

Effects of Curcumin on Gastric Microcirculation with Nonsteroidal Anti-inflammatory Drugs induced Gastric Injury in Rats

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ABSTRACT

Background: Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most commonly prescribed drugs worldwide. The mechanisms of NSAIDs induced gastrointestinal injury are multifactors. Curcumin is isolated from the rhizomes of the plant *Curcuma longa*. They are known anti-inflammatory properties.

Objective: The aim of this study was to investigate the effects of curcumin on gastric microcirculation and anti-inflammation action on rats with NSAIDs induced gastric injury.

Methods: Male Spraque-Dawley rats were randomly divided into three groups. Control group was fed olive oil and 5% NaHCO₃⁻ (vehicle). Group 2 (NSAIDs) was fed olive oil and indomethacin 150 mg/kg BW dissolve in 5% NaHCO₃⁻ at time 0th, 4th hr. Group 3 was fed curcumin 200 mg/kg BW dissolved in olive oil 0.5 ml 30 minute prior to indomethacin 150 mg/kg BW in 5% NaHCO₃⁻ at 0th, 4th hr. Leukocyte-endothelium interaction at postcapillary venule were record after acridine orange injection. Finally, the stomach was removed for histopathology grading for gastric mucosal injury.

Results: In NSAIDs group, the leukocyte adherence in postcapillary venule was significantly increased when compared to the control group (6.66 ± 2.33 vs 1.33 ± 0.20 cells/field, $p < 0.001$). And pretreatment with curcumin group, leukocyte adherence was reduced significantly compared to NSAIDs group (1.97 ± 0.30 vs 6.66 ± 2.33 cells/field, $p < 0.001$). In the NSAIDs group, the histopathology of stomach showed damaged with mild to severe mucosal inflammation and gastric lesions were erosive and ulcerative. In pretreatment with curcumin improved the stomach histopathology that showed only mild gastric mucosal injury and reduced erosive lesion of gastric mucosa.

Conclusion: Curcumin accomplishes the protective effect on NSAIDs-induced gastric mucosal injury. Pretreatment with curcumin could improve the leukocyte adherence in postcapillary venule and improve the gastric histopathology.

Key words : NSAIDs, curcumin, gastric injury, gastric microcirculation

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INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most commonly prescribed drugs worldwide. It is a well-known phenomenon that NSAIDs cause gastric mucosal damage resulting in outcomes ranging from nonspecific dyspepsia to ulceration, upper gastrointestinal (GI) bleeding and death - summarized by the term "NSAID gastropathy"⁽¹⁾. The mechanisms of NSAID-induced GI injury are not fully understood. Topical damage occurs in acidic NSAIDs such as acetylic-salicylic acid (ASA) and includes the accumulation of ionized NSAIDs in the gastric epithelial cell called "ion trapping" effect⁽¹⁾, the reduction of the hydrophobicity of the gastric mucosal surface and uncoupling of oxidative phosphorylation^(2,3). Disruption of the epithelial barrier allows back-diffusion of acid into the mucosa. By inhibiting cyclo-oxygenases (COX), NSAIDs block the formation not only of proinflammatory but also of gastroprotective prostaglandins⁽⁴⁾. This is a key element in NSAIDs gastropathy as prostaglandins maintain gastric mucosal blood flow and increase protective mucus as well as bicarbonate production. Inhibition of cyclo-oxygenases by NSAIDs is furthermore associated with an altered inflammatory mediator production⁽⁴⁾. As a consequence of COX-inhibition enhanced synthesis of leukotrienes may occur by shunting the arachidonic acid metabolism towards the 5-lipoxygenase pathway⁽⁵⁻⁷⁾. Leukotrienes are supposed to contribute to gastric mucosal injury by promoting tissue ischaemia and inflammation. Increased expression of adhesion molecules such as intercellular adhesion molecule-1 and increased neutrophil-endothelial adherence and activation⁽⁸⁻¹¹⁾.

There is evidence implicating 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 gastric and mesenteric venules. Indeed, using monoclonal antibodies that blocked NSAIDs-induced neutrophil adherence to vascular endothelium show significantly reduced the NSAIDs-induced gastric mucosal injury⁽¹²⁾. Neutrophils play an important role in the development of inflammation and tissue injury by releasing a variety of inflammatory mediators, including neutrophil elastase and reactive oxygen species. Since these inflammatory mediators are capable of producing tissue injury, they may be involved in the pathogenesis of indomethacin-induced gastric mucosal injury. Among

these mediators, neutrophil elastase has been demonstrated to play an important role in neutrophil infiltration and neutrophil-induced tissue injury⁽¹²⁾. 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⁽¹³⁻¹⁵⁾.

Curcuma, a genus in the plant family of Zingiberaceae, is the biological source for curcuminoids, including curcumin. *Curcuma longa*, the yellow tuberous root that is referred to as turmeric, was taken from India to Southeast Asia. It possesses a broad range of pharmacological activities including antioxidant, anti-carcinogenic and anti-inflammatory effects⁽¹⁶⁻¹⁸⁾. There are currently limited studies investigating the effect of curcumin on NSAIDs-induced gastric mucosal injury.

The aim of this study was to investigate the effects of curcumin on gastric microcirculation and anti-inflammation action determined by leukocyte adherence in postcapillary venule on rats with NSAIDs induced gastric injury.

MATERIALS AND METHODS

Animal preparation

Male Sprague-Dawley rats weighing 180-220 g purchased from the National Laboratory Animal Center, Mahidol University, Salaya, Nakorn pathom, were used in study. The rats were kept in a controlled temperature room at $25 \pm 1^\circ\text{C}$ under standard conditions (12 hour day-night rhythm). All rats were received well care in accordance with the Ethical Committee, Faculty of Medicine, Chulalongkorn University, Thailand.

Curcumin preparation

Curcumin in powder form (Cayman Chemical Company, USA) is dissolved in olive oil.

Experimental protocol

All rats were fasted, with free access to water ad libitum, for 22-24 hours before the experiment. They were randomly divided into three experimental groups.

Group 1 (Control, n = 6): Rats were fed olive oil 30 minute prior to 5% sodium bicarbonate 1 ml orally via an intragastric tube at time 0th, 4th hr.

Group 2 (NSAIDs, n = 6): Rats were fed olive oil

30 minute prior to indomethacin 150 mg/kg BW in 5% sodium bicarbonate 1 ml orally via an intragastric tube at time at 0th, 4th hr.

Group 3 (NSAIDs + curcumin, n = 6): Rats were fed curcumin 200 mg/kg BW dissolved in olive oil 0.5 ml 30 minute prior to indomethacin 150 mg/kg BW dissolved in 5% sodium bicarbonate 1 ml orally via an intragastric tube at time at 0th, 4th hr.

After 8th hr, the animals were anesthetized with intraperitoneal injection of pentobarbital (50 mg/kg body weight). After tracheostomy, carotid artery and jugular vein were cannulated for blood pressure measurement using polygraph and administration of fluorescent marker. The abdominal wall was incised and the stomach was extended and fixed. Then the leukocyte adherence in postcapillary venule of stomach was observed by in vivo microscopy.

Study of interaction between leukocytes and endothelial cells in postcapillary venule

For visualization of leukocytes, acridine orange was infused intravenously (0.5 mg/kg BW) as previously described⁽³⁰⁾.

The number of leukocyte adhesions was recorded using video recorder. Videotape of each experiment was played back and then leukocyte adherence was monitored. The leukocytes were markedly adhered to the postcapillary venule (about 15-35 µm in diameter). The location of leukocyte adherence in three areas was observed. Leukocytes were considered adherent to the vessel endothelium if they remained stationary for 30 or longer. Adherent leukocytes were expressed as the mean number of leukocyte adhesions per field of view.

$$\text{Mean number of leukocyte adhesions} = \frac{[\text{the number of (area 1 + area 2 + area 3) cells/field}]}{3}$$

Histopathological examination

The stomach was fixed in 10% formalin and embedded in paraffin. Sections was cut at a thickness of 5 µm and stained with hematoxylin and eosin (H & E) as previously described. Histopathological changes were observed under light microscope. Histopathological examination was performed by blinded pathologists and grading of gastric mucosal injury score according to Dixon criteria⁽¹⁹⁾.

Gastric mucosal injury was scored of neutrophil infiltration found in gastric mucosa

Score 0 = none

Score 1 = neutrophil infiltration found 1/3 of gastric mucosal layer

Score 2 = neutrophil infiltration found 2/3 of gastric mucosal layer

Score 3 = neutrophil infiltration found in the muscularis mucosae of gastric mucosal layer

Statistical analysis

All data were presented as means and standard deviation (SD). For comparison among all groups of animals, one way analysis of variance (one-way ANOVA) and Tukey post hoc comparisons were employed. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Interaction between leukocytes and endothelial cells

After gastric injury was induced by administration of indomethacin, leukocyte adherence to endothelial cells of postcapillary venules (15-35 mm in diameter) was observed under intravital fluorescence microscopy. The number of leukocytes adhered to postcapillary venules for 30 sec or longer was counted per each field of observation. The mean number of leukocyte adhesions in the NSAIDs group without treatment was significantly increased compared to the control group (6.66 ± 2.33 vs 1.33 ± 0.20 cells/field, $p < 0.001$).

In the pretreatment with curcumin group, the number of leukocyte adhesions was significantly decreased compared to the NSAIDs group (1.97 ± 0.30 vs 6.66 ± 2.33 cells/field, $p < 0.001$) (Figure 1 and Figure 2).

Histopathological examination

The histopathologic appearance of the stomach in the control group was normal gastric mucosa and no gastric mucosal injury (Figure 3a). In the indomethacin treated group, the histopathologic features showed mild to severe gastric mucosal injury (Figure 3b). Gastric lesions were erosive and ulcerative. In rats treated with indomethacin and curcumin improved the stomach histopathology that showed only mild gastric mucosal injury and small erosive lesion in gastric mucosa (Figure 3c). The summary of gastric mucosal injury score and gastric lesions were shown in Table 1.

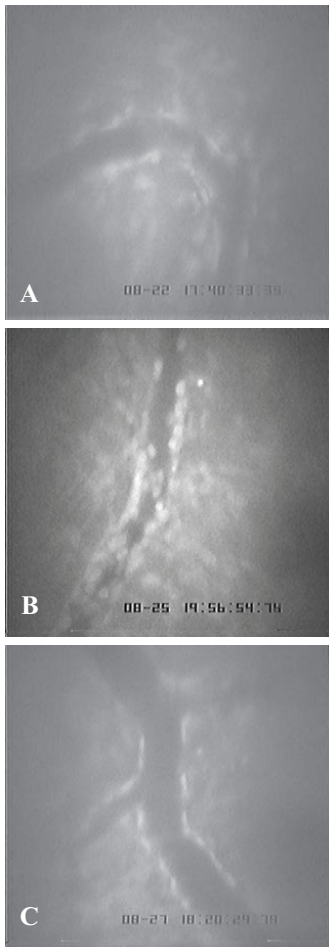


Figure 1. Intravital microscopic (40X) images of leukocyte adherence on vascular endothelium of postcapillary venules in control group (A), indomethacin group (B) and pretreatment with curcumin group (C).

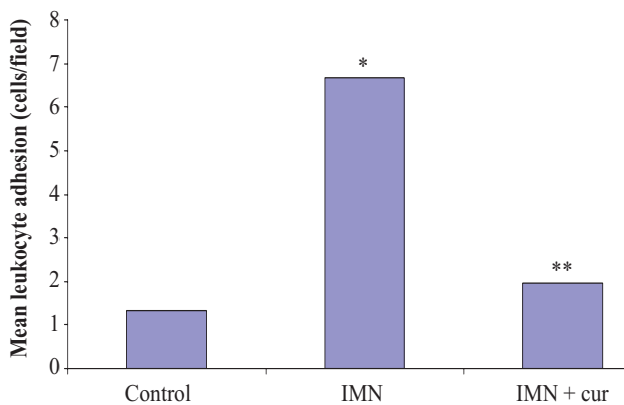


Figure 2. Mean leukocyte adhesion in all groups. All data are expressed as mean \pm SD. The mean leukocyte adhesion were significantly higher in the indomethacin treated group when compared with control group ($*p < 0.001$). Curcumin pretreatment decreased significantly mean leukocyte adhesion when compared with indomethacin group ($**p < 0.001$).

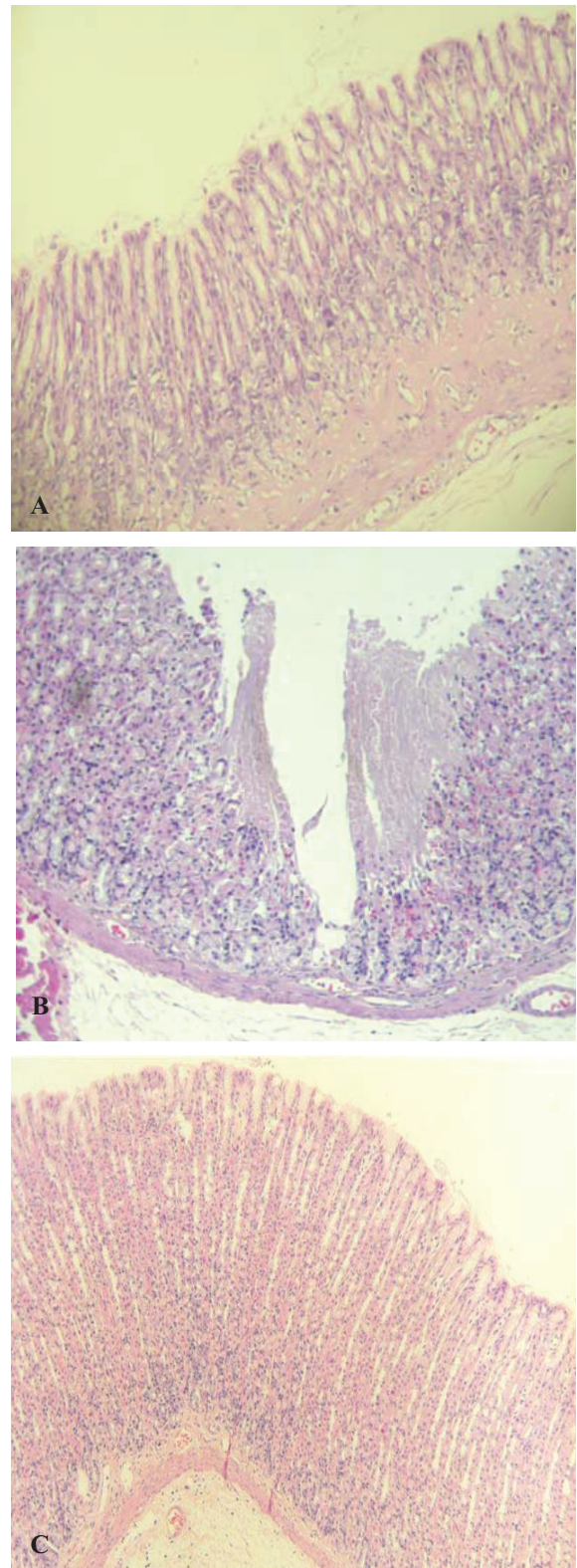


Figure 3. Hematoxylin-eosin stained stomach sections ($\times 100$). (A) Control group showed normal stomach histopathology. (B) Indomethacin treated group showed gastric ulcer and infiltration of inflammatory cells. (C) Curcumin treatment group showed the improvement in ulcer and inflammation.

Table 1. Summary of gastric mucosal injury score and erosion in all groups.

Experimental group	Gastric mucosal injury				Pathology		
	0	1	2	3	no erosion	erosion	ulcer
Control	6	0	0	0	6	0	0
IMN	0	3	2	1	0	5	1
IMN + curcumin	5	1	0	0	5	1	0

Data are expressed as the number of rats exhibiting the grade of gastric mucosal injury score and erosion indicated (each group, N = 6). The severity of gastric mucosal injury was grade by:

0 = no gastric mucosal injury

1 = neutrophil infiltration found 1/3 of gastric mucosal layer

2 = neutrophil infiltration found 2/3 of gastric mucosal layer

3 = neutrophil infiltration found in the muscularis mucosae of gastric mucosal layer

DISCUSSION

We investigated the effects of curcumin on indomethacin-induced gastric injury in rats. The results clearly demonstrate that pretreatment of curcumin prevents the ulcerogenic effect of indomethacin. In this study, after gastric injury was induced by NSAIDs, gastric inflammation increased leukocyte adherence to the endothelial surface of postcapillary venules and was characterized by the migration of macrophages and PMNs in the ulcer area. The migrated macrophages then released proinflammatory cytokines can up-regulate adhesion molecule expression on endothelial cells and leukocytes^(20,21) and cause leukocyte recruitment⁽²²⁾. Adhesion molecules on endothelial cells and leukocytes involve rolling, adhesion, and transmigration of leukocytes in gastric inflamed areas. It was reported that increment of PMNs may play an important role in the pathogenesis of NSAIDs-induced gastropathy⁽²³⁾. On the other hand, NSAIDs may enhance the expression of cell adhesion molecules on the surface of endothelial cells⁽²⁴⁾. Adhesion molecules play an important role in the recruitment of leukocytes to inflammation sites, leading to gastric mucosal injury^(24,25). It was also reported that leukocyte adhesion and aggregation can occlude microcirculation, resulting in ischemic mucosal injury^(24,26). Leukocyte infiltration in gastric mucosa can cause tissue damage leading the ulcerative lesion⁽²⁷⁾.

Curcumin is very rich in phenolics, they are known to possess antioxidant properties. Curcumin could reduce gastric injury was induced by NSAIDs. It has been reported that curcumin can decrease gastric injury that indomethacin inactivates gastric peroxidase to induce reactive-oxygen-mediated gastric damage and curcumin protects it by preventing per-

oxidase inactivation and scavenging reactive oxygen⁽²⁸⁾. All these studies showed that curcumin is an anti-inflammation substance because it can inhibit the activation of the major transcription factor NF- κ B. This transcription factors required for the expression of many proinflammatory genes, such as TNF- α , IL-1 β , iNOS and COX-2⁽²⁹⁾. There are currently limited studies investigation the effect of curcumin on anti-inflammation action in the gastric microcirculation on NSAIDs-induced gastric injury in rats.

In conclusion, Curcumin accomplishes the protective effect on NSAIDs-induce gastric mucosal injury on gastric microcirculation.

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