Reduced Amoxicillin Uptake into Human Gastric Mucosa when Gastric Juice pH Is High

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Amoxicillin when administered with gastric acid suppressors has been shown to be effective in eradication of *Helicobacter pylori* in 50 to 80% of subjects. The aim of this investigator-blind crossover study was to determine if gastric mucosal amoxicillin uptake was affected by increasing gastric juice pH. Fifteen male subjects (7 *H. pylori* positive and 8 *H. pylori* negative) were randomized to receive 150 mg of ranitidine twice a day, 300 mg of ranitidine twice a day, or no drug for 2 days prior to upper endoscopy. The last dose of ranitidine was given 60 min prior to upper endoscopy, and amoxicillin (500 mg) was given 30 min prior to upper endoscopy. The amoxicillin concentrations in mucosal biopsy samples, gastric juice, and serum were determined by a standard microbiological bioassay technique. Mean amoxicillin levels were greater in samples of antrum, fundus, and duodenum for volunteers who received no ranitidine than in those receiving 300 mg of ranitidine ($P < 0.05$) and those receiving 150 mg of ranitidine ($P < 0.05$ except for fundus). Amoxicillin levels in the antrum, fundus, and duodenum were negatively correlated with gastric juice pH ($P < 0.005$ for antrum; $P < 0.001$ for fundus and duodenum). There was no correlation between gastric juice pH and amoxicillin levels in either gastric juice or serum. The amoxicillin concentration in gastric juice was significantly higher with 300 mg of ranitidine than with no ranitidine ($P < 0.05$). Thus, lower gastric juice pH is associated with a higher rate of mucosal uptake of amoxicillin.

Helicobacter pylori infection is an important pathogenic factor in gastroduodenal diseases, including chronic type B gastritis, duodenal ulcers, gastric ulcers, and gastric adenocarcinoma (7, 13, 20). The bacteria reside in the gastric mucus, glands, and pits (8). *H. pylori* is sensitive in vitro to a large range of antibiotics, with the best monotherapy in vivo achieved by amoxicillin (4, 15–17). However, the rate of eradication of the organism by single antibiotic agents in vivo during clinical trials has been low (10). There is speculation as to the pH at which antibacterial agents would have to act to be most effective in vivo. Recent studies suggest that the addition of an agent used to suppress gastric acid to amoxicillin improves the efficacy of treatment of *H. pylori* gastritis (9, 11, 18). Hentschel et al. (9) reported eradication of *H. pylori* in 89% of subjects treated with 300 mg of ranitidine plus 750 mg of amoxicillin three times a day and metronidazole for 14 days. It has been postulated that an elevated gastric pH produced by gastric acid suppression results in optimization of the antibacterial effect of amoxicillin (21). The effect of gastric acid suppression with H_2 receptor antagonists on the uptake of amoxicillin has yet to be determined. This investigation aimed to determine the effect of increasing gastric juice pH on the uptake of amoxicillin in vivo into the human gastric antrum, fundus, and duodenum. The antisecretory agent used was ranitidine hydrochloride (Zantac), a potent histamine H_2 receptor antagonist.

MATERIALS AND METHODS

Subjects. Fifteen healthy male volunteers between 18 and 30 years of age were entered into the study. Details about age, medical histories, and previous medications were collected. Volunteers were excluded if they were already taking antibiotics or histamine H_2 receptor antagonists, bismuth preparations, antacids, or nonsteroidal anti-inflammatory drugs or if they had previously had gastrointestinal surgery or renal or hepatic dysfunction.

Administration of drugs. The volunteers received each of the following three drug regimens in a randomized order with a 1-week drug-free washout period between each regimen. Volunteers were instructed to take 150 mg of ranitidine twice a day, 300 mg of ranitidine twice a day (before breakfast and dinner), or no medication for 2 days prior to upper endoscopy. One hour prior to upper endoscopy, each volunteer was given the last dose of ranitidine. Volunteers fasted overnight prior to upper endoscopy and on the morning of the endoscopy. Ten milliliters of amoxicillin syrup (Amoxil; SmithKline Beecham) of 250 mg/5 ml was administered to each volunteer half an hour prior to upper endoscopy. Maximal gastric mucosal amoxicillin concentrations have been shown to occur approximately half an hour after oral administration (5).

Endoscopy. At endoscopy, five antral, two fundal, and two duodenal mucosal biopsy specimens were taken from each volunteer with a fiber-optic endoscope (Fujinon UGI FP2) and Fujinon biopsy forceps. Biopsy samples were obtained away from the pool of gastric juice in the fundus with the subjects in the left lateral position. Of the five antral biopsies, three were required to ascertain the presence or absence of *H. pylori*. One antral biopsy sample was used for a rapid urease test, one was placed in 0.25 ml of sterile saline for culture of *H. pylori*, and one was examined histologically as previously described (12). *H. pylori* determination was performed only at the first treatment visit. The two remaining antral biopsy samples along with two fundal and two duodenal biopsy samples were washed in sterile saline, transferred to plastic tubes containing 0.5 ml of 0.1 M phosphate buffer (pH 7), and stored at 4°C for 15 min until assayed. Biopsy samples in buffer were homogenized in sterile glass tissue grinders for 10 min, and the supernatant was removed for assay and protein content determination. The protein content of the biopsy samples was determined with the Coomassie blue dye binding assay (3). Dilutions of 8 mg of bovine serum albumin (Miles, Kaukakee, Ill.) per ml were used to construct a standard curve. The dilutions were 8, 4, 2, 0.5, 0.25, 0.125, 0.0313, and 0.0156 mg/ml. Each specimen which had been previously stored at -20°C was thawed, and 20 µl was added to 3 ml of Bradford reagent (100 mg of Page Blue G-90 [BDH Chemicals, Poole England]) dissolved in 50 ml of 95% ethanol (Ajax) and 100 ml of 85% (wt/vol) phosphoric acid (Ajax), diluted to 1 liter with distilled water. After 10 min, the A_{595} was measured on a spectrophotometer (Spectronic 1201; Milton Roy Co., New York, N.Y.).

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Gastric juice. A sample of gastric juice was collected with a sterile suction trap at endoscopy. The gastric juice was filtered (0.42-μm pore diameter Millipore filter), and the pH was determined with a Radiometer pH meter (PHM82). A 1-ml sample was adjusted to neutral pH with 0.1 M NaOH. A 1/100 dilution of this neutralized gastric juice sample was made with 0.1 M phosphate buffer (pH 7.0) prior to the amoxicillin assay.

Serum samples. Half an hour after administration of amoxicillin syrup, 10 ml of venous blood was obtained from each volunteer. The blood was centrifuged for 10 min (at 3,000 × g), and the serum was collected.

Amoxicillin bioassay. To determine the concentration of amoxicillin in the biopsy, gastric juice, and blood samples, a microbiological agar diffusion technique was employed as previously described (6). The intra-assay coefficient of variation was 10 to 14%, and the interassay coefficient of variation was 9 to 14%. The detection limit of the assay was 0.25 μg/ml. Recovery studies determined that 95% of the amoxicillin could be extracted from the tissue.

Standards. Amoxicillin standards were prepared by dissolving amoxicillin reference powder (Amoxil; SmithKline Beecham) in 0.1 M phosphate buffer (pH 6.0) to form a concentration of 100 mg/liter. A range of standard solutions of amoxicillin from 0.25 to 20 μg/ml was then prepared in 100% pooled human serum for use in the assay of serum specimens. The same concentration range was prepared for use in the assay of biopsy samples, but standards were prepared with 10% pooled human serum in 0.1 M phosphate buffer (pH 7). For gastric juice specimens, the standard concentrations used were 4 to 64 μg/ml in 10% pooled human serum.

Controls. Internal controls were prepared from a separate amoxicillin stock solution as described earlier. For serum and biopsy specimens, the control concentrations were 5 and 1 μg/ml. For gastric juice specimens, the control concentrations were 20 and 5 μg/ml.

Medium. One hundred ninety milliliters of Mueller-Hinton agar (Oxoid) was inoculated with 0.5 ml of *Bacillus subtilis* ATCC spore suspension (Difco) as the test organism, poured into plates (243 by 243 by 18 mm), and allowed to set on a level bench. Wells were cut in the agar with a sterile quarter-inch (0.64-cm) cork borer. Amoxicillin standards and biopsy, serum, and gastric juice samples were added in duplicate in 50-μl aliquots. Plates were incubated at 35°C overnight.

Calculations. The diameters of the zones of inhibition were measured to the nearest 0.05 mm with vernier calipers, and the duplicates were averaged. Concentrations of amoxicillin in gastric biopsy samples were calculated from standard curves as described previously (7). A standard curve was drawn by plotting the $\log_{10}$ of a standard concentration against the zone diameter with semilogarithmic paper. The concentration of each sample was determined from the standard curve. The concentration in each sample was then represented as micrograms per milligram of protein by division of the amoxicillin concentration by the protein content of that sample (for biopsy samples only).

Statistical analysis of data. The results shown are expressed as means ± standard deviations. To test for significant differences between concentrations of amoxicillin after no ranitidine, 150 mg of ranitidine, or 300 mg of ranitidine, an analysis of variance (ANOVA) and post-ANOVA (Tukey's test) were used. The assumptions of the ANOVA were that observations were independent of constant variance and that errors were normally distributed. Spearman's correlation coefficients were calculated to ascertain possible relationships between the amoxicillin concentrations in mucosal biopsy samples and gastric juice pH.

RESULTS

The mean age of the volunteers was 21.9 ± 0.9 years (range, 19 to 30 years), and the mean weight was 66.8 ± 2.7 kg (range, 57 to 92 kg). The volunteers were all healthy with no past history of gastroduodenal disease. Seven subjects were *H. pylori* infected, with both rapid urease and culture test results positive.

Amoxicillin levels in the gastric and duodenal mucosae. Amoxicillin levels in the antrum, fundus, and duodenum after each treatment regimen are shown in Fig. 1. For each type of sample, levels were significantly lower after ranitidine (150 or 300 mg) than with no ranitidine (except for fundal samples after 150 mg of ranitidine).

Amoxicillin levels in gastric juice. Amoxicillin levels in gastric juice half an hour after administration were over 40-fold greater than the levels in the mucosa. The gastric juice levels from subjects administered no ranitidine, 150 mg of ranitidine, or 300 mg of ranitidine were 2,122 ± 316, 1,700 ± 296, and 3,331 ± 316 μg/ml, respectively. The levels after 300 mg of ranitidine (but not after 150 mg of ranitidine) were significantly different from those in the absence of ranitidine (*P* < 0.05; ANOVA and Tukey's test). However, there was no sig-
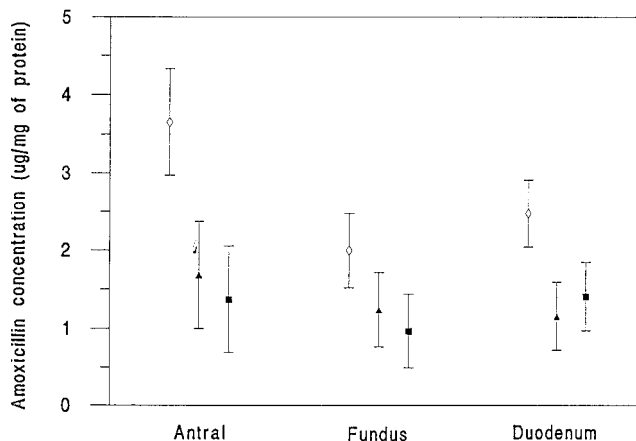

FIG. 1. Mean amoxicillin concentrations (± standard deviations) of subjects who received no ranitidine (◇), 150 mg of ranitidine (▲), and 300 mg of ranitidine (■) as measured in the antrum, fundus, and duodenum.

nificant correlation between gastric juice pH and amoxicillin levels in gastric juice.

Amoxicillin levels in serum. The mean levels of amoxicillin in the serum were similar after no ranitidine, 150 mg of ranitidine, or 300 mg of ranitidine: 2.57 ± 0.83, 2.35 ± 0.71, and 2.38 ± 0.50 μg/ml, respectively. There were no significant differences between these values (ANOVA), and there was no correlation between gastric juice pH and serum amoxicillin levels.

Amoxicillin levels and gastric juice pH. A significant inverse correlation was found between gastric juice pH and amoxicillin levels in the antrum, fundus, and duodenum (Fig. 2, 3, and 4 respectively).

DISCUSSION

This study was designed to determine if changes in gastric juice pH could influence the uptake of amoxicillin into the gastric and duodenal mucosae. Few studies have measured the levels of amoxicillin in the human gastric mucosa after oral amoxicillin administration (5, 15). This study reports for the first time amoxicillin concentrations measured after prior administration of the histamine H_2 receptor antagonist ranitidine.
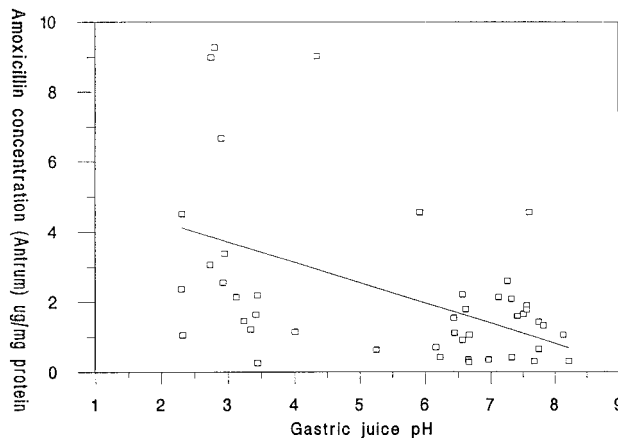

FIG. 2. Negative correlation between gastric juice pH and amoxicillin in the antrum. The correlation was statistically significant (*P* < 0.005).
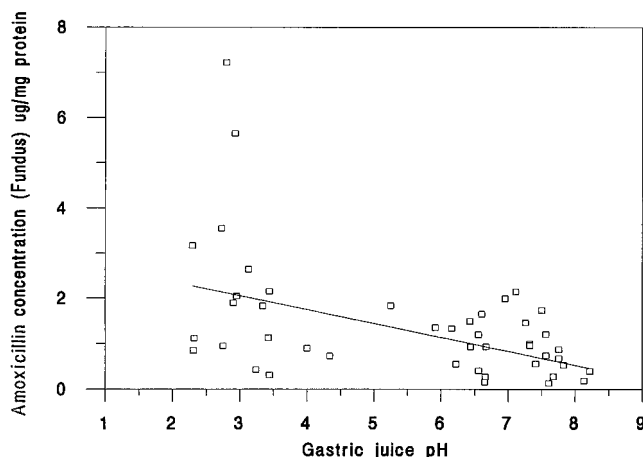
FIG. 3. Negative correlation between amoxicillin concentrations in the fundus and gastric juice pH. The correlation was statistically significant ($P < 0.001$).

Mucosal amoxicillin levels were highest in both antral and fundal areas of the stomach and in the duodenum when no ranitidine was administered (compared with 300 mg of ranitidine twice a day). This is in contrast to results of a study of clindamycin in which a 5.6-fold increase in clindamycin uptake was noted after administration of cimetidine, another H_2 receptor antagonist (24). Clindamycin is a weak base, and increasing intragastric pH would be expected to increase its uptake. Amoxicillin is acid stable and at low pH is less ionized and more active (1, 19). If the pH is increased, it is present as a zwitterion with a low lipid-water partition coefficient. Consequentially, tissue penetration may then decrease (14). This may partially explain the low mucosal amoxicillin levels attained when gastric juice pH was increased.

Amoxicillin uptake into the gastric mucosa is dependent on local penetration from luminal juices (5, 15). In the present study, no significant correlation was found between amoxicillin levels in the gastric juice and its pH, which suggests that the dispersion of amoxicillin in gastric juice is not altered at different pHs. Moreover, after 300 mg of ranitidine, the gastric juice amoxicillin concentration was elevated compared with that in the control, despite the fact that there was less tissue
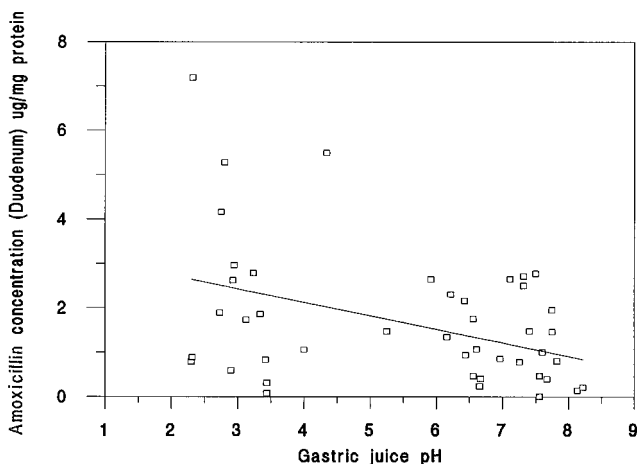

FIG. 4. Negative correlation between gastric juice pH and amoxicillin concentrations in the duodenum. The correlation was statistically significant ($P < 0.05$).

penetration with this dose of ranitidine. It is possible that an increase in gastric juice pH (elicited by ranitidine) brings about some mucosal change that limits amoxicillin uptake into the gastric mucosa. The gastric juice-mucosa interface has not been extensively investigated, and the effect on it of pH changes is not clearly defined.

In this study, amoxicillin suspension (rather than capsules) was used because of previous findings that administration of suspension leads to a relatively uniform and high concentration of amoxicillin in the mucosa (5). However, considerable variability in levels from subject to subject was still noted. Variable gastric emptying may be an important factor which could account for the inter- and intrasubject variability. In this study, attempts to control such variability were undertaken by the administration of drugs at the same time on each day after a defined fasting period.

A considerable discrepancy between the potent activity of amoxicillin against *H. pylori* in vitro and its effects on *H. pylori* in vivo has been noted. The addition of gastric acid suppressers with amoxicillin significantly increases eradication rates (21). However, the findings of the present study with ranitidine (rather than omeprazole) do not support the hypothesis postulated previously that increasing gastric juice pH per se will increase the activity of amoxicillin during treatment of *H. pylori*-associated gastritis (2, 21, 22). Other investigators have suggested that increasing intragastric pH may stop ammonia neutralization (which normally protects *H. pylori* from the acid environment), and this may have a direct antibacterial action (23). It is more likely that gastric acid suppressors may be important as they may interact synergistically with amoxicillin (21, 23).

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