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Curcumin Blocks Naproxen-Induced Gastric Antral Ulcerations through Inhibition of Lipid Peroxidation and Activation of Enzymatic Scavengers in Rats

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Curcumin is a polyphenol derived from the plant *Curcuma longa*, which is used for the treatment of diseases associated with oxidative stress and inflammation. The present study was undertaken to determine the protective effect of curcumin against naproxen-induced gastric antral ulcerations in rats. Different doses (10, 50, and 100 mg/kg) of curcumin or vehicle (curcumin, 0 mg/kg) were pretreated for 3 days by oral gavage, and then gastric mucosal lesions were caused by 80 mg/kg naproxen applied for 3 days. Curcumin significantly inhibited the naproxen-induced gastric antral ulcer area and lipid peroxidation in a dose-dependent manner. In addition, curcumin markedly increased activities of radical scavenging enzymes, such as superoxide dismutase (SOD), catalase, and glutathione peroxidase in a dose-dependent manner. Specifically, 100 mg/kg curcumin completely protected the gastric mucosa against the loss in the enzyme, resulting in a drastic increase of activities of radical scavenging enzymes up to more than the level of untreated normal rats. Histological examination obviously showed that curcumin prevents naproxen-induced gastric antral ulceration as a result of direct protection of the gastric mucosa. These results suggest that curcumin blocks naproxen-induced gastric antral ulcerations through prevention of lipid peroxidation and activation of radical scavenging enzymes, and it may offer a potential remedy of gastric antral ulcerations.

Keywords: Curcumin, gastric antral ulceration, lipid peroxidation, naproxen, oral gavage, radical scavenging enzymes

Introduction

Nonsteroidal anti-inflammatory drugs (NSAID) induce severe ulcerations as one of the most common side effects and these ulcerative lesions are the major limitation to their use as anti-inflammatory drugs [4–6, 31]. In this study, naproxen was chosen as the ulceration causing NSAID in rats, because it is used more frequently than other NSAIDs for arthritic patients, and also because the naproxen-induced gastric antral ulcer model is suitable in the human situation, where NSAID-induced gastric ulceration occurs mainly in the gastric antrum [5, 6, 19, 20, 25]. Naproxen is a NSAID with anti-inflammatory, antipyretic, and pain-relieving properties, which is known to induce erosions, antral ulcer, and petechial bleeding in the gastrointestinal tract as an adverse effect [5, 31]. According to previous reports, the development of gastric antral ulcerations induced by naproxen is mainly mediated through generation of oxygen free radicals and lipid peroxides [24, 36].

Curcumin is a natural phenolic component derived from the plant *Curcuma longa*, which is used in some cultures for the treatment of diseases associated with oxidative stress and inflammation [18]. Recently, great attention has been paid to the medical applications of curcumin in the treatment of human diseases [10, 26, 29]. Curcumin has been recognized as a promising anticancer drug owing to

its efficient induction of proliferation arrest and cell death, including apoptosis and necrosis in a variety of tumor cells [2, 14, 16, 17]. Curcumin exerts anticancer activity in human leukemia cells via diminishing ROS generation at low concentrations, so it exhibits a variety of pharmacological effects, including antitumor and anti-inflammatory, and apoptotic cell death [3, 27, 30, 37] and also prevents tumor-induced T cell apoptosis [28]. Furthermore, curcumin has been used in treatment of pancreatic cancer [7, 32], multiple myeloma [11, 22], Alzheimer's disease [34], and colorectal cancer [8]. Despite these diverse strides in investigations of different diseases, the protective effect of curcumin against naproxen-induced gastric antral ulcerations has not been studied well. Therefore, we investigated the protective effect of curcumin against naproxen-induced gastric antral ulcerations by measuring the amount of lipid peroxidation and by comparing activities of enzymatic scavengers, such as superoxide dismutase (SOD), catalase, and glutathione peroxidase.

Materials and Methods

Chemicals

Curcumin and naproxen were purchased from Sigma Chemicals (USA). Curcumin was dissolved in saline immediately before use and administered intragastrically to rats in a volume of 5 ml/kg. Naproxen was dissolved in distilled water and subsequently administered by oral gavage, with an appropriate feeding needle in a volume of 5 ml/kg.

Animals

Male Sprage-Dawley rats (200–250 g, 7 weeks old) were purchased from Daehan Biolink Co., Ltd. Rats were placed singled in cages with wire-net floors in a controlled room (temperature 22–24°C, humidity 70–75%, lighting regimen of 12 h light and 12 h dark), and they were fed a normal laboratory diet. Typically, rats were fasted for 18 h prior to studies. Following the first dose of naproxen, rats were provided with food for the remainder of the study. Rats were also allowed tap water throughout the study period. 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 Strategy

The optimal condition for induction of naproxen-induced gastric antral ulcers in rats was determined on the basis of our previous study [13]. To investigate the protective effect of curcumin against naproxen-induced gastric antral ulcerations, the rats were divided into six groups (n = 8 rats per group). The untreated normal rats received distilled water twice daily (at 07.00 h and 17.00 h) for 3 days, in comparable volume by oral route. The control rats received only 80 mg/kg naproxen twice daily (at 07.00 h and 17.00 h) for 3 days. Each of the remaining four test groups was treated with a vehicle (curcumin, 0 mg/kg) or three doses (10, 50, and 100 mg/kg) of curcumin for 3 days, and then treated with 80 mg/kg naproxen twice daily (at 07.00 h and 17.00 h) for 3 days. All the rats were killed under deep ether anesthesia 4 h after the naproxen treatment. 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 [9]. The quantitative analysis of protein was measured by Bradford protein assay.

Measurement of Lipid Peroxidation

Lipid peroxidation was determined by measuring the concentration of malondialdehyde (MDA) in the gastric mucosa according to the modified method of Ohkawa *et al*. [23]. The stomach homogenate was supplemented with 8.1% sodium dodecyl sulfate, 20% acetic acid (pH 3.5), and 0.8% TBA, 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. Lipid peroxidation was calculated from the standard curve using the MDA tetrabutylammonium salt. MDA concentrations were expressed as nM/g of tissue.

Measurement of SOD Activity

The activity of SOD in gastric mucosa of rats was determined according to the method of McCord and Fridovich [21]. 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 at 550 nm was started after adding xanthine oxidase. 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 units/mg of protein.

Measurement of Catalase Activity

The activity of catalase in gastric mucosa of rats was determined 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 minute 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 units/mg of protein.

Measurement of Glutathione Peroxidase Activity

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, 10 units/ml glutathione reductase). A sample of supernatant fluid with homogenate solution and 50 mM potassium phosphate buffer at pH 7.5 was prepared by centrifuging it at 1,000 ×*g* for 10 min at 4°C. The cuvette was subsequently filled with 900 μl of glutathione peroxidase assay buffer, 50 μl of NADPH assay reagent, and 50 μl of sample, and mixed by inversion. The reaction started when 10 μl of 30 mM *tert*-butyl hydroperoxide or 80% cumene hydroperoxide was added. Absorbance was recorded by the following program; Wavelength: 340 nm/ Initial delay: 15 sec/ Interval: 10 sec/ Number of readings: 6. The activity of enzyme was the sum of data using 30 mM *tert*-butyl hydroperoxide and 80% cumene hydroperoxide. The level of glutathione was expressed in terms of μM/min/mg of protein.

Histopathology

Stomach tissues were fixed in 10% neutral formalin and embedded in paraffin, and 4-μm-thick sections were prepared and stained with hematoxylin and eosin by standard procedures.

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 $p < 0.05$.

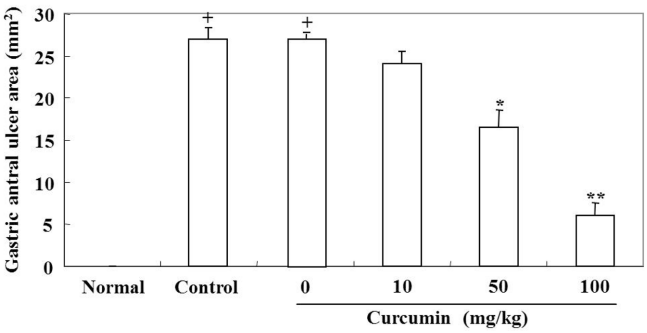

Fig. 1. Effect of curcumin on naproxen-induced gastric antral ulcer formation.

A vehicle (curcumin, 0 mg/kg) or three doses (10, 50, and 100 mg/kg) of curcumin were pretreated for 3 days, and then gastric antral ulcer was caused by 80 mg/kg naproxen treatment twice daily (at 07.00 h and 17.00 h) for 3 days. Curcumin significantly attenuated the gastric antral 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 group. $^{*}p < 0.05$ and $^{**}p < 0.01$, significantly different from the control group.

Results

Effect of Curcumin Treatment on Naproxen-Induced Gastric Antral Ulcer Formation

To verify the protective effect of curcumin on gastric antral ulcer formation induced by naproxen, a vehicle (curcumin, 0 mg/kg) or three doses (10, 50, and 100 mg/kg) of curcumin were pretreated for 3 days, and then treated with 80 mg/kg naproxen twice daily (at 07.00 h and 17.00 h) for 3 days. Gastric lesions were found to be primarily

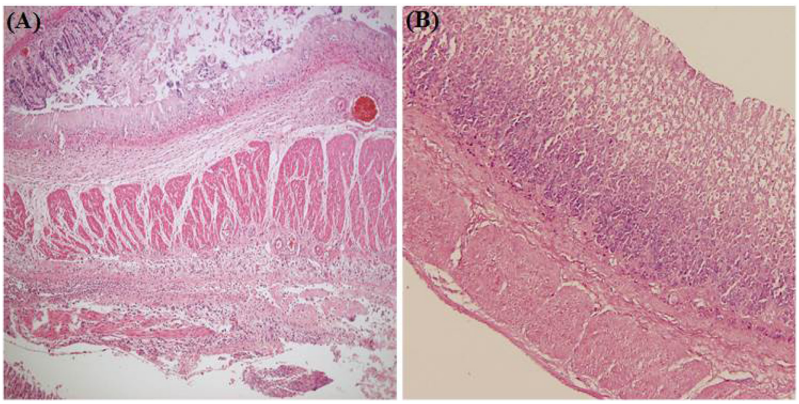

Fig. 2. Effect of curcumin on naproxen-induced gastric antral lesions in rats.

Curcumin (100 mg/kg) was pretreated for 3 days, and then gastric antral ulcer was caused by 80 mg/kg naproxen treatment twice daily (at 07.00 h and 17.00 h) for 3 days. (**A**) Gastric antral lesions in naproxen-treated rat. Gastric lesions are clearly visible in the gastric antrum. (**B**) Gastric antrum in curcumin-pretreated rat. Curcumin (100 mg/kg) inhibits naproxen-induced gastric antral lesions through direct protection of gastric mucosa.

in the form of antral ulcers and judged macroscopically by clear depth of penetration into the gastric mucosal surface in all test groups. As shown in Fig. 1, naproxen in the control and vehicle (curcumin, 0 mg/kg) groups showed drastic increase of the gastric antral ulcer area in the mucosa of stomach, compared with the untreated normal group ($^{+}p < 0.05$). On the other hand, curcumin significantly decreased the gastric antral ulcer formation in a dose-dependent manner, compared with the control group ($^{*}p < 0.05$, $^{**}p < 0.01$). Among the doses tested, 100 mg/kg curcumin was the most effective in inhibiting naproxen-induced gastric antral ulcer formation ($^{**}p < 0.01$).

To estimate the direct protective effect of curcumin against naproxen-induced gastric musosal lesions, histological examination was performed (Fig. 2). Naproxen induced gastric hemorrhagic lesions, affecting mostly the glandular portion of the mucosa (Fig. 2A). The lesions were long and thin in appearance, and were observed along the crests of the sides of rugal folds. In addition, naproxen caused mucosa cell necrosis, mucosa hemorrhagic erosion, and gastric pit disappearance in the stomachs of the control group. In contrast, curcumin pretreatment protected the gastric mucosa from naproxen-induced gastric lesions (Fig. 2B). Mucosa cell necrosis and mucosa hemorrhagic erosion occurred far less, and gastric pit disappearance was not observed. Mild to moderate villous atrophy was noted, and severe necrotic changes in mucus ridges were not observed. These results indicate that curcumin blocks naproxen-induced gastric antral ulcerations as a result of direct protection of the gastric mucosa.

Effect of Curcumin Treatment on Lipid Peroxidation and Radical Scavenging Enzyme Activities

To evaluate the gastroprotective mechanism of curcumin on naproxen-induced gastric antral ulcerations, the level of lipid peroxide and activities of scavenging enzymes were measured. As shown in Table 1, naproxen in the control and vehicle (curcumin, 0 mg/kg) groups significantly increased the level of MDA, as an index of lipid peroxidation, in comparison with the untreated normal group ($^{+}p < 0.05$). In contrast, curcumin reduced the level of MDA in a dose-dependent manner in comparison with the control group ($^{*}p < 0.05$, $^{**}p < 0.01$). Specifically, 100 mg/kg curcumin prominently decreased the level of MDA ($^{**}p < 0.01$), compared with control group.

In Table 2, activities of SOD, catalase, and glutathione peroxidase in the control and vehicle (curcumin, 0 mg/kg) groups were rapidly reduced in comparison with the untreated normal group ($^{+}p < 0.05$). However, all doses of curcumin increased the activities of these enzymes in a dose-dependent manner in comparison with the control group ($^{*}p < 0.05$, $^{**}p < 0.01$). Specifically, 100 mg/kg curcumin completely protected the gastric mucosa against the loss in the enzyme, resulting in a drastic increase of the activities of radical scavenging enzymes up to more than the level of the untreated normal group ($^{**}p < 0.01$).

Based on our present data, we suggest that curcumin effectively blocks naproxen-induced gastric antral ulcerations through inhibition of lipid peroxidation and activation of radical scavenging enzymes, such as SOD, catalase, and glutathione peroxidase.

Table 1. The lipid peroxide levels in all experimental groups.

Groups	LPO (MDA nM/g of tissue)
Normal	12.32 ± 0.96
Control	27.43 ± 2.49^{+}
Test 1	27.02 ± 2.64^{+}
Test 2	23.04 ± 1.76
Test 3	18.98 ± 1.82*
Test 4	12.46 ± 1.24**

Control: 80 mg/kg naproxen; Test 1: vehicle (curcumin, 0 mg/kg) + 80 mg/kg naproxen; Test 2: 10 mg/kg curcumin + 80 mg/kg naproxen; Test 3: 50 mg/kg curcumin + 80 mg/kg naproxen; Test 4: 100 mg/kg curcumin + 80 mg/kg naproxen. Values are expressed as means ± SEM. $^{+}p < 0.05$, significantly different from the untreated normal group. $^{*}p < 0.05$ and $^{**}p < 0.01$, significantly different from the control group.

Discussion

Cucumin, a major component of a dietary spice derived from the roots of *Curcuma longa*, has been recognized as a

Table 2. The activities of radical scavenging enzymes in all experimental groups.

Groups	SOD (units/mg of protein)	Catalase (units/mg of protein)	Glutathione peroxidase (μM/min/mg of protein)
Normal	5.64 ± 0.88	4.92 ± 0.83	14.46 ± 2.82
Control	1.96 ± 0.94^{+}	2.04 ± 0.48^{+}	4.08 ± 1.32^{+}
Test 1	2.04 ± 0.72^{+}	2.12 ± 0.52^{+}	4.26 ± 1.56^{+}
Test 2	3.02 ± 0.86	3.44 ± 0.56	8.83 ± 1.98
Test 3	4.98 ± 0.82*	5.04 ± 1.32*	14.98 ± 2.58*
Test 4	8.36 ± 0.93**	8.28 ± 1.46**	20.24 ± 2.89**

Control: 80 mg/kg naproxen; Test 1: vehicle (curcumin, 0 mg/kg) + 80 mg/kg naproxen; Test 2: 10 mg/kg curcumin + 80 mg/kg naproxen; Test 3: 50 mg/kg curcumin + 80 mg/kg naproxen; Test 4: 100 mg/kg curcumin + 80 mg/kg naproxen. Values are expressed as means ± SEM. $^{+}p < 0.05$, significantly different from the untreated normal group. $^{*}p < 0.05$ and, $^{**}p < 0.01$, significantly different from the control group.

naturally occurring antioxidant, and it exhibits prooxidant properties through affecting histone hypoacetylation under certain conditions [12]. Curcumin exhibits a variety of pharmacological effects, including anti-inflammatory activities via diminishing reactive oxygen species generation at low concentrations [3, 27, 28, 37]. Moreover, treatment of cell cultures and adult rodents with curcumin can protect neurons from being damaged and killed in models relevant to the pathogenesis of Alzheimer's disease, Parkinson disease, and stroke [33, 35]. This study was aimed to investigate the effect of curcumin on naproxen-induced gastric antral ulcerations in rats.

Generally, most NSAID-induced gastric damage occurs mainly in the corpus region of the stomach and tends to be mostly in the form of erosions rather than ulcers. This is unlike the situation in humans, where NSAID-induced gastric ulceration occurs mainly in the gastric antrum [19, 20, 25]. On the other hand, naproxen is used more frequently than other NSAIDs for arthritic patients, and naproxen-induced gastric ulceration occurs mainly in the gastric antrum [5, 6]. Therefore, we used a naproxen-induced gastric antral ulcer model, which is suitable for the human situation.

As our results, naproxen rapidly increased the gastric antral ulcer area and the lipid peroxide level. In contrast, curcumin showed a significant decrease of the gastric antral ulcer area and the lipid peroxide level in the stomach in a dose-dependent manner. Curcumin also markedly increased the activities of SOD, catalase, and glutathione peroxidase in a dose-dependent manner. In particular, 100 mg/kg curcumin was the most effective in inhibiting lipid peroxidation and activating radical scavenging enzymes. Based on our results, we demonstrate that curcumin inhibits lipid peroxidation and inactivation of radical scavenging enzymes induced by naproxen, and such inhibitory effect is directly involving its antioxidant property. Macroscopically, 100 mg/kg curcumin also obviously reduced the depth and severity of the naproxen-induced gastric antral lesions.

In conclusion, curcumin effectively blocks naproxen-induced gastric antral ulcerations through inhibition of lipid peroxidation and activation of radical scavenging enzymes, such as SOD, catalase, and glutathione peroxidase. Thus, we suggest that curcumin is a potent anti-antral ulcer and its use may offer an attractive strategy for curing gastric lesions in humans.

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