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Free Radical Biology & Medicine 40 (2006) 1397–1408
www.elsevier.com/locate/freeradbiomed

Original Contribution

Indomethacin inactivates gastric peroxidase to induce reactive-oxygen-mediated gastric mucosal injury and curcumin protects it by preventing peroxidase inactivation and scavenging reactive oxygen

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Received 7 July 2005; revised 28 November 2005; accepted 11 December 2005
Available online 17 January 2006

Abstract

We have investigated the mechanism of indomethacin-induced gastric ulcer caused by reactive oxygen species (ROS) and the gastroprotective effect of curcumin thereon. Curcumin dose-dependently blocks indomethacin-induced gastric lesions, showing 82% protection at 25 mg/kg. Indomethacin-induced oxidative damage by ROS as shown by increased lipid peroxidation and thiol depletion is almost completely blocked by curcumin. Indomethacin causes nearly fivefold increase in hydroxyl radical ($^{\bullet}OH$) and significant inactivation of gastric mucosal peroxidase to elevate endogenous H_2O_2 and H_2O_2-derived $^{\bullet}OH$, which is prevented by curcumin. *In vitro* studies indicate that indomethacin inactivates peroxidase irreversibly only in presence of H_2O_2 by acting as a suicidal substrate. 5,5-Dimethyl-pyrroline-*N*-oxide (DMPO) protects the peroxidase, indicating involvement of indomethacin radical in the inactivation. Indomethacin radical was also detected in the peroxidase–indomethacin–H_2O_2 system as DMPO adduct ($a^N = 15$ G, $a^H_\beta = 16$ G) by electron spin resonance spectroscopy. Curcumin protects the peroxidase in a concentration-dependent manner and consumes H_2O_2 for its oxidation as a suitable substrate of the peroxidase, thereby blocking indomethacin oxidation. Curcumin can also scavenge $^{\bullet}OH$ *in vitro*. We suggest that curcumin protects gastric damage by efficient removal of H_2O_2 and H_2O_2 -derived $^{\bullet}OH$ by preventing peroxidase inactivation by indomethacin.

Keywords: Curcumin; Indomethacin; Gastric ulcer; Reactive oxygen species; Gastric peroxidase; Antiulcer compound; Free radical

Introduction

Indomethacin (IMN), a nonsteroidal antiinflammatory drug (NSAID) widely used to treat arthritic diseases, causes gastric lesions [1] by inhibiting prostaglandin biosynthesis. Decreased prostaglandin level impairs almost all aspects of gastroprotection and increases acid secretion to aggravate the ulcer [2]. Other major factors include indomethacin-induced microvascular injury [3], neutrophil infiltration [4], induction of proinflammatory TNF-α expression [5,6], nitric oxide imbalance and apoptosis [6–8], and extracellular matrix damage by modulation of matrix metalloproteinases −9 and −2 [9].

Reactive oxygen species (ROS) also play a vital role in indomethacin-induced gastric damage [10–13]. Indomethacin causes gastric erosions with increased lipid peroxidation and decreased glutathione peroxidase activity [11]. Treatment with superoxide dismutase and catalase inhibits the lesions suggesting involvement of ROS in gastric damage [11]. Cultured gastric mucosal cells exposed to H_2O_2 undergo oxidative injury, which is protected by catalase and desferrioxamine [12]. Indomethacin also induces apoptosis by DNA fragmentation

Abbreviations: IMN, indomethacin; NSAIO, nonsteroidal antiinflammatory drug; TNF-α, tumor necrosis factor-α; ROS, reactive oxygen species; TBA, thiobarbituric acid; DMPO, 5,5-dimethyl-1-pyrroline-*N*-oxide; DTNB, 5,5-dithionitrobenzoic acid; DETAPAC, diethylene triaminepentaacetic acid; DMSO, dimethyl sulfoxide; TBARS, TBA reactive substances; GPO, gastric peroxidase; MSA, methanesulfonic acid; pnpp, *p*-nitrophenyl phosphate; LPO, lactoperoxidase; SOD, superoxide dismutase.

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doi:10.1016/j.freeradbiomed.2005.12.016

in cultured gastric mucosal cells by generating ROS, which is blocked by some antioxidants [13]. Among various ROS, H_2O_2 is relatively stable and membrane permeable and it kills the cell through various mechanisms including oxidative damage of lipid, protein, or DNA through site-specific generation of highly reactive $^{\bullet}OH$ [14]. A highly active peroxidase in the gastric mucosa plays a vital role in scavenging H_2O_2 to prevent oxidative damage [15,16]. Since catalase activity is very low, inactivation of this antioxidant enzyme is intimately related to the oxidative damage observed in stress related and *Helicobacter pylori*-mediated and nonmediated gastric ulcers [17–19]. However, the mechanism by which indomethacin generates ROS to cause mucosal damage despite the presence of peroxidase has not yet been clarified. The present study provides the mechanistic basis for ROS generation in the gastric mucosa through modulation of gastric peroxidase (GPO) activity by indomethacin.

Proton-pump inhibitors such as omeprazole, lansoprazole, etc. are now extensively used to control NSAIDs-induced gastroduodenal lesions [20,21]. The antiulcer effect of omeprazole is mediated through block of acid secretion by inactivation of H^+-K^+-ATPase [22]. It also offers an antiinflammatory effect [23] and antioxidant and antiapoptotic action [24] to block gastric ulcer. The superiority of omeprazole over ranitidine (H_2-receptor blocker) and misoprostol (prostaglandin E_2-analogue) for the treatment of NSAIDs-induced ulcers has been established [21]. Despite the well-documented efficacy and safety of omeprazole, some shortcomings have been reported [25–29]. Considering these limitations and the involvement of ROS in all forms of gastric ulcer [17–19], a search for an alternate nontoxic antioxidant with potent antiulcer activity was initiated. Curcumin (1,7-bis-[4-hydroxy-3-methoxyphenyl]-1,6-heptadiene-3,5-dione), a diferuloylmethane having two *o*-methoxy phenolic OH groups attached to the α,β-unsaturated β-diketone moiety (Fig. 1A inset), is a major bioactive yellow pigment of turmeric (*Curcuma longa*), a dietary constituent widely used as food preservative and spice and having use in traditional medicine. It has a wide spectrum of biological and pharmacological actions including antiinflammatory, antioxidant, antitumor, and anticarcinogenic properties [30,31]. Presence of both phenolic OH and CH_2 group of the β-diketone moiety in this natural compound has contributed remarkably to its potent antioxidant property [32,33]. Curcumin inhibits the generation of O_2^- and H_2O_2 in activated macrophages [34] and directly lowers O_2^- and $^{\bullet}OH$ [35]. Its antiinflammatory activity is mediated through block of expression of some inflammatory cytokines [36]. Considering the involvement of ROS and inflammation in all forms of gastric ulcer, the plausible antiulcer effect of curcumin was tested. The present study provides evidence that curcumin has potent antiulcer effect to block

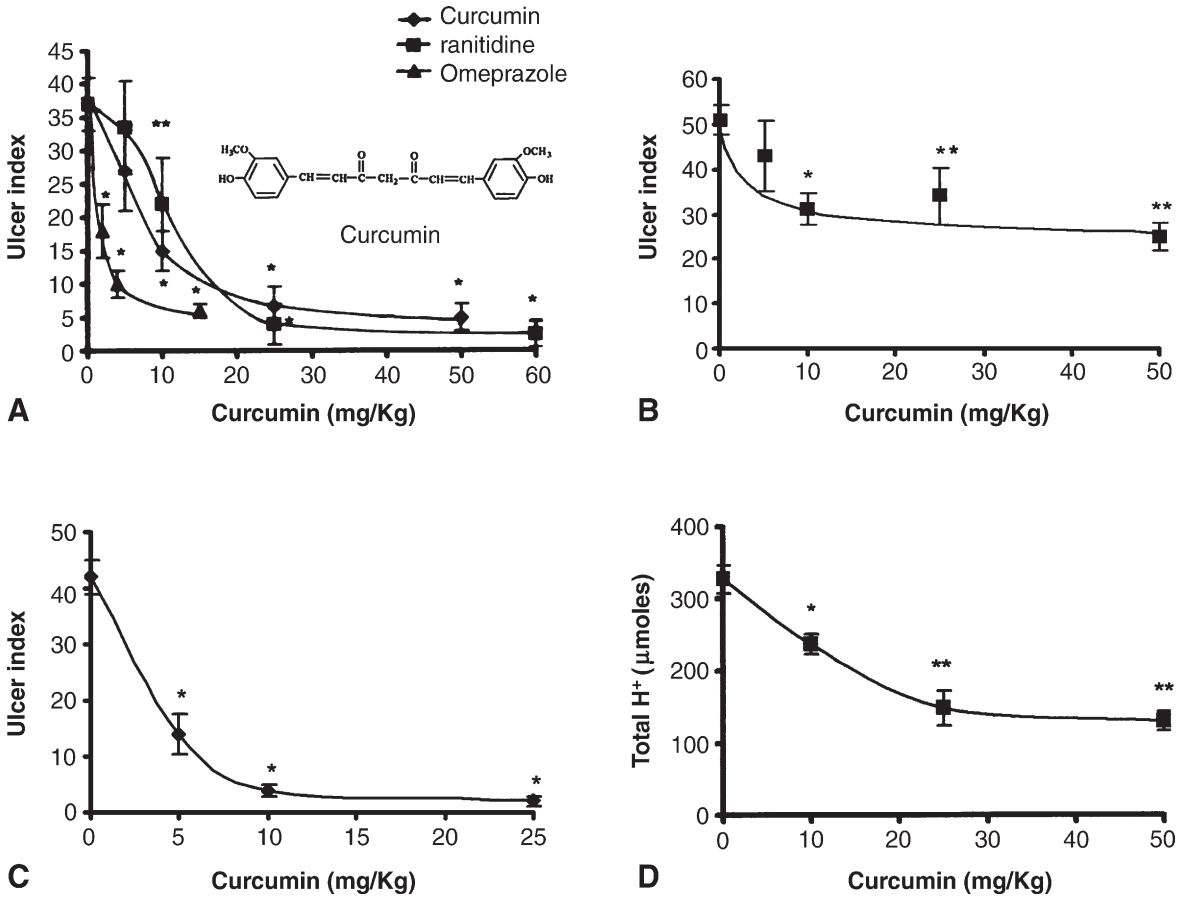

Fig. 1. Effect of curcumin on indomethacin-, stress-, and ethanol-induced gastric lesions and pylorus ligation-induced acid secretion. (A) Indomethacin-, (B) stress-, and (C) ethanol-induced gastric lesions and (D) pylorus-ligation-induced acid secretion. (A) $*p < 0.001$ and $**p < 0.01$; (B) $*p < 0.001$ and $**p < 0.05$; (C) $*p < 0.001$; (D) $*p < 0.05$ and $**p < 0.001$.