Potential protective effects of *Clostridium butyricum* on experimental gastric ulcers in mice

Fang-Yan Wang, Jia-Ming Liu, Hai-Hua Luo, Ai-Hua Liu, Yong Jiang

**Abstract**

**AIM:** To investigate the effects of *Clostridium butyricum* (*C. butyricum*) on experimental gastric ulcers (GUs) induced by alcohol, restraint cold stress, or pyloric ligation in mice, respectively.

**METHODS:** One hundred and twenty mice were randomly allocated into three types of gastric ulcer models (*n* = 40 each), induced by alcohol, restraint cold stress, or pyloric ligation. In each GU model, 40 mice were allocated into four groups (*n* = 10 each): the sham control group; model group (GU induction without pretreatment); *C. butyricum* group (GU induction with *C. butyricum* pretreatment); and Omeprazole group (GU induction with Omeprazole pretreatment).
effects of *C. butyricum* were evaluated by examining the histological changes in the gastric mucosal erosion area, the activities of superoxide dismutase (SOD) and catalase (CAT), the level of malondialdehyde (MDA), and the contents of interleukin (IL)-1β, tumor necrosis factor (TNF-α), leukotriene B4 (LTB4) and 6-keto-PGF-1α (degradation product of PGL) in the gastric tissue.

**RESULTS:** Our data showed that *C. butyricum* significantly reduced the gastric mucosal injury area and ameliorated the pathological conditions of the gastric mucosa. *C. butyricum* not only minimized the decreases in activity of SOD and CAT, but also reduced the level of MDA in all three GU models used in this study. The accumulation of IL1-β, TNF-α and LTB4 decreased, while 6-keto-PGF-1α increased with pretreatment by *C. butyricum* in all three GU models.

**CONCLUSION:** Our data demonstrated the protective effects of pretreatment with *C. butyricum* on anti-oxidation and anti-inflammation in different types of GU models in mice. Further studies are needed to explore its potential clinical benefits.

Key words: Inflammation; Probiotics; Oxidative stress; *Clostridium butyricum*; Gastric ulcer

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Core tip: In this study, we reported that the probiotic *Clostridium butyricum* (*C. butyricum*) pretreatment obviously attenuated gastric mucosal lesions induced by different stimulations. The oxidative stress- and inflammation-related parameters detected in this study showed that anti-oxidation and anti-inflammation participate in the underlying mechanism of *C. butyricum* protective effect on gastric mucosa. Our findings provide a potential protective method for the gastric mucosa and a novel application for *C. butyricum* in the clinic.

Wang FY, Liu JM, Luo HH, Liu AH, Jiang Y. Potential protective effects of *Clostridium butyricum* on experimental gastric ulcers in mice. *World J Gastroenterol* 2015; 21(27): 8340-8351. Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i27/8340.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i27.8340

**INTRODUCTION**

The stomach is an important organ for food digestion. In some cases, however, its digestive function may become too aggressive, resulting in self-digestion and gastric ulcers (GU)[1]. GU is commonly caused by an exposure to excessive amounts of various endogenous or exogenous factors, including excess secretion of gastric acids and pepsin, reactive oxygen species (ROS), stress, alcohol, non-steroidal anti-inflammatory drugs (NSAIDs), *Helicobacter pylori* (*H. pylori*) infection and smoking[2]. Although an estimated 10% of population in the world suffers from this disease[3], its severity is often not recognized, possibly because of its quick clinical recovery if timely treatment is started. Despite considerable progress with extensive application of gastroprotective agents, such as proton-pump inhibitor (PPI) and eradication therapy for *H. pylori*, some issues remain in the current therapy of GU. First, drug resistance of *H. pylori* may require changes in the treatment regimens[4]. Furthermore, antibiotics administered orally alter intestinal flora[4,5] and may cause drug resistance in some bacteria, leading to unexpected difficulties in the treatment of a variety infectious diseases. Second, the recurrence rate of GU is high with current therapies[6]. While the causes for GU relapses are complicated, infection by *H. pylori* may be an important contributor[6,7]. The weakened defensive barrier may also be a key factor contributing to recurrence[6]. However, only a few drugs are available that promote the repair of gastric mucosa. Third, knowledge of adverse effects of current drugs used to treat ulcers is accumulating[8]. For example, extended use of PPIs appears to cause an increased risk of fractures[9,10], hepatitis[11,12] and bacterial pneumonia[13,14]. Therefore, it is necessary to explore new therapies to prevent and treat GU.

*Clostridium butyricum* (*C. butyricum*) has been used as a probiotic to treat and prevent non-antimicrobial-induced or antimicrobial-associated diarrhea[15,16]. Its in vivo inhibition on *H. pylori* was reported by Takahashi et al[17]. The direct protective effects of *C. butyricum* on GU, however, are not clear.

This study was designed to test the hypothesis that pretreatment with *C. butyricum* could prevent lesions in the gastric mucosa. Clinical GU cases are often induced by alcohol overuse, stress, and excess of gastric acid and pepsin; therefore, we developed and used three GU models in mice induced by alcohol, cold stress or pylorus ligation, in this study. In each model, the pretreatment effects were evaluated by comparisons with no pretreatment and standard pretreatment with Omeprazole.

**MATERIALS AND METHODS**

**Drugs and chemicals**

*C. butyricum* was purchased from Miyarisan Pharmaceutical Co., Ltd (Nagano, Japan). Trypticase-phytone-yeast extract (TPY) liquid culture medium (Table 1) was obtained from Hope Bio-Technology Co., Ltd (Qingdao, China). Omeprazole was from Aosaikang Pharmaceutical Co., Ltd (Jiangsu, China). Detection kits for superoxide dismutase (SOD) and catalase (CAT) were purchased from Jiancheng Bioengineering Institute (Nanjing, China). The kits for malondialdehyde (MDA) detection and bicinchoninic acid (BCA) protein assay were bought from Beyotime Institute of Biotechnology (Shanghai, China). Enzyme-linked immunosorbent assay (ELISA) kits for IL-1β, TNF-α, leukotriene B4 (LTB4) and 6-keto-PGF-1α were
In each GU model and before induction, 40 mice were allocated into four groups (n = 10 each): sham control group; GU model group (GU induction without pretreatment); C. butyricum group (GU induction with C. butyricum pretreatment); and Omeprazole group (GU induction with Omeprazole pretreatment). The sham control group animals received only the culture medium, and the GU model group animals received the culture medium and alcohol administration, restraint cold stress or pyloric ligation, without any pretreatment.

In the C. butyricum group, the animals received C. butyricum pretreatment before GU model preparation. C. butyricum was cultured with TPY liquid culture medium in an anaerobic incubator. Ten hours later, C. butyricum at the logarithmic phase was used for intragastric administration of mice in C. butyricum group (dose = 5 × 10^8 CFU/mouse) for 5 d via an orogastric tube.

In the Omeprazole group, the animals were intraperitoneally administered with Omeprazole (13 mg/kg bw), 30 min before replication of GU models. The mice were sacrificed by cervical dislocation followed by isolation of the stomach from surrounding organs without any substantial connective tissues or vessels. The gastric specimens were used for lesion assessment, pathological observation and determination of parameters of oxidative stress and inflammation. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by Laboratory Animal Ethics Committee of Wenzhou Medical University (Permit number: wydw2012-0109). All surgery was performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering.

### Quantification of gastric hemorrhagic ulcer area and histological study
The stomachs of mice were fixed by injection of 1 mL 10% formalin solution, and digital images were taken by photo scanning. The ratio of gastric hemorrhagic ulcer area and total gastric area was calculated by the Image-Pro Plus (IPP) 6.0 software from Media Cybernetics (Bethesda, CA, United States). The specimens were embedded in paraffin, sectioned and stained with hematoxylin and eosin (HE). Pathological changes were identified under a Nikon Eclipse SOi light microscope (Nikon Co., Japan).

### Preparation of tissue samples
The stomachs of mice were washed mildly to remove food debris and then weighed using an electronic balance. Ice-cold normal saline with a volume corresponding to 9 × the tissue weight was added. As extensive lesions of the gastric mucosa existed in the acute GU models, the whole stomach was homogenized with a Potter-Elvehjem glass homogenizers, and centrifuged at 2400 × g for 15 min. The supernatant

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### Table 1  Trypticase-phytone-yeast extract liquid culture medium composition

| Components                  | Weight (g/L) |
|-----------------------------|--------------|
| Casein hydrolysate          | 1.0          |
| Soya bean peptone           | 5.0          |
| Yeast powder                | 2.0          |
| Glucose                     | 5.0          |
| L-Cysteine                  | 0.5          |
| KHPO₄                       | 2.0          |
| MgCl₂                      | 0.5          |
| ZnSO₄                      | 0.25         |
| CaCl₂                      | 0.15         |
| FeCl₃                      | 0.00001      |
| Tween-80                   | 1.0          |
| pH                          | 6.3 ± 0.1    |

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obtained from Westang Bio-tech Co., Ltd (Shanghai, China).

**Animals and GU models**

Male Institute for Cancer Research (ICR) mice, weighing 25-30 g, were provided by the Experimental Animal Center of Wenzhou Medical University (Wenzhou, China). All the animal procedures carried out in the present study were in accordance with the guidelines of the Animal Ethics Committee of Wenzhou Medical University.

Animals were given free access to tap water and food in air-conditioned animal quarters under a 12 h light-12 h dark cycle. 120 mice were randomly allocated into three types of gastric ulcer models (n = 40 each), induced by alcohol, restraint cold stress or pyloric ligation. All the models are classical and stable for the study of molecular mechanisms and screening of therapeutic or preventive drugs of GU.

Following the indicated pretreatments (see next paragraph), in the alcohol-induced GU model, mice were orally administered with pure alcohol 0.01 mL/g body weight (bw) and sacrificed by cervical dislocation 1 h later before the stomach tissues were collected for analysis.

In the cold-restraint stress-induced GU model, mice were lightly anesthetized by pentobarbital anesthesia (30 mg/kg bw, intraperitoneally) and fixed solidly to immobilization the mice. The bodies of mice under xiphoid were subjected to cold water (21 °C) for 20 h to induce a cold stress.

In the pyloric ligation-induced GU model, the abdomen of mouse was opened under light anesthesia with pentobarbital and the stomach was taken out under sterile conditions. The pyloric end was then isolated and ligated, followed by suture of the abdominal wall. The animals were sacrificed after the ligation for 20 h.

**Animal pretreatments**

In each GU model and before induction, 40 mice were allocated into four groups (n = 10 each): sham control group; GU model group (GU induction without pretreatment); C. butyricum group (GU induction with C. butyricum pretreatment); and Omeprazole group (GU induction with Omeprazole pretreatment). The sham control group animals received only the culture medium, and the GU group animals received the culture medium and alcohol administration, restraint cold stress or pylorus ligation, without any pretreatment.

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was collected for the assays of SOD, CAT and MDA.

**Measurement of SOD activity**
The activity of total SOD of gastric tissue was measured from gastric homogenate by the method of xanthine oxidase\[^{21}\], with absorbance at 550 nm using a 722N spectrophotometer from the Scientific Instrument Co., Ltd (Shanghai, China). The activity of SOD in per mg of tissue protein was calculated according to the formula supplied by the manufacturer.

**Determination of CAT activity**
The activity of CAT was determined at 405 nm with a 722N spectrophotometer from the Scientific Instrument Co., Ltd (Shanghai, China), through the ammonium molybdate colorimetric method\[^{22}\], according to the formula provided by the manufacturer.

**Measurement of MDA**
Lipid peroxidation in stomach samples was determined by measuring thiobarbituric acid reactive substances, as described by Ohkawa et al\[^{23}\]. The absorbance at 532 nm was measured with an ELx800 absorbance microplate reader (BioTek Instruments, Inc., Winooski, VT, United States). The concentration of thiobarbituric acid reactive substances was measured using a standard curve of malondialdehyde, and the results were expressed as \( \mu \text{mol MDA} / \text{g protein} \).

**Detection of IL-1\(\beta\), TNF-\(\alpha\), LTB4 and 6-keto-PGF-1\(\alpha\)**
IL-1\(\beta\), TNF-\(\alpha\), LTB4 and 6-keto-PGF-1\(\alpha\) of the gastric tissue were quantitated by the ELISA method. Briefly, 100 \( \mu \text{L} \) of homogenate was added to a 96-well plate that was coated with a corresponding primary antibody, and incubated for 3 h. The plate was washed twice with Phosphate Buffered Saline Tween-20 (PBST) and 100 \( \mu \text{L} \) of biotin conjugate was added to the plate, followed by an incubation of 45 min at room temperature. After washing to remove the non-specific binding, 100 \( \mu \text{L} \) of streptavidin-HRP was added and incubated for 45 min. Then the plate was washed with PBST and 100 \( \mu \text{L} \) of Chromogen was added to each well. Twenty minutes later, the reaction was stopped with stop solution. The absorbance was measured at 450 nm using an ELx800 absorbance microplate reader (BioTek Instruments). The levels of IL-1\(\beta\), TNF-\(\alpha\), LTB4 and 6-keto-PGF-1\(\alpha\) were calculated using standard curves, according to the instructions from the manufacturer.

**Protein assay of stomach samples**
The protein contents of stomach samples were detected by the bicinchoninic acid assay (BCA) method. The absorbance at 562 nm was detected by BioTek ELx800 Absorbance Microplate Reader (BioTek Instruments), according to the manufacturer's instruction. The protein concentration of the samples was calculated using a protein standard curve, and used to normalize the parameters of oxidative stress or inflammation in the stomach tissue.

**Statistical analysis**
All values were expressed as the mean \( \pm \) SD. Statistical differences were evaluated by Fisher's Least Significant Difference (LSD) test followed by a Dunnett's T3 multiple comparison test. A \( P \) value less than 0.05 was considered statistically significant.

**RESULTS**

**Histopathological observations**
The effects of pretreatment with *C. butyricum* were...
compared macroscopically to non-treated (Model group) and those pretreated with Omeprazole (Omeprazole group) (Figure 1). Quantitative comparisons of the gross morphology (Figure 1) were determined using the ratio of hemorrhagic area to total gastric area by the IPP 6.0 software (Figure 2). Alcohol overuse induced substantial gastric mucosal lesions, including many hemorrhagic injuries along the vessels of gastric mucosa. In some mice, shrinkage of the gastric wall was also observed. Restraint cold stress, also caused severe pathological changes within a larger hemorrhagic area, with a dark red appearance and excessive shrinkage. Pretreatment with *Clostridium butyricum* pretreatment significantly reduced gastric lesions in both alcohol and cold induced GU models (Figure 2, P < 0.01), with a comparable protective effect to Omeprazole, a standard treatment drug (Figure 2). The pylorus ligation model was not included in the comparison because the mucosal lesions were diffused in the group, which made it difficult to evaluate extent of injury and the effects of pretreatment.

To further examine the morphological lesions, we performed the conventional HE staining. Histological examination showed that the epithelial lining was intact, and the glandular cavity was clear and without any inflammatory cell infiltration in the gastric mucosa in sham control mice (Figure 3A, 3E and 3I). In the three GU models without pretreatments, marked histological changes were observed, including infiltration of neutrophils, lymphocytes and many erythrocytes into the mucosa. Some secretory glands were broken and many deciduous cells were observed in the glandular cavities (Figure 3B, 3F and 3J). Even mucosal desquamation was found in some gastric tissues from GU mice. Among the three GU models without pretreatments, the histological lesions in cold stress ulcer model were the worst (Figure 3F). Pretreatment with *Clostridium butyricum* alleviated the pathological changes (Figure 3C, 3G and 3K), with comparable results to pretreatment with Omeprazole (Figure 3D, 3H and 3L). All the results were consistent with the macroscopical changes described above.

**Oxidative stress in the gastric mucosa from GU mice**

Oxidative stress is a main contributor to the development of gastric mucosal lesions. Figure 4 shows the changes in the activities of SOD and CAT, and the content of MDA, in gastric tissues, which were used to evaluate the extent of oxidative stress. The data showed that in all three GU models, the activities of SOD and CAT decreased and the level of MDA increased (P < 0.01). Pretreatment with *Clostridium butyricum* attenuated those changes (Figure 4, P < 0.01), indicating that pretreatment with *Clostridium butyricum* reduced oxidative stress in GU mice. With pretreatment of Omeprazole, changes in the oxidative parameters were similar to those from pretreatment of *Clostridium butyricum*, although the activity of SOD in *Clostridium butyricum* group was higher than that of the Omeprazole group in the pylorus ligation model (P < 0.01, Figure 4G).

**Inflammation status of GU**

Excessive inflammation plays an important role in the development of gastric mucosal lesions induced by various proinflammatory stimuli; therefore, we performed cytokine detection to investigate the underlying mechanism of the protective effects of *Clostridium butyricum* on gastric mucosa. Changes of IL-1β, TNF-α and LTB4 in gastric tissues in three models with different pretreatments are shown in Figure 5. The levels of IL-1β, TNF-α and LTB4 were significantly elevated, by different magnitudes, in all three GU models. Pretreatment with *Clostridium butyricum* attenuated this elevation of proinflammatory factors (P < 0.01) (Figure 5). Overall, the effects of *Clostridium butyricum* pretreatment were similar to those of Omeprazole, despite a lower level of LTB4 in *Clostridium butyricum* group in the pylorus ligation GU mice (P < 0.01) (Figure 5). These data indicated that *Clostridium butyricum* ameliorated inflammatory reactions in the development of gastric mucosal lesions induced by different stresses in mice.

**PGE2 content of gastric tissue during GU**

As PGE2 and PGI2 are not stable under physiological conditions; therefore, the stable metabolite of PGI2, 6-keto-PGF-1α, was used to reflect changes in proinflammatory factors. Figure 6 shows that 6-keto-PGF-1α was initially at a high level under normal conditions whereas the different ulcer-inducing stress factors significantly reduced the level of 6-keto-PGF-1α in the gastric mucosa. Pretreatment with *Clostridium butyricum* markedly attenuated the decrease 6-keto-PGF-1α induced by stressful stimulations (P < 0.01), with
similar effects to Omeprazole (Figure 6).

**DISCUSSION**

*C. butyricum* was discovered by Dr. Miyairi in 1933\[^{16}\]. It has been reported to have therapeutical effects on colitis in rats\[^{25}\], inhibitory cytotoxic effects from *Clostridium difficile* *in vitro*\[^{16}\], and suppression of colonization by *H. pylori* *in vivo*\[^{17}\]. *C. butyricum* has been used clinically to treat antibiotic associated diarrhea caused by *H. pylori* eradication therapy\[^{26}\], possibly through the recovery of intestinal microbiota balance. To investigate whether *C. butyricum* has a potential protective effect from gastric mucosal lesions, we assessed the effects of *C. butyricum* pretreatments on gastric mucosa from injuries induced by alcohol, stress or excessive gastric acid and digestive enzymes. Our data showed that pretreatment with *C. butyricum* alleviated the level of MDA and improved the activities of SOD and CAT in different GU models of mice. On the other hand, inflammation is an important factor involved in gastric injury. In the current study, IL-1\(\beta\), TNF-\(\alpha\) and LTB\(_4\) were increased in GUs induced by alcohol, cold stress and pylorus ligation. This is consistent with previous reports\[^{28-30}\]. Administration of *C. butyricum* dramatically inhibited the elevation of IL-1\(\beta\), TNF-\(\alpha\) and LTB\(_4\), indicating that *C. butyricum* exerted potential protective effects on gastric mucosa by restraining excessive inflammation in GUs.

Prostaglandins (PGs) are important proinflammatory factors with complicated functions in the body. However, some PGs, such as PGE\(_2\) and PGI\(_2\), are critical in the mucosal defense of the stomach\[^{1,31}\] and play a key role in protecting gastric mucosa from injury in the development of GUs\[^{23-34}\]. NSAIDs cause gastric mucosal lesions, which may be explained by inhibiting the activity of cyclooxygenases (COXs) and thus reducing the production of PGs. Since PGI\(_2\) is the main subtype of PGs in gastric tissue, we quantified the contents of 6-keto-PGF-1\(\alpha\), a stable degradation product of PGI\(_2\)\[^{35,36}\] in the current study. Our results...
showed that the 6-keto-PGF-1α level was reduced in three different GU models. Pretreatment with *C. butyricum* significantly attenuated the decrease of 6-keto-PGF-1α, suggesting possible protective effects of *C. butyricum*. Therefore, it is possible that *C. butyricum* provides protection for gastric mucosa against injuries by reducing oxidative stresses, attenuating pro-inflammatory cytokines and increasing the level of PGJ2. Further studies are needed to test this hypothesis.

To further assess the effects of *C. butyricum*, the general conditions and behavior of mice in different GU models were observed and compared. We found that the behavior of alcohol-induced GU mice was improved by the pretreatment with *C. butyricum*, while the mice without pretreatment lay at the bottom of cage. In the cold-induced GU mice, pretreatment of *C. butyricum* alleviated the depression induced by cold stress. Similarly, in the pylorus ligation GU mice, pretreatment with *C. butyricum* eliminated the odor of gastric juice in these mice.

Previous studies have shown that *C. butyricum* prevents inflammation-related diseases via its metabolites\(^{25,27}\). It is reasonable to speculate that the metabolites of *C. butyricum* may play a role in protection of the gastric mucosa. Butyrate and hydrogen are the two major metabolic products of *C. butyricum*. As a primary energy source of colonic epithelial cells, butyrate promotes water and sodium absorption, tightens the junctions between epithelial cells and accelerates the repair of damaged epithelial cells\(^{28,29}\) in vitro. Butyrate has been reported to ameliorate inflammation in diversion colitis\(^{30-42}\), and attenuate

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**Figure 4** Effect of *Clostridium butyricum* on the activities of superoxide dismutase, catalase and the content of malondialdehyde in the gastric mucosa of mice with gastric ulcers induced by alcohol, cold stress or pylorus ligation. Gastric ulcer models were induced by alcohol (A-C), cold stress (D-F) or pylorus ligation (G-I), respectively. The activities of superoxide dismutase (SOD) (A, D, G) and catalase (CAT) (B, E, H), and the content of malondialdehyde (MDA) (C, F, I) in the gastric tissues of different gastric ulcers (GU) mice were detected. For each GU model, there were four different groups: Sham control group (1), Model group (without pretreatment) (2), *C. butyricum* pretreatment group (3) and Omeprazole pretreatment group (4). The data were expressed as mean ± SD (n = 6). \(a P < 0.01\) vs Model group; \(b P < 0.01\) vs Omeprazole group; \(c P < 0.01\) vs Sham control group.
Figure 5  Effect of Clostridium butyricum on the levels of IL-1β, TNF-α and LTB4 in the gastric mucosa of mice with gastric ulcers induced by alcohol, cold stress or pylorus ligation. Gastric ulcer models were induced by alcohol (A-C), cold stress (D-F) or pylorus ligation (G-I), respectively. The levels of IL-1β (A, D, G), TNF-α (B, E, H) and LTB4 (C, F, I) in gastric tissue of different GU mice were quantified. For each GU model, there were four different groups: Sham control group (1), Model group (without pretreatment) (2), Clostridium butyricum (C. butyricum) pretreatment group (3) and Omeprazole pretreatment group (4). The data were expressed as mean ± SD (n = 6). aP < 0.01 vs Model group; bP < 0.01 vs Omeprazole group; cP < 0.01 vs Sham control group.

Figure 6  Effect of Clostridium butyricum on 6-keto-PGF-1α in the gastric mucosa of mice with gastric ulcers induced by alcohol, cold stress or pylorus ligation. Gastric ulcer models were induced by alcohol (A), cold stress (B) or pylorus ligation (C), respectively. The content of 6-keto-PGF-1α in gastric tissue of different gastric ulcers (GU) mice was measured. For each GU model, there were four different groups: Sham control group (1), Model group (without pretreatment) (2), Clostridium butyricum (C. butyricum) pretreatment group (3) and Omeprazole pretreatment group (4). The data were expressed as mean ± SD (n = 6). aP < 0.01 vs Model (without pretreatment) group; bP < 0.01 vs Sham control group.
ROS production in intestinal epithelial Caco-2 cells stimulated by lipopolysaccharide and colonic mucosa from patients with Crohn’s disease. In addition, butyrate was shown to increase the expression and activity of COXs in human monocytes and ROS 17/2.8 osteoblastic cells, as well as human colon tumor tissue. In this study, we showed that C. butyricum reduced oxidative stress and inflammatory responses in different GU models. Furthermore, our other study (unpublished data) demonstrated that butyrate exerted protective effects on gastric mucosa challenged with ulcer-inducing stimuli, which provides an explanation for the anti-GU effect of C. butyricum.

C. butyricum is capable of producing a large amount of hydrogen; therefore, it has been used as a clean energy resource of hydrogen. Recently, hydrogen was used in therapeutics of various diseases as an anti-inflammatory gas, which opens an exciting approach for the treatment of inflammatory diseases. For example, a recent study showed that hydrogen could alleviate injury in stress-induced GU in mice. Thus, hydrogen might be a contributor to C. butyricum protective effects against GU. However, the metabolites of bacteria are highly dependent on survival environments. Unlike butyrate, which has a relatively stable turnover, hydrogen production is sensitive to the culture conditions. Although C. butyricum can survive and colonize in the stomach, the metabolites of C. butyricum may be different from those under optimized conditions. Therefore, further studies are required to identify whether butyrate and hydrogen are the crucial products of C. butyricum for the gastric mucosa protective effects.

C. butyricum might be a good choice in the future for pretreatment of GUs, especially with H. pylori infection. From the findings of this study, we designed a theoretical working model for C. butyricum (Figure 7), which includes three possible benefits of C. butyricum in clinical practice. First, C. butyricum directly inhibits H. pylori growth through antagonistic interaction, eliminating the most important cause of the occurrence and reoccurrence of GU. Furthermore, C. butyricum is suitable for patients suffering from GU but not sensitive to the therapy of eradication H. pylori. Second, C. butyricum attenuates gastric mucosal lesions independently, which provides long-lasting protection that prevents the occurrence and recurrence of ulcers. The main metabolic products, including butyrate and hydrogen, play crucial roles in the protective effects of C. butyricum. In addition, the probiotic C. butyricum can be used as a nutrition annexing agent for those sensitive to gastric mucosal lesions or infected by Helicobacter pylori, which is a

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**Figure 7** The hypothetical model of Clostridium butyricum protective effect on gastric ulcers induced by stressful stimuli. In addition to competitive inhibition of Helicobacter pylori, as reported by previous studies, Clostridium butyricum (C. butyricum) protects gastric mucosa from lesions through at least two different mechanisms: anti-oxidation and anti-inflammation. The metabolites of C. butyricum, such as butyrate and hydrogen, enhance the activity of antioxidases (superoxide dismutase (SOD) and catalase (CAT)), reduce the production of proinflammatory factors (interleukin (IL)-1, tumor necrosis factor (TNF)-α, leukotriene B4 (LTB4)) and promote the production of anti-inflammatory prostaglandins (PGI2), thus exerting protective effect on gastric ulcers by resolving peroxidation and alleviating exaggerated inflammation induced by various stresses.
prominent advantage lacking in the current clinical practice of GU treatment. Nonetheless, future investigations are needed to test these speculations.

In summary, pretreatment with *C. butyricum* reduced oxidative stress, inflammatory responses, and injury severity in mice with 3 different GU models in this study. These data may suggest a potential protective effect of *C. butyricum* on GU and support further effort to explore its possible clinical application.

ACKNOWLEDGMENTS

We thank Dr. W.Z. Martini from US Army Institute of Surgical Research at Sam Houston, TX, United States, for critical reading and editing of this manuscript. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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