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NITRIC OXIDE, SUPEROXIDE RADICALS AND MAST CELLS IN PATHOGENESIS OF INDOMETHACIN-INDUCED SMALL INTESTINAL LESIONS IN RATS

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We investigated the pathogenic mechanism of indomethacin-induced small intestinal lesions, in relation to nitric oxide (NO), superoxide radicals and mast cells. Rats received indomethacin (1—6 mg/kg) s.c. once daily for 3 days, and the small intestine was examined for lesions 24 hr after the final administration of indomethacin. Indomethacin caused hemorrhagic lesions in the small intestine, mostly in the jejunum and ileum, in dose- and time-dependent manners, with concomitant increase of mucosal microvascular permeability. This treatment also cuased an increase of inducible NO synthase (iNOS) activity with the expression of its mRNA, myeloperoxidase (MPO) activity as well as thiobarbituric acid reactants (TRBAS) in the mucosa, and the changes in iNOS activity preceded those in MPO activity and TRBAS as well as lesion development. These lesions induced by indomethacin were prevented by aminoguanidine (a selective inhibitor of iNOS), dexamethasone (an inhibitor of iNOS mRNA transcription), allopurinol (a xanthine oxidase inhibitor), hydroxyurea (a neutrophil reducing agent) and FR167653 (an inhibitor of interleukin-1/tumor necrosis factor-α production) as well as 16,16-dimethyl prostaglandin E_2ń Likewise, the severity of these lesions was also reduced by mast cell stabilizers FPL-52694 and disodium cromoglycate and a lipoxygenase inhibitor TMK-688, but not affected by tripelennamine (a histamine H_1-receptor antagonist) or methysergide (a serotonin receptor antagonist). These results suggest that: 1) the pathogenic mechanism of indomethacin-induced small intestinal lesions involves superoxide radicals as well as NO produced by iNOS, 2) the deleterious effect of NO may be accounted for by the cytotoxic action of peroxynitrite, produced from NO in the presence of superoxide radicals, and 3) the mast cells may also be involved in the process of small intestinal ulceration, although the mediator responsible remains undefined.

Key words: *indomethacin, intestinal lesions, nitric oxide (NO), inducible NO synthase (iNOS), mast cells*

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INTRODUCTION

Administration of nonsteroidal antiinflammatory drugs (NSAIDs) such as indomethacin to human or experimental animals causes gastrointestinal lesions as side effect. The inhibition of cyclooxygenase, leading to depletion of endogenous prostaglandins (PGs), may be a major factor in the pathogenesis of these lesions, because supplementation with exogenous PGs prevents the occurrence of gastrointestinal damage in response to indomethacin (1). However, there is not without controversy about the involvement of PG deficiency in the pathogenesis, i.e. there is no temporal relationship between changes in mucosal PG contents and development of intestinal lesions (2).

Several studies showed the pathogenic role of bile acid and bacterial flora in the formation of intestinal lesions induced by indomethacin (3—6). Recently, Whittle *et al.* (7) reported the importance of nitric oxide (NO) in the occurrence of microvascular injury in the rat jejunum caused by indomethacin. Miller *et al.* (8) demonstrated the expression of iNOS and formation of peroxynitrite in guinea pig ileitis. The deleterious role of NO in these lesions may be accounted for by a cytotoxic action of peroxynitrite, which is formed by NO and superoxide radicals. Overproduction of NO by iNOS may interacts with superoxide radicals to produce cytotoxic peroxynitrite, resulting in intestinal mucosal integrity (9). Others also proposed that mast cells play a key role in various inflammatory disorders including inflammatory bowel diseases (IBD)(10). Indeed, the number of activated mast cells was reportedly increased in tissues from IBD patients (11, 12).

In the present study, we investigated the pathogenic mechanism of indomethacin-induced small intestinal lesions in rats, especially, in relation to NO/iNOS, superoxide radicals as well as mast cells, and determined the time-sequential order of these changes in association with the occurrence of intestinal mucosal damage.

MATERIALS AND METHODS

Animals

Male Sprague Dawley rats (200～230 g, Nippon Charles River, Shizuoka, Japan) were used. The experiments were performed using 4～8 rats per group under unanesthetized conditions, unless otherwise specified.

Induction of Small Intestinal Lesions by Indomethacin

The animals were given indomethacin in doses of 1～6 mg/kg s.c. once daily for 3 days. FPL-52694 (100 mg/kg, i.p.), disodium cromoglycate (DSCG, 200 mg/kg, i.p.), tripelennamine (5

mg/kg, s.c.), methysergide (0.3 mg/kg, s.c.), TMK688 (30 mg/kg, s.c.) and FR167653 (60 mg/kg, i.p.) as well as 16,16-dimethyl prostaglandin E_2 (16, 16-dimethyl PGE_2; 10 μg/kg, s.c.) were given twice daily for 3 days, each 30 min before and 8hr after indomethacin, while aminoguanidine (20mg/kg, s.c.) and dexamethasone (3 mg/kg, s.c.) were given once daily for 3 days, 30 min before the indomethacin treatment. In some cases, the animals were pretreated with allopurinol (50 mg/kg) or hydroxyurea (200 mg/kg) i.p. for 1 or 2 days, respectively, before the first injection of indomethacin. The animals were killed 24 hr after the final injection of indomethacin under deep ether anesthesia, the lesion score, microvascular permeability, myeloperoxydase (MPO) as well as thiobarbituric acid reactants (TBARS) were measured.

Macroscopic Evaluation of Intestinal Lesions

Under deep ether anesthesia, both the jejunum and ileum were removed, and treated with 2% formalin for fixation of the tissues. Then they were opened along the mesenteric attachment, and examined for lesions under a dissecting microscope with square grids ($\times 10$). The area (mm^2) of macroscopically visible lesions was measured, summed per small intestine, and used as a lesion score. The person measuring the lesions did not know the treatments given to the animals.

Measurement of Microvascular Permeability

The microvascular permeability was evaluated during indomethacin treatment by measuring the amount of extravasated dye (Evans blue) in the small intestine according to methods described earlier (13). In each case, 1 ml of Evans blue (w/v) was injected intravenously 30 min before killing. Under deep ether anesthesia, the animals were killed by bleeding from descending aorta, the small intestines were removed, and the amount of dye that had accumulated in the intestinal mucosa in 30 min was measured. The extraction of dye was measured at 620 nm on a Hitachi spectrophotometer (U-2000, Ibaraki, Japan), and the amount of dye recovered from the intestinal mucosa was expressed as μg per 100 mg wet tissue.

Determination of Lipid Peroxidation and Meyloperoxydase Activity

Lipid peroxidation in the small intestinal mucosa was determined as thiobarbituric acid reactants (TBARS) during indomethacin treatment according to the modified method of Ohkawa *et al.* (14). In brief, the animals were killed under deep ether anesthesia and the small intestines removed. After rinsing the intestine with cold saline, the mucosa was scraped, weighed, and homogenized in 1 ml KCl per 100 mg wet tissue. The homogenate was supplemented with the mixture of TBARS and boiled at 100°C for 1 hr. The reactants were then supplemented with 5 ml of the mixture of n-butanol and pyridine, shaken vigorously for 1 min and centrifuged for 10 min at 4000 rpm. Absorbance was measured at 532 nm on a Hitachi spectrophotometer, and the results were expressed as nmol per 100 mg wet tissue. On the other hand, myeloperoxidase (MPO) activity was also measured in the same samples used for determination of TBARS, according to the method of Castro *et al.* (15). The mucosal scrapings were homogenized in phosphate buffer. The homogenized samples were subjected to freeze and thaw three times, and centrifuged at 2,000 rpm for 10 min at 4°C. After adding 5 μl of 3% H_2O_2 to the supernatant, changes in absorbance at 475 nm of each sample were recorded on a Hitachi spectrophotometer. MPO activity was obtained from the slope of the reaction curve, based on the following equation;

Specific activity (μmol H_2O_2/min/mg protein) = (OD/min)/OD/mol $H_2O_2 \times$ mg protein).

Measurement of NOS Activity

Small intestinal mucosal NOS activity was also measured during indomethacin treatment by determining the conversion of radiolabeled L-arginine to citrulline according to the method described by Bougton-Smith *et al.* (16). Under deep ether anesthesia, the animals were killed, and the small intestines removed. After rinsing the intestine with cold saline, the mucosa was scraped, and homogenized in ice-cold buffer (Tri-HCl 50 mM, sucrose 32 mM, dithiothereitol 1 mM, leupeptin 10 μg/ml and aprotinin 2 μg/ml), adjusted to pH 7.4 with HCl, followed by centrifugation for 20 min at 10,000 rpm at 4°C. The supernatant was incubated for 60 min at 37°C in reaction buffer comprising; Tris-HCl buffer 50 mM, $CaCl_2$ 1.25 mM, valine 12.5 mM, dithiothreitol 1 mM, L-arginine 10 μM, NADPH 100 μM, FAD 10 μM, FMN 10 μM and [^{3}H]-L-arginine 0.5 μCi/ml. The reaction was arrested by removal of substrate L-arginine with addition of aliquot of 50% suspension of Dawex (AG 50W-8, Na^+ form) in water, and each sample was centrifuged at 3,000 rpm for 5 min. After allowing the resin to settle, the supernatant was removed for estimation of the ratiolabeled products by scintillation counting. The activity of constitutive NOS (cNOS) was determined from the difference between in the presence and absence of 1 mM EGTA; thd activity of inducible NOS (iNOS) was determined in the presence of 1 mM EGTA. Sample protein content was estimated via spectrophotometric assay (Pieace protein assay kit), and NOS activity was expressed as pmol/min/mg protein.

Analysis of iNOS-mRNA by RT-PCR

The animals were killed under deep ether anesthesia 6 hr after the first indomethacin injection and the small intestines removed, and then frozen in liquid introgen and stored at-80°C until use. Intestinal tissue samples were pooled from 2—3 rats for extraction of total RNA, which was prepared by a single-step acid phenol-chloroform extraction procedure by use of TRIZOLE (GIBCO BRL, Gaithersburg, USA). Total RNA primed by random hexadeoxyribonucleotide was reversetranscribed with SUPERSCRIPT Preamplification system (GIBCO BRL). The sequences of sense and antisense primers for the rat iNOS were 5′-ACAACAGGAA-CCTACCAGCTCA-3′ and 5′-GATGTTGTAGCGCTGTGTGTCA-3′, respectively, givin rise to a 651 bp PCR product (17). For the rat glyceraldehyde-3-phosphate dehydrogenase (G3PDH), a constitutively expressed gene, the sequences were 5′-GAACGGGAAGCTCACTGGCATGGC-3′ for sense primer and 5′-TGAGGTTCCACCACCCTGT-TGCTTG-3′ for antisense primer, giving rise to a 310 bp PCR product (18). An aliquot of the RT reaction product served as a template in 35 cycles of PCR with 0.5 min of denaturation at 94°C, 0.5 min of annealing at 60°C and 1 min of extension at 72°C on a thermal cycler. A portion of the PCR mixture was electrophoresed in 2.0% agarose gel in TAE buffer (Tris buffer 40 mM, EDTA 2 mM and acetic acid 20 mM; pH 8.1), and gel was stained with ethidium bromide and photographed.

Preparation of Drugs

Drugs used in this study were indomethacin, aminoguanidine, tripelennamine (Sigma Chemicals, St. Louis, MO, USA), dexamethasone, methysergide (Nacali Tesque, Kyoto, Japan), alloprinol, hydroxyurea (Wako, Osaka, Japan), FPL-52694, DSCG (Fison/Fujisawa, Osaka, Japan), FR167653 (Fujisawa, Osaka, Japan), 16,16-dimethyl prostaglandin E_2 (Funakoshi, Tokyo, Japan) and Evans blue (Merck, Darmstadt, Germany). Indomethacin, dexamethasone, alloprinol and hydroxyurea were suspended in saline with a drop of Tween 80 (Wako) while FPL-52694, DSCG and FR167653 were suspended in carboxymethylcellulose (CMC) solution. 16,16-dimethyl

PGE_2 was first dissolved in absolute ethanol and then diluted with saline to a desired concentration. Other drugs were dissolved in saline. All drugs were prepared immediately before used and administered s.c. or i.p. in a volume of 0.5 ml/100 g body weight.

Statistics

Data are presented as the mean $\pm$ SE from 4 to 8 rats per group. Statistically analyses were performed using two-tailed Dunnett's multiple comparison test, and values of $P<0.05$ were regarded as significant.

RESULTS

Induction of Small Intestinal Lesions by Indomethacin

Repeated administration of indomethacin (1, 3 and 6 mg/kg) once daily for 3 days dose-dependently induced hemorrhagic lesions in small intestine, mostly in both the jejunum and ileum, with concomitant increase of musocal microvascular permeability; the lesion score at 3 and 6 mg/kg was 36.0 ± 7.4 and 216.6 ± 12.7 mm^2, respectively (*Fig. 1*). The intestinal lesions caused by 6 mg/kg of indomethacin occurred in a time-dependent manner; slight lesions were observed within 24 hr after the first injection, extending to severe hemorrhagic lesions after the second and third injections, the lesion score being 21.5 ± 6.9, 99.5 ± 9.9 and 216.6 ± 12.7 mm^2, respectively

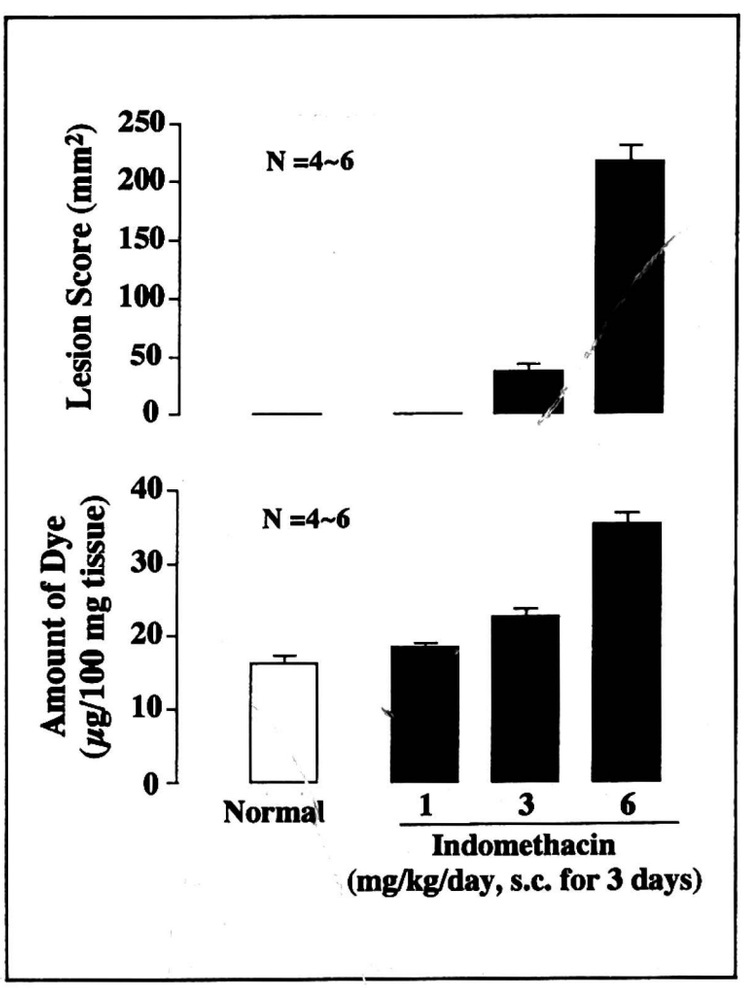


Fig. 1. Dose-response relationship for indomethacin in producing mucosal lesions (upper panel) and increasing microvascular permeability (lower panel) in the rat small intestine. Animals were given indomethacin (1, 3 and 6mg/kg) s.c. once daily for 3 days, and they were killed 24 hr after the final administration of indomethacin. Data are presented as the mean $\pm$ SE from 4~6 rats.

(*Fig. 2*). The increase of microvascular permeability induced by indomethacin at 6 mg/kg was also observed in a time-dependent manner, and the values were 23.2±0.9, 34.6±4.5 and 35.3±1.7 μg/100 mg tissue, respectively, at 24 hr after the first, second and the third injections.

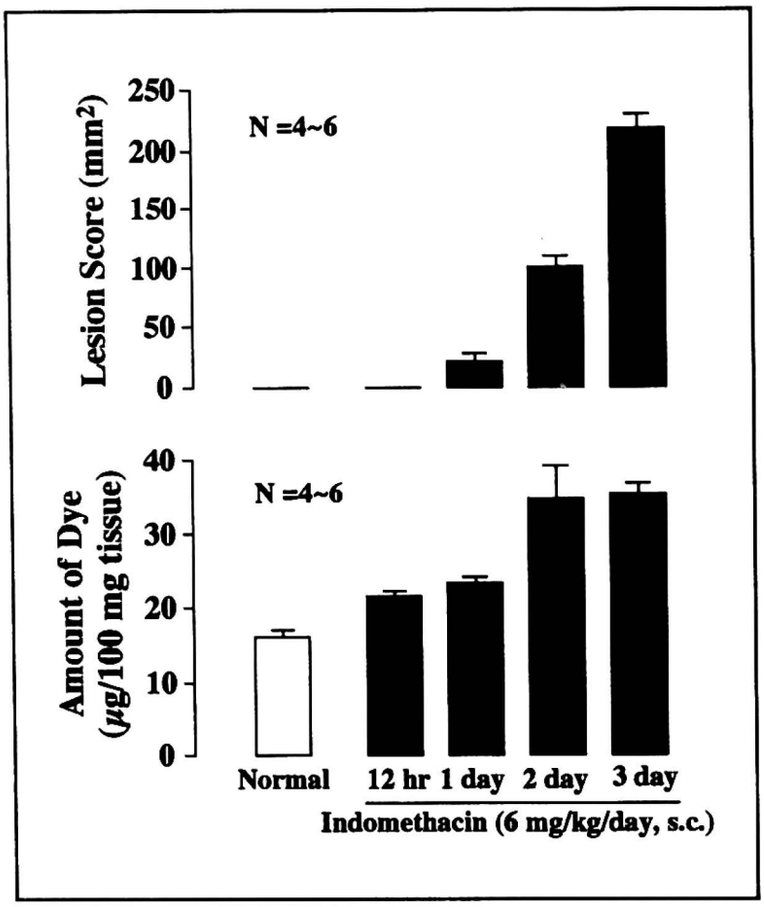
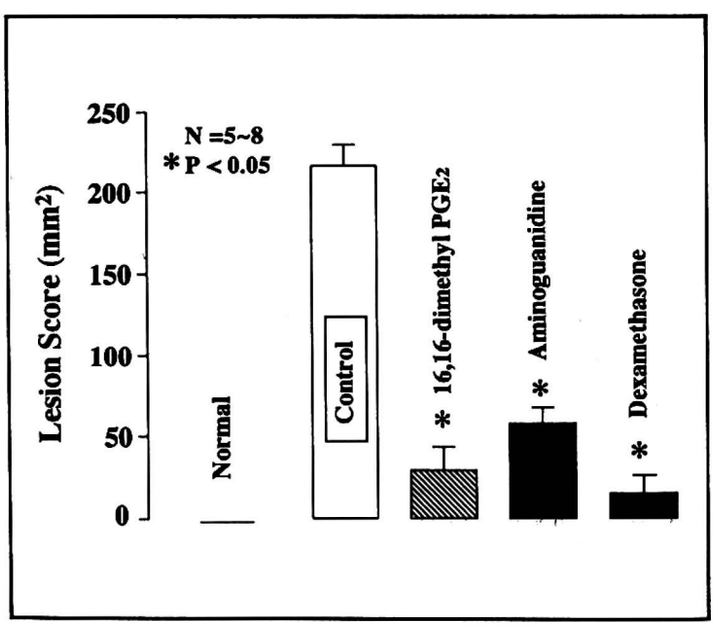


Fig. 2. Time course changes of lesion formation and microvascular permeability in the rat intestinal mucosa after indometchacin treatment. Animals were given indomethacin (6 mg/kg) s.c. once daily for 3 days, and they were killed 12 hr and 24 hr after the first injection, and 24 hr after the second and third injection. Data are presented as the mean±SE from 4~6 rats.

Effects of Various Drugs on Indomethacin-Induced Small Intestinal Lesions

The repeated administration of indomethacin at 6 mg/kg once daily for 3 days caused severe lesions in the small intestine, the lesion score being 216.6±12.7 mm^2. These lesions caused by indomethacin were significantly prevented by aminoguanidine and dexamethasone as well as 16,16-dimethyl PGE_2, the

Fig. 3. Effects of 16,16-dimethyl PGE_2, aminoguanidine and dexamethasone on the development of indomethacin-induced intestinal lesions in the rat. Animal were given indomethacin (6 mg/kg) s.c. once daily for 3 days, and they were killed 24 hr after the final administration of indomethacin. 16,16-dimethyl PGE_2 (10 μg/kg) was administered s.c. twice daily for 3 days 30 min before and 8 hr after indomethacin, while aminoguanidine (20 mg/kg) or dexamethasone (3 mg/kg) was given s.c. once daily for 3 days, each 30 min before indomethacin treatment. Data are presented as the mean±SE from 5~8 rats. * Statistically significant difference from control, at $P<0.05$.



inhibition being 73.2%, 92.6% and 86.3%, respectively (*Fig. 3*). Likewise, the severity of indomethacin-induced small intestinal lesions was significantly reduced by allopurinol (the xanthine oxidase inhibitor), hydroxyurea (the neutrophil reducing agent), and FR167653 (the inhibitor of interleukin-1 and TNF α production), the reduction being 49.0%, 81.8% and 35.4%, respectively

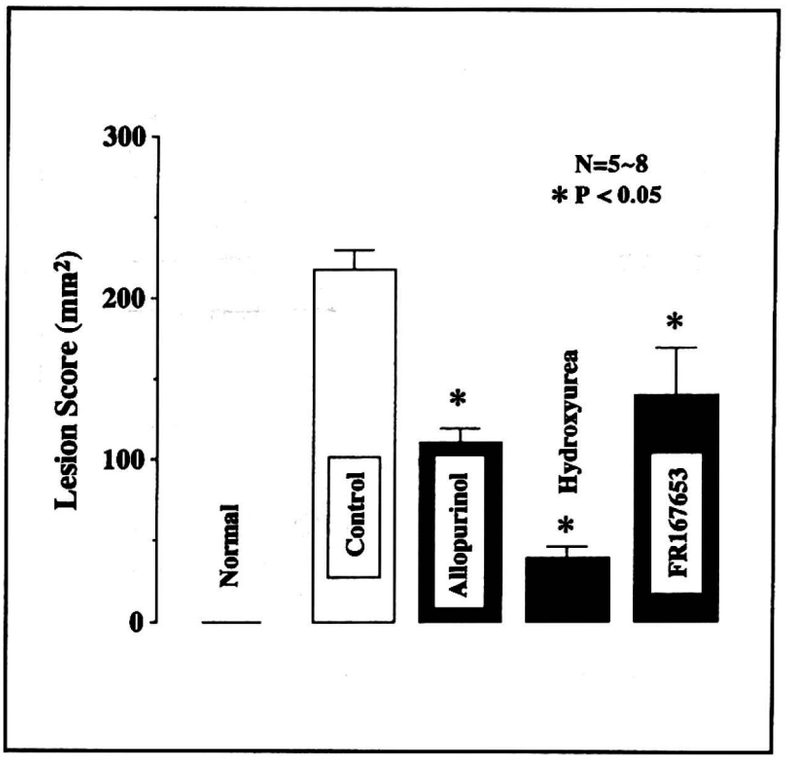


Fig. 4. Effects of allopurinol, hydroxyurea and FR167653 on the development of indomethacin-induced intestinal lesions in the rat. Animals were given indomethacin (6 mg/kg) s.c. once daily for 3 days, and they were killed 24 hr after the final administration of indomethacin. FR167653 (60 mg/kg) was given i.p. twice daily for 3 days 30 min before and 8 hr after indomethacin. Allopurinol (50 mg/kg) or hydroxyurea (200 mg/kg) was given i.p. for 1 or 2 days, respectively, before the experiment, and also administred for 3 days, each 30 min before indomethacin. Data are presented as the mean ± SE from 5~8 rats. * Statistically significant difference from control, at $P < 0.05$.

(*Fig. 4*). In addition, the development of intestinal lesions in response to indomethacin was also prevented by both FPL-52694 and DSCG the mast cell stabilizers or TMK688 the lipoxygenase inhibitor, the inhibition being 82.7%, 58.2%, and 56.4%, respectively. However, the intestinal ulcerogenic response induced by indomethacin was not significantly affected by either tripelennamine the histamine H_1-receptor antagonist or methysergide the serotonin receptor antagonist (*Fig. 5*).

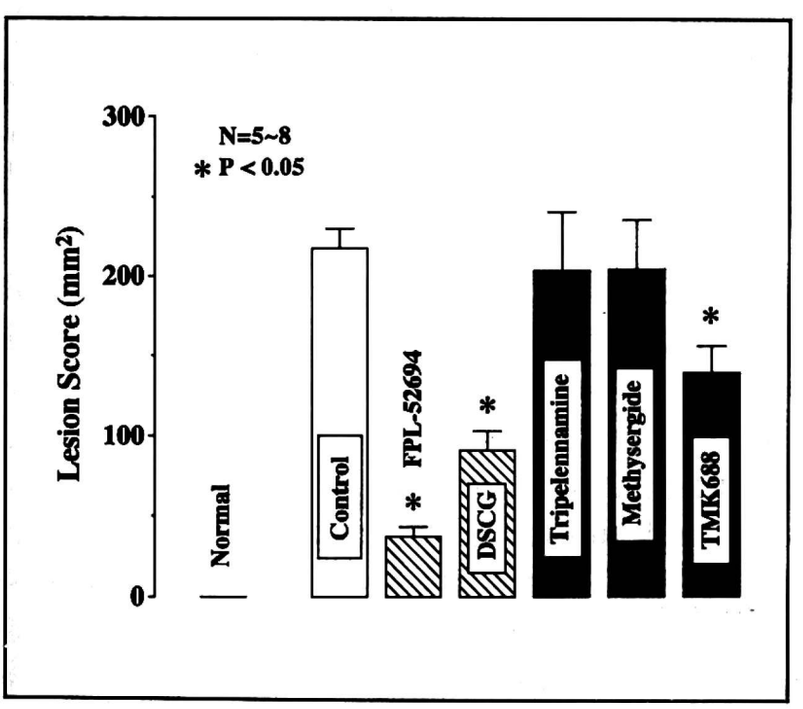


Fig. 5. Effects of FPL-52694, DSCG, tripelennamine, methysergide and TMK688 on the development of indomethacin-induced intestinal lesions in the rat. FPL-52694 (100 mg/kg, i.p.), DSCG (200 mg/kg, i.p.), tripelennamine (5 mg/kg, s.c.), methysergide (0.3 mg/kg, s.c.) or TMK688 (30 mg/kg, s.c.) was given twice daily for 3 days, each 30 min before and 8 hr after indomethacin. Data are presented as the means ± SE from 5~8 rats. * Statistically significant difference from control, at $P < 0.05$.

Biochemical Changes in Small Intestinal Mucosa after Indomethacin

NOS activity: In the normal intestinal mucosa, the iNOS activity was 1.8 ± 0.1 pmol/min/mg protein (*Fig. 6*). Following administration of indomethacin, the mucosal iNOS activity was markedly increased in a time-dependent manner, reaching approximately three times greater than normal values within 12 hr (5.7 ± 1.1 pmol/min/mg protein). The increase of iNOS activity was observed for 3 days during indomethacin treatment, the values observed at 24 hr after the first, second, and third injection being 6.5 ± 1.0, 4.5 ± 1.1 and 3.6 ± 0.2 pmol/min/mg protein, respectively. Pretreatment of the animals with dexamethasone almost totally inhibited the increase of iNOS activity in response to indomethacin, the values being 1.2 ± 0.1 pmol/min mg protein. As shown in *Fig. 7*, the iNOS mRNA was clearly detected in the intestinal mucosa at 6 hr after administration of indomethacin, and this expression was inhibited in the presence of dexamethasone. The increase of iNOS activity caused by indomethacin preceded the increase in the other parameters such as MPO activity and TBARS as well as the development of macroscopical lesions in the small intestine. On the other hand, the mucoal cNOS activity remained unchanged during indomethacin treatment.

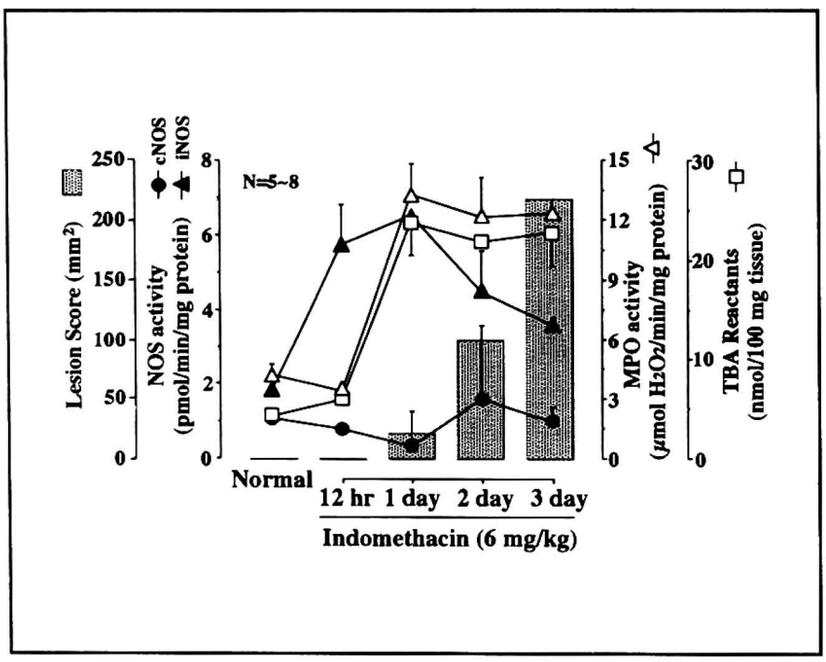


Fig. 6. Changes in NOS activity, MPO activity and TBARS as well as the development of intestinal lesions after administration of indomethacin in the rat. Animals were given indomethacin (6 mg/kg) s.c. once daily for 3 days, and they were killed 12 hr or 24 hr after the first injection, and 24 hr after the second and third injection. Values of NOS activity, MPO activity and TBARS are presented as the mean $\pm$ SE from 5~8 rats. The data of intestinal lesions are taken from *Fig. 2*. * Statistically significant difference from control, at $P < 0.05$.

MPO activity: MPO activity in the normal mucosa was 2.1 ± 0.3 μmol H_2O_2/min/mg protein (*Fig. 6*). Intestinal mucosal MPO activity remained unchanged for 12 hr after the first injection of indomethacin, but markedly increased to over 5 times greater than normal value within the next 12 hr; the values were 11.9 ± 1.7, 10.8 ± 1.4 and 11.3 ± 1.7 μmol H_2O_2/min/mg protein, respectively, at 24 hr after the first, second and third injection of indomethacin.

Lipid peroxidation: The amount of TBARS in the intestinal mucosa was minimal under normal conditions, the values being 8.4 ± 1.1 nmol/100 mg tissue (*Fig. 6*). The values remained unchanged for the initial 12 hr after the first

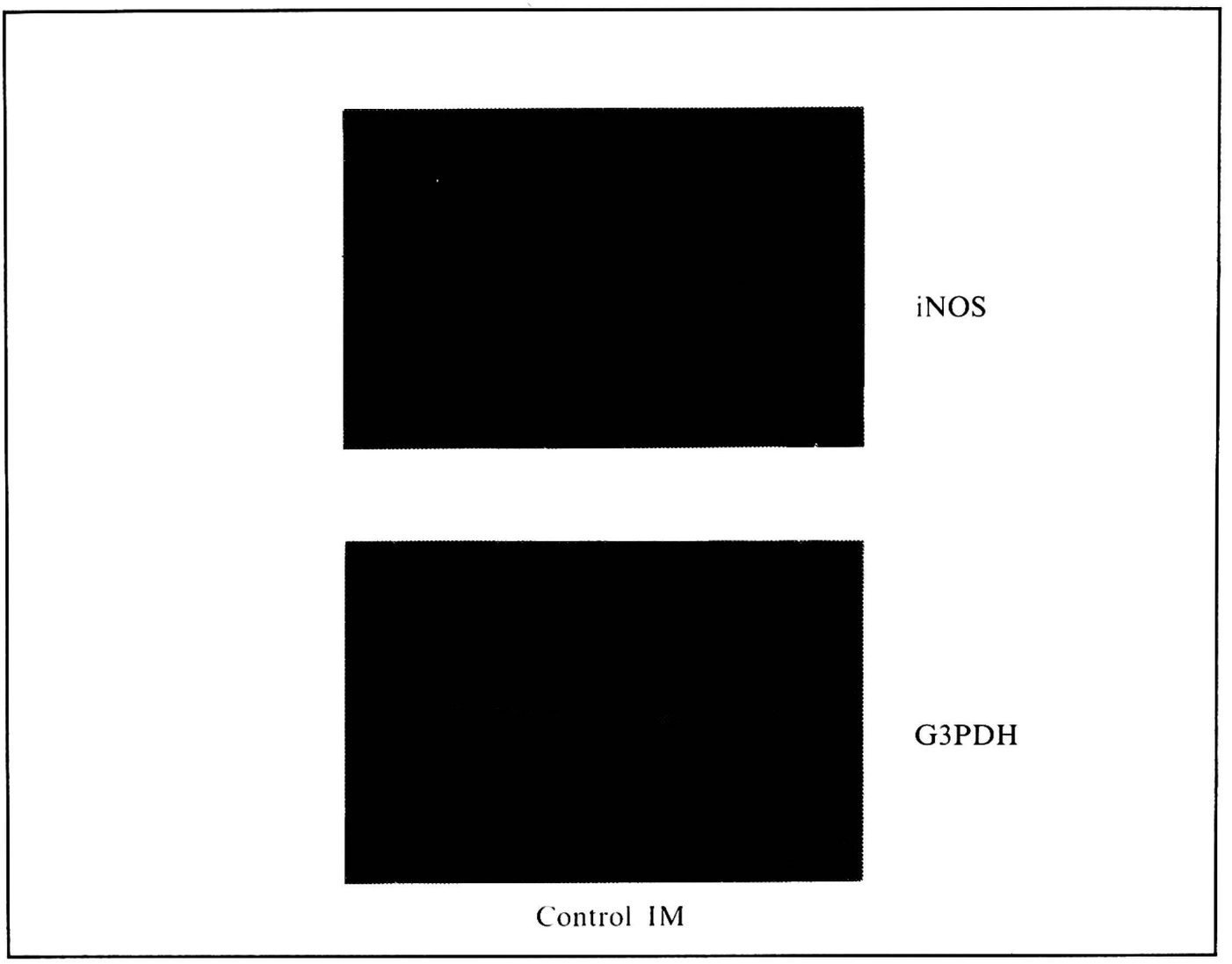


Fig. 7. Mucosal expression of iNOS mRNA in the rat small intestine after indomethacin (IM) treatment. The animal was given indomethacin s.c. in a dose of 6 mg/kg, and the small intestinal mucosa was excised 6 hr later, used for analysis fo iNOS mRNA by RT-PCR. Dexamethasone (3 mg/kg) was given s.c. 30 min before administration of indomethacin.

injection of indomethacin but, within the subsequent 12 hr, markedly increased to over 5 times of normal values, similar to MPO activity; the values being 26.6 ± 3.1, 24.3 ± 4.0 and 24.7 ± 1.5 nmol/100 mg tissue, respectively, at 24 hr after the firt, second, and third injection of indomethacin.

DISCUSSION

The present study showed that endogenous NO produced by iNOS plays a pathogenic role in the development of macroscopical lesions induced in the small intestine after repeated administration of indomethacin. These findings are in agreement with the previous report of Whittle *et al.* (7), who showed that indomethacin increased calcium-independent NO release followed by occurrence of the microvascular injury the rat jejunum. We further demonstrated in this study that the increase in mucosal iNOS activity preceded the elevation of MPO activity and TBARS as well as the occurrence of macroscopical lesions in

the small intestine, strongly suggesting a detrimental role of NO/iNOS in the developmental process of small intestinal lesions.

It is known that short-term and long-term administration of NSAIDs such as indomethacin can cause intestinal damage in human and laboratory animals (2, 6, 19, 20). Several factors have been postulated as the pathogenic element of intestinal lesions induced by indomethacin, including PG deficiency, bacteral flora, and NO (3—7). The depletion of endogenous PGs caused by indomethacin may be a critical factor in the pathogenesis of these lesions, inasmuch as exogenus PGs prevent gastrointestinal damage in response to indomethacin (1). However, since no temporal relationship was found between PG inhibition and lesion formation, and since aspirin was devoid of causing damage in the intestine, despite inhibiting PG biosynthesis, it is unlikely that PG deficiency alone is sufficient to initiate the processes which ultimately result in intestinal lesions (2). On the other hand, intestinal bacterial concentration has been shown to increase following indomethacin administration (6, 7, 21). Indeed, the antibiotics such as metranidazole or ampicillin prevented the intestinal inflammation induced in rats by indomethacin (3, 6). Recently, Boughton-Smith *et al.* (16) reported that bacterial endotoxin from *E. coli*, a predominant member of the rat gut bacterial flora, enhanced the intestinal permeability through increased expression of iNOS. Thus it is possible that the release of bacterial products such as endotoxin may contribute to indomethacin-induced intestinal damage through overproduction of NO by increasing the iNOS expression in the intestinal mucosa.

It is generally believed that NO produced by cNOS is important for housekeeping function in the gastrointestinal mucosa (22, 23). However, a detrimental role for NO produced by iNOS has been reported in the inflammatory disorders such as ulcerative colitis and compound 48/80- or serotonin-induced gastric lesions (24—27), indicating a dual action of NO, both protective and proulcerogenic effects in the gastrointestinal tract. In the present study, we observed that iNOS activity increased in the intestinal mucosa 12 hr after the first administration of indomethacin and that the changes of iNOS activity preceded the increase in MPO activity and TBARS as well as the development of macroscopic lesions. We also observed that the expression of iNOS mRNA was clearly detected in the intestinal mucosa 6 hr after the first injection of indomethacin. These findings were in agreement with a previous report of Whittle *et al.* (7), and further suggested that NO produced by iNOS is the most important element in the process of intestinal lesions following administration of indomethacin.

The present study also showed that the amount of TBARS as well as MPO activity in the intestinal mucosa were significantly increased when determined at 24 hr following the first injection of indomethacin, suggesting the involvement of superoxide redicals and neutrophils in the pathogenesis. Indeed, the

severity of indomethacin-induced intestinal lesions was significantly reduced by pretreatment of the animals with allopurinol a xanthine oxidase inhibitor as well as hydroxyurea a neutrophil reducing agent. We have previously reported that gastric lesions induced by compound 48/80 or serotonin were prevented not only by aminoguanidine but also by antioxidative agents such as superoxide dismutase and allopurinol, suggesting that both NO and superoxide redicals were involved in the pathogenesis of these lesions (26, 27). It is known that NO interacts with the superoxide radicals to produce a cytotoxic peroxynitrite, which causes a deleterious influence on the gastrointestinal mucosal integrity (28). Rachimilewitz *et al.* (29) reported the tissue inflammation by applying the peroxynitrite generating system in the rat colonic mucosa, while Miller *et al.* (8) demonstrated the expression of iNOS and the formation of peroxynitrite in guinea pig ileitis. Thus, it is assumed that a detrimental role of NO in indomethacin-induced small intestinal lesions may be explained by a cytotoxic effect of peroxynitrite produced from NO in the presence of superoxide radicals.

Another interesting finding in this study is that the intestinal ulcerogenic response to indomethacin was significantly prevented by mast cell stabilizers such as FPL—52694 and DSCG, suggesting the involvement of mast cells in the pathogenesis. Mast cells have been considered to play a key role in a variety of chronic inflammatory processes including IBD (10). McAuley & Sommers (11) and Dvorak *et al.* (12) reported the increase of the number of mast cells in tissues from IBD patients, while Balazs *et al* (30) showed the mast cell hyperplasia and its activation during intestinal inflammation. In addition, the severity of these intestinal inflammation was reduced by treatment with mast cell stabilizers (31, 32). Mast cells relase a variety of chemical mediators, such as histamine, serotonin, leukotrienes, cytokines and NO as well as superoxide radicals (33—37). In the present study, we observed that both 5-lipoxygenase inhibitor and IL-1 /TNF-α synthesis inhibitor partly prevented the development of intestinal lesions in response to indomethacin, while these lesions were not affected by either the histamine H_1-receptor antagonist or the serotonin receptor antagonist. Asako *et al.* (38) reported that indomethacin-induced leukocyte adhesion in mesenteric venules was prevented by leukotriene antagonist, indicating the role of 5-lipoxygenase produce in the activation of leukocyte. TNF-α has also been shown to activate the adhesion of leukocyte to endothelial cells (39) and to increase the iNOS expression in various cells (40). These results suggest that intestinal lesions induced by indomethacin may be partly modified by these mast cell-derived mediators. On the other hand, Mannaioni *et al.* (36) demonstrated that mast cells may be a source of NO in addition to superoxide radicals. Thus, it is possible that mast cells may also be involved in the pathogenesis of indomethacin-induced intestinal lesion, probably as the source cell for both NO and superoxide radicals.

In conclusion, the present results taken together suggest that: 1) the pathogenic mechanism of indomethacin-induced small intestinal lesions involves superoxide radicals as well as NO produced by iNOS, 2) the deleterious effects of NO may be accounted for by a cytotoxic action of peroxynitrite, produced from NO in the presence of superoxide radicals, and 3) the mast cells may also be involved in the process of small intestinal ulceration, although the mediator responsible remains to be identified.

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