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In situ gelling xyloglucan formulations for sustained release ocular delivery of pilocarpine hydrochloride

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Abstract

Thermoreversible gels formed in situ by aqueous solutions of an enzyme-degraded xyloglucan polysaccharide were evaluated as sustained release vehicles for the ocular delivery of pilocarpine hydrochloride. In vitro release of pilocarpine from gels formed by warming xyloglucan sols (1.0, 1.5 and 2.0% w/w) to 34 °C followed root-time kinetics over a period of 6 h. The miotic responses in rabbit following administration of xyloglucan sols were compared with those from in situ gelling Pluronic F127 sols and from an aqueous buffer solution containing the same drug concentration. Sustained release of pilocarpine was observed with all gels, the duration of miotic response increasing with increase of xyloglucan concentration. The degree of enhancement of miotic response following sustained release of pilocarpine from the 1.5% w/w xyloglucan gel was similar to that from a 25% w/w Pluronic F127 gel. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Ocular drug delivery; In-situ gelation; Xyloglucan gels; Pilocarpine hydrochloride; Sustained release

1. Introduction

The use of gels for the ocular administration of drugs offers many advantages compared with conventional eye-drops, mainly as a consequence of the more prolonged corneal contact time. Maurice (1987) has shown that the concentration of drug administered by liquid formulations can be reduced 10-fold within 4–20 min as a result of the normal protective mechanisms of the eye such as blinking and tear drainage. This rapid elimination of drug causes a short duration of the therapeutic effect. Moreover, drainage through the nasal-lacrimal duct may lead to absorption in the gastro-intestinal tract and the possibility of side effects.

Drainage rate may be decreased and therapeutic effect improved by increasing the viscosity of the formulation by addition of polymers such as cellulose ethers, or through the use of hydrogels or ointments (Sieg and Robinson, 1975). Experiments in rabbits and humans, for example, have

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demonstrated improved ocular drug bioavailability (Schoenwald and Boltralik, 1979) or enhanced therapeutic response (Saettone et al., 1980) from aqueous gels compared with conventional eye-drop formulations. Hydrogels are better tolerated by patients than ointments, they do not obstruct vision and when formulated as in situ gelling preparations offer the advantage of convenient administration. Residence times of several hours can be achieved using in situ gels (Hui and Robinson, 1985; Mazuel and Friteyre, 1992; Meseguer et al., 1993; Sanzgiri et al., 1993) and in the case of gellan formulations these may be extended to 20 h by adjustment of osmolarity (Carlfors et al., 1998).

Two main types of in situ gelling materials have been examined for their potential as vehicles for ocular delivery. Aqueous solutions (20–25% w/w) of high molecular weight ABA poly(oxyethylene)/poly(oxypropylene)/poly(oxyethylene) triblock copolymers such as Pluronic F127 form thermally reversible gels on administration to the eye and their use in the delivery of pilocarpine has been examined by Miller and Donovan (1982) and Desai and Blanchard (1998). Similarly, aqueous solutions of deacetylated gellan gum (an exocellular polysaccharide of microbial origin, commercially available as Gelrite™ or Kelcogel™) also form gels when instilled into the eye. The mechanism of gelation of this compound involves cations present in tear fluid, and gelation occurs at very much lower concentration, typically $<$ 2%. Gellan-based systems have been reported for the ophthalmic sustained delivery of methylprednisolone (Sanzgiri et al., 1993) and timolol (Rozier et al., 1989, 1997). It was shown, for example, that the ocular bioavailability of timolol in rabbit was increased by 3–4-fold compared with conventional timolol solution when administered from a gellan formulation (Rozier et al., 1989).

In the present paper, we have explored the potential of an alternative in situ gelling material of natural origin, xyloglucan, for the sustained ocular delivery of pilocarpine. Xyloglucan is a polysaccharide derived from tamarind seeds, which when partially degraded by β-galactosidase exhibits thermally reversible gelation in dilute aqueous solution, the sol–gel transition temperature varying with the degree of galactose elimination. The material used here had a percentage of galactose removal of 44% and exhibited a thermally reversible transition from sol to gel at temperatures of between 22 and 27 °C. We previously investigated the potential use of xyloglucan gels for rectal (Miyazaki et al., 1998), intraperitoneal (Suisha et al., 1998) and oral drug delivery (Kawasaki et al., 1999; Miyazaki et al., 2001). We now report an evaluation of the sustained release properties of xyloglucan gels formed in situ in the rabbit eye, using changes in pupil diameter as a monitor of pharmacologic response. Comparison has been made with the release characteristics of a Pluronic F127 gel formed in situ under identical conditions. Both the viscosity of the liquid formulations during instillation in the eye and the rheological properties of the gel subsequently formed, are important to the retention of the dosage form in the precornial cavity. For this reason, we have carried out comparative evaluations of these properties with those of the Pluronic formulation.

2. Materials and methods

2.1. Materials

Xyloglucan with a percentage of galactose removal of 44% (Lot. 9530L) was prepared as described previously (Shirakawa et al., 1998) and supplied by Dainippon Pharmaceutical Co., Osaka. Pilocarpine hydrochloride was obtained from Wako Pure Chemical Co. (Osaka, Japan) and Pluronic F127 was supplied by Sigma Chemicals (St. Louis, MO, USA).

2.2. Preparation of drug formulations

Xyloglucan sols of concentrations 1.0, 1.5 and 2.0% w/w were prepared by slowly adding a weighed amount of the enzyme-degraded xyloglucan to cold phosphate buffer pH 7.4. The mixture was slowly homogenised (Nihon Seiki Seisakusho homogeniser type HB) and an appropriate amount of pilocarpine hydrochloride was then

dissolved in the resulting solution to produce a final drug concentration of 1% w/v. Pluronic F127 sols (25% w/w) were prepared in a similar manner but stored overnight at 5 °C to ensure complete dissolution before the addition of pilocarpine hydrochloride. Sols were made isotonic by the addition of sodium chloride.

A 1% w/v solution of pilocarpine hydrochloride was prepared in pH 7.4 phosphate buffer and made isotonic by the addition of sodium chloride. All pilocarpine formulations were stored at 5 °C when not in use.

2.3. Measurement of viscosity of sols

The viscosity of xyloglucan and Pluronic F127 sols prepared in isotonic pH 7.4 phosphate buffer was determined with a cone and plate viscometer (TV-20H, model E, Tokimec Co., Tokyo) at 5 °C using a 1 ml aliquot of the sample.

2.4. Measurement of gel strength

Measurements of the comparative gel strengths of xyloglucan gels of concentrations 1.0, 1.5 and 2.0% w/w were carried out using a rheometer (CR-200D, Sun Scientific Co., Tokyo). A 30 g sample of the gel prepared in isotonic pH 7.4 phosphate buffer was contained in a 50 ml beaker maintained at constant temperature by a water jacket through which water was circulated at 34 °C (ocular surface temperature) from a thermostat bath. The beaker was raised at a rate of 60 mm min^{-1} so pushing a probe slowly through the gel. The changes in the load on the probe as a function of depth of immersion of the probe below the gel surface were measured for each gel.

2.5. Measurement of drug release rate from gels

The release rates of theophylline were measured at 34 °C using plastic dialysis cells similar to that described previously (Miyazaki et al., 1984). The capacity of each half-cell was 4 ml and the surface area of the membranes was 2.67 cm^2. The enzyme-degraded xyloglucan and Pluronic F127 formulations prepared in isotonic pH 7.4 buffer and loaded with a known weight of drug were placed in the donor compartment and an equal volume of simulated tear fluid of composition 0.67% NaCl, 0.2% $NaHCO_3$, 0.008% $CaCl_2$ $2H_2O$ (Rozier et al. 1989) was placed in the receptor compartment. The donor phase and the aqueous receptor phase were separated by a cellulose membrane (Viskase Sales Co., size 36/32). The assembled cell was shaken horizontally at the rate of 60 strokes min^{-1} in an incubator. The total volume of the receptor solution was removed at intervals and replaced by fresh release medium. The drug concentration of the sample was determined using a spectrophotometer at a wavelength of 215 nm.

2.6. In vivo release

Miotic studies were conducted using white male rabbits weighing 2.9–3.6 kg. The rabbits were kept in restraining boxes throughout the course of each experiment. All tests were performed in the same room under standard lighting conditions.

A metric ruler was fixed under the eye in the optical section of the iris. After 1 h of acclimatisation, the basal pupil diameter of the left eye was measured using a digital camera (CAMEDIA C-1400L, Olympus, Tokyo). The focus of the camera was adjusted to the iris. Each preparation containing the same dose (1% w/v) of pilocarpine was then tested in four animals by instilling a dose of 25 μl into the lower cul-de-sac of the left eye of four rabbits with a micropipette (Finnpipette no. 4500030, Labsystems, Helsinki). The gels were chilled prior to filling the pipette to facilitate this procedure. Both the aqueous and gel formulations were tested in each of the four rabbits. A minimum of 1 week elapsed between tests in the same rabbit.

Photographs of the pupil were taken at predetermined times and pupil size was determined from computer images using the ruler as a calibration scale. Effects of the formulations on pupil diameter were expressed as the change relative to basal pupil diameter. The AUC (mm min) of the tested formulations were calculated using the trapezoidal method. Data are presented as arithmetic means of four experiments $\pm$ S.E. The efficiency of the gel formulations was estimated by the peak miotic response (PR) and the area under

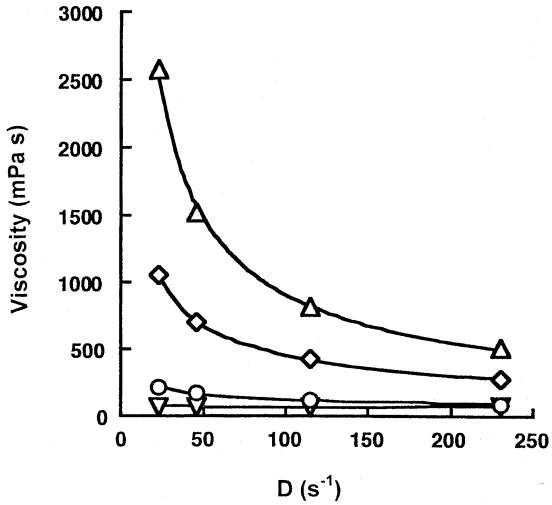

Fig. 1. Viscosity of xyloglucan sols of concentrations 1.0 (○), 1.5 (◇) and 2.0 (△) % w/w and 25% w/w Pluronic F127 sols (▽), in isotonic phosphate buffer pH 7.4 at 5 °C as function of shear rate, *D*.

the miosis-time-curve (AUC, miotic response) after administration of the respective preparation. The duration of miotic response (DR) was defined as the time interval between administration of the pilocarpine treatment and the time at which the pupil diameter returned to its normal pretreatment value.

3. Results and discussion

3.1. Viscosity and gelling properties of sols

Fig. 1 compares the shear dependency of the viscosity of 1.0, 1.5 and 2.0% w/w solutions of xyloglucan and a 25% w/w solution of Pluronic F127. The temperature of measurement was maintained at 5 °C to ensure that all preparations were in sol form. All of the xyloglucan solutions had higher viscosity than the Pluronic F127 sol at all shear rates, which is advantageous for their proposed usage in that leakage of solution from the eye during instillation would be minimised. The viscosity increased markedly with concentration, the higher concentrations showing shear thinning behaviour.

The gel strengths of xyloglucan gels of concentrations between 1.0 and 2.0% w/w were compared at 34 °C (temperature of the eye surface) using a simple method that measured the change in load of a probe pushed slowly through the gel. Although the values produced are not absolute values, they are useful for assessing the influence of concentration on the strength of the xyloglucan gels. Stress–strain plots (Fig. 2) of the xyloglucan gels were typical of those for elastic gels showing a sudden decrease of stress after the maximum, indicative of a brittle system. Values of gel

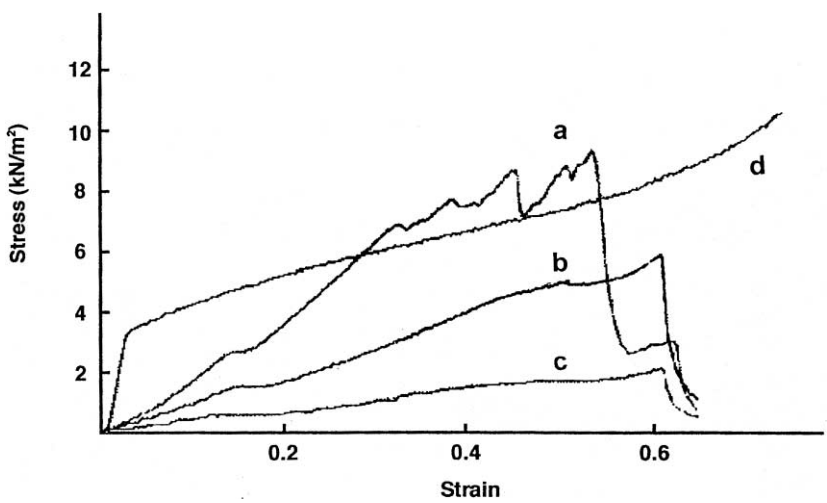

Fig. 2. Rheological properties of xyloglucan gels of concentrations (a) 2.0; (b) 1.5; (c) 1.0% w/w; and (d) 25% w/w Pluronic F127 gels in isotonic phosphate buffer pH 7.4 at 34 °C.

strength (in kN m^{-2}), taken as the stress at the point of collapse of the gel structure, were 2.08, 5.83 and 9.27 for xyloglucan concentrations of 1.0, 1.5 and 2.0% w/w, respectively. The observed increase of gel strength with concentration has been noted previously for xyloglucan gels (Miyazaki et al., 1998) and is a consequence of an increased density of the laterally stacked chains of the enzyme-degraded xyloglucan (Yuguchi et al., 1997). The Pluronic F127 gels are cubic gels formed by the packing of the micelles of this poly(oxyethylene)/poly(oxypropylene)/poly(oxyethylene) triblock copolymer and show different rheological behaviour to that of the xyloglucan gels. Nevertheless, Fig. 2 shows that the stress–strain characteristics of the 25% w/w Pluronic gel above its yield point most closely resemble those of the 2.0% w/w xyloglucan gel.

Thus, this simple comparative study of the rheological properties of xyloglucan and Pluronic F127 gels has shown that similar gel strengths can be achieved using much lower concentrations of xyloglucan with the added advantage of more viscous sols that would minimise the leakage of solution from the eye during instillation.

3.2. In vitro drug release

Fig. 3 shows the release of pilocarpine as a function of time from xyloglucan and Pluronic F127 gels loaded with an initial drug concentration, C_o, of 1% w/v and from an aqueous buffer solution pH 7.4 also containing 1% w/v of drug. As the pH of the aqueous phase of the gels was also 7.4 the extent of ionisation of the pilocarpine ($pK_{a2} = 7.1$) was the same in all preparations. Comparison of release profiles shows the expected sustained release effect of the gel vehicles, the most effective being the Pluronic gel.

The release data over the whole time period were analysed according to the treatment proposed by Higuchi (1962) for drug release from semisolid vehicles containing dissolved drug. For the initial 50–60% release the cumulative amount Q of drug released per unit surface area is proportional to the square root of time t:

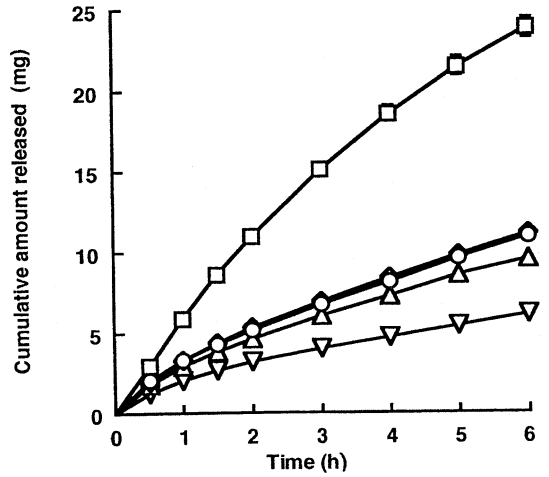

Fig. 3. Cumulative release of pilocarpine hydrochloride (initial concentration 1.0% w/v) as a function of time from xyloglucan gels of concentrations 1.0 (○), 1.5 (◇) and 2.0 (△) % w/w and from 25% w/w Pluronic F127 gel (▽) and isotonic phosphate buffer pH 7.4 (□). Each value is the mean ± S.E. of four determinations.

$$Q = 2C_o\left(\frac{Dt}{\pi}\right)^{1/2} \quad (1)$$

The plots of Q versus $t^{1/2}$ for the release of pilocarpine from xyloglucan and Pluronic gels were linear after a short lag period (Fig. 4) indicating diffusion-controlled release. Diffusion coefficients, D, calculated from the gradients of the plots of Fig. 3 were 7.96, 8.49 and 6.13×10^{-6} $cm^2\ s^{-1}$ for 1.0, 1.5 and 2.0% w/w xyloglucan gels respectively, and 2.18×10^{-6} $cm^2\ s^{-1}$ for the 25% w/w Pluronic gel.

3.3. In vivo release

The miotic responses to pilocarpine after the instillation to the rabbit eye of 25 μl of solutions of xyloglucan and Pluronic F127 and also buffer solution containing the same dose (1% w/v) of pilocarpine are compared in Fig. 5. It can be seen from this figure that at all times post-administration, the increase of pupil diameter was greater for the gel formulations than for the aqueous buffer solution, although the general shape of the profile was similar. Fig. 5a compares the miotic response following administration of pilocarpine from 1.0% w/w xyloglucan and 25% w/w Pluronic

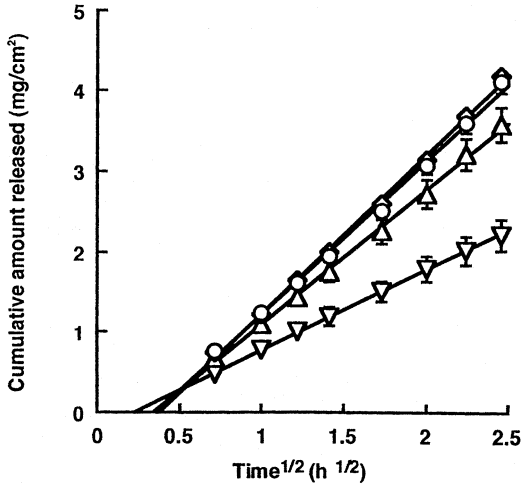

Fig. 4. Cumulative release per unit area, Q, for pilocarpine hydrochloride (initial concentration 1.0% w/v) as a function of square root time from xyloglucan gels of concentrations 1.0 (○), 1.5 (◇) and 2.0 (△) % w/w and from 25% w/w Pluronic F127 gel (▽). Each value is the mean ± S.E. of four determinations.

gels and from the aqueous solution and shows no significant differences at a 0.05 probability level. In contrast, Fig. 5b and c show significant differences in miotic responses at 0.01 and 0.05 probability levels for 1.5 and 2.0% w/w xyloglucan gels compared with the aqueous buffer solution. Table 1 shows a decrease in peak miotic response with increase of xyloglucan concentration and a concomitant increase in duration of response, indicating a more sustained release due to a greater diffusional resistance.

The individual areas under the curves (0–4.5 h), were calculated for each formulation using the trapezoidal rule. It is interesting to note the greater enhancement of response from the 2.0% w/w xyloglucan gel (1.45-fold increase) compared with that from the Pluronic gel which shows a 1.36-fold increase in miotic response. The difference in rank order of the in vitro and in vivo release characteristics of the xyloglucan and Pluronic gels may be a consequence of a greater susceptibility of the micellar gels of Pluronic F127 to dilution by tears than the xyloglucan gels which are formed by the lateral stacking of rod-like chains (Yuguchi et al., 1997).

4. Concluding remarks

Visual examination has shown that solutions of enzyme-degraded xyloglucan (2.0% w/w) form gels when instilled in the rabbit eye that remain in

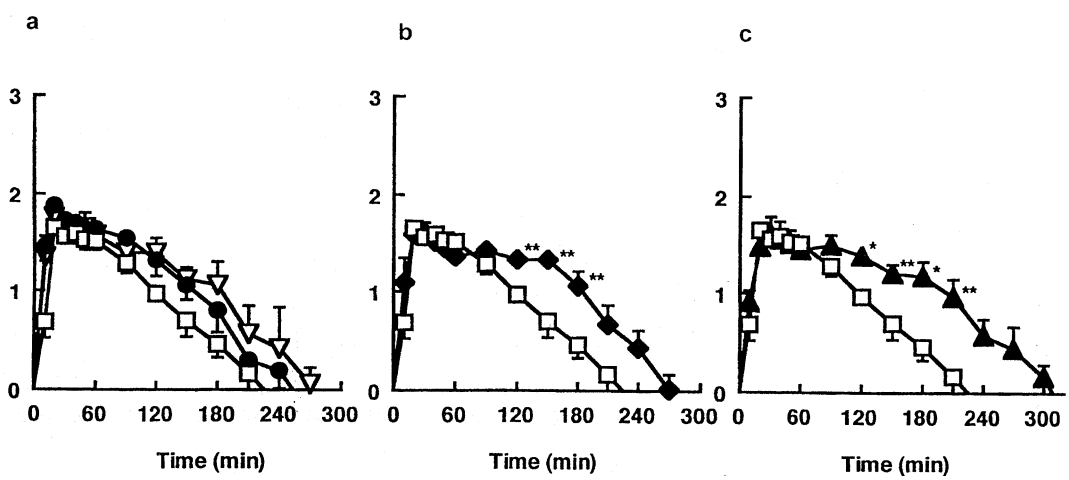

Fig. 5. Miotic response in rabbit eye to pilocarpine hydrochloride released from xyloglucan gels of concentrations 1.0 (●), 1.5 (◆) and 2.0 (▲) % w/w, 25% w/w Pluronic F127 gel (▽) and isotonic phosphate buffer pH 7.4 (□). Each value is the mean ± S.E. of four determinations. $^{*}P < 0.01$; $^{**}P < 0.05$.

Table 1
Miotic response of pilocarpine hydrochloride formulations

Dosage from	TP (min)[a]	PR (min)[b]	DR (min)[c]	AUC (0–270 min)(mm min)
Buffer solution	27.5 ± 7.5	1.72 ± 0.07	240.1 ± 29.9	214.9 ± 26.4
Xyloglucan gel				
1.0%	22.5 ± 2.5	1.88 ± 0.15	251.8 ± 14.0	275.9 ± 35.0
1.5%	22.5 ± 6.3	1.68 ± 0.11	274.1 ± 12.3	288.6 ± 19.5
2.0%	57.5 ± 21.0	1.67 ± 0.14	298.7 ± 13.7	312.1 ± 18.5[d]
Pluronic F127 gel				
25%	37.5 ± 10.3	1.79 ± 0.11	278.2 ± 19.6	292.6 ± 48.1

[a] TP, time required to achieve peak miotic response.
[b] PR, peak miotic response.
[c] DR, duration of miotic response.
[d] $P < 0.05$
Each value represents the mean ± S.E. of four determinations.

the eye for at least 6 h. Our results have shown a duration of significant miotic response following release of pilocarpine from the xyloglucan gels over a period of at least 4 h. The miotic response obtained with a 1.5% w/w xyloglucan formulation was similar to that from 25% w/w Pluronic F127. The much lower concentration of xyloglucan compared with Pluronic F127 required for in situ gelation and its approval for use as a food additive make it a more suitable material than Pluronic F127 for this purpose. Similarly, it has advantages over the gellan formulations previously examined for use in ocular delivery, which rely on uptake of cations present in the tear fluid for their gelation. Rapid gelation is essential to prevent loss of the formulation by drainage from the eye and for the gellan solutions this depends on the osmotic gradient between the gel and the surrounding environment, which must be carefully controlled by adjustment of solution osmolarity.

Acknowledgements

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