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Methylmethacrylate sulfopropylmethacrylate copolymer nanoparticles for drug delivery Part III: Evaluation as drug delivery system for ophthalmic applications

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Abstract

Copolymer nanoparticles were investigated as carrier systems for the topical ophthalmic application of the muscarinic agonists arecaidine propargyl ester (APE) and (S)-(+)-aceclidine in rabbits and compared to conventional eye drop preparations. The copolymer nanoparticles were prepared by free radical polymerization of methylmethacrylate (MMA) and sulfopropylmethacrylate (SPM). The in-vivo activity of the drug-containing carrier systems was tested by the measurement of the miotic effect observed after local administration in rabbits. It has been found that the copolymer nanoparticles were able to produce a significant increase of the ocular APE bioavailability as determined by the area under the miosis-time-curve (AUC). The nanoparticle preparations were tolerated without any irritating effect in the rabbit eye. Besides the copolymer nanoparticles, different formulations containing bioadhesive or viscosity-enhancing polymers with and without additional nanoparticles were tested. The administration of APE-loaded copolymer nanoparticles was found to be equivalent in efficacy to solutions containing the soluble polymers without nanoparticles. The combination of the nanoparticles with bioadhesive polymers further increased the ocular drug bioavailability. Hyaluronic acid alone or in combination with copolymer nanoparticles was observed to be the most effective soluble polymer for ophthalmic application by enhancing the AUC of miosis-time-curve 2-fold. Similar effects induced by the carrier systems were obtained with (S)-(+)-aceclidine. However, the magnitude of the enhancement of the miotic effect that is achievable by the binding of the drug to nanoparticles over free drug is much more pronounced with the short acting drug APE than with (S)-(+)-aceclidine. © 1997 Elsevier Science B.V.

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1. Introduction

The local therapy of ocular diseases is impeded by the protective mechanisms of the eye such as rapid tear turnover, lacrimal drainage and drug dilution by induced lacrimation. The relative impermeability of the cornea in combination with the rapid elimination of instilled aqueous solutions results in the fact that only 1–2% of the applied dose reaches intraocular tissues. As a consequence, topical application of aqueous solutions to the eye has to be considered as inefficient in many cases. Several approaches to improve the ocular drug absorption were undertaken by maximizing the precorneal residence time of the preparations. The most popular approach to extend drug residence time is the incorporation of soluble polymers into the aqueous eye drop preparations in order to increase the vehicle viscosity and, as a consequence, to reduce the solution drainage (Chrai and Robinson, 1974; Saettone et al., 1982). Besides such liquid preparations, gels and ointments were used as carrier systems. However, they possess several disadvantages such as a blurring of vision with associated discomfort for the patient. An alternative to preparations with an enhanced viscosity are colloidal carrier systems like liposomes or nanoparticles (Meisner and Mezei, 1995; Zimmer and Kreuter, 1995). They may be applied in liquid form like eye drop solutions. By interaction with the glycoproteins of the cornea and conjunctiva they can form a precorneal depot resulting in a prolonged release of the bound drug. Particularly, the combination of nanoparticles based on polyacrylic acid derivatives with cholinergic drugs used for the therapy of glaucoma was the subject of several publications (Harmia et al., 1986a; Diepold et al., 1989; Zimmer et al., 1994). It has been shown, that nanoparticles were capable of improving the pharmacodynamic drug responses such as miosis and reduction of the intraocular pressure (IOP).

The objective of this study was to evaluate the suitability of nanoparticles as carriers for the muscarinic agonists arecaidine propargyl ester (APE) and (S)-(+)-aceclidine and to compare the nanoparticle preparations to conventional systems such as aqueous eye drops and preparations with an enhanced vehicle viscosity. In comparison to the commonly used antiglaucoma drug pilocarpine, APE was shown to exhibit a higher potency in ocular studies in rats (Hagan et al., 1988) whereas (S)-(+)-aceclidine, the eutomer of the commercially available racemic drug (Lambrecht, 1976a,b; Ehlert et al., 1996), is a moderately potent muscarinic agonist, demonstrating similar activity as pilocarpine on intraocular pressure, miosis and accomodation in humans (Keren and Treister, 1980). It is also worth mentioning, that (S)-(+)-aceclidine is a very poor substrate for acetylcholinesterase (Lambrecht, 1982). The combination of nanoparticle carrier systems with highly potent drugs are expected to hold promise regarding a reduced application frequency and, therefore, to enable the improvement in the compliance of glaucoma patients.

2. Materials and methods

2.1. Reagents and chemicals

The arecaidine propargyl ester hydrobromide (APE·HBr) was synthesized by EMKA-Chemie (Markgröningen, Germany) according to a method previously described (Moser et al., 1989). S-(+)-aceclidine hydrobromide was synthesized in one of our laboratories according to the literature (Lambrecht, 1976a,b). Methylmethacrylate (MMA) (Merck-Schuchardt, Hohenbrunn, Germany) and sulfopropylmethacrylate-potassium (SPM) (Hüls, Marl, Germany) were used as monomers for the nanoparticle preparation. Ammonium persulfate was purchased from Hüls (Marl, Germany). Sodium hydrogen carbonate was purchased from Roth (Karlsruhe, Germany). Dichloromethane, mannitol, sodium chloride, sodium dihydrogenphosphate-monohydrate and disodium hydrogenphosphate-dodecahydrate were obtained from Merck (Darmstadt, Germany).

2.2. Preparation of nanoparticles

The copolymer nanoparticles were prepared as previously reported by free radical polymerization in water (Langer et al., 1996). An amount of 0.375 g SPM was dissolved in 75 ml water at 78°C, and 3.375 g MMA and 22.5 mg (= 0.03%) ammonium persulfate as polymerization initiator were added under stirring on a heated stirring plate at 400 rpm. The polymerization was carried out over a period of 24 h. The resulting suspensions were pooled and concentrated to obtain final polymer contents of about 25% (w/v) by using an Amicon Stirring Unit Series 8400 equipped with an Amicon YC05-filtration membrane (Amicon, Witten, Germany). The polymer contents of the resulting suspensions were determined by gravimetry.

2.3. Particle size by photon correlation spectroscopy (PCS)

The particle size of the resulting nanoparticles was determined by photon correlation spectroscopy (PCS) (De Jaeger et al., 1991). For the PCS study a BI-200SM Goniometer Vers. 2.0 (Brookhaven Instruments, Holtsville, NY) equipped with a 30 mW He–Ne laser and connected to a BI-2030AT Digital Correlator was used. The count rate was adjusted to a value of 20 kHz by diluting the samples with water. The measuring angle was fixed to 90° and the pinhole selection of the photomultiplier was set to 100 μm. The water used for dilution was filtered through a 0.22 μm filter unit (Millex-GS, Millipore, Molsheim, France). The particle size was expressed by the effective diameter (De Jaeger et al., 1991), and the width of the size distribution was characterized by the polydispersity index (Koppel, 1972). The software data analysis for calculating the size distribution of the nanoparticle samples was based on the fitting by non-negative constrained least-squares (NNLS) (Finsy et al., 1992).

2.4. Determination of APE

The gas chromatographic determination of APE was conducted by an analytical method reported earlier (Langer et al., 1997a). 100.0 μl of an aqueous APE solution, containing 0.10–8.5 μg APE·HBr, were transferred to a conical mini vial (Mini Vial 3.0 ml, Alltech, Unterhaching, Germany). 100.0 μl of a 0.004% aqueous solution of arecoline hydrobromide (internal standard), 600.0 μl of a 6% solution of sodium hydrogen carbonate and 1.0 ml of dichloromethane were added. The biphasic mixture was shaken for about 2 min. After separation of the phases, the organic layer was transferred to a second vial and evaporated under a gentle stream of nitrogen to a volume of about 30 μl. A 2 μl aliquot of each dichloromethane solution was injected to a GC system consisting of a HP 5890 Series II gas chromatograph equipped with a flame ionization detector system (FID) and a HP 3396A integrator (Hewlett-Packard, Bad Homburg, Germany).

2.5. Determination of (S)-(+)-aceclidine

The determination of (S)-(+)-aceclidine was performed in analogy to the gas chromatographic assay of APE described above. 100.0 μl of an aqueous (S)-(+)-aceclidine solution, containing between 10–100 μg aceclidine hydrobromide, were transferred to a conical mini vial. 100.0 μl of a 0.05% solution of arecoline hydrobromide (internal standard), 600.0 μl of a 6% solution of sodium hydrogen carbonate and 1.0 ml of dichloromethane were added. The sample preparation was carried out as described for the APE determination but without the evaporation step.

2.6. Drug loading

An amount of each drug, accurately weighted, was placed in a reaction cap (Greiner, Solingen, Germany) and an aliquot of the nanoparticle stock solution was added. In order to achieve isotonicity, mannitol or phosphate buffer, respectively, was added, followed by dilution of the suspensions with distilled water to 1.0 ml. If necessary, the pH of the preparations was adjusted to 6.2 prior to the dilution step. The preparations were shaken for a period of 3 h using an Eppendorf Thermomixer 5436 (Eppendorf-Netheler-Hinz GmbH, Hamburg, Germany).

After an equilibration time of 3 h an aliquot of each suspension was transferred to Microcon 10 microconcentrators (Amicon, Witten, Germany) followed by centrifugation at $10\,000 \times g$ for 1 h in an Eppendorf centrifuge 5417 (Gerätebau Eppendorf, Engelsdorf, Germany). The filtrates were assayed for the drug content as described above and the loading efficiency was calculated.

2.7. Preparation of the polymer solutions

The polymers polyacrylic acid (Carbopol 941®, BF Goodrich, Cleveland, OH), hyaluronic acid (Inst. f. Exp. Mikrobiologie, Friedrich-Schiller-Universität, Jena, Germany), methylhydroxyethylcellulose (Tylopur® MH 300, Hoechst, Frankfurt, Germany) and mucin (mucin type I-S from bovine submaxillary glands, Sigma, St. Louis) were dissolved in isotonic phosphate buffer pH 6.8, followed by the addition of the respective drug. The flow time of each preparation was determined in a Cannon-Fenske viscometer ($k = 0.0169$, Schott, Hofheim, Germany) and the density was measured in a DMA 48 density meter (AP PAAR, Graz, Austria). The dynamic viscosity at 20.0°C was calculated.

2.8. Miosis measurements

For the miosis study New Zealand white rabbits (Thomae, Biberach a.d. Riss, Germany), weight 3–4 kg, were used. All tests were performed in the same room under standard lighting conditions. A metric measuring tape (1 cm) was fixed under the eye in the optical section of the iris. After 20 min of acclimatization in restrainer boxes (Woetho, Teningen, Germany) the basal pupil diameter was measured by using a video system (Panasonic NV-M7, VHS-HQ Camcorder, Matsushita, Osaka, Japan) at a time interval of 15 min. The focus of the camera was adjusted to the iris. Each preparation was then tested in 6–10 animals by instilling a dose of 25 μl into the everted conjunctival sack of the left eye. After dosing, the lids were gently held together for 30 s in order to minimize loss of the dosage form. Pupil pictures were recorded at predetermined timepoints. The pupil size was measured using the still frame function of a video tape recorder (Toshiba V-711G, Toshiba, Mönchengladbach, Germany) and was calculated in relation to the metric measuring tape. Treatment effects on pupil diameter were expressed as the change relative to basal pupil diameter. The efficiency of a carrier system was estimated by the maximal miotic effect (E_{max}) and the area under the miosis-time-curve (AUC; miotic response) after administration of the respective preparation. The AUC (mm·min) of the tested vehicle was calculated using the trapezoidal rule. For statistical analysis the unpaired Student's t-test was performed. Data are presented as arithmetic means of n experiments ($\pm$ S.D., where appropriate).

3. Results and discussion

Cholinergic agonists, such as pilocarpine, ($\pm$)-aceclidine and carbachol, have been used for many years in the treatment of glaucoma. Their therapeutic action is based on their ability to contract the ciliary muscle. These contractions open the ocular angle and the trabecular meshwork, thereby promoting the drainage of aqueous humor and a decrease in intraocular pressure (Ehlert et al., 1996). Cholinomimetic drug-induced ciliary muscle contraction also causes unwanted spasm of accommodation, and stimulation of the iris sphincter muscle produces miosis, both of which limit the clinical usefulness of this drug class. The three functional responses to cholinomimetic drugs are all mediated by muscarinic M_3-receptors (Gabelt and Kaufman, 1992).

Arecaidine propargyl ester (APE) is a selective and highly potent muscarinic agonist that may offer new therapeutic perspectives (Wolf-Pflugmann et al., 1989). Although a lot of work has been done to characterize its pharmacological properties in vitro, only one paper has been published dealing with its topical administration to the eye (Hagan et al., 1988). In this earlier study the rat was used as an animal model. The miotic responses to APE were characterized by a rapid onset of action (10 min) followed by a rapid reduction in effect at all subsequent time points.

Most other agonists employed in the study of Hagan et al. (1988), such as pilocarpine, achieved their maximal effects 20–30 min after application. Consequently, it seemed promising to prolong the pharmacodynamic drug response by binding APE to a colloidal carrier system.

Prior to the administration of nanoparticle preparations the potency of APE after topical application to the rabbit eye was determined. For this purpose, APE was dissolved in phosphate buffer (pH 6.2) in concentrations between 0.1 and 2.0% (calculated as the free base) and the miotic response of the animals was recorded over a period of 120 min (Fig. 1). As already reported in the rat study (Hagan et al., 1988), the pharmacodynamic response was marked by a rapid onset of the miosis with a maximum after 10 min, after 30 min no statistical significant difference to the basal pupil diameter was observed. The miotic responses to APE were dose-dependent and linear regression analysis (Ross, 1990) led to an ED_{50} value of 8.1 $mg \cdot ml^{-1}$, corresponding to 0.20 $mg \cdot animal^{-1}$. Thus, the potency of APE in the rabbit eye was $\approx$470-fold lower than after administration to the rat eye (0.43 $\mu g \cdot animal^{-1}$; Hagan et al., 1988). A reason for this discrepancy may be the comparatively high esterase activity in ocular tissues of rabbits such as cornea, iris-ciliar body and aqueous humor (Lee, 1983; Lee et al., 1983). For the aqueous humor of rabbits it was shown that APE was cleaved, leading to arecaidine and propargyl alcohol (Langer et al., 1997a). In the following experiments, APE was used in a concentration of 1.0%, corresponding to a concentration of 1.45% APE · HBr.

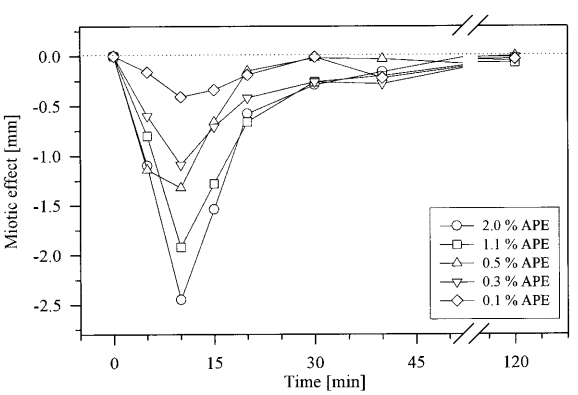

Fig. 1. Miotic responses after topical administration of aqueous APE solutions to the eyes of rabbits ($n \geq 5$). S.D. were omitted for the sake of clarity.

3.1. Influence of particle concentration on miotic responses

In order to obtain a nanoparticle carrier system for APE, methylmethacrylate sulfopropylmethacrylate copolymer nanoparticles were prepared by free radical polymerization. The content of the charged comonomer SPM was maintained at 10% of the total monomer throughout the experiments. After ultrafiltration, nanoparticles at different concentrations were loaded with APE and tested in the miosis study. A summary of the miotic activity data of these preparations is given in Table 1. It became obvious that a total polymer concentration of 10% was required to obtain a significant enhancement of the drug response, calculated as the area under the miosis-time-curve of the polymer preparation relative to the aqueous drug solution (AUC_{rel}). The binding to nanoparticles improved the miotic AUC by $\approx$50% (sample 5–7). This value was in accordance with earlier results concerning the in-vitro release of APE determined by dynamic dialysis (Langer et al., 1997b). In the earlier paper it was shown that a polymer concentration of 10% was more effective for achieving a prolonged release than a polymer content of 4%. Higher polymer contents led to no further increase in the miotic activity data (sample 7). The miotic peak time was found to be 10 min for all preparations. The addition of ionic components such as a phosphate buffer system instead of mannitol to achieve isotonicity (sample 6) reduced the loading efficiency of the carrier system. Despite the reduced drug-loading of the phosphate-containing system the miotic response was the same as with the mannitol containing system (sample 5). All nanoparticle preparations were tolerated without any irritating effect in the rabbit eye.

In former studies the binding of pilocarpine (2%) to polybutylcyanoacrylate (PBCA)-nanoparticles (Zimmer et al., 1994) and albumin-nanoparticles (Zimmer et al., 1995) yielded a comparable

Table 1
Summary of the miotic activity data of APE-loaded copolymer nanoparticles at different polymer concentrations ($n \geq 6$)

Sample no.	Preparation	Polymer concentration (%)	Particle size (nm)	Loading efficiency (%)	E_{max} (mm)	$AUC_{60\ min}$ (mm · min)	AUC_{rel}
1	Reference solution for no. 4	0	—	—	1.25 (0.45)	23.14 (9.51)	1.000
2	Reference solution for no. 5, 6	0	—	—	2.07 (0.51)	31.49 (8.68)	1.000
3	Reference solution for no. 7	0	—	—	1.68 (0.62)	21.40 (8.98)	1.000
4	Copolymer nanoparticles	4.0	107.1	31.7	1.23 (0.23)	22.00 (8.25)	0.950
5	Copolymer nanoparticles	10.0	101.1	53.6	2.43 (0.44)	47.33 (5.00)	1.503***
6	Copolymer nanoparticles (phosphate buffered prep.)	10.0	102.6	39.3	2.89 (0.30)	48.31 (12.31)	1.534**
7	Copolymer nanoparticles	22.2	109.9	58.5	1.87 (0.77)	32.25 (11.82)	1.507*

Numbers in parentheses represent S.D.
Level of statistical significance of differences from APE reference: *$p < 0.10$; **$p < 0.05$; ***$p < 0.01$, Students t-test.

increase in the miotic AUC relative to the drug reference. In order to enable a better comparison of the results with APE with the former studies that were performed with PBCA-nanoparticles, an effort was made to develop a PBCA-carrier system for APE. However, the incorporative and adsorptive loading of the drug APE to PBCA-nanoparticles prepared by an emulsion polymerization technique led to no statistical significant loading. Administration of APE-containing PBCA-nanoparticles to rabbit eyes led to comparable miotic effects to an aqueous APE solution (data not shown) because of the insufficient drug loading. A possible explanation for this insufficient loading is the much larger hydrophilicity of APE in comparison to the drugs formerly used in binding studies of nanoparticle preparations such as pilocarpine (Harmia et al., 1986b), betaxolol (Marchal-Heussler et al., 1990), progesterone (Li et al., 1986) and timolol (Harmia-Pulkkinen et al., 1989). This assumption was supported by the distribution coefficient (pH 6.2) of APE which is 0.08 compared to that of 0.24 of pilocarpine (Mitra and Mikkelson, 1988).

3.2. Viscosity enhancing polymers

The addition of soluble polymers to eye drop preparations is a common method for enhancing the ocular bioavailability of drugs. Most of the improvements in ocular drug absorption were observed in the viscosity range below 20 mPas. An optimal viscosity range of 12–15 mPas for ocular drug absorption in rabbit eyes was proposed by Patton and Robinson (1976). For this reason the miotic response after addition of soluble polymers to the aqueous APE solutions was compared to the nanoparticle carrier systems. The polymers employed either were mainly simple viscosity enhancers (methylhydroxyethylcellulose) or possessed additional bioadhesive properties (hyaluronic acid, polyacrylic acid, mucin). The concentrations of the polymers were chosen in order to yield similar viscosities as the nanoparticle preparation containing 10% of the copolymer (sample 5; $\eta = 12.7$ mPas; 20.0°C). The results are summarized in Table 2.

For a vehicle containing the mainly viscosity enhancing polymer methylhydroxyethylcellulose (sample 9) a 1.47-fold miotic AUC increase over the reference preparation was observed. The administration of preparations containing the bioadhesive polymers polyacrylic acid and mucin led to comparable results (samples 11, 12). No statistical significant differences between these polymer preparations were found. The AUC values for the cellulose derivative and the polyacrylic acid were statistically different from that of the reference solution and in the same order of magnitude as for the copolymer nanoparticle preparation. A comparable increase in the miotic response was observed for the mucin vehicle. This increase, however, was not found to be statistically significant compared to the aqueous solution. The reason for this was an irritating effect in the rabbit eye that led to redness and massive tear flow. As a result the evaluation of the miotic effect was only possible in a very small number of animals ($n = 3$). A similar eye irritation has already been described after the administration of pilocarpine in combination with mucin (Zimmer et al., 1995).

Addition of hyaluronic acid to APE (sample 10) produced a pronounced (1.81-fold) miotic AUC increase. The molecular weight of the hyaluronic acid used is of major importance for its efficacy. It was already shown (Camber and Edman, 1989; Saettone et al., 1991) that pilocarpine solutions prepared with high molecular weight sodium hyaluronate exhibited a greater miotic response than those prepared with lower molecular weight samples. In the present study hyaluronic acid of a molecular weight $2 \cdot 10^6$ Da was used representing a high molecular weight polymer. The pronounced miotic AUC increase confirmed former studies, that characterized hyaluronic acid as being superior to other viscosity enhancing polymers (Saettone et al., 1989).

In order to evaluate the bioadhesive properties of polysulfopropylmethacrylate which also is a polyanionic polymer such as polyacrylic acid and hyaluronic acid, sulfopropylmethacrylate was polymerized by free radical polymerization in absence of methylmethacrylate. In this case no particles but a clear polymer solution with an enhanced viscosity was obtained. The addition of

Table 2
Summary of the miotic activity data of APE preparations containing viscosity enhancing polymers ($n \geq 6$)

Sample no.	Preparation	Polymer concentration (%)	Viscosity (mPas)	E_{max} (mm)	$AUC_{60\ min}$ (mm · min)	AUC_{rel}
8	Reference solution for no. 9, 10	0.0	1.1	1.68 (0.62)	21.40 (8.98)	1.000
2	Reference solution for no. 5, 11, 12, 13	0.0	1.1	2.07 (0.51)	31.49 (8.68)	1.000
5	Copolymer nanoparticles	10.0	12.7	2.43 (0.44)	47.33 (5.00)	1.503***
9	Methylhydroxyethylcellulose (Tylopur® MH300)	0.75	12.5	1.88 (0.82)	31.36 (8.32)	1.465*
10	Hyaluronic acid	0.20	12.8	2.05 (1.17)	38.65 (17.11)	1.806**
11	Polyacrylic acid (Carbopol 941®)	0.22	17.9	2.74 (0.64)	45.28 (8.15)	1.438**
12	Mucin	4.5	13–17[a]	1.97[b] (0.97)	44.30[b] (32.06)	1.407[b]
13	Polysulfopropylmethacrylate	5.0	15.9	1.97 (0.49)	35.30 (7.54)	1.121

Numbers in parentheses represent S.D.
[a] (Zimmer et al., 1995).
[b] $n = 3$.
Level of statistical significance of differences from APE reference: *$p < 0.10$; **$p < 0.05$; ***$p < 0.01$, Students t-test.

Table 3
Summary of the miotic activity data of APE preparations containing combinations of viscosity enhancing polymers and copolymer nanoparticles ($n \geq 6$)

Sample no.	Preparation	Polymer concentration (%)	E_{max} (mm)	AUC_{60min} (mm · min)	AUC^{a}_{rel}	AUC^{b}_{rel}
14	Reference solution	0.0	2.51 (0.34)	30.94 (8.76)	1.000	0.668**
15	Copolymer nanoparticles	10.0	2.53 (0.56)	46.31 (9.53)	1.497**	1.000
16	Copolymer nanoparticles + hyaluronic acid	10.0 0.20	3.14 (0.77)	61.15 (15.64)	1.976**	1.320*
17	Copolymer nanoparticles + Carbopol 941®	10.0 0.25	2.49 (0.65)	50.43 (14.44)	1.630**	1.089
18	Copolymer nanoparticles + mucin	10.0 1.50	2.87 (0.60)	49.36 (13.28)	1.595**	1.066

Numbers in parentheses represent S.D.
[a] Values relative to APE reference solution.
[b] Values relative to copolymer nanoparticle preparation (sample 15).
Level of statistically significant differences from APE control preparation: $*p<0.05$; $**p<0.01$, Students t-test.

APE decreased its viscosity to 15.9 mPas. The administration of the polysulfopropylmethacrylate preparation to the animals led to no significant improvement in the miotic response (Table 2, sample 13). The reason for this may be that the cornea is covered by the hydrophilic, negatively charged mucin-glycocalyx that may not interact well with polymers exhibiting strong permanent negative charges. On the other hand polymers containing weak acidic functional groups such as carboxyl groups may be able for an interpenetration of their chains with the mucus which represents the main physical mechanism of bioadhesion (Peppas and Buri, 1985).

In conclusion, the administration of APE-loaded copolymer nanoparticles can be considered to be equivalent in efficacy to solutions containing viscosity-enhancing or medium bioadhesive polymers. This finding is in good agreement with results of Zimmer et al. (1995).

3.3. Combinations of nanoparticles and viscosity enhancing polymers

In a former study (Zimmer et al., 1995) it was shown, that the combination of nanoparticles with some soluble polymers induced a significantly improved pharmacological response when compared with either of the components, simple nanoparticles or soluble polymers without particles. The best results were observed with combinations of the nanoparticles with the bioadhesive polymers. For this reason, the copolymer nanoparticles of the present study were combined with the bioadhesive polymers hyaluronic acid, polyacrylic, acid and mucin (Table 3). The combinations with polyacrylic acid and mucin (samples 17, 18) led to a statistical significant increase in the miotic response in comparison with the aqueous reference solution (sample 14) and to a slight increase compared to the copolymer nanoparticles (sample 15). For the combination with nanoparticles the mucin concentration had to be reduced to 1.5% because of a strongly increasing viscosity at higher mucin concentrations. It is noteworthy that the coadministration of mucin and nanoparticles was tolerated without any irritating effect in the eye.

The addition of hyaluronic acid to the nanoparticles (sample 16) achieved the most pronounced increase (1.98-fold) in the miotic response (Fig. 2). It can be assumed that the coadministration of copolymer nanoparticles and bioadhesive polymers enabled an improved adhesion of the particles to the precorneal part of the eye and hence to a prolonged duration of the pharmacological drug effect.

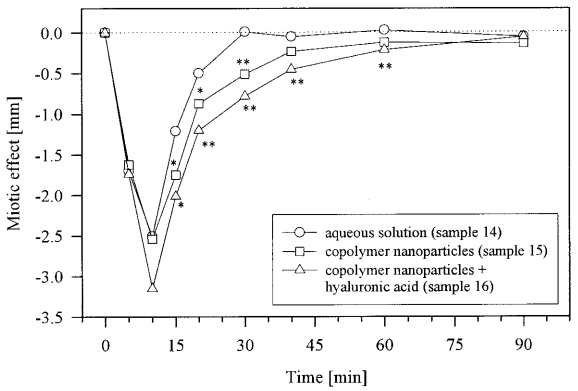

Fig. 2. Miotic effect after topical administration of different nanoparticle preparations of APE to the eyes of rabbits ($n \geq 6$). *$p < 0.05$; **$p < 0.01$, Students t-test. S.D. were omitted for the sake of clarity.

3.4. Carrier systems for (S)-(+)-aceclidine

In order to investigate the effect of the copolymer carrier systems on the miotic response to a drug with a prolonged cholinergic activity, (S)-(+)-aceclidine was chosen as a model compound. A comparison of the miotic effects of this compound with APE in an aqueous solution revealed the higher potency of (S)-(+)-aceclidine after topical administration (Fig. 3). For further studies with the carrier systems, (S)-(+)-aceclidine concentrations of 0.1% were used. The effects of the carrier system on the miotic response to the drug showed the same tendency as with APE (Table 4). Copolymer nanoparticles at a concentration of 10% (sample B) were able to induce a significant increase in the miotic AUC relative to the drug reference, whereas higher polymer contents led to no further increase in the miotic activity (sample C). The loading efficiency of the copolymer nanoparticles for (S)-(+)-aceclidine was calculated to be 12% (sample B) and 19% (sample C), respectively. Therefore, the loading efficiency was below that of APE. This was caused by the phosphate buffer components used for isotonicity and their competitive effect for the binding sites on the copolymer carrier. This competitive effect was much more pronounced at lower drug concentrations.

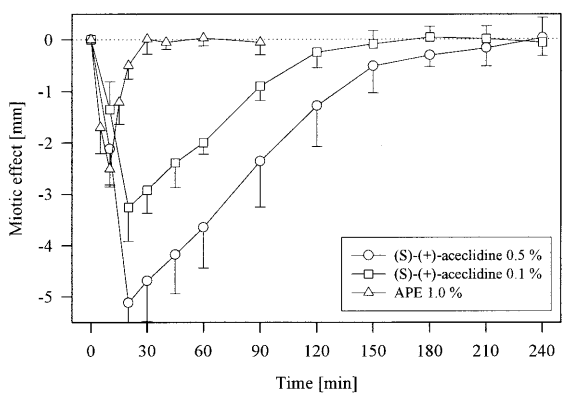

Fig. 3. Miotic effect after topical administration of aqueous solutions of (S)-(+)-aceclidine and APE to the eyes of rabbits ($n \geq 8$).

As for APE, hyaluronic acid alone (sample D) or in combination with copolymer nanoparticles (sample F) was the most effective soluble polymer for increasing the miotic AUC after administration of (S)-(+)-aceclidine (Fig. 4). In comparison to the reference solution both preparations (sample D + F) led to highly significant increases in the miotic AUC response. The combinations of polyacrylic acid (sample G) and mucin (sample H) with copolymer nanoparticles exhibited the same miotic effects as copolymer nanoparticles alone. Application of an aqueous mucin solution was not performed because of the above mentioned irritating effect. In general, it was observed that the influence of comparable carrier systems was much more pronounced in combination with the short acting drug APE than with drugs of a long lasting pharmacological activity such as (S)-(+)-aceclidine.

In summary, the results of the present study indicate, that the methylmethacrylate sulfopropylmethacrylate copolymer nanoparticles are suitable carriers for hydrophilic cationic drugs such as APE and (S)-(+)-aceclidine. These nanoparticles may offer new possibilities for ophthalmic drugs that do not bind well to nanoparticles made of the most frequently used polymers, the polyalkylcyanoacrylate and polymethylmethacrylate homopolymers. The copolymer nanoparticles caused no irritating effect in the rabbit eye confirming earlier results of Hoffmann et al. (1996). These authors studied the toxicity of polymers used for the preparation of nanoparticles towards different

Table 4
Summary of the miotic activity data of (S)-(+)-aceclidine preparations containing copolymer nanoparticles, bioadhesive polymers or combinations of them ($n \geq 6$)

Sample no.	Preparation	Polymer concentration (%)	E_{max} (mm)	AUC_{210min} (mm · min)	AUC^{a}_{rel}	AUC^{b}_{rel}
A	Reference solution	0.0	3.26 (0.66)	198.07 (35.34)	1.000	0.796**
B	Copolymer nanoparticles	10.0	3.80 (0.69)	248.73 (51.83)	1.256**	1.000
C	Copolymer nanoparticles	17.0	3.72 (0.63)	259.45 (58.73)	1.310**	1.043
D	Hyaluronic acid	0.20	4.05 (0.50)	289.58 (61.82)	1.462***	1.164
E	Polyacrylic acid (Carbopol 941®)	0.25	3.85 (0.89)	246.37 (63.33)	1.244*	1.003
F	Copolymer nanoparticles + hyaluronic acid	10.0 0.20	4.14 (0.89)	310.51 (84.52)	1.568***	1.248
G	Copolymer nanoparticles + Carbopol 941®	10.0 0.25	3.51 (0.91)	242.48 (69.08)	1.224	0.975
H	Copolymer nanoparticles + mucin	10.0 1.50	3.15 (0.64)	253.17 (84.29)	1.278	1.018

Numbers in parentheses represent S.D.
[a] Values relative to (S)-(+)-aceclidine reference solution.
[b] Values relative to copolymer nanoparticle preparation (sample B).
Level of statistically significant differences from (S)-(+)- aceclidine control preparation: *$p<0.10$; **$p<0.05$; ***$p<0.01$, Students t-test.

cell-lines (VH, HeLa, Vero, Ma104). It was shown that the introduction of sulfate functions into the PMMA-polymer chain did not result in a significant increase of the cytotoxicity in contrast to quarternary ammonium-functions that induced cytopathic effects on various cell-lines. Combination with soluble polymers such as hyaluronic acid may enable the manufacture of an effective carrier system for a number of eye drop preparations. Prolonged ocular residence times, mirrored by pronounced miotic effects, were observed for all hyaluronic acid-containing preparations. These findings confirm the positive properties of this polymer for ophthalmic applications.

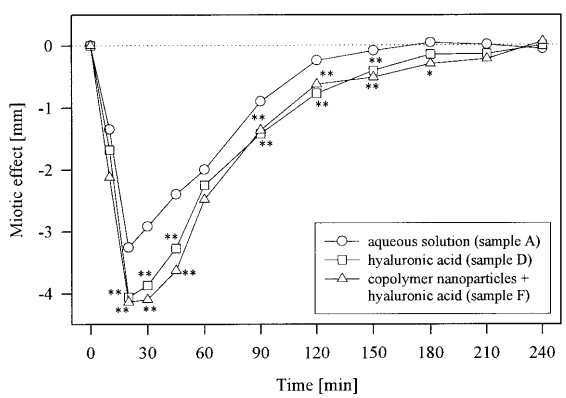

Fig. 4. Miotic effect after topical administration of different hyaluronic acid preparations of (S)-(+)- aceclidine to the eyes of rabbits ($n \geq 6$). *$p < 0.05$; **$p < 0.01$, Students t-test. S.D. were omitted for the sake of clarity.

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