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Effect of Sucralfate on Antibiotic Therapy for *Helicobacter pylori* Infection in Mice

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It has been documented that sucralfate, a basic aluminum salt, enhances the efficacies of antibiotics against *Helicobacter pylori*, resulting in eradication rates comparable to those associated with the use of proton pump inhibitors. However, its mechanism of action remains unclear. The aim of the present study was to investigate sucralfate's ability to complement antibiotic treatment of *H. pylori* infection in vivo. Four weeks following induced *H. pylori* infection, clarithromycin (CAM) and amoxicillin (AMPC) were administered orally to C57BL/6 mice for 5 days, both with and without sucralfate or lansoprazole. When sucralfate was concurrently given with CAM and AMPC at the maximum noninhibitory doses for the treatment of *H. pylori* infection, the bacterial clearance rates were comparable to those achieved by treatment with lansoprazole plus those antibiotics. The results of pharmacokinetic studies showed that lansoprazole delayed gastric clearance and accelerated the absorption of CAM, whereas sucralfate suppressed both gastric clearance and absorption. AMPC was undetectable in all samples. Scanning electron microscopy with a microscope to which a energy dispersive spectrometer was attached revealed that aluminum-containing aggregated substances coated the mucosa surrounding *H. pylori* in mice receiving sucralfate plus antibiotics, whereas the gastric surface and pits where *H. pylori* had attached were clearly visible in mice receiving lansoprazole plus antibiotics. The addition of sucralfate to the antibiotic suspension resulted in a more viscous mixture that bound to the *H. pylori*-infected mucosa and that inhibited the loss of CAM bioavailability in the acidic environment. Sucralfate delays gastric clearance of CAM and physically captures *H. pylori* through the creation of an adherent mucus, which leads to bacterial clearance.

Sucralfate has traditionally been classified as a topical site-protective or cytoprotective agent of ulcer-healing drugs with a high affinity for the gastric mucosa (18, 28, 29, 45). The healing rates following sucralfate treatment of ulceration are comparable to those associated with the use of H_2-receptor blockers and proton pump inhibitors (PPIs), and the relapse rates are low (16, 17, 20, 21, 23, 37). Sucralfate binds to the gastric mucosa, which results in the inhibition of pepsin activity (5, 18, 32) and the enhancement of prostaglandin synthesis (5, 7, 32). Furthermore, sucralfate therapy increases gastroduodenal mucus and bicarbonate secretion (6, 7) and suppresses acid diffusion (3). On contact with gastric acid, sucralfate becomes a highly condensed, viscous substance with the capacity to buffer acid and adheres to the surfaces of normal and defective mucosae (29, 45). A complex of sucralfate and extracellular mucus forms an impenetrable barrier locally (15, 45). The binding of sucralfate results from the electrostatic or ionic binding of the negatively charged molecules to the positively charged proteins in the mucus or ulcer crater (30). Although mucus glycoprotein is typically negatively charged, the damaged luminal mucus contains cellular debris, fibrin, and serum components, which offer potential sites to which sucralfate can bind (15). Binding of sucralfate is thus greater when chronic inflammation and ulceration are present (32).

Helicobacter pylori, an important etiologic factor for gastritis and peptic ulcer diseases, resides at the mucus-epithelial cell interface (4, 25). Evidence from experimental and clinical studies suggests that sucralfate has the potential to suppress *H. pylori* attachment (44) and urease activity (3, 41). More recent studies have shown that combination therapies consisting of sucralfate and antibiotics cure *H. pylori* infection in 80 to 90% of cases, comparable to the rates achieved with conventional eradication therapies containing PPIs (1, 22, 42, 43). Nevertheless, the clinical use of sucralfate for the treatment of *H. pylori*-associated diseases is uncommon, likely due to a lack of understanding of the mechanism of action of sucralfate and antibiotics given concomitantly.

The aim of the present study was to determine the in vivo action of sucralfate compared with that of lansoprazole, a PPI, in combination with antibiotics for *H. pylori* eradication by using a previously described model of *H. pylori*-induced gastritis in C57BL/6 mice.

MATERIALS AND METHODS

Animal model. Pathogen-free 6-week-old female C57BL/6J mice were purchased from Seac Yoshitomi (Fukuoka, Japan). The mice were housed in a specific-pathogen-free environment and were provided with food and water ad libitum. Experiments were performed according to the guidelines of the Ethical Committee for Animal Experiments at Oita University, Oita, Japan. The Sydney strain (strain SS1) of *H. pylori* (kindly provided by A. Lee, School of Microbiology and Immunology, University of New South Wales, Sydney, New South

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Wales, Australia) was grown in brucella broth containing 10% horse serum under microaerobic conditions (5% O_2, 10% CO_2, 85% N_2) at 37°C. The mice were inoculated with 0.5 ml of live SS1 (5×10^7 CFU/ml) by gastric intubation. They were deprived of food but were allowed free access to tap water for 24 h before they were killed.

Drug administration. Prior to drug treatments, the levels of immunoglobulin G against *H. pylori* in serum were determined by an enzyme-linked immunosorbent assay, as described previously (46). All mice used were seropositive. Four weeks following infection, mice were treated for 5 days with one of the following regimens: amoxicillin (AMPC) at 30 mg and clarithromycin (CAM) at 30 mg per kg of body weight daily (AC regimen); sucralfate (3, 30, or 300 mg/kg), AMPC at 30 mg/kg, and CAM at 30 mg/kg daily (SAC regimen); lansoprazole (3, 10, or 30 mg/kg), AMPC at 30 mg/kg, and CAM at 30 mg/kg daily (LAC regimen); and vehicle (3% hydroxypropyl starch) alone (control group). The drugs were suspended in 0.2 ml of 3% hydroxypropyl starch and were administered by gastric intubation.

Determination of gastric *H. pylori* levels. Gastric sections were obtained 5 days after the final drug administration. The specimens were gently pressed and ground in a coarse glass grinder with 5 ml of saline. The homogenate was then serially diluted and placed in a culture agar plate selective for *H. pylori*. The plates were incubated in a microaerophilic atmosphere at 37°C for 5 days, and the number of colonies was determined. The number of viable *H. pylori* cells was expressed as the number of CFU per stomach.

Pharmacokinetics of antibiotics. To evaluate the pharmacokinetics of the antibiotics tested, gastric sections and blood samples were obtained 0, 1, 3, 6, and 12 h after final drug administration. Stomach tissue samples were rinsed in sterile water, and the rinse solution was collected and stored for use as intragastric samples. The pH of the rinse solution was also evaluated. The remaining stomach tissue samples were gently homogenized in 5 ml of saline and then centrifuged at $400 \times g$ for 10 min at 4°C, and the supernatant was collected for use as tissue samples. The bioavailabilities of CAM and AMPC achieved when sucralfate was mixed with the antibiotics were measured in vitro. The concentrations of CAM and AMPC in these samples were determined by high-performance liquid chromatography, as described previously (19, 47). The limits of quantification for CAM and AMPC were 0.001 and 0.10 μg/ml, respectively.

SEM. To prepare for scanning electron microscopy (SEM), coded gastric mucosal specimens were fixed in Karnovsky's fixative (2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M sodium cacodylate [pH 7.4]) at 4°C for 12 h. After all specimens were rinsed in 0.1 M cacodylate buffer, they were placed in a 1% aqueous solution of tannic acid for 2 h and then in a 1% osmium tetroxide solution for 2 h. The specimens were then dehydrated through a graded series of ethanol and dried by the *t*-butyl alcohol freeze-dry method. Finally, the dried specimens were mounted on an aluminum stub with a silver paste, coated with gold, and examined under an Hitachi S-800 scanning electron microscope at 15 or 20 kV. Energy dispersive spectrometry (EDS) is a standard procedure used for the identification and quantification of the elemental compositions of sample areas. EDS-SEM analysis was performed by Hitachi High-Technologies Corp. (Tokyo, Japan).

Change of sucralfate viscosity in combination with antibiotics. Capillary rheology is a rheological method used to measure polymer viscosity over a range of shear rates. Rheology measurements are also useful for monitoring the course of a chemical reaction. The rheological properties of solutions of sucralfate and CAM or AMPC were determined at 37°C by using a double-cone-type viscometer (ReoStress 600; Haake, Karlsruhe, Germany), as described previously (34). The gap between the cone and the plate was 0.062 mm (as determined with a DC60/1 sensor system).

Statistical analysis. Differences in bacterial densities between groups were examined by the Mann-Whitney U test. A P value <0.05 was considered statistically significant.

RESULTS

Sucralfate and lansoprazole enhance antibiotic efficacy against *H. pylori* in vivo. The maximum noninhibitory dose of both CAM and AMPC in this model was 30 mg/kg (Fig. 1a). To evaluate the synergistic effect of sucralfate and lansoprazole on *H. pylori* antibiotic therapy, sucralfate or lansoprazole was administered at 3, 30, or 300 and 3, 10, or 30 mg/kg, respectively, in addition to the maximum noninhibitory doses of CAM and AMPC (Fig. 1b). Sucralfate monotherapy had no effect on *H. pylori* infection. The addition of sucralfate to CAM or AMPC increased the bacterial clearance rates in a dose-dependent manner. Sucralfate at a dose of greater than 30 mg/kg showed a synergistic effect on bacterial clearance. Similarly, lansoprazole alone failed to clear the bacteria, but its use with the antibiotics in combination enhanced the efficacies of the antibiotics against *H. pylori* in a dose-dependent manner.

Sucralfate delays gastric clearance and inhibits CAM absorption, whereas lansoprazole prolongs the intragastric residence time and accelerates absorption. As shown in Table 1, the SAC regimen increased the peak concentration (C_{max}) and delayed the half-life ($t_{1/2}$) of intragastric CAM to a level similar to that seen with the LAC regimen. For plasma CAM concentrations, the SAC regimen remarkably decreased the peak concentration and the area under the concentration-time curve over 12 h after dosing ($AUC_{0\text{-}12}$) compared with those achieved with the LAC and AC regimens. The addition of sucralfate also delayed the $t_{1/2}$ of CAM in plasma. No significant differences in tissue CAM concentrations were detected among the three regimens. The concentrations of AMPC in all samples were under the limits of measurement.

Sucralfate-antibiotic complexes coat the surfaces of gastric mucosae. On SEM, scattered aggregates, some of which formed sheets surrounding *H. pylori*, were found to coat the gastric mucosae of the mice receiving the SAC regimen (Fig. 2a and b). High magnification showed scattered particles bound to the surfaces of *H. pylori* cells (Fig. 2c and d). In mice receiving the LAC regimen, the gastric surface and pits where *H. pylori* had attached were clearly visible (Fig. 2e and f). The elemental composition of the aggregation was apparent when the energy dispersive spectrometer was attached. Although several peaks were observed for the gastric sections from mice treated with the LAC regimen, an additional aluminum peak was detected in the aggregation element from mice treated with the SAC regimen.

The sucralfate-CAM mixture becomes condensed and viscous. When sucralfate was added to a suspension of the antibiotics with vigorous stirring, particulate aggregation took place to produce a coarse dispersion. When sucralfate at 1,000 mg/ml and CAM at 160 mg/ml were suspended in hydroxypropyl starch, the initial viscosity was markedly greater than that of either sucralfate or CAM alone, as shown as Fig. 3a. The viscosity of the mixture decreased slightly at a low shearing rate but still remained high and increased at a high shearing rate. Neither sucralfate nor CAM alone showed the same behavior. This behavior did not occur with AMPC (Fig. 3b). The addition of lansoprazole to the antibiotics had little effect on viscosity (data not shown).

Sucralfate suppresses the degradation of CAM at low pH. We examined the mode of action of sucralfate in terms of the stabilities of the antibiotics in an acidic environment (Fig. 4). At pH 1.2, the bioavailability of CAM gradually decreased over time. However, the addition of sucralfate suppressed CAM degradation under the same acidic conditions. At neutral pH, CAM remained intact over time, but sucralfate addition appeared to facilitate the degradation of CAM. AMPC was stable at both pH 1.2 and neutral pH in both the presence and the absence of sucralfate.

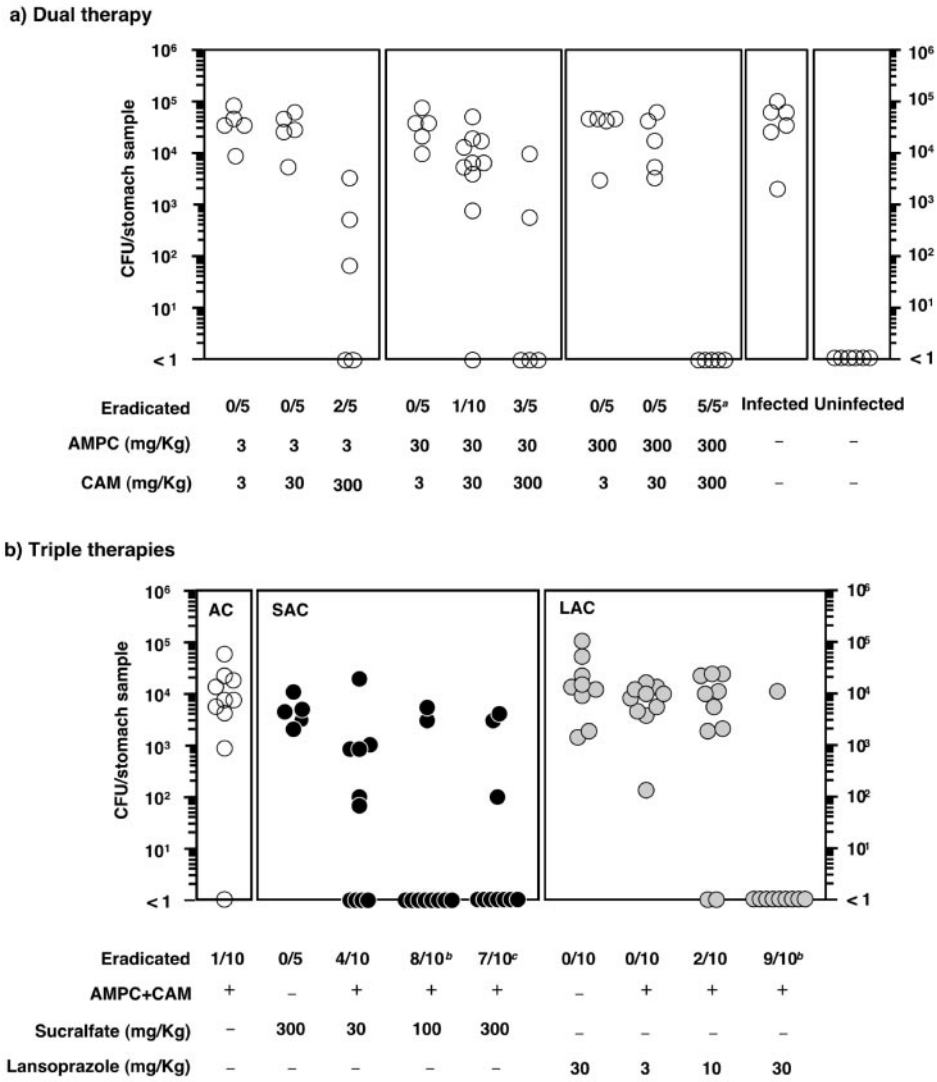

FIG. 1. Bacterial clearance by the addition of sucralfate and lansoprazole. Open circles, AC regimen; closed circles, SAC regimen; gray circles, LAC regimen. All mice studied were infected with *H. pylori*. [a], $P < 0.01$ for mice treated with the AC regimen versus untreated mice; [b], $P < 0.01$ for the SAC regimen or the LAC regimen versus the AC regimen; [c], $P < 0.05$ for the SAC regimen versus the AC regimen.

DISCUSSION

H. pylori is highly susceptible to several individual antibiotics in vitro (26). However, the bacterium is not easily eradicated by monotherapy (14, 35). The possible reasons for the discrepancy could be the low pH of gastric fluid, which accelerates the dissolution of antibiotics, as well as the limited time during which antibiotics are present in the stomach before gastric emptying. Moreover, the high concentrations of antibiotics necessary for bacterial killing are not readily achieved under the layer of gastric mucus.

PPI-containing antibiotic therapies have generally been considered acceptable for *H. pylori* eradication, although a PPI alone is insufficient for bacterial clearance (38, 44). The clearance of *H. pylori* during PPI-containing therapies can be explained by the increased antibiotic stability and the absorption at neutral pH induced by potent acid suppression (11, 13). In our mouse model, the intragastric pH increased when CAM and AMPC were coadministered with lansoprazole but not

TABLE 1. Pharmacokinetic parameters for CAM

Compartment and regimen	C_{max} (μg/ml)	$t_{1/2}$ (h)	AUC_{0-12} (μg · h/ml)
Intragastric			
AC	27.21	1.06	57.04
SAC	56.86	1.91	73.16
LAC	61.61	1.63	164.80
Tissue			
AC	4.76	1.13	8.22
SAC	3.97	1.46	9.58
LAC	5.02	3.18	18.58
Plasma			
AC	1.80	1.05	1.75
SAC	0.48	2.06	1.05
LAC	2.52	4.87	5.36

[a] In comparison with the AC regimen, the SAC and LAC regimens increased C_{max} and delayed $t_{1/2}$ for intragastric CAM concentrations. The SAC regimen decreased C_{max} and delayed $t_{1/2}$ for plasma CAM concentrations, whereas the LAC regimen increased C_{max} and $t_{1/2}$.

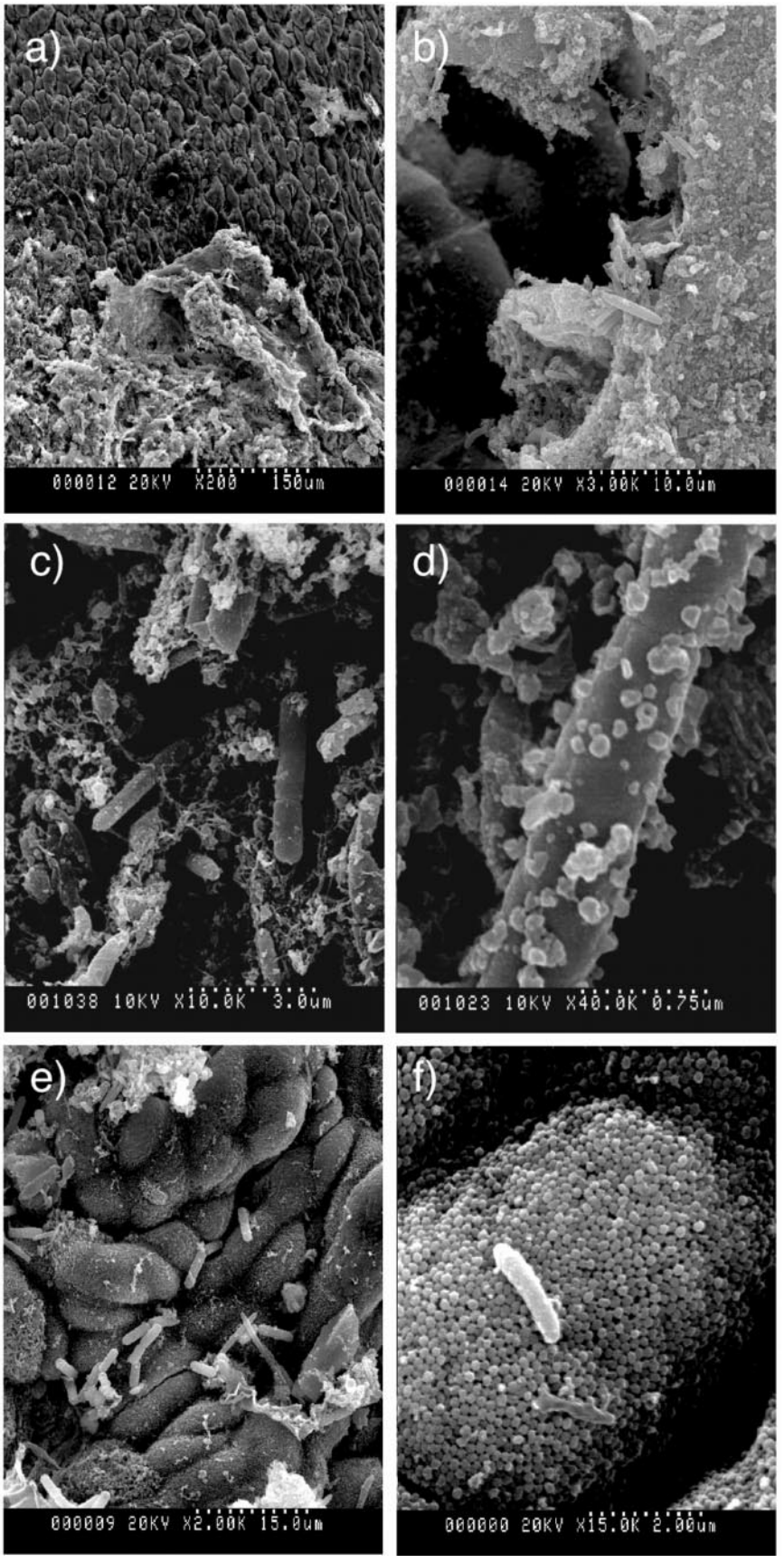

FIG. 2. Findings by SEM. Scattered aggregates, some of which formed sheets, coated the gastric mucosae (a and b). Particles on the surfaces of *H. pylori* cells were observed in mice receiving the SAC regimen (c and d). The gastric surface and pits where *H. pylori* had attached were clearly visible in mice receiving the LAC regimen (e and f).

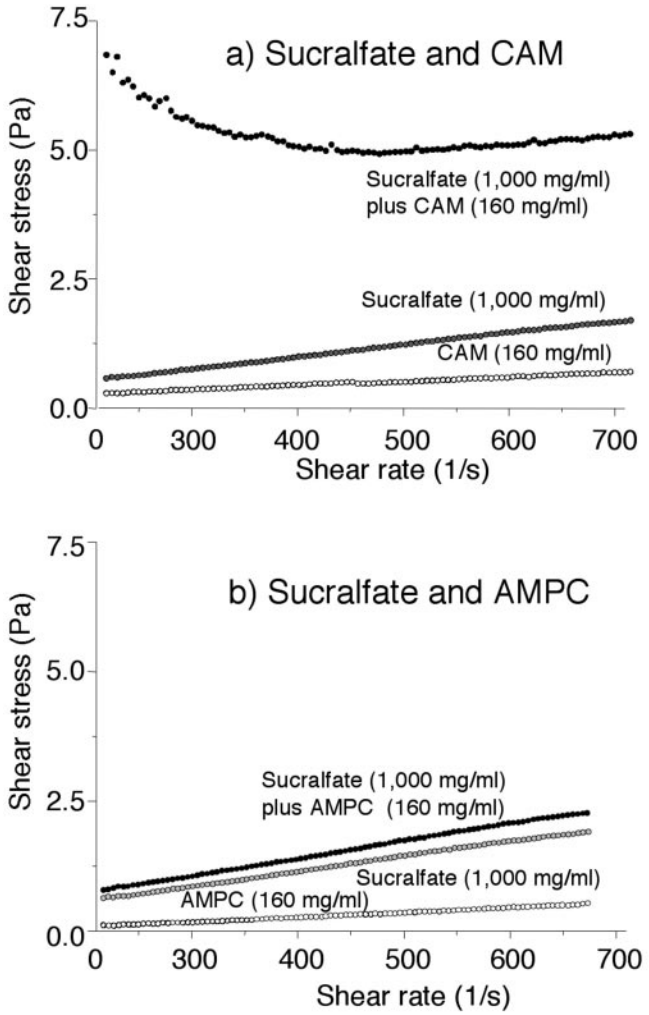

FIG. 3. Rheology measurements for CAM with or without sucralfate (a) and AMPC with or without sucralfate (b).

when they were coadministered with sucralfate (data not shown). Lansoprazole complemented antibiotic efficacy in a dose-dependent manner. High concentrations of CAM in stomach tissue and plasma were observed with the LAC regimen, concomitant with the suppression of acid secretion. Interestingly, the SAC regimen was as effective as the LAC regimen, despite a lack of systemic induction of acid suppression. Our in vitro studies, in which we followed National Committee for Clinical Laboratory Standards guideline M100-S9 (33), showed no additional direct antimicrobial effect of sucralfate (data not shown). After oral administration, the intragastric concentrations of CAM achieved with the SAC regimen were comparable to those achieved with the LAC regimen, whereas the concentrations in plasma were significantly lower than those achieved with the other regimens. The clearance of *H. pylori* achieved with the SAC regimen is likely due to the increased bioavailability of CAM in stomach.

Hydrochloric acid changes a coarse dispersion of sucralfate into a particulate aggregation and finally produces a sucralfate paste (31) which binds to the gastric mucosa (30, 32). Tarnawski et al. (45) have shown, using scanning and transmission electron microscopy, that sucralfate masses are scattered on the surface of gastric mucosa after sucralfate administration. Our scanning electron micrographs showed that the aggregates, some of which formed sheets surrounding the *H. pylori* cells, was in direct contact with the mucosal surface in the SAC regimen. The attachment of the energy dispersive spectrometer revealed that the aggregate consisted of aluminum, a major component of sucralfate. The variant form of sucralfate in the SAC regimen may be attributable to the physical changes that occur as a result of the addition of antibiotics. The rheological findings suggested that the increased binding capability resulted from the higher affinity between sucralfate and CAM but not between sucralfate and AMPC. The positively charged components of CAM, the dimethylamino groups, appear to increase the affinity of CAM for negatively charged sucralfate binding to the gastric tissue surface, resulting in a more widespread distribution of CAM in the stomach and a lower level of absorption into the circulation.

Sucralfate inhibited the loss of CAM bioavailability at low pH. An explanation for this protective effect against CAM degradation may be that bound sucralfate impedes the acid attack (29) and increases the level of bicarbonate secretion (6, 7). Sucralfate is not able to exert this beneficial property before it comes into contact with gastric acid (15). At neutral pH, the addition of sucralfate did not result in a protective effect. The barrier to acid diffusion created by sucralfate, together with its binding capabilities, might suppress the degradation of CAM. Furthermore, the creation of combinations of sucralfate and antibiotics, which capture the bacteria physically and interfere with bacterial attachment chemically (40), will improve the direct actions of antibiotics against *H. pylori* and may constrict the development of the drug-resistant organisms that are occasionally induced by these antibiotics when they are used at lower concentrations (9, 10). The efficacy of CAM is dependent on the time that its concentration is above the MIC for the organisms at the infection site (2).

Potent acid inhibition through the use of PPIs may induce secondary hypergastrinemia and accelerate the absorption of concomitantly administered drugs into the circulation (24). The withdrawal of potent acid suppressors is associated with subsequent acid rebound (27). The pharmacogenetics of CYP2C19 affect the efficacies of PPI-based therapies (8, 12). The failure of *H. pylori* eradication can occur in rapid metabolizers as a result of the fact that less drug is available after the administration of any given dose (8). Conversely, poor metabolizers may be at risk of overtreatment, with the accompanying increased incidence of adverse effects and unnecessary financial burden (12). The use of high doses of PPIs for *H. pylori*-infected elderly individuals should be further discussed, since they have corpusatrophic gastritis with a profound suppression of acid secretion, and their drug metabolism capacity is low (36, 39). These findings have led to a renewal of interest in the successful delivery of treatments to the *H. pylori* colonization site without potent systemic acid suppression.

Our data from studies with animals shows that sucralfate potentiates the effects of antibiotic therapy at the site where *H. pylori* resides. The creation of viscous mucus which physically traps *H. pylori* and inhibits the gastric clearance of CAM may lead to the satisfactory eradication of bacteria. Use of the combination of antibiotics and sucralfate may provide a site-

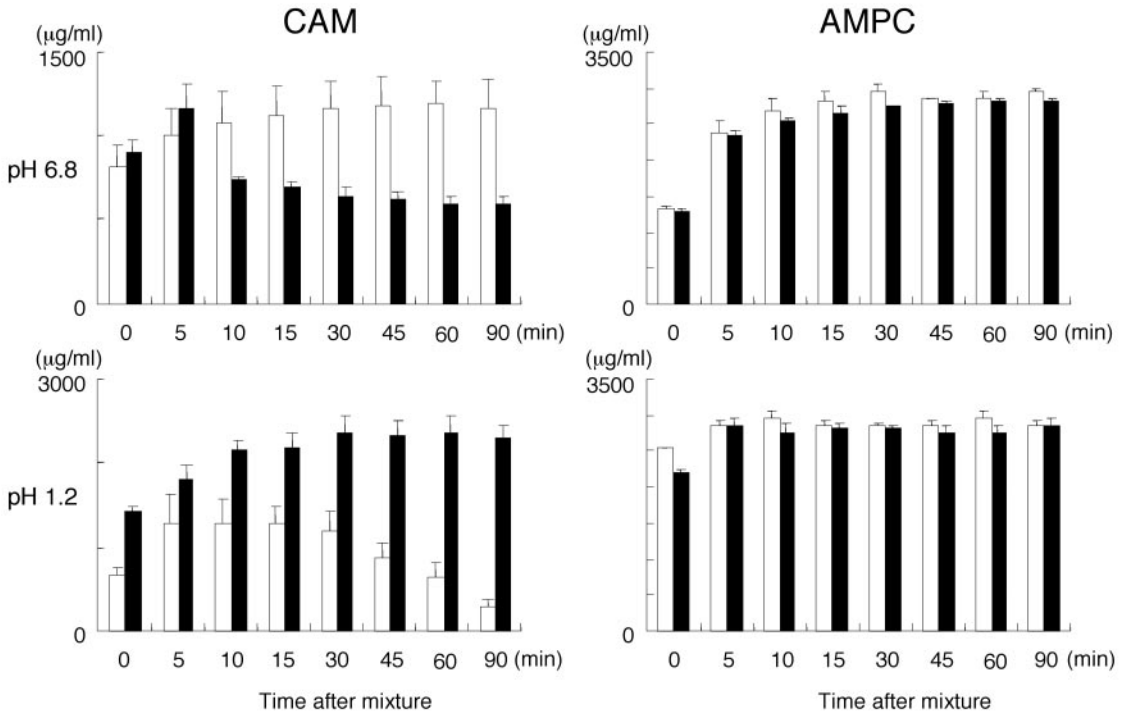

FIG. 4. Effect of sucralfate on the bioavailabilities of the antibiotics. The bioavailability of CAM gradually degraded in an acidic environment; however, the addition of sucralfate inhibited CAM degradation. AMPC was stable at both pHs, irrespective of the addition of sucralfate. Open bars, CAM or AMPC alone; closed bars, sucralfate mixed with either antibiotic. Data are presented as the means ± standard deviations for each group at each point. Each sample was tested in triplicate.

selective drug delivery system for the treatment of *H. pylori* infections.

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