Suppression of Gastric Acid Production by Proton Pump Inhibitor Treatment Facilitates Colonization of the Large Intestine by Vancomycin-Resistant *Enterococcus* spp. and *Klebsiella pneumoniae* in Clindamycin-Treated Mice[∇]

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Proton pump inhibitor treatment of clindamycin-treated mice elevated the gastric pH and facilitated the establishment of colonization of the large intestine by vancomycin-resistant *Enterococcus* spp. (75 to 80%, versus 20 to 25% for saline-treated controls) and *Klebsiella pneumoniae* (90%, versus 30% for saline-treated controls). These findings demonstrate a mechanism by which proton pump inhibitor therapy could contribute to the dissemination of nosocomial pathogens.

Several studies have demonstrated an association between medications that inhibit the production of stomach acid (e.g., proton pump inhibitors and histamine₂ blockers) and health care facility-associated pathogens, including vancomycin-resistant *Enterococcus* (VRE), *Candida* species, extended-spectrum β -lactamase-producing members of the family *Enterobacteriaceae*, and *Clostridium difficile* (1, 4, 11, 14). However, the mechanisms by which acid-suppressive medications may promote these organisms are not completely understood. For example, *C. difficile* spores are not killed by acid, and therefore, it has been suggested that effects other than the suppression of gastric acid may contribute to the association between infection due to this organism and treatment with proton pump inhibitors (10). We have previously shown that some health care facility-associated pathogens, including VRE and *Klebsiella pneumoniae*, are killed by exposure to acidic conditions and that killing may be enhanced by physiological concentrations of nitrite (15). In this study, we used a mouse model to test the hypothesis that proton pump inhibitor treatment facilitates the passage of ingested VRE and *K. pneumoniae* through the stomach, thereby promoting the establishment of colonization of the large intestines. Because the indigenous microflora of the colon inhibits colonization by ingested microorganisms, we postulated that proton pump inhibitor treatment would promote colonization by the pathogens only if proton pump inhibitors were given in combination with antibiotic treatment.

The experimental protocol was approved by the Cleveland Veterans Affairs Medical Center's Animal Research Committee. Female CF-1 mice (Harlan Sprague-Dawley, Indianapolis, IN) weighing 25 to 30 g were used in all experiments. Initial experiments were conducted to examine the effect of panto-

prazole treatment on the establishment of colonization by the pathogens. Mice were housed in individual cages with plastic filter tops to prevent cross-contamination among animals. One VanB-type VRE isolate (isolate C68), one VanA-type VRE isolate (isolate C38), and one extended-spectrum beta-lactamase-producing *K. pneumoniae* isolate (isolate P62) were studied (5, 9). Mice received subcutaneous pantoprazole (0.4 mg in 0.2 ml) or normal saline twice daily for 2 days. Food was removed 4 h before the final pantoprazole or saline doses, which were given in combination with one dose of subcutaneous clindamycin (4 mg); 2 h later the mice received 100 CFU of one of the pathogens in 50 μ l of normal saline by esophageal inoculation by use of a stainless steel gavage needle (Perfektum; Popper & Sons, New Hyde Park, NY). The rationale for administration of the pathogens in a small volume in a non-buffered solution was to limit rapid transit through the stomach and to avoid buffering of gastric acid; previous studies indicated that 100% of clindamycin-treated mice would develop intestinal colonization when the pathogens were administered in a large volume (0.5 ml) of buffered solution (5, 9). Stool pellets were collected at the baseline and 1 and 3 days after inoculation of the pathogens. The densities of the pathogens were measured as described previously (5, 9). If no organisms were detected, the lower limit of detection (~ 2.5 log/g) was assigned. For the purposes of the study, the presence of a detectable level of the pathogens on either day 1 or day 3 was considered to represent colonization. The experiments were performed with five mice per group and were repeated four times for VRE strain C68 ($n = 20$ total mice per group), three times for VRE strain C38 ($n = 15$ total mice per group), and two times for *K. pneumoniae* strain P62 ($n = 10$ mice per group).

To assess gastric acidity, mice ($n = 7$ mice per group) that were treated with pantoprazole or saline as described above were killed, the stomach was removed, and the pH of the stomach contents was measured. To assess whether pantoprazole promoted colonization in the absence of antibiotic treat-

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ment, mice ($n = 10$ per group) received pantoprazole and pathogen inoculation as described above, but clindamycin was not administered.

Additional experiments were conducted to examine whether proton pump inhibitor treatment promoted gastric overgrowth of the pathogens in mice with high-density intestinal colonization. To establish colonization, mice received subcutaneous clindamycin (0.4 mg) once in combination with the esophageal inoculation of 10^6 CFU of VRE strain C68 ($N = 14$ total mice) or *K. pneumoniae* strain P62 ($N = 10$ total mice) in 0.5 ml of phosphate-buffered saline (isolate C38 was not studied). The mice then received subcutaneous pantoprazole or saline, as described above, twice daily for 2 days. The mice were then killed, the stomach and cecum were sequentially removed, and quantitative cultures for the pathogens were performed as described above.

Data from replicate experiments were pooled for analysis. Chi-square tests were performed to compare the number of mice in each group developing detectable levels of pathogens in their stool. Student's t test was used to compare the pH values and the densities of pathogens in the stomach contents among the treatment groups. Computations were performed with Stata software (version 6.0; StataCorp, College Station, TX). A P value of <0.05 was considered significant.

Pantoprazole-treated mice had a significantly elevated gastric pH in comparison to the gastric pH of the controls (mean $\pm$ standard deviation [SD], 4.95 ± 0.71 and 1.94 ± 0.67 , respectively; $P < 0.001$). The pantoprazole-treated mice that received an esophageal inoculation of the pathogens without concurrent clindamycin treatment did not develop increased levels of pathogens in their stools in comparison to the levels in the saline-treated controls; neither group had detectable pathogens in their stools by day 3 after inoculation (data not shown). As shown in Fig. 1, pantoprazole treatment, when it was administered in combination with clindamycin, resulted in an increased frequency of the establishment of colonization of the large intestines with each pathogen in comparison to the frequency for the saline-treated controls (75% [15 of 20] for the controls versus 25% [5 of 20] for VRE C68, 80% [12 of 15] for the controls versus 20% [3 of 15] for VRE C38, and 90% for the controls [9 of 10] versus 30% [3 of 10] for *K. pneumoniae* P62; $P < 0.01$ for each comparison). For the mice that did develop detectable levels of pathogens in their stools, 100% had greater than $4 \log_{10}$ CFU/g, and much higher densities of the pathogens were typically present.

In mice with established high-density intestinal colonization with VRE strain C68 and *K. pneumoniae* strain P62, pantoprazole treatment promoted overgrowth in the stomach in comparison to the growth in the saline-treated controls (mean $\pm$ SD, $5.64 \pm 1.5 \log_{10}$ CFU/g for the controls versus $3.09 \pm 0.89 \log_{10}$ CFU/g for C68 [$P < 0.001$]; $5.46 \pm 2.1 \log_{10}$ CFU/g for the controls versus $2.62 \pm 0.63 \log_{10}$ CFU/g for P62 [$P = 0.019$]). Low levels of the pathogens (range, 2.8 to $4.7 \log_{10}$ CFU/g) were detectable in the stomach contents of 5 of 14 (36%) saline-treated control mice with high-density intestinal colonization with VRE strain C68, whereas VRE were not detectable in the stomach contents of the other 9 control mice. Pathogens were detectable in the stomach contents ($3.6 \log_{10}$ CFU/g) of only 1 of 10 (10%) saline-treated control mice with high-density colonization with *K. pneumoniae* P62. The density

FIG. 1. Establishment of colonization of the large intestine after esophageal inoculation of 100 CFU of vancomycin-resistant enterococci (A and B) and extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* (C) in mice receiving pantoprazole treatment versus establishment of colonization in controls receiving saline. Data for individual mice and mean values (horizontal lines) are shown.

of the pathogens in the cecal contents did not differ significantly between the saline-treated controls and the pantoprazole-treated mice (10.0 ± 0.58 versus $9.9 \pm 0.72 \log_{10}$ CFU/g for VRE C68 [$P = 0.79$]; 10.7 ± 0.38 versus $10.6 \pm 0.49 \log_{10}$ CFU/g for *K. pneumoniae* P62 [$P = 0.73$]).

In summary, we found that proton pump inhibitor treatment elevated the gastric pH and facilitated the colonization of the large intestine by VRE and *K. pneumoniae* in mice. Proton pump inhibitor treatment facilitated fecal colonization only when it was given in combination with clindamycin, suggesting that these agents may not independently promote colonization by pathogens. Rather, suppression of gastric acid may act as a cofactor that promotes colonization only in combination with other factors that disrupt the colonic microflora. Our results are consistent with those of clinical studies that have demonstrated a significant association between proton pump inhibitors (and antacids) and colonization or infection due to VRE (3, 8, 11). We are not aware of any previous studies that have associated proton pump inhibitors with stool carriage or infection with *K. pneumoniae* or other antibiotic-resistant gram-negative bacilli. However, proton pump inhibitor therapy has been associated with the overgrowth of gram-negative bacilli in the stomach (17). In addition, histamine₂ blocker treatment has been associated with colonization or infection with extended-spectrum β -lactamase-producing *Enterobacteriaceae* (1).

If our findings are applicable to patients, they have important clinical implications because acid-suppressive medications are widely used in health care facilities. In a survey of hospitalized patients, 54% were receiving acid-suppressive therapy, and 65% of the prescriptions were not indicated (12). Similarly, in a point-prevalence study conducted in our institution, we found that 61% of hospitalized patients were being treated with acid-suppressive medications, but only about half had a clear indication for therapy; more than 90% of the acid-suppressive medications were proton pump inhibitors (6). These data suggest that significant reductions in the use of acid-suppressive medications could easily be achieved without adverse effects on patients.

Our study has several limitations. First, our findings may overestimate the protection provided by gastric acid in patients because we challenged only fasting mice with the pathogens. The pH of the stomach rises significantly during and for 1 to 2 h after meals (7). Second, other factors such as the use of percutaneous enteral feeding may alter the gastric microflora of patients, despite the presence of a normal gastric acidity (13). Third, we tested only a relatively small inoculum (100 CFU) of the pathogens. A previous study found that healthy humans ingesting 100 CFU of *Pseudomonas aeruginosa* did not develop detectable levels of organisms in their stools; however, larger inocula ($\geq 10^6$ CFU) resulted in shedding in the stool for 1 to 6 days (2). Although foods such as salads may contain relatively large numbers of *P. aeruginosa* or *Enterobacteriaceae* isolates (10^3 to 10^4 CFU per serving) (16), we suspect that patients typically ingest relatively small numbers of nosocomial pathogens. Fourth, we challenged the mice with a single inoculum of the pathogens, whereas patients might be exposed to

repeated ingestions of pathogens. Finally, we administered only a single dose of clindamycin, whereas patients typically receive longer courses of treatment. However, we have previously shown that even short courses of antibiotics such as clindamycin may cause a prolonged disruption of the intestinal microflora (6). Despite these limitations, our findings suggest a potential mechanism to explain the associations between the use of acid-suppressive medications and health care facility-associated pathogens that have been observed. Future studies with hospitalized patients are indicated to determine the applicability of our findings to clinical situations.

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