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Anti-ulcer Effects of Chitin and Chitosan, Healthy Foods, in Rats

Mikio Ito, Ayako Ban and Masashi Ishihara

Department of Pharmacology, Faculty of Pharmacy, Meijo Unuversity, 150 Yagotoyama, Tenpaku-ku, Nagoya 468-4503, Japan

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ABSTRACT—In this study, we compared the effects of low molecular weight (LMW) chitosan (MW: 25,000 – 50,000), high molecular weight (HMW) chitosan (MW: 500,000 – 1000,000) and chitin on ethanol-induced gastric mucosal injury and on the healing of acetic acid-induced gastric ulcers in rats. Oral administration of LMW chitosan (250, 500 and 1000 mg/kg) dose-dependently prevented ethanol-induced gastric mucosal injury. Repeated oral administration of LMW chitosan (100, 200 and 400 mg/kg twice daily) also dose-dependently accelerated the gastric ulcer healing. However, the effects of HMW chitosan and chitin on the gastric mucosal injury formation and the gastric ulcer healing were less potent than those of LMW chitosan. LMW chitosan (250 and 500 mg/kg, orally) was ineffective in inhibiting gastric acid secretion in pylorus-ligated rats, although it had a weak acid-neutralizing action. LMW-chitosan (250, 500 and 1000 mg/kg orally) dose-dependently prevented the decrease in gastric mucus content induced by ethanol. These results indicate that of the three compounds, LMW chitosan has the most potent gastric cytoprotective and ulcer healing-promoting actions. In addition, gastric mucus-increasing action of LMW-chitosan may be, at least in part, related to the anti-ulcer effect of this compound.

Keywords: Chitin, Chitosan, Anti-ulcer action, Gastric cytoprotection

Chitin and chitosan are widely used as one of the healthy foods in many countries. As shown in Fig. 1, chitin is chemically a polymeric *N*-acetyl-D-glucosamine having a molecular weight of more than one million and is contained in the shells of crabs, shrimps, shellfishes and insects, etc. Chitosan is a polymeric D-glucosamine, a basic polysaccharide, and is produced by deacetylating chitin with 40% – 45% NaOH at 120°C (1). Chitin is insoluble in water, acid or alkaline solution. Chitosan is insoluble in water but can be solubilized in acid solution. It has been demonstrated that chitosan has numerous pharmacological actions including immunopotentiating (2, 3), anti-hypertensive (4), serum cholesterol-lowering (5 – 7), anti-bacterial (8, 9) and wound healing-promoting actions (10 – 12). However, there is yet no experimental evidence about whether or not chitin and chitosan have anti-ulcer action.

The aim of this study was to evaluate the effects of low molecular weight (LMW) chitosan (MW: 25,000 – 50,000), high molecular weight (HMW) chitosan (MW: 500,000 – 1,000,000) and chitin in comparison to those of cimetidine and sucralfate on ethanol-induced acute gastric mucosal injury and the healing of acetic acid-induced chronic gastric ulcers in rats. In this study, we demonstrated that LMW chitosan showed the most potent gastric cytoprotective and gastric ulcer healing actions. Therefore, to clarify the mechanism of the anti-ulcer action of LMW chitosan, we examined the effects of this compound on acid neutralization, gastric acid secretion and gastric mucus content.

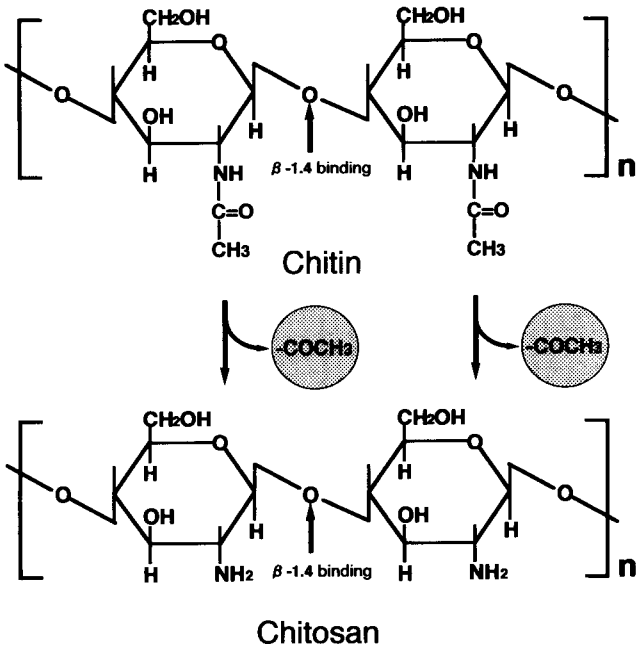

Fig. 1. Chemical structures of chitin and chitosan.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley strain SPF rats (Nippon SLC, Shizuoka), weighing 210–230 g, were used in the experiment. The animals were housed in an air-conditioned room at 23 ± 1°C.

Compounds

The compounds employed were LMW chitosan (MW: 25,000–50,000, deacetylation: 90.2%, viscosity in 0.5% acetic acid solution: 5.2 cP), HMW chitosan (MW: 500,000–1,000,000, deacetylation: 82.0%, viscosity in 0.5% acetic acid solution: 315 cP) and chitin (MW: >1,000,000). These compounds were supplied by Kimitsu Chemical Industries (Tokyo). Sucralfate (Ulcermin, Chugai Pharmaceutical Co., Ltd., Tokyo) and cimetidine (Sigma Chemical Co., St. Louis, MO, USA) were used as comparative anti-ulcer drugs. These compounds were suspended in 1% gum arabic. Furthermore, sodium bicarbonate (Wako Pure Chemical Industries, Ltd., Osaka) was used as a comparative antacid.

Measurement of ethanol-induced gastric mucosal injury

After rats were fasted for 24 h, absolute ethanol was administered in a volume of 1 ml per 100 g of body weight into the stomach of rats. Each test compound (LMW chitosan, HMW chitosan, chitin, sucralfate and cimetidine) was given orally in a volume of 1 ml per 100 g of body weight at 2 h prior to ethanol administration. Furthermore, as the control, vehicle (1% gum arabic) was given instead of each test compound. At 1 h after treatment with the necrotizing agent, the animals were killed under ether anesthesia, and then the stomach was removed and then opened along the greater curvature. The degree of gastric the mucosal injury was expressed as the mucosal lesion index (mm^2) as previously reported (13).

Measurement of acetic acid-induced gastric ulcers

The rats were allowed daily access to commercial food pellets between 9:00–10:00 a.m. and 5:00–6:00 p.m. throughout the experimental period from 3 days prior to ulcer induction (14). However, tap water was always supplied ad libitum. Gastric ulcers were induced in these rats by the injection of 20% acetic acid (v/v %) in a volume of 0.05 ml into the submucosal layer at the junction of the fundus and antrum in accordance with the method described by Takagi et al. (15). Each test compound was given orally, twice daily (LM chitosan, HM chitosan, chitin and sucralfate: 8:00 a.m. and 5:00 p.m.; cimetidine: 10:30 a.m. and 6:30 p.m.) for 14 consecutive days from the day (the 1st day) after acetic acid injection. Control animals were given the vehicle (1% gum arabic) instead of a test compound. On the 15th day, the animals were killed by rapid decapitation. The stomachs were removed, filled with 5 ml of 10% formalin and allowed to stand for 5 min. The stomachs were cut open along the greater curvature. The longitudinal and abscissal lengths of the upper, opened part of the ulcer were measured with a micrometer, which was set on a stereoscopic microscope; and the product of both lengths (mm^2) was expressed in terms of the ulcer index. After the ulcer size was measured, the stomach tissue was again immersed in 10% formalin for 24 h. The formalin-fixed tissue was then cut so that a little of the normal tissue surrounding the ulcer remained. Thereafter, the central part of the ulcer was cut vertically against the serosa along the long diameters. These tissues, cut in half, were embeded in paraffin and cut into 2- to 3-μm-thick sections. The sections were stained with hematoxylin and eosin. Histological measurements were performed under light micrography of the stained preparations as shown in Fig. 2. The healing effects of test compounds were evaluated by comparing the ulcer index, the defective area in the ulcerated region, the index for the decrease in the exposed floor and the index for the mucosal regeneration of each test drug with those of the respective control.

Measurement of antacid activity in vitro

Test compound was added drop by drop with continual stirring to 50 ml of 0.1 N HCl, and the maximum pH of the solution was measured by an automatic titrator (ABT-101; Tohadenpa, Tokyo).

Measurement of gastric acid secretion

The rats were deprived of food but allowed free access to water for 24 h. After fasting, each test compound was given orally. Control animals were given orally the vehicle only instead of test compound. At 1 h after the administration of test compound or the vehicle, the pylorus of each rat was ligated under ether anesthesia. The gastric contents were collected for 6 h after ligation. The volume of gastric juice was measured, the acidity was determined by the automatic titrator, and total acid output during the 1-h period was calculated.

Measurement of gastric mucosal injury and gastric mucus content after intragastric administration of ethanol

After rats were fasted for 24 h, absolute ethanol was administered in a volume of 1 ml per 100 g of body weight into the stomach. Each test compound was given orally in a volume of 1 ml per 100 g of body weight at 1 h prior to ethanol administration. Control animals were given orally the vehicle instead of test compound. At 5 h after ethanol treatment, the animals were killed under ether anesthesia, and the degree of gastric mucosal injury was expressed as the mucosal lesion index (mm^2) as described above. After

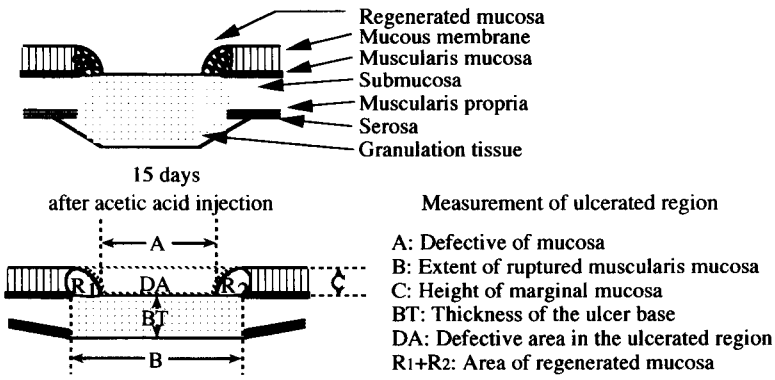

Fig. 2. Method for histological measurements. Schematic drawings of the vertical section in the ulcerated region on the 15th day after acetic acid injection.

the lesion index was measured, mucus content in gastric mucosa was determined by staining the mucus with 0.1% alucian blue by the method of Kitagawa et al. (16).

Statistical analyses

The results obtained are expressed as the mean ± S.E.M. The data were analyzed by one-way analysis of variance, and the statistical significance among groups was determined by Duncan's multiple-range test.

RESULTS

Effects of LMW chitosan, HMW chitosan, chitin, sucralfate and cimetidine on ethanol-induced gastric mucosal injury

Intragastric administration of absolute ethanol to control rats produced large hemorrhagic injury in the glandular stomach. LMW chitosan at oral doses of 250, 500 and 1000 mg/kg prevented the gastric mucosal injury by 87%, 95% and 99%, respectively (Fig. 3). HMW chitosan at oral doses of 250 and 500 mg/kg prevented the mucosal injury by 64% and 83%, respectively. Chitin (500 and 1000 mg/kg, orally) was also as effective as HMW chitosan in preventing the mucosal injury. Sucralfate, a comparative drug, at oral doses of 250 and 500 mg/kg prevented the injury by 93% and 99%, respectively. Cimetidine, another comparative drug, at an oral dose of 100 mg/kg prevented the mucosal injury by 61%.

Effects of LMW chitosan, HMW chitosan, chitin, sucralfate and cimetidine on the healing of acetic acid-induced gastic ulcers

Repeated oral administration of LMW chitosan for 14 consecutive days accelerated the healing of gastric ulcers in a dose-dependent manner (Fig. 4). Namely, LMW chitosan given at 100, 200 and 400 mg/kg twice daily decreased the ulcer index by 21%, 49% and 60%, respectively, and the defective area in the ulcerated region by 36%, 46% and 69%, respectively. In addition, LMW chitosan (100, 200 and 400 mg/kg twice daily) increased the index for the decrease in the exposed ulcer base by 27%, 52% and 68%, respectively, and the index for mucosal regeneration by 19%, 42% and 91%, respectively.

HMW chitosan given at an oral dose of 400 mg/kg twice daily decreased the ulcer index and the defective area in the ulcerated region by 23% and 34%, respectively (Fig. 5). Chitin given at an oral dose of 400 mg/kg twice daily decreased the defective area in the ulcerated region by 38% and increased the index for mucosal regeneration by 47% (Fig. 5). However, HMW chitosan and chitin at an oral dose of 200 mg/kg twice daily showed no apparent effect on ulcer healing.

Sucralfate (250 and 500 mg/kg twice daily orally) decreased the ulcer index by 40% and 62%, respectively, and the defective area of ulcerated region by 51% and 75%, respectively (Fig. 6). This drug furthermore increased the index for the decrease in the exposed ulcer base by 32% and 61%, respectively, and the index for mucosal regeneration by 53% and 66%, respectively. Cimetidine (100 mg/kg twice daily orally) decreased the ulcer index by 46% and the defective area of ulcerated region by 51% (Fig. 6). In addition, cimetidine increased the index for the decrease in the exposed ulcer base by 52% and the index for mucosal regeneration by 57%.

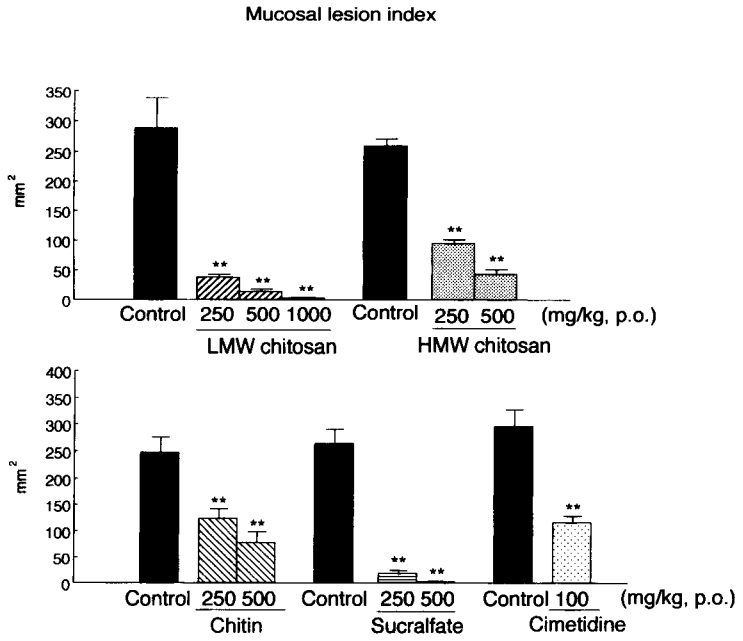

Fig. 3. Effects of LMW chitosan, HMW chitosan, chitin, sucralfate and cimetidine on ethanol-induced gastric mucosal injury in rats. Each test compound was given orally at 2 h prior to intragastric administration of absolute ethanol. The effects of test compounds on gastric mucosal injury were evaluated at 1 h after ethanol treatment. Each column denotes the mean ± S.E.M. for 8 rats. Significantly different from the respective control, **P<0.01.

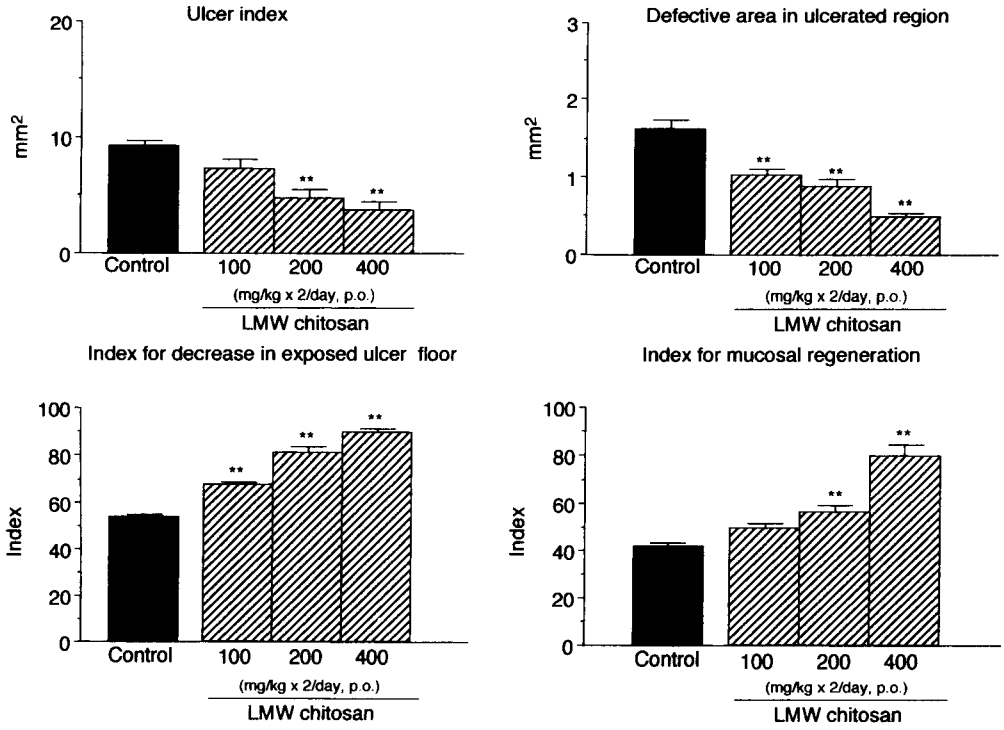

Fig. 4. Effects of LMW chitosan on the healing of acetic acid-induced gastric ulcers in rats. Each test compound was given orally, twice daily for 14 consecutive days beginning the first day after acetic acid injection. The effects of test compounds on ulcer healing were evaluated on the 15th day. Each column denotes the mean ± S.E.M. for 7 to 9 rats. Significantly different from the respective control, **P<0.01.

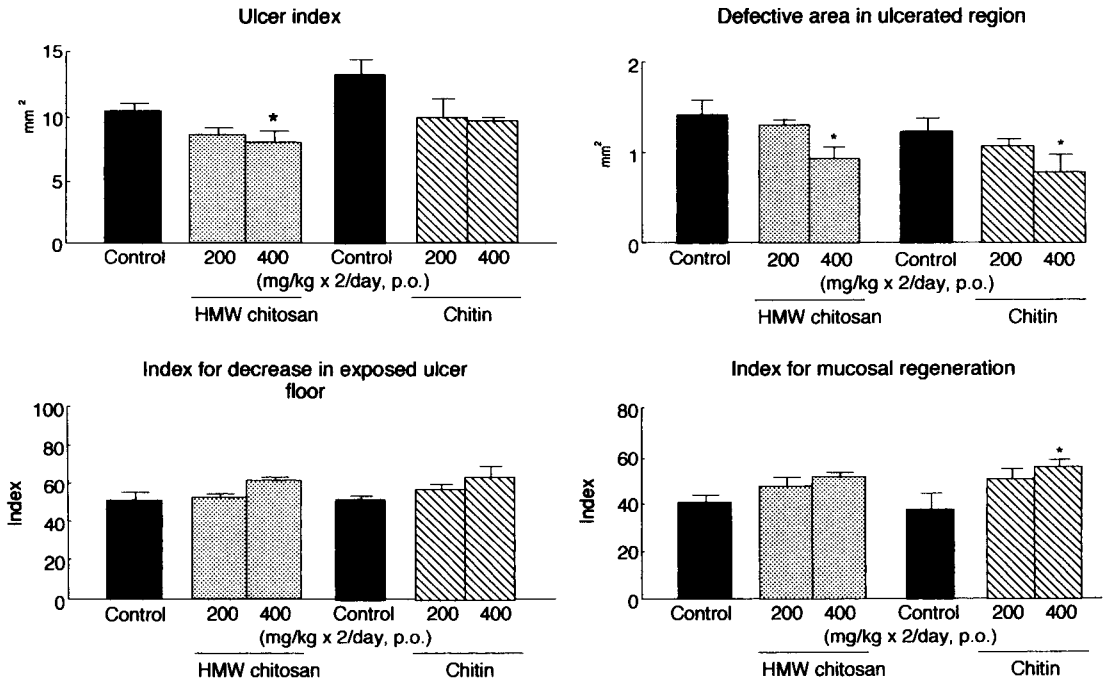

Fig. 5. Effects of HMW chitosan and chitin on the healing of acetic acid-induced gastric ulcers in rats. HMW chitosan or chitin was given orally, twice daily for 14 consecutive days beginning the first day after acetic acid injection. The effects of test compounds on ulcer healing were evaluated on the 15th day. Each column denotes the mean ± S.E.M. for 7 to 9 rats. Significantly different from the respective control, *P<0.05.

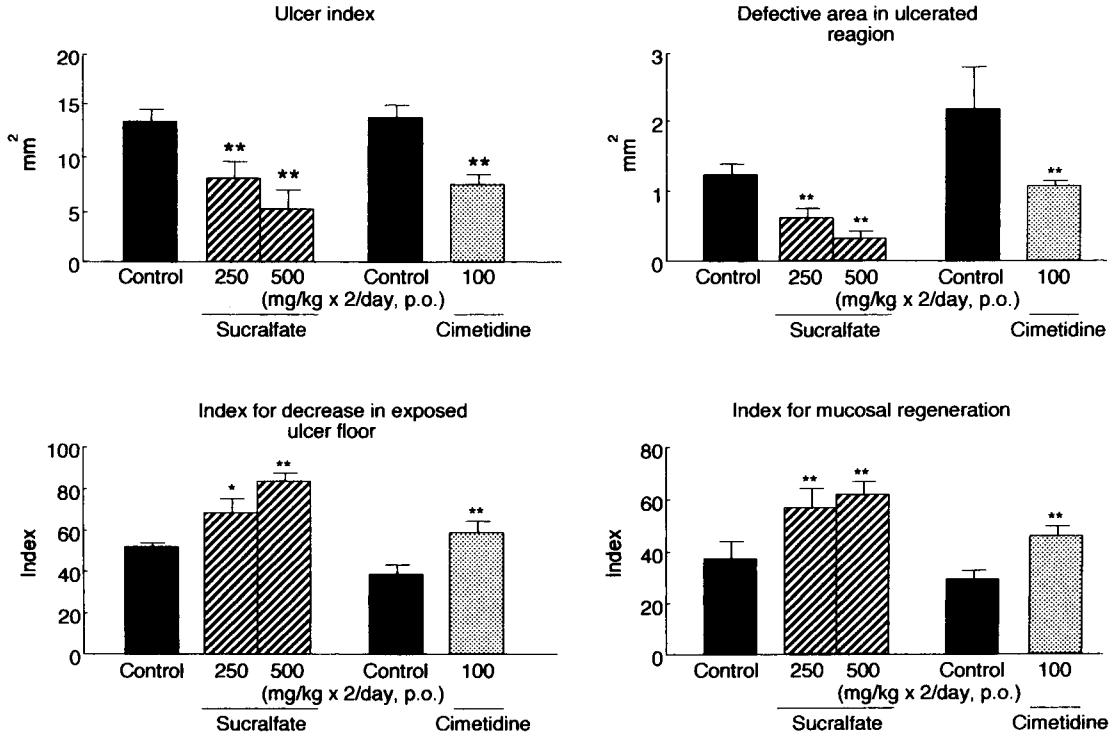

Fig. 6. Effects of sucralfate and cimetidine on the healing of acetic acid-induced gastric ulcers in rats. Sucralfate or cimetidine was given orally, twice daily for 14 consecutive days beginning the first day after acetic acid injection. The effects of both test compounds were evaluated on the 15th day. Each column denotes the mean ± S.E.M. for 7 to 9 rats. Significantly different from the respective control, *P<0.05, **P<0.01.

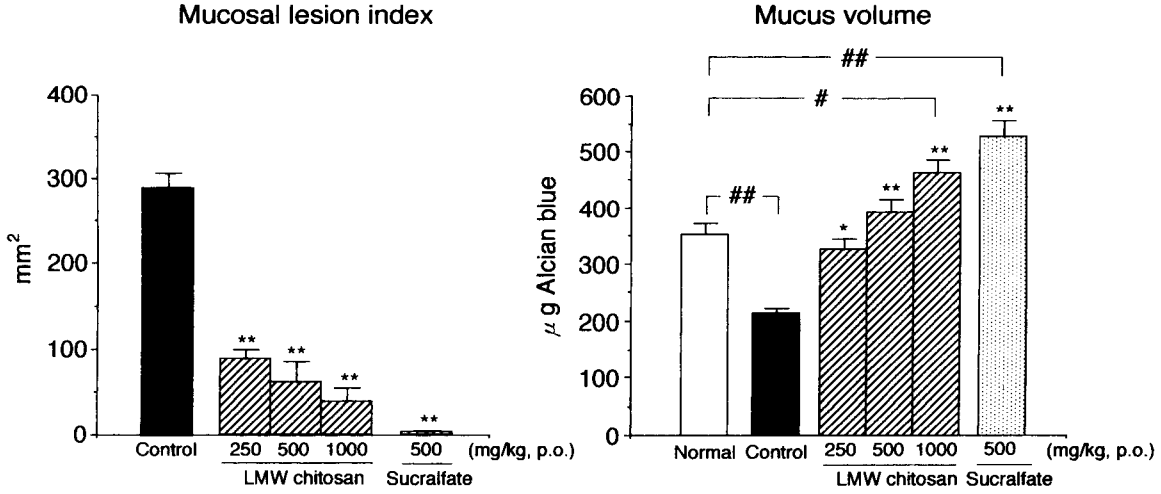

Fig. 7. Effects of LMW chitosan and sucralfate on ethanol-induced gastric mucosal injury and gastric mucus content after intragastric administration of ethanol in rats. LMW chitosan or sucralfate was given orally at 1 h prior to intragastric administration of absolute ethanol. The effects of both compounds on gastric mucosal injury were evaluated at 5 h after ethanol treatment. Each column denotes the mean ± S.E.M. for 8 rats. Significantly different from the respective control, *P<0.05, **P<0.01. Significantly different from normal rats (ethanol-untreated), #P<0.05, ##P<0.01.

Antacid activities of LMW chitosan, HMW chitosan and sodium bicarbonate in vitro

When test compound was added drop to drop with continual stirring to 50 ml of 0.1 N HCl, LMW chitosan elevated the pH from 1 to the maximum of 5.9 (data not shown). Sodium bicarbonate elevated pH of the HCl solution to the maximum of 8.5. However, HMW chitosan failed to elevate pH of the HCl solution.

Effects of LMW chitosan and cimetidine on gastric acid secretion

A single oral administration of cimetidine (100 mg/kg) significantly decreased the volume of gastric juice by 41% (control: 6.9 ± 0.8 ml/6 h vs cimetidine: 4.1 ± 0.3 ml/6 h, P<0.01) and total acid output by 49% (control: 107.8 ± 15.5 μEq/h vs cimetidine: 55.2 ± 5.0 μEq/h, P<0.01) (data not shown). However, LMW chitosan (250 and 500 mg/kg orally) was ineffective in decreasing the volume and total acid output.

Effects of LMW chitosan and sucralfate on gastric mucosal injury and gastric mucus content after intragastric administration of ethanol

The gastric mucus content in ethanol-treated control rats was about 40% lower than that in ethanol-untreated normal rats (Fig. 7, Right). LMW chitosan at oral doses of 250, 500 and 1000 mg/kg dose-dependently increased gastric contents in ethanol-treated rats by 153%, 185% and 218%, respectively. Especially, the mucus content in ethanol-treated rats given 1000 mg/kg of this compound was significantly higher than that of normal rats. The mucus-increasing effect of chitosan at 1000 mg/kg orally was as potent as that of sucralfate at 500 mg/kg orally. LMW chitosan (250, 500 and 1000 mg/kg orally) also prevented ethanol-induced gastric mucosal injury dose-dependently (Fig. 7, Left).

DISCUSSION

Prudden et al. (10) first reported that chitin has a wound healing-promoting action by local application of chitin powder on incised wounds of abdominal skin in rats. Since that time, as another clinical application of chitin, an artificial skin of chitin has been used for the treatment of burn injury. The agents used for the treatment of skin ulcers have also been applied as anti-ulcer agents (for examples azulen and solcoceryl) (17). Therefore, chitin and chitosan are also expected to have anti-ulcer action.

In the present study, of LMW chitosan, HMW chitosan and chitin, LMW chitosan exhibits the most potent gastroprotective and ulcer healing-promoting actions.

The mechanisms of the anti-ulcer actions of chitin and chitosan have not been well defined. Furthermore, it remains unclear whether the anti-ulcer actions of these compounds are due to their systemic or local actions. It is believed that chitin and chitosan may be primarily absorbed after they have been transformed into their oligosaccharides by chitinase and chitosanase secreted from intestinal bacterias and by lysozyme in intestinal juice. Their oligosaccharides absorbed from the intestine are finally hydrolyzed to their monosaccharides, *N*-acetyl-D-glucosamine and D-glucosamine, respectively, and may be

utilized for formation of granulation tissue and angiogenesis in the ulcerated part of acetic acid-induced gastric ulcers and may contribute to the ulcer healing. However, both monosaccharides were ineffective in preventing ethanol-induced gastric mucosal injury (M. Ito et al., unpublished data). This result suggests that the gastroprotective action of chitin and chitosan on ethanol-induced injury is mainly due to local action of both compounds. LMW chitosn (more than 500 mg/kg orally) almost completely prevented ethanol-induced gastric mucosal injury. In addition, LMW chitosan (100 – 400 mg/kg, twice daily orally) markedly accelerated the healing of acetic acid-induced chronic gastric ulcers. The gastrocytoprotective and ulcer-healing actions of LMW chitosan (500 mg/kg once or 400 mg/kg twice daily orally) were as potent as those of sucralfate (500 mg/kg once or twice daily orally) and more potent than those of cimetidine (100 mg/kg once or twice daily). As mentioned in the introduction, chitosan is a basic polysaccharide and is easily dissolved in acid solution, although chitin is insoluble in acid and alkaline solutions. Chitosan dissolved in the stomach by gastric acid has the viscous gel-forming properties, like those of mucus glycoprotein, and may protect gastric mucosa from acid (H^+) and pepsin. In addition, chitosan having an amino group forms polyvalent bridges between the positively charged chitosan polycations and negatively charged sulfated mucin or glycosaminoglycans formed on the ulcer floor and may protect gastric mucosa and the ulcerated area. Chitosan having an amino group also may neutralize H^+ in gastric juice and H^+ back-diffused into the mucosa. Sucralfate has been demonstrated to have anti-ulcer action partly by an adhesive action on gastric epithelial cells and by an H^+-neutralizing action because it contains aluminum in its chemical structure (18). Therefore, it is suggested that chitosan, like sucralfate, may locally exhibit anti-ulcer action, at least in part, by coating the gastric mucosa or the ulcerated area. In the present experiment, however, the anti-ulcer actions of HMW chitosan and chitin were very markedly weaker than those of LMW chitosan. When LMW and HMW chitosans were added in 0.1 N HCl to test their antacid actions, the former was easily dissolved and elevated pH in the solution gradually. However, the latter took a longer time to be dissolved than the former. In addition, the viscosity of the latter solution was very high and failed to elevate the pH of the solution. Consequently, most of the HMW chitosan given orally may have been transferred to the small intestine from the stomach before this compound is completely dissolved by gastric acid in gastric lumen. Therefore, it is possible that the difference in the effectiveness between LMW chitosan and HMW chitosan or chitin may be due to differences in the strength and duration of adhesiveness of these compounds to the mucosa or the ulcerated area.

In the present experiment, LMW chitosan (250 and 500 mg/kg, orally), unlike cimetidine (100 mg/kg, orally), was ineffective in decreasing gastric acid secretion in pylorus-ligated rats. LMW chitosan (250 – 1000 mg/kg, orally) as well as sucralfate (500 mg/kg, orally) significantly prevented the decrease in gastric mucus content induced by ethanol. These results suggest that gastric mucus-increasing and weak antacid actions may be partly related to the anti-ulcer effect of this compound. In addition, these results also suggest that LMW chitosan may be a new therapeutic agent of the sucralfate-type having a strong adhesive action on the gastric mucosa. However, further studies are needed to clarify the mechanisms of the anti-ulcer action of LMW chitosan.

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