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Disposition of timolol and inulin in the rabbit eye following corneal versus non-corneal absorption

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Summary

This study describes an investigation of the corneal versus non-corneal penetration routes of topically applied drugs in the eye. The time course of ocular distribution and disposition of topically applied timolol and inulin following corneal and non-corneal absorption was investigated. It was shown that non-corneal absorption may contribute significantly to drug penetration into intraocular tissues. Such 'productive' non-corneal drug penetration involves drug permeation across the conjunctiva and the underlying sclera. Drug absorbed by this route essentially bypassed the anterior chamber and distributed primarily in the uveal tract and vitreous humor. Non-corneal absorption may be important for drugs that are poorly absorbed across the cornea. This was demonstrated using inulin as a model of a large molecular weight compound with poor corneal permeability. Overall, 40% of the absorbed amount of inulin in the eye, and even larger proportions in selected ocular tissues could be attributed to non-corneal penetration. These studies suggest that anterior chamber levels may not be an appropriate measure of the ocular bioavailability for all drug delivery systems. The primary factor limiting the extent of non-corneal penetration of drugs into the eye appears to be the concurrent systemic loss of drug via the ocular vasculature.

Introduction

The development of ocular drug delivery systems has been hindered by the inability of most topically applied drugs to effectively permeate across the corneal membrane (Benson, 1974; Shell, 1982). This fact notwithstanding, almost all efforts in ophthalmic drug delivery have been aimed at optimizing corneal absorption, since the cornea has traditionally been regarded as the primary site for the intraocular entry of topically applied drugs (Doane et al., 1978; Brian et al., 1974; Maurice and Mishima, 1984). However, most of the currently available approaches do not lend themselves to the transcorneal delivery of high molecular weight polar compounds, such as many polypeptides and antibiotics (Beason, 1978; Maurice and Mishima, 1984; Stratford et al., 1983) and drugs that are inactivated by enzymes in the cornea (Ellis et al., 1972; Lee et al., 1982).

Although the ability of the conjunctiva to serve as a potential site for intraocular absorption has been noted (Lee and Robinson, 1979; Thombre

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and Himmelstein, 1984), few studies have been aimed at quantitatively examining non-corneal absorption as a delivery route for topically applied drugs. Drug absorption across the conjunctiva and sclera has been primarily regarded as non-productive, based on the assumption that drug entering these tissues is rapidly picked up by the local vasculature and does not contribute to intraocular drug levels (Patton and Robinson, 1976; Doane et al., 1978). It has often been cited that anterior chamber drug levels, which generally serve as the sampling compartment for measuring intraocular drug bioavailability, fall precipitously when drug access to the cornea is restricted (Doane et al., 1978; Brian et al., 1974). However, it should be noted that drug concentration in the aqueous humor does not always reflect that in the surrounding intraocular tissues (Miller et al., 1981). Furthermore, it has been suggested that drug entry into the eye via a non-corneal route may be one of the causes for such an observation (Francoeur et al., 1985).

An earlier report from this laboratory (Ahmed and Patton, 1985) indicated the existence of a non-corneal route of entry for topically applied timolol and inulin into the rabbit eye. Using an in vivo mechanical blocking technique that allowed restricted drug access to the cornea and the conjunctiva, it was demonstrated that the non-corneal route involved direct penetration of drug across the conjunctiva and sclera, bypassing the anterior chamber. In terms of topical ophthalmic drug delivery, the non-corneal absorption route may be important for drugs that are poorly absorbed across the cornea due to their physical–chemical properties.

The present study was designed to quantitatively evaluate the time course of ocular distribution and disposition of topically applied timolol and inulin following corneal and non-corneal absorption.

Materials and Methods

Materials

The levoisomer of timolol (carbon-14 labeled and unlabeled) was provided by Interx Research Corp. (Lawrence, KS) as its maleate salt. The liquid scintillation fluors Econofluor and Aquasol II, along with the tissue solubilizer Protosol, were purchased from New England Nuclear (Boston, MA). Unlabeled inulin was obtained from Sigma Chemical Co. (St. Louis, MO), and tritium-labeled inulin was obtained from the Amersham Corp. (Arlington Heights, IL). Xylazine (Rompun) was obtained from Bayvet Laboratories (Shawnee, KS), and ketamine hydrochloride was purchased from Parke Davis (Ann Arbor, MI). Sodium pentobarbital was obtained from Abbott Laboratories (Chicago, IL). All other chemicals used were analytical or reagent grade. The water used throughout these studies was deionized and charcoal filtered. Male, New Zealand rabbits were purchased from Small Stock Industries (Pea Ridge, AK), and housed in standard laboratory cages with no restrictions placed on food or water. At the time of experimentation, their ages ranged from 55 to 65 days.

Methods

(1) Preparation of dosing solutions

Stock solutions. The ^{14}C-labeled timolol maleate (spec. act. 110 μCi/mg) was stored until needed at $-20\,^{\circ}$C as received. The timolol molecule was labeled on both carbon atoms of the thiadiazole ring. The tritium-labeled inulin was received as a lyophilized powder (spec. act. 199 μCi/mg). The powder was dispersed in a 50/50 ethanol–water mixture and stored at $-20\,^{\circ}$C until needed.

Dosing solution. The timolol solutions used in these studies were prepared fresh for each experiment and discarded after use. An aliquot of the stock solution was combined with 500 μl of 95% ethanol in a microevaporation flask. After gentle mixing, the excess ethanol was evaporated under a stream of nitrogen. 'Cold' timolol maleate solution was prepared by dissolving unlabeled drug in a volume of isotonic Sorensen's phosphate buffer to give a final concentration of 0.65% (w/v) when added to the labeled drug. The final pH of the preparation was adjusted to 7.0. The dosing solution had an activity of 50 μCi/ml.

Labeled inulin solutions were prepared simi-

larly. However, due to the lower counting efficiency of the tritium label, a higher radioactivity of 150 μCi/ml was used.

Label purity and lability. The purity of timolol was established by the manufacturer (> 98%), and was used without further purification.

The lability of the tritium label to solvent exchange in phosphate buffer (pH 7.0) was checked at room temperature and at 33° C. This was done by preparing a labeled drug solution (0.65%), and dividing it into several 50 μl aliquots. From one of the aliquots, four 10 μl samples were taken and transferred to separate scintillation vials containing 10 ml of Aquasol II. These samples represented time zero values. The remaining aliquots were either incubated in a water-bath maintained at 33° C, or kept at room temperature. At hourly intervals over an eight hour period, one of these aliquots was evaporated to dryness under a stream of nitrogen and diluted by adding 50 μl of 'cold' inulin solution. Four 10 μl samples from each aliquot were counted in a liquid scintillation counter for 10 min.

An investigation of the stability of the tritium label and the chemical identification of inulin was also performed. A 100 μl aliquot of inulin containing both labeled (100 μCi) and unlabeled inulin (10% w/v) was incubated at 33° C for 8 h. At hourly intervals, 5 μl were spotted on a TLC plate (Avicel Cellulose, 250 μm, Analtech Inc.) and developed in a pre-equilibrated chamber using a *n*-BuOH : EtOH : H_2O (33 : 20 : 47) mobile phase. Five percent phenol–sulfuric acid in ethanol was used for visualization. First, the area of the spot, and then the rest of the coating material were scraped off into a liquid scintillation vial prefilled with 10 ml of Aquasol II and counted in a liquid scintillation spectrometer. In a separate experiment, a rabbit was anesthetized and 200 μl of the drug solution was placed in a reservoir over the cornea. Twenty minutes post-instillation, the animal was killed with an overdose of sodium pentobarbital, and 150 μl of aqueous humor were aspirated by limbal puncture. Ten 5 μl aqueous humor samples were directly spotted on TLC plates and developed as described above.

(2) In situ determination of the concentration vs time profile of timolol and inulin in the aqueous humor, cornea, lens, vitreous humor, sclera, conjunctiva, choroid-retina and iris-ciliary body

Animals were anesthetized with an intramuscular injection of ketamine (35 mg/kg) and xylazine (5 mg/kg). A 5 mm plug made out of polyethylene tubing (PE 50) was inserted into the puncta of each eye, and the globe of the eye was proptosed. A hollow, glass cylinder (o.d. 16 mm, i.d. 14 mm and 0.5 cm in length) was glued along the corneoscleral junction of one eye with a cyanoacrylate adhesive (Superglue). The diameter of the cylinder was sufficient, in all cases, to cover the entire corneal surface and overlapped slightly onto the conjunctiva ($\leq$ 1 mm). The eye was then returned to the socket. The other eye was left undisturbed. During the development of this procedure, the integrity of the glue-seal and the conjunctival membrane was repeatedly tested with dye solutions (fluorescein and Coomassie brilliant blue) placed both inside and outside the cylinder. It was determined that during the time course of these studies, no leakage through the seal occurred, nor was the conjunctival surface damaged. It was also verified that the puncta was completely blocked and no tear drainage was occurring, using the procedure described by Patton and Robinson (1976).

The rabbits were placed in plastic restraining boxes in their normal, upright position. Twenty-five μl of the drug solution were instilled under the upper lid of each eye with a Hamilton microsyringe. The eyelids were manipulated to ensure that the instilled drop spread inside the conjunctival sac.

Separate experiments were performed using the same protocol where the rabbits were either dosed in one eye (cornea open or blocked) and the tissue levels measured in both the dosed and undosed eye post-instillation, or where the animals were administered the entire dose intravenously and the intraocular tissue concentrations were measured. Studies were also conducted where drug levels in ocular tissues were measured in rabbits that were sacrificed and then dosed topically with the drug solution, both with and without corneal access.

(3) Collection of ocular tissues

At selected times post-instillation, the rabbits were killed with an overdose of sodium pentobarbital. Immediately following the death of the animal, the precorneal area was thoroughly rinsed with normal saline and gently blotted to remove excess fluid. Approximately 150 μl of aqueous humor were aspirated following limbal puncture. The iris-ciliary body, lens, cornea, the entire vitreous humor, bulbar conjunctiva and the sclera in toto were subsequently removed from each eye. The entire procedure took less than 3 min per eye. The iris-ciliary body, cornea, lens, conjunctiva and sclera were rinsed with 1 ml of saline, and gently blotted with Kimwipes to remove residual fluid. Tissue standards were prepared by spiking two of each tissue type with 25 μl of the drug solution. After obtaining their wet weights, the tissues were digested in Protosol. Scintillation fluors (Aquasol II for the aqueous humor and Econofluor for the tissues) were added and the samples stored overnight prior to counting in a liquid scintillation spectrometer (Beckman LS 7000). Values were converted to micrograms of drug based on total radioactivity and were normalized for the tissue weight.

(4) A check of possible surface and cross-contamination

To eliminate the possibility of ocular tissues coming in contact with the 'hot' eyelids during dissection, the entire eye was enucleated, thoroughly rinsed with normal saline, blotted and frozen in a dry-ice–acetone bath. Dissection was then performed on the frozen eyeball as described by Abel and Boyle (1976). This procedure allowed obtaining tissue samples, including the vitreous humor, as frozen units, thus minimizing tissue to tissue transfer of counts. The surgical instruments were wiped and rinsed between each surgery, and the rinses were counted for residual radioactivity.

Results

Technique validation

It was assessed experimentally that the tritium label on inulin was stable to solvent exchange. Furthermore, upon TLC analysis, it was observed that in excess of 90% of the ^{3}H-activity, both in the aqueous humor and in the labeled test solutions, was associated with intact inulin. Thus, it could be concluded that there was very little metabolism of inulin in the eye. The lack of significant metabolism of timolol in the eye has been confirmed by other investigators (Schmitt et al., 1980). The tracers, therefore, adequately reflected the amount of intact drug in the ocular tissues.

Timolol levels in various ocular tissues obtained by different dissection procedures are shown in Table 1. Based on results of the *F*-test, the observed drug levels in ocular tissues were independent of the dissection procedure. Furthermore, rinses of the surgical instruments taken at various times during and between dissections showed minimal residual counts. These results

TABLE 1

Comparison of timolol maleate levels [a] in ocular tissues following topical instillation measured using 3 different dissection procedures: eye in socket during surgery; entire eye enucleated prior to dissection; and entire eye enucleated and frozen prior to dissection

	Eye in socket	Eye – enucleated	Eye – enucleated and frozen
Aqueous humor	2.08 ± 0.493 (6) [b]	1.95 ± 0.091 (6)	1.83 ± 0.443 (4)
Cornea	34.0 ± 4.76 (6)	24.5 ± 1.47 (6) [c]	39.2 ± 9.11 (4) [d]
Lens	0.11 ± 0.024 (6)	0.12 ± 0.026 (6)	0.15 ± 0.027 (4)
Vitreous humor	0.01 ± 0.001 (6)	0.01 ± 0.002 (6)	0.01 ± 0.001 (4)
Iris-ciliary body	2.16 ± 0.297 (6)	2.34 ± 0.236 (6)	2.63 ± 0.275 (4)

[a] Twenty-five μl of a 0.65% timolol maleate solution, pH 7.0. The animals were anesthetized and their tear drainage duct was open.
[b] Mean ± S.E.M.; in parentheses is the number of determinations at 20 min post-instillation.
[c,d] These are the only values that are statistically different at the 95% confidence level.

support the fact that the radioactivity measured in the ocular tissues, in these studies, was not an artifact of the experimental procedure.

In vivo studies

The in vivo concentration vs time profiles for timolol in the rabbit aqueous humor, cornea, lens, vitreous humor, iris-ciliary body, sclera, conjunctiva and the choroid-retina tissues are shown for both when corneal contact was excluded (Fig. 1), and when drug access was allowed to the entire precorneal area (Fig. 2). These concentrations were measured after topical instillation of 25 μl of a 0.65% timolol maleate solution (pH 7.0) in

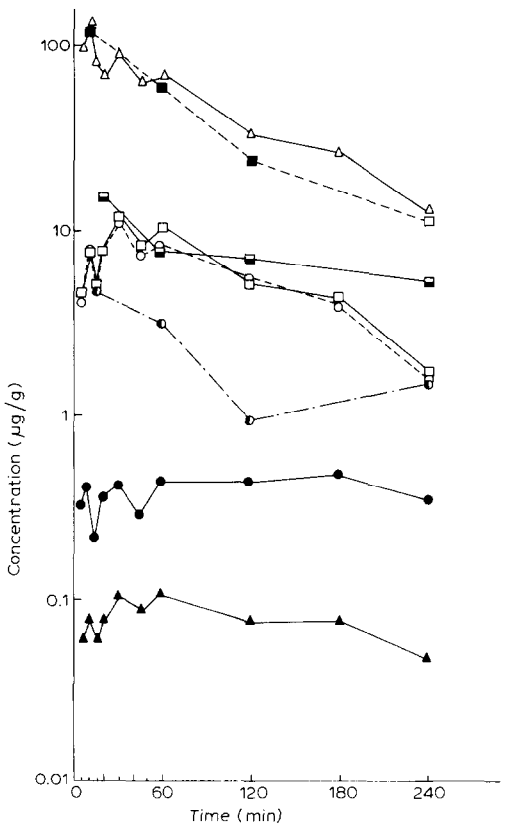

Fig. 1. Concentration vs time profiles for timolol following instillation of 25 μl of a 0.65% (w/v) timolol maleate solution in the eyes of 60-day-old anesthetized rabbits with blocked puncta and allowing for drug access to the entire precorneal area. △, cornea; ■, conjunctiva; ⬓, sclera; ◐, choroid-retina; □, iris-ciliary body; ○, aqueous humor; ●, lens; ▲, vitreous humor.

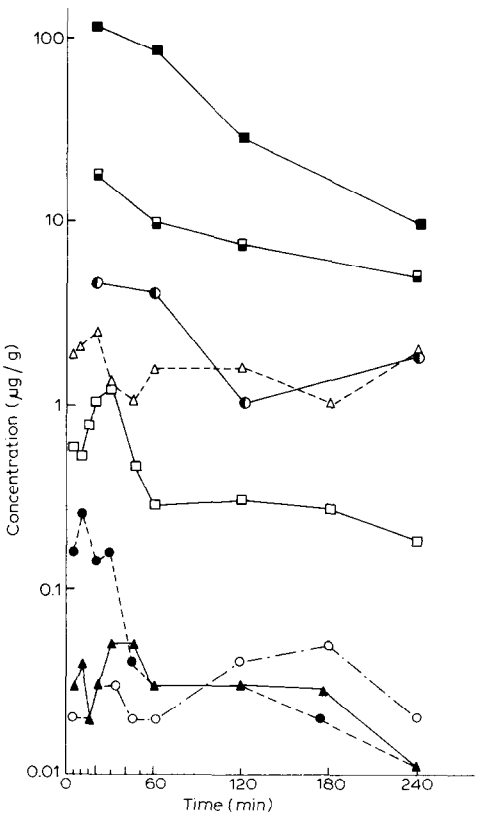

Fig. 2. Concentration vs time profiles for timolol following instillation of 25 μl of a 0.65% (w/v) timolol maleate solution in the eyes of 60-day-old anesthetized rabbits with blocked puncta and allowing for drug access only to the conjunctiva (i.e. blocking corneal access). △, cornea; ■, conjunctiva; ⊟, sclera; ○, choroid-retina; □, iris-ciliary body; ○, aqueous humor; ●, lens; ▲, vitreous humor.

anesthetized rabbits whose drainage ducts had been blocked. The concentrations at each time point represent the average of between 5 and 27 separate determinations. The coefficient of variation was $< 15\%$ in all cases.

In the case where corneal access was permitted, the highest timolol levels were observed in the cornea and the conjunctiva, followed by the sclera, iris-ciliary body, aqueous humor, choroid-retina, lens and the vitreous humor, respectively. The profiles for timolol in the iris-ciliary body and aqueous humor were superimposable, indicating that these two tissues behaved as a single compartment kinetically. Upon visual inspection, the

aqueous humor, lens, vitreous humor and iris-ciliary body all appeared to reach their maximum concentration approximately 30 min post-instillation. The peak concentration in the cornea occurred somewhat earlier, at about 10 min. The overall shape and rank-order of the concentration–time profiles were in agreement with those reported in unanesthetized rabbits with unobstructed drainage ducts (Francoeur et al., 1985). Therefore, the reduction in tear production due to anesthesia and obstruction of tear removal in these animals did not alter the distribution of the drug in the eye, although the actual concentrations were higher as expected.

When corneal access was blocked, the aqueous humor and corneal drug levels dropped to only a small fraction of those observed with corneal access. However, the conjunctival, scleral and choroid-retina levels remained essentially unchanged. The pattern of drug distribution in the eye also changed drastically when corneal drug contact was excluded. With restricted corneal access, drug levels in the iris-ciliary body were 10–50 times higher than those in the aqueous humor. The overall rank-order in terms of concentration in the absence of corneal access was: conjunctiva > sclera > choroid-retina > cornea > iris-ciliary body > lens > vitreous humor > aqueous humor. The maximum concentration in the lens, vitreous humor and iris-ciliary body was attained within 10–30 min post-instillation.

Based on a comparison of the maximum concentrations (Table 2), the non-corneal contributions to drug levels in the lens, vitreous humor and iris-ciliary body were 53%, 45% and 11% of that observed when corneal access was permitted. Using the areas under the concentration vs time profiles (0–240 min), these estimates were more moderate; 11%, 33% and 6%, respectively.

Similar in vivo studies were conducted using inulin as a test substance. Figs. 3 and 4 show the concentration vs time profiles for inulin in the aqueous humor, cornea, vitreous humor, iris-ciliary body, conjunctiva, sclera and choroid-retina in the case of drug contact with the entire precorneal area, and when drug access to the cornea was restricted. The dose and experimental conditions were identical to those described for timolol. Although the actual concentrations of inulin in ocular tissues were lower than those observed for timolol, the intraocular distribution of the two drugs was similar. Inulin levels were too low to detect in the lens. As with timolol, the aqueous humor levels of inulin diminished in the absence of corneal drug access. Table 3 lists values for some pharmacokinetic parameters for inulin calculated based on the observed drug levels in ocular tissues in the presence and absence of corneal drug access. Based on a comparison of the maximum concentrations, the non-corneal contributions to inulin levels in the iris-ciliary body and vitreous humor were 70–80% of those observed when corneal access was allowed. Statistically, these values did not differ significantly based on a

TABLE 2

Values of some observed pharmacokinetic parameters for timolol maleate in various ocular tissues after topical instillation when the drug solution can access the entire precorneal area versus the conjunctival surface only, in anesthetized rabbits with blocked tear drainage duct

	With corneal access			Without corneal access		
	T_{max} (min)	C_{max} (μg/g)	AUC_{0-240}	T_{max} (min)	C_{max} (μg/g)	AUC_{0-240}
Aqueous humor	30	11.5 ± 1.93 (5) [a]	1340	180	0.05 ± 0.014 (8)	7.6
Cornea	10	121 ± 10.1 (10)	10,927	20	2.61 ± 0.498 (7)	357
Lens	30	0.49 ± 0.100 (5)	101	10	0.26 ± 0.139 (6)	11
Vitreous humor	30	0.11 ± 0.006 (5)	20	30	0.05 ± 0.012 (8)	6.5
Iris-ciliary body	30	11.6 ± 0.60 (5)	1480	30	1.23 ± 0.039 (8)	90
Conjunctiva	20	116 ± 14.8 (8)	9654	20	108 ± 37.7 (8)	10,400
Sclera	20	15.5 ± 2.45 (8)	1981	20	18.8 ± 4.36 (8)	2017
Choroid-retina	20	5.01 ± 0.86 (4)	493	20	4.66 ± 0.95 (4)	548

[a] Mean ± S.E.M. The numbers in parentheses are the number of determinations.

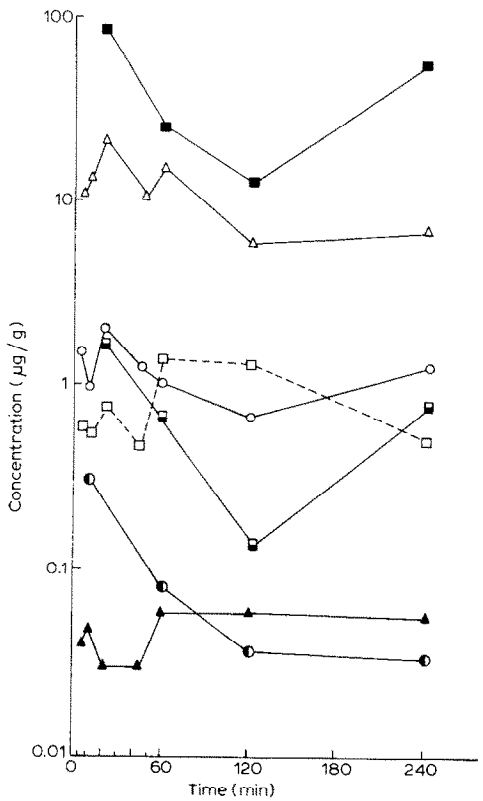

Fig. 3. Concentration vs time profiles for inulin following instillation of 25 μl of a 0.65% (w/v) inulin solution in the eyes of 60-day-old anesthetized rabbits with blocked puncta and allowing for drug access to the entire precorneal area. △, cornea; ■, conjunctiva; ◪, sclera; ◐, choroid-retina; □, iris-ciliary body; ○, aqueous humor; ▲, vitreous humor.

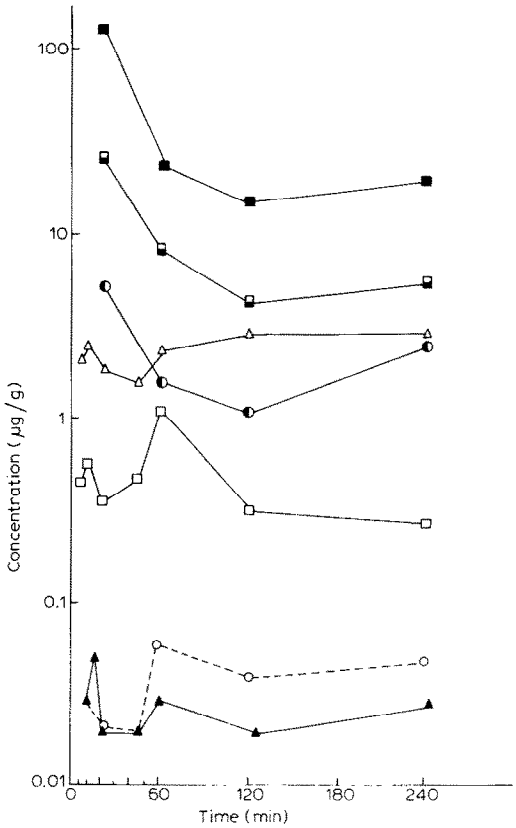

Fig. 4. Concentration vs time profiles for inulin following instillation of 25 μl of a 0.65% (w/v) inulin solution in the eyes of 60-day-old anesthetized rabbits with blocked puncta and allowing for drug access only to the conjunctiva (i.e. blocking corneal access). △, cornea; ■, conjunctiva; ◪, sclera; ◐, choroid-retina; ■, iris-ciliary body; ○, aqueous humor; ▲, vitreous humor.

two-tailed t-test ($P < 0.05$). Although more timolol than inulin was absorbed into the eye, the non-corneal contributions to intraocular levels for inulin were substantially higher than for timolol on a percentage basis, indicating a larger fraction of inulin entering ocular tissues via the non-corneal route. For both timolol and inulin, drug levels in the conjunctiva, sclera and the choroid-retina remained unchanged when drug access to the cornea was restricted.

In an effort to simulate a more realistic precorneal environment, and to assess the effect of tear drainage on the non-corneal contribution to drug levels in intraocular tissues, timolol and inulin levels were measured in anesthetized rabbits with unobstructed puncta. As expected, the drug concentrations in all tissues sampled, both in the presence and absence of corneal access, were lower when tear drainage was permitted. However, the relative non-corneal contributions to intraocular tissue levels remained essentially unchanged (Tables 4 and 5).

A substantial fraction of drug crossing the conjunctiva may be absorbed systemically (Urtti et al., 1985; Patton and Francoeur, 1978) and subsequently re-enter the eye from the general circulation (Maurice and Mishima, 1984). The contribution of systemic return to ocular drug levels was estimated by measuring drug levels in the contralateral eye after topical dosing. Likewise, the

TABLE 3

Values of some observed pharmacokinetic parameters for inulin in various ocular tissues after topical instillation when the drug solution can access the entire precorneal area versus the conjunctival surfaces only, in anesthetized rabbits with blocked tear drainage duct

	With corneal access			Without corneal access		
	T_{max} (min)	C_{max} (μg/g)	AUC_{0-240}	T_{max} (min)	C_{max} (μg/g)	AUC_{0-240}
Aqueous humor	20	2.10 ± 0.42 (9) [a]	260	60	0.06 ± 0.007 (8)	10
Cornea	20	22.8 ± 4.00 (9)	2340	240	3.17 ± 1.63 (6)	650
Lens		N.D. [b]			N.D.	
Vitreous humor	60	0.06 ± 0.009 (8)	12.4	10	0.05 ± 0.018 (6)	6.2
Iris-ciliary body	60	1.38 ± 0.40 (8)	230	60	1.13 ± 0.16 (8)	110
Conjunctiva	20	84 ± 26.9 (6)	8676	20	138 ± 27.2 (6)	7695
Sclera	20	16.4 ± 2.29 (6)	1437	20	26.5 ± 4.05 (6)	1950
Choroid-retinal tissue	20	3.01 ± 0.42 (6)	385	20	5.18 ± 1.00 (6)	451

[a] Mean ± S.E.M. The numbers in parentheses are the number of determinations.
[b] N.D. = not detectable.

TABLE 4

Values of some observed pharmacokinetic parameters for timolol maleate in various ocular tissues after topical instillation when the drug solution can access the entire precorneal area versus the conjunctival surface only, in anesthetized rabbits with unblocked tear drainage duct

	With access to entire precorneal area			With access to conjunctival surface only		
	T_{max} (min)	C_{max} (μg/g)	AUC_{0-240}	T_{max} (min)	C_{max} (μg/g)	AUC_{0-240}
Aqueous humor	20	6.93 ± 1.59 (8) [a]	346	120	0.06 ± 0.049 (8)	9
Cornea	20	39.1 ± 7.74 (8)	2780	20	0.51 ± 0.15 (8)	78
Lens	20	0.17 ± 0.041 (8)	28	120	0.04 ± 0.011 (8)	6.5
Vitreous humor	20	0.06 ± 0.012 (8)	5.7	20	0.02 ± 0.003 (8)	3.1
Iris-ciliary body	20	5.08 ± 0.51 (8)	258	20	0.40 ± 0.043 (8)	44

[a] Mean ± S.E.M. The numbers in parentheses are the number of determinations.

TABLE 5

Values of some observed pharmacokinetic parameters for inulin in various ocular tissues after topical instillation when the drug solution was allowed access to the entire precorneal area versus the conjunctival surface only, in anesthetized rabbits with unobstructed tear drainage ducts

	With access to entire precorneal area			With access to conjunctival surface only		
	T_{max} (min)	C_{max} (μg/g)	AUC_{0-240}	T_{max} (min)	C_{max} (μg/g)	$AUC_{0\rightarrow240}$
Aqueous humor	20	0.79 ± 0.10 (10) [a]	69	120	0.03 ± 0.003 (4)	5
Cornea	20	5.84 ± 0.68 (10)	446	20	0.47 ± 0.14 (10)	35
Vitreous humor	20	0.012 ± 0.003 (10)	1.4	60	0.015 ± 0.002 (4)	2.92
Iris-ciliary body	20	0.250 ± 0.080 (10)	33	60	0.260 ± 0.08 (4)	48

[a] Mean ± S.E.M. The numbers in parentheses are the number of determinations.

maximum contribution to intraocular levels expected via such a re-entry route was estimated by injecting the entire dose intravenously.

Timolol and inulin levels in the aqueous humor, cornea, lens, vitreous humor and iris-ciliary body at twenty minutes following the topical instillation of 25 μl of a 0.65% drug solution in the dosed eye (with restricted corneal access), contralateral (un-

TABLE 6

In vivo ocular levels of timolol maleate in the dosed (with restricted corneal access) and contralateral eye 20 min following topical instillation, and ocular levels following the systemic i.v. administration of 162.5 μg of timolol maleate in the rabbit

	Concentration (μg/g)		
	Dosed eye	Contralateral eye	i.v. administration
Aqueous humor	0.01 ± 0.004 (4) [a]	0.01 ± 0.001 (4)	0.01 ± 0.003 (10)
Cornea	1.55 ± 0.700 (4)	0.01 ± 0.010 (4)	0.02 ± 0.009 (10)
Lens	0.12 ± 0.011 (4)	0.001 ± 0.001 (4)	0.006 ± 0.001 (6)
Vitreous humor	0.03 ± 0.001 (4)	0.002 ± 0.001 (4)	0.009 ± 0.0007 (10)
Iris-ciliary body	1.25 ± 0.102 (4)	0.05 ± 0.009 (4)	0.06 ± 0.014 (10)

[a] Mean ± S.E.M. Sample size given in parentheses.

TABLE 7

The comparison of inulin levels in various ocular tissues and fluids in the dosed and the contralateral eye 20 min after topical instillation of 25 μl of a 0.65% inulin solution, pH 7.0

Tissue	Concentration (μg/g) [b]	
	Dosed eye	Contralateral eye
Aqueous humor	1.43 ± 0.288 (4) [a]	N.D.
Cornea	0.98 ± 0.255 (4)	N.D.
Lens	N.D.	N.D.
Vitreous humor	0.07 ± 0.018 (4)	N.D.
Iris-ciliary body	1.89 ± 0.389 (4)	0.015 ± 0.025 (4)

[a] Mean ± S.E.M. The numbers in parentheses refer to the sample size.

[b] The instilled solution contained 0.06 mCi/ml of ^{3}H-labeled inulin.

dosed eye), and the tissue levels of timolol following the intravenous administration of an equivalent dose are shown in Tables 6 and 7. This time point was selected since maximum drug concentrations in ocular tissues generally occur 20–30 min post-instillation. Results indicated that ocular levels of timolol and inulin due to systemic return were significantly lower than those observed following topical instillation in the absence of corneal access. This finding suggests that systemic return cannot be totally accountable for the tissue levels of the drugs. As expected, the extent of systemic return was less for inulin than for timolol due to its inability to effectively cross the blood–ocular barriers (Ross, 1951).

TABLE 8

The effect of local ocular blood circulation on the entry of timolol into the eye following topical instillation of 25 μl of a 0.65% timolol maleate solution, pH 7.0 – tissue levels 20 min after dosing

	Concentration (μg/g) [b]			
	Rabbits anesthetized (Intact circulation)		Rabbits dead (No circulation)	
	With corneal access	Without corneal access	With corneal access	Without corneal access
Aqueous humor	7.90 ± 0.61 (26) [a]	0.03 ± 0.005 (23)	9.40 ± 0.77 (7)	0.26 ± 0.17 (7)
Cornea	70 ± 5.66 (27)	2.6 ± 0.40 (27)	120 ± 12.7 (7)	2.3 ± 0.97 (7)
Lens	0.37 ± 0.16 (27)	0.14 ± 0.03 (27)	0.43 ± 0.07 (7)	0.09 ± 0.03 (7)
Vitreous humor	0.08 ± 0.006 (27)	0.03 ± 0.003 (27)	0.22 ± 0.036 (7)	0.21 ± 0.088 (7)
Iris-ciliary body	8.13 ± 0.63 (27)	1.03 ± 0.12 (27)	10.8 ± 1.40 (71)	1.31 ± 0.26 (7)

[a] Mean ± S.E.M. The numbers in parentheses refer to the sample size.

[b] The instilled solution contained 162.5 μg of timolol maleate per dose, with an activity of 0.05 mCi/ml.

An alternate explanation that may account for the observed drug levels in ocular tissues in the absence of corneal absorption may be by delivery via the ocular vasculature. Table 8 shows the results of a study where timolol was instilled in live animals (anesthetized, duct-blocked) and in dead animals (immediately after death, duct-blocked) either allowing for, or restricting drug access to the cornea. Observed results indicated that drug levels in the freshly killed animals were either comparable to, or significantly higher than the corresponding levels in live animals. This finding suggests that the ocular circulation does not serve as a delivery route for drugs to intraocular tissues, but may act as an exit route. Since these experiments were conducted within 30 min following the death of the animals, it is unlikely that the integrity of the corneal, conjunctival or scleral membranes was compromised in these studies.

Discussion

In order to systematically study the penetration routes of topically applied drugs, it is necessary to control drug contact with the eye. In this study, this was accomplished by means of a mechanical blocking technique wherein drug contact with the cornea was prevented in selected cases, and the resultant ocular tissue levels of drug were measured in the presence and absence of corneal access. The technique required the animals to be kept under general anesthesia for the duration of the experiment. An obvious concern was how the altered precorneal environment would affect the true in vivo representation of such a system. It has been shown that anesthesia reduces the extent of tear production and instilled solution drainage in rabbits (Chrai et al., 1973). Experiments were performed under two conditions: first, with the puncta blocked (mechanically) and then, with the puncta unblocked allowing for tear removal, albeit at a slower than normal rate. In the former case the drug solution was held in a reservoir inside the conjunctival sac, bathing either the entire precorneal area or the conjunctival surface exclusively. The advantage of this method was that it allowed examination of the parameters of major interest in this study, namely, corneal versus conjunctival and scleral (non-corneal) absorption, while eliminating parallel loss routes such as tear production and drainage. It also simulated a constant source of drug delivery as may be the case for some ocular insert devices such as the Ocusert (Shell, 1982). The latter case simulated a precorneal environment more representative of that expected after instillation of a conventional eye drop and also revealed how instilled solution removal would affect the extent and relative importance of corneal and non-corneal drug penetration into the eye.

Several possible pathways for the intraocular penetration of topically applied drugs are summarized in Fig. 5. Drug absorbed across the cornea primarily enters the anterior chamber and hence may distribute to surrounding ocular tissues, such as the lens and the iris-ciliary body (pathway 1). Conjunctival absorption may eventually lead to intraocular drug levels in several ways: (a) drug may be picked up by the conjunctival and episcleral blood vessels and delivered to other perfused intraocular tissues directly, or may re-enter the eye from the systemic circulation (pathway 3);

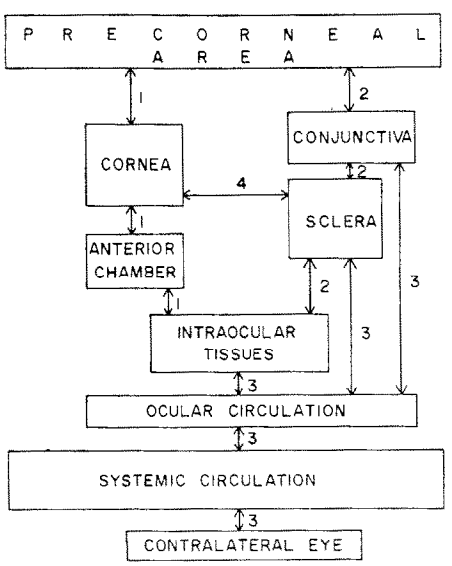

Fig. 5. Ocular penetration routes for topically applied drugs. 1 = transcorneal pathway; 2 = "productive" non-corneal pathway; 3 = systemic return pathway; 4 = lateral diffusion.

(b) drug absorbed by the conjunctiva may diffuse through the underlying sclera and enter intraocular tissue compartments (pathway 2); or (c) drug may diffuse laterally from the cojunctiva into the cornea, and hence, into the anterior chamber and other intraocular tissues (pathway 4).

Among the several possibilities that exist, whereby conjunctival absorption may lead to intraocular drug levels, it was shown that neither systemic return, nor direct delivery of drug to ocular tissues by the blood can account for the observed results. The corneal drug levels observed even when direct access to the cornea was prevented mechanically, suggested that drug may laterally diffuse into the cornea from the bulbar conjunctiva and the underlying sclera. However, the resultant aqueous humor levels were too low to account for the drug levels observed in the iris-ciliary body, which is generally thought to derive its drug from the aqueous humor. Therefore, by a process of elimination, it was concluded that productive non-corneal drug penetration involved drug absorption by the conjunctiva and diffusion through the underlying sclera. Drug penetrating the sclera has direct access to the base of the iris anteriorly, and the choroid and vitreous humor more posteriorly on the globe of the eye (Maurice and Mishima, 1984). Direct drug penetration into the eye is also supported by the rank-order in drug concentration measured in the various ocular tissues for timolol and inulin. The rank-order indicates that a concentration gradient exists for drug diffusion from the conjunctiva and sclera into the iris-ciliary body, choroid and the vitreous humor.

The total amount of drug absorbed into the eye by a particular route over a 4 h period was estimated from the corresponding sum of the area under the amount–time profile (AUC) in the individual ocular tissues. The AUC was calculated by multiplying the tissue concentration by the average tissue weight (Ahmed, 1985) and applying trapezoidal integration. For the purpose of directly comparing the extent of corneal versus non-corneal absorption, it was assumed that these two processes were mutually exclusive.

Considering the extent of drug penetration into the aqueous humor, lens, iris-ciliary body, vitreous humor, choroid and retina, a predominant fraction of drug absorbed through the cornea ($> 90\%$) was accounted for in the anterior chamber. In contrast, drug absorbed by the conjunctiva essentially bypassed the anterior chamber and distributed primarily in the uveal tract and the vitreous humor.

The amount of drug entering the eye by both routes together was almost 4 times higher for timolol than for inulin. However, the extent of intraocular penetration of timolol and inulin exclusively via the scleral route was similar, despite the higher permeability of timolol across the conjunctiva and sclera (Ahmed et al., 1985). This discrepancy can be explained by realizing that conjunctival absorption has associated with it both a non-productive and a productive component. Thus, a portion of the drug absorbed by the conjunctiva is removed by the vasculature, often causing the systemic side-effects that are associated with topically applied ophthalmic medications (Kumar et al., 1985; Schmitt et al., 1981). The extent of vascular clearance depends, at least in part, on the structure of the drug moiety (Landis and Pappenheimer, 1963). The high proportion of inulin entering the eye via conjunctival/scleral absorption may be attributed to the large molecular weight and high polarity of the compound, which results in its poor transcorneal permeability and low systemic loss (Keller et al., 1980; Stratford et al., 1985; Ahmed and Patton, 1985).

There are two salient conclusions that can be drawn from this study. First, non-corneal drug absorption, involving permeation of drug across the conjunctiva and sclera, may contribute significantly to drug penetration into intraocular tissues, and secondly, aqueous humor levels may not be an adequate measure of the effectiveness of some ocular drug delivery systems. The latter statement is particularly relevant considering that although aqueous humor levels are usually used as a measure of ocular bioavailability, for many ophthalmic drugs the intended site of action is not the anterior chamber but other intraocular tissue compartments (Maurice and Mishima, 1984). Concurrent systemic loss limits the usefulness of the conjunctiva as an intraocular entry site for many drugs. It seems likely that approaches that could

possibly reduce the extent of systemic loss, such as incorporating a vasoconstrictor in the topical formulation, may successfully increase non-corneal drug penetration into the eye. Further enhancement could be realized by fabricating drug delivery systems that maintain intimate contact and a high local drug concentration at the conjunctival surface. Liposomes have shown promise in this regard (Ahmed and Patton, 1986). In retrospect, it is likely that the non-corneal absorption route plays a more important role in the intraocular penetration of drugs injected subconjunctivally or from scleral implants, than has been realized based on an analysis of aqueous humor levels (Refojo, 1975; Conrad and Robinson, 1980; McCartney et al., 1965). The non-corneal absorption route may be particularly important for drugs that are poorly absorbed across the cornea due to their physical-chemical properties. This has been demonstrated using inulin as a model for a poorly absorbed, high molecular weight compound. There is further evidence that the distribution and activity of enzymes in the conjunctiva and sclera are markedly different from those in the cornea (Stratford and Lee, 1984). Therefore, another possible application of the non-corneal route may be for the intraocular delivery of drugs that are inactivated during their transcorneal transit.

Further studies aimed at identifying drug properties that may make this route significant; at ways of optimizing the non-corneal route by understanding the mechanism by which this occurs; and at potentially taking advantage of this route in the design and evaluation of ocular drug delivery systems may prove productive.

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