Curcumin prevents indomethacin-induced gastropathy in rats

Duangporn Thong-Ngam, Sakonwan Choochuai, Suthiluk Patumraj, Maneerat Chayanupatkul, Naruemon Klaikeaw

Duangporn Thong-Ngam, Sakonwan Choochuai, Suthiluk Patumraj, Maneerat Chayanupatkul, Department of Physiology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand

Naruemon Klaikeaw, Department of Pathology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand

Author contributions: Thong-Ngam D designed the study, performed the experiments, analyzed the data, and wrote the manuscript; Choochuai S performed the experiments and collected the data; Patumraj S provided analytical tools; Chayanupatkul M analyzed data and edited the manuscript; Klaikeaw N coordinated the pathological examination.

Supported by The Grant of Ratchadaphiseksomphot, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand [RA 53/52(2)]

Correspondence to: Duangporn Thong-Ngam, MD, Associate Professor, Department of Physiology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand. dr.duangporn@gmail.com

Telephone: +662-256-4267 Fax: +662-256-42672823
Received: March 22, 2011 Revised: February 9, 2012
Accepted: February 16, 2012
Published online: April 7, 2012

Abstract

AIM: To investigate the effects of curcumin on gastric microcirculation and inflammation in rats with indomethacin-induced gastric damage.

METHODS: Male Sprague-Dawley rats were randomly divided into three groups. Group 1 (control group, n = 5) was fed with olive oil and 5% NaHCO$_3$ (vehicle). Group 2 [indomethacin (IMN) group, n = 5] was fed with olive oil 30 min prior to indomethacin 150 mg/kg body weight (BW) dissolved in 5% NaHCO$_3$ at time 0th and 4th h. Group 3 (IMN + Cur group, n = 4) was fed with curcumin 200 mg/kg BW dissolved in olive oil 0.5 mL, 30 min prior to indomethacin at 0th and 4th h. Leukocyte-endothelium interactions at postcapillary venules were recorded after acridine orange injection. Blood samples were determined for intercellular adhesion molecule (ICAM)-1 and tumor necrosis factor (TNF)-α levels using enzyme linked immunosorbent assay method. Finally, the stomach was removed for histopathological examination for gastric lesions and grading for neutrophil infiltration.

RESULTS: In group 2, the leukocyte adherence in postcapillary venules was significantly increased compared to the control group (6.40 ± 2.30 cells/frame vs 1.20 ± 0.83 cells/frame, P = 0.001). Pretreatment with curcumin caused leukocyte adherence to postcapillary venule to decline (3.00 ± 0.81 cells/frame vs 6.40 ± 2.30 cells/frame, P = 0.027). The levels of ICAM-1 and TNF-α increased significantly in the indomethacin-treated group compared with the control group (1106.50 ± 504.22 pg/mL vs 336.93 ± 224.82 pg/mL, P = 0.011 and 230.92 ± 114.47 pg/mL vs 47.13 ± 65.59 pg/mL, P = 0.009 respectively). Pretreatment with curcumin significantly decreased the elevation of ICAM-1 and TNF-α levels compared to treatment with indomethacin alone (413.66 ± 147.74 pg/mL vs 1106.50 ± 504.22 pg/mL, P = 0.019 and 58.27 ± 67.74 pg/mL vs 230.92 ± 114.47 pg/mL, P = 0.013 respectively). The histological appearance of the stomach in the control group was normal. In the indomethacin-treated group, the stomachs showed a mild to moderate neutrophil infiltration score. Gastric lesions were erosive and ulcerative. In rats treated with indomethacin and curcumin, stomach histopathology improved and showed only a mild neutrophil infiltration score and fewer erosive lesions in the gastric mucosa.

CONCLUSION: The results indicate that curcumin prevents indomethacin-induced gastropathy through the improvement of gastric microcirculation by attenuating the level of ICAM-1 and TNF-α.

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Key words: Curcumin; Nonsteroidal anti-inflammatory drugs; Gastric damage; Gastric microcirculation; Intercellular adhesion molecule-1; Tumor necrosis factor-α

INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most commonly prescribed medications worldwide. However, NSAIDs have adverse effects on the gastric mucosa, resulting in various clinical presentations, ranging from nonspecific dyspepsia to ulceration, upper gastrointestinal bleeding, and death, summarized by the term “NSAID gastropathy”[9]. NSAIDs-induced gastric damage is the major side effect of this kind of drug[3].

The main action of NSAIDs is to inhibit prostaglandin synthesis. There is substantial evidence supporting the view that the ulcerogenic effect of this medication correlates with its ability to suppress prostaglandin synthesis[10,11]. Endogenous prostaglandins normally regulate mucosal blood flow, epithelial cell proliferation, epithelial restitution, mucosal immunocyte function, mucus and bicarbonate secretion, and basal acid secretion[12]. Therefore, decreases in prostaglandins, protective factors for ulcer formation, lead to gastric mucosal injury.

Animal studies have shown that neutrophil adherence to the endothelium of the gastric microcirculation is critical in NSAIDs injury[11]. Neutrophil adherence damages the mucosa by producing oxygen-free radicals, releasing proteases, and obstructing capillary blood flow. NSAIDs might induce the synthesis of tumor necrosis factor (TNF-α) and leukotrienes[9,10]. These inflammatory mediators subsequently stimulate neutrophil adherence by the upregulation of adhesion molecules[10].

NSAID administration in rats caused a rapid and significant increase in adhesion between neutrophils and vascular endothelial cells in both gastric and mesenteric venules[10-13]. This was dependent on intercellular adhesion molecule (ICAM)-1 expression on the endothelium and CD11/CD18 expression on the leukocyte[14,15]. Interestingly, Andrews et al[16] recently reported that administration of aspirin or indomethacin to rats resulted in a significant increase in ICAM-1 expression in the gastric microcirculation.

Curcuma, a genus in the plant family of Zingiberaceae, is the biological source for curcuminoids, including curcumin. Curcuma longa, the yellow tuberous root referred to as turmeric, was taken from India to Southeast Asia[16]. The yellow pigmented fraction of Curcuma longa contains curcuminoids, which are chemically related to its principal ingredient, curcumin[17]. It possesses a broad range of pharmacological activities, including antioxidant, anti-carcinogenic, wound-healing, and anti-inflammatory effects[17-19]. There are currently limited studies investigating the effect of curcumin on NSAIDs-induced gastric damage. The aim of this study was to investigate the anti-inflammation effect of curcumin on indomethacin-induced gastric damage in rats.

MATERIALS AND METHODS

Animal preparation and curcumin preparation

Male Sprague-Dawley rats weighing 180-220 g, purchased from the National Laboratory Animal Center, Mahidol University, Salaya (n = 18), Nakorn pathom, were used in this study. All rats were kept in a controlled temperature room at 25 ± 1 °C under standard conditions (12 h day-night rhythm). They were cared for in accordance with the Ethical Committee, Faculty of Medicine, Chulalongkorn University, Thailand. Curcumin powder (Cayman Chemical Company, United States) was suspended in olive oil.

Experimental protocol

All rats were fasted, with free access to water ad libitum, for 22-24 h before the experiment. They were randomly divided into three experimental groups. Group 1 (control, n = 6): Rats were fed with olive oil 30 min prior to 5% sodium bicarbonate 1 mL orally via an intragastric tube at time 0th and 4th h. Group 2 [indomethacin (IMN), n = 6]: Rats were fed with olive oil 30 min prior to indomethacin (150 mg/kg body weight in 5% sodium bicarbonate 1 mL orally via an intragastric tube) at time 0th and 4th h. Group 3 (IMN + Cur, n = 6): Rats were fed with curcumin (200 mg/kg body weight dissolved in olive oil 0.5 mL) 30 min prior to indomethacin [150 mg/kg body weight (BW) dissolved in 5% sodium bicarbonate 1 mL orally via an intragastric tube] at time 0th and 4th h.

After 8 h 30 min, animals were anesthetized with intraperitoneal injection of thiopental (50 mg/kg body weight). After tracheostomy, the carotid artery and jugular vein were cannulated for blood pressure measurement using a polygraph and for the administration of a fluorescent marker; acridine orange was infused intravenously (Sigma Chemical Co., United States, 0.5 mg/kg BW/min). The abdominal wall was incised and the stomach was extended and fixed. Leukocyte adherence in the stomach was observed by intravital fluorescence microscopy. At the end of the experiment, blood samples were collected for ICAM-1 and TNF-α determination using enzyme linked immunosorbent assay (ELISA) methods. The stomach was cut and fixed in 10% formalin solution to inspect the histopathology.

Study of the interaction between leukocytes and endothelial cells in postcapillary venule

It has been stated that NSAIDs-induced leukocyte adherence could contribute to the pathogenesis of gastric
mucosal injury. To visualize leukocytes, acridine orange was infused intravenously (0.3 mg/kg body weight). The number of leukocyte adhesions was recorded using a video recorder. Videotape of each experiment was replayed and leukocyte adherence was monitored. Most leukocytes were adhered to the postcapillary venule (about 15-30 μm in diameter). Leukocytes were considered adherent to the vessel endothelium if they remained stationary for 30 s or longer. Adherent leukocytes were expressed as the number of leukocyte adhesions per frame of view, as previously described.

Determination of serum cytokine levels
After the experiment, blood samples were taken by cardiac puncture, and allowed to clot overnight before centrifuging at approximately 2000 × g. Serum was stored at -80°C for determining ICAM-1 and TNF-α levels by ELISA kit (R and D systems).

Histopathological examination
Samples of the stomach were excised and transferred to formalin. The samples were subsequently processed by routine techniques before embedding in paraffin. Sections were cut at the thickness of 5 μm and stained with hematoxylin and eosin (HE), as previously described. One pathologist performed all the histopathological examinations. All histopathological changes were observed under a light microscope. The neutrophil infiltration score in each section was graded according to previously determined criteria.

Statistical analysis
All data were presented as mean ± SD. To compare data among all groups of animals, one-way analysis of variance (one-way ANOVA) and Duncan comparisons were employed. All statistical tests were performed using SPSS for Windows version 13.0 (SPSS Inc., Chicago, IL, United States). Differences were considered statistically significant at P < 0.05.

RESULTS

Histopathological examination
The histological appearance of the stomach in the control group (Figure 1) was normal. In the indomethacin treated group, the stomachs showed mild to moderate gastric mucosal injury. Gastric lesions were erosive and ulcerative. In rats treated with indomethacin and curcumin, the stomach histopathology improved and showed only mild gastric mucosal injury and reduced amounts of erosive lesions in the gastric mucosa. The summary of infiltration of inflammatory cells and gastric lesions are shown in Table 1.

Interaction between leukocytes and endothelial cells
After gastric injury was induced by the administration of indomethacin, leukocyte adherence to endothelial cells of postcapillary venules (15-30 μm in diameter) was observed under intravital fluorescence microscopy, 10-15 min after acridine orange injection (Figure 2). The number of leukocyte adherences significantly decreased after pretreatment with curcumin as compared to the IMN group (5.00 ± 0.81 cells/frame vs 6.40 ± 2.30 cells/frame, P = 0.027).

Changes in ICAM-1 levels
The levels of ICAM-1 increased significantly in the indomethacin treated group compared with the control group.
Pretreatment with curcumin markedly decreased the elevation of the ICAM-1 level compared with the indomethacin treated group (413.66 ± 147.74 pg/mL vs 1106.50 ± 504.22 pg/mL, P = 0.019) (Figure 3).

**Changes in TNF-α levels**

The level of TNF-α markedly increased in the indomethacin treated group compared with the control group (230.92 ± 114.47 pg/mL vs 47.13 ± 65.59 pg/mL, P = 0.009). Pretreatment with curcumin decreased the elevation of TNF-α levels compared with the indomethacin treated group (58.27 ± 67.74 pg/mL vs 230.92 ± 114.47 pg/mL, P = 0.013) (Figure 4).

**DISCUSSION**

In the present study, we investigated the effects of curcumin on indomethacin-induced gastric damage in rats. The results clearly demonstrated that curcumin administration prevented the ulcerogenic effect of indomethacin, possibly through its anti-inflammatory action. Evidence suggests that NSAIDs-induced gastric ulceration is a neutrophil-dependent process. NSAIDs administration to rats caused a rapid and significant increase in adhesion between neutrophils and vascular endothelial cells in both the gastric and mesenteric venules. Indeed, monoclonal antibodies that blocked NSAID-induced neutrophil adherence to vascular endothelium could significantly alleviate NSAID-induced gastric mucosal injury. Neutrophils play an important role in the development of inflammation and tissue injury by releasing a variety of inflammatory mediators. These inflammatory mediators are capable of producing tissue injury; therefore, they may be involved in the pathogenesis of indomethacin-induced gastric mucosal injury.

Furthermore, adhesion molecules expressed on activated neutrophils, such as CD11b and CD18, have been shown to play an important role in neutrophil-induced tissue injury. Moreover, NSAIDS are believed to have the effect on nuclear translocation of nuclear factor (NF)-κB, which modulates the expression of several adhesions molecules, including ICAM-1. ICAM-1, one of the major adhesion molecules, plays a pivotal role in the inflammatory reaction by increasing leukocyte adhesion to endothelium and promoting transendothelial migration of leukocytes to inflammatory sites. Another important mechanism that induces ICAM-1 expression is the increment of TNF-α levels. The inhibitory effect of NSAIDs on COX-2 leads reduced prostaglandin E2 (PGE2) levels. Thus, TNF-α production, which is normally inhibited...
April 7, 2012

In this study, authors investigated the protective effect of curcumin. However, it is not known whether curcumin’s anti-inflammatory effects will help prevent NSAIDs-induced gastroprophy in rats. Further studies on the expression of inflammatory mediators and adhesion molecules in the gastric mucosa are necessary to demonstrate the exact curative effect of curcumin on NSAID-induced gastric pathology. Clinical studies might also be needed to verify the protective effect of curcumin in humans.

In conclusion, NSAIDs could induce gastric injury through increases in inflammatory cytokines and leukocyte adhesions. Curcumin, an anti-oxidant herbal substance, could prevent these adverse events and might be used as a preventative method for NSAIDs-induced gastroprophy.

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S-Editor Gou SX L-Editor Stewart GJ E-Editor Zheng XM