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Study of ocular pharmacokinetics of in situ gel system for *S*(−)-satropane evaluated by microdialysis

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ABSTRACT

S(−)-Satropane is currently being developed to in situ forming ophthalmic gel, a new ophthalmic delivery system, for the treatment of glaucoma. To evaluate the pharmacokinetic profiles of *S*(−)-satropane, the microdialysis method was employed. The concentration of *S*(−)-satropane in dialysates was measured by using liquid chromatography/tandem mass spectrometry (LC–MS/MS). Unlike the common solution prepared in normal saline, in which the level of *S*(−)-satropane in aqueous humor increased rapidly after instillation and reached the maximal level (C_{max} of 1.508 ± 0.297 μg ml^{-1}) within 1 h, *S*(−)-satropane exhibited 3.2-fold greater C_{max} and 2.2-fold greater AUC_{0-3h} ($p < 0.05$) in the in situ forming gel. The results showed that the in situ forming gel system could improve the ocular bioavailability of *S*(−)-satropane.

1. Introduction

S(−)-satropane (*S*(−)3α-paramethylbenzenesulfonyloxy-6β-acetoxy-tropane, Fig. 1), derived from a lead compound named Baogongteng A (6β-acetoxy-2β-hydroxy-nortropane) [1], is under preclinical development. Clinical trials demonstrated that the therapeutic efficacy of Baogongteng A was similar to pilocarpine in the treatment of primary glaucoma [2]. In previous preclinical studies, *S*(−)-satropane has shown potent agonistic activities (Kd value of 0.22 ± 0.09 nM) [3] on muscarinic receptors and other advantages, therefore showing promise as a candidate for the treatment of primary glaucoma.

However, the ocular bioavailability of common aqueous formulation is less than 10%, due to the short precorneal contact time resulting from the lachrymation, the normal tear turnover and the drainage from the nasolacrymal duct [4]. In order to escape the ocular and systemic side effects induced by high concentration or frequent administration, in situ forming ophthalmic gel, a promising ocular drug formulation, was developed. Due to their elastic properties, hydrogel resisted ocular drainage leading to longer contact time. Better bioavailability could be achieved even with lower concentration in the gel vehicle. This would reduce side effects without loss of efficacy [5].

In this study, a pharmacokinetics method was established to understand the profiles of *S*(−)-satropane in rabbit aqueous humor after being dosed as in situ forming gel formulation.

2. Materials and methods

2.1. Animal model

Twelve New Zealand albino rabbits (two for pretests), weighing 2.5–3.0 kg (Certificate No. SYXK 2003–0026, Animal Center for Medical Sciences, Shanghai Jiaotong University), were anesthetized by ketamine hydrochloride (35 mg kg^{-1}) and xylazine (3.5 mg kg^{-1}) 30 min prior to the surgery and every hour thereafter throughout the experiment. On completion of the experiment, animals were euthanized with an intravenous injection of sodium pentobarbital (100 mg kg^{-1}) through the marginal ear vein. All procedures were performed in accordance with the institutional guidelines on the care and use of experimental animals set by College of Basic Medical Sciences, Shanghai JiaoTong University.

2.2. Materials

HPLC-grade methanol, acetonitrile and formic acid were obtained from Fisher Chemicals (Fair Lawn, NJ, USA). Phentolamine

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was purchased from Sigma–Aldrich (St. Louis, MO, USA). *S*(−)-Satropane (mp: 165–167 °C) was synthesized in the Department of Chemistry of Drug Research Institute in Medical College of Shanghai JiaoTong University with a purity of 98% [3].

2.3. Probe implantation

The nictating membrane was sutured to the eyelid. Pupils were dilated by topical instillation of 0.4% tropicamide prior to the probe implantation. A linear probe (MD-2005, Bioanalytical Systems, USA) was implanted into the aqueous humor through a 25 G needle inserted across the center of the anterior chamber to the other end of the cornea just above corneal scleral limbus. The sample-collecting end of the linear probe was inserted carefully into the bevel edge of the needle, then the needle was slowly retreated leaving the probe with the membrane in the middle of the anterior chamber. Penetrating polyethylene tubes were fixed to the surface of cornea with veterinary bonding glue (MR-5314, Bioanalytical Systems, USA) to prevent direct influx of drugs into the anterior chamber or leakage of aqueous humor along the tube. The probe was perfused with isotonic phosphate buffer saline (IPBS, pH 7.4) at a flow rate of 2 μl min^{-1} by a CMA/100 microinjection pump (Acton, MA, USA). The animals were allowed to stabilize for at least

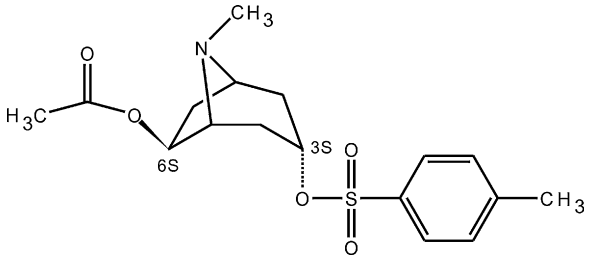

Fig. 1. Chemical structure of *S*(−)-satropane.

2 h before 50 μl of each formulation (1% *S*(−)-satropane in normal saline solution or in situ forming ophthalmic gel) was instilled into the eye. Dialysates were collected every 15 min within 2 h or every 20 min during the 3rd hour after instillation. All the dialysate samples were kept frozen −70 °C until they were analyzed.

2.4. Relative recovery

In vitro probe calibration was performed by placing the probe in IPBS solution, containing *S*(−)-satropane of a known concentra-

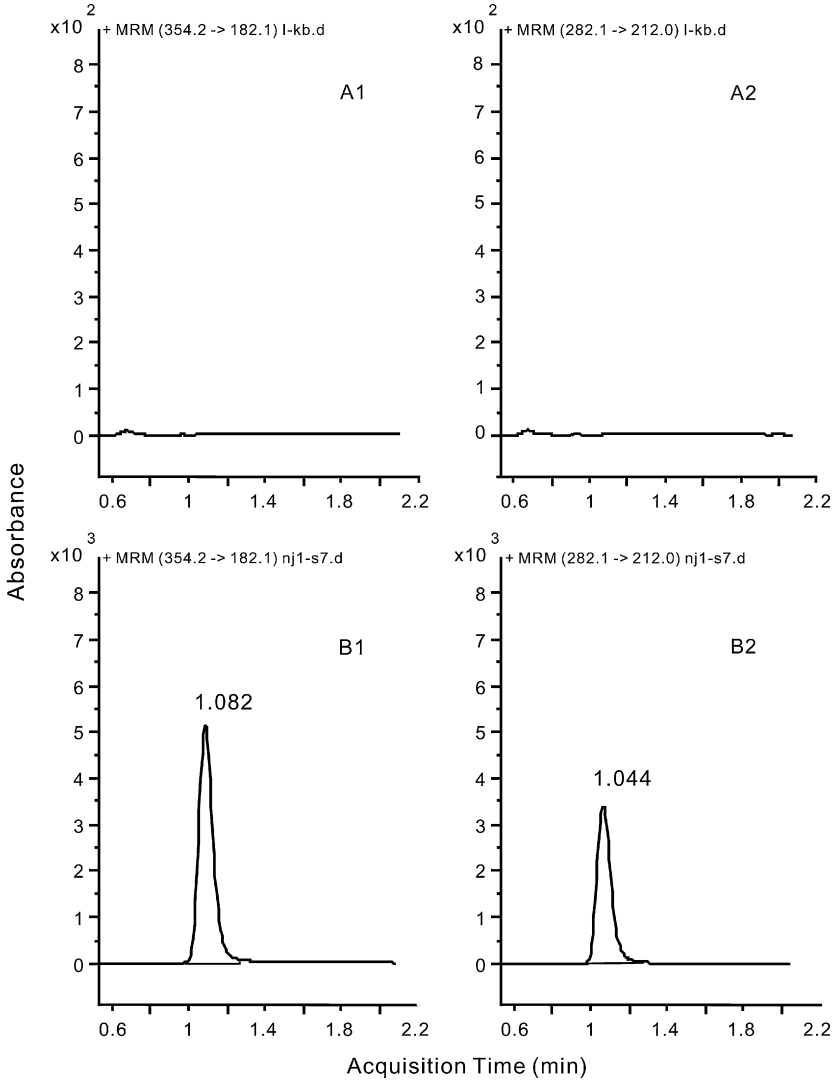

Fig. 2. Representative MRM chromatograms of the microdialysis samples of aqueous humor: (A) blank samples without *S*(−)-satropane (A1) and without the internal standard (IS, A2). (B) Samples after administration. B1: *S*(−)-satropane; B2: IS.

tion. The probe was perfused at a flow rate of 2 μl min^{-1} with IPBS and the dialysate was collected every 20 min for 2 h. Relative *in vitro* recovery of *S*(−)-satropane was estimated by the following formula: recovery = Cd/Cs, where Cd was the dialysate concentration and Cs was the known concentration of *S*(−)-satropane in IPBS. The concentration of *S*(−)-satropane in aqueous humor during the pharmacokinetic experiment was estimated by dividing the dialysate concentration with *in vitro* recovery.

2.5. Assay of S(−)-satropane

The LC–MS/MS system including an Aglient 6410 triple quadrupole mass spectrometer, a quaternary pump, an on-line degaser, an auto-sampler and a thermostatted column compartment (Agilent Technologies, USA) was used. The dialysate (20 μl) mixed with 10 μl internal standard (IS, 30 ng min^{-1} phentolamine) solution was separated on an Agilent ZORBAX SB-C18 (5 μm, 50 mm × 2.1 mm; Agilent Technologies, Santa Clara, CA, USA) column under the mobile condition of acetonitrile–water–formic acid (32:68:0.04, v/v/v) with a flow rate of 0.25 ml min^{-1} at 30 °C. The detection of *S*(−)-satropne was realized by multiple reaction monitoring (MRM) using transitions *m*/*z* 354 → 182. And an MRM transition from *m*/*z* 282 → 212 was performed for the IS.

2.6. Data analysis

Results were expressed as harmonic mean ± S.E.M. The kinetic parameters determined were compared by one-way analysis of variance (ANOVA). In all cases, $p < 0.05$ was considered statistically significant.

Drug concentration in aqueous humor was calculated from relative recovery and drug levels in dialysates. The aqueous concentration time data from each individual rabbit was analyzed by using a pharmacokinetic software package DAS 1.0 (Anhui, China). Pharmacokinetic parameters were determined by non-compartmental analysis. Area under aqueous concentration time curve (AUC_{AH}) was estimated by the linear trapezoidal method with extrapolation to infinite time. The slopes of the terminal phase of aqueous profiles were estimated by log-linear regression and the terminal rate constant (λz) was derived from the slope.

Terminal aqueous half-lives were calculated from the equation: $t_{1/2} = 0.693/\lambda z$. Maximum observed aqueous humor concentration of *S*(−)-satropane (C_{max}) and time of maximum observed concentration (T_{max}) of *S*(−)-satropane in the anterior chamber were obtained by data points of each rabbit.

3. Results

Under the current LC–MS/MS conditions, *S*(−)-satropane and IS were well separated from interference in the blank matrix. No interfering peaks from endogenous compounds were observed at the retention times of *S*(−)-satropane or IS. Representative chromatorgrams obtained from blank sample spiked with *S*(−)-satropane are shown in Fig. 2. The lower limit of quantification (LLOQ) values was 2 ng ml^{-1} and the method was linear over the concentration range from 2 to 500 ng ml^{-1}. In cases that *S*(−)-satropane concentration exceeded 500 ng ml^{-1}, samples were subjected to a dilution process to ensure the final concentration within the range mentioned above. Data below LLOQ would not be used in kinetic analysis, because of lack of confidence in their values.

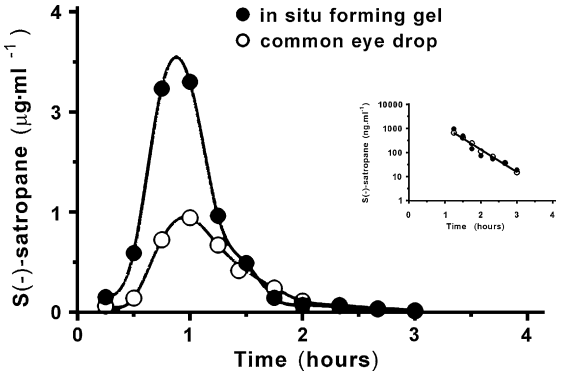

Fig. 3. Mean aqueous humor *S*(−)-satropane concentrations vs. time profile in five rats after instillation of 1% *S*(−)-satropane. Fifty microliters of the drug solution was instilled to a rabbit eye where a microdialysis probe was inserted. Each value is the harmonic mean ($n = 5$). Insert is the log-linear fit for the elimination portion of the data.

Relative recoveries for *S*(−)-satropane estimated in *in vitro* experiments were approximately 21.7%, which remained constantly throughout the whole experiment, and the coefficient of variation was less than 7.4% for *S*(−)-satropane.

Time–concentration profiles of *S*(−)-satropane were shown in Fig. 3 and Table 1. Concentrations in aqueous humor increased rapidly after instillation and reached maximal levels at 1 h. *S*(−)-Satropane seemed to be eliminated from aqueous humor according to first-order kinetics. Compared to in normal saline solution, *S*(−)-satropane exhibited a 3.2-fold greater C_{max} and a 2.2-fold greater $AUC_{0\text{-}3h}$, in the in situ forming ophthalmic gel while T_{max} remained unchanged. The ocular bioavailability of *S*(−)-satropane was significantly increased ($p < 0.05$). The $t_{1/2}$ of *S*(−)-satropane did not seem to be significantly different ($p > 0.05$).

4. Discussion

Logical uses of ophthalmic drugs based on the knowledge of pharmacokinetics for effective medication. Studies on ocular pharmacokinetics are being carried out either by obtaining a single sample of the ocular fluids from different animals or by direct serial sampling. These techniques would result in altered pharmacokinetics due to the introduction of relatively significant intra- and inter-subject variability, and loss of biological fluids [6–9]. Microdialysis has been proven to be beneficial over conventional sampling techniques in determining ocular pharmacokinetics [10,11].

The main disadvantage of microdialysis sampling is that the small volume of the analytes, normally combined with low concentration of analytes, becomes a challenging factor in quantization. Consequently, we established the method for analyzing *S*(−)-satropane. The liquid chromatography/tandem mass spectrometry (LC–MS/MS) method was chosen because of its high selectivity and sensitivity as well as the ability to conclusively identify the analytes [12]. The lower limit of quantification of *S*(−)-satropane in

Table 1
Pharmacokinetic parameters of *S*(−)-satropane in aqueous humor after topical administration

	$AUC_{0\text{-}3h}$ (μg ml^{-1} h)	C_{max} (μg ml^{-1})	T_{max} (h)	$t_{1/2}$ (h)	MRT (h)
Common eye drop	1.298 ± 0.112	1.508 ± 0.297	0.77 ± 0.13	0.36 ± 0.05	1.06 ± 0.07
In situ forming gel	2.820 ± 0.672*	4.804 ± 1.230*	0.79 ± 0.10	0.41 ± 0.05	0.83 ± 0.11

* $p < 0.05$ vs. common eye drop.

dialysate was 2 ng ml^{-1}, which was enough for determining the concentration of *S*(−)-satropane in aqueous humor.

Combined with the micro dialysis technique, the drug levels of *S*(−)-satropane could be determined for at least 3 h after instillation. Time–concentration curves were smooth enough to perform pharmacokinetic analysis and their elimination phase was fitted well to the first-order kinetic model, as shown in Fig. 2. Therefore, pharmacokinetic parameters calculated from less-variable data from much fewer animals were reliable and will be useful for clinical regimen.

As a candidate drug for the antiglaucoma therapy, *S*(−)-satropane requires a continuous and chronic administration. Improvement of ocular bioavailability can reduce the administration frequency or lower the drug concentration in ophthalmic preparation, meanwhile decrease undesired side effects. In situ gels could improve the ocular bioavailability by increasing the precorneal residence time [13,14]. In fact, a few ophthalmic drugs utilizing in situ forming gel solutions have been put on the market [15,16]. Compared to aqueous solution, the *S*(−)-satropane in situ forming eye gel exhibited 3.2-fold greater of the C_{max} and 2.2-fold greater of the AUC_{0-3h} (Table 1). These suggested more amount of drug could be absorbed into the eye before it was washed out of the conjunctiva sac by the normal tear turnover and the drainage from the nasolacrymal duct.

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