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Oral sustained delivery of theophylline from thermally reversible xyloglucan gels in rabbits

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Abstract

Thermally reversible gels formed in-situ following the oral administration of dilute aqueous solutions of an enzyme-degraded xyloglucan to rabbits were evaluated as sustained-release vehicles for the delivery of theophylline. In-vitro release of theophylline from gels formed by warming xyloglucan sols (0.5, 1.0 and 1.5% w/w) to 37°C followed root-time kinetics over a period of 4 h. Gels formed after oral administration to rabbits of chilled 1.5% w/w aqueous solutions of xyloglucan containing dissolved drug showed sustained-release characteristics with a maximum plasma concentration at 4.5 h. The theophylline bioavailability from a 1.5% w/w xyloglucan gel was 1.7–2.5 times that of commercial oral sustained-release liquid dosage forms containing an identical theophylline concentration. It was concluded that dilute solutions of the enzyme-degraded xyloglucan had suitable rheological properties and in-situ gelling characteristics for use as sustained-release vehicles for oral drug delivery. The in-vivo release characteristics of theophylline in a rabbit model suggested the potential for the use of these vehicles in humans for the oral delivery of this drug.

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Introduction

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. The tamarind seed xyloglucan is composed of three units of xyloglucan oligomers with heptasaccharide, octasaccharide and nonasaccharide, which differ in the number of galactose side-chains (Figure 1). When xyloglucan derived from tamarind seed is partially degraded by β-galactosidase the resultant product exhibits thermally reversible gelation, the sol–gel transition temperature varying with the degree of galactose elimination (Yuguchi et al 1997). Such gelation does not occur with native xyloglucan. In this study we have used a xyloglucan sample with a galactose removal of 44% that exhibits a thermally reversible transition from sol to gel at temperatures of 22–27°C over the concentration range 1–2% w/w (Miyazaki et al 1998). In this respect the gelation behaviour is similar to that observed with the poly(oxyethylene)–poly(oxypropylene)–poly(oxyethylene) triblock copolymer Pluronic F127, the potential for use in drug delivery of which has been widely studied (Miller & Donovan 1982; Morikawa et al 1987; Miyazaki et al 1984, 1986, 1992, 1995; Choi et al 1998; Jain et al 1991; Scherlund et al 2000). The mechanism of gelation of aqueous solutions of these two materials is of course different. Block copolymer gels are formed by the packing of micelles behaving effectively as hard spheres

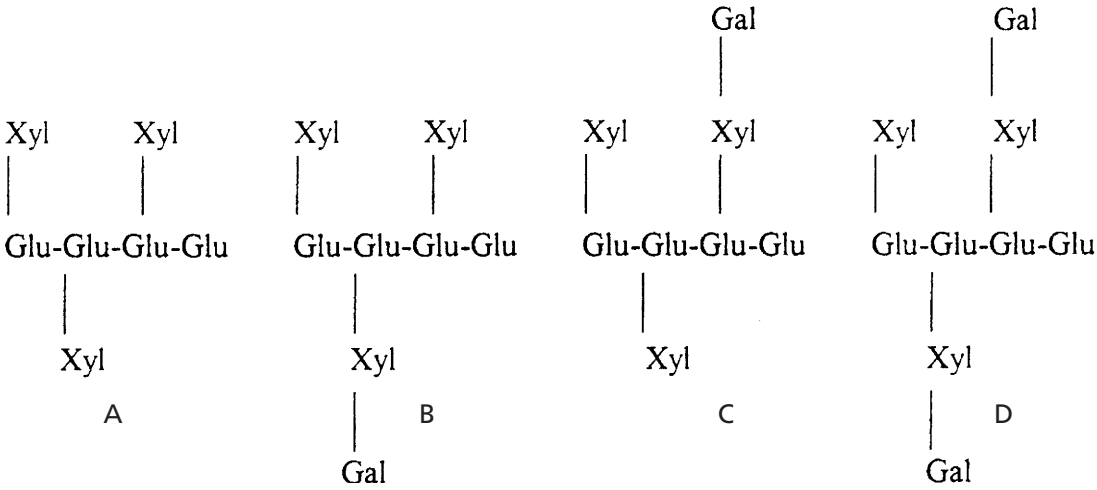

Figure 1 The unit structures of oligosaccharides from tamarind xyloglucan showing heptasaccharide (A), octasaccharides (B and C) and nonasaccharide (D).

(Booth & Attwood 2000), whereas the gelation of enzyme-degraded xyloglucan is by the lateral stacking of the rod-like chains (Yuguchi et al 1997). 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 over the concentration range 1–2% w/w (Miyazaki et al 1998) compared with 20–30% for Pluronic F127. 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.

Thermally reversible gelation is also exhibited by semi-dilute solutions of the nonionic cellulose derivative ethyl(hydroxyethyl)cellulose, EHEC (Carlsson et al 1990; Lindman et al 1990). A disadvantage of this system from a toxicological aspect is the need for inclusion in the formulation of an ionic surfactant. Although it is possible to reduce the quantity of surfactant required (Lindell & Engström 1993), this imposes some restrictions on the type of drug that can be delivered with this vehicle.

We previously reported the potential use of xyloglucan gels for rectal (Miyazaki et al 1998) and intraperitoneal (Suisha et al 1998) drug delivery, and more recently (Kawasaki et al 1999) we investigated the possibility of forming gels in-situ in the gastrointestinal tract of rats following the administration of dilute solutions of enzyme-degraded xyloglucan, using indometacin as a model drug. The gelation time of xyloglucan sols is sufficiently long (gelation of a 1% w/w sol at 30°C occurs in 6 min (Miyazaki et al 1998)) that it may be feasible to achieve in-situ gelation in humans by oral administration of chilled xyloglucan sols. The rationale for formulation as an in-situ gelling liquid rather than a preformed gel is primarily the ease of administration, a liquid being easier to swallow than a gel. This potential application was the impetus for this study, in which we have examined the in-vitro and in-vivo release of theophylline from xyloglucan gels and compared the bioavailability in rabbits with that from two commercial preparations, Theo-Dur Syrup and Theo-Dur Dry Syrup, selected because they are liquid oral sustained-release formulations of theophylline.

Materials and Methods

Materials

Xyloglucan with 44% galactose removal (Lot 9530L) was prepared as described previously (Shirakawa et al 1998) and supplied by Dainippon Pharmaceutical Co., Osaka. The commercially available products, Theo-Dur Syrup (20 mg mL^{-1}) and Theo-Dur Dry Syrup, were supplied by Mitsubishi Kasei Co., Tokyo. Theo-Dur Syrup is a colloidal suspension of microparticles of a cellulose derivative in concentrated sorbitol solution that is formulated to produce sustained release of theophylline. Theo-Dur Dry Syrup is a reconstituted suspension of microparticles (200 μm) of sustained-release matrix granulated with D-mannitol in water. Theophylline was obtained from Wako Pure Chemical Ind. Ltd, Osaka, and Pluronic F127 was a gift from BASF Wyandotte (Parsippany, NJ). All other reagents were of analytical grade.

Preparation of sols

A weighed amount of enzyme-degraded xyloglucan was slowly added to cold water or phosphate buffer, pH 7.4. The mixture was slowly homogenized (Nihon Seiki Seisakusho homogenizer type HB) and an appropriate amount of drug was then dissolved in the resulting solution. Sols containing marker dye were prepared with a 0.02% w/v solution of brilliant blue FCF.

Measurement of viscosity of sols

The viscosity of sols (drug-free) prepared in water was determined at 5 or 20°C with a cone and plate viscometer with cone angle 1° 34′ (TV-20H, model E, Tokimec Co., Tokyo) using 1 mL of the sample. Measurements on each sample were performed in triplicate, each taking approximately 30 s.

Measurement of gel strength

Measurements were carried out using a rheometer (CR-200D, Sun Scientific Co., Tokyo) as described previously (Kawasaki et al 1999). A 30-g sample of the gel (drug-free) prepared in water and equilibrated for 3 h was contained in a 50-mL beaker maintained at constant temperature by a water jacket through which water was circulated at 37°C 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 gels with concentrations over the range 0.5–1.5% w/w.

Measurement of drug release rate from gels

The release rates of theophylline were measured by using plastic dialysis cells similar to those described previously (Shirakawa et al 1998). 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 gel containing dissolved drug (20 mg) prepared in pH 7.4 buffer was placed in the donor compartment. To simulate in-vivo conditions, an equal volume of simulated gastric (pH 1.2) or intestinal (pH 6.8) fluid (as specified for the JP XIII disintegration test) was placed in the receptor compartment. The donor phase and the aqueous receptor phase were separated by a cellulose membrane (Viskase Sales Co., Chicago, IL, 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 274 nm.

Animal experiments

White male rabbits (2.3–3.6 kg) were fasted for 24 h before the experiments but were allowed free access to water. The chilled xyloglucan sol preparation was orally administered by a stomach sonde for rabbits (Natsume Seisakusho, Tokyo, Japan, KN-342) fitted onto a disposable syringe; the sol, sonde and syringe were stored in a refrigerator at 5°C before filling the syringe to facilitate this procedure. Gels containing theophylline were produced in-situ by administration of 4 mL of the enzyme-degraded xyloglucan solution, pH 7.4, containing 20 mg of dissolved drug. For intravenous administration, 20-mg doses of the drug in 4 mL saline solution were injected through the ear vein. Administration of Theo-Dur Dry Syrup (20 mg in 4 mL) and Theo-Dur Syrup (20 mg in 1 mL) was by means of the stomach sonde. At given intervals, 0.5-mL blood samples were taken from the ear vein and analysed as described below. The protocols for the experiments were previously approved by the Animal Ethics and Research Committee of the Health Sciences University of Hokkaido. The statistical significance of the results was assessed by the Student's *t*-test and results are presented as the mean $\pm$ s.e.m.

Analysis of plasma samples

The plasma samples were separated by centrifugation and assayed by HPLC (Shimazu LC-10A with a Shimazu SPD-10A detector at a wavelength of 274 nm). Assay of theophylline followed the method described by Schreiber-Deturmeny & Bruguerolle (1996) with minor modifications. To 0.05 mL of plasma was added 50 μL of caffeine solution (15 μg mL^{-1}) as internal standard and 20 μL of 20% perchloric acid, and the sample was vortex-mixed and centrifuged. The supernatant was passed through a Millipore filter (0.45 μm) and directly injected onto a 250 × 46 mm i.d. column packed with Inertsil-ODS. Elution was carried out with acetonitrile–tetrahydrofuran–concentrated acetic acid–distilled water (100:20:5:875) at a rate of 1 mL min^{-1} at 40°C.

Results and Discussion

Viscosity and gelling properties of sols

Figure 2 shows a comparison of the shear dependency of the viscosity of xyloglucan sols of concentrations 0.5, 1.0 and 1.5% w/w with Pluronic F127 sols and the

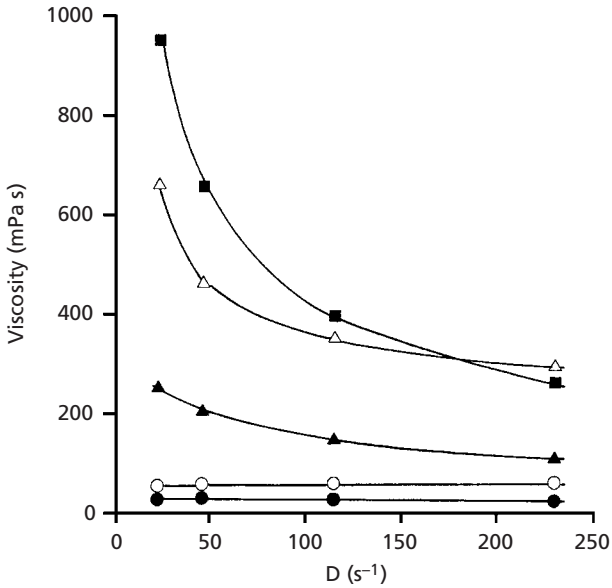

Figure 2 Viscosity of xyloglucan sols (drug-free) of concentrations 0.5 (●), 1.0 (▲) and 1.5 (■) % w/w (5°C), 25%w/w Pluronic F127 sols (○, 5°C), and Theo-Dur Syrup (△, 20°C) as a function of shear rate, D.

commercial preparation Theo-Dur Syrup. Measurements were performed under conditions representative of those of their proposed administration; the xyloglucan and Pluronic formulations were maintained in the sol form by measurement at 5°C whereas the commercial formulation was measured at 20°C.

The viscosity of the xyloglucan sols increased markedly with concentration. The 0.5% w/w sol had a similar viscosity to that of a 25% w/w Pluronic F127 sol and exhibited apparent Newtonian flow properties. In contrast, the viscosity of the 1.5% w/w xyloglucan sol was of similar magnitude and showed similar shear-thinning behaviour to the Theo-Dur Syrup, and oral administration would not therefore be expected to present any difficulty.

The rheological properties of xyloglucan gels at 37°C have been reported previously (Kawasaki et al 1999). Measurements on the xyloglucan sample of this study using the same experimental method gave values of gel strength (in kN m^{-2}) of 0.50, 2.12 and 3.53 for xyloglucan concentrations of 0.5, 1.0 and 1.5% w/w, respectively. The values obtained by this method are relative rather than absolute but serve to show the influence of xyloglucan concentration on gel strength. They are similar to those reported for the sample used previously and demonstrate the very weak nature of the gels formed by 0.5% w/w sols. The observed increase of gel strength with concentration is a consequence of an increased density of the laterally stacked chains of the enzyme-degraded xyloglucan (Yuguchi et al 1997).

In-vitro drug release

The release profiles of theophylline from xyloglucan gels loaded with 0.5% w/v drug were compared with those from Theo-Dur Dry Syrup (Figure 3). It was not possible to conduct a comparison of the release characteristics with those from the other commercial product, Theo-Dur Syrup, because of osmotic effects in the apparatus used. The receptor solutions were changed after 1 h from simulated gastric fluid at pH 1.2 to a simulated intestinal fluid at pH 6.8 to mimic gastrointestinal transit.

Release profiles for the 1.0 and 1.5% w/w xyloglucan gels were identical and lower than those for the respective 0.5% w/w gels, reflecting either the higher diffusional resistance of these gels compared with that of the weak gels formed at low xyloglucan concentration or higher convection in the less viscous 0.5% w/w gels during the release experiment in which the gels were shaken. Cumu-

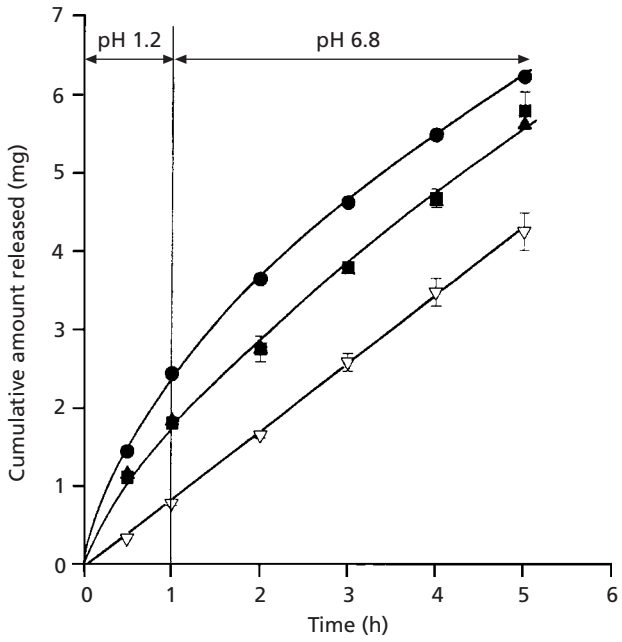

Figure 3 Cumulative release of theophylline as a function of time from xyloglucan gels of concentrations 0.5 (●), 1.0 (▲) and 1.5 (■) % w/w and from Theo-Dur Dry Syrup (▽). Release was into simulated gastric fluid, pH 1.2, for a period of 1 h and subsequently into simulated intestinal fluid, pH 6.8. Initial drug loading of gels was 20 mg of drug; surface area of membrane was 2.67 cm^2. Each value is the mean ± s.e. of 4 determinations.

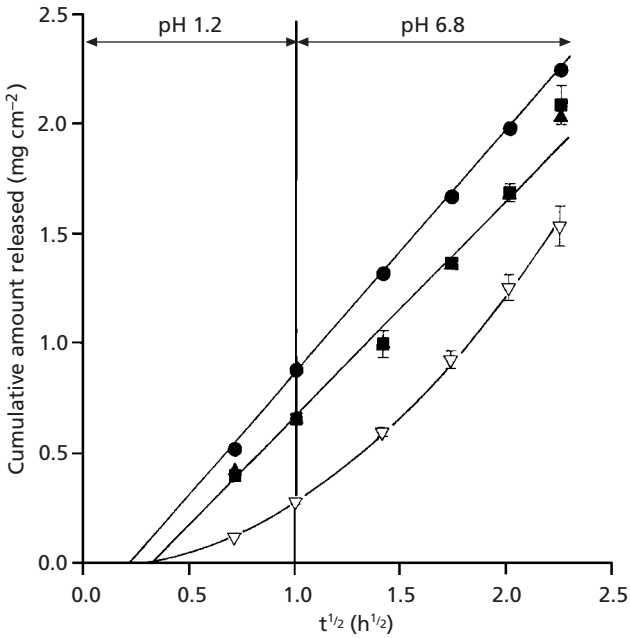

Figure 4 Cumulative release per unit area, Q, for theophylline as a function of square-root time from xyloglucan gels of concentrations 0.5 (●), 1.0 (▲) and 1.5 (■) % w/w and from Theo-Dur Dry Syrup (▽). Release was into simulated gastric fluid, pH 1.2, for a period of 1 h and subsequently into simulated intestinal fluid, pH 6.8. Each value is the mean ± s.e. of 4 determinations.

lative release from the xyloglucan gels was higher than that from the sustained-release Theo-Dur formulation, which increased linearly with time over 5 h.

It is interesting to note that there was no apparent discontinuity in the release profile of theophylline as the pH changed from 1.2 to 6.8. The drug is soluble in dilute acid but only carries a weak charge at pH 6.8 arising from the slight ionisation of its acidic group (pK_a = 8.6). Inspection of the solutions showed that the solubility at the higher pH was sufficient to maintain the drug in solution at the concentrations used in this study.

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:

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

where C_0 is the initial drug concentration and D is the diffusion coefficient. The plot of Q vs $t^{\frac{1}{2}}$ for the release of theophylline from 0.5% w/w xyloglucan gels was linear after a short lag period (Figure 4). Linearity of the plots for release from 1.0 and 1.5% w/w xyloglucan was not so convincing and there is a clear departure of the experimental data from the root-time plots at 5 h. As expected, release from the Theo-Dur Dry Syrup did not show root-time characteristics.

Diffusion coefficients calculated from the gradients of the plots of Figure 4 assuming linearity were 11.1×10^{-6} $cm^2\ s^{-1}$ for 0.5% w/w gels and 8.5×10^{-6} $cm^2\ s^{-1}$ for both 1.0 and 1.5% w/w gels (over 4 h). The D value for theophylline from 1.5% w/w gels was similar to that determined previously (Kawasaki et al 1999) for the release of diltiazem ($D = 7.6 \times 10^{-6}$ $cm^2\ s^{-1}$) from 1.5% w/w xyloglucan gels at 37°C.

In-vivo release

Plasma drug levels were determined following oral administration to rabbits of 20 mg theophylline from chilled 1.5% w/w solutions of xyloglucan (which gel in the stomach at 37°C) and from Theo-Dur Syrup, Theo-Dur Dry Syrup and an intravenous injection of an aqueous solution. Plasma levels at equivalent times were higher following administration of the gel formulation (Figure 5) and decreased to similar values to those achieved with commercial preparations after 12 h.

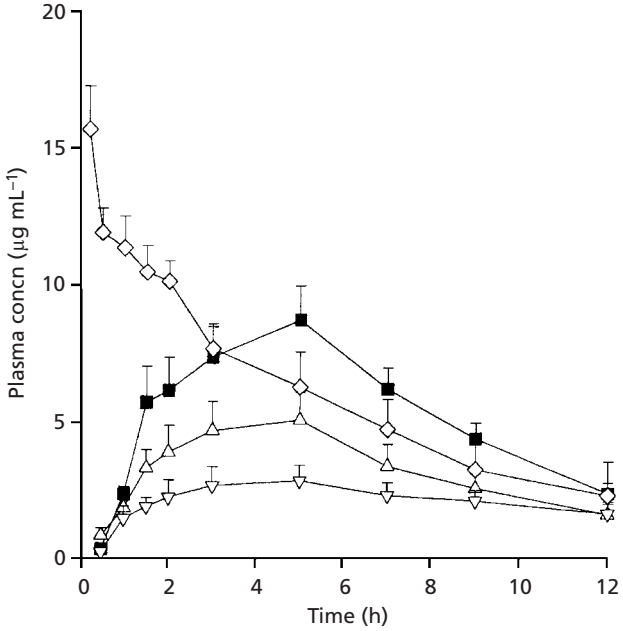

Figure 5 Plasma concentrations of theophylline in rabbits after intravenous injection of aqueous solution (◇) and oral administration from 1.5% w/w xyloglucan sols (■) and from Theo-Dur Syrup (△) and Theo Dur Dry Syrup (▽). All formulations contained 20 mg theophylline. Each value represents mean ± s.e. of 4 determinations.

Table 1 Comparison of bioavailability parameters in rabbits of theophylline administered from xyloglucan gels and commercial oral liquid dosage forms

Dosage form	t_{max} (h)	C_{max} (μg mL^{-1})	AUC (0–12 h) (μg h mL^{-1})	$AUC_{oral}/AUC_{i.v}$	MRT (h)
Intravenous injection	–	–	72.5 ± 11.7	–	4.1 ± 0.3
Xyloglucan gel (1.5% w/w)	4.5 ± 0.5	8.8 ± 1.3*	64.2 ± 8.6*	0.89	5.7 ± 0.1
Theo-Dur Syrup	4.3 ± 0.8	5.3 ± 0.9	38.7 ± 7.4	0.53	5.5 ± 0.3
Theo-Dur Dry Syrup	5.5 ± 1.3	3.2 ± 0.5	25.5 ± 5.0	0.35	6.1 ± 0.3

Each value represents the mean ± s.e. of 4 experiments. * $P < 0.01$ compared with Theo-Dur Dry Syrup.

The prolongation of release achieved following oral administration in chilled xyloglucan solutions indicates the in-vivo gelation of these solutions. Direct evidence of this was provided by visual observation of the contents of the stomach following administration of 4 mL of a 1.5% w/w xyloglucan gel (without drug) containing a marker dye (brilliant blue), which clearly showed the presence of a soft gel 5 h after dosing. It was not possible to detect the presence of the commercial preparations in the stomach after this length of time. The t_{max} of approximately 5 h and the sustained-release characteristics make this dosage form suitable for the administration of theophylline in the prevention of asthma attacks, which occur at their highest frequency at around 0500 h.

The area under the plasma concentration–time curve (AUC) and the mean residence time (MRT) were obtained from the plasma concentration–time data for each rabbit using a computer program for model-independent analysis (Yamaoka et al 1981) and are summarised in Table 1. The bioavailability of theophylline calculated from the ratio AUC ((oral/i.v.) × 100) was 89% for the 1.5% w/w xyloglucan gels compared with 53 and 35% for Theo-Dur Syrup and Dry Syrup, respectively. The mean $t_{\frac{1}{2}}$ value was 5.20 ± 1.92 h, which is in reasonable agreement with that of 5.22 ± 0.51 h, determined by Bouraoui et al (1995), for intravenous injection of theophylline (12 mg kg^{-1}) to rabbits. It is interesting to note that despite the differences in AUC and C_{max} of the xyloglucan and commercial preparations, the MRT values were similar. However, it should be noted that the plasma concentrations had not reached zero by the end of the measurement period (12 h), and this may affect the interpretation of these data. Theo-Dur syrup is a colloidal suspension of microparticles that is formulated to produce sustained release of theophylline as can be seen from the release profile in Figure 5. The sustained-release effect of the xyloglucan formulation is a consequence of the gel structure. However, the gel remains in the stomach for a longer time than the syrup and it is the balance of these two effects that is probably responsible for the similarity of the MRT values of the preparations.

Table 1 indicates a reasonable level of variability despite the probability that the gel masses formed in the stomach following oral administration in each of the 4 rabbits were unlikely to have been of regular shape and hence would have been of variable surface area. Our results, and those of our previous study in which a rat model was used (Kawasaki et al 1999), suggest that any variability in size and shape of the release depot was not sufficiently great to have a significant influence on the drug release profile.

Conclusion

This study has demonstrated that gels of enzyme-degraded xyloglucan formed in the stomachs of a rabbit model following administration of a chilled sol (1.5% w/w) and showed sustained-release characteristics with a maximum plasma concentration at 4.5 h. The theophylline bioavailability from a 1.5% w/w gel was 1.7 and 2.5 times that of the commercial preparations Theo-Dur Syrup and Theo-Dur Dry Syrup, respectively.

The bioavailability of theophylline from xyloglucan gels is similar to that achieved for this drug when released from other vehicles formed by in-situ gelation in the gastrointestinal tract of rabbits. For example, the bioavailabilities (0–12 h) in rabbits when 40 mg of theophylline was released from 1.0% w/v gellan gels and from 1.5% w/v alginate gels were 74.1% (Miyazaki et al 1999) and 82.4% (Miyazaki et al 2000), respectively. Liquid formulations of both of these materials contain calcium ions in complexed form, the release of which in the acidic environment of the stomach causes gelation. The gelation of xyloglucan does not, however, require the presence of H^+ ions and hence is of wider application. Moreover, the type of drug that can be delivered by the xyloglucan gels is not restricted by possible adverse

affects of the drug on gelation as in EHEC gels containing low surfactant content (Lindell & Engström 1993) and gellan formulations (Miyazaki et al 1999), where incorporation of some drug salts may cause gelation before administration.

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