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Evaluation of microdialysis sampling of aqueous humor for in vivo models of ocular absorption and disposition[1]

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Abstract

The dynamics of β-adrenergic-associated reductions in aqueous humor production for treatment of elevated intraocular pressure are not well understood. In particular, the relationship between ocular pharmacokinetics and pharmacodynamics has yet to be established. This study was undertaken to develop a procedure for examining the ocular absorption and disposition of topically administered ophthalmic β-adrenergic antagonists in individual animals. Dogs were anesthetized with isoflurane and a microdialysis probe was implanted in the anterior chamber of one eye and perfused with 0.9% saline at a rate of 2 μl min^{-1}. ^{3}H-propranolol was administered by intracameral injection or topically. Each dog received intracameral and topical propranolol, in alternate eyes on separate days, in a randomized cross-over fashion. Microdialysis probe effluent was collected every 5 min for ≥ 2.5 h; concentrations of propranolol were determined by liquid scintillation spectroscopy and were corrected for probe recovery of the substrate as determined by in vivo retrodialysis ($\sim 46\%$) to estimate aqueous humor concentrations. In separate experiments in rabbits, microdialysis probes were implanted in each eye. ^{3}H-propranolol was administered topically to one eye; the contralateral eye received intracameral ^{3}H-propranolol. Model-independent pharmacokinetic parameters for each treatment phase were calculated. The mean $\pm$ S.D. times to peak concentration of propranolol in aqueous humor were 86.6 ± 47.6 min in the dog and 54.1 ± 20.4 min in the rabbit. The terminal rate constant was 0.0189 ± 0.00429 min^{-1} in the dog vs. 0.00983 ± 0.00546 min^{-1} in the rabbit. Intraocular tissue availability of propranolol differed markedly between the dog ($n = 3$) and rabbit ($n = 3$) (~ 0.056 in the dog vs. ~ 0.55 in the rabbit). These results demonstrate the utility of microdialysis sampling for examination of ocular pharmacokinetics.

Keywords: Microdialysis; Ocular pharmacokinetics; β-adrenergic antagonists; Aqueous humor; Propranolol

1. Introduction

Characterization of regional disposition of xenobiotics in vivo has received increasing attention. Microdialysis has been employed as an analytic tool for regional sampling of fluids of brain,

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blood, liver [1], muscle, kidney [2], joint [3], and ocular tissue [4]. 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 [5]. Microdialysis sampling of ocular tissues (vitreous [4] and retina [6]) 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 [4].

β-adrenergic antagonists are administered topically in the treatment of ocular hypertension and glaucoma [7]. Studies attempting to correlate pharmacologic effect (i.e., decreased intraocular pressure) with concentrations of β-adrenergics in ocular tissues and aqueous humor have not been successful [8]. The cascade of events following the topical administration of β-adrenergics to the resultant decrease in aqueous humor production that ultimately results in decreased intraocular pressure has not been well characterized [9].

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 ophthalmics [10]. Aqueous humor, an ultrafiltrate of plasma [11], has low concentrations of proteins ($\sim 1\%$ of concentrations in plasma) [12] which can bind agents such as β-adrenergic antagonists [13]. Determination of drug concentrations in aqueous humor traditionally has been conducted with paracentesis sampling of multiple animals at each time point. Although repeated paracentesis sampling of individual animals has been used [14], 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 of tissues, as a complete concentration-vs.-time profile can be obtained in individual animals. The assessment of the regional disposition of β-adrenergic antagonists with microdialysis sampling may provide insight into the pharmacodynamics of decreased aqueous humor production as a function of xenobiotic concentrations.

The present study was conducted to evaluate the applicability of microdialysis sampling for determination of aqueous humor pharmacokinetics. Propranolol, a β-adrenergic antagonist, was selected as a probe to assess the regional disposition of topically administered ophthalmics.

2. Materials and methods

2.1. Reagents

For the initial dog experiments, DL-propranolol as the hydrochloride salt (5 μg μl^{-1} with respect to the base; Sigma Chemical Company, St. Louis, MO), in 0.9% normal saline with 49 μCi mg^{-1} or 17 μCi mg^{-1} of ^{3}H-propranolol was prepared aseptically for intracameral injection (150 or 700 μg), or with 33 μCi mg^{-1} for topical administration (75, 125 μg). The quantities of ^{3}H-propranolol required to obtain sufficient detection were estimated. For all remaining experiments, solutions for topical administration were prepared with DL-propranolol hydrochloride equivalent to 5 μg μl^{-1} propranolol base, containing 289 μCi mg^{-1} ^{3}H-propranolol hydrochloride (specific activity: 15–30 Ci $mmol^{-1}$, Amersham Life Science, Elk Grove, IL) in 0.9% normal saline. Solutions for intracameral administration were prepared as 5 μg μl^{-1} propranolol base with 18.3 μCi mg^{-1} ^{3}H-propranolol hydrochloride in 0.9% normal saline. Purity of ^{3}H-propranolol was $>95\%$ as assessed by thin layer chromatography. All other solvents and chemicals were reagent grade.

2.2. Animals

Four dogs (12–23 kg) were obtained from CNS Kennel (Kodak, TN). Three New Zealand white rabbits (3.9–5.5 kg) were obtained from Robinson Service (Winston-Salem, NC). The dogs were fed a standard diet and water ad libitum until the morning prior to surgery, when food intake was restricted to prevent potential aspiration of food during anesthesia. Rabbit diet was not restricted prior to surgery. All animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the University of North Carolina at Chapel Hill.

2.3. Dog experiments

Anesthesia was initiated with an i.v. injection of diazepam (5 mg kg^{-1}) and ketamine (35 mg kg^{-1}). The dogs then were intubated and anesthesia was maintained with isoflurane. Depth of anesthesia was evaluated by monitoring vital signs. The microdialysis probes (CMA/20, 10 mm probes, CMA/Microdialysis AB, Stockholm Sweden) were sterilized with ethylene oxide prior to use. The probe inlet and outlet lines were connected to a CMA/100 microsyringe pump and a CMA/140 fraction collector (CMA/Microdialysis AB), respectively. Probe recovery was assessed prior to surgical implantation by in vitro recovery from water [15].

2.4. Surgery

The surgery was performed under a Zeiss Universal S2 stereomicroscope (West Germany) with videocamera and photographic attachment for visualization via a monitor and documentation of the procedure on film. A fornix-based conjunctival flap was prepared at the superior limbus with microscissors. A 20-ga needle then was introduced into the anterior chamber in an oblique fashion from 3 mm posterior to the limbus and then removed. The microdialysis probe was introduced gently through the opening created by the needle until the probe tip was totally within and spanned the anterior chamber. A seal of the opening was made with the base of the probe. The probe was sutured via its attached anchor to the upper eyelid with 4-0 silk. Saline was perfused through the probe at a rate of 2 μl min^{-1} for a minimum of 20 min prior to drug administration.

2.5. Intracameral administration

Following implantation of the microdialysis probe and the stabilization period, 140 μl (700 μg) or 30 μl (150 μg) of dosing solution (5 μg μl^{-1}) was administered through a 30-ga needle inserted obliquely through the limbus into the anterior chamber at a site 90° from the probe insertion. Regurgitation of dosing solution was assessed with collection of back leakage on Weck Cell Surgical Spear adsorbent tips (Xomed Surgical Products, Jacksonville, FL); radioactivity was determined with a TRICARB liquid scintillation counter (Packard Instrument, Downers Grove, IL). Microdialysate collection was initiated immediately after drug administration, and samples were collected for ≥ 2.5 h. The 10 μl sample aliquots were placed in scintillation vials with 5 ml of Biosafe II scintillation cocktail (Research Products International Corp., Mount Prospect, IL). The limit of quantitation as determined by analysis of blanks with background subtraction was 0.001 μg ml^{-1}. Background was assessed as up to 3 standard deviations from blank scintillation counts. Following the sampling procedure, probe recovery was assessed in vivo with retrodialysis and the probe was removed. Verification of probe recovery following probe removal was performed using in vitro water recovery and water retro-recovery (recovery by difference).

2.6. Topical administration

A 15–40 μl aliquot of dosing solution (75, 125 or 200 μg) was placed near the center of the corneal surface or in the cul-de-sac. Microdialysis sampling was initiated immediately after dosing. Samples were collected for ≥ 2.5 h. Potential dosing loss through overflow of the cul-de-sac was assessed by collection on Weck Cell adsorbent tips and scintillation counting. Analysis of samples was performed as described for the intracameral experiments and probe recovery was assessed in the manner described above.

2.7. Rabbit experiments

Anesthesia was initiated and maintained with i.m. injections of ketamine (15 mg kg^{-1}) and xylazine (20 mg kg^{-1}). The surgical and propranolol administration procedures were identical to those used in the dog experiments. Potential dosing loss through intracameral dose regurgitation or topical overflow of the cul-de-sac was assessed by collection on Weck Cell adsorbent tips and scintillation counting.

2.8. Assessment of microdialysis probe recovery

Prior to probe insertion, the sterilized microdialysis probe was placed in sterile filtered saline containing ^{3}H-propranolol (0.04 μCi ml^{-1}). The recovery solution was placed in a water bath (34° to 38°C) and agitated. Saline was perfused through the probe at a rate of 2 μl min^{-1} and dialysate collected in 10 μl aliquots. In vitro recovery was conducted for 50 min. Two or more 10 μl aliquots of the standard were assayed along with microdialysis samples. In vitro recovery was calculated as:

$$\text{Recovery}(\%) = \frac{100 \cdot (\text{Mean DPM for samples no. } 6\text{–}10)}{\text{Mean DPM for standard aliquot}}$$

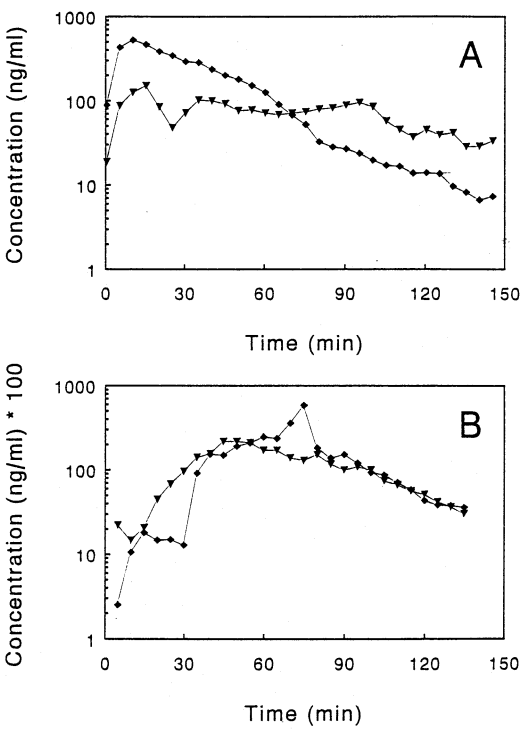

Fig. 1. Propranolol aqueous humor concentrations in a single dog after intracameral injection (A) or topical application (B) on two separate occasions (◆ = day 1; ▼ = day 2). Concentrations have been normalized for the dose, and are expressed per μg administered.

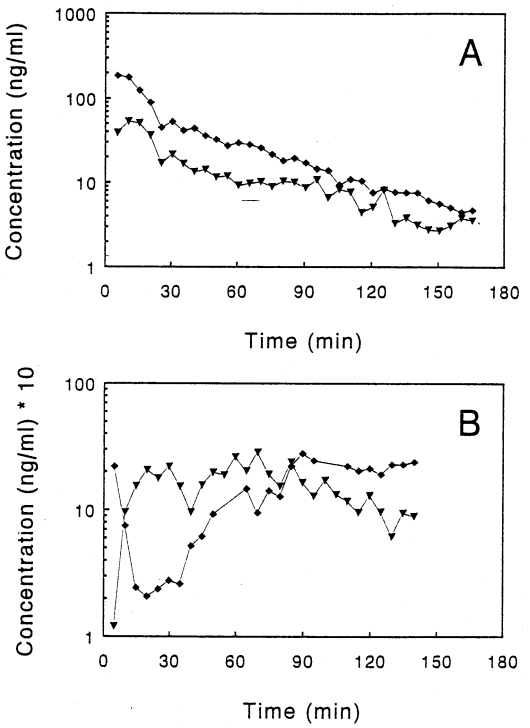

Fig. 2. Propranolol aqueous humor concentrations in two different dogs after intracameral injection (A) or topical application (B) (◆ = dog 1; ▼ = dog 2). Concentrations have been normalized for the dose, and are expressed per mg administered.

2.9. Recovery by in vivo retrodialysis

Following the microdialysis sampling of aqueous humor, the probe was retained in the anesthetized animal and was perfused with a solution of saline spiked with ^{3}H-propranolol (0.04 μCi ml^{-1}) at 2 μl min^{-1}. Microdialysate was collected in 10 μl aliquots for 65 min. Two or more 10 μl aliquots of the standard were assayed with the microdialysis samples. Retrodialysis recovery was calculated as:

$$\text{Recovery}(\%) = 100 \cdot (\text{Mean DPM for standard aliquot} - \text{Mean DPM for samples no. } 8\text{–}13)/\text{Mean DPM for standard aliquot}$$

Table 1
Propranolol disposition in aqueous humor after intracameral administration in the dog

Subject no.	Dose (μg)	AUC/Dose (μg min ml^{-1})[a]	CL_{AH} (ml min^{-1})	V (ml)	MRT (min)	λ_z (min^{-1})
A	150	12.6	0.0794	7.17	90.0	0.01500
C	612	2.30	0.435	22.8	52.5	0.0235
D	693	6.12	0.164	8.64	52.8	0.0176
Mean ± S.D.		7.00 ± 5.20	0.226 ± 0.189	12.9 ± 8.65	65.1 ± 21.6	0.0189 ± 0.00429

[a] Per μg administered.

2.10. Estimation of pharmacokinetic parameters

The area under the aqueous humor concentration vs. time curve (AUC_{AH}) and the first moment of the concentration-time curve ($AUMC_{AH}$) were estimated by the linear trapezoidal method with extrapolation to time infinity. Aqueous humor clearance (CL_{AH}), terminal rate constant (λ_z), concentration at peak (C_{max}), time to peak (T_{max}), mean residence time (MRT_{AH}) and intraocular biotissue availability (F_{AH}) were calculated according to relevant noncompartmental techniques [5]. All parameters are reported as mean ± S.D.

3. Results

Intra-animal reproducibility was examined in the dog. The dose normalized aqueous humor concentration vs. time profiles for a dog that received intracamerally administered propranolol in separate experiments is displayed in Fig. 1A. The aqueous humor exposure to propranolol was similar as reflected in similar areas under each curve (20.9 vs. 12.6 μg min ml^{-1} per μg administered). Reproducible concentration-time profiles also were observed in a second dog that received topically administered propranolol on separate occasions (Fig. 1B). The dose normalized concentration vs. time profiles after topical administration were nearly identical. Estimates for dose normalized AUC_{AH} (0.149 vs. 0.179 μg min ml^{-1} per μg administered) and terminal rate constant (0.028 vs. 0.032 min^{-1}) were similar for the two experimental trials.

To examine inter-subject reproducibility in the dog, two animals received intracamerally administered propranolol. The dose normalized concentration-time profiles are displayed in Fig. 2A. Although differences were noted in the observed aqueous humor exposure to propranolol for these two subjects, the disposition profiles in general were parallel. The pharmacokinetic parameters differed to a greater extent than was the case in the within-subject experiment, as would be expected (AUC_{AH} 6.11 vs. 2.19 μg min ml^{-1} per μg administered; CL_{AH} 0.164 vs. 0.460 ml min^{-1}). However, terminal rate constants (0.0189 vs. 0.0235 min^{-1}) were similar. In separate experiments, the same subjects received topically administered propranolol. Aqueous humor exposure to topically administered propranolol also was reproducible. Dose normalized profiles are displayed in Fig. 2B. Aqueous humor exposure was comparable in the two animals (0.0213 vs. 0.0219 μg min ml^{-1} per μg administered). Terminal decline was slower for the topically administered dose than for the intracameral dose in these subjects.

Aqueous humor pharmacokinetic parameters for dogs that received intracamerally administered propranolol are presented in Table 1. The dose normalized AUC_{AH}, CL_{AH}, and volume (V) were approximately 7 μg min ml^{-1} per μg administered, 0.2 ml min^{-1}, and 13 ml, respectively. Mean residence time (MRT) was $\sim$65 min, and the terminal rate constant was $\sim$0.0189 min^{-1} (corresponding to a half-life of $<$40 min). Parameters estimated for topical administration are presented in Table 2. Variability appeared to be greater in the calculated topical parameters as compared to the intracameral parameter estimates, and the intraocular tissue availability (F_{AH}) was $<$10% in all subjects.

Table 2
Propranolol disposition in aqueous humor after topical administration in the dog

Subject no.	Dose (μg)	AUC_{AH}/Dose (μg min ml^{-1})[a]	C_{max}/Dose (μg ml^{-1})[a]	T_{max} (min)	λ_z (min^{-1})	F_{AH}
B	125	0.149	0.00218	49.8	0.0279	0.0710
C	200	0.555	0.00324	140.4	0.00518	0.0907
D	200	0.0409	0.000284	69.7	0.00456	0.00668
Mean ± S.D.		0.248 ± 0.271	0.00190 ± 0.00150	86.6 ± 47.6	0.0123 ± 0.0135	0.0561 ± 0.0439

a Per μg administered.

The studies conducted in the rabbit were designed to assess intra-animal differences in propranolol aqueous humor disposition as a consequence of the route of administration. Microdialysis sampling was conducted simultaneously in each eye following intracameral administration to one eye and topical administration to the contralateral eye. The mean dose normalized concentration-time profile for intracameral and topical administration ($n = 3$) is displayed in Fig. 3. The terminal slopes produced by the two routes of administration were parallel, although a larger degree of variability was associated with the topical profile. The ocular aqueous humor pharmacokinetic parameters estimated for intracamerally administered propranolol in the rabbit are presented in Table 3; the parameters for topical administration are summarized in Table 4. Substantial inter-animal variability was observed for both intracamerally and topically administered propranolol ($n = 3$). However, the terminal rate constants for each administration route were quite similar (0.00770 ± 0.00504 vs. 0.00983 ± 0.00546 min^{-1}).

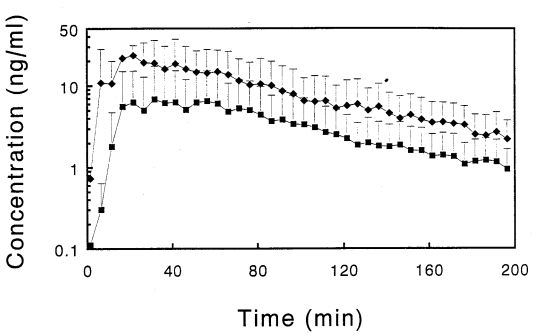

Fig. 3. Propranolol aqueous humor concentrations in rabbits after intracameral injection (◆) or topical application (■). Data represent mean ± S.D. ($n = 3$). Concentrations have been normalized for the dose, and are expressed per μg administered.

The relationship between in vivo and in vitro recovery of propranolol is shown in Fig. 4. The in vitro recoveries (~35%) were similar in dog and rabbit experiments. However, in vivo recovery tended to be lower in rabbit (~35%) as compared to the dog (~46%).

4. Discussion

Ocular pharmacokinetics of propranolol have been investigated in a limited number of studies using a rabbit model; propranolol ocular pharmacokinetics in dog have not been examined prior to the present study. Studies examining the corneal penetration of β-adrenergic antagonists as a function of lipophilicity were conducted in which propranolol was compared to twelve other drugs in vitro using isolated cornea and conjunctiva of albino rabbit [16]. In a second study, also utilizing albino rabbits, animals received 500 μg topical doses of β-adrenergic antagonists to assess the relationship between C_{max} or T_{max} in aqueous humor (obtained by paracentesis) and lipophilicity [17]. In that study, propranolol T_{max} was reported to be ~30 min; C_{max} was 8.59 ± 4.9 μg ml^{-1} (dose normalized: 0.0172 μg ml^{-1} per μg administered dose). In the present study, the dose normalized C_{max} observed for a 200 μg topically administered dose of propranolol in the rabbit ranged from 0.000564 to 0.0189 μg ml^{-1} per μg administered dose with T_{max} ranging from 31 to 72 min (mean 54.1 ± 20.4 min). Assuming that the

Table 3
Propranolol disposition in aqueous humor after intracameral administration in the rabbit

Subject no.	Dose (μg)	AUC_{AH}/Dose (μg min ml^{-1})[a]	CL_{AH} (ml min^{-1})	V (ml)	MRT (min)	λ_z (min^{-1})
A	466	0.529	1.89	103	54.6	0.00830
B	434	2.50	0.400	39.5	98.9	0.00530
C	445	3.32	0.300	22.1	73.8	0.0159
Mean ± S.D.		2.12 ± 1.44	0.863 ± 0.891	55.9 ± 41.4	75.8 ± 22.2	0.00983 ± 0.00546

[a] Per mg administered.

pharmacokinetics are linear, the upper estimate from the present study is comparable to the previously reported value, although the time to peak concentrations was longer but well within the estimated range reported for most drugs [18]. One possible explanation for the difference is that rabbits in the present study were anesthetized. Cummingham et al. [19] discussed the implications of anesthetic effects on intraocular pressure physiology in humans. A 25% reduction of intraocular pressure in children following i.m. administration of ketamine was observed [20]; other investigators noted intraocular pressure reductions following isoflurane administration [21]. It is possible that the anesthesia perturbed the aqueous humor turnover (i.e., decreased elimination) which would be reflected in changes in T_{max} (increased) and C_{max} (variable). Hussain et al. [22] estimated the terminal rate constant following a 250 μg topical dose of propranolol in unanesthetized rabbit (~ 0.019 min^{-1}), a value similar to the rate constants obtained in the dog in the present study (0.0189 ± 0.00429 min^{-1} after intracameral administration and 0.0123 ± 0.0135 min^{-1} after topical administration of propranolol), although somewhat higher than the values obtained in the anesthetized rabbit in the present study (0.00983 ± 0.00546 min^{-1} after intracameral administration and 0.00770 ± 0.00504 min^{-1} after topical administration).

Intra-animal ($n = 2$) and inter-subject ($n = 2$) differences in absorption and disposition kinetics were assessed, and differences in absorption and disposition kinetics between dog and rabbit ($n = 3$ respectively) also were examined. Corneal tissue in rabbit (0.35–0.45 mm) is thinner than in human (~ 0.52 mm center) [23] and dog (~ 0.62 mm) [24]. Therefore, it would be expected that greater corneal penetration of propranolol, and a subsequent higher intraocular tissue availability, would be observed. As noted, rabbit F_{AH} approached $\sim 90\%$ in one animal, and on average was 10-fold higher than in the dog. Typical aqueous humor availability of topically administered ophthalmics ranges from 1 to 10% in humans [18] and in previous animal studies [25]. Anterior chamber volume as well as depth for rabbit (volume 250 μl, depth 3.5 mm) and canine (volume 400 μl, depth 5 mm) [26] also may influence the observed ocular absorption/distribution kinetics of propranolol. Aqueous humor clearance of propranolol was higher in the rabbit than in the dog (0.863 ± 0.891 ml min^{-1} vs. 0.226 ± 0.189 ml min^{-1}). Rabbit iris/ciliary tissue was unpigmented (albino); in contrast, dog had pigmented iris/ciliary tissue. Highly lipophilic xenobiotics such as propranolol bind to melanin in iris/ciliary [10,27]. These conditions may result in decreased apparent aqueous humor clearance relative to non-pigmented tissues. The present study supports a possible pigment binding perturbation of clearance; further studies are required to confirm and elucidate this possible phenomenon.

The pharmacokinetics of drugs in aqueous humor are complex. Aqueous turnover, as well as availability of unbound substrate (i.e. tissue binding), complicate the assessment of ocular clearance. The volume of distribution (V) and ocular clearance (CL_{AH}) estimates obtained in the present study reflect these complexities. Anterior chamber volume in rabbit is estimated to be ~ 250–300 μl and ~ 400–600 μl [26] in the dog. Aqueous humor turnover is $\sim 1\%$ of anterior chamber volume (~ 2.5 or 4.0 μl min^{-1}). Propra-

Table 4
Propranolol disposition in aqueous humor after topical administration in the rabbit

Subject no.	Dose (μg)	AUC_{AH}/Dose (μg min ml^{-1})[a]	C_{max}/Dose (mg ml^{-1})[a]	T_{max} (min)	λ_z (min^{-1})	F_{AH}
A	116	0.476	0.00271	70.4	0.006610	0.898
B	107	1.81	0.0189	31.3	0.01320	0.722
C	200	0.112	0.000564	60.7	0.003303	0.0337
Mean ± S.D.		0.798 ± 0.892	0.00739 ± 0.0100	54.1 ± 20.4	0.00770 ± 0.00504	0.551 ± 0.456

[a] Per μg administered.

nolol ocular clearance and volume estimates were substantially higher than these physiologic values. In the anterior chamber environment, volume and clearance are not independent in the sense that drug clearance is a function of aqueous turnover and turnover rate is a function, in part, of anterior chamber volume. Mean residence time (MRT) may be a more appropriate parameter to use to communicate relative differences in drug disposition in aqueous humor. In the present study, aqueous humor exposure to propranolol was similar in the dog (MRT ~65 min) and the rabbit (MRT ~75 min).

A number of technical issues were managed in the development of this novel application of the continuous regional sampling of aqueous humor. Initial leakage of aqueous humor prior to probe insertion was associated with the creation of an opening for the insertion of the microdialysis probe into the anterior chamber. Miichi et al. [28] reported a 7- to 8-fold increase in mean protein concentrations in aqueous humor following cannulation of the anterior chamber in anesthetized rabbits. Tripath et al. [29] observed a ~4.5 fold increase in total proteins in human aqueous humor samples from patients with compromised blood/aqueous barrier function after paracentesis sampling. As with any paracentesis procedure, aqueous humor leakage is probable and was observed in the present study. As a consequence to the disruption of the blood-aqueous barrier, other responses including inflammation and the possible influx of plasma proteins can occur [28]. These conditions may impact directly on the unbound fraction of xenobiotic available for microdialysis sampling. Some of the observed variability in the developed pharmacokinetic parameters may be a result of influx of plasma proteins following the probe insertion procedure.

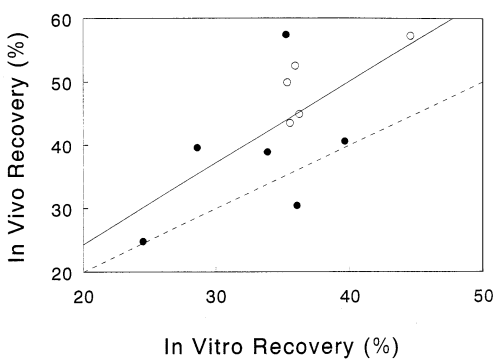

Fig. 4. In vivo vs. in vitro probe recovery in the dog (○) and the rabbit (●). Dotted line represents the line of identity; solid line indicates results of linear regression ($Y = 1.29X - 1.51$, $r^2 = 0.646$).

The in vitro recovery determinations were used to test the functioning of the probe prior to implantation into the animals. In vitro recovery also was performed post-use. The probe was placed in a conical tube with a micro stir bar. Agitation was not optimized; it is probable that there was an unstirred layer surrounding the probe tip, which could be reflected in decreased recovery relative to in vivo. In a limited number of experiments in dogs and rabbits, in vitro retrodialysis recovery was assessed; the recovery was conducted in the same manner as in vivo. These results were nearly identical to the in vivo recovery. Calculations for parameter estimates were based on in vivo retrodialysis recovery.

A possible explanation for the observation that rabbit in vivo recoveries were lower than in the dog is that the probe tip length was rather large

for the rabbit eye as compared to the dog eye. The tip spanned the entire rabbit anterior chamber. It is possible that the probe tip may have touched the iris and elicited fibrin formation which ultimately could decrease the probe recovery. Ideally, it is best to use a somewhat smaller probe tip size for the rabbit. A 10 mm probe tip size was chosen in order to maximize recovery.

5. Conclusions

The utility of microdialysis in regional sampling of aqueous humor has been demonstrated in the present study. The noncompartmental pharmacokinetic parameters developed with this procedure are comparable to reported estimates in the literature. Future refinements of the developed technique include studies to assess blood-aqueous barrier function by assessing the aqueous humor total protein levels during the procedure, and application in conscious animal models to assess the impact of anesthesia on the estimated pharmacokinetic parameters.

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