ABSTRACT—Gastroenteropathy is the most common among patients who use non-steroidal anti-inflammatory drugs (NSAIDs) for treatment of inflammatory disorders. It is known that rheumatoid arthritic patients are more susceptible to NSAID-induced gastropathy than other NSAID users. This article reviewed our recent studies concerning the influence of arthritic conditions on gastric ulcerogenic response to NSAID and healing response of chronic gastric ulcers in rats. Gastric lesions induced by indomethacin, one of the conventional NSAIDs, were markedly aggravated in arthritic rats. This increased ulcerogenic response in arthritic rats was attributable to nitric oxide production due to up-regulation of inducible nitric oxide synthase. In arthritic rat stomachs, cyclooxygenase (COX)-2 was also up-regulated, where COX-2 selective inhibitors such as rofecoxib or celecoxib provoked gross lesions, although they caused no damage in normal rats. In addition, the healing of chronic gastric ulcers was also delayed in arthritic rats because of less expression of various growth factors such as basic fibroblast growth factors or insulin-like growth factors. Based on these findings, it is concluded that arthritic conditions alter the mucosal ulcerogenic and healing responses in the stomach. Especially, caution should be paid on the use of COX-2 selective inhibitors in rheumatoid arthritic patients.

Keywords: Adjuvant arthritis, Rofecoxib, Celecoxib, Indomethacin, Gastric mucosal lesion

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly prescribed for treatment of rheumatoid arthritis (RA), osteoarthritis, acute pain and fever. It is well known, however, that these drugs have a momentous gastrointestinal toxicity as an adverse reaction. Indeed, these drugs induce damage in the gastrointestinal mucosa of experimental animals as well as humans. Depletion of endogenous prostaglandins (PGs) is important in the background for NSAID-induced gastric mucosal lesions (1, 2), yet the pathogenic mechanism of these lesions is not fully understood. Furthermore, it has been reported that the patients with RA are more susceptible to NSAID-induced gastropathy than other NSAID users. This observation has been reproduced in arthritic animal models, where gastric mucosal lesions induced by NSAIDs were markedly aggravated in comparison with normal animals (3, 4). However, the underlying mechanism for this phenomenon still remains unclear.

Selective cyclooxygenase (COX)-2 inhibitors have been recently developed as new gastric sparing anti-inflammatory drugs (5, 6). These new drugs have a lower gastric mucosal toxicity than the conventional NSAIDs that non-selectively inhibit both COX-1 and COX-2. However, the gastric ulcerogenic effect of selective COX-2 inhibitors in arthritic animals remains unknown. Moreover, it has been reported that NSAIDs impaired the healing of preexisting gastric ulcers (7). Thus, it is also of interest to determine how these drugs affect the healing response of chronic gastric ulcers in arthritic animals.

In this article, we review our recent studies concerning the influence of arthritis on the gastric ulcerogenic response to NSAIDs and healing of chronic gastric ulcers in adjuvant-induced arthritic rats (8 – 11).
reported that gastric mucosal lesions induced by conventional NSAIDs, such as indomethacin, naproxen and aspirin, were significantly aggravated in these rats when compared with normal rats (3, 4). Figure 1 shows the relationship between the severity of paw edema and the ulcerogenic response to indomethacin following the injection of Freund’s complete adjuvant (FCA) into the planter region of the right hindfoot. The paw edema in the right (injected) hindfoot was evident from the day following the injection of FCA and reached a maximum on days 14 – 17. In contrast, the paw volume of the left (uninjected) hindfoot was not changed for the first 7 days, but was apparently increased from 10 days after FCA injection and reached a maximum also on days 14 – 17. This result indicates that systemic inflammation develops following a single injection of FCA into the right hindfoot from 10 days later. Subcutaneous administration of indomethacin (25 mg/kg) induced hemorrhagic mucosal lesions in both normal and arthritic rat stomachs, but the severity of the lesions in the latter group was significantly higher than that in the former, depending on the degree of arthritis. We further observed that gastric mucosal lesions induced by oral administration of aspirin was also significantly aggravated in arthritic rats, suggesting that the increased mucosal susceptibility to NSAID is an universal phenomenon in arthritic rat stomachs. Rainsford (12), however, reported no significant difference between normal and adjuvant arthritic rats, in terms of either the incidence or the severity of gastric lesions following the single and repeated doses of aspirin. These discrepancies may be due to different experimental conditions, including the doses of NSAIDs used and the strain/species of animals. Especially, the strain of rats is important in determining the severity of arthritis; most strains of rats, including Sprague-Dawley, Wistar and F344, showed a low susceptibility to adjuvant arthritis, while DA and Lewis rats exhibited a high susceptibility to arthritis. Indeed, we observed in a preliminary study that the severity of arthritis in Wistar rats was moderate with an incidence of only 50 – 60%, while in both DA and Lewis rats, the severity was much higher than Wistar rats, with the incidence being 100%. It should also be noted that the aggravation of indomethacin-induced gastric lesions in arthritic rats was dependent upon the degree of arthritic changes, suggesting a cause-effect relationship between the systemic inflammation and the increased gastric mucosal susceptibility to indomethacin.

It is well accepted that gastric acid plays an important role in the pathogenesis of gastric mucosal lesions induced by NSAIDs such as indomethacin. Several investigators reported the increase in serum gastrin levels and acid secretion in arthritic rats (4, 13). We also confirmed that gastric acid output in arthritic rats was about two times higher than that in normal animals. Thus, it is easily speculated that the increase of gastric ulcerogenic response to indomethacin is attributable to the hyperacidity in the stomach. However, because a marked aggravation of these lesions was similarly observed in arthritic rats, even in the presence of exogenous acid to mask the hyperacid secretory condition normally observed in these rats, it is unlikely that the increased mucosal susceptibility to indomethacin in arthritic rats is associated with the increase of acid secretion.

Interestingly, the aggravation of indomethacin-induced gastric lesions in arthritic rats was significantly prevented by pretreatment of N\textsuperscript{G}-nitro-L-arginine methyl ester (L-NAME), a non-selective nitric oxide (NO) synthase (NOS) inhibitor, and aminoguanidine, a selective inducible NOS (iNOS) inhibitor, as well as dexamethasone, an inhibitor of iNOS mRNA transcription, although they did not have any influence on the severity of lesion observed in normal rats. Moreover, the distinct expression of iNOS mRNA was observed in the stomach of arthritic rats, accompanied with an increase in NO production. Although NO is considered to be important in maintaining the gastric mucosal integrity, we recently reported a detrimental role for NO produced by iNOS in inflammatory gastric lesions (14). These findings are not clearly explained.
strongly suggest that the increased ulcerogenic response to indomethacin in arthritic rats is mediated by endogenous NO, mainly produced by iNOS.

Recent studies have implicated neutrophils as culprits in the gastric damage associated with NSAIDs (15). We observed that anti-neutrophil serum significantly prevented the occurrence of indomethacin-induced gastric lesions in both normal and arthritic rats. These cells are recruited to a site of injury and participate in amplifying the inflammatory response by releasing several chemotaxins and produce further tissue injury by releasing reactive oxygen metabolites. On the other hand, it has been shown that the mechanism of NO toxicity in the gastrointestinal mucosa involves the superoxide radical which, by combination of NO, forms the high cytotoxic moiety peroxynitrite (16). Thus, it is possible that the increased susceptibility of arthritic rat stomachs to NSAIDs might be explained by production of peroxynitrite, resulting from the interaction of NO/iNOS and superoxide radicals.

**Selective COX-2 inhibitors:** The conventional NSAIDs that inhibit nonselectively both COX-1 and COX-2 activities cause gastrointestinal damage in experimental animals as well as humans (1, 2, 17). COX-1 is constitutively expressed in the gastrointestinal tract and maintains the mucosal integrity through continuous generation of PGs, whereas COX-2 is induced predominantly in certain inflammatory cells by various cytokines, endotoxins, tumor promoters and growth factors (18). It is believed that inhibition of COX-1 is critical in the ulcerogenic response to NSAIDs, while inhibition of COX-2 accounts for their anti-inflammatory action through suppression of PG production in inflammatory sites (19, 20). It has been demonstrated that new anti-inflammatory drugs with a several hundred-fold higher selectivity for COX-2 have minimal gastric toxicity in animals and humans (5, 6). Thus, the selective COX-2 inhibitors are thought to be clinically useful as safe anti-inflammatory drugs. Indeed, the selective COX-2 inhibitors such as rofecoxib and celecoxib, even at a higher dose (100 mg/kg), did not induce any damage in normal rat stomachs (Fig. 2). However, these selective COX-2 inhibitors caused gross lesions in the stomach of arthritic rats, similar to indomethacin, the conventional NSAID. Moreover, in the stomach of arthritic rats, PG generation was significantly enhanced with a marked expression of COX-2. Certainly, indomethacin reduced the mucosal PG content in both normal and arthritic rats. In contrast, the COX-2 inhibitor rofecoxib did not affect PG generation in normal rat stomachs, but significantly decreased PG content in the stomach of arthritic rats. It is thus likely that the increase in PG production in arthritic rat stomachs may be brought about by COX-2 activity. These findings all suggest that the COX-2 plays an important role in maintaining the integrity of gastric mucosa of arthritic rats. Adjunct arthritis is often used for animal models of RA, and these arthritic animals are known to suffer from chronic systemic inflammation and severe pain. Therefore, it is possible that the increases in COX-2 expression in the stomach occur in association with inflammation or stress caused by pain. Further study is certainly required to verify this point. Takahashi et al. (21) reported that COX-2 inhibitors caused gastric lesions in *Helicobacter pylori*-infected animals, in the stomachs of which COX-2 had been expressed. We also reported that NS-398, a selective COX-2 inhibitor, attenuated the adaptive gastric protection induced by a mild irritant, especially when COX-2 expression was observed in the stomach (22). Based on these findings, it can be speculated that selective inhibitors of COX-2 have deleterious influences on the stomach when a significant overexpression of COX-2 occurs under various conditions, including *Helicobacter pylori*-infection, severe arthritis and stressful stimulation.

**Alteration in healing response of chronic gastric ulcers**

It is known that NSAIDs at a low dose that do not by themselves induce any damage impair the healing response of chronic gastric ulcers. The deleterious effect of NSAIDs on the ulcer healing is attributable to the suppression of mucosal PG synthesis caused by COX inhibition. Indeed, daily administration of indomethacin has been shown to delay the healing of gastric ulcers induced in rats by acetic acid, and this effect was reversed by supplementation with an exogenous PG derivative, 16,16-dimethyl PGE₂ (7). Thus, these findings led us to speculate that the healing
process of gastric ulcers may also be modified in arthritic animals. Actually, the healing response of chronic gastric ulcers induced by thermal cauterization was significantly delayed in arthritic rats when compared with normal rats (Fig. 3). Certainly, the healing impairment effect of indomethacin was observed in both normal and arthritic rats, yet this effect was more pronounced in the latter group. Indomethacin impaired the healing response through suppression of PG synthesis. However, since PG synthesis in the arthritic rat stomach was increased due to COX-2 expression, it is unlikely that the delayed ulcer healing in these animals is attributable to a decrease of PG biosynthetic activity. In any case, it is interesting from the clinical point of view to note that arthritic conditions potentiate both the ulcerogenic and healing impairment effects of indomethacin in the stomach.

Konturek et al. (23) have shown that the inhibition of NO production delayed the healing of acetic acid-induced gastric ulcers in rats and suggested that endogenous NO is important in maintaining blood flow around the ulcer as well as angiogenesis in the granulation tissue. Akiba et al. (24) reported that aminoguanidine, a relatively selective iNOS inhibitor, reduced the severity of acetic acid-induced gastric ulcers in the early period but impaired the healing of these ulcers thereafter. The latter observation suggests that NO generated from iNOS, similar to PGs, play a role in the healing process of gastric ulcers, despite causing a deleterious influence on the developmental process of ulcers. However, in our study, the healing of these ulcers was not affected by the repeated treatment with either L-NAME or aminoguanidine in arthritic rats. Thus, it is also unlikely that the delayed ulcer healing in arthritic rats is associated with overproduction of endogenous NO.

On the other hand, the healing process of gastric ulcers is affected by intraluminal acid (25). Indeed, antisecretory agents, such as omeprazole, promoted the healing of acetic acid-induced gastric ulcers in rats (26). In arthritic rats, basal acid secretion is significantly increased when compared with normal rats. Several studies showed that acid hypersecretion resulted in retardation of gastric ulcer healing (25, 27). It is thus possible to speculate that the delayed ulcer healing in arthritic rats is accounted for by acid hypersecretion. We observed that daily administration of omeprazole significantly accelerated the healing of gastric ulcers in both normal and arthritic rats, and this healing promoting effect was more pronounced in the latter group. These findings suggest that the delayed ulcer healing in arthritic rats may be, at least partly, due to acid hypersecretion. However, since the ulcer score in arthritic rats treated with omeprazole was still significantly greater than that in normal rats, other factors should be involved in the mechanism for the delayed ulcer healing in arthritic conditions.

It is well known that various growth factors, such as basic fibroblast growth factor (bFGF), are also important in the healing of chronic gastric ulcers. Several studies have shown that bFGF exerts a healing promoting effect on experimentally-induced gastric and duodenal ulcers in rats (28, 29). In addition, daily administration of CS-23, a recombinant human bFGF, significantly accelerated the healing of gastric ulcers in both normal and arthritic rats, without any influence on the acid secretion or the severity of arthritis. This growth factor is a potent endothelial cell mitogen affecting the proliferation of other cell types, including fibroblasts, smooth muscle cells, and epithelial cells that are required for tissue replacement at the ulcer site (30). We previously reported that the decreased expression of bFGF impaired the healing of acute gastric lesions in experimentally induced diabetic rats (31). It is thus possible that the expression of this growth factor may be altered in arthritic rats. Indeed, the expression of bFGF in the rat gastric mucosa was markedly increased 3 days after ulceration, and this response was seen for 14 days thereafter. In arthritic rats, however, the increased expression of bFGF after ulceration was less marked in the stomach. In addition, other growth factors, such an insulin-like growth factor (IGF)-1, was also less expressed in the ulcerated stomach of arthritic rats, similar to bFGF. These findings suggest that the delayed ulcer healing in arthritic rats is partly attributable to less expression of these growth factors such as bFGF and IGF-1 in the gastric mucosa. At present, it remains unclear how the increased expression of the growth factors after ulceration is suppressed in arthritic rat stomachs. It has been recently reported that the decrease in salivary epidermal growth factor (EGF) in RA patients.

![Fig. 3. Changes in gastric ulcer area in normal and arthritic rats after ulceration. Arthritis was induced in DA rats by injection of FCA into the plantar region of the right hindfoot, while gastric ulcer was induced by thermal cauterization (70°C, 30 s) on day 7 after the FCA injection. Data are presented as the mean ± S.E.M. from 5–6 rats. *Statistically significant difference from the aged and batch-matched normal rats at P<0.05. Modified from ref. 10.](image-url)
increased the mucosal susceptibility to gastric ulceration (32). Further studies would be required to elucidate the possible involvement of other growth factors in the delayed ulcer healing and the mechanism for dysregulation of growth factors in arthritic conditions.

Summary
This review summarized our recent publications about the alterations in gastric ulcerogenic and healing responses observed in adjuvant-induced arthritic rats. First, NSAID-induced gastric mucosal lesions were markedly aggravated in rats with adjuvant-induced arthritis. The increased ulcerogenic response to NSAIDs was mediated by endogenous NO, mainly produced by iNOS. Secondly, arthritic conditions up-regulated COX-2 in the stomach, where selective COX-2 inhibitors produced apparent lesions, although these agents caused no damage in normal rat stomachs. Thus, caution should be paid on the use of selective COX-2 inhibitors for the treatment of RA. Thirdly, the healing of chronic gastric ulcers was significantly impaired in rats with arthritis. Daily administration of indomethacin caused further delay of gastric ulcer healing in arthritic rats. Impairment of the healing response in arthritic rats may be due to less expression of growth factors in the gastric mucosa such as bFGF and IGF-1. Given these findings, it is concluded that arthritic conditions increase the gastric ulcerogenic response to NSAIDs and impair the healing of chronic gastric ulcers. Further studies will be required to understand the relationship between NSAID-induced gastropathy and arthritis and also for the future development of new strategies for the treatment of RA.

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