Original Article

Effects of curcumin on *Helicobacter pylori* infection

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Background: Curcumin is a well-established natural molecule with significant biological and pharmaceutical effects. Its effects on *Helicobacter pylori* (*H. pylori*) infection have been repeatedly confirmed both in animal and human models. This study directly compared five different samples to evaluate if the effects are general or if they differ among samples.

Methods: Using a mouse model, we studied the effects of curcumin on lipid peroxide (LPO) level, myeloperoxidase (MPO) and urease activity, number of colonized bacteria, levels of anti-*H. pylori* antibodies, biofilm formation, IFN-γ, IL-4, gastrin and somatostatin levels in serum, and minimum inhibitory concentration. In addition, we evaluated the effects on biofilm production and antibacterial antibody response.

Results: In all tests, one sample (Sabinsa) was consistently the most active.

Conclusions: All curcumin samples showed some anti-*H. pylori* effects, but only some of the tested samples had significant activity.

Keywords: Biofilm; curcumin; *Helicobacter pylori* (*H. pylori*)

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Introduction

Helicobacter pylori (*H. pylori*) is a Gram-negative bacterium that selectively colonizes the human gastric epithelium and is epidemiologically linked to stomach and colorectal cancer (1). In addition, it is implicated in the etiology of gastritis and peptic ulcers. Antibody therapy coupled with other treatments is highly effective, but not without complications (2). Over 50% of the world population is infected with these bacteria. The resistance of these bacteria to common antibiotics has been related to the genetic variability and to its ability to develop biofilm (3). In addition, *Helicobacter* infection has been connected with development of allergies (4).

Lately, the focus of numerous investigations has switched to various herbal agents shown to have significant antibacterial activity against *H. pylori* (5). One of these agents is curcumin (6,7). In addition, curcumin also serves as a biofilm-disrupting agent (8), suggesting multiple roles of curcumin in inhibition of *H. pylori* infection.

Curcumin, commonly known as turmeric, is usually a mixture of three curcumoids (curcumin, demethocycurcumin, and bisdemethoxycurcumin) and volatile oil (9). Numerous studies have reported that curcumin has a wide range of biological activities including antimicrobial, antioxidant, antitumor (10), and anti-inflammatory effects. In addition, curcumin has some immunosuppressive activities (11) including expression of cytokines such as IL-1 and TNF-α (12,13). On the other hand, curcumin enhanced phagocytic activity of macrophages (14). In our study, we compared the antibacterial effects of five different types of curcumin.

Methods

Animals

Female, 8-week-old BALB/c mice were purchased from

Jackson Laboratory (Bar Harbor, ME, USA). All animal work was done according to the University of Louisville IACUC protocol. Animals were sacrificed by CO_2 asphyxiation.

Samples

Curcumin C3 complex 95% (sample #1) was purchased from Sabinsa (Sabinsa Corp., East Windsor, NJ, USA), curcumin powder 65% (sample #2) and curcumin 94% (sample #3) from Sigma (St. Louis, MO, USA), curcumin 95 (95%, sample #4) from Jarrow Formulas (Los Angeles, CA, USA), and curcumin 95% (sample #5) from Orcas Naturals (Landing, NJ, USA).

Bacteria

H. pylori strain ATCC43504 was purchased from ATCC (American Type Culture Collection, Manassas, VA, USA) and cultured on brain-heart infusion (BHI) agar (Sigma) supplemented with 7% sheep blood and incubated at 37 ℃ under microaerobic conditions.

Lipid peroxide (LPO) level and myeloperoxidase (MPO) activity

Gastric mucosal tissues were scrapped and homogenized in 10 mmol/L Tris buffer (pH 7.4). LPO levels were measured as described by Ohkawa *et al*. (15). MPO activity was determined by the modified method of Krawisz *et al*. (16).

Urease activity

Urease activity in the homogenized gastric tissue was performed as described by O'Riordan *et al*. (17).

Enumeration of colonized bacteria

Stomach samples were homogenized in phosphate buffer saline (PBS), cultured on the brucella agar plates incubated under microaerobic conditions. Five days after cultivation, colony counts were performed (18).

Anti-H. pylori antibodies

Serum anti-*H. pylori* IgG were measured using an enzyme-linked immunosorbent assay (ELISA). Isolates of *H. pylori* were used as an antigen at 25 μg protein/well. After incubation and washing, 100 mg of serum was added. Reaction was followed by incubation with horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG (Sigma, St. Louis, MO, USA). Optical density was measured using a STL ELISA reader (Tecan U.S., Research Triangle Park, NC) at 405 nm.

Biofilm formation

Bacteria were grown in glass tubes. BHI broths supplemented with 2% β-cyclodextrin (BCD) and 0.016% dimethyl sulfoxide (DMSO) were incorporated as blank and control, respectively. After 7 days of incubation, all culture medium was removed. The test tubes were washed twice with PBS, dried for 30 min at 60 ℃ and 10 mL of 0.1% crystal violet (Sigma) was added for 5 minutes. Unbound stain was discarded and the tubes were again dried for 30 min at 60 ℃. Bound crystal violet was decolorized with ethanol/acetone mixture (80:20, v/v). The level of biofilm formation was quantified by measuring the absorbance of the solution at 570 nm using a spectrophotometer (19).

ELISA

Serum levels of IFN-γ, IL-4, gastrin, and somatostatin were determined using an ELISA assay as described by Zhang *et al*. (20). Anti-IFN-γ and IL-4 Quantikine ELISA kits were purchased from B&D Systems (Minneapolis, MN, USA), anti-somatostatin ELISA kit was purchased from LSBio (Seattle, WA, USA), and anti-gastrin ELISA kit from Sigma. All kits were used according to manufacturer's instruction.

Minimum inhibitory concentration test

Technique using Mueller-Hinton agar (Oxoid, UK) described by Pattiyathanee *et al*. (19) was used.

Statistics

Student *t*-test was used to statistically analyze the data.

Results

Evaluation of the changes in IL-4 and IFN-γ levels showed that all samples significantly increased IL-4 serum levels (*Figure 1*), with the highest effects with samples #1 and #3 and all samples, with the exception of #4, significantly decreased IFN-γ levels (*Figure 2*). Somatostatin levels

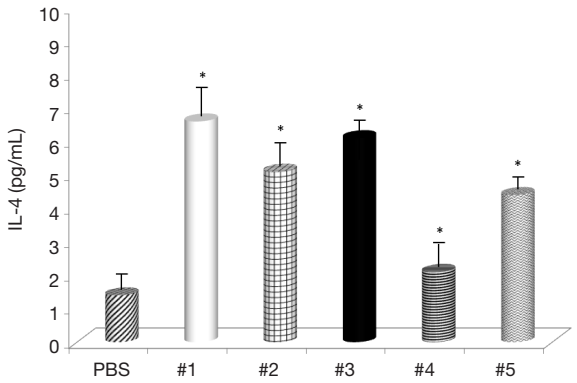

Figure 1 Effects of curcumin on serum levels of IL-4. Results represent mean from three experiments ± SD. *, represents significant differences between the control and curcumin-treated mice at P≤0.05 level.

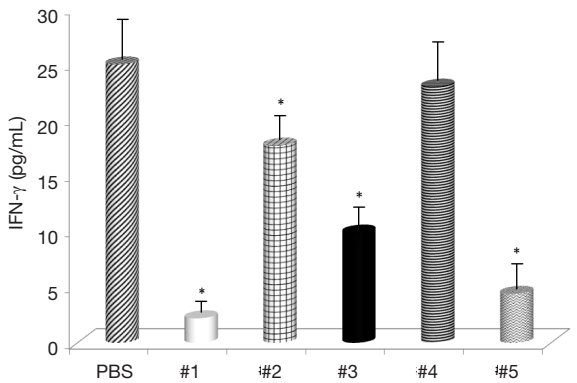

Figure 2 Effects of curcumin on serum levels of IFN-γ. Results represent mean from three experiments ± SD. *, represents significant differences between the control and curcumin-treated mice at P≤0.05 level.

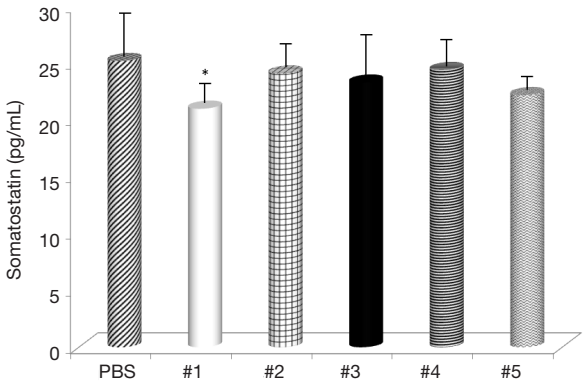

Figure 3 Effects of curcumin on serum levels of somatostatin. Results represent mean from three experiments ± SD. *, represents significant differences between the control and curcumin-treated mice at P≤0.05 level.

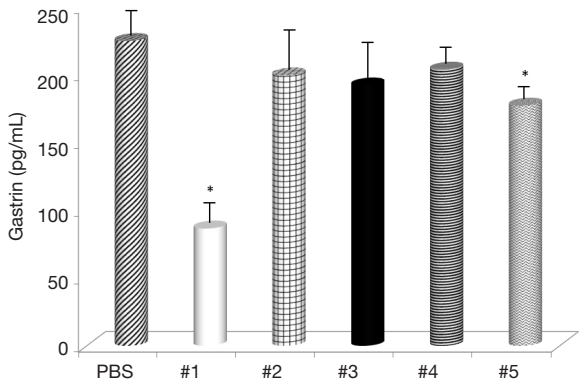

Figure 4 Effects of curcumin on serum levels of gastrin. Results represent mean from three experiments ± SD. *, represents significant differences between the control and curcumin-treated mice at P≤0.05 level.

significantly lowered only sample #1 (*Figure 3*); and gastrin levels showed significant effects only with samples #1 and #5 (*Figure 4*).

Gastric levels of LPO were significantly decreased by samples #1, #3, and #5 (*Figure 5*) and all samples, except sample #4, had decreased levels of MPO (*Figure 6*). The negative control mice levels (without infection) were 0.91 nmol/mg protein with LPO, and 0.49 units/mg protein with MPO activity.

The next part of the study was focused on bacterial enumeration and presence of urease. Urease was detected in all tested samples, but samples #1 and #3 significantly lowered the *H. pylori* counts (*Table 1*). We then studied the effects of tested samples on *H. pylori* formation. As summarized in *Table 2*, in higher concentration, we found strong effects of samples #1, #2, and #5 and in lower concentration, only sample #1 showed small effects.

The final part of this study measured direct effects of curcumin supplementation of levels of anti-*H. pylori* IgG. Results given in *Figure 7* show that all samples increased production of specific antibodies, with samples #1, #3 and #5 showing the strongest effects.

Conclusions

H. pylori is a highly mobile Gram-negative bacterium,

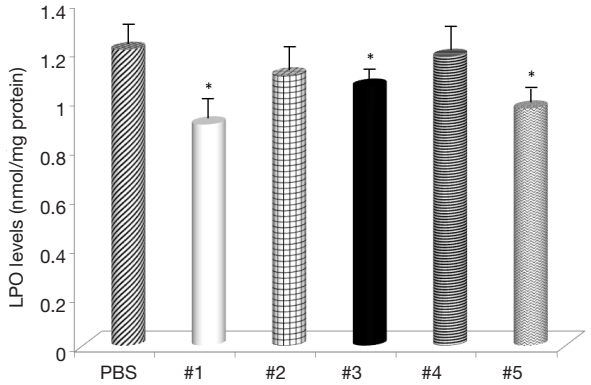

Figure 5 Effects of curcumin on LPO abundance in gastric mucosal tissue. Results represent mean from three experiments ± SD. *, represents significant differences between the control and curcumin-treated mice at P≤0.05 level. LPO, lipid peroxide.

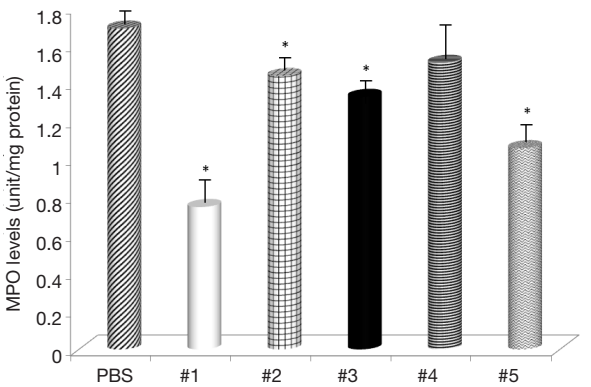

Figure 6 Effects of curcumin on MPO activity in gastric mucosal tissue. Results represent mean from three experiments ± SD. *, represents significant differences between the control and curcumin-treated mice at P≤0.05 level. MPO, myeloperoxidase.

Table 1 Bacterial enumeration and presence of urease

Group	CFU/g	Urease
Negative control	0	–
Positive control	58.9±7.2	+
#1	22.1±3.3*	+
#2	50.5±5.5	+
#3	41.7±4.9*	+
#4	50.6±6.7	+
#5	45.1±7.2	+

*, significant differences between sample and PBS at <0.05 level. Bacteria were counted 2 weeks after infection. Data are given as CFU/g ($\times 10^3$). –, no activity; +, activity.

Table 2 Effects of curcumin on *H. pylori* biofilm formation

Group	Concentration of samples		
	0	¼ MIC	½ MIC
Positive control	+++	N/A	N/A
#1	+++	++	–
#2	+++	+++	+
#3	+++	+++	+++
#4	+++	+++	+++
#5	+++	+++	++

Levels of individual biofilm observations are represented as: –, absent; +, just visible; ++, intermediate; and +++, extensive. Experiments were performed in triplicates. *H. pylori*, *Helicobacter pylori*; N/A, not available.

selectively colonizing in the human stomach. This colonization is etiologically associated with gastritis and peptic ulcers. In addition, an increased risk of gastric adenocarcinoma has been well established (21). With over 50% of the population (higher in developing countries) infected with *H. pylori*, various drugs have been routinely used for eradication of this infection. However, steadily increasing resistance to antibiotics, undesirable side effects, and raising costs have given rise to the recent surge of interest in alternative approaches (22,23).

Curcumin, the principle yellow pigment from the rhizome of turmeric (*Curcuma longa*), is known for a wide range of biological and pharmaceutical effects, most of all as an anti-inflammatory agent (24). Not surprisingly, curcumin has been evaluated as a potential anti-*H. pylori* agent. Curcumin supplementation was found to significantly downregulate MMP3 and MMP9 activities (25). A mouse study showed that orally-given curcumin caused significant inhibition of gastric inflammation induced by *H. pylori* infection (26).

With significant effects of curcumin on *H. pylori* infection, the aim of this study was to directly compare the antibacterial effects of five different types of curcumin to see if these effects are dependent on the individual type of curcumin. For our study we used samples with already well-established anti-inflammatory effects (27).

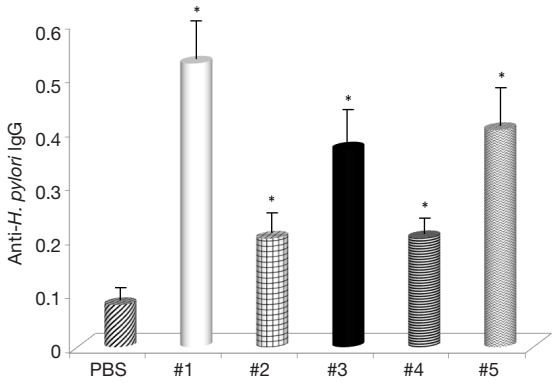

Figure 7 Effects of curcumin on anti-*H. pylori* IgG formation. Results represent mean from three experiments ± SD. *, represents significant differences between the control and curcumin-treated mice at P≤0.05 level. *H. pylori*, *Helicobacter pylori*.

To better explore the mechanisms of curcumin effects on this infection, IFN-γ, IL-4, and somatostatin represent good molecules for this purpose, as it is known that IFN-γ levels are elevated by *H. pylori* infection (28). IL-4 is an anti-inflammatory cytokine, unknown to be depressed by *H. pylori* (29) and somatostatin is a regulatory peptide needed for IL-4 mediated resolution of *H. pylori*-related gastritis (30). Gastrin is secreted by G cells from the pyloric antrum and is involved in the stimulation of gastric acid formation and release. As its levels are depressed as a result of *H. pylori* infection, we can speculate that the control of gastrin levels might result in modulation of gastritis. Significant effects of curcumin on all four molecules strongly support the hypothesis that curcumin can reduce effects of *H. pylori* infection.

The levels of LPO, an oxidative damage index, were decreased by supplementation with curcumin and in some cases almost reached the levels found in non-infected animals. These results suggested that curcumin treatment inhibited the *H. pylori*-induced increase in LPO abundance in the gastric mucosa and that curcumin does have antioxidant effects. LPO is considered to be a marker of oxidative membrane damage (31). Review of MPO activity indicated that curcumin addition attenuated neutrophil infiltration in the gastric mucosa, similar to the study using *Angelica keiskei* (32).

The next phase of our study focused directly on bacteria and their action. Enumeration of bacterial cells in the infected animal stomach showed that curcumin supplementation reduced the total amount of *H. pylori* bacteria. Urease-positive bacteria form thick biofilms in the stomach of the host (33). Curcumin was able to inhibit the growth of *H. pylori* at a MIC value of 18 μg/mL, which corresponded with the findings of others (19). When used at sub-inhibitory levels (½ and ¼ MIC), the effects on biofilm were observed.

Next, then analyzed the effects of curcumin supplementation on formation of anti-*H. pylori* IgG antibodies. The results showed that the same samples which reduced the number of bacteria also increased the formation of specific antibodies, supporting the hypothesis that curcumin has strong immunostimulating properties.

In summary, we directly compared the effects of five various samples of curcumin on *H. pylori*-induced gastritis. Similar to a previous study on inflammatory effects of curcumin (Vetvicka and Vetvickova, 2016), sample #1 (Sabinsa) was the most active of all samples in all of our tests, followed by samples #5 (Orcas Natural) and #3 (highly purified curcumin from Sigma). The two remaining samples were significantly less active, showing that despite the clear activity of curcumin in general, not every commercial sample of curcumin is highly active.

Acknowledgements

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: All animal work was done according to the University of Louisville IACUC protocol.

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