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RESEARCH PAPER

Oral Sustained Delivery of Paracetamol from In Situ Gelling Xyloglucan Formulations

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ABSTRACT

The purpose of this study was to evaluate the potential of a xyloglucan formulation with in situ gelling properties for the oral sustained delivery of paracetamol. Gelation of dilute aqueous solutions of the polysaccharide xyloglucan occurred in rabbit and rat stomachs as the orally administered chilled solutions attained body temperature. In vitro studies demonstrated diffusion-controlled release of paracetamol from the gels over a period of 6 hr. The bioavailabilities of paracetamol from the xyloglucan gels formed in situ in the stomachs of rabbits after oral administration of the liquid formulations were similar to that of a commercially available suspension containing an identical dose of paracetamol.

Key Words: Xyloglucan gels; Oral drug delivery; In situ gelation; Sustained release; Paracetamol.

INTRODUCTION

Paracetamol (acetaminophen) is widely used in the treatment of mild to moderate pain, and its antipyretic and analgesic efficacy has been established in many placebo-controlled trials in both adults and children. It is usually administered orally in tablet and liquid form, following which its absorption is rapid, predominantly from the small intestine. The use of a heat-sensitive melting gel containing κ-carrageenan and gelatin as gelling agents for the oral delivery of paracetamol, which achieved a high (90%) bioavailability in rabbits, has been reported by Endo et al.[1] In this article, we assess the potential for the sustained delivery of paracetamol of a xyloglucan formulation that forms gels in situ in the stomach.

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Xyloglucan polysaccharide derived from tamarind seed is composed of a (1–4)-β-D-glucan backbone chain that has (1–6)-α-D-xylose branches that are partially substituted by (1–2)-β-D-galactoxylose. Partial degradation (44%) by β-galactosidase yields a compound that in dilute aqueous solution exhibits sol to gel transition at temperatures of between 22° and 27°C.[2] We previously reported the potential use of xyloglucan gels for rectal[3] and intraperitoneal[4] drug delivery. The gelation time is slow, allowing sufficient time after oral administration for a chilled liquid preparation to reach the stomach before a soft gel depot is formed. We recently demonstrated sustained release of indomethacin[5] and cimetidine[6] from enzyme-degraded xyloglucan gels formed in situ by this method.

This study compares the in vitro and in vivo release characteristics of an in situ gelling xyloglucan formulation with those of a paracetamol solution, a control suspension, and a commercially available suspension (3 Months Plus Pain Relief Suspension, The Boots Co., UK) used for the oral administration of paracetamol to children.

MATERIALS AND METHODS

Materials

Xyloglucan with a percentage of galactose removal of 44% (lot 9530L) was prepared as described previously[7] and supplied by Dainippon Pharmaceutical Co., Osaka, Japan. Paracetamol was obtained from Yamanouchi Pharmaceutical Co., Tokyo, Japan. A commercially available product, Children's 3 Months Plus Pain Relief Suspension, was obtained from The Boots Co.

Preparation of Sols and Suspension

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 ultrapure water. The mixture was slowly homogenized (Automatic Homogenizer CM-200, Iuchi, Osaka, Japan), and an appropriate amount of paracetamol (1% w/v) was then dissolved in the resulting solution.

A 1% w/v control suspension was prepared for use in the in vitro release experiments (to avoid osmotic effects experienced with the commercial suspension) by dispersing paracetamol in a 0.57% w/v aqueous solution of sodium alginate. A 1% w/v solution of paracetamol was prepared in ultrapure water.

Measurement of Viscosity of Sols

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

Measurement of Drug Release Rate from Gels

The release rates of paracetamol were measured by using plastic dialysis cells similar to that described previously.[8] The capacity of each half-cell was 4 mL, and the surface area of the membranes was 2.67 cm^2.

Gels of xyloglucan loaded with 1% w/v of drug were placed in the donor compartment. 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 receptor solutions were changed after a given time interval from simulated gastric fluid at pH 1.2 to a simulated intestinal fluid at pH 6.8 to mimic gastrointestinal transit. 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 samples was determined using a spectrophotometer at a wavelength of 244 nm.

Animal Experiments

Rabbits

White male rabbits weighing 2.9–3.6 kg were fasted for 24 hr before the experiments, but allowed free access to water. The possibility of coprophagy was minimized by the fasting process, which ensured that very little food was present in the stomach (from

visual observation), and also through the use of a yoke. The chilled xyloglucan sol preparation (5 mL) containing 50 mg paracetamol was orally administered using a stomach sonde needle for rabbits (Natume Seisakusho, KN-342): the sol, sonde, and syringe were stored in a refrigerator at 5°C before filling the syringe to facilitate this procedure. A stomach sonde needle was also used for oral administration of the commercial paracetamol suspension (50 mg in 2.1 mL) and aqueous solution (50 mg in 5 mL). For intravenous administration, 50 mg doses of the drug in 5 mL saline solution were injected through the ear vein. At given intervals, 0.4 mL blood samples were taken from the ear vein and analyzed as described herein.

Rats

Male Wistar rats, weighing 250–300 g, were fasted for 24 hr with free access to water. The rats were anesthetized with an intraperitoneal injection of urethane and divided into two groups of four rats. One group received paracetamol gel preparation given orally as 1 mL xyloglucan solution containing the drug (10 mg) through a stomach sonde needle for rats (Natume Seisakusho, KN-348). A second group was dosed in a similar manner with a paracetamol aqueous solution (10 mg in 1 mL). At predetermined intervals, a blood sample was taken from the jugular vein of rats in each group.

The protocols for the animal experiments were previously approved by the Animal Ethics and Research Committee of the Health Sciences University of Hokkaido. Statistical significance of the results was assessed by the Student's *t*-test, and results are presented as the mean ± standard error of the mean.

Determination of Paracetamol

The plasma samples were separated by centrifugation and assayed by high-performance liquid chromatography (Shimazu LC-10A with a Shimazu SPD-10A detector at a wavelength of 254 nm; Shimazu, Japan). The assay of paracetamol was based on the method described by Ameer et al.,[9] with minor modifications. To 100 μL of plasma was added 300 μL of water, 100 μL of 2-acetoamidophenol solution (100 μg mL^{-1} in 20% methanol) as internal standard, and 7 mL ethyl acetate. The sample was mixed by shaking and centrifuged, after which 5 mL of the organic layer was evaporated to dryness under a nitrogen stream. The residue was reconstituted with 200 μL of 50% methanol, and aliquots of 20 μL were injected into a 150 × 4.6 mm i.d. column, packed with Inertsil-ODS. Elution was carried out with acetonitrile (pH 4.0):sodium acetate buffer (2:8) at a rate of 1.0 mL min^{-1} at 40°C.

RESULTS AND DISCUSSION

Viscosity of Sols

The rheological properties of the sols are of importance in view of their proposed oral administration. In the selection of the concentration of the gelling compound, a compromise is sought between a sufficiently high concentration for the formation of gels of satisfactory gel strength for use as a delivery vehicle, and a sufficiently low concentration to maintain an acceptable viscosity for ease of swallowing. Figure 1 compares the shear dependency of the viscosity of the commercial suspension with previous measurements on xyloglucan sols of a range of concentrations.[6] Additional measurements increased the range of xyloglucan concentrations to 2.0% for comparison with the control suspension. Measurements were performed under conditions representative

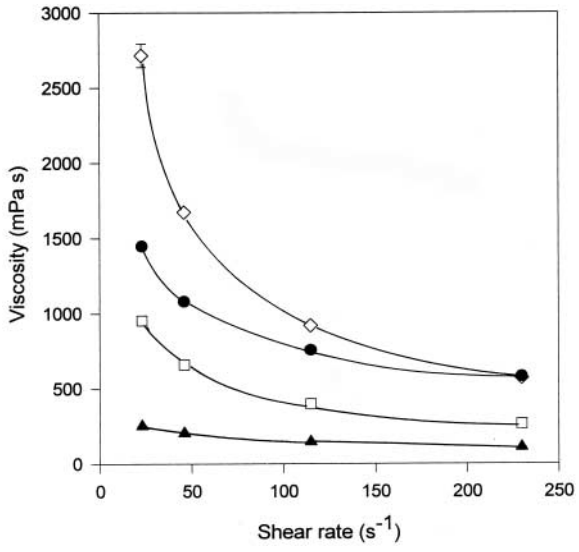

Figure 1. Viscosity of xyloglucan sols of concentrations (◇) 2.0%, (□) 1.5%, and (▲) 1.0% w/w (5°C), and (●) the commercial suspension of paracetamol (20°C).

of those of their proposed administration; the xyloglucan solutions were maintained in the sol form by measurement at 5°C, whereas the commercial formulation was measured at 20°C.

All concentrations showed evidence of shear thinning behavior, the effect being most pronounced at higher concentrations. The sols showed a marked increase of viscosity with concentration. The 2.0% xyloglucan sol had a significantly higher viscosity than the commercial suspension at all shear rates, which may be a disadvantage in oral administration; for this reason, we chose to conduct in vivo experiments on 1.5% xyloglucan gels, which have satisfactory gel strengths.[5]

In Vitro Drug Release

The release profiles of paracetamol from xyloglucan gels loaded with 1.0% w/v drug are compared with that from an aqueous solution in Fig. 2. Because of osmotic effects in the apparatus used, it was not possible to measure the release characteristics of the commercial suspension, and the control suspension of paracetamol (1% w/v) in 0.57% w/v sodium alginate was substituted for the purposes of comparison. The pK_a of paracetamol is 9.5,[10] and consequently there will be no change in the state of ionization of this acidic drug accompanying the pH change resulting from the change of receptor solution from simulated gastric fluid (pH 1.2) to simulated intestinal fluid at pH 6.8.

Comparison of the release profiles from the xyloglucan gels with that from an aqueous solution show a sustained release of paracetamol. The release data from gels and suspension over the whole time period were analyzed according to the treatment proposed by Higuchi[11] 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 from gels of initial drug concentration C_0 is proportional to the square root of time t:

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

Figure 3 shows linear plots of Q vs. $t^{1/2}$ for the release of paracetamol from the gels ($C_0 =$ 1% w/v) after a short lag period, indicative of

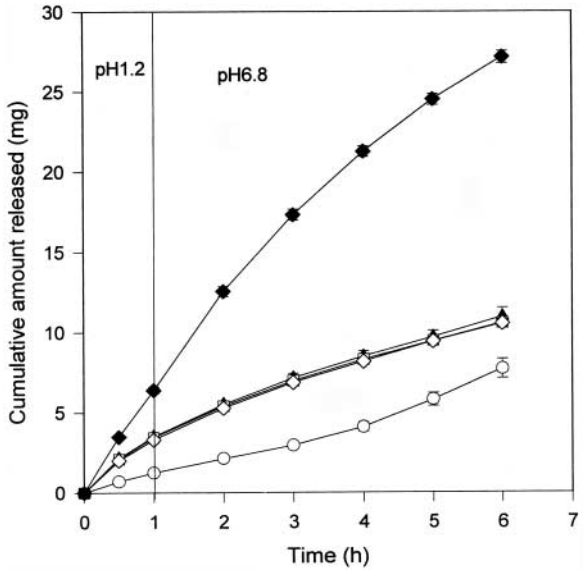

Figure 2. Cumulative release of paracetamol as a function of time from xyloglucan gels of concentrations (◇) 2.0%, (□) 1.5%, and (▲) 1.0% w/w, and from (◆) the paracetamol aqueous solution and (○) the control suspension of paracetamol. All formulations contain 1% w/v paracetamol. Release was into simulated gastric fluid (pH 1.2) for a period of 1 hr and subsequently into simulated intestinal fluid (pH 6.8). Each represents the mean ± SE of four determinations.

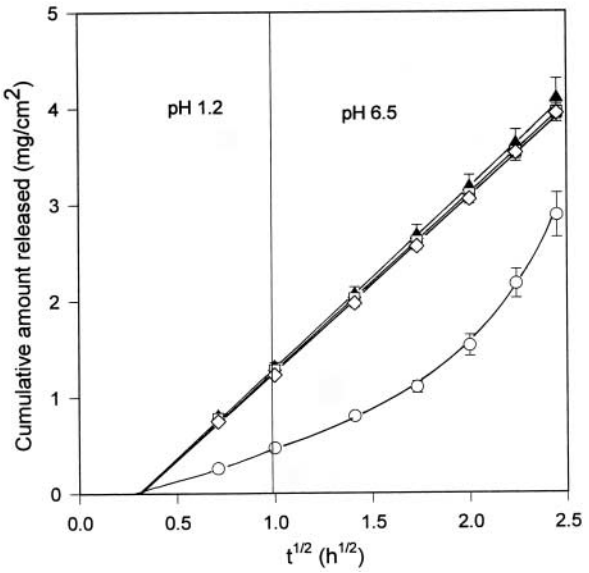

Figure 3. Cumulative release per unit area, Q, for paracetamol as a function of square root time from xyloglucan gels of concentrations (◇) 2.0%, (□) 1.5%, and (▲) 1.0% w/w, and (○) the control suspension of paracetamol. All formulations contain 1% w/v paracetamol. Release was into simulated gastric fluid (pH 1.2) for a period of 1 hr and subsequently into simulated intestinal fluid (pH 6.8). Each represents the mean ± SE of four determinations.

diffusion-controlled release. Diffusion coefficients, D, calculated from the gradients of the plots of Fig. 3 were 0.77, 0.72, and $0.74 \times 10^{-5}\,cm^2\,sec^{-1}$ for 1.0, 1.5, and 2.0% w/w gels, respectively. Release from the suspension did not conform to Eq. (1) as expected.

In Vivo Release

The release of paracetamol from a 1.5% w/w xyloglucan gel formed in situ in the rabbit stomach after oral administration of 5 mL of xyloglucan sol containing 50 mg paracetamol was monitored by the determination of plasma drug levels. Thermally reversible gelation of xyloglucan sols occurred as chilled sols reached body temperature. Gelation of these formulations was confirmed by visual observation of the stomach contents, which showed the presence of distinct gel blocks of regular shape (as discussed herein). Figure 4 compares paracetamol levels from the gels with those after oral administration of the commercial suspension (50 mg in 2.1 mL) and an aqueous solution of paracetamol (50 mg in 5 mL). The in vivo release curves from the xyloglucan gels had a similar profile to that of the commercial suspension.

The areas under the plasma concentration-time curve and the mean residence times were obtained from the plasma concentration-time data for each animal using a computer program for model-independent analysis[12] and are summarized in Table 1. Values of both parameters were similar for the gel formulation and the commercial suspension. The $t_{1/2}$ value was 2.91 ± 1.24 hr, which compares with a value of 2.6 hr following intravenous injection of paracetamol to human volunteers.[9] It is interesting to note the similarity of mean residence times of the gel and the commercial suspension. The sustained release effect of the gel formulation is a consequence of the resistance of the gel structure to the diffusion of drug, whereas that of the suspension arises from the reservoir effect of the suspension particles as they

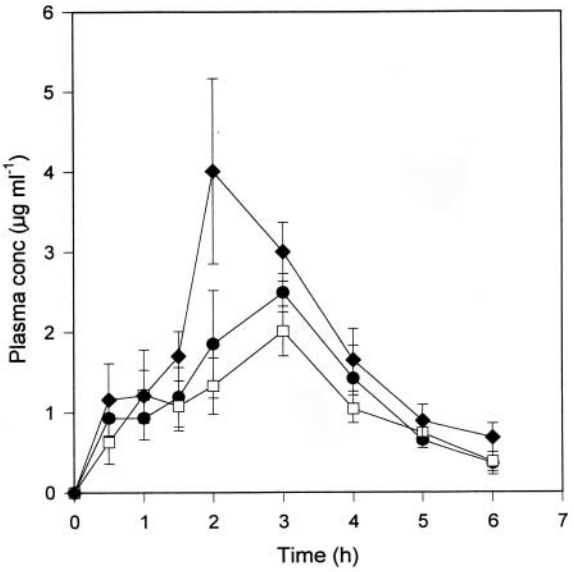

Figure 4. Plasma concentrations of paracetamol in rabbits after oral administration of (□) 1.5% w/w xyloglucan sols, (●) the commercial suspension of paracetamol, and (◆) the paracetamol aqueous solution. All formulations contained 50 mg paracetamol. Each value represents the mean ± SE of four determinations.

Table 1. Comparison of bioavailability parameters of paracetamol administered from the commercial suspension, aqueous solution, and intravenous injection of paracetamol, and from xyloglucan gels (1.5% w/w) formed in situ in rabbit and rat stomachs.

Dosage form	C_{max} (μg/mL)	t_{max} (hr)	AUC (0–6 hr) (μg hr/mL)	MRT (hr)
Rabbit				
Xyloglucan gel	2.29 ± 0.30	2.00 ± 0.58	6.45 ± 0.51**	2.92 ± 0.21
Paracetamol solution	4.33 ± 0.96	2.25 ± 0.25	10.93 ± 1.74	2.80 ± 0.09
Commercial suspension	2.93 ± 0.36	3.00 ± 0.41	7.65 ± 0.57	2.86 ± 0.15
Intravenous injection			11.75 ± 1.27	1.32 ± 0.19
Rat				
Xyloglucan gel	4.43 ± 0.88**	1.00 ± 0.20	12.18 ± 2.94	2.12 ± 0.03*
Paracetamol solution	11.89 ± 1.64	0.75 ± 0.14	21.05 ± 3.32	1.66 ± 0.14

Each value represents the mean ± SE of four experiments. AUC, area under the plasma concentration-time curve; MRT, mean residence time.
*$p < 0.01$, **$p < 0.05$ compared with paracetamol solution.

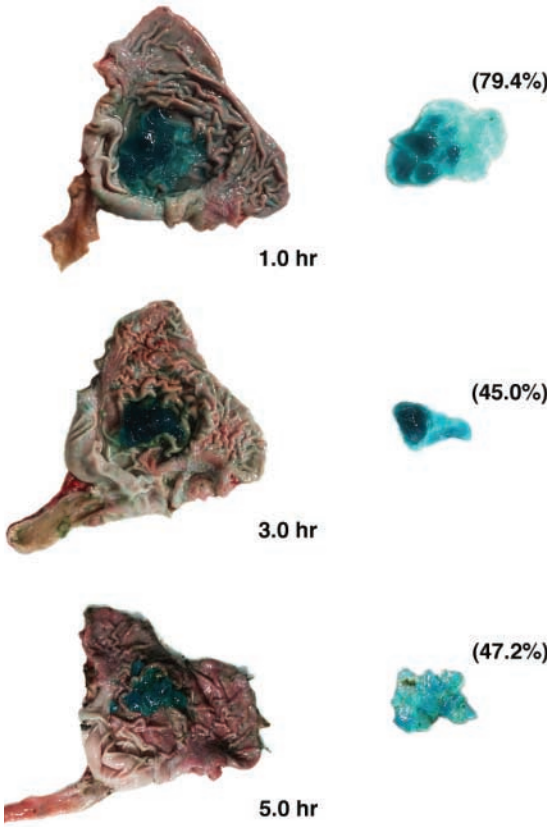

Figure 5. The presence of xyloglucan gel in rabbit stomach at 1, 3, and 5 hr after oral administration of a 1.5% w/w xyloglucan sol. *(See color insert at end of issue.)*

slowly dissolve in the intestine. Visual observation of the contents of the rabbit stomach after administration of a 1.5% w/w xyloglucan gel (without drug) containing a marker dye showed that approximately 79% of the soft gel remained at 1 hr after administration and approximately 46% at time periods of 3 and 5 hr after dosing (Fig. 5). The maintenance of the integrity of the gel over this time period is probably the cause of the prolongation of the release of paracetamol from the gel. No evidence of suspension particles, also marked with dye, could be detected at this time period after administration of the commercial suspension.

The more limited study of release in the rat model showed a similar enhanced residence time of the gel in the rat stomach compared with that of the paracetamol solution (Fig. 6), and observation of the rat stomach contents showed that approximately 26% of gel remained in the stomach at both 3 and 5 hr after administration.

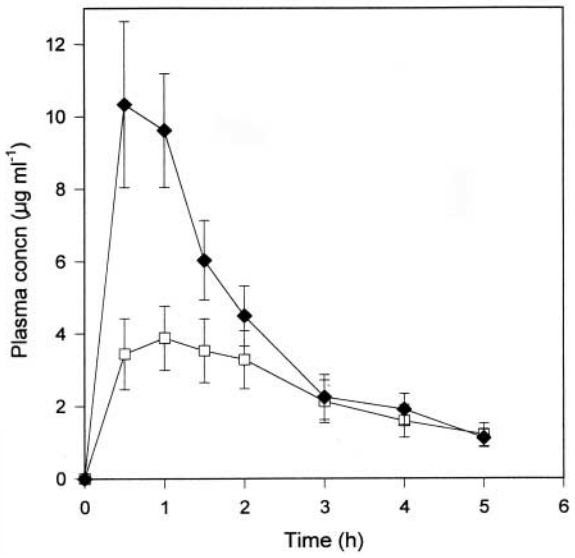

Figure 6. Plasma concentrations of paracetamol in rats after oral administration of (□) 1.5% w/w xyloglucan sols and (◆) the paracetamol aqueous solution. Both formulations contained 10 mg paracetamol. Each value represents the mean ± SE of four determinations.

CONCLUSIONS

We have demonstrated that similar bioavailabilities in rabbit to those of a commercial suspension for the oral administration of paracetamol can be achieved with an in situ gelling xyloglucan formulation. This formulation is a homogeneous liquid when administered orally and does not have the problems associated with the administration of suspensions. Our previous measurements[3] have shown that a 1.5% w/w xyloglucan sol would remain in a fluid state for approximately 4 min at body temperature, and difficulties in swallowing due to gelation are not envisaged after oral administration of a chilled solution. Xyloglucan gels are of wide application in drug delivery since gelation of the sols of this polysaccharide does not require the presence of H^+ ions as in gellan and alginate formulations.[6] Moreover, their use is not restricted by the nature of the drug, as is the case with gellan formulations in which incorporation of certain drug salts may cause gelation before administration. In addition, xyloglucan has advantages over triblock copolymers of poly(oxyethylene) and poly(oxypropylene), such as Pluronic F127,[13] and copolymers of poly(lactide-*co*-glycolide) and poly(ethylene glycol), such as ReGel™[14] that also form sol–gel reversible hydrogels, in that the

gelation of these synthetic materials occurs at much higher concentrations (typically 15–25%).

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