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Gastroprotective effects of a new zinc(II)–curcumin complex against pylorus-ligature-induced gastric ulcer in rats

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ABSTRACT

Zn(II)–curcumin, a mononuclear (1:1) zinc complex of curcumin was synthesized and examined for its antiulcer activities against pylorus–ligature–induced gastric ulcer in rats. The structure of Zn(II)–curcumin was identified by elemental analysis, NMR and TG–DTA analysis. It was found that a zinc atom was coordinated through the keto–enol group of curcumin along with one acetate group and one water molecule. Zn(II)–curcumin (12, 24 and 48 mg/kg) dose–dependently blocked gastric lesions, significantly reduced gastric volume, free acidity, total acidity and pepsin, compared with control group (*P*<0.001) and curcumin alone (24 mg/kg, *P*<0.05). Reverse transcriptase polymerase chain reaction (RT-PCR) analysis showed that Zn(II)–curcumin markedly inhibited the induction of nuclear factor-kappa B (NF-κB), transforming growth factor beta₁ (TGF- $β_1$) and interleukin-8 (IL-8), compared with control group (*P*<0.05). These findings suggested that Zn(II)–curcumin prevented pylorus–ligation–induced lesions in rat by inhibiting NF-κB activation and the subsequent production of proinflammatory cytokines, indicating a synergistic effect between curcumin and zinc. An acute toxicity study showed that mice treated with SDs of Zn(II)–curcumin (2 g/kg) manifested no abnormal signs.

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1. Introduction

(1,7-bis[4-hydroxy-3-methoxyphenyl]-1,6-hepta-Curcumin diene-3,5-dione) is the main constituent of the perennial herb Curcuma loga (known as turmeric). Traditional Indian and Chinese systems of medicine have reported the use of turmeric for wound healing [1]. Curcumin has a wide spectrum of pharmacological activities, including antioxidant [2], anti-inflammatory [3], antitumor [4], and anticardiovasculopathic properties [5]. Curcumin acts as a potent antiulcer compound, protecting against gastric mucosal injury, and suppresses the proliferation of Helicobacter *pylori* [6]. Curcumin is nontoxic to humans up to a dose of 10 g/day, with almost no adverse effects. It is considered to be a potential chemopreventive agent and has been used in clinical trials [7]. However, curcumin is only slightly absorbed in the gastrointestinal tract because it is poorly soluble in water (its maximum solubility is reported to be 11 ng/mL in plain aqueous solution) [8]. The oral bioavailability of curcumin is very low (only 1% in rats) [9]. Solid dispersions (SDs) of curcumin-polyvinylpyrrolidone K30 (PVP) in different ratios were prepared by coevaporation in ethanol solution to improve the dissolution and absorption of curcumin [10].

Under different pH conditions, curcumin can chelate various metal ions and form metal-curcumin complexes. This metal binding is mediated through the beta-diketone group of curcumin [11]. The Cu(II)-curcumin complex has free-radical-neutralizing capacity and antioxidant potential. Catalytic activity of the complex is greater than that of curcumin [12]. Manganese complexes of curcumin enhance its radical-scavenging activity, and are used as neuroprotective agents in vascular dementia [13]. A curcumin-gold complex (Au(cur)₂Cl) has antiarthritic properties in an adjuvantinduced rat model of polyarthritis [14].

Zinc plays an important role in cell-mediated immune functions. Zinc homeostasis is also important for the integrity of gastric mucosal cells. Zinc can halt the progression of gastrointestinal disease by free radical scavenging and interruption of the inflammatory process as an antioxidant and anti-inflammatory agent. A reduction in zinc content of the mucosa is observed in patients affected by ulcerative colitis, which is associated with an increase in reactive oxygen intermediates [15]. Zinc complexes have been shown to have antiulcer activity. Zinc-carnosine is an antiulcer drug commonly used in the treatment of gastric ulcers in Japan [16]. The zinc-indomethacin complex and the zinc-naproxen complex more significantly reduce these ulcerogenic effects compared with the parent drug, without affecting its therapeutic action [17,18]. Here, a Zn(II)-curcumin complex was synthesized using curcumin and zinc acetate, as an alternative to curcumin. SDs of Zn(II)-curcumin were produced using a spray-drying method to improve the absorbance

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of curcumin. Gastroprotective effects of the Zn(II)–curcumin complex were examined in a pylorus-ligature-induced model of gastric ulcer in rats.

2. Materials and methods

2.1. Reagents

Curcumin, 99% pure, was manufactured by Guangdong Zhongda Greenfield Bio-tech. Co. (Guangzhou, China). Polyvinylpyrrolidone K30 (PVP) was purchased from BASF Chemical Ltd. (New Jersey, USA). Lansoprazole tablets were obtained from Shengfan Pharmaceutical Company Limited (Henan, China). All other chemicals were of reagent grade.

2.2. Animals

Male Sprague Dawley rats (6-7 wk, 200-250 g) and male Swiss mice (6-7 wk, 18-22 g) were bred in-house with free access to food and water before use in this study. Animals were subjected to 12:12 h light:dark cycles and were maintained at room temperature of 25 °C. All procedures were carried out in accordance with guidelines approved by the Animal Ethics Committee of Sun Yat-sen University (Guangzhou, China).

2.3. Synthesis of Zn(II)-curcumin and SDs

The Zn(II)-curcumin complex was synthesized by mixing equimolar amounts of zinc acetate and curcumin in dry ethanol and refluxing the mixture for 3 h under a nitrogen atmosphere. The Zn(II)-curcumin complex precipitated, and the solid was separated by filtration and washed several times by water and ethanol to remove any unreacted curcumin and zinc acetate. Zn(II)-curcumin and PVP in a ratio of 1:6 (w/w) were added to an alcohol solution to produce a suspension by cryo-grinding under a nitrogen atmosphere (NS1001 High-Pressure Homogenizer, GEA Niro Soavi S.p.A. Inc., Parma, Italy). SDs of Zn(II)-curcumin/PVP were produced with a spray dryer (Laiheng Scientific Instruments, Beijing, China). The operating parameters were: inlet temperature, 70 °C; outlet temperature, 50 °C; feed rate, 2–3 mL/min; atomization air pressure, 2 kg/cm²; and inspiration, –280 mm WC. Curcumin SDs (1:6, w/w) were also produced with the same procedure.

2.4. Protocol for gastric ulceration and assessment of healing

Rats were randomly divided into seven experimental groups. Each group consisted of 10 animals. The normal and control groups received PVP vehicle (300 mg/kg, p.o.) throughout the course of the experiments. The treatment groups received different doses of Zn(II)-curcumin SDs (equivalent to Zn(II)-curcumin 12, 24, and

Table 1	
PCR primer and conditions.	

48 mg/kg, p.o.). The curcumin group received curcumin SDs (equivalent to curcumin 24 mg/kg, p.o.), and lansoprazole (7.8 mg/kg, p.o.) was used as the positive control for a period of 7 d. Animals were then fasted for 24 h, but with free access to water, before ulcer induction. All animals, other than those in the normal group, were anesthetized with ether, and their abdomens were incised and pyloric ligation was performed. The animals were deprived of water during the postoperative period. After pyloric ligation for 4 h, all the animals were killed under ether anesthesia. Their stomachs were removed rapidly, and the gastric contents were collected and centrifuged. After the volume of the supernatant was measured, free acidity and total acidity were measured separately by titration with 2 mM NaOH using 2% dimethyl-4-(phenyldiazenyl)benzenamine and phenolphthalein as the indicator, and expressed as mmol/L [19]. The ulcer index and protective percentage was calculated [20].

2.5. Reverse transcription-PCR (RT-PCR) to detect NF- κ B, TGF- β_1 , IL-8, and β -actin mRNAs

Stomach tissue samples from the rats were immersed in RNA Stabilization Reagent and stored at -70 °C. Total RNA was extracted with reagent, according to the protocol provided by the manufacturer, and quantified by measuring the absorbance at 260 nm. Complementary DNA was synthesized using 1 µg of total RNA from each sample in 20 µL of reaction buffer, using SuperScript II reverse transcriptase. cDNAs for NF- κ B, TGF- β_1 , IL-8, and β -actin were amplified by PCR using the primers listed in Table 1. PCR products were fractionated on 2% agarose gels and visualized by ethidium bromide staining.

2.6. Acute toxicity studies

Acute toxicity studies were performed on male Swiss mice, as described by Souza Brito [21]. Control and treated groups consisted of 12 animals each. The treated group received Zn(II)–curcumin SDs (14 g/kg, p.o.) and the control group received PVP (14 g/kg, p.o.). Animals were observed carefully at 30, 60, 120, 240, and 360 min after treatment based on Hippocratic screening. Mortality, body weights, and behavior of the mice were observed and recorded daily for 14 d after treatment. Possible macroscopic changes in the treated group were compared with those of the control group.

2.7. Statistical analysis

The values are expressed as the mean \pm S.D. for 10 animals in each group. The data were analyzed by SPSS/13 software. Hypothesis testing methods included one-way analysis of variance (ANOVA) followed by Dunnett's T3 multiple comparisons test. The significance levels were analyzed at *P*<0.001, *P*<0.01, *P*<0.05.

cDNA	Primers	PCR conditions					
		Melting	Annealing	Extension	Cycles		
NF-ĸB	5'-GGCAGCACTCCTTATCAA 3'-GCAACTGTAGGCATTTCTG	94 °C, 30 s	54 ° C, 30 s	72 °C, 30 s	30		
TGF-β1	5'-CCGCAACAACGCAATCTA 3'-GATGCTACCCTGCTGTGG	94 °C, 30 s	50 ° C, 30 s	72 °C, 30 s	40		
IL-8	5'-TGTGGGTCTGTTGTAGGG 3'-CGGTGGTAGAATGGAGTG	94 °C, 30 s	54 ° C, 30 s	72 °C, 30 s	40		
β-Actin	5'-GTCCCTGTATGCCTCTGG 3'-CAACTGTAGGCATTTCTG	94 ° C, 30 s	54°C, 30 s	72 °C, 30 s	30		



Fig. 1. Molecular structures of Zn(II)-curcumin complexes.

3. Results

3.1. Characterization of the Zn(II)-curcumin complex

Elemental analysis showed that the complex contained C 54.9% (54.2%), H 4.7% (4.7%), and Zn 13.1% (12.8%). Mass spectrometric analysis of the complex showed a molecular ion peak at 512.2 (509.6) mass units. The values in parentheses are the calculated values. Supporting evidence for the structure was obtained with thermal gravimetry and differential thermal analysis (TG–DTA). Initially, a 514.7 mg sample was loaded, and a weight of 20.1 mg was observed at 100–105 °C. The weight of loss corresponds to the loss of one water molecule in the above peak. This analysis confirmed the presence of one water molecule. Zn(II)–curcumin contained a zinc atom that coordinated through the keto-enol group of curcumin along with one acetate group and one water molecule. The molecular formula of the Zn(II)–curcumin complex was deduced as shown in Fig. 1.

3.2. Protective effects of Zn(II)-curcumin on 4 h pylorus-ligation-induced gastric acid secretion

As reported in Table 2 and Fig. 2, effects of Zn(II)-curcumin at dose of 12, 24, 48 mg/kg, once a day for 7 d prevented the development of acute gastric ulcer in dose related manner. Oral administration of Zn(II)-curcumin at 12-48 mg/kg decreased ulcer index by $30.4 \pm 9.4 - 17.1 \pm 5.8$ (53.3 - 73.7% protection) in comparison to control 65.1 ± 6.5 (*P*<0.001). Compared with the control rats, pretreatment with Zn(II)-curcumin at 12, 24, 48 mg/kg showed a significant reduction in gastric volume and free and total acidity (P<0.01, P<0.01, P<0.001). Zn(II)-curcumin showed strong antiulcer activity, causing a significant reduction of pepsin to 54.52 ± 6.36 , 45.90 ± 7.94 , and 38.87 ± 5.54 U when treated with 12, 24, and 48 mg/kg, respectively (P<0.01, P<0.001, P<0.001). Compared with curcumin at a dose of 24 mg/kg, Zn(II)-curcumin had significantly greater protective effects in reducing gastric lesions, gastric volume, and free, total acidity and pepsin (P<0.05 for all parameters). Zn(II)-curcumin combined the effects of curcumin and zinc synergistically. Compared with the control group, lansoprazole (7.8 mg/kg) was more potently reduced gastric lesions, gastric volume, and free, total acidity and pepsin (P<0.001 for all parameters).

3.3. Zn(II)-curcumin modulates the expression of NF- κ B, TGF- β_1 , and IL-8

As shown in Figs. 3–5, increased expression of NF-κB, TGF-β₁ and IL-8 was detected at the ulcer margin in the control group of rats compared with that in the normal gastric mucosa of the normal rat group (P<0.05). Pretreatment with Zn(II)–curcumin significantly inhibited NF-κB, TGF-β₁ and IL-8 mRNA expression compared with that in the control group (24 mg/kg, P<0.05; 48 mg/kg, P<0.01). Pretreatment with curcumin at a dose of 24 mg/kg significantly reduced the increased expression of TGF-β₁ and IL-8 (P<0.05), but had no significant effect on NF-κB expression compared with that in the control group. Lansoprazole (7.8 mg/kg) most potently reduced NF-κB, TGF-β₁, and IL-8 mRNA expression (P<0.01). Zn(II)–curcumin significantly inhibited pylorus-ligation-induced damage by inhibiting NF-κB activation and thus inhibited the production of proinflammatory cytokines.

3.4. Acute toxicity study

An acute toxicity study showed that animals treated with SDs of Zn(II)-curcumin at a dose of 14 g/kg (equivalent to 2 g/kg Zn(II)-curcumin) did not show any significantly abnormal signs, behavioral changes, body weight changes, or macroscopic findings at any time of observation. All animals, including the control group, were lethargic 3 h after oral administration. There was no mortality at the above mentioned doses at the end of 14 d observation period.

4. Discussion

NF-κB (a zinc-dependent transcription factor) appears to play a key role in the process of ulcer healing, because its activation is upregulated in rat gastric ulcers and the blockade of NF-κB activation resulted in impairment of ulcer repair [22]. NF-κB can be activated by hundreds of different stimuli, such as TNF- α and other proinflammatory cytokines (TGF- β_1 and IL-8) [23]. NF-κB activation followed by TNF- α release increases gastric ulcer formation of rat induced by phorbol-12-myristate-13-alphacetate [24]. Gastric ulcer model induced by 100% acetic acid to the serosal surface of rat stomach, the application of TGF- β_1 injected into the subserosa led to a significant acceleration of gastric ulcer [25].

Physiological levels of zinc inhibit NF- κ B in DU-145 and PC-3 human prostate cancer cells. Additionally, chelation of zinc with zinc chelator, *N*,*N*,*N*,',-tetrakis(2-pyridylmethyl) ethylenediamine (TPEN) abolishes this effect [26]. Zinc supplementation inhibits NF- κ B activation. The HUT-78 cells incubated in higher concentrations

Table 2

Effects of Zn(II)-curcumin on gastric lesion, volume, free acidity and total acidity of gastric juice obtained from pylorus-ligature rats.

Treatment	Dose (mg/kg)	Gastric lesion (mm)	Protective rate (%)	Pepsin (U)	Gastric volume (mL/100g)	Total acidity (µequiv./mL/4 h)	Free acidity (µequiv./mL/4 h)
Control (PVP)	300	65.1 ± 6.5	-	63.08 ± 5.97	5.26 ± 0.51	92.22 ± 5.52	64.10 ± 6.02
Zn(II)–curcumin	12 24 48	$\begin{array}{l} 30.4 \pm 9.4^{***} \\ 21.1 \pm 4.1^{***\#} \\ 17.1 \pm 5.8^{***} \end{array}$	53.3 63.1 73.7	$\begin{array}{l} 54.52 \pm 6.36^{**} \\ 45.90 \pm 7.94^{***\#} \\ 38.87 \pm 5.54^{***} \end{array}$	$\begin{array}{l} 4.35 \pm 0.27^{***} \\ 4.11 \pm 0.50^{***\#} \\ 3.86 \pm 0.29^{***} \end{array}$	$\begin{array}{l} 68.60 \pm 8.48^{***} \\ 63.60 \pm 4.62^{**\#} \\ 58.18 \pm 8.66^{***} \end{array}$	$\begin{array}{l} 51.24 \pm 7.82^{**} \\ 45.00 \pm 5.23^{**\#} \\ 36.82 \pm 6.24^{***} \end{array}$
Curcumin Lansoprazole	24 7.8	$\begin{array}{l} 31.6 \pm 9.6^{***} \\ 10.9 \pm 3.8^{***} \end{array}$	51.5 83.3	$\begin{array}{l} 56.30 \pm 7.84^{*} \\ 36.53 \pm 7.31^{***} \end{array}$	$\begin{array}{l} 4.60 \pm 0.47^{**} \\ 3.72 \pm 0.22^{***} \end{array}$	$\begin{array}{l} 70.02 \pm 5.68^{***} \\ 51.98 \pm 7.10^{***} \end{array}$	$\begin{array}{l} 52.70\pm9.10^{**}\\ 31.04\pm4.97^{***} \end{array}$

Data are represented as mean \pm S.D. (n = 10 per group).

Significance represented as ***P*<0.01 and ****P*<0.001 compared with control group.

 $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ compared with curcumin group.



Fig. 2. Effects of Zn(II)–curcumin on the inner surface stomach of rats induced by pylorus ligature. (A) Normal control group. (B) Control group treated pylorus ligature after pretreatment with PVP 300 mg/kg. (C) Curcumin group treated pylorus ligature after pretreatment with curcumin 24 mg/kg. (D–F) Zn(II)–curcumin group treated pylorus ligature after pretreatment with curcumin 24 mg/kg. (D–F) Zn(II)–curcumin 12, 24, 48 mg/kg. (G) Lansoprazole group treated pylorus ligature after pretreatment with lansoprazole 7.8 mg/kg.

of 50 and 100 mM zinc showed mild to moderate decreases in the levels of IL-2, and TNF- α production and mRNAs, and in NF- κ B activation compared to those incubated in 15 mM zinc medium [27]. Zinc deficiency leads to stress and the activation of macrophagesmonocytes, resulting in the increased generation of inflammatory cytokines such as TGF- β [28]. Supplements of zinc acetate for

patients with sickle cell disease (SCD) caused significant reductions in lipopolysaccharide-induced TNF- α and IL-1 β mRNA, compared with levels in the placebo-treated group [29]. Zinc–carnosine has also been shown to prevent acetic-acid-induced colitis in rats, through the induction of heat shock protein 72 and the suppression of transcription factor NF- κ B [16].



Fig. 3. Effects of Zn(II)–curcumin on NF-κB mRNA expression in pylorus-ligated rat stomach evaluated with RT–PCR. (1) Normal control group pretreatment with PVP (300 mg/kg, p.o.); (2) control group treated pyloric ligation pretreatment with PVP (300 mg/kg, p.o.); (3) curcumin group treated pylorus ligature after pretreatment with curcumin (24 mg/kg, p.o.); (4–6) Zn(II)–curcumin group treated pylorus ligature after pretreatment with Zn(II)–curcumin SDs (12, 24, 48 mg/kg, p.o.); (7) lansoprazole group treated pylorus ligature after pretreatment with lansoprazole (7.8 mg/kg, p.o.). Significance represented as *P < 0.05, **P < 0.01.



Fig. 4. Effects of Zn(II)–curcumin on TGF- β_1 mRNA expression in pylorus-ligated rat stomach evaluated by RT-PCR. (1) Normal control group pretreatment with PVP (300 mg/kg, p.o.); (2) control group treated pylorus ligation pretreatment with PVP (300 mg/kg, p.o.); (3) curcumin group treated pylorus ligature after pretreatment with curcumin (24 mg/kg, p.o.); (4–6) Zn(II)–curcumin group treated pylorus ligature after pretreatment with Zn(II)–curcumin SDs (12, 24, 48 mg/kg, p.o.); (7) lansoprazole group treated pylorus ligature after pretreatment with lansoprazole (7.8 mg/kg, p.o.). Significance represented as *P<0.05, **P<0.01.



Fig. 5. Effects of Zn(II)–curcumin on IL-8 mRNA expression in pylorus-ligated rat stomach evaluated by RT-PCR. (1) Normal control group pretreatment with PVP (300 mg/kg, p.o.); (2) control group treated pyloric ligation pretreatment with PVP (300 mg/kg, p.o.); (3) curcumin group treated pylorus ligature after pretreatment (24 mg/kg, p.o.); (4–6) Zn(II)–curcumin group treated pylorus ligature after pretreatment with Zn(II)–curcumin SDs (12, 24, 48 mg/kg, p.o.); (7) lansoprazole group treated pylorus ligature after pretreatment with lansoprazole (7.8 mg/kg, p.o.). Significance represented as *P < 0.05, **P < 0.01.

The anti-inflammatory activity of curcumin was associated with its ability to inhibit the production of pro-inflammatory cytokines such as TGF- β , IL-1 or TNF- α and inducible NO synthase [30]. Curcumin also inhibits the production by human peripheral blood monocytes and alveolar macrophages of IL-8, IL-1 β , and TNF- α , induced by inflammatory stimuli [31].

In this model, pretreatment with Zn(II)–curcumin significantly reduced gastric lesions, gastric volume, and free and total acidity compared with the effects of curcumin at a dose of 24 mg/kg (*P*<0.05). Effects of Zn(II)–curcumin was exerted by the augmentation of mucosal resistance and the attenuation of increased acid output and gastric juice secretion. Our data show that Zn(II)–curcumin also inhibited NF-κB, TGF-β₁, and IL-8 mRNA expression. Its antiulcer 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. These findings suggest that Zn(II)–curcumin prevents pylorus-ligation-induced damage by the mechanisms: reduction of NF-κB activation and subsequent production of proinflammatory cytokines.

In conclusion, this is the first evidence that oral administration of Zn(II)-curcumin has potent gastroprotective activity against pylorus-ligation-induced lesions in rats. These results also show that antiulcer and anti-inflammation activity of Zn(II)-curcumin is not only related to curcumin, but also attributable to zinc. Zn(II)-curcumin beneficial effects occur via the synergistic effects of curcumin and zinc.

Conflict of interest

None of the authors has a commercial interest, financial interest, and/or other relationship with manufacturers of pharmaceuticals, laboratory supplies, and/or medical devices or with commercial providers of medically related services.

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