Lactobacillus Alleviates Colitis Caused by Chemotherapy Via Biofilm Formation

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Abstract

Background: Severe colitis is a common side effect of chemotherapy in cancer patients. A widely consumed probiotic, *Lactobacillus*, has been reported to alleviate colitis. However, the gastric acid has a powerful bactericidal effect, which restricts to clinical trial success. In this study, we attempted to enhance the viability of probiotics in a gastric acid environment and improve the colitis induced by dextran sulfate sodium (DSS) and chemotherapeutic docetaxel.

Methods: We purified *Lactobacillus* from diverse brands of yogurt and estimated their growth at pH 6.8 and pH 2.0. In the further investigation, the bacterial biofilm formation was used to define the mechanism by which administration of LGG via oral gavage alleviates the colitis and intestine permeability of the mice induced by DSS and docetaxel. The potential benefit of probiotics on the treatment of breast cancer metastasis has been assessed as well.

Results: *Lactobacillus* growth was unexpectedly faster in the pH 2.0 than in the neutral pH medium during the first hour. However, its growth was greatly reduced after the first hour in the pH 2.0 medium but was maintained at neutral pH. As expected, *Escherichia coli* (E. coli) could not grow well in an acidic medium. *Lactobacillus rhamnosus* (LGG) administered in the fasting state via oral gavage significantly improved the preventive effect in the colitis caused by DSS and docetaxel. Further mechanistic investigations suggested that LGG reduced the permeability of the intestine and decreased the expression of proinflammatory cytokines, TNF α , IL-1 β , and IL-6, in colitis by biofilm formation. We examined the chemotherapeutic effect of docetaxel in a breast cancer model and found that increasing the docetaxel dose may reduce tumor growth and metastasis in the lung but did not benefit survival due to severe colitis. However, the LGG supplement significantly improved the survival of tumor-bearing mice following a high dose of docetaxel treatment.

Conclusions: Our findings provide new insights into the potential mechanism of probiotic protection of the intestine and provide a novel therapeutic strategy to augment the chemotherapeutic treatment of tumors.

Background

Colitis occurs in patients presenting with complex symptoms of acute abdominal pain and direct or rebound tenderness, possibly associated with neutropenia, fever, and/or diarrhea¹. The dextran sulfate sodium (DSS)-induced colitis mouse model² develops characteristics similar to those of ulcerative colitis patients. Two taxanes, paclitaxel and docetaxel³⁻⁵, are widely used to treat advanced breast cancer and other solid tumors in the clinic. However, patients receiving taxane-based chemotherapy may present with colitis symptoms. Taxane-induced colitis can cause serious consequences and restrict the use of chemotherapy. Treatment of taxane-induced colitis with intravenous fluids and antibiotics shortens the duration of symptoms, but there is still no effective way to prevent taxane-induced colitis.

The mucous layer overlaying the colonic epithelium consists of mucus glycoprotein (mucins or MUC) and trefoil factors (TFF) secreted by goblet cells, building up the first defense barrier between the luminal contents and mucosal cells in the gut ^{6,7}. Because of this barrier, pathogens and antigens cannot access the underlying epithelium and are thereby blocked from invading the body ⁸. In this context, inflammation is measured by increased myeloperoxidase activity, presence of proinflammatory cytokines, such as IL-1 β , IL-6, TNF α , IFN γ , and histological scores, indicating the severity of mucosal epithelial damage, glandular alterations, and the severity of lamina propria cellular infiltration ^{9,10}. It is critical to develop an effective treatment to reduce the colitis caused by paclitaxel-based chemotherapy.

There has been a growing interest in *Lactobacillus* as a probiotic that reduces host gut inflammation. Yogurt, the product of milk fermentation by *Lactobacillus*, has a beneficial impact on human health. *Lactobacillus* is a genus of rod-shaped, gram-positive, and anaerobic or microaerophilic bacteria that are “friendly” and normal residents in the oro-gastrointestinal, urinary, and genital tracts. It is mainly present in fermented milk like yogurt, cheeses, fruit juices, wine, and sausages. *Lactobacillus* is considered a probiotic to prevent and reduce diarrhea, help weight loss, improve lactose digestion in lactose-intolerant individuals, and increase levels of short-chain fatty acids, such as butyrate, which promote gut health. Gastric acid in the stomach activates an enzyme that breaks down proteins as well as kills microbes. Germs and bacteria, good or bad, are destroyed within 15 minutes in the highly acidic environment of pH 1.5 to 3.5 in the stomach ¹¹. Therefore, it was important to determine if *Lactobacillus* could survive in the acidic stomach environment. Increased survivability of probiotic lactobacilli in acidic conditions in the presence of glucose was previously reported ¹¹. One study reported that *Lactobacillus rhamnosus* (LGG), analyzed in a simulated environment with a pH of 2.0 in the presence of 19.4 mM glucose, showed enhanced survival after a 90 min exposure ¹². However, the dynamics of Lactobacilli in the gastric acid environment and the mechanism by which dietary *Lactobacillus* protects the intestine from inflammation caused by chemicals were still not clear.

Here we demonstrated the effect of *Lactobacillus* on reducing of inflammation in the DSS- and docetaxel-induced colitis mouse models and elucidated the protective mechanisms on the colonic barrier function.

Materials And Methods

Bacteria

Various *Lactobacillus* species were isolated from commercial yogurts, including Suki, Oikos, and Yoplait yogurt purchased from the supermarket (**Table 1**). *Lactobacillus rhamnosus* was purchased from ATCC (LGG, ATCC# 53103, Manassas, VA). All *Lactobacilli* were grown in the De Man, Rogosa, and Sharpe (MRS) broth (Hardy Diagnostics, Santa Maria, CA) at 37°C and incubated in a BactronEZ SHEL LAB anaerobic chamber (Sheldon Manufacturing, Inc. Cornelius, OR). MRS agar plates were made with 55g Lactobacilli MRS broth of dehydrated culture media and 15 g agar (Fisher Scientific) in one liter of water. The lysogeny broth (LB) medium and agar plates for *Escherichia coli* (E. coli) growth were purchased from Criterion and prepared according to the manufacturer's instructions.

Mice

BALB/c mice (6-8 weeks of age) were purchased from Jackson Laboratory. Mice were housed and fed in a specific pathogen-free animal house. All animal experiments were approved by the Institutional Animal Care and Use Committee of Nanjing Medical University.

Antibodies

The following rabbit anti-mouse antibodies used for Western blotting were purchased from Abcam: Claudin 5 (ab15106), Tjp2 (ab224314), Itgb5 (ab184312), GAPDH (ab181602), and horseradish peroxidase (HRP)-conjugated goat anti-rabbit antibody (ab270144).

***Lactobacillus* purification from yogurt**

Yogurt was diluted with 10 volumes of phosphate-buffered saline (PBS) and centrifuged at $700 \times g$ for 5 min at 4°C. The supernatant was washed once with an equal volume of cold PBS and centrifuged at $7,000 \times g$ for 15 min at 4°C. Pellets were then resuspended in an equal volume of medium (MRS broth and simulated gastric juice) at 37°C and incubated for 6 h with constant stirring.

Agar plate culture

After serial dilution in a maximum-recovery diluent, the bacteria were cultured on MRS agar and LB agar plates in an anaerobic chamber and a regular incubator at 37°C. The growth was monitored after 24~48 h and photographs were taken with the BioDoc-It™ Imaging System (UVP).

Bacterial growth monitoring

Samples were taken at 0, 0.5, 1, 2, 4, and 6 h, to measure the optical density (OD) at 600 nm using a spectrophotometer (BioTek) before and during incubation.

Biofilm formation assay

The overnight culture was diluted 1:100 in the fresh MRS medium containing 0.5% glucose to provide carbon and energy source and grown in a 96-well plate for 24 h at 37°C in the BactronEZ SHEL LAB anaerobic chamber. The quantitative assays were performed in 3 replicate wells for each treatment. After incubation, the culture was removed and washed twice with water to remove unattached cells and media components, significantly lowering the background staining. After 15 minutes of staining with 150 µl of 0.1% crystal violet (Sigma) diluted to 2.3% with H₂O, cells were washed twice with H₂O, and the plates were dried for several hours. The crystal violet stain was solubilized in 150 µl of 30% acetic acid, and optical density was read in a spectrophotometer (BioTek) at 550 nm.

Induction of Colitis by DSS and Docetaxel

Colitis was induced by feeding BALB/c mice 2.5% dextran sulfate sodium salt (DSS, MP Biomedicals, Santa Ana, CA) in drinking water until the end of the experiment. To generate colitis model by a chemotherapeutic agent, injectable solution of docetaxel (Sanofi) was purchased from the pharmacy in our hospital and was administered by intraperitoneal injection at 6mg/kg q4d for 28 days¹³⁻¹⁵. 5×10^{10} of LGG were administered to the mice (n=5 mice per group) by oral gavage with/without fasting 2 h daily for 7 days prior to the DSS or docetaxel administration. The PBS control colitis group served as the primary comparison with the LGG intervention group. Bodyweight, stool consistency, and fecal blood were monitored daily. The mice were euthanized on day 28 following 7 days of interventional feeding. Immediately after euthanasia by carbon dioxide inhalation, the abdominal skin was sprayed with 70% ethanol, and blood was taken by cardiac puncture. Next, the colons were quickly flushed with cold PBS (10 mM, pH 7.4) to remove feces and blood and the distal segments (1.0 cm) were fixed in 10% buffered formalin solution for histological examination.

***In vivo* intestinal permeability assay**

Fluorescein-5-isothiocyanate (FITC)-conjugated dextran (MW 4000; Sigma-Aldrich, St. Louis, MO) was administered at a concentration of 60 mg/100 g of body weight by oral gavage to study intestinal permeability *in vivo*. After 5 h, serum was collected retro-orbitally, and fluorescence intensity was determined with a fluorescence spectrophotometer (BioTek) at emission and excitation wavelengths of 485 nm and 528 nm, respectively. FITC concentration was measured from standard curves generated by serial dilution of FITC-dextran 4000, as described in the previously study¹⁶.

Histological analysis

Tissues were fixed overnight with buffered 10% formalin solution (SF93–20; Fisher Scientific, Fair Lawn, NJ) at 4°C and dehydrated by immersing in a graded ethanol series, 70%, 80%, 95%, 100% ethanol for 40 min each. Tissues were embedded in paraffin and subsequently cut into ultra-thin slices (5 mm) using a microtome, deparaffinized with xylene (Fisher), and rehydrated by decreasing concentrations of ethanol and PBS. Tissue sections were stained with hematoxylin and eosin (H&E), and slides were scanned with an Aperio ScanScope, as previously described¹⁷.

Enzyme-linked immunosorbent assay (ELISA)

The cytokine TNF α , IL-1 β , and IL-6 levels in mouse colon mucus were quantified using ELISA kits (eBioscience) according to the manufacturer's instructions. Briefly, excess binding sites were blocked with 200 μ l of 1x ELISA/ELISPoT Diluent (eBioscience) for 1 h at 22°C. The microtiter plates were coated with the anti-mouse TNF α , IL-1 β , or IL-6 antibody at 1:200 overnight at 4°C. After washing three times with PBS containing 0.05% Tween 20, the plates were incubated with the detection antibody in blocking buffer for 1 h at 22°C. The plates were washed three times and avidin conjugated with horseradish peroxidase (HRP), and substrate were added. Subsequently, absorbance at 405 nm using a microtiter plate reader (BioTek Synergy HT) was determined.

Labeling of bacteria with PKH26

Bacteria were labeled with PKH26 Fluorescent Cell Linker Kits (Sigma) in accordance with the manufacturer's instructions. After a wash with PBS, bacterial pellets were suspended in 250-500 µl of diluent C with 2-4 µl of PKH26 and subsequently incubated for 30 min at room temperature. After centrifugation for 5 minutes at 13,000x g, labeled LGG nanovectors were resuspended for further experiments.

RNA extraction.

Total RNA was isolated from murine tissues using an RNeasy mini kit (Qiagen) according to the manufacturer's instructions. In brief, 100 mg of the tissue was homogenized using a tissue grinder and was disrupted in QIAzol Lysis Reagent. Tissue. The homogenate was mixed with 140 ml of chloroform, centrifuged, the upper aqueous phase was mixed with 1.5 volumes of ethanol, and was loaded into the RNeasy spin column. The flow-through was discarded, and the column was washed with RWT and RPE, respectively. The flow-through was discarded after centrifugation, and the column was washed with RWT and RPE. Total RNA was eluted with RNase-free water, and the quality and quantity of the isolated RNA were analyzed with Nanodrop ND-1000 spectrophotometer (NanoDrop Technologies).

mRNA expression by Quantitative Real-Time PCR

For analysis of mRNA expression, 1 µg of total RNA was reverse transcribed by SuperScript III reverse transcriptase (Invitrogen) and quantitated using primers (Sangon, Shanghai) with SsoAdvanced™ Universal SYBR Green Supermix (BioRad); GAPDH was used for normalization. The primer sequences are listed in **Table 2**. qPCR was performed using the Applied Biosystems 7500 System, with each reaction performed in triplicate. Analysis and fold-changes were determined using the comparative threshold cycle (Ct) method. The change in mRNA expression was calculated as the fold-change. Gene expression was normalized to the control expression by calculating the $\Delta Ct = (Ct \text{ of control} - Ct \text{ of the gene})$. Setting the expression value of GAPDH to 1.0, the relative expression values were calculated as $2^{\Delta Ct}$. The change in miRNA or mRNA expression was calculated as fold-change.

Tight junction qPCR array

For analysis of tight junction genes mRNA expression, 2 µg of total RNA was reverse transcribed by SuperScript III reverse transcriptase (Invitrogen), and quantitation was performed using mouse Tight Junctions RT² Profiler™ PCR Array (Qiagen, PAMM-143) with SsoAdvanced™ Universal SYBR Green Supermix (BioRad); GAPDH was used for normalization. The data analysis was processed online at <https://dataanalysis2.qiagen.com/pcr>.

Western blot analysis

Samples were incubated in the SDS loading buffer at 95°C for 5 min and separated by 8% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), followed by electroblotting to

polyvinylidene difluoride membranes (Bio-Rad). After blocking nonspecific binding sites for 1 h in 5% nonfat dried milk in PBST (0.05% Tween 20 in PBS), the membrane was incubated for 1 h at room temperature or overnight at 4°C with the primary antibody. After three washes in PBST, the membrane was incubated with HRP-conjugated goat anti-mouse antibody for 45 min at room temperature, washed three times in PBST, and the signal was detected using enhanced chemiluminescence (ECL kit from Amersham Biosciences).

Quantification and statistical analysis

Unless otherwise indicated, all statistical analyses were performed with SPSS 16.0 software. Data are presented as mean $\pm$ standard deviation (SD). The significance of mean values between the two groups was analyzed using the Student's *t*-test. The differences between individual groups were analyzed by a one- or two-way ANOVA test. The differences were considered significant when the *p*-value was less than 0.05 or 0.01.

Results

Time-dependent effect of acidic pH on growth of *Lactobacillus* from yogurt

Lactobacillus supplement via the oral route must pass through stomach containing gastric acid that can kill most pathogenic germs¹⁸. The survival of different species of *Lactobacillus* purified from three brands of yogurts was investigated in gastric acid (**Table 1**). The bacteria were cultured in the MRS medium (**Supplementary Figure 1A**) on MRS agar plates (**Supplementary Figure 1B**) with pH 2.0, mimicking gastric acid pH¹⁹ and compared to those grown at neutral pH 6.8. The cell viability for the acid tolerance test was obtained at 0, 0.5, 1, 4, and 6 h. Unexpectedly, we found that *Lactobacillus* from all three sources of yogurts not only survived but even grew faster in the first hour in MRS with pH 2.0 compared to the medium with pH 6.8 (**Figure 1A**). The growth of *Lactobacillus* from all sources in simulated gastric acid quickly increased in the first hour, reaching the highest population at the end of the first hour, especially *Lactobacillus* from the OIKOS yogurt, and then reduced after 1 h. In contrast, all *Lactobacillus* species were in the lag phase growing slowly in MRS at pH 6.8 in the first hour, but the growth rates markedly improved after the first hour of incubation. This effect of pH on *Lactobacillus* growth was confirmed on MRS agar plates (**Figure 1B**). After 4 h, *Lactobacillus* in the pH 2.0 medium was irreversibly decreased and died, even after transferring to MRS agar plates with neutral pH (**Figure 1A, 1C**).

Next, we assessed the growth of LGG, the probiotic with widespread clinical use²⁰, in acidic conditions. Consistent with the *Lactobacillus* from yogurts, the LGG growth was fast in the first hour without a lag phase and restrained after 1 h in an acidic medium (**Figure 2D, 2E**). In the neutral pH medium, the LGG population was increased after 1 h of lag phase, surpassing the group in the acidic medium at 1.5 h. However, *E. coli* growth was significantly inhibited in the pH 2 medium compared to the group in the pH 6.8 medium at all time points (**Figure 2 F, 2G**). These data suggested that *Lactobacillus* is tolerant of the

gastric acid condition for at least 1 h; the low pH, rather than repressing, dramatically induces *Lactobacillus* growth for a short time.

To confirm our findings in vivo, LGG was labeled with the fluorescence dye PKH26²¹ and administered to BALB/c mice via oral gavage, and the gut bacteria were purified from the feces of the intestine. Fluorescence intensity analysis indicated that the LGG/PKH26 signal slightly increased, reaching the peak at 1 h after gavage in mice kept on a regular chow diet. However, the LGG/PKH26 signal in the intestine dramatically increased after 10 min gavage in mice with 6 h fasting (**Supplementary Figure 2**). Given 1-2 h time of solid diet in the stomach²², the LGG administered with solid chow diet could lead to repression of bacterial growth by gastric acid. However, more LGG could reach the intestine if bacteria were administered in the fasting condition.

LGG protects against DSS-induced colitis

LGG effects on colitis were investigated in DSS-induced colitis experimental mouse model established by oral administration of 2.5% DSS. Main clinical characteristics of colitis, including diarrhea, fecal bleeding, bodyweight loss, and colon shortening, were observed. The mice were fed with or without LGG (1×10^{10} /d/kg, body weight) for 7 days, and body weight and the length and histology of the intestine were estimated 14 days after DSS treatment (**Figure 2A**). As shown in **Figure 2B**, LGG significantly recovered bodyweight loss caused by DSS, and LGG administration in fasting as well as along with the chow diet in DSS-induced colitis could enhance protection from the bodyweight loss (**Figure 2B**). Also, DSS caused colon shortening, and LGG mitigated its effect; in particular, a significant enhancement in the protective role on the intestine was observed when LGG was administered in fasting (**Figure 2C**). H&E staining also confirmed more protection of histopathologic changes by LGG in fasting conditions (**Figure 2D**). Our findings supported that LGG could protect mice from DSS-induced colitis, and its administration in fasting enhanced the protective effect probably due to the easier passage of LGG to the intestine in fasting conditions.

LGG protects against chemotherapeutic agent-induced colitis

Chemotherapy-induced colitis may manifest in different clinical settings with serious sequelae, impacting patient care and outcomes. We sought to determine whether LGG could play a protective role in chemotherapeutic agent-induced colitis, as seen in DSS-induced colitis (**Figure 3A**). The results indicated that 21 d after administration of docetaxel (20 mg/kg weekly) in mice colitis could be reduced by LGG (**Figures 3B–3D**). Furthermore, mice fed LGG with fasting had a superior protective effect against the taxane-based chemotherapeutic agent docetaxel-induced colitis compared with mice fed LGG without fasting (**Figures 3B–3D**). Mice fed with LGG administration without fasting had a moderate bodyweight loss, but with fasting, LGG mitigated the body weight loss even more (**Figure 3B**). The length of the colon was shortened by docetaxel. However, LGG administration prevented colonic shortening and LGG with fasting exhibited further prevention (**Figure 3C**). The enhanced protection by LGG with fasting was also

confirmed by H&E staining (**Figure 3D**). These results indicated that LGG was protective against taxane-induced colitis, and fasting could enhance the LGG effect.

LGG alleviates gut permeability by up-regulating the expression of tight junction genes

Dysfunction of the gut epithelial barrier is a hallmark of inflammatory intestinal diseases. The intestinal epithelial barrier is maintained by tight junctions that connect adjacent epithelial cells and seal the paracellular space²³. We tested whether LGG may enhance the gut barrier function to protect the intestine from docetaxel-induced colitis. FITC-conjugated dextran was administered to mice by oral gavage. The fluorescence signal in the serum indicated that LGG could reduce the permeability of the intestine, and administration of LGG in fasting significantly improved gut permeability protection (**Figure 4A**). To address the molecular mechanism underlying gut permeability protection by LGG, we performed tight junction gene expression analysis using the mouse Tight Junctions RT² Profiler^{TM} PCR Array (Qiagen, PAMM-143). Eighty-four cell surface receptors involving tight junctions and cell adhesion in the intestine were analyzed. We found that 20 genes were upregulated and 8 genes were downregulated by LGG in docetaxel-treated mice (**Figure 4B**). These results were confirmed by performing qPCR of selected genes. The results suggested that LGG upregulated the expression of claudin 5 (Cldn5), tight junction protein 2 (Tjp2) and integrin beta-5 (Itgb5), consistent with the qPCR array results (**Figure 4C**). The expression of these genes at the protein level was confirmed by Western blotting (**Figure 4D**). Given the potential influence of LGG on the chemotherapeutic agent-induced alteration, we assessed the proinflammatory cytokines in the intestine using ELISA. The results showed that LGG reduced the expression levels of TNF α , IL-1 β , and IL-6 (**Figure 4E**).

LGG protects the intestine from colitis by biofilm formation

Given the biofilm release from microbial species against extreme environments²⁴, we hypothesized that *Lactobacillus* protected the intestine from colitis through the biofilm (**Figure 5A**). We, therefore, investigated biofilm formation by LGG using the crystal violet dye. The optical density measured at 550 nm indicated that LGG generated the biofilm in a dose-dependent manner (**Supplementary Figure 3**). The specificity of the optical density signal for the biofilm was confirmed by adding 10 μ M of aminoimidazole²⁵, the biofilm inhibitor 2, to the LGG medium. As expected, the biofilm formation was repressed by 2-aminoimidazole (**Supplementary Figure 4**). The protective effect of LGG on colitis through biofilm formation was examined by treating mice with docetaxel at 20 mg/kg weekly. LGG reduced colitis via biofilm formation; however, the biofilm inhibitor 2-aminoimidazole abolished the LGG effect (**Figure 5B**), indicating that docetaxel-induced colitis could be relieved by LGG-mediated biofilm formation.

We sought to define the role of *Lactobacillus* in the chemotherapy of tumors by determining the potential LGG benefits in breast cancer treatment with docetaxel. We first generated the breast cancer model using 4T1 cells and found that increasing the docetaxel dose from 10 mg/kg weekly (Lo) to 100 mg/kg weekly (Hi) significantly reduced the primary breast tumor size (**Figure 5C**), volume (**Figure 5D**), and metastasis nodules in the lung. However, the high dose of docetaxel was detrimental to the survival of tumor-bearing

mice due to severe colitis (**Figure 5E**). In the parallel group, LGG administration along with docetaxel reduced the tumor size and metastasis and significantly improved the survival of tumor-bearing mice treated with docetaxel (**Figure 5E**). Collectively, these observations suggested that LGG did not affect tumor size and metastasis directly but alleviated the colitis side effect of chemotherapy and improved the survival of tumor-bearing mice.

Discussion

Probiotics have long been used for maintaining enteric homeostasis and preventing diseases. However, stomach/gastric acid is known to kill germs and bacteria within 15 minutes when pH is less than 3. This study shows that *Lactobacillus* can tolerate pH 2.0 and grows well in the first hour at the acidic pH, but the acid tolerance cannot last more than one hour. In contrast, some pathogenic strains of *E. coli* cannot survive in gastric acid independent of exposure.

LGG of intestinal origin are considered intrinsically resistant to the acid environment and are often employed in fermented foods as probiotics, such as *L. acidophilus*, *L. casei*, and *L. bulgaricus*²⁶. Different brands of yogurts contain different strains of *Lactobacillus* (**Table 1**). Although there are differences between species and strains, these organisms generally exhibit increased sensitivity at pH values below 3.0^{27,28}. In this study, we observed that *L. acidophilus*, *L. casei*, and *L. bulgaricus* from different yogurt brands grew faster in the first hour, and no influence on growth was observed for 2 hours at pH 2.0 compared to the *Lactobacillus* growing in the pH 6.8 medium. Due to the gradient between the extracellular and cytoplasmic pH, cellular functions are inhibited and the cells die when the internal pH reaches a threshold value²⁹. In this case, fasting is necessary to reduce the retention time of food in the stomach after consumption.

Our results have been confirmed by another *Lactobacillus*, LGG, widely used in clinical therapies, including colitis³⁰. LGG, a significant probiotic strain with proven health benefits, showed better growth in acidic conditions in the first 1 h of incubation but restrained growth after 2 h. *E. coli* growth, on the other hand, was significantly inhibited in the pH 2.0 medium compared to the pH 6.8 medium independent of the incubation time. Our study showed acid tolerance of LGG consistent with the previous findings of the highest survival rate of LGG in human gastric and duodenum juice³¹. However, there are also few *E. coli* strains that survive in acidic conditions because these bacteria use a range of physiological, metabolic, and proton-consuming acid resistance mechanisms to survive acid stress in as low as pH 2.0³².

The intestinal tract consists of a diverse microenvironment with more than 500 species of bacteria. A single layer of epithelium separates these commensal microorganisms and pathogens from the underlying immune cells. Thus the epithelial barrier function is a key component in the defense arsenal required to prevent infections and inflammation³³. A sufficient number of probiotics may inhibit pathogenic bacterial adhesion, enhance barrier function, and interact with Toll-like receptors expressed on the intestinal epithelial cells and dendritic cells to produce cytokines/chemokines and further modulate T

cells³³. Probiotics can also produce bioactive metabolites, affect the nervous system, modulate gut motility, reduce pain, and are involved in gut-brain function³³.

Chemotherapy is a mainstay of primary treatment for advanced breast cancer. However, the side effects are major problems limiting the choice of specific chemotherapeutic drugs and doses. Paclitaxel and docetaxel, chemotherapy drugs used for solid tumors, including breast cancer, may cause ischemic colitis³⁴. In patients with breast carcinoma, colitis events were encountered with paclitaxel-based chemotherapy in both the neoadjuvant setting and treatment for metastatic breast carcinoma¹. Growing evidence suggests that probiotics may modify the intestinal microenvironment resulting in a decline in proinflammatory cytokines and reduction of colitis³⁵. However, the efficacy of probiotic treatment in chemotherapy-induced colitis is still debated. Histologic changes representing ischemic colitis and bowel perforation secondary to transmural necrosis have been reported³⁴. Our study has also shown the same histologic changes in colitis caused by docetaxel.

The underlying mechanism of colitis caused by paclitaxel-based chemotherapy is still not known. Both paclitaxel and docetaxel bind to the tubulin subunit, resulting in the formation of stable, nonfunctional microtubule bundles, and interfere with mitosis^{36,37}. Therefore, it was postulated that gastrointestinal necrosis or bowel perforation after paclitaxel administration is a direct drug effect because of the transient mitotic arrest due to nonfunctional microtubule bundles^{38,39}. The paclitaxel-induced colitis has histological features similar to ischemic colitis, characterized by a thinned attenuated surface epithelium, increased fibrosis, neutrophil infiltration, and focal hemorrhage^{40–42}. Colitis of paclitaxel-based chemotherapy can lead to cancer treatment discontinuation, intensive care unit (ICU) admission, and even colonic perforation.

Our study showed that LGG could relieve colitis caused by DSS and chemotherapeutic agent docetaxel, and fasting with LGG could enhance the effect. Reduction of colitis by LGG might allow increased chemotherapeutic dose, leading to enhanced therapeutic efficacy and better survival. Our findings demonstrated that LGG regulated the expression of tight junction genes, *Cldn5*, *Tjp2*, and *Itgb5*, providing insight into the molecular mechanisms by which LGG regulates intestine permeability. Both LGG groups significantly improved mRNA expression of *Cldn5*, *Tjp2*, and *Itgb5*, as evidence of the restoration of the epithelial barrier. The improvement of the barrier function was confirmed by the *in vivo* FITC-dextran 4000 permeability assay, showing statistical differences with the untreated control group. Previous studies of other probiotics have also reported that the improvement of the epithelial barrier function could contribute to the recovery of colonic damage^{43,44}.

As major proinflammatory cytokines, TNF- α and IL-1 β , induce apoptosis of epithelial cells, disrupt the epithelial barrier, and prolong inflammation⁴⁵. In our study, mice were euthanized at the end of LGG treatment to examine changes in intestinal response. We observed modest pathological changes, and colon length was significantly decreased by DSS or docetaxel in mice without LGG treatment. Additionally, TNF- α was reduced in the colon of mice treated with oral-gavaged LGG, further confirming

that colitis was relieved in the LGG-administered mice. The colonic IL-6 expression increased in the colitis model and its antibody showed beneficial effects ⁴⁶. In the present study, LGG administration significantly reduced the expression of colonic TNF- α , IL-1 β , and IL-6 in docetaxel-induced colitis, probably caused by the reduction in infiltration of activated monocytes and macrophages. One previous study also demonstrated that LGG improved colitis with goblet cells by reducing MUC2 expression ⁴⁷. LGG may accelerate ulcerative colitis recovery of the animals ⁴⁷, which is consistent with our study. Downregulation of intestinal trefoil factor TFF3 is caused by repression of transcription through TNF- α and NF κ B activation *in vitro*. This result is consistent with the view that probiotics have to survive the gastrointestinal environment in large quantity and adhere to intestinal cells to exert health functions ⁴⁸.

In general, lactic acid bacteria, especially the species belonging to the genus *Lactobacillus*, such as *L. acidophilus*, *L. rhamnosus*, *L. gasseri*, *L. fermentum*, and *L. plantarum* employ a variety of mechanism for protecting the intestine from inflammation, including phagocytosis regulation ⁴⁹, cell surface hydrophobicity adaption, and metabolic inhibitory activities against pathogens ⁵⁰ that are beneficial to health. However, these endogenous mechanisms may be ineffective when cells are exposed to a wide variety of chemical agents. Therefore, the addition of exogenous protectants is essential for probiotics to endure highly toxic chemical agents. Biofilm can protect microorganisms from harsh environmental conditions such as extreme temperature and pH, high salinity and pressure, poor nutrients, antibiotics, etc., by acting as a barrier ⁵¹. Our analysis of biofilm formation elucidated its role in the LGG anti-colitis effect. Furthermore, the biofilm inhibitor 2-Aminoimidazoles could abolish the probiotic effect on the intestine. 2-Aminoimidazoles are an emerging class of small molecules that inhibit and disperse biofilms in bacteria ²⁵. Herein we provided a novel biofilm function and an alternative mechanism to explain colitis protection by probiotics. Notably, our findings demonstrated that LGG alleviated the colitis side effect of chemotherapy and extended the survival of mice with advanced breast cancer following high-dose chemotherapy.

Conclusion

In conclusion, LGG exhibits accelerated growth at pH 2.0 in the first hour but fails to do so later, suggesting that LGG must be consumed on an empty stomach and not with food to exploit the probiotic activity. LGG is beneficial in conjunction with the chemotherapeutic agent docetaxel in breast cancer because of biofilm formation and regulation of gut tight junctions. This study provides a new therapeutic strategy for improving the chemotherapeutic efficacy in tumors.

Abbreviations

DSS: dextran sulfate sodium

E. coli : Escherichia coli

LGG : Lactobacillus rhamnosus

TFF : trefoil factors

PBS : phosphate-buffered saline

OD: optical density

FITC: Fluorescein-5-isothiocyanate

H&E: hematoxylin and eosin

HRP: horseradish peroxidase

Ct: comparative threshold cycle

SDS-PAGE: sodium dodecyl sulfate-polyacrylamide gel electrophoresis

SD: standard deviation

ICU: intensive care unit

TNF- α : tumor necrosis factor-alpha

IL-1 β : interleukin-1beta

IL-6: Interleukin 6

Declarations

Availability of data and materials

The datasets used and/or analyzed during the current study can be obtained from the corresponding author upon reasonable request.

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Contributions

S.W., Y.R. designed the study, analyzed, interpreted data, and X.Q. prepared the manuscript; X.Q., X.H., and X.Z. performed experiments and interpreted data; L.Z. did histological analysis; Z.L. and M.L. purified the bacteria.

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Ethics declarations

Conflict of interest

The authors declare no competing interests to this work.

Ethical approval

All animal experiments were approved by the Institutional Animal Care and Use Committee of Nanjing Medical University, China. This article does not contain any studies with human participants performed by any of the authors.

Informed consent

Not applicable.

Competing interests

All the authors declare that they have no competing interests.

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Supplementary Information

The document of supplementary materials is the supplementary data.

References

1. Li Z, et al. Colitis in patients with breast carcinoma treated with taxane-based chemotherapy. *Cancer*. 2004;101:1508–13. doi:10.1002/cncr.20546.
2. Okayasu I, et al. A novel method in the induction of reliable experimental acute and chronic ulcerative colitis in mice. *Gastroenterology*. 1990;98:694–702. doi:10.1016/0016-5085(90)90290-h.
3. Ibrahim NK, et al. Colitis associated with docetaxel-based chemotherapy in patients with metastatic breast cancer. *Lancet*. 2000;355:281–3. doi:10.1016/s0140-6736(99)06195-4.
4. Ravdin PM. Taxoids: effective agents in anthracycline-resistant breast cancer. *Semin Oncol*. 1995;22:29–34.
5. Crown J, O'Leary M. The taxanes: an update. *Lancet*. 2000;355:1176–8. doi:10.1016/s0140-6736(00)02074-2.
6. Salim SY, Söderholm JD. Importance of disrupted intestinal barrier in inflammatory bowel diseases. *Inflamm Bowel Dis*. 2011;17:362–81. doi:10.1002/ibd.21403.
7. Walker WA. Development of the intestinal mucosal barrier. *J Pediatr Gastroenterol Nutr*. 2002;34 **Suppl**(1):33–9. doi:10.1097/00005176-200205001-00009.
8. Mayer L. Mucosal immunity. *Pediatrics*. 2003;111:1595–600.
9. Jergens AE, et al. Induction of differential immune reactivity to members of the flora of gnotobiotic mice following colonization with *Helicobacter bilis* or *Brachyspira hyodysenteriae*. *Microbes Infect*. 2006;8:1602–10. doi:10.1016/j.micinf.2006.01.019.
10. Yan Y, et al. Temporal and spatial analysis of clinical and molecular parameters in dextran sodium sulfate induced colitis. *PLoS One*. 2009;4:e6073. doi:10.1371/journal.pone.0006073.

11. Elli M, et al. Survival of yogurt bacteria in the human gut. *Appl Environ Microbiol.* 2006;72:5113–7. doi:10.1128/aem.02950-05.
12. Charalampopoulos D, Pandiella SS, Webb C. Evaluation of the effect of malt, wheat and barley extracts on the viability of potentially probiotic lactic acid bacteria under acidic conditions. *Int J Food Microbiol.* 2003;82:133–41. doi:10.1016/s0168-1605(02)00248-9.
13. Scott RB. Cancer chemotherapy—the first twenty-five years. *Br Med J.* 1970;4:259–65. doi:10.1136/bmj.4.5730.259.
14. Bradshaw-Pierce EL, Steinhauer CA, Raben D, Gustafson DL. Pharmacokinetic-directed dosing of vandetanib and docetaxel in a mouse model of human squamous cell carcinoma. *Mol Cancer Ther.* 2008;7(9):3006–17. doi:10.1158/1535-7163.MCT-08-0370.
15. Chen E, et al. Clinical characteristics of colitis induced by taxane-based chemotherapy. *Ann Gastroenterol.* 2020;33:59–67. doi:10.20524/aog.2019.0431.
16. Teng Y, et al. Plant-Derived Exosomal MicroRNAs Shape the Gut Microbiota. *Cell Host Microbe.* 2018;24:637–52.e638. doi:10.1016/j.chom.2018.10.001.
17. Qiu X, et al. Tumor-derived nanovesicles promote lung distribution of the therapeutic nanovector through repression of Kupffer cell-mediated phagocytosis. *Theranostics.* 2019;9:2618–36. doi:10.7150/thno.32363.
18. Pienaar JA, Singh A, Barnard TG. Acid-happy: Survival and recovery of enteropathogenic *Escherichia coli* (EPEC) in simulated gastric fluid. *Microb Pathog.* 2019;128:396–404. doi:10.1016/j.micpath.2019.01.022.
19. Olivares A, Silva P, Altamirano C. Microencapsulation of probiotics by efficient vibration technology. *J Microencapsul.* 2017;34:667–74. doi:10.1080/02652048.2017.1390005.
20. Tractenberg RE, et al. Effects of Intravesical *Lactobacillus Rhamnosus* GG on Urinary Symptom Burden in People with Neurogenic Lower Urinary Tract Dysfunction. *Pm r.* 2020. doi:10.1002/pmrj.12470.
21. Teng Y, et al. Plant-Derived Exosomal MicroRNAs Shape the Gut Microbiota. *Cell Host Microbe* **24**, 637–652 e638, doi:10.1016/j.chom.2018.10.001 (2018).
22. Okabe T, Terashima H, Sakamoto A. A comparison of gastric emptying of soluble solid meals and clear fluids matched for volume and energy content: a pilot crossover study. *Anaesthesia.* 2017;72:1344–50. doi:10.1111/anae.14026.
23. Wawrzyniak P, et al. Nutritional Lipids and Mucosal Inflammation. *Mol Nutr Food Res.* 2021;65:e1901269. doi:10.1002/mnfr.201901269.
24. Turrini P, et al. The microbial community of a biofilm lining the wall of a pristine cave in Western New Guinea. *Microbiol Res.* 2020;241:126584. doi:10.1016/j.micres.2020.126584.
25. Gill RK, et al. Polysubstituted 2-aminoimidazoles as anti-biofilm and antiproliferative agents: Discovery of potent lead. *Eur J Med Chem.* 2017;138:152–69. doi:10.1016/j.ejmech.2017.06.043.

26. Tannock GW. A special fondness for lactobacilli. *Appl Environ Microbiol.* 2004;70:3189–94. doi:10.1128/aem.70.6.3189-3194.2004.
27. Rönkä E, et al. Probiotic and milk technological properties of *Lactobacillus brevis*. *Int J Food Microbiol.* 2003;83:63–74. doi:10.1016/s0168-1605(02)00315-x.
28. Jin LZ, Ho YW, Abdullah N, Jalaludin S. Acid and bile tolerance of *Lactobacillus* isolated from chicken intestine. *Lett Appl Microbiol.* 1998;27:183–5. doi:10.1046/j.1472-765x.1998.00405.x.
29. Konings WN, Poolman B, Driessen AJ. Bioenergetics and solute transport in lactococci. *Crit Rev Microbiol.* 1989;16:419–76. doi:10.3109/10408418909104474.
30. Ryu SH, et al. The Probiotic *Lactobacillus* Prevents *Citrobacter rodentium*-Induced Murine Colitis in a TLR2-Dependent Manner. *J Microbiol Biotechnol.* 2016;26:1333–40. doi:10.4014/jmb.1602.02004.
31. Faye T, Tamburello A, Vegarud GE, Skeie S. Survival of lactic acid bacteria from fermented milks in an in vitro digestion model exploiting sequential incubation in human gastric and duodenum juice. *J Dairy Sci.* 2012;95:558–66. doi:10.3168/jds.2011-4705.
32. Kanjee U, Houry WA. Mechanisms of acid resistance in *Escherichia coli*.
33. Ohland CL, Macnaughton WK. Probiotic bacteria and intestinal epithelial barrier function. *Am J Physiol Gastrointest Liver Physiol.* 2010;298:G807–19. doi:10.1152/ajpgi.00243.2009.
34. Ibrahim NK, et al. Colitis associated with docetaxel-based chemotherapy in patients with metastatic breast cancer.
35. Zaharuddin L, Mokhtar NM, Nawawi M. K. N. & Raja Ali, R. A. A randomized double-blind placebo-controlled trial of probiotics in post-surgical colorectal cancer. *BMC Gastroenterol.* 2019;19:131. doi:10.1186/s12876-019-1047-4.
36. Ringel I, Horwitz SB. Studies with RP 56976 (taxotere): a semisynthetic analogue of taxol. *J Natl Cancer Inst.* 1991;83:288–91. doi:10.1093/jnci/83.4.288.
37. Rowinsky EK, Cazenave LA, Donehower RC. Taxol: a novel investigational antimicrotubule agent. *J Natl Cancer Inst.* 1990;82:1247–59. doi:10.1093/jnci/82.15.1247.
38. Hruban, R. H., Yardley, J. H., Donehower, R. C. & Boitnott, J. K. Taxol toxicity. Epithelial necrosis in the gastrointestinal tract associated with polymerized microtubule accumulation and mitotic arrest. *Cancer* **63**, 1944–1950, doi:10.1002/1097-0142(19890515)63:10<1944::aid-cnrcr2820631013>3.0.co;2-# (1989).
39. Seewaldt VL, et al. A retrospective review of paclitaxel-associated gastrointestinal necrosis in patients with epithelial ovarian cancer. *Gynecol Oncol.* 1997;67:137–40. doi:10.1006/gyno.1997.4842.
40. Elsayed AG, Srivastava R, Pacioles T, Limjoco T, Tirona MT. Ischemic Colitis Associated with Paclitaxel and Carboplatin Combination. *Case Rep Oncol.* 2017;10:689–93. doi:10.1159/000479226.
41. Daniele B, Rossi GB, Losito S, Gridelli C, de Bellis M. Ischemic colitis associated with paclitaxel. *J Clin Gastroenterol.* 2001;33:159–60. doi:10.1097/00004836-200108000-00015.

42. Sodhi KS, Aiyappan SK, Singh G, Prakash M, Khandelwal N. Colitis and colonic perforation in a patient with breast carcinoma treated with taxane based chemotherapy. *Indian J Cancer*. 2011;48:134–5. doi:10.4103/0019-509x.76647.
43. Krishnan M, Penrose HM, Shah NN, Marchelletta RR, McCole DF. VSL#3 Probiotic Stimulates T-cell Protein Tyrosine Phosphatase-mediated Recovery of IFN- γ -induced Intestinal Epithelial Barrier Defects. *Inflamm Bowel Dis*. 2016;22:2811–23. doi:10.1097/mib.0000000000000954.
44. Kumar M, Kissoon-Singh V, Coria AL, Moreau F, Chadee K. Probiotic mixture VSL#3 reduces colonic inflammation and improves intestinal barrier function in Muc2 mucin-deficient mice. *Am J Physiol Gastrointest Liver Physiol*. 2017;312:G34-g45. doi:10.1152/ajpgi.00298.2016.
45. Grabinger T, et al. Inhibitor of Apoptosis Protein-1 Regulates Tumor Necrosis Factor-Mediated Destruction of Intestinal Epithelial Cells. *Gastroenterology*. 2017;152:867–79. doi:10.1053/j.gastro.2016.11.019.
46. Sommer J, et al. Interleukin-6, but not the interleukin-6 receptor plays a role in recovery from dextran sodium sulfate-induced colitis. *Int J Mol Med*. 2014;34:651–60. doi:10.3892/ijmm.2014.1825.
47. Wu H, et al. Lactobacillus acidophilus Alleviated Salmonella-Induced Goblet Cells Loss and Colitis by Notch Pathway. *Mol Nutr Food Res*. 2018;62:e1800552. doi:10.1002/mnfr.201800552.
48. Toscano M, De Grandi R, Pastorelli L, Vecchi M, Drago L. A consumer's guide for probiotics: 10 golden rules for a correct use. *Dig Liver Dis*. 2017;49:1177–84. doi:10.1016/j.dld.2017.07.011.
49. Rocha-Ramirez LM, et al. Probiotic Lactobacillus Strains Stimulate the Inflammatory Response and Activate Human Macrophages. *J Immunol Res* 2017, 4607491, doi:10.1155/2017/4607491 (2017).
50. Wang RM, Li N, Zheng K, Hao JF. Enhancing acid tolerance of the probiotic bacterium Lactobacillus acidophilus NCFM with trehalose. *FEMS Microbiol Lett*. 2018. doi:10.1093/femsec/fny217.
51. Rakoff-Nahoum S, Foster KR, Comstock LE. The evolution of cooperation within the gut microbiota. *Nature*. 2016;533:255–9. doi:10.1038/nature17626.

Tables

Table 1 Species of probiotic in the yogurts

	Suki	OIKOS	Yoplait
<i>L. acidophilus</i>		+	+
<i>L. casei</i>		+	
<i>L. bulgaricus</i>	+	+	

Table 2 Primer sequences used for gene expression analysis by qPCR

Primers	Forward (5'-3')	Reverse (5'-3')
Cldn5	TGCTGCCTTAATGTCCAGTG	CTTCCAGGAGGAAGGCAAC
Tjp2	GCACCATGCCTAAAGCTGTC	ACTCAACACACCACCATTC
Itgb5	GGCCAGCTCCGTCTATGATG	TGGATGTCTGAGCCATTAAGGA
Gapdh	GGTCGGTGTGAACGGATTG	GGAGTCATACTGGAACATGTAG

Figures

Figure 1

Effect of pH on growth of *Lactobacillus* from yogurt (A) 100 μ l of yogurt from Suki, Yoplait, and Oikos were grown in 1 ml of MRS *Lactobacillus* growth medium at pH 2.0 or 6.8. The proliferation of *Lactobacillus* at different time points (x-axis) was evaluated as absorbance at 600 nm wavelength (y-axis). (B, C) 50 μ l of the medium at 1 h (B, left) and 3 h (C, left) was spread on MRS agar plates and incubated in an anaerobic chamber for 48 h. Quantification of colonies on the plates shown as a bar graph (right panels). (D, F) 1.0 x 10⁸ μ l of LGG and *E. coli* were grown in 1 ml of MRS and LB media at pH 2.0 or 6.8. The proliferation of LGG and *E. coli* at different time points (x-axis) was evaluated as absorbance at 600 nm wavelength (y-axis). (E, G) 50 μ l of medium from LGG and *E. coli* at 1 h (up) and 3 h (bottom) was spread on MRS or LB agar plate and incubated for 24 h. Quantification of colonies on the plates shown as a bar graph (right panels). The graph represents three independent experiments with each bar as mean $\pm$ standard deviation (SD). Two-tailed Student's t-test, * p <0.05, ** p <0.01; pH 2.0 vs pH 6.8.

Figure 2

LGG-mediated protection against mouse colitis caused by DSS (A) Schematic representation of the treatment schedule for DSS-induced colitis. BALB/C mice were given 2.5 % DSS in autoclaved water throughout the experiment. Seven days after DSS administration, LGG ($5 \times 10^{10}/\text{kg}$) was administered to BALB/C mice by oral gavage twice a week with/without 2 h fasting. (B) Representative photographs of the colon (left panel) and quantification of colon length (right panel). (C) Body weight; (D) H&E-stained colon sections (400x magnification) from BALB/C mice treated as labeled in the figure. Data are representative of three independent experiments (error bars, SD). * $P < 0.05$, ** $P < 0.01$ (two-tailed t-test).

Figure 3

LGG-mediated protection against mouse colitis caused by the chemotherapeutic agent docetaxel (A) Schematic representation of the treatment schedule for docetaxel-induced colitis. BALB/C mice (n=5 mice per group) were administered with 6mg/kg docetaxel via intraperitoneal injection once four days. 7 days after docetaxel administration, LGG (5×10^{10} /kg) administered to BALB/C mice by oral gavage twice a week with/without 2 h fasting. 28 days after docetaxel administration, mice were euthanized. (B) Representative colons (left panel); quantification of colon length (right panel); (C) Body weight. (D) H&E-stained sections of the colon (400x magnification) from BALB/C mice treated as labeled in the figure. Data are representative of three independent experiments (error bars, SD). * $P < 0.05$, ** $P < 0.01$ (two-tailed t-test).

Figure 4

Regulation of intestine permeability and inflammation by LGG-induced tight junction gene expression (A) Mice (n=5) were treated with LGG via oral gavage twice a week with/without 2 h fasting followed by intraperitoneal injection of docetaxel. The mice were oral-gavaged with FITC-dextran 4000 (60 mg/100 g of body weight) and serum collected after 5 h to determine intestinal permeability. (B) Heat map showing a significant change ($P < 0.01$) in the cell junction pathway in colon tissues by LGG or E. coli using a qPCR array. Red and green colors represent elevated and decreased expression of mRNAs, respectively. (C) Analysis of tight junction genes using individual qPCR. (D) Analysis of tight junction proteins using Western blotting (left). Quantification of intensity in Western blots (right). (E) Analysis of cytokines in the intestine by ELISA. Data are representative of three independent experiments (error bars, SD). * $P < 0.05$, ** $P < 0.01$ (two-tailed t-test).

Figure 5

Protection of the intestine from colitis by LGG-mediated biofilm formation (A) Schematics represent biofilm formation by bacteria and molecular structure of biofilm inhibitor 2-aminoimidazole (AI). (B) Docetaxel (50 mg/kg) was administered to BALB/C mice ($n=5$ mice per group) via intraperitoneal injection every other day. Seven days after DSS administration, LGG (5×10^{10} /kg) and AI were administered to mice by oral gavage twice a week with/without fasting for 2 h. Representative colons (left panel); quantification of colon length (right panel). (C) 2×10^5 4T1 cells implanted into the mammary fat pad of BALB/c mice ($n=5$ each group). Seven days after inoculation of 4T1 cells, mice were treated with docetaxel (20 mg/kg, Lo; 100 mg/kg, Hi) and LGG via i.p. injection and oral gavage, respectively. Representative 4T1 breast primary tumor (left); Quantification of tumor volume (right). (D) Representative 4T1 lung (right, metastatic nodules indicated by arrows). Quantification of metastasis nodule number (> 1 m). (E) Survival of 4T1-bearing BALB/c mice treated with docetaxel and LGG. Data are representative of three independent experiments (error bars, SD). * $P < 0.05$, ** $P < 0.01$ (two-tailed t-test).

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