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Bioadhesion: The effect of polyacrylic acid on the ocular bioavailability of timolol

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Summary

The bioadhesive polymer, polyacrylic acid, was added to ophthalmic formulations and the effect on the ocular distribution of timolol evaluated. Ocular bioavailability of 0.5% Timoptol ® was measured in cornea, aqueous humor and iris + ciliary body, of albino rabbits and was compared to that of 0.5% timolol in isoviscous solutions of polyvinyl alcohol (PVA), polyacrylic acid (PAA) and timolol-polyacrylic acid salt (PAA salt). Ocular bioavailability of timolol was increased by each of the viscous solutions. These increases, assessed by measurement of AUC (0–4 h) in cornea, aqueous humor and iris + ciliary body ranged from 1.4- to 2.8-fold. The largest increases were obtained with the non-mucoadhesive polymer PVA. The bioadhesive PAA polymers modified the concentration vs time profiles of timolol and gave the highest timolol concentrations in iris + ciliary body at later sampling times.

Introduction

Topical administration of ophthalmic drugs in aqueous solution results in extensive drug loss, due primarily to tear fluid dynamics which occur in removing the solution from the eye (Lee et al., 1979). Usually, only a small fraction (1–3%) of the applied drug penetrates the cornea and reaches intraocular tissues (Patton and Robinson, 1976). Thus, for certain drugs, frequent administration of eyedrops is necessary to maintain an adequate level in the eye.

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For this reason, several approaches to extend the ocular residence time of topically applied medications have been reported and various ophthalmic vehicles, such as suspensions, ointments, inserts and aqueous gels, have been investigated (Lee and Robinson, 1986). These ocular drug delivery systems offer certain improvements over a conventional liquid dosage form but because of blurred vision (e.g., ointments) or lack of patient compliance (e.g., inserts), they are not universally accepted. Indeed, good ocular bioavailability after topical delivery of a drug to the eye remains a challenge yet to be satisfactorily resolved (Lee, 1990).

From the point of view of patient acceptability, a liquid dosage form is preferable. Ideally, this liquid should be able to sustain drug release and

to remain in contact with the front of the eye for extended periods of time. Bioadhesive polymers have been reported to attach, through non-covalent bonds, to the mucin layer of the conjunctival surface (Robinson, 1989). Such polymers have demonstrated promising improvements in the ocular bioavailability of the sparingly soluble drugs progesterone and fluorometholone (Hui and Robinson, 1985).

We have attempted to determine whether the addition of a mucoadhesive polymer could improve the ocular bioavailability of an ophthalmic agent. In this report, the ocular bioavailability of timolol has been studied using polyacrylic acid as the bioadhesive polymer. Since polymers increase the viscosity of the ophthalmic vehicle, the effects of the isoviscous non-mucoadhesive, polyvinyl alcohol were also compared with that of a simple aqueous solution of 0.5% Timoptol ®.

Materials and Methods

Ophthalmic vehicles

(A) Timoptol ®, 0.5% was used as a reference solution without addition of any bioadhesive agent.

Formulations containing 0.5% of timolol maleate (Merck & Co., West Point, PA) were prepared in the following solutions: (B) PAA: 0.6% polyacrylic acid (Mol.Wt. 250000, Aldrich-Chemie, Germany); (C) PAA salt: timolol-polyacrylic acid 0.6%. This was prepared by neutralizing the timolol base solution with the acidic polymeric groups from 0.6% polyacrylic acid; (D) PVA: 4.5% polyvinyl alcohol (Polyviol W 48/20, Wacker-Chemie, Germany).

Each of the formulations B–D contained 0.01% benzalkonium chloride as preservative and mannitol as isotonizing agent. The pH of the solutions was adjusted to 6.8 with 1 M NaOH to give a pH close to that of Timoptol ®.

Viscosity measurements

Viscosity of the test formulations was determined with a Rheomat 30 rotary viscosimeter (Contraves, AG, Zurich) equipped with a thermostated coaxial cylinder MS-O system. An Apple computer connected to a recorder gave shear stress vs shear rate values. The measurements were made at 33°C, which is the temperature of the eye surface. The shear rate was programmed to vary from 0.2 to 17 s^{-1} in 5 min.

Dialysis experiments

Dialysis was conducted by placing 0.5 ml of the test formulation into cellulose membrane tubing (Spectrapor no. 4, cut-off 12000–14000 Da, diameter 6.4 mm, thickness 0.0022 inch; Spectrum Medical Industries, CA). The membranes were closed at both ends with plastic clamps and immersed in 50 ml of dialysis solution pre-equilibrated at 33°C. This temperature was maintained throughout the test. Samples were rotated at 15 rpm for timed release experiments (Model 393, Hanson Research Co., CA). The formulations were dialysed against either de-ionized water or a 0.05 M pH 7.4 phosphate buffer. Aliquots (2.0 ml) were withdrawn at appropriate intervals and were replaced with an equal amount of fresh buffer. Timolol was analysed using a UV-visible recording spectrophotometer at 295 nm (Shimadzu). Each dialysis test was run in triplicate.

Treatment of animals

Albino rabbits (Charles River, France) were placed in wooden painted restraining boxes equipped with a chin-rest to minimize movement. Bilateral instillations of a 25 μl drop of each preparation were made into the conjunctival sac with a micropipette (Micropettor pipet. Scientific Manufacturing Ind., U.S.A.) and the lower lid brought gently up to meet the upper. Aqueous humor, cornea, iris + ciliary body were subsequently sampled as previously described (Grove et al., 1988).

Preparation of extracts for HPLC

Aqueous humor (120 μl) was deproteinized by addition of 18 μl of 20% trichloroacetic acid. The sample was placed in the refrigerator (4°C) for 30 min and then centrifuged in an Eppendorf 5412 S for 2 min. An aliquot of the supernatant was injected onto the chromatograph. The concentration of timolol was determined from a calibration

graph which was constructed from aliquots of a pool of aqueous humor, to which appropriate amounts of timolol were added and carried through the procedure.

The pre-weighed cornea was solubilized by transferring to a glass tube, adding 1 ml of 0.5 M KOH and heating for 30 min at 70°C in a Thermolyne Dri-bath (Pierce). A known quantity of internal standard was added to the cooled solution and extracted with 2×3.5 ml of ethyl acetate. After each extraction, the tubes were centrifuged and the organic phase transferred to another tube. The organic phases were pooled and evaporated to dryness under a stream of nitrogen.

The residue was reconstituted in 150 μl of mobile phase and an aliquot determined by HPLC. The iris + ciliary body was homogenized for about 1 min in a Kontes glass/glass homogenizer driven by an Akai motor set at 300 rpm. Timolol was extracted from the homogenate as described for the cornea. Calibration graphs were constructed by taking control tissues, adding known amounts of timolol, and carrying them through the procedure above.

Chromatography

A Varian Vista Model 5500 fitted with a 9090 automatic injector and a UV-200 detector was used, with a Hewlett Packard Integrator 3385A. Separation of timolol and its internal standard, isopropylaminohydroxypropoxymorpholinothiadiazole · HCl (L-714,440), was achieved by reverse-phase chromatography on a Brownlee Laboratories, Cyano 5 μm column (100×4.6 mm) fitted with the appropriate guard column (30×4.6 mm). Isocratic elution was carried out at 40°C with acetonitrile/0.06% phosphoric acid (30:70) using a flow rate of 1.2 ml/min. Under these conditions, when aqueous humor was assayed, the retention time of timolol was approx. 4.3 min, following direct injection of the trichloroacetic acid extract. When the iris or cornea were analysed and the sample dissolved in the mobile phase, the retention times were slightly increased to 6.0 and 6.7 min for L-714,440 and timolol, respectively. In all cases, good linear calibration plots were obtained.

Statistical analysis of results

A one-way analysis of variance was used for the comparison of timolol concentrations at each time point. An estimate for the AUC (area under the curve) for each treatment was calculated as a linear combination of the means for each time point using the trapezoidal rule. The standard error for each AUC allowed for the use of different animal populations at each time point. To determine how the treatments differed, the areas were listed in ascending order, and a Newman-Keul procedure (repeated application of the studentized range test) was applied (Winer, 1971).

Results and Discussion

Viscosity and rheological behavior of vehicles

The rheological behavior of an ophthalmic solution should be considered in relation to the tear fluid dynamics during blinking (Schoenwald et al., 1978; Bothner et al., 1990). The tear film is normally subjected to varying shear rates from 0 to 10000 s^{-1} (Bothner et al., 1990). Between blinks the tear film is essentially at rest and the shear rate is close to zero. It is well established that rabbits blink less frequently than man, i.e. about 4 times/h, compared with man who blinks 6–12 times/min. Bothner et al. (1990) reported that for high molecular weight polymers, viscosity will depend on the shear rate and, at low shear rates, viscosity will level off towards a constant value – the zero shear viscosity.

When comparing the ocular bioavailability of different formulations, a parameter often neglected is the viscosity of the vehicle. Recently, it has been demonstrated, in the albino rabbit, that ocular drug bioavailability is correlated with vehicle viscosity (Grove et al., 1990). Accordingly, to minimize the effects of viscosity, polymer concentrations were carefully adjusted in order to achieve the same viscosities. Since in the present studies, blinking was minimum, we chose to measure viscosity at low shear rates.

PVA, 4.5% showed a typical Newtonian behavior characterized by a constant viscosity of 45 ± 2 cps over the shear rate range studied (0–17 s^{-1}). Generally, at high shear rates, PAA shows a

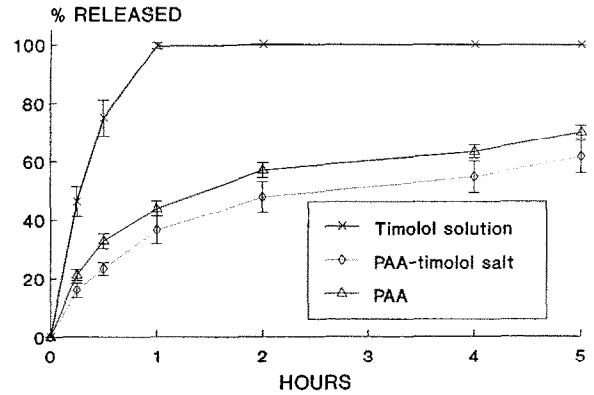


Fig. 1. Percentage release of timolol in 'in vitro' dialysis test of PAA formulations against deionized water. Error bars are ± S.D. ($n = 3$).

pseudoplastic behavior but, within this low shear range, 0.6% PAA had a constant viscosity of 45 cps. This is in agreement with the observations of Patton and Robinson (1975) who claimed that dilute polymer solutions generally exhibit or approximate Newtonian flow properties. Salt formation of timolol base with PAA had no effect on viscosity of the vehicle.

In vitro release

Diffusion of timolol from the formulations was compared with dialysis experiments using a 0.5% aqueous timolol solution as reference, against either deionized water or pH 7.4 phosphate buffer. Most of the timolol was released from the aqueous solution by 1 h (Figs 1 and 2).

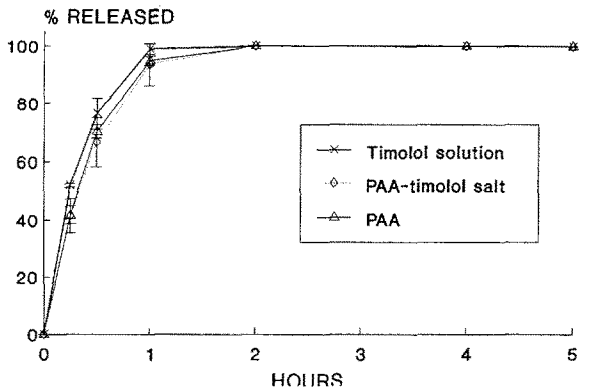


Fig. 2. Percentage release of timolol in in vitro dialysis test of PAA formulations against 0.05 M pH 7.4 phosphate buffer. Error bars are ± S.D. ($n = 3$).

Fig. 1 shows dialysis results obtained with deionized water. Timolol diffusion was retarded by the PAA vehicle. A release of 50% of the drug occurred by 4 h, but total release was not reached even after 24 h. No great difference in the timolol diffusion pattern was observed for both PAA formulations. The release from timolol maleate in PAA was only slightly faster than that from the timolol/PAA salt, indicating that ion exchange occurred between carboxylic groups of PAA vehicle and the maleate group of timolol.

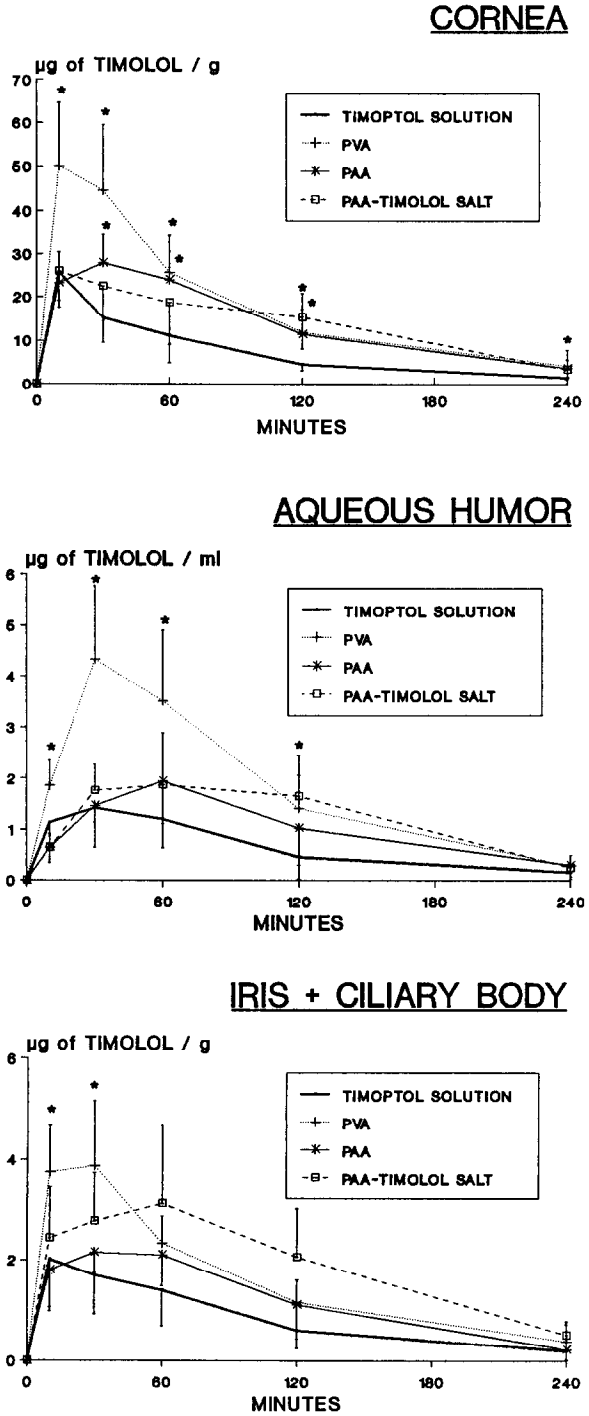
Using phosphate buffer (Fig. 2), practically no difference was noticed between the formulations, and timolol solution was completely dialysed by 2 h. Thus, timolol was displaced by sodium ions, confirming the ionic nature of the binding of timolol base to the anionic polymer in the PAA salt.

Ocular bioavailability

After instillation of the test formulations, aqueous humor, cornea and iris + ciliary body levels of timolol were measured and compared with values after instillation of 0.5% Timoptol ® aqueous solution.

The results are depicted in Fig. 3. Generally, timolol concentrations obtained with 0.6% PAA formulations and the 4.5% PVA formulation were higher than those after instillation of Timoptol ®. The ocular bioavailabilities, as assessed by AUC (0–4 h), are summarized in Table 1 for the three sites. Bioavailability was increased with each of the three polymer formulations ($P < 0.001$, Newman-Keul). The increases ranged from 1.4- to 2.8-fold.

The PVA formulation increased transcorneal penetration of timolol. Corneal concentrations of timolol were significantly higher at all time points (ANOVA $P < 0.05$). Similar significant differences were obtained in the aqueous humor (10–120 min) and iris + ciliary body (10 and 30 min). The 2-fold increases in AUC are in agreement with previous observations with pilocarpine and PVA (Patton and Robinson, 1975; Saettone et al., 1982) and another β-blocker, L-653,328 (Grove et al., 1990).



Compared to Timoptol ®, the two PAA formulations produced similar increases; bioavailability was statistically higher although not as large as that determined in the case of PVA (Table 1). The exception was the iris + ciliary body (Table 1), where the AUC was 7.93 μg h g^{-1} after PAA salt compared to 4.80 μg h g^{-1} after the PAA-timolol maleate solution.

Timolol concentrations in cornea, aqueous humor and iris + ciliary body were higher after instillation of PVA than those obtained with PAA at both 10 and 30 min, and in aqueous humor at 60 min (ANOVA $P < 0.05$). At the other time points, timolol concentrations after PVA or PAA instillation were essentially the same.

When we compared timolol concentrations obtained with both PAA formulations, there was no significant difference at any point, in cornea and aqueous humor. However, in the iris + ciliary body, at 120 and 240 min, timolol concentrations were significantly higher after instillation of the PAA salt. Comparable experiments have been carried out by Saettone et al. (1989a,b) with pilocarpine, ionically bound to a polyacrylic polymeric vehicle. Based on the AUC of the miosis response curve, they found that the polymer pilocarpine salt showed a significant increase in duration and intensity of activity. Davies et al. (1988) also reported increases in activity, using a PAA vehicle containing pilocarpine nitrate, not ionically bound to the polymeric vehicle.

Our data provide further evidence for the role of bioadhesion in ophthalmic formulations containing polyacrylic acid polymers. The present results demonstrate again that, in rabbits, the viscosity of the vehicle clearly increases the ocular bioavailability of an ophthalmic drug. Aqueous solutions of Timoptol ® and that containing the non-mucoadhesive polymer PVA generate maximum ocular concentrations at 10 or 30 min

Fig. 3. Concentrations of timolol in the cornea, aqueous humor and iris + ciliary body of albino rabbits given bilateral instillations of 25 μl of 0.5% timolol formulations. Values are means ± S.D. ($n = 12$). * Significantly higher ($P < 0.05$, ANOVA) than the corresponding timolol value.

TABLE 1

AUC (0–4 h) of timolol concentrations in ocular tissues of albino rabbits after instillation of various 0.5% formulations [a]

	(A) Timoptol ®	(B) PAA	(C) PAA-timolol	(D) PVA	Statistics [b]
Cornea	29.9	55.9	56.3	73.2	A B C D
Aqueous humor	2.71	4.06	5.03	7.29	A B C D
Iris + ciliary body	3.51	4.80	7.93	6.43	A B D C

[a] Expressed in $\mu g\ h\ ml^{-1}$ or $\mu g\ h\ g^{-1}$.

[b] Treatments compared separately for each region. Each ocular site had statistically significant differences among treatments ($P < 0.001$). Areas were placed in ascending order and compared statistically by the Newman-Keul test. Those not statistically higher ($P > 0.05$) are connected by a solid line. For more details see text.

post-instillation, but PVA did not radically alter the concentration vs time profile. This is consistent with previous observations using the β-blocker L-653,328 in a range of viscous solutions of hydroxyethyl cellulose (Grove et al., 1990).

In contrast, although polyacrylic acid produces higher ocular timolol concentrations than Timoptol ®, they are generally lower than those after PVA, and the concentration vs time profiles are flatter. This would be consistent with the slower release of timolol from PAA and the longer retention of the vehicle in the conjunctival sac by mucoadhesion. The dialysis experiments indicate that the timolol that is chemically bound to PAA would be released by displacement with tear-fluid ions. Since the volume of tear fluid in the rabbit is only 7 μl (Mishima, 1965) compared to the 25 μl instillate, there are not enough ions to displace timolol immediately for transcorneal penetration. Hence, a slow release of drug occurs with tear turnover and gives the high iris + ciliary body concentrations seen with the PAA salt formulation and a statistically significant higher AUC (Table 1). When the drug is not fully bound to the PAA the effect of mucoadhesion is less evident.

In conclusion, we have provided further evidence for the role of mucoadhesive polymers in ocular drug delivery. At present, the improvements in ocular drug bioavailability are modest and of the same order as those already reported using viscosifying agents. Bioadhesive vehicles for ophthalmic preparations should therefore be evaluated further.

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