Sustained Release Liquid Preparation Using Sodium Alginate for Eradication of *Helicobacter pyroli*

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We prepared a new liquid preparation for eradication of *Helicobacter pylori* (HP), and examined drug release *in vitro* and *in vivo*. The liquid preparation mainly consisted of a sodium alginate (AG) aqueous solution containing ampicillin (ABPC), an antibiotic drug, or methylene blue, a dye. Drug release was retarded by Ca pre-treatment (0.10 M, 20 s) of the AG preparation in *in vitro* drug release studies due to gel-formation at the liquid surface. In *in vivo* experiments, the AG preparations were administered orally to rats. The rats were divided into two groups, with or without pre-administration of ranitidine hydrochloride (RH, an H_2-blocker). The total remaining % of ABPC in the stomach was high in the rats administered the AG preparation compared to the ABPC solution. The AG preparation might float in the stomach without adhering to the gastric wall in the rats without pre-administration of RH. The total remaining % of ABPC at 30 min was almost 100% in the RH pre-administration rats administered the AG preparation, and about 80% of the drug existed in fraction 2 (implying adhesion of the preparation on the gastric mucus). At 60 min, the total remaining % in the AG preparation plus Ca (mean 87%) increased about 2-fold compared to that in the AG preparation alone (mean 44%). In this case, a large portion of the remaining ABPC also existed in fraction 2. This preparation may be useful for eradication of HP.

Key words *Helicobacter pylori*; sodium alginate; gel-formation; calcium; rat; ampicillin

Until recently, gastric and duodenal ulcers were believed to be caused by stress and hyperchlorhydria; however, recently it has been demonstrated that *Helicobacter pylori* (HP), a spiral bacterium, is closely associated with chronic gastritis and peptic ulcers.[1—3] HP is often observed to adhere to the antral epithelium of the human stomach and the gastric metaplasia in the duodenum. Gastric and duodenal ulcers are believed to develop as the result of damage to the gastric mucosa by cytotoxic substances (ammonia, cytotoxin, *etc.*) produced by HP.[4—6]

Although HP is highly sensitive to most antibiotics, including ampicillin (ABPC), gentamicin, tetracycline, *etc.*, *in vitro*, eradication of HP from patients is difficult even with the current best therapies (multidrug strategies). Conventional tablets or capsules are, in general, used for eradication therapy and these preparations do not remain in the stomach long. Therefore, it is difficult for the antibiotic concentrations in the gastric mucus, in which HP colonizes, to reach minimum inhibitory concentrations for HP. The antibiotics concentrations in the gastric mucus mainly depend on the degree of exsorption (drug transport/diffusion from blood to gastric lumen). Therefore, high doses of antibiotics must be administered to patients for long durations, and many patients suffer from adverse side effects derived from the drug therapy.[7—9] This problem might be overcome by a pharmaceutical technique, without resorting to an improvement of physical characteristics and/or antimicrobial activity of an antibiotic.

The absorption of an antibiotic into the mucus through the mucus layer (from the gastric lumen) is believed to be more effective for HP eradication than absorption through the basolateral membrane (from blood).[10,11] A preparation that spreads out, adheres to the gastric mucosal surface and continuously releases antibiotics should be highly effective against HP (Fig. 1). In this study, a liquid preparation using sodium alginate (AG), was prepared to eradicate HP. Alginic acid is a polysaccharide consisting of D-mannuronic acid and L-gluronic acid. AG solution spreads out and adheres to the gastric mucosal surface and is used for peptic ulcer disease to reduce the irritating effect of gastric acid in ulcers. AG solution forms a firm gel by adding an acid and di- and tri-valent metal ions (Ca, Ba, Sr, *etc.*).[12,13] If an acid or Ca solution is administered after taking AG solution containing an antibiotic, we expect that the drug would be released into the gastric mucus (HP exists) rapidly and continuously (Fig. 1).

MATERIALS AND METHODS

Materials Sodium alginate (AG 100; 100—150 cps at 25 °C, AG300; 300—400 cps at 25 °C, AG 500; 500—600 cps at 25 °C), Alloid G® (5%(w/v) AG aqueous solution) and Arcrane® (5%(w/v) AG aqueous solution) were purchased from Wako Pure Chemicals Ind., Ltd., Kyosei-Kaigen Co. and Tsuruhara Pharmaceutics, respectively. ABPC, Zantac® (ranitidine hydrochloride) and methylene blue (MB) were obtained from Sigma Chemical Co., Sankyo Co., Ltd. and Kanto Chemical Co., respectively. All other reagents used were of analytical reagent grade.

Alginate Liquid Preparation A 2 ml of 2%(w/v) AG aqueous solution containing ABPC (0.5%(w/v)) and MB (0.1%(w/v)) as marker were poured into a petri dish (d= 3 cm). The dish was immersed in a $CaCl_2$ solution (80 ml, 0—0.25 M) for 0—30 min and set in the dissolution apparatus (Fig. 2).

Viscosity Measurement The viscosity of each AG solution (1—5%(w/v)), Alloid G® and Arcrane® was determined at 8, 20, 37 and 50 °C by using of an E-type Viscometer (type ED, Tokyo Keiki, Japan).

Drug Release Study The release of ABPC or MB from the preparations was determined using a Japanese Pharma-

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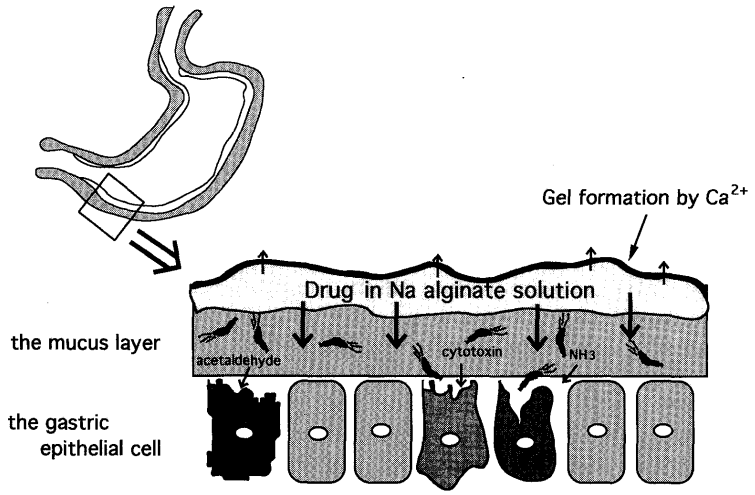

Fig. 1. Strategy for the Eradication of HP by Sustained Release Liquid Preparations Containing ABPC

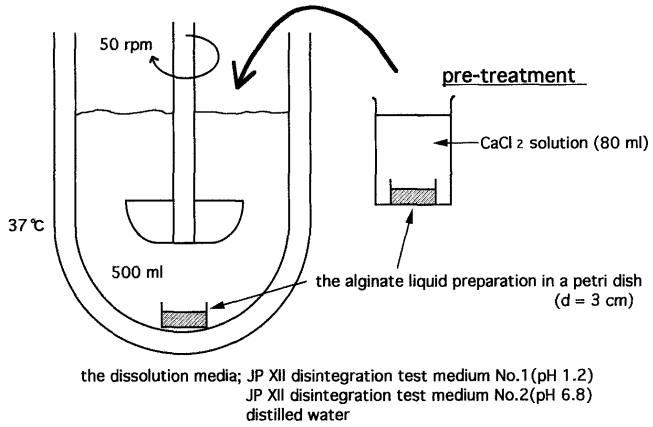

Fig. 2. Apparatus for Dissolution Test

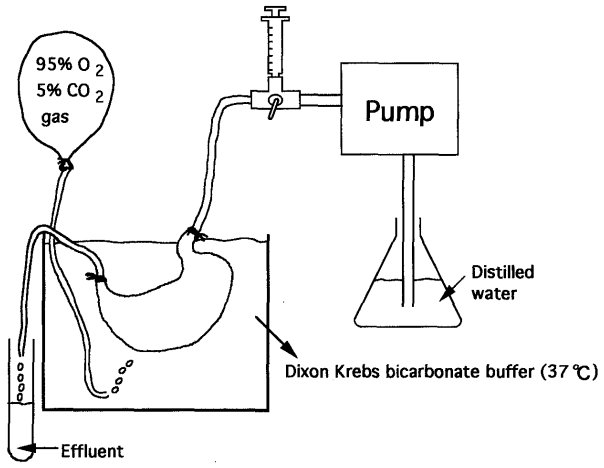

Fig. 3. Schematic of the Isolated Perfused Rat Stomach Preparation

copoeia (JP) XII dissolution test apparatus with a paddle stirrer at 50 rpm (Fig. 2). The dissolution medium used was 500 ml of distilled water, JP XII disintegration test medium No. 1 (pH 1.2) and No. 2 (pH 6.8). Aliquots of sample solutions were withdrawn at appropriate time intervals. The sample solutions were spectrophotometrically analyzed for MB at 664.5 nm, and by HPLC (column; Wakosil-II 5C18 HG, 4.6 mm×15 cm, Wako Pure Chemicals Co., Ltd.) for ABPC at 225 nm. The mobile phase was acetonitrile/0.067 M phosphate buffer (pH 4.6; 10/90, v/v) and the flow rate was 1.0 ml/min. The amount of AMPC released in the pre-treatment solution was also determined by HPLC as described above.

The Isolated Perfused Rat Stomach A male Wistar rat, weighing 250 to 300 g, was fasted overnight. The stomach was removed under ether anesthesia and was attached to the apparatus (Fig. 3). The stomach was first perfused with isotonic saline solution and was emptied by an injection of air. A 0.2 ml aliquot of an aqueous solution or the alginate liquid preparation containing 1 mg of ABPC was injected into the stomach through a three-way valve (Fig. 3). $CaCl_2$ solution was also injected through the valve, if necessary. After a 5 min equilibration, the stomach was perfused through the cardiac with distilled water at a constant flow rate of 0.1 ml/min. At the end of the experiments (0.5, 1 or 2 h following the perfusion), the stomach was removed from the apparatus and opened. The solution in the stomach was collected in a glass vessel. Then the stomach was rinsed with 20 ml of the JP XII disintegration test medium No. 1 (pH 1.2). The solution in the stomach and the rinsings were named fraction 1. The stomach was ultrasonicated for 30 s in JP XII disintegration test medium No. 2 (pH 6.8, 37 °C), and the resulting solution was named fraction 2. Finally, the stomach was homogenized in the No. 2 medium for 1 min (UL Trasonic Disruptor, Model UR-200P, Tomy Seiko Co., Ltd., Japan), and the homogenate was named fraction 3. Total remaining amounts of ABPC were obtained by adding the ABPC amounts in fraction 1—3. The concentrations of ABPC in each fraction were determined fluorometrically.[14] In some experiments, the inside of the stomach was observed with the naked eye.

Oral Administration A 0.2 ml aliquot of the aqueous or the alginate solution containing 2 mg of ABPC was administered through gastric tube to fasted rats (male Wistar, 250—300 g). The rats were divided into two groups, with or without pre-administration of ranitidine hydrochloride, an H_2-blocker. Ranitidine hydrochloride (Zantac®, 20 mg/kg, 1 ml/kg) was pre-administered to the rats 3.5 h before the solution was administered. Then the 0.10 M $CaCl_2$ solution was administered to some of them 5 min after the administration of the alginate liquid preparations. At the end of the experi-

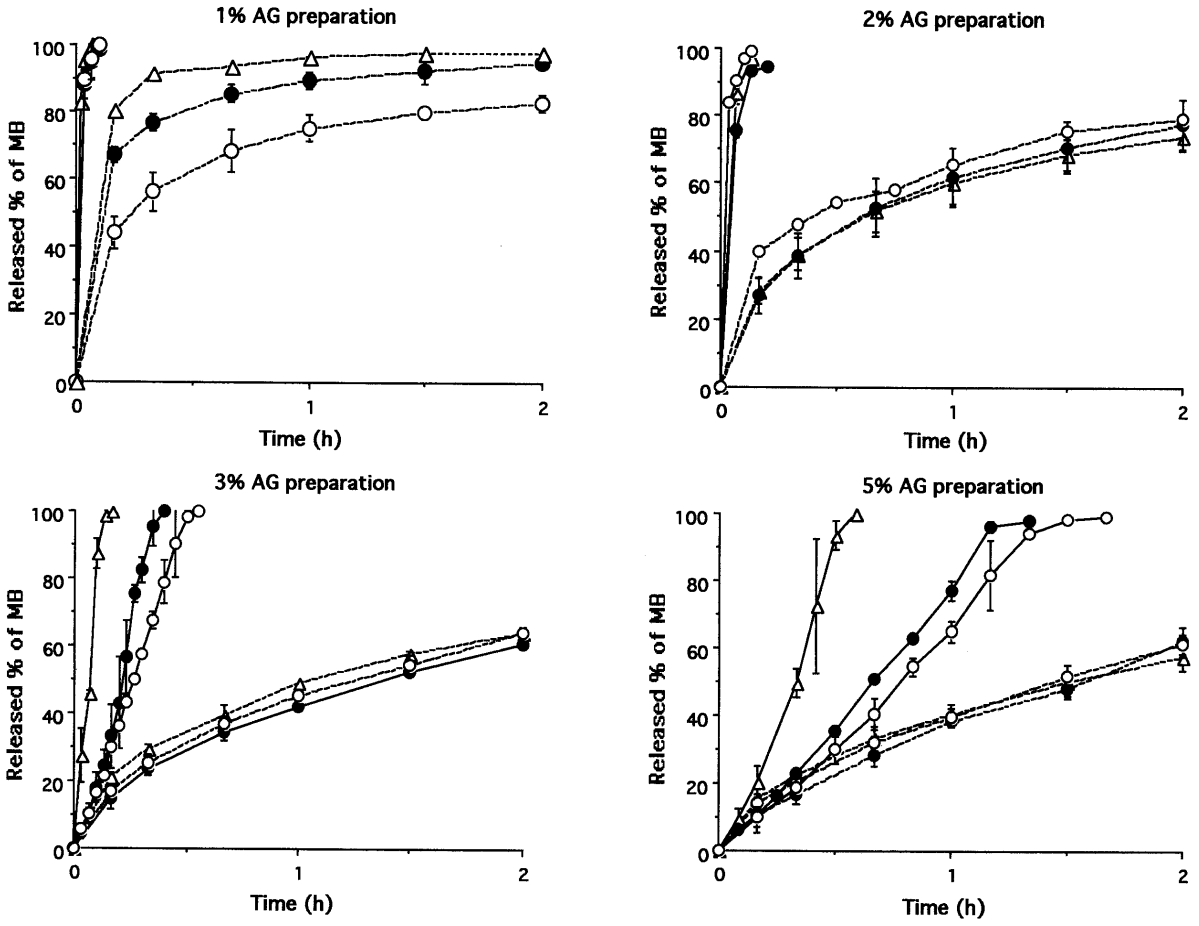

Fig. 4. Effect of Medium pH, and the Concentration and the Degree of Polymerization of Alginate on MB Release from the Alginate Liquid Preparations -----, JP1; ——, JP2. AG100 (△), AG 300 (●), AG 500 (○).

ments (0.5, 1 or 2 h following the administration), the stomach, of which cardiac and pylorus were closed by clamps, was removed quickly under ether anesthesia. In some experiments, blood samples (0.4 ml) were obtained from the jugular vein. The plasma was separated immediately by centrifugation and stored at −40 °C until assayed. ABPC concentrations in each fraction described above and plasma were determined fluorometrically.

RESULTS AND DISCUSSION

Effect of pH on Drug Release AG preparations were prepared from 1—5% of alginate solution, 3 different grades of AG, based on the degree of polymerization (AG 100, AG 300, AG 500) were used. Release profiles of MB from the preparations are shown in Fig. 4. The release of MB from the preparations made of 1% and 2% AG solution were not retarded in JP XII disintegration test medium No. 2 (pH 6.8 medium), regardless of the degree of polymerization of the alginate. However, the release from those of 3% and 5% AG solution was retarded in the pH 6.8 medium, depending on the degree of polymerization and the AG concentration. However, the release of MB for all AG grades was retarded in JP XII disintegration test medium No.1 (pH 1.2 medium). However, the degree of polymerization of the alginate did not affect the drug release except for 1% alginate. Drug release in pH 1.2 was retarded as the AG concentration increased. The sustained release in pH 1.2 compared to pH 6.8 medium was probably due to gelation of the surface of the AG preparations in pH 1.2 medium.

Viscosity of the AG Solution The viscosity of the alginate solution that patients can tolerate was determined by comparing the viscosity of the AG preparation with that of two commercial products (Alloid G® and Arcrane®) being used for the treatment of peptic ulcer by internal use. The viscosity of various AG preparations is shown in Fig. 5. The temperature of the commercial products soon after transfer from a refrigerator was 8 °C. The viscosity of Alloid G® was higher than that of Arcrane®, and it does not matter which of these two solutions to use. Therefore, when the viscosity of the preparation at 20 °C or 37 °C was lower than that of Alloid G® at 8 °C (790 cps), it was administratable. Although a number of AG preparations satisfied this requirement, the AG preparation made from 2% alginate solution of AG 300 was used in the following study, because the viscosity of the solution at 37 °C (487 cps) was closest to that of Alloid G® at 37 °C (368 cps).

Effect of Ca Treatment on Drug Release Alginate forms a gel by adding acid, Ca and other di- and tri-valent metal ions. The release profiles of ABPC from the AG preparations in various Ca concentrations and different pre-treatment times are shown in Figs. 6 and 7, respectively. ABPC release was retarded when the concentration of Ca in the pretreating solution was more than 0.10 M (Fig. 6). As shown in

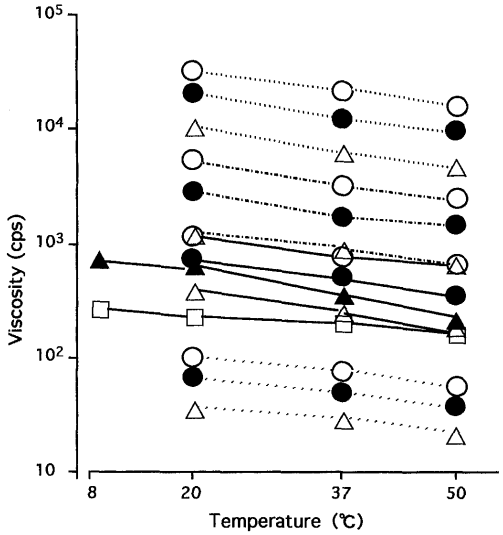

Fig. 5. Viscosity of the Alginate Liquid Preparations

AG 100 (△), AG 300 (●), AG 500 (○), Alloid G® (▲), Arcrane® solution (□). ·····, 1% AG; ——, 2% AG; – – – –, 3% AG; ·····, 5% AG.

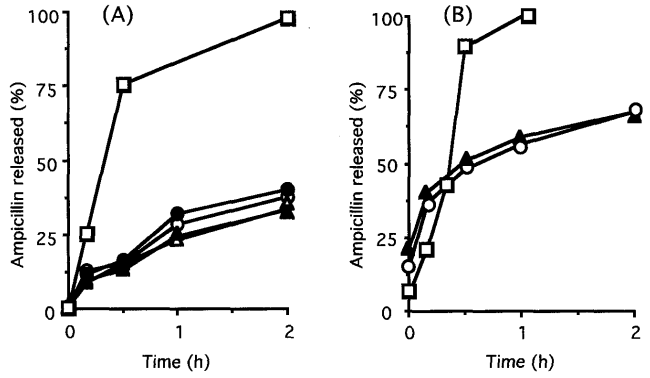

Fig. 6. Effect of Ca Pre-treatment Conditions on Release of ABPC from the Alginate Liquid Preparations in Distilled Water

(A) Pre-treatment time, 20 s. (B) Pre-treatment time, 10 min. Alginate concentration is 2% (w/v). The $CaCl_2$ concentrations used for the pre-treatment are as follows: ■, 0.01 M; □, 0.05 M; ▲, 0.10 M; △, 0.15 M; ●, 0.20 M; ○, 0.25 M. The symbols for 0.01 and 0.05 M overlap.

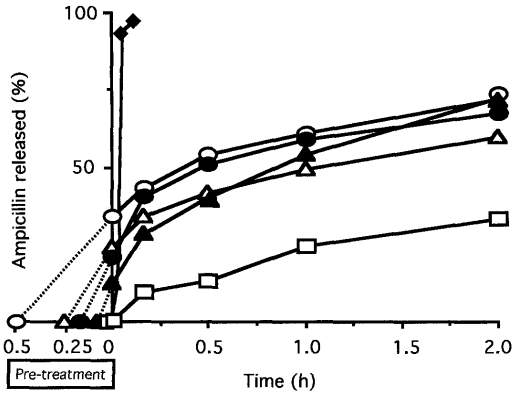

Fig. 7. Effect of Ca Pre-treatment Time on Release of ABPC from the Alginate Liquid Preparations in Distilled Water

Alginate concentration is 2% (w/v). The $CaCl_2$ concentration used for the pre-treatment is 0.10 M. ◆, without pre-treatment; □, 20 s; ▲, 5 min; ●, 10 min; △, 15 min; ○, 30 min.

Fig. 7, the longer the treatment time in 0.10 M $CaCl_2$ solution, the greater the loss of drug. The amount of drug the AG preparations lost during pre-treatment was indicated by Y-axis values at 0 on x-axis (expressing time). There was negligible drug loss when the pre-treating time was 20 s, and the release rates were nearly identical at all times (20 s—30 min). Film formation on the surface of the preparation to control the release rate of the drug took a minimum pretreatment time of 20 s and a Ca concentration of 0.10 M.

The Isolated Perfused Rat Stomach In order to evaluate the residence time of the preparation, isolated perfused rat stomachs were used. The remaining % of ABPC in the stomach at 60 min is shown in Fig. 8. When no Ca pre-treatment was performed, there was no significant difference between the aqueous ABPC solution and the AG preparation except for fraction 2 (implying adhesion of the preparation on the gastric mucus). Ca pre-treatment had no effect on the rats administered the aqueous solution, but approximately 3-fold increased the total remaining % (mainly fraction 2) at 60 min in the rats administered the AG preparation. The total remaining % of the drug was 8.3±1.2% (the AG preparation) even at 120 min (0.3±0.2% for the aqueous solution). Fraction 2 accounted for about 50% of the total remaining % at 120 min.

The color of MB inside of the stomach was observed in all cases studied (Ca pre-treatment group) up to 120 min, and the color was strong at 60 min compared to at 120 min. The color of the AG alone group was stronger than of the aqueous group. The distribution of the color was almost uniform in the AG group regardless of Ca pre-treatment, but not uniform in the aqueous group. It was confirmed that the AG preparation remained in the stomach (mainly on the gastric mucus) longer than in the aqueous solution.

***In Vivo* Study** Recently, antacid (H_2-blocker, proton pump inhibitor, *etc.*) have been used with antibiotics for HP eradication. The antacid was used to prevent the loss of the pharmacological activity of acid-sensitive antibiotics by elevating the gastric pH, and to control the subjective symptoms of peptic ulcers and accelerate recovery. To study the effect of the gastric pH on the residence time of the AG preparation and *in vivo* drug release, ranitidine, an H_2-blocker, was pre-administered in some experiments. The pH in the stomach was 1—2 in the rats without ranitidine administration. The pH in the stomach 3 h after the administration of ranitidine was 6—7. The remaining % of ABPC in rat stomach at 30 min and 60 min after oral administration of the AG preparation is shown in Fig. 9.

1) Without Ranitidine Pre-administration: The total remaining % of ABPC was very low 30 min following the administration of the aqueous solution (Fig. 9A). The total remaining % of ABPC greatly increased when the AG preparation alone was administered. Fraction 1 (implying the drug release from the preparation to the gastric fluid) accounted for about 80% of the total remaining % in the rats administered the AG preparation alone (AG alone group). The AG preparation might float in the stomach without adhering to the gastric wall, because the AG was insoluble in an acidic fluid (pH 1—2). The remaining % (total and the fraction 1) of the drug at 30 min decreased in the rats administered the AG preparation plus Ca (AG-Ca group) compared to the AG alone group (Fig. 9A). The reason is unclear, but the increase of the gastric fluid by the additional administration of the

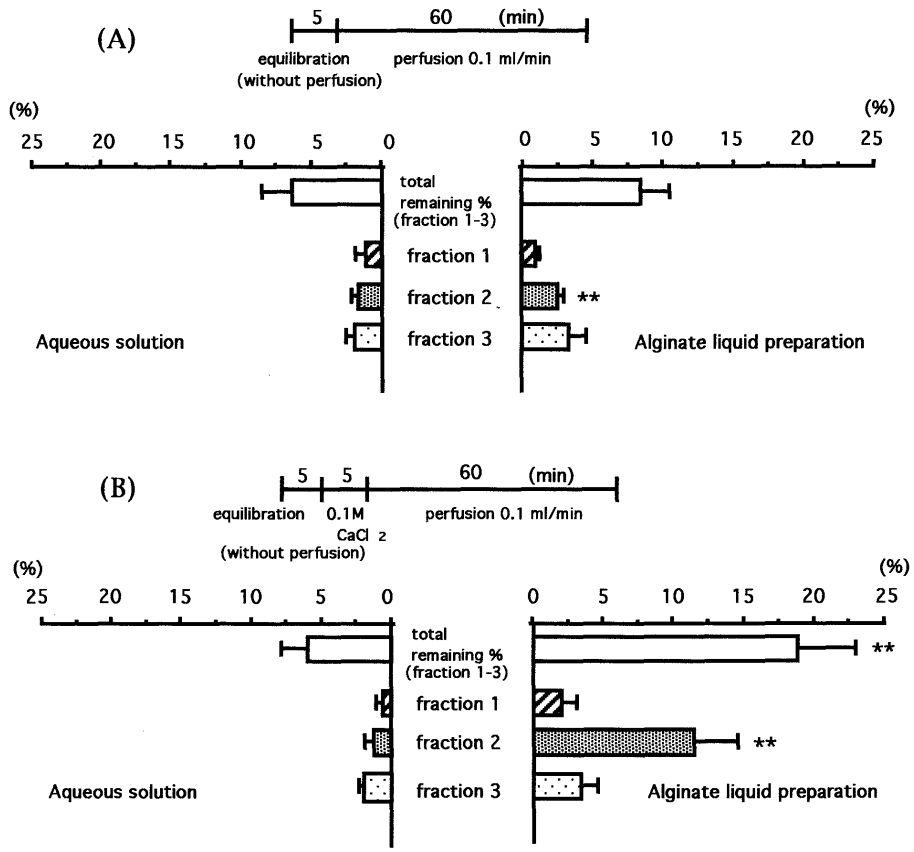

Fig. 8. Remaining ABPC in Isolated Perfused Rat Stomach

(A) Without Ca pre-treatment. (B) With Ca pre-treatment. Alginate concentration is 2% (w/v). Error bars are standard deviation (n=3—5). ** $p<0.01$ compared to aqueous solution (Student's t-test).

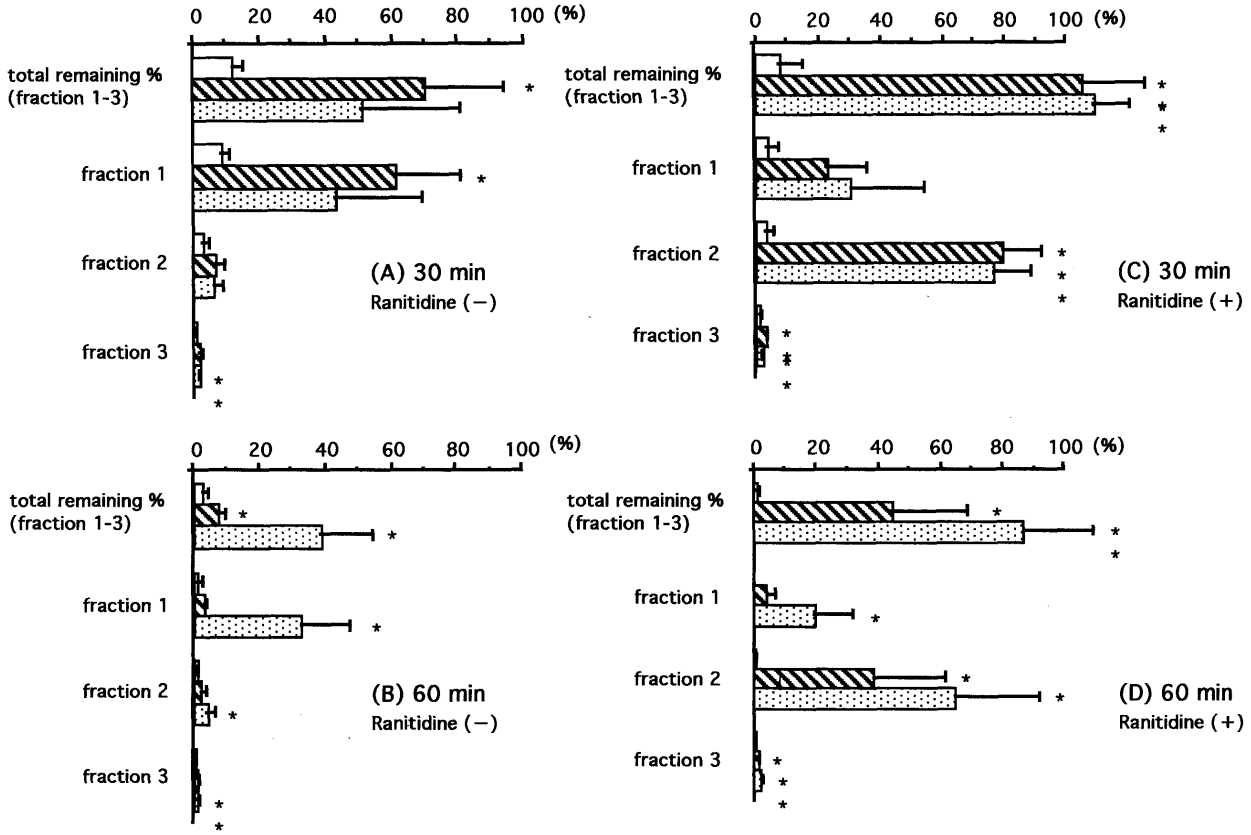

Fig. 9. Remaining ABPC in Rat Stomach after Oral Administration of the Alginate Liquid Preparations

(A), (B) Without ranitidine. (C), (D) With ranitidine. Alginate concentration is 2% (w/v). Error bars are standard deviation (n=3). □, the aqueous solution group; ▨, the AG alone group; ▩, the AG-Ca group. * $p<0.05$, ** $p<0.01$ compared to aqueous solution (Student's t-test).

$CaCl_2$ aqueous solution might cause the transport of a part of the AG preparations to the intestine. At 60 min after the administration of the aqueous solution, the remaining % of each fraction decreased compared to 30 min (Fig. 9B), and there was little drug in the stomach. The remaining % of each fraction in the AG preparation alone apparently was reduced at 60 min compared to at 30 min, and the total remaining % at 60 min was only about 8% of the administered dose. In the AG-Ca group, each remaining % also decreased at 60 min compared to at 30 min, but the total remaining % of the drug was relatively large (about 40%) and fraction 1 accounted for about 90% of the total remaining %. The AG preparation, dispersed and floated in the stomach fluid, formed a mass by gelation induced by the additional administration of Ca, and might not easily pass through the stomach. The inside of the stomach was dotted with MB at 30 min, which had disappeared at 60 min.

2) With Ranitidine Pre-administration: Since the total remaining % of ABPC in the rats administered the aqueous solution at 30 and 60 min were almost the same regardless of ranitidine pre-administration (Fig. 9C, D), ranitidine did not affect the gastric-emptying rate of the aqueous solution. When the AG preparations were administered with or without the additional administration of Ca, the drug amount adhering on the gastric wall (fraction 2) at 30 and 60 min greatly increased in ranitidine pre-administration group compared to the no pre-administration group. In the ranitidine pre-administration group, the AG preparation was easy to spread in the stomach, because the AG could dissolve in the gastric fluid (pH 6—7) in this study. The total remaining % of ABPC at 30 min was almost 100% in the rats administered the AG preparation regardless of the additional administration of Ca, and about 80% of the drug existed in fraction 2. At 60 min, the total remaining % in the AG-Ca group (mean 87%) increased about 2-fold compared to in the AG alone group (mean 44%). In this case, a large portion of the remaining ABPC also existed in fraction 2. Additionally, it was visually confirmed that the AG preparation remained almost uniform for a long time in the stomach regardless of Ca treatment *in vivo*. The AG preparation remained in the stomach longer and additional Ca administration retarded drug release to the lumen.

Plasma concentrations of ABPC are shown in Fig. 10. The plasma concentration of ABPC at 30 and 60 min in the aqueous solution was higher than in the AG preparation, and an initial rapid increase was observed in the aqueous solution experiments. The initial rapid increase was not observed when the AG preparation alone was administered, and was attenuated by additional Ca administration. The low plasma concentration of ABPC might result in a reduction in the incidence of side effects, but does not imply a reduction in pharmacological effects of the AG preparation, for ABPC could be supplied from the AG preparation to the gastric mucus.

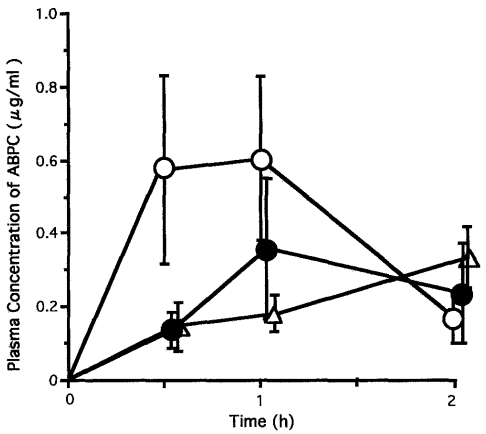

Fig. 10. Plasma Concentration of ABPC Following Oral Administration of the Alginate Liquid Preparations to the Rats Pre-administered Ranitidine

Alginate concentration is 2% (w/v). Error bars are standard deviation (n=3). ○, the aqueous solution group; ●, the AG alone group; △, the AG-Ca group.

In conclusion, the AG preparation used in this study widely spread in the stomach and released ABPC to the gastric wall rather than the gastric lumen by gel-formation on the surface of the preparation. This preparation might be a highly effective treatment for HP. However, drugs clinically used for the eradication of HP (amoxicillin, clarithromycin, *etc.*) should be tested. In addition, pharmacological studies using HP-infected model animals are also needed.

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