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Curative Effect of Selenium Against Indomethacin-Induced Gastric Ulcers in Rats

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Indomethacin is a nonsteroid anti-inflammatory agent that is known to induce severe gastric mucosal lesions. In this study, we investigated the effect of selenium on gastric mucosal lesions in rats. To confirm the curative effect of selenium against indomethacin-induced gastric ulcers, gastric ulcers were induced by oral administration of 25 mg/kg indomethacin, and then different doses (10, 50, and 100 μg/kg of body weight) of selenium or vehicle were treated by oral gavage for 3 days. Oral administration of indomethacin clearly increased the gastric ulcer area in the stomach, whereas selenium applied for 3 days significantly decreased the gastric ulcer area in a dose-dependent manner. In addition, selenium markedly reduced the increase of lipid peroxidation induced by indomethacin in the gastric mucosa and increased activities of radical scavenging enzymes such as superoxide dismutase, catalase, and glutathione peroxidase in a dose-dependent manner. These results reveal that selenium can heal indomethacin-induced gastric ulcers through elimination of the lipid peroxides and activation of radical scavenging enzymes.

Keywords: Anti-ulcer drug, gastric ulcers, indomethacin, oral administration, selenium

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used for their analgesic, antipyretic, and anti-inflammatory effects [5]. Gastrointestinal symptoms are the most common adverse events associated with NSAID therapy [8, 24]. In short or long term NSAID therapy, gastric ulcers, bleeding, and perforation are serious side effects that are observed and these ulcerative lesions are the major limitation to their use as anti-inflammatory drugs [2, 3, 14, 27]. Indomethacin is a noncorticosteroid drug with anti-inflammatory, antipyretic, and pain-relieving properties, which is known to produce erosions, ulcerative lesions, and petechial bleeding in the mucosa of stomach as serious side effects [10, 18]. According to previous reports, the oral administration of indomethacin in rats causes ulcerative lesions in the gastric mucosa [7, 13]. Furthermore, the development of the gastric mucosal lesions induced by indomethacin is mainly mediated through generation of oxygen free radicals and lipid peroxidation [6, 22, 25, 26, 28, 29].

Selenium is an essential nutrient of fundamental importance to human biology. It has important metabolic functions in animals, including protection of membrane lipids and macromolecules from oxidative damage produced by peroxides [23] and activation of important antioxidant proteins, thioredoxin reductase, and several selenoproteins [16, 17]. In addition, neuroprotective effects of selenium have been reported at an experimental level in both methamphetamine- and 6-hydroxydopamine-induced toxicities [12] as well as in positive clinical responses during therapy with selenium in neurodegenerative diseases [9]. However, currently, the curative properties of selenium on gastric ulcers are not well understood. In the present study, we hypothesized that selenium can heal gastric ulcers induced by indomethacin. Because indomethacin-induced gastric ulcers are caused by generation of oxygen free radicals and lipid peroxidation, we evaluated the curative effect of selenium against indomethacin-induced gastric ulcers by measuring the amount of lipid peroxidation and by comparing the activities of enzymatic scavengers such as SOD, catalase, and glutathione peroxidase.

Materials and Methods

Chemicals

Selenium and indomethacin were purchased from Sigma Chemicals (St. Louis, MO., USA). Selenium was dissolved in dimethyl sulfoxide (DMSO) immediately before use and administered intragastrically to rats in a volume of 5 ml/kg. Indomethacin was dissolved in 5% sodium

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bicarbonate and administered to rats in a dose of 25 mg/kg by orogastric gavage, with an appropriate feeding needle as a volume of 5 ml/kg. All chemicals were of the highest purity available.

Animals

Male Sprague-Dawley rats (200~250 g, 7 weeks old) were provided by Daehan Biolink Co., Ltd., Korea. Rats were placed in cages with wire-net floors in a controlled room (temperature 22~24°C, humidity 70~75%, light on at 06.00 h and off at 18.00 h; 12 h light and 12 h dark) and they were fed a normal laboratory diet. Rats were fasted for 24 h before experimental use, but allowed free access to tap water throughout. The animal experiment was performed in accordance with guidelines established by the Animal Care and Use Committee of Dong-Eui University and approved by the committee.

Experimental Procedure

To evaluate the curative effect of selenium against indomethacin-induced gastric ulcers, gastric ulcers were induced by a single oral dose of 25 mg/kg indomethacin. The concentration of indomethacin for induction of gastric ulcers was determined on the basis of our previous study [13]. Rats were divided into six groups (n=6 rats per group). The normal group received only 5% sodium bicarbonate orally in a volume of 5 ml/kg. The control group received only 25 mg/kg indomethin. Each of the remaining four groups was treated with a vehicle (selenium, 0 μg/kg) and three doses (10, 50, and 100 μg/kg) of selenium for 3 days after induction of gastric ulcers by pretreatment with 25 mg/kg indomethacin. The concentrations of selenium were selected on the basis of the preliminary results obtained from cytotoxicity studies using a broad concentration range for this reagent. All the rats were killed under deep ether anesthesia 4 h after the last oral administration of indomethacin/selenium. The rat stomachs were promptly excised, weighed, and chilled in ice-cold 0.9% NaCl. After washing with 0.9% NaCl, the mucosa was homogenized in 50 mM potassium phosphate buffer at pH 7.5. Mitochondria and cytosol fractions were prepared according to the method of Hogeboom [11]. The quantitative analysis of protein was measured by Bradford protein assay [4].

Malondialdehyde Levels

Lipid peroxidation was determined by measuring malonylaldehyde (MDA) production by using a thiobarbituric acid reaction [20, 21]. Briefly, the stomach homogenate was supplemented with 8.1% sodium dodecyl sulfate, 20% acetic acid (pH 3.5), and 0.8% thiobarbituric acid, and boiled at 95°C for 1 h. After cooling with tap water, the reactants were supplemented with *n*-butanol and pyridine [15:1 (v/v)] shaken vigorously for 1 min, and centrifuged for 10 min at 3,500 ×*g*. Absorbance was measured at 532 nm. The lipid peroxide level was calculated from the standard curve using the MDA tetrabutylammonium salt. MDA concentrations were expressed as nmol/g of tissue.

SOD Assay

SOD is a family of predominantly intracellular metalloproteins that catalyzes the dismutation of O_2^- to H_2O_2 in gastric mucosal cells [19, 22]. The activity of SOD in the gastric mucosa was measured according to the method of McCord and Fridovich [19]. The standard assay was performed in 3 ml of 50 mM potassium phosphate buffer at pH 7.8 containing 0.1 mM EDTA in a cuvette thermostated at 25°C. The reaction mixture contained 0.1 mM ferricytochrome *c*, 0.1 mM xanthine, and sufficient xanthine oxidase to produce a reduction rate of ferricytochrome *c* at 550 nm of 0.025 absorbance unit per minute. Tissue homogenate was mixed with the reaction mixture (50 mM potassium phosphate buffer, pH 7.8, containing 0.1 mM EDTA, 0.1 mM ferricytochrome *c*, and 0.1 mM xanthine). Kinetic spectrophotometric analysis was started by adding xanthine oxidase at 550 nm. Under these conditions, the amount of SOD required to inhibit the reduction rate of cytochrome *c* by 50% was defined as 1 unit of activity. The results were expressed as unit/mg of protein.

Catalase Assay

Catalase is a hemoprotein that catalyzes the decomposition of H_2O_2 to water in the gastric mucosal cell [1, 22]. The activity of catalase in the gastric mucosa was measured according to the method of Aebi [1]. The standard assay was performed in 3 ml of 50 mM potassium phosphate buffer at pH 7.0 (1.9 ml) containing 10 mM H_2O_2 (1 ml) and tissue homogenate (100 μl). Under these conditions, the amount of catalase required to decompose 1.0 μmol of H_2O_2 per min at pH 7.0 at 25°C was defined as 1 unit of activity. Absorbance was measured at 240 nm for 2 min, and the results were expressed as unit/mg of protein.

Glutathione Peroxidase Assay

Glutathione peroxidase is an important enzyme that plays a role in the elimination of H_2O_2 and lipid hydroperoxides in the gastric mucosal cell [22]. The activity of glutathione peroxidase in the gastric mucosa of rats was determined by a modified method of Lawrence and Burk [15]. The reaction mixture consisted of glutathione peroxidase assay buffer (50 mM potassium phosphate buffer, pH 8.0, 0.5 mM EDTA) and NADPH assay reagent (5 mM NADPH, 42 mM reduced glutathione, and 10 units/ml glutathione reductase). A supernatant of homogenate in 50 mM potassium phosphate buffer at pH 7.5 was prepared by centrifuging it at 1,000 ×*g* for 10 min at 4°C. Subsequently, 900 μl of glutathione peroxidase assay buffer, 50 μl of NADPH assay reagent, and 50 μl of the sample were added to the cuvette, and the contents were mixed by inversion. The reaction was started by adding 10 μl of 30 mM *tert*-butyl hydroperoxide or 80% cumene hydroperoxide. Absorbance was recorded by the following program: wavelength, 340 nm; initial delay, 15 s; interval, 10 s; number of readings, 6. The activity of enzyme was the sum of data obtained using 30 mM *tert*-butyl hydroperoxide and 80% cumene hydroperoxide. The level of glutathione was expressed in terms of μmol/min/mg of protein.

Statistical Analysis

All values were represented as means ± SEM. Data were analyzed by ANOVA according to the General Linear Model procedure. The means were compared by Tukey's Studentized Range (HSD) test to detect significant differences at $P<0.05$.

RESULTS

Selenium Shows Curative Effect Against Indomethacin-Induced Gastric Ulcers in Rats

To confirm the curative effect of selenium against indomethacin-induced gastric ulcers, gastric ulcers were induced by oral administration with a single dose of 25 mg/kg indomethacin, and then different doses (10, 50,

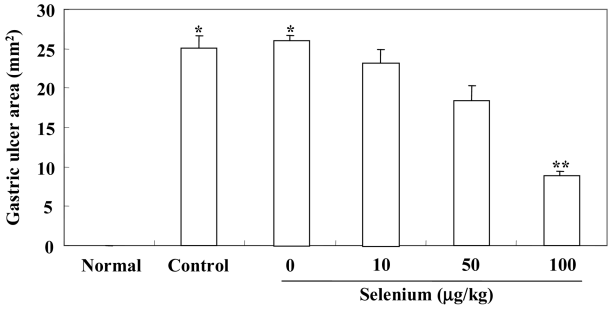

Fig. 1. Curative effect of selenium against indomethacin-induced gastric ulcers in rats.
Gastric ulcers were induced by oral administration of 25 mg/kg indomethacin, and then different doses (10, 50, and 100 μg/kg) of selenium or vehicle were treated by oral gavage for 3 days. Selenium treatment significantly decreased the gastric ulcer area in the mucosa of stomach in a dose-dependent manner, compared with the control group. Values are expressed as means ± SEM. **P*<0.05, significantly different from the untreated normal rats. ***P*<0.01, significantly different from the control rats.

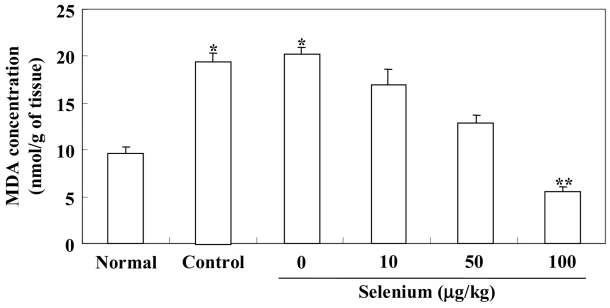

Fig. 2. Effect of selenium on lipid peroxidation induced by indomethacin in gastric mucosa.
The rats were treated with a vehicle (selenium, 0 μg/kg) and three doses (10, 50, and 100 μg/kg) of selenium for 3 days after induction of gastric ulcers by pretreatment with 25 mg/kg indomethacin. MDA production was estimated by using a thiobarbituric acid reaction. Selenium treatment markedly reduced the level of MDA in a dose-dependent manner in comparison with the control group. Values are expressed as means ± SEM. **P*<0.05, significantly different from the untreated normal rats. ***P*<0.01, significantly different from the control rats.

and 100 μg/kg) of selenium or vehicle were treated by oral gavage for 3 days. Gastric lesions were judged by measuring the gastric ulcer area on the gastric mucosal surface in all experimental groups. As shown in Fig. 1, oral administration of indomethacin significantly caused the increase of the gastric ulcer area in the mucosa of stomach, compared with the untreated normal group (**P*<0.05). In contrast, selenium treatment for 3 days markedly showed the decrease of the gastric ulcer area in a dose-dependent manner, compared with the control group. In particular, the gastric ulcer area was distinctly decreased by 60% at 100 μg/kg selenium, compared with the control group (***P*<0.01).

Because indomethacin-induced gastric ulcers are mediated by generation of oxygen free radicals and lipid peroxidation [6, 25, 26, 28], the curative effect of selenium against indomethacin-induced gastric ulcers was also evaluated by measuring the level of lipid peroxide and activities of scavenging enzymes in the stomach of all experimental groups. Fig. 2 shows the effect of selenium on lipid peroxidation induced by indomethacin in the gastric mucosa. In the case of lipid peroxidation, indomethacin treatment in the control and vehicle (selenium, 0 μg/kg) groups significantly increased the level of MDA in gastric tissue in comparison with the untreated normal group (**P*<0.05). On the other hand, selenium treatment for 3 days decreased the level of MDA in a dose-dependent manner in comparison with the control group. Specifically, 100 μg/kg selenium applied for 3 days showed a significant decrease in the level of MDA (***P*<0.01), compared with the control group.

Fig. 3 shows the effect of selenium on activities of radical scavenging enzymes such as SOD, catalase, and glutathione peroxidase in gastric musosa. Indomethacin treatment in the control and vehicle (selenium, 0 μg/kg) groups distinctly attenuated the activities of SOD, catalase, and glutathione peroxidase in comparison with the untreated normal group (**P*<0.05). In contrast, selenium treatment for 3 days increased the activities of these enzymes in a dose-dependent manner in comparison with the control group. Specifically, 100 μg/kg selenium applied for 3 days showed a drastic increase in the activities of radical scavenging enzymes, compared with the control group (***P*<0.01).

Based on our results, we concluded that selenium can heal indomethacin-induced ulcers through prevention of lipid peroxidation and activation of radical scavenging enzymes, such as SOD, catalase, and glutathione peroxidase.

DISCUSSION

NSAIDs have been widely used clinically as anti-inflammatory and analgesic agents [3, 27]. However, ulcerative lesions of the gastrointestinal tract are one of the major side effects of NSAIDs and are the major limitation to their use as anti-inflammatory drugs [2, 14]. Indomethacin is a noncorticosteroid drug that is known to induce ulcerative gastric damage. Indomethacin comprises polar lipids that have a high affinity for the lipophilic areas of cell membranes, where their polar groups trigger membrane disruption, with loss of structural phospholipids and membrane proteins. In addition, this leads to reduced hydrophobicity of the mucosal coat adherent to the mucosal cell surface. Such loss of hydrophobicity facilitates the entry of water-soluble agents of injury (*e.g.*, acid, pepsin, bile salts, *etc.*), which cause lipid peroxidation and also alter membrane fluidity [18]. As in previous reports, the oral administration of indomethacin in rats causes ulcerative lesions in the gastric mucosa [7, 13]. In particular, generation of oxygen free radicals and lipid peroxidation play a key role in the development of the gastric mucosal lesions induced by

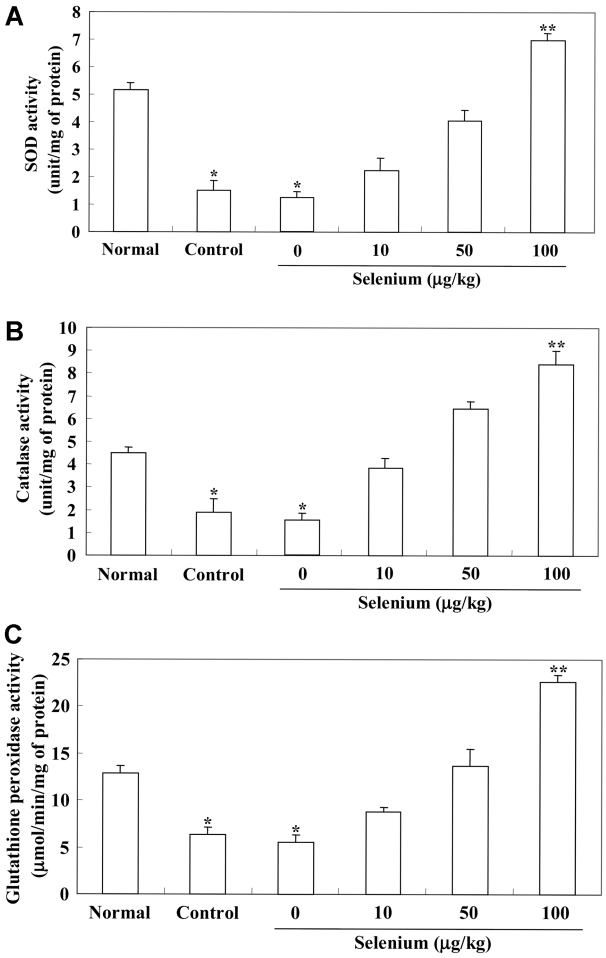

Fig. 3. Effect of selenium on activities of radical scavenging enzymes in gastric mucosa.

Gastric ulcers were induced by oral administration of 25 mg/kg indomethacin, and then different doses (10, 50, and 100 μg/kg) of selenium or vehicle were treated by oral gavage for 3 days. Selenium treatment distinctly increased the activities of SOD (**A**), catalase (**B**), and glutathione peroxidase (**C**) in a dose-dependent manner in comparison with the control group. Values are expressed as means ± SEM. **P*<0.05, significantly different from the untreated normal rats. ***P*<0.01, significantly different from the control rats.

indomethacin [6, 22, 25, 26, 28, 29]. In this study, we hypothesized that selenium shows a curative effect against indomethacin-induced gastric ulcers, and such curative effect may be directly involving its antioxidant property. Therefore, this study examined the curative effect of selenium against indomethacin-induced gastric ulcers by measuring the amount of lipid peroxidation and by comparing the activities of enzymatic scavengers, such as SOD, catalase, and glutathione peroxidase.

Oral administration with indomethacin significantly caused the increase of the gastric ulcer area and lipid peroxidation in the mucosa of stomach, whereas these increases were inhibited by selenium treatment for 3 days in a dose-dependent manner. Among the three doses of selenium tested, the highest dose (100 μg/kg) showed the best effect in reducing the gastric damage area and the lipid peroxide level. Moreover, indomethacin treatment markedly decreased the activities of SOD, catalase, and glutathione peroxidase in the gastric mucosa. According to the previous study, the radical scavenging enzymes, such as SOD, catalase, and glutathione peroxidase, provide defense against the oxidative tissue damage of gastric mucosa after administration of NSAIDs [22]. These enzymes play an important role in the elimination of oxygen free radicals and lipid hydroperoxides in the gastric mucosal cell [1, 19, 22]. Therefore, these results suggest that the inhibition of these enzymatic activities is, at least in part, responsible for oxidative tissue damage of gastric mucosa occurring after indomethacin treatment. On the other hand, selenium increased the activities of these enzymes in a dose-dependent manner. In particular, oral administration of 100 μg/kg selenium showed a drastic increase of activities of radical scavenging enzymes, up to more than the level of untreated normal rats.

In conclusion, this study reveals that selenium shows a curative effect against indomethacin-induced gastric ulcers through prevention of lipid peroxidation and activation of radical scavenging enzymes, and such curative effect is directly involving its antioxidant property. Therefore, we suggest that selenium is a powerful free radical quencher, and its use may offer an attractive strategy for curing gastric ulcers in humans.

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