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Curcumin Induces Recovery from Indomethacin-Induced Gastric Mucosal Lesions in Rats

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In the present study, the curative effect of curcumin on indomethacin-induced gastric mucosal lesions in rats was investigated. Indomethacin is a nonsteroidal anti-inflammatory drug (NSAID), with serious side effects, including erosion, ulcerative lesions, and petechial bleeding in the mucosa of the stomach. Gastric mucosal lesions were caused by oral administration of 25 mg/kg of indomethacin. Various doses (10, 50, and 100 mg/kg) of curcumin were treated for 3 days by oral gavage. Indomethacin clearly increased the gastric ulcer area in the stomach, and curcumin significantly decreased the gastric ulcer area in a dose-dependent manner. Curcumin also markedly inhibited lipid peroxidation in the gastric mucosa and activated radical scavenging enzymes, such as superoxide dismutase (SOD), catalase, and glutathione peroxidase, in a dose-dependent manner. These results suggest that curcumin can induce recovery from indomethacin-induced gastric mucosal lesions through inhibition of lipid peroxidation and activation of radical scavenging enzymes, such as SOD, catalase, and glutathione peroxidase. Curcumin appears to be a powerful free radical quencher, and it may offer an attractive strategy for healing gastric mucosal lesions in humans.

Key words : Curcumin, gastric mucosal lesions, lipid peroxidation, oral gavage, radical scavenging enzymes

Introduction

Indomethacin is a non-steroidal anti-inflammatory drug (NSAID) 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 effect [8, 15]. The oral gavage of indomethacin in rats causes ulcerative lesions in the gastric mucosa [6]. The development of the gastric mucosal lesions induced by indomethacin is mainly mediated through generation of oxygen free radicals and lipid peroxidation [4, 19, 22-25]

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 [14]. Recently, great attention has been paid to the medical applications of curcumin in the treatment of human diseases [10, 20, 21]. Curcumin has been recognized as a powerful anticancer drug due to its efficient induction of proliferation arrest and cell death including apoptosis and necrosis in a variety of tumor cells [2, 5, 7, 13]. However, the curative properties of curcumin against gastric mucosal lesions are not well understood. In this study, the curative effect of curcumin against indomethacin-induced gastric mucosal lesions in rats was evaluated 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

Curcumin and indomethacin were purchased from Sigma Chemicals (St Louis, MO., USA). Curcumin was dissolved in saline immediately before use and administered intragastrically to rats in a volume of 5 ml/kg. Indomethacin was dissolved in 5% sodium bicarbonate (Sigma, St Louis, MO., USA) and administered to rats in a dose of 25 mg/kg by oral gavage, with an appropriate feeding needle as a volume of 5 ml/kg.

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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 hr light and 12 hr dark), and they were fed a normal laboratory diet. Rats were fasted for 24 hr 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 design

Gastric mucosal lesions were induced by a single oral dose of 25 mg/kg indomethacin. The optimal concentration of indomethacin for induction of gastric mucosal lesions was determined on the basis of the previous our study [11]. 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 (curcumin, 0 mg/kg) or three doses (10, 50, and 100 mg/kg) of curcumin for 3 days after induction of gastric ulcers by pretreatment with 25 mg/kg indomethacin. All the rats were killed under deep ether anesthesia 4 hr after the last oral administration. 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 [3].

Malondialdehyde levels

Lipid peroxidation was determined by measuring malonylaldehyde (MDA) production by using a thiobarbituric acid reaction [17, 18]. 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 hr. 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 peroxide level was calculated from the standard curve using the MDA tetrabutylammonium salt. MDA (Sigma, St Louis, MO., USA) concentrations were expressed as nmoles/g of tissue.

Activities of enzymatic scavengers

The activity of SOD in the gastric mucosa was measured according to the method of McCord and Fridovich [16]. 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 min. 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 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 units/mg of protein.

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 µM 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 units/mg of protein.

The activity of glutathione peroxidase in the gastric mucosa of rats was determined by a modified method of Lawrence and Burk [12]. 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 sec/ Interval: 10 sec/ 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 μM/min/mg of protein.

Statistical analysis

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

Results and Discussion

Indomethacin is a non-steroidal anti-inflammatory drug (NSAID) that is known to cause gastric mucosal lesions. 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 [15]. Especially, generation of oxygen free radicals and lipid peroxidation play a important role in the development of the gastric mucosal lesions induced by indomethacin [4, 19, 22-25].

In this study, the optimal concentration of indomethacin for induction of gastric mucosal lesions was determined on the basis of the previous our study [11]. The concentrations of curcumin were selected on the basis of the preliminary results obtained from cytotoxicity studies using a broad concentration range for this reagent. Gastric mucosal lesions were caused by oral administration with 25 mg/kg of indomethacin, and then various doses (10, 50, and 100 mg/kg) of curcumin were treated for 3 days by oral gavage. Gastric lesions were judged macroscopically by clear depth of penetration into the gastric mucosal surface in all test groups. In Fig. 1, indomethacin significantly caused the increase of the gastric ulcer area in the mucosa of stomach, compared with normal group ($^{*}p<0.05$). However, curcumin markedly decreased the gastric ulcer area in a dose dependent manner, compared with control group ($^{**}p<0.01$).

The development of the gastric mucosal lesions induced by indomethacin is mainly mediated through generation of oxygen free radicals and lipid peroxidation [4, 22, 23, 24]. Therefore, the curative effect of curcumin against indomethacin-induced gastric mucosal lesions in rats was evaluated by measuring the amount of lipid peroxidation and by comparing the activities of enzymatic scavengers such as SOD, catalase, and glutathione peroxidase. Fig. 2 shows the effect of curcumin on lipid peroxidation induced by indomethacin in the gastric mucosa. Indomethacin considerably increased the level of MDA in gastric tissue in comparison to normal group ($^{*}p<0.05$), whereas curcumin decreased the level of MDA in a dose dependent manner

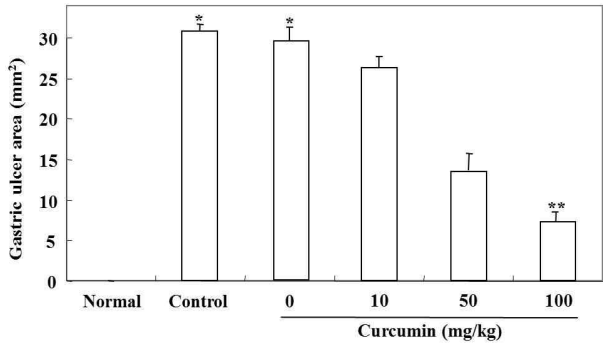

Fig. 1. Curcumin shows the curative effect against indomethacin-induced gastric mucosal lesions in rats. Gastric mucosal lesions were induced by oral administration with 25 mg/kg of indomethacin, and then various doses (10, 50, and 100 mg/kg) of curcumin were treated by oral gavage for 3 days. Curcumin significantly decreased the gastric ulcer area in the mucosa of stomach in a dose dependent manner, compared with control group. Values are expressed as means ± S.E.M. $^{*}p<0.05$, significantly different from the untreated normal group. $^{**}p<0.01$, significantly different from the control group.

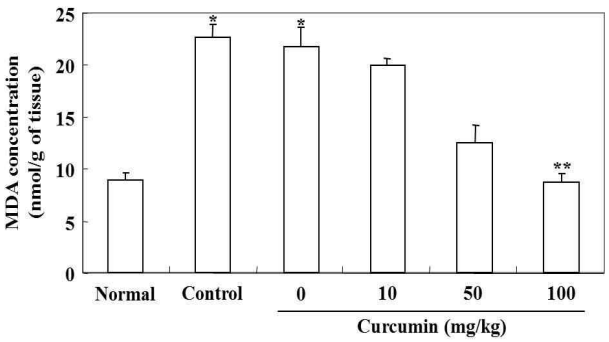

Fig. 2. Curcumin inhibited lipid peroxidation induced by indomethacin in gastric mucosa. MDA production was estimated by using a thiobarbituric acid reaction. curcumin distinctly reduced the level of MDA in a dose dependent manner in comparison to control group. Values are expressed as means ± S.E.M. $^{*}p<0.05$, significantly different from the untreated normal group. $^{**}p<0.01$, significantly different from the control group.

Table 1. The activities of radical scavenging enzymes at different concentrations of curcumin

Curcumin (mg/kg)	SOD (units/mg-protein)	Catalase (units/mg-protein)	Glutathione peroxidase (μmol/min/mg-protein)
Normal	5.82±0.38	6.02±0.24	15.63±0.62
Control	1.12±0.46*	1.54±0.22*	3.78±0.66*
0	1.38±0.42*	1.70±0.20*	4.45±0.58*
10	2.04±0.71	2.02±0.58	7.02±0.92
50	4.80±0.68	5.68±0.54	14.08±0.42
100	6.58±0.34**	7.86±0.61**	21.24±1.14**

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. *p<0.05, significantly different from the untreated normal group. **p<0.01, significantly different from the control group.

in comparison to control group (**p<0.01).

In Fig. 3, the effect of curcumin on activities of radical scavenging enzymes such as SOD, catalase, and glutathione peroxidase was tested. Indomethacin distinctly attenuated activities of SOD, catalase, and glutathione peroxidase in comparison to normal group (*p<0.05). 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. In contrast, curcumin increased activities of these enzymes in a dose dependent manner in comparison to control group (**p<0.01). At the highest dose (100 mg/kg) group of curcumin, the enzyme activities were enhanced by 4.6 fold over 0 dose group (Table 1).

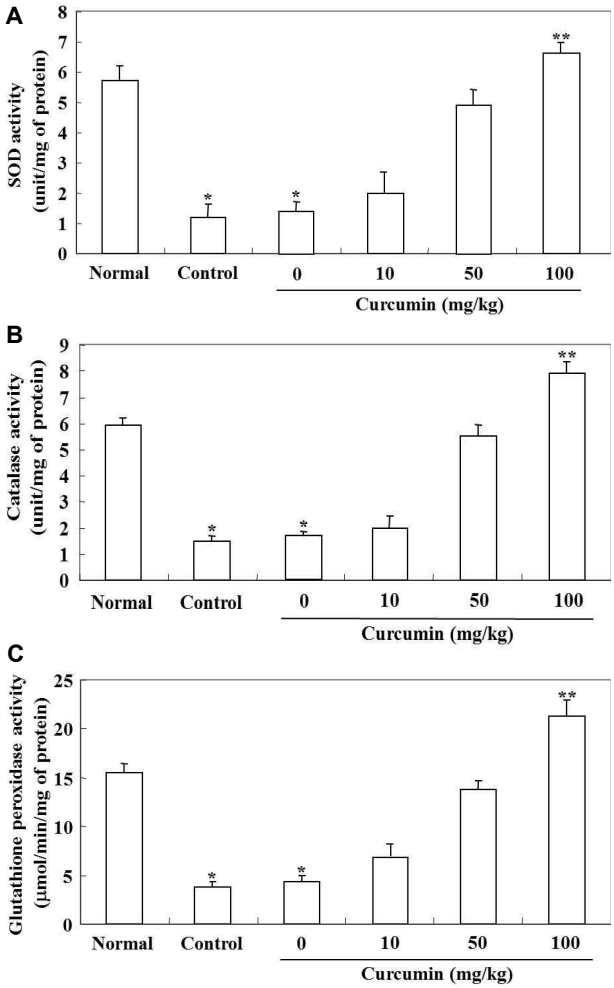

Fig. 3. Curcumin activated radical scavenging enzymes in indomethacin-induced gastric mucosal lesions. Curcumin markedly increased activities of SOD (A), catalase (B), and glutathione peroxidase (C) in a dose dependent manner in comparison to control group. Values are expressed as means ± S.E.M. *p<0.05, significantly different from the untreated normal group. **p<0.01, significantly different from the control group.

In conclusion, curcumin induce recovery from indomethacin-induced gastric ulcers through prevention of lipid peroxidation and activation of radical scavenging enzymes, and such effect is directly involving its antioxidant property. Therefore, we suggest that curcumin is a powerful free radical quencher, and its use may offer an attractive strategy for healing gastric mucosal lesions in humans.

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초록 : 커규민의 인도메타신 유도 위점막 손상에 대한 치료 효과

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인도메타신은 nonsteroid anti-inflammatory agent로 심각한 위점막 손상을 야기한다. 본 논문에서는 rat의 위점막 손상에 대한 커규민의 치료효과를 규명하였다. 인도메타신 유도 위점막 손상에 대한 커규민의 치료효과를 검증하기 위하여 인도메타신(25 mg/kg)의 경구투여를 통하여 위점막 손상을 유발한 후 다양한 농도(10, 50, 100 mg/kg)의 커규민을 3일간 경구 투여하였다. 실험 결과, 인도메타신의 처리는 위점막에 궤양부위를 증가시킨 반면, 3일간 커규민의 경구투여는 농도 의존적으로 궤양부위를 유의성있게 감소시켰다. 뿐만 아니라, 커규민은 인도메타신에 의해 유도되는 위점막에서의 지질과산화 증가를 상당히 억제시켰고 radical scavenging enzyme인 superoxide dismutase, catalase, glutathione peroxidase의 활성을 농도 의존적으로 증가시켰다. 이상의 결과들을 종합하여 볼 때, 커규민은 인도메타신 유도 위점막 손상을 지질과산화의 억제와 radical scavenging enzymes의 활성화를 통하여 치료한다.