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Validation of an ocular microdialysis technique in rabbits with permanently implanted vitreous probes: systemic and intravitreal pharmacokinetics of fluorescein

Banmeet S. Anand, Harisha Atluri, Ashim K. Mitra*

Division of Pharmaceutical Sciences, School of Pharmacy, University of Missouri-Kansas City, 5005 Rockhill Road, Kansas City, MO 64110-2499, USA

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Abstract

The purpose of this work is to validate a novel ocular microdialysis sampling technique in rabbits with permanently implanted vitreous probes. This objective is achieved by studying the vitreous pharmacokinetics of fluorescein following systemic and intravitreal administration. The rabbits were divided into two groups (groups I and II) based on whether or not they were allowed a recovery period following surgical implantation of probes. The integrity of the blood-retinal barrier was determined by the vitreal protein concentrations and the fluorescein permeability index. Vitreal protein concentrations returned to baseline 48 h after probe implantation and therefore experiments were conducted 72 h post-implantation of probes in rabbits where recovery period was allowed. The permeability indices for fluorescein after systemic administration in group I (without recovery period) and group II (with recovery period) indicated that the integrity of the blood-retinal barrier was maintained and were found out to be 0.55 ± 0.27 and 0.71 ± 0.38%, respectively, for the vitreous chamber. Following microdialysis probe implantation in the group II rabbits, the blood-retinal barrier integrity was not compromised. A novel microdialysis technique in rabbits with permanently implanted probes for studying the pharmacokinetics of posterior segment has been developed and characterized.

Keywords: Conscious; Microdialysis; Fluorescein; Ocular; Blood ocular barriers; Posterior segment

1. Introduction

Posterior segment of the eye is affected by various vision impairing pathological conditions such as viral retinitis, bacterial and fungal endophthalmitis, proliferative vitreo-retinal disorders, and diabetic retinopathy. Information regarding posterior segment drug kinetics is necessary for optimizing the dosing and development of ocular drug delivery systems. One of the major limitations in assessing ocular pharmacokinetics is the constraint that a single rabbit must be used for a single time point. Complete pharmacokinetic profiles are usually constructed by sacrificing 6–20 rabbits at each time point. A sufficient number of intervals must be selected to adequately characterize absorption, distribution and elimination processes (Schoenwald, 1990). Microdialysis has been proven to be beneficial over conventional sampling techniques in determining ocular pharmacokinetics by both reducing the number of subjects and providing statistically robust data. It has been applied in aqueous and vitreous drug disposition and delivery studies (Waga

* Corresponding author. Tel.: +1-816-235-1615; fax: +1-816-235-5190.
E-mail address: mitraa@umkc.edu (A.K. Mitra).

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et al., 1991, 1999; Stempels et al., 1993; Hughes et al., 1996; Waga and Ehinger, 1997; Rittenhouse and Pollack, 2000; Macha and Mitra, 2001b, 2002). An acute dual probe microdialysis technique for simultaneous sampling of vitreous and aqueous humor in rabbits has been previously established in our laboratory (Macha and Mitra, 2001a; Atluri and Mitra, 2003). This technique was validated by measuring intraocular pressure, protein concentration and fluorescein kinetics (Macha and Mitra, 2001a).

However a chronic ocular microdialysis technique would be advantageous over acute model since it can be applied to perform multiple experiments and study ocular kinetics for a longer duration. Chronic microdialysis techniques to sample vitreous and aqueous humor, respectively, have been established previously (Waga et al., 1991; Rittenhouse et al., 1999). Posterior segment microdialysis technique with permanently implanted probes has been previously reported for studying vitreous drug delivery and kinetics (Waga et al., 1991).

In this article we have reported the development and validation of an ocular microdialysis model for the posterior segment with permanently implanted probes, which is a definite improvement over the currently existing acute microdialysis technique. The development of this model is based on the procedures reported by Waga et al. (1991) except for the type of probes employed for the study. Our study aims at validating the method by studying vitreal kinetics of fluorescein following systemic and intravitreal administration in two separate groups of animals depending on the recovery period after probe implantation. Fluorescein was chosen as a model compound to investigate the blood-retinal barrier integrity after probe implantation. The protein concentration in the vitreous body was also determined at various time intervals in order to study the effect of probe implantation on the alteration of protein levels and the integrity of blood-retinal barrier.

2. Materials and methods

2.1. Materials

The linear microdialysis probes (MD-2000, 0.32 mm × 10 mm, polyacrylo nitrile membrane and 0.22 mm tubing) employed for vitreous sampling were purchased from Bioanalytical Systems (West Lafayette, IN). Microdialysis pump (CMA/100) for perfusing the isotonic buffer saline was procured from CMA/Microdialysis (Acton, MA). Surgical equipment was obtained from Henry Schein surgical equipment and sutures were purchased from Ethicon Inc. (Somerville, NJ). Ketamine HCl was supplied by Fort Dodge Animal Health and xylazine by Bayer Animal Health. Nembutal sodium was purchased from Abbott Laboratories (Abbott Park, Chicago, IL). Fluorescein sodium salt was obtained from Aldrich Chemical Company Inc. (Milwaukee, WI). Tropicamide was purchased from Martec Pharmaceuticals (Kansas City, MO). All other chemicals were obtained from Sigma Chemical Company (St. Louis, MO). The solvents and buffer components were of analytical grade and obtained from Fisher Scientific (St. Louis, MO).

2.2. Animals

Adult male New Zealand albino rabbits weighing between 2 and 2.5 kg were obtained from Myrtle's Rabbitry (Thompson Station, TN). This research was conducted strictly according to the experimental protocols listed in Association for Research in Vision and Ophthalmology (ARVO) guidelines on the use of animals.

2.3. Probe recovery

In vitro probe calibration was performed by placing the probe in isotonic phosphate buffer saline (IPBS) solution, pH 7.4, containing fluorescein of a known concentration. The probe was perfused at a flow rate of 2 μL/min with isotonic phosphate buffer saline and the dialysate was collected every 20 min for 1 h. Relative in vitro recovery of fluorescein is calculated by the following equation:

$$\text{Recovery} = \frac{C_d}{C_s}$$

where C_d is the dialysate concentration and C_s the known concentration of fluorescein in IPBS. In vivo retrodialysis method with fluorescein was employed for probe calibration in rabbits and the following equation was used:

$$\text{Recovery} = \frac{C_s - C_d}{C_s}$$

The concentration of the fluorescein in vitreous humor during the pharmacokinetic experiment was calculated by dividing the dialysate concentration with in vivo recovery above obtained.

2.4. Probe implantation

2.4.1. Animals without recovery period

The animals were anesthetized prior to the surgery by administering ketamine (50 mg/kg) and xylazine (5 mg/kg) intramuscularly. Pupils were dilated by topical instillation of 1% tropicamide prior to the probe implantation. A linear probe (Bioanalytical Systems, MD-2000) was inserted across the vitreous body with the aid of a 25G needle at about 3 mm below the corneal scleral limbus at 45° angle. The outlet of linear probe was placed into the needle at bevel edge, which was inserted into the vitreous. Then the needle was slowly withdrawn such that the probe remained fixed within the vitreous chamber. The microdialysis probe was perfused with isotonic phosphate buffer saline at a flow rate of 2 μL/min by a microdialysis pump. This group of animals was designated as group I.

2.4.2. Animals with recovery period

Animals were anesthetized as mentioned above. The eye was proptosed and a 25G needle was inserted into the eye approximately 8 mm below the scleral-limbus junction which exited at the opposite side. A linear probe (Bioanalytical Systems, MD-2000) was inserted into the needle and the probe was pulled through the eye by withdrawing the needle. The probe was positioned in such a way that the entire membrane area lies in the vitreous chamber and is slightly angled so as to avoid contact with the lens. After the microscopic inspection of the probe position, the probe was secured to the conjunctiva with a 6-0 surgical chromic gut suture. Subsequent to probe implantation in the eye, the area between the ears was shaved and a 4 mm incision was made using a sterile disposable scalpel. A subcutaneous tunnel from the area of the incision to the upper eyelid was cleared using forceps. The two ends of the forceps were then introduced through the subcutaneous tunnel and externalized through a small incision in the upper eyelid. Two ends of the probe were pulled through the tunnel with forceps. The outlet and inlet ends of the probe were exited completely through the area between the ears and the incision was then sutured by securing the probes with the skin using a 3-0 suture. Throughout the procedure IPBS was flushed through the probe with a microdialysis pump to ensure that the probe was intact. Once the skin sutures were in place the outlet and inlet ends were cut to the appropriate size so as to prevent the rabbit from accessing it. After the surgical procedure the animals were observed at least once daily for signs of irritation and inflammation. One to two drops of 0.1% dexamethasone phosphate were applied topically to counter any inflammation or redness of the sclera or conjunctiva. The probes were also flushed once a day (20 μl/min) with IPBS containing a cocktail of penicillin and streptomycin to prevent any bacterial growth in the eye and around the probe membrane. Only animals that showed no signs of infection or probe blockade were dosed for experiments. This group of animals with permanently implanted probes was designated as group II.

2.5. Protein determination

The effect of probe placement on the protein content of the vitreous humor was assessed as a function of time in order to determine optimum recovery time needed prior to initiation of a pharmacokinetic study. The protein content of the vitreous humor was determined up to a period of 5 days post-probe implantation. The probes were placed in the vitreous chamber of the rabbits as described in the animal surgical procedure under the section of animals with recovery period. The animals were sacrificed at appropriate time intervals and the eyes were immediately enucleated. Through a small incision in the sclera, vitreous humor (1–1.5 ml) was aspirated with a 1 μl tuberculin syringe. The vitreous humor was then centrifuged at 10000 rpm for 15 min to remove any cellular debris. Protein content of the vitreous humor was determined by the method of Bradford (Bradford, 1976) using bovine serum albumin as the standard (BioRad protein estimation kit, Hercules, CA). Protein concentration of the contralateral eye served as the control. Protein contents in the experimental and the control eyes were plotted against time.

2.6. Fluorescein kinetics

2.6.1. Systemic administration

Fluorescein was administered systemically (10 mg kg^{-1} body weight) into the rabbit marginal ear vein. Microdialysis vitreous samples were collected every 20 min over a period of 10 h. Blood samples were collected at 5, 30, 60, 120, 240, 360, 480 and 600 min. The animals were divided into two groups depending on the recovery period allowed after the probe implantation. Since the purpose of this experiment was to determine the integrity of the blood-retinal barrier following the surgical implantation of the probe. Animals in both the groups were kept anesthetized during the experiments for the ease of blood sampling. The analysis was done following extraction of the drug from the plasma as described previously (Larsen et al., 1988). The permeability index (PI) of vitreous chamber following systemic administration of fluorescein was calculated according to the following equation as described previously (Knudsen et al., 1992):

$$\mathrm{PI}_{\mathrm{vitreous}} = \frac{\mathrm{AUC}_{\mathrm{vitreous}}}{\mathrm{AUC}_{\mathrm{plasma}}}$$

where $\mathrm{AUC}_{\mathrm{vitreous}}$ and $\mathrm{AUC}_{\mathrm{plasma}}$ are the area under curves obtained after vitreous and systemic administration of fluorescein, respectively.

2.6.2. Intravitreal administration

The animals without recovery period were anesthetized during the experiment while on the other hand the animals with recovery period were kept conscious under a restrainer during the experiment. Fluorescein was administered intravitreally 20 min after probe implantation. A 100 μL volume of fluorescein (1 mg/ml) was administered into the vitreous body using a canula to which 30G needle was attached. The dialysate was collected every 20 min over a period of 10 h.

2.7. Analytical procedures

The analysis of fluorescein in microdialysis samples (vitreous and plasma) was performed by reversed phase liquid chromatography. The system was comprised of a Rainin Dynamax Pump SD-200 (Woburn, MA), a HP 1100 series fluorescence detector at λ_{ex} = 460 nm, λ_{em} = 520 nm and an Alcott autosampler Model 718AL HPLC (Norcross, GA). The column used was a C18 Phenomenex Luna column 4.6 mm × 250 mm (Torrence, CA). The mobile phase consisted of a mixture (50:50) of 0.1 M phosphate buffer and methanol set at a pH of 7.35. This method produced rapid and reproducible results and has been reported previously (Larsen et al., 1988).

2.8. Data analysis

All experiments were conducted at least in triplicate and the results are expressed as mean ± S.D. Student's *t*-test was used to detect statistical significance and $P < 0.05$ was considered to be statistically significant. The vitreous and plasma concentrations time data of an individual rabbit was analyzed using a pharmacokinetic software package WinNonlin, v2.1 (Pharsight, CA). Pharmacokinetic parameters were determined employing non-compartmental analysis, which applies a model independent approach. Area under the vitreous and plasma concentrations time curves were estimated by the linear trapezoidal method with extrapolation to infinite time. Slopes of the terminal phase of vitreous and plasma profiles were estimated by log-linear regression and the terminal rate constant (λ_z) was derived from the slope. Terminal vitreous and plasma half-lives were calculated from the equation: $t_{1/2} = 0.693/\lambda_z$.

3. Results

3.1. Probe recovery

Probe functioning was studied over a period of 7 days by examining the recovery of fluorescein in group II. The recovery of the probe from 0 to 7 days ranged from 97 to 100% of the control. Results indicated that the probe was functioning for at least 7 days and the recoveries on the seventh day did not vary significantly ($P < 0.05$) relative to the initial recovery at 1 h post-probe implantation. The in vitro probe recovery for the linear probe ranged from 48 to 52%. Recovery values obtained from in vivo retrodialysis method in rabbits ranged from 42 to 45%.

3.2. Protein determination

The total vitreous protein concentrations were determined in order to ascertain the integrity of the

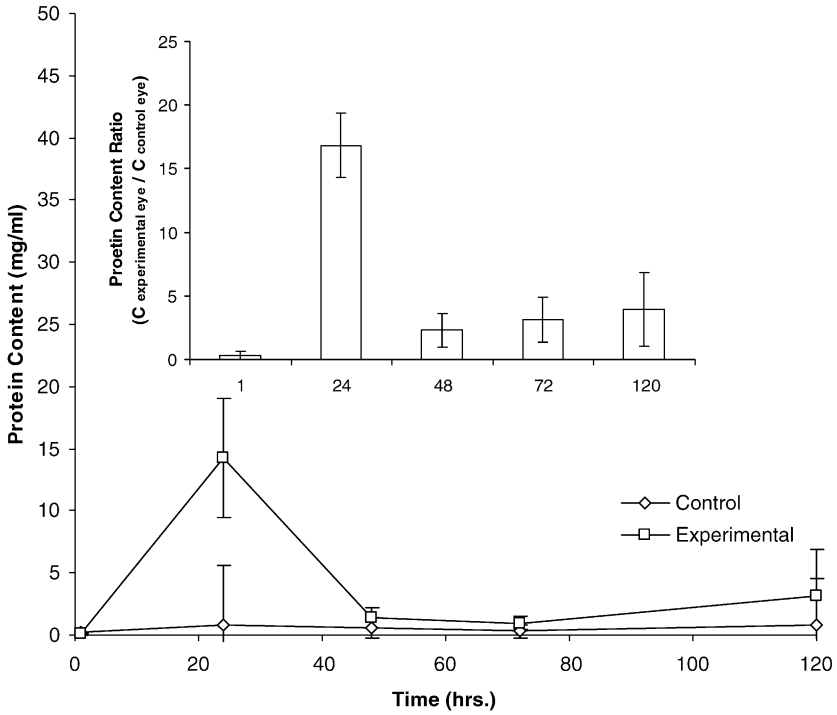

Fig. 1. Protein concentrations in the vitreous humor after implantation of the linear microdialysis probe. Comparison of control eye (◇) vs. the experimental eye (□). Inset, ratio of protein content of experimental eye to the control eye. Values are represented as mean ± S.D. ($n = 6$).

blood-retinal barrier and also to determine the initiation time of an experiment after the probe implantation in the conscious animals. As shown in Fig. 1 the total protein concentrations were maximum at 24 h post-probe implantation and declined with time ultimately reaching the baseline after 48 h. At any time point the ratio of the protein concentration of the experimental eye to the control eye was less than 20-fold (Fig. 1, inset), which would not have been the case had the blood-retinal barrier been compromised. Since the protein concentrations reached baseline at 48 h. All conscious animal experiments were performed 3 days post-implantation of the probes following surgery.

3.3. Fluorescein kinetics

3.3.1. Animals without recovery period (group I)

Figs. 2 and 3 depict the concentration–time profiles of fluorescein following systemic and intravitreal administrations in anesthetized rabbits without recovery period. The plasma and vitreous chamber concentration time profiles of fluorescein after systemic and intravitreal administration were analyzed with a non-compartmental model. Plasma and vitreous pharmacokinetic parameters are summarized in Table 1.

Intravitreal profile of fluorescein exhibited initial vitreous diffusive equilibration phase followed by elimination phase from the vitreous body (Fig. 3). The plasma elimination half-life of fluorescein was calculated to be 6.25 ± 2.6 h after systemic administration. Maximum concentration (C_{max}) of fluorescein in the plasma and vitreous chamber following systemic administration was calculated as 15.82 ± 4.1 and 0.039 ± 0.029 μg ml^{-1}, respectively. Permeability index (PI) of the vitreous chamber following systemic administration of fluorescein was calculated as 0.55±0.27% (Table 1). Vitreal elimination half-life of fluorescein following intravitreal administration was 3.17 ± 1.02 min. A similar half-life for the vitreous chamber following intravitreal administration has been reported previously (Macha and Mitra, 2001a). C_{max} of fluorescein in vitreous chamber following

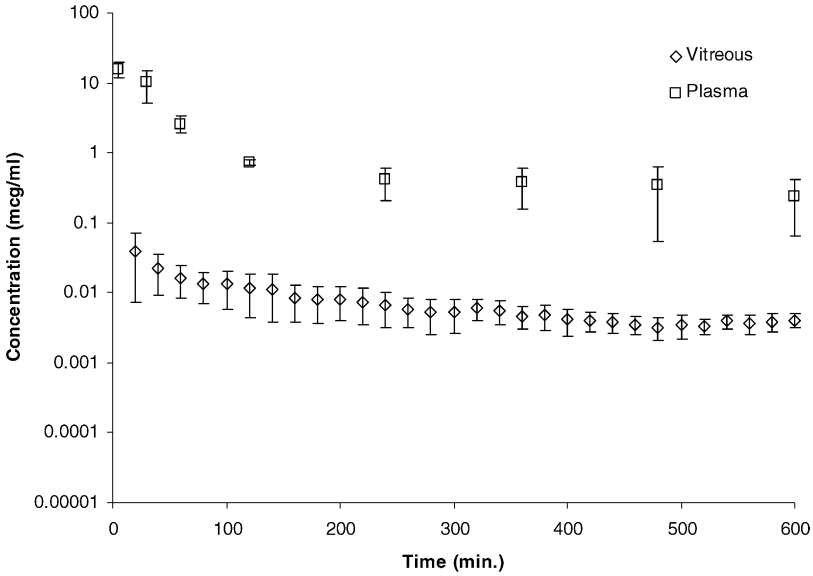

Fig. 2. Concentration–time profiles of plasma and vitreous chamber following systemic administration of fluorescein in anesthetized rabbits without recovery period. Values are represented as mean ± S.D. ($n = 6$).

intravitreal administration was calculated as 13.24 ± 7.75 $\mu g\,ml^{-1}$. The clearance (Cl) values from plasma and vitreous were calculated as 30.61 ± 9.15 and 0.025 ± 0.013 $ml\,min^{-1}$, respectively (Table 1).

3.3.2. *Animals with recovery period (group II)*

Figs. 4 and 5 depict the concentration–time profiles for fluorescein following systemic and intravitreal administration, respectively. The pharmacokinetic

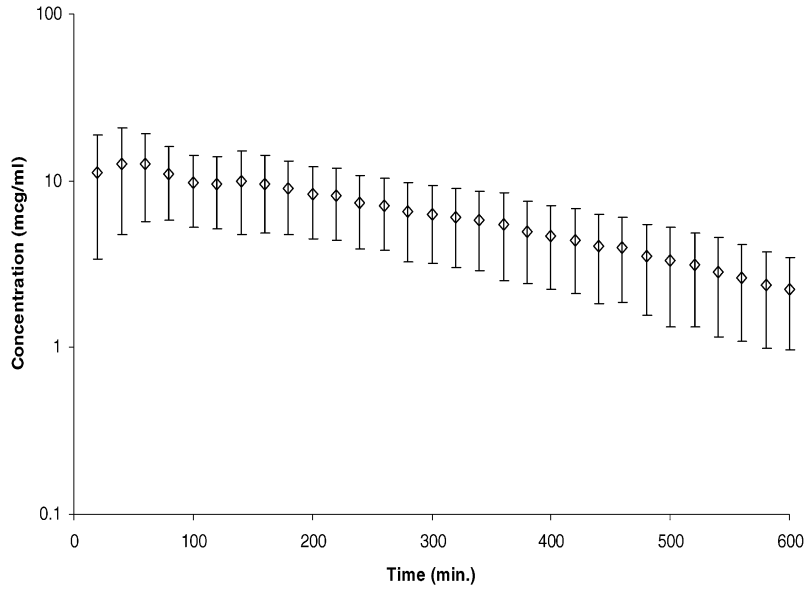

Fig. 3. Concentration–time profile of vitreous chamber following intravitreal administration of fluorescein in anesthetized rabbits without recovery period. Values are represented as mean ± S.D. ($n = 8$).

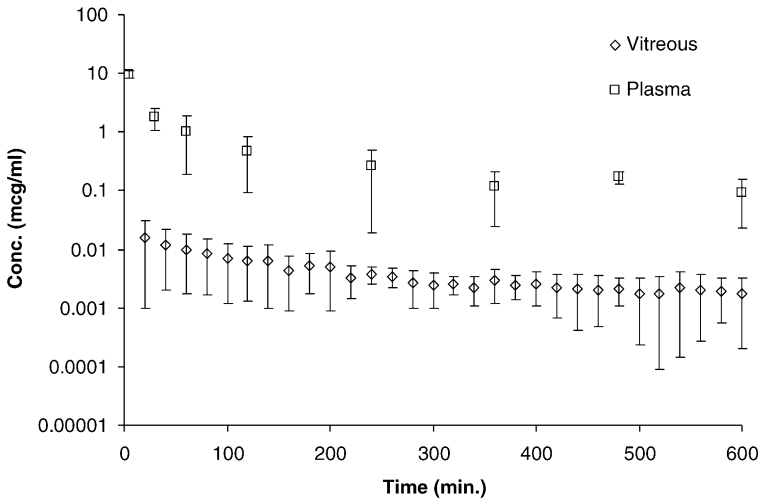

Fig. 4. Concentration–time profile of plasma and vitreous chamber following systemic administration of fluorescein in rabbits with recovery period. Values are represented as mean ± S.D. ($n = 6$).

parameters have been calculated by a non-compartmental model and are listed in Table 2. The elimination half-lives of the plasma and vitreous chamber following systemic and intravitreal administration were calculated to be 5.05 ± 1.72 and 2.56 ± 0.5 h, respectively, and were not significantly different ($P < 0.05$) from the half-lives calculate for anesthetized rabbits without recovery period (Table 2).

The PI of vitreous chamber upon systemic administration of fluorescein to conscious animals was calculated to be 0.71 ± 0.38%, which was not significantly different ($P < 0.05$) from the PI of vitreous chamber

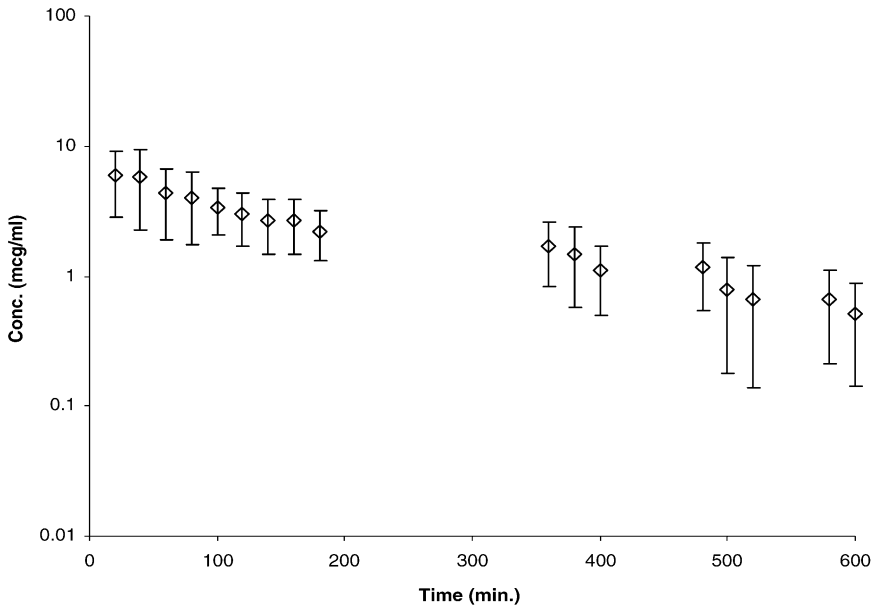

Fig. 5. Concentration–time profiles of vitreous chamber following intravitreal administration of fluorescein in conscious rabbits with recovery period. Values are represented as mean ± S.D. ($n = 6$).

Table 1
Pharmacokinetic parameters following systemic and intravitreal administration of fluorescein in group I rabbits without recovery period

Parameter	Systemic administration		Intravitreal administration
	Plasma	Vitreous	Vitreous
$t_{1/2}$ (h)	6.25 ± 2.6	–	3.17 ± 1.02
$AUC_{(0-t)}$ ($\mu g\,ml^{-1}\,min$)	891.55 ± 203.47	4.95 ± 2.42	4003.37 ± 1842.85
AUC_{inf} ($\mu g\,ml^{-1}\,min$)	1047.15 ± 309.82	6.43 ± 2.05	4670.24 ± 1987.54
Cl ($ml\,min^{-1}$)	30.61 ± 9.15	–	0.025 ± 0.013
PI	–	0.0055 ± 0.0027	–
C_{max} ($\mu g\,ml^{-1}$)	15.82 ± 4.1	0.039 ± 0.029	13.24 ± 7.75

Values are mean ± S.D. ($n = 3$–6).

Table 2
Pharmacokinetic parameters following systemic and intravitreal administration of fluorescein in group II rabbits with recovery period

Parameter	Systemic administration		Intravitreal administration
	Plasma	Vitreous	Vitreous
$t_{1/2}$ (h)	5.05 ± 1.72	–	2.56 ± 0.5
$AUC_{(0-t)}$ ($\mu g\,ml^{-1}\,min$)	380.38 ± 162.12	2.71 ± 1.46	1806.82 ± 898.67
AUC_{inf} ($\mu g\,ml^{-1}\,min$)	433.53 ± 167.17	3.17 ± 1.51	1924.28 ± 921.67
Cl ($ml\,min^{-1}$)	78.73 ± 33.96	–	0.082 ± 0.035
PI	–	0.0071 ± 0.0038	–
C_{max} ($\mu g\,ml^{-1}$)	9.76 ± 1.27	0.021 ± 0.018	7.91 ± 3.28

Values are mean ± S.D. ($n = 3$–6).

in anesthetized rabbits. The C_{max} of fluorescein in the plasma and vitreous chamber following systemic administration were calculated as 9.76 ± 1.27 and 0.021 ± 0.018 $\mu g\,ml^{-1}$, respectively, whereas C_{max} in the vitreous chamber following intravitreal administration was found to be 7.91 ± 3.28 $\mu g\,ml^{-1}$. The clearance (Cl) values from plasma and vitreous were calculated as 78.73 ± 33.96 and 0.082 ± 0.035 $ml\,min^{-1}$, respectively (Table 2).

4. Discussion

Topical and systemic administrations may not deliver effective therapeutic concentrations of the drug to the posterior segment of the eye (Lesar and Fiscella, 1985; Geroski and Edelhauser, 2000). Effective therapeutic concentrations in the vitreo-retinal tissues can be attained by direct intravitreal administration thereby reducing systemic load and toxicity. Intravitreal route of administration has proven to be relatively safe and effective (Baum et al., 1982). A major constraint in determining intravitreal kinetics is the inability to sample vitreous continuously. Microdialysis technique has proven to be an effective means of delineating intravitreal kinetics (Ben-Nun et al., 1989; Hughes et al., 1996; Waga and Ehinger, 1997; Waga et al., 1999; Waga, 2000). The present study is aimed at the development, validation and characterization of a microdialysis sampling technique in rabbits with permanently indwelling probes.

The main advantage of this animal model in comparison to the acute microdialysis model is the viability of probe for longer periods and its utility in performing multiple-dose and long term pharmacokinetic studies. In our studies the probe remained viable for 7 days and given proper care and maintenance the probes can even remain viable for up to 30 days. During the study period a topical anti-inflammatory agent, dexamethasone was administered to counter any inflammation of the sclera or conjunctiva. The animals responded very well to the treatment and there was no sign of inflammation after 2 days.

Alterations in the vitreal protein content may lead to a change in drug disposition for highly protein bound drug molecules. In the present study the protein levels

reached a maximum immediately after implantation of the probe and then declined with time, reaching baseline around 48 h. An earlier report has suggested that alterations in aqueous humor protein concentrations may dictate the ocular pharmacokinetics. Therefore proper caution must be exercised in the design of microdialysis experiments (Rittenhouse et al., 1999). In studying highly protein bound molecules an appropriate recovery period must be allowed in order for the protein levels to return close to baseline. In the present study all the experiments were performed at 72 h post-implantation, when the protein levels in the experimental eyes and the control eyes were similar and were at or near the baseline (Fig. 1). The protein levels examined in this study also may provide an insight into the integrity of the blood-retinal barrier. The ratio of protein concentration of the experimental eye to the control eye at all time points was less than 20-fold (Fig. 1, inset), indicating that there was no breakdown in the blood-retinal barrier following probe implantation.

The barrier property of blood-retinal barrier was also evaluated by determining PI of fluorescein. Fluorescein is an organic anion often used for in vivo evaluation of blood ocular barrier integrity. Integrity of the blood-retinal barrier has been determined by quantitative measurement of fluorescence in the vitreous cavity following systemic administration of fluorescein (Zeimer et al., 1983). The PIs of the vitreous chamber in animals without recovery period and with recovery period were calculated to be 0.55 ± 0.27 and $0.71 \pm 0.38\%$, respectively. Therefore PI values indicate restricted exchange of fluorescein between plasma and the vitreous chamber due to the intact blood-retinal barrier. Similar results have been obtained previously where a tighter barrier was observed to restrict molecular exchange between the plasma and vitreous chambers across the blood-retinal barrier (Knudsen et al., 1998).

Previously, a posterior segment conscious microdialysis model was developed by Waga et al., but no systematic comparison was made between the vitreous drug disposition in conscious and anesthetized animal models following intravitreal and systemic administration (Waga et al., 1991). Rittenhouse et al. have developed anterior chamber conscious microdialysis model to study the disposition of drugs in the anterior chamber. The effect of aqueous humor protein concentration and anesthesia on propranolol disposition was examined after topical administration (Rittenhouse et al., 1999). Anesthetic combination of ketamine and xylazine may produce suppressive effect on heart and respiratory rate (Sanford and Colby, 1980; Lipman et al., 1987) and may also exhibit effect on the intraocular pressure (Trim et al., 1985; Burke and Potter, 1986; Jia et al., 2000). Intraocular exposure of propranolol reflected by C_{max} and area under curve of aqueous humor (AUC_{AH}) values in the conscious rabbits was reduced relative to that in anesthetized rabbits. However the time to peak concentration (T_{max}) and the rate of elimination from the anterior chamber were similar in conscious and anesthetized rabbits. Such differences may be attributed to physiological effects caused by anesthesia in the anterior segment such as decreased aqueous humor drainage, reduced tear turnover and lower rate of blinking (Rittenhouse et al., 1999). In the present study however, the vitreous pharmacokinetic parameters were not found to be significantly different ($P < 0.05$) between conscious and anesthetized rabbits (Tables 1 and 2). It could possibly be due to lesser physiological effects of anesthesia especially the one altering the vitreous disposition. In addition, transport in the vitreous humor is caused by diffusion and convection and alteration in the ocular pressure due to the anesthesia affects the convective forces (Tsuboi and Pederson, 1988), which in turn alters the distribution of the high molecular weight compounds. Moreover such an effect is also predicted to be magnified in larger animals, such as humans (Xu et al., 2000). However this effect would not be applicable in the present study of rabbit intravitreal kinetics of fluorescein as it is a relatively low molecular weight compound.

In conclusion, the surgical procedure for the permanent implantation of the probes did not compromise the integrity of the blood-retinal barrier as evident by the comparative permeability indices of fluorescein upon systemic administration. The surgical procedure was very well tolerated by the animals in the group II as they did not show any major infection besides redness and slight inflammation of the eye. The probes in group II animals were functional for at least 7 days and did not show a significant difference in the recovery. The return of the protein levels to baseline at the end of 2 days makes it possible for the experiments to be performed after 3 days of the surgical procedure. In conclusion, the development and characterization

of this model provides a new technique for the study of drug pharmacokinetics in the posterior segment of the eye.

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