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EXPERIMENTAL PRODUCTION OF PEPTIC ULCER, GASTRIC DAMAGE AND CANCER MODELS AND THEIR USE IN PATHOPHYSIOLOGICAL STUDIES AND PHARMACOLOGICAL TREATMENT - POLISH ACHIEVEMENTS

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The common acid related diseases of the upper GI tract could be considered as primarily due to the defect in barrier function either of the gastric mucosal or duodenal epithelium leading to the formation of gastric or duodenal ulcers. An attempt was made in this chapter to discuss the history of peptic ulcer disease in humans and methods for the production of acute gastric lesions and ulcers in experimental animals with the special attention focused to the contribution of Polish scientists and investigators into this field. Early surgical advances in the management of peptic ulcers were emphasized that were then subsequently replaced by pharmacological treatment (histamine H₂-receptor antagonists, proton pump inhibitors) and considered as the major strategy against the acid disorders. This included the immense body of work performed by numerous group of investigators, including Polish researchers, to identify the effects of acid, bile salts, aspirin and other non-steroidal anti-inflammatory drugs (NSAID), stress, *Helicobacter pylori* (*H. pylori*) infection, prostaglandins (PG) and nitric oxide (NO) on the integrity of the gastrointestinal mucosa, which all were discussed in this chapter. The concept of major defensive mechanism in the stomach called "cytoprotection", originally proposed by Andre Robert is recalled in the relevance to the great contribution of polish scientist working at the Jagiellonian University in Cracow. These experimental studies gave a new insight into the mechanism of action of arachidonate cascade products such as PGs, thromboxanes and leukotrienes and had opened the new therapeutic avenues for the gastroprotective treatment of the acute gastric mucosal damage. Detailed studies revealed, however, that PG-induced cytoprotection offers a short-term protection against gastric lesions induced by corrosive agents but unfortunately this phenomenon gives a little, if any, impact to the process of ulcer healing. The experimental studies on healing of gastric ulcers that become supportive for the clinical trial in humans, performed also by polish pioneers and the effect of numerous growth factors (EGF, TGF α), NO inhibitors, cyclooxygenase- (COX)-1 and COX-2 inhibitors and new safer derivatives of NSAID releasing NO in protection and ulcer healing are discussed. Finally, the major discovery by Warren and Marshall of *H. pylori* that have been studied world-wide in

experimental animals including also Polish investigators, allowed for the better insight to the pathological consequences of this germ infection in the gastric mucosa and helped to establish the anti-*H. pylori* eradication therapy.

Key words: *peptic ulcers, prostaglandins, nitric oxide, growth factors, non-steroidal antiinflammatory drugs, Helicobacter pylori.*

INTRODUCTION

History of the peptic ulcer disease - early clinical and experimental observations

The elucidation of the peptic ulcer disease of the stomach and duodenum has accelerated dramatically in the last 20 years due to the identification of several techniques, which have facilitated the study of the gastric mucosa. Up to the late period of 19th century, the stomach was often not clearly recognized as a source of symptoms. From that earliest times chalk, charcoal and slop diets had been noted to provide the symptomatic relief from dyspepsia but little rational therapeutic intervention was available for the treatment of gastroduodenal ulcerations. In the 17th century chalk and pearl juleps were utilized for infant gastric disorders. The favorite theme of the 17th century physicians was dyspepsia due to gastric atony implicit in the title *De imbecillitate ventriculi*. This term was continued into the 18th century when it was equated with the "*hectic stomach*" (Arnold, 1743) and later with the term of "*embarrass gastrique*" utilized by French physicians. Throughout the 18th century there were various descriptions of gastric and intestinal diseases but no specific and logical remedies were recorded. In 1835, Cruveilhier had written extensively in Paris on the pathology of peptic ulcer disease and the conditions was referred to as Cruveilhier's disease. Cruveilhier, a pupil of the great surgeon of Paris, Dupuytren (1), subsequently become one of the foremost authorities on stomach ulcers in France. Cruveilhier pondered as to why an ulcer of the stomach might occur in a single place when the rest of the stomach was "*in a state of perfect integrity*". He made several notes with regard to haematemesis that are of interest from the point of current therapeutic strategies. Finally, his contribution includes the careful documentation of the each case history and autopsy and the detailed description of the pathology and sequeale of chronic and duodenal ulcerations. Dupuytren (1), first observed the relation between cases of burn and acute ulceration of the duodenum. In 1836 Dupuytren had noted the congestion of the various mucous membranes of the alimentary canal in the early stages of burns. He described in detail the ulceration and bleeding of the stomach and duodenum. He observed this relationship more than a decade prior to Curling (2), the former

published little of his work and his observations were not initially widely known. Formally the clear association between the ulceration of the duodenum with the special circumstances such as burn was first attributed to Curling in 1841 (2), who called attention to the connection between cases of cutaneous burn and acute ulcerations of the duodenum. It is important that these observations were later reproduced in animals by many experimental investigators to study pathomechanism of so-called "Curling ulcerations" (3).

Polish trace in the early concept of the peptic ulcer formation with some advises for the development of anti-ulcer strategy comes from the paper published in 1869 by A. Biesiadecki (1839-1889) in the *Przegląd Lekarski*, on the gastroduodenal ulcerations in humans (4). Biesiadecki believed that duodenal ulcerations develop as a result of not only hyperacidity but also due to impairment in the gastroduodenal microcirculation of the gut (4).

The issue of surgery of the stomach had been confounded for years by the problem related to sepsis and lack of adequate anesthesia. Nevertheless, by 1881 Billroth, Pean and Rydiger had resected the stomach and Wolfler had successfully developed the procedure of gastroenterostomy (5, 6). With the entering the new century, Moynihan (7) had transformed the treatment of peptic ulcer disease into an unique surgical discipline. He wrote extensively on the surgical management of gastroduodenal ulcer disease. In contrast to Cruveilhier's great contributions that were based on the description of the pathology of gastric ulcer, Moynihan defined many of the early surgical strategies for the peptic ulcer and became famous because of his contributions to gastric surgery (7). As the President of the Royal College of Surgeons of England, he has been considered as introductor of the science into surgical training programs. It is noteworthy that the anti-ulcer therapy at that time was very modest and consisted of the application of leeches, mustard plasters bleeding and various alcoholic concoctions which presumably allayed anxiety and relieved pain. Unfortunately, the consequences of gastric and vagal surgery produced a generation of patients with either significant vagotomy problems or postgastrectomy syndrome. Nevertheless, Pean, another pupil of Dupuytren's, was the first who made an outstanding contribution to the gastric surgery by resecting a pyloric gastric cancer in 1879. However, despite the fact that his operated patient received the blood transfusion, he died on the fifth postoperative day. Although the reason for the death of this patient was unknown, it is likely that the transfused blood incompatibility and sepsis were the major reasons, since the recognition of the blood groups was done about 40 years later.

L. Rydiger of Chelmo performed the second documented gastrectomy in 1880, which however was unsuccessful (6). Similarly to Pean, Rydiger made a partial resection of the pre-pyloric portion of the stomach in a patient with a gastric cancer. In the same year, few months later Billroth became the first surgeon who successfully had undertaken a gastrectomy, and his patient, a women, survived the period of 3 weeks postoperatively and died for another reason, namely hepatic metastases. It should be mentioned that the cumulative

operative survival from gastrectomy in two decades following Pean initial operation failed to exceed 50%.

The gastroenterostomy remained the standard choice for the first two to three decades of the twentieth century as the preferred surgical treatment of either peptic ulcer or gastric neoplasia.

H. Cushing was the first who pointed out the alterations in neural pathway regulating gastric physiology and who recognized that after posterior *cranial fossa* surgery duodenal ulcers often occur. This was the fundamental statement for the development of the association between vagi and peptic ulcer especially after I.P. Pavlov had delineated the importance of the neural regulation of gastric secretion in dogs. M.A. Latarjet of Lyons (8) first described vagotomy for the management of peptic ulcer disease and confirmed that it caused amelioration of symptoms as well as decrease in acid secretion. Working as an anatomist, he made an outstanding contribution to anatomy of the vagi to date. He had definitely explained the anterior and posterior vagal nerves of the gastric lesser curvature. Over the period of twenty years he wrote extensively on nerves of the colon, biliary tract and the pelvis in both men and women. His colleague, Wertheimer in 1921, provided the anatomic information necessary to enable Latarjet to successfully undertake vagotomy. In his thesis "*De l'énervation Gastrique*", he documented both anatomical and experimental work in regard to the vagal innervations of the stomach. The major message from his work was that cutting of vagal nerves causes impairment in gastric motility and gastric emptying resulting in a substantial inhibition of gastric acid secretion clearly indicating that the therapeutic vagotomy as a therapeutic treatment is not the optimal therapy for the peptic ulcer.

Dragstedt's team (9), which performed in 1943 a sub-diaphragmatic vagal resection on a patient with an active duodenal ulcer, continued this work. The major interest in research of this group was the pathogenesis of peptic ulcer disease (9). At this stage of medical sciences, he was fascinated by the existing contention that normal stomach does not digest itself. He postulated that the mucosal damage take place at night when people do not eat and that this nocturnal acid secretion was of nervous origin. He also noted that humoral stimulation could account for acid hypersecretion. This was in keeping with Edkins (10), who pioneered the study on gastrin released to blood stream by the mucous membrane in the antrum in response to contact with food and primary products of digestion. In dogs, Dragstedt excised and transplanted the antrum from a denervated stomach to its abdominal wall and found markedly reduced gastric secretion (9). When antrum transplanted to the colon, a marked rise in gastric acid secretion was observed, however, when it was re-implanted to the duodenum, normal gastric secretion resumed. With these studies, Dragstedt not only confirmed the findings of Edkins but also established the fundamental observation that gastrin secretion did not take place in an acid environment. His statement served as a background that for an existence of a feedback mechanism dependent on mucosal

pH for the control of secretion of gastrin and served as a rationale for the introduction of the surgical application of antrectomy. He claimed that gastric ulcers were caused by "abnormal" hormonal stimuli whereas duodenal ulcerations were of nervous origin due to pathological nervous stimuli transmitted by the vagi. Gastric ulcerations, he believed, might also result from gastric stasis, which caused prolonged antral contact with food that finally led to the hypersecretion of gastrin. He proposed a total vagotomy with gastroenterostomy to allow for gastric drainage in order to prevent the major complication in these patients such as gastric stasis.

Polish traces in experimental gastroduodenal ulcerations - from surgery to antisecretory agents

The recognition in the initial part of XXth century by Schwartz (11) that the mucosal damage was caused by acid (his famous statement *no acid-no ulcer*) and that this acid could be decreased luminal neutralization resulting in the amelioration of gastric injury changed the surgical way of thinking and open the new avenues of anti-ulcer strategies. This was the beginning for the antacid preparation, bland diets and constant milk infusion as therapeutic options. The subsequent identification of the histamine₂- receptor subtype and the development of agents specifically capable of blocking acid secretion by antagonism at this receptor by Black and his associates (12) revolutionized the management of the peptic ulcer disease and virtually obliterated surgery as a therapeutic option for peptic ulcer except in case of emergency.

It started to be evident that gastric acid plays an important role in the pathogenesis of the peptic ulceration and, therefore, the understanding of enhanced acidity in the mechanism of gastroduodenal ulcerations required an appropriate experimental model. First pathogenic candidate to study under experimental conditions was histamine, which that had been considered after Popielski (13), as a final chemo-stimulator of gastric parietal cell function and was believed for long time to play a central role in the pathogenesis of the peptic ulceration. In 1928 Buchner, Siebert and Mollay (14) were the first to produce an experimental ulcerations using histamine and to postulate that this might be due to the alteration in gastric microcirculation later widely accepted as a crucial mechanism responsible for the ischemic damage (*Fig. 1*). In experimental study, Hay, Varco, Code and Wangenstein (15) demonstrated the development of peptic ulceration following continuous gastric acid stimulation by the administration of histamine released from the beeswax and they concluded that histamine stimulated gastric acid could be considered as a primary cause of peptic ulcer disease. The Polish trace in histamine research at that time comes predominantly from the studies of K. Kowalewski, who was appointed as a professor in Bialystok but later emigrated and became the resident of Canada and worked at the University of Alberta. He was a pioneer in histamine research both in humans

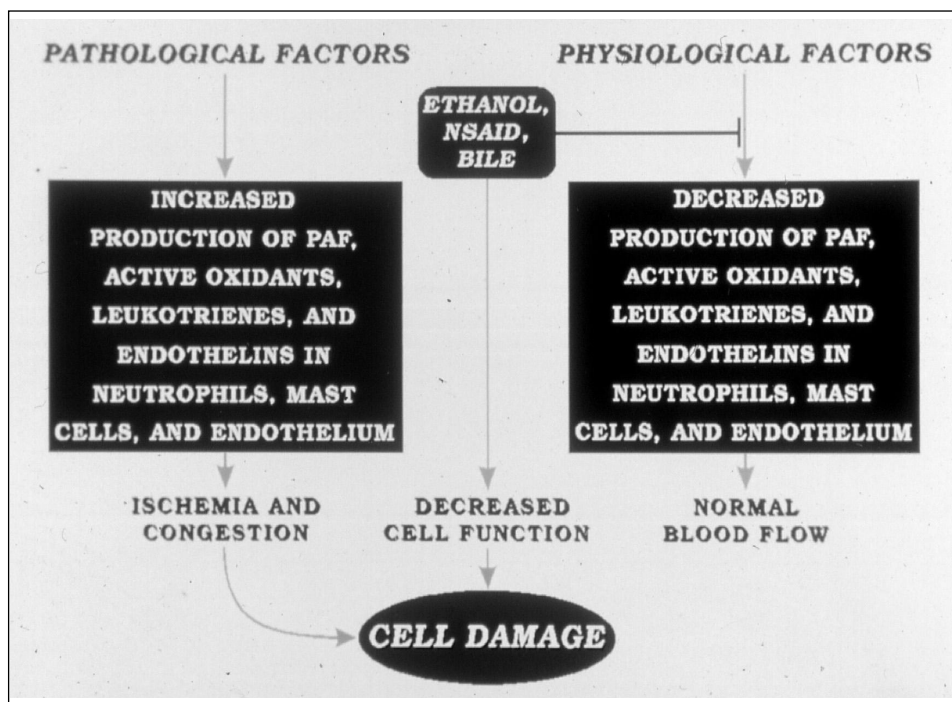


Fig. 1. Physiological factors involved in the mechanism of gastric integrity and mucosal defense against damage induced by pathological stimuli. Vascular factors such as active oxidants, mast cell products, endothelium derived leukotrienes and endothelins, both derived also by neutrophils, induce ischemia and vasocongestion ultimately leading to cell damage. Corrosive substances such as so called "barrier breakers" including ethanol, NSAID and bile salts activate neutrophils, reactive oxygen species and endothelial vasoconstrictive mediators decreasing cell function and facilitating mucosal cell damage.

and experimental animals (16 - 18), who first introduced the administration of gradual doses of histamine in humans, the study that preceded the popular clinical histamine test described 4 years later by Kay (19). In the field of experimental ulcerations Kowalski was the first who emphasized the importance of vascular factors in histamine-induced gastric lesions and to demonstrate that simultaneous administration of histamine and octapressin, an analogue of antidiuretic hormone (ADH, vasopressin) counteracted the gastric lesions induced by histamine (20).

In 1923, Mann and Williamson published the original experimental method of ulcer formation in dogs that served for the long time for the studying of subacute and chronic ulcers (21). According to their procedure, the duodenum was defunctionalized except for its role in carrying the bile and pancreatic juice into the terminal portion of ileum distant from the point where gastric contents enter the jejunum. In this experimental model, the subacute or chronic ulcers subsequently developed in majority of dogs. Most of the ulcers occurred in the

jejunum a few millimeters distal to its junction with the gastric mucosa. The finding of Mann and Williamson were rapidly confirmed in many parts of the world, and their procedure soon became the standard method of producing ulcers experimentally. The most important consequence of the Mann-Williamson operation was the demonstration that acid peptic gastric contents were required for the genesis and continued existence of this type of peptic ulcer (21). The Polish contribution to the scientific field on experimental gastric ulcers went after the end of II WW mostly from the work of Kaulbersz and his associates Bilski, Bugajski, Konturek, Radecki and Oleksy (22, 23). The major interest of Kaulbersz was in digestive physiology and the effect of high altitude resulting in hypoxia, on gastric secretory functions and formation of experimental gastric ulcerations including the experimental model of Mann-Williamson ulcerations in dogs. This was latter continued by Bilski, one of the Kaulbersz associates, who published evidence that hypoxia induced by decrease in the atmospheric pressure which corresponded to the altitude of 5500 m, enhanced the severity of Mann-Williamson ulcerations (23).

It is always the aim of the experimental researcher to develop models, which closely resemble the human disease under investigation. This was believed to give a further insight into etiology, the healing process and the therapy of the disease. Unfortunately, it became apparent from the history of ulcer disease that single ulcer model is not satisfactory. Shay and his associates (24) described an acute ulcerative process in the forestomach of the rat that could be produced simply by pyloric ligation. It need to be mentioned that these lesions appeared mainly in the non-secretory portion called rumen, lined with squamous epithelium that is subjected to corrosive action of gastric juice. The mechanism of this lesions was attributed primarily to the accumulation of acid and pepsin within the stomach despite the criticism that distention of the stomach and interference of blood circulation could also contribute to the formation of these lesions. Many investigators including Polish researchers have studied the Shay lesions technique extensively. Radecki (25), another associate of Kaulbersz, was the first who initiated this technique in Poland by studying the effect of duration of fasting and extension of the time of pyloric ligation on the severity of these lesions in rats. He showed that longer fasting and extension of the time after pylorus ligation increased both the amount of gastric acid and severity of these ulcerations. This technique was then continued by Kaulbersz's pupil, Konturek who successfully used this technique to study the effect of hypophysectomy and the removal of adrenal glands by adrenalectomy on the ulcers produced by Shay pylorus-ligation and histamine in rats and cats and on the urogastrone levels in urine of dogs subjected to adrenalectomy (26, 27). Konturek had firstly demonstrated that hypophysectomy and adrenalectomy decreased gastric acidity secretion and inhibited the formation of Shay- and histamine-induced gastric ulcerations (26). Moreover, Konturek's greater achievement was the demonstration of the potent gastric inhibitory and gastroprotective action of

urogastrone after successful extraction this hormone from urine. This original finding served as a basis for the latter discovery of gastroprotective and ulcer healing promoting actions of homologue of urogastrone, epidermal growth factor (EGF) that was found to be released from salivary glands and produced locally by ulcer-associated cell lineages (UACL) in response to the mucosal damage and accumulated at the ulcer margin thus accelerating the healing of these ulcerations (Fig. 2) (27).

Discovery of cytoprotection - real breakthrough in the concept of gastric mucosal defense

In 1979, the term of "cytoprotection" was introduced into gut literature by A. Robert, who described the unexpected and fascinating finding in rats that natural prostaglandins applied exogenously in the non-antisecretory doses, exhibit high activity in preventing the mucosal damage induced by necrotizing substances such as strong acids, base or concentrated bile including even the lesions caused by boiling water (Fig. 3) (28). Soon, Konturek and his associate Brzozowski

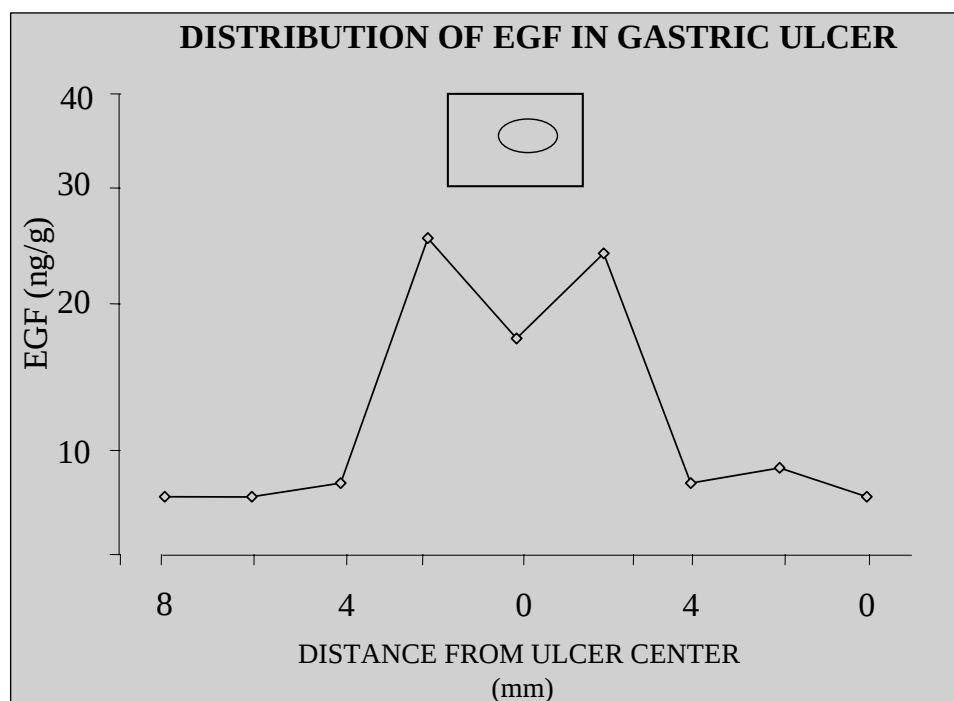


Fig. 2 A cartoon showing the distribution of EGF in the area of gastric ulceration performed by Polish investigators working at the Department of Physiology. This was the first demonstration that EGF is synthesized and accumulated, especially at the ulcer edge, supporting the essential role in the mechanism of acceleration of ulcer healing by this growth factor.

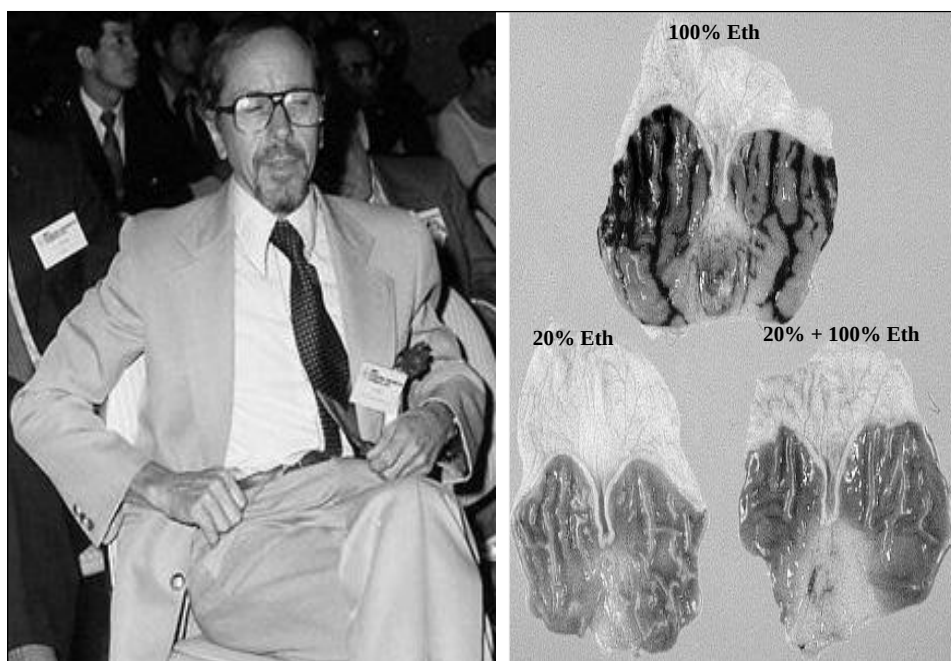


Fig. 3 Andre Robert working at Kalamazoo (Michigan) (left panel) pioneered the concept of gastric cytoprotection. According to his fascinating observation, prostaglandins applied in non-antisecretory doses attenuated the severe gastric lesions caused by necrotizing agents such as concentrated alcohol, bile salts and hyperosmolar solutions. He was the first to introduce the term "adaptive cytoprotection" in order to describe the phenomenon of prevention of gastric lesions induced by absolute ethanol in rats pretreated with mild irritant such as 20% ethanol (right panel).

conformed not only this finding but also documented, for the first time, that certain growth factors, especially EGF, could be considered as cytoprotective because they are capable of reducing aspirin-induced gastric ulcerations in rats and cats under the conditions where biosynthesis of endogenous PG was completely inhibited by the administration of aspirin (29). One important aspect of cytoprotection was so called "adaptive cytoprotection", the term that was also introduced originally by Robert and his associates (30) to describe the protective activity of endogenous prostaglandin generated within gastric mucosa by mild topical irritants such as 20 % ethanol or 5 mM NaCl to against severe mucosal damage induced by strong irritants such as 100 % ethanol or 25 % NaCl (*Fig. 4*). It should be emphasized that Konturek's group made an outstanding contribution to the concept of cytoprotection not only by extending Robert's observations but also by documenting that mild irritants offer the cross-protective response, e.g. 5% NaCl was effective in attenuation of damage induced not only by necrotizing 25 % NaCl but also by 100% ethanol, while 20% ethanol prevented

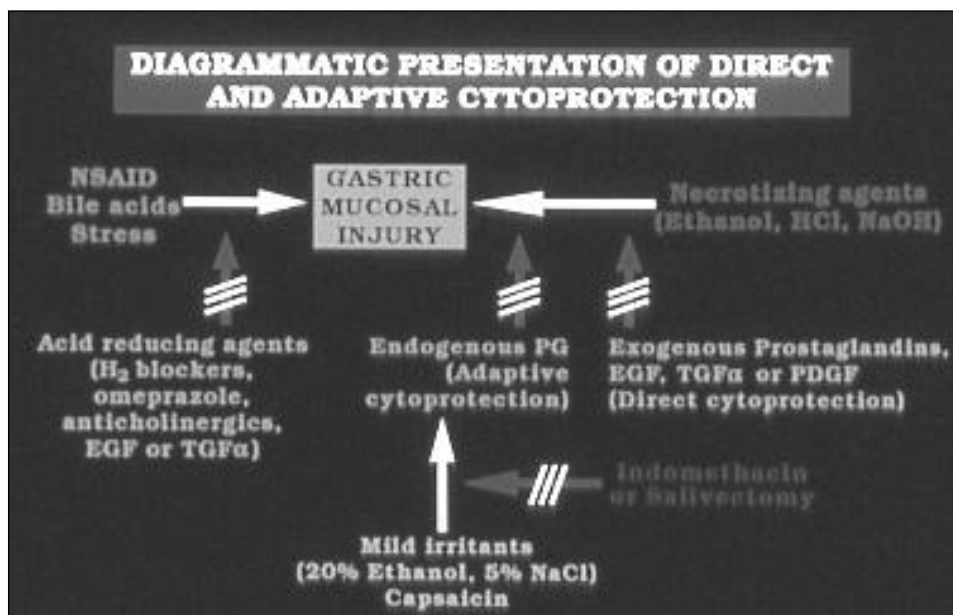


Fig. 4 The diagrammatic presentation of Andre Robert's concept of cytoprotection modified by Konturek *et al.* (31). The necrotizing agents such as 100% ethanol, 600 mM HCl and 200 mM NaOH induce gastric mucosal injury which could be reduced by pretreatment with exogenous prostaglandins (PG) and nitric oxide (NO) donors *via* direct cytoprotection. Mild irritants such as 20% ethanol, 5% NaCl, 5 mM taurocholate or capsaicin in low concentrations, prevent the lesions induced by necrotizing agents *via* adaptive cytoprotection involving an increase in the mucosal generation of endogenous PG and NO.

the damage caused by 25% NaCl (31). Moreover, they found using the bioassay technique to measure generation of prostacyclin (PGI₂) and PGE₂ in the gastric mucosa that pretreatment with mild irritant resulted in an enhancement in the generation of PGI₂ and PGE₂, indeed providing direct evidence on the involvement of endogenous prostaglandins in the mechanism of adaptive cytoprotection (32, 33). Polish researchers proposed that this protective mucosal mild-irritation acts locally because mild irritants failed to exhibit any protective activity when applied systemically (31).

The term of cytoprotection along with the prostaglandin contribution to the concept of cytoprotection, had been criticized by some researchers because it became apparent from detailed histological assessments that prostaglandins could afford protection to the deeper mucosal layers including regenerative zone of gastric glands but failed to prevent the injury to superficial mucosal cells, again, questioning the truly "cytoprotective" properties of these arachidonate metabolites. Besides prostaglandin also an important mediator nitric oxide (NO), was later implicated as mediator of adaptive cytoprotection (34) and in fact, some

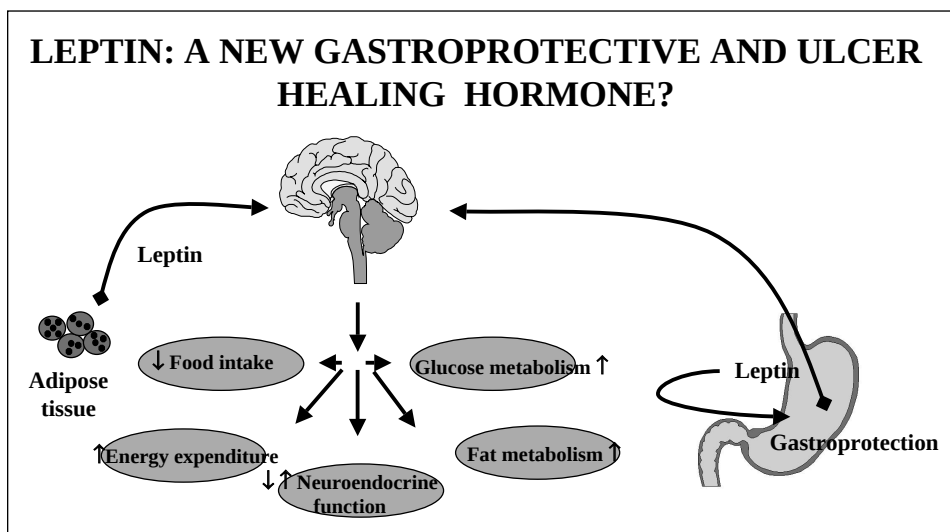


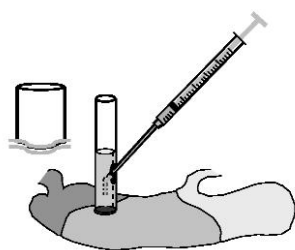
Fig. 5 A schematic representation of various physiological functions of leptin including role of this hormone in the control of food intake and metabolic processes. Recent experimental evidence indicates that leptin which is produced locally and released from the gastric mucosa, exhibits potent gastroprotective and ulcer healing activities.

reports suggested that prostaglandin might not be primary mediators of this mucosal adaptive cytoprotection (35).

Extensive experimental studies in the last decade revealed that NO released from vascular endothelium, sensory afferent nerves or gastric epithelium (36) is essential not only for adaptive cytoprotection but also for the gastroprotection evoked by many physiological factors including hormones such as cholecystokinin (CCK), gastrin and leptin (37, 38) and for healing of chronic ulcerations induced by acetic acid (39), the model originally described by Takagi and Okabe (40). Furthermore, Konturek and Brzozowski documented that gastrointestinal hormones such as CCK, gastrin and leptin, which regulates food intake and body energy expenditure, exhibit a potent gastroprotective activity against necrotizing injury induced by ethanol and mucosal damage caused by aspirin, *via* prostaglandin-independent mechanism (*Fig. 5*) (37, 38).

It is well known that in most cases of so-called ulcer models, the erosions appear that are very small and differ from histologically from the human chronic peptic ulcer with respect to pathomorphological appearance and healing characteristics. Only a few models of "real" peptic ulcers for studying the healing process are available, of which the acetic acid model introduced by Okabe and Pfeifer (41) has received wider attention (*Fig. 6*). Studying the time course of the healing process in this model at the Department of Physiology Jagiellonian University in Cracow, Polish researchers found that typical chronic gastric ulcers

Subserosal application of acetic acid solution



***Okabe and Pfeifer, 1971; modified
Konturek et al., 1987***

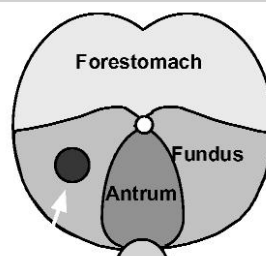


Fig. 6 The simplified cartoon showing the method of production of chronic gastric ulcerations originally introduced by Okabe and Pfeifer (41) and later modified by Konturek *et al.* (33), using the application of acetic acid onto serosal surface of the rat stomach. This widely used experimental model appears to be suitable for the determination of various physiological factors on ulcer healing and the screening of anti-ulcer drugs.

such as those induced by serosal application of acetic acid heal in progressive manner being completed in control animals after about 2 weeks (33, 42). Healing of these ulcers was accompanied by the increase of the gastric blood flow in the ulcer area and by the significant rise of both, plasma gastrin and proinflammatory cytokines such as $\text{TNF}\alpha$ and $\text{IL-1}\beta$ levels (43). With progression of ulcer healing, the blood flow at the ulcer margin and the elevated plasma $\text{IL-1}\beta$, $\text{TNF}\alpha$ and gastrin levels, declined gradually but their levels failed to reach the values recorded in intact rats (43). It was concluded that the hypergastrinemia observed during the early period of ulcer healing could be attributed to the remarkable suppression of gastric acidity and expression of growth factors such as EGF, $\text{TGF}\alpha$ and HGF which control the cellular proliferation and are well-known to exhibit antiseecretory activity as demonstrated by Konturek's group previously (*Fig. 7*) (29, 42).

Using these model with own modifications where acetic acid was placed onto the serosa of gastric wall instead of its intramuscular injection of this acid as in original method described by Okabe (*Fig. 6*) (41), Konturek and Brzozowski were the first to publish that the administration of NO-synthase inhibitors

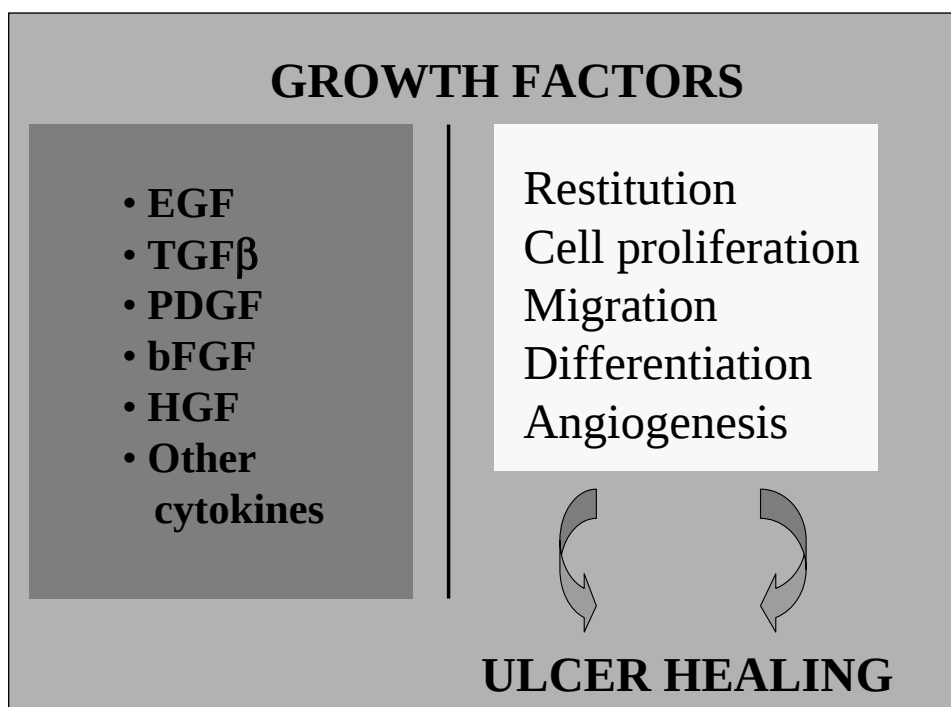


Fig. 7 Many growth factors including EGF, bFGF and HGF contribute to the mechanism of ulcer healing due to their stimulatory effect on the process of mucosal repair, cell migration and proliferation as well as angiogenesis.

abolished delayed healing of chronic gastric ulcers (39) and that hormones such as CCK, gastrin and leptin applied exogenously or released endogenously in response to fat or peptone meal, respectively, accelerated the healing process of these ulcerations and prevented the acute necrotizing injury induced by intragastric application of absolute ethanol (44, 45).

Prostaglandin cyclooxygenases and nonsteroidal anti-inflammatory drugs (NSAID) in the mechanism of the gastric mucosal integrity and ulcer healing and NSAID-induced gastropathy. New approach to limit adverse effects of NSAID?

Recent advances on the enzymatic pathways of arachidonate metabolism revealed that PG synthesis depends upon the activity of cyclooxygenase (COX), a rate-limiting enzyme in the synthesis of eicosanoids. Two isoforms of COX were identified in many cells; a constitutive enzyme designated as COX-1 and inducible isoform known as COX-2 (46). COX-1 appears to be responsible for the production of PG that is physiologically important for homeostatic functions, such as maintenance of the mucosal integrity and mucosal blood flow. Under

physiological conditions prostanoid synthesis depends upon the availability of arachidonic acid and the COX-1 activity, that is a major target for non-steroidal anti-inflammatory drugs (NSAID) causing mucosal damage in the stomach (47). Prostaglandin derived from the activity of the COX isoforms, especially COX-1, play an important role in mechanism of gastric integrity, gastroprotection and ulcer healing (28 - 33). Recently prostaglandin-derived from COX-2 were implicated in the protective and ulcer healing activities of growth factors by the demonstration that COX-2 is upregulated in the edge of gastric ulcer and that this is significantly enhanced by the treatment with growth factors (43, 48, 49). Moreover, endogenous prostaglandin derived from COX-1 and COX-2 are involved in the mechanism of mucosal recovery from ischemia/reperfusion-induced acute gastric erosions that subsequently progressed into deeper ulcerations and that healing of these ulcers is associated with an overexpression of COX-2 mRNA (48 - 50). Our notion (43) that the expression of COX-2 plays an important role in the healing of gastric ulcer is also in keeping with the recent observation by Gretzer *et al.* (51) who reported that PG derived from COX-2, but not only from COX-1, may be involved in adaptive cytoprotection induced by topically applied mild irritant, when larger area of mucosa is injured.

NSAID such as aspirin (ASA) are widely used but the major limitation of their clinical application are serious side-effects, including damage of gastrointestinal mucosa, aggravation of stress ulcerations and exacerbation of pre-existing gastric ulcerations (52 - 54). This deleterious action of conventional NSAID was attributed to their topical irritating effect, activation of neutrophils, fall in the microcirculation, enhancement in the motility induced by these agents and the reduction in mucosal generation of PGE₂, all these aspects being currently under investigation at the Department of Physiology Jagiellonian University in Cracow.

An interesting and practically important discovery related to the gastric damage induced by NSAID is an increase in mucosal tolerance or adaptation to the ulcerogenic action of these drugs that develops with their more prolonged administration. This remarkable attenuation of mucosal damage has been first demonstrated in rats and then confirmed in humans. Polish contribution to the field of gastric adaptation to repetitive ASA insult comes from the work of Konturek and Brzozowski (55), who found that aspirin caused a widespread initial injury, which was followed by the adaptation and increased tolerance to withstand further insult without significant injury (*Fig. 8*). They observed that following ASA ingestion, EGF which is normally present in saliva and gastric juice and exerts a potent mitogenic and gastroprotective activities, contributes significantly to the increased cellular proliferation observed during repetitive ASA insult, thus playing a major role in the mechanism underlying gastric mucosal adaptation (56). It was also concluded that this adaptation does not appear to be mediated by endogenous prostaglandins, since prolonged administration of ASA was accompanied by almost complete suppression of COX-1 and COX-2 activity in the gastric mucosa (*Fig. 8*). Furthermore,

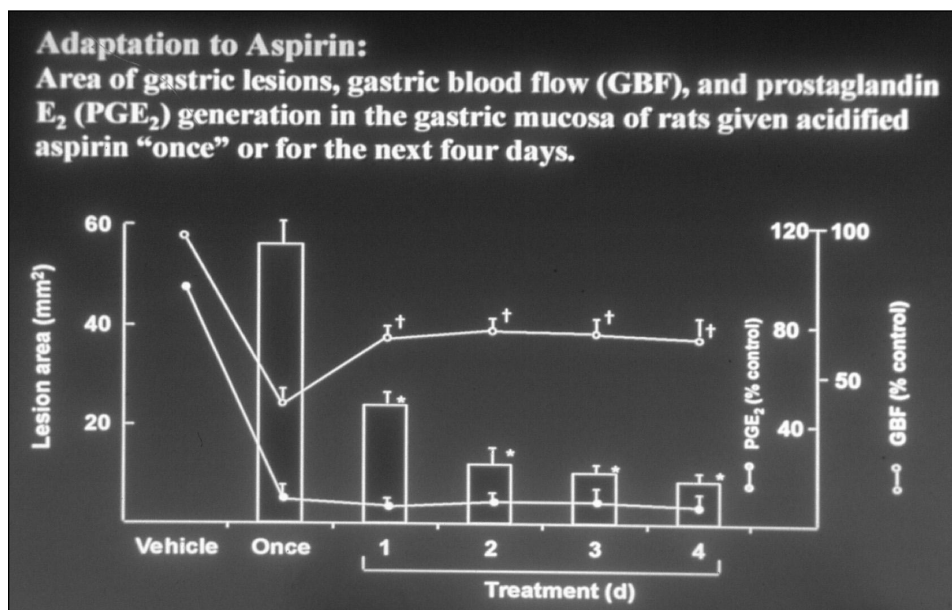


Fig. 8 The demonstration of phenomenon of gastric adaptation to the continuous administration of aspirin (ASA) in rats (adapted from reference (55) by Konturek *et al.*). Following 5 day treatment with ASA, the lesion area is decreased while the gastric blood flow (GBF) is enhanced despite the suppression of PGE₂ generation by this NSAID.

Brzozowski *et al.* (56) have demonstrated that the rat gastric mucosa adapts not only to topical ulcerogens such as acidified ASA, but also to other non-topical obnoxious factors such as stress caused by repetitive exposures to cold and restraint technique according to the method originally described by Takagi and Okabe (57). It is worthwhile to mention that the above mentioned Polish group showed for the first time that the ASA- and stress-adapted gastric mucosa showed enhanced resistance to subsequent challenge with topical irritants such as concentrated ethanol, 25% NaCl and diluted bile solutions (Fig. 9) (56).

Since gastrointestinal ulcerations are associated with the use of NSAID, the new strategy for treatment of inflammatory states include novel series of NSAID that consist of an NSAID linked to a NO-releasing moiety (58). The rationale behind the development of NO-NSAID that spare the gastric mucosa was that NO released from these compounds would counteract two events that occur subsequent to the suppression of PG synthesis by the NSAID, namely reduced gastric blood flow and an increased adherence of neutrophils to the vascular endothelium of the gastric microcirculation (58), thus sparing the gastric mucosa (Fig. 10).

In contrast to native NSAID, their NO-releasing derivative such as NO-aspirin (NO-ASA) constructed by adding an nitroxy-butyl moiety to aspirin was found to

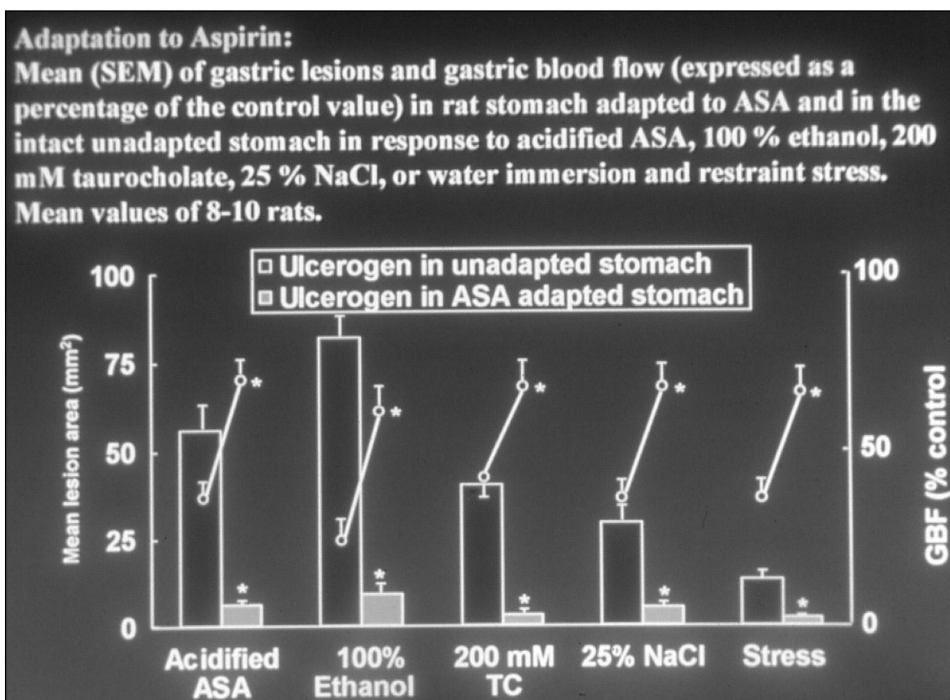


Fig. 9 The exposure of gastric mucosa to ASA resulting in the gastric adaptation to this ulcerogen strengthens the mucosal resistance to the mucosal damage induced by various topical and non-topical ulcerogens including 100% ethanol, 200 mM taurocholate (TC), 25% NaCl and stress (adapted from reference (56) by Brzozowski et al.).

exhibit lower gastric toxicity despite similar inhibition of both COX-1 and COX-2 activity in the gastric mucosa and exerting anti-thrombotic effects comparable to its parent NSAID (59, 60). Moreover, it was shown that NO-releasing NSAID by themselves exhibit only minimal ulcerogenic properties in the gastrointestinal tract, despite exerting a potent anti-inflammatory and analgesic action, similar to native NSAID (58 - 61). The major importance of NO in the prevention of mucosal damage or in preservation of ulcer healing is supported by previous studies showing that both endogenous NO released by capsaicin or NO originating from L-arginine, a substrate for NO-synthase (NOS), or that released from glyceryl trinitrate (62, 63) exert gastroprotective activity and accelerate healing mainly due to the maintenance of blood flow around the ulcer and enhancement of angiogenesis. The mechanism of protection and accompanying hyperemia induced by NO-releasing NSAID remains to be elucidated but it could be attributable to excessive local NO released from NO-derivatives of NSAID. This is supported by our evidence that a considerable amount of NO metabolites were detected in luminal content of the stomach of rats pretreated with NO-releasing NSAID and then exposed to stress, which finally resulted in

Gross appearance of the gastric mucosa exposed to stress or I/R with or without ASA and NO-ASA

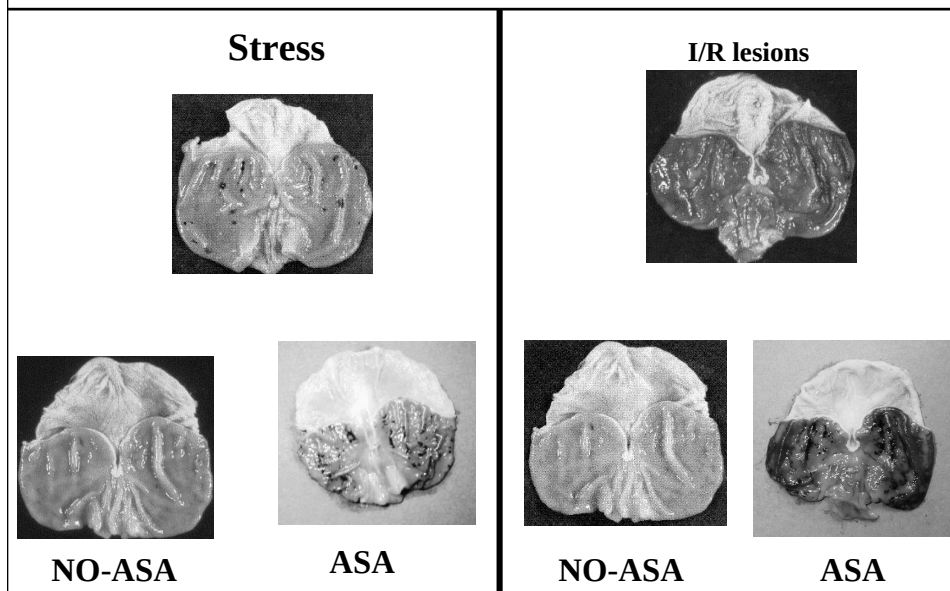


Fig. 10 New therapeutic approach to limit gastric damaging activity of NSAID such aspirin (ASA) by addition of NO-moiety to ASA. Pretreatment with native ASA aggravated the lesions induced by exposure of rats to stress or ischemia-reperfusion (I/R) while NO-ASA afforded gastroprotection against these lesions predominantly due to the excessive NO release from this NO derivative of ASA.

amelioration of stress-induced gastric lesions (*Fig. 11*) (64). In studies with chronic gastric ulcers, Brzozowski *et al.* (43, 64) found that classic NSAID such as indomethacin and ASA delay the healing of pre-existing ulcers induced by acetic acid mainly due to suppression of endogenous PG, the products of COX-1 and COX-2 activity while NO-ASA failed to alter the time course of this healing.

Furthermore, they noticed (43) that impairment in gastric blood flow (GBF) at an ulcer area and excessive cytokine expression and release as well as the fall in the mucosal antioxidizing enzyme activity induced by these NSAID may contribute to the delay in ulcer healing and the aggravation of stress-induced gastric damage (*Fig. 12*). The effects of both specific or nonspecific COX inhibitors on ulcer healing were fully restored by the addition to these inhibitors of minute dose of exogenous PGE₂ that by itself did not affect the alterations of ulcer healing and GBF at the ulcer margin. All these observations led to the conclusion that the deleterious effect of classic NSAID on ulcer healing can be reproduced by selective COX-1 and COX-2 inhibitors suggesting that both COX isoforms are important sources of PG during ulcer healing (43, 64).

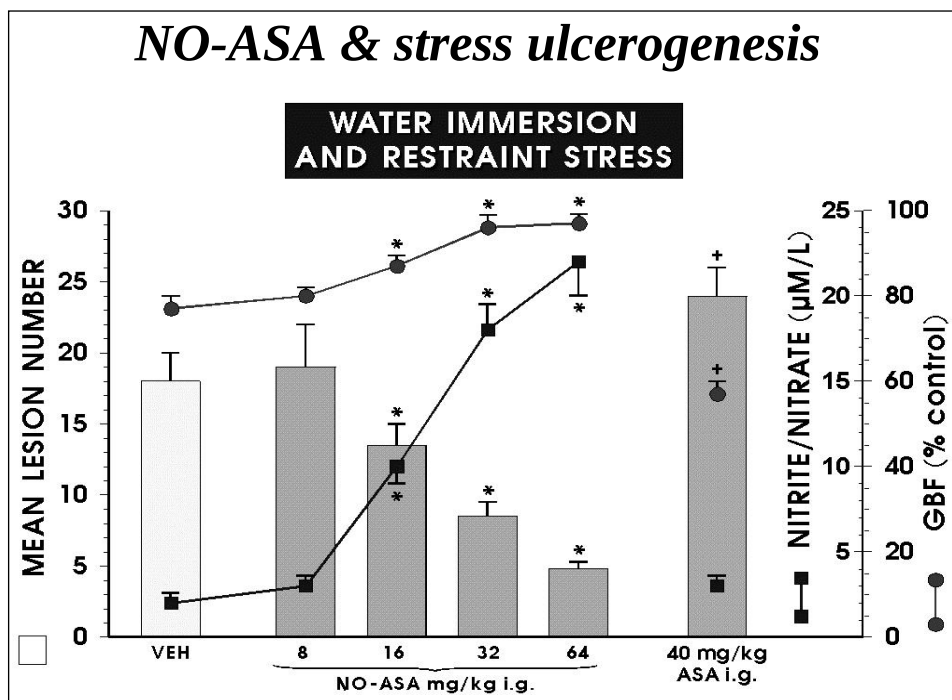


Fig. 11 The quantitative measurement of luminal nitrite/nitrate concentration and gastric blood flow (GBF) in rats pretreated with NO-ASA and native ASA and exposed 30 min later to water immersion and restraint stress. ASA augmented stress-induced gastric lesions but NO-ASA dose-dependently reduced these lesions and this effect is accompanied by the rise in GBF and luminal NO_x content.

Recently, Fiorucci and co-workers (65) have shown that administration of aspirin resulted in enhancement of apoptosis rate *via* upregulation of caspase system mediated by TNF α and NO-derivatives of NSAID counteracted these effects. It was suggested that NO-NSAID such as NO-ASA could spare the gastric mucosa and inhibit caspase activity, at least in part, through cGMP-dependent pathway (65) including the release of NO from these NSAID. We documented that both native aspirin and naproxen impaired significantly the healing of chronic gastric ulcers and attenuated both the gastric blood flow at ulcer margin and the PGE₂ generation in the intact as well as ulcerated gastric mucosa (64). This inhibition of COX-1 and COX-2 activity could contribute to the prolongation of ulcer healing by NSAID, since previous studies have shown that indomethacin, a non-selective COX inhibitor and NS-398 or rofecoxib, both highly specific inhibitors of COX-2, delayed healing of pre-existing chronic gastric ulcers and ischemia-reperfusion-induced acute erosions progressing into chronic ulcers (53, 54). Importance of COX-2 for ulcer healing was recently emphasized by the observation that expression of COX-2 mRNA is increased after induction of chronic gastric ulcers suggesting that COX-2 expression and

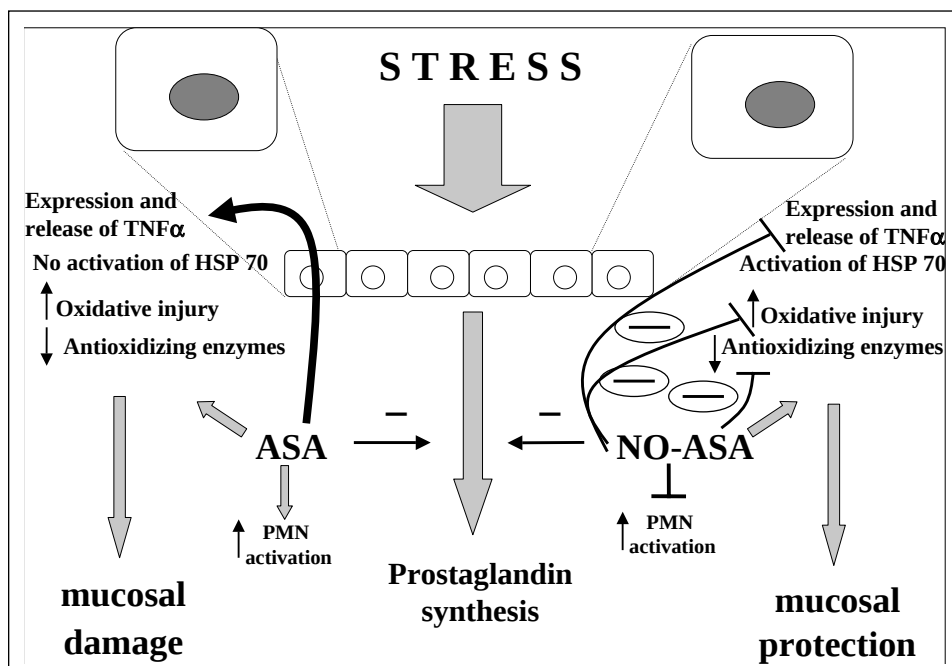


Fig. 12 The possible mechanisms by which NO-releasing ASA can afford protection of the gastric mucosa against stress-induced gastric lesions involve the counteracting effect of this NO-releasing compound on activation of polymorphonuclear leukocytes (PMN) and the fall in the activity of antioxidizing enzymes and the increase in the expression and release of proinflammatory cytokines induced by native ASA and the overexpression of heat shock proteins (HSP).

PG-derived from COX-2 may be crucial in the mechanism of ulcer healing (43, 61, 64).

Ischemia preconditioning refers to a phenomenon in which a tissue is rendered resistant to the deleterious effect of prolonged severe ischemia by previous exposures to brief moderate vascular occlusions (66). These protective effects of ischemia preconditioning were first described in the heart by Murry and coworkers in 1986 (67) but remained unknown whether similar adaptation to injury induced by ischemia-reperfusion exist in the stomach. Polish group working at the Department of Physiology Jagiellonian University in Cracow studied this phenomenon in the gastric mucosa subjected to brief 2-5 episodes of short ischemic preconditioning followed by prolonged ischemia-reperfusion that within 3 h causes gross and microscopic erosions in the stomach (*Fig. 13*) (68). It was found for the first time that few short ischemic episodes protects the gastric mucosa from the damage induced by prolonged ischemia-reperfusion via mechanism involving endogenous prostaglandins (PG) derived from COX-1 and COX-2, nitric oxide (NO) due to overexpression of iNOS and adenosine acting on A1 receptors (*Fig. 14*) (68). Moreover, using molecular techniques of RT-PCR

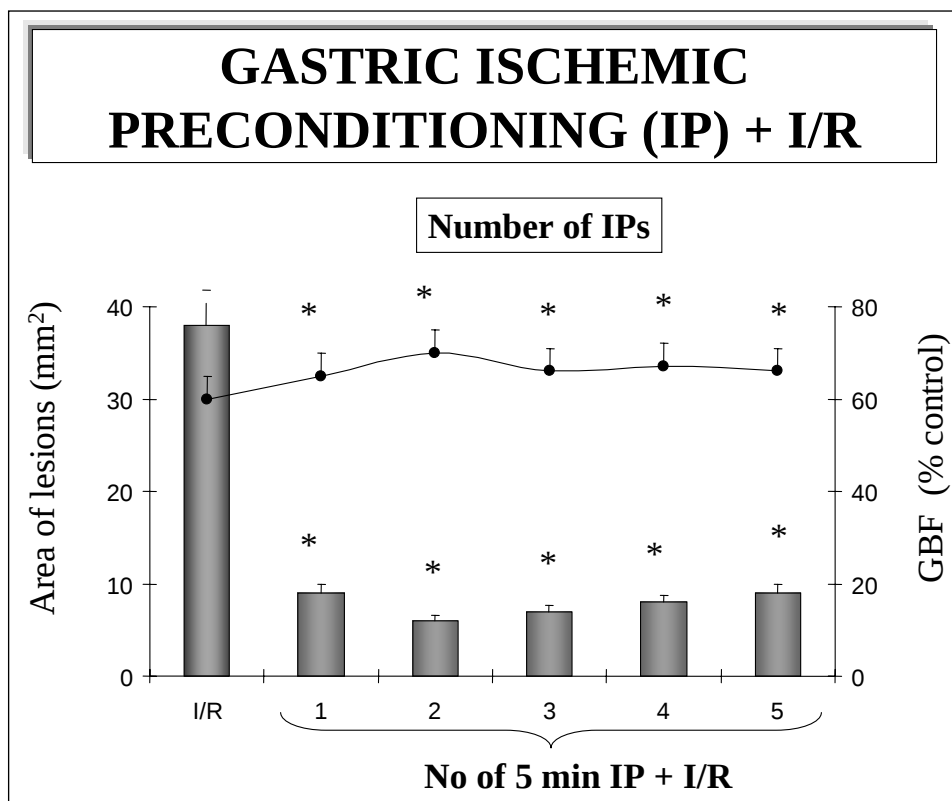


Fig. 13 The first demonstration that gastric ischemic preconditioning (IP) as reflected by 1-5 short (5 min) ischemic episodes of the stomach significantly attenuates gastric lesions caused by prolonged ischemia-reperfusion and that this effect is accompanied by the significant increase in the gastric blood flow (GBF) (adapted from reference (68) by Pajdo *et al.*).

and Western Blot, they showed directly COX-2 overexpression in the preconditioned gastric mucosa, at the levels of both, mRNA and protein, while signals for mRNA and protein of COX-1 were unchanged (68).

Experimental studies in the era of pathology of Helicobacter pylori infection of the gastric mucosa - Polish experience

H. pylori appears to be the most frequent cause of gastritis in man and *H. pylori*-induced gastritis appears to be the major risk factor of gastric and duodenal ulcers, gastric lymphomas, and gastric cancer (69). Polish internist W. Jaworski delivered the first description of spiral bacteria in the human gastric perfusate sediment from patients with gastric ulcer almost 100 years ago (70). The role of toxigenic *H. pylori* strains in the pathogenesis of peptic ulcer disease has not been fully explained but, as shown in previous studies, over 60% of *H. pylori* strains

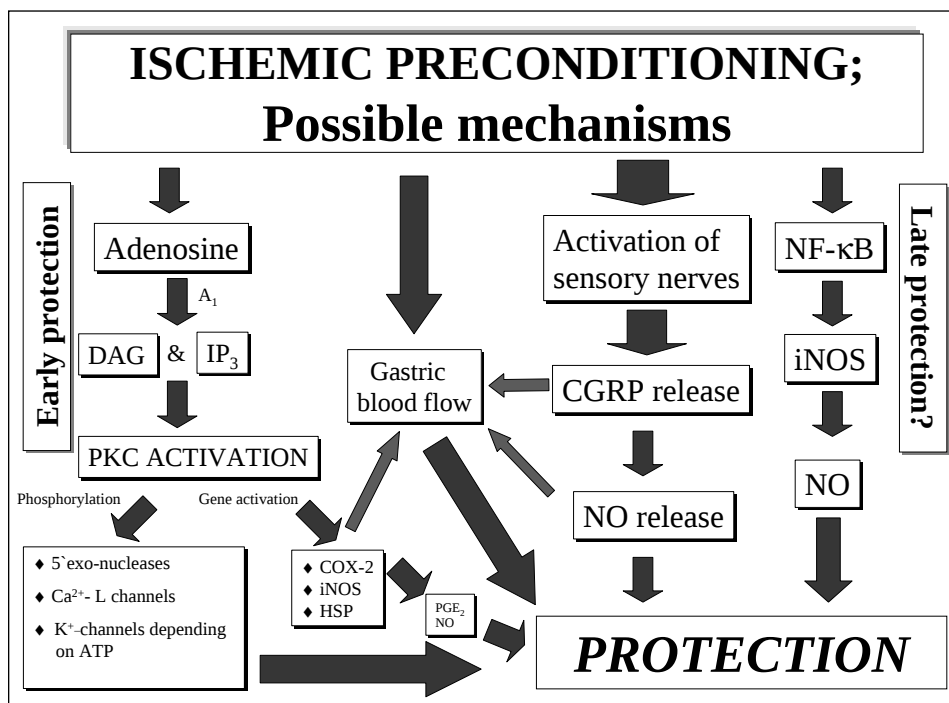


Fig. 14 The complexity of mechanisms of ischemic preconditioning. The studies performed in the Department of Physiology in Cracow revealed that gastric ischemic preconditioning involves numerous mediators including endogenous NO, PG and neuropeptides such as calcitonine gene related peptide (CGRP) released from the sensory afferent nerves.

produce toxins causing vacuolization of the cell and tissue damage due to direct Trojan horse type "injection" by bacteria of its cytotoxins into the mucosal cells (71, 72). This is supported by the fact that the most striking difference between patients infected with toxigenic and non-toxigenic *H. pylori* strains is that those who are infected with *H. pylori* expressing 120-130 kDa *cagA* product showed greater degeneration of the surface epithelium and a denser neutrophil infiltration and finally were more likely to develop peptic ulcers (69, 72).

The studies on various aspects of *H. pylori* infection and gastric pathology are, however, limited due to the absence of adequate animal model resembling *H. pylori* pathology in humans. In previous studies, the infection with *Helicobacters* (*H.*) such as *H. mustelae*, *H. felis* or *H. Heilmanii* in ferrets, cats, pigs, monkeys and Mongolian gerbils were reported to have some relevance to *H. pylori* infection in humans but these animal models do not mimic the *H. pylori* infection because of the lack of virulence factors of infecting germs such as *vacA* or *cagA* encoded cytotoxins required for the mucosal damage, mucosal inflammation and the ulcer formation (73). Furthermore, some of these animals are cost expensive and difficult to handle so there is a need for testing various aspects of this bug

infection and ulcer healing in more convenient animal models such as those with rats but the intact stomach in these animals difficult to infect. It is of interest, that water extracts of *H. pylori*, as shown by polish researchers, caused a profound delay in the healing of experimental pre-existing gastric ulcerations in rats (74).

Watanabe *et al.* (75) have shown that the gerbil stomach inoculated with *H. pylori* induced, in at 26th week of infection, active chronic gastritis, mucosal damage, and intestinal metaplasia. In their study, after 62 weeks upon inoculation, the adenocarcinoma had developed in the pyloric region of about 37% of the infected animals. Extensive study by Konturek *et al.* (76) showed a marked infiltration of inflammatory cells and formation of lymphoid follicles in the submucosa, especially in the transitional zone between the antrum and corpus of the Mongolian gerbil stomach infected with *H. pylori* that produced vacuolating cytotoxin and contained the cytotoxin-associated gene (*cagA*). According to our experience (76), the inoculation of gerbil stomach with human-originated *H. pylori* resulted in gastric infection and acquisition of these bacteria first in the antral mucosa and later in the gastric corpus, as assessed by *H. pylori* culture and rapid urease-test. Histopathology revealed that early mucosal lesions were seen already 2-4 weeks upon *H. pylori*-inoculation and consisted of chronic gastritis with increased mucosal foldings and elongated interfoveolar ridges and formation of multiple lymphoid follicles predominantly located in the gastric mucosa (Fig. 15). Typical adenomatous hyperplasia with cellular atypia was observed together with increased mitotic activity and apoptotic bodies' formation, while lamina propria was greatly reduced - leaving dilated gastric glands situated "back-to-back". This study (76) revealed for the first time at the experimental conditions, the functional aspects of *H. pylori*-infection in Mongolian gerbils that at early stages such as chronic gastritis, suppression of gastric secretion and gastric intraepithelial neoplasia together with impairment of both, gastric mucosal microcirculation and the gastrin-somatostatin link mimicking those observed in human gastric mucosa infected with *H. pylori*.

In conclusion, our study supports the notion that the Mongolian gerbil is an appropriate animal in which various gastrointestinal diseases such as gastritis and mucosal damage and functional and pathological changes that mimic human disease including gastric intraepithelial neoplasia could be studied. This model of functional alterations in gerbils infected by *H. pylori* that had been proposed by the group of polish researchers (76), could be helpful in clarifying the pathogenesis of this infection, especially in the early stage of bacteria colonization, and, at the same time, serving as a suitable animal model to study disorders linked to hyposecretion and the fall in the gastric microcirculation caused by *H. pylori* infection.

Concluding remarks

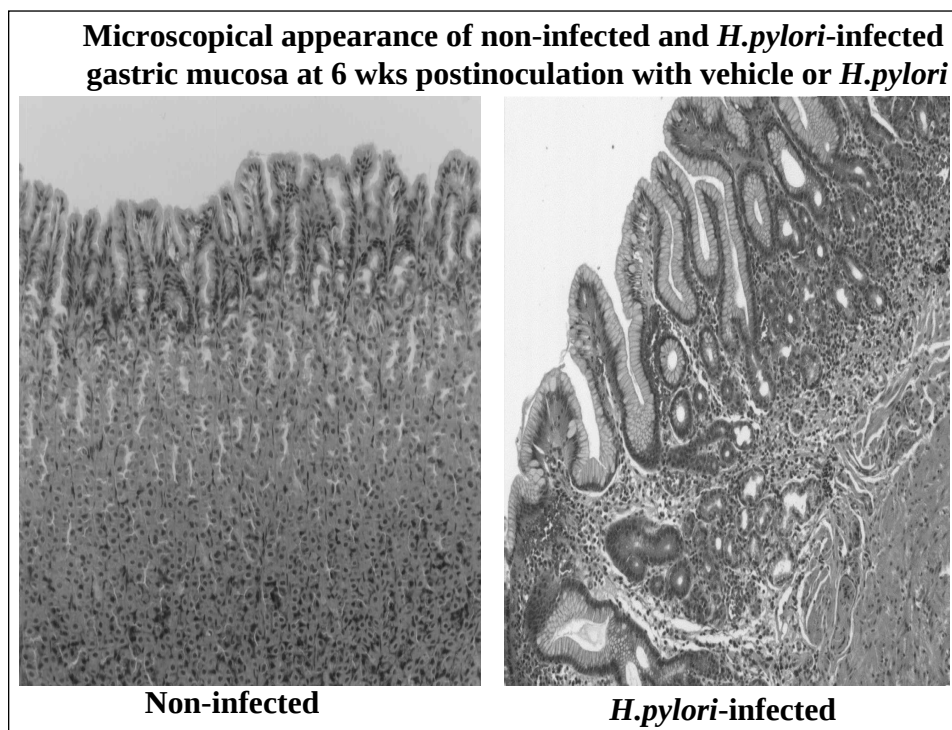


Fig. 15 H. pylori infection of the gastric mucosa in Mongolian gerbils. Histological appearance of normal oxyntic mucosa of the non-infected gerbil (left panel). Chronic gastritis with extensive foveolar hyperplasia of gastric oxyntic mucosa and chronic inflammatory infiltrate exhibiting polypous, thick mucosal folds and dilatation of hyperplastic gastric glands observed at 6 weeks postinoculation of gerbil stomach with *H. pylori* (right panel). H&E, magn. 100x.

Whilst acid and pepsin have long been recognized as pathogenic as regards the mucosa it become evident only recently that an infective component should be considered as critically relevant to the etiology of gastric and duodenal disease.

Along the history of peptic ulcer disease, as shown in this review, many efforts were done to create the adequate animal models which could serve as suitable to mimic ulcer disease in the human stomach. An attempt was made in this chapter to discuss the history of peptic ulcer disease in humans and methods for the production of acute gastric lesions and ulcers in experimental animals with the special attention focused to the contribution of Polish scientists and investigators into this field.

In summary, the Polish traces and their contribution to the understanding of the mechanism of peptic ulcer disease, which reflected the GI literature, include:

1) the identification of the mechanism of action of so-called "barrier breakers" such as acid, necrotizing substances such as ethanol and hyperosmolar solutions, bile salts, aspirin and other NSAID as well as non-topical ulcerogens such as

stress on the gastric mucosal integrity and functional changes in the gastric mucosa such as gastric blood flow, gastric secretion, prostaglandin generation and bicarbonate secretion;

2) the elucidation of the involvement of protective factors such as prostaglandins (PG), nitric oxide (NO) and neuropeptides released from sensory afferent nerves, to the mechanism of gastric mucosal defense including cytoprotection originally proposed by Andre Robert, as well as other defensive mechanisms such as cell restitution, mucosal repair after damage and the process of ulcer healing;

3) the gastroprotective and ulcer promoting actions of growth factors such as EGF, TGF α and hormones such as cholecystokinin, gastrin and leptin on the integrity of the gastrointestinal mucosa and accompanying changes in the gastric microcirculation and expression of key enzymes such as NO synthases and PG cyclooxygenases;

4) the studies on adverse effect of NSAID in the stomach and the evaluation of physiological mechanism of the phenomenon of gastric adaptation to NSAID and resistance of NSAID-adapted mucosa to the mucosal damage induced by corrosive agents;

5) the search for alternative therapy to classic NSAID using safer derivative of NSAID releasing NO, that exhibit less side effects in the GI tract than their parent drugs, thus sparing the gastric mucosa;

6) the pioneer work in the era of *H. pylori* to elucidate the various functional aspects of gastric infection with *H. pylori* in animal models including mice and Mongolian gerbils with the special focus to the functional changes accompanying this infection such as effect of this germ on gastric acid secretion, gastrin-somatostatin link and proinflammatory cytokines expression and release.

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