Probiotics in *Helicobacter pylori* gastric infection treatment: Mechanisms of action

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Introduction

Helicobacter pylori (*H. pylori*) infection is considered as the main cause of chronic gastritis,[1] peptic ulcer[2] and a risk factor for gastric malignancies.[3] According to several international guidelines, treatment consisting of two antibiotics and a proton pump inhibitor is regarded as a treatment of choice to eradicate *H. pylori*. However despite high efficacy of this regimen, some limits have been reported such as high cost, side effects and antibiotic resistance. For this reason triple therapy is not recommended in most infected subjects such as "healthy" asymptomatic carriers and dyspeptic patients without ulcers.[4]

Probiotics defined as live micro-organisms that when ingested in adequate amounts exert health benefits on the host have been used in the management of various medical disorders and particularly gastrointestinal pathologies. At present the most studied probiotics are lactic acid-producing bacteria particularly *Lactobacillus* species.[5] The most well documented effects of probiotics on gut diseases coming from clinical trials as well as from experimental studies concern acute infectious diarrhoea or pouchitis.[6] However, their use has been proposed and is still investigated against *H. pylori* infection. The clinical outcome of *H. pylori* infection is determined by several factors including the type of *H.*

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pylori strain, the extent of inflammation and the density of *H. pylori* colonization.[7] Further, the risk of peptic ulcer disease development and gastric cancer increases according to increasing level of infection.[8] Therefore, permanent or long-term suppression of *H. pylori* could decrease the risk of *H. pylori*-related diseases development. Consequently, there is increasing interest in developing low-cost large-scale alternative solutions to prevent or decrease *H. pylori* colonization. In this respect probiotics may close the therapeutic gap.

Mechanisms of action

The rationale of probiotic use, in a general way, is related to two general concepts concerning their mechanism of action. First probiotics are able to protect against abnormal growth of pathogenic intestinal micro-organisms and second they are able to enhance intestinal barrier function. We will develop paradigms related to these two concepts.

Antimicrobial activity

Probiotics may inhibit *H. pylori* growth by secreting antibacterial substances. This class of substances include compounds related to the bacteriocin family secreted by certain lactobacilli,[9] or other known substances as the endproducts of lactic acid fermentation, such as lactic and acetic acids and hydrogen peroxide.[10] Lactic acid in addition to its antimicrobial effect resulting from the lowering of pH could also inhibit *H. pylori* urease. It is important to note differences in the inhibitory effect on *H. pylori* between different lactobacilli strains. For example, *L johnsonii* La10 does not inhibit *H. pylori* although it produces similar lactic acid amount as *L johnsonii* La1.[11] On the other hand other strains, such as *L acidophilus*, *L casei* and *L lactis* exert an inhibitory effect on *H. pylori* by a lactic acid and pH-independent pathway.[12-14] The involvement of proteinaceous compounds in this inhibitory effect has been reported in the literature,[11-15] but their exact nature remains to be determined. In the case of *L subtilis* the antimicrobial substances secreted have been found similar to aminocumacins belonging to the isocoumarin group of antibiotics.[16]

The adhesion of *H. pylori* to epithelial cells is important in determining the outcome of *H. pylori*-associated diseases. In vitro studies report that *L johnsonii* La1, *L salivarius* and *L acidophilus* inhibit the attachment of *H. pylori* to intestinal HT-29 or to MKN 45 gastric lines.[11,14,17,18] There are several possible mechanisms by which probiotics can inhibit the adhesion of *H. pylori*. Certain lactobacilli (*L johnsonii* La1, *L acidophilus*...) can exert their antiadhesion activity by secreting antimicrobial substances[11,14] and others (L reuteri, W confuse...) can inhibit *H. pylori* growth by competing with adhesion sites.[17,19] For example, *L reuteri* inhibits the binding of *H. pylori* to specific glycolipid receptors asialo-GMI and sulfatide.[19] However, a non-specific rather than specific blockage of receptor sites is involved since lactobacilli can inhibit adhesion of large varieties of pathogens. In vivo

studies have shown that previous colonization by probiotics prevented or reduced *H. pylori* infection in germ-free mice.[18,20] Consequently, probiotics could prevent *H. pylori* colonization of the gastric mucosa by inhibiting its adhesion to epithelial cells.

Mucosal barrier enhancement

Gastric barrier including a physical (mucus, permeability...) and a functional (mucosal immunity) component represents the first line of defence against pathogens.

Reduced mucus secretion is frequently observed in *H. pylori*-associated gastritis. Further *H. pylori* suppresses MUC1 and MUC5A gene expression in human gastric cell line.[21] Strains such as *L rhamnosus* and *L plantarum* increase the expression of MUC2 and MUC3 genes expression[22] and the subsequent extracellular mucin secretion by colon cell cultures.[23] Trough this pathway these strains can restore the mucosal permeability of gastric mucosa or inhibit the adhesion of pathogenic bacteria including *H. pylori*.

The inflammatory response to gastric *H. pylori* infection is characterized by the release of proinflammatory mediators including chemokines and cytokines. The cytokine response is initially expressed by the release of interleukin 8 (IL-8) which results to neutrophils and monocytes migration to the inflamed mucosa. Probiotics could modify the immunologic response of the host by interacting with epithelial cells and modulating the secretion of anti-inflammatory cytokines which may result in reduction of gastric activity and inflammation.[24] In vitro *L salivarius* has been shown to inhibit the *H. pylori*-stimulated secretion of IL-8 by epithelial cells.[18] In vivo studies report that probiotic treatments can regulate the balance of pro-inflammatory and anti-inflammatory cytokines resulting in a reduction of gastric inflammation.[25,26] Further, a decrease in specific IgG antibodies to *H. pylori* infection following probiotic intake parallel to a reduction in gastric inflammation has been observed in animal studies.[27,18] Finally, probiotics have been shown to enhance the mucosal barrier by stimulating local IgA responses, leading to a mucosa-stabilizing effect.[28]

Clinical trials

Based on the resistance of certain lactobacilli to the low gastric pH, suggesting possible adhesion and transient residence in the human stomach, clinical trials have been undertaken in order to determine the effect of probiotic treatments on *H. pylori* gastritis. Nine fully published trials investigated this effect.[11,13,29-35] The most frequently used strain was *L johnsonii* La1, either in a fermented milk formula or as a free cell culture supernatant. Other probiotics used were *L casei*, *L brevis* and *L gasseri*. Two studies used yogurts containing mixtures of live probiotics. Seven of the nine studies showed a statistically significant effect of probiotic treatment on *H. pylori* gastritis. No study reported the eradication of *H. pylori* infection by probiotics.

Conclusions

In conclusion, animal and human studies show that probiotic intake improves *H. pylori* gastritis and reduces *H. pylori* density. This effect although statistically significant remains weak. On the other hand, no *H. pylori* eradication has been reported by probiotic treatment neither in human nor in animal studies. The putative mechanisms of beneficial effect of probiotics against *H. pylori*-associated gastric inflammation emerging from the literature depend on strain used and on host's immune status. They can be summarized as follows: (i) inhibitory effect of certain probiotics related to lactic acid and/or to other antimicrobial substances yet to be identified (ii) inhibition of the *H. pylori* adherence to epithelial cells preventing *H. pylori* colonization (iii) enhancement of gastric mucosa barrier by promotion of mucin secretion, and subsequent reduction of gastric mucosal permeability, as well as modulation of the immune response. Finally, long-term intake of probiotics may have a favourable effect on *H. pylori* infection in humans, particularly by reducing the risk of developing disorders associated with high levels of gastric inflammation.

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