A Novel Evaluation Method of Gastric Mucoadhesive Property *in Vitro* and the Mucoadhesive Mechanism of Tetracycline–Sucralfate Acidic Complex for Eradication of *Helicobacter pylori*

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Purpose. The gastric mucoadhesive property of tetracycline-sucralfate acidic complex (CO) was evaluated by using a novel method *in vitro* to compare with the *in vivo* test. The mucoadhesive mechanism of the acidic complex was also studied.

Methods. The gastric mucosa removed from a rat was placed covering the end of a plunger and secured in a disposable syringe. The acidic test medium was gradually infused in and then flowed out. Two different kinds of CO, tetracycline, or a physical mixture (PM) were introduced into the device to compare their mucoadhesive properties. The tetracycline content in the residue on the mucosa was measured. The results were compared with those of the *in vivo* test. The acidic response of CO and the protein binding capacity of a sucrose octasulfate group (SOS) in sucralfate or CO were evaluated.

Results. The mucoadhesive properties of CO were clearly superior to those of PM. The remaining amounts of tetracycline in each test sample, determined by the *in vitro* test, were in agreement with those of the *in vivo* test. The excellent mucoadhesive property of CO appeared to be caused by the rapid response to the acid and resulting mucoadhesive gel formation. Furthermore, the binding capacity of SOS to the protein was clearly greater than that of PM. The excessive acid treatment during the preparation of CO tended to decrease the mucoadhesive property.

Conclusions. CO appeared to be potentially useful for the eradication of *Helicobacter pylori* because of the direct delivery of tetracycline to the gastric mucosa for an extended period of time.

KEY WORDS: complex; *Helicobacter pylori;* mucoadhesion; sucralfate; tetracycline.

INTRODUCTION

Helicobacter pylori (*H. pylori*) infection is one of the most common bacterial infections all over the world. It can exist in the human stomach in spite of its acidic environment and predisposes the stomach to ulcers or cancers. In order to eradicate it, large amounts of antibiotics such as clarithromycin, metronidazole, amoxicillin, and tetracycline have been administered orally, but not without some side effects and the appearance of resistance (1–3). The mucoadhesive formulation as a drug delivery system appears to be one of the most effective eradication methods of *H. pylori,* because the preferred location of *H. pylori* is the epithelial cell surface of the gastric antrum (4).

Up until now, many pharmaceutical approaches for mucoadhering using various bioadhesive materials have been investigated (5–12). With respect to eradication of *H. pylori,* a potential of the liquid preparation of ampicillin using sodium alginate as a mucoadhesive material has been reported (13). Very recently, R. Hejazi and M. Amiji have reported the tetracycline-loaded chitosan microspheres for *H. pylori* eradication (14). The high loading capacity of tetracycline suggested the potential of the efficacy of eradicating the infection.

Sucralfate also displays an excellent mucoadhesive property (15–20) under acidic conditions as exist in the human stomach and has been well-known as a very safe medicine because of its nonabsorption characteristic. A sucralfate–tetracycline complex prepared as an aqueous mixture is a candidate of mucoadhesive preparation for eradication of *H. pylori* (21). Tetracycline must penetrate deeply into the whole mucus for the perfect eradication of the bacteria. For this purpose, the complex adheres to the gastric mucosa homogeneously and quickly before being eliminated from the stomach. Another role of the mucoadhesion is to separate the mucosa from the gastric lumen. Most of the TC released from the complex to the gastric lumen is eliminated in the small intestine. However, TC released into the mucosa interferes with the transfer to the gastric lumen again by the separation.

In this study, we prepared the sucralfate–tetracycline complex in an acidic condition and evaluated its mucoadhesive property in both *in vitro* and *in vivo* tests. For this purpose, a novel *in vitro* gastric mucoadhesive test was devised. The results of the mucoadhesive tests revealed the advantage of the acidic complex compared with the corresponding physical mixture. The mucoadhesive mechanism of the complex will be discussed based on the mucoadhesive tests.

MATERIALS AND METHODS

Materials

Sucralfate as a mucoadhesive material was supplied from Chugai Pharmaceutical Co. (Tokyo, Japan). The water content of sucralfate measured after loss of drying was 9.1%. Tetracycline was purchased from Sigma Co. (St. Louis, MO, USA). Bovine serum albumin (BSA) was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). BioRad protein assay reagent purchased from Bio-Rad Laboratories (CA, USA) was used with a 5-fold dilution with water. All other reagents used were of special reagent grade. McIlvain buffer (pH 4.0) was prepared by mixing 0.1 M citric acid and 0.2 M disodium hydrogen phosphate in the ratio 61.4:38.6. Clark Luks buffer (CLB, pH 1.6) with an ionic strength of 0.1 was prepared by adding a sufficient volume of water to 16 ml of 2 N HCl and 5.07 g of KCl to make 1000 ml. Sep-Pac C18 was purchased from Waters Assoc. (Milford, MA, USA). H_2SO_4–NaOH reagent was prepared according to the *Japanese Pharmacopoeia XIII* (JPXIII).

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Preparation of Tetracycline–Sucralfate Acidic Complex (CO)

Two preparations of tetracycline–sucralfate acidic complex (CO-A, CO-B) were produced as follows: Five grams of sucralfate, corrected for water content, was mixed with 95 ml (CO-A) or 90 ml (CO-B) of water in a vessel. Tetracycline dissolved with 1 M HCl [1 g in 5 ml (CO-A), 10 ml (CO-B)] was added to the mixture and stirred at 400 rpm using a propeller-type agitator with four blades for 24 h. Then, the mixture was entirely filtered, and the product obtained on the filter was remixed in an appropriate amount of fresh water to remove free tetracycline and filtered again. After duplicating this process, the resulting wet powdered product was freeze-dried. The freeze-dryer (Neocool, YAMATO Scientific Co., Japan) was operated for 3 days or longer after pre-freezing at –100°C.

Determination of Tetracycline and Sucralfate Content in CO

The aqueous mixture of CO (50 mg at 50 ml) was prepared so that it was homogeneous. Three milliliters of the mixture was removed and diluted to 50 ml with 2 M acetic acid–ammonium acetate buffer to extract the tetracycline. After gentle shaking, sucralfate was removed by centrifugation. Tetracycline content in CO was determined by measuring absorbance of tetracycline in the supernatant at 355 nm. The tetracycline content was corrected for the water content of CO determined by the loss after drying (105°C, 3 h). The sucralfate content in CO was determined by subtraction of the tetracycline content from the total amount.

Evaluation of Gastric Mucoadhesive Property of CO *in vitro*

The gastric mucoadhesive property *in vitro* was evaluated by the use of the specially designed device shown in Fig. 1. The device consisted of a micro feeder to supply a test medium to which we connected two plastic syringes (A and B) using a three-way cock. The micro feeder (C) was joined to the side of the cock. The syringe A (1 ml volume) for injection of each test sample was connected to the upper portion of the cock. Another syringe B (10 ml volume) used for simulation of the stomach was connected to the lower portion of the cock. The cock switched the route of the flow.

The stomach of a rat was removed and opened along the lesser curvature. The stomach was placed flush on the top of a plunger inside out and secured tightly by inserting it into syringe B. The plunger position was set so that the remaining volume was 2 ml from the surface of the mucosa as shown in the enlarged figure in Fig. 1. A hole leading to the outside was prepared beside syringe B at the position of 1 ml to collect the test medium.

JPXIII disintegration No. 1 fluid (pH 1.2) as the test medium was supplied to syringe B through the flow route junction by the micro feeder at the flow rate of 0.1 ml/min. After 1 min, the flow route from the micro feeder was switched to the syringe A side by the cock. Each test sample such as tetracycline, PM, CO-A, and CO-B was mixed with water for the test (6 mg of tetracycline in 1 ml of water) and was introduced from syringe A into syringe B at 0.4 ml rapidly. As soon as it was introduced, the test medium was supplied to syringe B again by switching the flow route to the micro feeder side. The introduced test medium flowed out of the side hole of syringe B and was recovered. Three hours later, the test was finished, and the inside of syringe B was washed out with 10 ml of water to mix with the recovered test medium. The adsorption of TC to the syringe was not observed. The tetracycline content in the recovered test medium was determined spectrophotometrically. Each surface of the mucosa was inspected after the test.

Evaluation of Gastric Mucoadhesive Property of CO *in vivo*

Eight-week-old male Sprague Dawley rats, weighing 256 to 275 g, were made to fast with free access to water overnight

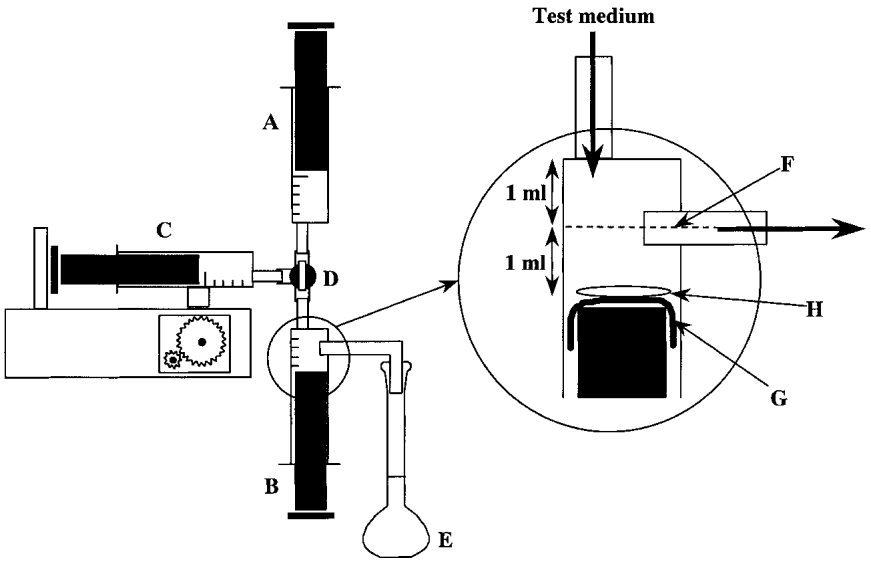

Fig. 1. Device for the evaluation of gastric mucoadhesive property *in vitro:* (A) syringe A for injection of the test samples, (B) syringe B to hold the gastric mucosa of a rat, (C) micro feeder to supply a test medium, (D) three-way cock, (E) flask to recover the test medium, (F) hole to the outside, (G) gastric mucous, (H) adhered sample.

prior to the experiments. The aqueous mixtures of the test samples (i.e., tetracycline, PM, CO-A, and CO-B) were prepared at the concentration of 10 mg of tetracycline in 1 ml of water. Each aqueous mixture was administered orally to the rat at 0.4 ml. After 3 h, the rats were sacrificed, and the excised stomach was dissected along the greater curvature. After an inspection of the surface of the mucosa, the residue on it including the mucosa was scraped carefully away using a plastic scraper. The mucus gel samples obtained were stored at −20°C until required to provide a measurement of tetracycline.

Determination of Tetracycline Amount in the Gastric Mucus and Mucosal Layer

The methods developed by Oka and co-workers (22,23) were modified and used with other methods (24,25). Each mucus and mucosal layer was put into a test tube with 1 ml of saline. They were blended twice with 2.5 ml of 0.1 M Na_2EDTA–MacIlvain buffer (pH 4.0) using a high-speed blender and then centrifuged. The supernatant was applied on a prepacked C_{18} cartridge (Sep-Pac C_{18}) activated with methanol, water, and 0.1 M Na_2EDTA–MacIlvain buffer. The cartridge was washed with 6 ml of water. Tetracycline was eluted with 5 ml of 0.01 M methanolic oxalic acid solution and collected in a graduated cylinder. The collected eluent was diluted with 0.01 M methanolic oxalic acid solution to make 7 ml in total. It was filtered using a membrane filter (0.45 μm) for the HPLC analysis.

For the determination of tetracycline content, 100 μl of each sample and standard solution were injected into an HPLC analysis system (pump, PU-980; detector, UV-970; auto-sampler 851-AS, Jusco Spectroscopic Co., Ltd., Japan). A UV detector was operated at 270 nm. The column used was a COSMOSIL 5C18-MS (5 μm, 4.6 × 150 mm, Nacalai tesque, Inc., Japan). Solution A was 0.02 M phosphate buffer (pH 3.0), and solution B was acetonitrile. Mixing of solution A and solution B in the ratio 85:15 was prepared for the mobile phase. The flow rate was 1 ml/min. The analysis was carried out at room temperature.

Evaluation of Acid Response of CO

One gram of CO-A was mixed with the 500 ml of water in a beaker. Under constant stirring, 0.1 N HCl was gradually introduced into the system (2 ml/min) using a perista pump. The change in pH of each aqueous mixture was monitored. Sucralfate alone and PM were tested to compare with CO-A. Their amounts were adjusted to the composition of sucralfate in CO-A.

Evaluation of the Adhesive Paste–Forming Capacity

Five milliliters of 0.1 M HCl was added to the aqueous dispersion of CO-B (250 mg at 15 ml) in a glassy dish (diameter, 150 mm; depth, 18 mm) under gentle shaking (shaking width, 30 mm; shaking speed, 60 strokes/min). During the 3 min of shaking, if the paste was not formed, 1 ml of 0.1 M HCl was added to the system, and the dish was shaken for 3 min more. The addition of acid every 3 min was repeated until the paste was formed and fixed to the surface on the bottom of the dish by adhesion. The adhesive paste–forming capacity was determined by taking into account the time required for fixing to the glass surface completely and the amount of the introduced acid to form an adhesive paste. The adhesive paste–forming capacity of CO-B was compared with PM.

Determination of Sucrose Octa-Sulfate Group Content in CO

Each test sample was exactly weighed at 300 mg of sucralfate content. H_2SO_4–NaOH (9.5 ml) reagent was added to these samples at 30°C (pH 1.3–1.5), and then they were completely dissolved by ultrasonic treatment. After the dilution with water to make 50 ml in total (pH 2.0–2.5), they were filtered using a membrane filter (0.45 μm). Potassium sucrose octa-sulfate was dissolved to a mobile phase for the standard solution. Fifty microliters of each sample and the standard solution were injected into the HPLC analysis system (detector, RI-71, Shodex Co., Ltd., Japan; pump, PU-980; auto-sampler 851-AS, Jusco Spectroscopic Co., Ltd., Japan). Sucrose octa-sulfate (SOS) was detected by the refractive index. The column used was a Unisil Q NH_2 (5 μm, 4.6 × 250 mm, GL science, Inc., Japan). For the mobile phase, an appropriate amount of ammonium sulfate (about 100–130 g) was dissolved in 1 l of water and was then adjusted to pH 3.5 with phosphoric acid. The flow rate was 1.2 ml/min. The analysis was carried out at room temperature.

Binding Characteristics of CO to BSA

The BSA standard solution was prepared by adding a sufficient volume of CLB to 400 mg of BSA to make 100 ml. Fifty milligrams and 80 mg of each sample (sucralfate, CO-A, CO-B, PM) was mixed with 10 ml of water. The mixing ratio of the physical mixture was determined by the tetracycline concentration of the CO. One milliliter of BSA was added to 200 μl separated from the previous mixture. Each mixture was incubated at 37°C for 30 min. After the reaction, 3 ml of CLB was added to the mixtures followed by filtration using a membrane filter (0.2 μm). Five milliliters of the protein assay reagent was added to 100 μl of each filtrate. The absorbance at 595 nm was measured, and the percentage of unbound BSA was determined by using a calibrated curve obtained from the same treatment of the BSA standard solution mentioned above.

RESULTS AND DISCUSSION

The Recovery of Tetracycline from Acidic Complex

Some solvents for dissolution of CO or extraction of tetracycline from CO were tested. Although a little tetracycline was extracted from CO by use of water, other solvents such as an acidic medium or the buffer solution significantly improved the efficiency of extraction as shown in Table I. In particular, tetracycline was almost completely extracted from CO by use of 2 M acetic acid–ammonium acetate buffer.

There was some concern that tetracycline was decomposed by the method of dissolving with an acidic medium. Therefore, the extraction by the buffer solution was consid-

Table I. Recovery Percentage of Tetracycline from CO

Medium for extraction of CO	Ultrasonic treatment	Recovery percentage of tetracycline in CO
Water	1 h	18.9
0.1 M HCl	1 h	90.4
1 M acetic acid–ammonium acetate buffer	1.5 h	87.8
2 M acetic acid–ammonium acetate buffer	1.5 h	90.4
2 M acetic acid–ammonium acetate buffer	non	99.9

ered more suitable to ensure the stability of tetracycline. Ultrasonic treatment seems not to be favorable because of attendant heating.

Gastric Mucoadhesive Property of CO

The upper photographs of Fig. 2 show the surface of the gastric mucosa after the *in vitro* study. A certain yellow residue was observed on the gastric mucosa tested by CO-A, CO-B (not shown in this figure), and PM. No residue of tetracycline was confirmed by the test of tetracycline alone, and the surface of the mucosa had been damaged by the acidic test medium. As shown in Table II, tetracycline alone flowed out from the system. The percentage of tetracycline detected in the recovered test medium of CO-A, CO-B, and PM was 41.3%, 50.4%, and 75.1%, respectively. In other words, about 60% of tetracycline in the CO-A, 50% in the CO-B, and 25% in the PM remained on the gastric mucosa.

The residue of tetracycline was observed in the *in vivo* test, too. As shown in the lower photographs of Fig. 2, residue of tetracycline could not be observed by the administration of tetracycline alone (E in the figure), but the yellow mass, which adhered to the mucosa, was observed (F in the figure) for the administration of CO-A. As shown in Table II, the amounts of tetracycline detected in and on the gastric mucosa of CO-A, CO-B, and PM were 77.75 μg, 49.68 μg, and 25.93 μg, respectively. Tetracycline was not detected when it was administered alone.

The results of the *in vitro* study were in total agreement with those of the *in vivo* study. Tetracycline alone did not adhere to the gastric mucosa, but the mucoadhesive property was recognized for the presence of sucralfate such as CO or PM. The acid pretreatment of tetracycline with sucralfate such as the CO preparation appeared to increase the mucoadhesive property of sucralfate. The amount of the residue of CO-B was less than that of CO-A, although it had been treated by a greater amount of acid. The excessive acid treatment during the complexation appeared to cause a decrease in the property. This indicated the requirement of the appropriate amount of acid during the preparation time of CO for the mucoadhesive property.

Most of the evaluation of the mucoadhesive property *in vitro* has been carried out by the measurement of the dynamic characteristics such as adhesive strength (8,11,12,26–31). The evaluation of the gastric mucoadhesive property should be carried out in consideration of the actual acid secretion. In particular, the mucoadhesive property of sucralfate appeared to be influenced by the amount of acid supplied because the mucoadhesive paste was formed by the reaction with the acid. The advantage of this device is that some conditions for the simulation of the stomach environment may be controlled. The rate of application of the acid can be controlled by the micro feeder (A). The expelling time of the stored volume of

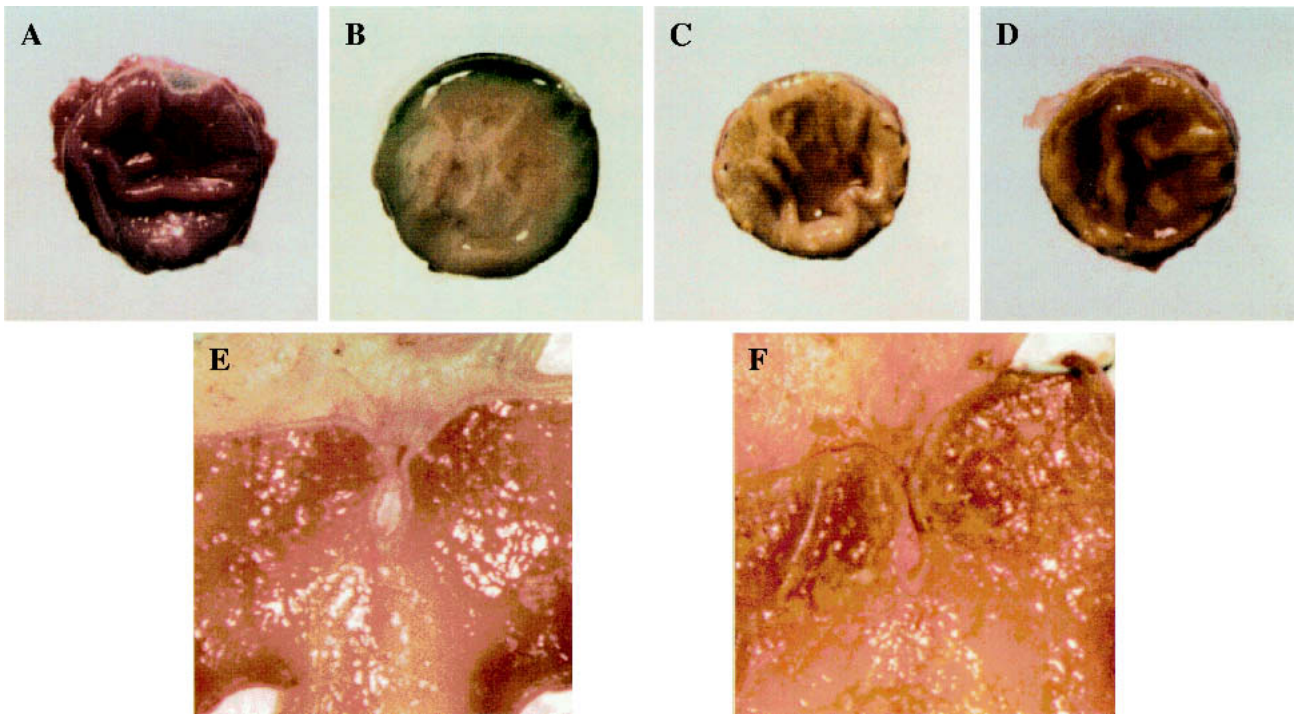

Fig. 2. Surface of the gastric mucosa after the mucoadhesive test. Results *in vitro* are shown in the upper photographs. (A) Before the test, (B) tetracycline alone, (C) physical mixture (PM), (D) acidic complex A (CO-A). The lower photographs show the results *in vivo*. (E) tetracycline alone, (F) CO-A.

Table II. The Percentage of the Tetracycline That Did Not Remain and Flowed Out of the Test Device *in Vitro* and the Amount of Tetracycline Adhered to the Gastric Mucosa *in Vivo* Test

	Tetracycline flowed out of the device *in vitro* (%)	Mucoadhered amount of tetracycline *in vivo* test (μg/head)
Tetracycline alone	102.9 ± 9.71	N.D.
CO-A	41.3 ± 5.07	77.8 ± 0.64
CO-B	50.4 ± 8.00	49.2 ± 17.0
PM	75.1 ± 4.56	25.9 ± 5.2

The results of the *in vitro* test are expressed as the mean ± SD (n = 4). *In vivo* results are expressed as the mean ± SE (n = 4 ~ 7). CO-A, complex A; CO-B, complex B; PM, physical mixture.

acid in the device can be controlled by the position of the plunger in the syringe C. Furthermore, the amount mucoadhered to a unit mucus area can be measured, because a constant surface area is secured in the device. This *in vitro* evaluation method appeared to be useful to estimate the mucoadhesive property on the gastric mucosa *in vivo* or when it was actually administered.

Acid Response of CO

The mucoadhesive property of CO comes from the sucralfate composition. The evaluation of the CO response to an acid is very important because the acid consumption of sucralfate causes its mucoadhesive property. The response to the acid was evaluated by its consumption rate when the acid was gradually added to the system in which the sample was mixed with water. Figure 3 shows the pH titration curves. A decreasing pattern for the pH of the non-sample case is plotted in the same figure for reference. The pH was naturally decreased with the introduction of HCl into the water alone. If the added acid was consumed by the sample, the pH should not decrease. When the acid was gradually introduced into the aqueous mixture of each sample, a turning point, which was caused by the sucralfate acid consumption, appeared in these curves. Interestingly, its appearance time from the start of the introduction of the acid was different for each sample. Those of sucralfate alone and CO-A were nearly equal, being about 2 and 3 min, respectively (arrows in the figure). With PM, the turning point appeared about 12 min after. The turning point indicates a mucoadhesive paste formation of sucralfate. The surface area of sucralfate for acid consumption appeared to be suddenly decreased by the paste formation. These results indicate that the mucoadhesion of CO is possible using a smaller amount of acid, and that the PM requires a greater amount of acid or time to mucoadhere.

The higher remaining percentage or larger mucoadhering amount of CO shown in the above *in vitro* and *in vivo* studies appeared to be closely related to its rapid response to the acid in the same fashion as sucralfate alone. If it was administered orally, CO-A potentially adheres to the gastric mucosa quickly, but PM might be expelled from the stomach before mucoadhesion because of its slow response to the acid.

The reason for the delay of the turning point is that the tetracycline in the PM might interfere with the response of sucralfate to the acid. Tetracycline evidently interferes with the neutralization property of sucralfate as shown in Fig. 3. Tetracycline particles might be coating the surface of sucralfate because only a small amount of tetracycline is dissolved in the initial pH of the system. The solubility of tetracycline increases with a lowering of the pH. The gradual lowering of the pH promotes the dissolution of tetracycline and the acidic complexation with sucralfate. After that, it leads to the reactivation of the acid consumption capacity of sucralfate. It appeared that these processes of PM caused the delay of the acid response.

The resultant quick paste formation of CO was evaluated based on the use of the adhering property to the glass surface. The property of SF under acidic conditions was often evaluated to calculate the mucoadhesive property of it (32). SF alone became sticky and adhered to the glass surface tightly when 4 ml of 0.1 M HCl was added. CO-B required only 11 min and consumed 8 ml of 0.1 M HCl for the formation of paste followed by fixation to the glass surface completely. On the other hand, PM required 58 min and 24 ml of acid consumption. These facts indicate that CO formed the adhesive paste more rapidly by using a smaller amount of acid than PM. In other words, even a small amount of acid required for forming paste of CO led to the rapid mucoadhesive capacity. In addition, there is concern that PM will leave the stomach before adhering to its mucosa.

Considering the explanation, acid consumption of CO produces the ionization of SF, which works directly to adhere to the mucus or to form an adhesive paste. On the other hand, with PM, the resultant ionization of SF was used for the neutralization of the system or binding to TC (preparation of CO) at first. This resultant large requirement of acid led to the greater requirement of time for forming the paste and delayed the following adhesion to the glass surface.

In the actual stomach, mucoadhesive characteristics of CO appeared to be influenced by the some personal physiological factors such as the secretion volume, secretion rate, pH of gastric juice, stomach activity, and so forth. Thus, it is impossible to determine rigidly the mucoadhesion time, re-

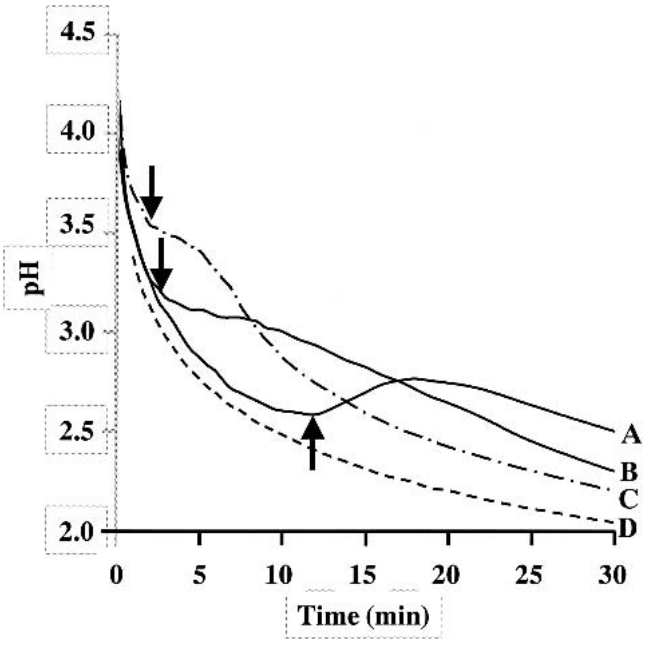

Fig. 3. pH titration curves: (A) PM, (B) CO-A, (C) sucralfate, (D) blank. Turning points are indicated by arrows in the figure.

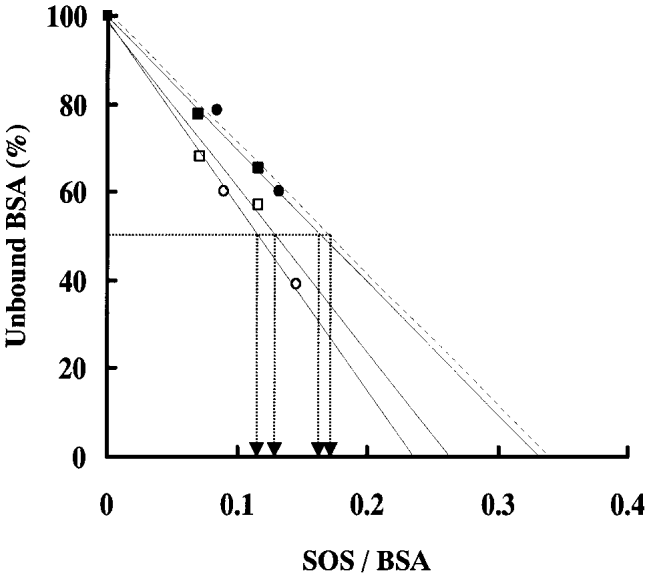

Fig. 4. Binding capacities to BSA per SOS unit in sucralfate (○), acidic complex A (CO-A) (□), acidic complex B (CO-B) (■), and PM (●).

tention time, and so forth. Despite this, however, CO must work more effectively than PM or the TC original powder.

Binding Characteristics of CO to BSA

It has already been demonstrated that sucralfate binds to the positively charged protein in the mucous layer by using its negatively charged SOS groups (15). To evaluate the capacity of mucoadhesion, the interactive property of the SOS groups with the positively charged protein such as BSA is usually studied (33). The results are shown in Fig. 4. The amounts of SOS in each sample for sucralfate, CO-A, and CO-B were 36.1%, 28.5%, and 28.4%, respectively. For the SOS content of PM, the value of sucralfate was used. The theoretical value [BSA completely remained (100%) when SOS was not added] is plotted in the same figure with the two experimental values. The plots of each sample were linear. The SOS/BSA ratio of sucralfate, CO-A, CO-B, and PM required for 50% binding to BSA were 0.117, 0.130, 0.165, and 0.172, respectively. These values increase according to the decrease of the binding capacity per SOS unit in each sample; that is, the capacity of sucralfate, CO-A, CO-B, and PM decreased in this order, and this order was in total agreement with the mucoadhesive capacity of those shown above.

If we observe the structure of sucralfate, a sucrose octasulfate group (SOS) has eight binding sites for protein. Aluminum hydroxide groups usually fill these sites. When sucralfate neutralizes the acid added during the preparation time of the CO, some aluminum hydroxide groups might partially dissociate from the sites. With CO, further acid addition during the mucoadhesive test *in vitro* or in the actual stomach appeared to promote more binding sites for the protein.

Interestingly, CO-A showed excellent BSA binding capacity, which was more than CO-B in spite of both of them containing similar amounts of SOS. Considering the mucoadhesive capacity of CO, the excess amount of acid added during the preparation time might decrease the capacity per SOS unit. It appeared that the decomposition of SOS itself was caused by excessive acid treatment.

On the other hand, the capacity of SOS in PM disappeared owing to the presence of tetracycline. It was suggested that the tetracycline interfered with the interaction between the SOS groups and BSA. Tetracycline might bind to not only aluminum moieties of sucralfate, but also to the SOS groups or BSA.

CONCLUSIONS

In this study, we proposed a novel evaluation method of the gastric mucoadhesive property *in vitro,* which was simulated in an actual stomach. Mainly, there are two characteristics of the device in this method. First, the mucoadhesive property is not evaluated by dynamics but by the actual mucoadhesive amount. Second, various environments in the stomach were simulated by changing some of the conditions of the device according to the requirements.

An excellent mucoadhesive property of tetracycline–sucralfate acidic complex was demonstrated by this method. Greater amounts of acidic-complexed tetracycline were retained on the gastric mucosa than just a simple physical mixture of tetracycline and sucralfate. The excellent mucoadhesive property was achieved by an appropriate amount of acid added during the preparation process of the acidic complex. The property tended to disappear owing to an excessive acid treatment. These results were in full agreement with the *in vivo* study. Considering the mechanisms, the excellent mucoadhesive property of the acidic complex appeared to be caused by the high sensitivity to acid and the resultant quick paste formation. Furthermore, the mucoadhesive potential of the sucrose octa-sulfate group in the acidic complex increased in spite of the decrease in its numbers.

The usefulness of the proposed novel evaluation method for the gastric mucoadhesive property and the advantage of the tetracycline-sucralfate acidic complex for *H. pylori* eradication were clarified.

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