Curcumin Regulates Expression and Activity of Matrix Metalloproteinases 9 and 2 during Prevention and Healing of Indomethacin-induced Gastric Ulcer*  

Snehasiki Swarnakar‡, Krishnendu Ganguly‡, Parag Kunda‡, Aditi Banerjee, Pallab Maity, and Anamika V. Sharma¶  
From the Department of Physiology, Indian Institute of Chemical Biology, 4, Raja S.C. Mullick Road, Jadavpur, Kolkata-700032, India

Matrix metalloproteinases (MMPs) are suggested to play a critical role in extracellular matrix degradation and remodeling during inflammation and wound healing processes. However, the role of MMPs in indomethacin-induced gastric ulcer and its healing process are not clearly understood. This study is aimed at determining the regulation of MMP-9 and -2 activities in indomethacin-induced gastric ulceration and healing. Indomethacin-ulcerated stomach extracts exhibit significant up-regulation of pro-MMP-9 (92 kDa) activity and moderate reduction of MMP-2 activity, which strongly correlate with indomethacin dose and severity of ulcer. The anti-inflammatory and antioxidant properties of curcumin, an active component of turmeric, suggest that curcumin may exert antiulcer activity through scavenging reactive oxygen species, by regulating MMP activity, or both. To test these possibilities, the effect of curcumin in indomethacin-induced gastric ulcer is examined by biochemical and histological methods. The results show that curcumin exhibits potent antiulcer activity in acute ulcer in rat model by preventing glutathione depletion, lipid peroxidation, and protein oxidation. Denudation of epithelial cells during damage of gastric lumen is reversed by curcumin through re-epithelialization. Furthermore, both oral and intraperitoneal administration of curcumin blocks gastric ulceration in a dose-dependent manner. It accelerates the healing process and protects gastric ulcer through attenuation of MMP-9 activity and amelioration of MMP-2 activity. Omeprazole, an established antiulcer drug does not inhibit MMP-9 while protecting indomethacin-induced gastric ulcer. We conclude that antiulcer activity of curcumin is primarily attributed to MMP-9 inhibition, one of the major pathways of ulcer healing.

Nonsteroidal anti-inflammatory drugs (NSAIDs),¹ stress, and Helicobacter pylori are the major causative factors for gastric ulcer where extracellular matrix (ECM) degradation plays a very important role in the development of the ulcer wound. Healing of ulcer encompasses a complex series of cell/matrix interaction involving cellular proliferation, migration, and differentiation. Wound formation and the healing thereof are dynamic processes of ECM remodeling that are mainly influenced by MMPs and tissue inhibitors of metalloproteinases (TIMPs). MMPs are a growing family of zinc-dependent endopeptidases that selectively degrade components of ECM (1). The catalytic activities of MMPs are highly regulated at multiple levels, including gene expression, spatial localization,zymogen activation, and inhibition by TIMPs (2). MMPs especially pro-MMP-2 (72-kDa gelatinase A) and pro-MMP-9 (92-kDa gelatinase B) as well as their active forms are responsible for regulating most of the turnover of matrix proteins because they together are capable of degrading basement membrane proteins like gelatin, collagen IV, collagen V, elastin, and fibronectin (2). Interestingly, MMP-9 remains as zymogen under identical cellular conditions where a majority of co-expressed pro-MMP-2 is activated via the cell surface mechanism (1–3). Although MMP-2 appears to be constitutively expressed by many cell types in culture, MMP-9 expression is induced by cytokines (4), growth factors, and cell/stroma interactions (5, 6).

Indomethacin, a NSAID, causes gastric lesions through a number of mechanisms including inhibition of prostaglandin synthesis, increased expression of interleukin-1 (IL-1), generation of reactive oxygen species (ROS), and induction of apoptosis (7–10). Increased lipid peroxidation, protein oxidation, and depletion of glutathione are the major indications of the oxidative damage of the gastric mucosal cells by indomethacin (8, 11). However, very little is known about the involvement of MMP and TIMP expression in NSAID-induced gastric ulcer (12–15). MMP-1 concentration is found to be significantly higher in H. pylori-induced ulcer compared with that of NSAIDs ulcer (12). The roles for ECM proteins and ECM degrading enzymes (i.e. MMPs) in gastric ulceration have been implicated in few reports during the last few years (15, 16), but the mechanistic basis is not very clear today. MMP-2 has been suggested to participate in the physiological turnover of the gastric ECM, whereas MMP-9 may be important in the early phase of indomethacin-induced chronic gastric ulcers (13). Very recently, Mori et al. (17) reported MMP-9 induction through activation of NF-κB in H. pylori-infected cultured gastric mucosal cells. Literature is also very scanty regarding the role of MMPs and TIMPs during the healing process of the gastric ulcer (18). The roles of MMPs in ulcer development and healing have been demonstrated only in the acetic acid-induced gastric ulcer model (18). The objective of the present study is to investigate in detail the expression and activities of MMP-9 and -2 during prevention and healing of indomethacin-induced gastric ulcer.

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
† To whom correspondence should be addressed. Tel.: 91-33-2473-0492 (ext. 224); Fax: 91-33-2473-5197; E-mail: snehasiktas@hotmail.com.
‡ Recipients of a Junior Research Fellowship from the Council of Scientific and Industrial Research (New Delhi, India).
¶ Recipient of Research Associateship from Council of Scientific and Industrial Research (New Delhi, India).
¹ The abbreviations used are: NSAID, nonsteroidal anti-inflammatory drug; MMP, matrix metalloproteinase; ECM, extracellular matrix; ROS, reactive oxygen species; TIMP, tissue inhibitors of metalloproteinase; IL, interleukin; TX, Triton X-100.
during indomethacin-induced gastric ulceration and its healing by curcumin.

Although the efficacy and safety of omeprazole used for the healing of gastroduodenal ulcers caused by NSAIDs are well established (19). Some shortcomings have also been reported (20, 21). The quality of healing remains crucial for preventing recurrence (22) because ulcer recurs with various adverse side effects when common antiulcer drugs, such as omeprazole, ranitidine, famotidine, etc. are used. Considering these limitations, a search for an alternative nontoxic antiulcer compound is always welcome. Curcumin (diferuloylmethane), a bioactive constituent from *Curcuma longa* possesses remarkable anti-inflammatory, antioxidant, and anticarcinogenic properties (23, 24). This compound has been shown to inhibit the expression of a sequence of inflammatory cytokines such as tumor necrosis factor-α, IL-1, or IL-8 (24). Curcumin is a potential scavenger of oxidized free radicals, and it also increases the level of glutathione during apoptosis (25). Because pro-inflammatory and pro-oxidant states are closely linked to ulcer development, a polyphenolic compound like curcumin having potent anti-inflammatory and antioxidant activities is anticipated to exert an antiulcer effect. Based on its ethnopharmacological studies and medicinal use in traditional practice (24), the present study is undertaken to examine the gastroprotective and ulcer healing effect of curcumin in indomethacin-induced gastric ulcer and its healing process with special emphasis on the regulation of MMP-9 and -2 activities. Evidence has been presented to show that curcumin not only protects gastric mucosal cell damage and oxidative insult, but it also regulates expression and activities of MMPs during protection and healing of indomethacin-induced gastric damage. Our studies have revealed for the first time the antiulcer activity of curcumin and its mechanism of action that blocks gastric damage by inhibiting the up-regulation of MMP-9 and down-regulation of MMP-2. Furthermore, characterization of ulcer prevention by omeprazole showed its properties to be distinct from curcumin and to involve the MMP-9-independent pathway. This finding identifies the MMP-9-dependent pathway in addition to the MMP-independent pathway for ulceration.

**MATERIALS AND METHODS**

**Chemicals**—Gelatin from porcine skin, indomethacin, curcumin, Triton X-100, protease inhibitors mixture, and 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium substrate solution from Sigma. The blots were processed using Adobe Photoshop version 6.0.

**Measurement of MMP Activity**—The mitochondrial membrane fraction from the fundic stomach homogenate was used for measurement of lipid peroxide content as thiobarbituric acid reactive species (24). Briefly, 1 ml of the membrane fraction was allowed to react with 2 ml of 15% trichloroacetic acid, 0.375% thiobarbituric acid, 0.25 M HCl reagent, heated in a boiling water bath for 15 min, cooled, and centrifuged. The absorbance of the supernatant was measured at 535 nm, and the number of nmol of thiobarbituric acid reactive species produced was determined from a standard curve using tetraethoxypropane as standard.

**Measurement of Protein Carbonyl Content**—Protein oxidation was measured as carbonyl content in the low speed supernatant of the fundic stomach homogenate (26). The fundic stomach from control, ulcerated, and curcumin-pretreated (40 mg kg⁻¹) indomethacin-treated rats were homogenized in 50 mM sodium phosphate buffer, pH 7.4, in a Potter-Elvehjem glass homogenizer for 2 min to get 20% homogenate. After centrifugation at 600 × g for 10 min, the proteins from 1 ml of the supernatant were precipitated with 10% TCA and allowed to react with 0.5 ml of 10 mM 2,4-dinitrophenylhydrazine for 1 h. After precipitation with 20% trichloroacetic acid, the protein was washed thrice with a mixture of ethanol-ethyl acetate (1:1), dissolved in 1.0 ml of a solution containing 6 μM guanidine HCl in 20 mM potassium phosphate adjusted to pH 2.3 with trisbuffer, centrifuged, and the supernatant was red for carbonyl content at 362 nm (e = 22,000 M⁻¹ cm⁻¹).

**Tissue Extraction and Gelatin Zymography**—The fundic part of the gastric mucosa was suspended in phosphate-buffered saline containing protease inhibitors, minced, and incubated for 10 min at 4 °C. After centrifugation at 12,000 × g for 15 min, the supernatant was discarded. The pellet was extracted in the lysis buffer (10 μl Tris-HCl pH 8.0, 150 mM NaCl, 0.1% Triton X-100, and 0.1% protease inhibitors) at 4 °C for 10 min and allowed to react with 0.5 ml of 10 mM 2,4-dinitrophenylhydrazine for 1 h. After precipitation with 20% trichloroacetic acid, the protein was washed thrice with a mixture of ethanol-ethyl acetate (1:1), dissolved in 1.0 ml of a solution containing 6 μM guanidine HCl in 20 mM potassium phosphate adjusted to pH 2.3 with trisbuffer, centrifuged, and the supernatant was red for carbonyl content at 362 nm (e = 22,000 M⁻¹ cm⁻¹).

**Indomethacin-induced Gastric Ulceration and Its Protection Studies**—Before ulcer induction animals of both control and experimental groups kept separately in standard controlled conditions were fasted for 24 h with free access to water. Acute gastric ulcers were induced by oral administration of indomethacin at a dose of 45 mg kg⁻¹ body weight and rats were sacrificed after 4 h of indomethacin treatment. The control group received the vehicle only, whereas the experimental group received indomethacin for gastric ulceration. After 4 h, the animals were sacrificed, and gastric lesions in the fundic stomach were scored and expressed as ulcer index (26) as follows: 0 = no pathology; 1 = a small pinhead ulcer spot; and 2–5 = a bandlike lesion of 2–5 mm length (26). The sum of the total scores divided by the number of animals is expressed as the mean ulcer index. Curcumin was administered intraperitoneally or orally 30 min prior to indomethacin treatment to see the gastroprotective effect. Omeprazole and Me₆SO were administered intraperitoneally at a dose of 15 mg kg⁻¹ body weight and 3 ml kg⁻¹ body weight, respectively.
Effect of Curcumin on MMPs in Gastric Ulcer

9411

48 mg kg⁻¹ body weight, and curcumin (40 mg kg⁻¹ body weight) was administered intraperitoneally prior to indomethacin treatment as described under "Materials and Methods." After 4 h the rats were sacrificed, and the stomachs were sectioned for histological studies. Shown are the histological appearances of control (A), indomethacin-treated (B), and curcumin-pretreated indomethacin-treated (C) gastric tissues stained with hematoxylin and eosin at ×20 magnifications. Epithelial and mucosal layers are shown by small and big arrows, respectively.

RESULTS

Histological Analysis of Gastric Mucosa during Ulceration and the Effect of Curcumin—Rats sacrificed 4 h after indomethacin administration showed hemorrhagic lesions covering the total glandular area of the stomach. Gastric lesions were observed macroscopically and expressed as ulcer index that became maximal at 4 h after administration of indomethacin at a dose of 48 mg kg⁻¹ body weight. Macroscopically visible gastric hemorrhagic lesions in the fundic stomach were not found in curcumin-pretreated (40 mg kg⁻¹ body weight) rats. Histological inspection of the tissue indicated that indomethacin caused exfoliation of the gastric epithelial cells along with disruption of mucosal layer of stomach compared with that of control (Fig. 1, A and B), and this evidence of ulcer was completely abolished in curcumin-pretreated indomethacin-treated tissue (Fig. 1C). Ulcer re-epithelialization with an appearance of intact mucosal layer was observed in healed tissue (Fig. 1C).

Effect of Curcumin on Oxidative Damage in Indomethacin-induced Gastric Ulcer—ROS is one of the major causative factors for gastric ulceration by indomethacin that creates oxidative damage by increasing membrane lipid peroxidation, protein oxidation, and glutathione depletion. Therefore, the effect of curcumin on oxidative damage of the gastric mucosa caused by indomethacin treatment was studied. Table I showed the antioxidant activity of curcumin in protecting gastric lesions through blocking of indomethacin-induced increase in lipid peroxidation and protein oxidation. The glutathione level, which was significantly decreased in indomethacin-treated samples, was completely restored to the control level by curcumin.

Altering of MMP-9 and -2 Activities in Gastric Mucosa during Ulceration—To compare MMP-9 and MMP-2 activities in gastric tissue extracts from indomethacin-treated and untreated tissues, the extracts were subjected to gelatin zymography. Indomethacin at different doses was administered to develop ulcer at different stages of severity. The result as shown in Fig. 2A indicated that increasing doses of indomethacin caused gradual increase in the mean ulcer index reaching maximum at 48 mg kg⁻¹ body weight. The gelatinolytic activity of pro-MMP-9 (92 kDa) was significantly increased with increasing doses of indomethacin reaching the maximum at 48 mg kg⁻¹ body weight (Fig. 2B), and similarly MMP-9 expression was correlated parallel with increasing doses of indomethacin as shown in Western blot (Fig. 2C). In contrast, under the same condition, the activities of both pro-MMP-2 and active MMP-2 were decreased (Fig. 2B). At the highest dose of indomethacin (48 mg kg⁻¹), although the mean ulcer index was 40, the index varied from as low as 10 to as high as 50. To ascertain any changes in the activity of MMPs at different stages of severity of ulcer, gelatin zymography was carried out with gastric tissue extracts from four different degrees of ulcer indices, e.g. 10, 20, 30, and 40. The changes in the activity of both MMPs were found well correlated with the increase in the severity of ulcer as measured by ulcer index (Fig. 3A). Fig. 3B represents the percentage of activity of MMP-9 and MMP-2 in different stages of ulcer. A 12-fold higher level of MMP-9 activity was observed in indomethacin-treated samples compared with those of untreated controls (Fig. 3B). Total activity of MMP-2 (both pro and active forms) appeared to be 2-fold lower in indomethacin-treated samples compared with those of untreated controls (Fig. 3B). Ulcer index value as well as MMP-9 activity (Fig. 4) was also measured at different time points after indomethacin administration. We found that the ulcer index was increased at 3–4 h in comparison with the earlier time points (Fig. 4A), and corresponding MMP-9 activity in the gastric tissue increased ~10–12-fold at 3–4 h after indomethacin administration (Fig. 4), with respect to the earlier time points.

Antiucler and Therapeutic Effects of Curcumin; Regulation of MMP-9 and -2 Activities—Fig. 5 shows that intraperitoneal administration of curcumin was highly effective in blocking indomethacin-induced gastric ulcer. ~90% protection of ulcer occurred with an intraperitoneal dose of 40 mg kg⁻¹ body weight curcumin (Fig. 5A). Curcumin dose-dependently blocked indomethacin-induced gastric lesions (Fig. 5A) through reduction of MMP-9 activity and enhancement of total MMP-2 (both pro and active forms) activity (Fig. 5B). This down-regulation of MMP-9 activity was due to attenuation of MMP-9 at protein level as judged by Western blot probed with anti-MMP-9 antibody (Fig. 5C). Based on prophylactic data we asked whether curcumin might be applicable for therapeutic purposes. As shown in Fig. 6 oral administration of curcumin was also highly effective in protecting ulcer through reduction of MMP-9 activity and enhancement of total MMP-2 (both pro and active forms) activity. At an oral dose of 60 mg kg⁻¹ body weight, curcumin exhibited 85% inhibition of ulcer index (Fig. 6A). It is interesting to note that only a 1.5 times higher dose of curcumin was required to block ulcer when given orally (Fig. 6A) compared with that done intraperitoneally (Fig. 5A). In addition, oral administration of curcumin dose-dependently blocked indomethacin-induced gastric lesions (Fig. 6A) and exerted significant inhibitory role on MMP-9 activity (Fig. 6B).
Effect of Curcumin on MMPs in Gastric Ulcer

Curcumin (40 mg kg$^{-1}$) was injected intraperitoneally 30 min prior to indomethacin administration (48 mg kg$^{-1}$ body weight). The control animals received only vehicle orally. Glutathione, lipid peroxidation, and protein oxidation were measured as described in the text. TBARS, thiobarbituric acid reactive substance. The results are expressed as the means ± S.E.

| Samples                        | Glutathione (nmol tissue) | Lipid peroxidation (nmol TBARS/mg protein) | Protein oxidation (nmol/mg protein) |
|-------------------------------|---------------------------|-------------------------------------------|------------------------------------|
| Control                       | 174 ± 7                   | 0.60 ± 0.05                               | 1.50 ± 0.01                        |
| Control + indomethacin        | 110 ± 12$^a$              | 0.88 ± 0.06$^b$                          | 2.83 ± 0.21$^a$                    |
| Control + curcumin + indomethacin | 171 ± 13$^b$            | 0.57 ± 0.04$^a$                          | 1.68 ± 0.06$^b$                    |

$^a$ p < 0.01 versus control.

$^b$ p < 0.001 versus control + indomethacin, using a t test to compare the mean.

The 12-fold up-regulation of MMP-9 activity caused by indomethacin treatment (i.e. zero dose of curcumin) almost completely disappeared (Fig. 6A) at the oral dose of 60 mg kg$^{-1}$ body weight where ulcer was inhibited by 85%.

Healing of Gastric Ulcer by Curcumin and Associated MMP-9 and -2 Activities—To test the effect of curcumin on ulcer healing and on MMP-9 activity, rats were treated with vehicle or curcumin after induction of ulcer. The experiment of Fig. 7A shows that curcumin not only protected gastric damage, but it could also stimulate the healing of ulcer caused by indomethacin. Time course studies on the autohealing of indomethacin-induced ulcer showed that ulcers started healing progressively after 8 h, and healing was almost complete by 24–30 h (Fig. 7A). Administration of curcumin enhanced ulcer healing quite remarkably because the ulcer index came down to 50% at initial 4 h, and maximum healing was reached at 8 h by curcumin, whereas autohealing required 30 h for the same (Fig. 7A). Side by side omeprazole, an established drug, was used in healing experiment to compare the healing efficiency of curcumin, which was found to be comparable with omeprazole (Fig. 7A). It is evident from Fig. 7B that time-dependent healing of indomethacin-induced ulcer correlated well with the gradual inhibition of MMP-9 and the increase of MMP-2 activity for both autohealing and curcumin healing. Curcumin significantly attenuated MMP-9 activity and ameliorated MMP-2 activity during healing, and the activities shown at 30 h during the autohealing process were now observed at 8 h (Fig. 7B).

Effect of Different Antioxidants and Omeprazole on MMP-9 Activity during Prevention of Indomethacin-induced Ulcer—The effect of different antioxidants on MMP-9 activity during protection of ulcer was tested. Like curcumin, Me$_3$SO protected gastric ulcer through reduction of MMP-9 activity, whereas omeprazole did not have any inhibitory role on MMP-9 activity (Fig. 8A). Both omeprazole and curcumin protected 90% of the ulcers that were induced by indomethacin (Fig. 8B), and

![Fig. 2. Effect of varying doses of indomethacin on gastric lesions and associated MMP-9 and -2 activities.](image)

![Fig. 3. Correlation of ulcer index with MMP-9 and -2 activities.](image)
Me₂SO, a scavenger for the hydroxyl radical, also had an appreciable (−80%) antiulcer effect (Fig. 8B). Ulcer inhibition by curcumin and Me₂SO were associated with attenuation of pro-MMP-9 activity. In contrast, omeprazole protected ulcers without inhibiting MMP-9 activity (Fig. 8B).

**DISCUSSION**

Gastropathy associated with the constant use of indomethacin, a nonselective cyclooxygenase inhibitor, acts through a number of mechanisms including suppression of prostaglandin E synthesis and increased expression of pro-inflammatory cytokines like IL-1 and tumor necrosis factor-α (7, 8, 9, 31). The mechanisms of diminishment of prostaglandin E production and the increment of cytokine expression are mediated through generation of ROS (8). In several systems, indomethacin-induced suppression of endogenous prostaglandin E production is associated with the inhibition of pro-MMP-9, and the decrease of MMP-9 activity (31, 32). Evidence has also been provided to show that IL-1-mediated pro-MMP-9 production is enhanced by indomethacin and that tumor necrosis factor-α induces the expression of MMP-9 via the NF-κB-mediated pathway (33, 34). Nevertheless it is clear from various studies that indomethacin increases MMP-9 activity either by prostaglandin-dependent or -independent pathways (32–36). Various combinations of cytokines present at inflammatory sites as well as their balance during different stages of inflammation may provide the signals for induction of MMP-9 activity. The up- and down-regulation of MMPs at the gene and protein levels are thought to be some of the underlying mechanisms for damage and regeneration of ECM during ulceration and healing processes, respectively. Our studies indicate that ulcerated gastric mucosal tissue of rat stomach exhibited significant increase of MMP-9 activity and decreased MMP-2 activity more than that of normal tissue. In addition, gastric lesions caused by indomethacin are due to overexpression of MMP-9 protein in damaged tissue. Lempinen et al. (13) showed that indomethacin causes enhancement of MMP-9 as well as MMP-2 activity in chronic gastric ulcers in the rat model. In contrast we have consistently seen decreased MMP-2 activity and increased MMP-9 activity by indomethacin in a time- and dose-dependent manner in the acute ulcer model. Our data are supported by the findings of Pan and Hung (37) that NSAIDs suppress the constitutive activity of MMP-2 gene expression by inhibiting its transcription. Nevertheless, it is clear from all the studies that changes of total MMP-2 (pro and active) activity signify involvement of MMP-2 in the ulceration of NSAID. One interpretation of these opposite expression patterns of MMP-2 and MMP-9 is that they are regulated by distinct mechanisms. In agreement with this, it is reported that MMP-2 and MMP-9 promoters differ markedly; MMP-9 but not MMP-2 promoters contain several putative AP-1 and NF-κB binding sites (36–38).

Omeprazole (a substituted benzimidazole derivative) is widely used to control gastric damage by stress and NSAIDs...
and is believed to offer its antiulcer activity through acid suppression, scavenging of 'OH radical, and blocking apoptotic cell death (26). Although omeprazole is extensively used, it has some side effects like neutrophil adherence, diarrhea, and recurrence of ulcers (22). Considering the disadvantages of this well-established drug, an alternative antiulcer agent is necessary at this junction. It is now generally agreed that among various mechanisms, oxidative damage of the mucosa by reactive oxygen species and by apoptotic cell death are the major causative factors for gastric ulceration (10, 11). Increased lipid peroxidation and protein oxidation along with the depletion of thiols are the major indications of ROS generation in gastric mucosa (8). Because ROS generation plays a major role in all types of gastric ulcer, the possible role of them in the regulation of MMPs and the effect of curcumin thereon is an interesting aspect for investigation. Because ROS are involved in the formation of indomethacin-induced gastric ulcer, polyphenolic substances like curcumin having potent anti-inflammatory and/or antioxidant properties are anticipated to exert protective effects on ulceration (8, 23, 24). Interestingly, our data document that curcumin offers gastroprotection by preventing oxidative damage caused by glutathione depletion, lipid peroxidation, and protein oxidation. It is evident from our histological studies that curcumin can completely reverse the damage of the surface epithelial cells and the mucosal layer of gastric lumen caused by indomethacin. However, the biochemical mechanism underlying the regulation of MMP activity and the manner in which ECM remodeling occurs by curcumin during healing process are yet unknown.

The present study first establishes the antiulcer activity of curcumin in indomethacin-induced gastric ulcer and its association with down-regulation of MMP-9 activity and up-regulation of MMP-2 activity. Attenuation of MMP-9 activity is due to the block of overexpression of this protein. In the present study we have found the ability of curcumin to protect gastric ulcer in a dose-dependent manner even when administered orally. The result shows that oral dose of curcumin (60 mg kg$^{-1}$) blocks 85% of gastric damage caused by indomethacin. Thus, it may be a promising alternative therapeutic agent for offering protection and healing of NSAIDs-induced gastric ulcer. Support for this idea is provided by the findings that curcumin is nontoxic to human up to 8 gm day$^{-1}$ when taken orally for 3 months (39). The other important finding is that healing of indomethacin-induced ulcer is greatly accelerated by curcumin through an MMP-dependent process. Autohealing of indomethacin-induced ulcer was achieved progressively with time and correlates well with the gradual attenuation of MMP-9 activity and increment of MMP-2 activity. Similarly, curcumin not only attenuates MMP-9 but also ameliorates MMP-2 activity while accelerating the healing process. It is probable that curcumin down-regulates MMP-9 via diminished transactivation because the 5'-flanking region of rat MMP-9 gene contains binding sequences for transacting molecule like Erks and NF-kB (36). We are interested to explore whether NF-kB is playing a role in the induction of MMP-9 gene during gastric ulceration or suppression of MMP-9 gene during protection or healing by curcumin. On the other hand, curcumin may up-regulate MMP-2 expression in a cytokine-mediated process during healing of gastric damage. Further studies will be required to test these possibilities. Because the inhibitory effect of indomethacin on angiogenesis via suppression of MMP-2 has been reported (40, 41), it is interesting to explore the plausible role of curcumin in the protection and healing of gastric lesions by regulating angiogenesis through MMP-2.
Finally, the above results raise the important question of whether MMP-9 inhibition necessarily means ulcer healing. Or, in other words, is MMP-9 alone diagnostic for ulcer healing? To prove the hypothesis, we used different antioxidants and an established drug, omeprazole, for blocking indomethacin-induced ulcer and looked for MMP-9 profile. Surprisingly, omeprazole protects gastric ulcers without affecting MMP-9 activity. On the other hand, MeSO, as well as curcumin caused significant MMP-9 inhibition during protection of ulcers. One interpretation of these data is that ulcer healing is associated with MMP-9-dependent as well as MMP-9-independent pathways. Additionally, autonealing data suggests that the MMP-9-mediated pathway for ulcer healing is the physiologically relevant one, and curcumin exerts accelerated healing with comparable efficiency like omeprazole in a MMP-9-dependent pathway. The possibility of recurrence of ulcer may be avoided by targeting the MMP-9-dependent pathway during the healing process.

In summary, the results of this study demonstrate that up-regulation of MMP-9 (~12-fold) in indomethacin-induced gastric ulcer is due to overexpression of this protein. The novel antioxidant activity of curcumin causes reversal of indomethacin-induced epithelial cell damage and the oxidative insult of the gastric lumen by preventing lipid peroxidation and protein oxidation. Furthermore, curcumin protects ulcer and stimulates the healing process by inhibiting MMP-9 activity and by increasing MMP-2 activity. Gastric ulcer healing is a multifaceted processes and action of curcumin in arresting the MMP-dependent pathway in ulcer wound healing may lead to better healing therapeutics. More studies are anticipated showing that curcumin is of particular clinical significance for the healing of ulcer disease.

REFERENCES

1. Egbeblad, M., and Verb, Z. (2002) Nat. Rev. Cancer 2, 161–174
2. Parks, W. C., and Mecham, R. P. (1998) Matrix Metalloproteinases, Academic Press, New York
3. Piedagnel, R., Murphy, G., Rone, P. M., and Lelontg, B. (1999) J. Biol. Chem. 274, 1614–1620
4. Magd, R., Murphy, T. J., and Galis, Z. S. (2003) J. Biol. Chem. 278, 32994–32999
5. Kondapaka, S. B., Fridman, R., and Reddy, K. B. (1997) Int. J. Cancer 70, 722–726
6. Xie, B., Luan, A., and Huberman, E. (1998) J. Biol. Chem. 273, 11576–11582
7. Miller, T. A. (1983) Am. J. Physiol. 245, G601–G623
8. Yoshikawa, T., Naito, Y., Kishi, A., Tomii, T., Kaneko, T., Inuma, S., Ichikawa, H., Yasuda, M., Takahashi, S., and Kondo, M. (1993) Gastroenterology 105, 732–737
9. Slomiany, B. L., Potrowski, J., and Slomiany, A. (1997) Scand. J. Gastroen-

terol. 32, 638–642
10. Fujii, Y., Matsura, T., Kai, M., Matsu, H., Kawasaki, H., and Yamada, K. (2000) Proc. Soc. Exp. Biol. Med. 224, 102–108
11. Miura, T., Muraoaka, S., and Fujimoto, Y. (2002) Biochem. Pharmacol. 63, 2069–2074
12. Menges, M., Chan, C. C., Zeitz, M., and Stallmach, A. (2000) Z. Gastroenterologie 38, 887–911
13. Lemppen, M., Inkinen, K., Wolf, H., and Ahonen, I. (2000) Eur. Surg. Res. 32, 169–176
14. Saariello-Kere, U., Vaalamo, M., Puolakkainen, P., Airolo, K., Parks, W. C., and Karjalainen-Lindsberg, M. L. (1996) Ann. Pathol. 148, 519–526
15. Shahin, M., Kunture, J. W., Pohle, T., Schwupper, D., Herbst, H., and Dom-schke, W. (2001) Microsc. Res. Tech. 53, 396–408
16. Ernst, H., Grunert, S., Schneider, H. T., Beck, W. S., Brune, K., and Hahn, E. G. (1995) Scand. J. Gastroenterol. 30, 847–853
17. Mori, N., Sato, H., Hayashibara, T., Senba, M., Geleziunas, R., Wada, A., Hirayama, T., and Yamamoto, N. (2003) Gastroenterology 124, 983–992
18. Baragi, V. M., Qiu, L., Gunja-Smith, Z., Woessner, J. F. J., Lesch, C. A., and Grisham, M. B. (1998) Am. J. Physiol. 275, 287–293
19. Biswas, K., Bandypadhyay, U., Chattopadhyay, I., Varadaraj, A., Ali, E., and Banerjee, R. K. (2003) J. Biol. Chem. 278, 10993–11001
20. Jaruga, E., Salvioli, S., Drobucki, J., Chru, S., Bandorowicz-Pikula, Sickora, E., Franceschi, C., Cassarizza, A., and Bartosz, G. (1998) FEBS Lett. 433, 287–293
21. Klingenberg-Knol, E. C., Neli, F., Dent, J., Snel, P., Mitchell, B., Prichard, P., Lloyd, D., Havu, V., Frame, M. H., Roman, J., and Wala, A. (2000) Gastroenterology 118, 661–669
Curcumin Regulates Expression and Activity of Matrix Metalloproteinases 9 and 2 during Prevention and Healing of Indomethacin-induced Gastric Ulcer
Snehasikta Swarnakar, Krishnendu Ganguly, Parag Kundu, Aditi Banerjee, Pallab Maity and Anamika V. Sharma

J. Biol. Chem. 2005, 280:9409-9415.
doi: 10.1074/jbc.M413398200 originally published online December 22, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M413398200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 40 references, 8 of which can be accessed free at http://www.jbc.org/content/280/10/9409.full.html#ref-list-1
Additions and Corrections

Vol. 279 (2004) 35287–35297
Dominant-negative inhibition of pheromone receptor signaling by a single point mutation in the G protein α subunit.
Yuh-Lin Wu, Shelley B. Hooks, T. Kendall Harden, and Henrik G. Dohlman

Pages 35291 and 35292, Figs. 5A and 6A: We failed to note that Dr. David Stone and co-workers had published experiments similar to those in Figs. 5A and 6A (See Refs. 28, 30, and 45). On the basis of those experiments, Dr. Stone and his colleagues had proposed that the mutated G protein α subunit must interact with the receptor in order to inhibit the pheromone signal. We apologize to Dr. Stone for this oversight.

Vol. 280 (2005) 9409–9415
Curcumin regulates expression and activity of matrix metalloproteinases 9 and 2 during prevention and healing of indomethacin-induced gastric ulcer.
Snehasikta Swarnakar, Krishnendu Ganguly, Parag Kundu, Aditi Banerjee, Pallab Maity, and Anamika V. Sharma

Page 9412, Table I: In the original submission, the data presented in Table I had been prepared by Dr. Ranajit K. Banerjee and Dr. Ishita Chattopadhyay and was based on previous work performed in Dr. Banerjee’s laboratory.

Vol. 280 (2005) 23251–23261
Presenilin/γ-secretase-mediated cleavage of the voltage-gated sodium channel β2-subunit regulates cell adhesion and migration.
Doo Yeon Kim, Laura A. MacKenzie Ingano, Bryce W. Carey, Warren H. Pettingell, and Dora M. Kovacs

Page 23251: The grant footnote should read “This work was supported by grants from the NIA/National Institutes of Health and the John Douglas French Alzheimer’s Foundation.”

Vol. 280 (2005) 27244–27250
Differential responses of the Nrf2-Keap1 system to laminar and oscillatory shear stresses in endothelial cells.
Tomonori Hosoya, Atsushi Maruyama, Moon-Il Kang, Yukie Kawatani, Takahiro Shibata, Koji Uchida, Eiji Warabi, Noriko Noguchi, Ken Itoh, and Masayuki Yamamoto

Dr. Eiji Warabi and Dr. Noriko Noguchi were inadvertently omitted from the author list. Their affiliation is: Laboratory for Systems Biology and Medicine, Research Center for Advanced Science and Technology, University of Tokyo, 4-6-1 Komaba, Meguro, Tokyo 153-8904, Japan.

Page 27250, “Acknowledgments”: As a result of adding Dr. Warabi to the author list, the “Acknowledgment” should now read: “We thank Dr. T. O’Connor for help in the preparation of the manuscript.”