

Role of bacterial overgrowth in the stomach as an additional risk factor for gastritis

Gregory Naylor MRCP, Anthony Axon MD FRCP

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Gastric bacteria can either be ingested or ascend from the distal bowel; however, their survival is usually limited by gastric acidity and motility. A reduction in gastric acid can result in bacterial overgrowth in the stomach and proximal small bowel, and the number of organisms rises as the intragastric pH rises.

The increased risk of noncardia gastric cancer seen in patients with hypochlorhydria may be explained by an excess of nitrites and N-nitroso compounds (NOCs). These compounds are found in the diet of populations with a high gastric cancer risk, but can also be produced by the organisms that exist in the hypochlorhydria stomach. It has long been hypothesized that nitrites and NOCs act as one of the triggers in the atrophy-metaplasia-dysplasia-carcinoma path. However, although indirect data have linked the premalignant changes of metaplasia and dysplasia to NOCs, direct measurement of gastric nitrites and NOCs has not confirmed such a link.

The role of *Helicobacter pylori* in bacterial overgrowth is mainly as a cause of hypochlorhydria resulting from atrophic gastritis, leading to a reduction in the parietal cell mass.

Acid-suppressing drugs can result in bacterial overgrowth and increased nitrites and NOCs, although there is no current evidence for an increased risk of gastric cancer in patients taking them. One explanation is that the stomach appears to be colonized by different organisms than those in patients with hypochlorhydria for other reasons. There is some evidence that bacterial overgrowth per se can cause gastric inflammation in mice; however, although in humans the degree of gastric inflammation is greater when overgrowth is more prominent this may simply reflect the greater degree of hypochlorhydria in patients with a more severe *H pylori*-induced inflammation.

Key Words: Bacterial overgrowth; Gastritis; Hypochlorhydria; N-nitroso compounds.

Bacterial colonization of the upper gastrointestinal tract is usually prevented by a combination of acid secretion and intestinal motility. The upper gastrointestinal tract normally has less than 10^4 colony forming units (CFU)/mL and 'bacterial overgrowth' has been defined as counts of more than 10^5 CFU/mL (1).

There are three possible sources of bacteria in the gastric lumen. They can be ingested with food, originate from the oropharyngeal cavity, or migrate from the small or large bowel

Rôle de la prolifération bactérienne dans l'estomac : facteur de risque supplémentaire de gastrite

La présence de bactéries dans l'estomac peut s'expliquer par leur ingestion ou leur remontée à partir de l'intestin distal, mais leur survie est généralement limitée par l'acidité ou la motilité gastriques. Toutefois, une diminution de l'acidité gastrique peut entraîner une prolifération de bactéries dans l'estomac et l'intestin grêle proximal, et le nombre de micro-organismes croît à mesure que s'élève le pH intragastrique.

L'augmentation du risque de cancer de l'estomac épargnant le cardia observé chez les patients atteints d'hypochlorhydrie peut s'expliquer par un surplus de nitrites et de composés N-nitroso (CNO). Ces composés se trouvent dans l'alimentation des populations ayant un risque élevé de cancer de l'estomac, mais ils peuvent également être produits par des micro-organismes qui survivent en milieu hypochlorhydrique. On croit, depuis longtemps, que les nitrites et les CNO agissent comme facteurs déclenchants dans la voie atrophie-métaplasie-dysplasie-carcinome. Toutefois, même si des données indirectes ont établi des liens entre les stades précancéreux que sont la métaplasie et la dysplasie, et ces composés, les mesures directes de nitrites et de CNO dans l'estomac, elles, n'ont pas permis d'établir ce type de lien.

Le rôle d'*Helicobacter pylori* dans la prolifération bactérienne constitue une des principales causes d'hypochlorhydrie attribuable à la gastrite atrophique, qui aboutit à une diminution de la masse cellulaire pariétale.

Les antiacides peuvent engendrer une prolifération bactérienne et accroître la présence de nitrites et de CNO, mais aucune donnée jusqu'à maintenant ne fait état d'un risque accru de cancer de l'estomac chez les patients qui en font usage. Une des explications serait que l'estomac semble colonisé par des germes différents de ceux observés chez les patients atteints d'hypochlorhydrie pour d'autres raisons. D'après certaines données, la prolifération bactérienne en soi peut causer une inflammation de l'estomac chez les souris; cependant, même si chez les humains un degré plus élevé d'inflammation de l'estomac est associé à une prolifération plus marquée, cela peut n'être que le reflet du degré supérieur d'hypochlorhydrie chez les patients souffrant d'une inflammation plus intense, causée par *H pylori*.

and enter the stomach by enterogastric reflux. There is a large variation in the ability of different organisms to survive in the acidic environment of the stomach (2). Most enteric pathogens have the ability to survive in an acidic environment to some extent. *Helicobacter pylori* (3) occupies a specific niche because it is able to colonize the stomach by using urease to produce an alkaline buffer zone of ammonia around itself. *Yersinia enterocolitica* also possesses a urease gene and can increase urease production as the pH decreases (4). *Escherichia*

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Centre for Digestive Diseases, Department of Gastroenterology, The General Infirmary at Leeds, Leeds, United Kingdom

Correspondence: Dr Anthony Axon, Centre for Digestive Diseases, Department of Gastroenterology, Room 190A, Clarendon Wing, The General Infirmary at Leeds, Great George Street, Leeds, LS1 3EX, United Kingdom. Telephone +44-113-392-2125, fax +44-113-392-6968, e-mail anthony.axon@leedsth.nhs.uk

TABLE 1
Studies of bacterial overgrowth, nitrite increase and nitrosation following treatment with acid-inhibitory drugs

Year (reference)	Number in study	Acid-inhibitory drug	Bacteria in gastric juice	Bacteriology test	Nitrite	Nitrosamine
1980 (11)	31	Cimetidine	Increase	Culture	NE	NE
1981 (48)	407	Cimetidine	Increase	Culture	Increase	Increase
1982 (44)	23	Cimetidine 1 g daily	Increase	Culture	Increase	Increase
1982 (50)	8	Cimetidine	Increase (resembling mouth flora)	Culture	NE	NE
1984 (51)	30	Cimetidine 400 mg BD	No evidence of bacterial overgrowth	H ₂ breath test	NE	NE
1984 (52)	4	Ranitidine	Increase (not nitrate reducing)	Culture	No increase	No increase
1984 (12)	10	Omeprazole 30 mg daily	Increase	Culture	Increase	Increase
1994 (53)	14	Omeprazole 20 mg daily	Increase	Culture	Increase	Increase
1996 (14)	47	Cimetidine 800 mg or omeprazole 20 mg daily	Increase in bacteria in gastric juice in 53% omeprazole, 17% cimetidine	Culture	No increase	No increase
1996 (54)	73	Cimetidine	Increase	Culture	NE	NE
2000 (55)	14	Omeprazole 20 mg daily	Increase	Culture	Increase	No increase
2000 (31)	29	Omeprazole	Increase	Culture	Increase	NE

BD Twice daily; NE Not examined

coli and *Shigella flexneri* can both survive exposure to a pH of 2.0 to 2.5. *Salmonella typhimurium* will not normally tolerate a pH of 3 but is able to mount an acid tolerance response that allows it to adapt and multiply at a lower pH (5). Differences in the ability to cope with an acidic environment may account for the infective dose of such enteric pathogens as *S typhimurium* being 10⁵ organisms while *S flexneri* may only need 10 organisms to be ingested. As the pH rises, so does the number of organisms. Gastric colonization is proportional to both the pH and the time spent above a pH of 3.8 (6,7).

Bacterial overgrowth is associated with an increased risk of noncardia gastric cancer as seen in patients with hypochlorhydria due to pernicious anemia (8), atrophic gastritis (9) or partial gastrectomy (10). The principal hypothesis of carcinogenesis is based on evidence that bacterial overgrowth results in the production of nitrite from nitrate with subsequent production of carcinogenic N-nitroso compounds (NOCs). The purpose of the present review is to explore the relationship between bacterial overgrowth and gastric mucosal inflammation.

CAUSES OF BACTERIAL OVERGROWTH

Reduction in gastric acid from any cause may result in bacterial overgrowth in the stomach and the small intestine. Autoimmune gastritis resulting from the production of parietal cell antibodies in pernicious anemia is the classical natural cause. Distal gastrectomy (removing the gastrin-producing G cells of the antrum) can also result in bacterial overgrowth. The introduction of pharmacological acid suppression, initially with H₂ receptor antagonists (11) and more recently with proton pump inhibitors (PPIs) (12), induces bacterial colonization of the stomach (Table 1). However, chronic infection with *H pylori* resulting in gastric atrophy and loss of parietal cell mass is the most common cause of hypochlorhydria and/or achlorhydria worldwide.

There appears to be a difference between the organisms found in the stomachs of patients with reduced acid due to autoimmune gastritis compared with those taking acid suppressing medication. In the former, coliforms predominate, while in the latter, Gram-positive organisms are found. The reasons for this are not clear but could relate to the shorter history of hypochlorhydria in acid suppression leading to colonization by organisms swallowed from the oropharynx (13,14).

H pylori status also appears to affect the degree of bacterial colonization (of non-*Helicobacter* species), especially in patients taking a PPI. *H pylori*-infected patients taking a PPI show a greater degree of colonization than do patients who are *H pylori*-negative. This is discussed in detail below.

CONSEQUENCES OF GASTRIC COLONIZATION

Enhanced nitrite production

Nitrites are required for the production of NOCs and can originate from three potential sources. They can be found in many foods such as cured meats, baked foods and cereals, while buccal and gastric bacteria can also convert dietary and/or salivary nitrate to nitrite. Twenty-five per cent of absorbed nitrate is secreted in the saliva and 40% of this is reduced to nitrite in the mouth. This appears to be due to reducing bacteria found in deep clefts at the back of the tongue. Swallowed nitrates can also be reduced to nitrites in the gastric lumen by colonizing bacteria. As the pH rises, the role of gastric bacteria as nitrite producers becomes more important, contributing 90% of nitrite load in a hypochlorhydria patient (15), and nitrite levels in gastric juice are also increased in these patients (16). Studies measuring gastric nitrite indirectly suggested a link between high concentrations of nitrite in gastric juice with an increased risk of gastric metaplasia, dysplasia and carcinoma, although this has not actually been confirmed by direct measurement (17). However, the persistence of nitrites in the stomach is dependent on the intragastric pH and the presence of ascorbic acid. At an acid pH and with ascorbic acid present, nitrites are rapidly reduced to nitric oxide, which can be detected in gas from the stomachs of individuals with normal acid secretion (18). Ascorbic acid is actively secreted by healthy gastric mucosa; however, its secretion is reduced in hypochlorhydric gastric juice. Hence, nitrites will persist because both of the factors needed for their conversion to nitric oxide (ascorbic acid and gastric acid) are decreased.

Production of NOCs

NOCs are composed of nitrosamides (R₁NNO-COR₂) and nitrosamines (R₁NNOR₂). They are recognized carcinogens in animals and can induce gastric cancer in rodents. They can be formed in the gastric lumen from the nitrosation of primary, secondary and tertiary amines, amino acids, peptides, amides, imides, bile acids, guanidines and urea.

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Correa's hypothesis of gastric carcinogenesis was based on epidemiological studies identifying achlorhydria and/or hypochlorhydria (19,20), diets high in nitrate or nitrite and low in vitamin C (21-26), and *H pylori* infection (27,28) as risk factors for cancer of the mid and distal stomach.

***H pylori* colonization and bacterial overgrowth**

The role of *H pylori* infection in bacterial overgrowth is complex. Acid suppression allows *H pylori* gastritis to spread from the antrum to the corpus. Inflammation of the corpus reduces parietal cell function further and can eventually lead to atrophy of the corpus mucosa and a permanent reduction in acid-producing capability or even achlorhydria. Mowat et al (29) studied the effect of *H pylori* status on pH and bacterial overgrowth during omeprazole treatment in 29 healthy volunteers. They simultaneously studied the effect of *H pylori* status on gastric ascorbic acid and nitrite levels during omeprazole treatment in a further 22 healthy volunteers. They concluded that acid suppression is more marked, bacterial overgrowth is greater, postmeal nitrite levels are higher and ascorbic acid levels are lower in *H pylori*-positive compared with negative patients. They found predominantly oropharyngeal organisms, some of which were capable of reducing nitrate to nitrite and of synthesizing NOCs.

H pylori infection can result in atrophic gastritis with an increased risk of gastric cancer. Whether this is due to a direct effect of the organism, a result of NOCs from bacterial overgrowth or a combination of the two is not yet known. *H pylori* infection has, however, been shown to enhance the carcinogenicity of nitrosamines in rodent models (30). Given the combination of bacterial overgrowth and gastric inflammation present in the stomach of *H pylori*-positive patients taking PPIs, there is some concern about the long term risks of gastric cancer, which are discussed below.

There does not appear to be a link between NOCs and pre-malignant changes in the gastric mucosa. Intestinal metaplasia is a precursor for gastric cancer and is known to be associated with *H pylori* infection, low ascorbic acid levels and bile reflux. However, intestinal metaplasia is not associated with elevated gastric nitrite or total NOC (17) and, at present, it appears that NOCs are pure carcinogens and do not contribute to 'precancerous' mucosal changes.

Acetaldehyde production and bacterial overgrowth

Acetaldehyde (31) is a known carcinogen (32). Bacterial overgrowth in the hypochlorhydric stomach enhances the production of acetaldehyde from ethanol after ingestion. Acetaldehyde levels are also higher in alcohol dehydrogenase deficient individuals (common in the Far East). Alcohol does not necessarily need to be ingested because bacterial and yeast overgrowth can result in endogenous ethanol production.

Treatment with a PPI increases acetaldehyde production from ethanol in the gastric juice, probably as a result of bacterial overgrowth. Several organisms are associated with acetaldehyde production, including *Neisseria*, *Stomatococcus* and *Streptococcus* species. Therefore, acetaldehyde could be considered together with NOCs as potential gastric carcinogens.

Effects of bacterial overgrowth on the gastric mucosa

The reduction of nitrates to nitrites and the subsequent production of NOCs has been the principal focus of the effect of bacterial overgrowth in the stomach. Could bacterial overgrowth affect the gastric mucosa by any other mechanisms?

Zavros et al (33), using a mouse model, studied wild type (gastrin secreting [G+]) and genetically achlorhydric (nongastrin secreting [G-]) mice. Initial assessment of gastric pathology in both groups revealed greater inflammation in the G- mice. There were also higher bacterial counts in the antrum of G- mice than in G+ mice. Groups of both genotypes were then treated with antibiotics until the feces showed no bacterial growth. The gastritis was then reassessed and found to have resolved in the G- mice. Treatment of the G+ mice with omeprazole led to an increase in bacterial count and an increase in gastritis that was reduced by subsequent antibiotic treatment (33). The bacterial species were typed and found to be similar in both genotypes. No *H pylori* or other urease-producing *Helicobacter* species were present and, hence, could not be a cause of the inflammation.

The authors concluded that bacterial overgrowth contributed to the inflammatory response in the stomach. It was noted that in both genetically and pharmacologically induced hypochlorhydria gastritis increased as bacterial overgrowth increased. However, there was a discrepancy in the magnitude of change of inflammation compared with the magnitude of change of bacterial numbers. This suggested that other factors, perhaps those related to bacterial metabolism, contributed to the degree of inflammation. Because NOCs were not measured, it is difficult to assess their role in this scenario, although there is no other evidence that they are pro-inflammatory.

If bacterial overgrowth contributes to inflammation in humans, we would expect to see evidence of it in patients receiving PPI treatment. Kuipers et al (34) studied the incidence of atrophic gastritis in *H pylori*-positive and negative gastroesophageal reflux patients treated with PPI or antireflux surgery. *H pylori*-negative patients were found to have a mild gastritis in seven of 46 patients before PPI treatment. After a mean follow-up of five years, moderate to severe corpus gastritis had developed in only two patients (4%). This was associated with atrophy and corresponded to an annual increase of 0.8%. There was no evidence of intestinal metaplasia. In comparison, the incidence of gastritis in the PPI-treated *H pylori*-infected group increased from 59% to 81% and atrophy from 0% to 31%. In the *H pylori*-negative surgical group, there was no evidence of inflammation or atrophy before treatment or after a mean follow-up of five years. The *H pylori*-positive surgical cohort showed no change in prevalence or severity of gastritis, and none of the patients developed atrophy. Is the 4% incidence of gastritis in the *H pylori*-negative PPI cohort evidence that bacterial overgrowth induces gastritis?

A study by Lundell et al (35) did not find any association between *H pylori* infection, PPI treatment and the development of gastric atrophy. There was no evidence of gastric inflammation in the *H pylori*-negative group either before or after treatment. There was a trend to atrophy in the *H pylori*-positive group that did not reach statistical significance, but other studies support Kuipers' results, suggesting an annual incidence of atrophy of 3.8% to 8.7% (35-40). Klinkenberg-Knol et al (36, 37) found an annual incidence of atrophy of 0.7% in *H pylori*-negative subjects on PPI treatment (4.7% in *H pylori*-positive subjects). There was an associated increase in the severity of gastritis in this cohort but the exact numbers are not given in the published results. These data suggest that severe gastric inflammation with associated atrophy occurs in less than 1% per year in *H pylori*-negative patients treated with a PPI. This does not support the hypothesis proposed by the Zavros et al (33) study,

which showed, in mice, that bacterial overgrowth alone can result in gastric inflammation.

Sanduleanu and colleagues (41,42) have addressed this issue in two papers. They have shown that non-*H pylori* flora (both luminal and mucosally associated) was significantly increased in those patients on acid suppression therapy who were infected with *Helicobacter* species than those who were not. In their second paper, they showed that the simultaneous presence of *H pylori* and non-*H pylori* bacteria was associated with a 20-fold increased risk of developing atrophic gastritis, suggesting a synergistic effect between the two infections. The weakness of their hypothesis, however, is that the greater the atrophy, the lower will be the acid secretion and, consequently, the greater the bacterial overgrowth. In other words, the increased bacterial overgrowth may reflect the higher degree of atrophy rather than vice versa. Nevertheless, the hypothesis is interesting and more research is needed in this area.

Bacterial overgrowth and bile acid metabolism

Bile reflux into the stomach is associated with an increased incidence of intestinal metaplasia (43). Some species of bacteria colonizing the stomach and small intestine during bacterial overgrowth are capable of deconjugating bile acids. Much of the attention has been focused on the role that bile plays in gastroesophageal reflux disease. The concentration of bile acids reaching the esophagus and their toxic effects are known to vary with pH depending on the degree of ionization (44). Unconjugated bile acids tend to precipitate in solutions with a

pH lower than 4. Esophageal perfusion studies in animal models have shown that unconjugated bile acids can cause mucosal damage in alkaline solutions (45,46); however, no equivalent data exist for the effect of unconjugated bile acids on the gastric mucosa. Bile acids can also act as a substrate for nitrosation to produce NOCs and there is evidence of genotoxicity from these products (47).

CONCLUSION

Non-*H pylori* bacterial colonization of the stomach occurs when the pH of the gastric juice rises. The number of bacteria present increases as the pH increases, and the resulting organisms can be of oropharyngeal or fecal origin. Decreased gastric acid secretion for any reason can lead to bacterial overgrowth; both H₂ receptor antagonists and PPIs are known to cause it. Many organisms metabolize nitrate to nitrite and others are capable of inducing nitrosation to NOCs. They may also increase acetaldehyde production and deconjugate bile salts. It is unclear whether these metabolic activities or others as yet undefined may increase the gastric inflammatory response in the human hypochlorhydric stomach. There is good evidence that gastric bacterial overgrowth per se causes inflammation in the stomachs of mice, and some data in humans show that the inflammatory response is greater when overgrowth is prominent, but this finding may merely reflect the greater degree of hypochlorhydria in those patients with a more severe *H pylori*-induced inflammation.

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