Suppression of Gastric Acid Secretion in Patients With Gastroesophageal Reflux Disease Results in Gastric Bacterial Overgrowth and Deconjugation of Bile Acids

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The aim of this study was to test the hypothesis that gastric bacterial overgrowth is a side effect of acid suppression therapy in patients with gastroesophageal reflux disease (GERD) and that the bacteria-contaminated gastric milieu is responsible for an increased amount of deconjugated bile acids. Thirty patients with GERD who were treated with 40 mg of omeprazole for at least 3 months and 10 patients with GERD who were off medication for at least 2 weeks were studied. At the time of upper endoscopy, 10 ml of gastric fluid was aspirated and analyzed for bacterial growth and bile acids. Bacterial overgrowth was defined by the presence of more than 1000 bacteria/ml. Bile acids were quantified via high-performance liquid chromatography. Eleven of the 30 patients taking omeprazole had bacterial overgrowth compared to one of the 10 control patients. The median pH in the bacteria-positive patients was 5.3 compared to 2.6 in those who were free of bacteria and 3.5 in the control patients who were off medication. Bacterial overgrowth only occurred when the pH was >3.8. The ratio of conjugated to unconjugated bile acids changed from 4:1 in the patients without bacterial overgrowth to 1:3 in those with bacterial growth greater than 1000/ml. Proton pump inhibitor therapy in patients with GERD results in a high prevalence of gastric bacterial overgrowth. The presence of bacterial overgrowth markedly increases the concentration of unconjugated bile acids. These findings may have implications in the pathophysiology of gastroesophageal mucosal injury. (J GASTROINTEST SURG 2000;4:50-54.)

KEY WORDS: Bacterial overgrowth, omeprazole, deconjugation

The primary treatment of gastroesophageal reflux disease (GERD) is acid suppression therapy using proton pump inhibitors. A significant proportion of patients require chronic or lifelong therapy for continuous relief of symptoms.[1] Although acid suppression therapy can relieve the symptoms of GERD and heal esophagitis, it also allows bacterial overgrowth in the normally sterile stomach.[2-6] At a normal resting gastric pH <3, ingested bacteria are destroyed within 10 minutes. In contrast, in gastric juice with a resting pH >4, bacterial growth is feasible[7] and when excessive can cause malabsorption,[8] nosocomial pneumonia,[9] or the formation of carcinogenic N-nitroso compounds.[10]

Bile acids have been shown to be an important pathophysiologic factor promoting esophageal mucosal injury.[11-13] In the human proximal gastrointestinal tract, they occur overwhelmingly in their conjugated form, bound to the amino acids glycine and taurine. Deconjugated or free bile acids, formed in the presence of colonic bacteria, are found largely in the distal gastrointestinal tract. Deconjugated bile acids have been shown to be more toxic to gastric mucosa and squamous epithelium than their conjugated counterparts and have significantly different physiochemical properties.[14] We hypothesize that gastric bacterial overgrowth in patients with GERD receiving acid suppression therapy will influence the conjugation

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status of bile acids within the refluxed gastric juice. If this were to occur, then refluxed gastric juice containing unconjugated bile acids would be expected to be highly toxic to the esophageal mucosa. This would result in continuous mucosal injury while the patient remained symptomatically improved.

PATIENTS AND METHODS

Study Population

The study population consisted of 30 patients with symptoms of GERD and increased esophageal acid exposure on 24-hour pH monitoring. There were 12 women and 18 men who had a median age of 54.4 years (range 23 to 73 years). All patients were treated with 40 mg of omeprazole for least 3 months prior to the examination of their gastric juice. The control group consisted of 10 patients, five women and 5 men, who also had symptoms of GERD and increased esophageal acid exposure. They were prohibited from taking any acid–suppressive medications for at least 2 weeks prior to the examination of their gastric juice.

Endoscopy and Gastric Fluid Aspiration

All patients underwent upper endoscopy after an overnight fast using a disinfected endoscope. Biopsy specimens were obtained from the antrum, cardia, and esophagus. *Helicobacter pylori* infection was confirmed by a positive Giemsa stain.

At the time of endoscopy 10 ml of gastric juice was aspirated with a sterile catheter passed through the biopsy channel of the endoscope (Washing pipe, Olympus, Melville, N.Y.). Five milliliters of the aspirate was injected into a transport tube for anaerobes (Anaerobe Systems, San Jose, Calif.) and cultured within 1 hour. The remaining 5 ml was stored at −20° C and subsequently assayed for bile acids using high-performance liquid chromatography (HPLC). The pH of the aspirated fluid was assessed at the time of endoscopy with a glass probe calibrated with standard solutions of pH 1, pH 4, and pH 7.

Bacterial Cultures

Bacteria were cultured using conventional plating methodology. All isolated anaerobic and aerobic organisms were identified according to standard procedures.[15] Aliquots of 0.01 ml in a 1:100 dilution were plated to establish colonies. For the identification of anerobes and aerobes, blood agar, phenylethyl-alcohol agar, McConkey agar, *Brucella* blood agar, and *Bacteroides fragilis* bile-esculin were used. Bacterial overgrowth was defined by more than 1000 bacteria/ml.

High-Performance Liquid Chromatography

Bile acids were quantified via a recently published modified high-performance liquid chromatography (HPLC) method.[16,17] Briefly, in addition to conventional HPLC technology, a postcolumn derivation step was added to improve the sensitivity and specificity. In this step the individual bile acids were reacted with the enzyme 3-α-hydroxy steroid dehydrogenase using nicotamide adenine dinucleotide (NAD) as cofactor. This resulted in the fluorescent species NADH (nicotamide adenine dinucleotide, reduced form) as its end product allowing the bile acids to be quantified using a Jasco 821-FP fluorescence spectrophotometric detector (Ciba Corning Diagnostics, Halstead, U.K.).

Statistical Analysis

Data for pH values and bile acid concentrations are expressed as medians and interquartile ranges. The overall differences in pH values and bile acid concentrations between the three groups were assessed using the Kruskal-Wallis test. Differences between two groups were analyzed with the Mann-Whitney U test. A P value <0.05 was considered to be statistically significant.

RESULTS

Eleven (37%) of the 30 patients taking proton pump inhibitors were found to having bacterial overgrowth. This compares to only one patient in the control group not taking acid-suppressive medication. When the patients taking proton pump inhibitors were divided into patients with and without bacterial overgrowth, the median pH of the bacteria-positive patients was significantly different from those who were free of bacteria, or the control group not taking acid-suppressive medication (Fig. 1). There was no significant difference in the prevalence of antral *Helicobacter pylori* infection among the groups.

One patient in the control group not taking medication had a gastric pH of 5.7 and bacterial overgrowth. Patients in either group with a gastric pH <3.8 were free of bacterial overgrowth. Table I shows the pH and the bacteria species found in the gastric aspirates.

Median concentrations for all bile salts varied from 68 to 92 μmol/L and did not differ among the three groups (Table II). Patients with bacterial overgrowth had significantly lower concentrations of taurine and glycine conjugates and a reversed ratio of a conjugated:unconjugated (1:3) bile salts when compared to patients on proton pump inhibitors without bacterial overgrowth (3:1) and the control patients off medication (4:1) (Fig. 2). This was statistically significant (P <0.001).

Table I. Gastric pH and bacterial flora in patients with bacterial overgrowth

Medication	pH	Species (>1000/ml)
No	5.7	*Streptococcus*
Yes	3.8	Yeast, *Lactobacillus bifidus*
Yes	6.2	*E. coli*, *Streptococcus*
Yes	5.3	*Neisseria*, *Streptococcus*, *Staphylococcus*
Yes	3.9	*Streptococcus*, *Microphilis*
Yes	6.9	*Neisseria*, *Lactobacillus bifidus*, *Streptococcus*
Yes	5.9	*Streptococcus*
Yes	4.3	*E. coli*, *Aeromonas*, *Candida albicans*
Yes	5.3	*Streptococcus*, *Neisseria*, *Staphylococcus*
Yes	4.2	*Streptococcus*, *Neisseria*
Yes	7.9	*E. coli*, *Staphylococcus*, *Streptococcus*
Yes	5.5	*Streptococcus*

Table II. Median bile acid concentrations

Bile acid	No medication (n = 10)	Medication with no bacteria (n = 19)	Medication with bacterial overgrowth (n = 11)	*P* value
Taurine conjugates	21.5 [6-68]	16 [9-55]	*1.5 [0-6]	*<0.01 vs. bacteria, control
Glycine conjugates	38 [0-138]	41 [7-76]	*7 [0-13]	*<0.01 vs. bacteria, control
Unconjugates	22 [2-34]	22 [2-34]	21 [12-65]	NS
TOTAL	92.5 [0-327.5]	68 [22-154]	69 [0-256]	NS
Ratio conjugated:unconjugated	3:1	4:1	*1:3	*<0.01 vs. bacteria, control

Values for bile acid conjugates are in μmol and [interquartile ranges]; group comparison by Mann-Whitney U test (NS = not significant).

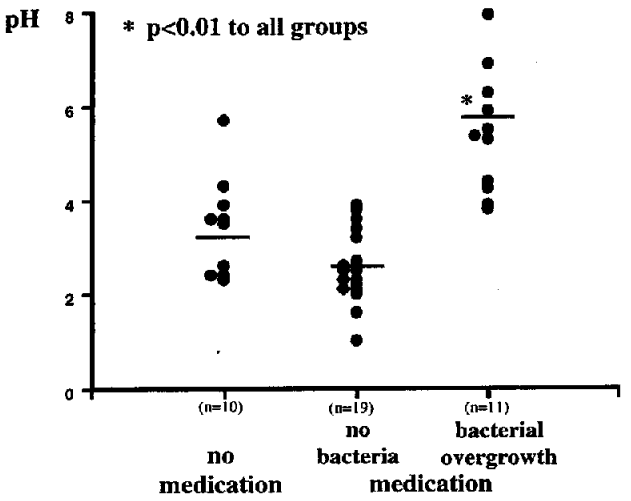

Fig. 1. pH of individual patients off acid suppression therapy (no medication), patients taking omeprazole with no bacteria, and patients taking omeprazole with bacterial overgrowth.

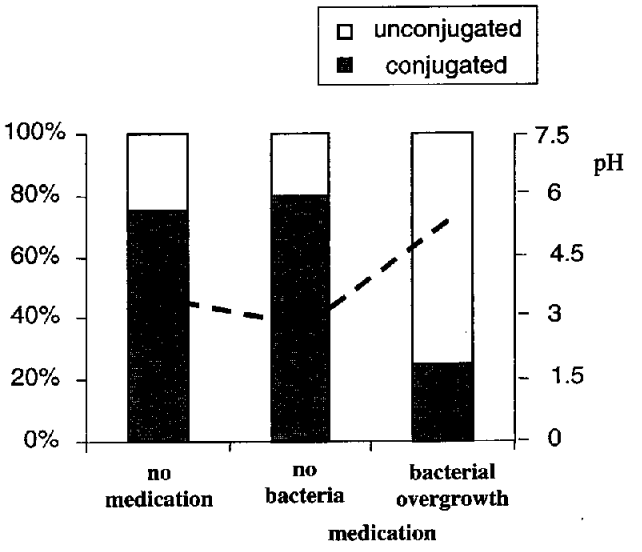

Fig. 2. Ratio of conjugated to unconjugated bile acids (bars) in relation to the median pH (hatched line) in the three groups. Off acid-suppression therapy (no medication), taking omeprazole with no bacteria, and taking omeprazole with bacterial overgrowth.

DISCUSSION

The side effects of acid suppression therapy, including the possibility of bacterial overgrowth, are well known.[2,3,6] Increased numbers of bacteria have been found in gastric aspirates of patients treated with acid suppression therapy for ulcer disease, whose pH was maintained at >4. At pH values <4, bacteria do not survive longer than 10 minutes.[7] Our results confirm this fact. The pH cut-off value for the presence of a significant number of bacteria in the present study was 3.8. No bacterial overgrowth was observed below pH 3.8.

Most bacterial species present in the aspirate of patients taking omeprazole are capable of deconjugating bile acids. Bile acid deconjugation ability has been demonstrated for *Lactobacillus*, *Streptococcus*, *Staphylococcus*, *Neisseria*, *Aeromonas*, and others. Bacteria found in our study not capable of deconjugation were *Escherichia coli* and *Candida albicans*. It is important to note that deconjugated as well as secondary bile acids have been shown to be more injurious to the gastric and esophageal mucosa than their conjugated counterparts.[18] Armstrong et al.[14] have shown, using an ex vivo gastric chamber model, that unconjugated bile acids produced greater injury (higher potential difference changes) than their conjugated counterparts. The findings were pH dependent, reflecting the pKa of the particular bile acid used.

We have shown that proton pump inhibitor therapy in patients with GERD results not only in a high prevalence of gastric bacterial overgrowth, but that the presence of bacterial overgrowth markedly increases the concentration of unconjugated bile acids, reversing the ratio of conjugated to unconjugated bile acids present in the foregut. Nehra et al.[17] have recently shown that unconjugated bile acids reflux into the esophagus. Using an improved sampling technique, they were able to show that a significant proportion of bile acids aspirated from the esophagus of patients with GERD are unconjugated. This was particularly true in the aspirates of patients with erosive esophagitis, stricture, or Barrett's esophagus.

Recent studies have been aimed at answering the question of whether bile acids have an effect on the molecular level. In vivo studies have shown that bile acids act as promoters of gastrointestinal cancer and that they enhance cell transformation in vitro. They are capable of activating protein kinase C[19] and inducing AP-1–mediated transcription.[20] Zhang et al.[21] and Theisen et al.[22] demonstrated that dihydroxy bile acids activate the transcription of the cyclooxgenase-2 gene.

For soluble bile acids to remain innocuous in a patient with chronic reflux managed by acid suppression therapy, they must remain completely ionized. This requires that the gastric pH be maintained at a level of 6 or 7, 24 hours a day, 7 days a week for the patient's lifetime. This is not only impractical but likely impossible unless very high doses of medication are used. Insufficient medication will allow the pH to drift down to 4 or 5 and cause cellular mucosal damage to occur while the patient remains relatively asymptomatic. The injury can result in mild to erosive esophagitis, ulceration, stricture, or the development of a columnar-lined esophagus with intestinal metaplasia, that is, Barrett's esophagus. The incidence of the latter has increased progressively since 1986 and initiates a sequence of mucosal changes that can ultimately lead to esophageal adenocarcinoma. It is unknown whether the movement toward cancer is due to mitogenesis secondary to chronic mucosal injury or mutagenesis from exposure to a direct mutagen. If bile salts are demonstrated to contribute to the development of malignancy, then early surgical intervention to reestablish an antireflux barrier should be encouraged.

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