Helicobacter pylori infection and ischemic heart disease: could experimental data lead to clinical studies?

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Abstract

Despite the remarkable advances made in primary prevention and treatment, ischemic heart disease (IHD) remains the leading cause of death and a significant cause of disability in developed countries. Since traditional cardiovascular risk factors failed to predict all cases of IHD, there is an intensive research to explore other potential etiologic factors. Among these, numerous studies have considered the theoretical link between IHD and chronic infections, including *Helicobacter pylori* (*H. pylori*). Considering that epidemiologic studies have produced conflicting results, due to geographical variations of IHD and *H. pylori* prevalence as well as heterogeneity of study designs, an alternative way to analyze this topic is to assess if consistency for a biological plausibility exists. In this review we critically analyzed the experimental data on this topic, to assess whether their results could lead future clinical studies.

Key words: *Helicobacter pylori*, thrombosis, ischemic heart disease, coronary artery disease, myocardial infarction.

Introduction

An impressive decline in mortality due to acute ischemic heart disease (IHD) has occurred in the last 50 years. This has been attributed to improvement in primary prevention and to major progress made in treatment.[1] Despite these remarkable advances, IHD remains the leading cause of death in developed countries[2] and the responsible for sudden cardiac death in postmyocardial-infarction patients.[3] Moreover, it occupies a significant position among the main causes of human disability.[4]

Since traditional cardiovascular risk factors consistently predict some but not all cases of IHD (about 20% of acute events occur in the absence of such factors), emerging risk factors are being explored.[5] In the last few decades many studies across several fields have explored the potential link between IHD and chronic infections[6-8], including *Helicobacter pylori* (*H. pylori*). Considering that epidemiologic studies have produced conflicting results, due to geographical variations of IHD and *H. pylori* prevalence as well as to heterogeneity of study designs [9-13], an alternative way to analyze this topic is to assess if a consistent biological plausibility exists. The aim of this review is to focus on experimental data obtained by investigating the relationship between *H. pylori* and IHD. This will be critically reviewed in 3 sections: 1) the bacterium, 2) the experimental findings both in the animal models and in humans 3) the potential clinical implications.

The bacterium

H. pylori is a gram negative spiral-shaped bacterium usually acquired during childhood.[14] The infection persists for years, often for a lifetime. Since the human stomach is an unfriendly place for most bacteria, in order to live in the gastric environment, *H. pylori* has developed a repertoire of acid resistance mechanisms. To colonize gastric mucosa, it penetrates the mucous layer through the acidic environment of the stomach (pH < 2) and to overcome this barrier the bacterium produces the enzyme urease, which

degrades urea to ammonia in the gastric epithelium. The excess of ammonia raises the pH neutralizing gastric acid and thereby allowing *H. pylori* to safely traverse the mucus layer to the epithelium surface.[15]

Currently, it is estimated that around half of the world population is infected with *H. pylori*. This bacterium is accepted as the most important cause of gastritis and peptic ulcer (PU) and a risk factor for gastric cancer.[16] Moreover, in the last few decades, several studies have reported on the link between chronic *H. pylori* infection and a variety of extragastric manifestations,[17] based on potential mechanisms involving a low-grade inflammatory state, molecular mimicry patterns, and interference with the absorption of nutrients.[18,19] Although the Maastricht IV/Florence Consensus Report of the European Helicobacter Study Group has considered causal associations of *H. pylori* in those with unexplained iron-deficiency anemia, idiopathic thrombocytopenic purpura (Grade A recommendation) and vitamin B12 deficiency (Grade B recommendation), other relationships could not be excluded.[20] The major interest to deeply research in this field is that the cure of the infection, consisting in a multidrug regimen based on antisecretive agents and antibiotics[21], is relatively easy and far less expensive than long-term treatment of either known risk factors for IHD or therapy for the acute events and the secondary prevention of IHD.

Experimental findings

Atherosclerosis is the principal underlying cause of IHD. As a general principle in the pathogenesis of most IHD cases, the epicardial coronary occlusion from atherosclerosis should be considered. Hence, the ischemic damage is almost always the result of an imbalance between supply and demand. Coronary atherosclerosis progress through stages of fatty-streak deposition in coronary arteries to development of fibro-fatty plaque, with the increase in size until it causes partial or total luminal obstruction. At any stage, the atherosclerotic lesion may be vulnerable to erosion or rupture, thereby exposing the subendothelial vessel wall to substances in the circulating blood. Vulnerable plaques have evidence of inflammation, with monocytes, macrophages, and sometimes T-cell infiltrates[22], together with thin

fibrous caps, derived from smooth muscle cells, which migrate from the media into the intima, and large lipid cores, released from necrotic foam cells monocyte-macrophages which migrate into the intima and ingest lipids. The inflammatory milieu in atherosclerosis is further exaggerated and sustained by the innate immune system involving T-cell activation.[23] Elevated bloodstream levels of the acute-phase proteins, such as C-reactive protein (CRP) and proinflammatory cytokines, in patients with cardiovascular events, provide more evidence suggesting the role of inflammatory processes.[24,25] Procoagulating factors reside within the plaque and, in the absence of counter-balancing antithrombotic factors and fibrinolytic activities within the endothelial cells, can lead to thrombosis.[26]

Whether and when *H. pylori* has a role in this multistep process has been investigated by several studies which are summarized and grouped on the basis of the main mechanism explored:

Studies on platelet aggregation and coagulation parameters

The issue of the role of infectious agents in recruiting platelets into thrombus has been widely examined.[27] Platelets derive from megakaryocite cytoplasm, and on activation change from the normal disc shape to a compact sphere with dendritic extension facilitating adhesion. Platelets are the first blood cells to arrive at the site of endothelial activation. By its glycoproteins (GPs) Ib and IIb/IIIA these cells engage surface molecules on the endothelium, which contributes to endothelial cell activation. Once activated, these cells express several types of leukocyte adhesion molecules, which can cause blood cell rolling along the vascular surface to adhere to the site of activation.[28] This step might play an important role in platelet aggregation leading to plaque vulnerability and eliciting acute ischemic events. Hence, the speculation that *H. pylori* infection could induce platelets and immune activation mediated by cytokines, such as interleukin (IL)-1, IL-6 and tumor necrosis factor (TNF)-α (Figure 1).[9]

It has been reported that some strains of *H. pylori* (namely 60190, 60190 VacA$^-$ and Tx30a) bound von Willebrand factor (vWF), a large multimeric GP (the basic vWF monomer is a 2050-amino acid protein), and interacted with GPIb to induce platelets aggregation in humans. Such aggregation was inhibited

completely by aspirin, GP IIb/IIIa antagonists and monoclonal antibodies, that prevented the vWF interaction with GPIb. Aggregation also required immunoglobulin G (IgG) directed against *H. pylori* and could be inhibited by an antibody to the platelets IgG receptor (FcγRIIA).[29] Moreover, studying endothelial function parameters, it has been shown that in patients with unstable angina and seropositivity to both *H. pylori* and *Chlamydia pneumonia* (*C. pneumonia*), antigenic kind of *H. pylori* was significantly correlated with an increased plasma concentration of vWF and plasminogen activator inhibitor type 1 (PAI-1).[30] The latter is a serine protease inhibitor (serpin) associated with elevated IHD risk.[31] However, another study did not find significant differences in platelet activation (measured also by P-selectin plasma level) or in inflammatory parameters after *H. pylori* eradication, in patients with recent acute coronary syndrome; nevertheless, cardiovascular events recurred in significantly less patients in whom the bacterium was absent or eradicated compared with subjects with persisting infection ($p = 0.01$).[32] In mice, *H. pylori* infection promoted the genesis of platelet aggregates. In particular, the bacterium evoked both marked increase in the flux of rolling leukocytes and the appearance of platelet and leukocyte-platelet aggregates in murine gastric venules; this process was abrogated by antibodies against L- or P-selectin.[33] The former participates in leukocytes rolling on the vessel wall as well as in the extravasation process during inflammation. The latter is an adhesion molecule restricted to the vascular system where it mediates the initial attachment of leukocytes to endothelial cells.[34] The same authors showed that circulating platelet aggregates and activated platelets were detected in 5 out of 5 *H. pylori* infected *versus* none uninfected humans.[33] Similar results were obtained by others groups.[35] Aguejouf *et al*. showed that, by damaging the mesenteric arteriole endothelium by Argon laser pulses, there was a significant increase in the number of platelet emboli ($p < 0.001$) and in the duration of embolization in mice chronically infected with *H. pylori versus* controls. A significant difference was also found in the fibrinogen levels between infected and uninfected mice.[36] Thus, on the basis of the available data, there are convergent evidences both in humans and in animal models that *H. pylori* could elicit platelet aggregation.[37]

The importance of finding increased thrombin generation rate and correlation of plasma prothrombin fragment 1+2 (comprising the first 271 residues of prothrombin) and TNF-α levels with *H. pylori* infection is uncertain, as these data have been obtained in subjects without IHD.[38] However, since thrombin plays a central role in haemostasis and thrombosis as well as in platelet function, fibrinolysis and vascular cell function[39], this issue is of paramount importance. Furthermore, the evidence that, after *H. pylori* eradication, fragment 1+2 and TNF-α levels decreased ($p < 0.05$) to the threshold observed in controls, suggests a potential role of the bacterium in the activation of the clotting system.[38]

Fibrinogen, a soluble 340 kDa plasma glycoprotein, is the ligand for GP IIb/IIIa to mediate platelet aggregation. Plasma fibrinogen is a well-known independent risk factor for arterial thrombotic disorders.[40] While some groups have reported a high level of fibrinogen in *H. pylori* infected patients[41,42], other studies failed to do so.[37,43] In a single-blind, randomized, prospective study, it has been shown that treatment for *H. pylori* and *C. pneumoniae* infections decreased fibrinogen plasma level ($p < 0.001$) in patients with IHD.[44] In a previous work, on *H. pylori*-positive patients with dyspepsia and/or PU, followed up for 5 years, fibrinogen decreased in a significant manner ($p < 0.0001$) after bacterial eradication (from 257 to 222 mg/dL).[45] Similar results were obtained by Yusuf and Mishra in elderly IHD subjects[46] and Ando *et al.*, who found, one year after eradication, a statistically significant fall in fibrinogen level that persisted throughout the 3 years' follow-up.[47] This contrasts with the findings of Elizalde *et al.*, who reported the benefit in preventing recurrence, after *H. pylori* eradication, in patients with acute coronary syndrome but failed to evidence a difference in plasma fibrinogen levels ($p = 0.8$).[32]

Studies on the immune response and antigen mimicry

The persistence of a chronic, low-grade inflammation, provides a vital clue for infectious pathogenesis of IHD. Chronic *H. pylori* infection induces a pro-inflammatory state, resulting into an increase in cytokines levels such as TNF-α, IL-1, IL-6, IL-8, γ interferon and soluble molecules like intercellular adhesion molecule (ICAM-1) and vascular cell adhesion molecule (VCAM-1). Furthermore, *H. pylori*

eradication leads to a reduction in cytokine levels.[48] The cytokines secreted by activated inflammatory cells can stimulate the endothelium, transforming its antiadhesive and anticoagulant properties into adhesive and procoagulant properties. Since dysfunction of endothelial cells is probably the earliest event in the process of lesion formation, the assessment of endothelial function may be a useful tool to better understand the physiopathology. But, is it possible that *H. pylori* participates in the genesis or in the consequences of endothelial dysfunction? Moreover, is this immune response able to maintain a pattern of chronicity? Some answers came from an elegant study conducted by Hatz *et al*. on gastric specimens. Considering that a prerequisite for trans-endothelial migration of leucocytes into the lamina propria of gastric mucosa is the primary adhesion of these cells to activated endothelium, the authors have shown that *H. pylori* was able to favor leukocyte-endothelial adhesion by increasing ICAM-1 and VCAM-1 levels.[49] Hence, whether the bacterium would be involved in extragastric manifestations such as IHD, the latter could be the consequence of a long-term inflammatory stimulus leading to a continual interaction between leukocyte and endothelial cells.

Strains of *H. pylori* are genetically diverse and have been divided into type I and type II according to the expression or not of cytotoxin-associated gene A (CagA) protein, which is encoded on the *cag* pathogenicity island.[50] The potential role of the more virulent *H. pylori* strain, named $CagA^+$, in the pathogenesis of IHD, has been investigated in epidemiological studies, with controversial outcomes.[51-54] From an experimental point of view, the ability of these strains to induce gastric epithelial IL-8 mRNA expression and IL-8 protein secretion results in neutrophil chemotaxis and activation with oxidative burst as an important pathogenetic factor. Thus, an intense immune response against $CagA^+$ *H. pylori* strains might play a role in the acute events of IHD.[55] Franceschi *et al.*, in an experimental study, have shown that anti-CagA antibodies reacted with cytoplasm and nuclei of smooth muscle cells in umbilical cord and atherosclerotic vessel sections, cytoplasm of fibroblast-like cells in intimal atherosclerotic plaques, and the cell membranes of endothelial cells. Anti-CagA antibodies also specifically immunoprecipitated two high molecular weight antigens of 160 and 180 kDa from both normal and atherosclerotic artery lysates.

[56] However, several studies have denied a higher prevalence of these strains in the case of IHD[9] and one has observed a significantly lower risk of cardiovascular mortality in subjects infected with *H. pylori* $CagA^+$ strains.[57] One speculative assertion might be that a constant stimulation of the immune system, in turn, might protect *H. pylori*-infected individuals from other acute infections that increase the risk of cardiovascular mortality.[58.59] Thus, the activation of the immune system alone by *H. pylori* may not be a sufficient pathogenetic mechanism able to induce the acute phase of IHD.

It is well-known that pathogen-triggered autoimmunity plays a role in early atherosclerosis, as antibodies to mycobacterial heat shock proteins (HSP)65 are associated with elevated levels of coronary calcification and correlate with *H. pylori* infection.[60] The lipopolysaccharide (LPS) of many *H. pylori* strains displays antigens similar to that of humans, such as Lewis x and y (the so-called antigen mimicry), which could induce antibodies that cross react with host tissues. This has been shown in humans, comparing anti-HSP60 levels in IHD patients and controls, and noticing that IgG level to HSP60 was more increased in the former than in the latter group.[61] Moreover, in a subset of patients with unstable angina, an intense immune response, for example against $CagA^+$, might act as a trigger for the precipitation of coronary instability via a molecular mimicry mechanism. Antibodies to CagA protein can act as autoimmune ones due, as described above, to cross-reactivity with the cytoplasm and the nuclei of myocytes existing in the coronary artery wall and cytoplasm of fibroblast-like cells in atherosclerotic plaques. Antigenic mimicry, such as immunological cross-reactivity between bacterial and HSPs, can lead to coronary calcification and early atherosclerosis. It has been shown that *H. pylori* HSP60, a 60 kDa oligomer composed of monomers that form a complex, arranged as two stacked heptameric rings, induced a specific T-helper 1 immune response associated with the progression of atherosclerosis in animal models.[62] To reinforce these data there was evidence that *H. pylori* eradication significantly reduced the progression of atherosclerosis in infected mice[9] and led to a significant fall in anti-HSP65 titres in patients with coronary atherosclerosis.[63]

Studies on atherosclerotic-related patterns

The link between *H. pylori* and atherosclerosis has been extensively explored by indirect (serological research for markers of metabolic alterations or systemic inflammation) and direct (bacterial research on the plaque) studies. Several researches have shown that *H. pylori* may induce atherogenesis though a low-grade persistent inflammatory stimulation[64,65]; however, other studies did not support this finding.[66] The potential implication of *H. pylori* into causing an atherogenic modified profile has been studied too. Based on some data, the levels of total cholesterol and low-density lipoprotein (LDL)[67] as well as of triglycerides[68] were increased in patients infected with *H. pylori*. Other studies showed lower high-density lipoprotein (HDL) cholesterol levels in *H. pylori*-infected patients compared to controls.[69] Huang *et al*. reported that more serious coronary atherosclerosis was observed in patients infected with *H. pylori* $CagA^+$ strains: in this case, the levels of total cholesterol, LDL, apolipoprotein B, CRP, oxidized LDL (oxLDL) were significantly elevated.[70] Some authors have proposed that the higher levels of cholesterol in *H. pylori*-infected patients may be a protective bacterial factor since it could enhance *H. pylori* resistance against antibiotic therapy.[71] The issue of the change in lipid profile after *H. pylori* eradication has been explored. An improvement has been obtained by some[45] but not all groups.[9] These results, although equivocal, suggest that *H. pylori* infection in different populations could be associated either with elevated total cholesterol or LDL and lower HDL and apolipoprotein A and B. The same consideration can be applied to triglycerides.[48]

The convergent role of inflammatory and metabolic pathways, stimulated by oral infection with *H. cinaedi* (a nongastric emerging *H. species*), in the pathogenesis of atherosclerotic plaque has been shown by Khan *et al*., who found that this infection significantly enhanced atherosclerosis in hyperlipidaemic mice. Aortic root lesions in infected mice showed increased accumulation of neutrophils and foam cells, which was due, at least partly, to bacteria-mediated increased expression of proinflammatory genes. Although the mice were asymptomatics, the detection of cytolethal distending toxin RNA of *H. cinaedi* indicated aorta infection. Moreover, *H. cinaedi* infection altered expression of cholesterol receptors and

transporters in cultured macrophages and caused foam cell formation. Also, infection induced differentiation of THP-1 monocytes into macrophages.[72]

Coronary artery calcium (CAC) levels reported by computer tomography scans are widely accepted as reproducible and reliable markers for IHD risk.[73] One study found that seropositivity for *H. pylori* IgG was significantly related to the presence of CAC.[74] This suggests that the bacterium could play a role in early atherosclerosis. The limitation of this study is due to the gap between *H. pylori* seropositivity and the possibility that this reflects a true infection.[75] A prospective, serological study on 5744 individuals, found that none of the five pathogens investigated or their accrual was associated with CAC incidence and progression.[76]

The fact that *H. pylori* DNA was identified in atherosclerotic plaques of patients with severe CHD would support the hypothesis of the association.[77] Some studies, using the polymerase chain reaction (PCR) technique have shown that a substantial proportion of coronary atherosclerosis plaques were colonized by *H. pylori.*[78-80] Kowalski *et al*. found 47.8% *versus* 0% of *H. pylori* DNA in coronary artery of patients with and without IHD, respectively.[77] Adiloglu *et al*., searching for *H. pylori* DNA in atherosclerotic plaques of 14 coronary endarterectomy specimens, detected it in 3 out of 14 specimens (21.4%) *versus* one out of 15 (6.7%) in the control group (left internal mammarian artery samples without any plaques). The difference was not statistically significant. With regard to the level of CRP, apolipoprotein B and IL-6, no significant difference was shown between *H.pylori* DNA positive and negative subjects. However, both CRP and IL-6 levels were higher in case of positivity.[81] These findings "revitalize" the question of the possible existence of the bacterium in the plaque and on its role. Revisiting the literature, *H. pylori* genomic material has been detected in the plaque only in sporadic occasions, in contrast to *C. pneumoniae,* the other agent often implicated in the pathogenesis of IHD. [78,82,83] Hence, due to the rarity of findings supporting a "direct" mechanism, the involvement of *H. pylori via* an indirect pathway has been proposed.[75]

Studies on homocysteinemia involvement

Chronic *H. pylori* infection strongly increases the risk of chronic atrophic gastritis.[84] Once the latter is present, gastric mucosa functions are impaired resulting in a reduced secretion of pepsinogen I (PGI) and II (PGII) and malabsorption of essential metabolites like vitamin B12[85], a condition accompanied with high serum homocysteine levels.[86] The increased pH of the gastric juice and the decrease of ascorbic acid levels lead to a reduced folate absorption. Low folate hampers the methionine synthase reaction determining an increased concentration of homocysteine in the blood. The last step, in the proposed causal pathway from *H. pylori* and chronic atrophic gastritis to coronary artery disease, is the impact of homocysteinemia, which induces damage to endothelial cells, atherosclerosis and finally, IHD.[87] Moreover, it has been reported that there may be an association between slow coronary flow, decreased plasma folate levels and increased homocysteinemia. This is interesting considering that the prevalence of *H. pylori* is increased in patients with slow coronary flow and there is a significant correlation between the former and folate reduction.[88]

Potential clinical implications

Several reports have demonstrated that antibiotic therapy can reduce relapse and mortality after an episode of acute myocardial infarction[89,90], thereby reinforcing the hypothesis of a link between bacterial infections and IHD.[91] Although encouraging, these data have been obtained from small pilot studies.[89,90] A double-blind, randomised, placebo-controlled trial, aimed to determine in patients with myocardial infarction or unstable angina whether antibiotics active against *C. pneumoniae* and *H. pylori* had beneficial effects on the development of cardiac death or re-admission with acute myocardial infarction or unstable angina. After 12 weeks, the proportion of patients treated with antibiotic regimen experienced an endpoint was 17.2% as compared with 27.2% in the placebo group ($p < 0.02$). By 52 weeks, these respective proportions were 25.8% and 38.9% ($p < 0.02$). There was no significant difference in total

mortality. The beneficial effect of antibiotics on cardiac events was independent of either *H. pylori* or *C. pneumonia* status. The authors concluded that the design of the trial did not allow conclusions to be drawn regarding the mechanism responsible for the positive effects observed.[92] Opposite results were obtained by the ANTIBIO Study, which did not observe a reduction in event rate 12 months after treatment with roxithromycin, in patients with acute myocardial infarction.[93]

Considering these controversial data, the need for extensive studies with appropriate epidemiological and clinical approaches is evident, to investigate a potential causal relationship between *H. pylori* infection and IHD. These should be prospective and randomised intervention trials, based on antimicrobial treatment and long-term follow-up for cardiovascular events, to evaluate an eventual delayed benefit. How might experimental data lead clinical studies? In this direction, experimental models could help to define under which conditions these trials should be performed. It may be that the optimal population would include patients with atherosclerosis and *H. pylori* infection, to assess if bacterial eradication could prevent the progression of a silent IHD or the trigger of an acute cardiac event. Nevertheless, the inclusion of patients with a previous history of acute myocardial infarction, would permit to assess if bacterial eradication could reduce its recurrence rate.[94,95] Obviously, the latter strategy should be applied considering the standard cardiology treatment. It is more exciting, but improbable, the possibility to assess if *H. pylori* eradication could induce a benefit in terms of primary prevention, in subjects without cardiovascular abnormalities. Moreover, since at least in the animal model a relationship between intestinal microbiota and myocardial infarction has been shown[96], and considering that the human bowel is the largest reservoir of microbes (it contains trillions of bacteria)[97] in the body, with remarkable relevance for the human health[98], this should be accompanied with the modulation of the physiologic intestinal microbiota.

Conclusions

Although currently available data do not prove in an unequivocal manner the role of *H. pylori* infection in the pathogenic mechanism of CHD, any potential relationship cannot be ruled out. Numerous risk factors contribute to IHD, a portion of which is still not known. From the data currently present in the literature, *H. pylori* might contribute with two alternative pathways. First, it could participle in initial steps of formation of atherosclerotic plaque. Second, the bacterium could trigger the acute onset of IHD by stimulating platelet aggregation.

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Legend

Figure 1: a proposed model of interaction between *Helicobacter pylori* and cytokines in the induction of a procoagulative condition.