New Therapeutic Strategy for Amino Acid Medicine: 
Prophylactic and Healing Promoting Effect of Monosodium 
Glutamate Against NSAID-Induced Enteropathy

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Abstract. We reviewed the effect of monosodium glutamate (MSG) on the development and 
healing of nonsteroidal anti-inflammatory drug (NSAID)-induced small intestinal lesions in rats. 
L Roxaprofen (60 mg/kg, p.o.) induced lesions in the small intestine within 24 h, accompanied by a 
decrease of Muc2 expression and an increase in enterobacterial invasion and inducible nitric oxide 
synthase (iNOS) expression. These lesions were prevented when MSG was given as a mixture of 
powdered food for 5 days before the loxoprofen treatment. This effect of MSG was accompanied 
by an increase in Muc2 expression / mucus secretion as well as the suppression of bacterial inva-
sion and iNOS expression. These intestinal lesions healed spontaneously within 6 days, but the 
process was impaired by the repeated administration of low-dose loxoprofen (30 mg/kg) for 5 days 
after the ulceration, with the decrease of vascular endothelial derived growth factor (VEGF) ex-
pression and angiogenesis. The healing-imposing effect of loxoprofen was prevented by feeding 
5% MSG for 5 days after the ulceration. These results suggest that MSG not only prevents loxo-
profen-induced small intestinal damage but also promotes a healing of these lesions; the former 
is functionally associated with the increase in Muc2 expression / mucus secretion and the suppres-
sion of bacterial invasion and iNOS expression, while the latter is associated with the stimulation 
of VEGF expression/angiogenesis.

Keywords: glutamate, loxoprofen, small intestinal damage, protective effect, 
healing-promoting effect, amino acid

1. Introduction

Glutamate is derived from the reaction of glutamic acid with sodium and is encountered mainly as an ingre-
dient of monosodium glutamate (MSG), a substance known to enhance the flavor of foods. On the other hand, 
glutamine, a derivative of glutamic acid, has been re-
ported to protect the stomach against stress- and non-
steroidal anti-inflammatory drug (NSAID)-induced 
damage and enhance the healing of acute or chronic 
gastric ulcers in experimental animals (1 – 4). In Japan, 
this amino acid is used to protect against gastritis and 
gastric ulcers. Glutamine is produced from glutamate and 
ammonia by glutamine synthase, while glutamate is 
generated from glutamine by glutaminase. However, the 
terrelationship of these amino acids in the protective 
action remains unknown.

We previously reported that both glutamine and MSG 
protected gastric epithelial cells against ammonia-induced 
cell death (5), and indeed, they were effective against 
H. pylori–induced diseases in vivo (6). Akiba et al. (7, 8) 
recently reported that MSG protected the duodenal mu-
cosa against acid by enhancing the secretion of mucus/ 
bicarbonate, probably mediated by multiple glutamate
receptors and suggested the importance of the chemosensing system to the duodenal mucosal defense. We also recently found that MSG protected the rat small intestine against the ulcerogenic action of loxoprofen, an NSAID frequently used in Asian countries, and accelerated the healing of these lesions (9). Anyhow, it is assumed that these amino acids exhibit a prophylactic effect against various ulcerogenic actions in the gastrointestinal tract and also a healing-promoting effect on preexisting lesions in these tissues.

In this article, we review, mainly based on our own publications (9, 10 – 12), the ulcerogenic effect of loxoprofen in the small intestine and the protective as well as healing-promoting effects of dietary MSG on loxoprofen-induced intestinal damage, including the mechanisms underlying these actions.

2. Effect of dietary MSG on loxoprofen-induced intestinal damage

NSAIDs are known to damage not only the stomach but the small intestine as well, yet the latter process has been little studied due to technical problems (13, 14). However, with the recent development of capsule-endoscopy and double-balloon endoscopy, we now know that NSAIDs damage the small intestine much more than previously thought (15).

Loxoprofen (10 – 60 mg/kg), administered orally to fed rats, dose-dependently produced multiple hemorrhagic lesions in the small intestine 24 h later, mainly in the jejunum and ileum, similar to other conventional NSAIDs, and the severity of the lesions generated by loxoprofen at 60 mg/kg was almost equivalent to that caused by indomethacin at 10 mg/kg (10, 11). Following the administration of loxoprofen at 60 mg/kg, the level of prostaglandin (PG) E2 in the intestinal mucosa significantly decreased 2 h later, the effect persisting for about 24 h (9). When the animals were pretreated with MSG (0.1% – 5% w/w) given as a mixture with a standard powdered diet (STD, 10 g/rat per day) for 5 days before the loxoprofen treatment, the severity of the intestinal lesions decreased in a concentration-dependent fashion (Fig. 1A). A significant effect was exhibited by MSG at 1% or greater, the lesion score at 5% being approximately 15% of that in the control group fed STD without MSG. Consistent with these findings, we previously reported that the intestinal ulcerogenic response to indomethacin was significantly suppressed by pretreatment with glutamine (1000 mg/kg) given twice daily for 5 days in rats, suggesting the beneficial influence of both glutamate/glutamine on NSAID-induced enteropathy (16).

3. Mechanism underlying MSG-induced intestinal protection

The mechanism responsible for NSAID-induced intestinal damage has also been studied extensively, and several functional changes are known to be involved, including intestinal hypermotility, a decrease in mucus secretion, an increase in inducible nitric oxide (NO) synthase (iNOS), and mucosal invasion by enterobacteria (17 – 21). Enterobacteria are known to play a major role in the pathogenesis of NSAID-induced intestinal ulceration (19). Enterobacteria that have invaded in the mucosa release endotoxin (lipopolysaccharide), which then upregulates the expression of iNOS and causes overproduction of NO (22). Meanwhile, neutrophils accumulate at the site of inflammation and cause production of superoxide radicals. NO reacts with the superoxide radicals, resulting in the formation of peroxynitrite, a very cytotoxic substance (23, 24). This sequence of events eventually leads to the development of intestinal lesions.

As expected, loxoprofen increased the invasion by enterobacteria in the intestinal mucosa when examined 24 h after the administration, similar to other conventional NSAIDs (9, 11). It was also shown that this process occurred as early as 3 h after the administration of indomethacin (21). Pretreatment of the animals with STD containing 5% MSG significantly suppressed the bacterial invasion following loxoprofen treatment. The number of enterobacteria in the mucosa was significantly decreased by MSG to values almost equivalent to that observed in normal animals fed STD without loxoprofen treatment. As shown in Fig. 1B, the expression of iNOS mRNA was upregulated in the intestinal mucosa 6 h after the loxoprofen treatment, consistent with previous results obtained with other NSAIDs (10, 11, 22). The upregulation of iNOS expression was markedly suppressed by pretreatment with 1% MSG for 5 days, confirming a close relationship between enterobacterial invasion and iNOS expression (22). The relation is also strongly supported by the finding that the upregulation of iNOS expression in the small intestine was not observed in rats fasted for 18 h before NSAID treatment.

The mechanism by which NSAIDs, such as loxoprofen, cause bacterial invasion remains unknown. However, previous studies suggest that a decrease in the secretion of mucus may contribute to bacterial invasion after indomethacin treatment (10, 11, 25). Consistent with these findings, loxoprofen decreased mucus production, as evidenced from the histological observation (Fig. 1C). The amount of PAS-positive substances was markedly reduced following the administration of loxoprofen, but this change was significantly reverted by feeding the animals STD containing 1% MSG for 5 days prior to the
loxoprofen treatment. Of interest, 1% MSG alone significantly increased the amount of PAS-positive substances as compared to normal rats. Furthermore, we found that loxoprofen downregulated the expression of Muc2 mRNA in the small intestine, and this response was also reverted by pretreatment with 1% MSG (Fig. 1B). Muc2, an important mucin gene, is known to play a major role in the dimerization of secretory mucin, an essential step in the formation of gastrointestinal mucus-gels (26). Thus, it is possible that MSG upregulates Muc2 expression/mucus secretion, thereby increasing the mucus gel’s thickness and hampering bacterial invasion following the administration of loxoprofen. Since glutamine, a related amino acid, is the precursor of hexosamine, an important component of mucus (27, 28), it would be reasonable that MSG also increases mucus production/secreton through the upregulation of Muc2 expression.

As observed in the small intestine following the administration of other NSAIDs (10, 11, 18), loxoprofen similarly increased neutrophil migration in the small intestine, in addition to bacterial invasion. The myeloperoxidase (MPO) activity in the normal intestinal mucosa was very low but increased markedly following the administration of loxoprofen 24 h later, reaching a level approximately 3 – 4 times higher than the control value. The increased MPO activity was significantly suppressed by feeding the animals a diet containing 1% MSG for 5 days prior. In panel C, PAS-positive mucus was quantified by image processing. Data are presented as the mean ± S.E.M. for 4 – 5 rats. Significant difference at $P < 0.05$; * from Normal; # from loxoprofen + STD. (data adopted after modification from Ref. 9)

**Fig. 1.** Effect of MSG on loxoprofen-induced small intestinal ulceration (A), the expression of iNOS and Muc2 mRNA (B), and the amount of PAS-positive substances in the small intestinal mucosa (C) in rats. Animals were given loxoprofen (60 mg/kg), p.o. and killed 24 h later. MSG was mixed into the standard diet (STD) in amounts of 0.1% – 5% (w/w) and given for 5 days before the administration of loxoprofen. Data are presented as the mean ± S.E.M. from 5 – 10 rats. *Significant difference from STD, at $P < 0.05$. In panel B, note that the expression of iNOS and Muc2 was increased and decreased, respectively, after loxoprofen treatment, but these responses were both reversed by feeding the animals STD containing 1% MSG for 5 days prior. In panel C, PAS-positive mucus was quantified by image processing. Data are presented as the mean ± S.E.M. for 4 – 5 rats. Significant difference at $P < 0.05$; * from Normal; # from loxoprofen + STD. (data adopted after modification from Ref. 9)

4. **Effect of dietary MSG on healing of loxoprofen-induced intestinal damage**

Loxoprofen (60 mg/kg), given p.o. to fed rats, produced multiple hemorrhagic lesions in the small intestine, but the wounds healed quite rapidly within 6 days; the lesions on day 6 were reduced to approximately 20.8% of their initial size observed 24 h after the administration.
of loxoprofen. Consistent with the macroscopic observation, the area of damage became smaller with time, and the damaged portion on day 6 was surrounded by granulation tissue and covered with a newly formed thin epithelium. Since in this model the lesions heal quite rapidly within a few days, one cannot easily detect the healing-promoting effect of drugs. So, it is necessary to impair the repair process in order to effectively detect the healing-promoting action. Concerning the healing of NSAID-induced intestinal damage, we previously reported that endogenous PGs produced by both cyclooxygenase (COX)-1 and COX-2 are involved in the healing of small intestinal lesions generated by indomethacin; COX-2 in the early phase and COX-1 in the late phase of the healing process (12, 29). Thus, we administered low doses (10 – 30 mg/kg) of loxoprofen once daily for 5 days starting 1 day after ulceration caused by loxoprofen at 60 mg/kg. Loxoprofen alone at such low doses rarely damaged the small intestine. As shown in Fig. 2A, loxoprofen at low doses impaired the healing of intestinal lesions generated by 60 mg/kg of loxoprofen, in a dose-dependent fashion, and a significant effect was observed at 30 mg/kg. Interestingly, the delayed healing of loxoprofen-induced intestinal damage was counteracted by feeding the animals STD containing MSG (1% and 5%) for 5 days after the ulceration, resulting in a significant reduction in the lesion score at 5% MSG (Fig. 2B). The healing-impairing effect of indomethacin was antagonized by the repeated administration of PGE2 or an EP4 agonist, suggesting the importance of PG supplementation to the healing process under such adverse conditions (12, 29). However, since MSG treatment did not affect PG production in the intestinal mucosa, it is unlikely that MSG promoted the delayed healing of the intestinal lesions through an amelioration of the PG deficiency.

Fig. 2. Effects of low-dose loxoprofen and MSG on the healing of small intestinal lesions caused by loxoprofen in rats. A: Effect of low-dose loxoprofen on the healing of small intestinal lesions caused by loxoprofen (60 mg/kg) in rats. Small intestinal lesions were produced by a single administration of loxoprofen (60 mg/kg), p.o., and the animals were given low doses of loxoprofen (10 – 30 mg/kg) once daily for 5 days starting from 1 day after the ulceration. Data are presented as the mean ± S.E.M. for 4 – 5 rats. *Significant difference from control, at P < 0.05. B and C: Effect of MSG on the delayed healing of loxoprofen-induced small intestinal damage (B) and the changes in VEGF protein expression in the small intestinal mucosa following loxoprofen treatment (C) in rats. Animals were given loxoprofen (60 mg/kg), p.o. once, followed by repeated administration of a low dose of loxoprofen (30 mg/kg) once daily for 5 days starting 1 day after the ulceration and killed 6 days after the ulceration. MSG was mixed into the standard diet (STD) in the amount of 1% or 5% (w/w) and given for 5 days starting 1 day post-ulceration. Data are presented as the mean ± S.E.M. for 5 – 10 rats. *Significant difference from STD, P < 0.05. In panel C, note that the VEGF expression examined on day 4 after the ulceration was decreased by a low dose of loxoprofen, but this response was reversed by feeding the animal STD containing 5% MSG during loxoprofen treatment. (data adopted after modification from Ref. 9)
5. Mechanism underlying MSG-induced healing-promoting effect

The healing of tissues involves multiple steps, including the formation of granulation tissue, contraction of the ulcerated tissue and re-epithelialization, and these processes are regulated by growth factors, such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor, which are known to be fundamental regulators of angiogenesis (30 – 32). We previously found that VEGF expression was increased in the small intestine from 1 day after the administration of indomethacin, peaking 3 days later (12). We also found that the changes in VEGF expression appeared to parallel those in mucosal PGE2 content in the small intestine. Furthermore, the up-regulation of VEGF expression was significantly suppressed by repeated treatment with indomethacin at a low dose (2 mg/kg) and this effect was antagonized by PGE2 or an EP4 agonist, suggesting the healing-promoting action of PGE2 to be associated with an increase in angiogenesis through the upregulation of VEGF expression via the activation of EP4 receptors (12, 29). Consistent with these findings, loxoprofen treatment at 30 mg/kg given once daily for 3 days downregulated VEGF expression in injured intestinal mucosa when examined 4 days after the ulceration (Fig. 2C). However, the decrease in VEGF expression was completely restored by feeding the animals STD containing 5% MSG for 3 days after the ulceration. On day 6 after loxoprofen treatment (60 mg/kg), the damaged portion was restituted by the growth of granulation tissue and a newly formed microvasculature, as represented by Factor VIII-positive cells (9). The angiogenic response was also suppressed by the repeated administration of loxoprofen (30 mg/kg) after the ulceration, and the degree of vascularization was lower than that in the control. However, the deleterious effect of loxoprofen (30 mg/kg) was antagonized by cotreatment with STD containing 5% MSG (9). Other studies have showed that glutamine prevents gut mucosal injury and improves mucosal recovery following lipopolysaccharide endotoxemia in rats (33), and glutamine enhances glucose-induced mesangial cell proliferation.

Fig. 3. Factors involved in the development and healing of NSAID-induced small intestinal damage, and the influences of dietary MSG on these processes. NSAIDs cause functional changes such as an increase in intestinal motility and a decrease in mucus secretion, followed by enterobacterial invasion in the mucosa. Endotoxin released from enterobacteria upregulates iNOS expression and NO production as well as inflammation, and by so doing results in damage to the small intestine. MSG increases mucus secretion through the upregulation of Muc2 expression and hampers the mucosal invasion by enterobacteria and so suppresses the upregulation of iNOS expression and neutrophil migration, eventually preventing damage to the small intestine. On the other hand, the healing of NSAID-generated small intestinal lesions is brought about by stimulating angiogenesis through the upregulation of VEGF expression. It is possible that dietary MSG, by upregulating VEGF expression, not only increases angiogenesis but also stimulates the proliferation/migration of epithelial cells, thereby accelerating the healing of small intestinal lesions.
Thus, other effects of MSG, such as stimulation of cell proliferation/migration, should also be considered as a mechanism involved in the healing-promoting action.

6. Summary and future prospects

The pathogenic mechanism of NSAID-induced small intestinal damage remains to be fully elucidated, yet there is no doubt that enterobacterial invasion plays the most important role in the pathogenesis of these lesions (19–22). This process is facilitated by a decrease in the secretion of mucus as well as abnormal intestinal hypermotility (21). Since mucus plays a crucial part in the innate host defense against intestinal pathogens and irritants, it is possible that the enhanced intestinal contraction plays a role in the pathogenesis of NSAID-induced intestinal damage by accelerating bacterial invasion in the mucosa. Alternatively, intestinal hypermotility causes mucosal hypoxia and microvascular injury due to smooth muscle contraction, leading to neutrophil infiltration and the release of various cytokines (35). Anyhow, these functional alterations weaken the intestinal barrier, resulting in bacterial invasion, which in turn increases iNOS expression and NO production, eventually resulting in NSAID-induced intestinal damage (21, 22).

As shown in this review, MSG upregulated Muc2 expression as well as mucus secretion and enhanced the intestinal barrier function, resulting in the suppression of bacterial invasion and iNOS expression, and by so doing prevented the development of intestinal lesions in response to loxoprofen (Fig. 3). In addition, it was also found that MSG counteracted the delayed healing of these intestinal lesions caused by repeated treatment with loxoprofen after ulceration, and the healing-promoting action may be at least partly accounted for by stimulation of angiogenesis via the upregulation of VEGF expression. At present, the precise mechanisms underlying the prophyllactic effects of MSG on NSAID-induced intestinal damage remain unknown, yet it is possible that dietary MSG not only protects the small intestine against NSAID-induced damage but promotes the healing of these lesions as well. Further study is required to examine the involvement of glutamate receptors in these actions of MSG and also the interrelationship between MSG and glutamine.

References

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