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Review

Impact of *Helicobacter pylori* and nonsteroidal anti-inflammatory drugs on gastric ulcerogenesis in experimental animals and in humans

Teresa Pawlik, Peter C. Konturek, Jan W. Konturek, Stanislaw J. Konturek*, Tomasz Brzozowski, Marta Cześnikiewicz, Malgorzata Plonka, Władysław Bielanski, Hany Areny

Department of Physiology, University Medical College, Ul. Grzegorzecka St. 16, 31-531, Cracow, Poland

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Abstract

Helicobacter pylori (*H. pylori*) and nonsteroidal anti-inflammatory drugs (NSAID) are the most common pathogens in the gastroduodenal mucosa in animals and humans, but their relationship in ulcerogenesis has been little studied. According to some authors, *H. pylori* infection in humans does not act synergistically with NSAID on ulcer healing, therefore, there is no need to eradicate the germ. This notion is supported by the finding that the eradication of *H. pylori* does not affect NSAID-induced gastropathy treated with omeprazole and that *H. pylori* infection induces a strong cyclooxygenase-2 expression resulting in excessive biosynthesis of gastroprotective prostaglandins, which should in turn counteract NSAID-induced gastropathy and heal the existing ulcer. Other investigators claim that *H. pylori* infection acts synergistically with NSAID on ulcer development, therefore, *H. pylori* should be eradicated, particularly at the start of long-term NSAID therapy. Maastricht 2-2000 consensus also recommends eradication prior to NSAID treatment, but this eradication does not appear to accelerate ulcer healing or to prevent the recurrent ulcers in NSAID users. Our studies in almost 6000 dyspeptic patients undergoing upper endoscopy and [^{13}C]-urea breath test (UBT) revealed that about 70% of these patients are *H. pylori* (+) and about 30.6% of these develop gastroduodenal ulcers. Of these ulcers, over 70% were *H. pylori* (+) positive, 12% NSAID (+), 8% were both *H. pylori* (+) and NSAID (+), while 22% ulcers were *H. pylori* (−) and NSAID (−) or ''idiopathic'' ulcers. Basically, our results support Hawkey's concept and this also agrees with our findings in the rat model showing that: (1) there is no synergistic interaction between *H. pylori* infection and NSAID on gastric ulcer development, (2) *H. pylori* and NSAID are independent risk factors for peptic ulceration, and (3) NSAID therapy in *H. pylori* positive patients attenuates the ulcer development possibly due to direct inhibitory action of these drugs on *H. pylori*.
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Keywords: *H. pylori*; Aspirin; Nonsteroidal anti-inflammatory drug; Peptic ulcer

1. Introduction

1.1. Pathogenesis of gastric ulcers induced by H. pylori infection and nonsteroidal anti-inflammatory drugs (NSAID)

Helicobacter pylori (*H. pylori*) infection and NSAID are generally considered as major ulcerogens responsible for gastroduodenal mucosal damage, but the mechanism underlying this damage appears to be quite different. *H. pylori* infection initially induces acute and then chronic active mucosal inflammation (bacterial gastritis—gastritis B). The acquisition of *H. pylori* in the stomach requires penetration of bacteria through an ''unstirred'' layer of mucus/HCO_3^- covering the surface epithelium to reach the surface and adhere directly to it (Konturek et al., 1999). This is facilitated by bacterial flagella allowing for rapid mobility of *H. pylori* within the mucus to reach the surface of epithelial cells and to permanently adhere to them via bacterial pseudopodia. Following adherence to surface epithelium, *H. pylori* acts directly on the cells to ''inject'' into them the bacterial proteins and cytotoxins, especially those encoded by the *cytotoxin-associated gene* (*cagA*), a marker for pathogenicity islands containing various genes with homology to the so-called type IV secretion system (Rektorschek et al., 2000; Stein et al., 2000; Odenbreit et al., 2000; Asahi

* Corresponding author. Tel.: +48-12-4211006; fax: +48-12-4211578.
E-mail address: mpkontur@cyf-kr.edu.pl (S.J. Konturek).

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et al., 2000) (Fig. 1). Once *cagA* protein and other cytotoxins of *H. pylori* have been "injected" or translocated into the mucosal cells, they result in tyrosine transphosphorylation along with other cytosolic proteins (Stein et al., 2000; Asahi et al., 2000; Covacci and Rappuoli, 2000) and enhance the rate of host cell growth and induction of potent cytokines such as interleukin-8 via nuclear factor (NF)-kB (NF-κB) (Asahi et al., 2000; Covacci and Rappuoli, 2000; Li et al., 1999). This *H. pylori*-host mucosal cell interaction resembles a "Trojan horse" for the host cell (Covacci and Rappuoli, 2000) because it causes not only the release of toxin cytokines such as interleukin-8 involving NF-κB (Li et al., 1999) (Fig. 1), but also results in direct damage to the host cells. In addition, *cagA* has been proposed to be an important risk factor for the development of peptic ulceration and even gastric cancer in *H. pylori*-infected gastric mucosa (Fallone et al., 2000; Konturek et al., 1999). The question remains contentious and studies on *cagA* and *vacA* as well as interleukin-1β polymorphism (Ji et al., 2000; El Omar et al., 2000) may provide additional information regarding the link between *H. pylori* and ulcerogenesis, carcinogenesis and other gastroduodenal diseases. Furthermore, *H. pylori* in the stomach stimulates the release of leukocyte chemoattractants [C5a, *N*-formyl peptides (fMLP), platelet-activating factor (PAF), leukotriene B_4 (LTB_4)], chemokins (e.g. interleukin-8) and tumor necrosis factor alpha (TNF-α) (Bourke and Jones, 2001). TNFα in turn up-regulates the expression of selectins and their ligands and integrin receptors (Fiorucci et al., 1999). These pro-adhesive factors are balanced by endogenous anti-adhesive substances including transforming growth factor alpha (TGFα) and nitric oxide (NO) induced by *H. pylori* from the infected mucosal and nonmucosal cells, including macrophages, leukocytes, miocytes, etc.

H. pylori in gastric mucosa, mainly in the gastric *antrum*, where this germ is usually present, induces chronic active gastritis (*antritis*) with overexpression of cyclooxygenase-2. Similarly, the infection of the margin of the ulcer infected by *H. pylori* causes overexpression of cyclooxygenase-2 without affecting cyclooxygenase-1 expression (Konturek et al., 2001) (Fig. 2). The excessive biosynthesis and release of "cytoprotective" prostanoids, particularly prostaglandin E_2, due to overexpression of cyclooxygenase-2 results from the activation of the whole arachidonate cascade starting from phospholipase-activated release of arachidonic acid from the phospholipid membrane, its transformation to prostaglandin G_2 and then prostaglandin H_2 and finally to prostaglandin E_2, prostaglandin I_2, prostaglandin $F_{2\alpha}$, prostaglandin D_2, and its prostaglandin J_2. The excessive amounts released of prostaglandin act via various membrane-bound receptors on target cells causing various physiological responses (Fig. 3). These effects of prostaglandins limit the extent of mucosal

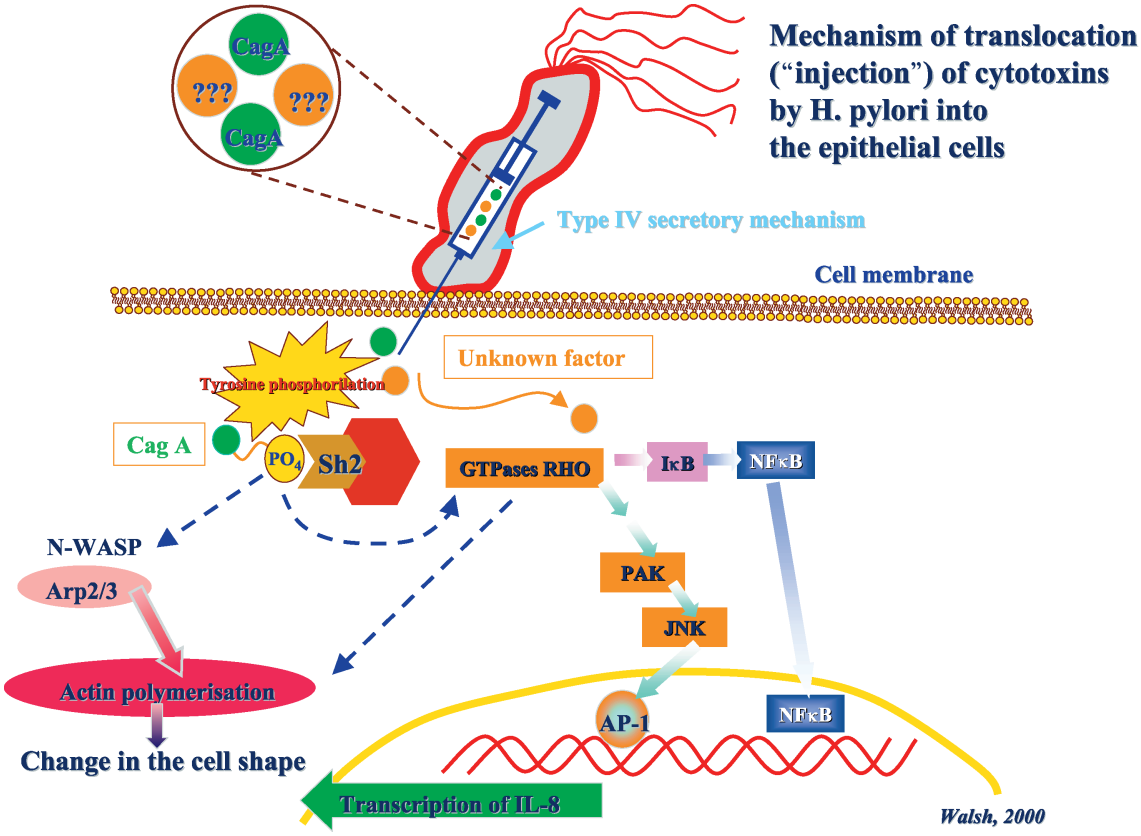

Fig. 1. *H. pylori*, as Trojan horse, injecting cytotoxins into epithelial cells and intracellular events leading to expression, production, and release of interleukin-8 (IL-8).

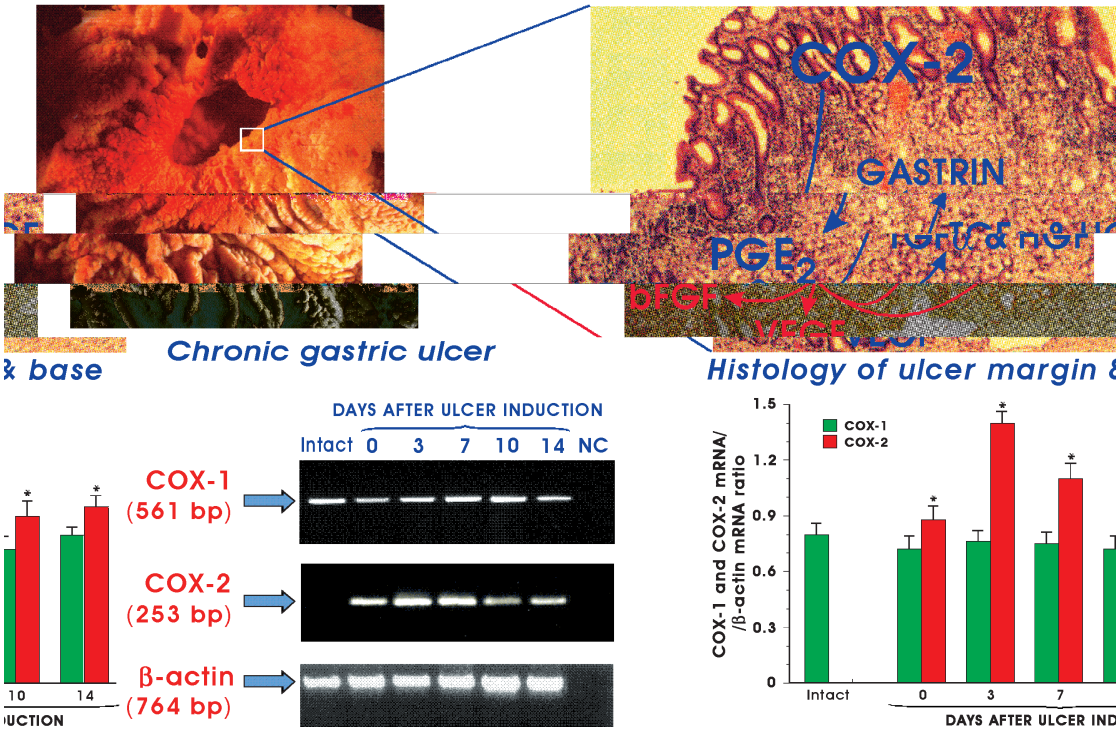

Fig. 2. The margin of gastric ulcer expressing cyclooxygenase-2 and producing large amounts of PGE_2 as well as various growth factors (TGFα. VEGF, and bFGF). The time course of expression of cyclooxygenase-1 and cyclooxygenase-2 at ulcer margin and their ratio as well as the PCR products in mucosal cells at various points of this time course (Konturek et al., 2001).

damage and accelerate mucosal repair and ulcer healing via stimulation of angiogenesis, expression of proliferating factors such as transforming growth factor α (TGFα) and hepatocyte growth factor (HGF) (Konturek et al., 1998b), resulting in proliferation of mucosal cells to replace those damaged by *H. pylori* and repair of mucosal lesions (Konturek et al., 1999) (see Fig. 2). It is of interest that *H. pylori*-induced gastritis is associated (at least at certain time periods after infection) with an increase in apoptosis attributed to the combined action of various noxious products (ammonia, cytotoxin, lipopolysaccharides, endotoxins, leukocyte attractive factor, etc.) released from the *H. pylori* itself or released by mucosa infected by this germ (cytokines, interleukin-8, interleukin-1β and others, especially TNF-α) (Konturek et al., 1998b). Hyperproliferation of mucosal cells, observed in chronically infected mucosa, may quite often be a secondary phenomenon initiated by excessive cell loss due to epithelium desquamation. It is of interest that such hyperproliferation of mucosal cells may also involve stimulation of apoptosis due to high cell turnover, and under special conditions, may eventually result in cancer development.

The NSAID present in the acidic gastric content exert their damaging action on the gastric mucosa in two ways: (1) a major systemic mechanism involving inhibition of cyclooxygenases and (2) by a local cyclooxygenase-independent mechanism (Fig. 4) by breaking the gastric mucosal barrier, penetrating the mucus layer to reach the surface of epithelial cells and diffusing into mucosal cells in the acidic gastric lumen (pK_a for aspirin is 3.5) by nonionic diffusion (Scheiman, 1996). During this diffusion, the cells of the surface epithelium loose their hydrophobicity and the ability to repel polarized substances such as HCl, while aspirin and other acidic NSAID diffuse and accumulate within cells. Here, they dissociate in the cytoplasm and being "trapped", they affect enzyme activity, uncouple oxidative phosphorylation and suppress the expression and production of heat shock proteins (HSP) that are normally responsible for cellular integrity (Wallace, 1997). The damaged surface epithelium swells and forms, with exfoliated cells, the "mucoid cap", allowing the penetration of luminal H^+ into the mucosa to release various inflammatory mediators such as leukotriene B_4 and histamine, to damage the microvascular wall, increasing its permeability, and to decrease the mucosal blood flow (Wallace et al., 1990; Wallace and Tigley, 1995) (Fig. 4).

Another mechanism of local action of NSAID is the release from mucosal cells of TNFα, which up-regulates adhesion molecules and activates neutrophils, leading to infiltration of the gastric mucosa, reduction in mucosal blood flow and the formation of acute erosions and ulceration. TNFα also activates proapoptotic caspases via stimulation of NF-κB leading to protection of the P53-DNA repair mechanism and enhancement of the apoptotic pathway (apoptosis is programmed cell death without surrounding inflammation). NSAID administration causes an

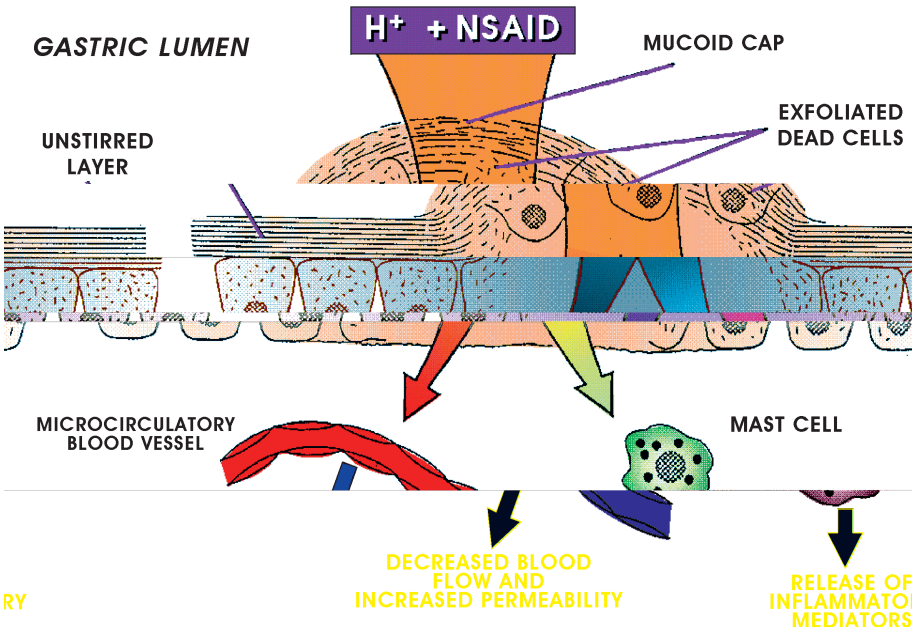

Fig. 4. The dependence of NSAID-induced topical gastric mucosal damage on gastric acidity (pH < 3.5) and consequence of non-ionic diffusion of acidic NSAID into mucosal cells with damage to these cells, disturbance of microcirculation and activation of mast cells to release inflammatory mediators (LTB_4, IL-Iβ, and TNFα).

in colonic mucosa involved in ulcerative colitis in dysplasia or neoplasia. Other prostanoids such as prostaglandin I_2 produced mostly by endothelium act via IP receptors that prevent platelet aggregation to cause vasodilatation, natriuresis, and gastric acid inhibition. Prostaglandin $E_{2\alpha}$ acts via FP receptors to induce uterus contraction, vasoconstriction, bronchospasm and reduction in gastric acid secretion. Prostaglandin D_2 uses DP receptors to increase gastric and renal blood flow, to inhibit gastric H^+ secretion and platelet aggregation. Prostaglandin D_2 can be transformed to J_2 that is the known stimulus of tissue repair and healing and to induce NO synthase. TXA_2 acts via TP receptors to increase the plasma Ca^{2+} level, resulting in platelet aggregation and potent vasoconstriction (see Fig. 3). The biological effects of cyclooxygenase products, prostaglandins, are mediated by specific membrane receptors termed *EP receptors* (EP_1, EP_2, EP_3, and EP_4) that are bound to membrane G-proteins linked to a different intracellular signal transduction pathway (Sugimoto et al., 2000). Binding of PGE_2 to EP_1 results in intracellular release of triphosphate inositol (IP_3) and diacylglycerol, binding to EP_2 and EP_4 activates adenylcyclase–cyclic adenosine monophosphate (cAMP), and to EP_3 inhibits the adenylcyclase–cAMP system (see Fig. 3).

In contrast to cyclooxygenase-1, cyclooxygenase-2 is overexpressed in inflammatory tissue, being an enzyme strictly inducible by cytokines, growth factors including gastrin, and tumor promoters (Hla et al., 1999). Cyclooxygenase-2 is responsible for excessive production of prostaglandin associated with inflammation, and this is due to a special large channel allowing arachidonic acid to remain longer and in close vicinity to an active center of the enzyme than in cyclooxygenase-2 (Fig. 5), while cyclooxygenase-1 is involved in the production of prostaglandin to maintain gastrointestinal mucosal integrity (Lipsky et al., 2000).

The regulation of prostaglandin biosynthesis is complex and depends not only on expression of cyclooxygenase-1 or cyclooxygenase-2 but also upon the availability of their substrate, arachidonic acid, released by phospholipases, particularly phospholipase-2, from the membrane phospholipids. Cytosolic phospholipase-2 ($cPLA_2$) is Ca^{2+}-dependent and selective for phospholipids containing arachidonic acid. Secretory phospholipase-2 ($sPLA_2$) exists in five distinct isoforms (Lambeau and Lazdunski, 1999) and one of them, known as inflammatory-type secretory phospholipase-2, is highly expressed in inflamed tissues such as *H. pylori*-induced gastritis through the action of proinflammatory cytokines, especially TNF-α (Sugimoto et al., 2000).

Since the function of prostanoids involves various receptor sites, mice with targeted gene disruption (knock-out mice) have been used to identify the real function of each prostanoid (Lambeau and Lazdunski, 1999; Sugimoto et al., 2000; Langenbach et al., 1999). Physiologically, prostaglandin E_2 is responsible for increasing gastric blood flow, renal blood flow, vasodilation, inhibition of gastric acid secretion, and natriuresis. Prostaglandin D_2 raises RBF and inhibits gastric acid secretion, prostaglandin I_2 was implicated in the inhibition of platelet aggregation and inhibition of gastric acid secretion. Prostaglandin $F_{2\alpha}$ was involved in the increase in uterine contraction and vaso- or bronchoconstriction. Thromboxane A_2 was held responsible for enhanced platelet aggregation and vasoconstriction.

Most conventional NSAID inhibit both cyclooxygenase-1 and cyclooxygenase-2 with a predominant action on

releasing excessive prostaglandin biosynthesis, is itself capable of inducing ulcer formation and, in humans, over 60% of these ulcers develop due to the *H. pylori* infection. According to our experience of total 5967 dyspeptic patients, about 10% could be attributed to NSAID (Konturek et al., 2002) (Fig. 6). Following *H. pylori* eradication, the NSAID ulcers do not heal at a higher rate than do those with preserved *H. pylori* infection (Hawkey et al., 1998b). The importance of prostaglandin originating from *H. pylori* infection is difficult to assess in *H. pylori*-infected NSAID users because these drugs are potent inhibitors of both cyclooxygenase-1 and cyclooxygenase-2, so it is unknown to what degree prostaglandin deficiency develops in gastric mucosa, though it was found that these drugs, despite their inhibition of cyclooxygenase activity, cause an enhanced expression of cyclooxygenase-2 in the *H. pylori*-infected stomach (Laine et al., 1995; Konturek et al., 2001).

1.3. Epithelium damage, lymphocyte involvement and neutrophil-mediated mucosal injury by H. pylori and NSAID

Colonization of gastric mucosa with *H. pylori* and its infection result from the ability of this germ to remain variable within the mucus covering this mucosa despite the hostile acid environment of gastric lumen that usually kills most of the ingested bacteria and "sterilizes" the gastric content. The potent urease activity of *H. pylori* has been successfully exploited in testing for the presence of active *H. pylori* infection using the [^{13}C]-urea breath test. Urease (UBT) represents a well-defined gastric colonization factor for this germ through the formation by alkaline ammonia (NH_3) on a "blanket" around the bacteria that neutralizes and counteracts the noxious effects of gastric luminal HCl. The urease-encoding gene present in *H. pylori* genome, termed *urel*, has been shown to be pH-sensitive and to function as urea channel (Weeks et al., 2000; Rektorschek et al., 2000), explaining how the viability of this particular bacteria can be preserved in the acid environment of the gastric lumen. The deletion of urel abolishes *H. pylori* acid resistance (Rektorschek et al., 2000), confirming that urease activity, which is strongly activated at lower pH, is a prerequisite of bacteria viability and growth in acidic gastric environment.

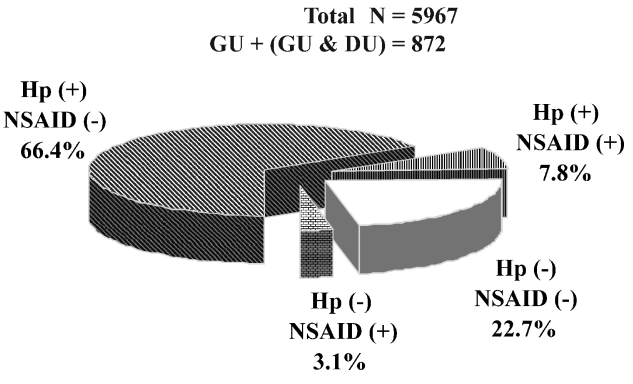

Fig. 6. Prevalence of gastric ulcer (N=872) in dyspeptic patients infected with *H. pylori*, using NSAID, both, or neither. Only *H. pylori* infection, but not NSAID, was effective to significantly increase in ulcer prevalence, while 66.4% of ulcer patients showed without *H. pylori* infection without NSAID use; there were about 22.7% without etiological factor, hence termed "idiopathic" ulcer (Konturek et al., 2002).

Other toxic substances produced by most *H. pylori* are lipopolysaccharides that are similar to blood group antigens, such as Lewis X (Le^x) or Lewis Y (Le^y). These LPS of *H. pylori* have been associated with host epithelial cells and implicated in *H. pylori*-induced gastric pathology (Gerhard et al., 1999; Wang et al., 2000). The blood group antigen that binds adhesin (BabA) of *H. pylori* encoded by $babA_2$ of this germ has been associated with the outcome of *H. pylori* infection and, in combination with *cagA* and *vacAS1*, has been shown to correlate strongly with the development of peptic ulcers or gastric cancer as compared with *cagA* and *vacAS1* alone (Bourke and Jones, 2001). According to our experience, small doses of *H. pylori*-LPS may induce gastroprotection against various topical irritants, accompanied by increased microcirculation, while larger doses have opposite effects, namely, they decrease both systemic blood pressure and the gastric blood flow, causing mild superficial injury to the gastric mucosa and prolong the healing of chronic gastric ulcers via induction and activation of cyclooxygenase-2 and iNOS, with excessive release of prostanoids and other products of arachidonate metabolism as well as NO (Konturek et al., 2002). It is of interest that the gastroprotective effects include the cerebral centers because the intracerebral administration of this LPS resulted in gastroprotection similar to that on peripherally application but disappearing after inactivation of afferent sensory nerves. In brief, it appears that the presence of *H. pylori* in the stomach acts on the gastric mucosa through activation of the brain–gut axis and the release of NO, probably due to the release of calcitonin gene-related peptide (CGRP) and other sensory neuropeptides. Evidence shows that endogenous opiates may also contribute to the protection involving the brain–gut axis because blockade of mμ-receptors with naloxone reverses the gastroprotection afforded by peripheral or central LPS (Brzozowski et al., unpublished). Thus, *H. pylori* strictly confined to the gastric mucosa appears to involve peripheral neural pathways as well as cerebral centers and the brain–gut axis.

1.4. Gastric immune response

H. pylori infection also induces a potent immune response in the host (Bourke and Jones, 2001; Zevering et al., 1999). Upon bacteria stimulation, native T-helper (Th) precursor cells differentiate both into Th_1 that are associated with cytokine (interleukin-2 and interferone-γ) production and a cell-mediated immune response, and into Th_2 that promote activation of B-lymphocytes and a humoral immune response including the release of various cytokines

(interleukin-4, interleukin-5, interleukin-6, and interleukin-10) (Ji et al., 2000; El Omar et al., 2000). Since *H. pylori* is a typical extracellular pathogen and noninvasive by nature, but associated with an exuberant humoral response, it is expected to induce a Th_2 response but, paradoxically, this germ induces antigen-specific T-cell clones in the gastric mucosa, producing a high level of interferone-γ and interleukin-4, reflecting a Th_1-type response (Israel and Peek, 2001). *H. pylori* also stimulates interleukin-12 which promotes Th_1 differentiation, suggesting that the bacterial gastritis (gastritis B) involves both Th_1 and Th_2 immune responses and perpetuates gastric inflammation from an acute superficial to a deeper active chronic gastritis and then gastric atrophy (Fallone et al., 2000). Infection with *H. pylori* strains producing cytotoxin encoded by *cagA*, *vacA*, *iceA*, and interleukin-8 released from the damage mucosal cells leads to intense atrophic gastritis (Zevering et al., 1999). Host cytokine polymorphisms such as that of interleukin-1 have been associated with an increasing risk of gastric carcinogenesis (El Omar et al., 2000; Bourke and Jones, 2001; Blaser, 2000), but this awaits confirmation.

An important feature of *H. pylori*-induced gastritis is an alteration in epithelial cell apoptosis (Blaser, 2000; Shirin and Moss, 1998; Jones et al., 1999; Konturek et al., 1998a) with compensatory cell proliferation, possibly mediated at least in part by excessive expression of growth factors (Konturek et al., 1998a) (see Fig. 2). Signaling through Fas death receptor has been postulated to mediate apoptosis either via activation of these receptors directly on gastric epithelial cells and/or through the production of cytokines such as TNFα (Houghton et al., 2000). An enhanced rate of apoptosis and subsequent widespread mucosal cell death may accelerate the progression of atrophic gastritis with a concomitant increase in the risk of distal gastric cancer, while reduced apoptosis and cell loss, leading to heightened retention of mutagenized cells, may also predispose to gastric cancerogenesis. Th_1 lymphocyte-derived cytokines such as interferon gamma (IFNγ) are synergistic with *H. pylori* to induce Fas ligand (FasL) in epithelial cells, resulting in *H. pylori*-induced apoptosis (Rudi et al., 1998).

In addition to direct mucosal cell damage by *H. pylori* and its mediators, there is increasing evidence for production by *H. pylori* of a neutrophil activation factor (Craig et al., 1992), resulting in mucosal infiltration with neutrophils that are probably involved in the release of interleukin-8 and formation of reactive oxygen species (Craig et al., 1992; Crabtree et al., 1994). NSAID also increase neutrophil activation and adherence to endothelium to liberate reactive oxygen species, proteases with obstruction of blood flow in the gastric mucosa vessels (Kitahora and Guth, 1987; Taha et al., 1995; Konturek et al., 1998b). Neutrophils have also been implicated in the pathogenesis of NSAID-induced gastropathy (Wallace et al., 1990), and the combination with *H. pylori* infection was suggested to aggravate NSAID-induced mucosal injury due to enhancement of interleukin-8 production and to release of oxygen-free radicals, but our experimental data and human results do not support this notion. According to our results, the interaction between *H. pylori* and NSAID, despite of the activation of gastric neutrophils and release reactive oxygen

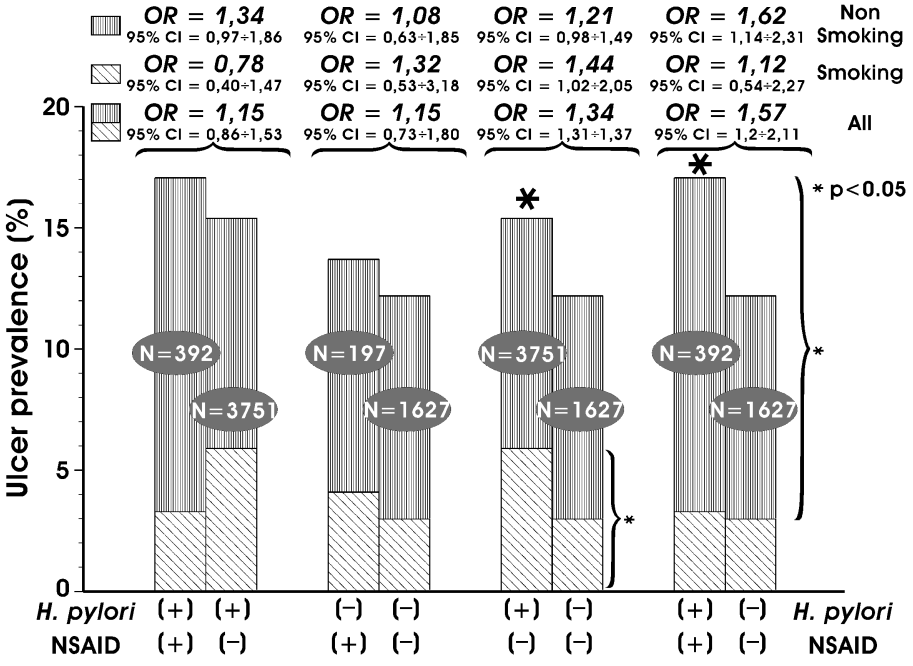

Fig. 7. Gastroduodenal ulcers ($N = 872$) in patients with *H. pylori* infection, with *H. pylori* infection plus NSAID use, with NSAID use only, and without *H. pylori* plus NSAID (idiopathic ulcers). Multivariate regression analysis shows that only *H. pylori* alone or combined with NSAID increases the ulcer risk when compared to that in patients without *H. pylori* and/or NSAID (Konturek et al., 2002).

species, results in significantly smaller incidence of mucosal lesions in *H. pylori*–NSAID users as compared with non-infected NSAID users only (Fig. 7).

1.5. Gastric acid secretion

Intragastric acidity and gastric acid secretory activity have been reported to be altered by *H. pylori* infection and by NSAID (Kitahora and Guth, 1987; Konturek et al., 1998b; Gerkens et al., 1977). Acute *H. pylori* infection in humans was reported to cause transient hypochlorhydria due to a direct action of *H. pylori*-derived inhibitor with the restoration within a few months of gastric acid secretory activity (Konturek et al., 1999), particularly in *antrum*-dominated chronic active gastritis (antritis) (El-Omar et al., 1997). This effect is accompanied by increased gastrin release due to reduction in somatostatin and impairment of paracrine inhibitory action on the G-cells (Konturek et al., 1999, 1998a). These functional secretory changes caused by antral inflammation with *H. pylori* disappear on eradication of *H. pylori* with a pantoprazol-based 1-week triple therapy (Fig. 8).

However, an extension of *H. pylori* infection to the gastric *corpus* and/or production of autoantibodies against parietal cells that bind and inactivate H^+–K^+–ATP-ase on the luminal surface of the acid secreting cells may result in gastric atrophy with permanent achlorhydria (Konturek et al., 1999). As gastric mucosal infection may last for decades and the bacteria colonize predominantly the antral portion of the stomach (*antritis*) or the *corpus* of the stomach (*corpusitis*) or both, pangastritis with mucosal atrophy may be accompanied by a severe decrease in gastric secretory activity, alteration in gastrin release (decrease in atrophic antritis and increase in corpusitis), and enhanced susceptibility to develop intestinal metaplasia, dysplasia, and gastric ulcer or cancerogenesis (McColl et al., 1997; El-Omar et al., 1997).

1.6. NSAID–H. pylori interaction on gastric mucosa

In contrast to *H. pylori*, NSAID use is expected to raise gastric acid secretion because of their ability to inhibit prostaglandin biosynthesis. However, this is usually not the case because breaking of the gastric mucosal barrier and reduction in blood flow occur in long-term users of NSAID (Kitahora and Guth, 1987). This results in the damage to surface epithelium (Ligumsky et al., 1983) and is followed by back-diffusion of luminal acid with subsequent reduction in gastric acid secretion and increased luminal pH, but this is not always the case due to inhibition of somatostatin by NSAID (Ligumsky et al., 1983). Nevertheless, luminal acid ($pH < 3.5$) is required for acidic NSAID, such as aspirin, to penetrate the mucosa and to cause its damage. NSAID suppress the natural cellular defence mechanism involving the release of HSP and enhanced apoptosis (Wallace, 1997).

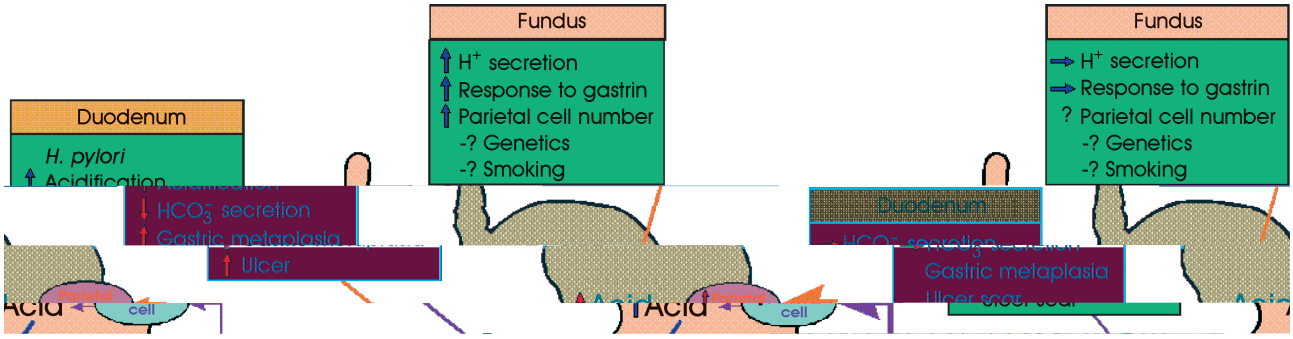

Fig. 8. The secretory, gastric and mucosal profile in *H. pylori*-infected duodenal ulcer patients before and after the eradication of *H. pylori*.

restored (enhanced), while plasma TNF-α showed markedly reduced values (Fig. 10).

These experimental studies (Konturek et al., 2002) provided evidence that *H. pylori* and aspirin, a prototype of NSAID or rofecoxib, a selective COX-2 inhibitor, are independent pro-ulcerogenic factors, acting via quite different mechanisms. *H. pylori* alone induces gastritis leading to a delay of healing despite accompanying overexpression of cyclooxygenase-2 and excessive release of prostanoids, indicating that the pro-ulcerogenic mechanism activated by *H. pylori* infection is overbalanced by the prostaglandin-related anti-ulcerogenic activity of *H. pylori*. Aspirin alone applied topically causes direct mucosal irritation and inhibition of prostanoid synthesis due to inhibition of both cyclooxygenase-1 and cyclooxygenase-2 activity, yet enhancing cyclooxygenase-2 expression and elevating plasma proinflammatory cytokines such as TNF-α and interleukin-1β. The combination of *H. pylori* infection with aspirin or rofecoxib application leads to enhancement of ulcer healing that could be interpreted that *H. pylori* infection with its potent cyclooxygenases-2 expression and prostaglandin release overcomes the damaging or ulcer healing prolonging effect of aspirin. An alternative explanation for this fascinating interaction could be that aspirin directly inhibits *H. pylori* growth and noxious action this germ in the stomach resulting in acceleration of ulcer healing infected with *H. pylori*. It is worth to mention that selective blockade of cyclooxygenase-2 with rofecoxib alone in rats with ischemia-reperfusion-induced gastric erosions into chronic gastric ulcers also delayed ulcer healing as reported previously (Brzozowski et al., 2001), but in *H. pylori*-infected stomach, rofecoxib or aspirin accelerated ulcer healing as compared to that observed when rofecoxib or aspirin was applied alone.

Results of these experimental studies (see Fig. 9) can be summarized as follows.

(1) NSAID with inhibitory efficacy on either cyclooxygenase-1 or cyclooxygenase-2 are highly ulcerogenic substances, but their ulcerogenic propensity is reduced when the gastric mucosa is concomitantly infected with *H. pylori*; this might be explained by partial reversal of NSAID-induced mucosal inhibition of prostanoid biosynthesis and reduction in NSAID-induced plasma cytokine release by *H. pylori* infection added to NSAID, and both exerting favorable effects on mucosal repair.

(2) *H. pylori* inoculation in rat stomach with preexisting gastric ulcers leads to mucosal infection, overexpressing cyclooxygenase-2 (without affecting cyclooxygenase-1 expression), and excessive prostanoid biosynthesis, which, however, does not appear to be sufficient to overcome the delay of ulcer healing caused by acute *H. pylori*-induced mucosal inflammation.

(3) Unexpectedly, specific inhibition of cyclooxygenase-2 (rofecoxib) also causes the delay of ulcer healing similar to that induced by aspirin (inactivated by both cyclooxygenase-1 and cyclooxygenase-2) and this is accompanied by a suppression of prostanoid biosynthesis stronger than that observed in aspirin-treated rats.

(4) The combination of aspirin or rofecoxib with *H. pylori* infection paradoxically reduced the ulcer size when compared with aspirin or rofecoxib alone, indicating that the interaction between specific cyclooxygenase-2 inhibitor and *H. pylori* on gastric mucosa is negative, which suggests that this infection in rofecoxib-treated animals, similar to that in aspirin-treated rats, counteracts in part the pro-ulcerogenic effect of this cyclooxygenase-2 inhibitor.

2.2. Clinical and endoscopic findings in humans regarding the interaction of infection of H. pylori and NSAID use

Our clinical studies included 5967 dyspeptic subjects (the largest number of patients ever examined for *H. pylori* infection and gastroduodenal ulcers in the same unit by the same investigators) who entered consecutively our trial in 1996–2000 and underwent [^{13}C]-urea breath test (UBT) and upper endoscopy. *H. pylori* prevalence averaged 73% and about 9.2% of subjects tested showed endoscopically gastric ulcerations, 17.2% duodenal ulcers, and 5.4% gastroduodenal ulcers. Among patients with gastric ulcers ($N=551$), 66.4% were *H. pylori*-positive, 7.8% were *H. pylori*-infected NSAID users, and 3.1% had ulcers associated with both *H. pylori* and NSAID use. Finally, 20% were *H. pylori*-negative and nonusers of NSAID. The latter group could be defined as "idiopathic" ulcers as no evidence for any major known ulcerogens such as *H. pylori* infection or NSAID could be found (see Fig. 7).

This group is of particular interest because the contribution of such idiopathic ulcers has increased gradually in the last years, at least in the Polish population, while the overall occurrence of peptic ulceration during the same period has gradually declined from 21% in 1996 to 10.5% in 2000 which was accompanied by a steady reduction in *H. pylori* prevalence in the Polish population, from about 74% in 1996 to 59% in 2000. NSAID were used by about 10% of study patients throughout the study period. The etiological factors responsible for these non-*H. pylori*, non-NSAID ulcers are unknown, but neither smoking nor family link (genetic factor) was found to be responsible as they were similar to those in patients with ulcers associated with *H. pylori* infection alone, or with NSAID use alone, and with *H. pylori* plus NSAID use. Physiological stress (Levenstein et al., 1999) and diet (Misciagna et al., 2000) have been suggested to contribute to the pathogenesis of non-*H. pylori*, non-NSAID ulcers, but this issue needs confirmation.

Our study does not support the results recently published by Huang et al. (2002), who based their conclusions on meta-analysis of 16 studies from various countries showing that *H.pylori*-infected NSAID users have a higher proportion of peptic ulcers than do patients with *H.pylori* alone or

NSAID alone. We found no evidence for such synergism between *H. pylori* and NSAID but, conversely, we found that such combination enhances ulcer healing possibly due to the fact that NSAID may directly suppress *H. pylori* growth and activity, resulting in elimination or, at least, limitation of the *H. pylori* contribution to gastroduodenal ulcerogenesis. Furthermore, according to Huang et al. (2002), no peptic ulceration can be observed in non-*H. pylori*, non-NSAID patients, while a study of Xia et al. (2001) with a similar to our number of symptomatic patients ($N = 8344$) entering the trial found a similar proportion of *H. pylori*-associated ulcers (66%) or ulcers associated with *H. pylori* plus NSAID (8.5%) and also that about 17% of their series were non-*H. pylori*, non-NSAID users, emphasizing that these idiopathic ulcers have distinct clinical and endoscopic characteristics. Our present results fully confirm existence of large proportion (20%) of such *H. pylori*-negative, NSAID-negative, or idiopathic ulcer. Thus, like in experimental animals, *H. pylori* infection in humans taking NSAID limits significantly peptic ulcerogenesis, but ulcers may also develop without infection with *H. pylori* or NSAID and these ulcer patients require special care to prevent their complications. Thus, *H. pylori* contributes to an increased ulcer risk when acting alone as also NSAID do, but an evidence for an antagonistic interaction between these two types of ulcerogens was found in our well-controlled series. *H. pylori* related to overexpression of cyclooxygenase-2 and an excessive increase in mucosal generation of prostanoids may appear to counteract the ulcerogenic efficacy of NSAID despite their ability to inhibit cyclooxygenase-2 and the suppression of prostanoid biosynthesis.

The similarity of gastric ulcerogenesis in the experimental animal model and in humans (showing negative interaction) is not clear, but humans infected with *H. pylori* taking NSAID may show a lower sensitivity of the stomach to the ulcerogenic action of NSAID due to gastric adaptation to these agents as described before (Konturek et al., 2001). Chan et al. (2001) proposed that the eradication of *H. pylori* before the start of long-term NSAID is required to prevent mucosal bleeding and this interaction between *H. pylori* and NSAID requires further studies.

In summary, (1) there is no evidence for a synergistic interaction between *H. pylori* infection and NSAID as claimed by Huang et al. (2002); (2) while about 69.4% of ulcers in dyspeptic patients might be attributable to *H. pylori* infection, both *H. pylori* prevalence and ulcer occurrence have shown a tendency to decline in the last few years when the diagnosis of *H. pylori* and eradication therapy are in common use; (3) a significant proportion of all ulcers (about 20%) appear to be non-*H. pylori*, non-NSAID (idiopathic) peptic lesions; (4) it is of interest that ulcer complications such as bleeding and perforation have also tended to decrease over the last few years but this requires confirmation.

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