International Journal of Pharmaceutics, 61 (1990) 241–249
Elsevier

IJP 02073

Controlled drug delivery devices for experimental ocular studies with timolol
2. Ocular and systemic absorption in rabbits

Arto Urtti [1,*], James D. Pipkin [1,2], Gerald Rork [1,2], Toshiaki Sendo [1,**], Ulla Finne [3] and A.J. Repta [1,2]

[1] *Department of Pharmaceutical Chemistry, The University of Kansas, Lawrence, KS 66045 (U.S.A.),* [2] *INTERx Research Corp., Merck Sharp & Dohme Research Laboratories, Lawrence, KS 66047 (U.S.A.) and* [3] *Department of Pharmaceutical Technology, University of Kuopio, P.O. Box 6, 70211 Kuopio (Finland)*

(Received 20 June 1989)
(Modified version received 22 December 1989)
(Accepted 23 December 1989)

Key words: Timolol; Ocular absorption; Systemic absorption; Controlled drug release; Silicone tubing; Ocular drug delivery; Pharmacokinetics; Radioreceptor assay

Summary

Controlled drug delivery was tested as a means to decrease the potentially dangerous systemic drug concentrations which are associated with timolol eyedrop therapy. L-Timolol (125 μg) was administered in 0.5% eyedrops (25 μl, pH 6.86) and in controlled release silicone tubing devices (dose 57.6 μg; release rate 7.2 μg/h for 8 h) in the eyes of pigmented rabbits. [^{3}H]Timolol tracer was used in ocular absorption studies and unlabeled timolol dosage forms in systemic absorption studies. [^{3}H]Timolol concentrations were determined in ocular tissues and tear fluid. Beta blocking activity in plasma was determined using a radioreceptor assay. Comparable timolol concentrations were achieved in the iris-ciliary body with silicone tubing devices (57.6 μg) and with eyedrops (125 μg). The relative ocular timolol bioavailability after controlled drug delivery was about 2-fold greater than from eyedrops. In plasma, peak beta-blocking activity was much higher after eyedrop administration (17.16 ± 2.40 ng/ml) than during controlled timolol delivery (< 1.0 ng/ml). The results indicate that controlled drug delivery is a viable alternative in improving the therapeutic index of glaucoma therapy with timolol.

Introduction

Timolol eyedrops are the most commonly used drug treatment in open-angle glaucoma. As systemic absorption of timolol may cause respiratory and cardiovascular side-effects (Nelson et al., 1986), it is important to minimize the systemic absorption of timolol. Recently, Chang et al. (1987) demonstrated that by using lipophilic prodrugs of timolol it is possible to increase the corneal penetration and ocular bioavailability of timolol without increasing the systemic drug absorption in the same proportion.

Prolongation of ocular contact and controlled drug release in tear fluid were shown to increase ocular bioavailability of several drugs (for references see Shell, 1984). However, it is important

Correspondence: J.D. Pipkin, INTERx Research Corp., Merck Sharp & Dohme Research Labs, 2201 West 21st Street, Lawrence, KS 66047, U.S.A.

* *Present address:* Department of Pharmaceutical Technology, University of Kupio, P.O. Box 6, 70211 Kuopio, Finland.

** *Present address:* Department of Hospital Pharmacy, School of Medicine, Kyushu University Hospital, Fukuoka 812, Japan.

not only to maximize ocular drug absorption, but also to minimize the systemic concentrations of ocularly applied drugs. Previously, the systemic peak concentrations of pilocarpine were significantly decreased by administering the drug in a controlled release device instead of eyedrops (Urtti et al., 1985).

In this study, ocular and systemic absorption of timolol after controlled delivery was compared with eyedrop administration. Controlled ocular delivery of timolol was achieved using recently developed silicone tubing devices (Urtti et al., 1990). The specific controlled release system selected for this study (release rate 7.2 μg/h for 8 h) delivers about the same dose as delivered by a clinically effective 0.25% timolol eyedrop. The aim of this study was to evaluate the utility of these silicone tubing devices in investigating whether it is possible to improve the ratio between ocular and systemic timolol concentrations with controlled release drug delivery system.

Materials and Methods

Preparation of dosage forms

In studies of systemic timolol absorption radiolabeled dosage forms were not used. [^{3}H]Timolol labeled dosage forms were used when ocular absorption of timolol was studied.

Timolol eye drops were prepared by dissolving 5.0 mg/ml L-timolol maleate (Merck Sharp & Dohme Research Laboratories, Rahway, NJ) in phosphate buffer (NaH_2PO_4, 5.40 mg/ml; Na_2HPO_4, 12.05 mg/ml). pH was adjusted to 6.86. [*propanol*-3-^{3}H]Timolol (timolol-O-CHT, Merck, Sharp & Dohme; specific activity, 1.6 Ci/mmol; radiochemical purity, 97%) was used as a tracer. After removing the ethanol solvent from the tracer by gentle evaporation under nitrogen gas, the radiolabeled material was dissolved in the above-mentioned timolol solution. The final radioactivity of the solution was 125–295 nCi/μl.

Timolol was administered at a controlled rate (7.2 μg/h) in rabbit eyes using reservoir-type silicone tubing devices that have been described previously (Urtti et al., 1990). Hemispheric pieces of cured Silastic™ Adhesive A (Dow Corning, Midland, MI) were attached to the ends of the devices to prevent ocular irritation by the cut edges of the devices.

Timolol maleate (27.4 mg/ml) was dissolved in 0.15 M borax and pH of the solution was adjusted to 8.64 at 32°C with 10% w/v HCl. The timolol maleate concentration in the solution was equivalent to 10.0 mg/ml of L-timolol free base. The timolol solution was labeled with [^{3}H]timolol as described for the eyedrops. The final radioactivity of the solution was 1.1–2.8 μCi/μl.

The silicone tubing devices were filled with 12.5 μl of either labeled or unlabeled timolol solution using a syringe with a fixed needle as described previously (Urtti et al., 1990). After filling, the devices were stored for 3 h at room temperature in 98% relative humidity (above lead nitrate) prior to use.

Absorption studies

Pigmented male Dutch belted rabbits, 1.3–2.4 kg, were used in the studies. The rabbits were kept in restraint boxes during the experiment. Eyedrops (25 μl) were gently instilled on the upper corneoscleral limbus of the rabbits. The devices were placed in the upper conjunctival sac of the rabbit eyes. The dosage forms were applied in only one eye of each rabbit.

Tear fluid samples were collected from the lower marginal tear strip using 1 μl disposable glass capillaries (Microcaps, Drummond Scientific, NJ). The tear samples were collected carefully to avoid any contact with the corneal epithelium. Suction was not used and the capillaries were allowed to fill by capillary action. When the capillary was not filled completely the sample volume was calculated by determining the filled portion of the capillary and the total length of the capillary tube. The capillaries were emptied into liquid scintillation bottles containing 15 ml of Aquasol II (New England Nuclear, Boston, MA). The capillaries were flushed several times with Aquasol II and finally placed in the scintillation vial. Recovery of the radioactivity from the capillaries was 98% using this procedure.

After 0.5, 2.5, 4.0 and 8.0 h the rabbits were killed by intravenous injection of T-61 euthanasia solution (American Hoechst, Somerville, NJ). The

eyes were proptosed and aqueous humor was withdrawn with a 27-gauge needle and syringe. The aqueous humor was placed in preweighed scintillation vials. After weighing, 10 ml of Aquasol II was added. The rest of the rabbit eye was enucleated and frozen in liquid nitrogen. The ocular tissues were dissected and weighed. The tissue samples were combusted using a tissue oxidizer (Model 306, Packard Instruments, Downers Grove, IL) for 2.5 min. The radioactivities of the tissue samples were determined in 15 ml of Monophase S scintillation cocktail (Packard). Radioactivities of all tissue samples were counted using a Beckman LS 7500 (Irvine, CA) liquid scintillation counter.

When systemic absorption of timolol was studied unlabeled dosage forms were administered in rabbit eyes as described above. Blood samples were collected from the cannulated ear artery. Plasma was separated and stored at −20 °C.

Beta-blocking activity in rabbit plasma was determined using a radioreceptor assay described previously by Wellstein et al. (1984). Binding of a hydrophilic β-antagonist [^{3}H]CGP12177 (specific activity, 31 Ci/mmol; radiochemical purity, 97.7%) or (−)-[^{3}H]CGP12177 (specific activity, 49 Ci/mmol; radiochemical purity, 95.5%) (both from Amersham International, Bucks, U.K.) to the β-receptors of rat reticulocyte membranes was determined in the assay. Reticulocytes were obtained from Wistar rats (80–120 g) of both sexes as described by Wellstein et al. (1984). Protein content of the reticulocyte suspension was determined using BCA Protein Assay Reagent™ (Pierce, Rockford, IL) according to the instructions of the manufacturer. The reticulocyte suspension was stored at −80 °C prior to use. In the assay, 20–100-μl aliquots of plasma were incubated for 1 h at 25 °C with 50 μl of reticulocyte suspension (400–600 μg protein), 30 nCi of [^{3}H]CGP12177 or (−)-[^{3}H]CGP12177 and phosphate buffer (pH 7.4, 310 mosM). The total incubation volume was 300 μl.

After incubation, 10 ml of the phosphate buffer was added to each incubation vial. Bound and free radioligand were rapidly separated by vacuum filtration using a Millipore filtration system and Whatman GF/F filters. The filters were rinsed twice under vacuum with 10 ml of phosphate buffer and placed in 5 ml of liquid scintillation cocktail (Lipoluma: Lumasolve: water; 10 : 1 : 0.2) in 6 ml polyethylene vials. After storing overnight in darkness the radioactivity of the samples was counted with an LKB Rackbeta 1215 liquid scintillation counter (Walac, Turku, Finland) for 5 min or until 10 000 counts.

Non-specific binding of radioligand to the membranes was determined by incubating the radioligand, reticulocytes and blank plasma in 10^{-5} M dl-propranolol (Sigma, St. Louis, MO). Standard concentrations of timolol (0.5–20.0 nM) were incubated with the same amounts of reticulocytes, radioligand and blank plasma that were used in the analysis of the samples. Separate standard samples were incubated with each run. The samples were run in triplicates and the mean values were used in calculations.

Specifically bound radioactivity (dpm) was calculated by subtracting the non-specific binding from the total radioligand bound. Standard curves for each run were generated by plotting the specifically bound radioactivity vs logarithm of timolol concentration. Standard curves were linear from 0.5 to 20.0 nM of timolol in the incubation vials. The results are given as timolol equivalents of beta-blocking activity in plasma, because both timolol and its possible active metabolites are measured.

Analysis of the results

$AUC_{0-8\,h}$ values for timolol in tear fluid, ocular tissues, and plasma were calculated using the trapezoidal method. When $AUC_{0-\infty}$ was calculated the residual area from $t = 8$ h to $t = \infty$ was estimated as $C_{8\,h}/K$, where K is the apparent first-order elimination rate constant (Gibaldi and Perrier, 1982) and $C_{8\,h}$ is the timolol concentration in the related ocular tissue. Apparent first-order elimination rate constants from the iris-ciliary body and aqueous humor (K_{icb}, K_{ah}) were calculated from the terminal slopes of the respective ln(tissue drug concentration) vs time plots (Table 1).

The fraction of timolol absorbed in the aqueous humor after eyedrop administration was calcu-

TABLE 1

Pharmacokinetic parameters used in calculations

Parameter	Value	Source
V_d	2.0 ml	Francoeur (1983)
K_{ah}	0.43 h^{-1}	calculated from terminal slope
K_{icb}	0.07 h^{-1}	calculated from terminal slope
K_{tf}	0.07 min^{-1}	Maurice and Mishima (1984)
V_{tf}	7 μl	Maurice and Mishima (1984)
S_c	1.8 cm^2	Ahmed et al. (1987)
P_c	12.6×10^{-6} cm/s	Huang et al. (1983)
		Chang et al. (1987)
		Ahmed et al. (1987)
Cl_{tf}	0.49 μl/min	calculated according to Eqn 4
Cl_{cj}	10.38 μl/min	Ahmed et al. (1987)

lated according to Eqn 1 (Patton and Francoeur, 1978):

$$F = \frac{AUC_{0-\infty}}{D} K_{ah} V_d \quad (1)$$

where V_d is the ocular volume of distribution for timolol (Table 1).

The steady-state flux of timolol in the aqueous humor (J_{ss}) during controlled drug delivery was calculated from the relationship (Baustian, 1987),

$$J_{ss} = V_d C_{ah,ss} K_{ah} \quad (2)$$

where $C_{ah,8\ h}$ was used as an approximation to the steady-state concentration of timolol in the aqueous humor. Transcorneal flux was determined by the corneal permeability of timolol (P_c), corneal surface area (S_c), and precorneal timolol concentration ($C_{pc,ss}$) (Table 1) according to the following equation.

$$J_{ss} = P_c S_c C_{pc,ss} \quad (3)$$

The contribution of tear turnover to the precorneal clearance was calculated as

$$Cl_{tf} = K_{tf} V_{pc} \quad (4)$$

where K_{tf} is the first-order rate constant for tear turnover and V_{pc} is volume of the tear fluid (Table 1). Precorneal clearance due to corneal absorption of timolol was calculated according to Ahmed et al. (1987),

$$Cl_c = P_c S_c \quad (5)$$

where P_c is the corneal permeability of timolol and S_c is the corneal surface area (Table 1). P_c was calculated as a mean of three determinations from the literature (Huang et al., 1983; Ahmed et al., 1987; Chang et al., 1987).

Curve-fitting procedures were performed using the MULTI program (Yamaoka et al., 1981) in which the Gauss-Newton algorithm was utilized.

Results and Discussion

Ocular absorption

Radioactivities in ocular tissues represent intact timolol, since timolol is not metabolized in rabbit eyes (Putterman et al., 1985). Although timolol is extensively metabolized systemically in rabbits (Schmitt et al., 1980), the radioactive metabolites from the systemic circulation only slightly affect ocular timolol concentrations (Salminen and Urtti, 1984).

Ocular administration of timolol in an eyedrop and in a controlled release device resulted in typical drug distribution in the pigmented rabbit eyes (Figs. 1 and 2; Table 2). Timolol concentrations were higher in the anterior tissues (cornea, conjunctiva, sclera, aqueous humor) than in the posterior tissues (lens and vitreous humor) (Table 2). However, timolol concentrations in the iris-ciliary body were higher than in other ocular tissues especially at later times after administration (Figs. 1 and 2; Table 2). This is due to the binding of timolol in the melanin pigmentation in the iris-ciliary body (Araie et al., 1982; Salminen and Urtti, 1984). Pigment binding is important therapeutically, because the ciliary body is the site of action of timolol (Katz and Berger, 1979).

Eyedrop administration resulted in a typical concentration profile in the ocular tissues (Fig. 1). Timolol administration in silicone devices resulted in essentially constant drug concentrations in the anterior tissues (i.e., cornea, conjunctiva, sclera) whereas in the aqueous humor, iris-ciliary body,

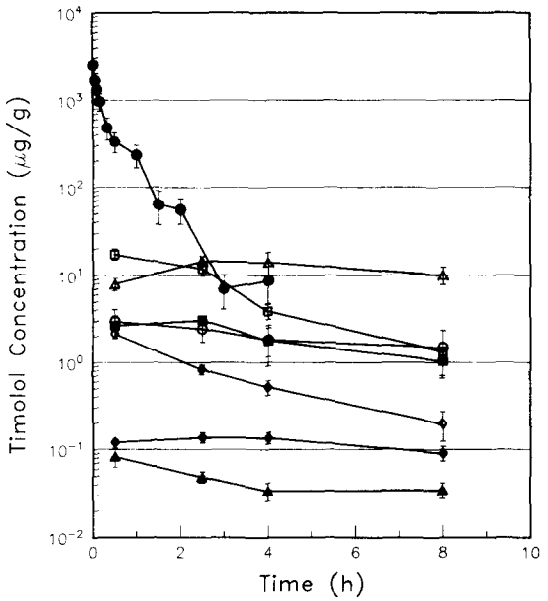

Fig. 1. Timolol concentration in ocular tissues of pigmented rabbits after topical ocular administration of 25 μl of 5 mg/ml solution. Means ± S.E. of timolol concentrations tear fluid (●), cornea (□), aqueous humor (◇), conjunctiva (○), anterior sclera (■), iris-ciliary body (△), lens (◆), and vitreous humor (▲) are presented. Numbers of experiments are listed in Table 2.

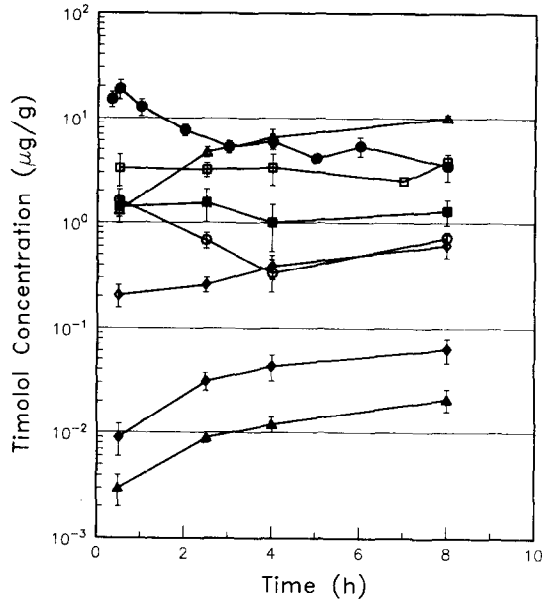

Fig. 2. Timolol concentrations in ocular tissues of pigmented rabbits after timolol administration in upper conjunctival sac in silicone tubing device with release rate of 7.2 μg/h. Means ± S.E. of drug concentrations are presented. For symbols see legend to Fig. 1. Numbers of experiments are listed in Table 2.

TABLE 2

Concentrations [a] (μg/g) of timolol in ocular tissues of pigmented rabbits after administration of 125 μg timolol in 25 μl eyedrop or in a silicone tubing device releasing timolol 7.2 μg/h

Tissue	Dosage form	0.5 h	2.5 h	4.0 h	8.0 h
Cornea	drop	17.38 ± 2.47	11.91 ± 1.98	3.92 ± 0.75	1.35 ± 0.12
	device	3.32 ± 1.12	3.21 ± 0.51	3.37 ± 1.12	3.86 ± 0.30
Aqueous	drop	2.16 ± 0.27	0.83 ± 0.10	0.52 ± 0.10	0.20 ± 0.07
humor	device	0.21 ± 0.05	0.26 ± 0.04	0.39 ± 0.10	0.62 ± 0.15
Iris-	drop	7.97 ± 1.26	14.58 ± 1.93	14.12 ± 4.06	10.03 ± 2.21
ciliary body	device	1.30 ± 0.31	4.74 ± 0.53	6.53 ± 1.28	10.15 ± 0.68
Conjunctiva	drop	3.00 ± 1.08	2.45 ± 0.72	1.84 ± 0.67	1.50 ± 0.83
	device	1.63 ± 0.43	0.69 ± 0.12	0.34 ± 0.12	0.73 ± 0.09
Sclera	drop	2.66 ± 0.75	3.09 ± 0.31	1.80 ± 0.88	1.05 ± 0.34
	device	1.41 ± 0.29	1.55 ± 0.53	1.01 ± 0.47	1.30 ± 0.35
Lens	drop	0.121 ± 0.009	0.138 ± 0.018	0.136 ± 0.020	0.091 ± 0.017
	device	0.009 ± 0.003	0.031 ± 0.006	0.043 ± 0.012	0.063 ± 0.017
Vitreous	drop	0.083 ± 0.020	0.049 ± 0.007	0.034 ± 0.008	0.035 ± 0.007
humor	device	0.003 ± 0.001	0.009 ± 0.001	0.012 ± 0.002	0.021 ± 0.005
n	drop	4	6	4	4
	device	5	4	4	4

[a] Means ± S.E. of *n* experiments are presented.

lens, and vitreous timolol concentrations increased gradually over the 8 h (Table 2, Fig. 2). Obviously, the timolol concentration in these tissues had not completely achieved steady-state levels at 8 h.

The apparent elimination rate constants of timolol from the aqueous humor and iris-ciliary body after eyedrop administration (K_{ah} and K_{icb}) were 0.43 and 0.07 h^{-1}, respectively (Table 1). Consequently, the value for the $AUC_{0-\infty}$ after eyedrop administration in the aqueous humor was 387 μg min ml^{-1} and 14234 μg min g^{-1} in the iris-ciliary body.

The fractional ocular absorption of timolol into the aqueous humor (F in Eqn 1) was 4.4% after administration of an eyedrop. During timolol administration with the silicone device, steady state was not achieved during 8 h. At 8 h, the timolol concentration was 0.62 μg/ml and the calculated flux (Eqn 2) was 0.53 μg/h, which is 7.4% of the drug release rate in vitro. This indicates that the ocular bioavailability of timolol was increased at least 1.7-fold, when the controlled release system was used.

TABLE 3

Concentrations (μg/ml) of timolol in tear fluid after administration of 125 μg timolol in 25 μl eyedrop or in a silicone tubing device releasing timolol 7.2 μg/h

Time (min)	Average timolol concentration (μg/ml) [a]			
	Eyedrop		Device	
1	2515 ± 300 [b]	15 [c]	–	
3	1686 ± 312	15	–	
5	1326 ± 265	14	–	
10	974 ± 217	13	–	
20	489 ± 138	13	15.1 ± 2.5 [b]	10 [c]
30	337 ± 89	14	18.9 ± 3.9	11
60	237 ± 67	12	12.8 ± 2.3	11
90	64 ± 26	8	10.0 ± 1.5	12
120	56 ± 18	11	7.7 ± 1.0	11
180	7.1 ± 3.0	6	5.3 ± 0.7	7
240	8.8 ± 4.0	7	6.0 ± 1.0	8
300	–		4.1 ± 0.4	4
360	–		5.4 ± 1.1	4
420	–		2.5 ± 0.7	4
480	–		3.5 ± 1.0	3

[a] Concentration is in equivalents of timolol free base.
[b] Means ± S.E. of n determinations are presented.
[c] Number (n) of determinations (eyes).

Precorneal kinetics

Timolol concentration in the tear fluid (C_T) after eyedrop administration (Table 3, Fig. 1) was best described by the triexponential equation: $C_T = 2.567e^{-0.451t} + 1.077e^{-0.035t} + 0.042e^{-0.003t}$, where t is the time (min). The initial rate constant for timolol elimination (0.451 min^{-1}) was in the same range with the elimination rate constant for pilocarpine from the rabbit tear fluid (0.6 min^{-1}) during the first 5 min after eyedrop instillation (Lee and Robinson, 1979; Thombre and Himmelstein, 1984). Due to the rapid decrease in drug concentration in the tear fluid, corneal drug absorption ceases in a few minutes (Chrai et al., 1974; Grass and Robinson, 1984) and the ocular surface attains pseudo-equilibrium with the precorneal drug. Probably, the same phenomenon also takes place in the conjunctiva. After the absorption processes have stopped, the drug is eliminated from the tear fluid solely via tear turnover. The elimination rate constant for timolol from the tear fluid during beta (0.035 min^{-1}) and gamma phases (0.003 min^{-1}) was, however, less than the rate constant for tear turnover (0.07 min^{-1}). This indicates considerable diffusion from the surrounding tissues back to the tear fluid (Figs. 1 and 2). This is expected because the rate constant for tear turnover (0.07 min^{-1}) is faster than the rate constant for timolol elimination from the aqueous humor (0.43 h^{-1}) and ocular tissues (Fig. 2). However, back diffusion to the tear fluid probably does not affect ocular drug concentrations significantly, because the volume of the tear fluid (7 μl) is negligible compared with ocular tissues and plasma. Consequently, when viscous ophthalmic vehicles are compared, drug concentrations in the tear fluid at later times may reflect initial loading of the cornea and conjunctiva with the drug and subsequent back diffusion rather than differences in vehicle retention on the ocular surface.

Administration of timolol in the silicone device resulted in slowly decreasing timolol levels in the tear fluid (Table 3, Fig. 2). Interestingly, even at 4 h timolol levels in the tear fluid were higher after eyedrop instillation than after silicone device administration (Table 3, Figs. 1 and 2). $AUC_{0-4\ h}$

after eyedrop administration was 748 μg h ml^{-1} and during controlled timolol delivery $AUC_{0-8\ h}$ was only 52 μg h ml^{-1}. Nevertheless, ocular bioavailability after controlled drug delivery was at least 1.7-times higher than after eyedrop administration (see Ocular absorption) and it is evident that the AUC of the drug in the tear fluid does not predict the ocular bioavailability. The higher AUC of drug in the tear fluid after instillation of an eyedrop is due to back diffusion of drug from the conjunctiva and cornea after drug absorption ceases. Constant low drug concentration in the tear fluid provides continuous flux of the drug in the eye. In this case, ocular bioavailability is entirely determined by the ratio of the corneal clearance (Cl_c) to the total drug clearance (Cl_{total}) from the tear fluid (Baustian, 1987). Using Eqns 4 and 5 and conjunctival clearance determined by Ahmed et al. (1987), the total precorneal clearance of timolol was calculated as $Cl_{pc} = Cl_{tf} + Cl_{cj} + Cl_c = (0.49 + 10.38 + 1.35)$ μl/min = 12.22 μl/min. Consequently, the maximum relative ocular bioavailability (Cl_c/Cl_{total}) of timolol after topical ocular administration is only 11.0%, which shows the negative impact of the nonproductive conjunctival absorption on ocular bioavailability.

Systemic absorption

As timolol is extensively metabolized in systemic circulation (Tocco et al., 1975), determination of tritium in plasma does not indicate the true beta blocking activity. Consequently, beta blocking activity was determined by a radioreceptor assay (Wellstein et al., 1984) after administering unlabeled timolol dosage forms.

Systemic absorption of timolol after administration of an eyedrop is shown in Fig. 3. Peak beta blocking activity in plasma reported as timolol equivalents was 17.16 ± 2.40 ng/ml after eyedrop administration (Table 4). Beta blocking activity in plasma was best described by the triexponential equation: $C_p = -25.64e^{-0.199t} + 27.06e^{-0.056t} + 2.71e^{-0.008t}$, where t is the time (min). The apparent first-order absorption rate constant was very rapid ($k_a = 0.199$ min^{-1}), which was also indicated by the early time of the peak drug concentration (Fig. 3, Table 4). After administration of the silicone device, timolol levels in plasma were below the radioreceptor assay detection limit (1.0 ng/ml) (Fig. 3, Table 4). Because elimination of timolol from rabbit plasma is very rapid (Fig. 3) timolol levels should reach steady-state in 8 h. Thus, it seems that by using controlled drug delivery it is possible to decrease the systemic peak concentrations of timolol (Fig. 3), while maintaining therapeutic drug concentrations in the ciliary body (Table 2).

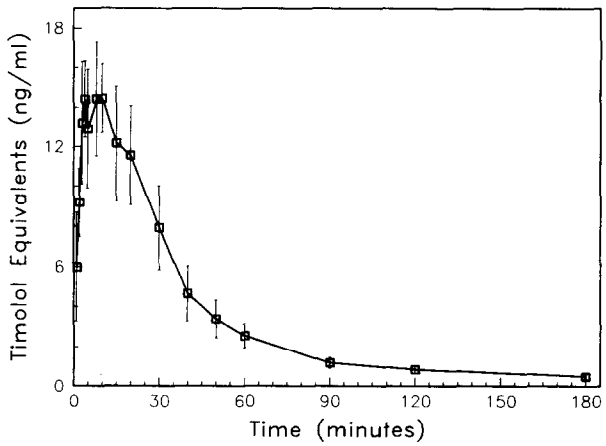

Fig. 3. Beta-blocking activity in rabbit plasma expressed as timolol equivalents (ng/ml) after topical ocular administration of 25 μl of 5 mg/ml timolol. Means ± S.E. of five determinations are presented.

Controlled delivery decreases the systemic concentrations of timolol in three ways. First, ocular bioavailability of timolol is increased about 2-fold, which enables reduction of the administered dose. Second, after instillation, part of an eyedrop enters the nasal mucosa (Hurwitz et al., 1975), which has a large surface area and high permeability to drugs (McMahon et al., 1987). Normally, tear

TABLE 4

Timolol equivalents [a] of beta blocking activity in rabbit plasma after administration of a silicone tubing device (release rate 7.2 μg/h) and after aqueous solution (0.5%, 25 μl) administration

Dosage form	t_{max} [b]	c_{max} [c]	n
Aqueous solution	9.2 ± 0.5	17.2 ± 2.4	5
Silicone device	–	< 1.0	6

[a] Means ± S.E. of n determinations are presented.
[b] t_{max} = time delay of peak concentration (min).
[c] c_{max} = peak concentration (ng/ml).

fluid does not enter the nasal mucosa (Doane, 1981). After administration of an eyedrop (approx. 36 μl) the instilled solution entered the nasal mucosa in all patients (Hurwitz et al., 1975), which is the most important site of systemic timolol absorption (Chang and Lee, 1987). Systemic absorption after device administration will take place mainly via the conjunctiva, since timolol in the normal tear fluid volume would not enter the nasal mucosa. Third, the concentration fluctuations observed after pulsed drug administration are eliminated. For drugs with a short half-life (e.g., timolol, $t_{1/2} = 3.5$ h (Genzo and Green, 1986)) and rapid absorption, pulsed drug delivery (e.g., via eyedrops) results in extensive fluctuation in peak drug levels in the plasma (Gibaldi and Perrier, 1982). Accordingly, vehicles from which drug releases rapidly (e.g., thickened eyedrops and gels) show only limited success in decreasing systemic peak drug concentration (i.e., fluctuation) (Kumar et al., 1986; Chang, 1987). On the other hand if the drug release from the vehicle is controlled, extensive improvement in ocular to systemic concentration ratio of timolol can be achieved (Tables 2 and 4).

Recently, several approaches have been tried to decrease the systemic toxicity of ophthalmic beta-blockers. Systemic absorption of ophthalmic timolol can be decreased slightly by preventing the drainage of the eyedrop from the ocular surface to the lacrimal passages (i.e., nasolacrimal occlusion) (Zimmerman et al., 1984; Kaila et al., 1986). Reduced eyedrop volume (Chang et al., 1986) and prolonged ocular contact of a viscous eyedrop (Chang and Lee, 1986) were not successful strategies to improve the ratio of ocular to systemic absorption of timolol. From viscous vehicles (e.g., thickened eyedrops and gels) drugs diffuse rapidly to the conjunctiva and nasal mucosa where the drug is absorbed systemically. Drug delivery is not controlled from viscous vehicles and fluctuations of timolol concentrations in plasma are not eliminated. Increased ocular absorption without corresponding enhancement of systemic absorption was achieved using prodrugs of timolol (Chang et al., 1987). Systemic beta-blocking activity was decreased also by using $beta_1$ selective beta-blockers, but even in this case hazardous systemic side-effects cannot be ruled out (Harris et al., 1986; Ball, 1987; Novack, 1987). Probably the best way to improve the therapeutic index of ophthalmic beta-blocker therapy is to combine pharmaceutical (controlled drug delivery), chemical (ideal permeability of the drug) and pharmacological (receptor selectivity) approaches.

Acknowledgements

We are grateful to Ms. Kristiina Kyyronen (M.Sc.) and Ms. Lea Pirskanen for skillful assistance during the study. This study was supported by a grant from INTERx Research Corporation, Merck Sharp & Dohme Research Laboratories, Lawrence, Kansas, U.S.A.

References

Ahmed, I., Gokhale, R.J., Shah, M.V. and Patton, T.F., Physicochemical determinants of drug diffusion across the conjunctiva, sclera and cornea. *J. Pharm. Sci.,* 76 (1987) 583–586.

Araie, M., Takase, M., Sakai, Y., Ishii, Y., Yokoyama, Y. and Kitagawa, M., Beta-adrenergic blockers: ocular penetration and binding to the uveal tract. *Jap. J. Ophthalmol.,* 26 (1982) 248–263.

Ball, S., Congestive heart failure from betaxolol. *Arch. Ophthalmol.,* 105 (1987) 320.

Baustian, C., *Ph.D. Thesis,* Department of Pharmaceutical Chemistry, The University of Kansas, 1987.

Chang, S.C., Prodrug and vehicle approaches to improve the therapeutic index of typically applied timolol in the pigmented rabbit. *Ph.D. Thesis,* School of Pharmacy, University of Southern California, 1987.

Chang, S.C., Bundgaard, H., Buur, A. and Lee, V.H.L, Improved corneal penetration of timolol by prodrugs as a means to reduce systemic drug load. *Invest. Ophthalmol. Vis. Sci.,* 28 (1987) 487–491.

Chang, S.C. and Lee, V.H.L., Nasal and conjunctival contributions to the systemic absorption of topical timolol in the pigmented rabbit: implications in the design of strategies to maximize the ratio of ocular to systemic absorption. *J. Ocul. Pharmacol.,* 3 (1987) 159–167.

Chrai, S.S., Makoid, M.C., Eriksen, S.P. and Robinson, J.R. Drop size and initial dosing frequency problems of topically applied drugs *J. Pharm. Sci.,* 63 (1974) 333–338.

Francoeur, M.L., *Ph.D. Thesis,* Department of Pharmaceutical Chemistry, The University of Kansas, 1983.

Gengo, F.M. and Green, J.A., Beta-blockers. In Evans, W.A., Schentag, J.J. and Jusko, W.J. (Eds), 2nd Ed., *Applied*

Pharmacokinetics; Principles of Therapeutic Drug Monitoring, Applied Therapeutics, Inc., Spokane, WA, 1986, pp. 735–781.

Gibaldi, M. and Perrier, D., *Pharmacokinetics*, 2nd Ed., Dekker, New York, 1982.

Grass, G.M. and Robinson, J.R., Relationship of chemical structure to corneal penetration and influence of low-viscosity solutions on ocular bioavailability. *J. Pharm. Sci.*, 73 (1984) 1021–1027.

Harris, L.S., Greenstein, M.D. and Bloom, A.F., Respiratory difficulties with betaxolol. *Am. J. Ophthalmol.*, 102 (1986) 274–275.

Huang, H.S., Schoenwald, R.D. and Lach, J.L., Corneal penetration behavior of beta blocking agents, II. Assessment of barrier contributions. *J. Pharm. Sci.*, 72 (1983) 1272–1279.

Hurwitz, J.J., Maisey, M.N. and Welham, R.A.N., Quantitative lacrimal scintillography I. Method and physiological application. *Br. J. Ophthalmol.*, 59 (1975) 308–312.

Kaila, T., Huupponen, R. and Salminen, L., Effects of eyelid closure and nasolacrimal duct occlusion on the systemic absorption of ocular timolol in human subjects. *J. Ocul. Pharmacol.*, 2 (1986) 365–369.

Katz, I.M. and Berger, E.T., Effects of iris pigmentation on response of ocular pressure to timolol. *Surv. Ophthalmol.*, 23 (1979) 395–398.

Kumar, V., Schoenwald, R.D., Barcellos, W.A., Chien, D.S., Folk, J.G. and Weingeist, T.A., Aqueous vs. viscous phenylephrine. 1. Systemic absorption and cardiovascular effects. *Arch. Ophthalmol.*, 104 (1986) 1189–1191.

Lee, V.H.L. and Robinson, J.R., Mechanistic and quantitative evaluation of precorneal pilocarpine disposition in albino rabbits. *J. Pharm. Sci.*, 68 (1979) 673–684.

Maurice, D.M. and Mishima, S., Ocular pharmacokinetics. In Sears, M.L. (Ed.), *Handbook of Experimental Pharmacology*, Vol. 69, Springer, Berlin, 1984, pp. 19–116.

McMahon, C., Hutchinson, L.E.F., Hyde, R. and Peters, G.E., Analysis of structural requirements for the absorption of drugs and macromolecules from the nasal cavity. *J. Pharm. Sci.*, 76 (1987) 535–540.

Nelson, W.L., Fraunfelder, R.T., Sills, J.M., Arrowsmith, J.B. and Kuritsky, J.N., Adverse respiratory and cardiovascular events attributed to timolol ophthalmic solution, 1978–1985. *Am. J. Ophthalmol.*, 102 (1986) 606–611.

Novack, G.D., Ophthalmic beta-blockers since timolol. *Surv. Ophthalmol.*, 31 (1987) 307–327.

Patton, T.F. and Francoeur, M., Ocular bioavailability and systemic loss of topically applied ophthalmic drugs. *Am. J. Ophthalmol.*, 85 (1978) 225–229.

Putterman, G.J., Davidson, J. and Albert, J., Lack of metabolism of timolol by ocular tissues. *J. Ocul. Pharmacol.*, 1 (1985) 287–291.

Salminen, L. and Urtti, A., Disposition of ophthalmic timolol in treated and untreated rabbit eyes. A multiple and single dose study. *Exp. Eye Res. 38* (1984) 203–206.

Schmitt, C.J., Lotti, V.J. and LeDouarec, J.C., Penetration of timolol into the rabbit eye. *Arch. Ophthalmol.*, 98 (1980) 547–551.

Shell, J.W., Ophthalmic drug delivery systems. *Surv. Ophthalmol.*, 29 (1984) 117–128.

Thombre, A.G. and Himmelstein, K.J., Quantitative evaluation of topically applied pilocarpine in the precorneal area. *J. Pharm. Sci.*, 73 (1984) 219–222.

Tocco, D.J., Duncan, A.E.W., Deluna, F.A., Hucker, H.B., Gruber, V.F. and Vandenheuvel, W.J.A., Physiological disposition and metabolism of timolol in man and laboratory animals. *Drug. Metab. Dispos.*, 3 (1975) 361–370.

Urtti, A., Pipkin, J.D., Rork, G. and Repta, A.J., Controlled drug delivery devices for experimental ocular studies with timolol. 1. In vitro release studies. *Int. J. Pharm.*, 61 (1990) 235–240.

Urtti, A. and Salminen, L., Concentration dependent precorneal loss of pilocarpine in rabbit eyes. *Acta Ophthalmol.*, 63 (1985) 502–506.

Urtti, A., Salminen, L. and Miinalainen, O., Systemic absorption of pilocarpine is modified by polymer matrices, *Int. J. Pharm.*, 23 (1985) 147–161.

Wellstein, A., Palm, D., Wiemer, G., Schafer-Korting, M., and Mutschler, E., Simple and reliable radioreceptor assay for beta-adrenoceptor antagonists and active metabolites in native human plasma. *Eur. J. Clin. Pharmacol.*, 27 (1984) 545–553.

Zimmerman, T.J., Kooner, K.S., Kandarakis, A.S. and Ziegler, L.P., Improving the therapeutic index of topically applied ocular drugs. *Arch. Ophthalmol.*, 102 (1984) 551–553.

Yamaoka, K., Tanigawara, Y., Nakagawa, T., and Uno, T., A pharmacokinetic analysis program (MULTI) for microcomputer. *J. Pharmacobiodyn.*, 4 (1981) 879–885.