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Enhanced Reduction of *Helicobacter pylori* Load in Precolonized Mice Treated with Combined Famotidine and Urease-Binding Polysaccharides

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The present study investigated the effect of a model urease-binding polysaccharide in combination with a histamine H_2 receptor antagonist on *Helicobacter pylori* colonization in vivo. Euthymic hairless mice were treated daily with dextran sulfate via drinking water and/or famotidine via intragastric gavage starting at 1 week postchallenge with a $CagA^+$ $VacA^+$ (type 1) strain of *H. pylori*. Treatment of precolonized mice for 2 weeks with dextran sulfate combined with famotidine yielded a group mean bacterial load (per 100 mg of gastric tissue) of $\log_{10}$ 1.04 CFU, which was significantly lower than those of the famotidine ($\log_{10}$ 3.35 CFU, $P < 0.01$) and dextran sulfate ($\log_{10}$ 2.45 CFU, $P < 0.05$) monotherapy groups and the infected nontreated group ($\log_{10}$ 3.64 CFU, $P < 0.01$). Eradication was achieved after 2 weeks of treatment in 50% or more of the test mice using drug combinations (1 or 2 weeks of famotidine plus 2 weeks of dextran sulfate) versus none in the monotherapy and positive control groups. The enhanced activity of the drug combination may be related to the daily pattern of transient acid suppression by famotidine inducing periodic bacterial convergence to superficial mucus sites penetrated by dextran sulfate from the lumen. Increased urease-dextran sulfate avidity was observed in vitro in the presence of famotidine and may partly account for the enhanced activity. With potential utility in abbreviating treatment time and eradication of antibiotic-resistant strains, the use of urease-targeted polysaccharides concurrently with a gastric acid inhibitor warrants consideration as an additional component of the standard multidrug chemotherapy of *H. pylori* infection.

Helicobacter pylori infection is present in up to 50% of the human population (4, 22) and is the most important cause of gastric ulcers and gastric malignancies in humans (6, 17). The epidemiological importance of this pathogen necessitates the development of inexpensive drugs with minimal side effects. Currently, a cost-effective approach to therapy of *H. pylori* infection is based on a combination of drugs largely involving a proton pump inhibitor (e.g., omeprazole) and one or two antimicrobials including antibiotics and bismuth (23). Most of the oral antimicrobials being used against *H. pylori* are active via the systemic route inasmuch as acidity within the luminal side of the gastric mucus either inactivates or renders them less effective. The acidic region of the mucus is the putative haven for the majority of *H. pylori* cells, since the presence of surface-adsorbed urease enzyme that generates ammonia within the peribacterial space requires an acidic microenvironment. The bacterium can survive with intact metabolic function anywhere from a lower limit of pH 3.5 (19), and the presence of urea in the medium greatly enhances its acid resistance resulting from the alkalinizing effect of ammonia from urealysis. Therefore, a therapeutic agent that specifically targets *H. pylori* within the acidic stratum of the gastric mucus is expected to complement other drugs whose optimal antibacterial activity is restricted to the perineutral mucus layer.

Considering the surface localization of urease on the bacterial outer membrane, persistence of colonization by *H. pylori* may be partly due to lectin-like adherence of urease to specific liaison sites in gastric mucin, particularly those within the acid-exposed layers on the luminal side of the mucus blanket. Our group recently described the bioadhesive function of the *H. pylori* urease enzyme (7). We have identified the adhesive specificity of urease for certain sugar-containing biomaterials, such as gastric mucin and sulfated cell membrane glycolipids, with which it exhibited maximal reactivity under acidic conditions (7; F. C. Icatlo, Jr., H. Goshima, N. Kimura, and Y. Kodama, unpublished data). We have likewise observed the in vivo suppressive effect of dextran sulfate (Icatlo et al., unpublished), a model urease-binding polysaccharide, on *H. pylori* gastric colonization using euthymic hairless mice as an infection model. Since this polysaccharide binds urease only under acidic conditions, the functional efficacy observed in vivo was presumably via capture of bacterium-associated urease within the acidic gastric mucus layer, resulting in bacterial aggregation and clearance via the constant washing action of the gut. In a previous study, the use of omeprazole was shown to modify the pH gradient structure in the rat gastric mucus (20). Given the acid-dependent suppressive effect of dextran sulfate on the *H. pylori* gastric load in mice, it is therefore of paramount clinical interest to profile the in vivo clearance of this bacterium when a modifier of gastric HCl secretion is concurrently administered. Specifically, the present study aimed to determine the efficacy of dextran sulfate treatment in combination with famotidine, a histamine H_2 receptor antagonist, on the gastric *H. pylori* load in a murine model of acute *H. pylori* infection developed earlier (10) by our group. We have focused on dextran sulfate as a model polymer because this polysaccharide is readily available and has demonstrated a comparatively strong affinity (7) for the urease enzyme.

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MATERIALS AND METHODS

***H. pylori* strain and cultivation conditions.** Type I ($CagA^+$ $VacA^+$) *H. pylori* strain NSP335 was originally isolated from a clinical patient with gastritis and handled as previously described during isolation, stock preparation, and propagation for mouse challenge (10). A second clinical isolate of *H. pylori* (strain 130) (7) was the source of the affinity-purified urease used in in vitro competitive-inhibition assays.

Experimental mice and maintenance conditions. The NS:Hr/ICR hairless mouse strain with the thymus intact, as described previously (10), was used as the host system for experimental infection with *H. pylori* at 7 to 8 weeks of age. The genetic stock of this strain is deposited (deposit IAR-NHI-9701) at the Imamichi Institute for Animal Reproduction, Toxicology Research Center, Ibaraki, Japan, and at the American Type Culture Collection (ATCC 72024). The animals were housed in a lightweight stainless steel autoclavable pentagonal isolator (8) with an automatic 12-h day-night light cycle. All of the procedures used in experiments related to the handling and treatment of animals were conducted in accordance with the *Institutional Animal Care and Use Committee Guidebook*, U.S. Department of Health and Human Services, National Institutes of Health (16). Animals were fed ad libitum with a diet (MF) manufactured by the Oriental Yeast Co., Tokyo, Japan.

Challenge of mice and treatment conditions. A total of 63 NS:Hr/ICR hairless mice were divided into five groups consisting of two control groups (negative or uninfected and positive or infected groups) and three treatment groups, namely, dextran sulfate (Pharmacia; molecular weight, 500,000), famotidine (Yamanouchi Pharmaceuticals), and combined dextran sulfate-famotidine groups. Dextran sulfate was administered via drinking water at a 2.5% concentration in distilled water for 2 weeks in both the single- and dual-drug treatment groups. Famotidine was given orally once a day at a rate of 2 mg/kg/mouse (1-ml volume) in the monotherapy group. In the two dual-drug treatment groups, famotidine was given for only 1 week while dextran sulfate was continued based on preliminary trials that showed no further increase in percent reduction with 2 weeks of famotidine treatment.

The challenge system used was based on an earlier procedure (10). Briefly, feed was withheld for 2 days prior to challenge. Except for mice in the negative control group that were given a mock-infected brain heart infusion broth culture, all mice were challenged with about 10^9 CFU of *H. pylori* NSP335 using a 72-h broth culture on days 0 and 1. Treatment with dextran sulfate via drinking water started on day 7 and continued for 2 weeks. At 1 and 2 weeks after the start of treatment, representative mice from each group were euthanatized for enumeration of *H. pylori* bacteria in the gastric tissue. All mice were observed daily for unusual appearance or behavior, and their gastrointestinal tracts were examined for gross changes at the time of necropsy. The pHs of the gastric luminal contents of selected mice (every other mouse) in each group were taken at necropsy with a digitally calibrated pH probe (Toa Electronics) introduced into the lumen of the excised stomach. To confirm the pH response and determine the duration of action of a single famotidine dose by intragastric gavage (2 mg/ml of distilled water/mouse), a group of 40 mice consisting of 20 mice infected for 1 week and 20 uninfected mice was subjected to similar gastric pH measurement at hourly intervals in the first 7 h and confirmation at 24 h postadministration (2 or 3 mice were tested at each pH reading time).

***H. pylori* identification and enumeration from gastric tissue.** *H. pylori* CFU isolated from gastric tissue were identified based on gram staining reactivity, morphology, and positive urease, catalase, and oxidase tests. The method used to enumerate *H. pylori* from gastric epithelial and mucus washings has been previously described (10). Briefly, mice were sacrificed and their stomachs were excised under aseptic conditions. The excised tissue was weighed and washed in a series of eight tubes containing phosphate-buffered saline (PBS). Washing of the tissue was done by top-speed vortex mixing in 20-ml tubes (10 times per tube) over a series of eight tubes. The resulting wash solution contained CFU representing the mucus-resident population of *H. pylori*. The gastric tissue was then dabbed with sterile filter paper to absorb water and weighed again (the weight difference represents the approximate mucus weight). To determine the titer of epithelium-adherent *H. pylori*, the washed gastric tissue was homogenized with a glass homogenizer and diluted in an appropriate volume with PBS. Samples from all tubes with washed mucus and homogenized tissue were taken and titrated using commercial blood agar plates (Hp Selective Medium; Eiken Co., Ltd., Tokyo, Japan). The plates were incubated for 5 days before final CFU counting.

In vitro competitive inhibition assay. To assess whether famotidine influences the ability of dextran sulfate to bind urease, an enzyme-linked ligand capture assay under acidic conditions was conducted as previously described (Icatlo et al., unpublished). Briefly, gastric mucin from a 2-month-old piglet was purified by two cycles of cesium chloride ultracentrifugation and Sepharose CL-4B size exclusion chromatography before labeling with *N*-hydroxysuccinimidobiotin. For in vitro assay, mixtures of dextran sulfate and famotidine (1:1 ratio by weight) in decreasing concentrations were mixed with an equal volume of biotinylated swine gastric mucin at 5 μg/ml in an adhesion medium consisting of 20 mM PBS and 0.05% Tween 20, pH 4.0. The mixtures were incubated in 96-well microtiter wells precoated (10 μg/ml) with affinity-purified (7) native *H. pylori* urease for 1 h at 37°C. After five washings with the adhesion medium, wells were heat fixed at 65°C and biotinylated mucin was probed with streptavidin-horseradish peroxidase conjugate (Zymed). Plates were then processed as for an enzyme-linked immunosorbent assay. Similar assays were conducted by using omeprazole (Yoshitomi Pharmaceuticals) in place of famotidine, and tests were carried out after activation of omeprazole in the pH 4.0 adhesion medium for 30 min at room temperature. Dextran with a molecular weight equivalent to that of dextran sulfate (Dextran T-500; Pharmacia Amersham) was used as a negative control. Quantitative analysis of bound biotinylated mucin, as well as calculation of percentage inhibition, was done exactly as described previously (Icatlo et al., in press). To determine whether famotidine or omeprazole can bind urease, an assay similar to that described above was conducted except that famotidine or omeprazole was incubated for 1 h at 37°C with immobilized urease and washed five times before labeled mucin was allowed to bind to urease.

Statistical analysis. Data were analyzed using Statcel, an add-in software program for Microsoft Excel (24). The differences between the mean bacterial counts of infected control and test groups were analyzed using one-way analysis of variance with Fisher's test for multiple comparisons. Differences in the frequency of *H. pylori* colonization among groups of mice were tested using the chi square test with Yates correction. P values of <0.05 were considered to indicate significant differences.

TABLE 1. pHs of gastric luminal contents of NS/Hr:ICR hairless mice[a]

Treatment group	Mean pH of gastric contents ± SD (no. of mice tested per group)	
	1 wk	2 wks
Negative control	3.6 ± 1.5 (4)	4.2 ± 0.6 (3)
Positive control	4.6 ± 0.6 (4)	4.7 ± 0.2 (3)
Famotidine	2.9 ± 0.7 (3)	4.3 ± 0.3 (3)
Dextran sulfate	5.3 ± 0.8 (3)	3.2 ± 1.2 (3)
1 wk of famotidine + 2 wks of dextran sulfate	5.0 ± 0.4 (3)	3.6 ± 0.4 (3)
2 wks of famotidine + 2 wks of dextran sulfate	—[b]	4.4 ± 0.5 (3)

[a] Gastric luminal contents were taken at necropsy after 1 and 2 weeks of daily oral treatment with dextran sulfate via drinking water and/or intragastric famotidine. At necropsy, readings were taken from randomly selected mice in the same groups of animals included in Table 2. For intragastric pH measurement, additional mice were included in the negative control group.

[b] —, not done.

RESULTS

Inasmuch as intragastric pH measurement tended to yield fluctuating real-time readings apparently due to the unbuffered character of the gastric luminal content, we recorded pH readings based on the most frequently occurring value (mode) within a 30-s time frame after an initial 60-s stabilization time. The pH continuously fluctuated within a general range of about 0.5 pH unit at reading time. Results shown in Table 1 do not clearly delineate the intragastric pH of negative control mice from those of the positive control and various treatment groups after 1 and 2 weeks of treatment. Among the famotidine- and/or dextran sulfate-treated groups, mean pH values at 1 or 2 weeks of treatment ranged from 2.9 to 5.3 and tended to cluster around 4.0. Furthermore, the mean intragastric pH of individual mice (from positive control or treatment groups) did not correlate with their respective *H. pylori* titer recovered after 1 week ($r = 0.18$) or 2 weeks ($r = 0.38$) of treatment.

With a single administration of 2 mg of famotidine given intragastrically per mouse, the temporal and quantitative responses among infected and uninfected mice were quite similar. The highest intragastric pHs among infected and uninfected mice were observed up to 4 h posttreatment, with an hourly mean pH of generally around 6.0. In some mice, values reached pH 6.1 (infected mice) or pH 6.6 (uninfected mice) within this period. These findings indicate that for approximately 80% of the daily time cycle (about 20 h), gastric acid output was at basal levels among the group of infected mice receiving a single daily famotidine dose.

TABLE 2. Therapeutic efficacy of combined famotidine and dextran sulfate[a]

Time and treatment group	Log_{10} CFU of *H. pylori* (geometric mean titer)/100 mg of tissue/mouse ± SD (% of tissue weight[b])			*H. pylori* eradication rate (no. of mice negative/no. tested)		
	Mucus	Epithelium[b]	Total	Mucus	Epithelium[b]	Total
First wk						
Negative control	0.0 (30.0)	0.0 (70.0)	0.0 (100)			
Positive control	4.28 ± 0.26 (34.4)	2.66 ± 0.18 (65.6)	3.87 ± 0.25 (100)	0/6	0/6	0/6
Famotidine	4.55 ± 0.67 (32.8)	2.41 ± 0.26 (67.2)	3.91 ± 0.45 (100)	0/6	0/6	0/6
Dextran sulfate	3.79 ± 0.34 (27.8)	0.50 ± 0.69[f,g] (72.2)	3.22 ± 0.28 (100)	0/8	5/8[j]	0/8
Dextran sulfate + famotidine	2.17 ± 1.74[f,g,i] (33.9)	0.26 ± 0.62[f,g] (66.1)	1.80 ± 1.48[f,g,i] (100)	2/6	5/6[k]	2/6
Second wk						
Negative control	0.0 (29.3)	0.0 (70.7)	0.0 (100)			
Positive control	4.09 ± 0.23 (37.0)	2.33 ± 0.26 (63.0)	3.64 ± 0.23 (100)	0/6	0/6	0/6
Famotidine	3.85 ± 0.22 (29.5)	1.29 ± 0.76[f] (70.5)	3.35 ± 0.29 (100)	0/6	1/6	0/6
Dextran sulfate	2.73 ± 1.23[e] (33.9)	0.98 ± 0.61[f] (66.1)	2.45 ± 0.75[e] (100)	1/8	2/8	0/8
Dextran sulfate + famotidine[c]	1.41 ± 1.72[f,g,h] (27.5)	0.14 ± 0.42[f,g,i] (72.5)	1.18 ± 1.45[f,g,i] (100)	5/9[j,l,m]	8/9[k,l,m]	5/9[j,l,m]
Dextran sulfate + famotidine[d]	1.33 ± 1.53[f,g] (32.1)	0.00 ± 0.0[f,g,k] (67.9)	1.04 ± 1.20[f,g,h] (100)	2/4	4/4	2/4

[a] Famotidine (oral, 2 mg/mouse/day) and dextran sulfate (2.5% in drinking water) were given to NS:Hr/ICR hairless mice for 1 to 2 weeks starting 1 week after challenge with *H. pylori* NSP335.
[b] Includes underlying tissues (tunica submucosa, muscularis, and serosa) during homogenization.
[c] Discontinued on the second week.
[d] Continued through the second week.
[e] $P < 0.05$ compared to positive control group, Fisher's test.
[f] $P < 0.01$ compared to positive control group, Fisher's test.
[g] $P < 0.01$ compared to famotidine group, Fisher's test.
[h] $P < 0.05$ compared to dextran sulfate group, Fisher's test.
[i] $P < 0.01$ compared to dextran sulfate group, Fisher's test.
[j] $P < 0.05$ compared to positive control group, chi-square test.
[k] $P < 0.01$ compared to positive control group, chi-square test.
[l] $P < 0.05$ compared to famotidine group, chi-square test.
[m] $P < 0.05$ compared to dextran sulfate group, chi-square test.

The therapeutic outcomes of the different modes of treatment after 1 and 2 weeks are presented in Table 2. For statistical analysis, the mean numbers of CFU per 100 mg of mucus or epithelial tissue were compared between groups. The total CFU values in Table 2 represent the sum of CFU counts in mucus and epithelial tissue according to their weight percentage composition; e.g., CFU values for mucus- and epithelium-associated bacteria were not summed as is but calculated according to their proportion (expressed as percentages of tissue weight in Table 2) within the whole gastric tissue examined. Famotidine monotherapy temporarily increased the CFU count after the first week of treatment but reversed colonization slightly after the second week. Dextran sulfate monotherapy yielded about 4.5- and 15-fold reductions in CFU counts after 1 and 2 weeks of treatment, respectively, with the 2-week group mean titer significantly lower than that of the positive control at this time point ($P < 0.05$). Combined treatment with dextran sulfate and famotidine yielded a significantly lower mean *H. pylori* load relative to the mean counts of the famotidine and dextran sulfate monotherapy groups at 1 and 2 weeks of treatment. Compared to the mean CFU count of the positive control group, those of the combined-treatment groups were 117-fold lower after 1 week ($P < 0.01$) and approximately 300- to almost 400-fold lower ($P < 0.01$) after 2 weeks of treatment without or with famotidine, respectively, during the second week. *H. pylori* was eradicated in about half of test mice given combined drugs (1 or 2 weeks of famotidine plus 2 weeks of dextran sulfate), with complete eradication of epithelium-associated bacteria among all of the mice given both dextran sulfate and famotidine for 2 weeks. All of the mice given monotherapeutic or combined drug regimens were alert, did not lose weight or exhibit any symptoms related to gastrointestinal or other disturbances, and did not exhibit gross lesions along the gastrointestinal mucosal wall upon necropsy.

Results of the in vitro assay of inhibition of the urease-mucin interaction by famotidine and dextran sulfate are shown in Fig. 1, with each value representing the mean of four independent trials. Interestingly, the dextran sulfate-famotidine combination exhibited much higher dose-dependent inhibitory activity than dextran sulfate, famotidine, or omeprazole. When combined with dextran sulfate the degree of potentiation by omeprazole solubilized in dimethyl sulfoxide (similar to that shown in Fig. 1; not shown) or suspended in pH 4.0 adhesion medium

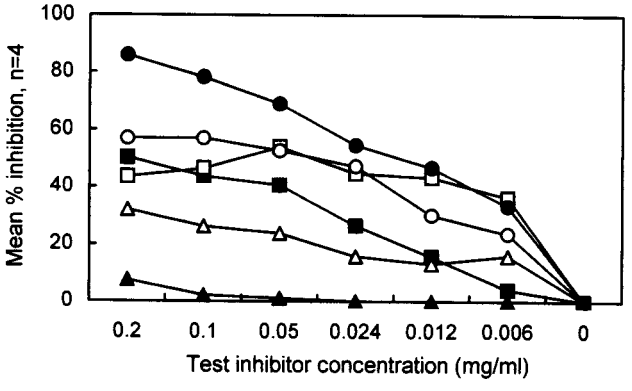

FIG. 1. Enhanced inhibition of biotinylated swine gastric mucin adherence to *H. pylori* native urease protein caused by dextran sulfate in the presence of famotidine. Dextran sulfate exhibited enhanced competitive activity in the presence of a weight-equivalent amount of famotidine using a pH 4.0 adhesion medium containing 20 mM PBS and 0.05% Tween 20. A similar percent inhibition was not observed when the dextran sulfate-omeprazole combination or single-component test inhibitors were used. Symbols: ●, dextran sulfate plus famotidine; ○, dextran sulfate plus omeprazole; ■, dextran sulfate; □, famotidine; △, omeprazole; ▲, dextran.

(shown in Fig. 1) was not as high as that of famotidine. For the in vitro assay, famotidine was not solubilized further in organic solvent for three reasons. Firstly, we used famotidine at 2 mg/ml as an oral dose and at 0.2 mg/ml or a lower concentration for the in vitro assay; these concentrations are roughly within the solubility range of this drug in water, which has been reported to be 0.1% at 20°C (15a). Secondly, in vitro results will allow direct comparison with in vivo conditions after intragastric dosing. Thirdly, the concentration of famotidine in solubilized form is not likely to reach 6 μg/ml or higher levels in serum after absorption from the gut, thereby ruling out a possible interaction with intragastric dextran sulfate via the systemic circulation. When assayed for direct adherence to urease by preincubation with urease and subsequent washing before the mucin adherence assay, famotidine or omeprazole adhered to urease but inhibited only about 10 to 30% of the labeled mucin at inhibitor concentrations of 6 to 200 μg/ml. Moreover, neither of these drugs inhibited mucin in a clear dose-dependent manner, thereby indicating nonspecific adherence to urease (data not shown).

DISCUSSION

The mean intragastric pHs of negative control mice shown in Table 1 are generally consistent with the reported values for this species variously observed at pHs 2.5 (2), 3.2 (3, 11), 3.8 (14), and around 4.0 (13). In the rat, 3 mg of famotidine/kg of body weight elevated the intragastric pH to about 5.4 from a resting pH of 3.0 (9) while 20 mg of famotidine/kg of body weight resulted in an intragastric pH increase to 6.5 from a similar resting pH (15). In the present study, famotidine was used in mice at about 80 mg/kg of body weight (2 mg/mouse), which was four times the above-mentioned dose for rats that yielded intragastrics pH of >6.0. The resulting intragastric pH elevation was confirmed by monitoring of the luminal pH after intragastric famotidine administration in a group of test mice (see Results). Inasmuch as the effect of famotidine lasted for roughly the same time (about >4 h) and the pH response magnitudes were similar (generally about pH 6.0) among infected and uninfected mice, infection with *H. pylori* did not appreciably alter the quantitative and temporal pH responses of mice to famotidine. The pH readings presented in Table 1 were taken about 24 h from the last administration of famotidine, that is, when the gastric acid secretion has reverted to the basal level. This explains why famotidine did not seem to influence pH readings at necropsy compared to groups not treated with this drug, as shown in Table 1. A trend toward increasing pH with longer *H. felis* infection time has been observed previously (13) but was not observed in this study and might be related to differences in the *Helicobacter* species used or in the degree of urease production by these isolates. The rapid reversion to basal acid secretion in mice observed in this study is consistent with that of humans, in whom intragastric acidity rebounds within the same day after a single famotidine administration (12).

The general elevation of intragastric pH to around 6.0 among infected mice within more than 4 h after famotidine administration (versus the pretreatment level of about pH 4.0; see Results) identified this pH response in mice as resulting from suppression of HCl secretion by famotidine and not due to ammonia production by urease enzyme activity. Gastric acid suppression presupposes a shift in the neutral and perineutral mucus layer within the mucus pH gradient toward the luminal side (20). Inasmuch as the neutral pH usually found near the epithelial surface (20) is apparently not favorable for the urease-producing *Helicobacter* spp. due to production of ammonia with resulting alkalinization arising from urealysis in a urea-containing mucus medium, acid suppression most likely induced most *H. pylori* cells to actively transmigrate toward a more superficial zone in the mucus during the acid suppression period postadministration of famotidine. This is supported by our finding (Table 2) that daily famotidine monotherapy resulted in a significant decrease ($P < 0.01$) in the mean count of epithelium-adherent *H. pylori* bacteria relative to that of the positive control group after 2 weeks of treatment with complete eradication from the epithelium in one of six mice at that time. This suggests that *H. pylori* cells adhering to surfaces of epithelial cells deep within the ion (acidic) channels of gastric glands converged (together with mucus-resident populations) toward more superficial sites in mucus when HCl secretions were disrupted via blockage of histamine H_2 receptors. The repellent effect of a neutral or nearly neutral pH on the bacterium is further supported by the finding that urease-producing *H. felis* exhibits a stratified distribution in the gastric mucus of mice, with virtually no bacterial cells observable within 5 μm of the gastric epithelial cell surface (21). However, the shift in the perineutral mucus stratum per se toward the lumen upon famotidine monotherapy has no obvious therapeutic benefit, as can be gleaned from Table 2, which shows that famotidine administered alone did not result in eradication of *H. pylori* in any test mouse after 2 weeks of treatment. This suggests that most bacterial cells were driven back to their preferred niche deeper within the mucus (but still on the acidic side) during the periodic reversion to basal acid secretion, taking note of the fact that navigation of the whole mucus depth via flagellar motility can be accomplished by this bacterium within seconds (21).

A 2.5% concentration of dextran sulfate given orally to mice was shown in a previous study to significantly reduce the gastric *H. pylori* load in infected mice and tended to eradicate the organism from the stomach (Icatlo et al., unpublished). This dose level allowed an approximate daily water intake of about 4 to 8 ml/mouse/day or 100 to 200 mg of dextran sulfate/mouse/day. Dextran sulfate exhibits a high degree of affinity for *H. pylori* urease from pH 4.5 down, with an optimum at pH 4.0. It also competes strongly with mucin or epithelial membrane sulfoglycolipids for the *H. pylori* urease binding site (Icatlo et al., unpublished). Inasmuch as the intragastric pHs among mice in the positive control group ranged from 3.8 to 5.2 (data not shown), with mean pH values of around 4.6 to 4.7 (Table 1), one may infer the feasibility of binding and aggregating urease-coated *H. pylori* by dextran sulfate during intervals of acidity fluctuating around pH 4.0. It may be that the epithelium-adherent *H. pylori* population is constantly renewed by mucus-resident bacteria and that decimation of the mucus-resident bacterial population by dextran sulfate monotherapy prevented full renewal of the epithelium-adherent population with bacteria coming from the mucus compartment. This may account for the tendency of monotherapeutic dextran sulfate to eliminate cell-associated bacteria shown in Table 2. In view of the observation that 150-kDa dextran diffuses in mucus with a diffusion coefficient similar to that of 150-kDa human immunoglobulin G (5), the mucus-penetrating ability of the dextran sulfate used in this study, with about thrice the molecular mass of human immunoglobulin G, can be deduced. Considering that >95% of *H. pylori* cells reside in the mouse gastric mucus (10), the high overall reduction of the mean gastric bacterial load in mice after 1 week of combined drug treatment or 2 weeks of monotherapy with dextran sulfate (Table 2) implies extensive penetration of the surface mucus gel and urease binding activity therein by the sulfated polymer.

Coadministration of dextran sulfate with famotidine resulted

in enhanced reduction ($P < 0.05$) of the gastric bacterial load, as well as bacterial eradication in 50% or more of the test mice (2 weeks of dextran sulfate plus 1 or 2 weeks of famotidine; Table 2), versus none in the monotherapy groups. The overall enhanced effect of combined-drug treatment may have been due to the daily pattern of alternating acid suppression and secretion induced by once-a-day famotidine treatment that tended to increase the frequency of bacterial capture by dextran sulfate along the acidic luminal side of the gastric mucus. Acid suppression most probably involved expansion of the pH 7.0 juxtaepithelial mucus zone that may have flushed out the bacterial cells from this region of the mucus toward more superficial sites (as mandated by the organism's unique acid-oriented physiology) since the superficial mucus margins are the only remaining acidic region when acid production is suppressed. With dextran sulfate penetrating the mucus from the luminal side, the onset and resumption of basal acid secretion (with the decline in famotidine activity) may have found most of these urease-coated bacteria in the acidic compartment of the mucus. Note that this is the time when the pH is around 4.0, which is optimal for urease-dextran sulfate interaction. Judging from the fastidious distribution of *H. felis* within a very narrow geographical zone of mucus from the epithelial surface (21), it is possible that these bacteria are capable of such constant fine tuning in their vantage position by flagellar locomotion. Reduction of the gastric bacterial load may have occurred in progressive stages, mainly during the daily phase of acid secretion. The relatively lower rate of reduction of the gastric *H. pylori* load when famotidine or dextran sulfate was used alone (monotherapy data, Table 2) may be indicative of the importance of herding the bacteria (by acid suppression) to more superficial fringes of the gastric mucus for subsequent and immediate capture by dextran sulfate once the luminal side of mucus becomes acidic. The apparent lack of difference in the therapeutic outcome with or without famotidine continuation in the second week (1 versus 2 weeks of famotidine plus dextran sulfate, Table 2) may be related to unequal mouse numbers or a treatment time that was not long enough to cause any difference in the eradication rate.

The observed enhanced activity of the above-described drug combination cannot be accounted for by direct bactericidal activity on *H. pylori*, since famotidine or dextran sulfate is microbiocidally inert in vitro at a concentration of 2 mg/ml (Icatlo et al., unpublished) or 2.5% (Icatlo et al., unpublished), respectively. To some extent, famotidine may have directly potentiated the urease-binding action of dextran sulfate, as demonstrated in Fig. 1. We observed enhanced inhibition of urease-mucin adherence with a 1:1 (by weight) mixture of famotidine and dextran sulfate at pH 4.0 in an in vitro assay (Icatlo et al., unpublished). Enhanced inhibition indicates a more stable and higher-affinity interaction between urease and dextran sulfate (and therefore between urease-coated bacteria and dextran sulfate) at pH 4.0 relative to the urease-mucin interaction in the presence of famotidine. This magnitude of in vitro potentiation of dextran sulfate was not observed with the omeprazole-dextran sulfate combination. The enhanced effect was observed at 6 μg/ml and higher concentrations that are not likely to be attained by famotidine in the circulation based on levels attained in human plasma after absorption from the gut (1) but could be attained in the mouse mucosa after intragastric gavage (at 2,000 μg/ml/mouse/day) with distilled water. The mechanism behind the additive effect is not known, but there seems to have been a direct but nonspecific effect of famotidine on the electrostatic charges or three-dimensional conformation of urease and/or dextran sulfate at pH 4.0, while famotidine itself does not participate in the adherence process, according to the results of our indirect binding assay (see Results). It is not clear to what extent this mode of synergism contributes to the observed enhancement of *H. pylori* clearance in vivo. A minor role is conceivable, since famotidine administration was done only once a day and famotidine in distilled water at the time of intragastric administration has a pH of 8.5 to 8.6, which may have been brought down to acidic or neutral level, depending on the amount of gastric juice or dextran sulfate-supplemented drinking water in the stomach just before and after famotidine treatment. Moreover, since omeprazole does not appreciably influence the direct urease-dextran sulfate interaction, as shown in Fig. 1, whereas the omeprazole-dextran sulfate combination results in enhanced *H. pylori* reduction in vivo (Icatlo et al., unpublished) with a pattern similar to that in Table 2, acid suppression emerges as the most likely major mechanism by which famotidine contributed to enhanced dextran sulfate activity during combined-drug treatment in this study.

The difference in basal intragastric acidity between mice and humans does not rule out extrapolation of the present approach to the chemotherapy of human *H. pylori* infection. At the daily dose used in this study, the suppressive effect of famotidine on gastric acid secretion was transitory and did not completely dissipate the gastric mucus pH gradient, as shown by a maximum intragastric pH of around 6.0 among famotidine-treated infected mice (see Results). A similar pH response magnitude is observed in humans after the intake of a single dose of 20 mg of famotidine or 20 mg of omeprazole (peak of about pH 5.9 for omeprazole and about pH 6.7 for famotidine), differing by the longer activity duration (around 7 h for famotidine or longer for omeprazole) (18). It is noteworthy that the 24-h mean intragastric pH when either of these drugs is used is elevated up to a 24-h mean of about 4.0 (18; http://www.cag.ucalgary.ca/sponsors/abbott/clinical_abstracts/clin.1.html/), which is optimal for the urease-dextran sulfate interaction (Icatlo et al., unpublished). The direct effect of famotidine illustrated in Fig. 1 might be more relevant to human patients because of their lower mean intragastric pH compared to mice and because a 40-mg once-a-day dose of famotidine powder (commercially available) may produce up to around 100 μg of famotidine/ml or higher concentrations in the human stomach when taken before bedtime. The generally lower intragastric pH in humans further suggests that urease molecules are more likely to be denatured (enzymatically inactivated) in *H. pylori*-colonized persons than in mice, while the colonized mouse intragastric pH does at times dip below pH 4.0 (Table 1). Any pH below this level irreversibly abolishes extracellular urease enzyme activity and therefore prevents ammonia production that shifts the pH toward neutrality, thus making the acid-driven urease-dextran sulfate interaction less likely to be reversed. Confirmation and optimization of the present drug combination concept in large-animal models with a basal acid secretion level comparable to that of humans is of clinical relevance and an immediate prospective goal of our group.

Our previous (Icatlo et al., unpublished) and current data indicate that it takes 2 to 4 weeks to achieve a >80% *H. pylori* load reduction in euthymic hairless mice when 2.5% dextran sulfate is used as a monotherapeutic drug. The time needed to attain and surpass this upper bacterial load reduction ceiling was cut down to 1 week by combining famotidine with dextran sulfate, as shown in Table 1. A shorter treatment period is significant, since the usually long duration of *H. pylori* chemotherapy compromises the eradication rate due to poor patient compliance. Another feature of the present chemotherapeutic concept is its applicability to treatment of *H. pylori* strains that

are resistant to conventional antimicrobials inasmuch as the action of dextran sulfate is independent of the bacterial metabolic response. Given that urease is an essential, abundant, and surface-accessible bacterial protein, *H. pylori* strain compatibility with this polymer is therefore broad. Unlike some systemic anti-*H. pylori* drugs, dextran sulfate is inert and does not depend on further biochemical transformation to an activated form within the host. The use of a locally acting urease-specific blocking agent on the mucosal surface avoids the systemic route and can therefore be tolerated by patients with forms of illness that limit systemic drug administration. The use of urease-binding polysaccharides is expected to be biocompatible not only with histamine H_2 receptor antagonists but also with other acid suppressors, such as omeprazole, a proton pump inhibitor. As noted earlier, combined omeprazole-dextran sulfate treatment of mice significantly reduced *H. pylori* loads with mouse negativity rates quite similar to those obtained with the famotidine-dextran sulfate combination as shown in Table 2 (Icatlo et al., unpublished). Thus, the differential mechanism of acid suppression by famotidine or omeprazole appears to be less relevant, whereas the resultant periodic shift in the mucus chemical gradient toward the neutral side (presumably affecting the localization of *H. pylori* cells in mucus when these drugs are used in a once-a-day regimen was apparently critical to the enhanced effect observed in vivo.

Using an established mouse model of infection in this study, we demonstrated in controlled experiments that the use of urease-targeted polysaccharides, in combination with a histamine H_2 receptor antagonist, significantly reduced the group mean titer of *H. pylori* in the gastric mucosa of precolonized mice in terms of both mucus-resident and epithelium-adherent populations and eradicated the bacterium in about half of the test mice. This unique drug combination holds potential for incorporation as an adjunct to the standard multidrug therapy for *H. pylori* infection. In particular, application of this novel approach to the clinical setting bears on the overall cost effectiveness of chemotherapy as it may abbreviate the treatment time and aid in the elimination of *H. pylori* strains resistant to certain antimicrobials.

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