Helicobacter Pylori, Zinc and Iron in Oxidative Stress-Induced Injury of Gastric Mucosa

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Abstract: A number of study have suggested a relationship between Helicobacter pylori infection and oxidative stress in the gastric epithelium. The oxidant- induced changes in zinc, iron, and vitamin C increase susceptibility to oxidative injury. Understanding of pathophysiologic mechanisms may provide new therapeutic strategies in treatment of oxidative injury of mucosa .

Key Words: *Helicobacter pylori*, zinc, iron, vitamin C, oxidative stress.

INTRODUCTION

Helicobacter pylori is a helical, or spiral-shaped, bacterium that lives in the stomach. Infection with *H. pylori* is associated with a number of diseases [1].

Gastric infection with *H. pylori* is well known to cause chronic gastritis and peptic ulcers, dramatically increasing the risk of gastric cancer and mucosa-associated lymphoid tissue (MALT) lymphoma. The urea breath test, stool antigen tests, and serological kits with a high accuracy should be used for the diagnosis of *H. pylori* infection. Triple therapy using a PPI with clarithromycin and amoxicillin or metronidazole given twice daily remains the recommended first choice treatment. Bismuth-containing quadruple therapy, if available, is also a first choice treatment option [2]. Histological observations in humans have indicated that the degree of *H. pylori* infection and the severity of mucosal injury are directly related to the extent of neutrophil infiltration into the mucosa.

Neutrophil counts were elevated in *H. pylori* positive mucosal biopsy specimens in comparison to *H. pylori* negative controls [3]. Reactive oxidative species (ROS) were increased in the gastric mucosa of persons with *H. pylori* – associated gastritis. The amount of ROS was directly correlated to bacterial load [4]. *H. pylori* can survive in a neutrophil-rich environment, but it is still unclear how these organisms evade phagocytic killing [5]. Maintaining metal homeostases is crucial for adaptation of *H. pylori* to gastric enviroment. The important metals for metabolism of *H. pylori* are Ni,Cu, Zn, Fe and Mg [6]. Nickel is a cofactor of two H. pylori enzymes. These are urease enzyme, required for gastric acid resistance [7] and hydrogenase enzyme [8].

Copper ions play an important role in bacterial metabolism, because they function as cofactors for electron transport, oxidases, and hydroxylases [9,10]. On the other hand, copper catalyzes the generation of toxic hydroxyl radicals *via* Fenton-like reactions [11], and this necessitates mechanisms to keep the concentration of cytoplasmic copper ions below toxic levels. Whereas copper import occurs nonspecifically [12,13] *H. pylori* controls the cytoplasmic copper concentration by efflux *via* the P-type ATPase CopA [14] which transports copper ions from the cytoplasm into the periplasmic space.

Magnesium (Mg^{2+}) is a cofactor of many enzymes involved in central biochemical pathways which are essential for bacterial viability [15,16]. Magnesium homeostasis was shown to be essential for H. pylori viability *in vitro* [17]. In order to overcome the Mg^{2+} limitation within the human host, pathogenic bacteria express specific Mg^{2+} uptake systems, the inner membrane protein CorA. Mg (2+) acquisition by CorA is essential for Helicobacter pylori *in vitro* [17].

Divalent cations (iron, zinc, cooper) inhibited urease activity of H. pylori while magnesium ion inhibited release of urease from H. pylori cells [18].

ZINC AS ANTIOXIDANT

Based on the hazardous effects of *H. pylori* on the gastrointestinal mucosa, addition of zinc complexes to antibiotic eradication therapy has been actively studied [19,20]. Zinc (Zn) is an essential mineral that stimulates activity of approximately 100 enzymes and supports a healthy immune system needed for wound healing and DNA synthesis [21]. It has been reported that Zn inhibits *H. pylori*-associated gastric mucosal inflammation [22]. Ishihara *et al*. [22] showed that the Zn component of the antiulcer drug polaprezinc (zinc complex of L-carnosine), significantly attenuated neutrophil activity, mononuclear infiltration and surface epithelial erosion in the gastric mucosa of mongolian gerbils infected with *H. Pylori* [23,24]. It is effective in the inhibition of superoxide generation by neutrophils and scavenging superoxide and the production of hydroxyl radicals [25]. *Helicobacter pylori*-associated inflammation activates various oxidant-producing enzymes in the host, such as NADPH oxidase, inducible nitric oxide synthase, cytokine inducible mangane superoxide dismutase (Mn-SOD). The NADPH oxidase is a plasma membrane associated enzyme which catalyzes production of superoxide radicals from oxygen using NADPH as the electron donor. Zinc is an inhibitor of this enzyme. The dismutation of superoxide radical to H_2O_2 is catalyzed

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by superoxide dismutase which contains both copper and zinc (Cu,Zn-SOD). Some oxidants lead to substantial increases in the concentration of Zn ions in epithelial cells of the acid-secreting gastric glands and mucus/HCO_3 secreting surface epithelium [26,27]. One such oxidant is monochloramine (NH_2Cl) which is produced from the reaction between hypochlorous acid (HOCl), generated by activated neutrophils, and ammonia (NH_3) generated by *Helicobacter pylori*. NH_2Cl oxidizes proteins responsible for regulating intracellular homeostasis of divalent cations such as Ca^{2+} and Zn^{2+}, and lead to sustained increases in concentration of these ions [26].

FENTON REACTION, VITAMIN C AND *HELICOBACTER PYLORI*

Iron overload results in iron toxicity, mainly due to the formation of hydroxyl radicals that strongly react with all kinds of biomolecules. The iron catalysed Haber-Weiss reaction, which relies on the Fenton reaction between ferrous iron and hydrogen peroxide, is the major mechanism of generating hydroxyl radicals in biological systems [28,29]. The hydroxyl radicals (·OH) are produced when superoxide and hydrogen peroxide react together. Generation of hydroxyl radicals from H_2O_2 is catalysed by iron and copper. Zinc is known to compete with both iron and copper for binding sites on the cell membrane thus decreasing the production of hydroxyl radicals [29]. Neutrophils secrete the iron-binding protein lactoferrin into phagosome and the extracellular enviroment. Investigation of the Fenton mechanism established that neutrophils do not have an endogenous transition metal and that release of lactoferin inhibits the reaction by complexing iron [30]. If target cells or molecules could provide iron to the neutrophils, the Fenton reaction will take place and generate the hydroxyl radicals. Although most of biological forms of iron are not catalytically active, neutrophils have been shown to produce hydroxyl radicals in the presence of proteolytically degraded transferrin or iron complexed to siderophore [31]. During *H. pylori* infection, the bacterium enhances gastric lactoferrin, which captures iron from transferrin. However, lactoferrin faces tough competition from bacterial iron-binding molecules, siderophore [32].This lactoferrin bound iron can be utilized by *H. pylori*, subsequently depleting host extracellular iron. This phenomenon of reduced host extracellular iron during infection is known as the hypoferremia of infection. *Helicobacter pylori* gastric infection has emerged as a new cause of refractory iron deficiency anemia but the pathogenetic mechanism of anemia is still unknown [33].

Additionally, *H. pylori* infection induced inflammation causes significantly enhanced consumption of vitamin C and reduces secretion of the vitamin into gastric lumen [34,35]. A number of studies have demnstrated that vitamin C levels of gastric juice are reduced in *H.* pylori gastritis. The successful eradication restores the juice/plasma ascorbic acid ratio [36-39] and decrease risk of gastric cancer in severe atrophic gastritis [40]. When exposed to bacteria, neutrophils oxidize extracellular ascorbic acid to form dehydroascorbic acid which is transported into the neutrophil and rapidly reduced to ascorbic acid by the enzyme glutaredoxin. As a result of recycling of extracellular ascorbic acid, the neutrophil internal concentration of ascorbic acid increases 10-fold [41]. High concentrations of ascorbic acid may enable these cells to quench oxidants generated during phagocytosis and thus protect neutrophils from oxidative damage [42]. It is not known however, how alternation of vitamin C concentration in gastric mucosa affects the function of these inflammatory cells. It could be that deficiency of vitamin C compromises their immune competence and makes them permissive to long-term infection of *H. pylori* in the stomach [34].

As neutrophils do not have endogenous transition metals [30] this would suggest that *H. pylori* promotes production of hydroxyl radicals *via* the Fenton reaction. Because ascorbic acid has transition metal-related prooxidant effects, high concentration of vitamin C could strongly enhance the Fenton reaction. Ascorbic acid accelerates free radical reactions by reducing Fe (III) to Fe (II). It has been shown that bacillary form of *H. pylori* generates the superoxide radical and the coccoid form generates preferentially the hydroxyl radical [43]. Also, *H. pylori* disrupt NADPH oxidase targeting such that superoxide anions are released into the extracellular milieu and do not accumulate inside *H. pylori* phagosomes [44].The ROS generated by both forms of *H. pylori* may have cytotoxic effects not only on the bacterium itself, but also on the gastric mucosal cells. Since bacteria are also capable of producing superoxide radicals [45] one could argue that secretion of bacterial superoxide is accelerating the oxidation of ascorbic acid into dehydroascorbic acid (DHA) and its transport into neutrophils. DHA is the biologically inactive form of vitamin C and does not have a prooxidative function related to transition metals. Since ascorbate itself may be consumed by oxidation in conditions where cells are exposed to peroxide or superoxide, bacteria may have adapted to an accumulation of vitamin C in neutrophiles through superoxide secretion. Hydrogen peroxide is the major reactive moiety involved in Fenton reaction. The higher cellular level of H_2O_2 contributes to the oxidation of Fe^{2+} into Fe^{3+} *via* the Fenton reaction. Increased Fe^{3+} causes augmented consumption of ascorbate for reduction to Fe^{2+} further decreasing the ability of the cell to reduce Fe^{3+}. All of this leads to decreased Fe^{2+} availability (Fig. (**1**)).

INFLAMMATION AND ZINC

The role of zinc as an antioxidant has been predicted on its ability to prevent the formation of disulfide bonds by either displacing or competing with cupric or ferric ions which trigger the formation of free radicals [46]. These findings suggest that inflammation is an important trigger for release of free iron in the upper digestive tract. Chronic zinc deprivation results in increased sensitivity to oxidative stress [47], while exposure of an organism to zinc on a long-term basis results in induction of some other substance, which is the ultimate antioxidant, as metallothionein [48].

In Ecuador where is a high prevalence of zinc deficiency, and the degree of inflammation in *H. pylori*-induced gastritis appears to be modulated by gastric tissue zinc concentration. *H. pylori* infection together with lower zinc concentration in gastric mucosa may lead to increased oxidative stress and be associated with increased inflammation [49] and with development of prenoplastic lesion in colonic mucosa [50,51]. Other studies have shown that patients with peptic ulcers have reduced levels of Zn^{2+} in their plasma, but elevated

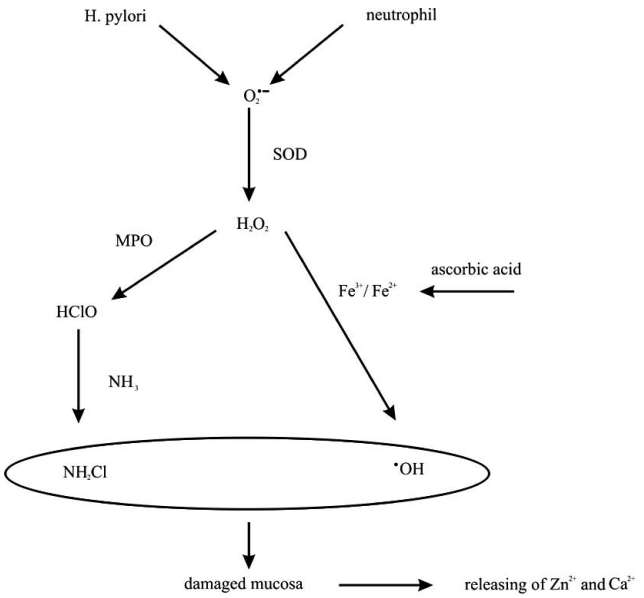

Fig. (1). Hydrogen peroxide (H_2O_2) have a key role in oxidative cell injury.

gastroduodenal mucosal zinc levels, suggesting that healing of the ulcer lesion is associated with a shift in Zn^{2+} from the plasma to the mucosa [52-54]. Recent studies have shown that dietary Zn supplementation attenuated *Helicobacter felis*-induced gastritis in mice [55] and that polaprezinc attenuated development of polymorphonuclear neutrophil (PMN) activity, mononuclear infiltration and surface epithelial erosion in gastric mucosa in Mongolian gerbils [22]. Indeed, the zinc component of polaprezinc inhibits *H. pylori*-induced PMN-mediated gastric inflammation by attenuating CD11b/CD18 expression on PMN and IL-8 production from gastric epithelial cells [24]. Cytotoxic cytokines such as TNF-α, IL-1β and IL-8 generate increased amounts of free radicals. In HL-60, a malignant human monocyte macrophage cell line, it has been shown that zinc decreased the production of cytotoxic cytokines. Polaprezinc (zinc complex of L-carnosine) showed an inhibitory effect against the growth of *H. pylori*. Divalent cations like Zn^{2+}, inhibit superoxide production in neutrophils and eosinophils [56]. Studies with dimethyl sulfoxide-differentiated HL-60 cells as model for NADPH oxidase –induced transport of vitamin C showed a reversible inhibition of the transport by free zinc ions. These data confirm suppression of the respiratory burst in the presence of free zinc ions [57].

TRACE ELEMENTS IN *H. PYLORI*-INDUCED OXIDATIVE STRESS

Helicobacte . pylori maintains a delicate balance between activating inflammatory processes and protecting itself from the negative consequences of inflammation. *H. pylori* has evolved complex strategies to maintain a mild inflammation of the gastric epithelium, while limiting the extent of immune effector activity. Severe disease associated with *H. pylori* might reflect loss of this control [58]. The driving force that transforms chronic gastritis ultimately into gastric cancer are oxygen radicals persistently produced in the gastric mucosa infected with *H. pylori* [3,59,60].

Although iron is vital for all living organisms iron is also potentially carcinogenic due to its catalytic effect on the formation of hydroxyl radicals while zinc has antioxidant properties. Metals such as iron can substitute for zinc and may responsible for metal-induced DNA damage and carcinogenesis, suggesting a close interrelation of two ions [61].

A high dietary iron intake may increase the risk of upper digestive tract cancer while high dietary zinc may decrease the risk. It is suggested that both iron and zinc play important roles in carcinogenesis of the upper digestive tract, probably through the mechanism of oxidative stress [62]. Iron homeostasis and oxidative stress defence are linked and H. pylori contains the ferric uptake regulator protein (Fur).

The Fur is a iron-responsive regulator that controls the iron metabolism in many bacteria, including *H. pylori*. It is directly or indirectly involved in the regulation of oxidative stress defense. Fur is a direct regulator of the *H. pylori* iron-cofactored superoxide dismutase Sod B, which is essential for the defense against toxic superoxide radicals [63]. A Positive correlation between *H. pylori* infection and iron deficiency has been shown [64-66]. The eradication of *H. pylori* infection without iron treatment led to the resolution of iron deficiency [64].

To avoid the negative effects of phagocytosis and the high levels of ROS generated by neutrophils, *H. pylori*, like many other bacteria, produce a number of ROS scavenging enzymes such as catalase and SOD [7].

Phagocytic cells inhibit the growth of intracellular pathogens by producing nitric oxide (NO) that affects bacterial replication by zinc release from metalloproteins involved in DNA synthesis [67]. NO production contributes to *H. pylori* pathogenesis, although it is potentially deleterious to the bacterium. *H. pylori* activates inducible NO synthase in the gastric mucosa [68] and bacterial urease has been implicated in iNOS activation [69]. One explanation of the anti-inflammatory activity of zinc was that endogenous zinc inhibited lipopolysaccharide (LPS) or IL 1 beta induced NO formation [70]. Zinc enhances the activity of contaminating LPS, even in substimulatory concentrations, and acts synergistically with LPS with respect to cytokine induction in leukocytes [71]. To avoid killing by NO, *H. pylori* produces an arginase that converts L-arginine to urea and L-ornithine [72]. Because L-arginine is also used by iNOS to produce NO, arginase can compete with iNOS for their common substrate and regulate NO synthesis. Additionally, nitric oxide - induced release of zinc from metallothionein may limit free radical membrane damage during inflammation [73].

CONCLUSION

Bacteria and PMNs act in concert to damage the gastric mucosa [63]. The oxidant-induced changes in zinc and iron ion equilibria increases susceptibility to oxidative injury of the gastric mucosa. Increasing Zn^{2+} and decreasing Fe^{2+} are signals of oxidative stress in the gastric mucosa, control of homeostasis of these trace elements during oxidative stress should be investigated. This may provide new therapeutic strategies applicable to treatment of injury of the gastric mucosa by oxidative stress.

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