

## Curcumin suppresses gastric NF- $\kappa$ B activation and macromolecular leakage in *Helicobacter pylori*-infected rats

Kawiya Sintara, Duangporn Thong-Ngam, Suthiluk Patumraj, Naruemon Klaikeaw, Tanittha Chatsuwana

Kawiya Sintara, Duangporn Thong-Ngam, Suthiluk Patumraj, Department of Physiology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand

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

Author contributions: Sintara K performed the majority of experiments and wrote the manuscript; Thong-Ngam D designed the study, analyzed the data and was involved in editing the manuscript; Patumraj S provided analytical tools and was involved in editing the manuscript; Klaikeaw N co-ordinated the pathological examination; Chatsuwana T provided the material for the *Helicobacter pylori* infection.

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Correspondence to: Duangporn Thong-Ngam, MD, Associate Professor, Department of Physiology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand. [thongngam007@yahoo.com](mailto:thongngam007@yahoo.com)

Telephone: +66-2-2527854 Fax: +66-2-2564267

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### Abstract

**AIM:** To investigate whether curcumin could attenuate nuclear factor (NF)- $\kappa$ B p65 expression and macromolecular leakage in the gastric mucosa of *Helicobacter pylori* (*H. pylori*)-infected rats.

**METHODS:** Twenty-five male Sprague-Dawley rats were equally divided into five groups: control rats (Control), control rats supplemented with 600 mg/kg curcumin, *H. pylori*-infected rats (*Hp*), *H. pylori*-infected rats supplemented with 200 mg/kg curcumin (*Hp* + cur I), and *H. pylori*-infected rats supplemented with 600 mg/kg curcumin (*Hp* + cur II). In *H. pylori*-infected groups, rats were inoculated with *H. pylori* suspension twice a day at an interval of 4 h for 3 d. Two weeks later, 200 or

600 mg/kg curcumin was given once daily to curcumin-supplemented groups for 7 d. On the day of the experiment, macromolecular leakage in gastric mucosa was examined by intravital fluorescence microscopy. The stomach tissue was removed to examine NF- $\kappa$ B p65 expression in gastric epithelial cells by immunohistochemistry.

**RESULTS:** The expression of NF- $\kappa$ B p65 in gastric epithelial cells and the macromolecular leakage from gastric mucosal microcirculation significantly increased in the *Hp* group compared with the Control group. The percentages of NF- $\kappa$ B p65 immunoreactive cells in Control and *Hp* groups were  $10.72\% \pm 2.10\%$  vs  $16.02\% \pm 2.98\%$ ,  $P = 0.004$ , respectively. The percentages of macromolecular leakage in Control and *Hp* groups were  $10.69\% \pm 1.43\%$  vs  $15.41\% \pm 2.83\%$ ,  $P = 0.001$ , respectively. Curcumin supplementation in *Hp* + cur I and *Hp* + cur II groups significantly decreased NF- $\kappa$ B p65 immunoreactive cells and macromolecular leakage compared with results in the *Hp* group. The percentages of NF- $\kappa$ B p65 immunoreactive cells in *Hp* + cur I and *Hp* + cur II groups were  $11.79\% \pm 2.13\%$  ( $P = 0.017$ ) and  $11.42\% \pm 1.68\%$  ( $P = 0.010$ ), respectively. The percentages of macromolecular leakage in *Hp* + cur I and *Hp* + cur II groups were  $12.32\% \pm 2.13\%$  ( $P = 0.025$ ) and  $12.14\% \pm 1.86\%$  ( $P = 0.018$ ), respectively.

**CONCLUSION:** *H. pylori*-induced gastric inflammation in rats is associated with increased NF- $\kappa$ B activation and macromolecular leakage which can be reduced by curcumin supplementation.

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**Key words:** Curcumin; *Helicobacter pylori*; Nuclear factor- $\kappa$ B p65; Macromolecular leakage

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## INTRODUCTION

*Helicobacter pylori* (*H. pylori*) is a spiral-shaped Gram-negative bacterium. The infection causes chronic gastritis and peptic ulcer diseases in patients. *H. pylori* infection is also related to mucosa-associated lymphoid tissue lymphoma and gastric cancer diseases in patients<sup>[1,2]</sup>. The pathogenesis of *H. pylori* infection is associated with the bacterial virulence factors. After *H. pylori* bacteria adhere to gastric epithelial cells, they inject their virulence factors into the host cells *via* a type IV secretory system<sup>[3]</sup>. The virulence factors can induce the activation of nuclear factor (NF)- $\kappa$ B in gastric epithelial cells<sup>[4]</sup>.

NF- $\kappa$ B is an important regulator of many cellular processes including the control of the immune response and inflammation<sup>[5,6]</sup>. NF- $\kappa$ B is a dimeric complex composed of the five mammalian Rel proteins, p65, c-Rel, p50/NF- $\kappa$ B1, p52/NF- $\kappa$ B2, and RelB, in almost any combination. In resting cells, the inhibitors of NF- $\kappa$ B (I $\kappa$ B) form complexes with NF- $\kappa$ B. Upon stimulation, specific intracellular signalling pathways are activated, leading to the activation of the I $\kappa$ B kinase complex (IKK complex). The activated IKK complex phosphorylates the I $\kappa$ B at specific amino acids for the poly-ubiquitination of these NF- $\kappa$ B inhibitors. The ubiquitination of I $\kappa$ B and its subsequent degradation by a proteasome are required for NF- $\kappa$ B activation. NF- $\kappa$ B is now free to translocate into the nucleus and regulate NF- $\kappa$ B-dependent gene expression<sup>[7]</sup>. The target of activated NF- $\kappa$ B includes the genes encoding proinflammatory cytokines and chemokines that are the causes of *H. pylori*-induced gastric inflammation<sup>[4,8,9]</sup>.

In *H. pylori*-associated gastric inflammation, inflammatory mediators could induce vascular damage. A previous study demonstrated that *H. pylori*-infected patients showed erythema, edema, and vasodilation as well as neutrophil infiltration in the mucosa<sup>[10]</sup>. Our previous study suggested that leukocyte adhesion in postcapillary venules was increased in *H. pylori*-infected rats. Moreover, the degree of leukocyte adhesion was correlated with the level of the proinflammatory cytokine, tumor necrosis factor (TNF)- $\alpha$ <sup>[11]</sup>. In addition, previous studies have demonstrated that water-soluble extracts of *H. pylori* induced leakage of macromolecules from rat gastric mucosal microcirculation<sup>[12-14]</sup>.

Curcumin (diferuloylmethane) is an active ingredient of *Curcuma longa* (turmeric) and is pharmacologically safe for human and animals. Curcumin has many biological activities, including anti-inflammatory properties<sup>[15]</sup>.

Most of the anti-inflammatory effects can be explained by the efficient inhibition of NF- $\kappa$ B mediated by this substance<sup>[16-18]</sup>. Recently, a previous study showed that curcumin can inhibit NF- $\kappa$ B activation in *H. pylori*-infected gastric epithelial cells<sup>[19]</sup>. Curcumin is also a potent antibacterial agent against *H. pylori* as shown in *in vitro* study<sup>[20]</sup>. In contrast, curcumin did not eradicate *H. pylori* in *H. pylori*-infected patients<sup>[21]</sup>.

However, it is not clear whether curcumin has any *in vivo* effects in *H. pylori*-induced gastric inflammation. Therefore, we examined the anti-inflammatory effect of curcumin, which may reduce mucosal macromolecular leakage through the suppression of gastric epithelial NF- $\kappa$ B p65 expression induced by *H. pylori* infection in rats.

## MATERIALS AND METHODS

### Experimental design

Male Sprague-Dawley rats (National Laboratory Animal Center, Mahidol University, Nakorn pathom, Thailand) weighing 200-250 g were used. All experiments and procedures carried out on the animals were approved by the Ethics Committee of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand. Rats were housed in a controlled temperature room at 25  $\pm$  1  $^{\circ}$ C under standard conditions (12-h day-night rhythm). Twenty-five rats were divided into five groups (five rats each) as follows.

Control rats (Control): rats were fed normal saline (1 mL/rat) orally *via* intragastric tube twice a day at an interval of 4 h for 3 consecutive days. Two weeks later, 0.1% DMSO (1 mL/rat) was given once daily to the rats by intragastric tube for 7 d.

Control rats supplemented with 600 mg/kg curcumin (Cur): rats were fed normal saline (1 mL/rat) orally *via* intragastric tube twice a day at an interval of 4 h for 3 consecutive days. Two weeks later, 600 mg/kg curcumin (95% purified curcumin, Cayman Chemical, Ann Arbor, MI, USA) dissolved in 0.1% DMSO (1 mL/rat) was given once daily to the rats by intragastric tube for 7 d.

*H. pylori*-infected rats (*Hp*): rats were inoculated with *H. pylori* suspension according to Thong-Ngam *et al.*<sup>[22]</sup>. Briefly, *H. pylori* suspension (10<sup>10</sup> CFU/mL; 1 mL/rat) was given to the rats by intragastric tube twice a day at an interval of 4 h for 3 consecutive days. Two weeks later, 0.1% DMSO (1 mL/rat) was given once daily to the rats by intragastric tube for 7 d.

*H. pylori*-infected rats supplemented with 200 mg/kg curcumin (*Hp* + cur I): 2 wk after *H. pylori* inoculation, 200 mg/kg curcumin dissolved in 0.1% DMSO (1 mL/rat) was given once daily to the rats by intragastric tube for 7 d.

*H. pylori*-infected rats supplemented with 600 mg/kg curcumin (*Hp* + cur II): 2 wk after *H. pylori* inoculation, 600 mg/kg curcumin dissolved in 0.1% DMSO (1 mL/rat) was given once daily to the rats by intragastric tube for 7 d.

### *H. pylori*

*H. pylori* strains used for all experiments were originally

obtained from peptic ulcer patients who visited the King Chulalongkorn Memorial Hospital. The bacteria were grown in Brucella broth (pH 7.0) supplemented with 10% goat serum for 24 h at 37°C in an automatic CO<sub>2</sub>-O<sub>2</sub> incubator (85% N<sub>2</sub>, 10% CO<sub>2</sub>, and 5% O<sub>2</sub>).

### Animal preparation

The method of preparing animals for *in vivo* fluorescent microscopy was adapted from a previous study<sup>[14]</sup>. The animal was anesthetized with thiopental (General Drug House, Thailand; 60 mg/kg, intraperitoneal). A constant level of anesthesia was maintained throughout the experiment by a supplement dose (20% of original dose) every 30-45 min<sup>[11]</sup>. A tracheotomy was performed. The arterial blood pressure was recorded in the common carotid artery using a pressure transducer (Nihon Kohden, Tokyo, Japan). The abdominal cavity was opened *via* a midline laparotomy. A 1.0 cm incision was made using an electrical microcautery device (Hyfrecator plus<sup>®</sup>, Conmed, Utica, NY, USA) at the posterior wall, being parallel to the "limiting ridge" of the exteriorized stomach<sup>[12]</sup>. Next, the stomach was gently extended and placed on a designed board. The incision in the anterior wall was opened using microclamps and covered with Saran wrap to allow visualization of the posterior mucosal surface<sup>[12]</sup>. The animals were terminated after studying intravital fluorescent videomicroscopy.

### Intravital fluorescent videomicroscopy

Observations were made from the glandular portion of the posterior mucosa. Fluorescence tracer [0.3 mL of 0.5% fluorescein isothiocyanate (FITC)-labeled dextran (FITC-dx, MW = 250000, Sigma-Aldrich, USA)] was injected into the jugular vein<sup>[23]</sup>. The posterior mucosal microcirculation was visualized under an intravital fluorescence videomicroscope (Nikon Optiphot-2, Nikon, Tokyo, Japan), and examined under × 20 objective lens (Nikon). The selected area included the characteristic honeycomb-like network of mucosal capillaries and at least one postcapillary venule (PCV; diameter 15-30 μm)<sup>[24]</sup>. Five minutes after FITC-dx administration, a recording was performed as a baseline using a video-recorder (Sony SVT-124p, Sony, Tokyo, Japan). Thirty minutes later, recording was performed again.

### Measurement of macromolecular leakage in gastric mucosa

Based on the recorded video images, we measured macromolecular leakage from the PCV in the selected area. Computerized image analysis (GLOBAL LAB<sup>®</sup> image II program, USA) was used to measure fluorescence intensity in the interstitial space and in the PCV at both time points during the experiment. The fluorescence intensities between outside and inside vessels ( $I_{out}/I_{in}$ ) at baseline and 30-min time points were measured<sup>[25]</sup>. The molecular leakage in percentage was calculated using the equation: Macromolecular leakage (%) =  $[(I_{out}/I_{in}) \text{ at } 30 \text{ min} - (I_{out}/I_{in}) \text{ at baseline}] / [(I_{out}/I_{in}) \text{ at baseline}] \times 100$ .

### Assessment of *H. pylori* infection

The presence of *H. pylori* infection in individual rats was

determined by urease test and histological examination by a pathologist. After studying intravital fluorescent microscopy, the rat was terminated by injection of an overdose of thiopental. Then the stomach was removed and longitudinally dissected along the greater curvature. A 2 mm<sup>3</sup> segment of gastric mucosa from the antrum was immediately cut and placed in the urease test tube.

Regarding histological examination, the stomach was fixed with 4% paraformaldehyde in phosphate-buffered saline, pH 7.4 at room temperature for 24 h. The tissue was processed, embedded in paraffin, and cut at 5 μm thickness. The sections were stained with hematoxylin and eosin, and microscopically examined for the presence of *H. pylori*. The presence of *H. pylori* was also detected by Warthin-Starry staining in unclear cases. The level of bacterial colonization was recorded by using a grading system as follows, score 0: no bacteria detected; score 1: mild colonization in some gastric crypts; score 2: mild colonization in most gastric crypts; score 3: moderate colonization in all gastric crypts; and score 4: dense colonization in some gastric crypts. The results were presented as the bacterial colonization scores for each group. Moreover, gastric inflammation was classified by using the Sydney system<sup>[26]</sup>. The infiltrations of mononuclear and polymorphonuclear leukocytes in the gastric mucosa defining the inflammatory scores were recorded. Score 0 to 3 represented normal, mild, moderate, and marked histopathology changes, respectively.

### Immunohistochemistry

The stomach sections were deparaffinized with xylene and gradually dehydrated in ethyl alcohol. Next, antigen retrieval was performed by immersing the sections in citric acid buffer (pH 6.0) in a microwave oven for 13 min. Endogenous peroxidase activity and nonspecific binding were blocked with 3% hydrogen peroxide (Merck, Hohenbrunn, Germany) for 5 min and 3% normal horse serum (Gibco, Carlsbad, CA, USA) for 20 min, respectively. After that, the sections were incubated with polyclonal antibody against the p65 subunit of NF-κB (sc109; Santa Cruz Biotechnology, Santa Cruz, CA, USA) at a dilution of 1:100 in a humidified chamber for 1 h at room temperature. Then the sections were incubated with biotinylated anti-rabbit immunoglobulin (DAKO, Glostrup, Denmark) in the humidified chamber for 30 min. The reaction was visualized using the substrate diaminobenzidine (DAKO). The sections were then counterstained with hematoxylin.

Under light microscope, the expression of NF-κB p65 was cytoplasmic with scattered positive nuclear staining<sup>[27]</sup>. Thus, NF-κB p65 immunoreactive cells were defined as those with dark brown stain in their nuclei. To quantify, one thousand gastric epithelial cells were counted for each rat under × 40 objective lens. All counting was manually performed by an investigator who was blinded to the treatment groups. The data were shown as percentage of immunoreactive cells calculated from this equation: Percentage of immunoreactive cells (%) = (number of immunoreactive cells × 100)/1000.



**Table 1** Demonstrated level of *Helicobacter pylori* colonization and gastric inflammation scores

Group	No.	Level of <i>H. pylori</i> colonization					Score of gastric inflammation			
		0	1	2	3	4	0	1	2	3
Control	5	5	-	-	-	-	5	-	-	-
Cur	5	5	-	-	-	-	5	-	-	-
<i>Hp</i>	5	-	5	-	-	-	-	3	2	-
<i>Hp</i> + cur I	5	-	5	-	-	-	3	2	-	-
<i>Hp</i> + cur II	5	-	3	2	-	-	3	2	-	-

Level of *Helicobacter pylori* (*H. pylori*) colonization: Score 0 = no *H. pylori* detected; Score 1 = mild colonization in some gastric crypts; Score 2 = mild colonization in most gastric crypts; Score 3 = moderate colonization in some gastric crypts; Score 4 = dense colonization in some gastric crypts. Score of gastric inflammation: Score 0 = normal gastric tissue; Score 1 = mild inflammation and histopathology changes; Score 2 = moderate inflammation and histopathology changes; Score 3 = marked inflammation and histopathology changes. Control: Control rats; Cur: Control rats supplemented with 600 mg/kg curcumin; *Hp*: *H. pylori*-infected rats; *Hp* + cur I: *H. pylori*-infected rats supplemented with 200 mg/kg curcumin; *Hp* + cur II: *H. pylori*-infected rats supplemented with 600 mg/kg curcumin.

### Statistical analysis

All data were presented as mean  $\pm$  SD. The means were compared by one-way analysis of variance (one-way ANOVA) followed by LSD post hoc test. Correlation between the percentages of NF- $\kappa$ B p65 immunoreactive cells and macromolecular leakage was analyzed using Pearson's correlation. All the statistical tests were performed using the computer program SPSS, version 13.0, for Windows (SPSS Inc., Chicago, IL, USA). Probability value of less than 0.05 was considered to be statistically significant.

## RESULTS

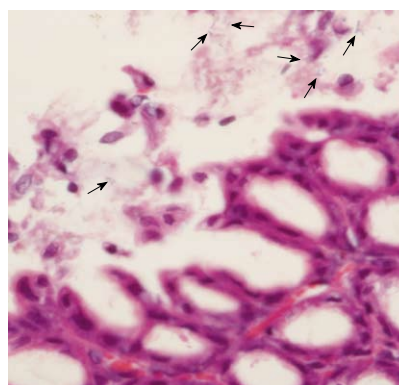
### *H. pylori* colonization and histological changes

*H. pylori* infection in rats was judged based on a urease test and histological examination. From histological examination, *H. pylori* colonization was observed in the *Hp* group (Figure 1) as well as in the *Hp* + cur I and *Hp* + cur II groups, whereas colonization was not observed in the Control and Cur groups. Figure 2 demonstrates histological differences between Control and *Hp* groups. The data are shown in Table 1.

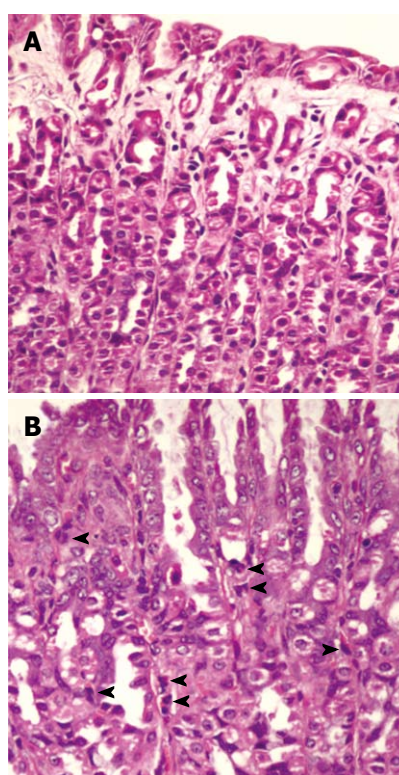
### Effects of *H. pylori* infection on NF- $\kappa$ B p65 expression and role of curcumin

NF- $\kappa$ B p65 expression in gastric epithelial cells was studied using immunohistochemistry (Figure 3). *H. pylori* infection increased NF- $\kappa$ B p65 expression in gastric epithelial cells. The percentage of immunoreactive cells significantly increased in the *Hp* group (16.02%  $\pm$  2.98%) compared with the Control group (10.72%  $\pm$  2.1%,  $P = 0.004$ ) (Figure 4A).

The expression of NF- $\kappa$ B p65 in gastric epithelial cells was diminished by curcumin supplementation in both *Hp* + cur I and *Hp* + cur II groups. The percentage of immunoreactive cells significantly decreased in *Hp* + cur I (11.79%  $\pm$  2.13%,  $P = 0.017$ ) and *Hp* + cur II



**Figure 1** Histological examination (HE stain,  $\times$  1000) of *Helicobacter pylori*-infected rats. *Helicobacter pylori* (arrows) in the gastric mucosa identified by the pathologist.



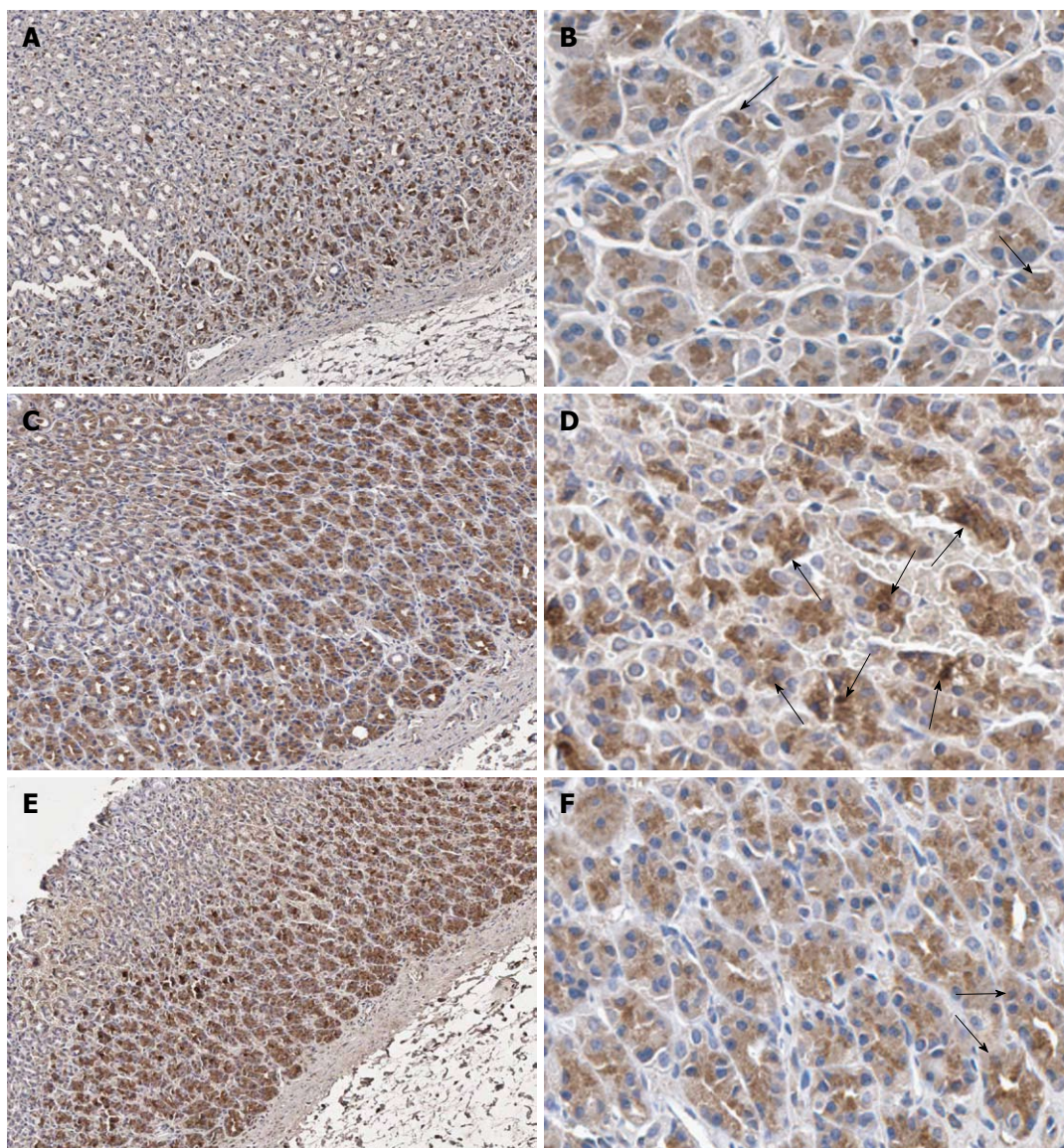
**Figure 2** Pathological changes of rat gastric mucosa in control (A) and *Helicobacter pylori*-infected rats (B) (HE stain,  $\times$  400). A: Normal gastric mucosa; B: *Helicobacter pylori*-infected gastric mucosa showing inflammatory cell infiltration in the lamina propria (arrowheads).

(11.42%  $\pm$  1.68%,  $P = 0.010$ ) compared with the *Hp* group (Figure 4A). However, there was no significant difference between the number of immunoreactive cells in *Hp* + cur I and *Hp* + cur II. Curcumin administration in the Cur group did not alter the baseline NF- $\kappa$ B p65 expression (Cur group, 9.47%  $\pm$  3.46%,  $P = 0.447$ ) in gastric epithelial cells.

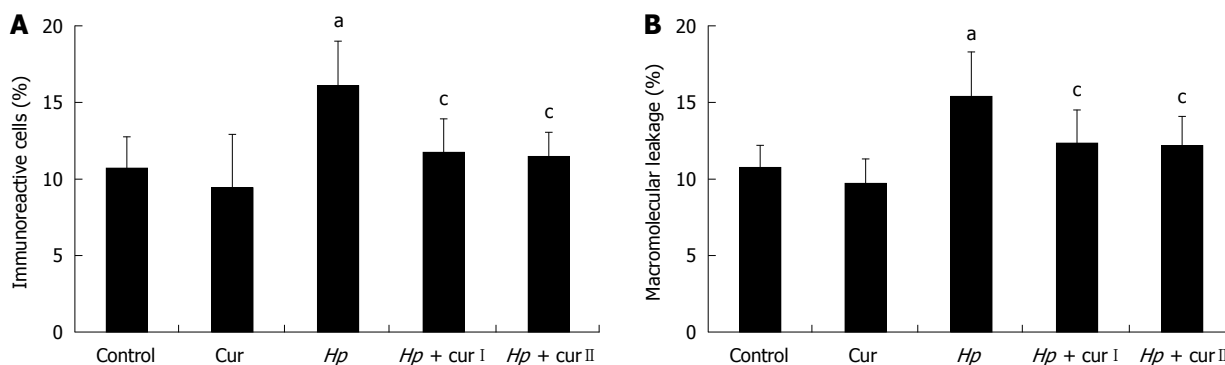
### Effects of *H. pylori* infection on macromolecular leakage and role of curcumin

The macromolecular leakage was studied by intravital fluorescent videomicroscopy. The captured images of





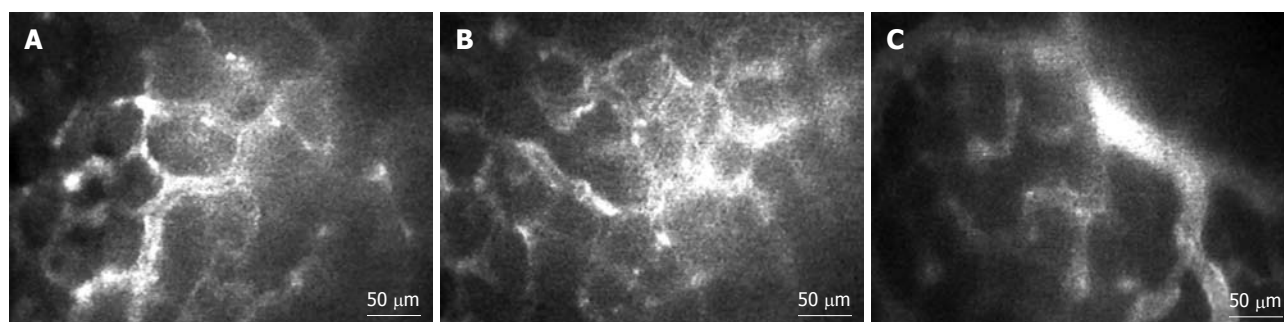
**Figure 3** Immunohistochemical staining of nuclear factor- $\kappa$ B p65 antibody in representative tissue specimens. A, B: Control rats; C, D: *Helicobacter pylori* (*H. pylori*)-infected rats; E, F: *H. pylori*-infected rats supplemented with 200 mg/kg curcumin. Nuclear counterstaining was performed with hematoxylin. The examples of immunoreactive cells are those with dark brown stain in their nuclei (arrows). Images were obtained at  $\times 100$  (A, C and E) and  $\times 400$  (B, D and F).



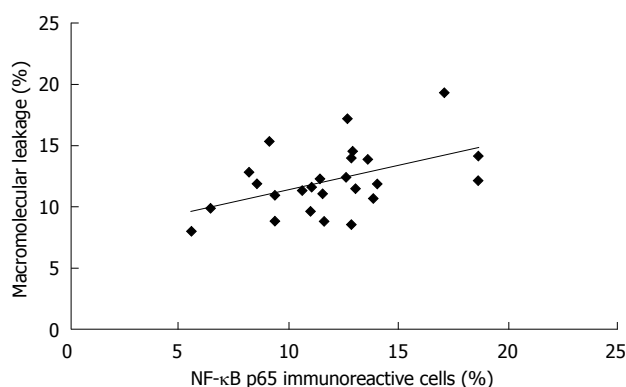
**Figure 4** mean  $\pm$  SD of the percentage of nuclear factor- $\kappa$ B p65 immunoreactive cells (A) and macromolecular leakage (B) in all experimental groups. <sup>a</sup> $P < 0.05$  vs control rats (Control); <sup>c</sup> $P < 0.05$  vs *Helicobacter pylori* (*H. pylori*)-infected rats (Hp). Cur: Control rats supplemented with 600 mg/kg curcumin; Hp + cur I: *H. pylori*-infected rats supplemented with 200 mg/kg curcumin; Hp + cur II: *H. pylori*-infected rats supplemented with 600 mg/kg curcumin.

gastric mucosal microcirculation from Control, Hp, and Hp + cur I groups at the 30-min time point are shown

in Figure 5. *H. pylori* infection led to a significant increase of macromolecular leakage in the Hp group (15.41%  $\pm$



**Figure 5** Intravital fluorescent microscopic images ( $\times 200$ ) demonstrate macromolecular leakage from vessels to the interstitial space at 30-min time points. A: Control rats; B: *Helicobacter pylori* (*H. pylori*)-infected rats; C: *H. pylori*-infected rats supplemented with 200 mg/kg curcumin.



**Figure 6** Correlation between the percentage of nuclear factor- $\kappa$ B p65 expression in gastric epithelial cells and the macromolecular leakage ( $r^2 = 0.2228$ ,  $P = 0.017$ ). NF- $\kappa$ B: Nuclear factor- $\kappa$ B.

2.83%) compared with the Control group ( $10.69\% \pm 1.43\%$ ,  $P = 0.001$ ) (Figure 4B).

Oral treatment with curcumin over 1 wk attenuated *H. pylori* infection-induced macromolecular leakage significantly in both *Hp* + cur I ( $12.32\% \pm 2.13\%$ ,  $P = 0.025$ ) and *Hp* + cur II ( $12.14\% \pm 1.86\%$ ,  $P = 0.018$ ) groups (Figure 4B). However, there was no significant difference between the extent of macromolecular leakage in *Hp* + cur I and *Hp* + cur II. In addition, macromolecular leakage showed no significant differences between the Control group and the Cur group (Cur group,  $9.74\% \pm 1.5\%$ ,  $P = 0.463$ ).

### Relationship between NF- $\kappa$ B p65 expression and macromolecular leakage

The percentages of NF- $\kappa$ B p65 immunoreactive cells and of macromolecular leakage from the same rat in all groups were plotted against each other (Figure 6). Interestingly, the level of NF- $\kappa$ B p65 expression was moderately correlated with the degree of macromolecular leakage ( $r^2 = 0.2228$ ,  $P = 0.017$ ).

## DISCUSSION

The present study demonstrated that *H. pylori* infection activated NF- $\kappa$ B in gastric epithelial cells. This result corresponds to previous observations studied in both *in vitro* and *in vivo* models<sup>[4,19,28]</sup>. NF- $\kappa$ B is an important transcription

factor which activates many genes involved in inflammatory and immune responses<sup>[5,6]</sup>. Activated NF- $\kappa$ B induced by *H. pylori* infection upregulates cytokine production and is associated with gastric inflammation<sup>[9]</sup>. Clinically, NF- $\kappa$ B was also seen to be activated in the stomach of patients with *H. pylori*-induced gastritis<sup>[29-31]</sup>. Therefore, gastric epithelial NF- $\kappa$ B activation may play an important role in the initiation of *H. pylori*-induced gastric inflammation.

From the results, this study can also demonstrate an increased macromolecular leakage after *H. pylori* infection. This alteration is in good agreement with previous reports<sup>[12,14]</sup>. Several mechanisms may contribute to the increased macromolecular leakage. A previous study demonstrated that the transmigration of activated neutrophils expressing a specific protein could regulate endothelial permeability, allowing macromolecules to leak<sup>[32]</sup>. Furthermore, many proinflammatory cytokines such as TNF- $\alpha$  and interleukin (IL)-1 $\beta$  could be directly modulating vascular permeability<sup>[33-35]</sup>. Neutrophil transmigration and proinflammatory cytokine production were also suggested in a NF- $\kappa$ B-dependent manner in gastric mucosa during *H. pylori* infection<sup>[4,8,36-38]</sup>. *H. pylori* infection may directly influence the leakage *via* the transportation of *H. pylori* toxin and activation of NF- $\kappa$ B in endothelial cells. Previously, *in vitro* studies indicated that endothelial cells infected with *H. pylori* have changes in protein expression and function<sup>[39,40]</sup>. Our results substantiate these findings by showing that increased NF- $\kappa$ B p65 expression in gastric epithelial cells is accompanied by increased macromolecular leakage during *H. pylori* infection. Thus, the increased macromolecular leakage may result from inflammatory mediator production and vascular permeability changes through *H. pylori*-induced NF- $\kappa$ B activation.

Our experiments show that curcumin supplementation can suppress *H. pylori*-induced gastric inflammation, as indicated by decreased NF- $\kappa$ B p65 expression in gastric epithelial cells and decreased macromolecular leakage in the gastric microcirculation. The activation of NF- $\kappa$ B is essential for transcription of many genes involved in inflammatory and immune responses influencing gastric inflammation induced by *H. pylori* infection. In the present study, NF- $\kappa$ B p65 expression in the nucleus indicated that curcumin may possibly suppress the translocation of activated NF- $\kappa$ B into transcriptional sites. *H. pylori*-



induced NF- $\kappa$ B activation affects recruitment of neutrophils and vascular permeability that reflect gastric inflammation. Curcumin decreased these parameters, indicating that curcumin could decrease gastric inflammation.

Inhibition of *H. pylori* growth was unlikely to be a mechanism that contributed to the effect of curcumin observed in this study, since positive results regarding *H. pylori* infection were still obtained from both urease test and histological examination after curcumin treatment in *Hp* + cur I and *Hp* + cur II animals. Recently, Di Mario *et al*<sup>[21]</sup> demonstrated that 7-d treatment with curcumin significantly improved gastric inflammation in *H. pylori*-positive patients despite *H. pylori* persistence. However, eradication by curcumin may be dependent on a high dose of curcumin, the safety of which has to be confirmed in animals and humans<sup>[41]</sup>.

A previous study demonstrated that curcumin at the doses of 200 mg/kg and 600 mg/kg had an anti-inflammatory property<sup>[42]</sup>. In this study, 200 mg/kg curcumin was a sufficient dose for reducing gastric epithelial NF- $\kappa$ B p65 expression and mucosal macromolecular leakage. The possible mechanism cited was that curcumin inhibited *H. pylori*-induced NF- $\kappa$ B activation. This finding corresponded to an earlier *in vitro* study showing that *H. pylori*-induced NF- $\kappa$ B activation and the subsequent release of IL-8 were inhibited by curcumin<sup>[19]</sup>. In addition, the correlation between NF- $\kappa$ B p65 expression and macromolecular leakage found in our study suggests that *H. pylori*-induced mucosal macromolecular leakage may be mediated *via* NF- $\kappa$ B activation in gastric epithelial cells. Thus, the decreased macromolecular leakage may be explained by the reduction of inflammatory mediators due to epithelial NF- $\kappa$ B inhibition by curcumin.

In conclusion, the present study showed that *H. pylori* infection induced gastric epithelial NF- $\kappa$ B activation and increased mucosal macromolecular leakage. Curcumin supplementation may exert its anti-inflammatory effect by reducing macromolecular leakage through the suppression of NF- $\kappa$ B p65 expression in gastric epithelial cells. Hence, curcumin might be a novel therapeutic strategy against gastric inflammation induced by *H. pylori* infection.

## COMMENTS

### Background

The pathogenesis of *Helicobacter pylori* (*H. pylori*) infection is associated with bacterial virulence factors. The virulence factors can induce the activation of nuclear factor (NF)- $\kappa$ B in gastric epithelial cells, causing gastric inflammation and inducing vascular damage. Curcumin has many biological activities, including anti-inflammatory properties resulting from inhibition of NF- $\kappa$ B.

### Research frontiers

Curcumin (diferuloylmethane) is an active ingredient of *Curcuma longa* (turmeric) that has many biological activities mediated by the efficient inhibition of NF- $\kappa$ B. *H. pylori* infection induces gastric epithelial NF- $\kappa$ B activation and increases mucosal macromolecular leakage. The hotspots of this study indicate that curcumin supplementation may exert its anti-inflammatory effect by reducing macromolecular leakage through the suppression of NF- $\kappa$ B p65 expression in gastric epithelial cells.

### Innovations and breakthroughs

A previous study showed that curcumin is a potent antibacterial agent against *H. pylori* and can inhibit NF- $\kappa$ B activation in *H. pylori*-infected gastric epithelial cells *in vitro*. However, it is not clear whether curcumin has any *in vivo* effects

on *H. pylori*-induced gastric inflammation. Therefore, in this study, the authors examined the anti-inflammatory effect of curcumin, which was shown to reduce mucosal macromolecular leakage through the suppression of gastric epithelial NF- $\kappa$ B p65 expression induced by *H. pylori* infection *in vivo* in rats.

### Applications

Curcumin might be a novel therapeutic strategy against gastric inflammation induced by *H. pylori* infection.

### Peer review

The merits of the manuscript are in showing the conclusion that curcumin can reduce the gastric inflammation reflected in attenuated levels of NF- $\kappa$ B and leakage of dextran.

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