Helicobacter pylori bacteria: Tools for Eradication

By: Rachel Olivier, MS, ND, PhD

The mammalian intestinal tract contains a complex, dynamic, and diverse society of nonpathologic bacteria. Undeniably, the number of bacteria that colonize the human body is so large that researchers have estimated that of the total number of cells in the human body, estimated at 10^{14} (100 trillion) cells, only 10% are not bacteria and belong to the human body proper.[1] Indeed the human large intestine is portrayed as a complex microbial ecosystem.

Many of these bacteria serve beneficial purposes in the intestinal tract, such as assisting with digestion, and in the making and processing of both short-chain fatty acids (SCFA)[2, 3, 4] and amino acids, in addition to their vital association with the intestinal epithelial cells, which affords immune homeostasis.[5] Additionally, recent evidence has singled out the epithelial cell-bacterial interaction, and its function in inhibiting the inflammatory signaling cascades via its action on blocking the activation of NF-κB.[5] In spite of these beneficial functions, the intestinal tract may become populated with noncomensal flora, one of these species being *Helicobacter pylori* (*H. pylori*) (formerly *Campylobacter pylori*), resulting in gastric distress and suboptimal gastric function. Bacterial imbalance in the colon has been described in IBD,[6] as well as in association with the development of a colitogenic flora.[5] Colonization of the gastric mucosa by non-commensal flora, including *H. pylori*, has been associated with diminished gastric health and associated illnesses.[7] An added compounding factor associated with *H. pylori* infection is iron deficiency anemia, established as an independent risk factor in both adolescent children[8] and adults.[9]

The bacterium *H. pylori* is characterized as a flagellated, curved or S-shaped gram-negative rod[10] that is able to penetrate the gastric mucosa and colonizes the gastric epithelium of humans, resulting in persistent infection with possible complications.[11] The parietal cells of the stomach secrete profuse amounts of hydrochloric acid, which serves to maintain the acidic environment stomach, characteristically at a pH of less than two (<2).[12] Despite this highly acidic environment, *H. pylori* is able to persist, thought to be due primarily to its characteristic spiral morphology, and its high motility.[13] Acute *H. pylori* induced gastritis is associated with hypochlorhydria,[14] and colonization is speculated to have the ability to modify the net gastric acidity, but virtue of the substances the bacterium secretes.[15,16] *H. pylori* infestation is associated with a dysregulation in the function of the gastric epithelial barrier,[17] as well as with increased epithelial permeability.[18] Once acquired, *H. pylori* can inhabit the human stomach for years, decades, or possibly for life.

By design the stomach is able to withstand a highly acidic state. As noted above the optimum pH in the stomach is below 2.0. In addition to its digestive nature, this low pH serves several other purposes, including the ability to destroy incoming bacteria, yeasts or parasites, the ability to release intrinsic factor for the absorption of vitamin B12, the capability to absorb minerals, to trigger the release of enzymes and hormones by the pancreas and intestines, and to trigger the enzyme pepsin, which is utilized in the breakdown of proteins into amino acids. Besides these, a more debilitating consequence resulting from the lack of or suboptimal gastric pH is undigested proteins, which make their way into the intestines. These proteins in turn trigger an immune response, which results in the production of inflammatory substrates. Consequently, this may ultimately result in food allergies or more seriously "leaky gut,"[19] which is correlated to the consequent production of inflammatory cytokines and prostaglandins. Thus, the root cause of infection with *H. pylori* may indeed be a deficiency in the production of hydrochloric acid.[19]

As a result of the common use of both acid suppressing drugs and non-steroidal anti-inflammatory drugs (NSAID), an escalating incidence in the colonization by *H. pylori* has been observed. The allopathic medical community associates the colonization of *H. pylori* with the development of peptic ulcers, gastroduodenal inflammation, and gastric carcinoma,[20, 21] in addition to disease conditions such as ankylosing spondylitis,[22] inflammatory bowel disease,[23] and idiopathic thrombocytopenic purpura.[24] The risk for colonization is also increased with immunosuppressant type therapies, including the use of drugs such as corticosteroids or prednisone.[25, 26] Oxidative stress is another factor implicated in the pathogenesis of *H. pylori*, said to result as a consequence of the associated cellular damage due to colonization of the bacterium. This activity is postulated to result due to either an increase or decrease in the expression of specific proteins, which are associated with cell proliferation, carcinogenesis, cytoskeletal function and cellular defense mechanisms.[27]

In regards to NSAID use, *H. pylori* infection is indicated as an independent risk factor for the development of peptic or bleeding ulcers, with additive implications indicated when these factors are correlated.[28] Added to the use of acid suppressing drugs, *H. pylori* infection is associated with lower acid secretion, attributed to the induction of an immune response, resulting in the synthesis and production of interleukin 1 (IL-1), which is a potent inhibitor of gastric acid secretion.[29] The overuse of acid suppressants, which in reality may offer little to no benefit for the patient, further compounds this scenario. In fact it has been documented that patients on proton pump inhibitors demonstrated benefits from the approved indications of these drugs in only 37% of the cases.[30]

The fact that *H. pylori* can live and survive in the hostile acidic environment of the gastric mucosa is proposed to result as a consequence of a pH gradient across its cell envelope.[31] It also possesses the ability to produce unusually high levels of urease, which is presumed to be critical for its colonization. High levels of urease result by virtue of its capability to hydrolyze urea, resulting in the production of ammonia. As a consequence of its ammonia producing ability, a thin acid-neutralizing layer, or "cloud" is formed around the bacterium, which in turn protects it from destruction by the acidic environment of the gut.[32] The production of urease, therefore, has been associated with a protective effect on the bacterium.[33]

There has been a growing interest in a phytochemical approach to the eradication this bacterium, primarily due to the decline in efficacy of the currently used drugs for treatment. Specific botanical preparations have documented benefits in supporting gastrointestinal health, as well as in mitigating *H. pylori*.

Phytochemical Support for Bacterial Eradication

Berberine. Berberine is isolated as the main alkaloid derived from the roots and bark of *Berberis vulgaris*, a deciduous shrub, native to Central and Southern Europe, Northwest Africa and Western Asia. Significant antimicrobial activity against a variety of organisms, including bacteria, viruses, fungi, parasites (including *Giardia lamblia,* and *Entamoeba histolytica*) protozoans, (including *Trichomonas vaginalis*[34]) helminths, and chlamydia has been documented with Berberine extracts and decoctions.[35, 36]

The inhibitory effect of berberine on the growth of *H. pylori* is well recognized[37] and its antiproliferative action, in a dose-dependent manner, has been confirmed. The later attribute has been correlated to its action via the mitochondrial/caspase-dependent pathway.[38] *In vitro* studies have demonstrated berberine's ability to interact with nucleic acids, in particular DNA.[39] Berberine has also been demonstrated to induce G0/G1 cell cycle arrest in an animal model,[40] to selectively inhibit the cell cycle at G2/M in a cell line model (Balb/c 3T3),[41, 42] and to decrease experimentally induced apoptosis in a concentration- and time-dependent manner.[43, 44]

Wild Indigo (*Baptisia tinctoria*). *Baptisia tinctoria*, commonly known as Wild Indigo, is an herbaceous perennial herb. The herb is noted for both its astringent[45] and immune stimulating properties.[46,47] Traditionally used by North American Indians for its antiseptic properties, it is also a noted antibacterial cleansing agent.[48,49] It has been associated with the promotion of normal cellular metabolism and in the support of healthy tissues.[50]

The chemical constituents of the root consists of glycoproteins, quinolizidine alkaloids, including cytisine and anagyrine, isoflavanoids, hydroxycumarins, and polysaccharides, including arabinogalactans.[51] Use has been associated with supporting the body's natural resistance to microorganisms and toxins via its activation of macrophages, and due to an increased production of interleukin-1. In animal studies the polysaccharide and glycoprotein fraction was demonstrated to produce an immune-stimulating effect.[51] Other research supports the use of arabinogalactan components for their anti-inflammatory and immunostimulating properties.[52]

Licorice (*Glycyrrhiza glabra*). Licorice is a perennial herb or sub-shrub containing numerous active compounds, including flavonoids, triterpene saponins, isoflavonoids and hydroxycoumarins. Of these, the component possessing the greatest activity is the triterpenoid saponin glycyrrhizin (GL), having demonstrated antiviral[53, 54, 55, 56] antimicrobial[57] and antifungal[58,59] properties.

In animal studies GL was demonstrated to stimulate interferon gamma production by T-cells,[60] resulting in an antiviral effect,[61] and to augment the activity of natural killer cells.[62] In the human intestinal tract GL is converted primarily to its biologically active metabolite glycyrrhetinic acid (GA), and to a lesser extent to glycyrrhetinic acid-3-O-beta-D-glucuronide (GAMG) by the intestinal microflora.[63,64] Both GL and GA have demonstrated anti-inflammatory properties.[63,65] GL has also been demonstrated to impair the growth of *H. pylori in vivo*, via the inhibition of the activity of arylamine N-acetyltransferase (NAT family of enzymes).[66] This family of enzymes functions to catalyze the transfer of acetyl groups from acetyl-coenzyme A to an aromatic amine, heterocyclic amine or hydrazine compound.[67] In a separate *in vitro* study GA, at a concentration of ≤50mg/L, was demonstrated to inhibit 79% (23/29) of the *H. pylori* stains tested, including two clarithromycin-resistant strains, noting both concentration- and strain-dependent bactericidal effects of GA.[65] A more recent *in vitro* study utilizing PC12 cells demonstrated that treatment with GA resulted in a neuroprotective effect, by virtue of a decrease in ROS, via the elevation of glutathione peroxidase and catalase, with a corresponding decrease in mitochondrial membrane potential. The authors concluded that GA treatment may play a role in modulating both the intracellular antioxidant system and mitochondria-induced apoptosis, resulting in cellular protection from ischemic injury.[68]

Clove (*Syzygium aromaticum*). Clove is a plant indigenous to the Moluccan Islands of Indonesia. It is cultivated on these islands, as well as in other tropical regions, including Tanzania, Madagascar and Brazil. Its active compounds include its volatile oils, flavonoids, tannins, triterpines and steroids, including beta-sitosterol.[69] The main component eugenol, comprising 85-95%, has been associated with the prevention of lipid peroxidation[70] and is recognized as a strong scavenger of active oxygen radicals.[71] It is considered an effective antimicrobial agent and has use as an antiseptic.[72]

A number of studies have demonstrated clove's activity against various bacterial species,[73] including *Bacillus subtilis*,[74] *Campylobacter jejuni*, *Salmonella enterides*, *Staphylococcus aureus* and *E. coli*.[75] An aqueous infusion of cloves was demonstrated to restrict cellular invasion, resulting in a notable reduction in the incidence of skin papilloma in animals, as well as in the multiplicity of the growth, in a dose dependent manner. At the most effective oral dose (100 μl/day), no adverse or toxic effects were noted.[76] It has also been specifically demonstrated to inhibit the growth of *H. pylori*.[77] Clove is approved by the German Commission E for use as a topical antiseptic and as an anesthetic for inflammation.[78]

Slippery Elm (*Ulmus fulva*). Slippery Elm has been used for centuries by North American Indians for skin irritations including, wounds, boils, ulcers, burns, and skin inflammation, as well as orally for the relieve of coughs, sore throats, diarrhea, and stomach problems.[79] The powdered inner bark has mucilaginous qualities, which is indicated for relief of irritation of the mucus membranes, and may be useful for treating irritation or ulceration of the stomach lining and duodenum.[80] Its mucilaginous characteristics also contribute to its noted action, that of acting as a coating. In this regard it acts to soothe the mouth, throat, stomach, and intestines.[79]

Barberry (*Berberis vulgaris*). *B. vulgaris* is native to most of Europe. Its root bark contains several isoquinoline alkaloids including berberine, berbamine, and oxyacanthin. The root bark is also a source of vitamin C (citric acid), and contains chelidonic, malic and tartaric acids.[81] It possesses mild diuretic qualities,[82] and also has confirmed anti-inflammatory properties.[83] Furthermore, studies have validated the anti-inflammatory action of berberine, demonstrating a significant down-regulation in the expression of pro-inflammatory genes including TNF, IL-1, IL-6, MCP-1, iNOS and COX2 with treatment.[84, 85]

Berberine, a major alkaloid of Barberry possesses anti-inflammatory properties, having demonstrated to effectively inhibit, in a dose- and time-dependent manner, COX-2 transcriptional activity in a malignancy cell model,[86] as well as to reduce prostaglandin E2 (PGE2) production. The latter effect was noted to also result in a reduction of COX-2 protein production.[87] In a separate study berberine was demonstrated to decrease both the expression and protein binding on the hypoxia-response element of the vascular endothelial growth factor (VEGF). VEGF is a critical growth factor in tumor angiogenesis.[88] Based on *in vitro* studies, it was proposed that berberine's action *in vivo* may be 'in abolishing the angiogenic function of vascular endothelial cells, preventing them from responding to the call for angiogenesis, and may also prevent hypoxic tumor cells from inducing angiogenesis,' thus retarding cellular proliferation. In fact hypoxia induced VEGF expression was demonstrated to be completely inhibited by berberine.[89]

Myrrh (*Commiphora molmol*). *C. molmol* is a highly valued botanical medicine in Ayurveda, the Indian system of medicine, as represented by the wide variety of Ayurvedic formulas containing Myrrh. In Arab medicine, *C. molmol* is used as part of a polyherbal formulation to improve digestion and to treat gastrointestinal maladies.[90, 91, 92] Its action is attributed to its positive effect on inflammation.[93]

In studies utilizing myrrh, a wide range of inhibitory action against both Gram (+) and Gram (-) bacteria has been demonstrated.[94] Myrrh, in a dose-dependent manner, was also demonstrated to protect the gastric mucosa against the necrotizing effects of various agents. This protective action was attributed to its positive effect on mucus production, as well as to its ability to increase both nucleic acid production, and the concentration of non-protien sulfhydryl compounds, noted for both their involvement in maintaining gastroduodenal integrity, as well as in the protection they offer against chemically-induced lesions in cells, tissues and organs.[95, 96, 97] These actions were associated to Myrrh's ability to scavenge free radicals, as well to its thyroid-stimulating and prostaglandin-inducing properties.[98] Endogenously produced prostaglandins have been attributed to functioning as activators of potassium ATP channels, which has been demonstrated in part to mediate gastroprotection.[99]

Oregon Grape (*Berberis aquifolium*). The major components of Berberis are berberine, a yellow colored isoquineoline alkaloid, beramine and oxyacanthine, which are both white alkaloids, along with phytosterin, gum and sugar. As noted above the medicinal value of Berberis is thought to be due to its high content of isoquineoline alkaloids, especially berberine, which is postulated to have antibiotic activity.[100] Its actions are noted in promoting excretion and secretion, improving digestion and assimilation, and in stimulating the lymphatic system. It is documented as having an 'invigorating power over the gastric functions.'[101]

Select Minerals as Beneficial Adjuncts for Bacterial Eradication.

Bismuth citrate. Bismuth is a naturally occurring mineral that has documented activity against *H. pylori*.[102, 103] Bismuth salts have been used extensively for the alleviation of gastrointestinal irritation, as well as for irritation of the stomach and bowels.[104] Standard preparations of bismuth are used to decrease the flow of fluids and electrolytes into the bowel, to reduce inflammation within the intestine, and may serve to alleviate the diarrhea causing organism.[105] Preparations are recognized as safe when taken as directed.[106]

Bentonite clay. Bentonite is an absorptive and colloidal clay, demonstrated to be a very effective absorber of toxins.[107] In one study a hepato-nephro (liver-kidney) protective effect

against aflatoxins was demonstrated with bentonite use, indicating that it 'diminished most of the deleterious effects of the aflatoxin.' This effect was attributed the suppressive effect it exerted against chromosomal aberrations.[108]

The inflammatory response to *H. pylori* is well documented, implicated to result in cellular proliferation and gastric mucosal damage. *H. pylori* components have been demonstrated to act directly on gastric epithelium and to induce an increased release of cytokines.[109] These actions have been primarily attributed to the up-regulation of inflammatory markers, including COX-2 and IL-1β.[110] To curtail this deleterious effect on the gastric mucosa, a comprehensive blend of phytochemical nutrients, known to have a positive impact on the gastrointestinal tissues, may aide in healing and repairing these tissues. The eradication of *H. pylori* is indeed beneficial, specifically when coupled with gastric complications, as recently demonstrated in a Japanese trial involving 544 patients with early gastric cancer. In this study the eradication of *H. pylori* was associated with a greatly reduced risk for recurring gastric cancer.[111] Select herbs and minerals are recognized as possessing anti-pylori activity, noted to downregulate inflammatory markers associated with *H. pylori* infestation and correlated to gastric inflammation. Added to this, the use of specific herbal components has demonstrated a positive effect in reducing the antigens associated with *H. pylori*.

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