Leading article

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PHYSIOLOGICAL, IMMUNOHISTOCHEMICAL AND MOLECULAR ASPECTS OF GASTRIC ADAPTATION TO STRESS, ASPIRIN AND TO H. PYLORI—DERIVED GASTROTOXINS

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Gastric mucosa is continuously exposed to various aggressive factors such as stress, ulcerogenic drugs including aspirin-like agents, gastrotoxic bacteria, particularly Helicobacter pylori (Hp) and many other exogenous and endogenous irritants. The maintenance of mucosal barrier depends upon the activation of pre-epithelial (mucus-alkaline secretion), epithelial (surface active phospholipids, mucosal cell restitution and proliferation) and post-epithelial (mucosal microcirculation) lines of mucosal defence. The mucosa exposed to aggressive factors develops acute lesions, which usually heal completely within few days, but following repeated exposures to hostile environment it adapts to survive the challenge of noxious agents. This adaptation may be of short term (adaptive cytoprotection) and follows the exposure to “mild” irritants that activate local mucosal biosynthesis of protective prostaglandins (PG) and nitric oxide (NO) and stimulate sensory nerves and mucosal cell migration and proliferation through enhanced expression of growth factors such as EGF, TGFα and trefoil peptides. The fact that exogenous PG, NO-donor agents, growth factors and capsaicin, stimulating sensory nerves, protect the mucosa against strong necrotizing agents (direct cytoprotection), supports the notion that endogenous PG, NO, growth factors and sensory nerves are involved in the complex process of adaptive cytoprotection. With repeated insults of ulcerogens such as stress, aspirin, Hp-derived gastrotoxins, especially ammonia, a long-term adaptation develops which is mediated mainly by overexpression of EGF and TGFα and their common receptor (EGFR) with subsequent increase of mucosal cell proliferation and enhanced healing of mucosal lesions. The failure of mucosal adaptation seems to play a pivotal role in the pathogenesis of gastric lesions and peptic ulcerations.

Key words: cytoprotection, prostaglandins, growth factors, stress, aspirin

INTRODUCTION

The mucosa lining the gastrointestinal tract, especially the stomach, is exposed to a variety of noxious factors of exogenous and endogenous origin and the maintenance of mucosal integrity requires continuous activation of various defence lines of the mucosa to counteract effect of these noxious factors.
The mucosal damage, occurring immediately after the exposure of the mucosa to noxious conditions, includes massive desquamation of surface epithelium, the formation of deep hemorrhagic erosions or ulcerations such as those observed after severe stress, intake of non-steroidal antiinflammatory agents (NSAID) such as aspirin (ASA) (1). The mucosal lesions may be either due to impaired mucosal defence or to exaggerated aggression of noxious factors. The mucosal defence includes mucus-alkali secretion, mucosal blood flow, surface hydrophobicity depending upon the presence of membrane phospholipids, epithelial regenerative capacity and biosynthesis of protective prostaglandins (PG), nitric oxide (NO) and growth factors such as epidermal growth factor (EGF), transforming growth factor alpha (TGFα) and trefoil peptides. The increased aggression may be caused by stress, ingestion of NSAID, infection with bacteria such as Helicobacter pylori (Hp) and other topical irritants and ulcerogens including an excessive acid and pepsin secretion, bile reflux into the stomach and gastric ischemia (Fig. 1).

Fig. 1. Diagram of aggravating causes and defence mechanisms against mucosal damage and peptic ulceration.

Gastrointestinal mucosa exposed to hostile environment is capable of adapting to survive the challenge of everyday life and to withstand the action of various noxious factors. An exposure to aggressive forces immediately activates the mucosal defence lines resulting in adaptive cytoprotection, while repeated
exposures to various ulcerogens lead to gastric adaptation. The impairment or loss of the adaptive properties of gastric mucosa may result in acute mucosal lesions or chronic peptic ulcer formation.

**Adaptive cytoprotection**

The original concept of cytoprotection pioneered by A. Robert (2, 3) proposed that PG applied on the gastric mucosa at a dose many times smaller than that affecting gastric acid secretion protect this mucosa from becoming necrotic when it is subsequently exposed to such necrotizing substances as 100% ethanol, 0.6 M HCl, 0.2 M NaOH, hypertonic saline solution (25% NaCl) or even boiling water (4). This finding suggested that certain natural PG, that are known to be present in the gastric mucosa and gastric juice, could prevent the formation of gastric ulcers without interfering with gastric acid secretion (Fig. 2).

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**Fig. 2.** Mean area (± SEM) of gastric lesions induced by 100% ethanol in rats pretreated with vehicle saline, intragastric (i.g.) PGE₂ (20 or 40 µg/kg), 20% ethanol (1 ml i.g.), indomethacin (5 mg/kg i.p.) plus 20% ethanol or indomethacin (5 mg/kg i.p.) followed by 100% (1.5 ml i.g.) as well as basal gastric acid secretion from gastric fistula rats pretreated (but without 100% ethanol) similarly as the groups of animals given 100% ethanol. Means ± SEM 10 tests on 10 rats. Asterisk indicates significant decrease below the value in vehicle saline animals. Cross indicates significant increase above the value recorded in rats pretreated with i.g. PGE₂, at 20 or 40 µg/kg.
In our study, PGE\(_2\) given intragastrically (i.g.) at doses 20 or 40 \(\mu\)g/kg caused a dose dependent reduction in the area of gastric lesions induced in rats by intragastric application of 1,5 ml of 100% ethanol. In rats equipped with chronic gastric fistula, PGE\(_2\) given i.g. failed to affect gastric acid secretion. Similar protection of gastric mucosa was observed in rats pretreated with 20% ethanol (1 ml i.g.) and then given 30 min later 100% ethanol. When the rats were first injected with indomethacin 5 mg/kg i.p. and then 60 min later given 20% ethanol, followed 30 min by 100% ethanol there was a marked increase in the area of gastric lesions as compared to that obtained with 20% ethanol plus 100% ethanol. In control experiments, indomethacin given in the same dose 90 min before 100% ethanol increased significantly the area of mucosal lesions. Gastric acid output in our gastric fistula rats was not significantly affected by 20% ethanol, the combination of the 20% ethanol plus indomethacin or indomethacin alone.

Besides PG, other factors present in the gastric mucosa, particularly growth factors such as EGF, TGF\(\alpha\) and trefoil peptides, exhibit gastroprotective activity similar to that attained with PG (5,6) (Fig. 3). The spectrum of cytoprotective activity of exogenous PG is, however, much broader than that of growth factors because they are capable of preventing the mucosal damage even when given by intragastric route through which growth factors are less effective. In fact, EGF given i.g. at a dose of 100 \(\mu\)g/kg partly reduced the mucosal damage only when acidified aspirin or water immersion and restraint stress were used to induce this damage (Fig. 4). It is of interest that the cytoprotective activity of 16,16-dimethyl PGE\(_2\) and EGF against the damage induced by indomethacin was observed also in vitro when isolated gastric glands were employed and the cell viability and LDH release were used as an index of cell protection (6).

Since PG of E and I series have been identified in the gastric mucosa and gastric juice, it was hypothesized that their formation by constitutive cyclooxygenase (COX-1), probably expressed by epithelial and endothelial cells of gastric mucosa, contributed to the mucosal integrity in the presence of hostile environment in the stomach (Fig. 5). This is supported by a well-known finding that the inhibition of COX-1 by aspirin or indomethacin resulted in mucosal damage and enhanced the injurious action of other irritants such as 100% ethanol. It was reasoned that the constant biosynthesis and release of PG in the gastric mucosa exerted a cytoprotective effect against the potentially noxious agents. The possibility that the formation of gastric PG may be increased by irritating the mucosa by certain substances present in the gastric lumen either naturally or following i.g. administration of so called “mild” irritants also has been explored (8). As shown on Fig. 2, “mild” irritants such as 20% ethanol, which by itself did not induce gross mucosal damage, protected the stomach from necrosis when the stomach was later exposed to a necrotizing substance such as 100% ethanol. Since this protection was almost completely
Fig. 3. Mean area of gastric lesions induced by 100% ethanol in rats pretreated with vehicle saline and various doses of intragastric PGE₂ or s.c. EGF, TGFα, PDGF, hSP or bFGF (12.5—100 μg/kg). Means ± SEM of 8—10 rats. Asterisk indicates significant decrease below the control value obtained with vehicle saline.

Fig. 4. Mean area of gastric lesions in rats pretreated with vehicle saline or EGF given intragastrically (i.g.) or subcutaneously in a dose of 100 μg/kg 30 min before the exposure of the stomach to 100% ethanol, acidified 100 mM taurocholate, acidified (150 mM HCl) aspirin (ASA — 200 mg/kg) or 3.5 h of water immersion of stress. Means ± SEM of 10 rats. Asterisk indicates significant decrease below the vehicle control value. Cross indicates an increase above the value obtained with pretreatment with s.c. EGF plus ulcerogen.
Fig. 5. Schematic diagram for the conversion of arachidonic acid from the phospholipids to eicosanoids via the constitutive cyclooxygenase (COX-1) under physiological conditions to secure gastroprotection or via inducible cyclooxygenase (COX-2) under pathological conditions such as endotoxemia. Note that growth factors and cytokines may activate phospholipase to trigger the arachidonic acid cascade or to activate the inducible COX-2. Endotoxins such as lipopolysaccharides may activate COX-2 directly.

Fig. 6. Adaptive cytoprotection: cross-cytoprotection. As mild irritants were used ethanol (12.5—20%), NaOH (0.05—0.75 M) or HCl (0.1—0.3 M) given intragastrically 30 min before i.g. administration of strong irritants or boiling water. Means ± SEM of 8—10 rats. Asterisk indicates significant decrease below the value obtained with vehicle saline. (adapted from Robert et al. (7).)
prevented by the pretreatment with indomethacin, which when given alone caused only little change in gastric acid output and mucosal integrity, it was concluded that mild irritants protected the stomach by stimulating COX-1 and the release of PG in the stomach. This was confirmed by actual measurement of mucosal generation of PG (7, 8).

Thus, COX-1 is a constitutive enzyme present in gastric mucosal cells where it functions to produce physiologically important PG. In contrast, COX-2 is an enzyme that is rapidly induced at the site of inflammation and responsible for the production of large amounts of proinflammatory PG and other mediators such as cytokines or proteases. Large quantities of proinflammatory PG generated by COX-2 stimulants such as lipopolysaccharides (LPS) may be damaging to the gastric mucosa and this can be prevented by the application of specific COX-2 inhibitors (9). Using specific molecular method such as reverse transcriptase polymerase chain reaction (RT-PCR), it was observed that COX-1 transcripts dominated in the intact gastric mucosa, where COX-2 was absent. Following the administration of LPS or after exposure to stress, there was a rapid induction of COX-2 with an excessive generation of proinflammatory PG and other inflammatory mediators resulting in tissue damage that can be attenuated by the pretreatment with specific COX-2 inhibitors (9).

Adaptive cytoprotection can occur either when the mild irritant and the necrotizing agent are either the same substance (such as protection by 20% ethanol against 100% ethanol) and this is called “homo-cytoprotection” or when the two substances are different (e.g. protection by 20% against 25% NaCl) and this is called “cross-cytoprotection” (3, 4, 7) (Fig. 6).

Although the cytoprotective activity of PG against various irritants in the stomach is well documented, the extent of this protection and its mechanisms are still controversial. PG and mild irritants do not appear to prevent widespread destruction of surface epithelium when the mucosa is exposed to necrotizing substances. For this reason cytoprotection was redefined by Ito et al., (10) and now it refers more specifically to the prevention of the deeper necrotic and hemorrhagic lesions rather than of the surface epithelium. An immediate consequence of the destruction of much of the surface epithelium is the process of rapid restitution and healing (6, 10, 11). PG (11) as well as growth factors (12) do not prevent the widespread destruction of the surface epithelium but stimulate the cell migration and, thus, enhance the restitution of the surface epithelium (Fig. 7).

Exposure of the mucosa to ethanol and other necrotizing substances without addition of PG or EGF caused vasocongestion, stasis and thrombus formation in the subepithelial microvasculature. PG and growth factors appear to preserve the microvascular integrity (vasoprotection) and to reduce the underlying vasocongestion so that they maintain the capability of the epithelial cells to migrate from the preserved regeneration zone of gastric glands to the
Fig. 7. Time course of mucosal damage and restitution of surface epithelium after exposure to single insult of ethanol alone and in combination with EGF (100 μg/kg s.c.) or PGE₂ (100 μg/kg i.g.).

Fig. 8. Effects of ig administration of vehicle saline (1.5 ml), 100% ethanol (1.5 ml), acidified (150 mM HCl) plus aspirin (ASA) 100 mg/kg, or exposure to 3.5 h of water immersion and restraint stress on basal gastric acid secretion and luminal release of TGFα and EGF in conscious rats with the chronic gastric fistula. Means ± SEM of 8—10 rats. Asterisk indicates significant change as compared to vehicle control.
injured surface epithelium (11, 12). The physiological role EGF in cytoprotection has been proved by demonstrating that the removal of the major endogenous source of EGF by excision of salivary glands greatly augmented stress-induced gastric lesions (13) and abolished adaptive gastroprotection (14).

Acute mucosal damage was reported to enhance local mucosal expression of EGF, TGFα and their receptors, especially at the proliferating zone of gastric mucosa (15). Furthermore, EGF and TGFα as well as PG generated in large amounts in the gastric juice were proposed to inhibit gastric acid secretion in the injured oxyntic mucosa (15). This inhibition was almost complete when the oxyntic mucosa was exposed to 100% ethanol or acidified ASA but only partial in rats exposed to stress (Fig. 8). Such local inhibition of acid secretion probably limited the extent of gastric damage due to the removal of acid as an aggressive factor. It was accompanied by an increase in mucosal blood flow involving the β-adrenergic receptors and improving the supply of oxygen and nutrients to the mucosa as well as eliminating the damaging agent from the mucosa and providing material for biosynthesis of the protective mucus-alkaline secretion. Thus, it is likely that both PG and growth factors contribute to mucosal repair by reactive hyperemia, the migration of viable cells and rapid re-epithelialization of mucosal defects (Fig. 9).

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**Fig. 9.** Schematic presentation of the involvement of prostaglandins (PG) and growth factors (EGF and TGFα) in the gastric acid inhibition, gastric hyperemia mediated by β-adrenoreceptors, the migration of surface epithelium (restitution) and increased mucus secretion observed after exposure of the gastric mucosa to the action of topical irritant.
Adaptive cytoprotection induced by hypertonic NaCl was shown to be accompanied by an increased mucosal blood flow that was found to be mediated predominantly by PG (16) as originally proposed by Robert (7). Fig. 10 is a schematic representation of direct and adaptive cytoprotection. Recent studies have shown that besides endogenous PG, an important role in adaptive cytoprotection is played by the arginine-NO system, growth factors such as EGF and sensory nerves and sensory neuropeptides such as calcitonin-gene-related peptide (CGRP).

The importance of endogenous PG, NO, EGF, TGFα, SP and CGRP and sensory nerves has been documented in numerous studies by showing that the pretreatment with indomethacin (to suppress COX-1), L-NNA (to block NO synthase), salivectomy (to remove the major source of endogenous EGF) and capsaicin (to inactivate sensory nerves) attenuated or reversed adaptive protection induced by mild irritants (13—16).

In agreement with these findings, vasodilators such as nitroglycerine acting via releasing NO, have been shown to protect the mucosa against acute
Fig. 11. Effects of intragastric (i.g.) administration of various doses of nitro-glycerine 30 min before 100% ethanol (1.5 ml) or acidified (150 mM) aspirin (SA) (100 mg/kg) on gastric mucosal lesions and gastric blood flow (expressed as percent of control value). Means ± SEM of 8—10 rats. Asterisk indicates significant increase above, while cross indicates significant decrease below the control value in vehicle pretreated rats.

Fig. 12. Effects of topically applied capsaicin (0.5 mg/kg i.g.) alone or in combination with L-NNA (40 mg/kg i.v.) or with L-arginine (300 mg/kg i.v.) plus L-NNA (40 mg/kg iv) on gastric lesions and gastric blood flow expressed as percent of control value in rats without or with pretreatment with indomethacin (5 mg/kg i.p.). Means ± SEM of 8—10 rats. Asterisk indicates significant change as compared to the vehicle control. Cross indicates significant change as compared to the values recorded in corresponding groups of rats without pretreatment with indomethacin.
damage by 100% ethanol or acidified aspirin. This protective effects of nitroglycerine were accompanied by enhanced gastric blood flow suggesting that NO affords gastric protection by increasing gastric microcirculation (Fig. 11). Other studies emphasized the role of NO in direct and adaptive cytoprotection (17, 18). Capsaicin, the pungent ingredient of chilli pepper has also been found to protect the mucosa against experimental injury in animals (19, 20). In rats, this gastroprotection induced by capsaicin, similarly to that promoted by mild irritants, is probably of adaptive nature and can be significantly attenuated by the pretreatment with either L-nitro-arginine (L-NNA or L-NMMA) or indomethacin (20) suggesting that both NO and PG are implicated in this protective mechanism (Fig. 12).

It should be mentioned, that while small amounts of NO exhibit protective activity due to the stimulation of guanylate cyclase and the increase of cyclic

Fig. 13. Mechanisms of cytoprotective or cytotoxic effects of NO produced, respectively, in small physiological amounts by constitutive NO synthase and in large amounts by inducible NO synthase. Large amounts of NO may interact with O$_2$ to form a potent oxidant, ONOO$^-$, peroxynitrate radical causing lipid peroxidation and cell damage.
GMP in myocytes leading to vasodilatation, excessive amounts of NO either released by the administration of large doses of NO-donors such as S-nitroso-N-acetylpenicillamine (SNAP) or released by the activation of inducible NO synthase (e.g. by endotoxins) may result in the mucosal damage (21). This noxious effect of excessive NO could be attributed to the peripheral vasodilatation, hypotension and gastrointestinal hemorrhage with marked increase in the mucosal permeability and leakage of plasma into the gut lumen. NO may interact with superoxide anion (O₂\textsuperscript{−}) produced by activated neutrophils to form free radicals such as peroxynitrate radicals (ONOO\textsuperscript{−}) that result in lipid peroxidation and mediate some of the cytotoxic influence of NO on the gastrointestinal mucosa observed in such conditions as septic shock and endotoxemia (21) (Fig. 13).

Our recent study employing lipopolysaccharide (LPS) originating from Hp showed, however, that this endotoxin applied parenterally actually protected gastric mucosa against the damage provoked by 100% ethanol and this was accompanied by an increase of gastric blood flow. Both these effects were probably mediated by endogenous NO, because they were accompanied by enhanced expression of constitutive and inducible NO synthase as detected by RT-PCR (22). Only larger doses of LPS, which produced systemic hypotension and the fall in gastric blood flow failed to protect gastric mucosa against the ethanol damage (Fig. 14.). The importance of endogenous NO in gastroprotection afforded by lower doses of LPS is also supported by our finding that the pretreatment with L-nitro-arginine (L-NNA) to suppress the constitutive NO synthase or with dexamethasone to block the induction of NO synthase abolished the protection afforded by this substance (22).

Several anti-ulcer drugs such as sucralfate and other aluminium-containing antacids appeared to act through the stimulation of mucosal release of NO and probably also PG (23,24). It has been proposed that the release of hexa-aquoaluminium cation, especially at lower intragastric pH, is responsible for the protection afforded by aluminium containing drugs probably acting via the stimulation of constitutive NO synthase in the gastric epithelial cells and local release of NO that improved mucosal blood flow and mucosal cell restitution.

In general, the major property of adaptive cytoprotection induced by mild irritants, capsaicin, NO-donors and certain anti-ulcer drugs is that 1) it is accompanied by gastric hyperemia probably mediated by PG, NO or CGRP; 2) it is a short-lasting phenomenon and disappears after 1.5—2 h; 3) its common initial cause (at least in case of mild irritant) is the subtle superficial mucosal damage caused by the action of mild irritants; 4) it serves in a short-term adaptation of the stomach to withstand the damaging action of
subsequent strong irritants or necrotizing substances; and 5) it may occur in response to LPS originating from bacteria such as Hp to protect gastric mucosa and to enhance the gastric mucosal microcirculation via activation of NO-synthase and production of NO.
Gastric adaptation to stress

Gastric stress lesions usually arise as a complication of burn, sepsis, major surgery, trauma to the central nervous system etc. The major factors implicated in the pathogenesis of stress ulcerations include the increase of aggressive forces such as acid-pepsin secretion and gastric motility, activation of neutrophils and increased formation of free radicals with decrease of mucosal defence lines, such as mucus-alkaline secretion, gastric blood flow, mucosal cell proliferation and mucosal restitution (25—27) (Fig. 15).

![Diagram of stress and factors involved in gastric ulcer formation](image)

Fig. 15. Stress and the factors involved in gastric ulcer formation.

We found that “mild” stress such as 1 h immobilization is capable of preventing the formation of acute gastric lesions induced by longer (7 h) stress suggesting that adaptive cytoprotection may also be triggered by stress. According to our experience, the exposure to 1 hour of water immersion and
restraint stress (WRS) significantly reduced the number of lesions provoked by strong 7 h-lasting stress as well as by 100% ethanol, and this reduction was accompanied by the increase in gastric blood flow (Fig. 16). This confirms that mild stress is capable of protecting gastric mucosa in a similar way as mild irritants i.e. by increasing mucosal blood flow probably mediated by local production of PG and NO. This is supported by the fact that the pretreatment with indomethacin (5 mg/kg i.p.) to inhibit COX-1 activity or with L-NNA (40 mg/kg i.v.) to suppress the NO synthase, abolished almost completely the protection afforded by mild stress. As activation of neutrophils was thought to play a crucial role in the mucosal infiltration and the production of oxyradicals in the mucosa of stressed animals, it is likely that PG and NO released by mild stress reduced the activation of neutrophils and thus removed the noxious actions of oxyradicals. As the consequence of the neutrophil/endothelium interaction during strong stress there is a dramatic decrease in gastric

Fig. 16. Gastric lesions and gastric blood flow (GBF) in rats in response to mild 1 h stress (WRS) alone, to strong 7 h stress alone, to the combination of 1 h stress and 7 h stress, to 100% ethanol, to 1 h stress and 100% ethanol, to indomethacin plus 1 h stress plus 7 h stress and to L-NNA plus 1 h stress plus 7 h stress. Means ± SEM of 8—10 rats. Asterisk indicates significant change as compared to the values obtained with 1 h stress. Cross indicates significant change to the value obtained with 7 h of stress or to 100% ethanol. Double asterisks indicate significant increase above the values obtained with 1 h plus 7 h stress or 1 h stress plus 100% ethanol.
microcirculation leading to local ischemia and vascular damage with mucosal hemorrhages. Mild stress applied before the exposure to strong stress reversed the fall in mucosal blood flow caused by this strong stress or by necrotizing substance such as 100% ethanol resulting in the improvement in gastric mucosal circulation and mucosal integrity.

Repeated stress insults were found to increase the tolerance of gastric mucosa to stress ulcerogenesis and to attenuate the formation of acute gastric lesions (28). When the exposure to stress was repeated every other day, there was not only the gradual decrease in the number of stress ulcerations but also a gradual increase in gastric blood flow and DNA synthesis (Fig. 17). This decrease in the formation of stress-induced lesions developed fully after 6 days of the repeated daily exposures to stress and lasted for about 6 days and then the sensitivity of the mucosa to stress-lesions was restored indicating that, unlike adaptive cytoprotection, the gastric adaptation is a long-term phenomenon.

The pretreatment with indomethacin to suppress mucosal biosynthesis of endogenous PG almost completely abolished the adaptation to repeated stress insults (28). This removal of the ability of gastric mucosa to adapt to stress was accompanied by the decrease in gastric blood flow suggesting that endogenous PG may contribute to stress adaptation via enhancement of gastric microcirculation.

Our recent studies on the mucosal expression of PG synthesizing enzymes demonstrated that COX-1 and COX-2 mRNA were detected by RT-PCR at all time intervals after exposure to stress (29). The mechanism of gene expression of COX-1 and COX-2 during recovery from the stress and mucosal adaptation to the stress is not clear, but growth factors that are overexpressed after the stress might be involved in the stimulation of COX-expression. It is reasonable to assume, therefore that these growth factors trigger a gene for COX. It is of interest that EGF and TGFα are not the only growth factors expressed in the mucosa recovering from stress, but recently also trefoil peptides, especially spasmolytic peptide (SP), was found to be overexpressed (as detected by immunohistochemistry and RT-PCR) in the mucosa recovering from the stress lesions (30). Exogenous human SP (hSP) was shown in our study not only to prevent the formation of acute ethanol-induced gastric lesions (see Fig. 3) but also to accelerate healing of stress ulcers and this finding together with the observation of enhanced expression of this growth factor in gastric mucosa after the stress support the notion that it contributes to the repair of gastric mucosa after stress ulcers (30). Thus, our studies, provide evidence for the coexpression and cooperation of PG (29) and growth factors (30, 31) in the repair of stress lesions and in mucosal adaptation to repeated exposures to stress. (Fig. 18).
Fig. 17. (A) Repeated exposures to 3.5 h water immersion and restraint stress every other day results in gradual diminution of the number of gastric lesions and this is accompanied by gradual and significant increase in gastric blood flow and DNA synthesis. Means ± SEM of 8—10 rats. Asterisk indicates significant change as compared to the control values recorded when the animals were exposed to the stress for the first time ("once"). (B) Immunocytochemistry of EGF (I), TGFα (II) and spasmolytic peptide — SP (III) in the intact mucosa (L-left panel) and after four repeated expositions to water immersion and stress (R-right panel).
Brzozowski et al., 1996

Fig. 18. (A). Mean area of gastric lesions and gastric blood flow in rats without adaptation or with adaptation to 3.5 h of water immersion and restraint stress by repeated stress insults every other day for 8 days and then exposed again to 3.5 h stress or to 100% ethanol, 200 mM taurocholate, 25% NaCl or acidified aspirin. Means ± SEM of 8—10 rats. Asterisk indicates significant change as compared with the value obtained after application of these irritants in rats non-adapted to stress. (B) Mucosal expression of β-actin, EGF, TGFα and SP in intact mucosa (line a) and after 0, 2, 4, 6, 12, 24, 36 and 48 h (lines b, c, d, e, f, g, h i) after stress.
Another factor that seems to be involved in the stress-induced gastric adaptation is sensory innervation. The functional ablation of sensory nerves by parenteral administration of a large dose of capsaicin (125 mg/kg s.c.) resulted in a marked decrease of the ability of the mucosa to adapt to the stress insults (28). Administration of exogenous CGRP in capsaicin-deactivated rats restored the ability of the mucosa to adapt to stress insults confirming the role of sensory nerves in this adaptation probably via release of sensory neuropeptides such as CGRP. This peptide is probably responsible for hyperemia during adaptation to stress and may contribute to the enhancement of the mucosal tolerance to repetitive stress insults. The hyperemia induced by CGRP during adaptation probably acts via specific receptors and can be blocked by CGRP$_{7-27}$, an antagonist of these receptors. As shown in our studies (32) this hyperemia depends, at least in part, upon endogenous nitric oxide (NO), because the suppression of NO synthase by L-NNA markedly reduced the mucosal blood flow without the aggravation of the mucosal lesions in response to repeated stress insults.

Single exposure to stress in salivectomized rats was shown in our study (33) to cause significantly greater increase in the gastric mucosal damage than in rats with preserved salivary glands and this was accompanied by a marked fall in the level of EGF in the gastric lumen. With repeated stress insults, the luminal content of EGF in sham-operated animals gradually increased and the number of gastric lesions was reduced in these animals due to gastric adaptation (32). In contrast, salivectomized animals showed significantly lower luminal EGF-contents and significantly greater number of gastric lesions in response to the repeated stress insults. Oral administration of EGF (added to drinking water) significantly reduced the number of gastric lesions after the exposure to repeated stress insults in salivectomized rats confirming the possible implication of this peptide in the gastric adaptive process. The important role of growth factors is supported by our findings (31), that following single exposure to stress an enhancement of expression of EGF, TGF$_{a}$ and their common receptor (EGFR) occurs with a subsequent increase in DNA synthesis and mucosal cell proliferation. With repeated insults of stress, the rate of mucosal repair, and mucosal expression and luminal release of growth factors are highly increased and this may contribute to gastric adaptation to stress.

Gastric adaptation to stress not only attenuated the formation of stress-induced gastric lesions but also enhanced the mucosal resistance to the injury induced by strong irritants such 100% ethanol, 25% NaCl, acidified aspirin or 200 mM taurocholate (Fig. 18). This strengthening of mucosal integrity of stress-adapted mucosa and increased resistance to strong irritants is mediated by increased mucosal blood flow due to increased release of PG and NO and by enhanced regeneration of mucosal cells accompanied by an increased luminal and mucosal contents of EGF and TGF$_{a}$ and mucosal overexpression of their common receptor EGFR (32).
Fig. 19. Schematic presentation of various mechanisms involved in gastric adaptation to stress including mucosal production of PG, excitation of sensory nerves with release of CGRP acting on endothelium to release NO, EGF released by salivary glands and EGF and TGFα released by the gastric mucosa. PG, NO and EGF are probably responsible for the vasodilatation and inhibition of neutrophil activation, while EGF and TGFα — for the enhancement of cell migration and proliferation in the mucosa adapted to stress.

These studies demonstrate that: 1) the stomach is capable of adapting to repeated stress insults by reducing the activation of neutrophils and enhancing of gastric blood flow probably mediated by sensory nerves, PG, CGRP, NO
and growth factors including EGF, TGFα and trefoil peptides 2) the stress-adapted mucosa is more resistant to the damage by strong irritants and this enhanced mucosal resistance to damage may result, in part, from the enhanced mucosal regeneration mediated by overexpression of EGF, TGFα, EGFR and trefoil peptides (Fig. 19).

**ADAPTATION TO NSAID**

Aspirin and other NSAID are ulcerogenic in animals (31—33) and in humans (34—36). The NSAID-associated mucosal damage principally consist of mucosal hemorrhages, acute erosions and less frequently of acute or chronic gastroduodenal ulcers.

The pathogenesis of NSAID gastropathy is poorly understood but it has been causally linked to the direct damaging effect of NSAID on the mucosal cells as well as to the inhibition of COX-1 and subsequent reduction in the biosynthesis of cytoprotective PG that are potent stimulants of gastric mucus-alkaline secretion, surface active phospholipids and mucosal cell proliferation. NSAID-induced gastropathy has also been associated with the vascular endothelial damage, the activation of neutrophils and the formation of intravascular thrombi leading to mucosal damage and ischemia associated with the formation of oxyradicals and, finally, chronic ulcers. (38—42) (Figs. 20 and 21).

![Figure 20: Mechanisms involved in acute gastric mucosal injury caused by NSAID.](image-url)
In addition to neutrophil/endothelium interaction, an important role in the NSAID-induced gastropathy seems to be played by disordered motility of the stomach due to central vagal stimulation and the deficiency of PG in the muscle layer of the gastric wall. Gastric hypermotility may result in microcirculatory disturbances with sequential events including the neutrophil/endothelium interaction and the formation of inflammatory mediators and oxyradical production similarly to the neutrophil hypothesis of NSAID-induced damage (38—42).

Neutrophil activation and infiltration in the mucosa is a hallmark feature of NSAID gastropathy and is reflected by increased myeloperoxidase (MPO) activity. Neutrophils recruited at the site of gastric mucosal injury participate in amplifying the inflammatory response by releasing several chemotaxins and by producing noxious reactive oxygen metabolites. Immediately after administration of aspirin, the gastric mucosa becomes infiltrated by neutrophils and exhibits increased biosynthesis of leukotriene $B_4$. In addition, there is an increase in the blood neutrophils which show increased activity with respect to the platelets (42) (Fig. 21).
Following repeated application of aspirin, there was a decrease in the area of gastric lesions and in neutrophil-endothelial adherence as well as a decline in neutrophil infiltration together with the fall in LTB₄ formation (41—43). This suggests that the changes in activation of neutrophils and their interaction with endothelial cells combined with mucosal infiltration by these cells play a crucial role both in the development of early mucosal damage and in the mucosal adaptation to repeated NSAID-insults (43—48) (Fig. 22).

It is of interest that like repeated exposures to stress (see Fig. 18) also repeated application of aspirin leads to an increase in mucosal tolerance to the ulcerogenic action of this drug. The attenuation of mucosal damage has been first demonstrated in rats (43—45) and then confirmed in humans (46—48). This adaptation to aspirin, like that to stress, enhanced the mucosal resistance to injury induced also by strong irritants (49) (Fig. 23).

Gastric adaptation to NSAID requires initial mucosal damage by these agents and cannot be observed after parenteral administration of these agents, with very low doses of NSAID that caused only negligible mucosal damage (Fig. 22). With the application of larger doses producing greater mucosal damage a quick adaptation of the mucosa to aspirin developed (43—45).
Fig. 23. Mean area of gastric lesions and gastric blood flow in rats without adaptation or with adaptation to acidified aspirin by repeated aspirin insults every day for 4 days and then exposed again to acidified aspirin or to 100% ethanol, 200 mM taurocholate, 25% NaCl or 3.5 h stress with water immersion and restraint. Means±SEM of 8—10 rats. Asterisk indicates significant change as compared with the value obtained after application of these irritants in rats non-adapted to aspirin.

It appears that gastric adaptation to NSAID is accompanied by increased DNA synthesis (49) and mucosal regeneration as demonstrated by enhanced bromo-dioxyuridine uptake and DNA synthesis (Fig. 24). This increased cell proliferation in the mucosa adapted to NSAID probably results from the overexpression of EGF, TGFα and their common receptors in the gastric mucosa (49) (Fig. 25). Recently, Romano et al. (50) measured mucosal content of growth factors by radioimmunoassay and showed that the predominant factor mediating the gastric adaptation to aspirin in rats and monkey is TGFα rather than EGF. Thus, increased mucosal expression of EGF and/or TGFα and EGFR is probably the major mechanism implicated in the increased mucosal cell proliferation and the restoration of mucosal integrity despite the repeated insults of aspirin and other NSAID. These polypeptide growth factors initiate mucosal repair, regeneration and cell proliferation.

Also in humans, aspirin treatment continued for 14 days caused mucosal damage as detected by endoscopy and measured by gastric microbleeding (47). Stachura et al. (48) reported that the adhesion of leukocytes to endothelial cells
Fig. 24. The uptake of bromo-dioxyuridine (BrdU) and DNA synthesis expressed as DPM of tritiated thymidine incorporated to DNA in rats given intragastrically acidified aspirin (200 mg/kg i.g) only once or after 1 day or 4 consecutive days. Means ± SEM of 8—10 rats. Asterisk indicates significant increase above the value recorded in rats after single administration of acidified aspirin.

Fig. 25. Expression of EGF and TGFα in the gastric mucosa as measured by intensity of immunostaining in rats non-adapted and in rats adapted to aspirin by its repeated administration every day for 5 consecutive days. Means ± SEM of 8—10 rats. Asterisk indicates significant increase above the value recorded in rats non-adapted to aspirin.
in the gastric mucosa was observed after 3 days of aspirin treatment but then declined. With prolonged aspirin treatment, an increase in the DNA synthesis and mucosal hyperplasia were observed mainly in the antral mucosa. The hyperplastic surface epithelium in healthy human volunteers treated for 14 days with aspirin exhibited an increased expression of TGFα, which probably played a crucial role in hyperplasia of gastric surface epithelium (48).

Since adaptation occurring during prolonged administration of aspirin is accompanied by almost complete inhibition of COX-1 in the gastric mucosa (43, 44), it may be concluded this adaptation does not appear to be mediated by endogenous PG.

The fact that the adapting mucosa exhibits remarkable regenerative changes and a concomitant increase in expression of growth factors and their common receptors suggest that these factors may be involved in the mechanism of mucosal adaptation. Wright et al. (51) reported that EGF may be secreted in the margin of gastrointestinal ulcerations from the EGF-expressing novel cell lineage. It is likely that the gastric mucosa responds to aspirin-induced damage also by an excessive local production of EGF that together with other growth factors such as TGFα and trefoil peptides (15, 52) initiate the cell proliferation and mucosal repair.

Gastric adaptation to Helicobacter pylori and its gastrotoxins

*Helicobacter pylori* (Hp) is now recognized as the causative agent of chronic superficial gastritis and a major risk factor in the pathogenesis of peptic ulcer disease (53—55). Hp is highly adapted to survive in gastric acidic milieu and it is capable of inducing both the inflammatory changes in the gastric mucosa and the alterations in gastric acid secretion (Fig. 26). The ability of Hp to survive in acidic conditions has been linked with its activity of urease which hydrolyzes urea to alkaline ammonia directly protecting the Hp from injury by luminal acid (55—57). Survival of Hp *in vitro* at the pH of less than 4 is markedly enhanced in the presence of urea and Hp mutants defective in the production of urease subunits are substantially more sensitive to low pH (<4) than wild-type Hp strains (57). Inhibition of urease activity in the stomach using acetohydroxamic acid, a specific inhibitor of urease activity significantly reduced the colonization of the mouse stomach by *H. felis* (58). Furthermore, urease negative Hp strains failed to colonize the stomach of gnotobiotic piglets (59). It has been suggested, therefore, that urease activity facilitates colonization of the stomach and thus contributes to gastric adaptation to Hp.

Survival of Hp in the stomach has also been attributed to the ability of bacteria to colonize the mucus layer overlying gastric epithelium (60). There is a pH gradient across the gastric mucus layer that ranges from pH 2 on the luminal side to nearly neutral pH at the epithelial cell surface. Multiple
flagellae of Hp facilitate the penetration and movement of bacteria within the viscous mucus layer, permitting their escape from extremely low pH and avoiding clearance by gastric peristalsis. Microaerobic metabolism enables the organism to exist within gastric mucus, where oxygen concentration is lower than in the gastric lumen. Although the mucus layer affords protection from low pH, this is not absolute, and the Hp must survive highly acidic pH during the earliest stage of gastric colonization before reaching a deeper layer of the mucous coat and attaching to the surface of mucosal cells.

An important factor in the adaptation of Hp to gastric acidic milieu may be the ability of bacteria to influence gastric acid secretion (Fig. 27). In this respect, it is noteworthy that initial acute infection with Hp leads to hypochlorhydria which has been observed in volunteers infected experimentally with these bacteria (62, 63), as well as in patients inadvertently infected with Hp during gastroduodenal intubation (64). At this early stage of infection the stomach adapts to bacteria by developing, acute superficial pangastritis and this is accompanied by transient achlorhydria that resolves within few weeks. In the meantime bacteria colonize the stomach and, thus, survive the hostile intragastric conditions. During the later phase of infection,
Hp colonizes mostly the antral portion and the stomach adapts by developing chronic active (bacterial) type B gastritis that may be accompanied by an increased gastric secretion and the formation of duodenal ulcer. When the bacteria colonizes the entire stomach, pangastritis with chronic inflammation and intestinal metaplasia may develop leading to the reduction in gastric secretory capacity and to the formation of gastric cancer or lymphoproliferative gastritis (see Fig. 26).

**Fig. 27.** Gastric acid secretion in Hp infected humans at early phase of infection when acute gastritis develops and at later phases with chronic active gastritis and chronic atrophic gastritis.

It is not clear how the initial Hp infection transiently inhibits gastric acid secretion. To colonize the stomach, the Hp strain has to be motile and urease-positive (53). Hp directly affects the function of parietal cells as its constituents such as LPS and tetradecanoic acid, directly inhibit acid secretion. The organism occasionally may be seen within the canaliculi of the parietal cells in vivo (53, 54, 60). It is also possible that increased release of various cytokines in the Hp infected mucosa such as interleukin IL-1β, IL-6, IL-8 or tumor necrosis factor alfa (TNFα) could play an important role in inducing hypochlorhydria (65, 66).

Following acute infection accompanied by acute gastritis and hypochlorhydria, a chronic infection with compensatory hypergastrinemia and increased gastric acid secretion occur in some patients (54). In most of Hp-positive patients, basal and maximal acid outputs (pentagastrin-stimulated)
Fig. 28. Excessive release of gastrin and increased gastric acid secretion due to deficient action of luminal acid and endogenous CCK on D-cells releasing somatostatin and controlling the G-cells releasing gastrin.

remain unchanged but there is a loss of normal inhibitory control of gastrin release and gastric acid secretion by antral acidification (67, 68) or distension pressure (69) (Fig. 28). Acid response to gastrin-releasing peptide (GRP) or meal in Hp-positive patients is also several times higher than that in Hp-negative subjects (70, 71). The chronic active gastritis induced by Hp infection is a reflection of gastric adaptation to bacteria being associated with reversible increase of plasma gastrin level and gastric acid secretion in response to GRP and/or meal stimulation. This hypergastrinemia resolves after about a year upon eradication of Hp infection confirming its dependency upon the gastric infection by Hp (70). Furthermore, normal gastric acid inhibition by endogenous cholecystokinin (CCK) in Hp-infected patients appears to be impaired due to the deficiency of somatostatin release from antral D cells by endogenous CCK, the alteration that also can be completely reversed following the eradication of Hp (72, 73) (Fig. 28). Thus, in duodenal ulcer patients with chronic Hp infection, several disturbances of gastric secretory mechanisms occur; 1) increased gastrin release by antral mucosa due to the impaired inhibition of this hormone release by antral acidification or distension, 2) the deficiency of somatostatin release, 3) an exaggerated acid response to stimulation by endogenous gastrin (G-17) that is selectively released by gastric antrum in Hp-infected stomach.
These results suggest that increased gastrin release and enhanced acid secretion are probably the key factors in the pathophysiology of duodenal ulcer disease in Hp-infected stomach. An elevated plasma gastrin with subsequent increase in parietal cell mass determines the excessive acid output in Hp-infected duodenal ulcer patients. The sequence of events that follows the Hp-infection includes; 1) the reduction in the number and impairment of antral D cells with increased ratio of G-cells to D-cells, 2) the decrease of somatostatin production with subsequent elevation of basal and postprandial plasma gastrin, 3) trophic effects of gastrin with subsequent increase of parietal cell mass and 4) increased acid output and increased mucosal cell proliferation (74—76). Increased cell proliferation in Hp-infected stomach and the inflammation of the mucosa may increase the gastric mutation rate, which alongside mutagens, decreased ascorbinic acid content and may be a major factor in the development of gastric dysplasia and gastric cancer. This sequence of events is supported by the observation that after the Hp eradication, the acid output and plasma gastrin returned to the levels observed in Hp-negative subjects and mucosal inflammatory changes disappeared.

As Hp colonizes predominantly the antral portion of the stomach, the gastric antrum plays a pivotal role in gastric secretory alterations in Hp infection via enhancement of gastrin release from the G-cells. Hypergastrinemia in Hp-infected patients occurs under basal conditions and following GRP or meal stimulation but returns to baseline values following the eradication of Hp (70—72). This hypergastrinemia consists of elevated plasma gastrin G-17, the form of hormone released predominantly in the antral mucosa in response to a GRP or meal (70—74). As antral alkalinization is known to enhance gastrin release it was initially believed that ammonia generated by Hp-urease produced an alkaline environment in the vicinity of G-cells to stimulate this gastrin release (77). The fact that the inhibition of urease activity with acetohydroxamic acid failed to suppress serum gastrin in Hp-positive patients militates, however, against the major role of ammonia in the stimulation of gastrin release. An alternative hypothesis has been proposed that specific products of Hp inflammation such as cytokines including IL-1β, IL-6, IL-8, TNFα, IFNγ determine gastrin hypersecretion. This is supported by the finding that following eradication of Hp, when inflammation declines, basal and meal-induced plasma gastrin levels are significantly reduced (72). This reduction could be also attributed to the excessive release of EGF and TGFα that was observed in our studies following successful eradication of Hp in duodenal ulcer patients (78).

Previous studies demonstrated that the infection of gastric mucosa with Hp and the presence of urease, especially in patients with peptic ulcer disease (79) was an important source of a potent gastrotoxic substance, ammonia (79—84) (Fig. 29). The significant correlation between the severity of gastric
Fig. 29. Hp infection results from the action on the gastric mucosa of various pathogens such as ammonia, cytotoxin and endotoxins (LPS) as well as from the inflammatory immune reactions in the mucosa.

(79—84) (Fig. 29). The significant correlation between the severity of gastric inflammation and the gastric luminal concentration of ammonia suggested that this substance contributed to Hp-associated gastric injury. Indeed, ammonia was shown to exert the cytotoxic effects on various cultured cell lines in the concentration-dependent manner (80, 81) and on the gastric mucosa in vivo in animals (82, 86). Furthermore, the presence of ammonium ions was found to be required for the full activity of Hp strains producing toxins such as vacuolating toxin (vacA) acting at low pH and resulting in the expression of inflammatory mediators such as interleukins and TNFα (86).

Although the deleterious effect of ammonia on the gastric mucosa has been confirmed in the rat stomach using urea in the presence of exogenously administered urease (87), it is not clear whether ammonia generated in amounts similar to those recorded in Hp infected stomach is capable of affecting gastric mucosal integrity. Our recent study confirmed earlier reports that ammonia applied topically resulted in a concentration-dependent damage to the mucosa but it appears that only higher concentrations of this substance caused gastric lesions (88). This deleterious action of locally produced ammonia on the gastric mucosa was attributed to the production of oxygen free radicals such as monochloramines due to an interaction of neutrophil-derived hypochlorous acid and ammonium ion (89). Ammonia applied to the mucosa in lower
concentrations (6.2—30 mM/L) similar to those recorded in Hp-infected stomach actually induced adaptive cytoprotection that prevented the development of growth mucosal damage by high concentration (250 mM/L) of ammonia or 100% ethanol (87) (Fig. 30). This prevention was also observed when the mixture of urea plus urease was instilled into the stomach just before the application of 100% ethanol or damaging concentration of ammonia (250 mM/L) (Fig. 30). Like other forms of adaptive cytoprotection, ammonia-induced protection is probably mediated by local release of nitric oxide because the pretreatment with L-nitro-arginine analogs (L-NAME) greatly attenuated this adaptive protection afforded by low concentration of ammonia (89). (Fig. 31).

![Graph](image1.png)

**Fig. 30.** Mean area of gastric lesions and gastric blood flow in adaptive cytoprotection achieved with lower concentration of ammonia administered 30 min before 100% ethanol in rats without or with pretreatment with L-NAME. Means ± SEM of 8—10 rats. Asterisk indicates significant difference as compared to the values obtained after 100% ethanol alone. Cross indicates significant change as compared to the values obtained in corresponding groups of rats without pretreatment with L-NAME.

It is of interest that gastric mucosa can adapt to repeated applications of higher concentrations of ammonia (Fig. 32). First exposure of the mucosa to high concentrations of ammonia led to excessive cell desquamation and deep hemorrhagic necrosis. These changes were gradually reduced following repeated ammonia insults and at the same time, a marked mucosal regeneration was observed. Like in case of adaptation to stress or aspirin, there
was a minimal dose of ammonia which damages the gastric mucosa and induces gastric adaptation (Fig. 32).

The mechanism of this gastric adaptation is not clear but the finding of regenerative changes in the mucosa adapted to ammonia and overexpression of EGF and its receptors suggest that EGF plays a crucial role in gastric adaptation to ammonia (Fig. 33). This increased mucosal expression of growth factors and their receptors has been documented semiquantitatively by immunohistochemistry and by detection of signal for EGF mRNA using RT-PCR technique (88). It is not excluded that EGF and EGFR are epiphenomena related to the excessive mucosal regeneration that follows initial damage by topical irritant such as ammonia. Since the ammonia produced by Hp urease in the stomach occurs in rather low concentration it may actually exhibit gastroprotective rather than damaging effects on the mucosa and when applied repeatedly it strengthens the mucosal resistance to other irritants due to adaptive mucosal changes. Such gastric adaptation induced by repeated exposures to stress, topical aspirin or ammonia enhance mucosal resistance to the damage by strong corrosive substances such as 100% ethanol (31,32).
**Fig. 32.** Mean lesion area and gastric blood flow in the rat stomach exposed to ammonia in various concentrations applied once or every day during 5 consecutive days. Asterisk indicates significant decrease in lesion area below the value obtained after single exposure to ammonia. Cross indicates significant increase in blood flow above the value recorded after single exposure to ammonia.

**Fig. 33.** Expression of EGF, TGFα, and EGF receptors as determined by immunohistochemistry (staining intensity from 0 = no expression to 3 = highest expression) in the gastric mucosa after administration of ammonia once, for 1 or 5 days. Asterisk indicates significant increase above the value obtained after first ("once") administration of ammonia.
according to our data, ammonia released locally in Hp infected stomach may have rather favourable than deleterious influence on mucosal integrity and strengthens the mucosal barrier as well as promotes gastric adaptation to Hp infection.

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