[Chem. Pharm. Bull. 36(10)4033—4038(1988)]

Sustained-Release and Intragastric-Floating Granules of Indomethacin Using Chitosan in Rabbits[1)]

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(Received February 24, 1988)

The release rate of indomethacin from chitosan granules was compared with that of conventional commercial indomethacin capsules and a sustained-release capsule. In contrast with the rapid release of a commercial conventional capsule form, sustained release from the chitosan granules was observed. Furthermore, the release rate could be controlled by changing the mixing ratio of drug and chitosan.

The potential of chitosan granules as an oral sustained-release dosage form of indomethacin was investigated in rabbits. When a conventional commercial capsule was administered orally to rabbits, the plasma concentration reached the maximum level 1 h after administration. In the case of the granules with a 1 : 2 mixture of drug and chitosan, the chitosan granules did not give a sharp peak of plasma concentration, but produced a sustained plateau level of indomethacin. The area under the plasma concentration curve (AUC) (0—8 h) value of chitosan granules showed a slightly higher value than that of commercial capsules. This may be due to the slow rate of release from the chitosan granules and the longer residence time in the stomach.

Thus, in terms of decrease in the peak of plasma concentration and maintenance of indomethacin concentration in plasma, the chitosan granules were superior to the conventional commercial capsules. Indomethacin granule preparations using chitosan may be practically useful as oral preparations with reduced side effects and with prolonged action.

Keywords——chitosan granule; indomethacin; sustained release; intragastric floating; oral administration; rabbit

Chitin [(1→4)-2-acetamido-2-deoxy-β-D-glucose], a naturally occurring structural polysaccharide, is distributed widely in nature, and chitosan [(1→4)-2-amino-2-deoxy-β-D-glucan] is easily prepared from the chitin of crabs and lobsters by *N*-deacetylation with alkali.

In the previous papers,[2,3)] the possible use of chitosan as a new vehicle for sustained-release preparations was examined. Chitosan was useful for the preparation of granules which exhibit sustained release of indomethacin *in vitro*.[3)] When granules prepared from chitosan were placed in an acid medium at pH 1.2, they gradually swelled and floated.

Recently, a sustained-release orally administered product was achieved by using a formulation which floats on the gastric medium.[4)] The ability of the chitosan granules to float on the acid medium may permit extensive use of chitosan in the formulation of sustained-release preparations of various drugs.

Herein, we describe the rate of drug release from chitosan granules compared with that of conventional commercial indomethacin capsules and the sustained-release capsule. Granules composed of different weight ratios of drug and chitosan (1 : 0.5, 1 : 1, 1 : 2) were used. We have also investigated *in vivo* indomethacin release after oral administration of the granules to stomach-emptying controlled rabbits.

Experimental

Materials——Chitosan, Flonac N for chromatography use, was kindly supplied by Kyowa Yushi Co., Tokyo, and used after being passed through a 42 mesh screen. Indomethacin was obtained from Sigma Chemical Co., St. Louis. A conventional commercial indomethacin capsule (Indacin, 25 mg) and a sustained-release commercial indomethacin capsule (Indacin-R, 25 mg) were products of Nippon Merck Banyu Co., Tokyo.

Preparation of Chitosan Granules——The chitosan granules containing indomethacin were prepared by the method described in a previous paper.[3] Indomethacin (0.5 g) was dissolved in 5 ml of methanol at 60 °C and chitosan was added to the drug solution. After evaporation of the solvent at 60 °C, the residue was dissolved in 5 ml of acetic acid (10% w/v). The gelatinous chitosan-drug mixture was sucked into a glass syringe, and extruded onto a glass plate. After drying overnight at room temperature, the chitosan gel cord was cut into pieces and dried for an additional 8 h at 80 °C *in vacuo*. The final chitosan granules were 0.5—0.7 mm in diameter and 1—3 mm in length.

Measurement of Release Rate——Drug release from the chitosan granules was determined by the use of a JP XI dissolution apparatus according to method I (rotating basket method) in 900 ml of JP XI pH 7.2 phosphate buffer–water (1 : 4) at an agitation speed of 100 rpm. The temperature was maintained at 37±0.5 °C. Chitosan granules, equivalent to 25 mg of indomethacin, were encapsulated in hard gelatin capsules (JP XI, no.3). The drug concentration of the sample was determined with a spectrophotometer at 265 nm. All experiments were carried out in triplicate and average values were plotted.

Release patterns were also measured with the U.S.P. dissolution tester by the pH shift method,[5] in consideration of the change of pH in the gastrointestinal fluids.

Animal Experiment——The stomach-emptying-controlled rabbits[6] were fasted for 24 h before drug administration and placed in a stainless-steel case for fixing rabbits. Chitosan granules, equivalent to 25 mg of indomethacin, were encapsulated in hard gelatin capsules (JP XI, no.3) and administered orally to the male rabbits (weighing approximately 3.5 kg). At given intervals, a 1 ml blood sample was taken from the ear vein. The plasma samples were separated by centrifugation and assayed for indomethacin by using an high performance liquid chromatography (HPLC) technique[7] with slight modifications.[8]

Results and Discussion

Release of Indomethacin from Chitosan Granules

In the previous paper,[3] release profiles as well as dissolution profiles of indomethacin in the release medium at pH 7.5 were investigated. In contrast with the rapid dissolution of indomethacin in powdered form, sustained but almost complete release from chitosan granules was observed. The purpose of the present study was to investigate in more detail the *in vitro* release rate of indomethacin from chitosan granules by means of the pH shift method.[5] The rate of drug release from the chitosan granules was compared with that of conventional commercial indomethacin capsules and the sustained-release capsule. The effect of chitosan content on the drug release kinetics was also studied.

In contrast with the rapid release of a commercial conventional capsule form, sustained release from the chitosan granules was observed in all the drug : chitosan ratio (Fig. 1). Only small differences were observed between the release patterns of the drug from the chitosan granules and the commercial sustained-release capsule. For studying the effect of chitosan content on the drug release kinetics, granules composed of different weight ratios of drug and chitosan (1 : 0.5, 1 : 1, 1 : 2) were used. Increasing chitosan content in the granules resulted in a decrease in the release rate of indomethacin. The total amount of indomethacin released during the test period (6 h) was 95.4, 79.5, and 71.3% of the dose for the granules with 1 : 0.5, 1 : 1, and 1 : 2 mixtures, respectively. Although the drug was not completely released from the granules during the test, the results indicate that the chitosan gels serve as a rate controlling barrier. The release rate of indomethacin can be modified by changing the mixing ratios of drug and chitosan.

Further, the drug release profile from chitosan granules containing indomethacin was compared with the obtained by the pH shift dissolution test method.[5] The release characteristics of indomethacin from chitosan granules are shown in Fig. 2, together with the results for the commercial conventional capsules and the commercial sustained-release

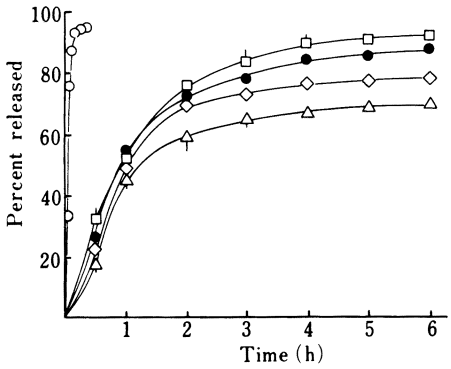

Fig. 1. Release Behavior of Indomethacin from the Test Preparation in the Release Medium, pH 7.2, as Determined by the JP XI Rotating Basket Method

○, conventional commercial capsule; ●, commercial sustained-release capsule; chitosan granules prepared from 1 : 0.5 (□), 1 : 1 (◇) and 1 : 2 (△) mixtures of drug and chitosan.

A sample equivalent to 25 mg of indomethacin was added to 900 ml of release medium. Each value represents the mean ± S.E. of 3 experiments.

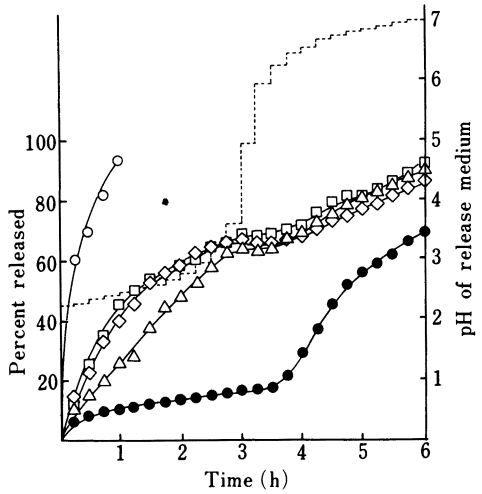

Fig. 2. Release Behavior of Indomethacin from the Test Preparations and pH Change (----) of the Release Medium in the pH Shift Release Test Method

Symbols: see Fig. 1.

A sample equivalent to 25 mg of indomethacin was added to 900 ml of release medium. Each value represents the mean of 3 experiments.

capsules. Conventional capsules disintegrated immediately and indomethacin was released rapidly in the acid medium. The release of indomethacin from the chitosan granules was rapid at acidic pH and slow at neutral or alkaline pH. This result is attributed to the good gel-forming ability of chitosan at low pH, and poor gel-forming ability at high pH. The release rate of the drug from chitosan granules was larger than that of commercial sustained-release capsules. Increased swelling and gel-formation at the low pH would facilitate the movement of drug molecules out of the chitosan granules.

Plasma Concentration of Indomethacin after Oral Administration

Plasma imdomethacin concentration data after oral administration of chitosan granules to rabbits were compared with those of commercial indomethacin capsules and commercial sustained-release capsules (Fig. 3). Chitosan granules using 1 : 0.5 and 1 : 2 mixtures of drug and chitosan were selected for the *in vivo* study. Table I summarizes the parameters of bioavailability obtained from Fig. 3; the area under the plasma concentration curve (AUC), up to 8 h post-administration, was calculated by moment analysis.[9]

The absorption of indomethacin from commercial capsules was very rapid, and the mean maximum plasma concentration (C_{max}) was higher than that of the commercial sustained-release capsules. Then indomethacin was eliminated rapidly from the plasma. Chitosan granules with a 1 : 0.5 mixture showed a curve displaced slightly to the right, but the peak height was the same as in the case of the commercial indomethacin capsules. On the other hand, it was evident that there was a distinct difference in plasma concentration response between conventional commercial capsules and chitosan granules of the 1 : 2 mixture. As compared with the commercial conventional capsules, both the chitosan granules and the commercial sustained-release capsules showed lower peak plasma levels and more prolonged plasma levels. Both the reduction of the peak plasma level and the prolongation of the plasma

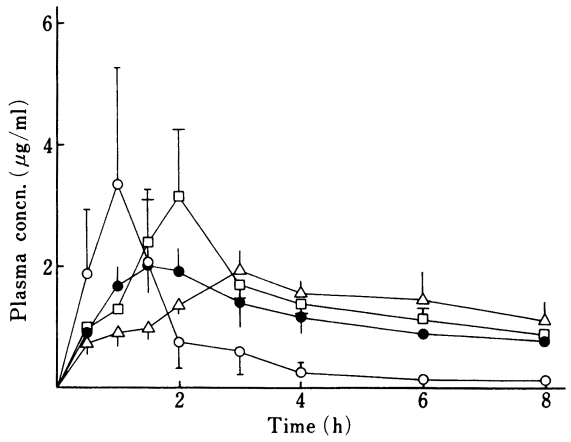

Fig. 3. Plasma Concentration of Indomethacin after Oral Administration to Rabbits

Symbols: see Fig. 1.
Each preparation contained 25 mg of the drug in the capsule forms. Each value represents the mean ± S.E. of 3 rabbits.

TABLE I. Bioavailability Parameters in Rabbits

Preparation	Drug–chitosan ratio	C_{max} [a] (μg/ml)	T_{max} (h)	AUC (0—8 h) [a] (μg·h/ml)
Commercial conventional capsule	—	5.76 ± 1.22	0.5—1.5	9.39 ± 1.45
Commercial sustained-release capsule	—	2.59 ± 0.20 [c]	1—2	9.37 ± 0.90
Chitosan granules	1 : 0.5	3.62 ± 1.01	1.5—2	11.63 ± 1.80
Chitosan granules	1 : 2	2.23 ± 0.19 [b]	3—6	10.65 ± 1.33

a) Each value represents the mean ± S.E. of 3 rabbits. *b*) Significantly different from the conventional commercial capsule; $p<0.05$, *c*) $p<0.10$.

levels observed in chitosan granules with a 1 : 2 mixture may be attributed to the retardation of the rapid release of the drug from the granules at the early stage. The mean AUC (0—8h) value after administration of the drug–chitosan (1 : 2) mixture (10.65 μg·h/ml) was slightly larger than that of the commercial capsule (9.39 μg·h/ml).

The absorption behavior of indomethacin from chitosan granules with a 1 : 2 mixture was similar to that from the sustained-release capsules. This observation indicates that indomethacin was absorbed to a similar extent despite the difference in release rates (Fig. 2) from the commercial sustained-release capsules. This may be due to the slow rate of release from the chitosan granules (Fig. 1) and longer residence time in the stomach. Although the advantages of chitosan have been discussed elsewhere, one unique advantage of the chitosan granules is that they gradually swelled and floated on the acid medium at pH 1.2, as shown in a previous report.[3]

Thus, in terms of decrease in the peak of plasma concentration and maintenance of indomethacin concentration in plasma, the chitosan granules are superior to the conventional capsules. This indicates that chitosan granules composed of a 1 : 2 mixture might be an effective sustained-release preparation with prolonged action.

General Discussion

Since chitosan has a good biocompatibility and is inexpensive, it might be suitable for use in the preparation of dosage forms of commercial drugs. Recently, chitosan has been reported to have some useful pharmaceutical applications.[10]

The present results revealed that the gel-forming property and floating property of

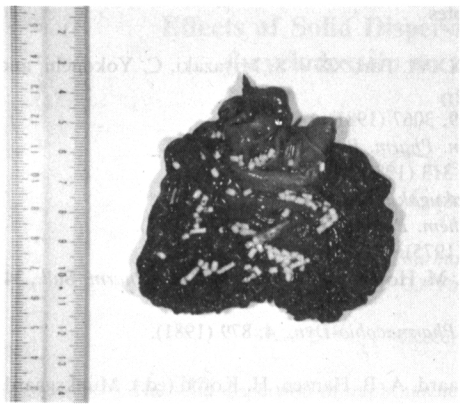

Fig. 4. Chitosan Granules Composed of a 1 : 2 Mixture Retained in the Stomach at 3 h after Oral Administration to a Rabbit

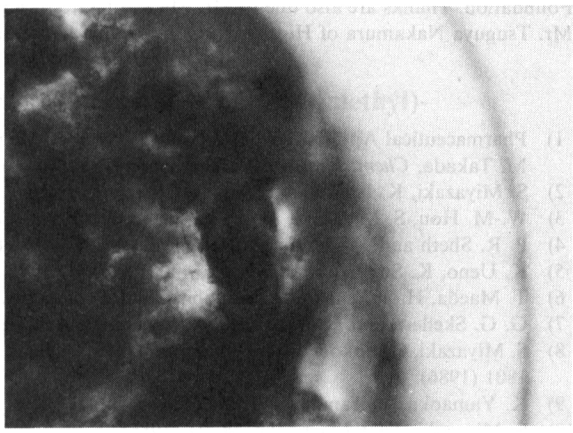

Fig. 5. Photomicrograph of Cross Section of the Gel-Layer at the Surface of Chitosan Granules Composed of a 1 :.1 Mixture (× 100)

chitosan granules at the low pH range may permit extensive use of chitosan in the formulation of sustained-release preparations of various drugs. There are several dosage forms available for the oral delivery of drugs. The transit time of single-unit formulations is highly variable.[11] However, as the subunits of multiple-unit formulations can pass individually through the gastrointestinal (GI) tract, they offer a possibility of achieving a longer-lasting and more reliable source of drugs. Accordingly, a granular formulation was chosen as a sustained-release dosage form.

Recently, several approaches have been tried to extend GI transit time by prolonging the residence time of drug delivery systems in the stomach.[12] A sustained-release orally administered product was achieved by using a formulation which floats on the gastric medium.[13] The hydrodynamically balanced system is an oral dosage form that is designed to prolong the residence time of the dosage form within the GI tract.[4] It is a formulation of a drug with gel-forming hydrocolloids meant to remain buoyant on the stomach contents.

A unique characteristic of the chitosan granules was that they gradually swelled and floated on the acid medium (pH 1.2).[3] The ability of the chitosan granules to float on an acid medium may permit extensive use of chitosan for most drugs where sustained release from the dosage form is desired by the oral route. Figure 4 is a photograph of the stomach of a rabbit at 3 h after oral administration of chitosan granules with a 1 : 2 mixture. It was found that the chitosan granules swelled and were retained in the stomach for at least 3 h.

In addition, chitosan granules have a gel-layer forming property in the low pH range, and the gel might prevent irritation to the stomach. Figure 5 is a photomicrograph of the cross section of the gel-layer at the surface of a chitosan granule. When a granule prepared from chitosan was placed in an acid medium, it adsorbed water to form a gel-layer starting from the granule surface. Furthermore, chitosan has antacid and antiulcer activities.[14] These characteristics may be useful for preventing drug irritation in the stomach, since the clinical usefulness of indomethacin is restricted by the gastrointestinal side effects. These results suggest that sustained-release chitosan granules containing drugs have potential as a method of drug delivery which reduces the side effects.

Acknowledgement The authors wish to acknowledge financial support from the Uehara Memorial

Foundation. Thanks are also due to Mr. Michiatu Sato of Kyowa Yushi Co. for generous gifts of chitosan, and to Mr. Tsuguya Nakamura of Higashi-Nippon-Gakuen University for his assistance.

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