Approaches to the Prevention and Treatment of Helicobacter Pylori Infection

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Current therapies to heal peptic ulcers and eradicate Helicobacter pylori generally rely on a combination of antibacterial agents and anti-secretory drugs. The major factors affecting the outcome of these eradication therapies are the selection of antibiotic(s), daily dose, the dosage regimen(s) selected and duration of dosing and patient non-compliance due to side effects or number or tablets to be taken. Future therapies will seek to maximize effectiveness through taking account of these factors.

The only new drug to be introduced in recent years uniquely for the eradication of H. pylori is ranitidine bismuth citrate, which when combined with a single antibiotic (clarithromycin) or two antibiotics has been shown to be highly effective (even against H. pylori “resistant” to clarithromycin, treated with the simple dual therapy).

Awareness of the increasing resistance of Helicobacter pylori to antibiotics, particularly the nitromidazoles and macrolides, acquired either during or before therapy is becoming a significant concern. Thus, there is a need for therapies that either prevent acquisition of resistance or are effective against organisms already resistant. At present there is no suitably-effective single drug available for use as monotherapy. It is also known that single or multiple antibiotics are only capable of eradicating H. pylori by enhanced or potentiated efficacy due to the suppression of gastric acid or by addition of bismuth. For this reason, the concept of monotherapy against H. pylori is not possible with currently-available drugs.

Other approaches in therapy for H. pylori infection include a “therapeutic” vaccine (immunotherapy) in which the IgA-mediated mucosal immune response is boosted to cause elimination of the organism. Issues relating to the success of this approach involve the choice of H. pylori antigen(s), a suitable adjuvant such as a soluble protein or an attenuated live organism, and the nature of the vaccination programme. Another novel approach being pursued involves the use of “anti-adhesive” agents to prevent or remove H. pylori adherence to the gastric mucosa (or mucus). This may have an advantage of providing a selective agent which may prevent gastritis due to the adherent bacteria, but may have to be used as part of combination eradication therapy.

Further into the future, more “rational” therapies are likely to be developed based on greater knowledge of the organism (genome sequence/metabolic pathways) and its ecological environment. However, the concepts of monotherapy and vaccines are major therapeutic challenges and agents for the clinic from on-going basic research are unlikely to be seen over the next five years due to the long duration of the drug development process.

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Abbreviations: RBC, ranitidine bismuth citrate.
**HELIcobacter pylori — THE ORGANISM**

*H. pylori* has evolved over the millennia to survive in a specialized micro-environment within the human upper gastrointestinal tract, where it is found closely associated with gastric epithelium. In addition to the stomach, the organism can also be found on metaplastic gastric epithelium in the duodenum and on ectopic gastric mucosa at other sites. Within the stomach, *H. pylori* preferentially inhabits the gastric antrum although bacteria may also be found in the fundus. For example, changes in intragastric distribution of *H. pylori* have been noted during treatment with omeprazole [1, 2]. The bacteria are often found around intracellular junctions and adhering to epithelial cell membranes [3], and it has been reported that *H. pylori* may occasionally penetrate cells [4], although this is controversial.

As with other pathogens, an understanding of the factors allowing colonization and subsequent pathogenicity within the host will enable new therapeutic approaches to be developed. The virulence factors which enable *H. pylori* to colonize and subsequently persist or flourish within the host include its motility, adaptive enzymes, and the ability to adhere to gastric epithelium. Certain enzymes and toxins may also play a role in the pathogenesis of various diseases, for example, urease, phospholipases and cytotoxins. *H. pylori* is well-adapted for motility in gastric mucus [5] being curved or spiral, and having two to six polar, sheathed flagella, enabling rapid passage through both gastric juice and gastric mucus [6]. The organism is able to survive in gastric juice prior to penetrating the protective layer of mucus over the gastric epithelium by means of its abundant urease, generating bicarbonate and ammonium ions around the organism to form a protective micro-environment. In addition to its role as a survival factor, urease activity may be associated with tissue damage due to the production of ammonia [7-10]. All *Helicobacter* species produce large amounts of catalase which protects the bacteria from toxic metabolites associated with lysis of neutrophils [5].

Selective colonization of gastric, mucus-secreting, epithelial cells by *H. pylori* is facilitated by its ability to attach to specific phospholipids and glycoproteins within the gastric mucosa. In addition, in persons with blood group O, the Lewis B blood group antigens mediate attachment of the organism to the mucous cells [11]. Other putative receptors for *H. pylori* binding are extracellular matrix components, such as laminin, fibronectin, collagen types I, IV and V, vitronectin, and heparan sulphate [12-15]. Adhesion of *H. pylori* at contact sites on gastric epithelium has been categorized as adhesion pedestals, indentation sites, or abutment adhesions although gastric cell injury occurs irrespective of the morphologic type of attachment [16]. Enzymes from *H. pylori*, for example, urease, phospholipases A₂ and C, and proteases may be responsible for subsequent damage.

Study of the molecular mechanisms by which *H. pylori* causes disease suggests that isolates of *H. pylori* can be divided into two major types. Type I bacteria express a vacuolating cytotoxin (VacA) and a cytotoxin-associated antigen (CagA). Type II bacteria express neither VacA nor CagA. Duodenal ulcer patients are almost invariably infected by Type I *H. pylori* [17-19]. The vacuolating toxin in cytotoxic strains of *H. pylori* induces vacuoles in epithelial cells which results in cell death. The gene for the cytotoxin protein (VacA), whilst present in all strains of *H. pylori*, produces an active cytotoxin in only about 50-60 percent of isolates in vitro [19-21].

It appears therefore that peptic ulceration, and possibly gastric cancer, may result only from infection by strains of *H. pylori* producing both VacA and CagA. In tissue culture of gastric epithelial cells, these strains induce secretion of interleukin-8 [22] which may account for the relationship between severity of disease (i.e., inflammation and subsequent ulceration) and presence of CagA-, VacA-positive bacteria.
ISSUES FACING ERADICATION OF H. PYLORI

The association between infection by H. pylori and peptic ulcer disease has been well recognized over recent years, and approximately 95 percent of patients with duodenal ulcer and up to 90 percent of those with gastric ulcer are likely to be infected [23]. The management of peptic ulcer disease has undergone a remarkable evolution over the past ten years with the recognition that eradication of H. pylori markedly reduces ulcer recurrence and thus diminishes the need for continuous anti-secretory therapy in most patients [24-26]. However, there is considerable confusion at present concerning appropriate diagnostic procedures and therapeutic options. This latter aspect has arisen due to the inability of monotherapy with existing antibiotics to eradicate the organism in most patients. Eradication regimens are becoming increasingly "added-on" to ulcer-healing therapies in which antibiotics are added to anti-secretory agents. The early absence of well-controlled clinical trials has resulted in a wide array of proposed dual, triple and even quadruple therapy regimens, many of which have not been adequately tested.

The last decade has seen a large number of "eradication studies," although many of these have been published only as abstracts or without adequate details to evaluate their merits. A number of reviews of these data have been published [27-34] which allows the following general observations. Early studies were dominated by therapy with a bismuth salt, often for four weeks, plus two antibiotics for the first week or two. Combinations of colloidal bismuth subcitrate with metronidazole and tetracycline have consistently provided high eradication rates of over 90 percent [35, 36]. Unfortunately, these triple therapies are often associated with poor patient compliance due to complex dosing schedules with large number of tablets [37], frequent side-effects [30, 38], and resistance to metronidazole [39].

Such limitations led to investigators studying two week, dual therapy options, of which the most widely reported has been omeprazole plus amoxicillin. Eradication rates with this regimen have ranged from 0 to 90 percent, with pooled data suggesting an average rate of about 60 percent [33]. Interest in this combination has declined as recent properly-controlled trials have shown low eradication rates [40-42]. Effort subsequently focused on the novel macrolide clarithromycin as the antibiotic of choice in dual therapy regimens, with pooled eradication rates of about 66 percent reported when given in combination with omeprazole [33]. However, current research trends show a revived interest in short duration (seven day) triple therapies based on proton pump inhibitors plus two antibiotics, with pooled eradication rates of about 85 percent [33].

During the next few years it is likely that existing and new combination therapies will be refined and defined through randomized, double-blind clinical trials of sufficient size to determine optimal therapeutic regimens.

Increasing consideration is being given to developing agents or therapies to eradicate H. pylori in asymptomatic carriers of the organism in addition to patients with peptic ulcer, since H. pylori is now accepted as a risk factor in the development of gastric carcinoma [43]. Such new therapies may need to work without the need for acid-suppression. These new agents may represent the "magic bullet" against H. pylori, but they are unlikely to be available this decade.

FACTORS AFFECTING ERADICATION

Drug-related factors such as daily dose, dosing frequency, duration of therapy, pretreatment with certain agents, administration before or after food, and antibacterial potency have major bearings on the efficacy of any eradication regimen. Also patient-related factors such as disease state, concurrent illness, adverse events, smoking, ethnic origin, and socio-economic status have also been suggested as being important. The impact of
patient non-compliance during therapy with short-duration eradication regimens may be critical to success, but has yet to be evaluated. However, much discussion has been given over recent years to organism-related factors. These have included organism sub-types, route of transmission, location within the stomach and gastro-intestinal tract, morphological variants, virulence factors, pathogenic mechanisms, resistance mechanisms, and response to environmental factors.

While modification of drug dose, dosing frequency and duration of therapy of each component of an eradication regimen may improve efficacy, there are many other factors which may affect the outcome. For example, the distribution of *H. pylori* in the stomach (antrum, fundus, corpus) and at sites of gastric metaplasia in the duodenum may vary between duodenal and gastric ulcer disease. Moreover, the ability of any antibiotic to reach and to kill the organism depends on various physico-chemical and metabolic considerations, especially whether topical or systemic activity is needed. Hence, differences in the location, histology, pH, and other physico-chemical factors of the micro-environment in the vicinity of the organism could lead to differences in eradication.

Optimization and standardization of clinical trial design will be particularly important in advancing our understanding of the relative merits of different eradication therapies. Attention has to be given to appropriate comparator or control treatments administered in a randomized and blinded fashion. Analysis of results should be based on carefully defined observed or intent-to-treat patient populations, rather than per protocol analysis which may give misleadingly high eradication results. Much of the existing literature is confused about which population the analyses are based upon.

It is apparent that although optimisation of current therapeutic strategies may lead to greater *H. pylori* eradication rates and lower ulcer recurrence rates, such combination therapies are not ideal. More rational research to understand the organism, its environment, and its relationship to gastrointestinal disease is likely to facilitate the development of a safe and effective monotherapy.

**FUTURE APPROACHES TO ERADICATION OF *H. PYLORI* INFECTION**

Potential advances in the therapy of *H. pylori*-related disease may come from a number of alternative approaches. A better understanding of how the infection is transmitted may provide simple methods of prevention. It is also clear that there are host factors that determine individual susceptibility to disease, but this has received little attention to date. Publications and patents for various novel therapies aimed at eradicating infection are now emerging. Thus, anti-adhesive agents, novel anti-microbial agents, certain dietary factors, bacteriocins, urease inhibition/bicarbonate removal, aggressive "topical" therapy, colostral antibodies and vaccines are all attracting interest. Although it is difficult to assess which of these approaches will be the most effective, most interest at conferences appears to be directed at vaccine development and novel agents.

**Vaccines**

The recognition that peptic ulcer is caused by an infectious agent suggests that the disease may be prevented by vaccination [44]. Preliminary experiments in animals were reported by Czinn et al. [45]. Oral immunization of germ-free mice with sonicated *H. felis* plus cholera toxin resulted in elevated serum, gastric and intestinal anti-*H. felis* antibody. 70 percent of the immunized animals were subsequently protected from acute infection. More recently mice have been immunized against *H. felis* infection by vaccines from disrupted cells of *H. pylori* or *H. pylori* urease preparations [46, 47]. Results of recent early clinical trials with a recombinant urease vaccine in healthy volunteers have been encouraging [48].
Virulence and pathogenicity factors of *H. pylori* may be suitable antigen candidates for the development of a vaccine. Major consideration is being given to the urease and flagellae (two subunits, FlaA and FlaB) although the membraneous sheath around the flagella may prevent the FlaA protein being useful as a vaccine. Identification of the ulcerogenic Type I strain of *H. pylori* suggests the possibility that vaccine development may be targeted only toward those bacteria responsible for severe disease. Thus mice were immunised with antigens peculiar to Type I bacteria and subsequently exposed to cytotoxic strains. Immunization with purified VacA protected mice from infection with a Type I strain of *H. pylori* but not from infection with Type II strains [49]. At present only one gene (*hpaA*) coding for an adhesion subunit protein has been characterized and cloned [50]. The future development of vaccines to prevent adhesion of *H. pylori* to mucosal cells will rely on identification of the genes for other proteins mediating adhesion of the bacteria.

Although most research with vaccines has focused on the “classical” prophylactic approach, recent publications have shown that vaccines may have a “therapeutic” effect in actually eradicating pre-existing infection with *H. pylori* [51-53]. A major challenge in the perceived race to get a good vaccine for mucosal immunity is obtaining a safe and effective adjuvant, and a genetically detoxified *E. coli* enterotoxin may provide this [54]. Research is also continuing into antigen-coated particles, attenuated *Vibrio cholerae*, *Salmonella typhi* and *Shigella dysenteriae*, as well as commensal bacteria, being developed as live vaccines.

**Anti-adhesive agents**

Oligosaccharide components of membrane glycoproteins and glycolipids act as bacterial adhesion ligands for *H. pylori* on epithelial cells. Soluble oligosaccharides mimicking these epithelial ligands may disrupt bacterial attachment. The oligosaccharide, NE-0080, detached bound bacteria from epithelial monolayers and, in gnotobiotic piglets infected with pig-adapted *H. pylori*, reduced colonization in the majority of treated animals, and this agent is currently undergoing clinical investigation [55]. Similarly, fucosylated glycosides inhibit adherence of bacteria to mucosal surfaces. Compounds containing a terminal L-fucose inhibited *H. pylori* binding to human gastric tissue in an in situ adherence assay. Moreover, bismuth, in addition to its bactericidal properties, may influence bacterial adhesion in vitro [56-59] and in vivo [60]. Lectins are also capable of preventing binding of *H. pylori* to colostral IgA [61]. Binding was inhibited by those with sialic acid residues whereas non-sialylated carbohydrates had no effect.

**Novel agents**

In addition to on-going clinical trials of broad-spectrum antibiotics, generally combined with acid-suppression, several companies have reported an interest in some novel agents. Thus, agents such as an antimicrobial, cationic peptide used as a food additive (nisin) from AMBI [62], a nitrothiazole (nitazoxanide) from Romark Laboratories [63], and a lipid preparation (Helicore™) from NovaVax, Inc. [64], are all undergoing early clinical trials.

**Prevention of transmission of infection**

The mode or modes of transmission of *H. pylori* are still uncertain. Socio-economic status has repeatedly been shown in many countries to have a strong inverse relationship with *H. pylori* infection; family size, and hence overcrowding, the sharing of a bedroom and of a bed being the key factors [65]. Thus reduced transmission may be achieved by general improvement in living standards.
Endoscopes are highly contaminated after use in *H. pylori*-positive patients. Inadequate cleaning procedures in the past may therefore have led to iatrogenic transmission [66]. Guidelines now recommend extensive manual cleaning of all parts of an endoscope and submersion in glutaraldehyde for at least two minutes.

**Host factors**

Although the precise aetiology of gastric and duodenal ulceration is not established, a number of risk factors are associated with the development of peptic ulcer disease in the majority of patients. For example, these include excess gastric acid secretion, genetic predisposition, cigarette smoking, and concomitant disease (hepatic cirrhosis, chronic pulmonary failure, chronic renal failure). The interrelationship between such factors and *H. pylori* infection in the pathogenesis of peptic ulceration has received little attention. It has been suggested that all strains of *H. pylori* have the ability to induce ulceration if other conditions are met, and a research priority should be the identification of those host factors which allow changes in the mucosal environment caused by colonization with any strain of *H. pylori* to initiate duodenal ulceration [67].

**Dietary factors**

The influence of diet on the development of peptic ulceration has been recognised [68] and may provide clues for future therapies. Observations that gastric bacterial metabolism may be inhibited by long-chain fatty acids [69-72] led to experiments showing that two common dietary polyunsaturated fatty acids ($\omega$-3 linolenic acid and linoleic acid) disrupted the cell membrane of *H. pylori*, leading to lysis [73]. There has also been a report that *H. pylori* was cleared in six of eight patients who received two months’ treatment with a polyunsaturated fatty acid supplement (2 g linoleic/linolenic acid), although the mode of action was unclear [74].

Medium-chain monoacylglycerol esters are bactericidal for *H. pylori* and *H. felis* in vitro, and for the latter, also in vivo [75]. The mechanism of antibacterial activity is unknown but disruption of cell membrane permeability and inhibition of amino acid uptake have been suggested [76, 77].

Pasteurised and skimmed milk inhibited *H. pylori* following acidification with hydrochloric acid to pH less than 5.5, or after digestion with trypsin [78]. The acid-released inhibitor is unknown, whereas the trypsin-released inhibitor is derived from alpha and kappa fractions of caseins. Lactoferrin, a glycoprotein found in mammalian milk, is also able to inhibit the growth of *H. pylori* [79].

Lactic acid-producing bacteria form substances that have bactericidal activities against *H. pylori* [80], and it was shown in a recent randomized, double-blind clinical study, that a whey-based *Lactobacillus acidophilus* culture supernatant (4 x 50 ml/day) suppressed the growth of *H. pylori* in 20 volunteers [81].

Other natural dietary constituents reported to possess anti-*H. pylori* properties in vitro include extract of black and green tea [82], varieties of Chinese herbal medicines [83], and Manuka honey [84].

**Urease inhibition/carbonate removal**

Although urease is essential for colonization, inhibition of its activity, by the potent agent flurofamide, did not cause eradication of *H. mustelae* in an animal model [85]. Although benzimidazole proton pump inhibitors reduce urease activity in cell extracts and in intact *H. pylori* [86], their effect on growth of *H. pylori* may be independent of the inhibitory action of these agents against urease [87].
Compounds removing bicarbonate, ammonium ions or urea from the gastric milieu may eliminate the protective micro-environment from *H. pylori* thus rendering it susceptible to the antibacterial properties of gastric acid. Substances investigated include dibasic magnesium compounds, dialdehyde polysaccharides, calcium and magnesium salts and zeolites. Combined ammonia and bicarbonate scavengers were particularly effective when used concomitantly.

"Topical" therapy

A novel approach to eradicate *H. pylori* by high luminal concentrations of antibiotics has been reported from Japan [88]. Using this technique, a concentrated solution of sodium bicarbonate containing a proteolytic agent to dissolve gastric mucus, and a bactericidal cocktail of bismuth subnitrate, amoxycillin and metronidazole was instilled into the stomach via a nasal tube, after pre-treatment with lansoprazole and the proteolytic agent. Importantly, leakage of the solution was prevented by a balloon catheter inflated in the duodenum. The solution was kept in the stomach for one hour and the position of the patient changed every 15 min to expose the entire mucosa, and the solution was removed by suction after the procedure. *H. pylori* infection was reported to be eradicated in 24/25 (96 percent) of patients, with the one failure due to balloon failure!

Novel drug formulations may offer greater gastric retention times thus increasing exposure of *H. pylori* to an antibiotic. Such a formulation comprising a benzene-based antimicrobial, muco-adherent cellulose derivative and a chelating agent has been described. Suggestions of mucosalhesive agents, floating devices, mucolytic agents to enhance drug penetration, acid-stable antibiotics, and agents which markedly slow gastric emptying to prolong gastric residence time for an antibiotic have all been proposed.

Colostral antibodies

Antigen-specific antibodies from cow’s milk may target a broad range of pathogens in the gastrointestinal tract [89]. Orally administered bovine immunoglobulins with activity against highly purified *H. pylori* antigens may eradicate the organism [90]. The efficacy of an immunoglobulin concentrate derived from mammary gland secretions of animals immunised with *H. pylori* has also been assessed in gnotobiotic piglets. There was a reduction or elimination of *H. pylori*-induced pathological changes and bacterial colonization of various gastric epithelial regions.

**WHAT IS NEW AND CURRENTLY AVAILABLE IN ERADICATION THERAPY**

Ranitidine bismuth citrate (RBC) is the first and only compound specifically introduced for the eradication of *H. pylori*. It is a novel compound with unique physicochemical properties. For example, when compared with bismuth citrate it is some 50,000-fold more soluble, inhibits human pepsin isoenzymes in vitro, and is more effective at inhibiting the growth of *H. pylori* both in vitro and in vivo [91]. Moreover, RBC kills *H. pylori* rather than just having a bacteriostatic effect. This rate of killing is significantly increased in a synergistic manner when antibiotics such as clarithromycin or metronidazole are added to low concentrations of RBC. Clinical studies have demonstrated that this may contribute to efficacy, Peterson et al. [92] reported 82 percent eradication with two weeks dual therapy of RBC 400 mg twice daily plus clarithromycin 500 mg three times each day, whereas RBC alone gave no eradication, and clarithromycin alone eradicated the organism in only 36 percent of patients treated. A simpler dosing regimen of both agents has been shown to eradicate *H. pylori* in 96 percent of patients after two weeks therapy of RBC 400 mg twice daily plus clarithromycin 500 mg twice daily [93]. Moreover, a one week therapy of RBC twice daily plus two antibiotics twice daily has also been shown to be highly effective [94, 95].
Apart from RBC, it is unlikely that there will be any new medicines for the eradication of *H. pylori* over the next few years due to the long lead time for drug development and approval. Optimization of current therapeutic approaches using two week dual or one week triple therapy regimens involving proton pump inhibitors or RBC are therefore likely to feature in the evolution of eradication therapy in the immediate future.

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