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

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Indomethacin is a nonsteroidal 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
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 indomethacin. 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 malondialdehyde (MDA) production by using a thiobarbituric acid reaction [20, 21]. Briefly, the stomach homogenate was supplemented with 8.1% sodium (MDA) production by using a thiobarbituric acid reaction [20, 21]. Lipid peroxidation was determined by measuring malonylaldehyde production by using 30 mM tert-butyl hydroperoxide or 80% cumene hydroperoxide. Absorbance was recorded by the following program: wavelength, 540 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.

**SOD Assay**

SOD is a family of predominantly intracellular metalloproteins that catalyzes the dismutation of O$_2^-$ to H$_2$O$_2$, 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$_2$O$_2$ 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$_2$O$_2$ (1 ml) and tissue homogenate (100 µl). Under these conditions, the amount of catalase required to decompose 1.0 µmol of H$_2$O$_2$ 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$_2$O$_2$ 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 x 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-buty1 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-buty1 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,