

Improved Ocular: Systemic Absorption Ratio of Timolol by Viscous Vehicle and Phenylephrine

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Increasing the ocular absorption of timolol relative to its systemic absorption is important clinically because ophthalmic timolol may cause serious respiratory, cardiac, and central nervous system side effects. The authors evaluated the effects of phenylephrine coadministration and solution viscosity on the aqueous humor:plasma and iris ciliary body:plasma ratios of peak timolol concentrations after ocular application. Timolol eye drops (5 mg/ml, 25 μ l) were administered to the eyes of pigmented rabbits. Coadministered phenylephrine (0.8–8.2 mg/ml) decreased the systemic peak concentrations of timolol significantly. Since ocular absorption of timolol was not affected by phenylephrine, the ocular:systemic concentration ratios were improved four- to fivefold. Phenylephrine slows down the systemic absorption of timolol by constricting the conjunctival and nasal capillaries. The ratios of the aqueous humor:plasma and iris ciliary body:plasma peak concentration of timolol were improved three- to ninefold in the presence of sodium carboxymethylcellulose compared with nonviscous eye drops. The improved ocular penetration is probably due to the longer corneal contact, and the decreased rate of systemic absorption may be caused by the slower spreading of the solution on the nasal mucosa. Compared with timolol eye drops, the ratio of the eye:plasma peak timolol concentrations was improved tenfold by using viscous eye drops with phenylephrine. Systemic concentrations of ophthalmic timolol and possibly related side effects can be decreased when timolol is instilled in a viscous vehicle with a low phenylephrine concentration. Invest Ophthalmol Vis Sci 31:1827–1833, 1990

Although systemic absorption of ocularly administered timolol may cause respiratory and cardiovascular side effects,¹ the factors affecting the systemic absorption of timolol are not well known. Due to the side effects, it is important to minimize the systemic concentrations of timolol with ophthalmic dosage-form design. The ratio of ocular:systemic concentrations of ophthalmic drugs is used to describe the relative safety of the ocular delivery systems of a drug in terms of its systemic side effects.²

When the ocular bioavailability of the drug is increased, therapeutic drug levels can be achieved in the eye with a smaller topical dose; this also decreases serum concentrations of ophthalmic drugs. Ocular bioavailability of several drugs³ has been increased by administering them in viscous polymeric solutions. The effects of viscous solutions on both ocular and systemic absorption of timolol have been described in only one study.⁴ Increased vehicle viscosity improved the aqueous humor:plasma absorption ratio of ophthalmic timolol almost fivefold.⁴ The increased ocu-

lar bioavailability was due to the longer corneal contact of the viscous solution, and the reduced systemic drug load may have been caused by the slower spreading of the solution on the nasal mucosa.

Other approaches undertaken to maximize the ocular:systemic concentration ratio of timolol include administration of the drug as a prodrug^{2,4} and in sustained-release devices instead of in eye drops.⁵ With eyelid closure and nasolacrimal-duct occlusion, it is possible to reduce the serum concentrations of timolol in clinical practice slightly.⁶ Respiratory side effects might be decreased by administering a selective β_1 -adrenergic blocker, like betaxolol, instead of timolol.⁷

In a previous study we showed that epinephrine pretreatment significantly reduces the systemic peak concentrations of timolol.⁸ This was due to epinephrine-induced conjunctival and nasal vasoconstriction which slowed down the systemic absorption of timolol.⁸ To maximize the ratio of the ocular:systemic concentrations of timolol, we investigated absorption of timolol from eye drops with mucoadhesive⁹ viscosity-inducing polymer (sodium carboxymethylcellulose, NaCMC) and/or a vasoconstricting additive (phenylephrine). Our objective was to combine the increased ocular absorption with increased viscosity and the decreased rate of systemic timolol absorption with vasoconstriction.

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Supported by a grant from INTER, Research Corporation, Merck, Sharp & Dohme Research Laboratories, Lawrence, Kansas.

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Materials and Methods

Animals

Mixed-breed pigmented rabbits of both sexes (2.0–4.3 kg) were used in these studies. Before the test, the animals were housed singly in cages under standard laboratory conditions: 10-hr dark/14-hr light cycle and $20.0 \pm 0.5^\circ\text{C}$ temperature. All animals were treated in accordance with the ARVO Resolution on the Use of Animals in Research.

Preparation of Instilled Eye Drops

Timolol solutions were prepared by dissolving l-timolol maleate (6.84 mg/ml; Merck, Rahway, NJ) in phosphate buffer. The concentration was equivalent to 5 mg/ml of timolol base. In the case of timolol-phenylephrine eye drops, 0.8, 1.6, 4.1, or 8.2 mg/ml of l-phenylephrine (1.0, 2.0, 5.0, or 10.0 mg/ml of the hydrochloride salt; Ega Chemie Steinheim/Albuch, West Germany) and 6.84 mg/ml of timolol maleate were dissolved in phosphate buffer. The eye drops containing timolol maleate (6.84 mg/ml) and NaCMC (10.0 or 20.0 mg/ml; Tamro, Helsinki, Finland) with or without phenylephrine hydrochloride (1.0 mg/ml) were prepared in phosphate buffer. The apparent viscosity of solutions with pseudoplastic NaCMC was calculated at a rate of shear of 150.4 s^{-1} (64 rpm/min). The rheologic data of the preparations were determined at 20°C , using a Rotovisco RV 2 viscometer (Haake, Karlsruhe, West Germany). The apparent viscosities of solutions containing 10.0 or 20.0 mg/ml of NaCMC were 41 cP and 330 cP, respectively. The pH of all eye drops was adjusted to 7.5 with 5 N NaOH.

In ocular-absorption studies timolol eye drops were labeled with ^3H -timolol (timolol-O-CHT, specific activity 8.5 Ci/mmol and radiochemical purity > 99%; Merck). A methanol solvent of ^3H -timolol was evaporated under nitrogen gas, and the tracer was dissolved in the formulations. Radioactivity of the final solution was about $0.26\text{ }\mu\text{Ci}/\mu\text{l}$. Tritium does not change from the tracer to water during 1 week. Only fresh solutions were used in the experiments.

Absorption Studies

The rabbits were kept in restraint boxes during the experiments and could move their heads and eyes freely.

When systemic absorption of timolol was studied, the eye drops were instilled in one eye of a rabbit. In ocular-absorption studies, the eye drops were applied to both eyes of the rabbits on the upper corneoscleral limbus. During instillation, the upper lid was gently pulled away from the eye, and $25\text{ }\mu\text{l}$ of timolol solution was applied. Blood samples were collected from

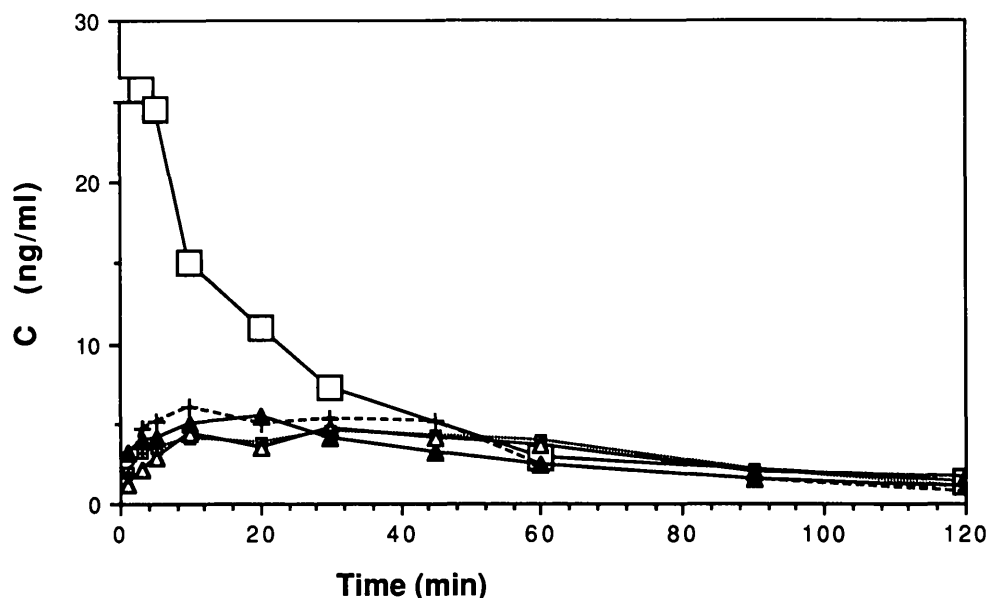
the cannulated ear artery of each rabbit. Plasma was separated and stored at -20°C until assayed.

The beta-blocking activity of timolol and possible metabolites of timolol in rabbit plasma were determined as described earlier.^{8,10} In the assay, β_2 antagonists and l- ^3H -CGP 12177 (specific activity 48.8 Ci/mmol, radiochemical purity 99.1%, Amersham International, Buckinghamshire, UK) compete for receptor binding in rat reticulocytes. The reticulocytes were separated from rat blood as described previously.¹⁰ In the assay, $60\text{ }\mu\text{l}$ or $100\text{ }\mu\text{l}$ of plasma, $50\text{ }\mu\text{l}$ of reticulocyte suspension (0.5 mg protein), and 30 nCi of radioligand were incubated in phosphate buffer (pH 7.4, 310 mOsm, ad $300\text{ }\mu\text{l}$) for 1 hr at 25°C . Bound and free radioligand were separated by vacuum filtration through Whatman GF/F glass fibre filters (Whatman, Maidstone, UK). Filters were washed with phosphate buffer before they were placed in 5 ml of Lipoluma:Optisolv (both Lumac, Schaesberg, The Netherlands):water (10:1:0.2) scintillation cocktail. After storing vials overnight in darkness, the retained radioactivity was determined using liquid scintillation counting (Rackbeta 1215; Wallac, Turku, Finland) for 5 min or until 10,000 counts.

Nonspecific binding of the radioligand was determined by incubating the radioligand, reticulocytes, and blank plasma in 10^{-5} M of racemic propranolol. Specific binding (96–98%) was calculated as total binding minus nonspecific binding. Standard concentrations of timolol (0.5–20.0 nM) were incubated with each run. Both samples and standards were run in triplicate, and the mean values were used in calculations. The coefficient of variation of the assay was 10.1%, and the assay sensitivity was 0.6 ng/ml of timolol in plasma. Linear standard curves for each run were generated by plotting specifically bound activity against the logarithm of the timolol concentration. Although timolol does not have active metabolites in human plasma, possible active metabolites of timolol in rabbit plasma might compete for the binding sites. Consequently, the results are expressed as timolol equivalents of beta-blocking activity (ng/ml) in plasma. Phenylephrine did not displace the radioligand from beta receptors at concentrations of 1–20 nM.

In the ocular-absorption studies, rabbits were killed by intravenous injection of T-61 euthanasia solution (American Hoechst, Sommerville, NJ) at 0.5 and 4.0 hr after instillation of timolol. Aqueous humor was withdrawn from the proptosed eyes with a needle and syringe and placed in preweighed 6-ml polyethylene vials for liquid scintillation counting. After the vials were weighed, 5 ml of ACS (Amersham) scintillation liquid was added. The proptosed eyes were enucle-

Fig. 1. Means ($n = 5-11$) of timolol equivalents in rabbit plasma after topical ocular administration of 25 μ l of timolol (5 mg/ml) without ($\square$) and with 0.8 ($\blacktriangle$), 1.6 ($+$), 4.1 ($\triangle$) and 8.2 ($\square$) mg/ml of phenylephrine in the same eyedrop. For the sake of clarity the error bars were omitted.



ated, frozen in liquid nitrogen, and stored at -20°C until dissected. Cornea, iris ciliary body, conjunctiva, and sclera were separated. Then 100 μ l of distilled water was added to each tissue and the tissues were dissolved in 1 ml of Optisolv at $40-50^{\circ}\text{C}$. After the solubilized tissue samples were cooled, 10 ml of isopropanol:Lipoluma mixture (0.3:10) was added to the samples. The iris ciliary-body samples were first bleached with 0.3 ml of hydrogen peroxide (35%) for 15–30 min. To eliminate chemiluminescence, 100 μ l of 0.1 N HCl was added to the iris ciliary-body samples after addition of the scintillation liquid. After storing in the dark overnight the ^3H -timolol in the tissues was determined by liquid scintillation counting for 10 min or until a count of 12,000. Linear

standard curves were generated by plotting the radioactivity (DPM) of standard solutions against timolol concentration. The DPM values of the samples were converted to micrograms using the standard curve.

Analysis of the Data

Areas under the curves ($\text{AUC}_{0-3 \text{ hr}}$) of beta-blocking activity in plasma were calculated using the trapezoidal method.¹¹ Peak beta-blocking activity (C_{max}) in the plasma was determined from actual data points. Ocular:systemic concentration ratios were obtained by dividing timolol concentrations in the aqueous humor and iris ciliary body by the peak drug concentration in plasma. Mean residence time (MRT) of

Table 1. Pharmacokinetic parameters of timolol equivalents (ng/ml) in plasma after topical ocular instillation of timolol

Instilled eyedrop	Conc. of PHE/NaCMC* (mg/ml)	C_{max} (ng/ml)	$\text{AUC}^{\dagger} 0-3 \text{ hr}$ (hr*ng/ml)	$\text{MRT}^{\ddagger}$ (min)	n
TIM	none	30.7 ± 3.6	13.0 ± 0.9	40.5 ± 3.3	7
TIM-PHE	1.0/none	$6.6 \pm 0.8^{\dagger\dagger}$	$6.3 \pm 0.7^{\dagger\dagger}$	$56.5 \pm 2.6^{\dagger\dagger}$	11
TIM-PHE	2.0/none	$9.0 \pm 3.2^{\dagger\dagger}$	$6.6 \pm 1.0^{\dagger\dagger}$	49.2 ± 3.3	10
TIM-PHE	5.0/none	$6.2 \pm 1.1^{\dagger\dagger}$	$7.4 \pm 0.9^{**}$	$65.7 \pm 5.2^{**}$	7
TIM-PHE	10.0/none	$5.0 \pm 1.0^{\dagger\dagger}$	$7.4 \pm 1.5^{\dagger}$	$60.3 \pm 5.9^{**}$	5
TIM-NaCMC§	none/10.0	$20.6 \pm 1.0^{\dagger\dagger\dagger}$	11.7 ± 1.1	$40.3 \pm 1.1^{\dagger\dagger}$	5
TIM-NaCMC	none/20.0	$12.5 \pm 2.8^{**\S\S}$	$8.3 \pm 0.8^{**\S\S}$	52.7 ± 7.2	5
TIM-PHE-NaCMC§	1.0/10.0	$6.5 \pm 1.0^{\dagger\dagger}$	$8.3 \pm 1.3^{\dagger}$	$61.0 \pm 2.0^{\dagger\dagger}$	8
TIM-PHE-NaCMC	1.0/20.0	$7.6 \pm 0.6^{\dagger\dagger}$	$8.9 \pm 0.7^{**}$	$66.7 \pm 3.1^{\dagger\dagger}$	5

Timolol (5 mg/ml, 25 μ l) was administered without (TIM) and, with phenylephrine (TIM-PHE), with sodium carboxymethylcellulose (TIM-NaCMC), and with phenylephrine and sodium carboxymethylcellulose (TIM-PHE-NaCMC) in the same eyedrop. Means $\pm$ SEM of n determinations are presented.

* The concentration of phenylephrine hydrochloride/sodium carboxymethylcellulose.

† Area under the beta-blocking activity vs. time curve.

‡ Mean residence time of timolol equivalents in plasma.

§§ Pseudoplastic. The apparent viscosities of the solutions were 41 cP§ and 300 cP|| (rpm = 64 min $^{-1}$).

† $P < 0.05$, $^{**}P < 0.01$, $^{\dagger\dagger}P < 0.001$ if compared with TIM.

$^{\dagger\dagger\dagger}P < 0.001$ compared with similar eyedrop with PHE.

$^{\S\S}P < 0.05$ compared with TIM-NaCMC (10.0 mg/ml) (unpaired 2-tailed student t -test).

timolol in plasma was calculated using the equation $AUMC_{0-3 \text{ hr}}/AUC_{0-3 \text{ hr}}$, where $AUMC_{0-3 \text{ hr}}$ is the area under the time*timolol concentration against time curve.¹² Statistical significance of the differences were tested with the Mann-Whitney U-test or with the two-tailed unpaired student t-test.

Results

Timolol was absorbed rapidly into the systemic circulation after eye-drop administration, as suggested by the early time of peak concentrations (Fig. 1) and by the high systemic bioavailability.⁴ When a vasoconstrictor, phenylephrine (0.8–8.2 mg/ml), was administered in the same eye drop with timolol, the systemic peak concentrations ($C_{\max}$) of timolol were decreased by about 70–80% (Table 1, Fig. 1). Also the MRT of timolol in the systemic circulation was prolonged, and the total amount absorbed systemically (AUC) was decreased significantly (Table 1). Ocular

absorption of timolol was not affected by administration of phenylephrine (Fig. 2). When the $C_{\max}$ were decreased at the same time, phenylephrine coadministration improved the aqueous humor:plasma and iris ciliary body:plasma ratios of peak timolol concentrations four- to fivefold (Table 2).

The $C_{\max}$ of timolol were decreased significantly when the timolol solution was thickened to 41 cP with NaCMC (10.0 mg/ml) (Table 1, Fig. 3). Increasing the viscosity of vehicle to 330 cP reduced the systemic drug absorption further (Table 1). The MRT of timolol was not affected by NaCMC (Table 1) and its systemic bioavailability (AUC) was reduced only with NaCMC concentration of 20.0 mg/ml (Table 1). Timolol concentrations in the eye at 0.5 and 4.0 hr were increased about two- to threefold in the presence of NaCMC (20.0 mg/ml) (Fig. 2). Finally, the aqueous humor:plasma and iris ciliary body:plasma ratios of peak timolol concentration were three to nine times higher with thickened eye drops (NaCMC,

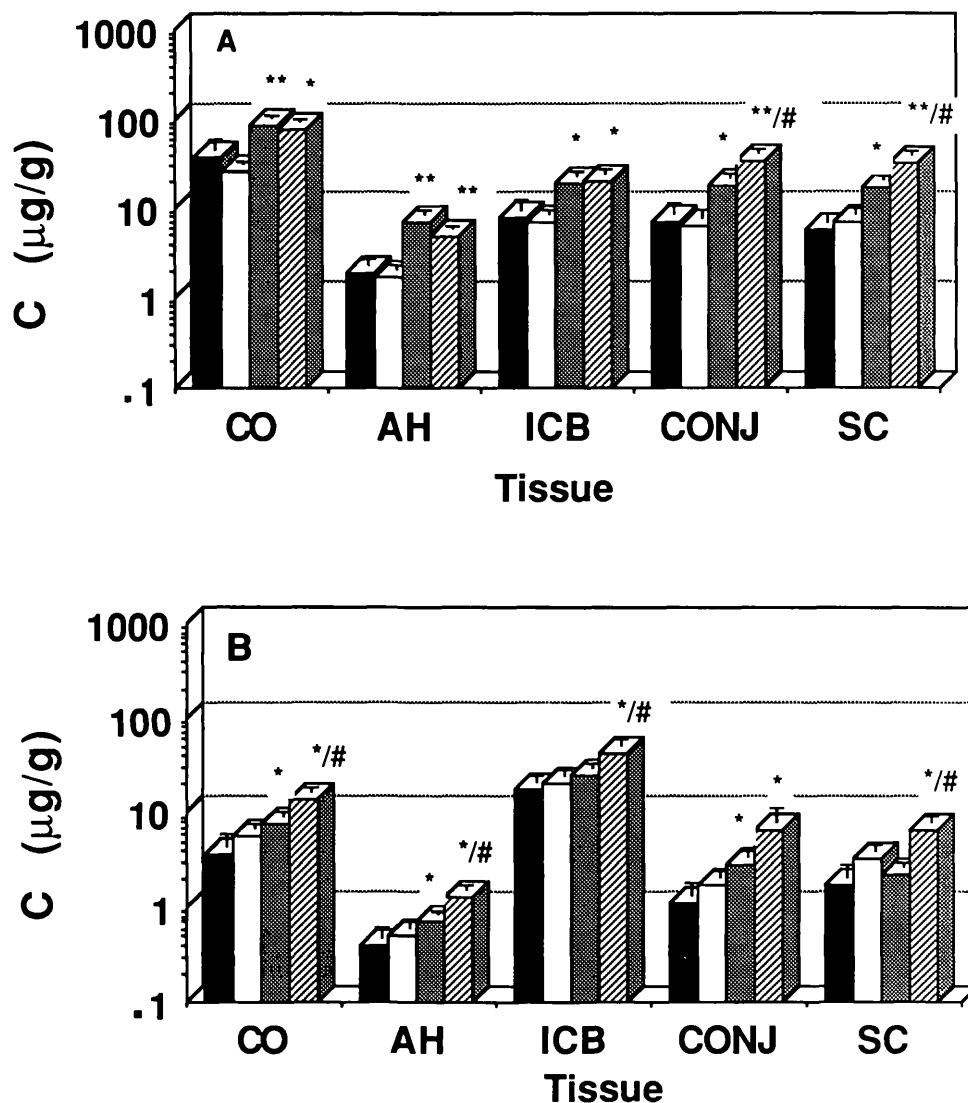


Fig. 2. Timolol concentrations in the cornea (CO), aqueous humor (AH), iris-ciliary body (ICB), conjunctiva (CONJ), and anterior sclera (SC) 0.5 hr (A) and 4.0 hr (B) after ocular instillation of timolol (5 mg/ml, 25 µl). Timolol was administered without (TIM) (solid bars) and with 4.1 mg/ml of phenylephrine (TIM-PHE) (open bars), with 20.0 mg/ml of sodium carboxymethylcellulose (TIM-NaCMC) (shaded bars), and with 0.8 mg/ml of phenylephrine and 20.0 mg/ml of NaCMC (TIM-PHE-NaCMC) (striped bars) in the same eyedrop ($n = 4-6$). $*P < 0.05$, $**P < 0.01$ if compared with TIM. $\#P < 0.05$ if compared with similar eyedrop without phenylephrine (Mann-Whitney's U-test).

Table 2. Ocular (C_{ocu})/systemic ($C_{\text{pl,max}}$) ratios of peak timolol concentrations of ocularly applied timolol 0.5 hr and 4.0 hr after instillation of 25 μl timolol (5 mg/ml)

Instilled eyedrop	$C_{\text{ocu}}/C_{\text{pl,max}}$		
	Aqueous humor, 30 min	Iris-ciliary body	
		30 min	240 min
TIM	60	270	570
TIM-PHE	280	1140	3240
TIM-NaCMC	545	1500	1980
TIM-PHE-NaCMC	615	2630	5555

Timolol was administered without (TIM) and with 4.1 mg/ml of phenylephrine (TIM-PHE), with 20.0 mg/ml of sodium carboxymethylcellulose (TIM-NaCMC) and with 0.8 mg/ml of phenylephrine and 20.0 mg/ml of sodium carboxymethylcellulose (TIM-PHE-NaCMC) in the same eyedrop.

20.0 mg/ml) than with nonviscous timolol eye drops (Table 2).

Compared with timolol-NaCMC (20.0 mg/ml) eye drops, timolol concentrations in the conjunctiva at 0.5 hr, in the sclera at 0.5 and 4.0 hr, and in the iris ciliary body at 4.0 hr were increased (Fig. 2) and the C_{max} of timolol decreased by administering phenylephrine (0.8 mg/ml) in the same eye drop with timolol and NaCMC (20.0 mg/ml) (Fig. 3). The best ocular:systemic concentration ratios of timolol were gained by combining phenylephrine-induced slow systemic absorption and the improvement of ocular absorption by NaCMC (Tables 1, 2). In this case the aqueous humor:plasma and iris ciliary body:plasma ratios of peak timolol concentrations were improved about tenfold compared with timolol in plain phosphate buffer (Table 2).

Discussion

Since timolol is not metabolized in the rabbit eye,¹³ the concentrations of ^3H -timolol in the ocular tissues represent intact timolol. The sampling times of ocular tissues (0.5 and 4.0 hours) were chosen, because the peak drug concentrations in aqueous humor are typically achieved 0.5 hours after eye-drop administration,¹⁴ and in the pigmented iris ciliary body, the highest concentrations of timolol are achieved 4.0 hours after instillation of the eye drop.¹⁵ The pH of the administered solutions was adjusted to 7.5, because the ocular absorption of timolol is better at this pH than at the pH 6.9 of commercial timolol eye drops.¹⁶

The decreased peak levels and the prolonged MRT of timolol equivalents in plasma after phenylephrine coadministration suggest that phenylephrine prolongs the systemic absorption time of timolol. This is indicated because the MRT of a drug is defined as the sum of the mean absorption time (MAT) of given drug and the constant MRT of intravenously injected drug.^{11,12} The prolonged systemic MAT is probably due to the phenylephrine-induced vasoconstriction in the conjunctiva of the eye and nose, which are the main sites of the systemic absorption of ocularly applied timolol.¹⁷ In the previous studies we demonstrated that administration of another vasoconstricting agent, epinephrine, 5 minutes before timolol solution reduced peak beta-blocking activity in rabbit plasma by about 80%.^{8,16} Epinephrine pretreatment, like phenylephrine coadministration, did not affect the ocular concentrations of timolol, but it prolonged the MRT and reduced the AUC of timolol in rabbit plasma.¹⁶ Because lipophilic timolol penetrates easily across the conjunctiva and nasal mucosa,^{17,18} the re-

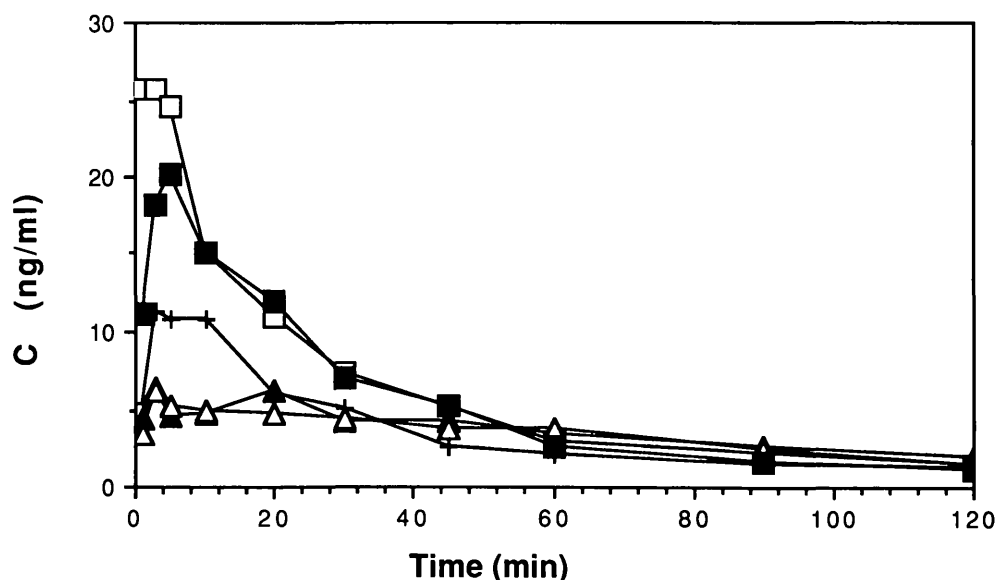


Fig. 3. Means ($n = 5-11$) of timolol equivalents in rabbit plasma after topical ocular administration of timolol (5 mg/ml, 25 μl) without ($\square$) and with 10.0 ($\blacksquare$) and 20.0 (+) mg/ml of sodium carboxymethylcellulose (NaCMC) and after ocular instillation of timolol with phenylephrine (0.8 mg/ml) and 10.0 mg/ml ($\triangle$) or 20.0 mg/ml of NaCMC ($\blacktriangle$) in the same eyedrop. For the sake of clarity the error bars were omitted.

duced conjunctival and nasal blood flow caused by phenylephrine or epinephrine affects timolol absorption. It is known that drug absorption may be limited by the local blood flow when drug penetration across the epithelium is rapid, and the underlying tissue has a rich flow of blood.¹⁹ The short half-life of timolol in rabbit plasma²⁰ also makes the peak timolol concentrations susceptible to changes in absorption rate.

The increased concentrations of ocular drug in the presence of NaCMC (20.0 mg/ml) are probably due to prolonged corneal contact of the vehicle, which is caused by the increased viscosity and mucoadhesiveness⁹ of the vehicle. In the case of viscous formulations, phenylephrine further increased the concentrations of timolol in the conjunctiva at 0.5 hours, in the sclera at 0.5 and 4.0 hours, and in the iris ciliary body at 4.0 hours. This is probably due to the prolongation of the phenylephrine-induced vasoconstriction in the conjunctiva of the eye. Conjunctival vasoconstriction may have increased timolol absorption through a noncorneal (conjunctiva-sclera-iris ciliary body) route.²¹ The corneal route, however, is much more important for timolol absorption.²¹

The most significant relative improvement in ocular bioavailability was observed for vehicles in the viscosity range 1–15 cP.^{22,23} Further increases in vehicle viscosity do not improve ocular bioavailability significantly. On the other hand, systemic bioavailability and $C_{\max}$ of ocularly applied timolol are reduced significantly when the viscosity of the instilled solution is increased from 2.7 cP to 70 cP.⁴ In our study, the peak timolol concentrations in plasma were decreased by 33% with NaCMC-solution at 41 cP compared with eye drops without NaCMC (Table 1). Increasing the viscosity of a vehicle from 41 cP to 330 cP would probably improve the ocular drug absorption only slightly,^{22,23} but it reduced the systemic drug absorption significantly (Table 1). The decreased systemic absorption was probably caused by the slower spreading of the solution to the nasal mucosa and slower drug diffusion in the viscous vehicle.

The nonviscous timolol solution used in this study (pH 7.5) and the timolol solution of pH 6.9 (like the commercial eye drops) produced similar ocular:systemic ratios for peak timolol concentrations.¹⁶ Consequently, the formulations used in this study might also produce similar improvement factors in ocular:systemic ratios of timolol concentration when compared with commercial timolol eye drops. Because we showed the maximum decrease in $C_{\max}$ was achieved with the lowest phenylephrine concentration (0.8 mg/ml) (Table 1, Fig. 1), it may be possible to reduce the systemic concentrations of timolol significantly also by using smaller concentrations of phenylephrine. Also lower solution viscosities com-

bined with phenylephrine may cause similar improvements in ocular:systemic concentration ratios of timolol as shown in this study. This is suggested by the maximal decrease in $C_{\max}$ with both viscous eye drops with phenylephrine (Table 1).

It is clinically important to minimize the peak levels of timolol in systemic circulation, because the systemic side effects, known to emerge soon after administration of timolol eye drops,^{24–26} are associated with the peak beta-blocking activity in plasma. These results suggest that with phenylephrine-induced conjunctival and nasal vasoconstriction the $C_{\max}$ of timolol and the rate of systemic drug absorption can be reduced substantially. A combination of the decreased systemic absorption with phenylephrine and improved ocular absorption by mucoadhesive polymer may be an effective way to reduce the systemic side effects of timolol if the instilled concentration of phenylephrine is safe and does not affect intraocular pressure. Long-term efficacy of vasoconstriction in reducing systemic absorption of ocularly applied drugs still is not known, and the clinical efficacy and acceptability of this approach also need to be studied further.

Key words: ocular absorption, systemic absorption, timolol, phenylephrine, viscosity

Acknowledgments

The authors thank Markku Taskinen and Lea Pirskanen for their skillful technical assistance.

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