration of racemic anisodamine in rabbits (dose: 50 mg/kg, five rabbits)

Pharmacokinetic parameters	Non-stereoselective disposition				Stereoselective disposition	
	(6*S*, 2′*S*)-	(6*R*, 2′*R*)-	(6*S*, 2′*R*)-	(6*R*, 2′*S*)-	(6*R*, 2′*R*)-	(6*S*, 2′*R*)-
$AUC_{0\sim\tau}$ (μg min/ml)	295.7 ± 16.66	296.3 ± 24.79	441.8 ± 38.28	444.6 ± 37.46	239.0 ± 45.18	352.4 ± 64.70
$AUC_{0\sim\infty}$ (μg min/ml)	303.1 ± 14.65	303.9 ± 21.98	453.2 ± 33.36	455.6 ± 33.27	243.5 ± 42.92	359.8 ± 61.16
$t_{1/2}$ (min)	46.65 ± 12.70	47.44 ± 12.65	48.49 ± 12.20	46.77 ± 12.58	40.78 ± 9.405	42.55 ± 9.808
MRT (min)	54.64 ± 5.178	54.85 ± 7.118	53.99 ± 7.448	54.48 ± 7.179	55.98 ± 3.486	56.72 ± 4.165
CL (ml/min kg)	33.04 ± 1.641	33.03 ± 2.492	33.22 ± 2.527	33.05 ± 2.514	41.75 ± 7.361	42.31 ± 7.191
Vd_{ss} (ml/kg)	1811 ± 264.8	1824 ± 381.4	1806 ± 391.6	1812 ± 384.4	2349 ± 557.9	2414 ± 584.1

the absence of (6*S*, 2′*S*)- and (6*R*, 2′*S*)-anisodamine, the two remaining enantiomers also showed similar pharmacokinetic characteristics.

4. Conclusion

In this paper, pharmacokinetic properties of individual anisodamine enantiomers have been studied for the first time. Since anisodamine is a racemic mixture of two pairs of enantiomers, a capillary electrophoresis method for the simultaneous separation of these enantiomers in plasma samples has been firstly developed and validated. Using a 75 mM phosphate buffer containing 25 mM CM-γ-CD at pH 2.5, good resolution was achieved on a 45-cm uncoated fused-silica capillary at the voltage of 20 kV and 25 °C. Compared to the chiral HPLC method, enantioseparation by CE requires no expensive stereo specific separation column and no sample derivatization. Moreover, acceptable levels of accuracy and precision of the assay have been shown.

After oral and intravenous administration of racemic anisodamine, plasma samples were collected and extracted with ethyl acetate. The most important phenomenon we observed was that in different rabbits, anisodamine enantiomers showed non-stereoselective disposition or stereoselective disposition, regardless of the administration routes. For the rabbits with non-stereoselective disposition, all enantiomers could be detected, and the plasma profiles and the pharmacokinetic parameters obtained for the individual enantiomers were virtually identical. For the rabbits with stereoselective disposition, (6*S*, 2′*S*)- and (6*R*, 2′*S*)-anisodamine were below the established LOD, while the two remaining enantiomers also had similar pharmacokinetic profiles. Further investigations remain necessary to find out the underlying mechanism about the stereoselective disposition of (6*S*, 2′*S*)- and (6*R*, 2′*S*)-anisodamine in some rabbits.

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