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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

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decreasing gastric acid secretion and enhancing the mucosal defensive mechanism through suppression of iNOS-mediated inflammation.

Keywords: Curcumin; Bisdemethoxycurcumin; Inflammatory cytokines; Gastric ulcer; Inducible nitric oxide synthase (iNOS); Tumor necrosis factor-alpha (TNF-α)

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-α), 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^- 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-α 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-α and PGE_2 production in an *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-α 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 *C. longa* whereas curcumin can be found in different *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-α cytokine), in a gastric ulcer model system was elucidated and compared with curcumin.

Materials and methods

Medicinal plant

Dried powder (1 kg) of *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 ($3\,l \times 3$), 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 $25 \pm 1\,^{\circ}C$ with a controlled 12-h light–dark cycle and maintained on standard rodent chow and tap water *ad libitum*. The rats