Aggravation of Cold-Restraint Stress–Induced Gastric Lesions in Adjuvant Arthritic Rats: Pathogenic Role of Inducible and Endothelial Nitric Oxide

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Abstract. It was reported previously that non-steroidal anti-inflammatory drugs (NSAID)–induced gastric damage was markedly aggravated in rats during arthritis, and this response was mediated by the overproduction of nitric oxide (NO) derived from endothelial NO synthase (eNOS) in addition to inducible NO synthase (iNOS). The present study examined the gastric ulcerogenic response to cold-restraint stress in adjuvant arthritic rats, particularly in relation to NO/NOS isozymes. Exposure of normal rats to cold-restraint stress (13°C) produced slight gastric damage 3 h later, but the ulcerogenic response was markedly aggravated in arthritic rats. Pretreatment with \(N^G\)-nitro-L-arginine methyl ester (L-NAME) (a nonselective inhibitor of NOS) slightly increased the cold-restraint stress–induced gastric lesions in normal rats, but dose-dependently prevented the aggravation of these lesions in arthritic rats. The increased ulcerogenic response in arthritic rats was significantly suppressed by 1400W (a selective inhibitor of iNOS) and L-iminoethyl ornithine (L-NIO) (a selective inhibitor of eNOS), but not by \(N^G\)-propyl-L-arginine (L-NPA) (a selective inhibitor of nNOS), and almost totally abolished by the co-administration of 1400W and L-NIO. The mucosal expression levels of eNOS and iNOS but not nNOS mRNAs were enhanced in arthritic rats compared with normal rats. The aggravation of stress-induced gastric lesions in arthritic rats was also significantly suppressed by pretreatment with glutathione. These results suggest that the gastric ulcerogenic response to cold-restraint stress is enhanced in arthritic rats, similar to that induced by NSAIDs, and this phenomenon may be causally associated with the upregulation of eNOS/NO in addition to iNOS/NO.

Keywords: nitric oxide (NO), endothelial NO synthase (eNOS), inducible NO synthase (iNOS), gastric damage, cold-restraint stress

Introduction

Rheumatoid arthritis (RA) is a systemic and chronic disease characterized by persistent inflammation of multiple joints. It was reported previously that the gastric ulcerogenic responses to non-steroidal anti-inflammatory drugs (NSAIDs), including indomethacin, aspirin, and selective cyclooxygenase (COX)-2 inhibitors, were markedly aggravated in rats with adjuvant arthritis (1–3). These findings are interesting from a clinical point of view because RA patients are reportedly more susceptible to NSAID-induced gastropathy than other NSAID users (4). It was further revealed that the increased gastric ulcerogenic response to NSAIDs in arthritic rats is mediated by overproduction of nitric oxide (NO) derived from the inducible isozyme of NO synthase (NOS) (1). NO plays an important role in the regulation of various cellular functions in cardiovascular, neuronal, and immune systems as well as in the gastrointestinal tract (5). NOS, which produces NO from the precursor L-arginine, exists as three isozymes: neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS). We have recently found that the aggravation of damage in arthritic rats is mediated...
by not only iNOS/NO but also by eNOS/NO (6). Because a constitutive isozyme of NOS (cNOS), including eNOS, is believed to play an important role in maintaining the mucosal integrity of the gastrointestinal tract (7), it is interesting that NO produced from the protective isoform, eNOS, acts harmfully in the mucosa of arthritic rats.

It remains unknown whether gastric ulcerogenic responses to other noxious stimuli, such as stress, are similarly aggravated in arthritic rats. The exposure of animals to cold-restraint stress induces gastric lesions, accompanied by a lowering of body temperature, an increase in acid secretion, and an increase in motility (8–10). The blood levels of thyroid-stimulating hormone (TSH), a pituitary hormone that is released by thyrotropin-releasing hormone (TRH), increased during cold-restraint stress in association with the decrease in body temperature (9, 11). The central administration of TRH antiserum reduced the severity of stress-induced gastric damage, suggesting that TRH plays a pathogenic role in the gastric ulcerogenic response to cold-restraint stress (9, 10, 12, 13). Nishida et al. (14) reported that water-immersion restraint stress induced an increase in iNOS activity and a decrease in cNOS activity in the gastric mucosa, and this phenomenon was closely related to the development of gastric damage. Thus, it is possible that the gastric ulcerogenic response to stress may also be modified in arthritic rats.

The present study examined the gastric ulcerogenic response to cold-restraint stress in arthritic rats in relation to NOS isozymes.

Materials and Methods

Drugs

The drugs used were heat-killed Mycobacterium tuberculosis (H37Ra; Difco, Detroit, MI, USA), paraffin oil (Wako, Osaka), indomethacin, N\(^{\text{2}}\)-nitro-L-arginine methyl ester (L-NAME), aminoguanidine (Sigma Chemicals, St. Louis, MO, USA), N\(^{\text{6}}\)-propyl-L-arginine (L-NPA), 1400W (Cayman Chemical, Ann Arbor, MI, USA), L-iminoethyl ornithine (L-NIO; A.G. Science, San Diego, CA, USA), and glutathione [reduced form] (GSH; Nacalai Tesque, Kyoto). L-NIO was first dissolved in ethanol and then diluted with saline to the desired concentration, while the other drugs were dissolved in saline. All drugs were prepared immediately before use and given in a volume of 1 ml/200 g body weight.

Animals

Male Dark Agouti (DA) rats (140–160 g; SLC, Shizuoka) were used. The animals were fed standard rat chow and tap water ad libitum. All experimental procedures were approved by the Experimental Animal Research Committee of the Kyoto Pharmaceutical University.

Induction of adjuvant arthritis

Adjuvant arthritis was induced by injection of 50 μl of Freund’s complete adjuvant (FCA: 10 mg/ml heat-killed Mycobacterium tuberculosis H37Ra suspended in paraffin oil) into the plantar region of the right hindpaw. Normal rats were housed in the same manner for the same period of time, so that age- and batch-matched normal and arthritic rats were used in all of the subsequent experiments. The severity of arthritis was assessed by measuring the paw volume (edema) using a plethysmometer (Ugo-Basile, Comerio-Varese, Italy). Because paw edema in the left (uninjected) hindpaw was observed from 10 days and reached a maximum at 14 days after the injection of FCA, the animals were used at 14 days after injection in all experiments as arthritic rats. Normal and arthritic rats were deprived of food but allowed free access to tap water for 18 h before the experiments.

Induction of gastric damage by cold-restraint stress

The animals were kept in a Bollman cage and placed in a cold room for 3 h, where the ambient temperature was 13°C. This treatment was referred to as a mild stress, because only slight damage occurred in normal rats using this treatment. At the end of stress treatment, the animals were sacrificed under deep ether anesthesia; and the stomachs were removed, inflated by injecting 7 ml of 2% formalin, immersed in 2% formalin for 10 min to fix the gastric wall, and opened along the greater curvature. The area (square millimeters) of each region of hemorrhagic damage developed in the glandular mucosa was measured using a dissecting microscope with a square grid (×10), summed per stomach, and used as a lesion score. The investigator measuring the lesion sizes was kept unaware of the treatment given to the animals. The effects of the following drugs on the development of gastric damage induced by cold-restraint stress were examined in both the age-matched normal and arthritic rats on day 14 after FCA injection: L-NAME, a non-selective inhibitor of NOS (3, 10, and 30 mg/kg); aminoguanidine, a relatively selective inhibitor of iNOS (50 mg/kg); L-NPA, a selective inhibitor of nNOS (10 mg/kg); L-NIO, a selective inhibitor of eNOS (30 mg/kg); 1400W, a selective inhibitor of iNOS (10 mg/kg); and GSH (200 mg/kg).
were given s.c. twice at 18 and 1 h before the onset of stress. The doses of these drugs used in this study were selected on the basis of previously published studies (6, 15 – 8).

Western blotting analyses for NOS isozymes

Under deep ether anesthesia, the animals were sacrificed and the stomachs were removed and incised along the greater curvature. Then, the corpus mucosa was scraped using two glass slides, frozen in liquid nitrogen, and stored at −80°C until use. Each sample was homogenized in lysis buffer (pH 7.4) containing 50 mmol/L Tris-HCl, 150 mmol/L NaCl, 50 mmol/L dithiothreitol, 1 mmol/L EDTA, protease inhibitor cocktail tablet (Complete mini; Roche, Penzberg, Germany), and 1% Triton X-100. After centrifugation at 20,000 × g for 30 min at 4°C, the protein concentration in the supernatants was determined using a BCA protein assay kit (Pierce, Rockford, IL, USA). An appropriate volume of the sample was mixed with an equal volume of sample buffer (pH 6.8, 100 mmol/L Tris-HCl, 4% SDS, 20% glycerol, 10% 2-mercaptoethanol, and 0.02% bromophenol blue), heated at 95°C for 5 min, subjected to SDS-PAGE (50 μg per 10 μl in each lane) using 7.5% acrylamide gels, and transferred electrophoretically to PVDF membranes. The membranes were incubated with goat polyclonal anti-human nNOS (NOS 2) antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA), rabbit polyclonal anti-human eNOS (NOS 3) antibody (Santa Cruz Biotechnology), rabbit polyclonal mouse iNOS (NOS 2) antibody (BD Transduction Laboratory, Franklin Lakes, NJ, USA), or mouse monoclonal anti-β-actin antibody (Sigma Chemicals) and then treated with horseradish peroxidase–conjugated goat polyclonal anti-goat IgG antibody (Santa Cruz Biotechnology), horseradish peroxidase–conjugated rabbit polyclonal anti-goat IgG antibody, horseradish peroxidase–conjugated rabbit polyclonal anti-rabbit IgG antibody (Santa Cruz Biotechnology), or horseradish peroxidase–conjugated rat polyclonal anti-mouse IgG1 antibody (Santa Cruz Biotechnology), respectively. The immunocomplex was visualized by an enhanced chemiluminescence detection system (NEN, Boston, MA, USA) and photographed (VersaDoc 5000; Bio-Rad Laboratories, Hercules, CA, USA). The integrated density of the bands was quantified using ImageJ 1.42q analysis software (National Institutes of Health, Bethesda, MD, USA).

Immunohistochemical study

Under deep ether anesthesia, the animals were sacrificed, the stomachs were removed and washed in phosphate-buffered saline (PBS), and then immersed in 4% paraformaldehyde for 48 h. After the treatment with 10% and 20% sucrose solution, the tissue sample was embedded in O.C.T. compound (Miles, Elkhart, IN, USA) and frozen rapidly in carbon dioxide gas. Cryostat sections (CM1510; Leica, Wetzlar, Germany) were cut serially at a thickness of 20 μm were mounted on MAS-coated slides (Matsunami, Osaka). For the fluorescent immunohistochemical study of eNOS and iNOS, the slides were incubated with rabbit polyclonal anti-human eNOS (NOS 3) antibody (Santa Cruz Biotechnology) or rabbit polyclonal mouse iNOS (NOS 2) antibody (BD Transduction Laboratory) and then treated with Alexa Fluor 488–labeled donkey polyclonal anti-rabbit IgG antibody (Molecular Probes, Eugene, OR, USA) or Alexa Fluor 546–labeled donkey polyclonal anti-rabbit IgG antibody (Molecular Probes), respectively. Immunofluorescence was observed using a confocal laser scanning microscope (LSM-510; Carl Zeiss MicroImag, Inc., Thornwood, NY, USA). For the immunohistochemical study of nitrotyrosine, the slides were incubated with rabbit anti-human nitrotyrosine polyclonal antibody (Upstate, Lake Placid, NY, USA) after the deactivation of endogenous peroxidase with 0.3% H2O2. The immunocomplex was visualized by the avidin–biotin–peroxidase method using a Vectastain ABC-peroxidase kit (Vector, Burlingame, CA, USA). Sections were counter-stained with hematoxylin.

Statistical analyses

Data are presented as the mean ± S.E.M. from 6 rats per group. Statistical analyses were performed using a two-tailed unpaired t-test and Dunnett’s multiple comparison test, and values of P<0.05 were considered to be significant.

Results

Gastric ulcerogenic responses to cold-restraint stress in normal and arthritic rats

The exposure to cold-restraint stress (13°C) for 3 h produced only slight damage in the gastric mucosa of normal rats, the damage score being 1.2 ± 0.5 mm² (Fig. 1A). The gastric ulcerogenic response was markedly enhanced in arthritic rats, and the damage score reached 45.5 ± 3.0 mm². Figure 1B shows the macroscopic observations of gastric damage induced by cold-restraint stress for 3 h. Severe hemorrhagic damage was observed particularly in the corpus mucosa of arthritic rats, while damage was barely found in the gastric mucosa of normal rats.
Effects of L-NAME and aminoguanidine on cold-restraint stress–induced gastric damage in normal and arthritic rats

In normal rats, prior administration of L-NAME (a nonselective inhibitor of NOS: 3–30 mg/kg) dose-dependently aggravated the severity of stress-induced gastric damage, the damage score at a maximal dose (30 mg/kg) being 6.0 ± 1.2 mm², which was significantly greater than that (1.5 ± 0.6 mm²) of the control (vehicle alone). However, aminoguanidine (a relatively selective inhibitor of iNOS, 50 mg/kg) did not affect the ulcerogenic response (Fig. 2). On the other hand, the aggravation of damage induced by cold-restraint stress in arthritic rats was dose-dependently prevented by pretreatment with L-NAME, the damage score at 30 mg/kg being 9.8 ± 1.2 mm², which was almost equivalent to that in normal rats. Likewise, aminoguanidine (50 mg/kg) also significantly suppressed the increased ulcerogenic response in arthritic rats, although the effect was less than that of L-NAME, the damage score was 22.5 ± 2.0 mm², which was still significantly greater than that in normal rats.

Effects of selective NOS inhibitors on cold-restraint stress–induced gastric damage in normal and arthritic rats

In normal rats, the severity of gastric damage induced by cold-restraint stress was significantly aggravated by prior administration of L-NPA (a selective inhibitor of nNOS, 10 mg/kg), the damage score reached 5.7 times greater than that of the control, but damage was not affected by L-NIO (a selective inhibitor of eNOS, 30 mg/kg) and 1400W (a selective inhibitor of iNOS) (Fig. 3). In contrast, the enhanced ulcerogenic response to cold-restraint stress in arthritic rats was significantly prevented by L-NIO and 1400W but not by L-NPA, the inhibition being 53.0%, 42.6%, and 16.5%, respectively. The concurrent administration of L-NIO and 1400W almost totally abolished the aggravation of damage in arthritic rats; the inhibition being 85.2%, which was almost equivalent to that of L-NAME at 30 mg/kg.

Expression of NOS isozymes in the gastric mucosa of normal and arthritic rats

Protein expression levels of nNOS, eNOS, and iNOS were analyzed by Western blotting (Fig. 4). Although the expression levels of nNOS and eNOS were clearly expressed in the gastric mucosa of both normal and arthritic rats, the expression level of eNOS but not
nNOS was markedly enhanced in arthritic rats (2.30 ± 0.24-fold and 0.89 ± 0.06-fold, respectively). In contrast, the mucosal expression of iNOS was hardly detected in normal rats, but was markedly upregulated in arthritic rats (3.3 ± 0.23-fold). On the other hand, exposure to cold-restraint stress slightly, but not significantly, reduced the expression of nNOS in both normal and arthritic rats, while it had no effect on the expression of eNOS or iNOS in these rats.

**Immunohistochemical study of eNOS, iNOS, and nitrotyrosine in the gastric mucosa of normal and arthritic rats**

The expression of eNOS was observed mostly in the vasculature throughout the gastric mucosa in both normal and arthritic rats, but the expression level was markedly enhanced in arthritic rats, particularly around the surface of the gastric mucosa (×200) (Fig. 5: A and B). In contrast, the expression of iNOS was hardly seen in the gastric mucosa of normal rats, whereas strong iNOS expression was found in the epithelial and inflammatory cells in the gastric mucosa of arthritic rats (Fig. 5: C and D).

On the other hand, immunohistochemical staining for nitrotyrosine was not found in the gastric mucosa of normal rats, but the level of staining was slightly increased by exposure to cold-restraint stress for 3 h (Fig. 6: A and B). In contrast, positive immunostaining for nitrotyrosine was clearly detected at the epithelial cells in arthritic rat stomachs, and this staining was markedly enhanced by the exposure to stress (Fig. 6: C and D).

**Effects of GSH on cold-restraint stress–induced gastric damage in normal and arthritic rats**

Prior administration of GSH (200 mg/kg) reduced the severity of stress-induced gastric damage in normal rats, although not significantly, the inhibition being 36.3% (Fig. 7). However, this treatment significantly prevented the aggravation of these lesions in arthritic rats, the inhibition being 64.1%.

**Discussion**

The present study showed that the gastric damage provoked by cold-restraint stress was markedly worsened in adjuvant-induced arthritic rats when compared with normal rats. We previously reported that the gastric ulcerogenic response to NSAIDs, including indomethacin and aspirin as well as selective COX-2 inhibitors, was
markedly enhanced in arthritic rats (1–3). It is assumed that the susceptibility to gastric damage induced by not only NSAIDs but also stress is higher in arthritic rats than normal rats.

We also previously demonstrated that the increased ulcerogenic response to indomethacin in arthritic rats was mediated by the overproduction of NO mainly derived from iNOS (1). Indeed, the expression of iNOS was upregulated in the gastric mucosa of arthritic rats, and the increase in ulcerogenic response was significantly prevented by either l-NAME or aminoguanidine. However, because the protective effect of aminoguanidine was less marked than that of l-NAME, it was speculated that other NOS isozymes are also involved in the increased ulcerogenic response in arthritic rats. Recently, we found using several highly selective inhibitors of various NOS isozymes that the aggravation of damage in arthritic rats was significantly prevented by 1400W (a selective inhibitor of iNOS) and l-NIO (a selective inhibitor of eNOS) but not by l-NPA (a selective inhibitor of nNOS) (6). These findings suggested that the overproduction of NO derived from not only iNOS but also eNOS may be involved in the increased ulcerogenic response to indomethacin in arthritic rats.

In the present study, it was observed that the aggravation of gastric damage induced by cold-restraint stress in arthritic rats was significantly prevented by both l-NAME and aminoguanidine. Similar to the results
obtained in NSAID-induced gastric damage (6), the effect of l-NNAME was more potent than that of aminoguanidine; l-NNAME at 30 mg/kg almost totally prevented the aggravation of damage while aminoguanidine, even at 50 mg/kg, only partially prevented the aggravated response. We recently reported that aminoguanidine (10 – 50 mg/kg) significantly suppressed the aggravation of indomethacin-induced gastric damage in arthritic rats (17). This agent showed the maximal suppressive effect at 30 mg/kg in arthritic rats, but failed to further increase the efficacy at higher dose (50 mg/kg). Thus, the present results together with previous findings clearly suggest that the aggravation of stress-induced gastric damage in arthritic rats is mediated by overproduction of NO derived from not only iNOS but also other isoforms of NOS.

Interestingly, the exacerbation of these lesions in arthritic rats was significantly suppressed by 1400W and l-NIO but not by l-NPA. These agents, 1400W, l-NIO, and l-NPA, have been often used as a selective inhibitor of iNOS, eNOS, and nNOS, respectively (6, 15 – 18). We reported that 1400W at the doses used in the present study almost totally prevented the development of iNOS/NO-mediated gastric injury under ischemia/reperfusion (17). However, 1400W even at a high dose (10 mg/kg) did not totally prevent the aggravation of stress-induced gastric damage in arthritic rats. On the other hand, l-NIO is known to be a selective inhibitor of eNOS, yet the selectivity is not so high. Indeed, this agent at high dose (>30 mg/kg) reportedly suppressed the iNOS/NO-mediated response (16). However, l-NIO given together with 1400W almost totally prevented the aggravation of stress-induced gastric damage in arthritic rats as effectively as l-NNAME. These results suggest that the increased gastric ulcerogetic response to cold-restraint stress in arthritic rats is also mediated by both iNOS/NO and eNOS/NO.

The present study confirmed that the mucosal protein expression of iNOS was only barely detected in normal rats but apparently enhanced in arthritic rats. In addition, it was found that the mucosal expression of eNOS and nNOS was clearly observed even in normal rats, and the expression level of eNOS but not nNOS was markedly upregulated in arthritic rats. Immunohistochemical analysis showed that eNOS expression was mostly observed in the vasculature of the gastric mucosa in normal rats but markedly enhanced in arthritic rat stomachs, particularly in the vasculature around the surface of the mucosa. These findings suggest that the increased gastric ulcerogetic response in arthritic rats is accounted for by the upregulation of eNOS/NO in addition to iNOS/NO. Although eNOS was believed to be a constitutively expressed enzyme, recent studies demonstrated that the expression of this enzyme is also regulated depending on pathophysiological conditions (19). Indeed, the upregulation of eNOS was observed in the gastric mucosa (20) and the colonic mucosa (21) during ulcer healing. However, it remains unknown how eNOS expression in the stomach is enhanced during arthritis. It is possible that systemic inflammatory responses may cause the upregulation of eNOS. Further study is needed to clarify this point.

Nishida et al. (14) reported that water-immersion stress caused a decrease in eNOS activity and an increase in iNOS activity in the stomach and these changes were closely related to the development of gastric damage. They also showed that stress-induced gastric damage was prevented by aminoguanidine but aggravated by \(N^2\)-monomethyl l-arginine (a non-selective inhibitor of NOS). In the present study, although cold-restraint stress slightly decreased the mucosal expression of nNOS in the stomach, this stress, unlike water-immersion stress, did not by itself induce the upregulation of iNOS. These differences in iNOS expression between these two studies may be due to the severity of stress. Cold-restraint stress used in the present study was mild in terms of gastric ulcerogenicity, compared with water-immersion stress, and produced only slight damage in the normal rat stomach.

Consistent with the findings of Nishida et al. (14), in the present study it was observed that cold-restraint stress–induced gastric damage in normal rats was significantly exacerbated by pretreatment with l-NNAME. It was also found that the worsening effect of l-NNAME was mimicked by l-NPA but not by l-NIO or 1400W. Therefore, it was assumed that nNOS/NO may play a protective role in the gastric mucosa against stress. Cold-restraint stress causes gastric hypersecretion and hypermotility through central release of TRH (9, 10, 12, 13). We previously reported that inhibition of NO synthesis enhanced the hypermotility and hypersecretory responses to hypothermia and a TRH analog, respectively (22, 23). Thus, it is possible that these functional changes induced by the inhibition of nNOS/NO production may account for the exacerbation of stress-induced gastric damage in normal rats. On the other hand, we observed that l-NPA, similar to l-NNAME, significantly worsened the gastric ulcerogetic response to stress in normal rats but had no effect on the ulcerogetic response in arthritic rats. Certainly, it is possible that l-NPA may also exacerbate gastric damage in arthritic rats when l-NIO was given together with 1400W. Further study is needed to elucidate the role of nNOS/NO in the mucosal defense in arthritic rats under stress conditions.

It is known that NO interacts with superoxide (\(O_2^-\)) to produce the cytotoxic oxidant peroxynitrite (ONOO\(^-\)),
which has a deleterious influence on gastrointestinal mucosal integrity (24 – 27). The present study showed that the positive immunostaining for nitrotyrosine, a marker of damage by OONO$, was markedly enhanced in the gastric mucosa of arthritic rats compared with normal rats, suggesting the involvement of ONOO$ in the mechanism of the increased gastric ulcerogenic response to cold-restraint stress in arthritic rats.

Several studies demonstrated that NO plays a dual role in the ulcerogenic response of the gastrointestinal mucosa depending on the NOS isozymes; a protective effect of NO derived from nNOS and eNOS (5, 7) and a proulcerogenic effect of NO derived from iNOS (28, 29). As shown in the present study, it is interesting that eNOS/NO is upregulated in arthritis and acts as an ulcerogenic factor in the stomach, similar to iNOS/NO. Endogenous non-protein sulphydryls (SH), mainly present as intracellular GSH, are known to play an important role in the regulation of various physiological functions in the body (30). Several reports showed the protective role of SH in the stomach against various ulcerogenic stimuli (31 – 34). Ueshima et al. (35) reported that indomethacin-induced gastric lesions were exacerbated NO-induced cell injury in vitro. Furthermore, GSH reacts with NO to generate potential NO donors, such as S-nitrosoglutathione (GSNO), which exhibit physiological and protective effects in cardiovascular and gastrointestinal systems (37, 38). Thus, it is possible that GSH may hamper the noxious effect of ONOO$. In the present study, it was observed that GSH, given exogenously, suppressed the aggravation of cold-restraint stress–induced gastric damage in arthritic rats. We previously reported that mucosal non-protein SH contents in the stomach were significantly reduced in arthritic rats (6). These results together suggest that the formation of GSNO may be decreased in the arthritic rat stomach, where excessive production of NO even derived from eNOS may exhibit a proulcerogenic but not a protective effect.

Taken together, the present study showed that the aggravation of gastric damage induced by cold-restraint stress in adjuvant arthritic rats is accounted for by the up-regulation of eNOS/NO, in addition to iNOS/NO. It is assumed that eNOS/NO may act as a proulcerogenic factor in the stomach of arthritic rats.

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