Zinc(II)–curcumin accelerates the healing of acetic acid-induced chronic gastric ulcers in rats by decreasing oxidative stress and downregulation of matrix metalloproteinase-9

Xue-Ting Mei, Dong-Hui Xu *, Si-Ka Xu, Yan-Ping Zheng, Shi-Bo Xu

Laboratory of Traditional Chinese Medicine and Marine Drugs, School of Life Sciences, Sun Yat-Sen University, Guangzhou 510275, China

Abstract

Gastric ulcers form as a result of a multifaceted process which includes acid secretion, reactive oxygen species generation and extracellular matrix (ECM) degradation. The aim of this study was to investigate the possible mechanisms underlying the anti-ulcerogenic effects of the Zn(II)–curcumin complex, a curcumin derivative, on the healing of acetic acid-induced gastric ulcers in rats. The severely ulcerated gastric mucosa of control animals had a lower glutathione level (GSH) and superoxide dismutase activity (SOD), and increased malondialdehyde (MDA) content compared to sham operated rats (P<0.001). Zn(II)–curcumin solid dispersions (equivalent to 12, 24 and 48 mg/kg) dose-dependently reduced the gastric ulcer index, significantly increased SOD activity and GSH levels, and reduced the MDA content to control animals (P<0.001). In conclusion, these results confirm that the Zn(II)–curcumin complex possesses an enhanced mucosal barrier defense activity compared to curcumin alone, due to its synergistic ability to decrease oxidative stress and attenuate MMP-9-mediated inflammation.

1. Introduction

Peptic ulcers are a serious problem in humans and are highly prevalent worldwide, with an annually increasing incidence. Gastric bleeding and ulceration have often been attributed to an increase in gastric acid secretion, which damages the gastric mucosal barrier and penetrates the mucus layer, allowing endogenous and exogenous aggressors to induce injury to the epithelial cell surface (Heeba et al., 2009; Goldstein et al., 2006). Oxygen free radical production and lipid peroxidation play a crucial role in the development of gastric ulceration (Silva et al., 2013; Jaiswal et al., 2011), and gastric mucosal damage is directly associated with extracellular matrix (ECM) degradation, in which the zinc-dependent matrix metalloproteinases (MMPs) play a crucial role (Wang et al., 2012).

Zinc, an essential trace metal, has been shown to facilitate the healing of small intestinal mucosal damage. Zinc deficiency significantly impairs wound healing by delaying fibroblast proliferation and collagen synthesis (Zalewski et al., 2005). As a free radical scavenger and anti-inflammatory agent, zinc can halt the progression of gastrointestinal disease and interrupt the associated inflammatory processes. Zinc complexes have also been shown to exert potent antiulcer activity (Clara Gerosa et al., 2008). Zinc-carnosine is an anti-ulcer drug commonly used in the treatment of gastric ulcers in Japan (Odashima et al., 2006). Pre-treatment with zinc sulfate (22, 44 and 88 mg/kg body weight, i.p.) was found to reduce reserpine-induced gastric ulceration in rats by inducing lysosomal stabilization (Pfeiffer et al., 1980). The zinc-indomethacin complex demonstrated more significant attenuated ulcerogenic effects than the parent drug indomethacin, and was 2.55 times more potent than the corresponding physical mixture of indomethacin and zinc sulfate (Singla and Wadhwa, 1995). Anti-inflammatory studies in the carrageenan-induced hind paw edema model indicated that the zinc–aspirin complex was 2.64 times more potent than aspirin, and 1.73 times more potent than a physical mixture of aspirin and zinc sulfate (Singla and Wadhwa, 1998).

In our previous work, Zn(II)–curcumin solid dispersions (SDs) (equivalent to 12, 24 and 48 mg/kg Zn(II)–curcumin, p.o.) showed a significantly higher antiulcer activity than curcumin SDs alone (equivalent to 24 mg/kg curcumin, p.o.) in a rat model of pylorus ligature-induced gastric ulcers, by inhibiting NF-κB activation and the subsequent production of proinflammatory cytokines.
Male adult Sprague–Dawley rats (6–7 wks, 180–200 g) were housed in a facility at the Laboratory of Traditional Chinese Medicine and Marine Drugs, School of Life Sciences, Sun Yat-sen University which is approved by the Guangdong Experimental Animals Association (Guangzhou, China), under conditions of constant temperature (23 ± 1 °C) and relative humidity (50 ± 5%) with a 12:12 h light: dark cycle (lights on at 07:00 h) before and during the experiment. All experiments were previously approved by the Institutional Ethics Committee of Sun Yat-sen University (Guangzhou, China) and were carried out in accordance with international standards and ethical guidelines on animal welfare.

2.2. Drugs and reagents

Curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] and Zn(II)–curcumin (greater than 99% purity) were manufactured by Guangdong Zhongda Greenfield Bio-technology Co., (Guangzhou, China). Polyvinylpyrrolidone (PVP) was purchased from BASF Chemical Ltd. (New York, USA). Lansoprazole tablets were obtained from Shengfan Pharmaceutical Company Ltd., (Henan, China). Synthesis of Zn(II)–curcumin and solid dispersions (SDs) followed methods previously described by our laboratory (Singla and Wadhwa, 1998). All other chemicals were of reagent grade.

2.3. Acetic acid–induced ulcer model

Sprague–Dawley rats were divided into seven experimental groups with 10 animals in each group: normal group (300 mg/kg PVP vehicle, p.o.), control group (300 mg/kg PVP vehicle, p.o.), Zn(II)–curcumin SDs groups (equivalent to 12, 24 and 48 mg/kg Zn(II)–curcumin, p.o.), curcumin SDs group (equivalent to 24 mg/kg curcumin, p.o.) and lansoprazole group (7.8 mg/kg lansoprazole, p.o.). Gastric ulcers were induced in all animals, except for the normal group, by luminal application of acetic acid as described by Silva et al. (2013). Briefly, the rats were starved for 24 h and a laparotomy was performed under anesthesia with xylazine/ketamine acetic acid as described by Silva et al., 2013. The rats were then sutured closed and the abdomens were then sutured closed and the rats were allowed to recover with free access to food and water. Two days after the induction of ulcers, daily treatment with the various drugs for 10 days, the rats were killed by cervical dislocation and the stomachs were immediately removed. The stomach tissues were split longitudinally and pinned onto a card. An independent observer scored the macroscopic appearance of the gastric mucosa. The ulcer area was calculated as follows: \( A = \pi (a^2 - b^2) \), where \( a \) was the long axis and \( b \) the short axis. The ulcer area was expressed as an ulcer index.

2.4. Measurement of SOD activity, and GSH and MDA levels

After the macroscopic analyses, SOD activity, and GSH and MDA levels were determined in the rat stomach tissues. The stomach tissues were homogenized with liquid nitrogen using a mortar and pestle, then 0.5 g tissue was mixed with 4.5 ml of liquid nitrogen using a mortar and pestle, then 0.5 g tissue was mixed with 4.5 ml of homogenization buffer (Tris 10 mM, pH 7.4), homogenized on ice using an Ultraturrax homogenizer (IKA Werke GmbH & Co. Deutschland, Germany) for 15 min, centrifuged at 1000 g at 4 °C for 20 min. The supernatants were used for the determination of biochemical analyses.

SOD activity was measured using the generation of superoxide radicals produced by xanthine and xanthine oxidase, which react with nitro blue tetrazolium (NBT) to form a formazan dye (Suckow et al., 1985). SOD activity was measured at 560 nm as the degree of inhibition of this reaction by the tissue homogenate supernatant. SOD activity leading to 50% inhibition was considered as 1 unit per milligram protein. The GSH level was determined using the method described by Moron (Moron et al., 1979). Briefly, the homogenate was precipitated with 25% trichloro acetic acid (TCA) and centrifuged. The supernatant was used for GSH estimation using freshly prepared DTNB solution. The intensity of the yellow color formed was read at 412 nm using a spectrophotometer and compared with glutathione standards. MDA content was determined using the method described by Okawa et al. (1989). Half a milliliter of homogenate was mixed with 3 ml of H2PO4 solution (1%, v/v) followed by the addition of 1 ml of thiobarbituric acid solution (0.67%, w/v). Then the mixture was heated in a water bath at 95 °C for 45 min. The colored complex was extracted into n-butanol, and the absorption at 532 nm was measured using tetramethoxypropane as standard. MDA levels were expressed as nmol TBA per mg of protein.

2.5. Reverse transcriptase (RT)–PCR analysis of MPP–9

Total cellular RNA was extracted from the gastric mucosa using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s protocol, and quantified by measuring absorbance at 260 nm. Complementary DNA (cDNA) was synthesized from 1 μg total RNA in 20 μl reactions using SuperScript II reverse transcriptase (Invitrogen). The cDNA (1 μl) was amplified by RT–PCR in 20 μl reactions using primers for MPP–9 (sense, 5’–CTGGATATGCATCAGTGC–3’ and anti-sense, 5’–CTGTTCGGCAGTTTACAG–3’) and β-actin (sense, 5’–TCCAACTGTTGACAGC–3’ and anti-sense, 5’–AGGGCTACGGAGACAAC–3’) over 35 cycles of denaturation (94 °C for 30 s), annealing (54 °C for 30 s) and extension (72 °C for 60 s). The PCR products were analyzed by electrophoresis using 2% agarose gels and visualized by ethidium bromide staining.

2.6. Histological analysis

After the stomach specimens for RT–PCR were obtained, the remaining tissues (margin of the ulcer) were immediately immersed and fixed in 4% paraformaldehyde in phosphate buffer (pH 7.6) for 48 h at 4 °C. Paraffin sections (5 μm thick) were prepared and stained with Mayer’s hematoxylin and eosin according to standard procedures. Tissue preparations were observed and microphotographed under a light microscope.

2.7. Statistical analysis

All values are expressed as the mean ± S.D. of the 10 animals in each group. The data was analyzed using SPSS version 13 software (SPSS, Chicago, IL, USA). Hypothesis testing methods included one-way analysis of variance (ANOVA) followed by Dunnett’s T3 multiple comparisons test. Significance was assessed at the levels of \( P < 0.001 \), \( P < 0.01 \), and \( P < 0.05 \).

3. Results

3.1. Zn(II)–curcumin protects against acetic acid–induced gastric lesions

Macroscopic appearances and microscopic analysis of gastric tissue are shown in Figs. 1 and 2, respectively. Rats treated only with PVP (Fig. 1A) showed normal glandular stomach. Fig. 1B shows that revealed deep ulceration, severe edematous changes in gastric mucosa in control group induced by 60% acetic acid and daily treated with PVP. By contrast, rats treated with Zn(II)–curcumin or curcumin showed less severe ulceration and less edema in gastric mucosa induced by 60% acetic acid (Fig. 1C–F). Almost normal gastric mucosa were observed in lansoprazole treated group (Fig. 1G).

As shown in Fig. 2A, histologically, normal gastric epithelium and lamina propria were observed in the normal sham-operated group. Following the application of 60% acetic acid, gastric ulcers with extensive destruction of surface epithelium, subepithelial vasocongestion, and swelling and spoiling of the epithelial cells in the glandular stomach were observed in the control group (Fig. 2B). Additionally, acetic acid induced massive areas of necrotic epithelial destruction, submucosal edema, areas of hemorrhage and inflammatory cell infiltration, and the ulcers were scarred due to the migration of transitional zone cells into the ulcerated areas.

Zn(II)–curcumin (12, 24 and 48 mg/kg) significantly reduced the severity of gastric ulcers and lead to well developed marginal regeneration of the epithelium, compared to the control group (Fig. 2C–E). In curcumin (24 mg/kg) treated, mild disruptions of the epithelium, submucosal edema, areas of hemorrhage and inflammatory cell infiltration, and the ulcers were scarred due to the migration of transitional zone cells into the ulcerated areas.

X.-T. Mei et al. / Food and Chemical Toxicology 60 (2013) 448–454
As shown in Table 1, oral administration of Zn(II)–curcumin at dose of 12, 24 and 48 mg/kg decreased the gastric ulcer index to 35.37 ± 8.76, 28.02 ± 6.86 and 22.64 ± 7.08 mm² (21.3%, 37.7% and 49.6% protection) in comparison to control 44.96 ± 9.02 mm² (P < 0.05, P < 0.001, P < 0.001, respectively). At a dose of 24 mg/kg, Zn(II)–curcumin exerted a greater anti-ulcerogenic effect and lead to a significantly lower gastric ulcer index (28.02 ± 6.86 mm²) than curcumin (35.91 ± 8.92 mm²; P < 0.05). Lansoprazole (7.8 mg/kg) demonstrated similar potency to reduce the gastric ulcer index (24.14 ± 9.14 mm²; P < 0.001 compared to the control group).

Fig. 1. Gross appearances of acetic acid-induced gastric ulcers in (A) Normal group daily treated with PVP (300 mg/kg, p.o.) for 10 days, (B) Ulceration of control group induced by acetic acid and daily treated with PVP (300 mg/kg, p.o.) for 10 days, (C–E) Ulceration of Zn(II)–curcumin group induced by acetic acid and daily treated with Zn(II)–curcumin SDs (12, 24 and 48 mg/kg, p.o.) for 10 days, (F) Ulceration of curcumin group induced by acetic acid and daily treated with curcumin (24 mg/kg, p.o.) for 10 days, (G) Ulceration of Lansoprazole group induced by acetic acid and daily treated with Lansoprazole (7.8 mg/kg, p.o.) for 10 days.
3.2. Zn(II)–curcumin increases the SOD activity and GSH level, and reduces MDA content in acetic acid-induced gastric tissue of rats

Increased oxidant stress was observed in the ulcerated gastric mucosa of the control group, indicated by a significant decrease in SOD activity and the level of GSH, as well as a significant increase in lipid peroxidation (MDA content) compared to the sham-operated group (all \( P < 0.001 \)).

As shown in Table 1, SOD activity in the mucosa was significantly lower (55.6 ± 6.4 U/mg protein) in the acetic acid-treated animals from the control group than the normal group (123.9 ± 15.1 U/mg protein; \( P < 0.001 \)). Zn(II)–curcumin treatment at 12, 24 and 48 mg/kg significantly increased the SOD activity to 70.4 ± 16.0, 84.3 ± 20.0 and 108.7 ± 23.9 U/mg protein (\( P < 0.05 \), \( P < 0.001 \) and \( P < 0.001 \), respectively. Additionally, the administration of 24 mg/kg Zn(II)–curcumin lead to a higher SOD activity.

Fig. 2. Photomicrographs of acetic acid-induced gastric ulcers in (A) Normal group daily treated with PVP (300 mg/kg, p.o.) for 10 days, (B) Ulceration of control group induced by acetic acid and daily treated with PVP (300 mg/kg, p.o.) for 10 days, (C–E) Ulceration of Zn(II)–curcumin group induced by acetic acid and daily treated with Zn(II)–curcumin SDs (12, 24 and 48 mg/kg, p.o.) for 10 days, (F) Ulceration of curcumin group induced by acetic acid and daily treated with curcumin (24 mg/kg, p.o.) for 10 days, (G) Ulceration of Lansoprazole group induced by acetic acid and daily treated with Lansoprazole (7.8 mg/kg, p.o.) for 10 days. The H.E. stained slides were visualized under a bright field microscope with 20 x magnification.
than administration of curcumin at the same concentration (84.3 ± 20.0 vs. 66.7 ± 13.4 U/mg protein; *P < 0.05), indicating that Zn(II)–curcumin had a stronger anti-oxidative effect than curcumin alone.

GSH levels in the gastric mucosa of acetic acid-treated animals in the control group were significantly lower than that of the normal group (2.76 ± 0.66 vs. 4.93 ± 0.74 mg GSH/g protein; **P < 0.01). Administration of 12, 24 and 48 mg/kg Zn(II)–curcumin significantly increased the GSH level in a dose-dependent manner (P < 0.01, P < 0.01 and P < 0.001, respectively compared to the control group).

The MDA content in the gastric mucosa of the acetic acid-treated rats from the control group was higher than the normal sham operated group (4.10 ± 0.93 vs. 2.06 ± 0.70 nmol TBA/mg protein; **P < 0.01). The inhibitory effects of curcumin and Zn(II)–curcumin on lipid peroxidation are shown in Table 1. Treatment with 12, 24 and 48 mg/kg Zn(II)–curcumin significantly decreased the MDA content to 2.88 ± 0.85, 2.37 ± 0.55 and 2.08 ± 0.41 nmol TBA/mg protein (P < 0.01, P < 0.001 and P < 0.001, respectively, compared to the control group). Furthermore, Zn(II)–curcumin (24 mg/kg) lead to a significantly lower MDA level than curcumin at the same dose (2.37 ± 0.55 vs. 3.00 ± 0.74 nmol TBA/mg protein; **P < 0.01). Zn(II)–curcumin (24 mg/kg) lead to a similar increase in SOD activity and GSH level, and similar reduction in the MDA content as 7.8 mg/kg Lansoprazole (P < 0.05).

### 3.3. Matrix metalloproteinase 9 (MMP-9)

Matrix metalloproteinases play a major role during ulceration and ulcer healing, particularly in the remodeling and reassembly process. As shown in Fig. 3, the expression level of MMP-9 mRNA was 9-fold higher in the acetic acid-treated rats from the control group, compared to the normal group (P < 0.001), indicating that the model successfully induced elevated MMP-9 expression levels in the gastric mucosa. Curcumin (24 mg/kg) lead to a slight, but not significant reduction in MMP-9 mRNA expression, compared to the control group. Administration of 12, 24 and 48 mg/kg Zn(II)–curcumin significantly reduced MMP-9 mRNA expression in a dose-dependent manner (P < 0.05, P < 0.01 and P < 0.05, respectively compared to the control group). Zn(II)–curcumin (48 mg/kg) lead to a similar reduction in MMP-9 mRNA expression as 7.8 mg/kg Lansoprazole (P < 0.05).

### 4. Discussion

In this study, we used a model of acetic acid-induced gastric ulcers in rats, which resembles human ulcers in terms of both the pathological features and healing mechanisms, to study the effects of curcumin and Zn(II)–curcumin on the rate and mechanism of gastric ulcer healing. The application of acetic acid to the gastric mucosa induced a state of acute stress, which induced the development of gastric ulcers. In turn, acetic acid-induced gastric ulceration resulted in chronic oxidative stress, which was indicated by decreased SOD activity and GSH levels, and increased lipid peroxidation (MDA). Additionally, increased expression of MMP-9 mRNA was detected in the ulcerated gastric mucosa. Curcumin, an intense yellow dye extracted from *Curcuma longa*, has been safely used to treat a variety of inflammatory and digestive disorders since ancient times in China (Ravindran et al., 2007). Curcumin has already been demonstrated to act as a potent antiulcer and antioxidant compound, and significantly prevented ROS generation and accelerated the healing of gastric ulcers in rats (Toorkey and Karolink, 2009). Administration of curcumin (40 and 80 mg/kg) show the gastroprotective effect in acetic acid-induced chronic gastric ulcer in rats (Mahattanadul et al., 2009).

Zn(II)–curcumin (12, 24 and 48 mg/kg) demonstrated a significant and dose dependent gastroprotective effect. Zn(II)–curcumin

---

**Table 1**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg)</th>
<th>Ulcer Index (mm²)</th>
<th>SOD (U/mg)</th>
<th>GSH (mg GSH/g)</th>
<th>MDA (nmol TBA/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>–</td>
<td>0 ± 0</td>
<td>123.9 ± 15.1</td>
<td>4.93 ± 0.74</td>
<td>2.06 ± 0.70</td>
</tr>
<tr>
<td>Control</td>
<td>–</td>
<td>44.96 ± 9.02</td>
<td>55.6 ± 6.4</td>
<td>2.76 ± 0.66</td>
<td>4.10 ± 0.93</td>
</tr>
<tr>
<td>Zn(II)-curcumin</td>
<td>12</td>
<td>35.37 ± 8.76</td>
<td>70.4 ± 16.0</td>
<td>3.81 ± 0.90</td>
<td>2.88 ± 0.85</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>22.64 ± 7.08</td>
<td>84.3 ± 20.0</td>
<td>4.03 ± 0.90</td>
<td>2.37 ± 0.55</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>20.73 ± 5.86</td>
<td>108.7 ± 23.9</td>
<td>4.30 ± 0.79</td>
<td>2.08 ± 0.41</td>
</tr>
<tr>
<td>Curcin</td>
<td>24</td>
<td>35.01 ± 8.92</td>
<td>66.7 ± 13.4</td>
<td>3.70 ± 0.94</td>
<td>3.00 ± 0.74</td>
</tr>
<tr>
<td>Lansoprazole</td>
<td>7.8</td>
<td>24.14 ± 9.14</td>
<td>104.1 ± 16.0</td>
<td>4.21 ± 0.73</td>
<td>2.27 ± 0.69</td>
</tr>
</tbody>
</table>

Data are mean ± S.D. (n = 10 per group).

*P < 0.05 compared with the control group.

**P < 0.01 compared with the control group.

***P < 0.001 compared with the control group.

*P < 0.05 compared with the curcumin group.

---

**Fig. 3.** Effects of daily oral Zn(II)–curcumin on MMP-9 mRNA-expression in ulcerated rats induced by acetic acid and evaluated with RT–PCR. (A) Normal group daily treated with PVP (300 mg/kg, p.o.) for 10 days, (B) Control group with ulcerated mucosa induced by acetic acid and daily treated with PVP (300 mg/kg, p.o.) for 10 days, (C) Curcumin group induced by acetic acid and daily treated with curcumin (24 mg/kg, p.o.) for 10 days, (D and F) Zn(II)–curcumin group induced by acetic acid and daily treated with Zn(II)–curcumin SDs (12, 24, 48 mg/kg, p.o.) for 10 days, (G) Lansoprazole group induced by acetic acid and daily treated with Lansoprazole (7.8 mg/kg, p.o.) for 10 days. Significance represented as *P < 0.05, **P < 0.01, ***P < 0.001 compared to the control group, *P < 0.05 compared to the curcumin group.
(48 mg/kg) significantly protected against acetic acid-induced ulcers (49.7% reduction in the gastric ulcer index), and had a similar potency to the antulcer drug lansoprazole (7.8 mg/kg, 46.4% reduction in the gastric ulcer index). In our previous work, pre-treatment with Zn(II)–curcumin significantly reduced gastric lesions, gastric volume, and free and total acidity in pylorus-ligature-induced gastric ulcer of rats compared to curcumin at a dose of 24 mg/kg (p < 0.05) (Mei et al., 2009). Effects of Zn(II)–curcumin was exerted by the augmentation of mucosal resistance and the attenuation of increased acid output and gastric juice secretion. Zn(II)–curcumin also inhibited NF-κB, TGF-β1, and IL-8 mRNA expression. Its antioxidant effect might be attributable to its capacity to reduce gastric acid secretion and enhance the mucosal defense mechanism through the suppression of NF-κB-mediated inflammation. Administration of Zn(II)–curcumin also resulted in a down-regulating the expression of TNF-a and IL-6 mRNA in ethanol-induced acute gastric ulceration in rats, suggesting that Zn(II)–curcumin effectively enhance the mucosal defense mechanism through the prevention of proinflammatory cytokine-induced oxidative damage (Mei et al., 2012). Zn(II)–curcumin also showed a significantly higher anti-ulcerogenic effect than curcumin against the development of cold-restraint stress-induced gastric ulcers in rats, by leading to increased heat shock protein 70 (HSP70) mRNA expression and attenuating increased iNOS mRNA expression in the mucosa. Zn(II)–curcumin also exerted aghigher antidepressant effect than curcumin in the forced swimming test, tail suspension test and 5-hydroxy-γ-tryptophan-induced head twitch test in mice (Mei et al., 2011). Oxygen-derived free radicals are implicated in the mechanism of chronic ulceration in the gastric mucosa, whereas free radical scavenging has been implicated in gastric ulcer healing. Therefore, we extended this investigation to examine biochemical indicators of oxidative stress in gastric ulcers, including SOD, GSH and MDA, to investigate the different efficacies of curcumin and Zn(II)–curcumin on gastric healing. SOD and GSH are known to play an important role in protecting against oxidative gastric mucosal injury by decreasing ROS production (Chattopadhyay et al., 2006). SOD activity and GSH act as enzymatic defenses against ROS-induced lipid peroxidation in tissues. GSH prevents tissue damage by maintaining ROS at low cellular concentrations (Sekhar et al., 2011). Reduced levels of GSH may reflect an increase in the consumption of GSH by oxidative stress scavenging. Increased GSH levels can significantly inhibit MMP-9 activity and function. MDA represents an end-product of the per-oxidation of polyunsaturated fatty acids and related esters within cell membranes, and is currently regarded as a reliable index of ROS-induced mucosal injury (Maes et al., 2011). Curcumin could attenuate increased lipid peroxidative damage, and prevent depletion of GSH, GSH-Px and CAT activities, but did not alter the activity of copper, zinc–superoxide dismutase (Cu, Zn–SOD) which serves as markers of renal injury and urinary excretion during gentamicin-induced oxidative stress (Farombi and Ekor, 2006). Oral administration of 1–25 mg/kg polaprezinc (PZ), which consists of l-carnosine and zinc, could prevent 100% ethanol-induced gastric mucosal lesions with a dose-dependent manner. Zinc chloride (ZnCl2) at dose of 27 mg/kg significantly reduced gastrointestinal lesion by elevated gastric mucosal SOD and CAT activities in rats 1 h before or after oral administration of ethanol (Ineu et al., 2013). In this study, the SOD activity and GSH levels decreased, and MDA content increased in the gastric tissue of acetic acid-treated rats, confirming that acetic acid induced the formation of ROS. Curcumin alone lead to a small increase in SOD activity and GSH levels, and small reduction in the MDA content; however, Zn(II)–curcumin lead to a significantly higher increase in SOD activity and GSH levels, and significantly lower MDA content than curcumin. These findings suggest that the potent ability of orally administered Zn(II)–curcumin to promote the healing of chronic gastric ulcers is due, at least in part, to protection against free radical-induced oxidative stress, and additionally, that the increased ability of Zn(II)–curcumin to scavenge the free radicals produced by acetic acid metabolism is due to the synergistic effects of curcumin and zinc.

Gastric ulceration is often associated with a dysregulation of ECM remodeling in gastric tissue. MMPs are a family of at least 25 zinc-dependent endopeptidases which selectively degrade ECM components and are involved in ECM remodeling (Ratzinger et al., 2010). Tissue repair processes such as wound healing and regeneration require ECM synthesis, deposition and degradation, and elevated MMP activity has been implicated in diseases such as gastric ulcers. MMP-9, a 92 kDa gelatinase B, expressed in the endothelial cells of injured tissues, is responsible for degradation of the ECM. Chronic gastric ulceration causes matrix MMP-9 and −3 augmentation (Ganguly and Swarnakar, 2012). ROS production in gastric ulcers has been shown to upregulate the transcription and activity of MMPs via activation of mitogen-activated protein kinases (MAPKs) (Hwang et al., 2011). Antioxidants can significantly arrest the upregulation of proMMP-9 secretion and inflammatory cell infiltration to protect ethanol-induced gastric ulcer (Singh et al., 2007). Indomethacin is known to activate MMP-2 and −9 expression and secretion in gastric tissue (Ganguly et al., 2005), and it was previously reported that curcumin prevented upregulation of MMP-2 and -9 expression and activity in a rat model of indomethacin-induced gastric ulcers (Swarnakar et al., 2005). Curcumin also reduced the expression of MMP-9 in orthotypically implanted pancreatic tumors and ovarian tumors in nude mice (Lin et al., 2007); and it has been suggested that the ability of curcumin to inhibit MMP-9 transcription is linked to inhibition of NF-κB nuclear translocation (Saja et al., 2007). In this study, the expression of MMP-9 was significantly upregulated in the gastric mucosa of the rats in the control group. Curcumin did not have a significant effect on MMP-9 mRNA expression in this model; however, we observed that Zn(II)–curcumin (12, 24 and 48 mg/kg) offered significantly more gastroprotection that curcumin alone, and lead to significantly reduced MMP-9 mRNA expression. It was previously shown that zinc could affect the expression of MMPs. Pre-treatment of rats with zinc sulfate (50 mg/kg body weight) significantly decreased indomethacin induced activation of MMP-2 and MMP-9 (Sivalingam et al., 2011). Zinc could prevent against indomethacin-induced gastrointestinal disease by free radical scavenging, due to its ability to stabilize sulfhydryl groups (Varghese et al., 2009). ZnCl2 (30 μM) also attenuated ethanol- and acetaldehyde-induced production of tissue inhibitors of metallo-proteinases (TIMP-1 and TIMP-2) and decreased MMP-2 activity (Agnieszka et al., 2009). Collectively, this data suggests that Zn(II)–curcumin can effectively halt the progression of gastrointestinal damage due to the synergistic effects of curcumin and zinc on free radical scavenging and the inflammatory process.

Proton pump inhibitors such as famotidine and lansoprazole are the most popular drugs used for the therapeutic control of gastrointestinal ulcers. Upregulation of HSPs is crucial for preventing the pathogenesis of stress-induced gastric mucosal damage (Otaka et al., 2009). Previous experiments have shown that administration of lansoprazole (≥ 50 mg/kg) decreases the absolute and relative weights of the thymus in rats (Youssef et al., 2009), and reduced the expression of HSP72 after famotidine-treatment (Wada et al., 2006). These results show that proton pump inhibitors are not suitable for the treatment of peptic ulcers in children or stress ulcers in adolescents. In our previous work, Zn(II)–curcumin exerted a significantly more potent anti-ulcerogenic effect than curcumin against the development of cold-restraint stress-induced gastric ulcers in rats, by leading to increased HSP70 mRNA expression.

In conclusion, this report provides evidence that orally administered Zn(II)–curcumin exerts a potent gastroprotective
effect against acetic acid-induced gastric ulcers. Zn(II)–curcumin prevented acetic acid-induced damage via at least two mechanisms: elevation of antioxidant activity and inhibition of MMP-9 expression, indicating that the anti-ulcerative activity of Zn(II)–curcumin is related to the synergistic effects of curcumin and zinc.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. NCT-04-0808); The Program of the Fok Ying Tung Education Foundation of China (No. 91036).

References


