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Research Article

The Kinetics of Timolol in the Rabbit Lens: Implications for Ocular Drug Delivery

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This study examines the uptake and distribution of timolol in the rabbit lens following topical instillation using a heuristic approach. The implications of anisotropic drug diffusion through the lens are presented here and discussed in the context of actual *in vivo* data. The dynamics of timolol in the lens involve an initial, rapid uptake of the drug by the capsule and epithelium followed by slower, anisotropic diffusion through the cortex body. Kinetically, the capsule and epithelium can be treated as a separate compartment which is distinct from the cortex and which serves to provide a concentration gradient for subsequent diffusion of timolol into the dense interior structures of the lens. Model simulations support the hypothesis that the preferred route of penetration of timolol into the vitreous humor via the lens is the diffusion of drug around the capsule/epithelium and peripheral cortical layers. It is also shown that due to the high and increasing diffusional resistance toward the center of the lens, as well as the diminishing drug concentrations in the capsule and epithelium, steady-state levels in the lens may be extremely difficult to achieve in some therapeutic situations. This phenomenon could have a significant impact on the success or failure of a drug treatment involving the lens and ocular tissues.

KEY WORDS: diffusion; timolol; lens; ocular drug delivery; model.

INTRODUCTION

Recently, there has been considerable interest in the delivery of drugs to the lens of the eye. Much of this interest has evolved from studies showing the ability of aldose reductase inhibitors to prevent monosaccharide-induced lens degeneration in diabetis (1–3). Rational drug therapy to treat lens disorders, such as secondary cataract formation, requires an in-depth understanding of the underlying principles that govern the biodisposition of substances in the lens. Therefore, characterization of the permeability or transport properties of the lens would clearly be valuable to establish a rational framework for successful drug delivery. In addition, a knowledge of these parameters would improve our understanding of the role of the lens in the distribution of drugs designed to exert their effect in other ocular tissues.

To date, most studies have focused on the *in vitro* uptake of solutes by the lens in an effort to examine lens structure and physiology (4–7). However, relatively few studies have examined the *in vivo* exchange of drugs between the lens and surrounding intraocular fluids or drug distribution within the lens (8–10). The objectives of this work were to quantitate the uptake and distribution of topically applied timolol in the rabbit lens and to develop a physical model in order to understand the factors governing the kinetic disposition of timolol within the lens, as well as their relevance to ocular drug delivery.

MATERIALS AND METHODS

Materials

Timolol maleate and ^{14}C-timolol (sp act, 0.1 mCi/mg) were provided by the InteRx-Merck Research Corp., Lawrence, Kansas, and used without further purification. Protosol, Econofluor, and Aquasol II were purchased from New England Nuclear, Boston, Mass. Male, New Zealand rabbits (Small Stock Industries, Pea Ridge, Ark.) were housed in standard laboratory cages with no restrictions placed on food or water. At the time of use, the animals were 55 to 65 days old (~2.0–2.4 kg).

Experimental Methods

Rabbits were sacrificed with an intravenous injection of sodium pentobarbital. Lenses with the capsule intact were obtained by the posterior approach from enucleated eyes as described by Kinsey and Reddy (4). After removal, the lenses were gently blotted, weighed, and transferred to glass vials prefilled with 5 ml of incubation medium. The medium composition was as follows: 134 m*M* NaCl, 1.4. m*M*

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$MgCl_2 \cdot 6H_2O$, 1.5 m*M* $CaCl_2 \cdot 2H_2O$, 20.0 m*M* $NaHCO_3$, 5.0 m*M* Na_2CO_3, 5.0 m*M* KH_2PO_4, and 10 m*M* dextrose (11,12). Tracer amounts of radiolabeled timolol were added to the medium, along with an appropriate amount of nonradioactive drug to provide a total drug concentration of 1.2 μg/ml. Incubations were carried out at 33.5°C up to a period of 4 hr. The pH of the medium was maintained at 7.57 by bubbling 5% CO_2–95% O_2 through the medium. At the end of the incubation period the lenses were removed from the medium, blotted dry, transferred to tared glass scintillation vials, and reweighed. The tissues were then digested (Protosol, NEN), diluted with a fluor (Econofluor, NEN), and analyzed for radioactivity in a scintillation counter.

According to a procedure described previously (13), aqueous solutions of timolol maleate containing both labeled (300 μCi/ml) and unlabeled drug at a total concentration of 0.65% (w/v) were prepared in Sorensen's phosphate buffer. The solutions were rendered isotonic by adding NaCl, and the final pH was adjusted to 7.4 with 5 *N* NaOH. The timolol solutions used in these studies were prepared fresh for each experiment and discarded after use. Unanesthetized rabbits were placed in plastic restrainers in their normal, upright position. Twenty-five microliters of the drug solution was instilled inside the conjuctival sac in each eye. At selected times postinstillation, the rabbits were sacrificed with an intravenous injection of sodium pentobarbital. The precorneal area was thoroughly rinsed with normal saline and gently blotted to remove the excess fluid. Following enucleation, approximately 150 μl of aqueous humor was aspirated by limbal puncture. The lens with the capsule intact and the vitreous humor were removed by a posterior approach. Care was taken not to rupture the lens capsule during the initial dissection but subsequently the capsule was removed as described previously by Kinsey and Reddy (4). No efforts were made in these preliminary experiments to separate the anterior from the posterior capsule or to strip the epithelium off the anterior capsule. The aqueous humor, vitreous humor, capsule, and lens cortex samples were placed in separate, tared 20-ml glass scintilation vials. Tissue standards were prepared by spiking two of each tissue type with 25 μl of the drug solution. After obtaining the wet weights, the tissues were digested in Protosol at 60°C. After digestion was complete (~48 hr), an appropriate amount of scintillation fluors (Aquasol II, Econofluor, NEN) were added, and the samples were stored overnight prior to counting in a liquid scintillation spectrometer. In all cases the counting efficiency did not vary more than 2% between samples for a particular type of tissue. The count rates obtained from the respective tissue standards were used to convert the count rate of the samples to microgram equivalents of drug, and the values were normalized for the tissue weight.

RESULTS

In Vitro Kinetic Uptake of Timolol

Figure 1 shows the uptake profile of timolol in the lens. The accumulation profile was biphasic, with an initial rapid uptake phase followed by a slow, prolonged accumulation phase. Equilibrium uptake was not achieved within the 4-hr incubation period. The observed concentration (C_t) versus time profile in the lens was empirically fitted to a biphasic uptake model represented by Eq. (1) to obtain estimates for the drug uptake rate constants (k_1 and k_2) and the equilibrium drug concentration in the lens (C_∞):

$$C_t = C_\infty - Ae^{(-k1t)} - Be^{(-k2t)} \quad (1)$$

In Eq. (1), the sum of the preexponential A and B equals C_∞. The observed and model-predicted lens concentration profiles are compared in Fig. 1. The value of C_∞ was estimated as 1.02 μg/g. Following least-squares regression, the rate constants associated with the rapid uptake phase (k_1) and the slower accumulation phase (k_2) were calculated to be 0.072 and 0.0051 min^{-1}, respectively.

The value of the tissue-to-medium distribution ratio, *R* ($=C_\infty/C_m$), was determined to be 1.1. Assuming an average water content of 66% in the lens, the distribution volume for timolol in the lens as defined by Thoft and Kinoshita (14) was estimated to be 169% of the lens water. A distribution volume larger than the total water content of the lens suggests that timolol diffuses into the intracellular space of the lens fibers and may also accumulate in the epithelial cells. This may be attributed to the substantial lipid solubility of the timolol molecule (15).

In Vivo Kinetic Disposition

The average timolol concentrations in the aqueous humor (AH), lens capsule and epithelium (CE), lens cortex and nucleus (CN), and vitreous humor (VH) after topical drug instillation in the eye are shown in Fig. 2. The highest drug

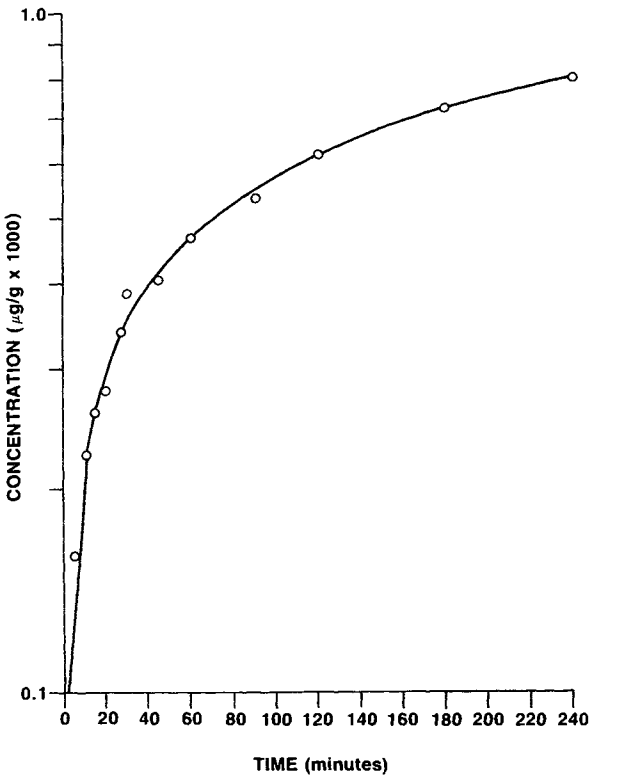

Fig. 1. *In vitro* uptake of timolol by the lens. (○) Observed; (——) fitted.

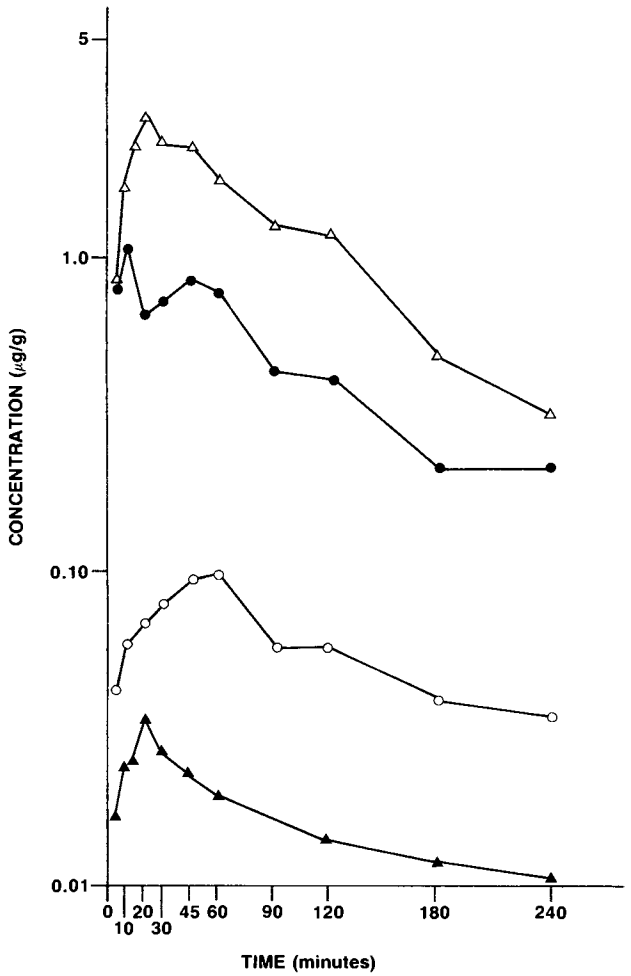

Fig. 2. *In vivo* concentration versus time profiles for timolol in the aqueous humor, capsule-epithelium, cortex-nucleus, and vitreous humor following topical drug instillation in rabbit eye. (△) Aqueous humor; (●) capsule-epithelium; (○) cortex-nucleus; (▲) vitreous humor.

levels were observed in the AH, followed in decreasing order in the CE, CN, and VH. Peak timolol concentrations were observed within 10–20 min postinstillation in the AH, CE, and VH and at 60 min in the CN. The AH and CE concentration profiles were similar in shape and were consistent with the behavior of rapidly equilibrating, well-mixed compartments (16). Since the capsule and epithelium were collected *in toto*, it was not possible to discriminate experimentally between the anterior and the posterior capsule drug levels or to isolate the contribution of the epithelium to the capsule drug levels. Since the VH drug concentrations were negligible (<5%) compared with those in the AH, the primary source of the drug detected in the CE was attributed to the drug within the AH. This in turn implies that the posterior capsule in contact with the vitreous humor contributes minimally to the CE drug levels.

The values of observed timolol levels in the CE and CN, and their sum, which represents the total amount of drug in the entire lens, are tabulated in Table I. It is noted that even when averaged over the entire capsule and epithelium weight, the drug levels in the CE were 4- to 18-fold higher than those in the CN on a per weight basis. Furthermore, the

Table I. Distribution of Timolol in the Lens After Topical Instillation of 25 μl of a 0.65% (w/v) Timolol Maleate Solution in the Rabbit Eye[a]

Time (min)	Timolol Concentration (μg/g)		
	Lens Cortex	Lens Capsule	Lens
5	0.041 (0.007, 11)	0.791 (0.179, 11)	0.064 (0.011, 11)
10	0.058 (0.004, 11)	1.042 (0.265, 11)	0.091 (0.020, 11)
20	0.067 (0.010, 8)	0.647 (0.152, 8)	0.089 (0.012, 8)
30	0.078 (0.012, 9)	0.703 (0.093, 9)	0.097 (0.012, 9)
45	0.091 (0.010, 12)	0.850 (0.123, 12)	0.115 (0.011, 12)
60	0.095 (0.007, 8)	0.772 (0.206, 8)	0.133 (0.030, 8)
90	0.057 (0.005, 5)	0.433 (0.178, 5)	0.068 (0.009, 8)
120	0.057 (0.003, 7)	0.403 (0.029, 7)	0.061 (0.005, 7)
180	0.038 (0.005, 5)	0.209 (0.060, 5)	0.045 (0.005, 5)
240	0.034 (0.006, 7)	0.213 (0.101, 7)	0.036 (0.002, 7)

[a] Weight of the lens cortex = 0.2553 ± 0.0028 g. Weight of the capsule = 0.0096 ± 0.0006 g. The numbers in parentheses are the standard error of the mean and the sample size, respectively.

fractional contribution of the CE to total lens drug levels were highest initially, plateauing off at later time points (Fig. 3). These results together with the *in vitro* findings clearly point to a nonhomogeneous kinetic disposition of timolol within the lens. In particular, there appears to be rapid drug uptake by the CE and possibly the peripheral cortical layers of the lens, followed by a slower distribution and penetration of the drug into the cortex and nucleus. Similar observations have been presented for the diffusion of flourescein (9) and horseradish peroxidase and lanthanum (17) in the lens.

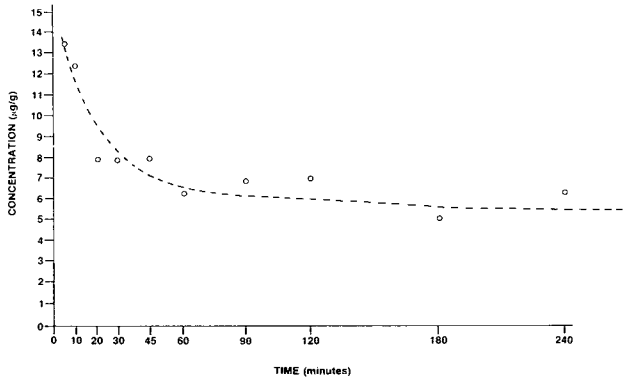

Fig. 3. Fractional contribution of the capsule and epithelium to the total drug level in the lens as a function of time.

MODEL

A physical model depicted schematically in Fig. 4 was developed to facilitate the understanding of the drug disposition kinetics in the lens. It has been shown previously (9) that the anterior surface of the lens represents a barrier to drugs due to the presence of the epithelium. Therefore, assuming that timolol enters the CE from the AH by passive diffusion in response to a concentration gradient and that it is simultaneously depleted from the CE by diffusion into the CN, a mass balance for drug in the CE may be written as follows:

$$V_c dC_c/dt = P_o(C_{AH} - C_c/R_c) - K_c C_c \qquad (2)$$

where C_c represents the drug concentration in the CE, V_c is the volume of the CE, P_o is the AH-to-CE mass transfer coefficient, R_c is the AH-to-CE distribution ratio, and K_c is the first-order rate constant for the removal of drug from the CE. The cortex and VH were not included as compartments because their drug concentrations were very low compared with the CE. A value of 1.1 was assigned to R_c on the basis of the *in vitro* data. Equation (2) was solved numerically employing the nonlinear regression analysis program NONLIN (18). Reasonable agreement between the model-simulated and experimental data observed over a 4-hr period (Fig. 5) lends support to the model assumption that the CE may be treated as a well-mixed compartment.

In contrast, the transport of timolol in the CN is complicated because the lens fibers become more densely packed toward the nucleus (19). Once drug penetrates the surface, it may diffuse rapidly through the less dense outer segments and, simultaneously at a slower rate, into the more dense nucleus. Hence, the concentration and diffusion coefficient of diffusing species in the lens may vary with distance from the surface as demonstrated by Bassnett *et al.* (20). Utilizing this information, a diffusion model was proposed

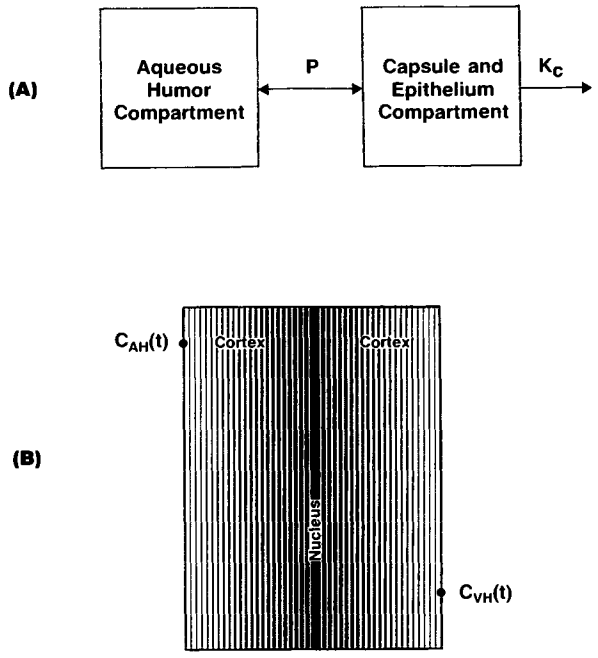

Fig. 4. Schematic for the proposed diffusion model.

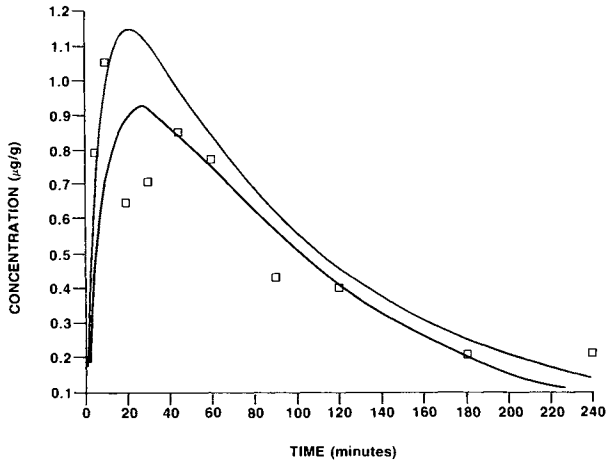

Fig. 5. Comparison of observed and model-simulated timolol concentration in the lens epithelium and capsule following topical drug instillation.

for the penetration of timolol in the CN. To circumvent some of the mathematical complexity the following assumptions were made: (a) the geometry of the lens was approximated to be planar, (b) the diffusion coefficient of timolol was assumed to decrease exponentially as a function of distance from the surface of the lens toward the center, and (c) the driving force for timolol penetration into the CN on the anterior boundary was attributed to the drug concentration in the CE as predicted by solving Eq. (2), while an empirical polyexponential fit of the VH concentration profile was used to define the posterior boundary condition.

Accordingly, the model equations are obtained by considering the steady-state mass balance for timolol in the CN:

$$\partial C_{CN}/\partial t = \partial/\partial \times [D(x)\partial C_{CN}/\partial x], \qquad O < x < h_{CN} \qquad (3)$$

where $D(x)$ is the position-dependent diffusion coefficient, x is the distance in the CN, and t is the time after instillation.

The initial condition is that of zero timolol concentration or

$$C_{CN}(x,O) = O \qquad (4)$$

and the conditions at the anterior and posterior boundaries of the CN are given by

$$C_{CN}(O,t) = C_{CE}(t) \qquad (5)$$

$$C_{CN}(h_{CN},t) = C_{VH}(t) \qquad (6)$$

of the slab representing the CN.

The exact functional form for the variation of the diffusion coefficient with distance within the CN is difficult to determine experimentally. In the simulations presented here, the following functional form was assumed:

$$D(x) = D_o \exp[-4\beta(x/h_{CN} - x^2/h^2_{CN})] \qquad (7)$$

where β is a model parameter whose magnitude governs how rapidly the timolol diffusion coefficient decreases with distance from the surface of the lens. When β is zero, the current model is reduced to a homogeneous diffusion problem in a slab geometry with time-dependent boundary conditions. It should be noted that $D(x)$ is symmetric about the center-

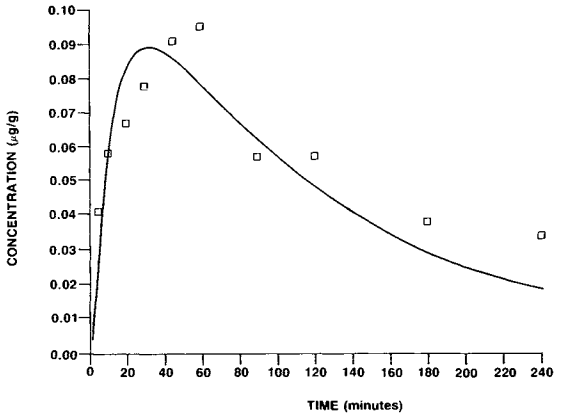

Fig. 6. Observed and model-simulated profiles for timolol in the lens cortex and nucleus.

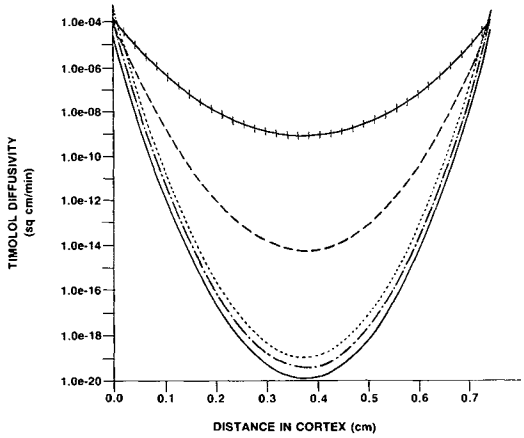

Fig. 7. Variation of timolol diffusivity in the lens cortex and nucleus as a function of D_o and β. (-++-) D_o, 3.97×10^{-5}; β, 35.7; (- - - -) D_o, 1.19×10^{-4}; β, 35.7; (· · · · ·) D_o, 3.57×10^{-4}; β, 35.7; (— · — ·) D_o, 1.19×10^{-4}; β, 23.8; (——) D_o, 1.19×10^{-4}; β, 11.9.

line of the slab geometry, which is consistent with the lens anatomy.

The average drug concentration in the cortex and nucleus at time t is given by

$$C_{CN,avg}(t) = \frac{1}{h_{CN}} \int_0^{h_{CN}} C_{CN}(x,t)dx \qquad (8)$$

Equations (3)–(8) were normalized and solved numerically applying the method of finite differences (21). Parameter values of 0.75 cm for the thickness (h) of the CN, 2.0×10^{-6} cm^2/sec for D_o, and 35.7 for β resulted in the best (least-squares) approximation to the experimental data. The good agreement observed between the model-simulated and the experimental data (Fig. 6) indicates that the model adequately accounted for both the rapid uptake of drug by the outer cortical layers and the slower penetration of drug into the interior of the lens.

DISCUSSION

There are numerous reports implying that lens pharmacokinetics are unique or complex and certainly not well appreciated vis-à-vis ocular therapy (9,13,18,20). The desire to investigate the lens uptake and distribution of timolol in the present study evolved out of a need to explain how the lens influenced the intraocular distribution of drugs within the eye. The current model was developed after the recognition that the stratified structure of cortex plays an important role in the distribution of drugs within the lens as well as in the transport of drugs into other ocular tissues.

The model presented here successfully describes the timolol concentration profile in the lens in a manner consistent with the overall physiology of the crystalline lens. As in the case of fluorescein (9), it is shown that the distribution of timolol in the lens is nonhomogeneous and that the diffusion is highly anisotropic. Therefore, this feature appears to be dictated largely by the structure of the lens more so than the nature of the chemical entity. In addition, it is shown that due to the higher drug concentrations, the capsule and epithelium represent the major component of driving force for drug movement into the interior of the lens while the vitreous contributed very little in this respect. Whether this observation holds true for other drugs (e.g., more polar or charged compounds which may not readily partition into the capsule and epithelium) remains to be tested.

Furthermore, the model allows a quantitative reconciliation of the kinetics of timolol distribution into the lens, provides some insight into the delivery of drugs into the lens, and demonstrates how the lens may affect intraocular drug disposition. Also, since the current model is based on phys-

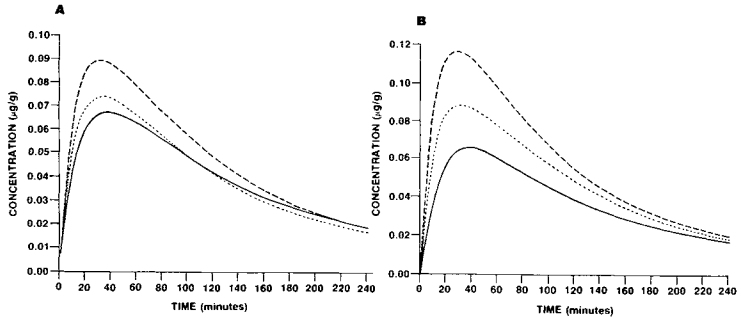

Fig. 8. Simulated timolol levels in the lens cortex and nucleus for selected values of D_o and β. A: (- - - - -) D_o, 1.19×10^{-4}; β, 11.9; (· · · · ·) D_o, 1.19×10^{-4}; β, 23.8; (——) D_o, 1.19×10^{-4}; β, 35.7; B: (- - - - -) D_o, 3.97×10^{-5}; β, 35.7; (· · · · ·) D_o, 1.19×10^{-4}; β, 35.7; (——) D_o, 3.57×10^{-4}; β, 35.7.

icochemical principles, these simulations may allow the establishment of design procedures for the delivery of drugs specifically targeted to the lens. However, before model-based conclusions can be accepted, the sensitivity of the predicted results to perturbations in the model parameters must be determined. This is best achieved by analyzing simulations with parameter values over realistic ranges. A sensitivity analysis is especially important in the case of those parameters which are difficult to estimate experimentally (e.g., D_o and β of the present model).

Figure 7 shows the variation of timolol diffusivity in the lens as a function of distance within the cortex for selected values of D_o and β. The rapid drop in the magnitude of the diffusion coefficient with distance from the surface of the lens reflects the increased resistance afforded by the dense packing of the inner fibers. In fact, for most practical purposes, the value of the diffusion coefficient is effectively zero within a relatively short distance from the surface of the lens, and the innermost layers of the cortex are virtually impenetrable. However, a diffusing substance can traverse the lens along the peripheral fibers of the cortex (9), which is a path that offers less resistance to drug transport.

The rapidity with which the diffusion coefficient decreases with distance within the lens is governed by the magnitude of β. Higher values of β result in the most rapid decrease in diffusion coefficient and indicate a greater degree of structure-related nonhomogeneity in the lens. On the other hand, D_o is the diffusion coefficient in the outmost cortical layers and its value determines the maximum drug transport rate at the surface of the lens. The magnitude of D_o is bounded and cannot exceed the diffusion coefficient of timolol in water, which is estimated to be 8.34×10^{-6} cm^2/sec (13).

Figure 8A and B show simulated timolol levels for the selected, arbitrary values of D_o and β. For a constant value of β (35.7), the effect of increasing D_o is to increase the average concentrations in the CN corresponding to a greater ease of drug penetration at the outer surfaces of the lens. On the other hand, simulated results obtained as a function of β while maintaining a constant D_o show a more complex relationship. At early times, the average concentration increases with β since the drug cannot readily penetrate into the dense nucleus. However, at later times, after the concentration gradient for drug uptake into the CN is reduced, drug which has previously penetrated into the dense structures tends to remain within those dense structures, resulting in average concentrations that are higher as a function of β.

This phenomenon is further illustrated in Figs. 9A and B, which show the extent of penetration of timolol into the cortex and nucleus of the lens as a function of distance and time for two different values of β. The notable features are as follows: (a) there is a rapid drop in drug concentration as a function of distance from the outer surface reflecting increased resistance to diffusion at the lens center; (b) the concentration–distance profiles become flatter with time with peak levels nearer the center, reflecting a process of extremely slow drug penetration into the lens; and (c) drug penetrates only into the peripheral cortical layers, without reaching a steady concentration throughout the lens. It is also apparent that if the lens is treated as highly nonhomogeneous (high β), the depth of drug penetration is lower compared to the case in which the lens is treated as being slightly nonhomogeneous (low β).

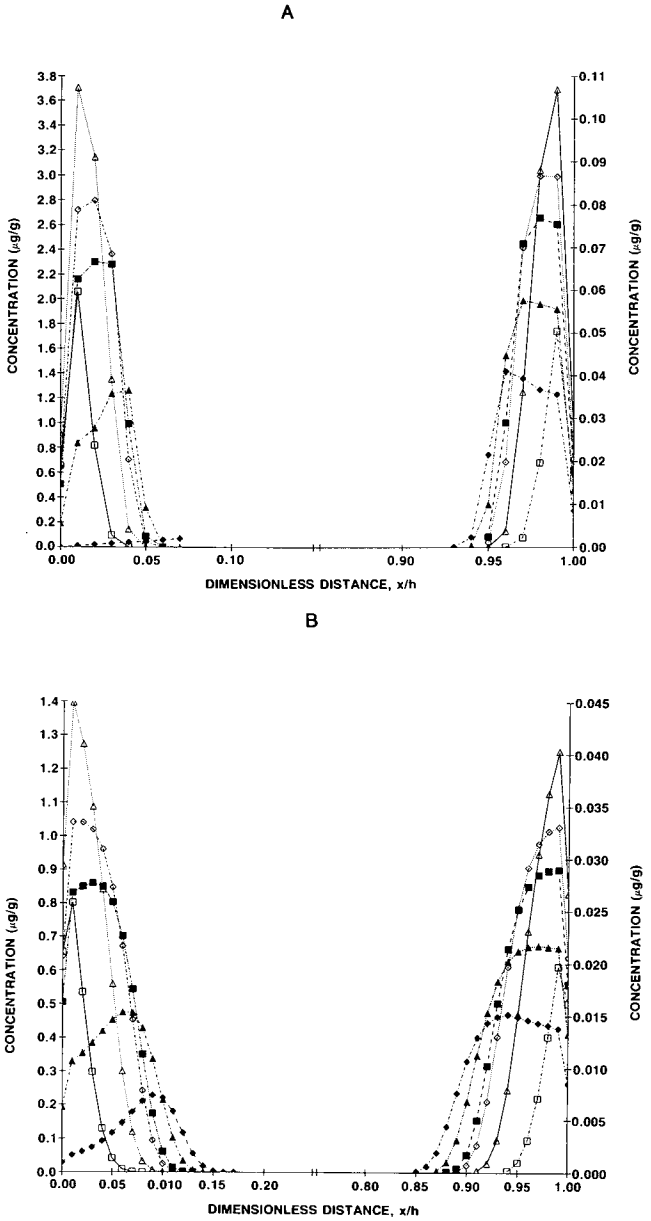

Fig. 9. Simulated timolol concentration profiles in the lens cortex and nucleus as a function of time and distance for selected values of D_o and β. (A) D_o, 1.19×10^{-4}; β, 35.7; (B) D_o, 1.19×10^{-4}; β, 11.9. □, 5 min; △, 20 min; ◇, 45 min; ■, 60 min; ▲, 120 min; ◆, 240 min.

The time shift for peak drug concentration as a function of distance traversed into the lens may partially explain why peak drug levels in the vitreous humor or deeper ocular tissues like the retina are often observed prior to peak drug levels in the lens. Specifically, due to the nonhomogeneous kinetic distribution of timolol within the lens as demonstrated by this study, the drug present in the vitreous humor probably is the result of material diffusing along the outer cortical edges of the lens. The time frame for such a process would be totally consistent (10–20 min) with the time observed for peak drug levels in the vitreous humor and retina (22). Similarly, the results may also, to some extent, explain

the basis for a noncorneal route of penetration proposed for topically applied drugs (23).

This study also emphasizes some salient points in relation to ocular drug delivery in general. First, for drugs targeted to the lens, the driving force must be continually present in order to achieve steady state and possibly efficacious levels of a pharmacologically active substance. Otherwise, the drug will preferentially diffuse around the outer layers of the lens without penetrating the center. The data suggest, in fact, that for drugs exhibiting rapid depletion from the aqueous and vitreous humor, high levels in the lens may never be achieved. This is important because if the concentration in the aqueous humor is reduced rapidly, the drug that is in the peripheral layers of the cortex will easily diffuse out of the lens. Therefore, if the lens or epithelia are the intended site of action, the dynamics of distribution could prevent the drug from reaching its target. Conversely, if drug concentration in the anterior chamber is maintained for a long period of time so as to promote drug penetration into the internal dense structures of the lens, drug will remain in the lens for a prolonged duration even to the extent that it may function as a reservoir. This may be feasible by employing rate-controlled ocular drug delivery systems (24). An alternate but less desirable method would be to load the lens by very frequent pulse dosing. Finally, if the target site is some ocular tissue other than the lens, it should be obvious that the dosing strategy would have a profound effect on the short- and long-term drug levels.

In conclusion, the lens plays an important role in determining the temporal and spatial patterns of drug disposition in the eye. Ocular delivery of drugs requires a thorough understanding of the underlying mechanisms for drug uptake and distribution in the lens and presents a formidable challenge to the formulation and fabrication of effective drug delivery systems.

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