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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 beta$_1$ (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.

1. Introduction

Curcumin (1,7-bis[4-hydroxy-3-methoxyphenyl]-1,6-heptadiene-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)$_2$Cl) has antiarthritic properties in an adjuvant-induced 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/cm^2; 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 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-κB, 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-κB, 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 ± 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 $P < 0.001$, $P < 0.01$, $P < 0.05$.

Table 1
PCR primer and conditions.

cDNA	Primers	PCR conditions			
		Melting	Annealing	Extension	Cycles
NF-κB	5′-GGCAGCACTCCTTATCAA 3′-GCAACTGTAGGCATTTCTG	94 °C, 30 s	54 °C, 30 s	72 °C, 30 s	30
TGF-β_1	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