Comparative antiulcer effect of Bisdemethoxycurcumin and Curcumin in a gastric ulcer model system

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Abstract

The antiulcer effect of bisdemethoxycurcumin, a yellow pigment found mainly in rhizomes of Curcuma longa, was compared with curcumin in gastric ulcer model systems to validate its clinical application as a remedy for peptic ulcer. Western blot analysis of mouse macrophage cell line RAW 264.7 activated with lipopolysaccharide showed that bisdemethoxycurcumin inhibited inducible nitric oxide synthase (iNOS) production significantly but had no effect on tumor necrosis factor-alpha (TNF-α) production, whereas curcumin showed stronger suppression of iNOS protein production and inhibited TNF-α protein production significantly. However, bisdemethoxycurcumin and curcumin possessed similar potency in scavenging nitric oxide generated from mouse macrophage cell line RAW 264.7. Reverse-transcriptase polymerase chain reaction (RT-PCR) analysis showed that both curcuminoids inhibited the induction of iNOS dose-dependently at the transcriptional level and curcumin also appeared to inhibit the induction of TNF-α at post-transcriptional level. In an animal model, intraduodenal administration of bisdemethoxycurcumin (5–80 mg/kg body wt.) showed a strong inhibitory effect on gastric acid secretion in pylorus-ligated rats whereas curcumin (5–20 mg/kg body wt.) showed a less inhibitory effect, with maximum potency at a dose of 20 mg/kg body wt. Moreover, oral administration of bisdemethoxycurcumin at doses of 20–80 mg/kg body wt. twice daily for 10 days showed a significant curative efficacy in accelerating the healing of acetic acid-induced chronic gastric ulcer and promotion of mucosal regeneration in the ulcerated portion in a dose-related manner with potency equal to curcumin. In contrast, the curative potency of curcumin tended to decrease at doses over 160 mg/kg body wt./day. Western blot analysis in ulcerated gastric mucosa showed that bisdemethoxycurcumin dose-dependently reduced the increased protein expression level of iNOS but not TNF-α. These results indicated that bisdemethoxycurcumin directly accelerates gastric ulcer healing with potency equal to curcumin. Its antiulcer effect might be due to its properties of...
Introduction

Loss of control of gastric inflammatory response can lead to an inappropriate recruitment of leukocytes into the gastric mucosa and, in turn, to gastric mucosal injury. A pro-inflammatory cytokine, tumor necrosis factor-alpha (TNF-\(\alpha\)), secreted by activated macrophages, has been found to play a crucial role in regulating immune response and in promoting the release of other pro-inflammatory mediators (Holzer, 2001; Pavlick et al., 2002). Furthermore, sustained overproduction of nitric oxide (NO) generated from inducible nitric oxide synthase (iNOS) expressed in activated macrophages and endothelium, can lead to a modulation of leukocyte infiltration, a cyclo-oxygenase-2 (COX-2)-dependent production of inflammatory prostaglandins (PGs), and an interaction of NO with leukocyte-derived \(O_2^-/C0_2^-\) that forms other potent cytotoxic oxidants (Pavlick et al., 2002). Likewise, the suppression of the inflammatory reaction by inhibiting excess generation of pro-inflammatory cytokines TNF-\(\alpha\) and iNOS-derived NO supports gastric mucosal defense and promotes the onset of gastric ulcer healing (Shimizu et al., 2000; Holzer, 2001).

The powdered rhizome of the medicinal plant Curcuma longa L. (Zingiberaceae), known commonly as turmeric, has been used safely for centuries to treat a variety of inflammatory, biliary and digestive disorders in several traditional folklore prescriptions. Recently, a clinical study with turmeric powder treatment in patients with peptic ulcer found a beneficial effect in healing peptic ulcer after 12 weeks of treatment (Prucksunand et al., 2001). Analytical studies have so far revealed that the three main curcuminoids isolated from turmeric are curcumin, demethoxycurcumin and bisdemethoxycurcumin. These three curcuminoids have been shown to be a good inhibition of the COX-2 enzyme (Ramsewak et al., 2000). It was also found that curcumin was a more potent inhibitor of iNOS gene expression than bisdemethoxycurcumin. More recently, all three curcuminoids were found to be effective inhibitors of TNF-\(\alpha\) and PGE\(_2\) production in an \textit{in vitro} system, with curcumin and demethoxycurcumin being the most and the least effective compound, respectively (Lantz et al., 2005). We reported previously that curcumin directly accelerates ulcer healing in a chronic gastric ulcer model induced by acetic acid in rats via a mechanism involving its inhibition of gastric acid secretion and its anti-inflammatory activity against iNOS and TNF-\(\alpha\) production (Mahattanadul et al., 2006a, b). The potential ulcer healing activity of other curcuminoids in turmeric has not been examined systematically. It has been reported that bisdemethoxycurcumin is found mainly in rhizomes of \textit{C. longa} whereas curcumin can be found in different \textit{Curcuma} species (Hansel, 1997). Therefore, the antiulcer potency of bisdemethoxycurcumin on gastric ulcer healing, including its mechanisms of action on gastric acid secretion and on the production of pro-inflammatory mediators (iNOS enzyme and TNF-\(\alpha\) cytokine), in a gastric ulcer model system was elucidated and compared with curcumin.

Materials and methods

Medicinal plant

Dried powder (1 kg) of \textit{Curcuma longa} rhizome (voucher specimen nos. 21.1.410.1.458 Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand) was macerated in hexane (3 l) followed by ethyl acetate (3 l/C\(_2\)3), to produce hexane and ethyl acetate extracts. The ethyl acetate extract was taken to dryness under reduced pressure, and was then subjected to silica gel vacuum chromatography using a chloroform–methanol mixture as an eluent (gradient elution from 100% to 98% chloroform). Following this procedure, 45.5 g of curcumin (yield: 4.55% w/w) and 3.5 g of bisdemethoxycurcumin (yield: 0.35% w/w) were obtained. The purity of the curcumin and bisdemethoxycurcumin was confirmed by comparison of their spectroscopic data and m.p. with literature values (Roughley and Whiting, 1973; Kosuge et al., 1985) and by HPLC analysis (TSK-gel ODS-80Tm column; mobile phase: methanol:water, 60:40; flow rate: 1 ml/min; detection: 420 nm). The purity of curcumin and bisdemethoxycurcumin were 99% and 100%, respectively.

Animals

Male Wistar rats weighing 180–220 g each was housed under normal laboratory conditions at 25 ± 1 °C with a controlled 12-h light–dark cycle and maintained on standard rodent chow and tap water \textit{ad libitum}. The rats...
were deprived of food with access to water ad libitum for 24 h before the experiments. All animal studies were carried out according to the guideline of the Animal Care and Use Committee of Prince of Songkla University, Thailand; the Principles of Laboratory Animal Care (NIH publication number 85–23, revised 1985) and the guideline of the Animal Investigation Committee, Chiba University, Japan.

Chemicals

RPMI 1640 medium was purchased from Kojin Bio (Japan). Fetal bovine serum (FBS) was purchased from Gemini Bio Products (USA). Penicillin–streptomycin solution and Tris–HCl were purchased from Invitrogen Corp. (USA). Anti-iNOS and anti-TNF-α antibodies were purchased from Cell Signaling Technology, Inc. (USA). Goat anti-mouse and donkey anti-rabbit IgG, HRP-conjugate secondary antibody were purchased from Santa Cruz Biotech Inc. (USA). Agarose (molecular biological grade), Bio-Rad Dc protein assay reagent and Immune-Star chemiluminescent reagent were purchased from Bio-Rad (USA). RNA extraction reagent (TRIzol™) was purchased from Molecular Research Center Inc. (USA). RNA extraction kit (RNAeasy mini) and one-step reverse-transcribed polymerase chain reaction kit were purchased from Qiagen Inc. (USA); GeneRuler 50 bp DNA Ladder was purchased from Fermentas (USA). Aminoguanidine; cimetidine; lipopolysaccharide (LPS) derived from Escherichia coli; monoclonal mouse anti-β-actin clone AC-15; mouse iNOS, TNF-α and β-actin oligonucleotide primers; precision plus dual protein standards and all other chemicals were purchased from Sigma–Aldrich (USA).

Cell culture and stimulation

Mouse macrophage RAW 264.7 cell line was purchased from JCRB Cell Bank (Japan). These cells were cultured in 24 well plates (2 × 10⁵ cells/well) at 37°C in RPMI 1640 medium containing 10% FBS and 1% penicillin/streptomycin sulfate in a humidified atmosphere of 5% CO₂. When the cells reached a density of approximately 80% confluence, they were incubated in medium (using RPMI 1640 without FBS) containing 1 µg/ml of LPS with various concentrations of bisdemethoxycurcumin and curcumin dissolved in DMSO. The final concentration of DMSO in cell culture was fixed at less than 0.1% in order to avoid cell toxicity. Cells used for Western blot analysis were washed with cold phosphate buffered saline (PBS) after 16 h of incubation and then harvested using homogenate buffer (50 mM Tris–HCl, pH 7.5, 150 mM NaCl, 1 mM sodium orthovanadate, 1% Triton X-100, 1% protease inhibitor cocktail and 1 mM PMSF). Cells used for reverse-transcriptase polymerase chain reaction (RT-PCR) analysis were washed with cold PBS after 16 h of incubation and the RNA was then extracted with TRIzol™ reagent and purified by using RNAeasy mini kit. The concentration and purity of total RNA were determined spectrophotometrically. The ratio of absorbance at 260–280 nm was 1.8–2.0.

Determination of nitrite production

About 100 µl of cell culture medium containing 1 µg/ml of LPS with various concentrations of bisdemethoxycurcumin and curcumin dissolved in DMSO was mixed with 100 µl of Griess reagent [equal volumes of 1% (w/v) sulfanilamide in 5% (v/v) phosphoric acid and 0.1% (w/v) naphtylenediamine HCl] and incubated at room temperature for 10 min. The absorbance at 550 nm was then measured using a microplate reader. The amount of nitrite in the samples was measured with the sodium nitrite serial dilution standard curve to determine nitrite production.

Detection of protein expression for iNOS and TNF-α in macrophage cell line RAW 264.7 activated with lipopolysaccharide

After protein determination of the cell lysate using the Bio-Rad Dc protein assay reagent according to the manufacturer’s instructions, 50 µg of macrophage cellular protein was subjected to 8–15% SDS-polycrylamide gel electrophoresis. Proteins were transferred onto a nitrocellulose membrane by electric blotting with 200 mA for 30–60 min. The immunoblot was incubated for 30 min with blocking solution [TBS-T (10 mM Tris–HCl, pH 7.5, 50 mM NaCl, 1 mM KCl, 10% Tween 20) containing 5% skim milk powder]. Blots were then washed three times with TBS-T solution, followed by incubation overnight at 4°C with a 1/1000 dilution of monoclonal anti-iNOS and TNF-α antibodies, and 1/10,000 dilution of anti-β-actin in TBS-T solution containing 1% BSA and 0.5% FBS. After three washing steps with TBS-T solution, the membranes were incubated for 1 h at room temperature with secondary antibodies: goat anti-rabbit IgG, HRP-conjugate immunoglobulin for iNOS; rabbit anti-goat mouse IgG, HRP-conjugate immunoglobulin for TNF-α; and goat anti-mouse IgG, HRP-conjugate immunoglobulin for β-actin, followed by three more washing steps with TBS-T solution. Detection was performed using the enhanced chemiluminescence detecting reagent on the image scanner, LAS 1000 Plus (Fuji Film Ltd., Japan) according to the manufacturer’s instructions.
Detection of messenger RNA (mRNA) for iNOS and TNF-α in macrophage cell line RAW 264.7 activated with lipopolysaccharide

RT-PCR was performed using a commercially available one-step reverse-transcribed polymerase chain reaction kit. Briefly, 2 µg of RNA were mixed with 5 x one-step RT-PCR buffer, one-step RT-PCR enzyme mix, dNTP mix, 5 x Q solution, and primers according to the manufacturer’s recommendation in a 50-µl volume. The amplification was carried out with a GeneAmp PCR system Dice thermal cycler (Takara Bio Inc., Japan). The sense and antisense primers used for mouse β-actin as an internal standard were 5’-CAT-GAAGGTGTGACGGACATCCGT-3’ and 5’-CCTA-GAAGCATTTGCGGTCAGATG-3’, respectively. The sense and antisense primers used for mouse iNOS were 5’-ATTGGCAACATCAGGTCGGCCATCACT-3’ and 5’-GCTGTGTGTCACAGAAGTCTCGAATCTC-3’, respectively. The sense and antisense primers used for mouse TNF-α were 5’-ATGAGCACAGAAAGCATGATG-3’ and 5’-TACAGGCTGTCACTCGAATT-3’, respectively. After initial denaturation for 15 min at 95°C, 30 amplification cycles were performed for iNOS (1 min of 95°C denaturation, 1 min of 60°C annealing, and 1.5 min of 72°C extension) and TNF-α (1 min of 95°C denaturation, 1 min of 55°C annealing, and 1 min of 72°C extension), and followed by a final PCR temperature of 72°C for 10 min. A portion of the PCR mixture was then electrophoresed on a 2% agarose gel containing ethidium bromide in TBE buffer (20 mM Tris–HCl, pH 7.4, 2 mM EDTA, pH 8.0). The bands were visualized by UV irradiation and photographed.

Effect of bisdemethoxycurcumin on chronic gastric ulcer induced by topical application of acetic acid

Gastric ulcer was induced by acetic acid treatment in rats according to the method of Okabe et al. (1971). The abdomen of a rat anesthetized with pentobarbital sodium (50 mg/kg body wt., i.p.), was opened and a cylindrical plastic mold (6 mm diameter) was tightly placed upon the anterior serosal surface of the stomach wall (antrum). Acetic acid (100%, 0.06 ml) was then poured into the mold and allowed to remain for 60 s. The acetic acid remaining on the surface was wiped away with a filter and was gently washed with normal saline. The opened abdomen was then closed and the rat was fed normally. Either 1% CMC (5 ml/kg, p.o. bid), bisdemethoxycurcumin (20–80 mg/kg body wt., p.o. bid) or curcumin (20–80 mg/kg body wt., p.o. bid) was administered to the rat for 10 consecutive days, beginning on the 4th day after the operation. The rat was killed on the 14th day after the treatment and the ulcer was examined macroscopically and histologically for the following parameters: ulcer index (mm²) = length (mm) × the width of the ulcer (mm):

% Curation = [(UIcontrol at 4th day – UItreatment at 14th day)/UIcontrol at 4th day] × 100

%Mucosal regeneration index = regeneration of the mucosal layer/(defect of the mucosa + regeneration of the mucosal layer) × 100

% Healing index = (1 – defect of the mucosa/distance of ruptured muscularis mucosa) × 100

Determination of gastric acid secretion

A pylorus ligation was carefully done in fasted rats under anesthesia with pentobarbital sodium (50 mg/kg body wt., i.p.). The rats received either 1% CMC (5 ml/kg body wt., intraduodenally (i.d.)), bisdemethoxycurcumin (5–80 mg/kg body wt., i.d.) or curcumin (5–80 mg/kg body wt., i.d.), immediately after the ligation. Four hours following the treatment, the rats were killed and the gastric juice was collected and centrifuged. After measuring the volume of the supernatant, the total acid output was analyzed by titration with 2 M NaOH using 2% phenolphthalein as an indicator and expressed as μEq/ml or μEq/h.

Effect of bisdemethoxycurcumin on the level of protein and mRNA expression of iNOS and TNF-α in the gastric ulcerated mucosa

Each gastric ulcerated mucosa or gastric control mucosa was scraped off the underlying muscularis externa and serosa, and placed in a homogenate buffer (100 mg sample/1 ml buffer). The sample was homogenized for two 30-s bursts of a Polytron homogenizer under ice-cold condition and then centrifuged at 11,000 g for 10 min at 4°C. After protein determination, 50 μg of macrophage cellular protein was subjected to 8–15% SDS-polyacrylamide gel electrophoresis, followed by electro-blotting onto nitrocellulose membranes as described previously.

Statistics

All data were expressed as mean ± SEM. Comparisons between groups were made by one-way analysis of variance (ANOVA) followed by the Dunnett test. Values of p < 0.05 were considered to be statistically significant.
Results

Effect of bisdemethoxycurcumin on LPS-induced NO production in macrophage cell line RAW 264.7

The production of nitrite was measured using the method of Griess. Bisdemethoxycurcumin as well as curcumin significantly reduced NO production in a dose-dependent manner (Fig. 1).

Effect of bisdemethoxycurcumin on LPS-induced iNOS and TNF-α protein and mRNA Expression in macrophage cell line RAW 264.7

From Western blot analysis, neither iNOS nor TNF-α protein expression in unstimulated RAW 264.7 was detectable. The protein expression level of these cytokines was markedly augmented in response to LPS (1 μg/ml) (Figs. 2 and 3). Co-incubation of macrophages with LPS plus bisdemethoxycurcumin or curcumin significantly inhibited iNOS protein induction in a dose-dependent manner after 16 h of incubation (Fig. 2). However, curcumin showed a greater degree of inhibition than bisdemethoxycurcumin. In addition, curcumin but not bisdemethoxycurcumin showed a significant inhibitory effect on TNF-α protein induction (Fig. 3). The concentration that produced the maximum inhibitory effect without any affect on the expression of the housekeeping gene β-actin of both curcuminoids was 10 μM. The RT-PCR analysis showed that the decrease in iNOS protein levels from both curcuminoids correlated with a decrease in iNOS mRNA expression (Fig. 4). However, curcumin exhibited a minimal inhibitory effect on the level of TNF-α mRNA expression (Fig. 4).

Effect of bisdemethoxycurcumin on gastric acid secretion

Cimetidine (100 mg/kg body wt., i.d.) showed the most potent efficacy in reduction of gastric juice volume, acid output and acid concentration (Table 1). Aminoguanidine (30 mg/kg body wt., s.c.) significantly
inhibited the acid output, whereas very little change was observed in the acid concentration. Curcumin (5–20 mg/kg body wt., i.d.) reduced the acid output dose-dependently and slightly but significantly depressed the acid concentration. The antisecretory effect of curcumin was rather weakened at doses over 20 mg/kg body wt. On the other hand, bisdemethoxycurcumin (5–80 mg/kg body wt., i.d.) strongly reduced the acid output and acid concentration.

**Effect of bisdemethoxycurcumin on chronic gastric ulcer induced by topical application of acetic acid**

Cimetidine (100 mg/kg body wt./day, p.o., twice a day), aminoguanidine (30 mg/kg body wt./day, s.c. daily), curcumin (20–80 mg/kg body wt., p.o., twice a day) and bisdemethoxycurcumin (20–80 mg/kg body wt., p.o., twice a day) significantly decreased the index of acetic acid-induced chronic gastric ulcer after 10 days treatment and also promoted the mucosal regeneration in the ulcerated portion (Table 2). The curative potency of bisdemethoxycurcumin was almost the same as that of curcumin and was slightly higher than that of aminoguanidine. The ulcer curative potency of curcumin tended to decrease at doses greater than 160 mg/kg body wt./day.

**Effect of bisdemethoxycurcumin on the level of protein and mRNA expression of iNOS and TNF-α in the gastric ulcerated mucosa**

The protein expression of iNOS and TNF-α was detected by Western blot analysis. In rats with gastric ulcer, an increased expression of these cytokines was detected at the ulcer margin in the vehicle-treated rat, as compared with the normal gastric mucosa (Figs. 5 and 6). Treatment with aminoguanidine significantly
Each value represents the mean positive iNOS inhibitor control. (vehicle control) 1% Methylcellulose

Discussion

TNF-α but had no effect on the protein expression level of reduced the increased protein expression level of iNOS but had no effect on the protein expression level of TNF-α. Treatment with bisdemethoxycurcumin dose-dependently reduced the increased protein expression level of iNOS but not TNF-α. On the other hand, treatment with curcumin dose-dependently reduced the increased protein expression level of iNOS and TNF-α to normal levels.

Table 1. Effect of bisdemethoxycurcumin and curcumin on gastric acid secretion in rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (/kg body wt)</th>
<th>No. of rats</th>
<th>Volume (ml)</th>
<th>Acid output (meEq/h)</th>
<th>Acid conc. (meEq/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1% Methylcellulose (vehicle control)</td>
<td>5 ml/kg i.d.</td>
<td>10</td>
<td>4.40 ± 0.44</td>
<td>0.063 ± 0.001</td>
<td>0.055 ± 0.006</td>
</tr>
<tr>
<td>Bisdemethoxycurcumin</td>
<td>5 mg/kg/5 ml i.d.</td>
<td>10</td>
<td>2.66 ± 0.36*</td>
<td>0.028 ± 0.005*</td>
<td>0.040 ± 0.005*</td>
</tr>
<tr>
<td></td>
<td>10 mg/kg/5 ml i.d.</td>
<td>10</td>
<td>1.68 ± 0.10*</td>
<td>0.018 ± 0.002*</td>
<td>0.039 ± 0.002*</td>
</tr>
<tr>
<td></td>
<td>20 mg/kg/5 ml i.d.</td>
<td>10</td>
<td>2.16 ± 0.16*</td>
<td>0.021 ± 0.003*</td>
<td>0.038 ± 0.003*</td>
</tr>
<tr>
<td></td>
<td>40 mg/kg/5 ml i.d.</td>
<td>10</td>
<td>2.39 ± 0.34*</td>
<td>0.024 ± 0.006*</td>
<td>0.038 ± 0.005*</td>
</tr>
<tr>
<td></td>
<td>80 mg/kg/5 ml i.d.</td>
<td>10</td>
<td>2.60 ± 0.24*</td>
<td>0.026 ± 0.004*</td>
<td>0.039 ± 0.003*</td>
</tr>
<tr>
<td>Curcumin</td>
<td>5 mg/kg/5 ml i.d.</td>
<td>10</td>
<td>3.70 ± 0.56</td>
<td>0.043 ± 0.007</td>
<td>0.046 ± 0.004</td>
</tr>
<tr>
<td></td>
<td>10 mg/kg/5 ml i.d.</td>
<td>10</td>
<td>3.89 ± 0.39</td>
<td>0.035 ± 0.005*</td>
<td>0.037 ± 0.004*</td>
</tr>
<tr>
<td></td>
<td>20 mg/kg/5 ml i.d.</td>
<td>10</td>
<td>3.04 ± 0.25*</td>
<td>0.030 ± 0.005*</td>
<td>0.040 ± 0.006*</td>
</tr>
<tr>
<td></td>
<td>40 mg/kg/5 ml i.d.</td>
<td>10</td>
<td>3.34 ± 0.39*</td>
<td>0.043 ± 0.01*</td>
<td>0.048 ± 0.006</td>
</tr>
<tr>
<td></td>
<td>80 mg/kg/5 ml i.d.</td>
<td>10</td>
<td>3.60 ± 0.37</td>
<td>0.049 ± 0.008</td>
<td>0.052 ± 0.004</td>
</tr>
<tr>
<td>Cimetidine</td>
<td>100 mg/kg/5 ml i.d.</td>
<td>9</td>
<td>1.65 ± 0.17*</td>
<td>0.001 ± 0.001*</td>
<td>0.003 ± 0.001*</td>
</tr>
<tr>
<td>Aminoguanidine</td>
<td>30 mg/kg/5 ml s.c.</td>
<td>9</td>
<td>3.41 ± 0.26*</td>
<td>0.045 ± 0.007*</td>
<td>0.052 ± 0.006</td>
</tr>
</tbody>
</table>

All treatments were conducted using male Wistar rats (180–220 g each) immediately after surgery, and gastric secretion was collected for 4 h after pylorus ligation. Cimetidine was used as the positive antisecretory (a histamine H2 receptor antagonist) control and aminoguanidine was used as the positive iNOS inhibitor control.

Each value represents the mean ± SEM.

*p<0.05 compared with the vehicle control-treated rats (Dunnett test).

reduced the increased protein expression level of iNOS but had no effect on the protein expression level of TNF-α. Treatment with bisdemethoxycurcumin dose-dependently reduced the increased protein expression level of iNOS but not TNF-α. On the other hand, treatment with curcumin dose-dependently reduced the increased protein expression level of iNOS and TNF-α to normal levels.

Discussion

The results obtained from the study confirmed previous reports that bisdemethoxycurcumin was as active as curcumin in directly scavenging nitric oxide generation (Sreejayan and Rao, 1997) but was less potent than curcumin in suppression of iNOS (Gafner et al., 2004) and TNF-α gene expression (Lantz et al., 2005) in activated mouse macrophage cell line RAW 264.7. In addition, bisdemethoxycurcumin exerts potent inhibitory activity on iNOS expression at the transcriptional level, but not TNF-α. Curcumin, on the other hand, results in a greater degree of inhibition on both iNOS and TNF-α expression at the transcriptional and post-transcriptional level, respectively. Moreover, bisdemethoxycurcumin, at doses of 40–160 mg/kg body wt., directly accelerates gastric ulcer healing by mechanisms involving its inhibitory activity on gastric acid secretion and on iNOS production. The antiulcer potency of bisdemethoxycurcumin is equal to curcumin, with stronger inhibitory potency on gastric acid secretion but weaker suppressing potency on the induction of iNOS. Thus, the methoxy group was not essential for the nitric oxide scavenging activity but the substitution overproduction of nitric oxide by iNOS of activated macrophage in the development of ulcer and the involvement of gastric acid in aggravating gastric ulcers (Mahattanadul et al., 2006a, b). It has been observed that, after ulcer induction, cNOS activity decreased while iNOS activity increased 6 h after ulcer induction in the inflammatory cells at the ulcer base and ulcer...
mucosa, thereby eliminating iNOS-positive inflammatory cells in the regenerating epithelium but also in ulcer healing by producing NO not only in the inflammatory process and ulcer generation but also in ulcer healing by producing NO not only in the inflammatory process and ulcer.

marginal, with the maximal level of expression at day 3 and declining by day 6. A significant level of expression was found at day 10 after ulcer induction (Akiba et al., 1998). In addition, activated and accumulated inflammatory cells in the lesions were found to be destroyed by apoptosis to promote resolution of acute inflammation and during the healing process (Savill, 1997). Apoptosis was also found to play an important role in remodeling the inflamed site by destruction of myofibroblasts. It was found that administration of aminoguanidine reduced ulcer size 3 days after ulcer induction significantly, but without further reduction 1 week after induction, because of reversible inhibition of iNOS activity without any effect on iNOS expression (Akiba et al., 1998). Additionally, this treatment increases antiulcer control with antisecretory activity, iNOS inhibitory activity and iNOS plus TNF-α inhibitory activity, respectively. All measuring parameters were determined as described in the section “Materials and methods”. Each value represents the mean ± SEM.

* p < 0.05 compared with the vehicle control-treated rats (Dunnett test).

Fig. 5. Densitometric analysis of iNOS protein expression assessed by Western blot and expressed as each cytokine per β-actin ratio in normal gastric mucosa (lane 1); in mucosa around ulcer treated with vehicle (lane 2); curcumin 40 mg/kg body wt., p.o. bid (lane 3); curcumin 80 mg/kg body wt., p.o. bid (lane 4), bisdemethoxycurcumin 40 mg/kg body wt., p.o. bid (lane 5), bisdemethoxycurcumin 80 mg/kg body wt., p.o. bid (lane 6), or aminoguanidine 30 mg/kg body wt., s.c. daily (lane 7). Aminoguanidine and curcumin were used as the positive iNOS inhibitor control. Mean ± SEM of 6 rats; *p < 0.05 compared with the vehicle (Dunnett test).

Table 2. Curative effect of bisdemethoxycurcumin and curcumin on acetic acid-induced chronic gastric ulcer in rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (/kg body wt)</th>
<th>No. of rats</th>
<th>Ulcer index (mm² ± SEM)</th>
<th>Curation (%)</th>
<th>Healing index (%)</th>
<th>Mucosal regeneration index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1% Methycellulose (vehicle control)</td>
<td>5 ml/kg p.o. bid</td>
<td>10</td>
<td>30.05 ± 3.78</td>
<td>51.30</td>
<td>33.10 ± 1.94</td>
<td>33.98 ± 2.50</td>
</tr>
<tr>
<td>Bisdemethoxycurcumin</td>
<td>20 mg/kg/5 ml p.o. bid</td>
<td>9</td>
<td>20.89 ± 4.79*</td>
<td>66.14*</td>
<td>38.56 ± 5.48*</td>
<td>42.70 ± 3.22*</td>
</tr>
<tr>
<td></td>
<td>40 mg/kg/5 ml p.o. bid</td>
<td>9</td>
<td>14.69 ± 2.42*</td>
<td>76.19*</td>
<td>41.25 ± 2.80*</td>
<td>46.71 ± 3.49*</td>
</tr>
<tr>
<td></td>
<td>80 mg/kg/5 ml p.o. bid</td>
<td>9</td>
<td>8.61 ± 1.59*</td>
<td>86.05*</td>
<td>43.79 ± 3.22*</td>
<td>49.47 ± 3.19*</td>
</tr>
<tr>
<td>Curcumin</td>
<td>20 mg/kg/5 ml p.o. bid</td>
<td>10</td>
<td>10.18 ± 1.04*</td>
<td>83.50*</td>
<td>36.30 ± 4.06</td>
<td>43.53 ± 2.90*</td>
</tr>
<tr>
<td></td>
<td>40 mg/kg/5 ml p.o. bid</td>
<td>10</td>
<td>9.21 ± 2.62*</td>
<td>85.07*</td>
<td>45.44 ± 4.35*</td>
<td>57.99 ± 4.52*</td>
</tr>
<tr>
<td></td>
<td>80 mg/kg/5 ml p.o. bid</td>
<td>10</td>
<td>11.80 ± 2.11*</td>
<td>80.88*</td>
<td>39.50 ± 2.73*</td>
<td>41.53 ± 2.37*</td>
</tr>
<tr>
<td>Cimetidine</td>
<td>100 mg/kg/5 ml p.o. bid</td>
<td>10</td>
<td>10.85 ± 1.45*</td>
<td>82.42*</td>
<td>47.00 ± 3.19*</td>
<td>49.37 ± 2.95*</td>
</tr>
<tr>
<td>Aminoguanidine</td>
<td>30 mg/kg/5 ml s.c. od</td>
<td>10</td>
<td>20.17 ± 1.62*</td>
<td>66.69*</td>
<td>43.11 ± 4.06*</td>
<td>45.57 ± 2.69*</td>
</tr>
</tbody>
</table>

Either 1% CMC, bisdemethoxycurcumin, curcumin, cimetidine and aminoguanidine was administered to male Wistar rats (180–220 g each) for 10 days from the 4th day after production of acetic acid-induced gastric ulcer. Cimetidine, aminoguanidine and curcumin were used as the positive antulcer control with antisecretory activity, iNOS inhibitory activity and iNOS plus TNF-α inhibitory activity, respectively. Mean ± SEM of 6 rats; * p < 0.05 compared with the vehicle control-treated rats (Dunnett test).
In conclusion, the results of the present study indicate that bisdemethoxycurcumin as well as curcumin may be clinically beneficial as a potential therapeutic agent for the improvement and acceleration of the healing process in chronic gastric ulcer by mechanisms involving its inhibition of gastric acid secretion and promoting an increase in the mucosal defensive mechanism through suppression of iNOS-mediated inflammation. Since the curative potency of curcumin tends to decrease at doses greater than 80 mg/kg body wt./day, presumably through some curative mechanisms differently modified by curcumin dosage, the present findings provide further insight into the appropriate combination ratio of the amount of curcumin and bisdemethoxycurcumin in treatment of peptic ulcer.

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