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Forum Review

Amelioration of Oxidative Stress with Ensuing Inflammation Contributes to Chemoprevention of *H. pylori*-Associated Gastric Carcinogenesis

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ABSTRACT

The gastric inflammatory response provoked by *Helicobacter pylori* (*H. pylori*) consists of infiltrations by neutrophils, lymphocytes, and macrophages, resulting in varying degrees of epithelial cell damage. *H. pylori*-associated inflammation not only activates various oxidant-producing enzymes such as NADPH oxidase and inducible nitric oxide synthase, but also lowers the antioxidant ascorbic acid in the stomach. Reactive oxygen metabolites and nitrogen metabolites generated by these enzymes react with each other to generate new or more potent reactive species. The specific types of cellular damage resulting from reactive oxygen metabolites include lipid peroxidation, protein oxidation, and oxidative DNA damage. All of these oxidative products can result in biochemical changes leading to cancer. A positive association has been demonstrated between *H. pylori* infection and gastric adenocarcinoma with increased oxidative stress. Therefore, appropriate treatment to reduce oxidative stress would be expected to prevent subsequent gastric carcinogenesis through lessening of *H. pylori*-associated inflammation. This review will provide evidence that antiinflammatory regimens can decrease the development of tumors and the amelioration of gastric inflammation might lead to chemoprevention strategies by the attenuation of oxidative stress. *Antioxid. Redox Signal.* 6, 549–560.

INTRODUCTION

GASTRIC CARCINOGENESIS has been considered to reflect a multistep process of morphological abnormalities accompanied by the progressive accumulation of genetic alterations (24, 30, 43, 52, 54, 62). Although the underlying mechanisms driving progression from normal to neoplastic mucosa is still obscure, epidemiological studies provide a positive association between chronic *Helicobacter pylori (H. pylori)* infection and the subsequent development of adenocarcinoma of the mid- or distal stomach (2, 30, 47, 52, 62). In addition to these epidemiological analyses, experimental studies using Mongolian gerbils (60, 66) or C57BL/6 mice (13, 22) have shown that *H. pylori* infection results in a high risk for gastric cancer as carcinogen or promoter.

H. pylori infection induces active inflammation with infiltration of neutrophils, which are a major source of oxygen-derived free radicals that could cause DNA damage to the adjacent cells (12, 15, 40). It is well known that intake of fresh vegetables containing antioxidants such as vitamin C or β-carotene reduces the risk for gastric cancer. *H. pylori* was found to reduce the systemic availability of dietary vitamin C (54), and an intervention study has shown that either the eradication of *H. pylori* or supplements of vitamin C and β-carotene brought about the regression of cancer precursor lesions (7, 37, 42, 53, 67). In this review, we will discuss why

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reactive oxygen metabolites (ROM) are suspected to be involved in gastric inflammation and ensuing carcinogenesis and whether the amelioration of oxidative stress and ensuing inflammation contributes to chemoprevention of *H. pylori*-associated gastric carcinogenesis.

OXIDATIVE STRESS IN *H. PYLORI*-INDUCED GASTRIC INFLAMMATION

ROM in H. pylori *infection*

Oxidative stress through an excessive release of ROM is one of the major fundamental mechanisms of tissue destruction. A positive association was found between ROM production and the infective loads of *H. pylori* (2, 41, 50, 51). ROM production in gastric mucosa is enhanced by the infection of specific cytotoxin association gene A (*cag*A)-positive *H. pylori* species resulting in extensive accumulation of neutrophils in patients with gastric ulcer (48, 50). These findings are consistent with results of a recent study showing a relationship between gastric inflammation and oxidative stress in patients with chronic gastritis even in the absence of peptic ulcer (41).

Primary oxygen-derived free radicals are superoxide anion ($O_2^{-\bullet}$) and hydroxyl radical ($OH^\bullet$), which are generated from a variety of sources in both physiological and pathophysiological conditions. As an initial inflammatory response to the pathogen, *H. pylori* has been demonstrated to cause infiltration of neutrophils and macrophages. These cells, on interaction with proinflammatory molecules, such as cytokines, immune complexes, or bacterial products, undergo a so-called respiratory burst (44, 46). This process involves a sudden stimulus-induced activation of the membrane-bound enzyme NADPH oxidase, which in turn evokes the release of large amounts of oxygen-derived free radicals, resulting in oxidative stress. In the mitochondrion, an electron transfer takes place from NADPH to oxygen inside and outside cells, and the oxygen molecules that receive an electron become $O_2^{-\bullet}$, which is rapidly converted to hydrogen peroxide (H_2O_2) by spontaneous dismutation or enzymatic activity of superoxide dismutase (SOD), and $OH^\bullet$, which is formed nonenzymatically in the presence of Fe^{2+} as a secondary reaction. As much as 1–5% of the total oxygen consumption by normal tissue may thus be transformed into $O_2^{-\bullet}$. Although $O_2^{-\bullet}$ generation by phagocytes is essential for an effective host defense mechanism against bacterial infection, its continuous abnormal overproduction during inflammatory processes may also cause extensive tissue destruction (34, 61).

Inflammatory phagocytes also generate and release remarkable amounts of H_2O_2. Instead of being neutralized to water, H_2O_2 can also be metabolized by the enzyme, myeloperoxidase, to catalyze the oxidation of chloride by H_2O_2 to yield hypochlorous acid (HOCl). HOCl is estimated to be 100–1,000 times more toxic than $O_2^{-\bullet}$ or H_2O_2. Among them, $OH^\bullet$ is considered to be the most reactive inflammation-relevant ROM, generated during the inactivation of copper/zinc SOD by H_2O_2 (60). *H. pylori* has been proposed to be resistant to the antimicrobial action of ROM produced by polymorphonuclear leukocytes, and such resistance has been attributed to bacterial antioxidant species production. Consequently, excessive amounts of free radicals can start lethal chain reactions, which can inactivate vital enzymes, proteins, and other important subcellular elements needed for cell survival and lead to cell death. These also could lead to gene modifications that are potentially mutagenic or carcinogenic (9).

Reactive nitrogen metabolites (RNM) in H. pylori *infection*

Recently, considerable interest has been generated concerning the role of RNM in cellular redox reactions in inflammation status. Inducible nitric oxide synthase (iNOS) contributes to cytotoxicity of phagocytes, utilizing L-arginine, NADPH, and O_2 as substrates, producing nitric oxide (NO), NADP+, and citrulline (43). The toxicity of NO during chronic inflammation occurs by the oxidation of NO with superoxide to form peroxynitrite ($ONOO^-$) and nitrosating species such as nitrates (NO_3^-), nitrites (NO_2^-), and N_2O_3. Mannick *et al.* (37) found induced iNOS expression and nitrotyrosine in *H. pylori* gastritis.

H. pylori-derived NH_3 and HOCl produce monochloramine (NH_2Cl), which is exceptionally reactive and toxic because of its high lipophilic property and low molecular weight. NH_2Cl freely penetrates biological membranes to oxidize intracellular components. Suzuki *et al.* (55) demonstrated that NH_2Cl (0.001–0.01 m*M*) significantly increased cytoplasmic oligonucleosomes, in amounts suggesting DNA cleavage, one of the important markers of apoptosis. In addition to NH_2Cl, recent studies have demonstrated that gastric epithelial cells possess an isozyme of gp91-phox, mitogen oxidase 1 (Mox1), and essential components for the phagocyte NADPH oxidase (p67-, p47-, p40-, and p22-phox), and these gastric pit cells also produce ROM via activation of nonphagocytic NADPH oxidase in response to *H. pylori* (41). The same group recently extended their study and found that lipid A of *cag*A-positive *H. pylori* may be a potent stimulator for innate immune response of gastric mucosa, examples being stimulation of the toll-like receptor 4 (TLR-4) cascade and Mox1 oxidation in gastric pit cells (28). Our laboratory found that *H. pylori* infection increased the expression of TLR-2 or TLR-4 (Fig. 1), and polymorphisms of TLR-4 were found to be a major determinant of the pathologic response to *H. pylori* infection, *i.e.*, progression to chronic atrophic gastritis or duodenal ulcer (27).

CELLULAR TARGETS OF OXIDATIVE ATTACK IN GASTRIC MUCOSA

Membrane lipid peroxidation in the H. pylori *infection*

Free radical-mediated lipid peroxidation is believed to be an important cause of cellular membrane destruction and cell damage. As cell membranes are partially composed of phospholipids, which are rich in polyunsaturated fatty acids, these membranes can be readily attacked by ROM, producing fatty acid radicals and lipid hydroperoxides (41, 45).

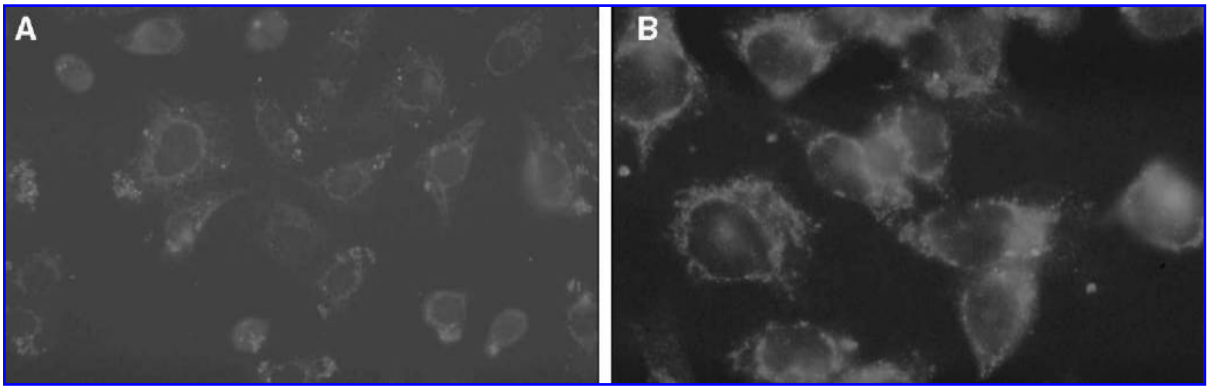

FIG. 1. TLR-4 expression was significantly increased on the cell surface after *H. pylori* infection in human gastric epithelial cells (AGS). (A) TLR-4 expression in control cells. **(B)** TLR-4 expression after *H. pylori* infection. Using TLR-4 antibody (Santa Cruz Biotechnology) at a dilution of 1:100 for 2 h at room temperature visualized by Cy™ 3-conjugated goat anti-rabbit IgG antibody at a dilution of 1:100 for 30 min at room temperature, expression of TLR-4 was examined by fluorescence microscopy. Magnifications: ×400.

The most obvious consequence of membrane lipid peroxidation is the disruption of various membrane functions, including transport processes, maintenance of ion and metabolite gradients, and receptor-mediated signal transduction. Accumulation of lipid peroxidation products, such as alkanes and aldehydes, in *H. pylori*-infected gastric mucosa reflected the presence of increased oxidative stress (20, 41). It has been shown that levels of thiobarbituric acid-reactive substances are higher in patients with *H. pylori* infection or in *H. pylori*-infected Mongolian gerbils (41, 42). Interestingly, these levels were ameliorated successfully by either the antioxidant polaprezinc (57) or eradication of *H. pylori* (11). These data demonstrate that oxidative stress is closely associated with gastric mucosal damage by *H. pylori* infection, rather than being a secondary event associated with infection (41).

Glutathione (GSH) of gastric mucosa in the H. pylori *infection*

GSH, the major nonprotein thiol, is an important endogenous antioxidant peptide that is found in particularly high concentrations in the stomach and liver. Interestingly, concentrations of GSH are much higher (up to 7–8 m*M*) in the glandular gastric tissue than in other portions of the gastrointestinal tract (41). Immunohistochemical studies have revealed that glutathione peroxidase is located in the gastric mucosal cells, epithelial cells, and parietal cells. GSH is present in cells mainly in the reduced form and plays a role of a free radical scavenger.

Recently, it was reported that the level of GSH was significantly lower in *H. pylori*-infected patients than in *H. pylori*-negative patients (41, 51, 64), and a study using gerbils demonstrated that the content of GSH was significantly elevated at 12 weeks following *H. pylori* inoculation without any change in glutathione peroxidase activity (56). These conflicting findings could be explained by differences in species or duration of *H. pylori* infection. It has been definitely shown that *H. pylori* directly reduces intracellular GSH and impairs GSH metabolism of gastric epithelial cells, suggesting that preservation of intracellular GSH might be an important determinant of outcome in *H. pylori* infection. In addition, diminished GSH levels in the gastric mucosa may be associated with gastric carcinogenesis, because GSH is involved in the direct repair of oxidative DNA damage and *H. pylori*- associated gastric carcinogenesis has been shown to be affected by the levels of intracellular GSH (41).

Oxidative damage on cellular proteins following H. pylori *infection*

Proteins are the most abundant cell constituents, and are also one of the important targets of ROM. Moreover, even a relatively minor structural modification of a single protein by oxidation can lead to marked functional changes with deranged biological activity *in vivo* (9). Because protein oxidations frequently introduce new functional groups, such as hydroxyls and carbonyls, they contribute to altered function and possible degradation of cells.

ROM may cause protein fragmentation, cross-linking, and unfolding. Another permanent modification that can adversely affect protein function is the nitration of protein-bound tyrosine by peroxynitrate, which may be involved in phosphorylation and signal transduction pathways, although protein nitration and inactivation might also occur through a peroxynitrate-independent, myeloperoxidase-catalyzed pathway. Finally, *H. pylori* infection is significantly associated with dysfunction of cellular proteins and molecular chaperones involved in oxidative stress (9, 25, 41).

Oxidative DNA damage in H. pylori *infection*

Both nuclear and mitochondrial DNA are known targets of ROM attack. Many types of DNA modifications such as gene mutations can result in malignant transformation or cell death by oxidative stress (12, 20). Through the generation of nitrating and oxidizing agents, *H. pylori* infection could lead to increased rates of DNA damage and, as a consequence, apoptosis in gastric mucosa, from which eventually atrophic gastritis could appear because of loss of stem cells. Accelerated rates

of proliferation may be the stimulus to increase apoptosis or vice versa, and an imbalance between apoptosis and proliferation may explain the diverse clinical outcomes of infection, including neoplasia (14, 20, 32).

Interestingly, *H. pylori* infection increased the levels of 8-hydroxydeoxyguanosine (8-OHdG), which might be closely associated with gastric carcinogenesis. The increased levels of 8-OHdG were proven to be lower after the successful eradication of *H. pylori*. Furthermore, inflammation observed after *H. pylori* infection may result in oxidative stress, resulting in increased formation of 8-OHdG (20). These facts may explain the association between *H. pylori* infection and gastric carcinogenesis, which will be discussed further in the following paragraphs.

GASTRIC INFLAMMATION INDUCED BY *H. PYLORI*

The gastric inflammatory response induced by *H. pylori* consists of neutrophils, lymphocytes, plasma cells, and macrophages along with varying degrees of epithelial cell degeneration and injury (25, 50). As the invasion of the gastric mucosa by *H. pylori* is reported to be rare, other potential mechanisms for induction of inflammation must be postulated. One possibility is that *H. pylori* secretes substances that stimulate mucosal inflammation. For example, urease has been detected within the lamina propria, and the urease complex of *H. pylori* stimulates chemotaxis by both monocytes and neutrophils and activates mononuclear cells. Another mechanism by which *H. pylori* may induce inflammation is through direct contact with gastric epithelial cells and by stimulating release of cytokines such as interleukin-8 (IL-8).

Currently, *cag*A protein was reported to be the type IV secretory system for cytokine release (41, 48). Clinically, gastric epithelium from *H. pylori*-infected individuals demonstrates enhanced levels of IL-1β, IL-2, IL-6, IL-8, and tumor necrosis factor-α (TNF-α) (17, 31, 49). IL-8 is a potent neutrophil-activating chemokine, and expression of this protein is localized to gastric epithelial cells *in vivo*. The human IL-8 gene contains several binding sites within its promoter region, including a nuclear factor-κB (NF-κB) binding motif and a binding site for *c-jun*, which together comprise the transcription factor activator protein-1 (AP-1) (41, 49). Several studies have demonstrated that contact between *H. pylori* and gastric epithelial cells results in brisk activation of NF-κB, which is followed by increased IL-8 expression (Fig. 2) (29). Interestingly, increased DNA binding of NF-κB after *H. pylori* injection could be decreased after the administration of the antioxidative and antiinflammatory drug, rebamipide (1, 15, 19, 21). Therefore, *H. pylori* provoked gastric inflammation through several cytokines, whose expression is controlled by gene transcriptional factors such as NF-κB or AP-1.

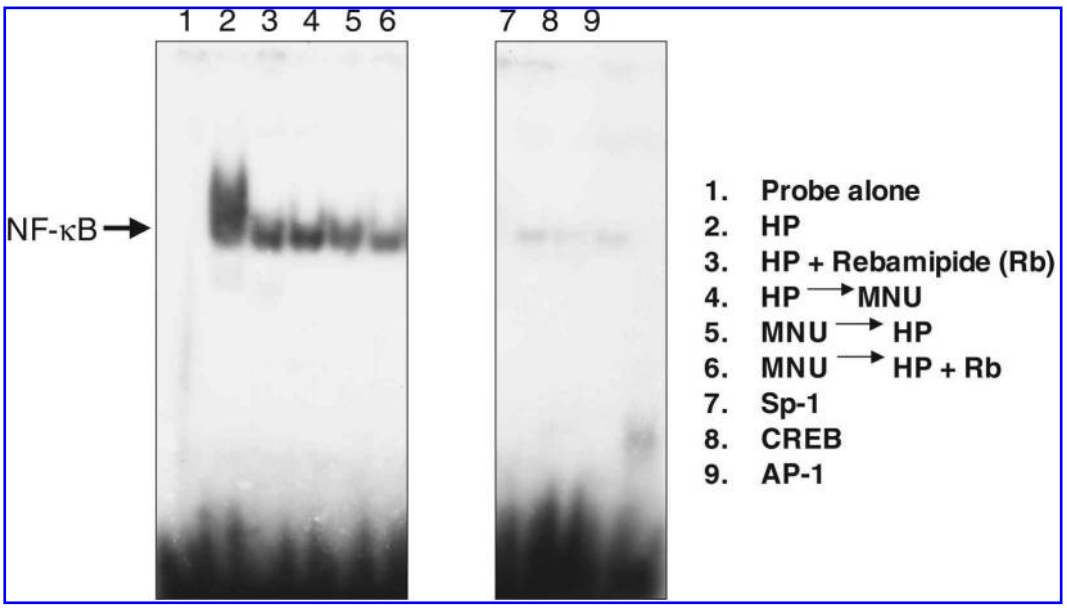

FIG. 2. Electrophoretic mobility shift assay for NF-κβ. *H. pylori* infection triggered intracellular signaling pathways and led to increased induction of several mediators involved in the propagation of inflammations. The experiment demonstrated that *H. pylori* infection significantly increased DNA-binding NF-κB activity. Interestingly, the antioxidative and antiinflammatory drug, rebamipide, showed significant decreases of NF-κB DNA-binding activity, and other transcription factors like AP-1 or Sp-1 were also responsible for *H. pylori* infection. Lane 1, probe alone; lane 2, *H. pylori* (HP) alone; lane 3, HP + rebamipide (Rb); lane 4, HP → *N*-methylnitrosourea (MNU); lane 5, MNU → HP; lane 6, MNU → HP + Rb; lane 7, Sp-1; lane 8, cyclic AMP-response element binding protein; lane 9, AP-1.

H. PYLORI INFECTION AND OXIDATIVE STRESS PROMOTE GASTRIC CARCINOGENESIS

H. pylori, first isolated in 1983 by Drs. Warren and Marshall, is a noninvasive, non–spore-forming, and spiral-shaped Gram-negative microaerophilic bacterium measuring ~3.5 × 0.5 μm (65). In 1994, a working group of the WHO International Agency for Research on Cancer concluded that *H. pylori* infection is a class I, that is, definite carcinogen in humans and that the bacterium plays a causal role in the development of gastric cancer (24). Epidemiological and biological findings demonstrated a close link between gastric cancer or mucosal associated lymphoid tissue lymphoma and chronic infection with *H. pylori*, although the mechanism through which a formation occurs remains obscure (30, 33, 47) (Fig. 3).

Persons infected with *H. pylori* are approximately four times more likely to develop gastric cancer than those who are not infected (25). As a plausible mechanistic explanation connecting *H. pylori* infection and gastric cancer, an increase in cell proliferation or an alteration in apoptosis, which may be secondary to loss of growth inhibition by p53 and blocking to apoptosis, have been suggested (40). Recently, we demonstrated that the role of *H. pylori* infection is as a promoter of gastric carcinogenesis rather than as an initiator in experiments using a mouse model. For instance, the incidence of gastric adenocarcinoma was 80% in mice at 50 weeks after *H. pylori* inoculation followed by treatment with *N*-methylnitrosourea (MNU), whereas the incidence was only 27% in mice treated with MNU only, a well known carcinogen for rodent gastric tumors (21, 22).

OTHER FACTORS INVOLVED IN GASTRIC CARCINOGENESIS BY *H. PYLORI*

CagA pathogenicity island

Several factors have been proposed as possible virulence determinants, inducing vacuolating cytotoxin gene A (*vac*A), *cag*A, and lipopolysaccharide, in the pathogenesis of *H. pylori* infection. In particular, the *cag* pathogenicity island (PAI), an ~40-kb region of possibly extraneous origin, is found in ~50–60% of *H. pylori* isolates in western countries and in >90% of such isolates in oriental countries (5, 25, 35, 41).

Extensive studies of the *cag*A gene have demonstrated that the *cag*A protein is associated with peptic ulcer disease, gastric cancer, and mucosa-associated lymphoid tissue lymphoma. In Western populations, persons carrying *cag* PAI have an enhanced risk for developing atrophic gastritis, noncardiac gastric adenocarcinoma, and peptic ulcer. However, in East Asian

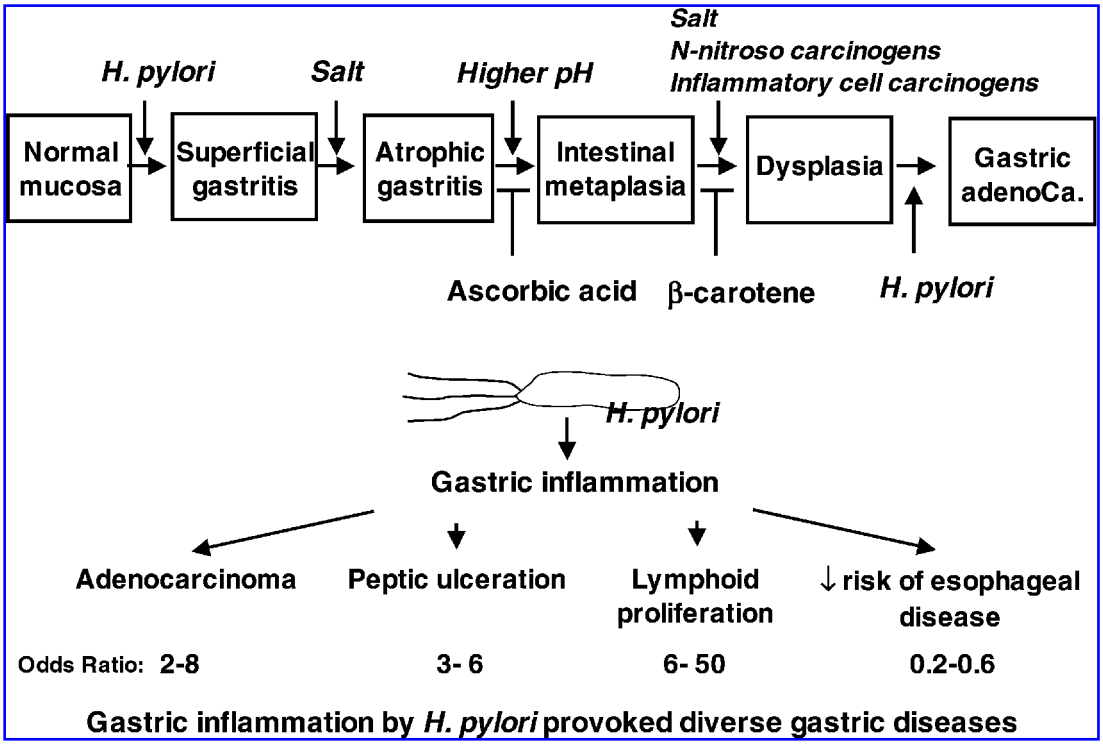

FIG. 3. Sequential changes of gastric pathology after *H. pylori* infection. *H. pylori* infection mostly provoked chronic gastritis, which progressed to intestinal metaplasia and gastric adenoma, all of which are now considered as precancerous lesions for gastric cancer. This is why *H. pylori* is defined as a definite class I carcinogen. Also, gastric inflammation provoked by *H. pylori* infection can increase the odds ratio for gastric tumors (7, 21).

populations, the relationship of *cag* PAI with *H. pylori*–related disease is more difficult to establish. Recent reports suggest that *cag*A protein can be phosphorylated on tyrosine residues by an unidentified host cell kinase. However, disruption of *cag*A does not affect nuclear factor (NF-κB) or mitogen-activated protein kinase activation or even IL-8 release, indicating that an independent bacterial factor might be injected into the gastric epithelial cell by the *cag* PAI, and that a gene other than *cag*A may be used as a marker for *cag* PAI virulence (35, 48, 49).

Recently, the ability of two *H. pylori* isolates to induce differential host response *in vivo* and *in vitro* was examined, and then a *H. pylori* whole genome microarray was used to identify bacterial determinants related to pathogenesis. Hybridization to the *H. pylori* whole genome microarray revealed differences in the ability of *H. pylori* strains to induce epithelial cell responses to an intact *cag* PAI (25).

IL-1β polymorphism

H. pylori increases IL-1β production, which amplifies the inflammatory response to *H. pylori* infection in accordance with the inhibition of gastric acid secretion by IL-1β. By the way, interestingly the overproduction of IL-1β depends on the presence of an IL-1β polymorphism, and *H. pylori*-positive individuals who had haplotype that up-regulated IL-1β production, such as IL-1β-511T/31C IL-1RN*2, showed an increased risk for gastric cancer. That is, the effect of *H. pylori* infection on the risk for gastric cancer depends on the severity of inflammation, which is fundamentally determined by underlying host genetic factors. These findings can explain why only a small proportion of patients with *H. pylori* infection develop gastric cancer (41).

REVERSIBILITY OF OXIDATIVE DNA DAMAGE BY ERADICATION OF *H. PYLORI*

ROMs are known to induce critical damage in DNA, causing DNA strand breaks and modifying bases. One of the oxidative DNA changes that occur, an increased 8-OHdG level, can cause misincorporation during replication and subsequently lead to G-T transversions. With respect to misreading of 8-OHdG containing DNA by DNA polymerase, it is interesting to note that 8-OHdG is incorporated into RNA instead of UTP on the poly[d(A-T)Πd(A-T)] template during RNA synthesis by RNA polymerase. Thus, the formation of 8-OHdG is proportional to that of other DNA modifications produced by oxygen radicals, regardless of the organism, type of oxygen radical-forming agent, and conditions of exposure (15, 27).

Recently, 8-OHdG has become accepted as a sensitive marker for reflecting oxidative DNA damage in particular affected organs (27). In our previous studies (15, 20), we measured 8-OHdG content of DNA from human gastric mucosa with or without *H. pylori* infection and observed changes in 8-OHdG contents after eradication of *H. pylori* (Fig. 4). The

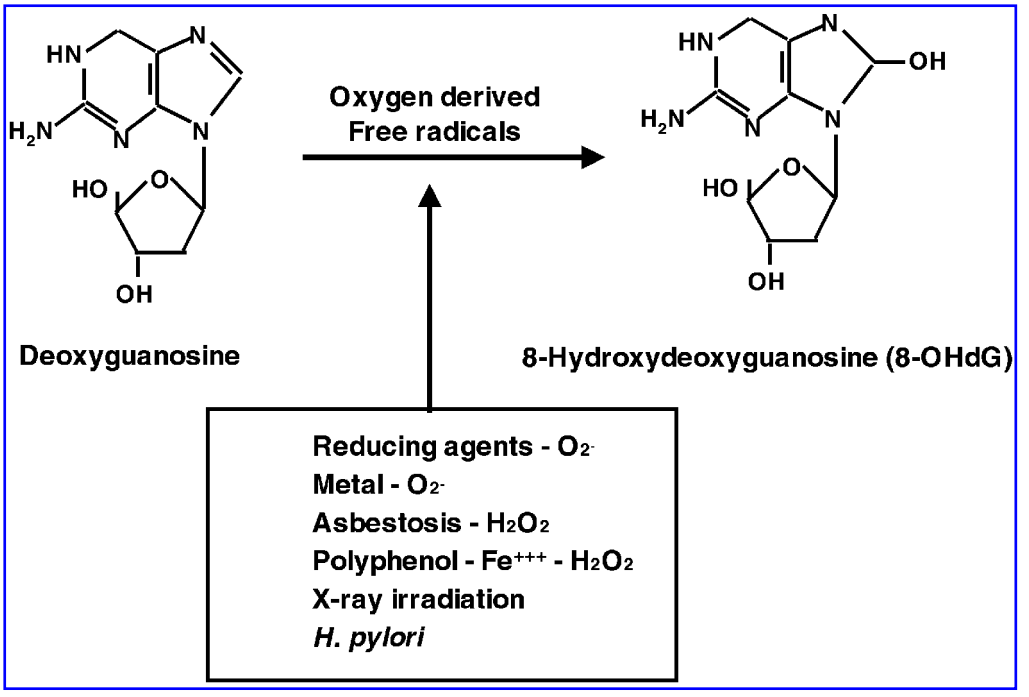

FIG. 4. Formation of 8-OHdG from deoxyguanosine. Increased formation of 8-OHdG has been regarded as a marker for oxidative DNA damage, and *H. pylori* also is known to contribute to the increased formation of 8-OHdG. After eradication of *H. pylori* using a triple regimen including proton pump inhibitor and two antibiotics, the levels of 8-OHdG were significantly decreased, suggesting that the DNA damage after *H. pylori* infection might be reversible and the removal of etiologic agents is very important (Table 1) (15).

TABLE 1. 8-OHdG CONTENT OF GASTRIC MUCOSAL DNA ACCORDING TO *H. PYLORI* STATUS

	8-OHdG (8-OHdG/10^5 dG)
Normal volunteers	
H. pylori (−) (n = 28)	4.31 ± 2.33
Patients with gastritis due to *H. pylori*	
Before eradication (n = 59)	10.40 ± 7.25*
After eradication (n = 41)	2.42 ± 1.22†

*$p < 0.05$ (versus normal volunteers).
†$p < 0.01$ (versus before eradication).

8-OHdG content of *H. pylori*-positive gastritis patients contained 2.4-fold higher levels of 8-OHdG than those of *H. pylori*-negative volunteers ($p < 0.05$). The 8-OHdG content of patients whose H. pylori was eradicated was significantly decreased after 2 weeks of eradication medications (Table 1). We also found suppression of transforming growth factor-β1 expression directly in the gastric epithelium, which enables unlimited oxidative bursts in recruited macrophages by *H. pylori* infection (19).

In the presence of inflammation-associated oxygen free radicals, NO can form potentially genotoxic nitrating species, such as peroxynitrite, and other nitrosating species, such as nitrosonium ion. NO is cytotoxic at high concentrations, so the levels of NO generated by macrophages after iNOS induction contribute to killing of intracellular and extracellular microorganisms. When we examined the iNOS score, a significant decrease was found after eradication of *H. pylori*-positive gastritis (Table 2). Grading of chronic gastritis, neutrophil infiltrations, and the density of *H. pylori* were all defined according to the Updated Sydney System (1996), and the iNOS score was determined using a scoring system considering intensities and distribution of iNOS-positive cells. NO in *H. pylori*-infected gastric mucosa can be produced in a sustained manner by host macrophages and polymorphonuclear leukocytes infiltrating the gastric mucosa (46). In addition, dietary nitrites and *H. pylori*-related intraluminal ammonia production are other sources of RNM. *H. pylori* is generally located in the mucus layer adjacent to the surface epithelium of the gastric mucosa, but because *H. pylori* is highly adherent to and capable of invading epithelial cells *in vitro,* it is probable that a variety of *H. pylori* antigens would then be presented to the lamina propria immune cells by these gastric epithelial cells.

TABLE 2. APOPTOTIC INDEX AND iNOS SCORE ACCORDING TO *H. PYLORI* STATUS

Apoptotic index	
Before eradication	3.72 ± 1.14
After eradication	1.17 ± 1.06*
iNOS score	
Before eradication	10.34 ± 6.79
After eradication	1.13 ± 1.14*

*$p < 0.01$ (versus before treatment).

Apoptosis represents physiological programmed cell death that plays important roles, including the removal of senescent cells, deletion of cells with genetic damage, and maintenance of gastric epithelial tissue homeostasis (9). Recently, we observed changes in apoptosis in gastric biopsies obtained before and after treatment of *H. pylori* infection by transferase-mediated dUTP nick end labeling, 4,6-diamidino-2-phenylindole staining, and DNA fragmentation (20). We found that the apoptotic index was significantly decreased after eradication of *H. pylori* (Table 2). Biomarkers have been attracting interest because the accurate assessment of such stress is necessary for investigation of various pathological conditions, as well as to evaluate the efficacy of interventional treatment. Therefore, 8-OHdG production, iNOS expression, and apoptotic index could be good biomarkers for assessing oxidative stress.

Besides these changes, *H. pylori* infection leads to changes in many factors that are important in the pathogenesis of gastric cancer, including the vitamin C content of gastric juice, epithelial cell proliferation, and ornithine decarboxylase activity.

BALANCE OF REDOX SIGNALING

A paradox exists: free radicals are known to be potentially dangerous, but they also have beneficial actions because ROM are needed for signal transduction pathways that regulate cell growth, and to maintain reduction–oxidation (redox) status (8). The proposal that the free radical system can be used to kill tumor cells selectively does not necessarily contradict the belief that free radicals damage DNA leading to mutagenesis and carcinogenesis.

Interestingly, it appears as though the actions of free radicals on normal and tumor cells are diametrically opposite. When free radicals attack normal cells, DNA damage can occur, leading to the development of tumors, whereas when the same free radicals are produced in excess in tumor cells, there is a totally unexpected but highly beneficial action, namely, elimination of those cells (8). In the latter instance also, the mechanism of action appears to be damage to DNA, but the end results differ depending on whether the target is normal or tumor cells. This idea is supported by Dormandy (10), who proposed that "excessive peroxidation and free radical activity may need inhibiting" especially in normal cells to prevent carcinogenesis, but "inadequate peroxidation and free radical activity need boosting" to kill tumor cells, and "normal peroxidation and free radical activity should be left alone."

Furthermore, both free radicals and lipid peroxides seem to suppress Bcl-2, enhance p53 expression, and induce telomere shortening, thus arresting tumor cell growth, and also cause apoptosis. For chemoprevention of *H. pylori*-associated gastric carcinogenesis, the first intervention might be the eradication of the microorganism, but the second option might be the maintenance of homeostasis of gastric mucosa by either scavenging oxygen-derived free radicals or preserving antioxidant capability, resulting in antiinflammatory conditions in the gastric mucosa (Fig. 5).

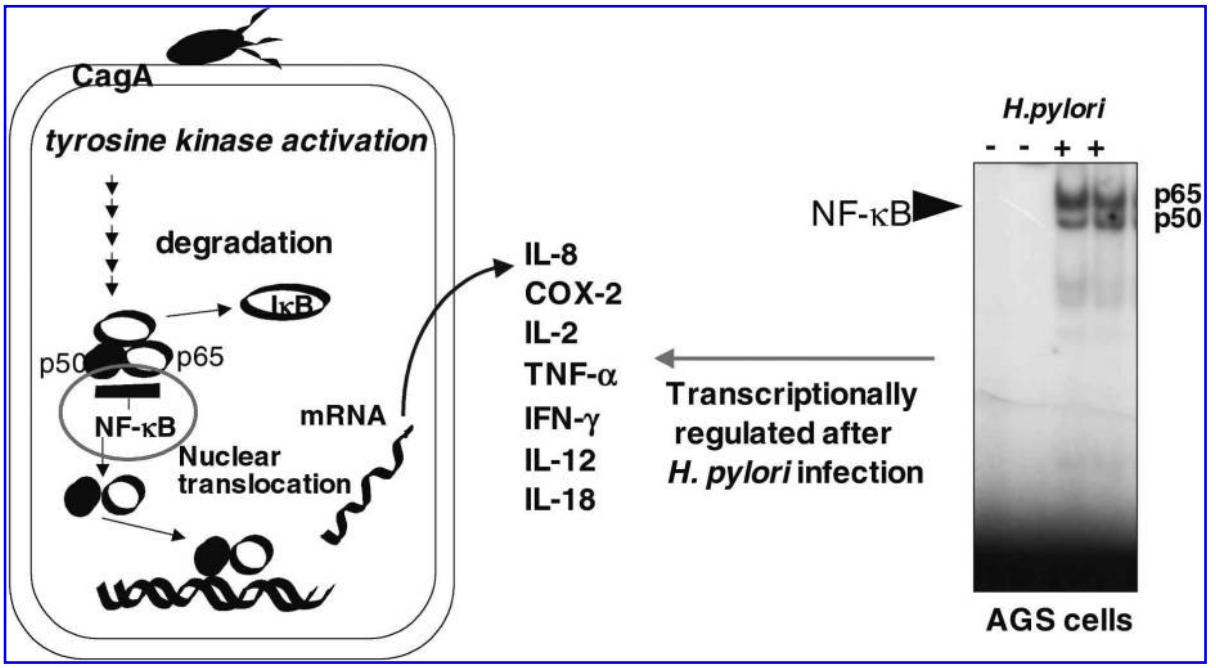

FIG. 5. Transcriptional regulation of inflammatory related cytokines by *H. pylori* infection. *H. pylori* infection significantly increased the DNA binding of transcription factor like NF-κB for increasing the synthesis of inflammatory related cytokines or mediators. After *H. pylori* infection, increased mRNA of IL-8, COX-2, IL-2, TNF-α, and interferon-γ (IFN-γ) could be observed. Among them, IL-8 and IFN-γ were known to be important cytokines involved in perpetuation of gastric inflammation and carcinogenesis.

H. PYLORI, CYCLOOXYGENASE-2 (COX-2) INDUCTIONS, AND GASTRIC CARCINOGENESIS

The COX enzyme converts arachidonic acid to prostaglandin G_2 (PGG_2) by inserting two oxygen molecules and then reducing this intermediate to PGH_2. PGH_2 is an unstable metabolite that is converted to an array of PGs, including PGE_2, prostacyclin (PGI_2), and thromboxanes, which have both autocrine and paracrine functions through the action of specific PG synthases. Two isoforms of COX enzyme have been identified. COX-1 is constitutively expressed in many tissues as a housekeeping enzyme, and COX-2 is an inducible isoform, whose expression is stimulated by growth factors, cytokines, tumor promoters, and *H. pylori* infection. COX-2 expression is up-regulated in both *H. pylori* infection and gastric adenocarcinomas (32, 54). *H. pylori* infection induced excessive production of PGE_2 during gastric tumor cell growth (45). Epidemiological studies have revealed that the use of nonsteroidal antiinflammatory drugs (NSAIDs) such as aspirin can reduce the risk of cancer, and genetically manipulated-animals have shown that both COX-1 and COX-2 disruptions decreased the tumor yield, although the mechanisms by which COX-1 and COX-2 deficiency decrease tumorigenesis are still unknown (58, 59). Based on the well documented pharmacological action of aspirin and other NSAIDs, it can be presumed that the beneficial effects of NSAIDs either in *H. pylori* infection or in tumors may be mediated by the inhibition of PG biosynthesis (21, 33, 63).

An expanding body of studies has shown that COX prevents apoptosis by generating an antiapoptotic product, PGs, as well as by removing a proapoptotic substrate, arachidonic acid. Cao and Prescott (4) suggested the downstream signaling pathways of COX-2 in apoptosis as follows: the Bcl-2 mediated pathway, the NO pathway, and production of ceramide. Bcl-2 has been shown to have a positive correlation with COX-2 induction, which was reversed by a COX-2 inhibitor.

Bcl-2 prevents peroxidation of certain intermediates and then blocks cell death (4) as a downstream pathway of both PG and arachidonic acid. Molecular cross-talk between the COX-2 pathway and NO contributes to tissue homeostasis. Recently, McGinty *et al.* (38) found that COX-2 expression inhibited apoptosis induced by withdrawal of nerve growth factor in pheochromocytoma cells. Intracellular accumulation of free arachidonic acid has been shown to elevate the level of a small lipid messenger, ceramide. Because ceramide has been identified to be an effective inducer of apoptosis, removal of arachidonic acid by COX-2 might prevent apoptosis by reducing the concentration of ceramide.

Several experiments also have demonstrated that COX inhibitors reduced tumor cell migration, cell adhesion, tumor invasiveness, and angiogenesis using both *in vivo* (16, 21, 58) and *in vitro* experiments (17). Either the activation of metalloproteinase-2 or reduction of E-cadherin expression has been proposed as the critical mediator of the effects of COX-2 in cell motility, but the underlying mechanism of those actions awaits to be explored (36). Classically, COX-1 has been believed to exert important functions in normal tissue homeostasis, but to have minimal effects on inflammation

TABLE 3. INCIDENCES AND MULTIPLICITIES OF GLANDULAR STOMACH TUMORS OF MICE ACCORDING TO GROUP

Group and treatment	*Total no. of mice*	*Effective no. of mice*	*No. of tumor-bearing mice (% incidence)*	*Tumor multiplicity**
MNU → Hp	20	16	11 (68.8)	2.62 ± 0.36
MNU → Hp + AG	20	17	12 (70.6)	1.41 ± 0.24†
MNU → Hp + NSD	20	18	7 (38.9)†	0.44 ± 0.12†
MNU alone	10	10	1 (10.0)	0.10 ± 0.10
Hp alone	15	13	0	0
Hp alone + AG	5	4	0	0
Hp alone + NSD	5	5	0	0

MNU, *N*-methylnitrosourea; Hp, *H. pylori* infection; AG, aminoguanidine; NSD, nimesulide.
*Mean ± SE.
†Significantly different from the value of MNU → Hp ($p < 0.05$).

and carcinogenesis. A recent study found that COX-1 null mice showed significantly reduced intestinal tumorigenesis, a phenotype similar to COX-2-deficient mice. Therefore, COX-1 inhibition may have a similar importance in cancer prevention. Our recent experiment (Table 3) documented that NSAID administration can block either the development or progression of *H. pylori* and carcinogen-induced gastric carcinogenesis. *In vivo* experiments revealed that the main action of this chemoprevention is mediated by up-regulation of apoptosis (14, 15, 20).

PERSPECTIVES

The host–parasite interaction between the human stomach and *H. pylori* triggers a number of molecular and cellular events, such as inflammatory reactions and oxidative stress, which disrupt gastric homeostasis and may ultimately lead to gastric inflammation or cancer. We (Fig. 6) and other groups have demonstrated that some gastroprotective drugs, such as rebamipide, ecabet sodium, and polaprezinc, might regulate the interaction between *H. pylori* infection and gastric

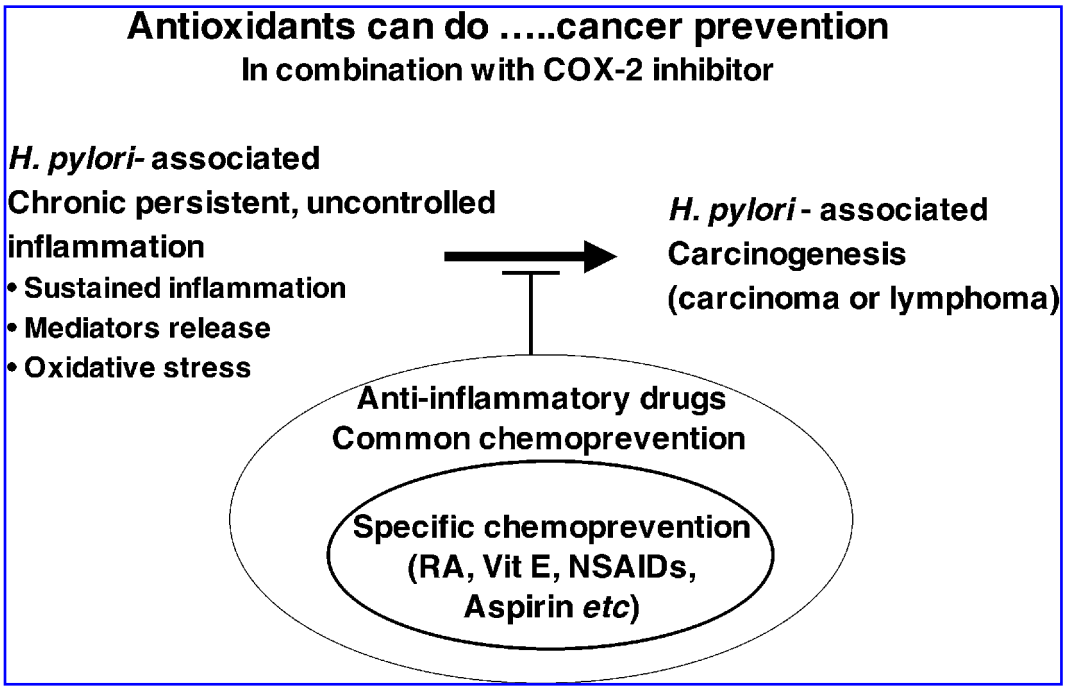

FIG. 6. Perspective of chemoprevention in *H. pylori*-associated gastric cancer by antioxidant and antiinflammatory drugs like nimesulide or rebamipide. Retinoic acids (RA) or rexinoids are well-known chemopreventive drugs in various cancers, such as leukoplakia, breast cancer, and aerodigestive tumors. According to the results in the our study (21), the long-standing administration of antioxidants or antiinflammatory drugs like nimesulide or rebamipide may be proposed as chemopreventive agent, because these drugs retarded the development of *H. pylori*-associated gastric carcinogenesis in the mouse model.

inflammation. Well-known mechanisms of these drugs are antioxidative properties and the modulation of gastric inflammation. Interestingly, these drugs can halt the progression of gastritis into precancerous lesions like chronic atrophic gastritis and intestinal metaplasia. Either new therapeutic pathways or molecules that can modulate inflammatory reactions imposed by *H. pylori* and can attenuate oxidative stress might be feasible candidates for chemoprevention of gastric cancer.

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ABBREVIATIONS

AP-1, activator protein-1; *cag*A, cytotoxin association gene A; COX, cyclooxygenase; GSH, glutathione, reduced form; *H. pylori, Helicobacter pylori*; H_2O_2, hydrogen peroxide; HOCl, hypochlorous acid; IFN-γ, interferon-γ; IL, interleukin; iNOS, inducible nitric oxide synthase; MNU, *N*-methylnitrosourea; Mox1, mitogen oxidase 1; NF-κB, nuclear factor-κB; NH_2Cl, monochloramine; NO, nitric oxide; NSAID, nonsteroidal antiinflammatory drug; $O_2^{-\bullet}$, superoxide anion; OH•, hydroxyl radical; 8-OHdG, 8-hydroxydeoxyguanosine; PAI, pathogenecity island; PG, prostaglandin; RNM, reactive nitrogen metabolites; ROM, reactive oxygen metabolites; SOD, superoxide dismutase; TLR, toll-like receptor; TNF-α, tumor necrosis factor-α; *vac*A, vacuolating cytotoxin gene A.

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