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Review articles

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PROSTAGLANDINS AND ULCER HEALING

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Exogenous prostaglandins (PG) applied in small gastroprotective doses fail to affect healing of gastro-duodenal ulcers but accelerate the healing when used in larger gastric inhibitory doses that appear to enhance COX-2 expression and PGE_2 generation in the ulcer area. COX-1 and COX-inhibitors delay ulcer healing, particularly when both COX isoforms are suppressed such e.g. by indomethacin. Dexamethasone, that decreases the expression of COX-2 and mucosal generation of PGE_2, delays ulcer healing that can be reversed by the addition of small dose of exogenous PGE_2. Proton pump inhibitors (PPI) such as omeprazole and PGE analogs, accelerate ulcer healing mainly due to potent inhibition of gastric acid secretion, but they also augment the COX-2 expression and enzyme activity in the ulcerated mucosa. Endogenous PG generated at ulcer margin appear to be involved in ulcer healing promoted by growth factors and gut hormones such as gastrin or CCK and melatonin acting, at least in part, through increase of induction of COX-2 and local release of PGE_2 in the ulcer area . The ulcer healing activity of growth factors (e.g. EGF, TGFα, HGF) and certain gut hormones (gastrin, CCK) as well as melatonin, can be attenuated by treatment with COX-1 or COX-2 inhibitors which suppress the release of PGE_2 but enhance the expression of COX-2. It is concluded that endogenous PG originating mainly from upregulated COX-2 at the ulcer margin play crucial role in ulcer healing by exogenous PG, PPI, growth factors, gut hormones and melatonin, while COX-1 and COX-2 inhibitors delay ulcer healing by suppressing PG generation, and increasing COX-2 expression in the ulcer area.

Key words*; peptic ulcer, prostaglandins, cyclooxygenase, omeprazole, growth factors*

INTRODUCTION

Peptic ulcer is generally considered as a result of the imbalance between the gastroduodenal mucosal defense mechanisms and damaging factors breaching in

This article is dedicated to Professor Kornel Gibinski, MD, mult h.c. MD for his pioneering archievements in clinical gastroenterology at his 90th Birthday Anniversary.

the mucosa and extending through the *muscularis mucosae* into the submucosa and deeper. The recognition by Schwartz (1) in the first decade of the twentieth century that the formation of gastroduodenal ulcers is caused by the aggressive action of acid (his famous *dictum* was, "no acid-no ulcer") and that the decrease of gastric acid favors the repair of gastric lesions, changed the surgical way of thinking and opened new avenues of anti-ulcer strategies. The discovery of cytoprotective effects of prostaglandins (PG) on gastric mucosa in experimental animals (2) raised a hope that these PG may be an ideal remedy for ulcer prevention and healing, but subsequent clinical trials failed to support their clinical usefulness in ulcer therapy, mainly due to side effects. With the identification of the histamine-2 receptor subtype and the development by Black and his associates (3) of agents specifically capable of blocking acid secretion by antagonism of this receptor revolutionized the management of peptic ulcer disease and virtually obliterated surgery as a therapeutic option for peptic ulcer disease, except that in the case of an emergency and complications. With discovery of drugs that inhibit H^+,K^+ATPase, the proton pump of the parietal cell (4), the most effective inhibitors of gastric acid secretion so called proton pump inhibitors (PPI) are available and widely used in ulcer therapy (5).

Although gastric acid and pepsin are requisites for ulcer formation (1) and appropriate acid suppression is required for optimal ulcer healing (6, 7), the most notable aggressive factor in pathogenesis of peptic ulcerations appears to be *Helicobacter pylori* (*H. pylori*) (8-10). Most of peptic ulcers have been associated either with gastric *H. pylori* infection or with ingestion of nonsteroidal anti-inflammatory drugs (NSAID). Numerous studies showed that peptic ulcer recurred infrequently when either *H. pylori* infection or NSAID use is eliminated (10). With the recognition of important role of *H. pylori* in pathogenesis of peptic ulcer, its healing and recurrence, the antisecretory therapy has been combined with antimicrobial treatment in order to accelerate ulcer healing, to reduce ulcer complications and to prevent ulcer recurrence (10).

Following the discovery of cytoprotective activity of PG, stable PGE analogues were obtained, suggesting that they could be useful in the treatment of peptic ulcer, particularly that they were found to be effective gastric acid inhibitors in humans (11). Indeed, several clinical trials (12-14) documented that these analogues were effective in accelerating healing rate of gastroduodenal ulcers not only accompanying NSAID therapy, when the deficiency of endogenous PG exists (15), but also in NSAID-independent peptic ulcerations (12-14). It was found that PGE_1 stable analog misoprostol, significantly lowered the frequency of gastroduodenal ulcers occurring in patients with long term therapy of NSAID (15). This analogue was, however, effective in enhancing peptic ulcer healing mainly by gastric acid inhibition than by cytoprotective activity, indicating that cytoprotection, exerting so dramatic preventive action against acute gastric lesions in experimental animals (2), plays no part in healing of chronic peptic ulcer that involves mucosal repair (16). Although in patients at

high risk for recurrent gastric ulcer, the use of cotherapy with misoprostol was found to be almost equally affective as PPI such as lanzoprazole or omeprazole (17, 18), exogenous PGE or its stable analogues are not widely used in peptic ulcer therapy because of their diarrhogenic and abortifaciant effects. This, "unfulfilled promise" (16), regarding the clinical usefulness of prostaglandin in peptic ulcer therapy, does not exclude the possibility that endogenous PG generated by cyclooxygenase (COX)-1 or COX-2 in the ulcer area are implicated in the pathogenesis and healing of peptic ulcerations.

The purpose of this article is to overview the mechanisms of ulcer healing in experimental model of acetic acid-induced chronic gastric ulceration in rats, especially the role of endogenous PG generated by COX-1 and COX-2.

Role of exogenous and endogenous PG in healing of peptic ulcers

Healing of peptic ulcer is an active and complex process including the reconstruction of the mucosa by formation of granulation tissue at the ulcer base, formation of new vessels (angiogenesis) and re-establishment of glandular architecture (19). PG generated especially at an ulcer margin by COX-2, appear to play a crucial role in ulcer healing through triggering the cell proliferation, promotion of angiogenesis and restoration of mucosal integrity. Unlike COX-1, which is constitutively expressed in intact gastric mucosa to produce PG that regulate mucosal blood flow and epithelial secretion of mucus and bicarbonate, PGs from COX-2 influence epithelial proliferation and endothelial-leukocyte adherence. COX-2 has been shown to be induced in ulcerated and inflamed gastric mucosa (20-24).

In this study, we used an experimental ulcer model obtained by serosal application of 100% acetic acid on the area of 28 mm^2 for 25 s according to our modified method (25) (*Fig. 1*). Histologically, such acute ulcer develops immediately after serosal application of acetic acid on mid portion of the stomach and involves the entire mucosa and submucosa to become chronic within 2-3 days. It heals spontaneously depending on initial size within 2-4 weeks without perforation or penetration to surrounding organs. After recovery from surgery, the animals start normal chow diet next day after ulcer induction and can be treated either with vehicle (saline) or various substances such as PG, PPI, COX-inhibitor, growth factors or gut hormones. The animals were then lightly anesthetized with ether after 3, 7, 10 or 14 days upon ulcer induction, the abdomen was opened and the gastric mucosal blood flow at the ulcer margin was determined using H_2-gas clearance technique. The stomach was then opened and the area of gastric ulcers was determined using planimetry. In addition, the large (50 mg) of biopsy samples were taken from the ulcer margin and intact mucosa and immediately frozen in liquid nitrogen for further studies of gene or protein expression of COX-1, COX-2. The blood samples were also taken for the assessment of plasma levels of gastrin, melatonin and cytokines as described before (20, 26). Each

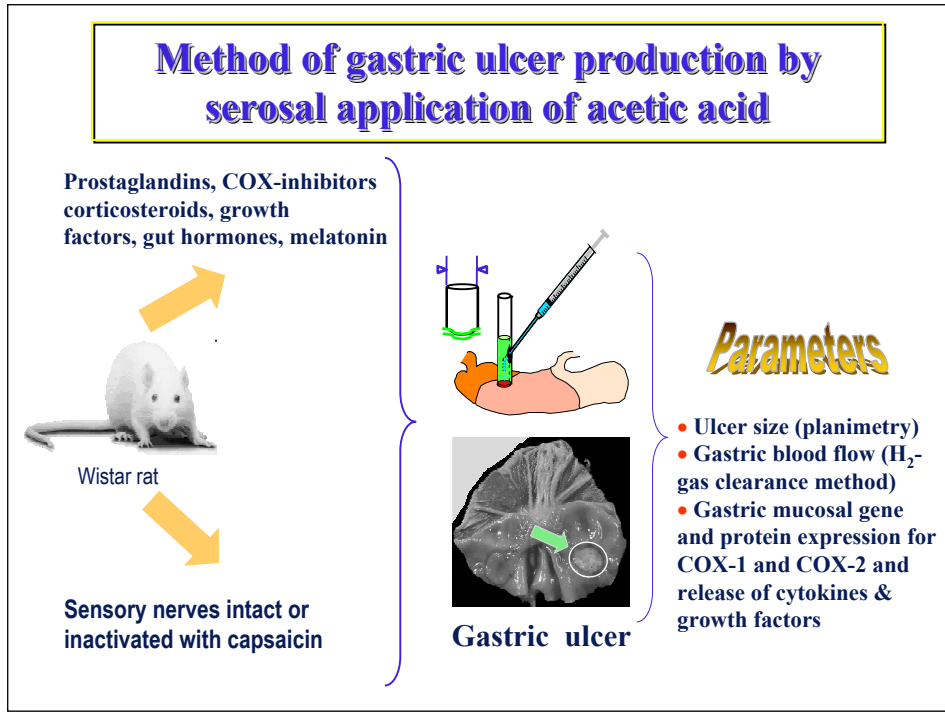

Fig. 1. Production of gastric ulcer by serosal application of acetic acid in rats treated daily with prostaglandins (PG), proton pump inhibitors, COX-1 or COX-2 inhibitors, growth factors, gut hormones or melatonin without and with pretreatment with neurotoxic dose of capsaicin. At the end of experiments the animals were anesthetized and the area of gastric ulcers were measured by planimetry, mucosal blood flow was measured by H_2 gas clearance and biopsy samples were taken for the determination of mucosal generation of PGE_2 and expression of COX-1 or COX-2.

experimental group included 6-10 animals that were fasted about 24 h before the anesthesia. The studies were approved by Institutional Ethic Committee of the Jagiellonian College of Medicine, Cracow, Poland.

As shown on *Fig 2*, in rats with chronic acetic acid-induced gastric ulcer the mRNA expression for COX-1 was similar in the intact mucosa and at ulcer margin with gastritis as well as in the ulcer base. It did not change significantly also following healing of gastric ulcer. In contrast, the expression for COX-2 markedly increased both in the margin of ulcer as well as in ulcer itself and disappeared following the ulcer healing. PGE_2 generation rose significantly at the ulcer margin and the ulcer base as compared to the intact mucosa to decline after ulcer healing. These results indicate that the induction of gastric ulcer and accompanying gastritis induce dramatic rise in expression of COX-2 as reported previously (20).

It is of interest that PG generated from COX-1 tonically suppress COX-2 activity in the GI tract. COX-2 is rapidly up-regulated after COX-1 inhibition,

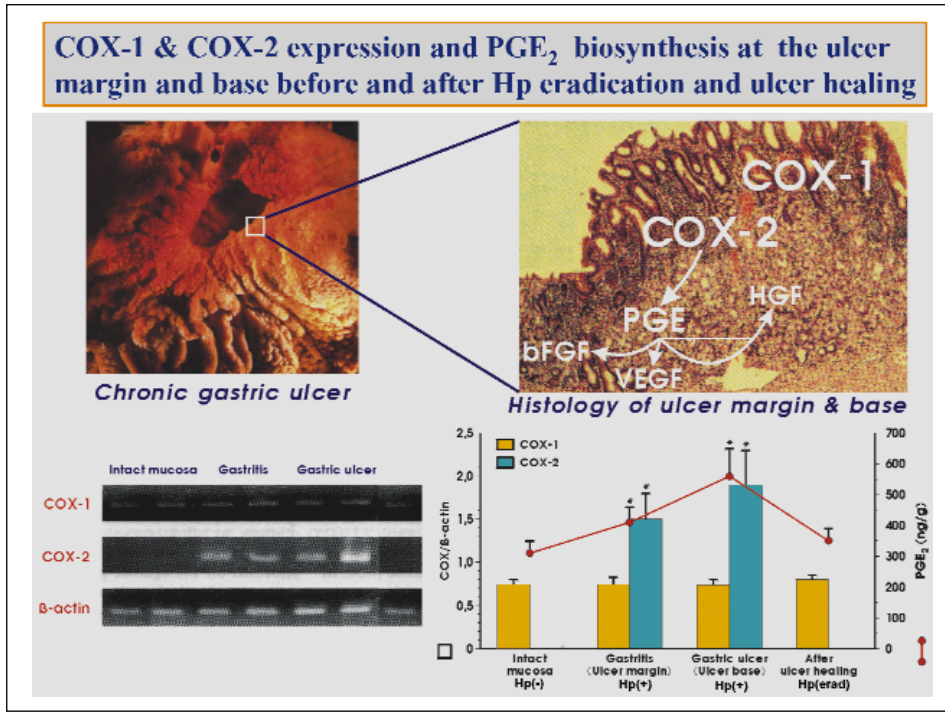

Fig. 2. Macrosopic and microscopic pictures of chronic gastric ulcer in rats induced by serosal application of acetic acid (upper panel). COX-1 and COX-2 expression and mucosal generation of PGE_2 in *H. pylori* negative intact gastric mucosa, at the ulcer margin *H. pylori* postive with gastritis, at the *H. pylori* positive ulcer base and in the area of healed *H. pylori* eradicated ulcer. COX-2 generated PGE_2 stimulates the expression of growth factors in the mucosa.

when the mucosa is exposed to potentially damaging agents or when the mucosal injury or ulceration occurs. Recent studies showed that PGE_2 release by fibroblasts at the ulcer margin expressing COX-2 is accompanied by the release of growth factors in the ulcer area that may contribute to mucosal repair and angiogenesis (26) (*Fig. 3*).

The question remains what is the effect of exogenous PG on ulcer healing and COX-1 and COX-2 expression in the ulcer area. We demonstrated before (25) that the small non-antisecretory dose of 16,16 dimethyl PGE_2 ($dmPGE_2$) failed to affect the healing and these results have not been included. In this study we used larger dose of $dmPGE_2$, which in experiments with chronic gastric fistula rats caused significant inhibition of gastric acid secretion. Such larger dose of this PGE_2 analogue (50 µg/kg/d) was found in the present study to be as effective in the acceleration of ulcer healing as omeprazole used in equipotent gastric inhibitory dose (40 mg/kg/d). In vehicle-treated rats, the ulcer area gradually decreased, the reduction in ulcer area being significant at day 7 and 10 to

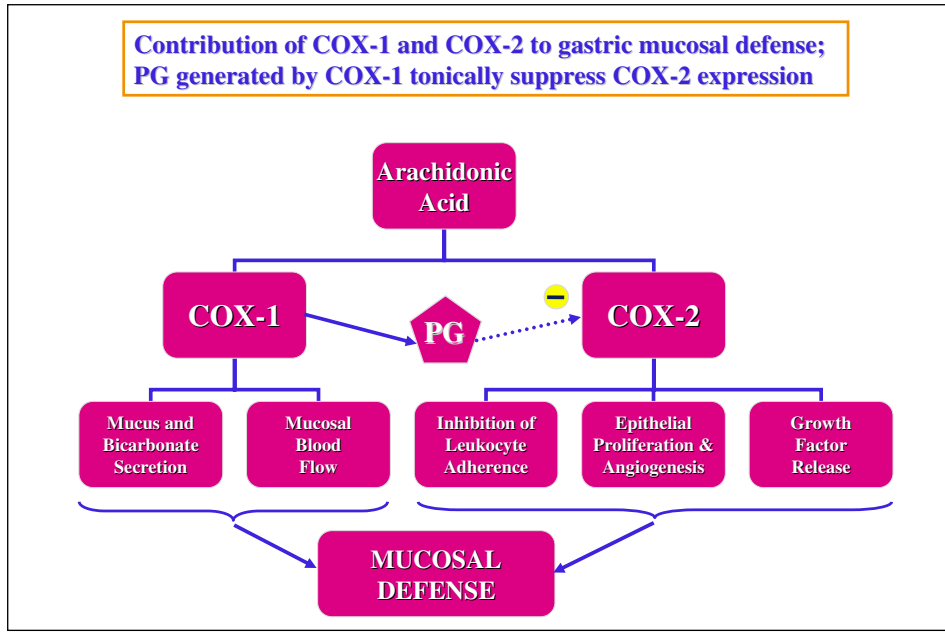

Fig. 3. Contribution of COX-1 and COX-2 to various aspects of gastric mucosal defense. PGE_2 generated by COX-1 tonically suppresses COX-2 activity.

disappear almost completely at day 14 (*Fig. 4*). Thus, we can conclude that exogenous PGE analogue, applied in larger dose, was equally effective in acceleration of ulcer healing as omeprazole administered in equipotent gastric inhibitory dose.

The question remained whether the ulcer healing effect of exogenous PGE analogue and omeprazole, representing PPI, is only due to the inhibition of gastric acid secretion or whether these agents also affect COX-PG system in the ulcerated mucosa. *Fig. 5* shows that administration of exogenoms PGE_2 analogue or omeprarole in gastric inhibitory dose caused significant increase in mucosal generation of PGE_2, especially in the first days of drug application. As shown on *Fig.6*, COX-1 showed similar expression in the intact mucosa and at ulcer margin in rats without or with administration of $dmPGE_2$ or omeprazole. In contrast, COX-2, which showed only negligible expression in the intact mucosa was pronounced at the ulcer area even of vehicle-treated controls, but treatment with $dmPGE_2$ or omeprazole resulted in further significant elevation of COX-2 expression in the ulcerated mucosa (*Fig. 7*). Thus, the excessive generation of PGE_2 in $dmPGE_2$- or omeprazole-treated rats originated from the upregulation of COX-2 at the ulcer margin by the tested agents.

The mechanism of COX-2 upregulation at an ulcer margin, particularly following treatment with $dmPGE_2$ or omeprazole is unknown, but we suspect

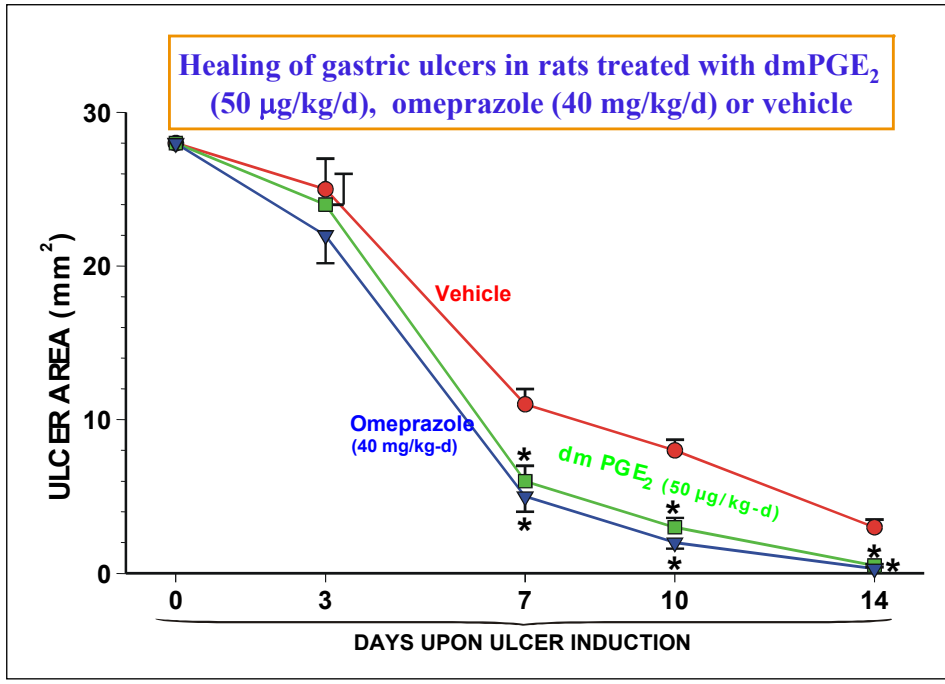

Fig. 4. Healing of gastric ulcers in rats treated with intragastric vehicle (saline), dmPGE$_2$ (50 μg/kg/d) or omeprazol 40 mg/kg/d. Mean ±SEM of 6 experiments on 6 rats. Asterisk indicates significant decrease below the value recorded in vehicle-treated animals (unpublished results).

that this could be attributed, at least in part, to the hypergastrinemia that was found to be accompanying the production of gastric ulceration itself and the administration of gastric inhibitory dose of dmPGE$_2$ or omeprazole (*Fig. 8*). The upregulation of COX-2 by gastrin, released in higher amounts following administration of gastric inhibitors such as lanzoprazole, has been suggested before by Tsuji *et al.* (28), who reported that the protective effects of this PPI, against ethanol-induced gastric damage could be attributed to the upregulation of COX-2 due to the action of gastrin released in excessive amounts because the blockade of specific gastrin receptors abolished lanzoprazole-induced enhancement of PGE$_2$-generation and the upregulation of COX-2. The acceleration of ulcer healing combined with upregulation of COX-2 and elevated generation of PGE$_2$ in our tests with dmPGE$_2$ could also be attributed to hypergastrinemia resulting from gastric inhibition by this PGE$_2$-analogue as reported previously(11-13). An alternative explanation could be that exogenous stable PGE analogue by itself could directly increase the COX-2 expression and activity in similar fashion to that exerted by this PGE analogue in prostate cancer cells (29) but this possibility requires confirmation in the model of gastric ulceration (*Fig. 7*).

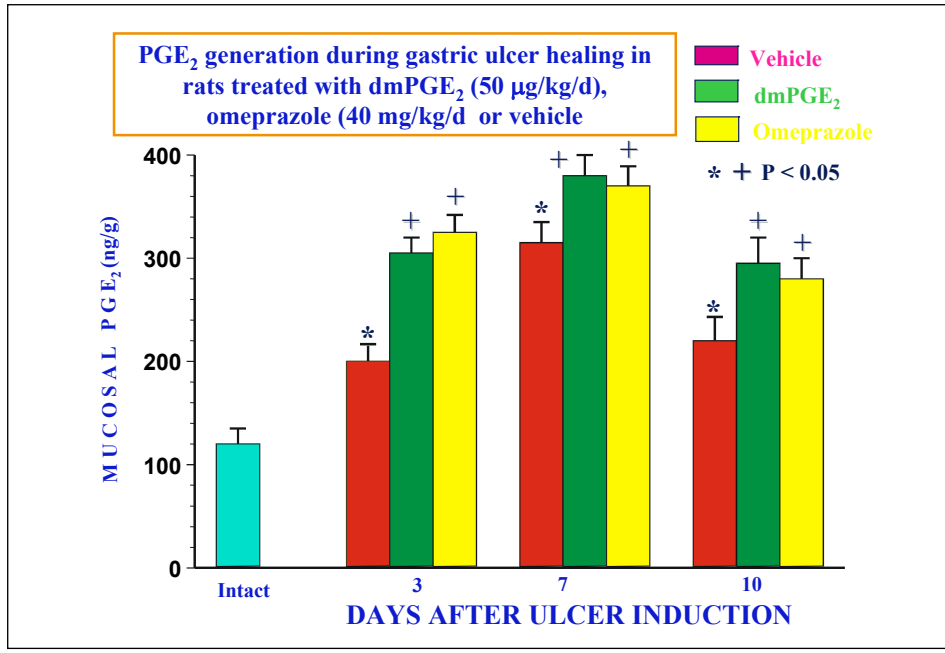

Fig. 5. Mucosal PGE_2 generation during ulcer healing in rats treated with $dmPGE_2$ (50 µg/kg/d), omeprazole (40 mg/kg/d) or vehicle (unpublished results).

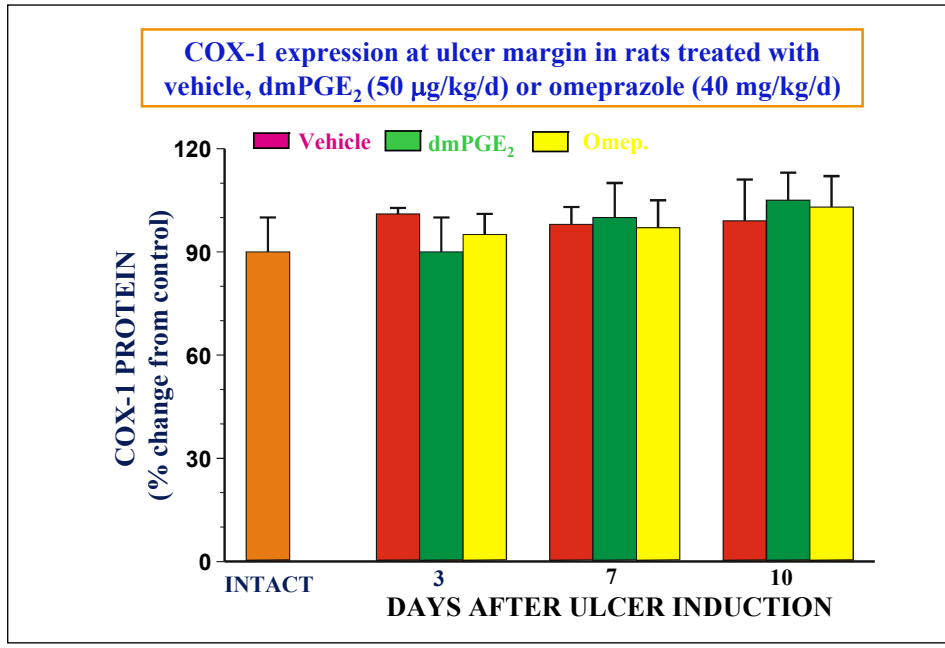

Fig. 6. COX-1 expression in intact gastric mucosa and at ulcer margin of rats treated with vehicle, $dmPGE_2$ or omeprazole at day 3, 7 and 10 upon ulcer induction (unpublished results).

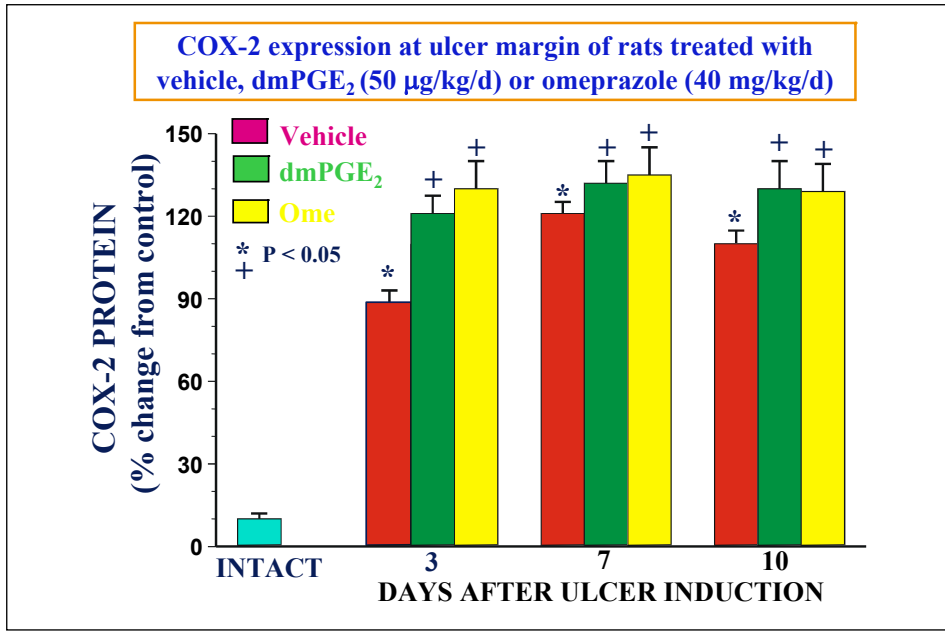

Fig. 7. COX-2 expression in intact gastric mucosa and at ulcer margin of rats treated with vehicle, $dmPGE_2$ or omeprazole at day 3, 7 and 10 upon ulcer induction. Asterisk indicates significant increase above the value recorded in gastric mucosa of intact rats. Cross indicates significant increase above the value recorded in vehicle-treated rats (unpublished results).

In summary, the ulcer healing efficacy of omeprazole (and probably other PPI) and exogenous potent PGE analogues could be attributed not only to their gastric acid inhibitory action but also to the upregulation of COX-2 in the ulcer area (*Fig. 8*).

If endogenous PGE_2 generated by the upregulated COX-2 at the ulcer margin, contributes to ulcer healing it is expected that the inhibition of COX-1 and/or COX-2 should delay ulcer healing as shown by other studies (30) including our own (20). This delay in ulcer healing by NSAID has been associated with the inhibition of endothelial cell proliferation and the reduction in angiogenesis at the ulcer site. In the present report we confirmed that specific inhibitor of either COX-1 (SC-56) or COX-2 (NS-398) as well as nonspecific inhibitor of both COX-1 and COX-2 (indomethacin) delayed ulcer healing (*Fig. 9*). This delay in ulcer healing by COX-inhibitors was accompanied by expected strong reduction in PGE_2 generation, especially in ulcerated gastric mucosa normally exhibiting more pronounced release of PGE. The inhibition of PGE_2 generation was more impressive after the application of indomethacin than of specific COX-2 inhibitor (NS-398) because the former agent is known to inhibit non-specifically both COX-1 and COX-2 activity, and, therefore, is more effective inhibitor of PGE_2 generation than NS-398 (*Fig. 10*). This remains in agreement with studies of

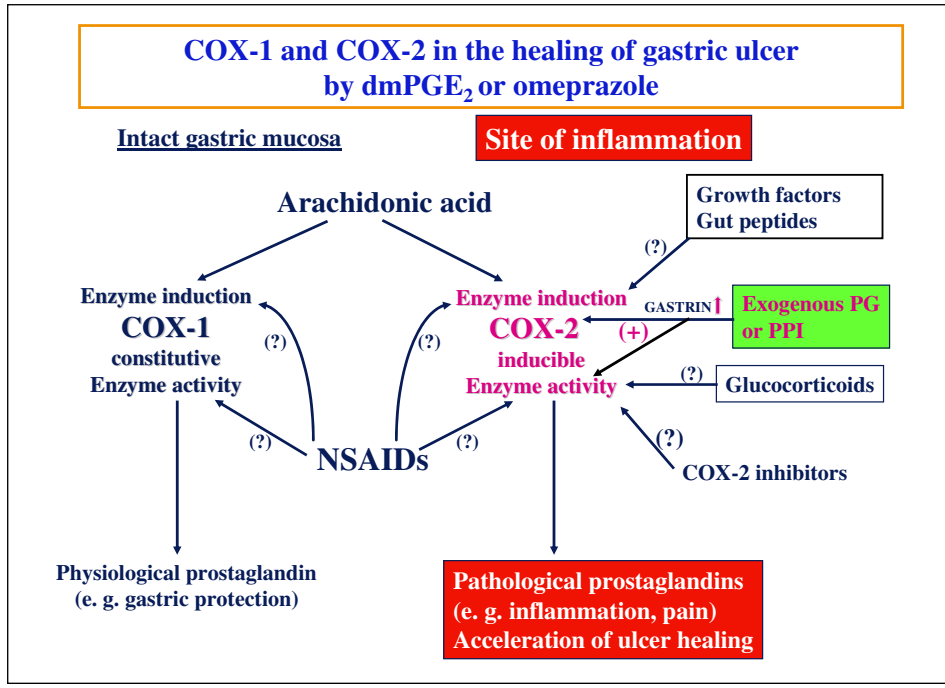

Fig. 8. Schematic presentation of the arachidonic metabolism *via* COX-1 and COX-2 under physiological and pathological conditions such as gastric ulcerations treated with exogenous dmPGE$_2$ or proton pump inhibitor (PPI) such as omeprazole. Enhanced release of plasma gastrin is possibly responsible for the upregulation of COX-2 at the ulcer margin and accelerated ulcer healing.

Wallace and Devchand (26), who proposed that selective inhibition of only COX-1 or only COX-2 activity results in rather small mucosal damage, but suppression of both isoforms of COX, as achieved with indomethacin, causes significantly more pronounced mucosal damage. As endogenous PGE$_2$ release was more suppressed by indomethacin than by NS-398, it is obvious that the COX-2 expression was more enhanced with indomethacin than with NS-398.

Davies et al (31) were first to observed significant upregulation of COX-2 in the rats following the administration of aspirin and suggested that diminished mucosal generation of PGE$_2$ by this NSAID was responsible for triggering this upregulation of COX-2. In this study we found that marked reduction in PGE$_2$ generation due to inhibition of COX-1 and COX-2 activity was not accompanied by any change in expression of COX-1 (*Fig. 11*). In contrast, the COX-2 expression was significantly upregulated in tests with indomethacin but not with NS-398 that produced smaller fall in PGE$_2$ generation. (*Fig. 12*). It is of interest that even small doses of exogenous dmPGE$_2$ given to rats treated with COX-1 or COX-2 inhibitors restored completely the healing of gastric ulcers and increased

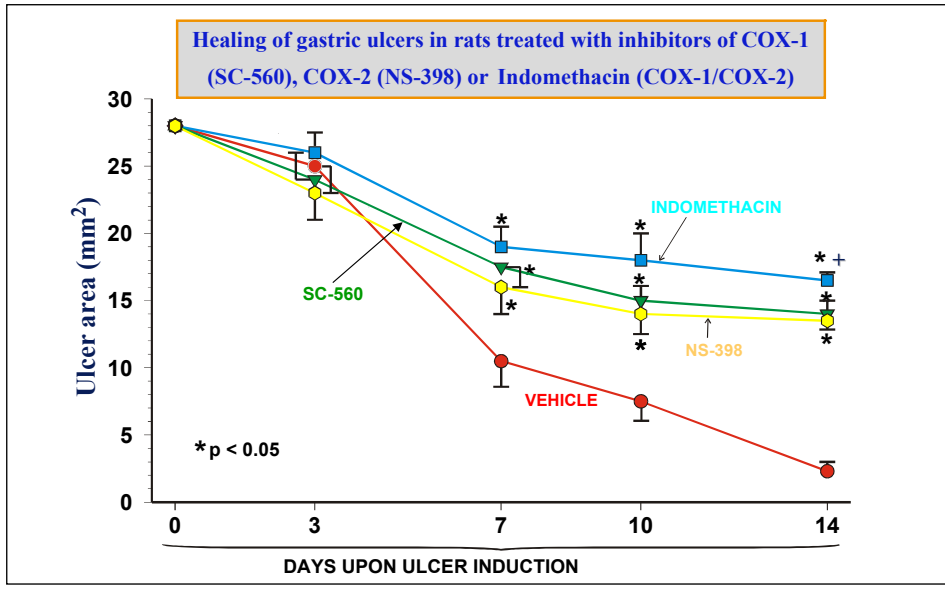

Fig. 9. Mean area of gastric ulcers measured at day 0, 3, 7, 10 and 14 upon ulcer induction in vehicle-treated control rats and those treated with indomethacin, SC-560 or NS-398. Mean ± SEM of 6 experiments on 6 rats. Asterisk indicates significant increase, above the value in vehicle-treated rats (unpublished data)

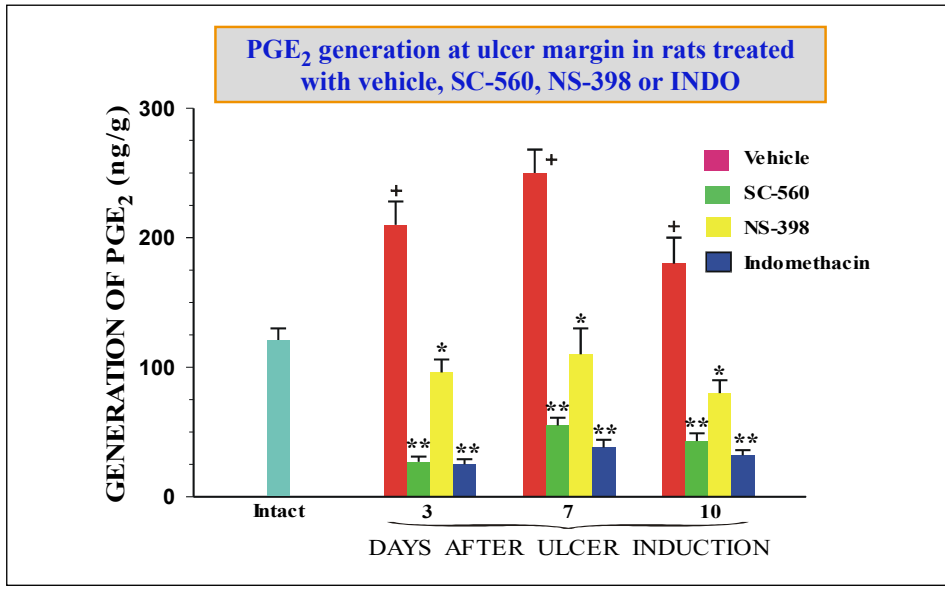

Fig. 10. PGE_2 generation in the ulcerated and non-ulcerated gastric mucosa of rats treated with vehicle, SC-560, NS-398 or indomethacin. Mean ± SEM of 6 experiments on 6 rats. Single asterisk indicates significant decrease, below the value in vehicle-treated rats. Double asterisks indicate significant decrease below the value obtained after inhibition of COX-2 by NS398. (unpublished data)

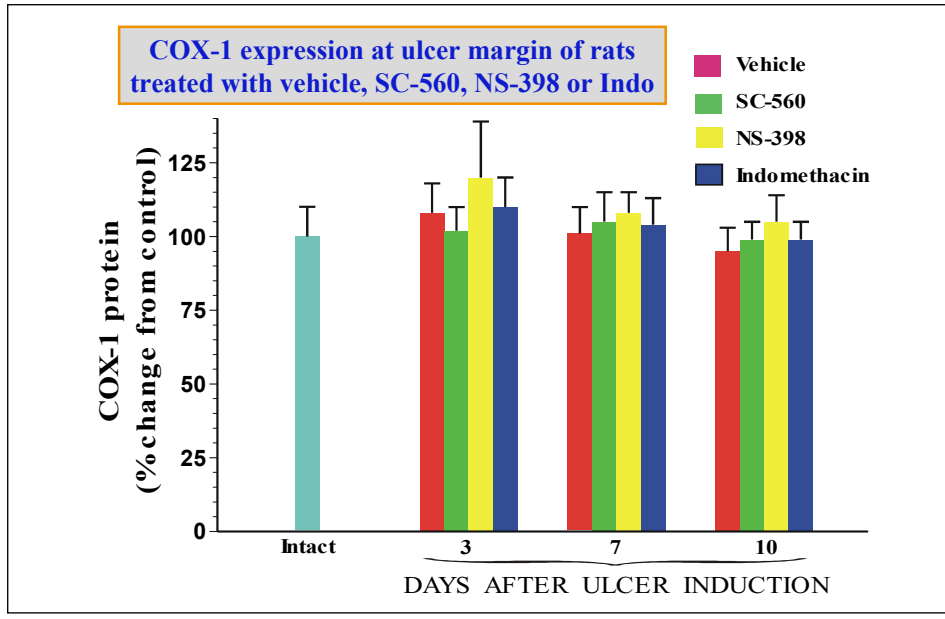

Fig. 11. COX-1 expression in intact gastric mucosa and at ulcer margin of rats treated with vehicle, SC-560, NS-398 or indomethacin or at day 3, 7 and 10 upon ulcer induction (unpublished results).

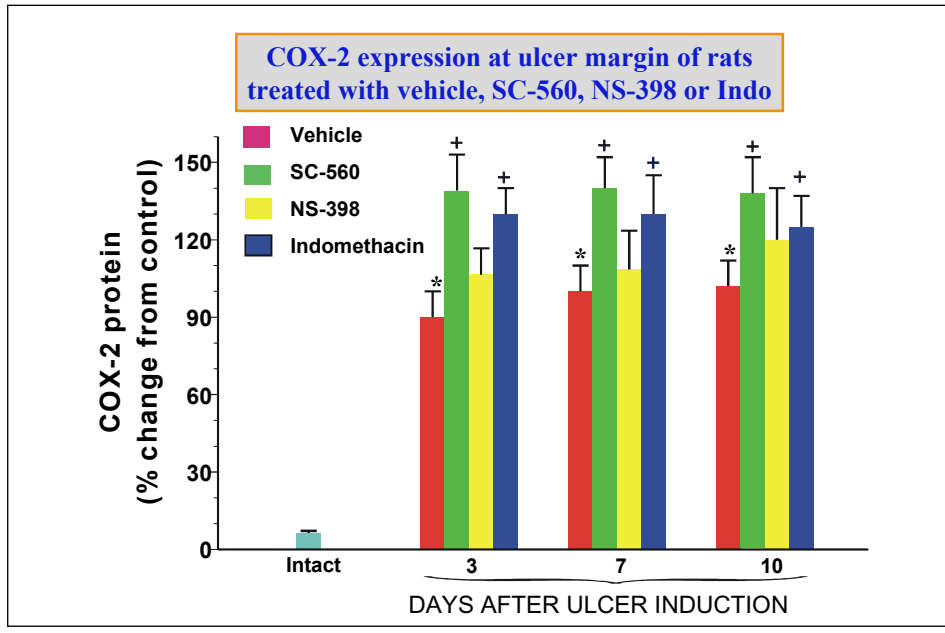

Fig.12. COX-2 expression in intact gastric mucosa and at ulcer margin of rats treated with vehicle, indomethacin or SC-560 or NS-398 at day 3, 7 a dn10 upon ulcer induction. Asterisk indicates significant increase above the value recorded in gastric mucosa of vehicle-treated rats. Cross indicates significant increase above the value recorded in vehicle-treated rats (unpublished results).

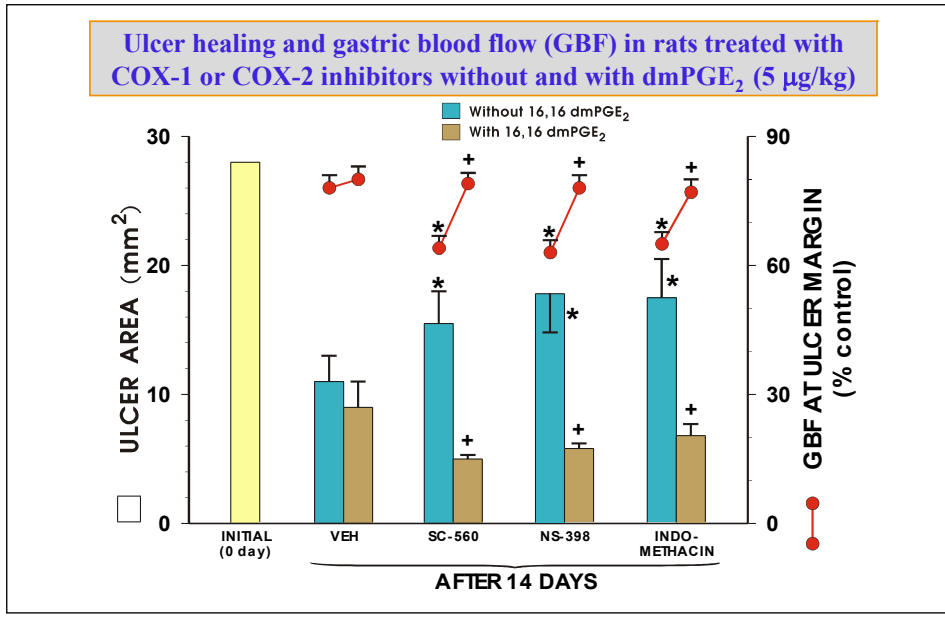

Fig. 13. It is of interest that minute amount of exogenous $dmPGE_2$ (5 µg/kg/d) administered to rats receiving vehicle, SC-560, NS-398 or indomethacin abolished the delay in ulcer-healing and restored gastric blood flow caused by pretreatment with COX-1 or COX-2-inhibitor or by indomethacin. Asterisk iondicates significant change as compared to vehicle-treated rats, Cross indicates significant change as compared to the value obtained in SC-560, NS-398 or indoemthacin administration. (unpublished results)

mucoal blood flow (*Fig. 13*). These observations lead to hypothesis that inhibition of COX-1 activity is associated with delay of ulcer healing and that decrease in local PGE_2 release is combined with an increase in COX-2 expression in the ulcerated mucosa (*Fig. 14*). Thus, inhibition of COX-1 and COX-2 activity by nonselective inhibitors such as indomethacin reduces COX activity and elevates the expression of COX-2 at the ulcer area, while specific COX-2 inhibitor does not affect the expression of this COX isoform (*Fig. 14*).

Effects of corticosteroids on peptic ulcer healing

The ulcerogenic effect of corticosteroids in the stomach is controversial. While some investigators suggested that there is no association between corticosteroids therapy and ulcerogenesis, others emphasized an increased risk of peptic ulcer and its complications (33-37) or reported that peptic ulcer is rather rare complications of corticosteroid therapy (34) and prophylaxis should be considered only in patients with increased risk factors such as concurrent NSAID therapy or previous history of peptic ulceration (35,36). Animal experiments with

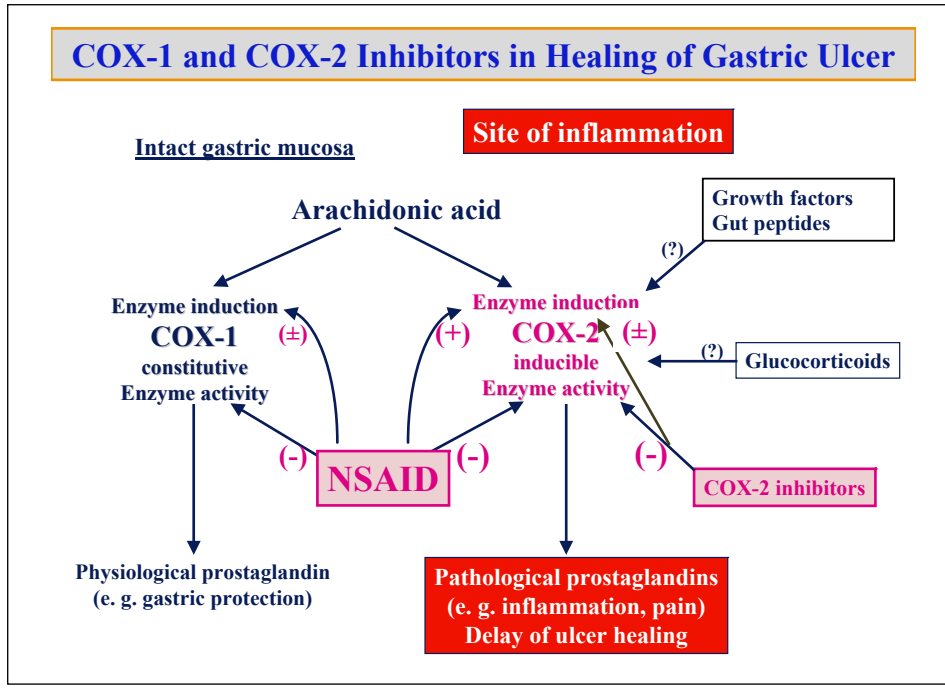

Fig. 14 Schematic presentation of the arachidonic metabolism *via* COX-1 and COX-2 under physiological and pathological conditions such as gastric ulcerations treated with nonspecific NSAID or specific COX-2 inhibitors;

gastric ulcers showed that hydrocortisone (37), dexamethasone (38) or prednisolone (39) delayed gastric ulcer healing and this healing could be improved by the addition of exogenous PGE (37, 39).

In this preliminary study we used non-ulcerogenic dose of dexamethasone (38) and confirmed that such dose delayed gastric ulcer healing rate after 7 and 10 days of treatment (*Fig. 15*). Addition of dmPGE at a small dose (10μg/kg/d), that by itself failed to affect ulcer healing rate when given alone (data not shown), reversed the delay of healing caused by dexamethosone. We confirmed that dexamethasone reduced the generation of PGE_2 (*Fig. 16*) and decreased both expression and activity of COX-2 but not COX-1 at the ulcer area (*Figs 17* and *18*). We can conclude, therefore, that corticosteroids such as dexamethasone at the dose used delayed ulcer healing most likely due to the inhibition of both expression and activity of COX-2 (*Fig. 19*). Our results coincide with previous reports showing that dexamethasone at non-ulerogenic dose (38) causes an inhibition of both COX-1 and COX-2 activity and this is required for induction by this corticosteroid of gastric mucosal damage (40). The mechanism by which dexamethasone induced reduction in COX-2 expression and attenuated PGE_2

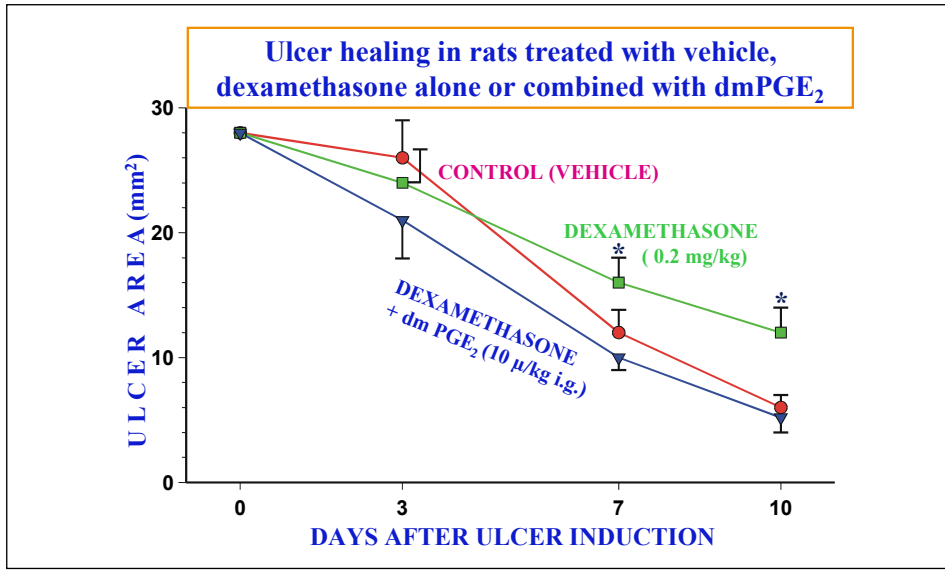

Fig. 15. Mean area of gastric ulcers measured at day 0, 3, 7 and 10 upon ulcer induction in vehicle-treated control rats and those treated with vehicle, dexamethasone (0.2 mg/kg) alone and dexamethasone combined with $dmPGE_2$ (10 µg/kd) Mean ± SEM of 6 experiments on 6 rats. Asterisk indicates significant increase, above the values in vehicle-treated rats. (unpublished results)

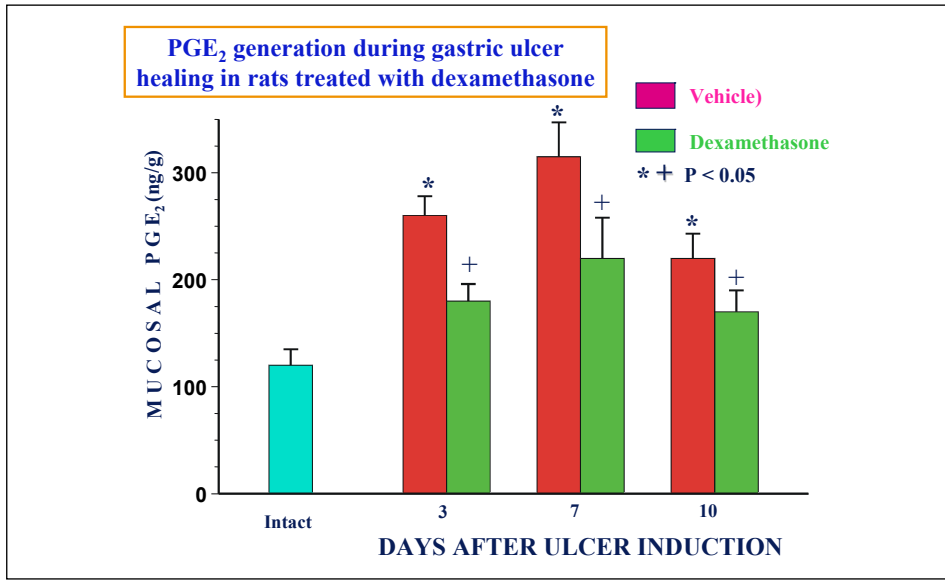

Fig. 16. Mucosal generation of PGE_2 in intact rats and in those with gastric ulceration treated with vehicle and dexamethasone (0.2 g/kg/d) at day 3, 7 and 10 upon ulcer induction. Mean ± SEM of 6 experiments on 6 rats. Asterisk indicates significant increase above the value recorded in intact mucosa. Cross indicates significant decrease below the value recorded at ulcer margin in vehicle-treated rats (unpublished results).

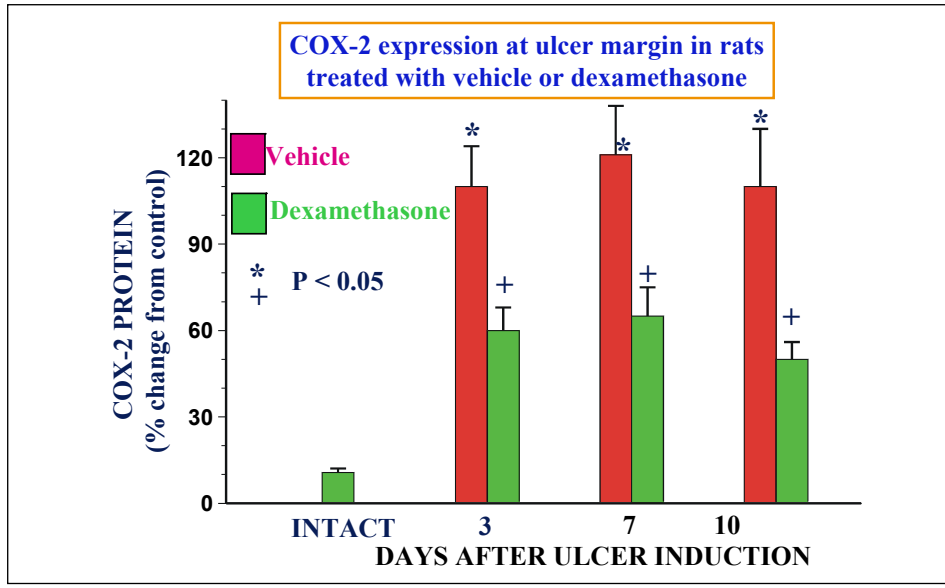

Fig. 17. COX-2 expression in the mucosa of intact rats and at the ulcer margin of rats treated with vehicle or dexamethasone. Mean ± SEM of 6 experiments on 6 rats. Asterisk indicates significant increase above the value recorded in intact mucosa. Cross indicates significant decrease below the value recorded in vehicle-treated rats (unpublished results).

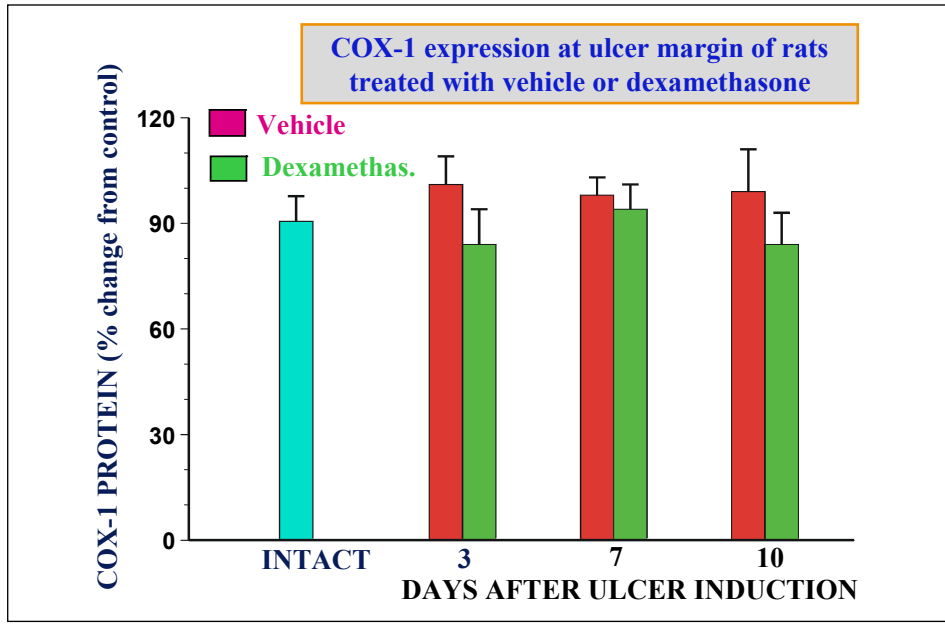

Fig. 18. COX-1 expression in intact gastric mucosa and that at ulcer margin of rats treated with vehicle or dexamethasone at day 3, 7, and 10 upon ulcer induction. Mean ± SEM of 6 experiments on 6 rats (unpublished results)

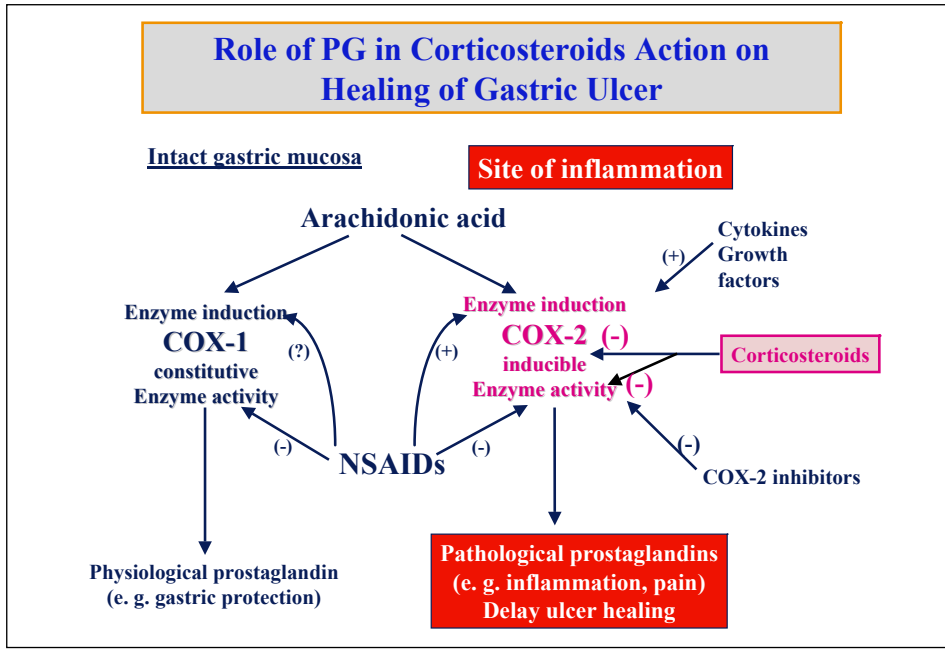

Fig. 19. Schematic presentation of the inhibitory action of corticosteroids on COX-2 induction and COX-2 activity in gastric mucosa of rats with gastric ulcer.

formation could delay ulcer healing is not obvious, but it could be related to the decrease in the proliferation of gastric epithelial cells (41) and angiogenesis (42), that are normally enhanced by COX-2-derived PGE_2 and associated with induction of hepatocyte growth factor expression (41). Depletion of mucosal PGE_2 by dexamethasone seems to play a key role in delay of ulcer healing as supplementation with $dmPGE_2$ returned the ulcer healing rate back to normal level observed in vehicle-treated animals. In conclusion, ulcer production by acetic acid activate the repair system in the gastric mucosa including epithelial cell proliferation and angiogenesis at ulcer margin that are mediated by COX-2-PGE_2 and hepatocyte growth factor production. The interference of dexamethazone in COX-2-PGE_2 system appears to deter the above repair mechanisms leading to worsening of the ulcer healing process (*Fig. 19*).

Effects of growth factors and gut hormones on ulcer healing

It is well-known that ulcer healing involves local expression of various growth factors in the ulcer area such as epidermal growth factor (EGF), transforming growth factor (TGFα), hepatocyte growth factor (HGF) and basic fibroblast growth factor (bFGF) as well as gastrin (27). According to our observations besides COX-PG system, the most effective in the acceleration of ulcer healing

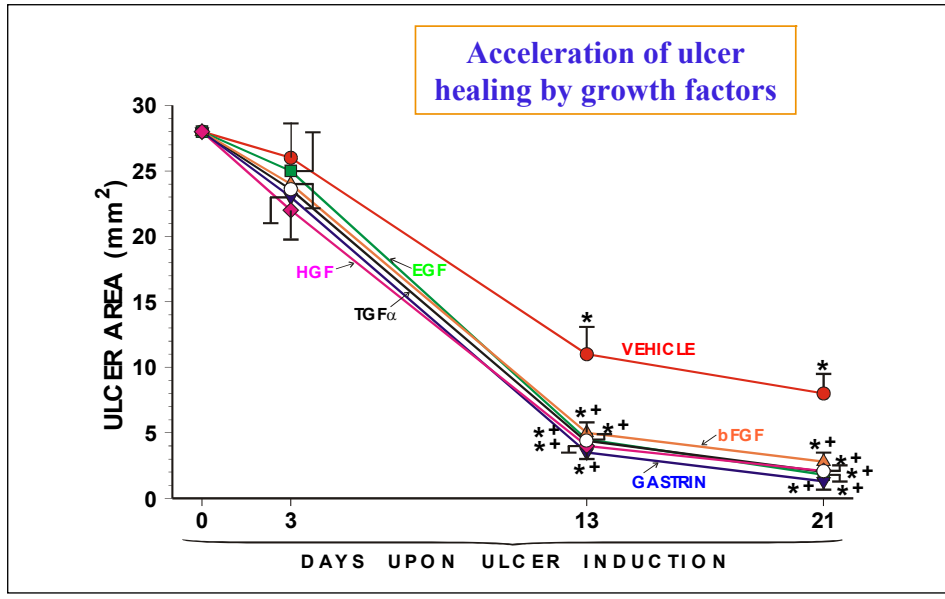

Fig. 20. Mean area of gastric ulcers in rats with daily treatment with various growth factors and gastrin at a dose of 10 µg/kg/d injected i.p. Asterisk indicates significant decrease below the value obtained at day 0. Cross indicates significant decrease below the value obtained in rats treated with vehicle.

are growth promoting factors such as EGF, HGF, TGFα and bFGF (*Fig. 20*). These growth factors were found to be expressed at the ulcer margin during ulcer healing and could contribute to the healing process (43). It is of interest that expression of these growth factors, coincides with the inhibition of gastric acid secretion and increased mucosal blood flow at the ulcer margin as well as hypergastrinemia (44). Administration of gastrin, that increases gastric acid secretion, also accelerates ulcer healing (see *Fig. 20*), indicating that the healing effect of this hormone is unrelated to its gastric acid stimulation. Furthermore, gastrin receptors (CCK_2-receptors) were found to be expressed in the regenerative mucosal ulcer margin as demonstrated by RT-PCR and autoradiography (45), reinforcing the concept that in addition to growth factors, gastrin also contributes to the mucosal cell proliferation at the ulcer margin (46, 47). It is of interest that the acceleration of ulcer healing by growth factors or gastrin can be attenuated by local application of antibodies against these growth factors or gastrin (27), emphasizing the specificity of their ulcer healing promotion through stimulation of mucosal growth and angiogenesis at the ulcer margin. It may be important to stress that treatment with growth factors does not affect the gene expression of COX-1, but elevates COX-2 expression in the ulcerated mucosa (*Fig. 21*). As acceleration of ulcer healing by growth factors can be delayed by the

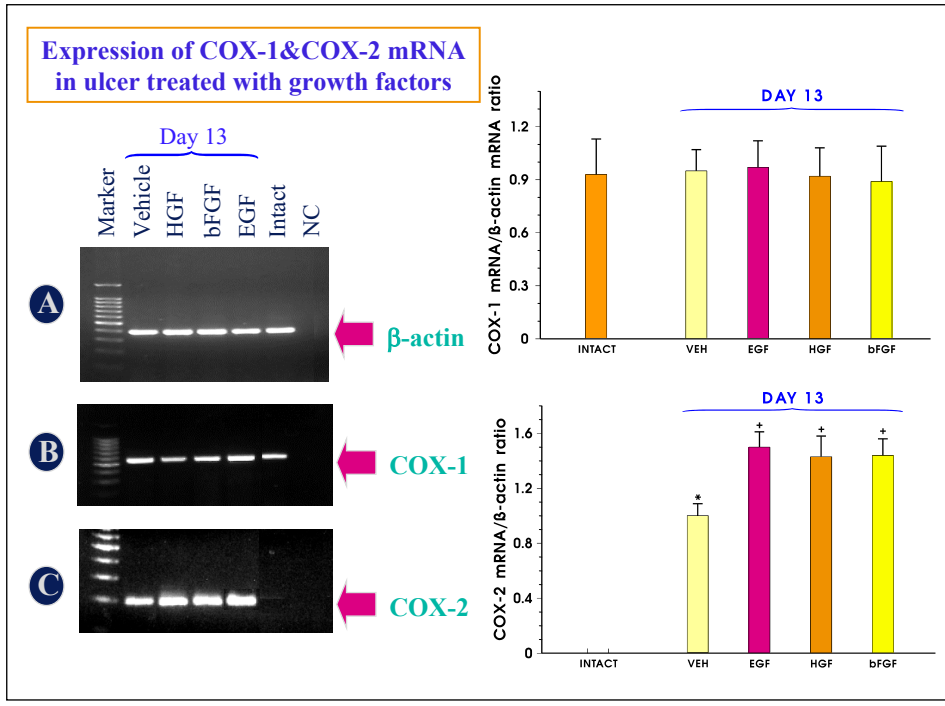

Fig. 21. Gene expression of COX-1 and COX-2 (presented as ratio of COX to beta-actin) in the intact gastric mucosa and at the gastric ulcer margin in rats treated for 13 days with EGF, HGF and bFGF. Asterisk indicates significant increase above the value recorded in intact mucosa. Cross indicates significant increase above the value obtained in vehicle-treated mucosa.

administration of COX-1 and COX-2 inhibitors such as indomethacin and is accompanied by the upregulation of COX-2 at the ulcer margin, it is reasonable to conclude that COX-2 derived PG mediate the acceleration of ulcer healing by various growth factors expressed at the ulcer margin (*Fig. 22*).

The list of ulcer healing factors includes several others factors of gastrointestinal origin such as cholecystokinin (CCK), gastrin releasing peptide (GRP), ghrelin, leptin, somatostatin and insulin (50-53). As shown on *Fig. 23*, the ulcer healing activity of various gut hormones is accompanied by the stimulation of mucosal growth (except somatostatin) and depends, in most instances, on the mucosal generation of PG due to elevation of COX-2 expression (*Fig. 23*).

The ulcer healing action of various gut hormones controlling food intake such as CCK, leptin or ghrelin is independent on gastric acid secretion but involves the activation of brain-gut axis *via* stimulation of peripheral sensors and afferent nerves. This is supported by the finding that inactivation of sensory nerves with neurotoxic dose of capsaicin attenuated the ulcer healing effects of these appetite-regulating gut hormones (49-53).

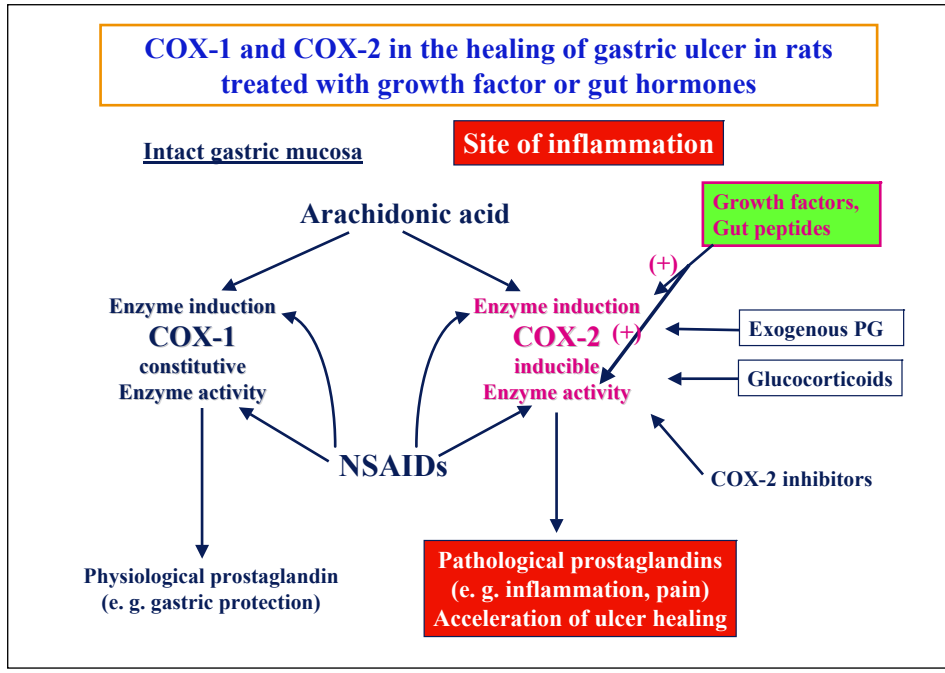

Fig. 22. Schematic presentation of the mechanism of stimulatory action of growth factors and certain gut hormones such as gastrin on the induction of expression and activity of COX-2 that contribute to the stimulation of ulcer healing effects of these substances.

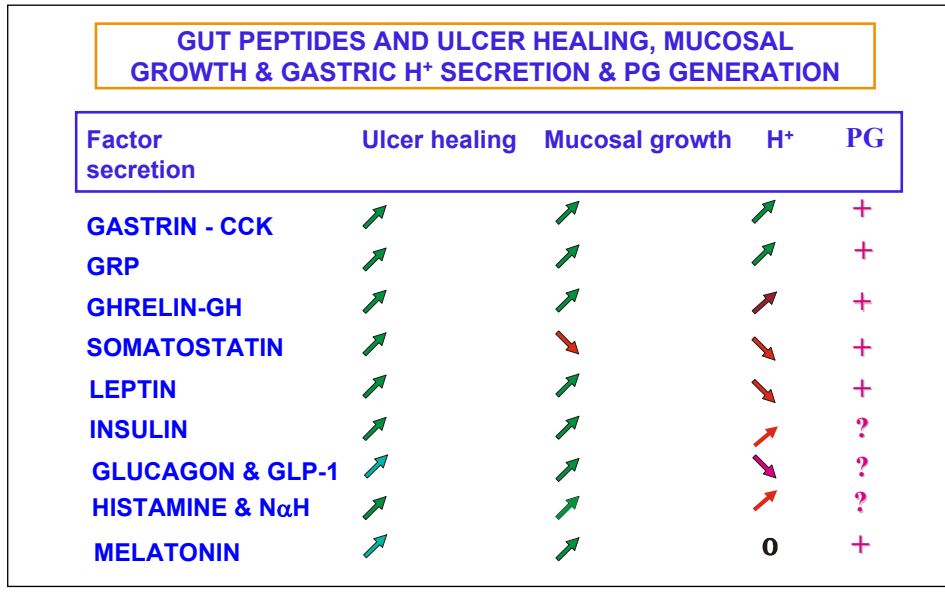

Fig. 23. List of gut hormones affecting the ulcer healing, mucosal growth, gastric acid secretion and prostaglandin generation in the gastric mucosa

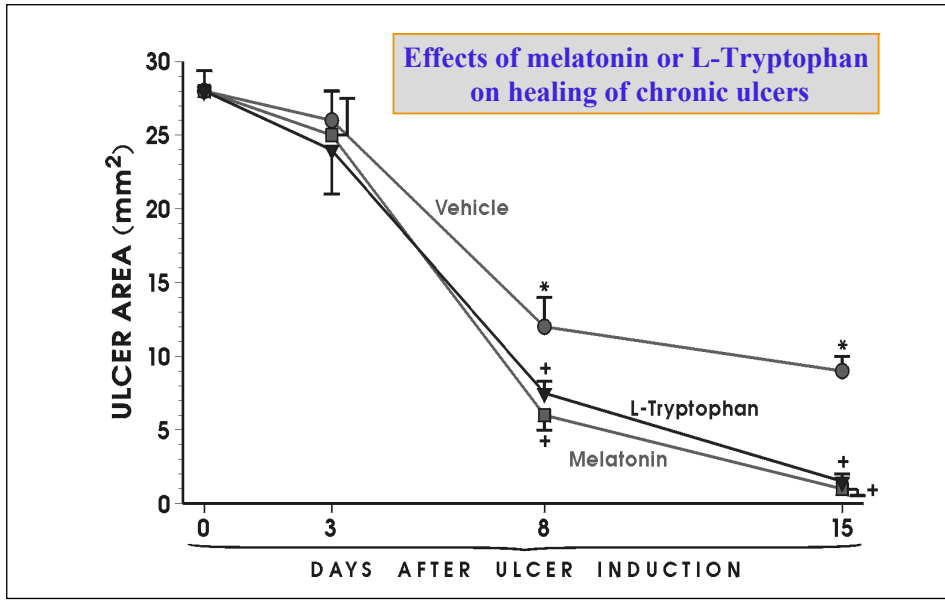

Fig. 24. Time sequence of healing rate of preexisting ulcers by melatonin and its precursor, L-tryptophan. Asterisk indicates significant difference compared to initial value at day 0. Cross indicates significant difference compared to vehicle control. (unpublished results)

Melatonin that was thought to originate primarily from the pineal glands, but recently it has been detected in large amounts in the digestive organs, such as stomach, gut and the pancreas (54, 55). Although its gastroprotective activity has been attributed to scavenging of reactive oxygen species and increase of antioxidative enzymes (56), we found that this indole, as well as its substrate L-tryptophan, accelerates ulcer healing (*Fig. 24*), at least in part, by activation of COX-PG system because it was accompanied by the increase in gastric mucosal generation of PGE_2 and the inhibition of COX-1/COX-2 system by indomethacin delayed the ulcer healing promoted by melatonin (*Fig. 25*). Furthermore, the deactivation of sensory nerves with neurotoxic dose of capsaicin reversed the healing acceleration by metalonin and L-tryprophan and supplementation with calcitonin-gene related peptide (CGRP), a neuropeptide that is deficient in such sensory deactivated animals, restored the healing effects of melatonin and its precursor (*Fig. 26*). Its is, therefore, reasonable to assume that melatonin enhances ulcer healing through the activation of brain-gut axis and stimulation of afferent sensory nerves (54-58).

As indicated in the introduction, *H. pylori* infection and NSAID are known to delay ulcer healing in humans but it is not clear how these two factors interact on the healing process of experimental ulcer. Using our acetic acid model in rats with or without *H. pylori* infection, we found that infection significantly delays ulcer

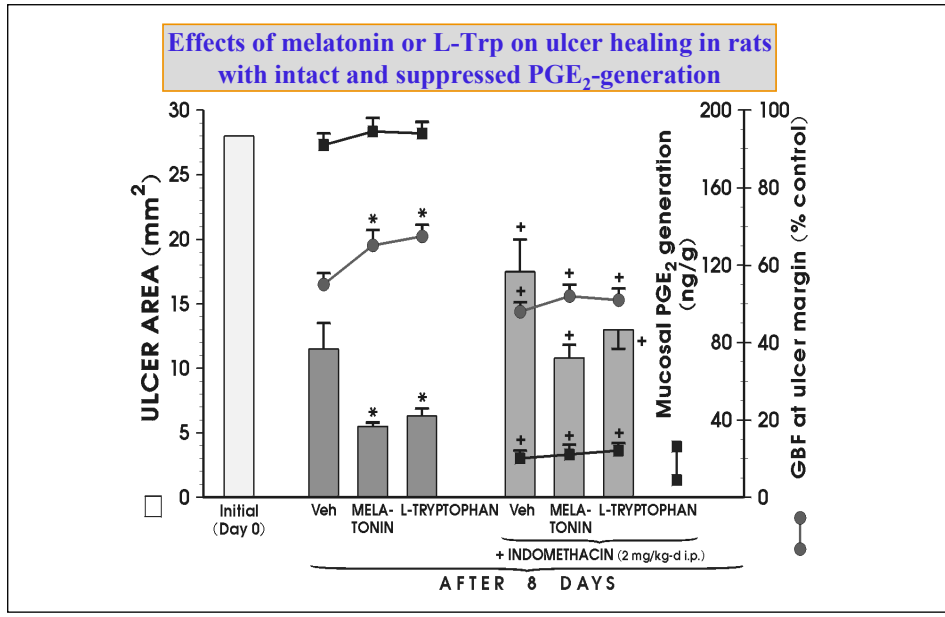

Fig. 25. Effects on melatonin and its substrate, L-tryptophan, on the area of gastric ulcers, mucosal blood flow (GBF) and mucosal generation of PGE_2 after 8 days upon ulcer production in rats without an with treatment with indomethacin. Mean ± SEM of 6 experiments on 6 rats. Asterisk indicates significant decrease below the value in vehicle-treated rats. Cross indicates significant change as compared to the values recorded in rats without pretreatment with indomethacin. (unpublished results)

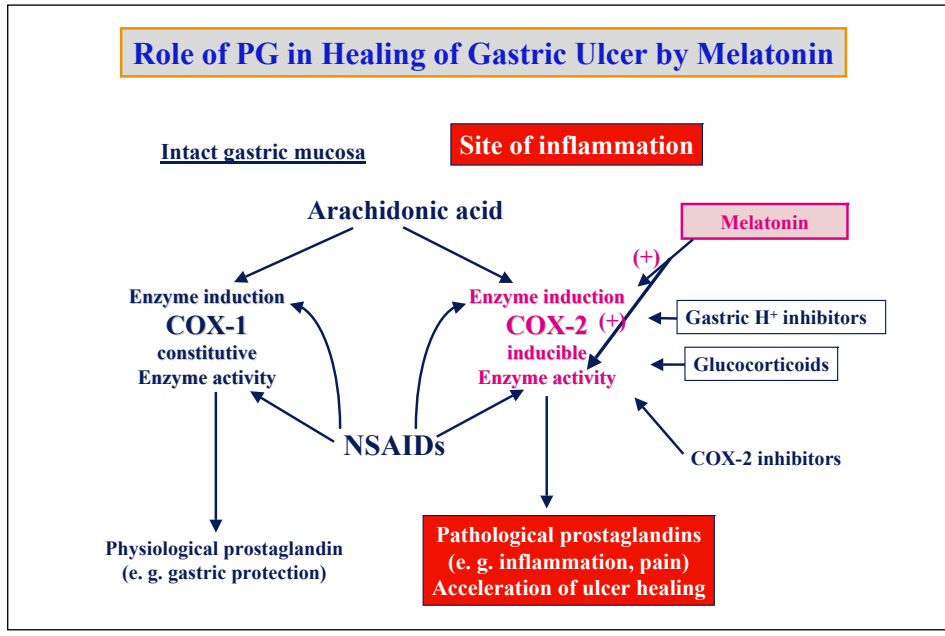

Fig. 26. Role of COX-1 and COX-2 expression in melatonin induced acceleration of ulcer healing.

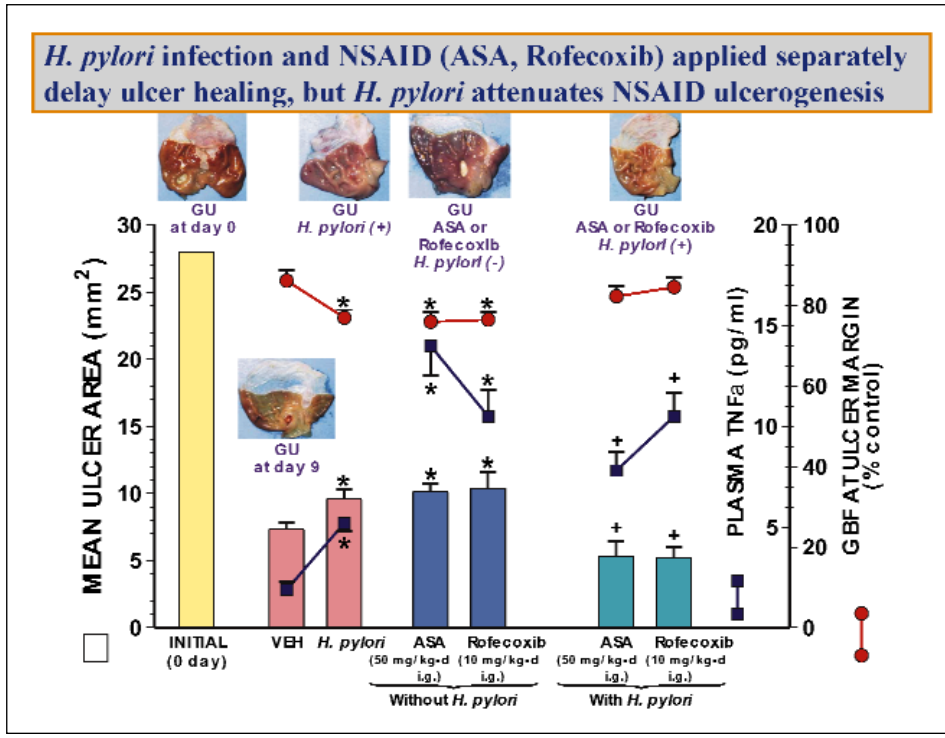

Fig. 27. Mean ulcer area, gastric blood flow (GBF) at the ulcer margin and plasma level of tumor necrosis factor (TNFα) in rats given saline (control) or inoculated with *H. pylori* without or with administration for 9 days of aspirin, rofecoxib. Asterisk indicates significant change as compare to the value recorded in in vehicle-treated rats. Cross indicates significant change as compared to the value obtained in rats treated with aspirin or rofecoxib but not inoculated with *H. pylori*. (unpublished results)

healing as compared with vehicle control (*Fig.27*). Similar effects are exerted by the application of aspirin (50 mg/kg/d) or rofecoxib (10 ng/kg/d). With the combination of *H. pylori* infection and addition of aspirin or rofecoxib, the ulcer area was significantly reduced as compared to that obtained with aspirin or rofecoxib alone without *H. pylori* infection. The increased ulcerogenicity of aspirin or rofecoxib in rats without *H. pylori* infection could be simple attributed to the inhibiton of PG generation with subsequent decrease in mucus alkaline secretion, attenuation of mucosal blood flow and neutrophil adherence to vascular epithelium. The reduced ulcerogenicity of aspirin and rofecoxib in *H. pylori*-infected rats could result from activation of the inflammatory cascade, release of various cytokines and most important from the increased expression of COX-2 and enhanced generation of PGE_2 by the presence of *H. pylori* in the ulcer area but this requires further documentation. Clinical implication of this finding would

be that the eradication of *H. pylori* in NSAID ingesting patients should not be recommended except when ulcer complications occur, but this is the controversial issue requiring further studies.

In conclusion, PG of E series are involved in mucosal repair and healing at least in part, due to increased expression and activity of COX-2 in the ulcer area. Exogenous PGE enhance ulcer healing only at higher gastric inhibitory dose and exerts similar stimulatory action on COX-2 activity and its expression at ulcer margin to that of proton pump inhibitors. Both nonspecific NSAID and specific COX-2 inhibitor reduce the activity of COX-1 and COX-2, while enhancing the expression of COX-2. Corticosteroids inhibit both the expression and activity of COX-2 and this is probably the major mechanism of their ulcerogenic effects. Growth factors and certain gut peptides such as gastrin and CCK or melatonin accelerate ulcer healing due to stimulation of mucosal cell proliferation and, at least in part, expression and activity of COX-2-PG system in the ulcer area as well as activation of brain gut-axis. The infection of gastric mucosa with *H. pylori* while increasing by itself the ulcerogenesis, appears to reduce the ulcerogenicity of NSAID in our experimental model possibly due to increase in the expression and activity of COX and release of PG in the ulcer area.

REFERENCES

1. Schwarz K. Uber penetrierende Magen- and Jejunalgeschwure, 1910.
2. Robert A, Nezamis JE, Lancaster C et al. Cytoprotection by prostaglandins in rat. Prevention of gastric necrosis produced by alcohol, HCl, hypertonic NaCl, and thermal injury. *Gastroenterology* 1979; 77: 433-443.
3. Black JW, Duncan AM, Durant CJ, Ganellin CR, Parsons EM. Definition and antagonism of histamine H2 receptors. *Nature* 1972, 236, 385-390.
4. Sachs G. Physiology of the parietal cell and therapeutic implications. *Pharmacotherapy* 2003; 23: 68S-73S.
5. Anersson T. Pharmacokinetics, metabolism and interactions of acid pump inhibitors. Focus on omeprazole, lanzoprazole and pantoprazole. *Clin Pharmacokinet* 1996; 31: 9-28.
6. Jones DB, Howden CW, Burget DW, Kerr GD, Hunt RH. Acid suppression in duodenal ulcer: a metaanalysis to define optimal dosing with antisecretory drugs. *Gut* 1987; 28: 1120-1127.
7. Hunt RH, Cederberg C, Dent J, et al. Optimizing acid suppression for treatment of acid-related diseases. *Dig Dis Sci* 1995; 40: 24S-49S.
8. Marshall BJ. Helicobacter pylori. *Am J Gastroenterol* 1994; 89: (Suppl. 1), S116-128.
9. Watanabe T, Higuschi K, Tominaga K, Fujiwara Y, Arakawa T. Peptic ulcer recurrences after successful eradication of Helicobacter pylori - clinical characteristics and managements. *Nippon Rinsho* 2004; 62: 495-498.
10. Kurata JH, Nogawa AN. Meta-analysis of risk factors for peptic ulcers; Nonsteroidal anti-inflammatory drugs, Helicobacter pylori and smoking. *J Clin Gastroenterol* 1997; 24: 2-17.
11. Konturek SJ, Kwiecien N, Swierczek J, Oleksy J, Sito E, Robert A. A comparison of methylayed prostaglandin E analogues given orally in the inhibition of gastric responses to pentagastrin and peptone meal in man. *Gastroenterology* 1976; 70: 683-687.

12. Gibinski K, Rybicka J, Mikos E, Nowak A. Double-blind clinical trial on gastroduodenal ulcer healing with prostaglandin analogues. *Gut* 1977: 18: 636-639
13. Rybicka J, Gibinski K. Methylated prostaglanndin E_2 analogues for healing of gastroduodenal ulcers. *Scand J Gastroenterol* 1987; 13:155-159.
14. Poynard T., Pignon JP. Acute treatment of duodenal ulcer analysis of 293 randomized clinical trials. Paris, John Libbey Eurotext, 1989, p. 7.
15. Graham DY, White RH, Moreland LW et al. Duodenal and gastric ulcer prevention with *misoprostol* in arthritis patients taking NSAID. Misoprostol study group. *Ann Intern Med* 1993; 19: 257-262.
16. Hawkey CJ, Walt RP. Proostaglandins for peptic ulcer. A promise unfulfilled. *Lancet* 1986; 2: 1084-1087.
17. Goldstein JL, Huang B, Amer F, Christopulos NG. Ulcer recurrence in high-risk patients receiving nonsteroidal antiinflammatory drugs plus low-dose aspirin: results of a post HOC subanalysis. *Clin Ther* 2004; 26: 1637-1643.
18. Graham DY. Critical effect of Helicobacter pylori infection on the effectiveness of omeprazole for prevention of gastric or duodenal ulcer in chronic NSAID users. *Helicobacter* 2002; 7: 1-8.
19. Perini RF, Ma L., Wallace J. Mucosal repair and COX-2 inhibition. *Curr Pharm Design*. 2003; 9: 2207-2211.
20. Brzozowski T, Konturek PC, Konturek SJ et al. Classic NSAIDand selective cyclooxygenase (COX)-1 and COX-2 inhibition in healing of chronic gastric ulcers. *Microsc Res Tech* 2001; 53: 343-353.
21. Mizuno H, Sakamoto C, Matsuda K et al. Induction of cyclooxygenase 2 in gastric mucosal lesions and its inhibition by the specific antagonist delays healing in mice. *Gastroenterology* 1997; 112: 387-397.
22. Takahashi S, Shigeta J, Inoue H et al. Localization of cyclooxygenase-2 and regulation of its mRNA expression in gastric ulcers in rats. *Am J Pharmacol Gastrointest Liver Physiol* 1998; 275: G1137-G1145.
23. Tatsuguchi A, Sakamoto C, Wada K et al. Localization of cyclooxygenase 1 and cyclooxygenase 2 in Helicobacter pylori related gastritis and gastric ulcer tissues in humans. *Gut* 2000; 46: 782-789.
24. Mizuno H, Sakamoto C, Matsuda K, Wajda K et al. Induction of cyclooxygenase-2 in gastric mucosal lesions and its inhibition by specific antagonist delays healing in mice. *Gastroenterology* 1997; 112: 387-397.
25. Konturek SJ, Stachura J, Radecki T, Drozdowicz D, Brzozowski T. Cytoprotective and ulcer healing properties of prostaglandin E_2 in rats. *Digestion* 1987; 38: 103-113.
26. Wallace JL, Devchand PR. Emerging roles for cyclooxygenase-2 in the gastrointestinal mucosal defense. *Br J Pharmacol* 2005; 145; 275-282.
27. Brzozowski T, Konturek PC, Konturek SJ et al. Effect of local application of growth factors on ulcer healing and mucosal expression of cyclooxygenase 1 and 2. *Digestion* 2001; 64: 15-29.
28. Tsuji S, Sun WH, Tsuji M et al. Lanzoprazole induces mucosal protection through gastric receptor-dependent up regulation of cyclooxygenase-2 in rats. *J Pharmacol Ther* 2002; 303: 1301-1308.
29. Tjandrawinata RR, Hughes-Fulford M. Upregulation of cyclooxygenase-2 by product-prostaglandin E_2. *Adv Exp Med Biol* 1997; 407: 163-170.
30. Tibble J, Sigthorsson G, Caldwell C, Palmer RH, Bjarnason I. Effects of NSAID on cryoprobe-induced gastric ulcer healing in rats. *Aliment Pharmacol Ther* 2001; 15: 2001-2008.
31. Davies NM, Sharkey KA, Asfaha S, MacNaughton WK, Wallace JL. Aspirin causes rapid upregulation of cyclooxygenase-2 expression in the stomach of rats *Aliment Pharmacol Ther*. 1997; 11:1101-1108.

32. Conn HO, Blitzer BI. Nonassociation of adrenocorticosteroid therapy and peptic ulcer. *N Engl J Med* 1976; 294: 473-479.
33. Messer J, Reitman D, Sachs HS, Smith H Jr, Chalmers TC. Association of adrenocorticosteroid therapy and peptic ulcer disease. *N Engl J Med* 1983; 309: 21-24.
34. Ellershaw JE, Kelly MJ. Corticosteroids and peptic ulceration. *Palliat Med* 1994; 8: 313-319.
35. Conn HO, Poynard T. Corticosteroid and peptic ulcer: meta-analysis of adverse events during steroid therapy. *J Intern Med* 1994; 236: 619-632.
36. Pecora PG, Kaplan B. Corticosteroids and ulcers is there an association. *Ann Pharmacother* 1996; 30: 870-872.
37. Kuwayama H, Matsuo Y, Eastwood GL. Effects of prostaglandins on hydrocortisone-induced delayed healing of chronic gastric ulcers in the rat. *J Clin Gastroenterol* 1991; 13: 554-557.
38. Luo JC, Shin VY, Liu ES et al. Non-ulcerogenic dose of dexamethasone delays gastric ulcer healing in rats. *J Pharmacol Exp Ther* 2003; 307: 692-698.
39. Carpani de Kaski M, Rentsch R, Levi S, Hodgson HJ. Corticosteroids reduce regenerative repair of epithelium in experimental gastric ulcers. *Gut* 1995; 37: 613-616.
40. Wallace JL. Glucocorticoid-induced gastric mucosal damage: inhibition of leukotriene, but not prostaglandin biosynthesis. *Prostaglandins* 1987; 34: 311-323.
41. Takahashi M, Ota S, Hata Y. Hepatocyte growth factor as a key to modulate anti-ulcer action of prostaglandins in the stomach. *J Clin Invest* 1996; 98: 2604-2611.
42. Ghosh AK, Hirasawa N, Niki H, Ohuchi K. Cyclooxygenase-2-mediated angiogenesis in carageeniu-induced granulation tissue in rats. *J Pharmacol Exp Ther* 2002; 295: 802-809.
43. Konturek PC, Brzozowski T, Konturek SJ, Ernst H, Drozdowicz D, Hahn EG. Expression of epidermal growth factor and transforming growth factor alpha during ulcer healig. Time sequence study. *Scand J Gastroenterol* 1997; 32: 6-15.
44. Konturek SJ. Role of growth factors in gastroduodenal protection and healing of peptic ulcers. *Gastroenterology Clinics of North America* 1990; 19: 41-65.
45. Schmassmann A, Reubi JC. Cholecystokinin-B/gastrin receptors enhance wound healing in rat gastric mucosa. *J Clin Invest* 2000; 1066: 1021-1029.
46. Li H, Helander HF. Hypergastrinemia increases proliferation of gastroduodenal epithelium during gastric ulcer healing in rats. *Dig Dis Sci* 1996; 41: 40-48.
47. Brzozowski T, Konturek PC, Konturek SJ et al. Involvement of cyclooxygenase (COX)-2 products in acceleration of ulcer healing by gastrin and hepatocyte growth factor. *J Physiol Pharmacol* 2000; 51: 751-753.
48. Brzozowska I, Targosz A, Sliwowski Z et al. Healing of chronic gastric ulcers in diabetic rats treated with native aspiryn, nitric oxide (NO)-derivative of aspirin and cyclooxygenase (COX)-2 inhibitor. *J Physiol Pharmacol* 2004; 55: 773-790.
49. Brzozowski T, Konturek PC, Konturek SJ et al. Acceleration of ulcer healing by cholecystokinin (CCK): role of CCK-A receptors, somatostatin, nitric oxide, and sensory nerves. *Reg Pept* 1999; 82: 19-33.
50. Konturek SJ, Brzozowski T, Dembinski A, Warzecha Z, Konturek PC, Yanaihara N. Interaction of growth hormone-releasing factor and somatostatin on ulcer healing and mucosal growth in rats: role of gastric and epidermal growth factor. *Digestion* 1988; 41: 121-128.
51. Takeuchi K, Hirata T, Yamamoto H et al. Effects of S-0509, a novel CCKB/gastrin receptor antagonist on gastric acid secretion and experimental duodenal ulcers in rats. *Aliment Pharmacol Ther* 1999; 13: 87-96.
52. Konturek PC, Brzozowski T, Burnat G et al. Role of brain-gut axis in healing of gastric ulcers. *J Physiol Pharmacol* 2004; 55: 179-192.

53. Harsch IA, Brzozowski T, Bazela K et al. Impaired gastric ulcer healing in diabetic rats: role of heat shock protein, growth factors, prostaglandins and proinflammatory cytokines. *Eur J Pharmacol* 2003; 481: 249-260.
54. Jaworek J, Brzozowski T, Konturek SJ. Melatonin as an organoprotector in the stomach and the pancreas. *J Pin Res* 2005; 38: 73-83.
55. Brzozowska I, Konturek PC, Brzozowski T et al. Role of prostaglandins, nitric oxide, sensory nerves and gastrin in acceleration of ulcer healing by melatonin and its precursor, L-tryptophan. *J Pineal Res* 2002; 3, 149-162.
56. Bandyopadhyay D, Biswas K, Bhattacharyya M, Reiter RJ, Banerjee RK. Gastric toxicity and mucosal ulceration induced by oxygen-derived reactive species: protection by melatonin. *Curr Mol Med* 2001; 1, 501-513.
57. Cabeza J, Motilva V, Martin MJ, de la Lastra CA. Mechanisms involved in gastric protection and melatonin against oxidant stress by ischemia-reperfusion in rats. *Life Sci* 2001; 68: 1405-1415.
58. Konturek PC, Brzozowski T, Burnat G, Kwiecien S, Pawlik WW, Hahn EG, Konturek SJ. Role of brain-gut axis in healing of gastric ulcers. *J Physiol Pharmacol* 2004; 55: 179-192.

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