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INTERACTION OF NONSTEROIDAL ANTI-INFLAMMATORY DRUGS (NSAID) WITH *HELICOBACTER PYLORI* IN THE STOMACH OF HUMANS AND EXPERIMENTAL ANIMALS

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Helicobacter pylori (*H. pylori*) and non-steroidal anti-inflammatory drugs (NSAID) are major pathogenic factors in peptic ulcer disease but whether these two factors exert synergistic or antagonistic action on the gastric mucosa has been a subject of controversy. The classic concept states that there is an increased ulcer occurrence and bleeding in patients with both *H. pylori* infection and NSAID use. However, the question whether the *H. pylori* eradication therapy in NSAID users reduces the occurrence of peptic ulcer has not been fully addressed. Studies on secondary prevention of NSAID-associated ulcers in *H. pylori* patients have indicated that *H. pylori* eradication results in impaired ulcer healing with an effect on the rate of peptic ulcer occurrence. On the other hand, the treatment of *H. pylori* in patients with no prior history of chronic NSAID therapy has been shown to decrease the risk of peptic ulcer. Studies in experimental animals revealed for instance, that the *H. pylori* infection augments the gastric mucosal damage induced by NSAID in Mongolian gerbils. In rats with preexisting chromic gastric ulcers, *H. pylori* infection attenuated significantly the aspirin-induced inhibition of ulcer healing and accompanying fall in the gastric blood flow at the margin of these ulcers, suggesting negative interaction between aspirin and *H. pylori* on ulcerogenesis. Accumulated evidence in humans and animals shows that both aspirin and *H. pylori* upregulate the expression of cyclooxygenase (COX)-2 both at mRNA and protein levels at the ulcer margin, but failed to influence significantly that of COX-1. It was, therefore, proposed that *H. pylori* may in fact, antagonize, aspirin-induced delay of ulcer healing due to suppression of acid secretion by the enhancement in PGE_2 possibly derived from COX-2 expression and activity and to the overexpression of growth factors such as TGFα and VEGF. The present review summarizes and further addresses the issue of the interaction between these two major ulcer risk factors determined in the stomach of humans and experimental animals.

Key words: *Helicobacter pylori, nonsteroidal anti-inflammatory drugs, aspirin, peptic ulcer, cyclooxygenase, gastric adaptation*

Pathophysiology of NSAID and H. pylori

Consumption of NSAID such as aspirin is associated with the development of gastric erosions or ulcers *via* several mechanisms including a release of salicylic acid which is not ionized by gastric acid (1, 2). Salicylic acid enters and accumulates in the gastric mucosal cells and undergoes ionization. It inhibits cell metabolic functions and permeates H^+ ion back diffusion leading to gastrointestinal damage (3). The world-wide use of NSAID such as ASA, e.g. for the treatment of musculoskeletal disorders, is associated with well-known gastrointestinal complications such as dyspepsia, gastric and/or duodenal erosions and ulcers and the formation of peptic ulcers (4). Among these complications the most important are bleedings, representing the most frequent serious adverse effect resulting from the intake of NSAID. In particular NSAID cause gastric erosions and delay ulcer healing through various mechanisms including: a) significant inhibition of biosynthesis of prostaglandins (5, 6) and suppression of both cyclooxygenase (COX)-1 and COX-2 activity, b) reduction in cell regeneration and inhibition of ulcer contraction (7) and c) decrease in mucosal blood flow in the

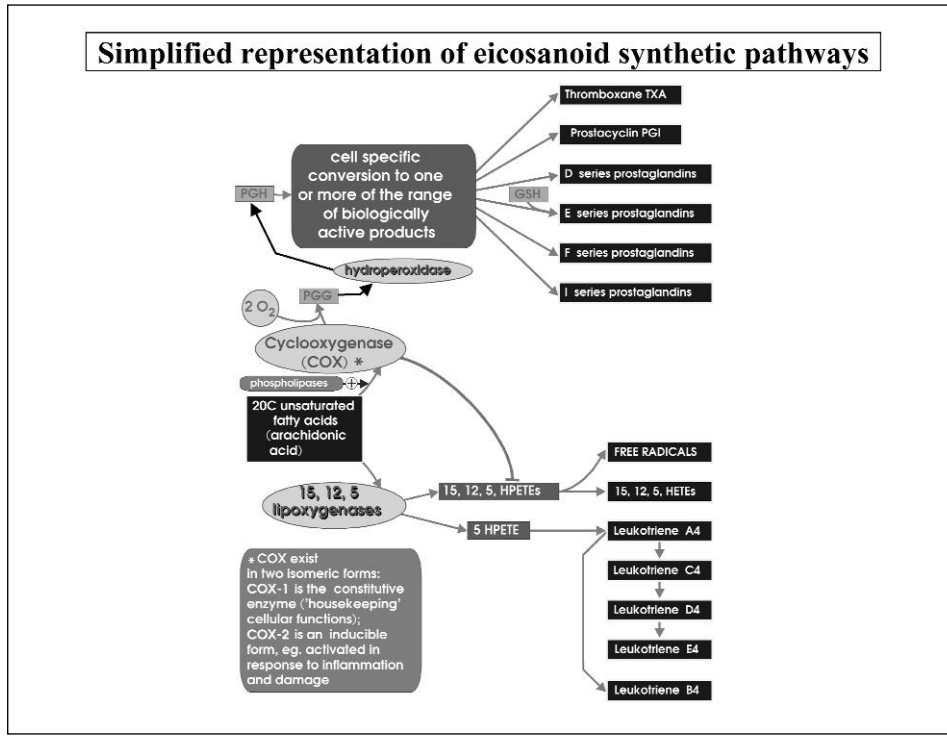

Fig. 1. Schematic representation of arachidonate synthetic pathways and their products derived from the cyclooxygenase (COX) and 15, 12 and 5 lipoxygenase enzymatic isoforms. Two COX-enzymes, namely constitutive COX-1 and inducible COX-2 play an important role in the pathomechanism of gastrointestinal integrity, gastroprotection, mucosal repair and ulcer healing.

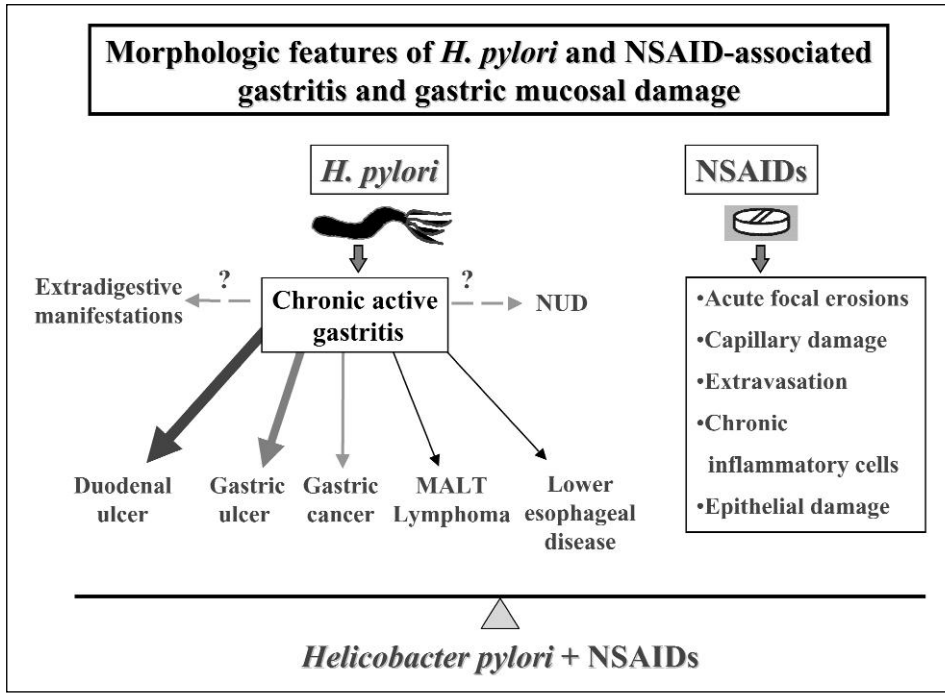

Fig 2. Consequences of concomitant actions of *H.pylori*- and NSAIDs in the mechanism of gastropathy induced by these two factors. *Bacterium* induces chronic gastritis and gastroduodenal ulcers and may lead to MALT-lymphoma and in some cases even to gastric cancer. NSAIDs, which besides *H.pylori* are considered as an independent risk factor of peptic ulcer disease, share with *bacterium* some features of gastric pathology including acute gastric epithelial damage, the impairment of the microcirculation and the development of chronic inflammation.

ulcer margin (8). The inhibition of the COX-1 enzyme impairs the production of protective prostaglandins (PG) and suppresses platelet production of thromboxane, which increases bleeding when an active GI bleeding site is present (9).

H. pylori is now generally accepted as a major cause of chronic gastritis and the important risk factor of peptic ulcer disease, increasing incidence of MALT lymphoma and in some cases of gastric cancer (10). Various pathogenic factors originating from *H. pylori* have been implicated in damaging effect of this bacteria on the gastric mucosa, the most important being cytotoxins released by *H. pylori*-strains expressing vacuolating cytotoxin A (*vacA*) and cytotoxin-associated gene A (*cagA*) proteins, *H. pylori*-derived lipopolysaccharides and the enhanced generation of free oxygen radicals and ammonia, the product of germ urease (6,10). *H. pylori* infection induces a substantial inflammatory reaction in the gastric mucosa with recruitment of leukocytes and overexpression and release of proinflammatory cytokines. Interestingly, this infection causes overexpression of COX-2 mRNA leading to enhanced biosynthesis of endogenous PG in the gastric mucosa (10,12).

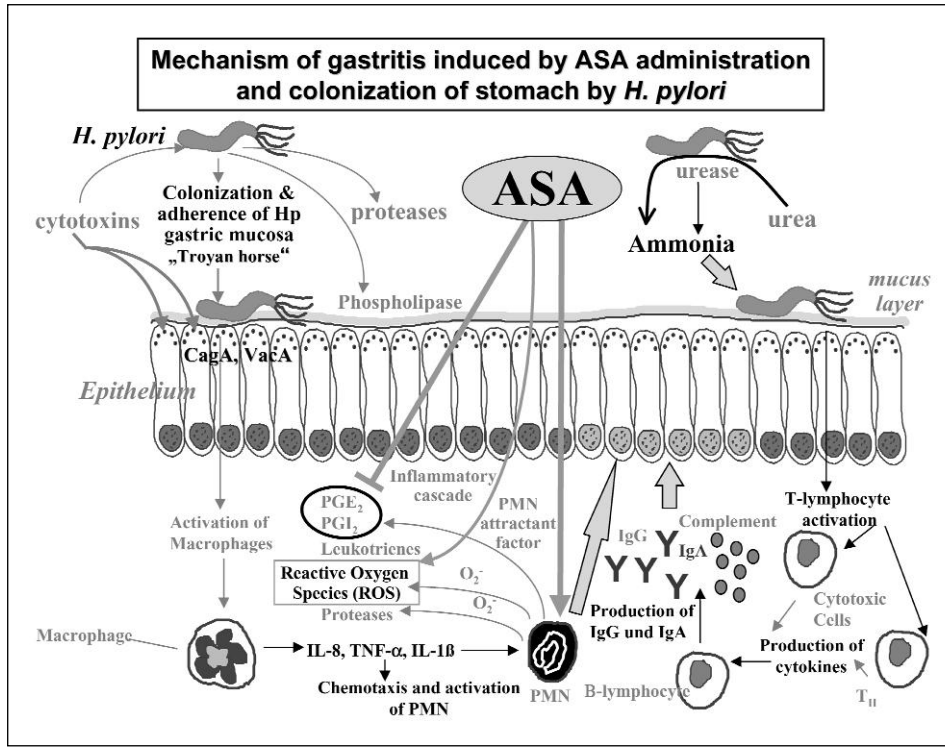

Fig. 3. Mechanism of acute and chronic damage induced by NSAIDs such as aspirin (ASA) and *H.pylori* colonizing gastric mucosa. ASA attracts polymorphonuclear (PMN) cells and triggers production of reactive oxygen species (ROS) while inhibiting of the COX enzyme-derived prostaglandins (PGE_2 and PGI_2). *H.pylori* acts as a "Troyan horse" adhering to the surface epithelial cell compartment and injecting cytotoxins and ammonia responsible for the aquisition of the bacteria in acidic envinroment of the stomach and triggers the activation of neutrophils and inflammatory response mediated by proinflammatory cytokines (IL-8, TNF-α and IL-1β).

Effect of NSAID on gastric mucosa of naive users infected with H. pylori

It is generally accepted that *H. pylori* and NSAID are major pathogenic factors in peptic ulcer disease (13), however, the results of studies on the interaction between NSAID and *H. pylori* are controversial and confused (14). If *H. pylori* gastritis enhances the risk of ulcer bleeding in NSAID users, then *H. pylori* eradication should substantially reduce such a risk in this setting, especially in patients with peptic ulcer. Indeed, in systemic review (15) the synergism for the development of peptic ulcer and ulcer bleeding between *H. pylori* infection and aspirin as a representative of NSAID, was proposed. In particular, the presence of *H. pylori* infection enhanced 3-5 fold the risk of peptic ulcer in aspirin users and by about 18 fold in subjects not taking aspirin. Recent meta-analysis review based on 16 studies of 2625 NSAID without secretory treatment claimed a clear

synergism for the development of mucosal ulceration by *H. pylori* infection and NSAID intake (16). It was concluded that the risk of peptic ulcer is approximately 60-fold higher in *H. pylori* positive NSAID users compared to *H. pylori* negative subjects not taking aspirin (16). Generally, since both *H. pylori* and NSAID are responsible for the mucosal damage they could be reasonably considered to increase the risk of development of uncomplicated and complicated peptic ulcer, however, data from several studies do not always confirm such an assumption (17-19). In agreement with this notion, Chan *et al.* (20) have shown that these two well-established risk factors for peptic ulcer disease exert a synergistic effect resulting in the increasing risk of this disease. However, this issue was not studied carefully, since peptic ulcer disease in NSAID users infected with *H. pylori* was less frequently diagnosed than in those taking NSAID without *H. pylori* infection (21). Furthermore, conflicting results were obtained from controlled randomized trials that examined whether *H. pylori* eradication could influence ulcer healing in individuals subjected to NSAID therapy (15, 17) and whether this eradication could reduce the development of peptic ulcer disease in NSAID takers (20, 22). As a broad generalization, *H. pylori* positive healthy individuals without ulcer history, benefit from *H. pylori*-eradication therapy at the start of NSAID therapy (15).

Effect of H. pylori eradication in NSAID users without a history of peptic ulcer complication

Previous study have demonstrated, that the deleterious effect of NSAID in patients with past or current peptic ulcers on the ulcer healing in *H. pylori* positive subjects treated with omeprazole, could be attenuated by *H. pylori* eradication (22) but the results of this study are difficult to interpret because of maintenance treatment with antisecretory agents such as omeprazole with greatly enhanced activity in *H. pylori* infected subjects. This first large clinical trial of *H. pylori* eradication (HELP study) raised several questions for the benefit of such an intervention (22). In this trial (22) almost 300 *H. pylori* positive chronic NSAID users with past or current peptic ulcers or NSAID-associated dyspepsia who continued a minimum dosage of NSAID for at least 6 months were randomized to receive *H. pylori* eradication therapy with omeprazole, amoxycillin and clarythromycin or omeprazole plus placebo antibiotics for 1 week. In addition, all patients received omeprazole 20 mg daily for 3 weeks followed by an additional 4-week therapy with omeprazole course in cases with endoscopically detected peptic ulcers. The probability of resolving peptic ulcer at 6 month was identical in *H. pylori* eradication versus omeprazole-controls but interstingly, the ulcer healing was significantly impaired in the *H. pylori* eradication (about 70% of ulcer healed) vs omeprazole-control group (100% ulcers healed) (22). It should be emphasized that *H. pylori* eradication therapy was given in this study to subjects with ulcers or at high risk of ulcers who had been already on long term consumption of NSAID. Moreover, these patients were treated for the period up to 8 weeks with

omeprazole, which significantly influenced the ulcer healing. Impairment of ulcer healing in *H. pylori* eradicated subjects could be attributed to the lower activity of the PPI in *H. pylori* eradicated stomach since more potent antisecretory activity of this inhibitor was observed at the presence of *H. pylori* infection (23). Previous study revealed that beneficial effect of PPI such lansoprazole is enhanced in the presence of *H. pylori* infection than in *H.pylori* negative individuals (24).

Hawkey *et al.* (22), concluded that the eradication of *H. pylori,* as shown in this clinical trial, retards the healing of gastric ulcers in NSAID users who were treated with omeprazole. This implies that *H. pylori* acts to increase the risk at the start of NSAID treatment (12) but with prolong NSAID therapy, *H. pylori* may exert protective influence against NSAID-induced ulcerogenesis, possibly by stimulating mucosal prostaglandins and other protective factors.

Interaction of H. pylori with NSAID in gastric mucosa of experimental animals

It is of interest that some aspects first documented in humans were confirmed in experimental animals with preexisting gastric ulcers inoculated with live *H.*

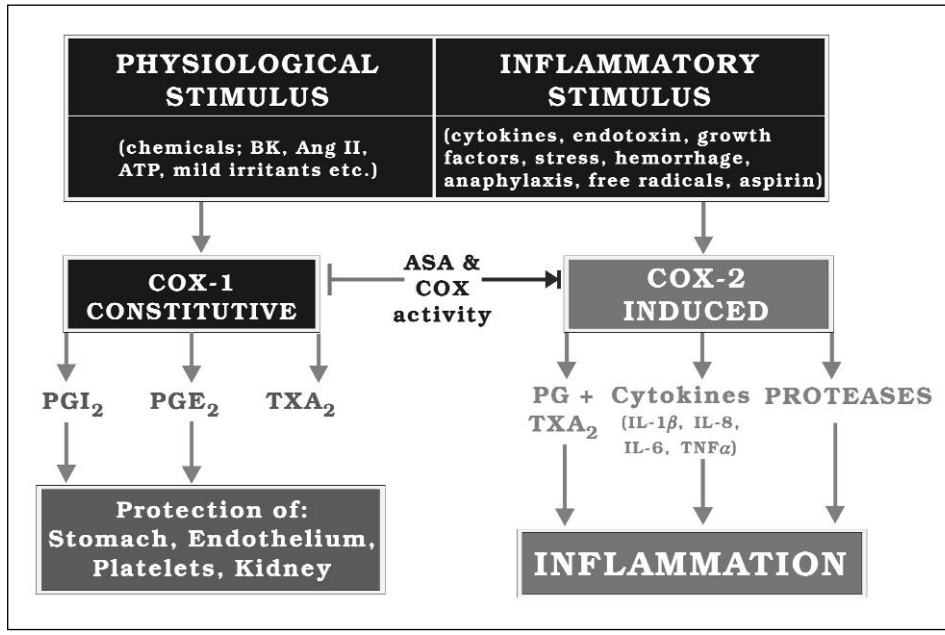

Fig. 4. Simplified demostration of contribution of COX-1 and COX-2 enzyme activities and their products such as PGs and tromboxane A_2 (TXA_2) to the maintenance of gastric mucosal integrity including protection (COX-1) and adverse processes (inflammation mediated by COX-2) of different organs including stomach. Physiological stimuli such as vasodilators or mild irritants were reported to influence the COX-1 activity and exert gastroprotective influence whereas various cytokines and proteases are known to stimulate COX-2 mediated proinflammatory action. Both, the COX-1 and COX-2 activities are suppressed by ASA and other NSAIDs.

pylori and administered with aspirin (25). First, an attempt was made in that study to determine whether *H. pylori* colonizes non-ulcerated and ulcerated rat gastric mucosa and whether the effect of these two major ulcer risk factors, aspirin and *H. pylori,* applied alone or in combination, can influence gastric acid secretion and gastric blood flow at the ulcer margin as well as gene expression for COX-1, COX-2 and growth factors such as transforming growth factor alpha (TGFα) and vascular endothelial growth factor (VEGF).

The healing of chronic ulceration is a complex process that includes filling of the mucosal defect with granulation tissue, cell proliferation at the ulcer margin, and an adequate blood supply delivering oxygen and nutrients to the ulcer area (26). The ulcer healing is accelerated by various growth factors, including TGFα and VEGF (27, 28). TGFα is normally expressed in the gastric mucosa to maintain the physiological functions of this mucosa, but its expression is significantly increased in the ulcerated gastric mucosa. TGFα, like epidermal growth factor (EGF), acts *via* epidermal growth factor-receptor (EGFr) accelerating the cell proliferation, migration and inhibiting of gastric acid secretion (29, 30). VEGF is a heparin-binding glycoprotein that occurs in five isoforms, which are generated as a result of alternative splicing from a single vascular endothelial growth factor gene. VEGF acts specifically on vascular endothelial cells to increase vascular permeability and to stimulate endothelial cell proliferation, migration and tube formation (angiogenesis). VEGF also increases blood flow and prevents endothelial cell apoptosis acting as the major angiogenic factor that was proposed to contribute to the ulcer healing and showing enhanced expression at the ulcer margin (31, 32), but its interaction with *H. pylori* or its products has not been clarified. Our study (25) demonstrated that gastric inoculation of rats with *H. pylori* at the start of the treatment with aspirin, partly reduced the retarding effect of this drug on ulcer healing. It is of interest that the *H. pylori*-infected mucosa not involved in gastric ulcer failed to show inflammation, confirming the observations by other investigators (33) that mild or moderate mucosal inflammation in rats infected with *H. pylori* is rather limited to the ulcer area in these species and provides a useful model for studying the pathogenesis of *H. pylori* infection, that, however, may not exactly reflect the human *H. pylori* infection. This notion is supported by the fact that *H. pylori* used in our study (25) was isolated from duodenal ulcer patient that showed antrum-predominant gastritis and acid hypersecretion leading to ulcer formation in humans. Moreover, rats infected with *H. pylori* exhibited hypochlorhydria rather than acid hypersecretion suggesting that these data, at least in terms of secretory changes, could not be easy extrapolated into the human scenario of *H. pylori* infection (25). Both, *H. pylori* and aspirin separately delayed healing of preexisting gastric ulcers but their combination failed to prolong the ulcer healing more than that achieved by application of aspirin or *H. pylori* infection alone (25). This could be due to suppression of acid secretion by bacteria itself or to the antisecretory activity of *H. pylori* derived

lipopolysaccharides (LPS) that may result in limitation of the local action of acid-dependent ulcerogen such as aspirin on ulcer healing. In addition, the fall of the gastric microcirculation in *H. pylori*-infected mucosa paralleled with hyposecretion in these animals, both being possibly attributed to *H. pylori*-cytotoxins as well as to LPS and ammonia released from this bacteria (34). This *H. pylori*-induced gastric hypochlorhydria, which was not seen in animals treated with aspirin alone, could result in hypergastrinemia observed in *H. pylori*-infected animals suggesting that gastrin originally recognized for its trophic effect on gastric mucosa, may also contribute to the acceleration of ulcer healing observed in rats treated concomitantly with *H. pylori* and aspirin.

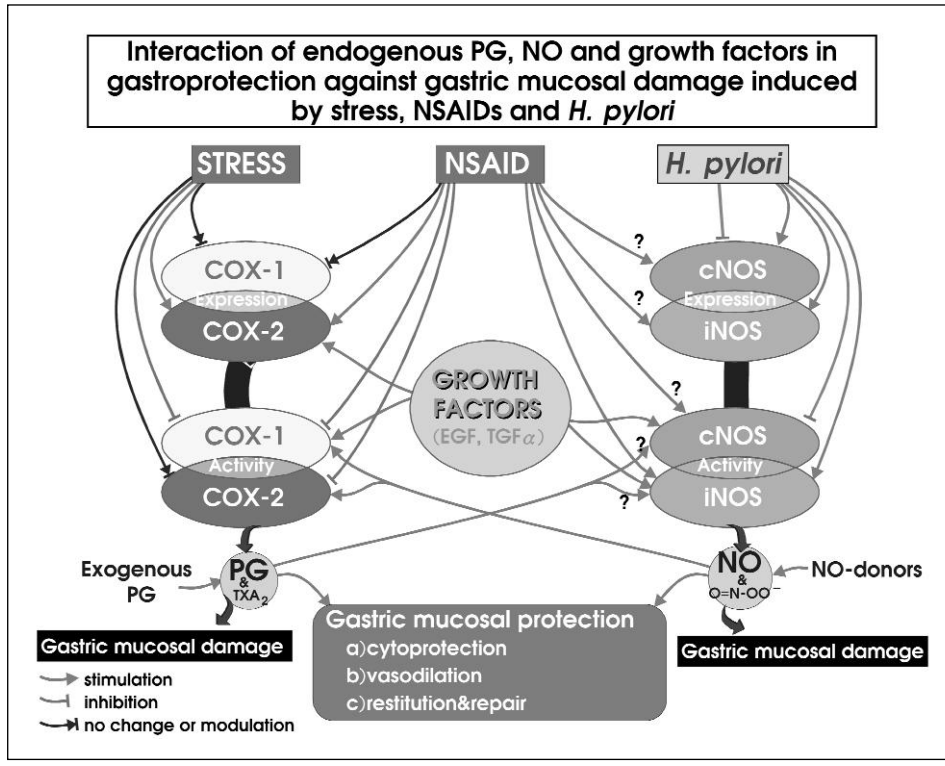

Fig. 5. Complex interactions between three independent risk factors of peptic ulcer disease such as stress, NSAIDs and *H.pylori* in the mechanism of gastric mucosal protection and ulcerogenesis. NSAIDs including ASA upregulate COX-2 expression, possibly compensating the suppression of COX-1 and COX-2 activity induced by this drug. *H.pylori* inhibits gene expression of constitutive nitric oxide (cNOS) while enhancing the expression of inducible NOS (iNOS) that may lead to overproduction of NO and excessive generation of toxic radical peroxynitrate involved in the gastric cell inflammatory response and cellular damage. Growth factors such as EGF, TGFα and VEGF contribute to gastroprotection by stimulation of COX and NOS enzymes expression and activities and by facilitating fast restitution process and mucosal repair of the gastric mucosa exposed to stress, or damaged by NSAIDs and *H.pylori*

This notion partly supports clinical observation of Hawkey *et al.* (22) that in NSAID users with peptic ulcer disease taking conventional acid suppressive therapy with omeprazole, *H. pylori* eradication was associated with a significant delay in healing of gastric ulcers as compared to that in long-term NSAID users who were not *H. pylori*-eradicated. One possible explanation for the antagonistic effects of the combined treatment with aspirin and *H. pylori* on the ulcer healing as compared to *H. pylori* or aspirin applied alone could be the overexpression of COX-2 by *H. pylori* with consecutive elevation of prostaglandin E_2 production in the gastric mucosa. This rapid upregulation of COX-2 mRNA in response to aspirin has been recognized before and could represent a compensatory response to inhibition of COX-2 activity and gastric prostaglandin synthesis (35). In agreement to this hypothesis (35), a non-selective COX inhibitor such as aspirin induced overexpression of COX-2 at the level of mRNA and protein but suppressed the COX-1 and COX-2 enzyme activities as documented by the profound decrease in the generation of PGE_2 in the gastric mucosa in aspirin-treated animals and humans.

Effectiveness of H. pylori eradication in chronic NSAID users with a peptic ulcer complication

A particular subgroup of NSAID subjects with a history of upper gastrointestinal bleedings or other complications represent those patients that are at a high risk for the recurrent bleeding during continued aspirin therapy especially when they are *H. pylori* infected (36). These patients should undergo concurrent therapy with PPI or eradication of *H. pylori*. According to accumulated evidence in large clinical trial enrolling almost 400 *H. pylori* positive NSAID users with previous ulcer bleeding, omeprazole was more effective than *H. pylori* eradication in the prevention of ulcer bleeding recurrence in patients taking naproxen for 6 months (the probability of recurrent bleeding about 4% in omeprazole group *vs* 18 % in those with *H. pylori* eradication). These data (36) indicate that *H. pylori* eradication that was reported to prevent effectively the recurrence of gastrointestinal bleeding in chronic aspirin users, actually appears to be less effective in those taking different NSAID other than aspirin, possibly due to the fact that this group of patients requires long-term antisecretory therapy with a PPI.

H. pylori has been also found to increase the risk of upper gastrointestinal bleeding even in chronic users taking a low prophylactic dose aspirin (37). In another more recent studies, *H. pylori* was implicated to increase the risk of upper gastrointestinal bleedings in NSAID users even when other factors predisposing to bleeding were considered (38, 39). All these observations confirmed that NSAID and *H. pylori* are independent risk factors for peptic ulcer and bleeding from peptic ulcer (36 - 39).

Gastric adaptation to NSAID in H. pylori infected gastric mucosa – the question unanswered?

An interesting, practical, and important discovery related to the gastric damage induced by NSAID is an increase in mucosal tolerance or adaptation to the ulcerogenic action of these drugs that develops with their more prolonged administration. This remarkable attenuation of mucosal damage had been first demonstrated in rats and then confirmed in humans. Aspirin caused a widespread initial injury, which was followed by the adaptation and increased tolerance to withstand further aspirin insult without significant injury. Interestingly, this remarkable ability of the gastric mucosa to withstand the prolonged exposure to the ulcerogenic action of aspirin does not depend upon the PG biosynthesis because this generation is suppressed with the first dose of aspirin and remained suppressed during continuous administration of this NSAID (40). For instance, gastric adaptation to aspirin does not appear to be mediated by endogenous PG, since prolonged administration of this NSAID was accompanied by almost complete suppression of COX-1 and COX-2 activity in the gastric mucosa.

Recent study indicates that *H. pylori* impaired the gastric adaptation to aspirin in humans as evidenced by persistent microbleeding, suggesting that *H. pylori* enhances the gastric toxicity of this NSAID (41). In all subjects, aspirin-induced gastric damage that reached maximum on day 3 while in those infected with *H. pylori*, this damage was maintained at a similar level up to day 14. After *H. pylori* eradication, the damage was significantly lessened both in endoscopy and histology at day 14 and accompanied by increased mucosal expression and luminal release of TGFα (41). Prostaglandin E_2 generation was significantly greater in *H. pylori*-positive subjects than after *H. pylori* eradication, but aspirin treatment resulted in >90% reduction of this generation independent of *H. pylori* status (40). It was concluded that gastric adaptation to aspirin was impaired in *H. pylori*-positive subjects, but eradication of this *bacterium* restored this process (41). Findings in animal model of *H. pylori* infection appeared to be contradictory to those observations in humans but the difference could be easy explained by the divergence in experimental conditions and the fact that experimental studies considered mostly animals with chronic gastric ulcers, while human studies recruited human volunteers without previous ulcer history (25, 41). Since gastric adaptation in experimental animals is triggered by direct contact of the gastric mucosa with ulcerogen such as aspirin applied in injurious dose (42), it is apparent that the question as to whether *H. pylori* infection influences the phenomenon of gastric adaptation to aspirin could not be properly addressed in rat model with chronic gastric ulcers.

Recent studies revealed that intragastric aspirin when administered repeatedly induces acetylation of COX-2 which is upregulated during continued treatment with this NSAID resulting in the local generation of 15-(R)-epi-lipoxin A4, also termed

"aspirin-triggered lipoxin" (ALT) (43, 44). ALT exerts gastroprotective activity in the stomach and was also implicated in the enhanced gastric mucosal resistance to aspirin-induced mucosal injury in animal gastritis model (43). Moreover, lipoxins contribute to the aspirin-induced gastric adaptation in experimental animals (44). The involvement of ALT in *H. pylori*-infected patients with the concomitant NSAID intake remains to be studied but the possibility that these COX-2 products could play an important role in the limitation of the synergistic influence of the NSAID on the gastric mucosa of *H. pylori*-infected individuals can not be ruled out and should be further evaluated in an appropriate clinical trial.

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