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Xyloglucan gels as sustained release vehicles for the intraperitoneal administration of mitomycin C

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Abstract

Thermoreversible gels formed by a xyloglucan polysaccharide derived from tamarind seed were evaluated as a sustained-release vehicle for the intraperitoneal administration of mitomycin C (MMC). The in vitro release of MMC from gels prepared from galactoxyloglucan that had been partially degraded by β-galactosidase to eliminate 44% of galactose residues followed root-time kinetics over 5 h. Intraperitoneal administration of MMC in a 1.5% (w/w) xyloglucan gel to rats resulted in a broad concentration–time profile for this drug in both the ascites and the plasma over a 3-h time period, compared with a narrow peak and rapid disappearance from both sites when this drug was given i.p. as a solution.

Keywords: Xyloglucan gels; Mitomycin C; Intraperitoneal administration; Drug delivery; Thermoreversible gels

1. Introduction

Mitomycin C (MMC) is a clinically significant antineoplastic antibiotic and has been administered intraperitoneally (i.p) in solution for carcinomatous peritonitis (Hagiwara et al., 1987). However, MMC administered in solution form is rapidly absorbed through the serosal surface into blood plasma and it is difficult to keep a high concentration of the drug in the peritoneal cavity for effective control of the local lesion (Sasaki and Ogita, 1980). Attempts have been made to achieve sustained intraperitoneal delivery of MMC to overcome this problem. Drug delivery systems devised to sustain the release of MMC have included activated carbon particles (Hagiwara et al., 1987), and poly(L-lactic acid) microcapsules (Iwa

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et al., 1985). More recently, we have evaluated the potential of gels of the oxyethylene–oxypropylene–oxyethylene triblock copolymer, Pluronic F127, as a sustained-release vehicle for i.p. administration of MMC in the treatment of Sarcoma-180 ascites tumour. A prolongation of the life-span of tumour-bearing mice following injection of MMC in gels was noted and MMC in Pluronic gel was therapeutically more active than free drug (Miyazaki et al., 1992). However, the high concentration of block copolymer (20–30%, w/w) required for gelation and its possible toxicity, particularly when intended for i.p administration (Abe et al., 1990) has led to a study of the potential of natural polymers as vehicles for drug delivery.

The present study is an examination of the potential use of a gel formulation prepared using a xyloglucan polysaccharide for i.p. administration of MMC. Xyloglucan polysaccharide derived from tamarind seeds is composed of a (1–4)-β-D-glucan backbone chain which has (1–6)-α-D-xylose branches that are partially substituted by (1–2)-β-D-galactoxylose. When partially degraded by β-galactosidase, the resultant product exhibits thermally reversible gelation in dilute aqueous solution. Such gelation does not occur with native xyloglucan. A detailed study of the sol–gel transition temperature as a function of the degree of galactose elimination has been reported by Yuguchi et al. (1997) and Shirakawa et al. (1998). The gelation behaviour is similar to that observed with Pluronic F127, with a sol–gel transition on heating from refrigerator temperature or cooling from a high temperature (so-called cold and hot gelation, Schmolka, 1972). We have recently shown that enzyme-degraded xyloglucan forms gels at concentrations of between 0.5 and 2% (w/w) at gelation temperatures decreasing over the range 20–25°C with increasing concentration (Miyazaki et al., 1998). Time-resolved small-angle X-ray scattering studies (Yuguchi et al., 1997) have shown that enzyme-degraded xyloglucan forms gels by the lateral stacking of the rod-like chains. In this respect these gels differ from block copolymer gels which are formed by packing of micelles behaving effectively as hard spheres (Tanodekaew et al., 1993; Luo et al., 1993). An important difference between the gelation properties of the xyloglucan gels and block copolymers such as Pluronic F127 from a toxicity viewpoint is that the xyloglucan polysaccharide forms gels at much lower concentrations. In addition, xyloglucan is approved for use as a food additive and, although there may be the possibility of some partial degradation in vivo, it is clear that any such degraded material does not possess any significant toxicity in humans. Preliminary acute toxicity studies on rats are reported in the present study and no detectable toxicity effects have been observed.

In a previous study (Miyazaki et al., 1998) we examined the potential of gels of enzyme-degraded xyloglucan as vehicles for rectal drug delivery. We now report a study of their possible use for i.p. drug administration. The absorption characteristics of MMC when administered intraperitoneally to rats in aqueous solution and in xyloglucan gels are compared and related to the corresponding ascites concentrations.

2. Materials and methods

2.1. Materials

Galactoxyloglucan with a percentage of galactose removal of 44% was prepared as described previously (Shirakawa et al., 1998). Removal of the required percentage of β-D-galactosidase residues was carried out by reacting a 3% aqueous solution of xyloglucan with a 0.09% aqueous solution of the enzyme β-galactosidase from *Bacillus circulans* (Biolacta N5, Daiwa Kasei K.K., Osaka, Japan). The conditions required for 44% removal are pH 6.0 and 55°C for 16 h. The reaction was terminated at this time by heating to 90°C for 30 min to inactivate the enzyme. The enzyme-degraded xyloglucan was precipitated from this solution by addition of ethanol.

Mitomycin C (MMC) was supplied by Kyowa Hakko Kogyo Co., Tokyo.

2.2. Preparation of gels

A weighed amount of enzyme-degraded xyloglucan was slowly added to cold phosphate

buffer at pH 7.2. The mixture was homogenised (Nihon Seiki Seisakusho homogeniser type HB) for 1 h at 1000 rpm. An appropriate amount of MMC was then dissolved in the resulting solution.

2.3. Measurement of drug release rate from gels

The release rate of MMC was measured by 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 3.14 cm^2. The xyloglucan gel, prepared in pH 7.2 buffer and loaded with a known weight of drug, was formed in the donor compartment and an equal volume of pH 7.2 phosphate buffer was placed in the acceptor compartment. The gel 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 in an incubator. The total volume of the receptor solution was removed at certain intervals and replaced by fresh buffer solution. The drug concentration was determined spectrophotometrically at a wavelength of 360 nm.

2.4. Animal experiments

Male Wistar rats weighing 200–280 g were divided into two groups of four rats. The xyloglucan sol preparation was administered intraperitoneally to the rats of one group through a needle (Terumo 27Gx3/4) fitted on to a disposable syringe: the sol was cooled prior to filling the syringe to facilitate this procedure. The MMC gel preparation was given as 1.5% (w/w) xyloglucan solution containing the drug (2 ml/kg, 5 mg/kg body weight). The rats of the control group were injected with MMC solution in pH 7.2 phosphate buffer. At predetermined intervals, a blood sample was obtained by heart puncture. Five ml of saline were injected into the peritoneal cavity and a peritoneal fluid (ascites) sample was also collected at the same time.

2.5. Measurement of MMC in plasma and ascites

MMC levels in plasma and ascites were determined by HPLC (Shimadzu LC-6A with a Shimadzu SPD-6A detector at a wavelength of 360 nm) using the method of Li et al. (1993) with modification. To 0.5 ml of plasma or ascites were added 20 μl of *p*-aminoacetophenone solution as internal standard. The drug was extracted with 4 ml of dichloromethane by mechanical shaking for 10 min. The drug organic phase was separated by ultracentrifugation and evaporated with nitrogen gas. The residue was reconstituted with 0.2 ml of methanol and injected on to a 25 cm × 4.6 mm i.d. column, packed with Inertsil ODS-2. Elution was carried out with a mixture of acetonitrile and 0.02 M potassium dihydrogen phosphate (2:8, v/v) at a rate of 1.0 ml/min at 50°C.

3. Results and discussion

3.1. In vitro release of MMC

The cumulative release of MMC from an aqueous solution of concentration 0.025% (w/v) and from xyloglucan gels with gel concentrations of 0.5, 1 and 1.5% (w/w) is compared in Fig. 1 at constant temperature (37°C) and at an initial MMC concentration, C_0, of 0.025% (w/v). Release data 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:

$$Q = 2C_0(Dt/\pi)^{1/2} \quad (1)$$

Plots of Q vs. $t^{1/2}$ were linear after a short lag period (Fig. 2), indicating that drug release was controlled by diffusion through the gel matrix, as might be expected for this water-soluble drug which will partition predominantly in the water channels between the aggregated chains. Diffusion coefficients, D, calculated from the gradients of the plots of Fig. 2 were 8.97, 7.28, and 3.92×10^{-6} cm^2/s at gel concentrations of 0.5, 1.0 and 1.5% (w/w), respectively. The decrease of D with

increase of gel concentration is probably a result of narrowing of the water channels within the gel structure through which drug diffusion may occur.

It is interesting to compare the release of MMC from the xyloglucan gels with that from Pluronic F127. The data reported by Miyazaki et al. (1992) for the release of MMC from a 25% (w/w) Pluronic F127 gel was obtained using the same equipment as in the present study with a dialysis membrane of the same type and identical surface area. Analysis of these data using Eq. (1) shows that the release of MMC from Pluronic F127 gels is also diffusion controlled; the drug in this gel diffusing through water channels between the packed micelles of the cubic phase matrix. The diffusion coefficient for MMC when released from the 25% (w/w) Pluronic gel loaded with an initial drug concentration of 0.05% (w/v) ($D = 3.61 \times 10^{-6}$ cm^2/s) was approximately the same as that for release from a 1.5% (w/w) xyloglucan gel at an initial drug loading of 0.025% (w/v).

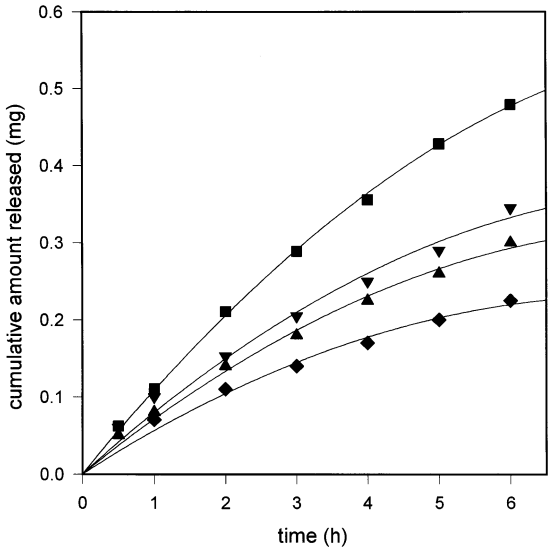

Fig. 1. Cumulative amount of MMC released as a function of time at 37°C from xyloglucan gels of concentrations (▼) 0.5, (▲) 1 and (◆) 1.5% (w/w) containing an initial MMC concentration of 0.025% (w/v) and from (■) 0.025% (w/v) solution of MMC

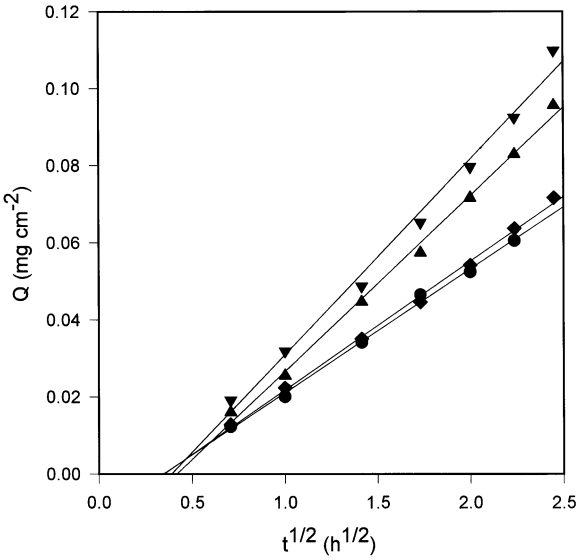

Fig. 2. Cumulative amount of mitomycin C released per unit area as a function of root time at 37°C from xyloglucan gels of concentrations (▼) 0.5, (▲) 1 and (◆) 1.5% (w/w) containing an initial MMC concentration of 0.025% (w/v) and from (●) 25% (w/w) Pluronic F127 gel containing an initial MMC concentration of 0.05% (w/v).

3.2. In vivo release of MMC

Fig. 3 shows plots of the ascites concentration of MMC as a function of time after i.p. administration for drug injected in solution form and as a MMC-loaded gel. Comparison of the two profiles shows a rapid appearance of drug in the ascites when given as a solution, with a peak concentration at $t \leq 15$ min, and almost complete removal after 1 h. In contrast, the drug concentration in the ascites when MMC was administered in a gel remained at approximately 30 μg/ml over the time period 15 min to 1 h following administration and drug was still present (5 μg/ml) after 3 h.

Fig. 4 compares plasma concentrations of MMC after i.p. administration in the two dosage forms. The MMC concentration reached a peak at 30 min when given in solution and decreased rapidly thereafter, with only trace amounts detectable in plasma samples taken at 3 h. The concentration profile when administered in gel state was much broader with detectable quantities of drug after 6 h.

Examination of the contents of the abdomen of rats after i.p. administration of MMC showed the presence of gel at a time interval of at least 6 h. The long residence time of the gel in the abdomen is probably the reason for the prolongation of the release of MMC when administered in a gel compared with that from a solution.

3.3. Toxicity studies

The acute toxicity of the enzyme-degraded xyloglucan was evaluated on the basis of changes in body weight after i.p. administration of 4 ml of 1.5% (w/w) sols (without drug) in a preliminary study. Increase of body weight over a 14-day period of three rats following an i.p. injection of the sol was not significantly different to that of a control rat.

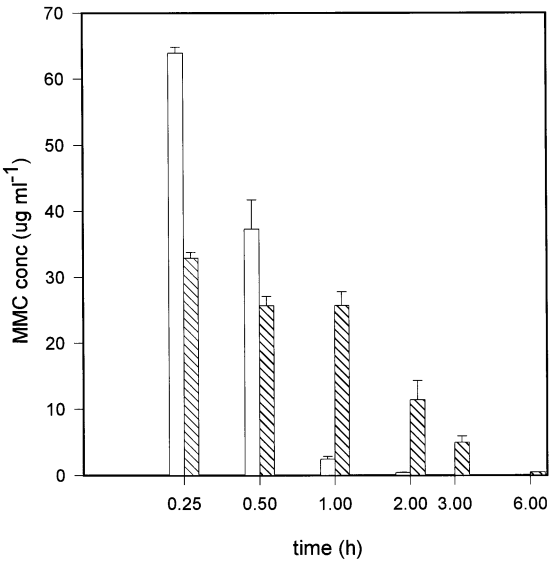

Fig. 3. Ascites concentration of MMC as a function of time after i.p. administration to rats of MMC in 1.5% (w/w) xyloglucan gel (shaded) and buffer solution. Each value represents the mean ± S.E. of four experiments ($p < 0.05$ for gel sample at 2 h and $p < 0.001$ for gel samples at 0.25 and 1 h, compared with solution).

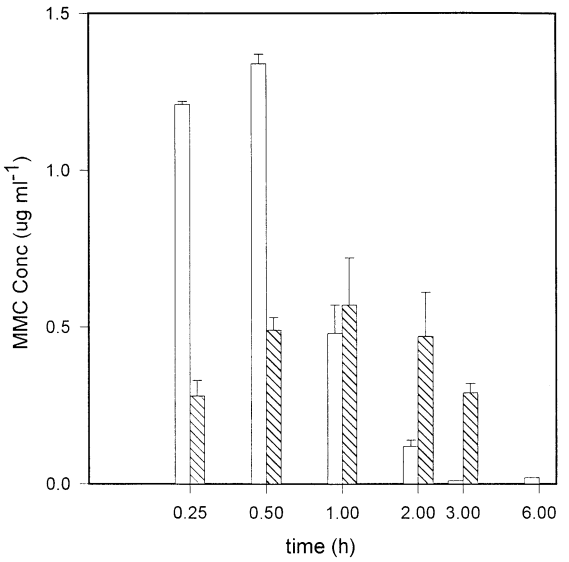

Fig. 4. Plasma concentration of MMC as a function of time after i.p. administration to rats of MMC in 1.5% (w/w) xyloglucan gel (shaded) and buffer solution. Each value represents the mean ± S.E. of four experiments ($p < 0.05$ for gel sample at 2 h and $p < 0.001$ for gel samples at 0.25 and 1 h, compared with solution).

4. Conclusion

The results of this study have shown that the in vitro release of mitomycin C from the enzyme-degraded xyloglucan gels is diffusion controlled over a time interval of 5 h; the diffusion coefficient decreasing with increase of gel concentration. Comparison of release rates of MMC from this gel with those from a Pluronic F127 gel shows similar rates of release from a 1.5% (w/w) xyloglucan gel and a 25% (w/w) Pluronic gel. Intraperitoneal injection of MMC in a xyloglucan gel resulted in a broad concentration–time profile for MMC in both the ascites and the plasma, compared with a narrow peak and rapid disappearance from both sites when this drug was given i.p. as a solution.

Xyloglucan gels have many advantages for use as a vehicle for drug delivery in that they are non-toxic and form at low concentrations. However, there is no published information on their biodegradability, which may limit their suitability for use in i.p. injection, although not for the oral,

rectal, ophthalmic or nasal administration of drugs.

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