Evaluation of Mucoadhesive Polymers in Ocular Drug Delivery. I. Viscous Solutions

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The potential of a mucoadhesive polymer as an ophthalmic vehicle is evaluated within the rabbit. Precorneal clearance of a mucoadhesive polymer solution (Carbopol 934P) is compared to that of an equiviscous nonmucoadhesive poly(vinyl alcohol) solution (PVA) and buffer (PBS). The precorneal retention of the Carbopol 934P, as studied by lacrimal dacryoscintigraphy, is shown to be significantly greater ($P < 0.05$) than that of PVA, which, in turn, is significantly greater than that of PBS. The effect of the polymer solution on the bioavailability of pilocarpine is subsequently assessed by measuring the relative miotic response intensities produced by a 1% solution of the drug. Carbopol 934P solution produces a significant increase ($P < 0.05$) in bioavailability as compared to PVA and PBS. The bioavailability from PVA is significantly greater ($P < 0.05$) than that from PBS. Studies evaluating vehicle–drug association indicated no binding of the drug to the polymer.

KEY WORDS: ocular drug delivery; mucoadhesion; poly(acrylic acid); ocular retention; pilocarpine; miosis.

INTRODUCTION

One of the major problems in ocular drug delivery is to provide and maintain an adequate concentration of the drug in the precorneal area. Most ophthalmic drugs are administered topically in the form of eye drops, a dosage form consisting of buffered, isotonic, aqueous solutions or suspensions of the drug. However, eye drops provide low bioavailability because of the efficient precorneal elimination processes resulting in most of the instilled solution being lost due to drainage within the first 15–30 sec postinstillation (1). The precorneal retention of solutions can be enhanced by the inclusion of viscosity enhancing agents such as poly(vinyl alcohol) and methyl cellulose (2,3). This approach is based on the concept that viscosity is the major factor influencing the ocular retention of the vehicle and subsequently the bioavailability of incorporated drug. However, other various physicochemical properties of the viscosity enhancing polymers, such as surface spreading and adsorptive characteristics, have been postulated to exert an influence (4–6).

The capacity of polymers to adhere to mucin-epithelial surfaces have been utilized to design drug delivery systems for application to mucous rich environments including that of the precorneal region. The pioneering work of Hui and Robinson (7) demonstrated that the ocular bioavailability of the drug progesterone was significantly increased by inclusion within a bioadhesive suspension, and recent reports by both Gurny *et al.* (8) and Saettone *et al.* (9) have confirmed the beneficial properties of mucoadhesive polymers in increasing both the precorneal retention of viscous solutions and the bioavailability of incorporated drug.

A wide range of both water-soluble and insoluble polymers has been investigated for the ability to bind to mucin/epithelial surfaces, and the adhesive capacity of poly(acrylic acid) has been demonstrated (10,11). The aims of the present investigation are

(a) to evaluate the potential of a poly(acrylic acid) to increase the precorneal residence of ophthalmic solutions, with the use of lacrimal dacryoscintigraphy, and
(b) to study the effect of the mucoadhesive polymer on the bioavailability of the model drug pilocarpine by measuring pupillary responses induced in rabbits.

MATERIALS

The following materials were used as received: Carbopol 934P (B. F. Goodrich & Co., Ltd., UK), poly(vinyl alcohol), fully hydrolyzed, MW 86,000 (Aldrich Chemical Company Ltd., UK), pilocarpine nitrate (Smith & Nephew Research Ltd., UK), and indium-113m, obtained by elution of a sterile 370-MBq generator (Amersham International, UK). All other reagents were of at least AnalaR grade and obtained from B.D.H. Chemicals Ltd., UK. All water used was glass distilled.

METHODS

Vehicles

Carbopol 934P solutions were prepared in filtered distilled water and then adjusted to pH 7.4 by titration with 1 *M* NaOH. The solutions were rendered isotonic by the addition of 0.9% (w/v) NaCl. Polyvinyl alcohol (PVA) solutions were prepared in filtered Sørenson's modified phosphate buffer (PBS). All polymer solutions were sterilized by autoclaving at 121°C with a holding time of 15 min.

Viscosity Determinations

Rheological characterization of the sterilized polymer solutions was undertaken at 33°C using a Rheomat RM-30 rotary viscometer (Contraves A.G.) employing the MS-O measuring system.

Polymer Radiolabeling Technique

Thirty microliters of a sodium acetate solution (0.05%, w/v, in 0.4 *M* HCl) was added to 6 ml of eluate containing approximately 250 MBq of indium-113m chloride. This was subsequently neutralized by the addition of 0.04 *M* NaOH and rotary evaporated (Buchi, Switzerland) at a temperature of 85°C to a volume of less than 0.1 ml prior to mixing with the test polymer solution.

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Scintigraphic Precorneal Clearance Assessment

Five unanesthetized, preconditioned male NZW albino rabbits (3–4 kg) were used in a randomised manner in all experiments. The precorneal clearance was measured using a gamma-camera (IGE Maxicamera, 400T) fitted with a 3-mm pinhole collimator and tuned to detect gamma-radiation of energy within the range 343–443 keV. The test animals were held stationary in restraining boxes and positioned such that the eye was 5 cm from the collimator aperture. This allowed for the acquisition of a clear image situated within a predetermined area in which the radiation count rate variation is less than 10% for a uniform distribution of activity. The polymer concentrations chosen for the *in vivo* study were 0.49% (w/v) Carbopol 934P and 6.0% PVA, each giving rise to solution viscosities of 60 mPa · sec at unit shear rate. The eye drop, 20 μl of labeled polymer solution or PBS in which an indium-113m–DTPA complex had been dispersed, was instilled into the lower fornix of the conjunctival sac and the eye manually blinked to distribute the solution over the cornea. A dynamic protocol of 6 × 5- and 16 × 15-sec frames was employed, followed by a series of static images taken at various time intervals up to 3270 sec postinstillation. The images were stored on computer for later analysis (Link Analytical System, MAPS 5,000).

For image analysis, three anatomical regions of interest (RoI) were defined: the cornea, inner canthus, and lacrimal sac, together with a fourth region which encompassed the total area of observation. To correlate the data, the activity associated with each RoI in each new frame of study was normalized to the activity associated with the new total area of observation. Graphs of percentage activity remaining in the corneal region as a function of time were plotted and calculation of the AUC taken as a measure of the corneal residence of the instilled solution. Drainage rate constants and $t_{0.5}$ values were determined by least sum of squared errors analysis (MINIM, R. D. Purves) and the percentage total residence associated with the initial rapid drainage phase calculated from the determined AUC for both the initial and the basal clearance phases.

Bioavailability Study

From previously determined dose–response studies, a submaximal concentration of pilocarpine nitrate (1%) was formulated in sterile PBS and equiviscous (60 mPa · sec at unit shear rate) solutions of Carbopol 934P and PVA on the morning of the *in vivo* study.

The procedure used for the bioavailability study was as described for the clearance study using six male NZW rabbits (3–4 kg), with a rest period of not less than 3 days allowed for each rabbit between successive studies. The test animals were positioned in restraining boxes in the normal upright position in a room with constant light intensity and devoid of distractions. All rabbits were acclimatized to the laboratory testing conditions for 30 min prior to initiating the study. Pupil diameter measurements were taken photographically at various time intervals postinstillation of 20 μl of each formulation, and the measurements standardized to a scale placed next to and in the same plane as the pupil. The changes in pupillary diameter were converted to miotic response intensity values (IR_t) according to the equation $IR_t = (I_o - I_t)/I_o$, where I_o is the average baseline diameter and I_t the pupil diameter at time t. Peak miotic response intensities (maximum change in pupil diameter), duration of action (time for pupil to return to baseline value), and area under the miotic response intensity–time curve, AUC (calculated via the trapezoidal rule), were evaluated. The results were statistically analyzed using Duncan's test for variability.

Pilocarpine–Polymer Association Studies

Binding of pilocarpine (2%, w/v) to the polymer solution was investigated by equilibrium dialysis at 33°C using a dianorm apparatus (Diachema, A.G., Zurich) employing a semipermeable cellulose membrane with a molecular weight cutoff of 5000 Da. The presence of pilocarpine in the dialysate was assayed by UV spectrophotometry (λ_{max}, 300 nm) after initial studies had shown no binding of the drug to either the cellulose membrane or the Teflon cells.

RESULTS

The precorneal clearance of ophthalmic solutions is significantly affected by the viscosity of the instilled solution, with changes in viscosities at values less than 15 mPa · sec having a greater effect than changes at values in excess of 15 mPa · sec (3). Thus, equiviscous solutions of Carbopol 934P and PVA were required for the *in vivo* precorneal clearance study having viscosities in excess of 15 mPa · sec (to avoid different clearance kinetics due to small viscosity changes), at a very low rate of shear, i.e., similar to that experienced in the rabbit eye. The pseudoplastic behavior of both polymers was more pronounced for Carbopol 934P, which also exhibited greater viscosity-enhancing properties than PVA. Curve fitting (least squares regression analysis) permitted the extrapolation of the viscosity curves to unit shear rate and the calculation of the polymer concentration exhibiting a viscosity of 60 mPa · sec. For PVA the concentration is 6.0% (w/v) and for Carbopol 934P, a concentration of 0.49% (w/v).

After 5 hr of dialysis, 97 and 99% of the ^{113m}In remained associated with the PVA and Carbopol 934P, respectively, thus demonstrating the effectiveness of the labeling procedures.

By defining regions of interest around the cornea, inner canthus, and lacrimal sac, the movement of the isotope between these anatomical regions could readily by quantified. The activity in the lacrimal sac reflects drainage of materials down the nasolacrimal apparatus and is not relevant to precorneal drainage. It was, therefore, considered sufficient to measure clearance parameters in the cornea and inner canthus alone. Figures 1 and 2 show the activity profiles of the various test solutions for the cornea and inner canthus.

The clearance of the solutions from the precorneal region is biphasic (Fig. 1), with an initial rapid phase (30–60 sec) followed by a much slower drainage phase. Greater than 50% of the PBS solution was cleared within the first 30 sec postinstillation. At 270 sec, corresponding to the termination of the dynamic study, approximately 80% of the PBS, 75% of the PVA, and 60% of the Carbopol 934P solution had been lost from the corneal region, while approximately 9, 14, and 24%, respectively, remained associated with the cornea at 3270 sec, corresponding to the termination of the study. Analysis of the activity–time profiles for the inner canthus

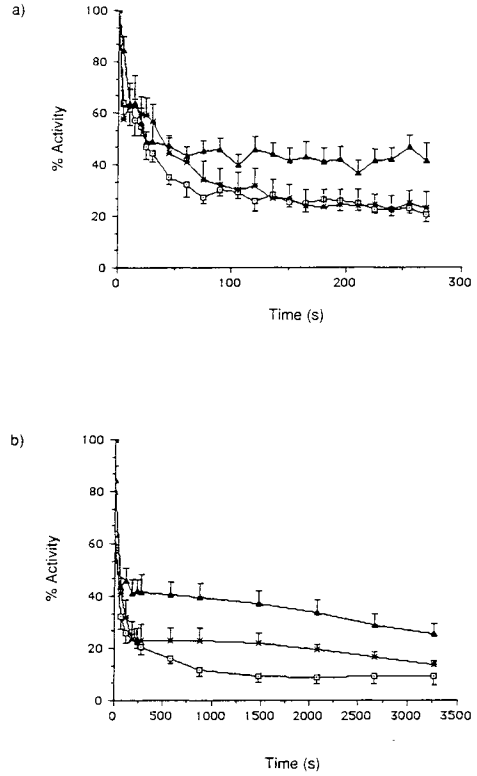

Fig. 1. Precorneal drainage profiles of PBS (□), PVA (*), and Carbopol 934P (▲). (a) 0–270 sec; (b) 0–3270 sec. Values represent the mean ± SE of five experiments.

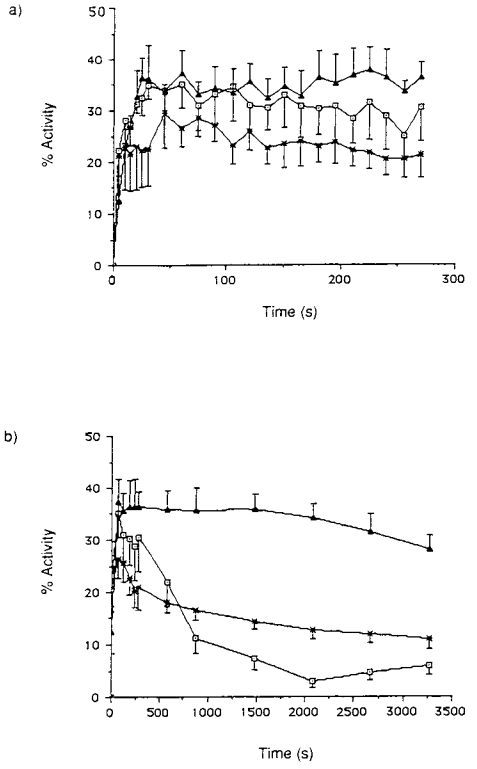

Fig. 2. Inner canthal activity profiles of PBS (□), PVA (*), and Carbopol 934P (▲). (a) 0–270 sec; (b) 0–3270 sec. Values represent the mean ± SE of five experiments.

(Fig. 2) also confirms this rapid initial precorneal clearance phase, with the maximum activity associated with this region being reached within the first minute, followed by steady drainage and accumulation of the solution within the lacrimal sac.

Calculation of the corneal AUC (0–3270 sec) was taken as a measure of the corneal residence of the instilled solution. Analysis of variance (5% confidence limits) of the AUC using Duncan's test for variability demonstrated that the precorneal retention of the Carbopol solution is significantly greater than that of the PVA solution, which in turn is significantly greater than that of PBS. Similarly, the inner canthal retention of the Carbopol 934P is significantly greater than that of the PVA which in turn is greater than that of the PBS. Consideration of the percentage activity remaining at the termination of the experiment further demonstrates the enhanced precorneal retention of the polymer solution with Carbopol 934P > PVA > PBS ($P < 0.05$). A similar trend was observed for the inner canthal region, with the viscous solutions being cleared more slowly and showing a "plateau" effect as compared to the PBS solution. Table I summarizes the main clearance parameters.

The results of the miosis study are shown graphically in Fig. 3 and the main activity parameters are summarized in Table II. The area under the miotic response intensity–time curve (AUC) for each preparation is of particular importance, as it is indicative of the bioavailability of pilocarpine from each vehicle. Administration of 1% pilocarpine nitrate in a Carbopol 934P solution produced a statistically significant increase ($P < 0.05$) in bioavailability, as determined by the AUC, compared to an equiviscous PVA solution and PBS. The bioavailability from the PVA solution is in turn significantly greater than from PBS. The duration of action associated with the Carbopol was prolonged as compared to that observed for PBS ($P < 0.05$). Peak miotic response intensities for PVA and Carbopol 934P were similar but significantly different to those produced by PBS.

Investigation of the binding of pilocarpine by the polymer solution demonstrated no association of the drug with the polymer, an unexpected result when consideration is given to the charge on the drug molecule and that on the polymer at physiological pH. Thus, the beneficial effects of the Carbopol 934P in promoting the availability of pilocarpine as compared to an equiviscous solution of PVA is likely to be due to its enhanced precorneal retention rather than to association of pilocarpine with the Carbopol 934P.

DISCUSSION

Many workers in the field of ophthalmic vehicles have investigated the influence of various polymers on the availability of ophthalmic medication. Most attention has been directed toward the influence of solution viscosity, although it has been reported that the maximum bioavailability increase that can be obtained in rabbits by increasing the viscosity of the vehicle is approximately twice that of a nonviscous solution (3). However, other physicochemical properties of the viscolyzer may also influence both the corneal retention and the bioavailability from ophthalmic solutions (4,12).

Table I. Summary of Clearance Parameters (±SE)

	PBS	PVA	Carbopol 934P
Cornea			
% remaining after 3270 sec	9.00 ± 3.17	13.60 ± 1.33	23.50* ± 4.16
AUC relative to PBS	1.00 ± 0.19	1.80* ± 0.56	2.90* ± 0.75
kd_1 (min^{-1})	2.28 ± 1.38	1.26 ± 0.21	5.10 ± 1.75
$t_{0.5}$ (min)	0.30 ± 0.17	0.55 ± 0.11	0.14 ± 0.05
kd_2 (min^{-1})	0.03 ± 0.01	0.01 ± 0.004	0.01 ± 0.002
$t_{0.5}$ (min)	23.10 ± 9.9	77.00 ± 13.4	68.00 ± 21.9
% of total residence associated with initial phase	3.00 ± 1.78	1.46* ± 0.65	0.25* ± 0.11
Inner canthus			
% of max. remaining after 3270 sec	16.5 ± 3.71	37.7* ± 5.6	63.7* ± 3.49
AUC relative to PBS	1.0 ± 0.18	1.4 ± 0.05	3.2* ± 0.29

* Values statistically significant with respect to PBS ($P < 0.05$).

Recently, polymers capable of adhering to the mucin coating of the corneal and conjunctival surface have been recognized as potential viscosity enhancing agents for use in ophthalmic solutions (8,9). Park and Robinson (10) demonstrated that solutions of poly(acrylic acid) were able to interact with cultured, human conjunctival epithelial cells. In the present study, the adherence capacity of the poly(acrylic acid) Carbopol 934P to the precorneal region of the rabbit has been substantiated by lacrimal dacryoscintigraphy, with Carbopol 934P solutions demonstrating a prolonged precorneal residence as compared to an equiviscous solution of PVA.

The bioavailability studies conducted have also demonstrated the superiority of Carbopol 934P as an ophthalmic vehicle despite the additional loss factors experienced upon incorporation of pilocarpine, as discussed by Lee and Robinson (13). Increases of 43 and 23% in the bioavailability and duration of pilocarpine action relative to an equiviscous PVA solution and 145 and 65%, respectively, as determined relative to PBS were observed. Morimoto *et al.* (14), examining the effect of a poly(acrylic acid) gel on the nasal absorption of insulin and calcitonin, postulated that the enhancement produced was due to polymer effects on increased water influx through the intracellular channels. This may form a plausible explanation for the increased ocular bioavailability of pilocarpine, a water-soluble molecule, as observed in this study. However, studies on the mechanism of corneal penetration of pilocarpine (15) have revealed a transcellular route of absorption for both the cationic and the nonionized form of pilocarpine. Thus, the mechanism of increased water influx due to poly(acrylic acid) may not account for the observed effects.

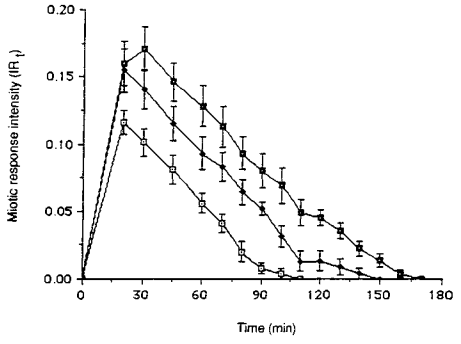

Fig. 3. Miotic response intensities elicited by PBS (⊡), PVA (♦), and Carbopol 934P (□). Values represent the mean ± SE of six experiments.

Saettone and co-workers (6,9,16) have also examined the effects of poly(acrylic acid) and PVA on the ocular bioavailability of ophthalmic drugs. These workers reported an increased duration of action and bioavailability of similar magnitude to that observed for the respective polymers in the present study. However, a poly(acrylic acid) gel (9) which had been demonstrated to possess mucoadhesive properties and to form a stable film for up to 2 hr was unable to increase the bioavailability parameters as compared to a poly(acrylic acid) solution (16). Failure to increase further the bioavailability parameters by employing a gel rather than a solution as observed previously (16) was attributed to the high solubility of pilocarpine, which would allow it to leach out rapidly from the vehicle, thus partially voiding any effect due to vehicle-mediated increased precorneal retention. When the study was repeated employing the less soluble drug tropicamide, it was found that the duration of action and the AUC were further increased as compared to an aqueous solution. Thus, it appears that rapid leaching of the drug from the formulation may reduce any benefits attained from the prolonged precorneal residence of the vehicle.

Investigation of pilocarpine–polymer binding revealed no association of the drug to either polymer. Thus, the prolonged precorneal residence provided by the Carbopol 934P

Table II. Main Activity Parameters of Pilocarpine Solutions (±SE)

	Peak miotic response intensity (IR_t max)	AUC (relative to PBS)	Duration of action (min)
PBS	0.121 ± 0.010	1 ± 0.1	97 ± 4.9
PVA	0.158 ± 0.016	1.7 ± 0.17	130 ± 6.3
Carbopol 934P	0.177 ± 0.016	2.45 ± 0.25	160 ± 5.7

solution may not necessarily provide a prolonged precorneal residence of pilocarpine. However, examination of the bioavailability data does show a slight delay in time to reach maximum response and an increase in maximum miotic response intensity, an observation which does suggest an enhanced precorneal residence of the drug. These observations may be attributed to the time for diffusion to occur or, as is observed from the drainage profiles, a decrease in percentage removed in the initial rapid drainage phase. However, the beneficial effects of increased precorneal residence may not be fully exploited due to the rapid diffusion of the drug from the vehicle and subsequent drainage from the precorneal area. A delivery system which would allow the drug to remain associated with a vehicle exhibiting enhanced precorneal retention may, therefore, provide for an attractive ocular drug delivery system.

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