Helicobacter (Campylobacter) pylori and acid peptic diseases

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ABSTRACT: *Helicobacter pylori* is a spiral-shaped Gram-negative bacteria implicated as a cause of histological gastritis, contributing to peptic ulcer disease and perhaps playing a role in gastric cancer in humans. The organism is found worldwide; the prevalence of infection increases with age; and colonization probably persists for life. Diagnostic approaches that have been used include tissue stains, culture of stomach biopsy specimens, labelled-urea breath tests and serology. It is too early to advocate treatment for all infected individuals; the benefits and risks have yet to be carefully studied and assessed. *Can J Gastroenterol* 1990;4(6):237-242

Key Words: *Campylobacter pylori*, Gastritis, Gastric cancer, *Helicobacter*, Peptic ulcer

Helicobacter pylori et les affections gastro-duodénales

RESUME: *Helicobacter pylori* est une bactérie Gram négatif en forme de spirale responsable des gastrites histologiques, contribuant aux ulcères gastro-duodénaux et jouant peut-être un rôle dans le cancer de l'estomac chez l'homme. Cet organisme se trouve partout dans le monde; la prévalence de l'infection augmente avec l'âge et la colonisation existe probablement à vie. Les colorants histologiques, la culture des pièces de biopsie de l'estomac, les tests respiratoires et la sérologie figurent parmi les approches diagnostiques. Il serait précoce de recommander le traitement de toutes les personnes infectées, l'étude et l'évaluation attentives des avantages et des risques étant encore à faire.

CURVED OR SPIRAL-SHAPED Bacteria have been observed on the gastric mucosa of animals and man for almost a century. Until recently, they were generally viewed as probable contaminants or commensals. Over the past seven years the conditions for successful cultivation have been defined (1,2), and reports of association with active gastritis, duodenal ulcers and gastric ulceration have led to a reconsideration of their role in human disease (1,3-5).

MICROBIOLOGY

Campylobacter pylori, recently transferred to a separate genus and now known as *Helicobacter pylori* (6), is a curved S-shaped Gram-negative rod (oxbow shapes are occasionally seen in vitro), 2.5 µm long and 0.5 µm wide (7). The organism has a tuft of four to six unipolar sheathed flagella, making it very motile; it uses a 'corkscrew' action to propel itself through highly viscous materials. The cells have smooth surfaces with rounded ends. Biopsy specimens can be transported in sterile

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nonbacteriostatic isotonic saline or 20% glucose; in the latter solution, organism viability is sustained for 5 h at 4°C (8). Plating should be done as soon as possible onto nonselective chocolate and 5 to 10% sheep or horse blood agar or, if contamination is a problem, onto a selective medium (eg, Skirrow's) (2,8,9). After three to five days of incubation at 37°C in a moist microaerophilic environment (eg, Campypak; Oxoid, Basingstoke, United Kingdom), smooth, convex, circular, greyish, translucent 1 to 2 mm colonies appear (2). Growth in broth is enhanced by a large surface area with agitation. The organisms are positive for urease, catalase, oxidase, gamma glutamyl and leucine aminopeptidases and hydrogen sulphide; and negative for indole, hippurate and nitrate (2).

For identification purposes, all strains are resistant to nalidixic acid and sensitive to cephalothin by disk diffusion. In vitro, *H pylori* is susceptible to cephalosporins, bismuth, tetracyclines, chloramphenicol, aminoglycosides, erythromycin, ciprofloxacin, ofloxacin, oxolinic acid, rifampin, metronidazole and the penicillins. It is resistant to vancomycin, trimethoprim-sulfamethoxazole, the H₂ blockers cimetidine and ranitidine, antacids, carbenoxalone and sucralfate (10). Recently, a new noncultivable spiral bacterium has been found in gastric mucosa. While the numbers are small, they seem to be associated with a chronic active type B gastritis. A proposal for their name is *Gastrospirillum hominis* (11).

EPIDEMIOLOGY

H pylori has been found in every country studied (12). In Canada and the United States the prevalence of infection increases with chronological age (2,7,13). *H pylori* infection is rare in asymptomatic children, averages 20% in healthy young adults, and is seen in 50% of 50-year-old subjects. There are also ethnic differences in the prevalence of *H pylori* colonization of the antrum. As determined by a positive urea breath test, 60% of asymptomatic middle-aged Chinese are infected with *H pylori* versus only 24%

of an age-matched North American population (13). There is no gender predilection. *H pylori* gastritis has been reported in patients with acquired immune deficiency syndrome (AIDS) (14). Person-to-person transmission may occur (15,16), but this possibility requires further study. One recent study documented patient-to-patient transmission of *H pylori* via the fiberoptic endoscope (17). The possibility of a natural or experimental model of *H pylori* infection in monkeys and the gnotobiotic piglet exists (18,19).

PATHOLOGY

H pylori colonizes native gastric epithelium or the metaplastic gastric epithelium seen in the duodenum adjacent to ulcers, Barrett's esophagus and Meckel's diverticulum. However, two recent reports found that the organism also inhabits subgingival plaque and dental pulp (20,21). Bacterial colonization of the oral cavity and release of viable organisms in saliva may account for some cases of person-to-person transmission and for colonization of antral mucosa in the same patient.

Generally, *H pylori* are found within the mucous layer or beneath it adherent to the apical plasma membrane of antral epithelial cells. The organisms appear to congregate preferentially at or near the intercellular junctions and occasionally penetrate the intercellular spaces (22). *H pylori* are rarely seen intracellularly (22), and then only contained in phagocytic vacuoles. The mucous layer may protect *H pylori* from gastric acid while the breakdown of urea (via urease production) to the ammonium ion may help to promote the alkaline conditions which are conducive to its survival.

Histologic study reveals a loss of gastric mucus, sporadic epithelial cell necrosis and neutrophil invasion of the epithelial layer associated with acute and chronic inflammatory cells in the lamina propria. With electron microscopy the surface epithelial cells are edematous, degenerated and depleted of mucus granules; there is also a loss of microvilli. In these areas, *H pylori* attaches to the eukaryotic cell surface at a region of pedestal formation (23). The

receptor for *H pylori* on red blood cells, tissue culture cells, pig stomach and human antrum is a novel glycerolipid (24). Bacterial adhesion may be mediated by cell surface fibrillar protein antigens (adhesins) (25).

There are a number of factors which could account for *H pylori*-induced damage to gastric tissue (26). These include: interference with gastric mucus production which thereby facilitates injury by gastric acid; ammonia production mediated via the urease released by *H pylori*, which may damage cells by increasing the back-diffusion of hydrogen ions into mucosal cells (26); and toxins (27). In addition, extracellular proteases, phospholipases and lipases may be released by *H pylori* and degrade gastric mucus (28,29). Therefore, proteolytic and lipolytic activity of *H pylori* could damage both elements of mucosal defence, ie, the mucus layer and the cell membranes of the gastric epithelium. However, additional studies confirming this pathogenic mechanism are required (30).

CLINICAL MANIFESTATIONS

H pylori has been linked to a variety of disease states in humans.

Chronic active (type B) gastritis: Increasing evidence suggests that *H pylori* is the etiologic agent of type B gastritis. Chronic active gastritis (symptomatic or asymptomatic) is closely associated with *H pylori* infection (31-34). The antral mucosa is most often affected. Clearing of the bacteria is usually associated with a normalization in histological findings (35). Experimental self-feeding (36,37) or inadvertent infection with the organisms (38,39) results in typical gastric inflammation.

Autoimmune (type A) gastritis and secondary (eg, Crohn's disease, drug-induced and bile reflux) gastritis are not associated with *H pylori* infection (31,32,40,41).

Duodenal ulcer disease: The prevalence of *H pylori* colonization of inflamed antrum in patients with endoscopically proven duodenal ulcers is fairly high (greater than 70%), but this may simply reflect the previously recognized close association between duodenal ulcers and antral inflamma-

tion (42). Indeed, there are a number of discordant facts in the link between duodenal ulcers and infection with *H pylori*: the prevalence of *H pylori* increases with age though duodenal ulcer disease is not predominantly a disease of the elderly; ulcer craters heal even though bacteria persist; acid hypersecretion (Zollinger-Ellison syndrome) leads to duodenal ulcer disease without *H pylori* present; and there is no gender predilection for *H pylori* infection whereas duodenal ulcer disease predominates in males. Therefore, other factors must also contribute to the pathogenesis of duodenal ulcer disease.

Compelling evidence that *H pylori* infection plays a role in duodenal ulcer disease comes from the data of antimicrobial treatment trials (43-45). Antibiotics, bismuth and H_2 antagonists heal duodenal ulcer disease at the same rate; however, when H_2 blocker therapy is discontinued, ulcer recurrence is frequent and rapid. When *H pylori* is cleared with antibiotics and bismuth compounds, duodenal ulcer disease recurrence is significantly reduced (45). Ulcers that do return after antimicrobial therapy is discontinued are associated with recurrent *H pylori* infection (43,45).

The genesis of duodenal ulcers may require increased exposure to acid in the duodenum leading to formation of metaplastic gastric epithelium (46) which then becomes colonized by *H pylori*; this causes further inflammation and with further acid contact an ulcer results. Alternatively, Levi et al (47) proposed that *H pylori* colonization of the antrum results in an inappropriate release of gastrin from G cells in the stomach, excess acid production by parietal cells and duodenal ulceration from delivery of excess acid into the duodenum.

Family members (symptomatic or asymptomatic) of duodenal ulcer patients have a greater prevalence of *H pylori* infection (48). These findings suggest person-to-person spread or a common point source of infection. However, bacterial restriction endonuclease digest analysis of the isolates did not reveal identical organisms within families (49).

Benign gastric ulcer: For a number of reasons, *H pylori* does not appear to be the sole cause of gastric ulcer. The prevalence of *H pylori* infection in gastric ulcer is only 60 to 80%. Nonsteroidal anti-inflammatory drugs account for most gastric ulcers and are not associated with an increased frequency of *H pylori* colonization.

Barrett's esophagus: When gastric epithelium is found in the esophagus, *H pylori* can colonize these metaplastic sites (50). However, the clinical importance is not yet clear (51).

Nonulcer dyspepsia: Self-feeding experiments with *H pylori* causes transient dyspeptic symptoms consistent with nonulcer dyspepsia (36,37). The prevalence of infection with nonulcer dyspepsia is 40 to 60%, which is similar to that found in an age-matched asymptomatic control population. Antimicrobials provide irregular relief of nonulcer dyspepsia symptoms, although the inflammatory reaction is decreased if *H pylori* is present (52). Currently, therefore, there is no compelling evidence that *H pylori* is an important causative factor in nonulcer dyspepsia.

Gastric cancer: Chronic gastritis is considered the precursor lesion of gastric carcinoma (53). Whether these lesions progress from superficial active gastritis due to *H pylori* is not known. Preliminary reports suggest, however, that there is an association between the presence of these organisms and both gastric dysplasia and carcinoma (54,55). In economically advanced countries, infection with *H pylori* and the development of gastritis usually occurs in middle age (31). However, in other countries infection with *H pylori* occurs more frequently with onset of colonization at a younger age (56). It is possible that individuals in a population with a high frequency of gastric carcinoma might become infected with *H pylori* at an early age.

LABORATORY DIAGNOSIS

Culture: Culture of biopsies of gastric mucosa will identify 90% of persons infected. Distribution of infection may be patchy, so cultures should be obtained from at least two antral sites to

enhance the recovery of *H pylori*. The organism has rarely been isolated from the body of the stomach unless antral-type mucosa is present or there is mucosal inflammation and bacterial colonization of the antrum (57).

Negative cultures can result from inadequate sampling (ie, biopsy of non-antral epithelium), delay in transport of specimens, swallowed topical anesthetic (eg, benzocaine) (58), prior therapy with bismuth compounds or antibiotics, and residual disinfectant (eg, glutaraldehyde) present on the biopsy forceps and biopsy of nongastric epithelium.

H pylori bacteremia has not been described, perhaps because it does not occur or current commercial blood culture systems are inadequate for its detection (59).

Histology: *H pylori* detected by various tissue stains correlates well with culture results (32,34). Gram stain of 'touch' preparations of biopsies is a rapid and inexpensive approach to diagnosis that correlates well with results of both culture and other stains (60). The bacteria can be detected in tissue with hematoxylin-eosin, Giemsa, acridine orange, crystal fast violet and silver stains such as the Warthin-Starry, Dieterle and modified Steiner methods which facilitate the detection of bacteria especially when present in small numbers. Monoclonal antibodies against *H pylori* have been used to detect the organism in clinical specimens by the immunoperoxidase technique (61).

Biochemical tests: Tests which assay for preformed bacterial urease permits the rapid detection of *H pylori* in biopsy samples (62). In these tests tissue is incubated with urea as a substrate and phenol red as a colour indicator of changes in pH. If *H pylori* is present in adequate numbers, bacterial urease hydrolyzes the urea, which raises the pH and changes the phenol red indicator from yellow to pink. Recently, a Minitek (BBL Microbiology Systems, Maryland) urea disk test has been evaluated which is both sensitive and cost effective (63). Slants of Christensen's urea agar or broth can also be used for the detection of urease. Rapid urease tests correlate fairly well

with other methods of *H pylori* detection (62); however, the presence of *Proteus* species as oral contaminants can result in false positive reactions (8).

Noninvasive breath tests: Bacterial urease forms the basis of noninvasive breath tests (13). A test meal is given with ^{13}C - or ^{14}C -labelled urea to delay gastric emptying. If *H pylori* is present in the stomach, the urea is hydrolyzed and radiolabelled carbon dioxide detected in expired breath samples within 1 h.

Serology: Circulating and local antibodies to *H pylori* have been measured by agglutination, complement fixation and enzyme-linked immunosorbent assay. There is a strong correlation between high serum IgG and IgA antibody levels to *H pylori*, gastritis and a positive culture (34,48,64). Antibodies can also be detected in gastric juice and saliva.

Although titres of *H pylori*-specific antibody may decrease after *H pylori* infection has been eliminated (65), the levels tend to remain elevated for years.

Therefore, serology indicates present or past infection and is useful for seroprevalence studies (48,56).

TREATMENT

In vitro susceptibility of *H pylori* does not reliably predict efficacy in the eradication of the organism in humans (66). Many single and combination antimicrobial regimens can suppress bacterial growth, but they are less effective in permanently clearing the organism. Fifty to 90% of culture-positive patients will become culture-negative immediately after treatment with colloidal bismuth and amoxycillin, singly or in combination. However, only 40% are still culture-negative one month after treatment is discontinued (67). This indicates that relapse or recurrence of infection is common. Restriction endonuclease digest analysis shows that these recurrences are usually due to relapses with the same strain of organism (68). This may in some cases be related to a reservoir of the organism harboured

in dental plaque and dental pulp (20,21,69), or simply to inhibition without eradication of bacteria from the stomach. Higher doses, repeated or more prolonged courses of single, double or triple antimicrobials (bismuth and two other agents) may eliminate *H pylori* (70,71). However, such regimens are associated with increased costs and adverse effects including diarrhea and pseudomembranous colitis. Therefore, the benefits versus potential risks must be carefully considered. The development of safe, convenient and effective therapy will facilitate the investigation of the role of *H pylori* in gastroduodenal disease. It is important to define which patient populations and disease entities related to *H pylori* infection will benefit from therapy. Currently, physicians must temper treatment with common sense and not subject individual patients to possibly ineffective or toxic regimens nor encourage the spread of resistance to clinically useful antimicrobial agents.

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