Microdialysis Evaluation of the Ocular Pharmacokinetics of Propranolol in the Conscious Rabbit

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Purpose. This study was conducted to assess the effects of anesthesia and aqueous humor protein concentrations on ocular disposition of propranolol.

Methods. Rabbits were anesthetized and a microdialysis probe was inserted into the anterior chamber of one eye; the contralateral eye served as a control. At timed intervals after probe placement, a 100-μl sample of aqueous humor was aspirated from each eye to determine protein concentration. *In vitro* protein binding parameters were used to simulate the impact of protein concentration on propranolol disposition. To assess the influence of anesthesia, probes were implanted in the anterior chamber of each eye. After >5-day stabilization, conscious and anesthetized rabbits (n = 3/group) received a 200-μg topical dose of [^{3}H]DL-propranolol in each eye; propranolol was assayed in probe effluent.

Results. Changes in aqueous humor protein concentrations were observed following probe insertion. Simulations demonstrated that the unbound propranolol AUC (~2.4-fold) in aqueous humor should be reduced due to protein influx. Intraocular propranolol exposure in anesthetized rabbits was ~8-fold higher than in conscious rabbits, and ~1.9-fold higher than in rabbits without a post-surgical recovery period.

Conclusions. Anesthesia and time-dependent aqueous humor protein concentrations may alter ocular pharmacokinetics, and must be taken into account in the design of microdialysis experiments.

KEY WORDS: propranolol; β-adrenergic antagonist; ocular pharmacokinetics; microdialysis; aqueous humor; protein binding; anesthesia.

INTRODUCTION

Microdialysis has been used in vitreous (1–3), retina (4,5) and aqueous humor (AH; 6–8) in order to estimate ocular bioavailability of regionally administered ophthalmics. With microdialysis, complete AH concentration-time profiles can be obtained from individual animals; the number of animals required for pharmacokinetic studies consequently is reduced. In previous work in anesthetized rabbits, microdialysis sampling of AH was used to assess the disposition of propranolol, a β-adrenergic antagonist (7). Drugs of this class are used in the treatment of glaucoma and ocular hypertension (9). Anterior chamber disposition of topically administered β-adrenergic antagonists is important in assessing the pharmacodynamics of AH formation and intraocular pressure (IOP) reduction (9,10).

Since protein concentrations in AH are low (<1%) relative to plasma (11), protein binding in AH should be minimal. However, because probe placement may compromise the blood-aqueous barrier (12), protein concentrations in AH immediately following probe placement may be increased. Drugs that bind extensively to plasma proteins (*e.g.*, propranolol) might exhibit altered ocular pharmacokinetics under these conditions (13). A conscious rabbit model, which allows complete recovery of the animal from anesthesia and the return of basal AH protein concentrations, is the logical next step in development of microdialysis procedures for ocular pharmacokinetics.

The present study was conducted in order to develop a conscious rabbit model for the ocular disposition of propranolol. To evaluate the performance of the model, effects of AH protein concentrations and anesthesia on propranolol disposition were examined.

METHODS

Materials

Human serum albumin (HSA), human α_1 - acid glycoprotein (AAG), and unlabeled DL-propranolol were obtained from Sigma Chemical Co. (St. Louis, MO). Human serum was prepared from pooled blood samples obtained from healthy volunteers under an IRB-approved protocol. [^{3}H]DL-propranolol hydrochloride (>95% pure, specific activity 15–30 Ci/mmol) was obtained from Amersham Life Science (Elk Grove, IL) and Biosafe II scintillation cocktail was obtained from Research Products International Corp. (Mount Prospect, IL). Ultrafiltration units (Amicon Inc., Beverly, MA) were used for protein binding determinations and for preparation of AH samples for TLC. Silica gel TLC plates (Alltech Associates Inc., Deerfield, IL) were used to assess the presence of propranolol metabolites. CMA/20 microdialysis probes (CMA/AB Microdialysis, Stockholm, Sweden) were used for all *in vivo* experiments. All other chemicals used were reagent grade.

Animals

New Zealand white rabbits (2.5–3.5 kg; Robinson Service, Winston-Salem, NC) were allowed a standard diet and water *ad libitum*. Animals were handled according to the Principles of Laboratory Animal Care (NIH publication #85-23), and the protocol was approved by the University's Institutional Animal Care and Use Committee.

Protein Binding Experiments

Standards were prepared by evaporating appropriate volumes of propranolol ethanolic solutions (labeled and unlabeled), yielding concentrations of 28 to 28000 ng/ml (0.225 μCi/ml) after reconstitution with protein-containing buffer (pH 7.4 phosphate buffer containing 1 mg/ml AAG and 40 mg/ml HSA) or human serum. For ultrafiltration, standards (500 μl) were placed in ultrafiltration devices and centrifuged (160×g, 37°C) for 2.5 min (protein-buffer) or 6 min (human serum). Aliquots (20–30 μl) of standard and ultrafiltrate were assayed by liquid scintillation spectroscopy. Ultrafiltration of propranolol in non-protein-containing buffer was associated with negligible nonspecific

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binding. For microdialysis, a CMA/20 probe with a 10-mm polycarbonate membrane was placed into a 1.5-ml aliquot of standard, and the solution was agitated with a micro stir bar in a 37°C water bath. Normal saline was perfused through the probe (2 μl/min), and 5-min effluent fractions were collected for >10 fractions. Probe effluent was assayed as described above. Probe recovery was assessed *in vitro* (14), and calculated as:

$$\%\text{Recovery} = 100 \cdot \frac{\text{Mean dpm of last 4 effluent fractions}}{\text{Mean dpm of protein free standard aliquots}}. \quad (1)$$

Unbound fraction as determined by microdialysis was corrected for probe recovery.

AH Protein Time Course

Rabbits (n = 16) were anesthetized with i.m. ketamine (50 mg/kg) and xylazine (10 mg/kg). A microdialysis probe (CMA/20, 10-mm polycarbonate membrane) was inserted into one eye as described previously (7). Saline (2 μl/min) was perfused through the probe. The contralateral eye was used as a control. At 0 (immediately following probe placement), 30, 90 ,and 150 min, a single AH sample (~100 μl) was aspirated from each eye with a 27-ga needle for determination of total protein (15).

Ocular Propranolol Pharmacokinetics

A custom-designed CMA/20 microdialysis probe was constructed with a 4-mm polycarbonate membrane (20,000-dalton cut-off) on a 6-mm shaft containing a 90° bend in the shaft followed by an additional 3 mm of shaft to the probe anchor. A 2.5-mg/kg i.v. dose of flunixin meglamine injection (Fort Dodge Labs. Inc., Fort Dodge, Iowa) was administered 30 min before surgery to prevent intracameral fibrin formation. Following anesthesia with i.m. ketamine (50 mg/kg) and xylazine (10 mg/kg), a microdialysis probe was placed into the anterior chamber of each eye. A limbal-based conjunctival flap was prepared superior nasally or temporally, ~3 mm from the limbus. A conjunctival pocket (10–12 mm) was prepared by posterior dissection. 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. The probe was introduced as described previously (7), the anchor was sutured to the sclera with 7-0 Vicryl (Ethicon Inc., Somerville, NJ), and the conjunctiva was sutured over the anchor. Exterior wound surfaces were treated with flurbiprofen sodium 0.03% ophthalmic solution (Bausch & Lomb, Tampa, FL), dexacidin ophthalmic suspension (CibaVision, Atlanta, GA), and proparacaine hydrochloride 0.5% ophthalmic solution (Bausch & Lomb, Tampa, FL). Animals were used for experimentation after >5 days recovery. Slit-lamp or stereozoom photomicrographs of rabbit ocular anterior segment were taken after recovery to estimate fibrin formation and the condition of the eye prior to use of the rabbit in experiments. In order to compare the intraocular disposition of propranolol in the conscious versus the anesthetized rabbits, animals received identical post-operative care prior to the experiment.

DL-propranolol hydrochloride, equivalent to 5 mg/ml propranolol base containing 222 μCi/mg [^{3}H]DL-propranolol in normal saline, was prepared aseptically. Conscious rabbits (n = 3) were placed in acrylic rabbit restrainers (PLAS LABS, Lansing, MI) that permitted free movement of the head. Following a 1-hr equilibration period with perfusion of buffer through the probe, 40 μl of propranolol solution was placed in the lower cul-de-sac with a micropipettor. In general, the rabbits closed their eyes without blinking after propranolol administration. Potential loss of propranolol after administration was estimated throughout the experiment by collection of overflow on Weck-Cell Surgical Spear absorbent tips (Xomed Surgical Products, Jacksonville, FL) (7). Immediately post-dose, 40−μl fractions of effluent were collected every 20 min. A 20-μl to 40-μl aliquot of each fraction was assayed as described above Anesthetized rabbits (n = 3) received initiation and maintenance i.m. doses of 50-mg/kg ketamine and 10-mg/kg xylazine prior to receiving propranolol as described for the conscious rabbits. Probe recovery was estimated by aspiration of ~100 μl of AH during collection of the final fraction:

$$\%\text{Recovery} = 100 \cdot \frac{\text{dpm of last fraction}}{\text{dpm of aspirate}}. \quad (2)$$

To assess the presence of propranolol metabolites, AH aliquots from each animal were pooled, placed in ultrafiltration devices, and centrifuged at 13000g for 5 min to remove protein. The ultrafiltrate was evaporated to dryness under nitrogen and reconstituted in 50 μl ethanol. An aliquot was placed on a TLC plate, which was exposed to glacial acetic acid:1-butanol:water, 45:180:75 v/v/v). Following development, 1-cm segments were scraped from the plate and analyzed by liquid scintillation spectroscopy. Aliquots of AH aspirate for each subject also were assayed for protein (15).

Data Analysis

Protein Binding Experiments

Protein binding data were fit by nonlinear least-squares regression (WinNonlin, SCI, Apex, NC) with the following equation (16):

$$C_{bound} = \frac{n \cdot P \cdot K_a \cdot C_{unbound}}{1 + K_a \cdot C_{unbound}} \quad (3)$$

where n = number of binding sites, P = protein concentration, K_a = equilibrium association constant, and $C_{unbound}$ and C_{bound} = unbound and bound propranolol concentrations. Parameter sensitivity and model misspecification analyses were performed, and Akaike's information criterion (AIC) was used to identify optimal model structure.

AH Protein Experiment

Single-factor ANOVA was used to assess the time-dependence of AH protein concentrations following probe implantation. A paired t-test was used to ascertain differences in control versus treated eyes.

Simulations of Ocular Disposition in the Presence of Altered AH Protein Concentrations

AH propranolol concentration-time data for an anesthetized rabbit that received intracamerally-administered propranolol to one eye (700 μg) and topical propranolol (200 μg) to the fellow eye was used for the simulation study. Protein binding parameters (K_a, n) were used to calculate the fraction unbound (f_u) over time:

$$f_u = \frac{C_{unbound}}{\frac{K_a \cdot n \cdot P \cdot C_{unbound}}{1 + K_a \cdot C_{unbound}} + C_{unbound}}. \quad (4)$$

Two additional data points for terminal decline (180 and 210 min) were added for simulation purposes and the projected protein concentrations for the additional time points were estimated according to the calculated half-life for loss of AH protein. The estimated f_u was multiplied by the corresponding total concentration at each time point. AH area under the concentration vs. time curve (AUC_{AH}) was calculated for simulations of unbound concentration in the presence of changing protein concentrations versus a constant unbound fraction (0.93) over the interval.

Pharmacokinetic Experiments

AUC_{AH} was estimated by the linear trapezoidal method with extrapolation to infinite time. Peak AH concentrations of propranolol (C_{MAX}), time to peak (T_{MAX}), and terminal rate constant (λ_z) were calculated with noncompartmental techniques (17). All relevant parameters (C_{MAX}, AUC_{AH}) were dose-normalized. Individual AH pharmacokinetic parameters for each eye were calculated. Ross *et al.* (10) demonstrated that no detectable propranolol was observed in the contralateral eye following the topical administration of a 500-μg dose to New Zealand white rabbits. These results demonstrate that no return of propranolol from the systemic circulation could have contributed to the measured concentrations in the fellow eye following topically administered propranolol. Therefore, for the present study, each eye was treated as independent for the purpose of statistical analysis.

RESULTS

Propranolol f_u was assessed by ultrafiltration and contrasted with estimates obtained by microdialysis. Good agreement was observed between the two techniques (Fig. 1). A constant f_u of ~12% was observed in protein-buffer for propranolol concentrations up to 2800 ng/ml. At higher concentrations, f_u increased with increasing concentration to ~40% at 28,000 ng/ml. While unbound fractions were lower, in general, in human serum compared to protein-buffer medium, good agreement still was observed between ultrafiltration and microdialysis.

The fit of a binding isotherm to data obtained from human serum is presented in Fig. 2. The equilibrium association constant estimated from ultrafiltration data was 0.0364 ± 0.00622 μM^{-1} versus 0.0453 ± 0.00692 μM^{-1} by microdialysis. The binding capacity (n·P) was 155 ± 15 μM (ultrafiltration) versus 145 ± 12 μM (microdialysis). Saturable binding was evident with both experimental techniques.

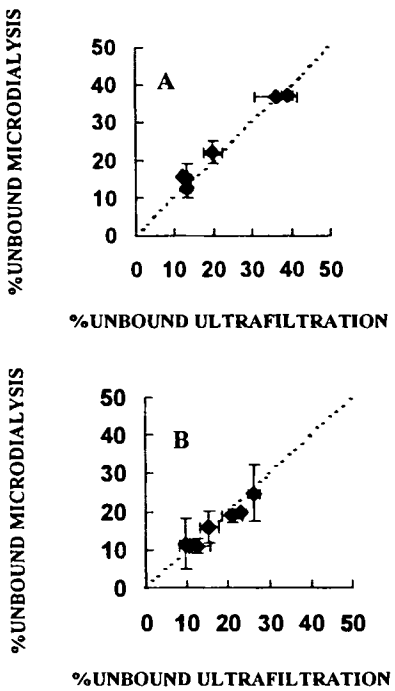

Fig. 1. Relationship between microdialysis and ultrafiltration estimates of unbound fraction for propranolol in a protein-buffer solution (A) or in human serum (B). Bars indicate ±SD (n = 2–6 per point, bars indicated for n = 3–6); line is the line of identity.

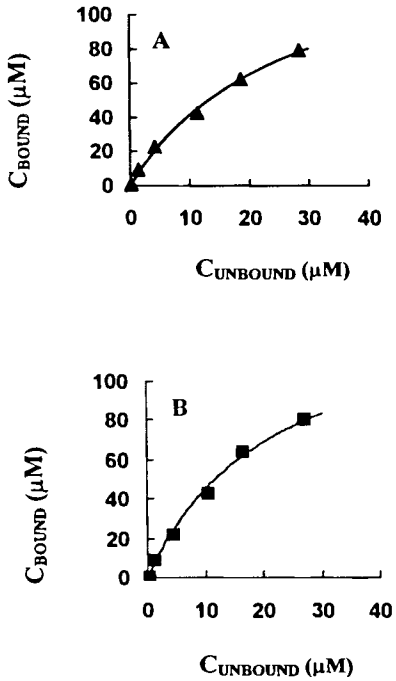

Fig. 2. Fit of a binding isotherm (solid line) to averaged (n = 2–6 per concentration) protein binding data in human serum based on ultrafiltration (A) or microdialysis (B).

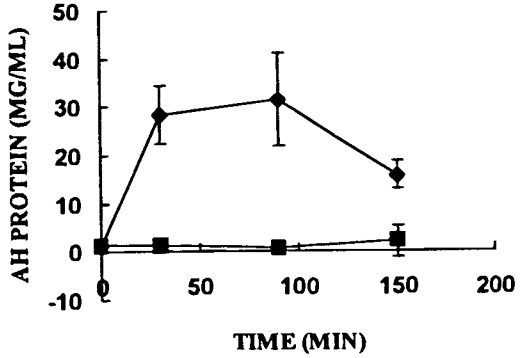

Fig. 3. Aqueous humor protein concentrations in rabbits following probe placement into the anterior chamber (▲) versus controls (no probe placement, (■)). Bars indicate ±SD (n = 4 per time point; at time 0 min, n = 2 per group).

The protein concentration-time profiles in treated eyes versus control eyes (no probe) are presented in Fig. 3. Peak protein concentrations were reached 30–90 min following probe placement; at peak, AH protein approached concentrations in plasma (~30 mg/ml). By 150 min, protein concentrations had declined to half of peak. AH protein concentrations in treated eyes were significantly elevated at all time points after probe implantation compared to the control eye ($p < 0.0005$). The time-dependent changes in AH protein concentrations were statistically significant ($p < 0.001$).

Simulations of the effect of time-dependent AH protein on the intraocular disposition of propranolol are presented in Fig. 4. Simulations demonstrated that, at the lower propranolol concentrations typically encountered with topical administration, reduced AUC_{AH} for unbound propranolol should be observed due to elevated AH protein. For the simulated propranolol AH concentration-time profile with a constant unbound fraction (0.93) at basal AH protein concentration, AUC_{AH} for unbound propranolol was 2.4-fold higher than that for the disposition modulated by time-dependent AH protein concentrations. For intracamerally administered propranolol, because of the relatively high intraocular concentrations achieved, a smaller alteration due to time-dependent AH protein concentration was observed (~1.5-fold lower AUC_{AH}).

The averaged dose-normalized AH propranolol concentration-time profiles in acutely anesthetized rabbits undergoing probe placement (7) versus rabbits that were anesthetized following >5 day recovery from surgery are compared to data from conscious rabbits (also following a >5 day recovery) in Fig. 5. Differences in the magnitude of dose-normalized AUC_{AH} and C_{MAX} were observed. The averaged dose-normalized AUC_{AH} for anesthetized rabbits with time-dependent AH protein (*i.e.*, no recovery period) was ~1.9 fold lower than in anesthetized rabbits with a >5-day recovery period, in agreement with the simulations described above (2.4-fold). The ocular pharmacokinetic parameters for conscious rabbits are presented in Table I. The intraocular exposure to propranolol was decreased (~8-fold) relative to that in anesthetized rabbits ($p < 0.001$). Time to peak (T_{MAX}) and the rate of terminal decline in propranolol concentrations were similar, however, between the two experimental preparations. AH protein concentrations of rabbits allowed to recover for >5 days were lower on average (~2.9 mg/ml; Table II) than in those animals with no recovery period (~30 mg/ml; Fig. 3). The protein concentrations in rabbits allowed to recover were slightly elevated compared to those in anesthetized rabbits immediately post-probe implantation (~2.9 mg/ml versus ~1.4 mg/ml). There appeared to be less variability in AUC_{AH} and C_{MAX} in both conscious and anesthetized rabbits following a >5-day recovery period compared to the acute surgical preparation (7).

TLC analysis of AH after topical administration of propranolol confirmed that no appreciable propranolol metabolites were present. The parent compound accounted for 94% of radioactivity, comparable to the purity of the authentic compound (Fig. 6).

DISCUSSION

Microdialysis is a well-accepted technique for estimating unbound substrate concentrations *in vivo* (16,18). Its application in compartments such as the eye may be complicated due to

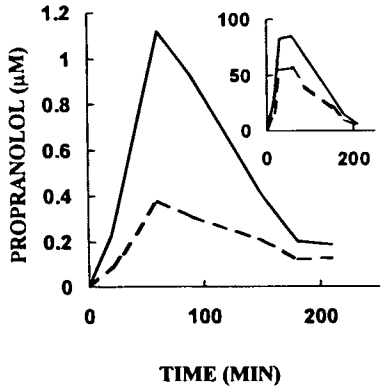

Fig. 4. Simulated aqueous humor propranolol concentration-time profiles following a 200-μg topical dose of propranolol with a constant unbound fraction of 0.93 (solid line) versus a time-dependent unbound fraction (dashed line). Inset: concentration-time profiles following a 700-μg intracameral dose of propranolol with a constant unbound fraction (solid line) versus a time-dependent unbound fraction (dashed line).

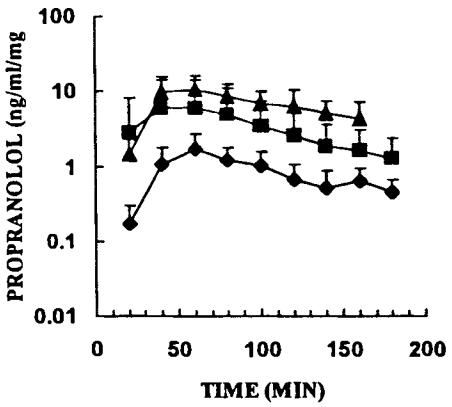

Fig. 5. Averaged dose-normalized aqueous humor propranolol concentration-time profiles following a 200-μg topical dose in conscious rabbits with >5-day recovery (◆, n = 6), rabbits with >5-day recovery period but anesthetized prior to topical administration (▲, n = 5), and previously reported data (7) in anesthetized rabbits with no recovery period (■, n = 3).

Table I. Propranolol Disposition in AH After Topical Administration in the Conscious Rabbit

Subject*	Dose (μg)	AUC_{AH}/Dose ($ng \cdot min \cdot ml^{-1} \cdot \mu g^{-1}$)	C_{MAX}/Dose ($ng \cdot ml^{-1} \cdot \mu g^{-1}$)	T_{MAX} (min)	λz (min^{-1})	Protein (mg/ml)
1A	188	112	0.92	60	0.012	2.57
1B	196	54†	0.68	80	N/A	N/A
2A	200	344	3.14	60	0.011	3.06
2B	200	202	2.03	60	0.0094	1.36
3A	158	211	2.32	60	0.012	5.81
3B	200	120	1.18	60	0.016	1.57
Mean ± SD	190 ± 17	198 ± 94	1.71 ± 0.95	63 ± 8	0.012 ± 0.0024	2.87 ± 1.78

* A and B refer to different eyes in the same subject.
† AUC to t = 150 min: not included in the mean.

Table II. Effects of Recovery Period and Anesthesia on Propranolol Intraocular Disposition

Parameter	Conscious >5-day recovery (n = 6)*	Anesthetized >5-day recovery (R; n = 5)*	Anesthetized no recovery[b] (NR; n = 3)*	Ratio (R/NR)
AUC_{AH}/Dose ng · min/ml/μg)	198 ± 93	1520 ± 465[a]	798 ± 892	1.9
C_{MAX}/Dose (ng/ml/μg)	1.71 ± 0.90	12.08 ± 4.74[a]	7.39 ± 10.00	1.6
T_{MAX}(min)	63 ± 8	65 ± 38	54 ± 20	1.2
λ_z (min^{-1})	0.012 ± 0.0024	0.010 ± 0.0026	0.0077 ± 0.0050	1.3
Protein (mg/ml)	2.87 ± 1.78	2.95 ± 2.32	N/A	N/A

* Mean ± SD.
[a] $p < 0.001$ vs. conscious rabbit.
[b] Data obtained from Rittenhouse *et al.* 1998 (7).

the invasiveness of the technique (i.e., the requirement for continual anesthesia) and physiologic changes secondary to probe placement. The present study was undertaken to assess these limitations, and to develop a physiologically relevant model for investigations of ocular pharmacokinetics.

Nonlinear least-squares regression analysis provided an excellent description of the protein binding data with use of a model that incorporated a single class of saturable binding sites. The estimate of K_a(0.0364–0.0453 μM^{-1}) was comparable to that (0.0304 μM^{-1}) obtained by Glasson *et al.* (19). Propranolol binding to AAG is capacity-limited, while binding to albumin is not saturable (19). Serum contains a number of proteins (*e.g.* lipoproteins) in addition to albumin which bind propranolol nonsaturably (19). In the present study, the propranolol f_u was lower in human serum than that in a protein-buffer solution containing only HSA and AAG at physiologic concentrations, as expected.

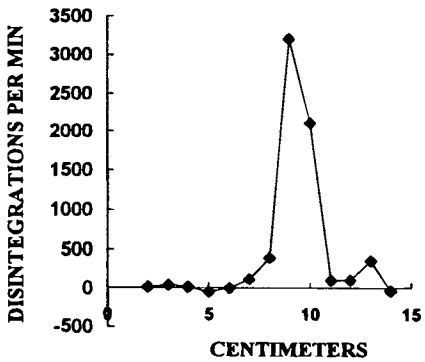

Fig. 6. TLC analysis of ^{3}H-propranolol in pooled aqueous humor samples.

In secondary AH, which forms following paracentesis sampling of naive rabbit eye (20), concentrations of proteins are. AH protein concentrations at 30–90 min following paracentesis range from ~30–40 mg/ml (20,21). In the present study, AH protein concentrations were ~29–32 mg/ml. The observed terminal decline in AH protein in the present study provided an estimate for ocular AH protein turnover (~60 min; terminal rate constant of ~0.0114 min^{-1}); the rate of efflux of the elevated protein concentrations from AH probably is accounted for solely by AH turnover. Based on this estimate, basal AH protein concentrations would be obtained after ~5 hr. A longer recovery period is recommended to allow replenishment of AH fluids lost following paracentesis of the eye for probe implantation and to ensure the return to basal intraocular pressure. Since placement of probes into the anterior chamber might initiate inflammatory cascades (22), these experiments also were conducted to ascertain whether AH protein would remain elevated for prolonged periods of time. A slight elevation in AH protein was observed >5 days post-recovery. This elevation in concentration (~2.9 mg/ml) is not expected to influence propranolol intraocular disposition. This condition, however, may be indicative of inflammation present due to the intraocular device. Waga *et al.* (1) reported that microdialysis probes

inserted in the vitreous of rabbits were well-tolerated for up to 30 days. Results of the present study indicate that the CMA/20 custom designed probes also were well-tolerated for at least 18 days in xthe anterior chamber.

In previous work (7), microdialysis probes were placed into the anterior chamber of the rabbit. After a minimum of 30 min, while anesthesia was maintained, propranolol was administered topically to each eye and the time-course of AH propranolol was determined. The AH protein time-course presented herein provides evidence that protein concentrations are time-dependent from 30 to 150 min [*i.e.*, the duration of the study of Rittenhouse *et al.* (7)]. Simulations were used to link the AH protein time-course determined in the present study with the propranolol AH time-course from the previous study (7) to assess whether AH protein concentrations can perturb the pharmacokinetics of propranolol in AH. This analysis suggested that the effects on propranolol disposition are appreciable: there was good agreement between simulations and the *in vivo* data. Anesthetized rabbits that received topically-administered propranolol immediately following probe implantation had a lower intraocular exposure to propranolol relative to rabbits that were allowed to recover for >5 days prior to the initiation of anesthesia and topical dosing of propranolol (Table 2, ~1.9 fold lower).

Ross *et al.* (10) reported a propranolol AH C_{MAX} of ~5000 ng/ml (~10 ng/ml/μg dose) in anesthetized rabbits with paracentesis sampling, comparable to the present results in anesthetized rabbits (~12 ng/ml/μg). In conscious rabbits, the propranolol AH C_{MAX} has been reported to be in the range of 8 (24) to 17 (23) ng/ml/μg. The dose-normalized C_{MAX} in the conscious rabbit presented in this paper were much lower than these values (~1.7 ng/ml/μg). Two possible explanations can be proposed for the differences between these studies. The first possibility is that extensive intraocular metabolism occurs following topical administration of propranolol to the rabbit. Loss of parent and formation of metabolite possessing intraocular distributional characteristics different from the parent may have resulted in loss of detectable radiolabeled substrate. We examined this possibility using TLC of AH samples. Based on this analysis, >94% of the radioactivity in AH was associated with parent (comparable to purity of the administered dose). Thus, this scenario does not explain the apparently lower AH propranolol concentrations in conscious rabbits presented in this study as compared to those reported in the literature. A second possible explanation is that artifactually higher intraocular propranolol exposure was encountered due to the methods used to obtain AH samples in the previous studies. Although propranolol was administered to conscious rabbits in previous studies, the procedure used to collect samples involved sacrifice by i.v. pentobarbital (24,25) prior to obtaining AH aspirate. This procedure may have introduced artifacts to AH turnover (cessation) as well as decreased loss due to reduced tear turnover and decreased blink reflex (26) which, in turn, would result in increased AH concentrations. This increased exposure would be a direct function of the time post-euthanasia at which AH aspirate was obtained. In order to examine this possibility, a limited study was conducted. Propranolol (400 μg) was administered topically to each eye of a conscious rabbit, and microdialysate was collected for up to 40 min. At 40 min, ketamine (50 mg/kg) and xylazine (10 mg/kg) i.m. were administered with the continued collection of microdialysate. AH propranolol concentrations were increased after anesthesia. The average dose normalized AUC_{AH} for propranolol (n = 2) was ~1070 ng-min/ml/μg, ~5.4-fold higher than that in conscious rabbits (Table 2) but only ~1.4-fold lower than that in anesthetized rabbits. These preliminary results support the hypothesis that the currently available methods for evaluation of ocular pharmacokinetics may not capture true substrate disposition. Results obtained in these studies demonstrate the large artifactual effects of systemic anesthesia on ocular disposition of topically administered propranolol. It appears that a closer examination of the currently available methods used to assess the ocular pharmacokinetics of ophthalmics is warranted. The conscious rabbit model with anterior chamber microdialysis sampling may be a meaningful method for the accurate and real-time characterization of ocular pharmacokinetics.

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