Effect of antioxidants on the immune response of *Helicobacter pylori*

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Antioxidants are substances capable of inhibiting oxidation. In chronic diseases, inflammatory response cells produce oxygen free radicals. Oxygen free radicals cause DNA damage, and this may lead to gene modifications that might be carcinogenic. Chronic *Helicobacter pylori* infection causes the production of DNA-damaging free radicals. In recent years, various groups have studied the effects of antioxidants, especially on *H. pylori*-associated gastric cancer. In most of the studies, it has been shown that *H. pylori* infection does affect the level of antioxidants measured in the gastric juice, but there are also controversial results. Recent experimental studies, both in vivo and in vitro, have shown that vitamin C and astaxanthin, a carotenoid, are not only free radical scavengers but also show antimicrobial activity against *H. pylori.* It has been shown that astaxanthin changes the immune response to *H. pylori* by shifting the Th1 response towards a Th2 T-cell response. Very few experimental studies support the epidemiologic studies, and further studies are needed to describe the effect and the mechanism of antioxidants in the *H. pylori* immune response.

Keywords *Helicobacter pylori*, antioxidants, immune response

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Antioxidants, due to their important role in chronic processes such as cardiovascular diseases, cancer, inflammatory bowel disease and Alzheimer's disease, as shown by many multidisciplinary research groups, have become an important research topic [1–3]. Antioxidants are substances capable of inhibiting oxidation that can easily give away an oxygen molecule without requiring much energy. An ideal antioxidant should be stable and should be effective over a wide pH range. Oxidation is the addition of oxygen or the removal of hydrogen. The chain reaction of oxidation requires a minimum amount of oxygen. An antioxidant can stop the chain reaction by giving away an electron without changing its stability [4,5]. Fruit and vegetables are the main source of antioxidant nutrients, especially vitamin C, vitamin E and carotenoids [6].

In chronic diseases such as *H. pylori* infection, the active inflammatory response is induced with neutrophilic infiltration. These neutrophils, macrophages and/or monocytes produce oxygen free radicals that can cause DNA damage to the adjacent cells. The DNA damage provoked by oxygen free radicals could have very harmful consequences, leading to gene modifications that are potentially carcinogenic [7]. Free radicals play an important role in the pathogenesis of gastroduodenal mucosal inflammation, peptic ulcer disease, and probably, even gastric cancer [8]. Factors that are suspected to influence the progression of this chain of events are: infection with *H. pylori*, a major cause of chronic gastritis; excessive salt intake, which is known to induce atrophy in experimental animals; increased cell replication, which potentiates the effect of gastric carcinogens; and carcinogens acting on the gastric epithelium, such as *N*-nitroso compounds and free oxygen radicals [3]. Infection with *H. pylori* causes gastritis, and with excessive salt intake, atrophy evolves,

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which is the first stage of carcinogenesis. Antioxidants have the potential to neutralize DNA-damaging free radicals, which are generated by various factors, such as chronic *H. pylori* infection, and might therefore lower the risk of gastric cancer [9]. Most of the antioxidant studies, performed with vitamin C (ascorbic acid), reveal that *H. pylori*-infected patients have lower gastric ascorbic acid levels. Ascorbic acid might act to reduce the risk of gastric cancer in two ways: (1) it is a good nitrite scavenger and could thereby reduce the endogenous formation of carcinogenic *N*-nitroso compounds in the stomach lumen by its presence in gastric juice; and (2) high mucosal concentrations of ascorbic acid may be important in limiting free radical-mediated damage within the epithelium, such as *H. pylori*-associated gastritis [10]. After the oral intake of vitamin C, the reduced form of ascorbic acid (AA) is oxidized to dehydroascorbic acid (DHAA). DHAA is absorbed in the small intestine and then reduced again by blood cells to AA. From the blood, it is secreted into the stomach and excreted in the urine. In the stomach at a pH above 3, AA is oxidized to DHAA [11]. In subjects with *H. pylori* infection, AA becomes impaired, the vitamin C concentrations in gastric juice become subnormal, and a significant proportion is found to be the inactive oxidized form of DHAA [12–14] (Figure 1).

Most of the human studies related to gastric cancer show that the cancer risk is reduced by ascorbic acid intake in a dose-dependent way [15]. In *H. pylori*-infected patients, the ascorbic acid levels are lower than in the normal population [14,16,17], but in some studies there was no change in ascorbic acid levels according to their *H. pylori* status [18]. These controversial findings could be due to the methods employed for measuring the ascorbic acid or vitamin C levels in different studies. Gastric juice vitamin C levels are associated closely with gastric pathology, but there is no association with plasma levels [12]. *H. pylori* infection has been associated with reduced levels of both vitamin C and ascorbic acid in the gastric juice but not in the plasma [14]. Lipid-soluble antioxidants, like vitamin E, either have no obvious role in protecting mucosal cells from free radical damage or they do indeed have scavenging potential against free radicals. However, they can be rapidly converted back to their original form by redox or other processes [19]. In a study by Zhang et al. it was shown that vitamin C is not only an antioxidant and a free radical scavenger, but it also shows antimicrobial activity both in vitro and in vivo. The minimal inhibitory concentration values of vitamin C for 64 *H. pylori* strains were tested by the agar dilution method, and vitamin C showed an inhibitory effect dependent on pH, whereas vitamin E showed no effect on *H. pylori* growth. The in vivo experiment performed on Mongolian gerbils suggested that a high dose of vitamin C had an inhibitory effect on *H. pylori* colonization in the gerbils' stomachs [20]. In the study performed by Wang et al. the effects on *H. pylori* infection of the lipid-soluble antioxidant astaxanthin, which is a carotenoid, found in seafood, especially salmon, and a water-soluble antioxidant, vitamin C, were tested both in vivo and in vitro. Mice treated with astaxanthin-rich meals or vitamin C showed significantly lower colonization levels and lower inflammation scores than those that were untreated. Lipid peroxidation was also significantly decreased compared to the untreated group. Both astaxanthin and vitamin C showed an inhibitory effect on the in vitro growth of *H. pylori* [21].

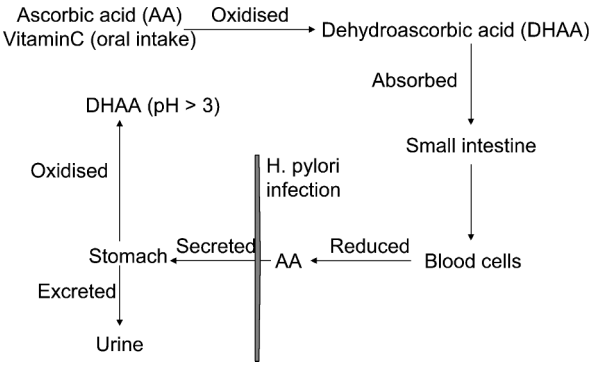

Figure 1 The route of ascorbic acid, after oral intake.

The pathogenesis of *H. pylori* infection is partly due to the immunologic response. The infiltration of neutrophils results in free radicals, which initiate a membrane peroxidation cascade that leads to mucosal damage, disrupting the integrity of the biological membranes. The immune response is polarized to a Th1 cell-mediated response with release of interferon gamma (IFN-γ), which activates phagocytic cells and contributes to mucosal damage [22,23]. In the study by Bennedsen et al. the effect of antioxidants on the immune response to *H. pylori* was evaluated. It was shown that mice treated with astaxanthin showed a significant

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increase in interleukin-4 release, which indicates a shift towards a Th2 T-cell response. The observed shift of the Th1/Th2 balance following treatment was probably the result of the downregulation of Th1 cells and upregulation of Th2 cells by the antioxidant astaxanthin; another possible mechanism of action is that it neutralizes reactive free oxygen metabolites in the mucosa [24]. The preliminary chemotaxis and oxidative burst results of investigations on astaxanthin, algamel (a powder from a plant that contains astaxanthin naturally), vitamin E and dimethylsulfoxide in *H. pylori* show that the compounds tested had no effect on chemotaxis. However, astaxanthin and algamel had a negative effect on oxidative burst, which supports the findings of the epidemiologic studies (L. P. Andersen, personal communication).

Further studies are needed to describe the effect and the mechanism of antioxidants in the *H. pylori* immune response. Antioxidants may not represent the only answer for the treatment of *H. pylori* infection, but the studies do show that they might constitute a supportive treatment.

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