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Study on the Ocular Pharmacokinetics of Ion-Activated In Situ Gelling Ophthalmic Delivery System for Gatifloxacin by Microdialysis

Zhidong Liu
Department of Pharmaceutical, Shenyang Pharmaceutical University, Shenyang, P. R. China and Research Center of Traditional Chinese Medicine, Tianjin University of Traditional Chinese Medicine, Tianjin, P. R. China

Xing-Gang Yang, Xiang Li, and Weisan Pan
Department of Pharmaceutical, Shenyang Pharmaceutical University, Shenyang, P. R. China

Jiawei Li
Department of Experimental Department, Tianjin University of Traditional Chinese Medicine, Tianjin, P. R. China

The poor bioavailability and therapeutic response exhibited by conventional ophthalmic solutions due to rapid precorneal elimination of the drug may be overcome by the use of gel system. The present work was conducted to evaluate the relative bioavailability of ion-activated in situ ophthalmic gel of gatifloxacin by microdialysis. The conventional ophthalmic solution of gatifloxacin was used as reference. The AUC of test group is 3.8-fold vs. the reference group (1.4316 ± 0.1327 μg·mL^{-1}·h vs. 0.3756 ± 0.0380 μg·mL^{-1}·hr) ($P < 0.05$), and the C_{max} of test group vs. the control group is 3.0-fold (0.3363 ± 0.0634 μg·mL^{-1} vs. 0.1112 ± 0.0151 μg·mL^{-1}) ($P < 0.05$). The T_{max} of test group is longer than that of reference group (2.0 ± 0.67 hr vs. 0.667 ± 0.17 hr) ($P < 0.1$), and K_e of test group is lower than that of reference group. The developed formulation has a higher bioavailability and longer residence time in aqueous humor than conventional ophthalmic solutions. The developed system is a viable alternative to conventional eye drops.

Keywords gatifloxacin; ion-activated; in situ gelling; ophthalmic delivery system; pharmacokinetics; microdialysis

INTRODUCTION

Characterization of regional disposition of xenobiotics in vivo has received increasing attention. Microdialysis has been employed as an analytical tool for regional sampling of fluids of brain, blood, liver (Kurata, et al., 1995), muscle, kidney (Ekstrom, Andersen, Warren, Giercksky, & Slordal, 1996), joint (Sluka, Jordan, Willis, & Westlund, 1994), and ocular tissue (Ben-Nun, Joyce, Cooper, Cringle, & Constable, 1989). It is theorized that pharmacokinetic parameters developed via regional sampling correspond more closely to biophase xenobiotic concentrations: xenobiotic disposition in plasma may not parallel disposition at the site of action (Gibaldi & Perrier, 1982). Microdialysis sampling of ocular tissues (vitreous (Ben-Nun et al., 1989)and retina (Louzada-Junior et al., 1992)) has been reported. Disposition of ophthalmic anti-infectives was examined in vitreal tissue because of perceived difficulties in the pharmacokinetic characterization of drugs administered to reach vitreous (Ben-Nun et al., 1989).

The anterior chamber, which contains the aqueous humor, is a relevant sampling site for estimation of the ocular absorption and regional disposition of topically administered ophthalmic (Acheampong, Shackleton, & Tang-Liu, 1995). Aqueous humor, an ultrafiltrate of plasma (Vaughan & Riordan-Evaoza, 1992), has low concentrations of proteins (~1% of concentrations in plasma; Cole, 1977) which can bind agents such as β-adrenergic antagonists (Evans, Schentag, & Jusko, 1992). Determination of drug concentrations in aqueous humor traditionally has been conducted with paracentesis sampling multiple animals at each time point. Although repeated paracentesis sampling of individual animals has been used (Miller et al., 1991), the standard approach requires single-subject sampling as a terminal procedure. In order to obtain a sufficient sample pool to characterize pharmacokinetics reliably, a large number of animals is required. Microdialysis provides an important advance to the regional sampling tissues, as a complete concentration vs. time profile can be obtained in individual

Address correspondence to Weisan Pan, Department of Pharmaceutics, Shenyang Pharmaceutical University, Shenyang 110016, P. R. China. E-mail: lonerliuzd@163.com

animals. The assessment of regional disposition of β-adrenergic antagonists with microdialysis sampling may provide insight into the pharmacodynamics of decreased aqueous humor procedure as a function of xenobiotic concentration.

The present study was conducted to evaluate the relative bioavailability of an ion-activated in situ gel of gatifloxacin.

MATERIALS AND METHODS

Materials

Gatifloxacin was purchased from HuBei Qianjiang Pharmaceutical Manufacture (Qianjiang, China). Gatifloxacin ophthalmic solution (ZhuNing®) was purchased from AnHui ShuangKe Pharmaceu-tical manufacture (AnHui, China). Lidocaine hydrochloride injection was purchased from Shanghai Hefeng Pharmaceutical Co. Ltd. (Shanghai, China).Ofloxacin ophthalmic solution was purchased from HuBei Qianjiang Pharmaceutical manufacture (Qianjiang, China). Sodium alginate (Kelton®, SA) was kindly gifted by ISP. HPMC (Methocel E50LV) was kindly gifted by Colorcon (UK). All the other chemicals were of analytical grade.

Animals

New Zealand White rabbits, weighing 2.5–3.0 kg, were offered by Animal Experimental Center of Shenyang Pharmaceutical University. The animals, housed in standard cages in light-controlled room at (19 ± 1) ^{o}C and (50 ± 5) % RH, were given a standard pellet diet and water ad libitum. The animals were treated and used as indicated in the publication "Guide for the care and use of laboratory animals" (NIH Publication No. 92–93 revised 1985).

Preparation of Formulation

The formulation were prepared by dispersing alginate 1.0 g and HPMC 2.0 g in 75 mL distilled, deionized water with continuous stirring until completely dissolved. Gatifloxacin was dissolved in hydrochloric acid and the pH was adjusted using sodium hydroxide. Benzalkonium chloride (BKC) was then added to the above solution. The drug solution was added to the alginate/HPMC solution under constant stirring until uniform solution was obtained. Distilled, deionized water was then added to make the volume up to 100 mL (Liu, Li, Nie, Liu, Ding, & Pan, 2006).

Chromatographic Analysis

Gatifloxacin were assayed by reversed-phase HPLC (Shimadzu LC-10ATvp, Japan). A C_{18} column was used with the mobile phase of acetonitrile-0.3 mol·L^{-1} triethylamine (1:4, adjusted pH to 4.5 with phosphate acid) at the detection wavelength of 293 nm. The flow rate was 1.0 mL·min^{-1}. The calibration curve was linear in the range of 0.045 ~ 1.800 μg·mL^{-1}. The sensitivity was 10 ng.

Surgery

Rabbits (n = 4) had been treated with ofloxacin 0.3% ophthalmic solution for 4-days before surgery. Then the animals were anesthetized with injection lidocaine hydrochloride injection. A custom-designed LM-10 microdialysis probe (Bioanalytical System) was implanted into the anterior chamber of each eye as described (Lonnroth, Jansson, & Smith, 1987). Probe inlet and outlet lines were tunneled beneath the conjunctiva, under the upper eyelid, and exited between the ears. The leads were protected with a latex glove pocket affixed to the top of the head (Figure 3). The probe was introduced as described previously (Duchêne & Wouessidjewe, 1996), the anchor was sutured to the sclera with 7-0 Vicryl, and conjunctiva was sutured over the anchor. Exterior wound surfaces were treated with ofloxacin 0.3% ophthalmic solution. Animals were used for experimentation after > 5 days recovery. Slit-lamp was taken after recovery to estimate fibrin formation and the condition of the eye prior to use of the rabbit in experiments.

Recovery by In Vivo Retrodialysis

Conscious rabbits (n = 4) were placed in rabbit restrainers (Figure 5) which permitted free movement of the head. Following a 1-hr equilibration period with perfusion of saline through the probe, different concentration standard gatifloxacin saline solutions (0.090, 0.180, 0.360, 0.540, 0.720, and 0.900 μg·mL^{-1}) were perfused through the probe at a rate of 3-μL·min^{-1}, and dialysate were collected for 15 min after 30 min of perfusion. A 20-μL aliquot of each fraction was analyzed by HPLC. In vivo recovery was defined as (Higuchi, 1960): $R = (C_{in}-C_{out})/(C_m-C_{out})$ (C_{in}, the concentration of standard solutions; C_{out}, the concentration of dialysate; and C_m, the concentration in aqueous humor). A linear equation was plotted by ($C_{in}-C_{out}$) vs. C_{out}, and the slope of the line gives the recovery (R). Figure 1 illustrated the linear regression

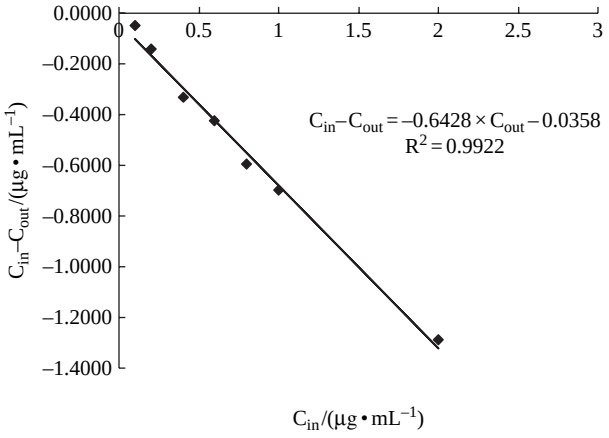

FIGURE 1. In vivo recovery of microdialysis probe in aqueous humor (n = 4). Figure 1 illustrated the linear regression between perfusate (C_{in}) and dialysate (C_{out}): $C_{in}-C_{out} = -0.6428C_{out} - 0.0358$ (R^2 = 0.9922), so the in vivo recovery (r) is 64.28% ± 8.23.

between perfusate (C_{in}) and dialysate (C_{out}); $C_{in} - C_{out} = -0.6428\ C_{out}$—0.0358 ($R^2$ = 0.9922), so the in vivo recovery (r) is 64.28% ± 23.

Pharmacokinetics Studies

A total of 40-µL of gatifloxacin gel as test or gatifloxacin ophthalmic solution as reference was placed in the lower culde-sac with a micropipette. In general, the rabbits closed their eyes without blinking after gatifloxacin administration. Immediately post-dose, 30-µL fractions of effluent were collected every 10 min for 1 hr, then 60-µL collected every 20 min for 6 hr. A 20-µL aliquot of each fraction was assessed by HPLC.

Statistical Analysis

The data obtained are expressed as $M \pm SD$. The pharmacokinetics parameters were statistically evaluated by *t*-test. Differences were considered to be significant at $P < 0.05$.

RESULTS AND DISCUSSION

Recovery

In vitro, there are a number of parameters effecting on recovery, which can be investigated. These parameters include perfusion flow-rate, temperature, perfusate composition, characteristics of the drug, characteristics of the semipermeable membrane, and the surface of the semipermeable membrane. All parameters that influence in vitro recovery will also influence in vivo recovery. However, in vivo, tissue characteristics will play an important role and may ultimately determine the recovery. In vivo recovery depends on diffusion in three regions: probe lumen, dialysis membrane and the periprobe environment (Benveniste, Hansen, & Ottosen, 1991; Bungay, Morrison, & Dedrick, 1990; Morrison et al., 1991). The first two regions can be characterized in vitro. Diffusion in probe lumen is limiting only with the use of very low flow rates. Diffusion through the dialysis membrane is limiting only when transport through the periprobe environment is rapid. Rapid diffusion through the periprobe environment occurs in most flowing system (like blood). In tissues, effective diffusion through the extracellular fluid determines the recovery of the microdialysis probes (Benveniste et al., 1991; Morrison et al., 1991).

In this study, in vivo recovery is higher than reported (Kay, Robert, & Gary, 1998): the recovery was 35%. The difference exists in probe lumen and perfusion flow-rate.

Pharmacokinetics Studies

The area under the aqueous humor conentration vs. time (AUC) was estimated by the linear trapezoidal method with extrapoltion to infinite time. Concentration at peak (C_{max}), time to peak (T_{max}), and terminal rate constant (K_e) were calculated with noncompartmental techniques (Gibaldi, 1998). Individual aqueous humor parameters for each eye were calculated. All parameters were reported as mean ± SD. After the study, no visible ocular damage to the cornea is visible (Figure 4).

Aqueous humor pharmacokinetic parameters were presented in Table 1. The *AUC* of test group is 3.8-fold vs. the reference group ($P < 0.05$), and the C_{max} of test group vs. the control group is 3.0-fold ($P < 0.05$). The T_{max} of test group is longer than that of reference group ($P < 0.1$), and K_e of test group is lower than that of reference group.

As shown in Figure 2, gatifloxacin could be still detected at 8 hr after adminstration in the test group, otherwise, it could only be detected at 5.3 hr after adminstration in the reference group. So the developed formulation has longer resident time in aqueous humor than conventional ophthalmic solutions.

The preparation was composed of Alginate and HPMC. Sodium alginate used as gelling agent forms a low viscosity, free-flowing liquid at concentrations suitable for gel formation upon exposure to divalent cations in the lacrimal fluid. HPMC was used as an enhancing- viscosity agent. The developed formulation exhibited pseudoplastic rheology and prolonged the precorneal retention time of drug than that of conventional ophthalmic solution (Liu et al., 2006).

CONCLUSION

Pharmacokinetic study indicated the ion-activated in situ gel of gatifloxacin has a higher bioavailability and longer residence time in aqueous humor than conventional ophthalmic solutions of gatifloxacin, and which is a viable alternative to conventional eye drops by its ability to enhance bioavailability through its longer precorneal residence time and ability to sustain release of the drug. Also important is the ease of administration afforded and decreased frequency of administration resulting in better patient compliance.

TABLE 1
Pharmacokinetics Parameters of Gatifloxacin in Aqueous Humor After Topical Administration in Conscious Rabbit (n = 4)

Drug	AUC/(µg·mL^{-1}·hr)	C_{max} (µg·mL^{-1})	T_{max} /hr	K_e/hr^{-1}	$t_{1/2}$/hr
Reference	0.3756 ± 0.0380	0.1112 ± 0.0151	0.667 ± 0.17	0.3961 ± 0.19	2.09 ± 1.10
Test	1.4316** ± 0.1327	0.3363** ± 0.0634	2.0* ± 0.67	0.1959 ± 0.03	3.59 ± 0.56

*$P < 0.10$
**$P < 0.05$.

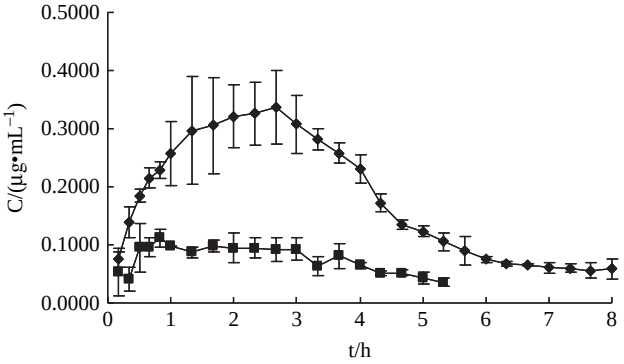

FIGURE 2. Aqueous humor gatifloxacin concentration-time profiles following a 40-μL topical dose in conscious rabbits (n = 4). □: test; ■: reference.

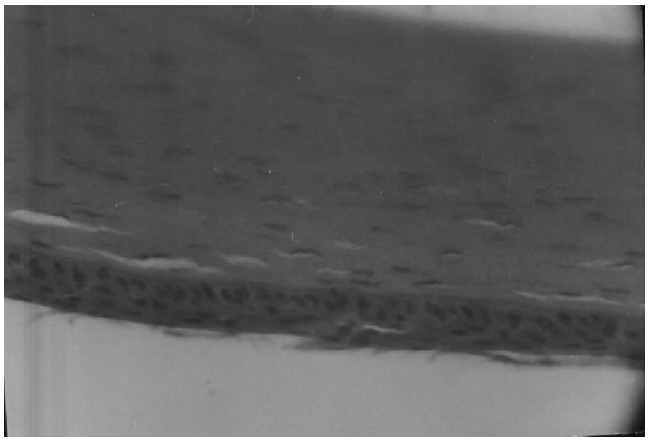

FIGURE 4. The photograph of rabbit cornea after pharmacokinetics study (40×).

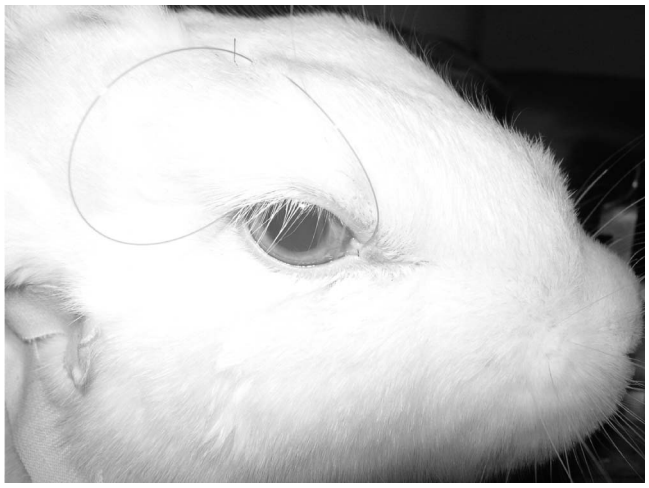

FIGURE 3. The photograph of microdialysis probe implantation in the ocualr.

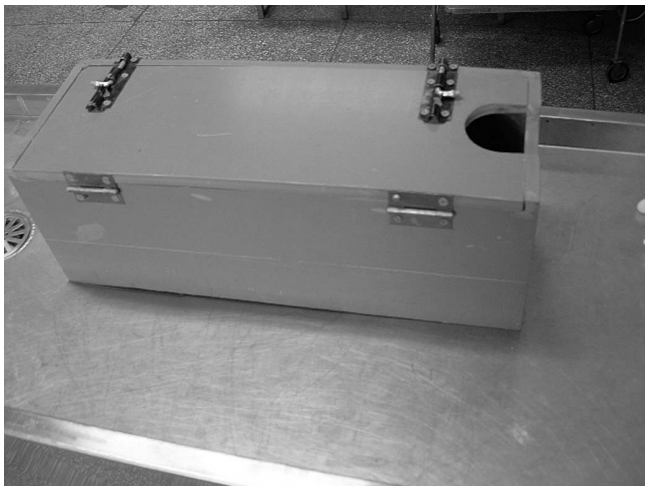

FIGURE 5. The photograph of rabbit restrainer.

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