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ORIGINAL ARTICLE

Basic Study

Probiotic BIFICO cocktail ameliorates *Helicobacter pylori* induced gastritis

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Abstract

AIM: To determine the protective effect of triple viable probiotics on gastritis induced by *Helicobacter pylori* (*H. pylori*) and elucidate the possible mechanisms of protection.

METHODS: Colonization of BIFICO strains in the mouse stomach was determined by counting colony-forming units per gram of stomach tissue. After treatment with or without BIFICO, inflammation and *H. pylori* colonization in the mouse stomach were analyzed by hematoxylin and eosin and Giemsa staining, respectively. Cytokine levels were determined by enzyme-linked immunosorbent assay and Milliplex. The activation of nuclear factor (NF)-κB and MAPK signaling in human gastric epithelial cells was evaluated by Western blot analysis. Quantitative reverse transcription-polymerase chain reaction was used to quantify TLR2, TLR4 and MyD88 mRNA expression in the mouse stomach.

RESULTS: We demonstrated that BIFICO, which contains a mixture of *Enterococcus faecalis*, *Bifidobacterium longum* and *Lactobacillus acidophilus*, was tolerant to the mouse stomach environment and was able to survive both the 8-h and 3-d courses of administration. Although BIFICO treatment had no effect on the colonization of *H. pylori* in the mouse stomach, it ameliorated *H. pylori*-induced gastritis by significantly inhibiting the expression of cytokines and chemokines such as TNF-α, IL-1β, IL-10, IL-6, G-CSF and MIP-2 ($P < 0.05$). These results led us to hypothesize that BIFICO treatment would diminish the *H. pylori*-induced inflammatory response in gastric mucosal epithelial cells *in vitro via* the NF-κB and MAPK signaling pathways. Indeed, we observed a decrease in the expression of the NF-κB subunit p65 and in the phosphorylation of IκB-α, ERK and p38. Moreover, there was a significant decrease in the production of IL-8, TNF-α, G-CSF and GM-CSF ($P < 0.05$), and the increased expression of TLR2, TLR4 and MyD88 induced by *H. pylori* in the stomach was also significantly reduced following BIFICO treatment ($P < 0.05$).

CONCLUSION: Our results suggest that the probiotic cocktail BIFICO can ameliorate *H. pylori*-induced gastritis by inhibiting the inflammatory response in gastric epithelial cells.

Key words: BIFICO; Gastritis; *Helicobacter pylori*; Nuclear factor-κB; Inflammation; Toll-like receptors; *Bifidobacterium longum*; MAPK

Core tip: We investigated the effects of a traditional probiotic pharmaceutical cocktail in China, composed of the viable bacteria *Enterococcus faecalis*, *Bifidobacterium longum* and *Lactobacillus acidophilus*, on *Helicobacter pylori* (*H. pylori*)-induced gastritis in experimental mice and found that it could ameliorate *H. pylori*-induced gastritis by inhibiting the epithelial cell inflammatory response.

Yu HJ, Liu W, Chang Z, Shen H, He LJ, Wang SS, Liu L, Jiang YY, Xu GT, An MM, Zhang JD. Probiotic BIFICO cocktail ameliorates *Helicobacter pylori* induced gastritis. *World J Gastroenterol* 2015; 21(21): 6561-6571 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i21/6561.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i21.6561

INTRODUCTION

Helicobacter pylori (*H. pylori*) is a common human pathogen that colonizes approximately 50% of the world's population[1,2]. This bacterium can persist for decades in its preferred niche, the gastric mucosa, despite triggering vigorous host innate and adaptive immune responses[3]. *H. pylori* infection causes chronic gastritis, which is asymptomatic in the majority of carriers but is considered to be a major risk factor for the development of gastric and duodenal ulcers and gastric malignancies[2]. Between 10% and 15% of individuals suffering from *H. pylori*-induced gastritis develop peptic ulcer disease (PUD), and approximately 1% progress to gastric cancer (GC)[4]. Although successful eradication of *H. pylori* cures the majority of those diagnosed with gastritis and PUD, the prevalence of strains resistant to currently available antimicrobial agents has increased dramatically in recent years. Therefore, alternative treatment approaches, including novel methods to eradicate *H. pylori* or to reduce *H. pylori*-induced inflammation in the stomach, need to be investigated.

The gastric mucosa is the first barrier of defense against *H. pylori* infection. Direct interaction of *H. pylori* with gastric epithelial cells stimulates pattern recognition receptors (PRRs), such as toll-like receptors (TLRs) and downstream signaling pathways. The inflammatory cytokines released upon PRR activation recruit the innate immune cells residing in the gastric lamina propria under steady state conditions[3,5,6]. As a result, *H. pylori* can induce significant inflammation of the gastric mucosa.

The stomach is not a sterile organ and is estimated to support a community of up to 200 bacterial species[7]. However, when present, *H. pylori* is usually numerically dominant and readily visible in gastric biopsy tissue sections as helical rod-shaped organisms covering the gastric epithelium and surrounded in mucus. In a conducive environment, the stomach bacterial community forms hierarchies in which only a selected group of bacteria occupy the mucosal layer and epithelium, and non-selected bacteria are expelled from the mucosal surface. Competition within the bacterial community plays a pivotal role in the prevention of pathogenic bacterial invasion. Therefore, it is reasonable to hypothesize that supplementation with probiotic bacterial strains could inhibit the colonization of *H. pylori* and the resulting gastritis by preventing *H. pylori* access to the mucosal surface. Indeed, several probiotics including *Lactobacillus* spp., *Saccharomyces* spp., *Bifidobacterium* spp., and *Bifidobacterium clausii* have been studied for their impact on *H. pylori* infection[8-13].

BIFICO capsules, which contain a mixture of the viable bacteria *Enterococcus faecalis* (EF), *Bifidobacterium longum* (BL), and *Lactobacillus acidophilus* (L), were approved as an over-the-counter (OTC) drug product in October 2002 by the current Chinese regulatory authority, the State Food and Drug Administration (SFDA)[14]. This product is indicated for the treatment of disorders caused by an imbalance of normal intestinal flora. In this study, we investigated the effect of BIFICO capsules on an *H. pylori* SS1-infected mouse model and demonstrated that BIFICO treatment ameliorates *H. pylori*-induced gastritis by

inhibiting TLR activation.

MATERIALS AND METHODS

Animal care and use statement

The animal protocol was designed to minimize pain or discomfort to the animals. The animals were acclimatized to laboratory conditions (23 ℃, 12 h/12 h light/dark, 50% humidity, *ad libitum* access to food and water) for two weeks prior to experimentation. Intragastric gavage administration was carried out with conscious animals, using straight gavage needles appropriate for the animal size (15-17 g body weight: 22 gauge, 1 inch length, 1.25 mm ball diameter). All animals were euthanized by barbiturate overdose (intravenous injection, 150 mg/kg pentobarbital sodium) for tissue collection.

Reagents

Antibodies against phospho-ERK (9101) and phospho-IκB-α (9246) were purchased from Cell Signaling Technology. Antibodies against p65 (sc8008), PCNA (proliferating cell nuclear antigen) (sc56), ERK (sc-154) and IκB-α (sc-371) were obtained from Santa Cruz Biotechnology.

Mice

C57BL/6 female mice (6-8-wk-old) were obtained from Shanghai Slac Laboratory Animal Co. LTD (China). All procedures in this study were carried out in compliance with National Institutes of Health guidelines for the Care and Use of Laboratory Animals, and the protocol was approved by the Animal Ethics Committee of Tongji University (Permit Number: TJmed-012-65). The mice were anesthetized with intraperitoneal pentobarbital sodium and sacrificed by cervical dislocation.

Bacterial and cell growth

The *H. pylori* reference strain SS1 was used in this study. The bacteria were grown in a microaerobic humidified atmosphere at 37 ℃ on 10% lysed sheep blood Columbia agar. After 48-72 h, bacteria were harvested in PBS (pH 7.4) and re-suspended to a concentration of 6 × 10^8 colony-forming units (CFU)/mL using the McFarland standard kit. GES-1 cells were cultured in DMEM supplemented with 10% FBS (Hyclone) and 100 μg/mL penicillin/streptomycin.

Animal infections

Female C57BL/6N mice were housed at the animal facility of Tongji University under standard conditions in sterile cages. Mice were given drinking water containing 0.6 g/L of penicillin/streptomycin for 8 d prior to the experiments. The mice were then inoculated intragastrically with 10^9 CFU *H. pylori* every 48 h for a total of 6 times. Two weeks after infection, the mice were administered BIFICO (*Lactobacillus acidophilus*, 10^7 CFU; *Bifidobacterium longum*, 10^7 CFU; *Enterococcus faecalis*, 10^7 CFU) once a day for 7 d. Animals were sacrificed at 3, 4, and 5 wk post-infection. Age-matched uninfected mice were included as controls in all experiments.

Inactivation of H. pylori for host cell interaction experiments

The equivalent of 2.5 × 10^7 cells from a culture were washed and resuspended in 1 mL of PBS in a six-well plate. *H. pylori* was exposed to four doses of 100000 μjoules/cm^2 in a CL-1000 UV crosslinker (UVP, Upland, California, United States) with agitation between each dose to treat cells evenly.

Epithelial cell-H. pylori interaction

The equivalent of 2.5 × 10^7 cells from a culture were washed extensively to remove any traces of protein or polysaccharide. UV-inactivated *H. pylori* was exposed to confluent human gastric epithelial cells (GES-1) with or without BIFICO coculture at an MOI of 5. The cells were lysed after 15, 30, 45 and 60 min of stimulation to assay for inflammatory signal activation. After 24 h of stimulation, the cell supernatants were collected for cytokine assays.

Western blot

Epithelial cells were serum-starved overnight, stimulated, and lysed in lysis buffer containing 150 mmol/L NaCl, 50 mmol/L HEPES pH 7.4, 1 mmol/L EDTA, 1% Nonidet P-40, and protease inhibitors to obtain cell lysate extracts or buffer containing 10 mmol/L HEPES pH 7.9, 1.5 mmol/L $MgCl_2$, 10 mmol/L KCl, 0.5 mmol/L DTT, and 0.05% NP40 to obtain nuclear extracts. The nuclear extracts were prepared in extraction buffer (5 mmol/L HEPES, 1.5 mmol/L $MgCl_2$, 0.2 mmol/L EDTA, 0.5 mmol/L DTT, 6% glycerol, pH 7.9). Cell lysate extracts and nuclear extracts were subjected to SDS-PAGE and then analyzed using the indicated antibodies.

Cytokine measurements

The levels of TNF-α, IL-8, GM-CSF, and G-CSF in supernatants of *H. pylori*-stimulated GES-1 cells were measured with Ready-SET-GO enzyme-linked immunosorbent assay (ELISA) kits (eBioscience). All samples were measured in triplicate according to the manufacturer's protocol.

Determining cytokine expression

Homogenized stomach tissue extracts were analyzed simultaneously for cytokines and chemokines using the Milliplex MAP mouse cytokine and chemokine magnetic bead panel (Millipore) according to the manufacturer's instructions. Standard curves were generated for each cytokine and chemokine with the standards included in each kit. The median fluorescence intensity for each analysis was calculated with a four- or five-point

logistic parameter curve. Cytokine and chemokine measurements below the detection level of the assay were calculated using a default value of 0 pg/mL for the particular analysis.

Reverse transcription-polymerase chain reaction analysis

Total RNA was extracted from stomach tissue with 1 mL of TRIzol reagent according to the manufacturer's instructions (Invitrogen). Next, 1 μg total RNA was reverse-transcribed using the ReverTra Ace qPCR RT Kit (FSQ-101) according to the manufacturer's instructions (Toyobo). A LightCycler (LC480, Roche) and a SYBR reverse transcription-polymerase chain reaction (RT-PCR) kit (QPK-212, Toyobo) were used for quantitative real-time RT-PCR analysis. Expression levels were normalized to those obtained for the control gene GAPDH (glyceraldehyde phosphate dehydrogenase).

Statistical analysis

The statistical methods of this study were reviewed by Zi-Sheng Ai from Tongji University School of Medicine. At least two biological replicates were performed for all experiments unless otherwise indicated. Differences between groups were analyzed by Student's *t*-test. *P* values less than 0.05 were considered statistically significant.

RESULTS

Colonization of BIFICO strains in the mouse stomach

To determine whether BIFICO strains are able to colonize the mouse stomach, bacterial colonization analysis was carried out. Mice were infected intragastrically with 1×10^7 bacteria per mouse. Colonization was determined by counting CFU per gram (CFU/g) of stomach tissue. Two independent colonization experiments were performed; the first was an 8-h time point after a single-dose administration, and the second was a time course colonization experiment with CFU counts on days 6, 7, and 8 after consecutive administration of strains for 5 d. Data shown in Figure 1 suggest that BIFICO strains are tolerant to the stomach environment and are able to survive both the 8-h and 3-d courses of administration.

BIFICO ameliorates H. pylori-induced gastritis in mice

Gastric mucosa samples from control and BIFICO-treated mice were analyzed. For hematoxylin and eosin (HE) staining, inflammatory cell evasion was detected throughout the observation period. However, a significant decrease in mouse gastric mucosal inflammation was observed in the BIFICO-treated group at all time points (Figure 2A).

Analysis of cytokines and chemokines relevant to gastritis showed that BIFICO treatment significantly inhibited the *H. pylori* infection-induced up-regulation and secretion of TNF-α, IL-1β, IL-10, IL-6, G-CSF and MIP-2.

BIFICO treatment does not suppress the colonization of H. pylori in the stomach

Because competition within the bacterial community plays a pivotal role in the prevention of pathogenic bacterial invasion, it is necessary to determine whether supplementation with BIFICO strains reduces the colonization of *H. pylori* in the mouse stomach. *H. pylori* colonization was detected in gastric samples by histopathologic evaluation with Giemsa staining at 3, 4 and 5 wk after the first administration. The difference in *H. pylori* populations between the gastric samples of mice in the BIFICO pretreatment group and the control group was not significant at the indicated times (Figure 3). These results suggest that BIFICO does not suppress the colonization of *H. pylori* in the mouse stomach.

H. pylori-induced NF-κB and MAPK signaling is suppressed by BIFICO

The NF-κB and MAPK pathways play a central role in *H. pylori*-induced gastritis. To investigate the mechanism underlying the inhibitory effect of BIFICO, we used inactivated *H. pylori* to activate the NF-κB and MAPK pathways. Western blot analysis showed that *H. pylori* infection stimulates the NF-κB and MAPK inflammatory cascade in GES-1 cells, resulting in a significant increase in p65 and phosphorylation of IκB-α, ERK and p38 (Figure 4A and B). All of these inflammatory responses to *H. pylori* were inhibited by the BIFICO strains (Figure 4A and B).

Similar results were observed in the inflammatory cytokine analysis. ELISAs were performed to measure IL-8, TNF-α, G-CSF and GM-CSF expression in the supernatants of GES-1 cells treated with or without BIFICO. Co-culture with BIFICO significantly inhibited the up-regulation of each of these cytokines and chemokines (Figure 4C). Taken together, these results suggest that BIFICO diminishes the *in vitro* inflammatory response stimulated by *H. pylori via* the NF-κB and MAPK signaling pathways.

H. pylori-induced increases in TLR2, TLR4 and MyD88 expression in the mouse stomach are suppressed by BIFICO

Because both commensal and pathogenic bacteria are recognized by a number of PRRs, we next investigated the PRRs involved in BIFICO-mediated suppression of inflammation. Quantitative RT-PCR analyses were carried out to measure the mRNA levels of TLR2 (Figure 5A), TLR4 (Figure 5B) and MyD88 (Figure 5C). The *H. pylori*-induced increases in the mRNA levels of TLR2 (Figure 5A), TLR4 (Figure 5B) and MyD88 were inhibited by BIFICO, suggesting that the TLR/MyD88 pathway is involved in the BIFICO-mediated down-regulation of the *H. pylori*-induced immune response in gastric mucosal epithelial cells.

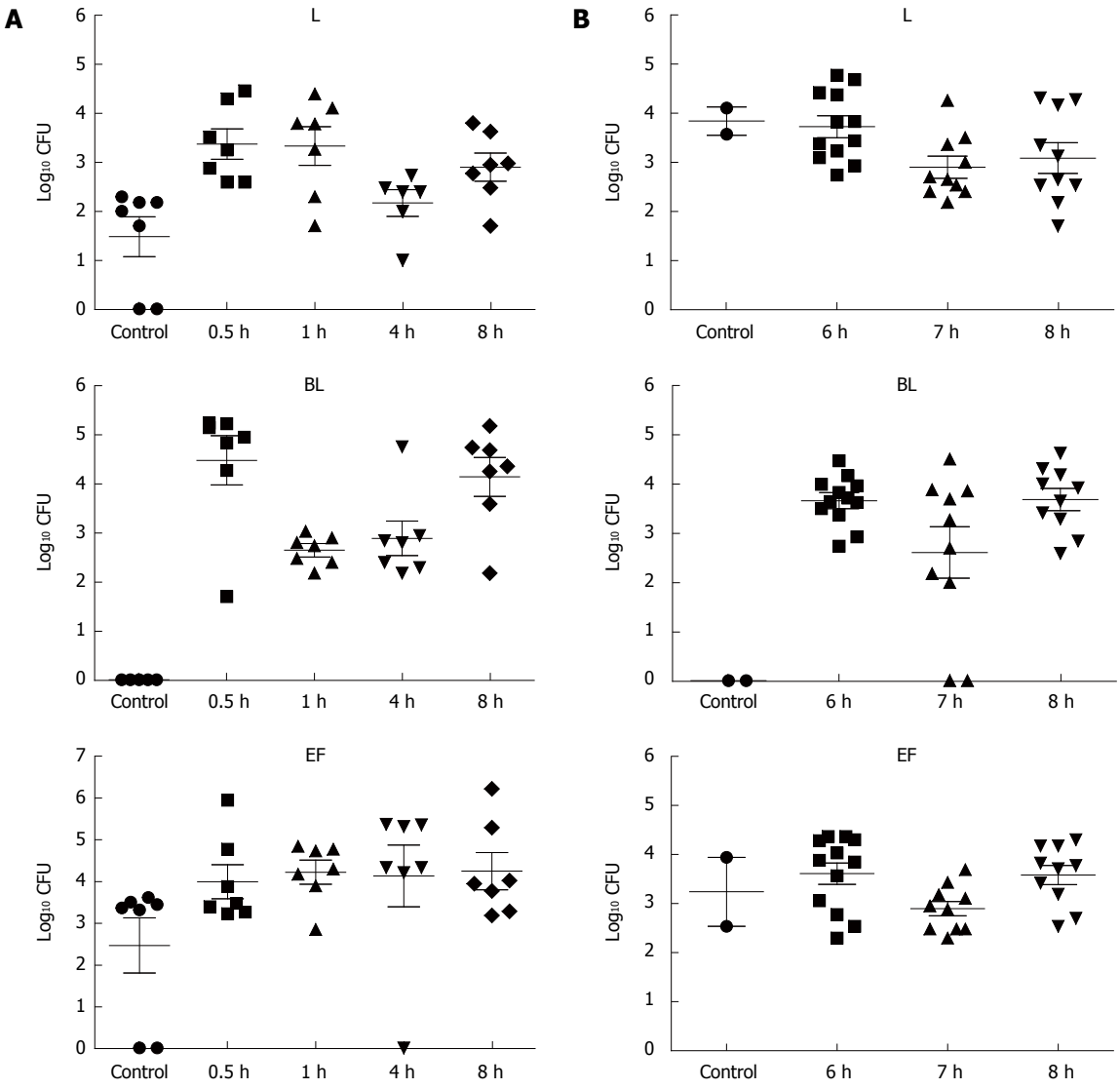

Figure 1 Colonization of BIFICO strain in mouse stomach. A: Stomach loads of *Lactobacilli acidophilus* (L), *Bifidobacteria longum* (BL) and *Enterococci faecali* (EF) at the indicated times after mice were administrated with a single dose of BIFICO (L, 107 CFU; BL, 107 CFU; EF, 107 CFU); B: Stomach loads of L, BL and EF at the indicated times after mice were administrated with BIFICO (L, 107 CFU; BL, 107 CFU; EF, 107 CFU) once a day for 5 d.

DISCUSSION

In this study, we demonstrated that the probiotic BIFICO cocktail could ameliorate *H. pylori*-induced gastritis. Specifically, colonization with probiotic strains of BIFICO attenuated histopathological changes and gastritis in *H. pylori* SS1-infected mice. BIFICO treatment resulted in reduced levels of cytokines and chemokines relevant to gastritis, such as TNF-α, IL-1β, IL-10, IL-6, G-CSF and MIP-2. However, BIFICO did not suppress colonization of *H. pylori* in the stomach. BIFICO suppressed the inflammatory response stimulated by *H. pylori in vitro via* the NF-κB and MAPK signaling pathways. In addition, our results show that the TLR/MyD88 pathway is involved in BIFICO-mediated down-regulation of the *H. pylori*-induced immune response in gastric mucosal epithelial cells.

BIFICO capsules, which are composed of viable *Enterococcus faecalis*, *Bifidobacterium longum* and *Lactobacillus acidophilus*, were approved as a prescription drug by the Ministry of Health of China in April 1995 and were then approved as an OTC drug product in October 2002 by the current Chinese regulatory authority, the SFDA. This product is indicated for the treatment of acute and chronic diarrhea caused by an imbalance of normal intestinal flora, mild to moderate diarrhea, indigestion and abdominal distention. More than 700 million BIFICO capsules have been consumed by over 8000000 Chinese patients since 1995. These patients ranged in age from 1 mo to 75 years and received BIFICO capsules for the treatment of a range of clinical disorders including diarrhea, irritable bowel syndrome (IBS), ulcerative colitis (UC), newborn jaundice and others.

Because competition in the bacterial community plays a pivotal role in the prevention of *H. pylori* invasion, it is believed that supplementation with

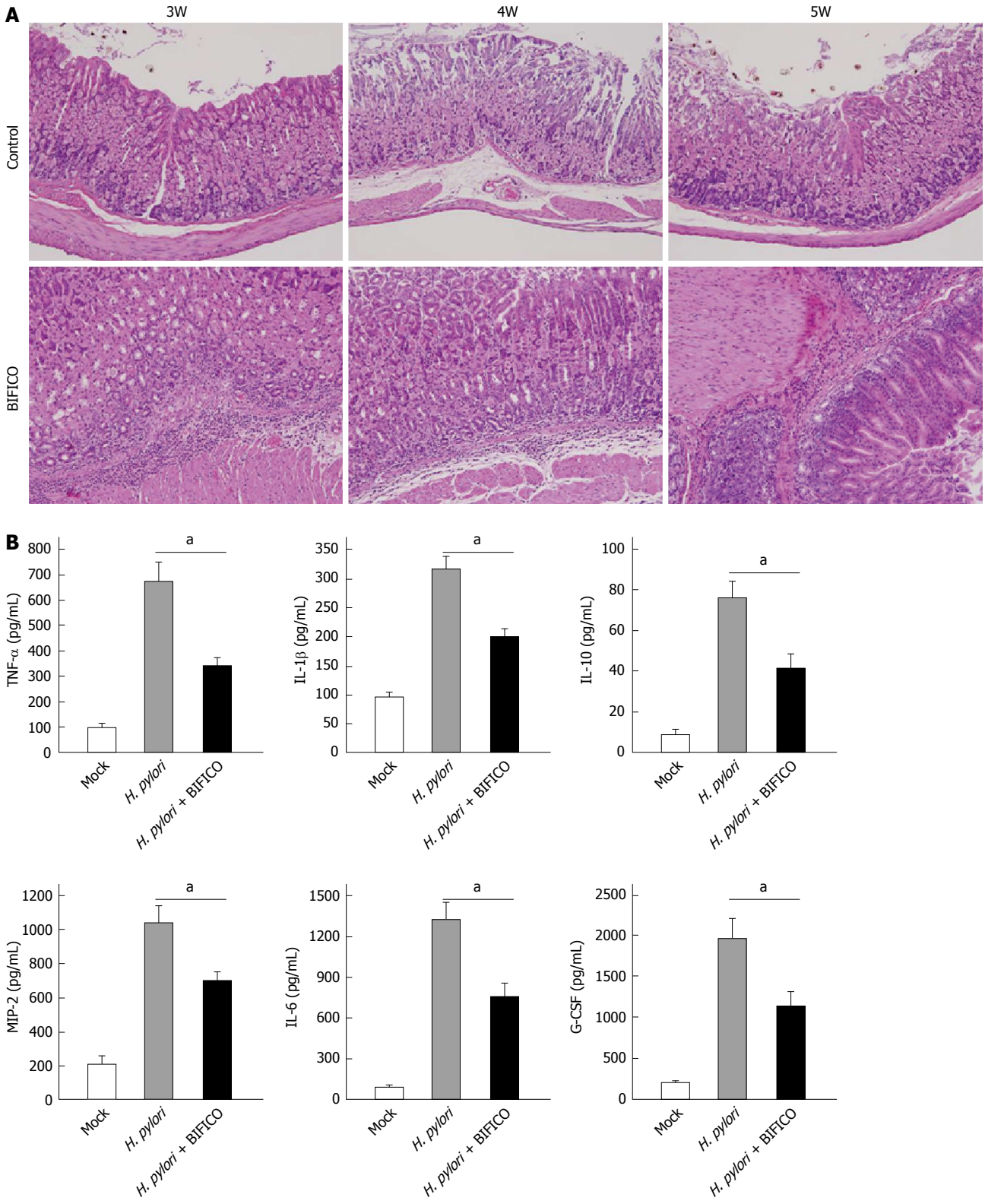

Figure 2 BIFICO ameliorates *Helicobacter pylori* induced gastritis in mice. A: Stomach histopathology of mice with *Helicobacter pylori* (*H. pylori*) infection was analyzed with hematoxylinand eosin (HE) staining at the indicated times after treatment with BIFICO; B: Production of cytokines and chemokines including TNF-α, IL-1β, IL-10, IL-6, G-CSF and MIP-2 in mouse stomach stimulated with *H. pylori*. The levels of cytokines and chemokines in the extracts of homogenized tissues were measured with the MILLIPLEX Mouse Cytokine and Chemokine Magnetic Bead Panel. Data shown are representative of three independent experiments; $^{a}P < 0.05$, *H. pylori vs H. pylori* + BIFICO (*t* test).

probiotic bacterial strains may inhibit the colonization of *H. pylori* and subsequent gastritis by preventing *H. pylori* access to the mucosal surface. In recent years, the application of probiotics in treating *H. pylori* infection has become an active area of research. Several probiotics, including *Lactobacillus*

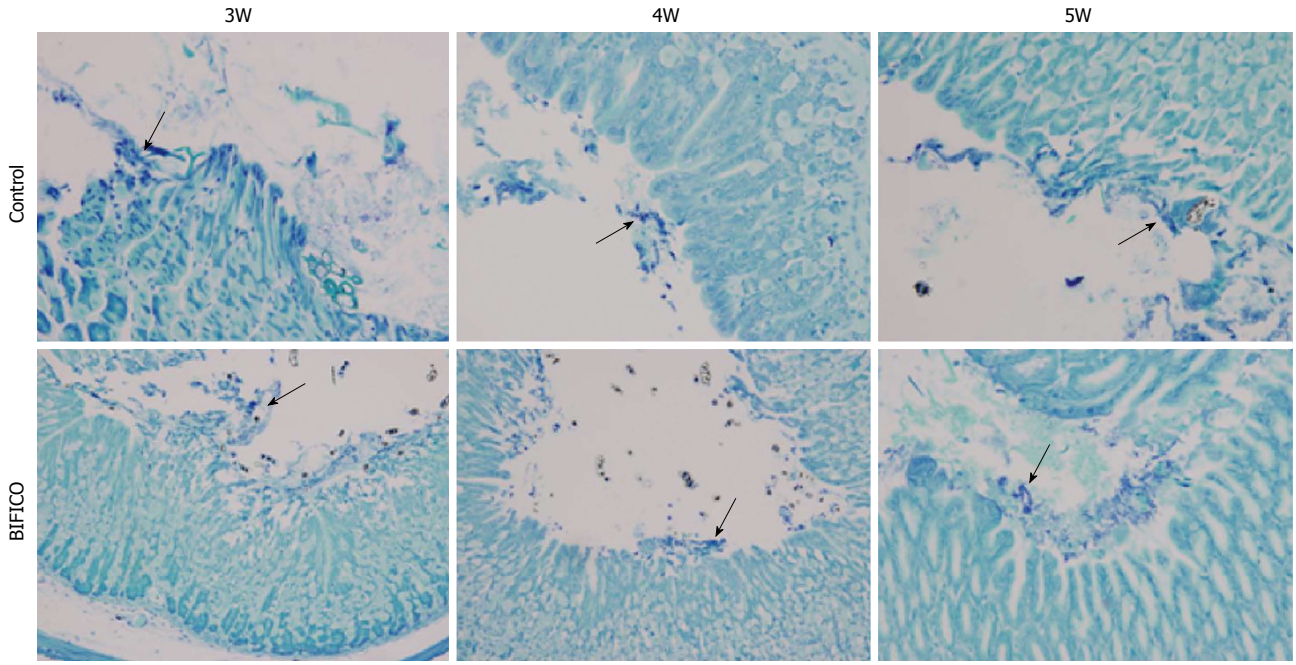

Figure 3 BIFICO does not suppress *Helicobacter pylori* colonization in mouse stomach. Stomach histopathology of mice with *Helicobacter pylori* infection was analyzed with Giemsa staining at the indicated times after treatment with BIFICO.

spp., *Saccharomyces* spp., *Bifidobacterium* spp. and *Bifidobacterium clausii*, have been studied for the treatment of *H. pylori* infection. However, it remains unclear whether probiotics can improve the eradication of *H. pylori* more effectively than standard triple therapy[8-10,15]. To evaluate the clinical efficacy of BIFICO capsules, a multi-center, positive parallel controlled clinical trial was carried out. A total of 85 *H. pylori*-positive patients were randomly assigned to a 7-d triple therapy based on rabeprazole (20 mg b.i.d.), clarithromycin (500 mg b.i.d.) and amoxicillin (1000 mg b.i.d.) (RCA group: 44 subjects) or to the same regimen supplemented with a 14-d therapy of BIFICO capsules (3×10^7 CFU of each strain, b.i.d.) before and after triple therapy (RCAB group: 41 subjects). Analysis of the outcome of this study was based on clinical improvement, and *H. pylori* eradication was confirmed based on the (13)C-urea breath test. However, these results were not consistent with those observed in the mouse model. Although colonization of *H. pylori* was not significantly suppressed in the *in vivo* study, eradication of *H. pylori* was improved in the clinical trial. In the RCA group, eradication was successful in 59.1% of patients (26 out of 44 patients), and in the RCAB group, the eradication rate increased to 82.9% (34 out of 41 patients). The frequency of side effects, including abdominal pain, abdominal distension, nausea and diarrhea, in the RCAB group was lower than that in the RCA group (data not shown).

Recent studies have compared the beneficial effects of probiotic mixtures and single strains against a wide range of disorders, and the results have demonstrated that probiotic mixtures are more effective than single-strain treatments for a variety of end points such as inhibition of pathogen growth and atopic dermatitis[16-21]. Specifically, it has been demonstrated that probiotic mixtures containing multiple strains of more than one genus are even more effective than multistrain probiotics[21]. As a cocktail probiotic capsule, BIFICO contains three strains of probiotic species belonging to the genera *Lactobacillus*, *Enterococcus* and *Actinobifida*. After more than 20 years of clinical use in China, BIFICO has been demonstrated to be more effective than single-strain preparations in treating diarrhea, indigestion and abdominal distention (unpublished observations). The mechanisms underlying these enhanced beneficial effects of the probiotic combination are summarized below. First, *Enterococcus faecalis* contained in the preparation increases the likelihood of colonization of *Bifidobacterium longum*. In addition, strains of *Enterococcus faecalis* are oxygen scavengers and create anaerobic conditions that potentially enhance the growth and survival of the strict anaerobic *Bifidobacterium longum*. Second, the synergistic health-promoting effects of individual strains exist in BIFICO probiotic mixtures. Growth of probiotic organisms is necessary to maintain sustainable numbers at certain sites in the gastrointestinal tract. In particular, the growth of *Bifidobacterium longum* strain can be stimulated by *Lactobacillus acidophilus*, which is known to produce certain growth factors such as bifidogenic growth factors, amino acids and free peptides. Finally, BIFICO capsules containing more than one strain belonging to different genera are able to produce a wider variety of antimicrobial moieties, such as weak organic acids, bacteriocins, hydrogen,

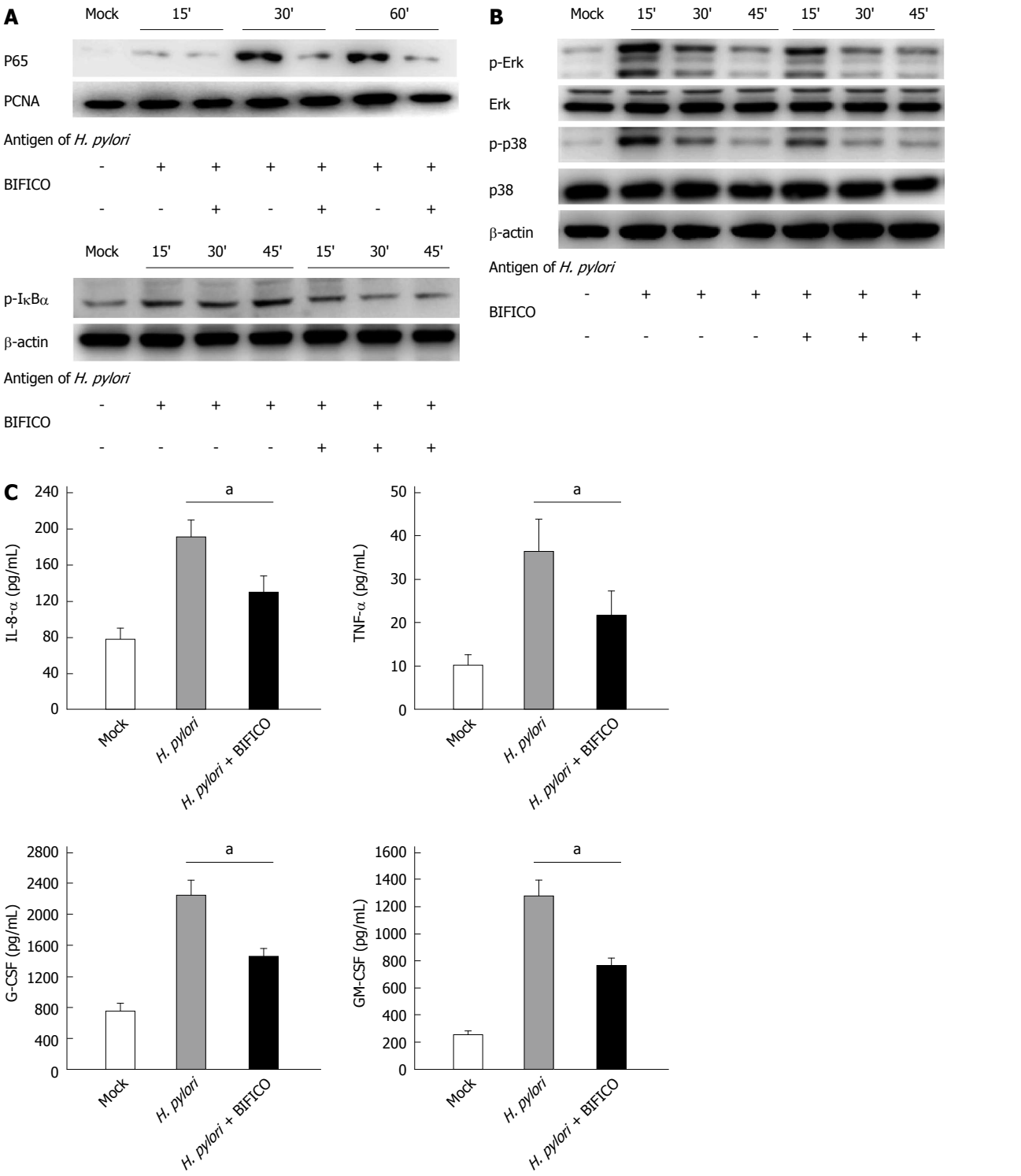

Figure 4 Nuclear factor-κB and MAPK signaling activation in human gastric epithelial cell GES-1 induced by *Helicobacter pylori* is suppressed by BIFICO. A, B: Epithelial GES-1 cells treated with or without BIFICO were stimulated by inactivated *Helicobacter pylori* (*H. pylori*) (MOI = 5) for the indicated times. The cell lysates (A) and nuclear extracts (B) were analyzed by immunoblotting with the indicated antibodies. A set of representative results from three independent experiments was presented; C: ELISA results for IL-8, TNF-α, G-CSF, and GM-CSF in supernatants of GES-1 treated with or without BIFICO, which were stimulated with inactivated *H. pylori* for 24 h. Data shown are representative of three independent experiments. SD is indicated; $^{a}P < 0.05$, *H. pylori vs H. pylori* + BIFICO (*t* test).

coaggregation molecules, biosurfactants, and stimulate sIgA production and mucus secretion by the host. As described above, BIFICO capsules, which have been demonstrated to be more effective than single-strain preparations, may also have synergistic effects for the treatment of *H. pylori* infection.

H. pylori harbors conserved PAMPs that can be recognized by epithelial cells and innate immune cells *via* four distinct classes of innate PRRs[3,5,6]. These PRRs differ in their subcellular localization, in addition

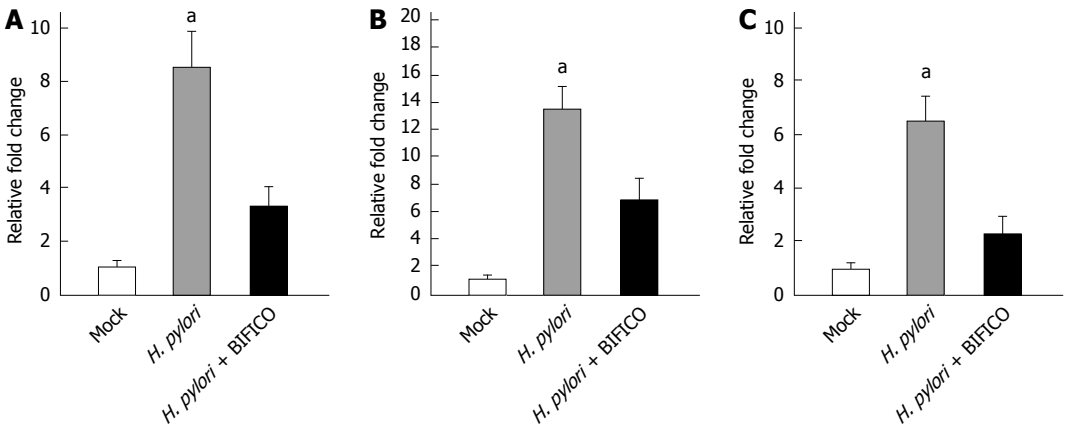

Figure 5 TLR2, TLR4 and Myd88 overexpression in mouse stomach induced by *Helicobacter pylori* is suppressed by BIFICO. A-C: Quantitative reverse transcription-polymerase chain reaction analysis of TLR2 (A), TLR4 (B) and Myd88 (C) mRNAs in mouse stomach stimulated with *Helicobacter pylori* (*H. pylori*). [a]$P < 0.05$, *H. pylori vs H. pylori* + BIFICO (*t* test).

to their downstream signaling pathways and ligand specificity. The best-defined among the four classes of PRRs are the TLRs. TLRs bind diverse classes of PAMPs, among which are the ligands for TLR2 (lipoteichoic acid and lipoproteins), TLR3 (double-stranded RNA and polyinosinic polycytidylic acid), TLR4 [lipopolysaccharide (LPS)], TLR5 (flagellin) and TLR9 (unmethylated CpG). Studies have suggested that TLR2[22,23] and TLR4[24,25] are the main sensors of *H. pylori* LPS. Colonization of gastric epithelial cells by *H. pylori* activates these TLRs, which predominantly activate anti-inflammatory signaling pathways including the NF-κB and MAPK pathways[5,26], leading to increased production of the proinflammatory cytokines TNF-α, IL-1, IL-6 and IL-8[27,28]. In our study, we demonstrated that BIFICO treatment diminished the inflammatory response of gastric mucosal epithelial cells stimulated by *H. pylori in vitro via* NF-κB and MAPK signaling and reduced the release of subsequent inflammatory cytokines including IL-8, TNF-α, G-CSF and GM-CSF. Furthermore, *H. pylori*-induced overexpression of TLR2, TLR4 and MyD88 in the mouse stomach was suppressed by BIFICO treatment, suggesting that the TLR/MyD88 pathway is involved in BIFICO-mediated down-regulation of *H. pylori*-induced immune responses in gastric mucosal epithelial cells.

In conclusion, we investigated the effect of BIFICO treatment on *H. pylori*-induced gastritis. BIFICO is a clinical drug consisting of three species of probiotic strains. Although unable to reduce the colonization of *H. pylori*, BIFICO was able to diminish the inflammatory response of gastric mucosal epithelial cells stimulated by *H. pylori* and the subsequent inflammatory cytokine release. However, because gastric epithelial cells are exposed to a myriad of commensal and pathogenic bacteria, we cannot exclude the possibility that other signaling cascades other than TLR signaling may be stimulated by *H. pylori* infection. Nevertheless, our results show that NF-κB activation could be reduced by *BIFICO treatment*. Further studies are necessary to further elucidate these beneficial effects of BIFICO capsules, and a better understanding of how probiotics affect epithelial inflammation could lead to therapeutically relevant strategies for effective *H. pylori* treatment. Thus, these results could form the basis for future clinical studies for microbiota manipulation to ameliorate *H. pylori*-induced infection worldwide.

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COMMENTS

Background

Helicobacter pylori (*H. pylori*) is a common human pathogen that colonizes approximately 50% of the world's population. Chronic gastritis caused by *H. pylori* infection is considered to be a major risk factor for the development of gastric and duodenal ulcers and gastric malignancies. Although successful eradication of *H. pylori* using currently available antimicrobial agents is able to cure the majority of patients diagnosed with gastritis and peptic ulcer disease, a dramatic increase in resistant strains has prompted a need for alternative treatments.

Research frontiers

H. pylori is usually the dominant species within a community of bacterial species in the stomach and may induce gastritis under conditions of bacterial imbalance or immune dysfunction. Because competition in the bacterial community plays a pivotal role in preventing the invasion of pathogenic bacteria, several probiotics including *Lactobacillus* spp., *Saccharomyces* spp., *Bifidobacterium* spp. and *Bifidobacterium clausii* have recently been studied in *H. pylori* infection. However, a combination of multiple probiotics may be more powerful to prevent invasion by pathogenic *H. pylori* and subsequent inflammation.

Innovations and breakthroughs

This study demonstrates for the first time that a traditional probiotic pharmaceutical (BIFICO) in China composed of *Enterococcus faecalis*, *Bifidobacterium longum* and *Lactobacillus acidophilus* triple viable bacteria, which was approved for treating intestinal disorders, can suppress *H. pylori*-induced gastritis in experimental mice. Although BIFICO treatment had no effect on the colonization of *H. pylori* in the stomach, it diminished the inflammatory response of gastric mucosal epithelial cells stimulated by *H. pylori via* the TLR/MyD88 pathway.

Applications

Due to its inhibitory effects on *H. pylori*-induced gastritis, BIFICO can potentially be used to treat patients with *H. pylori* infection and the associated inflammation.

Terminology

BIFICO is a traditional probiotic pharmaceutical in China composed of *Enterococcus faecalis*, *Bifidobacterium longum* and *Lactobacillus acidophilus* triple viable bacteria, which was previously approved for the treatment of intestinal disorders. The TLR/MyD88 pathway is stimulated in the immune response to pathogens; specifically, innate immune cells recognize pathogen-associated molecular patterns *via* Toll-like receptors, which activates inflammatory signaling pathways and the production of proinflammatory factors.

Peer-review

This is an interesting study where the authors have investigated the effects of a traditional probiotic pharmaceutical, BIFICO, on *H. pylori*-induced gastritis *in vivo*. The results suggest that BIFICO, which is commonly used to treat intestinal disorders, could be beneficial for preventing *H. pylori*-induced gastritis.

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