

Protective Effects of Ginger against Aspirin-Induced Gastric Ulcers in Rats

Zhongzhi Wang, Junichi Hasegawa, Xinhui Wang, Akiko Matsuda, Takahiro Tokuda, Norimasa Miura and Tatsuo Watanabe*

*Division of Pharmacotherapeutics, Department of Pathophysiological and Therapeutic Science, and *Division of Integrative Physiology, Department of Functional, Morphological and Regulatory Science, School of Medicine, Tottori University Faculty of Medicine, Yonago 683-8503, Japan*

We investigated the mechanism underlying the protective effects of ginger against gastric damage induced by aspirin in rats. Gastric mucosal lesions were produced by orally administering 200 mg/kg aspirin suspended in 1% carboxymethylcellulose solution to pyloric-ligated male Wistar rats. Ginger powder (200 mg/kg) markedly reduced the aspirin-induced gastric hemorrhagic ulcer area. The total acidity of gastric juice was not significantly influenced by aspirin or ginger. Ginger powder did not affect the aspirin-induced reduction in mucosal prostaglandin E₂ (PGE₂) content; however, it did ameliorate the aspirin-induced increases in mucosal activity of the inducible form of NO synthase (iNOS) and plasma tumor necrosis factor (TNF)- α and interleukin (IL)-1 β levels. In the next experiment, high and low doses of 6-gingerol and 6-shogaol were used instead of ginger powder in the same experimental model to examine their roles in the anti-ulcer mechanism of ginger. Both 6-gingerol and 6-shogaol reduced aspirin induced ulcer formation, mucosal iNOS and plasma TNF- α and IL-1 β levels. In conclusion, ginger powder prevents the aspirin induced gastric ulcer formation by reducing mucosal iNOS activity and the plasma levels of inflammatory cytokines but does not affect gastric juice or acid production or mucosal PGE₂ content. This protective effect of ginger powder against gastric ulcers may be attributable to both gingerol and shogaol.

Key words: aspirin; gastric damage; ginger powder; inflammatory cytokine; inducible form of NO synthase activity

Aspirin is a potent nonsteroidal anti-inflammatory drug (NSAID) that is used for the treatment of rheumatoid arthritis and related diseases as well as the prevention of cardiovascular thrombotic diseases. Gastric ulcer associated with the use of aspirin is a major problem. Many factors such as gastric acid and pepsin secretion, gastric microcirculation, prostaglandin E₂ (PGE₂) content (Laine et al., 2008), and proinflammatory cytokines interleukin (IL)-1 β and tumor necrosis factor (TNF)- α (Santucci

et al., 1995; Appleyard et al., 1996) play important roles in the genesis of gastric mucosal damage, and its subsequent development (Wang et al., 2007; Wallace, 2008). It has been reported that increases in NO synthase (NOS) activity is involved in the gastrointestinal mucosal defense and also in the pathogenesis of mucosal damage (Muskara et al., 1999; Wallace et al., 2000).

Ginger (*Zingiber officinale*) has been used as a spice and an ingredient of Chinese traditional

Abbreviations: IL, interleukin; iNOS, inducible form of NOS; LPS, lipopolysaccharide; NIH, National Institute of Health; NOS, NO synthase; NSAID, nonsteroidal anti-inflammatory drug; PGE₂, prostaglandin E₂; TNF, tumor necrosis factor

stomach medicine for thousands of years. In traditional medicine, ginger has been used to treat many inflammatory conditions and associated pain (Altman and Marcussen, 2001). The major pungent constituents of ginger, 6-gingerol and 6-shogaol, have been shown to have many interesting pharmacological effects, such as anti-oxidant, anti-tumor promoting and anti-inflammatory effects (Surh, 2002; Kim et al., 2005; Young et al., 2005). However, the mechanism underlying the protective effects of ginger against gastric damage is unclear. Here, we investigated the antiulcer effects of ginger in aspirin-induced gastric ulcer model rats.

Materials and Methods

Animal experiment using ginger powder

The experimental protocol was approved by the ethics committee on animal experiments in Tottori University, and the experiments were carried out in accordance with the guidelines for animal experiments in the same facility.

Male Wistar rats at 8 weeks of age (weighing 300–350 g) were purchased from Shimizu Laboratory Supplies (Kyoto, Japan). They were acclimated in an air-conditioned room at 25°C and 55% humidity and given standard chaw for a few days. Before surgery, the rats were fasted for 24 h and allowed free access to drinking water. Then, the animals were anesthetized using pentobarbital (30 mg/kg body weight, intraperitoneally), the abdomen was opened, and the pyloric end of stomach was ligated without causing any damage to its blood supply. The stomach was then replaced, and the abdominal wall was closed in two layers with sutures.

After they had fully recovered from the anesthesia, the animals were divided into 4 groups of five animals each. Group 1 orally received 3 mL of 1% carboxymethylcellulose in water (vehicle) by gavage. Group 2 orally received ginger powder (200 mg/kg body weight) suspended in 3 mL of 1% carboxymethylcellulose in water. Group 3 orally

received aspirin (200 mg/kg body weight) suspended in 3 mL of 1% carboxymethylcellulose in water. Group 4 orally received aspirin together with ginger powder suspended in 3 mL of 1% carboxymethylcellulose in water. At 4 h after the test drug administration, the animals were anesthetized, and then blood was collected from the heart, and the stomach was removed after the esophageal end had been tied (Jainu and Devi, 2006; Jainu et al., 2006). The stomach was cut open along the greater curvature, and the contents were collected in tubes and centrifuged at $200 \times g$ for 10 min. The resultant supernatant was used for the estimation of acid and gastric juice output. The stomach was then washed with warm saline, and the inner surface was photographed to allow the measurement of the area covered by hemorrhagic ulceration. Next, the gastric mucosal tissues were removed, frozen in liquid nitrogen and stored at -80°C . The total acid content of the gastric juice was determined by titrating it with 0.01 N NaOH, using phenolphthalein as indicator, and was expressed as mEq/4 h/100 g (Jainu et al., 2006; Wang et al., 2007). The acidity of gastric juice was calculated as total acid content/gastric juice volume in mEq/mL (Khushtar et al., 2009).

In animal experiments using 6-gingerol and 6-shogaol, the same procedures as were used in the first experiment to produce the pylorus ligated rats were repeated using high dose (2 mg/kg) and low dose (1 mg/kg) 6-gingerol, and high dose (1 mg/kg) and low dose (0.5 mg/kg) 6-shogaol, instead of ginger powder suspended in 3 mL of 1% carboxymethylcellulose in water. The doses of 6-gingerol and 6-shogaol used in the experiment were determined by those found in normal ginger (Schwertner and Rios, 2007).

Quantification of the gastric hemorrhagic ulcer area using NIH image

The photographs of the stomach were digitized and converted to binary images through gray scale imaging (Khan, 2004). Using the National Institute of Health (NIH) image-J software, the area of gastric hemorrhagic ulcers (mm^2) was calculated.

Histological studies

Gastric tissue samples from each group were fixed in 10% formalin for 24 h. The specimens were then embedded in paraffin, sectioned and stained with hematoxylin and eosin, before being evaluated by light microscopy.

Measurement of mucosal PGE₂

Frozen gastric mucosal tissue (1 g) was added to 5 mL homogenization buffer (0.1 M phosphate (pH 7.4), containing 1 mM EDTA and 10 µM indomethacin) and homogenized. The lysate was then centrifuged in a microcentrifuge at 16,000 × *g* for 15 min at 2°C to 8°C. The supernatant was transferred to a new tube, and its total protein content was analyzed using the advanced protein assay. PGE₂ concentrations were investigated using the PGE₂ ELISA Kit (R&D Systems, Minneapolis, MN).

Measurement of iNOS activity

Individual specimens of the gastric mucosa were homogenized in sample buffer containing 10 mM EDTA and centrifuged at 13,000 × *g* at 4°C for 5 min. The supernatant was transferred to a new tube and had a total protein content of 10 µg/µL. Gastric mucosal iNOS activity was measured with the NOS-detect assay kit (Agilent Technologies, Santa Clara, CA).

Determination of plasma TNF-α and IL-1β levels

Blood samples in EDTA-containing vials were centrifuged at 1000 × *g* for 10 min at 4°C. The levels of IL-1β and TNF-α were determined by ELISA according to the manufacturer's instructions (Assay Designs, Ann Arbor, MI; Bender MedSystems, San Diego, CA).

Reagents

Aspirin was purchased from Sigma Chemical (St. Louis, MO). Carboxymethylcellulose sodium salt, 6-gingerol and 6-shogaol were purchased from Wako Pure Chemical Industries (Osaka, Japan). The ginger powder was a generous gift from Yawata Bussan (Yonago, Japan). It has been confirmed that 100 g of ginger powder contains 0.68 g of 6-gingerol (Japan Food Research Laboratories, Tokyo, Japan).

Statistical analysis

All values are expressed as the mean ± SEM. All results were analysed by one way analysis of variance followed by the proper post-hoc test by SPSS 11.0 J (SPSS Japan, Tokyo). *P* < 0.05 was considered statistically significant.

Results

Aspirin-induced gastric hemorrhagic ulcer formation

The macroscopic findings of the opened stomach are shown in Fig. 1a. Hemorrhagic gastric ulcers covered with coagulated blood were more apparent in the aspirin administered group (Aspirin, Group 3) than the control (Control, Group 1). Ginger powder alone (Ginger, Group 2) had no effects on the stomach, and the coadministration of ginger powder with aspirin inhibited aspirin-induced ulcer formation (Aspirin + Ginger, Group 4). Figure 1b shows the mean area of gastric hemorrhagic ulcers in each group. Even in the control conditions, pin-point ulcers were sometimes seen. Aspirin-induced ulcer formation was completely inhibited by the coadministration of ginger powder (*P* < 0.01).

Histopathological findings of gastric mucosa

The histopathological findings of the gastric mucosa are shown in Fig. 2. The gastric mucosa obtained from the control (Group 1) rats showed

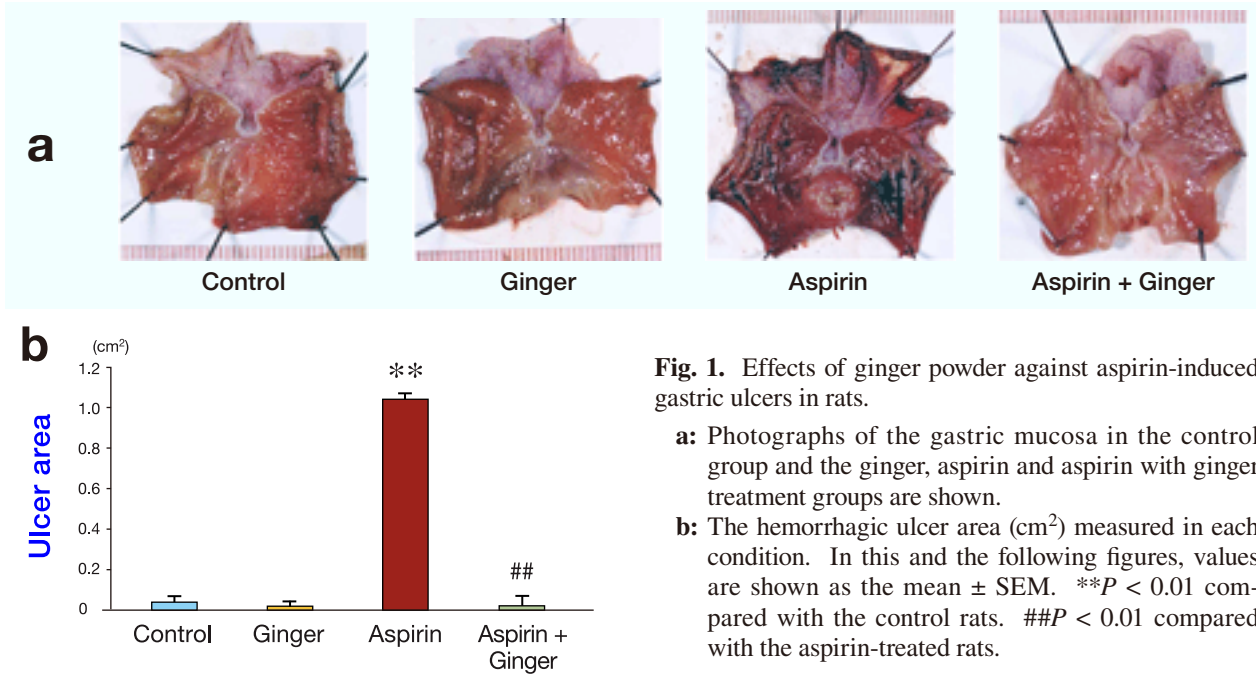


Fig. 1. Effects of ginger powder against aspirin-induced gastric ulcers in rats.

a: Photographs of the gastric mucosa in the control group and the ginger, aspirin and aspirin with ginger treatment groups are shown.

b: The hemorrhagic ulcer area (cm²) measured in each condition. In this and the following figures, values are shown as the mean ± SEM. ***P* < 0.01 compared with the control rats. ##*P* < 0.01 compared with the aspirin-treated rats.

an intact cellular architecture (not shown). Ulcers combined with distorted gastric glands, a damaged mucosal epithelium, inflammatory exudates and cellular debris were found in the stomachs of the aspirin-treated rats (Fig. 2a, Aspirin). The protection against these histopathological changes induced by the coadministration of ginger powder

to rats resulted in the maintenance of glandular organization and the structure of the muscularis mucosa (Fig. 2b, Aspirin + Ginger). The histological examination of the ginger powder alone-treated rats showed a normal cellular architecture in the gastric mucosa with no pathological changes (not shown).

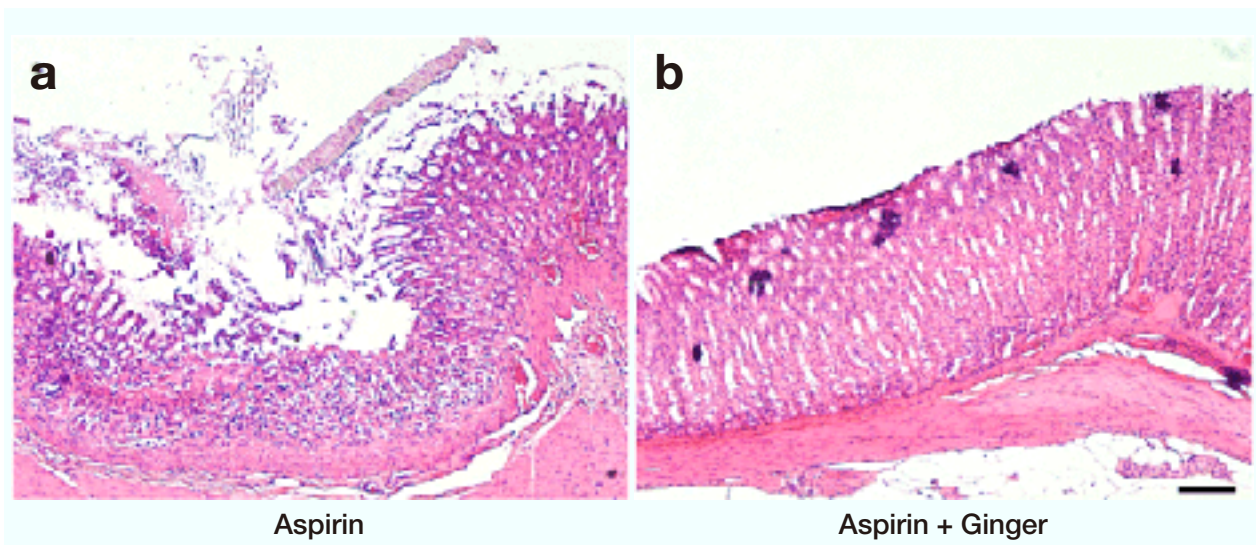


Fig. 2. Histological examinations of gastric mucosal tissue. Ulcer formation with distorted gastric glands, a damaged mucosal epithelium and cell debris are shown in **a** (Aspirin); however, the coadministration of ginger with aspirin protected against these changes, as shown in **b** (Aspirin + Ginger) (hematoxylin and eosin stain). Bar = 500 μm.

Gastric juice and acid production

Both ginger and aspirin reduced gastric juice production /100 g body weight. However this effects disappeared by coadministration of ginger and aspirin. The total acidity showed no significant differences among these conditions (Table 1).

Mucosal PGE₂ levels

The gastric mucosal PGE₂ contents measured in each condition are compared in Fig. 3. Aspirin reduced the mucosal PGE₂ content, and the coadministration of ginger powder failed to inhibit the aspirin-induced reduction of PGE₂ content.

Mucosal iNOS expression

Aspirin administration led to a significant increase in gastric mucosal iNOS activity in the ulcerated rats as compared to that measured in the control or ginger administered rats (Fig. 4). The coadministration of ginger powder resulted in a significant decrease in gastric mucosal iNOS activity compared with that observed in the aspirin alone group ($P < 0.05$). No differences were seen in iNOS lev-

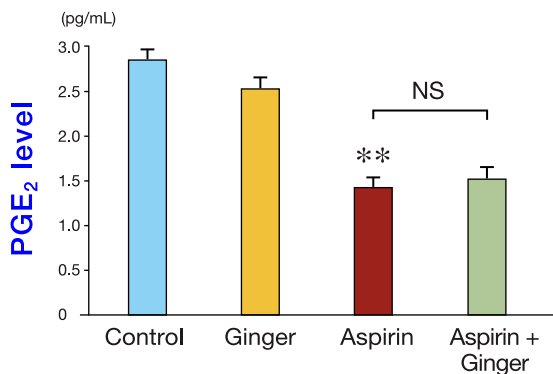


Fig. 3. Effects of ginger powder on the PGE₂ level of the gastric mucosal tissue. Aspirin administration reduced the PGE₂ level, and the coadministration of ginger powder (200 mg/kg) for 4 h failed to inhibit the reduction in the PEG₂ level. ** $P < 0.01$. NS, not significant; PGE₂, prostaglandin E₂ (pg/mL).

Table 1. Effects of ginger powder on gastric mucosal factors in experimental gastric ulcer model rats

Group	Parameter	
	Volume of gastric juice (mL/100 g)	Acidity (mEq/mL)
Control	6.15 ± 1.3	52.54 ± 11.54
Ginger	3.03 ± 0.27*	47.85 ± 12.40
Aspirin	3.80 ± 0.42*	46.71 ± 0.37
Aspirin + Ginger	6.20 ± 0.72#	50.25 ± 7.02

Values are shown as the mean ± SEM.

* $P < 0.05$ compared with the control rats.

$P < 0.05$ compared with the aspirin group.

els between the ginger powder treated Group 2 and Group 1 (control).

Expression of TNF- α and IL-1 β in plasma

The plasma concentrations of TNF- α and IL-1 β in the rats are shown in Fig. 5. The concentrations of TNF- α and IL-1 β were significantly increased after the administration of aspirin compared with those seen in the control rats (Aspirin, Figs. 5a and b). Ginger powder attenuated these increases in the plasma levels of TNF- α and IL-1 β , even after the coadministration of aspirin in rats (Ginger + Aspirin, Figs. 5a and b).

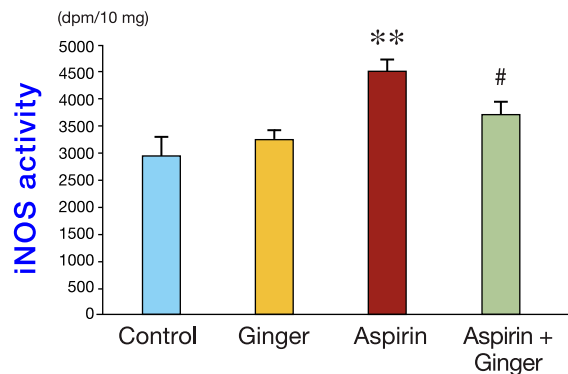


Fig. 4. Effects of ginger powder in iNOS activity in the gastric mucosa. The coadministration of ginger powder (200 mg/kg) inhibited the increase in iNOS activity induced by aspirin alone. ** $P < 0.01$ compared with the control rats. # $P < 0.05$ compared with the aspirin-treated rats. iNOS, inducible form of NO synthase (dpm/10 mg).

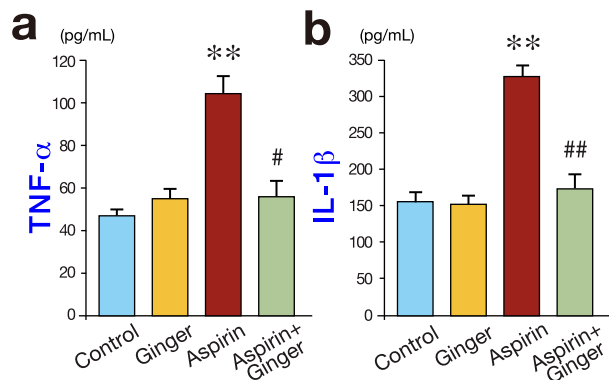


Fig. 5. Effects of ginger powder on the plasma levels of TNF- α and IL-1 β . The aspirin-administered rats showed marked increases in their plasma TNF- α and IL-1 β levels. The coadministration of ginger powder completely inhibited these marked increases in TNF- α (a) and IL-1 β (b) levels. * $P < 0.05$, ** $P < 0.01$ compared with the control rats. # $P < 0.05$, ## $P < 0.01$ compared with the aspirin administered rats. TNF- α , tumor necrosis factor- α ; IL-1 β , interleukin-1 β .

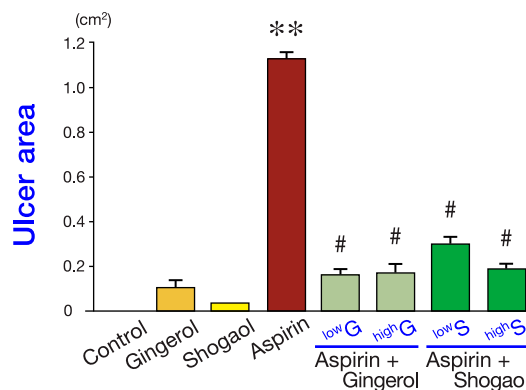


Fig. 6. Effects of 6-gingerol and 6-shogaol on the gastric mucosal lesion formation induced by aspirin in rats. Both 6-gingerol and 6-shogaol inhibited the gastric ulcer formation induced by aspirin. The animals were treated alone with 2 mg/kg 6-gingerol (Gingerol), 1 mg/kg 6-shogaol (Shogaol), 200 mg/kg aspirin (Aspirin); and treated combinedly with Aspirin and 1 mg/kg 6-gingerol (^{low}G), 2 mg/kg 6-gingerol (^{high}G), 0.5 mg/kg 6-shogaol (^{low}S), 1 mg/kg 6-shogaol (^{high}S). ** $P < 0.01$ compared with the control. # $P < 0.05$ compared with the aspirin-induced rats.

Effects of 6-gingerol and 6-shogaol in the presence of aspirin

High and low doses of 6-gingerol and 6-shogaol, the major constituents of ginger powder, had no adverse effects on the stomach and inhibited the ulcer formation induced by aspirin (Fig. 6). 6-Gingerol and 6-shogaol exerted inhibitory effects on the aspirin induced increases in mucosal iNOS activity (Fig. 7) and plasma TNF- α and IL-1 β levels (Fig. 8) in the same manner as ginger powder.

Discussion

Ginger has been used as an ingredient of Chinese traditional stomach medicines for thousands of years. The anti-ulcerative effects of ginger have previously been investigated in experimental gastric ulcer models (Yamaha et al., 1988; al-Yahva et al., 1989; Khushtar et al., 2009). However, the mechanism underlying the protective effects of ginger against gastric damage is unclear. In the present study, the anti-ulcerative effects of ginger powder were investigated in aspirin-induced gastric

ulcer model rats. Aspirin has been reported to reduce the gastric juice pH and increase the volume of gastric juice (Wang et al., 2007), or decrease the volume of gastric juice and its acid output (Jainu et al., 2006). In the present study, the volume of gastric juice and acid output/100 g body weight for 4 h reduced by aspirin and recovered by the coadministration of ginger with aspirin. The acidity of gastric juice was not significantly changed by any treatments. Our results suggest that the changes in the volume of gastric juice and acid production induced by aspirin are not a major factor in ulcer formation or the protective effects of ginger powder seen in these experimental ulcer model rats.

Prostaglandins have protective effects against various gastric injury models (Wallace, 1992; Brzozowski et al., 2005). Aspirin has been shown to reduce the mucosal PGE₂ content (Takeuchi et al., 1986; Lichtenberger et al., 2007; Wang et al., 2007). The lack of attenuation of the decrease in gastric mucosal PGE₂ content after the coadministration of ginger powder also reveals that the restoration of the PGE₂ level in the gastric mucosa is not

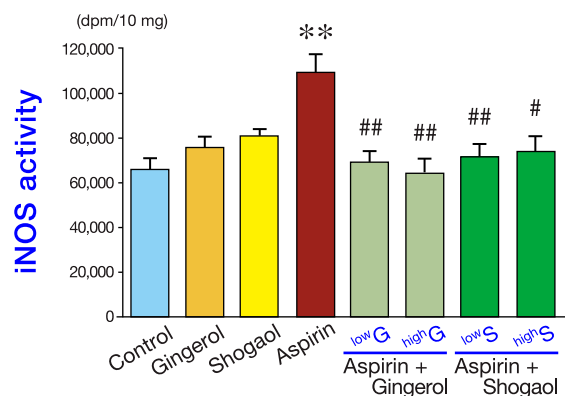


Fig. 7. Effects of 6-gingerol and 6-shogaol on iNOS activity in the gastric mucosa. The effects of aspirin on the iNOS activity in the gastric mucosa were compared in the absence and the presence of high and low doses of 6-gingerol or 6-shogaol. ** $P < 0.01$, compared with the control rats. # $P < 0.05$, ## $P < 0.01$ compared with the aspirin-treated rats. iNOS, inducible form of NO synthase. The definition of groups, refer to the legends for Fig. 6.

the mechanism underlying the protective effects of ginger powder in this aspirin induced ulcer model. This is not unexpected because the reduction of the gastric mucosal PGE₂ concentration induced by aspirin does not necessarily participate in gastric ulcer generation (Takeuchi et al., 1986; Lichtenberger et al., 2007).

One of the mechanisms by which aspirin damages the gastric mucosa is the increased production of NO due to the overexpression of iNOS (Kontureck et al., 2006). NO is a mediator not only of gastrointestinal mucosal defense (Calatayud et al., 2001), but also of its damage (Muscara and Wallace, 1999). It has been shown that different concentrations of NO have completely opposite effects in the same tissue (Wallace and Millor, 2000). In general, the mucosal and endothelial NOS isoforms produce low amounts of NO. However, the high quantity of NO produced by iNOS damages the epithelium (Piotrowski et al., 1999; Wallace and Miller, 2000). The excessive release of NO from gastric epithelial cells induced by aspirin has been reported to exert detrimental effects (Whittle, 2003; Hsu and Liu, 2004). Inhibiting aspirin-induced increases in iNOS expression in the gastric

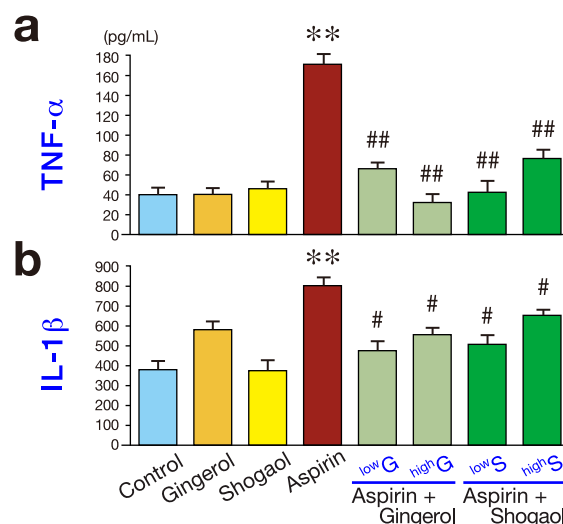


Fig. 8. Effects of 6-gingerol and 6-shogaol on the plasma levels of TNF- α and IL-1 β . The effects of high and low doses of 6-gingerol or 6-shogaol were examined during their coadministration with aspirin. The increases in plasma TNF- α (a) and IL-1 β (b) levels induced by aspirin were reduced by the coadministration of these constituents. ** $P < 0.01$ compared with the control. # $P < 0.05$, ## $P < 0.01$ compared with the aspirin-treated rats. The definition of groups, refer to the legends for Fig. 6.

mucosa leads to a reduction in gastric mucosal damage (Konturec et al., 2006). In the present study, ginger powder reduced iNOS activity and inhibited the production of gastric ulcers, even in the presence of aspirin.

Inflammation and neutrophil infiltration are also important in the pathogenesis of the gastric damage induced by NSAIDs (Wallace et al., 1990; Lee et al., 1992; Trevethick et al., 1993; Souza et al., 2004). The inflammation induced in the gastric mucosa by aspirin is accompanied by increased TNF- α production (Naito et al., 2001; Jainu and Devi, 2006), which augments neutrophil-derived superoxide generation (Kwiecień et al., 2002) and stimulates IL-1 β production, leading to neutrophil accumulation (Kokura et al., 2000; Odashima et al., 2006). In the present study, the levels of TNF- α and IL-1 β were increased by aspirin administration, and the coadministration of ginger powder inhibited the increases in TNF- α and IL-1 β without ulcer formation progressing. These effects re-

sembled those induced by pretreatment with *Cissus quadrangularis* extract for 7 days (Jainu and Devi, 2006).

Then, the anti-inflammatory effects of the main constituents of ginger were examined using the same experimental protocol. In the previous study, 6-gingerol, 6-shogaol, 8-gingerol and 10-gingerol have been identified as the principal components of ginger powder (Chen et al., 1986). 6-Shogaol, a dehydration product of 6-gingerol, is found in ginger powder but not in fresh ginger powder. 6-Shogaol appears to be formed from 6-gingerol during thermal processing and long-term storage (Chen et al., 1986). In general, the ability of compounds was related to both the length of the side chain and to the parent compound (gingerol versus shogaol). According to the relatively similar potency for several effects, their contribution on the effects of ginger may depend on their amounts. Therefore, several studies have focused on the effects of major components, 6-gingerol and 6-shogaol. The anti-inflammatory effects of 6-gingerol have been examined in the acetic acid-induced writhing response in mice and carrageenan-induced rat paw edema model (Young et al., 2005). Lipopolysaccharide (LPS) induced PGE₂ production was inhibited by treatment with either gingerol or shogaol (Lantz et al., 2007). Shogaol has been reported to markedly inhibit the LPS-induced expression of iNOS and COX-2 (Pan et al., 2008) and to have no effect on COX-2 expression (Lantz et al., 2007), whereas gingerol was found to inhibit LPS-induced COX-2 expression (Lantz et al., 2007). Both gingerol and shogaol have been shown to inhibit TNF- α mediated actions at the cellular level (Lantz et al., 2007; Isa et al., 2008). In the present study, both constituents of ginger showed protective effects against aspirin-induced gastric ulcers together with anti-inflammatory effects. We failed to show the dose dependency on the anti-inflammatory effects of these constituents; however, the rather high concentrations of these compounds used may explain this result.

From these results, ginger powder is suggested to protect the stomach against the ulcer formation

induced by aspirin by reducing iNOS activity in the gastric mucosa and inflammatory cytokine (TNF- α and IL-1 β) expression. These effects of ginger powder seem to be derived from the actions of gingerol and shogaol, the main ingredients of ginger.

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Corresponding author: Junichi Hasegawa, MD, PhD