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Kinetic disposition and distribution of timolol in the rabbit eye. A physiologically based ocular model

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Summary

This paper presents a physiologically-based mathematical model which describes the disposition of timolol in the rabbit eye. In vitro uptake experiments were utilized to obtain estimates for the various transport and equilibrium tissue distribution coefficients. Two approaches were used to evaluate the uptake data. Initially, the ocular tissues and incubation medium were considered as well-stirred compartments. Alternatively, the iris and lens were viewed as geometric membranes, where the uptake of timolol was governed by simple passive diffusion of drug through the entire tissue. The results clearly indicate that the compartmental treatment of the lens is inappropriate. Consequently, the characterization of ocular drug distribution in the lens should involve a consideration of diffusion through the entire structure. No discernable differences, however, were observed for the iris using either approach, suggesting that the compartmental view may be a valid approximation in this case. In vivo concentration–time profiles were constructed for the cornea, iris, lens, aqueous and vitreous humors following topical dosing with a solution of timolol. Good agreement between the model-predicted and experimental data is observed for both the iris and aqueous humor. However, the magnitude of the iridal transport parameter, estimated from the uptake studies, was not sufficient to

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account for the early peak concentration observed for the iris. In this and most other ocular models, drug is assumed to enter the iris predominantly by exchange with the aqueous humor. To explain the relatively early peak time, an alternate route for drug entry is proposed. The existence of such a pathway is consistent with other reports in the literature, as well as the rapid peak levels observed here for both the vitreous humor and lens.

Introduction

A decrease in intraocular pressure following the administration of a beta-blocker was first reported in 1967, when it was observed that an i.v. injection of propranolol produced ocular hypotension in seven patients (Phillips et al., 1967). Since that time, a number of beta-blocking agents have been screened as potential drugs for the treatment of glaucoma. Thus far, the beta-blocker most widely used has been timolol.

Several mathematical models have been reported in the literature which describe the ocular pharmacokinetics of pilocarpine in the rabbit (Himmelstein et al., 1978; Lee and Robinson, 1979; Makoid and Robinson, 1979; Miller et al., 1981). However, only one attempt has been made thus far to adapt or develop similar types of systems for timolol (Araie et al., 1982). Timolol has rapidly emerged as one of the primary drugs used in the treatment of glaucoma. Accordingly, it is important to understand those factors which determine its kinetic behavior or ultimate distribution pattern in the eye.

The concentration–time profile of timolol in several ocular tissues of the rabbit has been measured following both intravenous and topical dosing (Schmitt et al., 1980; Araie et al., 1980). In one study (Schmitt et al., 1980), the ocular tissue concentrations were determined simultaneously by GLC and a liquid scintillation technique. Comparison of these data suggested that there is an appreciable systemic metabolism of timolol, but none of importance in the eye. Aqueous humor levels of several different beta-blockers following topical dosing have been compared (Schmitt et al., 1981). The rate of appearance in the aqueous humor was greater for those compounds possessing a higher octanol : buffer (pH 7.4) partition coefficient. The time of maximum aqueous humor concentration, however, did not correlate well with the apparent partition coefficient, suggesting that differences exist in the intraocular disposition characteristics of each agent.

The main objective of this paper, therefore, is to attempt to characterize and understand some the physical processes or phenomena which are ultimately responsible for the intraocular disposition of timolol. For this purpose, a physiologically-based model has been developed to describe the kinetic behavior of timolol in the rabbit eye. It should be possible to utilize the knowledge thus acquired to provide insight into the in vivo performance of timolol in the rabbit, and will be generally applicable to the improvement of ophthalmic drug delivery in man.

Materials and Methods

Materials

The levoisomer of timolol (radio- and unlabeled) was provided by INTER$_x$ Research Corporation (Lawrence, KS) as its maleate salt, and was used without further purification. The timolol molecule was labeled with carbon-14 (spec. act. 0.1 mCi/mg) on the two carbon atoms of the thiadiazole ring. Protosol, Econofluor, Aquasol-II, and all other scintillation supplies were purchased from New England Nuclear (Boston, MA). All solvents and chemicals utilized were at least reagent grade.

The rabbits were white, albino, New Zealand males, 55–65 days old. The animals were housed in standard laboratory rabbit cages. No restrictions were placed on their food and water intake prior to experimentation.

Method

In vitro incubation of ocular tissues

As necessary, approximately 25–50 μg of [^{14}C]timolol maleate was accurately weighed on a Cahn-21 microbalance (Cahn Instruments, Cerritos, CA) and dissolved in 1 ml of USP ethanol. This stock solution and the remaining solid material were stored at -20°C until needed.

The timolol solutions used in the in vitro studies were prepared fresh from the above stock solution and discarded after use. Normally, a microliter-sized aliquot was taken from the stock solution and diluted with 500 μl of ethanol in a microevaporation flask. The excess solvent was evaporated off under a stream of nitrogen. Following the evaporation, the material remaining in the flask was redissolved in a specific volume of isotonic 0.067 M sodium phosphate buffer (pH 7.57). This solution was later diluted as described below. The specific activity of the final experimental solution was adequate to ensure a reasonable number of counts per minute (i.e. > 1000) for each tissue sample to be analyzed, using a liquid scintillation technique.

Rabbits were sacrificed with an overdose of sodium pentobarbital administered in the marginal ear vein. Following the death of the animal, the eyeball was proptosed by applying finger pressure on each side of the globe. To maintain the eyeball in this position during the isolation procedure, Kelly forceps were attached to the eyelids below the globe. Sequentially, the iris and lens were each carefully excised with dissecting scissors and forceps. The tissues were rinsed with ice-cold normal saline, and the excess clinging drops were gently removed with a Kimwipe. Each tissue was placed in a tared 20 ml glass scintillation vial. The vials were reweighed and stored on ice (less than 30 min) until used.

The physiological medium utilized for the incubation of the ocular tissues is described in Table 1. Generally, the medium was prepared 1 liter at a time and discarded when evidence of either microbial growth or precipitation occurred. Just prior to use, a sufficient volume of the medium was placed in a beaker and warmed to 33.5°C in a water bath. The pH was adjusted to and maintained at 7.57 by purging the solution with $O_2 : CO_2$ (95 : 5) gas.

TABLE 1

PHYSIOLOGICAL MEDIUM USED FOR THE IN VITRO INCUBATION OF OCULAR TISSUES

Ingredients	Concentration (mM)
NaCl	134.0
$MgCl_2 \cdot 6H_2O$	1.4
$CaCl_2 \cdot H_2O$	1.5
$NaHCO_3$	20.0
Na_2CO_3	5.0
KH_2PO_4	5.0
dextrose	10.0
water	q.s.

Using a Gilson pipettor, 5 ml of the medium were added to the tared vials containing the tissues. The pipettor had been calibrated beforehand to accurately deliver 5 ml at the specified temperature. The headspace of each vial was layered with a blanket of the $O_2 : CO_2$ gas before recapping. All of the samples were then preincubated in the 33.5°C water bath to allow temperature equilibration. At the end of 10 min, 100 μl of the buffered timolol solution (pH 7.57), prepared above, were added to each vial. The headspaces were relayered with $O_2 : CO_2$ (95 : 5), and the samples were replaced in the water bath for the designated incubation period.

The initial concentration of timolol in the medium was calculated to be 1.2 μg/ml (3.8×10^{-6} M), and did not change appreciably over the course of the experiment. The pH of the incubation medium did not vary by more than ± 0.05 units over the course of 4 h. At preselected times, the tissues were removed from the incubation medium and reweighed. The amount of timolol taken up was then determined by a liquid scintillation technique.

In vivo ocular concentration versus time profiles

Throughout these studies the rabbits were held in plastic restrainers in the normal upright position. Animals were dosed topically with 25 μl of a solution containing

TABLE 2

FORMULATION OF THE EXPERIMENTALLY-INSTILLED TIMOLOL MALEATE SOLUTION

Ingredients	quantity per ml
Timolol maleate equivalent to	8.82 mg
(Timolol)	(6.46 mg)
Sodium phosphate monobasic (monohydrate)	5.40 mg
Sodium phosphate dibasic (anhydrous)	12.06 mg
Benzalkonium chloride (17% solution)	0.00071 ml
Sodium hydroxide U.S.P. q.s.	pH 7.0
Water q.s.	1.0 ml

timolol. The formula of the instilled solution is listed in Table 2. Except for the final concentration of timolol (i.e. 0.65% w/v), this formulation is identical to that of the commercially available product Timoptic (Merck, West Point, PA). Radiolabeled [^{14}C]timolol was added to the solution such that each ml contained 300 μCi. The final concentration of the instilled drop took into account the presence of both unlabeled and carbon-14 timolol.

At various post-instillation intervals up to 4 h, the rabbits were sacrificed with an injection of pentobarbital. Immediately following the death of the animal, the precorneal area was thoroughly rinsed with normal saline. Approximately 150 μl of aqueous humor were aspirated, following limbal puncture with a 1 ml tuberculin syringe. The iris, lens, cornea and entire vitreous humor were surgically excised or removed from each eye and transferred to tared glass scintillation vials. The vials were reweighed and treated according to the procedure described below.

Analysis of ocular tissues by liquid scintillation

Varying amounts of Protosol (lens, 2 ml; vitreous humor, 4 ml; cornea and iris, 1 ml) were added to each tissue sample in 20 ml glass scintillation vials. The samples were tightly capped and heated for not more than 72 h in a 55°C water bath. Periodically, the vials were vortexed and the caps were retightened. When the tissue digestion was complete, the samples were allowed to cool to room temperature before adding 10 ml of Econofluor. It was not necessary to digest the aqueous humor prior to counting. Consequently, 100 μl of each collected aqueous humor sample were transferred directly to a scintillation vial containing 10 ml of Aquasol-II. For individual experiments, at least two standards were prepared in a similar manner for each type of tissue or fluid by directly spiking blank tissues with 10–100 μl of the experimental timolol solution. Likewise, the appropriate amount of Protosol was added to the standards. The samples and standards were then stored overnight in the dark before counting in order to minimize any photo and/or chemiluminsence.

Following storage, the samples and standards were counted for at least 5 min using a Beckman LS-7000 liquid scintillation system. Blank samples were included to correct for any background counts. Quenched carbon-14 commercial standards were utilized to evaluate the degree and consistency of quenching for each type of tissue. In all cases, the counting efficiency did not vary by more than 2% between samples for a particular type of tissue. The tissue weights and cpm of each sample were subsequently used to calculate the micrograms of timolol present per gram of tissue.

Results and Discussion

In vitro uptake of timolol by the iris and lens

The average timolol concentrations of the iris and lens at the different time points are recorded in Table 3. The iris appeared to exhibit the highest rate of uptake, followed by the lens. Changes in weight during the incubation were less than 5% for both the lens and iris.

TABLE 3

IN VITRO UPTAKE OF TIMOLOL BY THE IRIS AND LENS

Time (min)	Average timolol [a] concentration (μg/mg of tissue)$\times 10^3$	
	Iris	Lens
5	1.85 (0.095) [b] 4 [c]	0.159 (0.005) 5 [c]
10	2.10 (0.083) 7	0.224 (0.009) 4
16	2.19 (0.094) 7	0.258 (0.003) 5
20	2.64 (0.15) 6	0.278 (0.008) 7
30	2.40 (0.15) 7	0.388 (0.006) 4
45	2.83 (0.30) 5	0.405 (0.005) 7
60	2.51 (0.30) 8	0.469 (0.005) 5
90	3.14 (0.22) 6	0.534 (0.009) 7
120	2.92 (0.045) 6	0.621 (0.021) 5
180	3.25 (0.11) 7	0.724 (0.007) 9
240	3.10 (0.086) 6	0.796 (0.021) 6

[a] Concentration is in equivalents of timolol free base.

[b] Numbers in parentheses represent the standard error of the mean.

[c] Number of individual tissues incubated at that time point.

If the isolated ocular tissues and the incubation medium are treated as well-mixed, homogenous compartments, the following equation may be applied:

$$C_{tissue}(t) = C_{tissue}(\infty)[1 - e^{-kt}] \quad (1)$$

which describes, mathematically, the time course of timolol uptake by the tissue. The timolol concentration in the tissue following equilibration with the medium is $C_{tissue}(\infty)$, and the apparent rate constant, k, is equal to PA/RV_{tissue}. The term PA is the product of the permeability coefficient, P, and the surface area, A. This lumped parameter is generally referred to as a mass transfer coefficient, and represents the rate-limiting clearance for timolol across the interfacial membrane or barrier separating the medium and tissue. Due to the indeterminant nature of A in most biological systems, no attempt is usually made to decompose the product into its individual area and permeability contributions. Since the concentration of timolol in the applied medium remained constant, the equilibrium tissue-to-medium (T/M) distribution ratio, R, can be calculated with a knowledge of $C_{tissue}(\infty)$:

$$R = \frac{C_{tissue}(\infty)}{C_{medium}} \quad (2)$$

To obtain estimates for k and $C_{tissue}(\infty)$, the uptake data for each ocular tissue was fit to Eqn. 1 using the computer program NONLIN (Meltzler et al., 1974). The final estimates along with their standard deviations are shown in Table 4. Also included in Table 4 are the calculated values of the T/M ratio, R, and the mass

TABLE 4

PARAMETER ESTIMATES OBTAINED BY LEAST-SQUARES ANALYSIS DESCRIBING THE UPTAKE OF TIMOLOL BY OCULAR TISSUES. THE DATA WERE FITTED TO EQN. 1 USING THE PHARMACOKINETIC COMPUTER PROGRAM, NONLIN

	$C_T(\infty)$ (μg/g)	V_{tissue} [a] (mg)	k (min^{-1})	Regression correlation coefficient	R [b]	PA [c] (μl/min)
Lens	0.75 (0.047) [d]	258.5 (19.2)	0.0187 (0.0031)	0.98	0.625	3.02
Iris	2.85 (0.11)	36.2 (4.7)	0.141 (0.029)	0.95	2.38	12.16

[a] Average weight of the isolated ocular tissue.

[b] Equilibrium tissue-to-medium distribution coefficient calculated from Eqn. 2 ($C_M = 1.20$ μg/ml).

[c] Mass transfer coefficient calculated from the relationship, $PA = kV_TR$.

[d] Number in parentheses refer to the standard deviation.

transport coefficient, PA. A density of unity was assumed for each tissue in all cases. The results are illustrated graphically in Figs. 1 and 2, where the drawn curves are the computer-generated values calculated by fitting the data to Eqn. 1. The highest T/M ratio was observed for the iris, the lowest for the lens. The rank order of the mass transfer coefficients, PA, for the two tissues was the same as the T/M ratios. Statistical comparisons were not made, as the variance of this parameter (PA) can not be precisely determined.

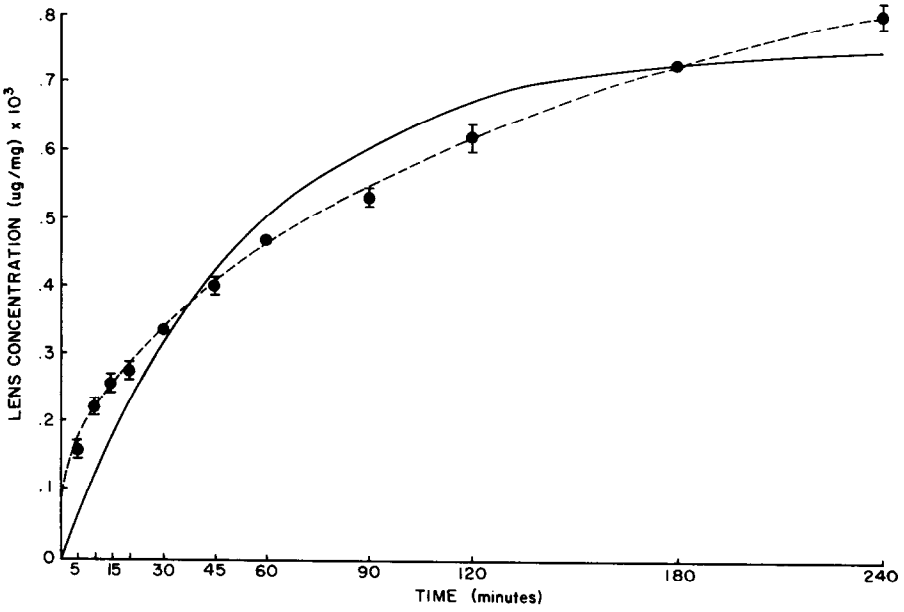

Fig. 1. Comparison of computer-predicted and experimental timolol uptake by the isolated lens. Predicted values were calculated by NONLIN using Eqn. 1 (———) and Eqn. 9 (— — —). Error bars represent the S.E.M.

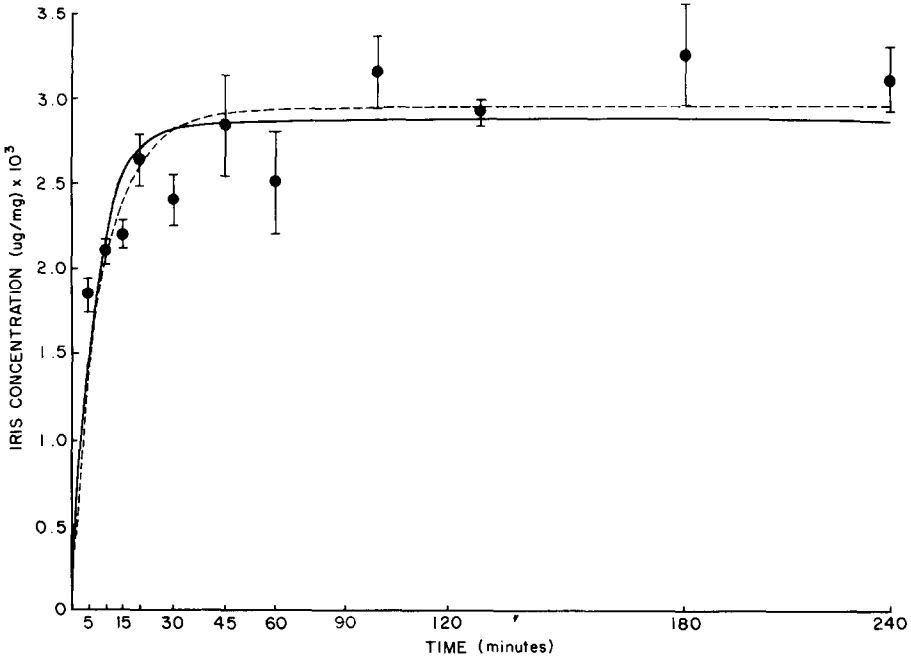

Fig. 2. Comparison of computer-predicted and experimental timolol uptake by the isolated iris. Predicted values were calculated by NONLIN using Eqn. 1 (———) and Eqn. 6 (— — —). Error bars represent the S.E.M.

Situations where drug movement or uptake is controlled by passive diffusion through the entire tissue can be described mathematically using Fick's Second Law:

$$\frac{\delta c}{\delta t} = D \cdot \frac{\delta^2 C}{\delta x^2} \tag{3}$$

where concentration depends on both time and position. The above equation would only apply to those tissues resembling a plane sheet. However, analogous equations exist for other simple geometrical shapes such as a sphere or cylinder. Solutions to all these equations under a number of different initial and boundary conditions are readily available (Crank, 1975).

If the iris is assumed to be a plane sheet of thickness, 2h, exposed on both surfaces to a constant concentration and having no drug present initially, the solution to Eqn. 3 can be shown to be:

$$M(t) = M(\infty)\left\{1 - \sum_{n=0}^{\infty} \frac{8}{(2n+1)^2 \pi^2} \cdot \exp\left[-D(2n+1)^2 \pi^2 t/4h^2\right]\right\} \tag{4}$$

where M(t) is the total mass of drug in the tissue at time, t, and M(∞) represents the total mass after equilibration with the applied phase or medium.

TABLE 5

PARAMETER ESTIMATES OBTAINED BY LEAST-SQUARES ANALYSIS DESCRIBING THE UPTAKE OF TIMOLOL BY THE IRIS AND LENS. THE DATA WERE FITTED TO EITHER EQN. 4 OR 5 USING THE PHARMACOKINETIC PROGRAM, NONLIN

	$C_T(\infty)$ ($\mu g/g$)	k' [a] (min^{-1})	Regression correlation coefficient	R [b]	D (cm^2/s)
Lens [c]	1.13 (0.062) [e]	0.00311 (0.00044)	1.00	0.94	1.31×10^{-6}
Iris [d]	2.91 (0.11)	0.101 (0.022)	0.85	2.42	1.07×10^{-7}

[a] For a plane sheet, $k' = D\pi^2/4h^2$; for a sphere, $k' = D\pi^2/a^2$ (see Eqns. 4 and 5).

[b] Equilibrium tissue-to-medium distribution ratio (calculation based on $R = M(\infty)/V_{tissue} \cdot C_{medium}$).

[c] Assumed that a = 0.5 cm.

[d] Assumed that h = 0.0125 cm.

[e] Numbers in parentheses refer to the standard deviation.

Analogously, if we assume the lens to be represented by a sphere, constrained by the same boundary and initial conditions, the solution becomes:

$$M(t) = M(\infty)\left\{1 - \frac{6}{\pi^2} \cdot \sum_{n=1}^{\infty} \frac{1}{n^2} \cdot \exp\left[-Dn^2\pi t/a^2\right]\right\} \tag{5}$$

where a is the radius, and M(t) and $M(\infty)$ are as defined above. The equilibrium tissue-to-medium (T/M) distribution ratio, R can then be calculated from Eqn. 2 with a knowledge of $M(\infty)$ and the average tissue volume.

Using NONLIN, the timolol uptake data was fitted to Eqns. 4 and 5. Estimates obtained for $C_T(\infty)$ and the diffusivity are listed in Table 5, while the results are also plotted in Figs. 1 and 2. Comparison with the earlier findings of the compartmental analysis obtained for the iris is inconclusive. There is no significant improvement in either the correlation coefficient or the pattern of residuals. Additionally, there is no marked change for the estimates of $C_T(\infty)$ and R for either treatment.

Both the correlation coefficient and residual scatter for the lens data, however, were noticeably improved. The final predicted T/M ratio suggested that 4 h is not sufficient time for steady-state to be attained between the lens and its contiguous fluids (i.e. aqueous and vitreous humors). This is entirely consistent with the in vivo data previously reported for pilocarpine (Miller et al., 1981), and here for timolol.

A significant amount of literature exists describing the ocular disposition of topically applied drugs in the rabbit eye. Many of these studies have evaluated their results or data in the context of mathematical models. The predominant approach in these ocular models has been to divide the eye into separate compartments. This type of approach makes many assumptions, foremost being that the compartments are well-stirred phases. However, the results of this study clearly indicate that treatment of the crystalline lens as a well-stirred compartment is inappropriate. Consequently, the characterization of ocular drug disposition in the lens should

include a consideration of simple passive diffusion through the entire tissue. No discernable difference using either approach was observed for the iris, tacitly suggesting that a compartmental approach may be a valid approximation in this case.

In vivo concentration versus time ocular profiles

The concentration versus time profiles for the cornea, iris, lens, aqueous and vitreous humors are collectively illustrated in Fig. 3. These levels were determined following the topical instillation of a solution (25 μl) containing 0.65% timolol. The concentration at each time point represents the average of between 5 and 13 individual measurements (i.e. eyes). The mean values along with their standard deviations are compiled in Table 6.

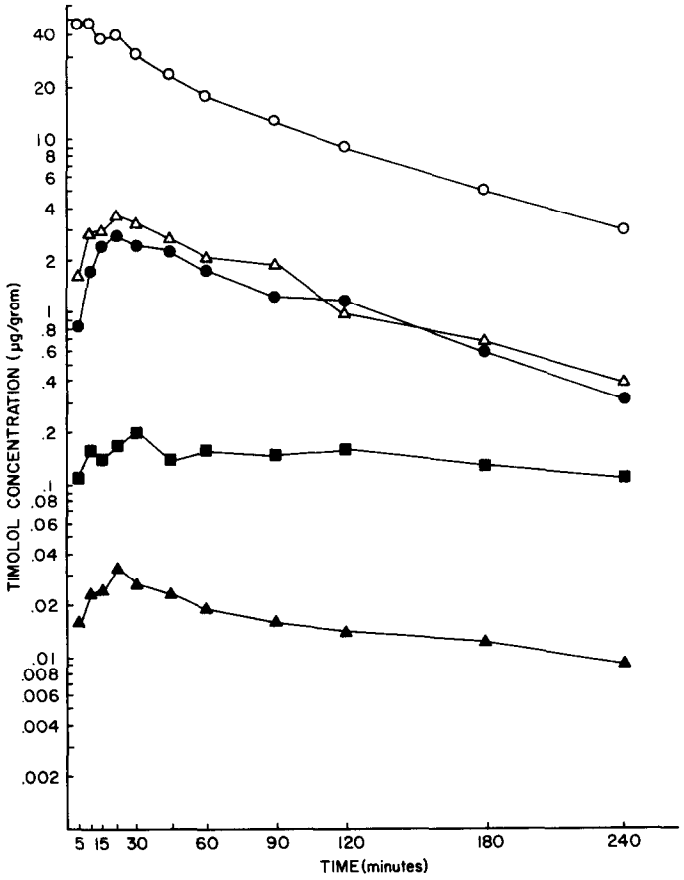

Fig. 3. Concentration versus time profiles following the topical instillation of 25 μl of a 0.65% timolol solution in the eyes of 60-day-old albino rabbits. Cornea (○), iris (△), aqueous humor (●), lens (□), vitreous humor (▲).

TABLE 6

AVERAGE CONCENTRATION OF TIMOLOL IN OCULAR TISSUES AT VARIOUS TIMES FOLLOWING THE TOPICAL ADMINISTRATION OF 25 μl OF A 0.65% (w/v) SOLUTION

Time (min)	Timolol concentration[a] (μg/g)				
	Cornea	Iris	Aqueous humor	Lens	Vitreous humor
5	45.78(8.32)[b] 10[c]	1.66 ≈ (0.36)[b] 11[c]	0.83 ≈ (0.17)[b] 8[c]	0.16(0.03)[b] 8[c]	0.016(0.003)[b] 12[c]
10	45.56(16.13) 9	2.91[d](1.03) 12	1.73[d](0.66) 10	0.16(0.05) 11	0.023(0.007) 9
15	37.82(5.74) 9	2.97[d](0.32) 8[c]	2.43[d](0.48) 7	0.14(0.03) 12	0.024(0.005) 11
20	40.56(8.27) 11	3.62[d](0.69) 10[c]	2.80[d](0.59) 8	0.17(0.02) 8	0.033(0.010) 13
30	31.00(6.46) 11	3.25[d](0.64) 12	2.46[d](0.38) 7	0.20(0.09) 9	0.026(0.005) 12
45	24.07(8.92) 10	2.69(0.87) 11	2.25(0.74) 10	0.14(0.06) 12	0.023(0.009) 11
60	18.03(3.44) 8	2.06(0.53) 11	1.75(0.28) 9	0.16(0.06) 11	0.019(0.003) 10
90	12.96(3.05) 10	1.87[d](0.22) 9	1.22[d](0.26) 9	0.15(0.03) 12	0.016(0.002) 9
120	9.11(3.71) 9	0.98(0.15) 8	1.17(0.60) 10	0.16(0.07) 10	0.014(0.004) 8
180	5.11(1.72) 6	0.66(0.20) 6	0.48(0.22) 6	0.13(0.02) 6	0.012(0.002) 7
240	3.02(1.05) 6	0.39(0.09) 5	0.31(0.09) 6	0.11(0.02) 6	0.0089(0.001) 6

[a] Timolol concentration is in equivalents of the free base.
[b] Numbers in parentheses refer to the standard deviation.
[c] The numbers of determinations at that particular time point.
[d] Time points where the iris concentration is significantly different than the aqueous humor concentration (based on two-tailed, t-test, $\alpha = 0.05$).

The highest concentrations of timolol were observed in the cornea; followed by the iris, aqueous humor, lens and the vitreous humor. The kinetic profiles for the iris and aqueous humor appear, upon inspection, to be very similar. Using the two-tailed Student t-test, the drug concentration at the 5, 10, 15, 20, 30 and 90 min time points for the iris and aqueous humor were found to be significantly different ($P < 0.05$). There was no significant difference ($\alpha = 0.05$), however, at the remaining times.

The iris, vitreous and aqueous humors all reached their peak concentration at approximately 20 min post-instillation. The maximum concentration in the cornea occurred somewhat earlier (at approximately 10 min); while the peak level of timolol in the lens was achieved between 20 and 30 min following instillation of the drug solution. The distribution and/or elimination portion of the tissue profiles all appeared to be multiphasic, with the overall shape and rank order of the curves agreeing quite well with that reported in similar studies (Araie et al., 1980; Schmitt et al., 1980).

Development of the model

The rabbit eye is represented in this proposed model by the four compartments depicted in Fig. 4. According to this scheme, drug, which is exchanged between two adjacent compartments, must traverse an interfacial barrier separating them. The transport of timolol across this interface is assumed to be symmetric and to occur only by passive diffusion. Furthermore, the movement of drug within the tissue itself is considered to be fast, relative to the diffusion across the compartmental interface.

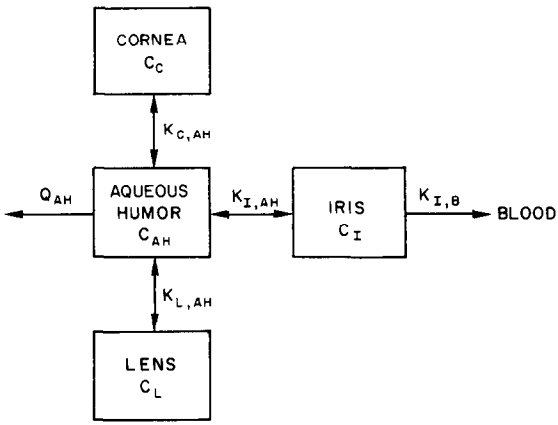

Fig. 4. Schematic representation of the proposed pharmacokinetic model describing the disposition of timolol in the rabbit eye.

The degree of resistance associated with each interface is defined by the product of a permeability coefficient and the surface area. This product or mass transport coefficient has units of volume per time and is designated in this model by the letter K. The transport coefficient is also viewed as being independent of both time and concentration.

This model attempts to incorporate the influence of the iridial blood flow on the kinetic behavior of drug distribution in the iris. The approach taken is essentially that developed by Rinkin (1955) and is described elsewhere (Francoeur, 1983). The final equation relating the blood flow rate and the capillary permeability is almost identical to that derived in the "parallel tube" model, describing drug clearance from the liver (Wintler et al., 1973). The iris-to-blood mass transfer coefficient, $K_{I,B}$ is given by:

$$K_{I,B} = Q_I(1 - e^{-P_I/Q_I}) \tag{6}$$

where Q_I represents the iridal blood flow rate, and P_I is the mass transfer coefficient of its capillary walls.

The cornea will be treated here as a single compartment. Studies have shown, that following topical administration, the timolol concentrations of the corneal epithelium were in the same range as those of its stroma endothelium (Araie et al., 1982). Based on these data, this compartmental view would appear to be a reasonable first approximation. To simplify the model and eliminate the complication of precorneal disposition factors, an empirical equation describing the average corneal concentration of timolol will be used as the 'driving force' for drug movement into the eye. This equation was obtained by treatment of the experimental data with the pharmacokinetic computer program NONLIN and is given below:

$$C_C = 24.75\, e^{-0.0088t} + 33.38\, e^{-0.035t} - 58.13\, e^{-0.46t} \tag{7}$$

where C_C is the timolol concentration of the cornea at any time, t.

The aqueous humor is also assumed to be homogeneous with respect to its drug concentration. As the aqueous humor is produced, it flows from the posterior to the anterior chamber, and out through the porous tissue at the irido-scleral angle. The convection currents associated with this bulk flow serve to mechanically mix the aqueous compartment. Once drug reaches the aqueous humor it can undergo exchange with the adjacent structures including the iris and the lens. Additional drug is lost via the normal aqueous turnover described above. Taking all of these processes into account, a mass balance can be written for timolol in the aqueous humor:

$$\frac{dV_{AH}C_{AH}}{dt} = K_{C,AH}\left(\frac{C_C}{R_C} - C_{AH}\right) + K_{L,AH}\left(\frac{C_L}{R_L} - C_{AH}\right)$$

$$+ K_{I,AH}\left(\frac{C_I}{R_I} - C_{AH}\right) - Q_{AH}C_{AH} \qquad (8)$$

where V_{AH} is the volume of aqueous humor, and Q_{AH} is the rate of its turnover.

Each of the transport coefficients in Eqn. 8 describe the proportionality factor between the interfacial flux and the concentration gradient for the exchanging compartments. The concentration of timolol in any two compartments, however, would not necessarily be equal. Differences in intrinsic solubility, binding or the operation of specialized transport systems are all examples of phenomena which could result in such a situation. To account for this, equilibrium tissue distribution coefficients are incorporated into Eqn. 8 for the iris, lens and cornea. It is not necessary in this approach to specify quantitatively the factors which contribute to R, as long as the interfacial transport remains rate-limiting.

The kinetic similarity in the aqueous humor and the iris concentration–time profiles infer that the iris may also be approximated as a well-mixed compartment. In this and most ocular models, timolol enters the iris only through exchange with the aqueous humor. Drug in the iris may then diffuse into the iridal capillary bed and be swept into the general circulation. The following mass balance equation represents this situation:

$$\frac{dV_IC_I}{dt} = K_{I,AH}\left(C_{AH}\frac{C_I}{R_I}\cdot I\right) - K_{I,B}C_I \qquad (9)$$

where V_I is the volume of the iris and $K_{I,B}$ (Eqn. 6) is the coefficient describing the clearance of timolol by the blood.

The systemic blood is not included formally as a compartment since its timolol concentration was not actually measured in these experiments. Studies in other laboratories have shown that the concentration of timolol in the blood, under similar conditions, is very small in comparison to that of the iris following the topical administration of a drug solution to the rabbit eye (Araie et al., 1980; Araie et al., 1982; Schmitt et al., 1980). With this in mind, the blood will be treated as a 'sink' for drug removal from the iris.

The in vitro data discussed earlier indicates that a compartmental view of the lens is inappropriate, and that diffusion through the entire tissue should be considered. Kinetic studies performed with fluorescein further corroborate these findings (Kaiser and Maurice, 1964). For mass balance purposes, nevertheless, the lens is included here as a hypothetical compartment. An empirical equation describing the average lens timolol concentration–time profile will be incorporated into the model. This equation was obtained by treatment of the actual experimental data with the computer program, NONLIN and is given below:

$$C_L = 0.172(e^{-0.00166t} - e^{-0.241t}) \tag{10}$$

It is not currently known whether or not the vitreous humor can be adequately treated as a well-stirred compartment. Several distribution phases are observed in the vitreous humor concentration–time profile for timolol (see Fig. 3), suggesting a multiplicity of routes by which drugs may enter the vitreous. The lens, retina, ciliary body and posterior chambers are all possible pathways for drug entry. In terms of total mass, though, the amount of timolol in the vitreous humor at any given time is negligible compared to those levels measured in the other ocular tissues. For these reasons, the vitreous humor has not been included in this model.

Estimation of model parameters

In order to evaluate the proposed model, it is necessary to estimate those parameters defined in Eqns. 8 and 9. These equations can then be solved in conjunction with Eqns. 7 and 8; and the results can be compared with the actual in vivo data. These differential equations were solved using a fourth-order Runge-Kutta algorithm coupled with Hamming's predictor corrector numerical method in a computer program (Carnahan et al., 1976).

The rate of aqueous humor turnover, Q_{AH} represents the minimum rate at which timolol can be lost from the anterior and posterior chambers. Normally, 1% of the aqueous is replaced each minute (Sears, 1981; Ruskell et al., 1964). However, timolol has been demonstrated to decrease this turnover by approximately 30% in man (Araie and Takase, 1981). For the purposes here, a comparable effect will be assumed for the rabbit, and Q_{AH} will be assigned a value of 2.18 μl/min.

The equilibrium tissue distribution coefficients are considered in this model to be independent of concentration. The in vitro tissue-to-medium distribution ratios were fixed as estimates for R_I, R_L and R_C and it was not necessary to correct these values for aqueous humor binding, as dialysis experiments showed no significant interaction between timolol and aqueous humor (Francoeur, 1983).

The blood flow rate in the rabbit iris has been determined to be approximately 32 μl/min, and does not appear to be altered by the presence of beta-blockers (Sakai et al., 1981). This value, therefore, represents the maximum rate at which timolol can be cleared from the iris into the systemic circulation. Accordingly, the iridal clearance ($K_{I,B}$) should fall somewhere between 0 and 32 μl/min, depending on whether loss of the drug into the capillary blood is flow- or permeability-limited. $K_{I,B}$ was initially fixed at 2 μl/min as the apparent partition coefficient of timolol at

TABLE 7

PARAMETERS USED IN THE SOLUTION OF THE DIFFERENTIAL EQUATIONS WHICH DESCRIBE THE MODEL

Parameter	Value	Source
V_{AH}	311 μl	Kinsey and Reddy (1964)
V_I	36.24 mg	Francoeur (1983)
R_I	2.11	Experimental
R_C	2.38	Francoeur (1983)
R_L	0.94	Experimental
$K_{C,AH}$	8.0 μl/min	Francoeur (1983)
$K_{I,AH}$	23.0 μl/min	Model [a]
$K_{L,AH}$	26.8 μl/min	Model [a]
Q_{AH} [b]	2.18 μl/min	Araie and Takase (1981) [b]
$K_{I,B}$	7.3 μl/min	Model [a]

[a] Evaluated in terms of the model.

[b] Assumes $V_{AH} = 311$ μl and that Q_{AH} (normal) = 1%/min (Kinsey and Reddy (1964)).

[c] Recent work by Araie (ARVO Invest. Ophthalmol. Vis. Sci., 25 (1984) 974) suggests that aqueous humor turnover in rabbits may not be affected by timolol.

physiological pH and temperature is only about 2 (Baustian and Mikkelson, 1982); and as such, a permeability-limited situation would be anticipated.

All of the transfer coefficients, including K_I, were adjusted in terms of the model until good agreement was achieved between the data and simulations. The permeability coefficients calculated from the previous in vitro uptake studies (Francoeur, 1983) were used as initial estimates for $K_{I,AH}$, $K_{C,AH}$ and $K_{L,AH}$. It should be mentioned that the true physical meaning of the parameters R_L and $K_{L,AH}$ are confounded by the fact that the treatment of the lens as a well-stirred compartment is not valid. The final estimates for all of the model parameters as well as their source are compiled in Table 7.

Conclusions

Figs. 5 and 6 compare the model-predicted and experimental data for the aqueous humor and iris. Good agreement is observed for both structures. Thus, the proposed model provides good predictability over the 4-h study for both the iris and aqueous humor concentration–time profiles.

The in vitro uptake studies for timolol in the isolated iris, lens and cornea were conducted with the hope that estimates for the in vivo transport coefficients could be obtained for each of these ocular tissues. In the context of this model, $K_{I,AH}$ was found to be 23 μl/min compared to only about 12 μl/min as estimated from the in vitro data. The two-fold increase in $K_{I,AH}$ from its initial guess was required in order to match the early peak time observed for the iris. It must be recognized that the physiological and metabolic performance of isolated tissues may be diminished from

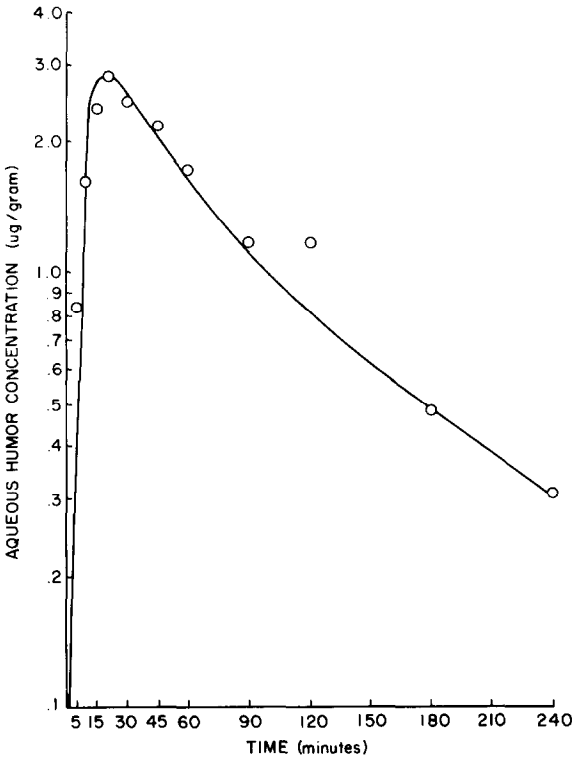

Fig. 5. Comparison of model-predicted and experimental aqueous humor concentrations, following the instillation of 25 μl of 0.65% timolol solution.

that seen in the intact animal. If the uptake or transport of timolol by the iris is in any way dependent on its metabolic integrity, a deterioration in cellular viability could possibly explain these results.

However, indirect evidence provides an alternate explanation for the relatively early peak times measured in the iris. In this and in most ocular models, it is assumed that drug enters the iris only through exchange with the aqueous humor. Another pathway, however, is possible and has been demonstrated for pilocarpine and epinephrine (Doane et al., 1978; Bienfang, 1973). Blockage of the cornea resulted in only a 5–7-fold reduction of pilocarpine levels measured for the iris, presumably due to diffusion across the conjunctiva and sclera. Timolol, having an apparent partition coefficient (i.e. in octanol) very similar to that of pilocarpine (Mitra and Mikkelson, 1983), could also be expected to enter the iris by the same route across the conjunctiva/sclera.

The portion of drug crossing the conjunctiva that is not lost to the local circulation can then diffuse into the sclera. Once in the sclera the drug would be available to various parts of the globe, including the anterior chamber and the uveal tract. The highly vascularized uvea could distribute the drug throughout various parts of the eye, including the retina and vitreous humor. Further evidence for this

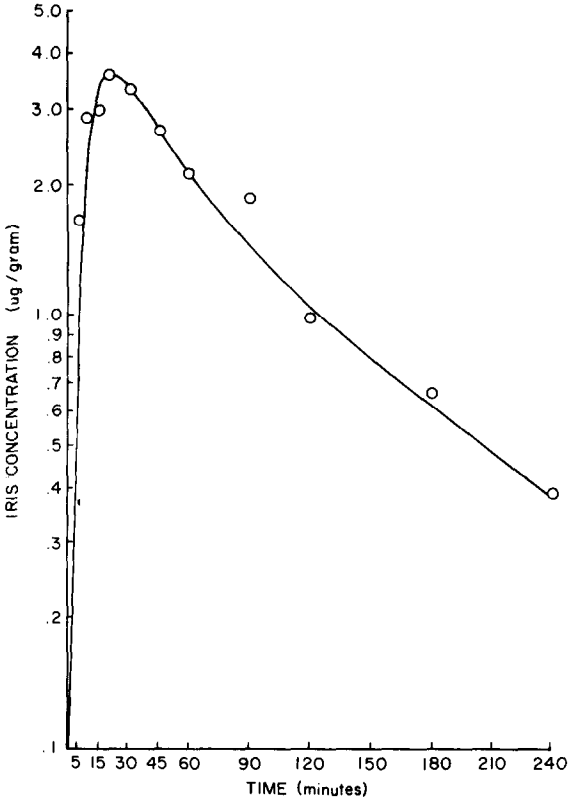

Fig. 6. Comparison of model-predicted and experimental iris concentrations following the instillation of 25 μl of 0.65% timolol solution.

kind of pathway exists, as levels comparable to that measured in the aqueous humor are found in the choroid-retina, following topical instillation of timolol (Schmitt et al., 1980). It is difficult to visualize significant amounts of drug reaching the retina by serial diffusion across the cornea, aqueous humor, lens and then vitreous humor. Certainly, another pathway must exist, and could help explain the early peak times observed in the iris for timolol as well as for pilocarpine (Miller et al., 1981).

As discussed previously, a compartmental view of the lens is inappropriate, and the simple diffusion of drug through the entire tissue should be considered in addressing drug disposition. Modeling of the lens using such an approach is currently in progress.

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