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Selected polysaccharides at comparison for their mucoadhesiveness and effect on precorneal residence of different drugs in the rabbit model

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Abstract

Mucoadhesive polysaccharides may prolong the residence of ophthalmic drugs in precorneal area. In this article, the mucoadhesiveness of arabinogalactan, tamarind seed polysaccharide, hyaluronan, hydroxyethylcellulose is compared in vivo, by the polymer residence time in rabbit tear fluid, and in vitro, by the polymer-induced increase of viscosity of a mucin dispersion. Polymer residence is prolonged by increased viscosity but shortened by reflex tearing caused by excessive viscosity. Tamarind seed polysaccharide is the most effective in prolonging the residence of ketotifen and diclofenac in precorneal area; hence, it is the optimal eyedrop additive as it is mucoadhesive while not increasing viscosity excessively.

Key words: *Corneal contact time; diclofenac; eyedrops; ketotifen; precorneal residence; mucoadhesive polysaccharide*

Introduction

Eyedrops are currently the dosage forms of choice for the topical treatment of ocular diseases, essentially because they are the best accepted by patients. Treatment with eyedrops, however, poses the issue of a poor bioavailability because the precorneal area, that is, the site of drug action/absorption, is rapidly cleared of drugs by protective mechanisms of the eye, such as blinking, basal and reflex tearing, and nasolacrimal drainage. This implies the need of frequent instillations, and hence, the risk of side effects. Increasing ocular bioavailability, thereby decreasing the frequency of instillations, remains a stimulating challenge for the formulators of eyedrops. An approach to the task has been the reduction of drainage rate by increasing the viscosity of the preparation[1–6] or resorting to mucoadhesive polymers[7]. Mucomimetic polysaccharides, such as xyloglucan [tamarind seed polysaccharide (TSP)], hyaluronic acid (HA), hydroxyethylcellulose (HEC), which are currently used in commercial artificial tears for the treatment of the dry eye syndrome, may as well prolong the residence of ophthalmic drugs in the precorneal area in virtue of their mucoadhesive properties. The ability of a polymer to improve ocular bioavailability of drugs by adhering to the ocular surface and binding the drug to it is a more promising property than the polymer viscosifying power, so far as fluid solutions are better tolerated than viscous ones[8].

In this work, the above polysaccharides have been put to comparison on the ground of their adhesiveness to the ocular surface and their ability to prolong the residence of the antiallergic ketotifen or the antiinflammatory diclofenac in the precorneal area of the rabbit eyes. Also included in the comparative study has been arabinogalactan (AG), a natural polysaccharide contained in *Larix occidentalis* (western larch), which has been found by Burgalassi et al. to be biocompatible in the eye, mucomimetic, and mucoadhesive[9].

Concerning the chemical or structural characteristics of the polymers involved in the study we wish to sketch out the following. TSP is a nonionic, neutral, branched polysaccharide consisting of a cellulose-like backbone carrying

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xylose and galactoxylose substituents[10]. HA is known to be a polymer of disaccharides, themselves composed of D-glucuronic acid and D-*N*-acetylglucosamine. HEC is known to be a derivative of cellulose carrying nonionic hydroxyethyl ether substituents. AG is a nonionic highly branched polysaccharide of the 3,6-β-D-galactan type, the side chains of which consist of β-galactose and β-arabinose residues[11].

In addition to factors pertaining to the polymer, such as molecular weight, functional groups, molecular conformation or chain flexibility, and mobility, mucoadhesion is influenced by environmental factors that determine polymer charge, hydration, or swelling degree[12,13]. Hence, we have attempted to compare the mucoadhesiveness of the different polymers in vivo by determining their time of residence in the precorneal area of the rabbit eyes. Such a time is influenced by the effects of environmental factors, such as, for example, reflex tearing possibly induced by the polymer, on the mucoadhesion strength, and this makes the comparisons more significant from a practical standpoint than those based on in vitro or ex vivo procedures. However, comparisons based on precorneal clearance may be biased by the viscosity of the applied polymer solution which may not be correlated with the polymer mucoadhesiveness while still influencing the clearance. Therefore, care was taken to distinguish mucoadhesion from viscosity effects. For this purpose, the rank order of polymer precorneal clearance resulting from in vivo tests was compared with that of mucin–polymer interactivity resulting from an in vitro method based on the measurement of the effect of mucin–polymer interaction on the viscosity of a system of porcine gastric mucin and polysaccharide in solution[14]. Such a method suffers from several limitations, as highlighted by Hägerström and Edsman[15], nevertheless it has been used for its inherent simplicity and also because it has been profitably adopted by other authors in recent years[9]. The results from this in vitro procedure in conjunction with those from the in vivo tests were expected to provide reliable indications on the most suitable of the polysaccharides under study to be introduced into eyedrops as an ocular bioavailability-enhancing excipient.

The rabbit model has also been used to assess the effects of the polysaccharides on the time of residence of ketotifen fumarate (KF) or diclofenac sodium (DS) in the tear fluid. Each of these drugs is the active principle of commercial eyedrops, the former for the treatment of allergic conjunctivitis, the latter to treat inflammation and swelling of the eye following cataract surgery.

Molecular drug–polymer binding is of great relevance to the drug residence time in tear fluid. Such a binding has been quantified by the time-honored method of the dynamic dialysis, basically consisting in measuring the polymer effect on the drug permeation rate across a membrane permeable to the drug, impermeable to the polymer[16].

It must be recognized that the precorneal clearance determined in rabbits is not representative of that in humans, mainly because of differences in blinking frequency[5,17–19]. Such differences may be reflected in differences in shear thinning of tear film, mucoadhesion of polymer, and ultimately in drainage of drugs. Nevertheless, if the effects of blinking can be considered to be similar for the different preparations tested, then the rabbit model is deemed robust for the comparative purposes of this work.

Materials and methods

Materials

Ketotifen fumarate (Sifavitor S.p.A., Lodi, Italy), diclofenac sodium (Corden Pharmachem NV, Landen, Belgium), tamarind seed polysaccharide, MW 700 kDa (Opocrin S.p.A., Modena, Italy), hyaluronic acid, MW 950 kDa (Contipro, Dolní Dobrouč, Czech Republic), and hydroxyethylcellulose, MW 1300 kDa (Natrosol®, Hercules Italia S.p.A., Milan, Italy) all were kindly gifted by Farmigea S.p.A. (Pisa, Italy). Arabinogalactan, MW 10–120 kDa (Fiberaid®), fluorescein isothiocyanate (FITC), and porcine gastric mucin type III were purchased from Sochim International S.p.A. (Milan, Italy), Fluka, and Sigma, respectively. All other chemicals and solvents were of reagent grade.

Fluorescein isothiocyanate labeling of polysaccharides

A previously described procedure was followed[20,21]. A solution of FITC in dimethyl sulfoxide (1 mL, 2 mg/mL) was added to an aqueous solution of AG, TSP, HA, or HEC (20 mL, 2 mg/mL), and the mixture was incubated at 4°C for 8 hours. Next the solution was passed through a column of Sephadex G15 in order to clear the labeled polymer of nonreacted FITC, then it was lyophilized. In no case did the Sephadex column retain any fluorescence, in all cases indicating the absence of nonreacted FITC and the complete labeling of polymer. Hence, the fluorophore bound to the polymer could be calculated at 5% of the total mass (0.13 mmol/g).

Preparation of ophthalmic drops

For comparing the polysaccharides for their adhesiveness to the ocular surface, ophthalmic drops containing 0.2%, 0.5%, or 0.7% (wt/vol) of each FITC-labeled polymer were prepared. The vehicle of FITC–TSP, FITC–HA, and FITC–HEC was 0.0375 M phosphate buffer, pH 7.4, made isotonic with sodium chloride (phosphate buffered saline, PBS). FITC–AG could not be dissolved in PBS, so the relevant ophthalmic drops were prepared by mixing separately prepared aqueous solutions of polymer with

sodium chloride solutions to obtain isotonic solutions containing the designed concentrations of FITC–AG.

Medicated ophthalmic drops were prepared, containing 0.7 mg/mL KF or 1 mg/mL DS and 0.2% (wt/vol) or 0.7% (wt/vol) of AG, TSP, HA, or HEC. The vehicle, in the cases of TSP, HA, or HEC, was PBS. Each drug was dissolved in the vehicle before adding the polymer. In the case of AG, a solution of drug and sodium chloride was prepared, which was mixed with an aqueous solution of AG to give the desired concentrations of drug, polymer, and salt. For controls, polymer-free ophthalmic drops containing 0.7 mg/mL KF or 1 mg/mL DS in PBS were prepared. The isotonicity of ophthalmic drops was checked by a microosmometer (Hermann Roebling, Berlin, Germany). The solution containing 0.7 mg/mL KF and 0.7% (wt/vol) HEC in PBS was clear after its preparation at ambient temperature, but at 35°C, that is, the temperature of the eye surface, it became milky with a fine precipitate, which indicated an incompatibility of ingredients under physiological conditions. Therefore, this formulation was not retained for further studies.

Viscosity measurements

Rheograms of nonmedicated ophthalmic drops prepared as described above with nonFITC-labeled polymers were recorded at 35°C with a Haake RS1 rheometer equipped with coaxial cylinders Z40 (rotor) and Z41 (stator). Data were acquired and analyzed using Rheo Win Pro software (Haake, Thermo Fisher Scientific Inc., Karlsruhe, Germany). The AG solutions of 0.2%, 0.5%, and 0.7% (wt/vol), and the TSP solutions of 0.2% (wt/vol) showed a Newtonian behavior. The viscosity of these systems at 35°C was measured by the Ostwald viscometer (Effeci, Milan, Italy), which yielded more accurate values. Means of at least three measurements are shown in Table 1. The coefficient of variation of measurements never exceeded 0.4%. The viscosity of distilled water at 35°C was assumed to be 0.7194 mPas[22]. The TSP solutions of 0.5% and 0.7% (wt/vol), and the HA and HEC solutions of 0.2%, 0.5%, and 0.7% (wt/vol) showed a pseudoplastic behavior (rheograms not shown). For these systems, the viscosity values were measured at the shear rate of 200 s^{-1} because at rates of this magnitude the dependence of viscosity on shear rate was minimal in all cases tested. Viscosity measurements showed insignificant differences between FITC-labeled and unlabeled polymers.

In vitro comparative evaluation of polysaccharide mucoadhesiveness

According to Hassan and Gallo[14], the viscosity coefficient, η, of a hydrophilic dispersion of mucin and a mucoadhesive polymer results from the additive contributions of the viscosity coefficients of mucin, η_m, and polymer, η_p, and a viscosity component because of mucin–polymer interaction, η_{mp}. After measuring η, η_m, and η_p the interactive component was calculated as follows:

$$\eta_{mp} = \eta - \eta_m - \eta_p, \quad (1)$$

where η_{mp} values, determined at the rate of shear of 200 s^{-1}, were used for a comparative evaluation of polymer mucoadhesiveness.

Dispersions containing 15% (wt/vol) mucin and 0.2%, 0.5%, or 0.7% (wt/vol) polysaccharide in PBS (TSP, HA, HEC) or in saline (AG) were tested. The dispersions were prepared by adding 2 mL of polymer solution, at fourfold the final concentration, to 6 mL of a 20% (wt/vol) mucin dispersion in the same solvent. The mucin–polymer systems and the dispersion of mucin alone showed a pseudoplastic rheological behavior. Their viscosity coefficients were measured at 35°C by the Haake RS1 rheometer at the shear rate of 200 s^{-1}, that is, the same as for the pseudoplastic polymers alone.

Table 1. Comparison between the polysaccharide effect on the viscosity of the mucin polysaccharide dispersion and the mean residence time of polysaccharide in tear fluid of rabbits.

Polymer	C_p (%, wt/vol)	η_p[a] (mPas)	η_{mp}[a] (mPas)	η_{mp}/η[a] (%)	MRT ± SE (minutes)
AG	0.2	0.73*	16.7	6.2	3.02 ± 0.50
	0.5	0.73*	−0.1	0.0	2.68 ± 0.51
	0.7	0.73*	−15.6	−7.7	4.23 ± 0.37
TSP	0.2	1.85*	44.7	20.4	7.35 ± 0.77
	0.5	4.45	63.5	22.1	10.42 ± 1.70
	0.7	9.88	94.0	32.7	18.09 ± 1.55
HA	0.2	2.11	32.7	15.7	6.50 ± 1.16
	0.5	15.0	54.0	18.6	14.48 ± 2.56
	0.7	53.5	140.3	38.0	11.51 ± 1.76
HEC	0.2	2.88	45.1	20.3	8.49 ± 0.98
	0.5	12.3	82.7	26.3	14.68 ± 2.79
	0.7	59.7	146.1	38.5	13.44 ± 2.37

C_p, polysaccharide concentration; η_p, polysaccharide viscosity; η_{mp}, viscosity component of the mucin polysaccharide dispersion because of mucin–polymer interaction; η_{mp}/η, contribution of the interactive component to the viscosity of the mucin polysaccharide system; MRT, mean residence time of polysaccharide in tear fluid of rabbits. Values marked by * refer to a Newtonian and nonmarked ones to a pseudoplastic behavior (shear rate, 200 s^{-1}). [a]Viscosity measurements carried out at 35°C.

Measurement of elimination kinetics from tear fluid of rabbits

Male New Zealand albino rabbits weighing 3.0–3.5 kg, maintained under standard stabulation conditions,

were used. They were treated as prescribed in the 'Guide for the Care and Use of Laboratory Animals' (NIH Publication No. 92–93, revised 1985). All experiments were carried out under veterinary supervision, and the protocols were approved by the Ethical Scientific Committee of the University.

Elimination kinetics of polysaccharides

The nonmedicated ophthalmic drops of FITC–TSP, FITC–HA, FITC–HEC, or FITC–AG were tested. One drop of each solution (50 μL) was instilled into the lower conjunctival sac by a Gilson pipette (Franceschi, Pisa, Italy) with care to avoid spillage. Tear fluid samples were collected at various intervals from the lower marginal tear strip using 1.0-μL disposable glass capillaries (Microcaps, Drummond Scientific Co., Broomall, PA, USA), which were flushed with 1.0 μL of water. After further dilution with 100 μL of water the samples were analyzed for the polymer. For each polymer at each concentration eight elimination curves were obtained, each determined in a single eye of different animals. The analysis of samples was carried out by a fluorimetric method (spectrophotofluorimeter, Perkin-Elmer LS 45, Milan, Italy). For FITC–TSP, FITC–HA, and FITC–HEC the excitation was at 494 nm, the emission at 510 nm; for FITC–AG the excitation was at 485 nm, the emission at 508 nm. Calibration curves were constructed using six standard aqueous solutions per polymer in the concentration range of 0.01–0.2 μg/mL for FITC–TSP, FITC–HA, and FITC–HEC, and of 0.04–0.8 μg/mL for FITC–AG. In all cases the fluorescence versus concentration plot was linear in the concentration range of the standards ($r^2 > 0.99$).

Elimination kinetics of drugs in the presence of polysaccharides

The medicated ophthalmic drops were tested, following the procedure described under Elimination Kinetics of Polysaccharides for the nonmedicated ones, except that the withdrawn tear fluid samples were diluted with 50 instead of 100 μL of water because the high-performance liquid chromatography (HPLC) methods used for the analysis of the drugs had detection limits higher than the fluorimetric method used for the polysaccharides. The HPLC apparatus (Perkin-Elmer, Milan, Italy) consisted of Series 200 pump, 20 μL Rheodyne injector, UV detector, and Turbochrom Navigator HPLC software for data integration. A Spheri-5 RP18, 250 × 4.6 mm, 5-μm column was used.

For the analysis of KF, the mobile phase (flow rate 2.0 mL/min) was acetonitrile/water/triethylamine/glacial acetic acid (50:50:0.2:0.1) and the UV detection was set at 301 nm. Standard curves were constructed by analyzing six standard KF solutions in PBS. The standard curves produced on different days were all linear ($r^2 > 0.99$) in the concentration range from 0 to 10 μg/mL (limit of determination, about 0.02 μg/mL). The retention time was 6.3 minutes.

For the analysis of DS, the mobile phase (flow rate 1.5 mL/min) was acetonitrile/water/glacial acetic acid (50:46:4) and the UV detection was set at 276 nm. Standard curves were constructed by analyzing six standard DS solutions in PBS. The standard curves produced on different days were all linear ($r^2 > 0.99$) in the concentration range from 0 to 25 μg/mL (limit of determination, about 0.03 μg/mL). The retention time was 5.8 minutes.

With either KF or DS samples the concentration of each unknown analyzed on a given day was determined using the standard curve produced on the same day.

Elimination data treatment

The concentration in tear fluid (C_{TF}) versus time data, obtained with the nonmedicated and the medicated ophthalmic drops as described under Elimination Kinetics of Drugs in the Presence of Polysaccharides, were used to calculate the mean residence time (MRT) of each polysaccharide, or drug, in tear fluid. This parameter resulted from the ratio, AUMC/AUC, between the area under momentum curve ($C_{TF}t$ versus t curve) and the area under the C_{TF} versus t curve. AUMC and AUC were calculated by the linear trapezoidal rule between time 0 and the time when C_{TF} dropped below the minimum quantifiable value. For each elimination curve the corresponding MRT was calculated, thus, for each case studied eight MRT values were obtained, of which mean and *SE* were calculated. Difference significance between two means was evaluated by the Student's t-test ($P < 0.05$).

With the medicated ophthalmic drops also the maximum residence time (RT_{max}) of the drug at quantifiable concentrations in tear fluid was reported. This time corresponded to the last point of the C_{TF} versus time plot for the drug. In this plot for each time interval the mean of eight C_{TF} values obtained with different animals was reported. The minimum quantifiable C_{TF} value was 1.1 μg/mL for KF, 1.5 μg/mL for DS, considering the necessity to dilute the withdrawn samples at least 1:50 (vol/vol).

Measurement of drug–polymer interactions

A previously described method, based on the dynamic dialysis technique[16,23], was used to determine the drug–polymer interactions in the ophthalmic drops. Drug flux through a porous cellulose membrane permeable to the drug, impermeable to the polymer (Spectra/Por®, cut-off 3500 Da, Spectrum Laboratories Inc., Rancho Dominguez, CA, USA) under quasi-steady state conditions was measured at 35°C in the presence or absence of the polymer in the donor phase. The polymer and initial drug concentrations in the donor were equal to those used in the in vivo tests for the determination of the elimination kinetics of drugs in the presence of

polysaccharides. The composition of the donor medium was the same as that of the ophthalmic drops under study, with an ionic strength similar to that of the lachrymal fluid. Sink conditions were ensured in the receptor medium, which contained the same solutes at the same concentrations as the donor, except for the permeant and the polymer, in order to prevent volume variations because of osmosis. The receptor was spectrophotometrically analyzed for KF, at 301 nm, or DS, at 276 nm. The regression for the fitting of dialysis data, expressed as permeant concentration in the donor versus time, to first-order kinetics was always significant ($r^2 \geq 0.99$, $n \geq 8$). This allowed calculation of the dialysis rate constant. Under the above experimental conditions, a reduction of the dialysis rate constant caused by a polymer was considered as a sign and a measure of drug–polymer interactions. The fraction of bound, interacting permeant, f_B, was expressed by the following equation[23]:

$$f_B = 1 - \frac{k_p}{k_a}, \qquad (2)$$

where k_p and k_a represent the dialysis constants in the presence and in the absence of polymer, respectively. Values of f_B were calculated, by Equation (2), only for those cases where k_p and k_a were significantly different on the basis of the Student's t-test ($P < 0.05$).

Results and discussion

Comparative assessment of polysaccharide mucoadhesiveness

In an attempt to assess the rank order of polysaccharide adhesiveness to the eye surface, the mucin–polymer interactivity, determined in vitro by viscosity measurements, was compared with the MRT of polymer in the tear fluid of rabbits. All ophthalmic drops when instilled in rabbit eyes were biocompatible and caused no apparent irritation signs, such as conjunctival/corneal edema and/or hyperhemia.

In Table 1, the viscosity component because of mucin–polymer interaction, η_{mp}, and the percent contribution of this interactive component to the viscosity of the mucin polysaccharide system, η_{mp}/η, are listed for each mucin polysaccharide dispersion and compared with the MRT of polysaccharide in the tear fluid of rabbits. As can be observed, for all polymers except AG the interactive component gives an important contribution to the overall viscosity of the dispersion. Such a contribution increases with increasing polymer concentration, as expected. These results are in agreement with the recognized mucoadhesiveness of the polysaccharides under study. The case of AG is anomalous in that the relevant interactive component is little significant, in fact, it becomes negative at the polymer concentration of 0.7% (wt/vol), possibly because of slight conformational changes of mucin chains induced by AG. In agreement with the comparatively low in vitro mucoadhesiveness of AG at the concentrations studied, the MRT values for this polysaccharide, shown in Table 1, are the lowest of all polymers tested.

For the concentration of 0.2% (wt/vol) the differences among the MRT values for TSP, HA, and HEC are statistically insignificant. Because the viscosity of TSP at this concentration is lower than that of HA or HEC, it can be stated that the in vivo mucoadhesiveness of TSP of 0.2% (wt/vol) is not lower than that of HA or HEC of 0.2% (wt/vol). This is confirmed by the relevant data in Table 1 showing that, at this concentration, the in vitro interactivity of TSP with mucin is stronger than that of HA and similar to that of HEC.

For the concentration of 0.5% (wt/vol), the rank order of mucin–polymer interactivity is HEC>TSP>HA, whereas the mean values of MRT are in the order HEC≈HA>TSP. It must be considered, in this respect, that the MRT value can be directly influenced by the viscosity of the solution, in addition to the mucoadhesiveness of the polymer, and that, at this concentration, the viscosity of HEC or HA is markedly higher than that of TSP. Then, at the concentration of 0.5% (wt/vol), the rank order of mucoadhesiveness is more accurately established by the method in vitro than by the in vivo evaluation of the MRT values, because the latter cannot discriminate between mucoadhesiveness and viscosity effects.

At the concentration of 0.7% (wt/vol) the order of in vitro mucin–polymer interactivity, resulting from the data in Table 1, that is, HEC≈HA>TSP, disagrees with that of the MRT values, which is TSP>HEC≈HA. This cannot be explained by a direct influence of the solution viscosity on MRT because, at this concentration, the viscosity of TSP is much lower than that of HEC or HA. The above discrepancy between in vitro and in vivo data could be explained by admitting that, although no appreciable irritation of the rabbit eyes was ever observed, when the viscosity of the ophthalmic drops was too high, such as in the cases of HEC and HA of 0.7% (wt/vol), reflex tearing was particularly abundant and caused dilution of polymer in tear fluid, with consequent decrease of both viscosity of such a fluid and polymer mucoadhesion. As a result, the MRT value would drop below that resulting from instillation of less viscous eyedrops. Thus, in such cases as those of HEC and HA of 0.7% (wt/vol), the particular conditions of the in vivo environment make up an uncontrollable variable that makes in vitro methods, such as the present one, inadequate to comparatively predict mucoadhesion and residence time of different polymers in the precorneal area.

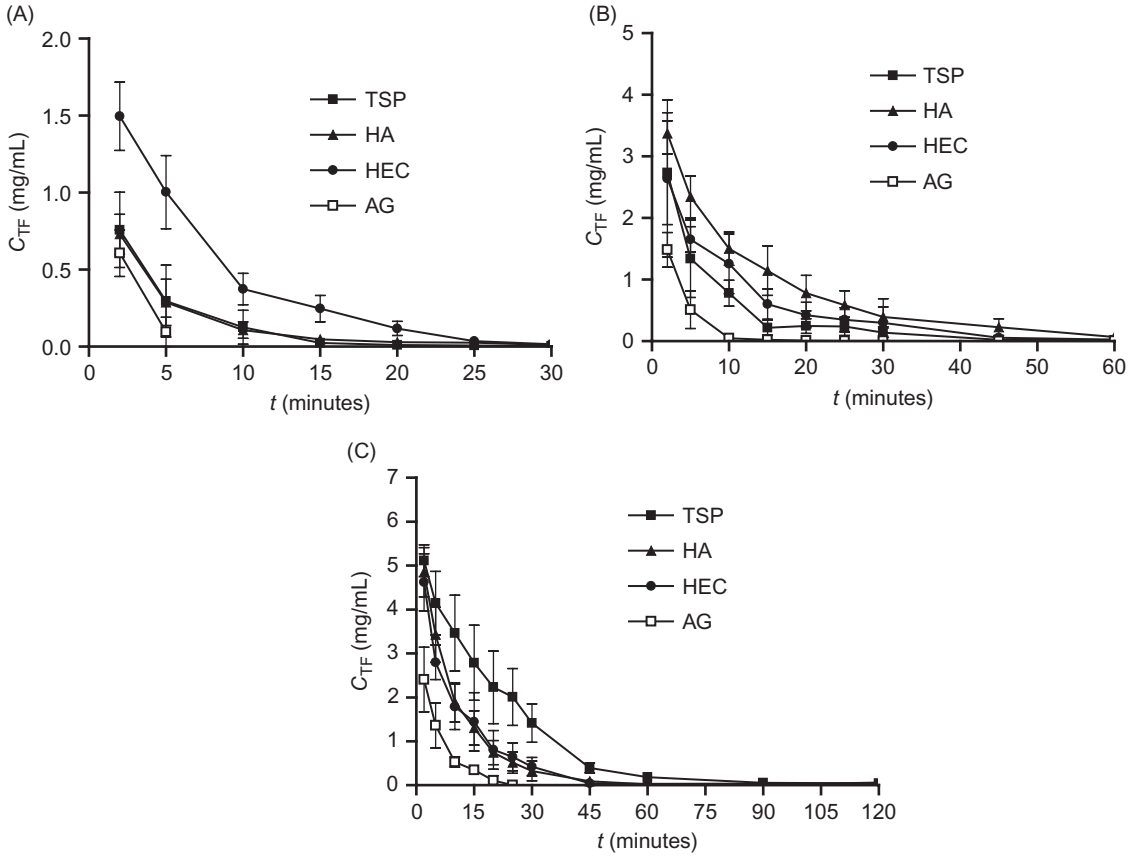

Figure 1. Profiles of polysaccharide elimination from tear fluid of rabbits following instillation of ophthalmic drops containing 0.2% (wt/vol) (A), 0.5% (wt/vol) (B), and 0.7% (wt/vol) (C) polymers.

The curves of polymer elimination from tear fluid are shown in Figure 1A–C. They generally show an exponential decay in line with the usual trend of xenobiotic clearance from precorneal area. Exceptionally TSP of 0.7% (wt/vol), which shows the highest MRT value of all polymers in Table 1, also shows an almost constant elimination rate in Figure 1C. Such a unique behavior is believed to be because of a comparatively strong adhesion of this polysaccharide to the ocular surface at this concentration.

Polysaccharide effect on drug residence in tear fluid of rabbits

Case of ketotifen fumarate

The KF concentration of 0.7 mg/mL in ophthalmic drops was equal to that contained in the commercial product Ketoftil (Farmigea, Italy). The polysaccharides tested were AG, TSP, HA, and HEC at the concentration of 0.2% (wt/vol), and AG, TSP, and HA at 0.7% (wt/vol). HEC was not tested at the latter concentration because a phase separation was observed in the solution containing KF and HEC in PBS. The relevant drug elimination profiles can be seen in Figure 2, while the residence times in tear fluid are shown in Table 2, where the drug fraction bound to polymer, as determined by dynamic dialysis, is also reported.

It appears from data in the table that the effects of the polymer concentration of 0.2% (wt/vol) on either the MRT or RT_{max} values for KF are generally insignificant except with TSP, in the presence of which the MRT value is significantly higher than that for the control. On the other hand, at the concentration of 0.7% (wt/vol) the polymers show enhancing effects on both MRT and RT_{max} with respect to the control, the strongest effect being exerted by TSP. An effect of TSP to slow down KF elimination from tear fluid is also apparent in Figure 2B, where the relevant C_{TF} versus time profile shows a plateau at 4–8 minutes, quite unlike all other cases. It results from a comparison of MRT values in Tables 1 and 2 at corresponding polymer concentrations that the residence of each polymer, except AG, in the tear fluid of rabbits is longer than that of the drug. This can be given the following explanation. TSP, HA, and HEC have shown an adhesiveness to the eye surface that should slow down their removal from precorneal area. These polymers, being hydrophilic, would retain/stabilize tear fluid on the eye surface, thereby slowing down

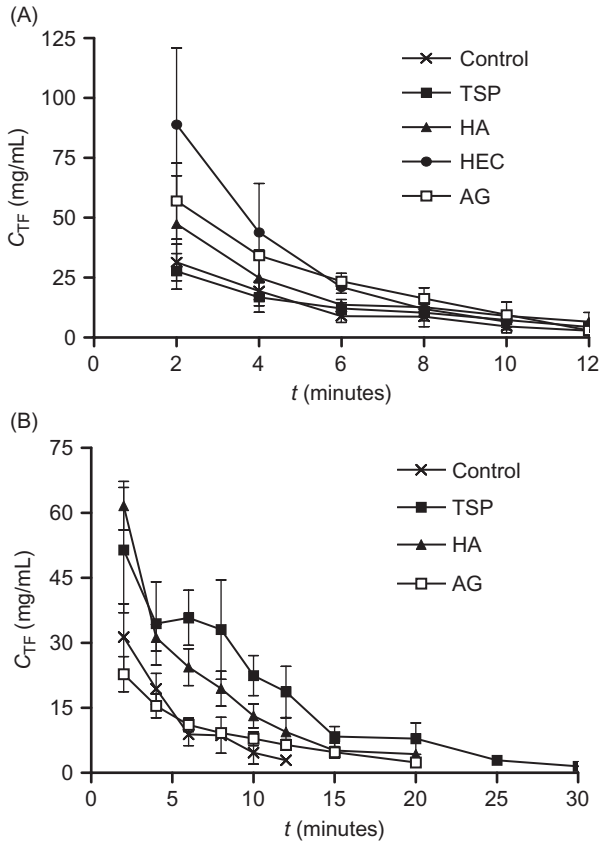

Figure 2. Profiles of KF elimination from tear fluid of rabbits following instillation of medicated ophthalmic drops containing 0.7 mg/mL drug and 0.2% (wt/vol) (A) or 0.7% (wt/vol) (B) of different polysaccharides.

Table 2. Polysaccharide effect on the residence time of KF in tear fluid of rabbits.

Polymer	C_p (%, wt/vol)	MRT ± SE (minutes)	RT_{max} (minutes)	f_B (%)
Control	—	5.07 ± 0.46	12	—
AG	0.2	5.16 ± 0.20	12	NS
	0.7	5.60 ± 0.74	20	NS
TSP	0.2	5.94 ± 0.29*	12	8
	0.7	9.14 ± 0.38*	30	9
HA	0.2	5.74 ± 0.38	12	12
	0.7	6.97 ± 0.62*	20	12
HEC	0.2	4.42 ± 0.33	10	NS

C_p, polymer concentration in eyedrops; MRT, mean residence time; RT_{max}, maximum residence time at measurable concentrations (≥1.1 μg/mL); f_B, drug fraction bound to polymer; NS, not significant. *Value significantly different from control ($P < 0.05$).

drainage and ultimately prolonging drug precorneal residence. Nevertheless the residence time of the drug is not expected to be so long as that of the mucoadhesive polymer, considering that the f_B values in Table 2 in no case point to any strong drug–polymer binding. The free, unbound drug molecules could diffuse in the tear film even in the presence of a mucoadhesive polymer and be removed from the precorneal area. Anyway, this diffusion flux is likely to be significantly slower than the convective drainage that would occur in the absence of polymer. To account for the lack of strong interactions of KF with the polymers under study, it should be considered that TSP, HEC, and AG are nonionic polysaccharides. As for HA, the carboxyls of the glucuronic acid units of this polymer may be thought to be strong interactive sites for ketotifen free base. However, ketotifen was in fact used as the complex with fumaric acid and this can be the reason of such a weak KF–HA binding as that shown in Table 2.

As far as AG is concerned, this polysaccharide has not appeared particularly mucoadhesive at the concentrations tested, in fact, its precorneal residence, shown in Table 1, is shorter than that of KF alone, shown in Table 2.

Case of diclofenac sodium

Diclofenac is chemically quite different from ketotifen, for example, the former is an acid, the latter a base. Yet, the data on DS residence in precorneal area, shown in Table 3, and on DS elimination kinetics from such an area, shown in Figure 3, show some similarities with the corresponding data for KF discussed under case of Ketotifen Fumarate. Thus, as already observed with KF, also with DS it appears that only in the case of TSP is the polymer effect on drug MRT statistically significant at 0.2% (wt/vol) polymer concentration, and that, at 0.7% (wt/vol) polymer concentration, TSP exerts the strongest effect of all polymers on drug MRT. The values of this parameter as shown in Tables 2 and 3 for TSP of 0.7% (wt/vol) are similar for KF and DS. The same similarity is observed when HA of 0.7% (wt/vol) is the polymer. Also worth stressing is the similarity between the elimination profiles of KF and DS in the presence of 0.7% (wt/vol) TSP, shown in Figures 2B

Table 3. Polysaccharide effect on the residence time of DS in tear fluid of rabbits.

Polymer	C_p (%, wt/vol)	MRT ± SE (minutes)	RT_{max} (minutes)	f_B (%)
Control	—	5.55 ± 0.21	20	—
AG	0.2	6.00 ± 0.24	20	NS
	0.7	6.33 ± 0.65	20	10
TSP	0.2	6.47 ± 0.61*	20	13
	0.7	8.11 ± 0.89*	35	12
HA	0.2	5.85 ± 0.42	20	9
	0.7	6.85 ± 0.44*	35	21
HEC	0.2	6.25 ± 0.58	20	NS
	0.7	6.05 ± 0.30	20	18

C_p, polymer concentration in eyedrops; MRT, mean residence time; RT_{max}, maximum residence time at measurable concentrations (≥1.5 μg/mL); f_B, drug fraction bound to polymer; NS, not significant. *Value significantly different from control ($P < 0.05$).

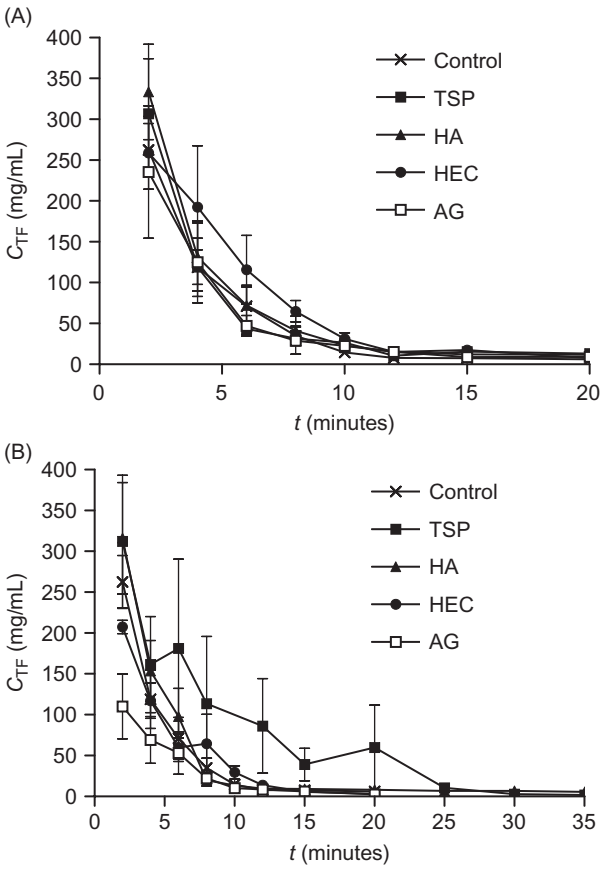

Figure 3. Profiles of DS elimination from tear fluid of rabbits following instillation of medicated ophthalmic drops containing 1.0 mg/mL drug and 0.2% (wt/vol) (A) or 0.7% (wt/vol) (B) of different polysaccharides.

and 3B. These observations suggest that a polymer, if mucoadhesive, exerts a paramount influence on the residence time of a drug in the precorneal area, irrespective of the chemical nature of the drug.

A consideration of the f_B values in Table 3 suggests that in none of the cases tested was the molecular drug–polymer binding so strong as to be determinant to the polymer effect on drug MRT. DS was present as an anion and this suggests no strong ionic interactions with any of the polymers studied. It was probably the stabilization of tear film by the mucoadhesive polymer that mostly delayed drug elimination from the eye surface compared to the polymer-free control, as illustrated under case of Ketotifen Fumarate.

Conclusions

The comparative evaluation of the ability of the different polysaccharides studied to resist removal from tear fluid has suggested that the optimal polymer to be used as an additive in ophthalmic drops should be mucoadhesive without increasing the viscosity of the solution to an excessive extent. TSP of 0.7% (wt/vol) has shown to possess more of these properties than the other polymers at comparison, in fact, it has exhibited the highest MRT value. HA and HEC, although mucoadhesive, increase the solution viscosity to an excessive degree, and this, in addition to worsening the patient compliance, could induce an anomalous reflex tearing with consequent acceleration of precorneal clearance. AG, at the concentrations at which TSP, HA, and HEC were tested, has not exhibited any particular mucoadhesive properties. This polysaccharide, nevertheless, did not increase the solution viscosity, so it has the potential to be used at much higher concentrations. In virtue of its mucoadhesiveness, TSP of 0.7% (wt/vol) is supposed to stabilize the tear film. Perhaps, this is the main reason why the MRT and RT_{max} of the two different drugs tested in this work were significantly prolonged by TSP of 0.7% (wt/vol). These results have shown a prospect of future ophthalmic formulations of KF and DS allowing a decreased frequency of instillations.

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