Anti-inflammatory effects of tegoprazan, a novel potassium-competitive acid blocker, in mice colitis model

Mi Jeong SON

Department of Medical Science

The Graduate School, Yonsei University

Anti-inflammatory effects of tegoprazan, a novel potassium-competitive acid blocker, in mice colitis model

Mi Jeong SON

Department of Medical Science

The Graduate School, Yonsei University

Anti-inflammatory effects of tegoprazan, a novel potassium-competitive acid blocker, in mice colitis model

Directed by Professor Jae Hee Cheon

The Master's Thesis
submitted to the Department of Medical Science,
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree
of Master of Medical Science

Mi Jeong Son

December 2020

This certifies that the Master's Thesis of
Mi Jeong Son is approved.

Thesis Supervisor : Jae Hee Cheon

Thesis Committee Member : Jae Jun Park

Thesis Committee Member : Hyun Jung Lee

The Graduate School
Yonsei University

December 2020

ACKNOWLEDGEMENTS

I would like to thank my thesis supervisor, Professor Jae Hee Cheon, who allowed me to finish my thesis.

I would also like to appreciate Dr. Seung Won Kim for his guidance.

Especially, special gratitude goes to my parents, who have provided unconditional love and care. Words cannot express my appreciation for your dedication and sacrifice.

Last but not least, Thank you to all the individuals who helped and trusted me.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Anti-inflammatory effects of tegoprazan, a novel potassium-competitive acid blocker, in mice colitis model

Mi Jeong Son

Department of Medical Science
The Graduate School, Yonsei University

(Directed by Professor Jae Hee Cheon)

Inflammatory bowel diseases (IBD), including both ulcerative colitis (UC) and Crohn's disease (CD) are autoimmune diseases characterized by progressive inflammatory condition that affects the damage in the gastrointestinal tract. The etiology of IBD remains unclear, but it is thought that IBD results from abnormal intestinal immunity and altered gut microbiota caused by environmental factors such as diet and infection in genetically susceptible individuals. Specifically, gut microbiota can influence intestinal functional integrity, barrier strength, and permeability regulation, thereby affecting the immune response, which has been linked to the development of IBD.

Proton pump inhibitors (PPIs) are widely used medication for gastric acid inhibition. However, long-term PPI administration can alter the composition of the intestinal microbiome, which worsens the severity of disease in IBD patients.

The present study demonstrated that tegoprazan significantly ameliorates the symptoms of DSS-induced colitis such as body weight loss, diarrhea, bleeding, colon shortening, and histological damage and enhanced intestinal epithelial barrier function by upregulating tight junction protein expression. Tegoprazan significantly improved the symptoms of colitis, even in a

DNBS-induced colitis model. Above all, tegoprazan alleviated gut microbiota dysbiosis and enhanced the growth of *Bacteroides vulgatus* (*B.vulgatus*). *B. vulgatus* alleviated intestinal inflammation by inhibiting the epithelial adhesion of pathogenic bacteria.

Especially, unlike rabeprazole, Tegoprazan did not have adverse effects such as gut dysbiosis. These results suggest that Tegoprazan has potential as a therapeutic agent for IBD.

Key words: inflammatory bowel disease, tegoprazan, potassium-competitive acid blocker, proton pump inhibitor, *bacteroides vulgatus*, microbiome

Anti-inflammatory effects of tegoprazan, a novel potassium-competitive acid blocker, in mice colitis model

Mi Jeong Son

Department of Medical Science
The Graduate School, Yonsei University

(Directed by Professor Jae Hee Cheon)

I. INTRODUCTION

Inflammatory bowel diseases (IBD), including both ulcerative colitis (UC) and Crohn's disease (CD), are autoimmune diseases characterized by progressive inflammatory condition that affects the damage in the gastrointestinal tract, resulting in various clinical symptoms.[1] UC is usually restricted to the colon and characterized by transmucosal inflammation in continuous pattern. In contrast, CD may affect any part of the GI tract from the mouth to the anus and is characterized by transmural inflammation, skip lesions, and granuloma formation.[2] The incidence rates and prevalence of IBD are the highest in North America and Europe, but several recent epidemiological studies have shown that the incidence of IBD has increased over the past decades in Asia countries, including Korea.[3,4] The etiology of IBD remains unclear, it is thought that IBD results from abnormal and continuing immune response to the microbes in the gut, catalyzed by the individual's genetic susceptibility.[5]

The human gastrointestinal (GI) tract harbors a complex and dynamic population of microorganisms, the gut microbiota, which plays a fundamental role in maintaining our health. The gut microbiota plays a major role in maintaining immune and metabolic homeostasis and protecting against pathogens. The microbiota provides numerous benefits for the host,

such as metabolism, de novo synthesis of nutrients, and immune system development.[6] It also helps maintain host resistance by modulating the intestinal barrier integrity through the mucus layer, tight junction proteins, and antimicrobial peptides.[7] But the imbalance of gut microbiota, also known as 'dysbiosis,' leads to epithelial dysfunction and intestinal permeability. These epithelial defects may represent a primary dysfunction in Crohn's disease and may perpetuate chronic mucosal inflammation in ulcerative colitis.[8,9]

Proton pump inhibitors (PPIs) are used worldwide to treat acid-related disorders such as gastroesophageal reflux disease (GERD), peptic ulcers, and *Helicobacter pylori* infection. They inhibit H+, K+-adenosine triphosphatase (H+/K+-ATPase) in the parietal cells, involved in the final step of gastric acid secretion.[10] Although PPIs have a good safety profile, several PPI-related adverse effects have been reported, including bone fractures,[11] pneumonia,[12] *Clostridium difficile* infection,[13] and imbalance in gut microbiota composition.[14] Recent studies have also found that long-term use of PPIs exacerbates the severity of IBD patients and increases the risk of IBD-related hospitalization or surgery.[15] The exact cause of these adverse effects remains unclear, but it is thought that the reduction of gastric acid secretion is a significant risk factor. Gastric acid is essential for maintaining homeostasis within the gastrointestinal tract by preventing bacterial overgrowth.[16] But PPIs reduce the acidity of the stomach, allowing the survival of microbes normally killed by the gastric environment, which can increase the risk of gut dysbiosis.[14]

To overcome these limitations of PPIs therapy, potassium-competitive acid blockers (P-CABs), a new class of gastric acid suppressants, have been developed.[17] Unlike PPIs, P-CABs directly inhibit gastric H+/K+-ATPase in a K+-competitive and reversible manner and do not require the acid activation process for inhibition.[18,19] Also, P-CABs have a stronger and longer-lasting effect on gastric acid suppression than traditional PPIs such as

rabeprazole and omeprazole.[20] Tegoprazan, a novel potassium-competitive acid blocker (P-CAB), was recently approved in Korea before entering the world market. Presently, tegoprazan is widely prescribed as a treatment for GERD, eradicating *Helicobacter pylori*, and treating peptic ulcers.[21] However, it has not been investigated whether gastric acid suppression by P-CAB affects the exacerbation of IBD.

In this study, we aimed to clarify whether PPIs-induced worsening of intestinal damage and dysbiosis were due to the acid-suppressive effect or a specific drug class effect of PPIs. Furthermore, we investigated the therapeutic effects of tegoprazan against colitis in mice modes and determined whether it has the potential as a therapeutic agent for IBD.

II. MATERIALS AND METHODS

1. Animals

Male C57BL/6 mice, 6–8 wks old, were purchased from OrientBio (Sungnam-si, Gyeonggido, South Korea) and allowed to acclimate for one week before starting the experiment. Mice were maintained at an ambient temperature of 22°C under a 12-hour light/dark cycle in a specific pathogen- free (SPF) facility.

All experiments using animals were approved by Yonsei University Institutional Animal Care and Use Committee (IACUC; Approval No. 2018-0304, 2020-0160) and were carried out following the guidelines of the IACUC.

2. Dextran sulfate sodium (DSS)-induced colitis model

Experimental ulcerative colitis was induced by providing 2% (w/v) DSS (M.W. 36-50 kDa; MP Biomedicals, Santa Ana, CA, USA) in distilled drinking water for 5 days, followed by normal drinking water until the end of the experiment. Mice were randomly divided into four groups.

i) Control group (CON)

ii) DSS + vehicle group (DSS)

iii) DSS + tegoprazan treated group (DSS+TEGO)

iv) DSS + rabeprazole treated group (DSS+RPZ)

The control group received normal drinking water without DSS during the entire experimental period. Tegoprazan (30 mg/kg) and rabeprazole (30 mg/kg) were administered orally twice daily throughout the experiment. All drugs were dissolved in 0.5% (w/v) methylcellulose (Sigma-Aldrich, St. Louis, MO, USA). On day 9, blood was collected from the heart of anesthetized mice, and the entire colon was removed by autopsy for further studies.

To further determine whether *B. vulgatus* can attenuate the severity of

colitis, experimental colitis was induced by DSS as previously described. Mice were randomly divided into six groups.

i) Control group (CON)

ii) *B. vulgatus group* (BV)

iii) DSS + vehicle group (DSS)

iv) DSS + *B. vulgatus* group (DSS+BV)

v) DSS+tegoprazan group (DSS+TEGO)

vi) DSS + *B. vulgatus* + tegoprazan group (DSS+BV+TEGO)

B. vulgatus ($5x10^8$ CFU/mouse/day) and tegoprazan (30 mg/kg BID) administered by oral gavage until the end of the experiment.

3. Dinitrobenzene sulfonic acid (DNBS)-induced colitis model

To investigate the effect of tegoprazan in Crohn's disease, experimental colitis was induced by rectal administration of DNBS (Sigma-Aldrich, St. Louis, MO, USA). Mice were fasted for 24 hrs before the induction of colitis and lightly anesthetized by inhalation of isoflurane. Then, a polyethylene catheter was inserted into the rectum, and 5 mg of DNBS dissolved in 100 μL of 50% ethanol was administered into the colon. The control group was administered with 50% ethanol alone. Mice were randomly divided into four groups.

i) Control group (CON)

ii) DNBS + vehicle group (DNBS)

iii) DNBS + tegoprazan treated group (DNBS+TEGO)

iv) DNBS + rabeprazole treated group (DNBS+RPZ)

The control group received normal drinking water without DSS during the entire experimental period. Tegoprazan (30 mg/kg) and rabeprazole (30 mg/kg) were administered orally twice daily throughout the experiment. All drugs were dissolved in 0.5% (w/v) methylcellulose (Sigma-Aldrich, St. Louis, MO, USA). On day 5, the entire colon was removed by autopsy for further studies.

4. Assessment of colitis

A. Disease activity index (DAI)

Mice were checked daily for colitis development by monitoring weight loss, rectal bleeding, and stool consistency (summarized in Table 1). The percentage of body weight (BW) loss was calculated relative to the initial BW (Day 0) using the following method: [(Weight on day X−Initial weight)/Initial weight] × 100.

Table 1. Disease activity index (DAI) scoring system

Score	Body weight loss (%)	Stool consistency	Rectal bleeding
0	0-1%	Normal	Normal
1	1-5%	Moist and sticky	Visible blood
2	5-10%	Soft	Slight bleeding
3	10-20%	Diarrhea	Gross bleeding
4	>20%	-	-

B. Histological analysis

Colon tissues were fixed in 10% neutral formalin solution overnight, embedded in paraffin, and stained periodic-acid Schiff reagent (PAS). Images were acquired using a light microscope (Olympus BX41; Olympus Optical, Tokyo, Japan). The severity of symptoms was determined by scoring the extent of bowel wall thickening, crypt damage, and the infiltration of inflammatory cells. (summarized in Table 2).[22] Goblet cell loss of colon tissues was also evaluated using ImageJ software.

Table 2. Histological scoring system

Score	Severity of inflammation	Extent of injury	Crypt damage
0	None	None	None
1	Slight	Mucosal	Basal 1/3 damaged
2	Moderate	Submucosal	Basal 2/3 damaged
3	Severe	Transmural	Only surface epithelium intact
4	-	-	Entire crypt and epithelium lost

5. *In vivo* intestinal permeability assay

Intestinal permeability was evaluated by measuring paracellular permeability to 4 kDa fluorescein isothiocyanate (FITC)-dextran (Sigma-Aldrich, St. Louis, MO, USA) on the day of sacrifice. Mice were administered 150 μl of 80 mg/ml FITC-dextran was administered orally, and blood was collected 4 hrs after administration. Fluorescence intensity was measured using a fluorescent microplate reader (excitation, 490 nm; emission, 520 nm; Varioskan Flash; Thermo Fisher Scientific, Waltham, MA, USA).

6. Extraction of RNA and quantitative real-time reverse-transcription polymerase chain reaction

Total RNA was extracted using TRIzol Reagent (Life Technologies, Carlsbad, CA, USA) and Ribospin™ total RNA purification kit (GeneAll biotechnology, Seoul, Korea). 1 μg of RNA was reverse-transcribed using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions.

Amplification was performed using StepOne Plus real-time PCR system (Applied Biosystems, Foster City, CA, USA) for 45 cycles using the following

thermocycling steps: 95°C for 30 secs, 59-61°C for 30 secs, and 72°C for 40 secs. Gene expression levels were calculated after normalization to the standard housekeeping gene *bactin* using the fold change method. Primers used for real-time PCR are listed in Table 3.

Table 3. Primers used for real time PCR

Gene	Sequence (5'-3')
	Mouse
Tnfa	F: CAAAGGGAGAGTGGTCAGGT
	R: ATTGCACCTCAGGGAAGAGT
Il1b	F: GCAACTGTTCCTGAACTCAACT
	R: ATCTTTTGGGGTCCGTCAACT
Il6	F: TTGCCTTCTTGGGACTGATG
	R: CCACGATTTCCCAGAGAACA
Muc2	F: GGTCCAGGGTCTGGATCACA
	R: GCTCAGCTCACTGCCATCTG
Kcnj1	F: GGTAAGACGGTGGAAGTGGA
	R: TTTGGGTGTCGTCTGTTTCA
Kcnma1	F: GACGTTCTGAGCGTGACTG
	R: TGGTGGAGCAATCATTAACAGAG
Zo1	F: ACCCGAAACTGATGCTGTGGATAG
	R: AAATGGCCGGGCAGAACTTGTGTA
Occludin	F: CTCTCAGCCAGCGTACTCTT
	R: CTCCATAGCCACCTCCGTAG
bactin	F: AGTGTGACGTTGACATCCGT
	R: TGCTAGGAGCCAGAGCAGTA

F: forward primer, **R**: reverse primer

7. Immunohistochemistry (IHC)

Colon sections were deparaffinized in xylene and ethanol and rehydrated in water. Antigen retrieval using sodium citrate buffer (pH 6.0) was performed. Sections were quenched in 0.3% hydrogen peroxide to block endogenous peroxidase activity and blocked in 3% bovine serum albumin (BSA) diluted in PBST for 30 mins at room temperature. The sections were incubated with primary antibody diluted in blocking buffer overnight at 4°C, and biotinylated secondary antibody was applied for 30 mins at room temperature. The VECTASTAIN® Elite ABC-HRP Kit (Vector Laboratories, Inc., Burlingame, CA, USA) was used, and staining was visualized using the DAB Substrate Kit (Vector Laboratories, Inc., CA, California, USA). Slides were counterstained with hematoxylin, dehydrated, mounted, and then observed using a microscope.

8. Metagenome analysis of microbiome

According to the manufacturer's instructions, bacterial genomic DNA was obtained from cecal tissues using a SPIN for Soil Kit (MP Biomedicals, Santa Ana, CA, USA). Extracted DNA was amplified using bar-coded primers flanking the V3-V4 region of the 16S rRNA gene by ChunLab Inc. (Seoul, Korea). Microbiome data was analyzed with the 16S-based Microbial taxonomic profiling (MTP) platform of EzBio-Cloud (ChunLab Inc., Seoul, Korea). All 16S rRNA sequences were deposited in the ChunLab's EzBioCloud Microbiome Database.[23]

9. Cell culture

Human colon carcinoma cell lines HT-29 (Korea Cell Line Bank, Seoul, South Korea) and Caco-2 (ATCC, Manassas, VA, USA) were maintained at 37°C in a humidified incubator of 5% CO_2. HT-29 cells were cultured in RPMI 1640 medium (HyClone™, LOGAN, UT, USA) containing 10% heat‑inactivated fetal bovine serum (FBS) (Ab frontier, Seoul, Korea) and 1%

penicillin- streptomycin solution (GenDEPOT, Katy, TX, USA). Caco-2 cells were cultured in DMEM medium (HyClone™, LOGAN, UT, USA) containing 10% FBS and 1% penicillin-streptomycin solution. Cell viability was checked with trypan blue staining under the microscope.

To evaluate intestinal barrier function, Caco-2 cells were used, and barrier damage was induced by treatment with 40 ng/ml TNF-α (R&D systems, Minneapolis, MN, USA) for 48 hrs.

10. *In vitro* intestinal permeability assay

To establish an *in vitro* model of the intestinal epithelial barrier, Caco-2 cells were plated into the upper chamber of the Transwell system (0.4 μm pore, Corning, Corning, NY, USA). Epithelial permeability was assessed by analysis of transepithelial electrical resistance (TEER) and paracellular flux of FITC-dextran.

The electrical resistance of the Caco-2 cell monolayers cultured on transwell chamber was assessed using a Millicell-ERS instrument (Millipore, Bedford, MA, USA).

FITC-dextran was added to the upper chamber at a final concentration of 1 mg/ml. 2 hrs after the addition of FITC-dextran, medium from the lower chamber was collected and fluorescence intensity was measured using a fluorescent microplate reader.

11. Immunofluorescence analysis

Caco-2 cells were fixed 10% neutral buffer formalin for 30 mins at room temperature and washed with PBS. Cells were blocked for non-specific binding with 5% BSA for 1 hr at room temperature and incubated with rabbi anti-E-cadherin antibody (1:200; Cell Signaling Technology, Inc., Beverly, MA, USA) diluted in blocking buffer overnight at 4°C. Cells were then washed with PBS, incubated with the secondary antibody, Alexa Fluor 488 goat anti-rabbit

IgG H&L (1:200; Abcam, Cambridge, UK) for 1 hr at room temperature, and nuclear stained with diamidino-2-phenylindole (DAPI, 300 nM) for 3 mins. Confocal images were obtained using a Zeiss LSM700 confocal microscope (Carl Zeiss AG, Oberkochen, Germany).

12. Western blotting

Caco-2 cells were lysed with Pierce RIPA buffer (Thermo Fisher Scientific, Rockford, IL, USA) supplemented with Halt Protease & Phosphatase Inhibitor Cocktail (Thermo Fisher Scientific, Rockford, IL, USA). After measuring protein concentration with Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, Massachusetts, USA), proteins were separated according to their molecular weight by SDS polyacrylamide gel electrophoresis and subsequently transferred to polyvinylidene fluoride (PVDF) membranes.

Membranes were blocked for 1 hr with Tris-buffered saline plus 0.1% Tween-20 (TBST) supplemented with 5% skimmed milk and then incubated with primary antibody (anti-ZO-1; 1:1,000, anti-E-cadherin; 1:1,000) overnight at 4°C. Membranes were washed and incubated with HRP-conjugated secondary antibodies (anti-rabbit, 1:2,500; anti-mouse, 1:2,500) for 1 hr at room temperature. For detection of protein bands, ECL™ Prime Western Blotting Detection Reagent (Amersham, Buckinghamshire, UK) was used, and signals were pictured using LAS 4,000 mini (Fujifilm, Tokyo, Japan).

13. Cytometric Bead Array (CBA)

The concentrations of cytokines in plasma were measured using CBA Mouse Th1/Th2/Th17 Cytokine Kit (BD Biosciences, San Jose, CA, USA) according to the manufacturer's protocol. Samples were analyzed with flow cytometry (FACS Verse, BD Biosciences, San Jose, CA, USA). The cytokine levels were normalized to total protein concentration.

14. Bacterial strains and growth conditions

B. vulgatus strains were kindly provided by Dr. Sangsun Yoon, Yonsei University. *Salmonella enterica subsp. enterica serovar Typhimurium (S. typhimurium)* GFP strains were purchased from American Type Culture Collection (ATCC; Manassas, VA, USA).

B. vulgatus strains were cultivated on Gifu Anaerobic Media (GAM) broth (KisanBio, Seoul, Korea) under anaerobic conditions. *S. typhimurium* strains were routinely grown at 37°C in Nutrient broth containing 100 μg/ml ampicillin under aerobic conditions.

15. Bacterial adhesion assay

HT-29 cells were plated in 12-well plate in fresh medium supplemented with 10% FBS. *B. vulgatus* and *S. typhimurium* were treated to the cells for 3 hrs and the ratio of bacteria to cell was 50:1. Cells were washed and removed from the plates by exposure to 100 μL of 0.1% Triton-X 100 for 10 mins. 900 μL of Nutrient broth was added to the plates and the cell lysates were resuspended. Cell lysates were serially diluted and plated on Nutrient agar plates with 100 μg /ml ampicillin to enumerate colony-forming unit (CFU) of adhered bacteria.

16. Statistical analysis

GraphPad Prism Software (GraphPad Inc., La Jolla, CA, USA) was used for statistical analyses. The significance of differences between conditions was assessed using one-way analysis of variance (ANOVA) or Student's t-test. *P* values < 0.05 were considered significant.

III. RESULTS

1. Tegoprazan attenuates symptoms of DSS-induced colitis

DSS-induced colitis has a phenotype similar to that of human UC. To investigate the anti-inflammatory effects of tegoprazan, colitis was induced in mice by administering 2% DSS in distilled drinking water for 5 days.

Compared with the control group, all DSS treated groups showed colitis symptoms such as weight loss, diarrhea, bloody stools, and shortening of the colon. However, these symptoms were alleviated in mice administered tegoprazan. The tegoprazan-treated group exhibited similar weight loss to the DSS group until day 5, but from day 6, the relative body weights were significantly increased in the tegoprazan-treated group compared with the DSS group (Fig. 1A). The increased DAI score in the DSS group was remarkably decreased in the tegoprazan-treated group (Fig. 1B). Also, the colon length of the DSS group (6.6 ± 0.2 cm) was significantly reduced compared with the control group (8.2 ± 0.2 cm), whereas this phenomenon was alleviated by tegoprazan (8.3 ± 0.4 cm) (Fig. 1C and D). However, there were no significant differences between the DSS group and the rabeprazole-treated groups regarding weight loss, DAI, and colon length. These results suggest that tegoprazan ameliorates the symptoms of colitis caused by DSS, unlike rabeprazole.

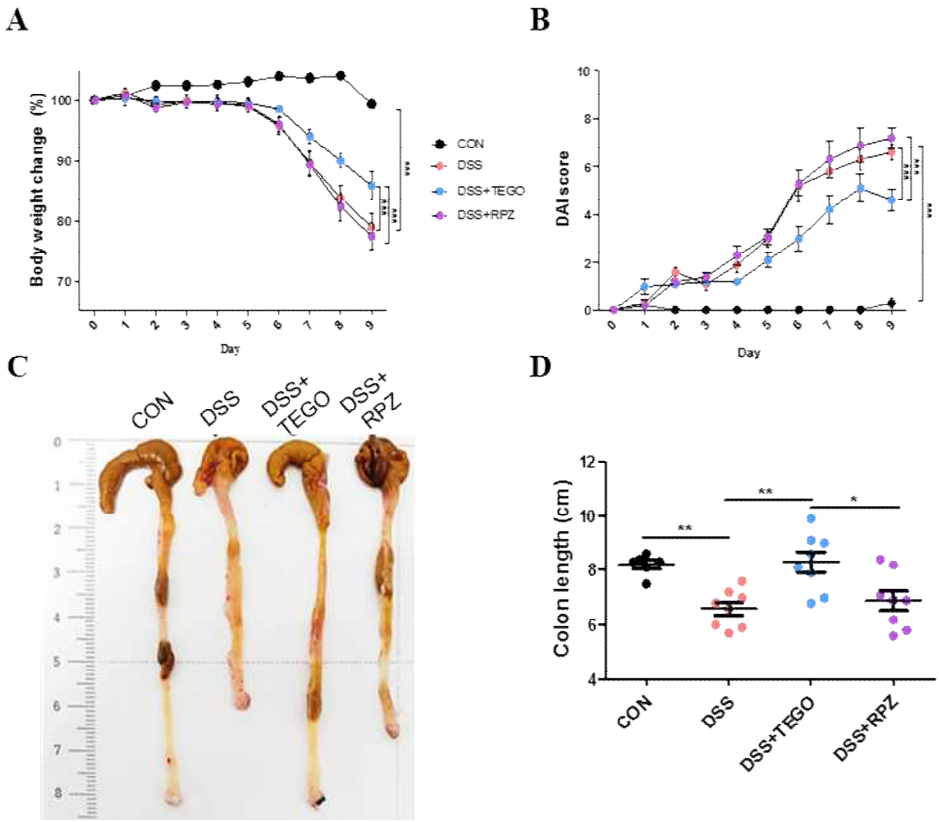


Figure 1. Effect of tegoprazan on clinical symptoms of DSS-induced colitis. (A) Body weight change (%). (B) Disease activity index (DAI). (C) Representative images of colons. (D) Colon length. Data represent the mean ± S.E.M. Significance is indicated by $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ using two-way ANOVA or one-way ANOVA followed by Tukey's test**.**

2. Tegoprazan ameliorates DSS-induced colonic damage

The control group maintained normal colonic structures. However, the DSS group elicited deteriorating pathological alterations, such as epithelial cell destruction, crypt deformation, mucosa inflammation, and cell infiltration, resulting in significantly higher histological scores than the control group. By contrast, the mice treated with tegoprazan showed remarkably ameliorated intestinal injury, reduced infiltration of inflammatory cells, and significantly decreased histological scores compared with the DSS group. However, the rabeprazole-treated group showed no marked protection against colon damage, as indicated by high histological scores (Fig. 2A and B).

The DSS group also exhibited severe goblet cell loss, whereas the tegoprazan-treated group displayed significantly increased goblet cells, which secretes mucus (Fig. 2C). Consistently, the mRNA expression level of Muc2, produced by goblet cells, was upregulated in the tegoprazan-treated group compared with the DSS group (Fig. 2D).

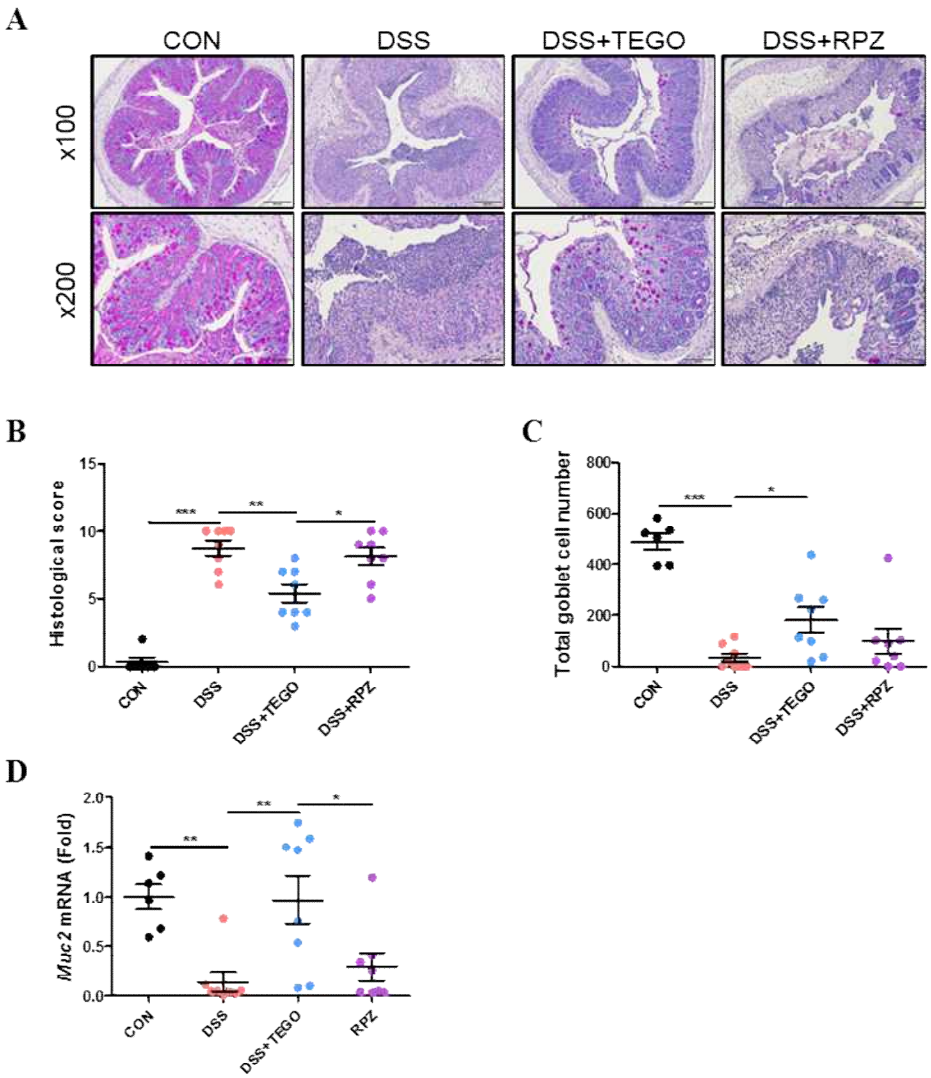


Figure 2. Effect of tegoprazan on colon damage. (A) Representative image of periodic acid-Schiff (PAS)-stain. Scale bars = 200 μm for 100× and 100 μm for 200× magnification. (B) Histological score. (C) Total goblet cell number. (D) mRNA expression level of *Muc2*. Data represent the mean ± S.E.M. Significance is indicated by *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ using one-way ANOVA followed by Tukey's test or Dunnet's test**.**

3. Tegoprazan reduces the expression level of pro-inflammatory cytokines

Pro-inflammatory cytokine is considered to play an essential role in the pathogenesis of IBD.[24] To determine the effect of tegoprazan on inflammatory cytokine, qRT-PCR was performed. The mRNA expression levels of *Tnfa*, *Il1b,* and *Il6* were significantly increased in the DSS group. In the tegoprazan-treated group, these cytokine expression levels were significantly reduced, and their levels were similar to the control group. Also, similar results were found in secretory K^+ channel expression. The mRNA expression levels of *Kcnj1* and *Kcnma1* were upregulated in the DSS group compared with the control group. However, administration of tegoprazan reduced these increased expression levels induced by DSS (Fig. 3). These results suggest that tegoprazan relieves colitis by reducing pro-inflammatory factors.

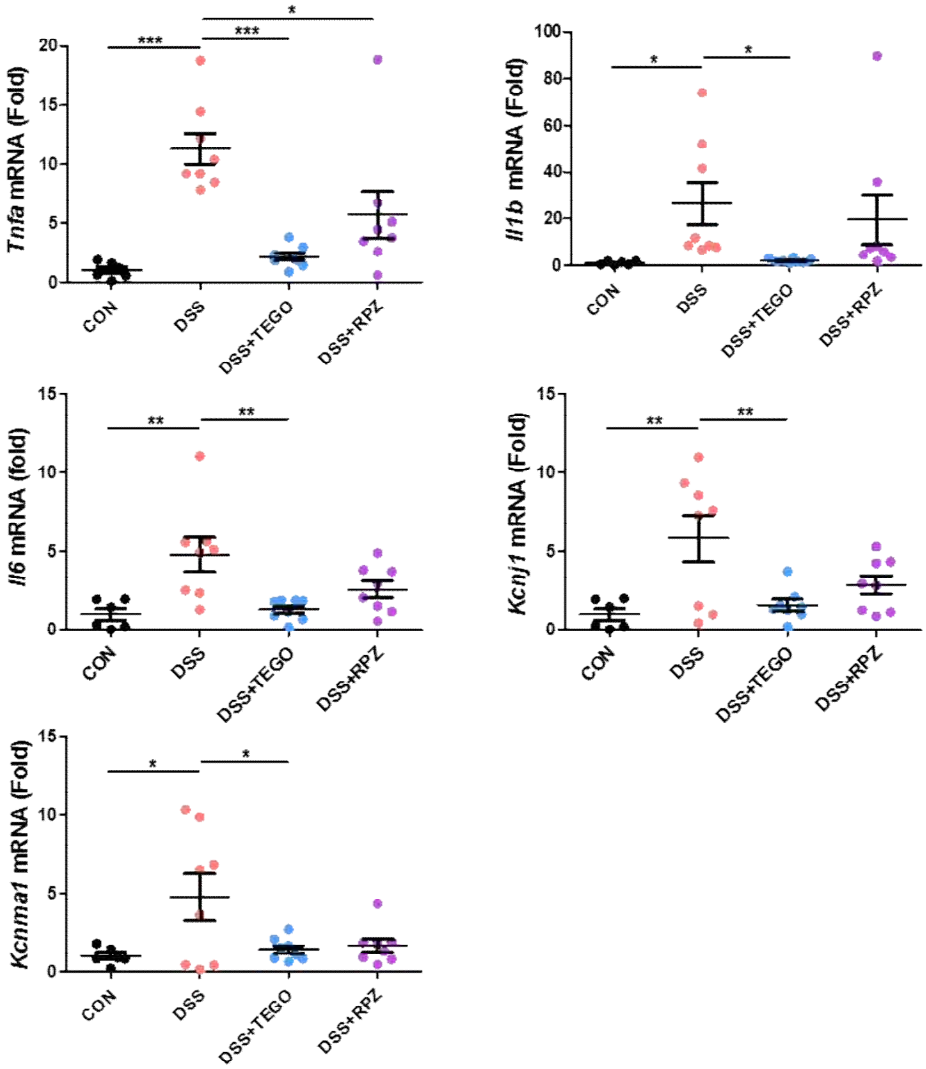


Figure 3. Effect of tegoprazan on colon-derived cytokine levels. mRNA expression level was measured by qRT-PCR. Data represent the mean ± S.E.M. Significance is indicated by *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ using one-way ANOVA followed by Tukey's test or Student *t*-test**.**

4. Tegoprazan prevents intestinal permeability and loss of tight junction proteins

Impaired epithelial barrier results in increased intestinal permeability, leading to the development of chronic inflammation.[25] To characterize the protective effects of tegoprazan on epithelial permeability. The intestinal permeability was measured by FITC-dextran assay. As shown in Fig. 4A, intestinal permeability was markedly increased in the DSS group compared with the control group, but it was significantly reduced in tegoprazan-treated mice. However, there was no significant change observed between the DSS group and the rabeprazole-treated groups.

Because tight junctions regulate intestinal epithelial permeability, the expression of tight junction molecules, which are important for intestinal barrier function, were assessed. Initially, mRNA levels of specific tight junction molecules were analyzed. qRT-PCR results revealed that mRNA levels of *Zo1* and *Occludin* in the tegoprazan-treated group were significantly higher than the DSS group. (Fig. 4B). Furthermore, the protein level of Zo-1, which is essential for barrier formation, was investigated via immunohistochemistry. The DSS group showed significantly lower Zo-1 protein level than the control group. Consistently with the mRNA level, the protein level of Zo-1 was remarkably recovered in the tegoprazan-treated group (Fig. 4C and D). These findings implicate tegoprazan may protect the intestinal epithelial tight junction barrier and inhibit the increase in intestinal permeability.

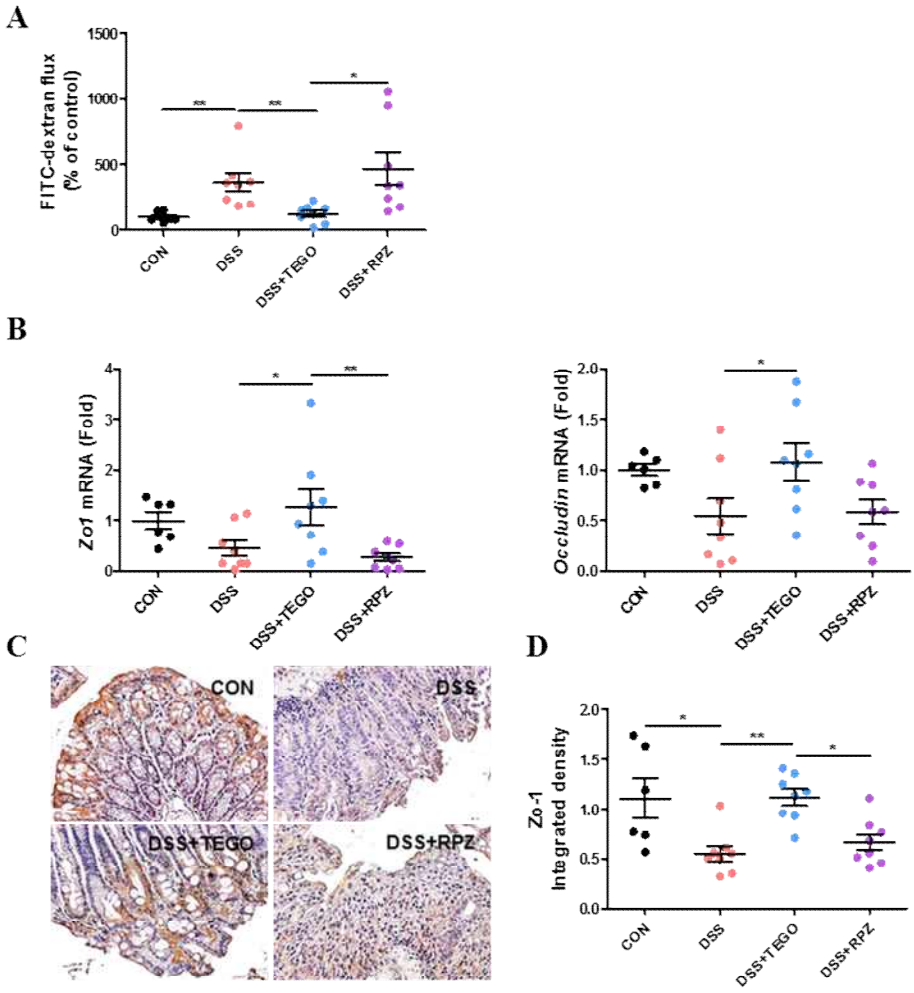


Figure 4. Effect of tegoprazan on intestinal epithelial barrier function. (A) FITC-dextran flux (arbitrary unit), plotted as percent of control. (B) mRNA expression level of *Zo1* and *Occludin*. (C) Representative immunohistochemical images of Zo-1 protein. (D) Quantification of the integrated density of Zo-1 (DAB-stained region). Data represent the mean ± S.E.M. Significance is indicated by $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ using one-way ANOVA followed by Dunnett's test or Student *t*-test.

5. Tegoprazan restores the intestinal barrier function *in vitro*

To further address molecular mechanisms of the protective role of tegoprazan in intestinal barrier function, in vitro Caco-2 cells culture system was used.[26] Caco-2 cells are widely used as a model of the intestinal epithelial barrier. Caco-2 cell monolayers were treated with 40 ng/mL TNF-α for 48 hrs to disrupt the epithelial barrier, and then measured transepithelial electrical resistance (TEER). TNF-α-induced reduction in the TEER level was significantly suppressed by administration of tegoprazan (Fig. 5A).

Also, the paracellular permeability of Caco-2 cell monolayers was evaluated by FITC-dextran assay. TNF-α significantly increased FITC-dextran flux, and this increased FITC-dextran flux was significantly decreased by co-treatment with tegoprazan (Fig. 5B).

Consistently, immunostaining and western blot with anti-E-cadherin and ZO-1 antibodies showed that tegoprazan remarkably enhanced tight junction expression in the Caco-2 monolayer (Fig. 5C-E).

These data indicate that tegoprazan suppresses the TNF-α-induced disruption of the epithelial barrier function of Caco-2 cell monolayers. These in vitro results are consistent with the observation in vivo that tegoprazan reduces DSS-induced colitis in part by maintaining high junction integrity of the epithelial mucosa.

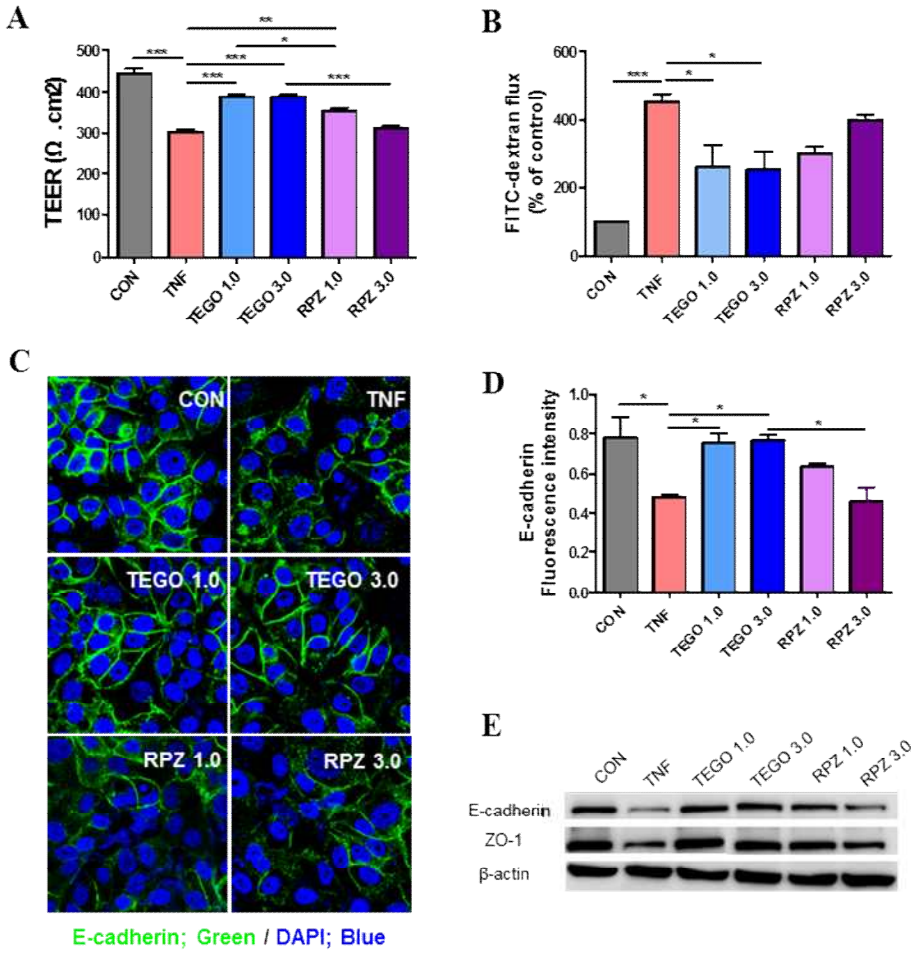


Figure 5. Effect of tegoprazan on the barrier function *in vitro.* (A) TEER measurement. (B) FITC-dextran flux (arbitrary unit), plotted as percnt of control. (C) Immunofluorescence of E-cadherin in Caco-2 cell monolayers. (D) Quantification of the fluorescence intensity of E-cadherin (arbitrary unit). (E) Representative image of western blot of E-cadherin and ZO-1. CON, treated with DMSO; TNF, treated with 40 ng/ml TNF- α, TEGO 1.0, treated with 40 ng/ml TNF- α and 1.0 uM tegoprazan; TEGO 3.0, treated with 40 ng/ml TNF- α and 3.0 uM tegoprazan; RPZ 1.0, treated with 40 ng/ml TNF- α and 1.0 uM rabeprazole; RPZ 3.0, treated with 40 ng/ml TNF- α and 3.0 uM rabepazole.

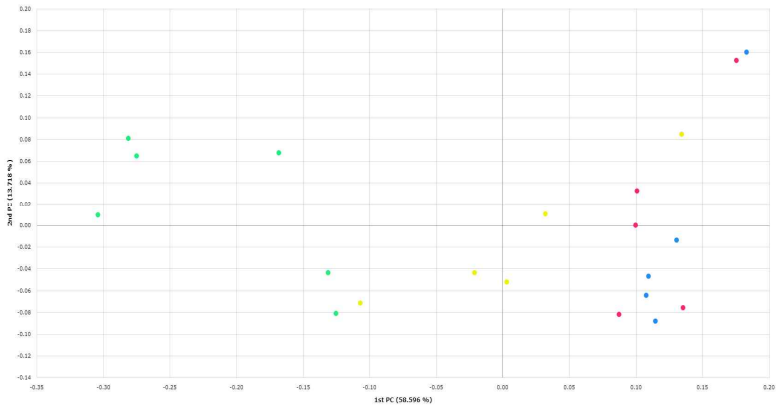
Data represent the mean ± S.E.M. Significance is indicated by *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ using one-way ANOVA followed by Tukey's test.

6. Tegoprazan modulates the structure of gut microbiota and enriches for certain gut bacteria

It is well-known that microbes and the immune system interact with each other.[27] To investigate whether tegoprazan attenuates DSS-induced colitis by altering the gut microbiota, cecal tissue samples from all groups of mice to 16S rRNA metagenome analysis were performed. Both principal coordinate analysis (PCoA) and unweighted pair group method with arithmetic (UPGMA) dendrogram indicated that the cecal microbiota structure of the DSS group was significantly different from that of tegoprazan treated group (Fig. 6A and B). The tegoprazan-treated group also showed significantly increased richness of cecal microbiota assessed using the number of observed operational taxonomic unit (OTU) and Chao index (Fig. 6C and D).

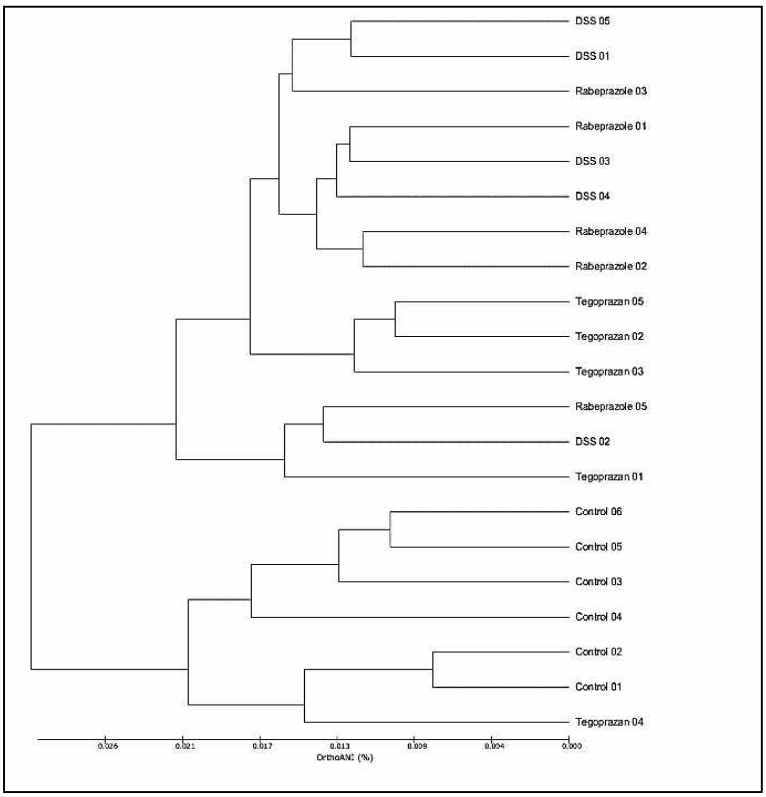
The normal gut microbiota consists of two major phyla, namely *Firmicutes* and *Bacteroidetes*.[28] At the phylum level, the relative abundance of *Bacteroidetes* decreased while *Proteobacteria* increased in all DSS treated groups. (Fig. 7A and B, Table 4). However, administration of tegoprazan restored the abundance ratio of *Bacteroidetes* reduced by DSS to a level similar to that of the control group. Furthermore, at the species level, the bacterial composition of the tegoprazan group differed from the DSS group. The abundance ratio of *B. vulgatus* decreased by DSS, tegoprazan administration was significantly increased the abundance ratio of *B. vulgatus*. But, rabeprazole did not affect the abundance ratio of *B. vulgatus* in the intestine. Of note, the rabeprazole significantly increased the relative abundance of phylum *Proteobacteria* and species *Escherichia coli* (*E.coli*) (Fig. 7A-D, Table 4 and 5). Taken together, these results show that tegoprazan alleviates gut microbiota dysbiosis and promotes the proliferation of certain bacteria.

A



CON; Green / DSS; Red / TEGO; Yellow / RPZ; Blue

B



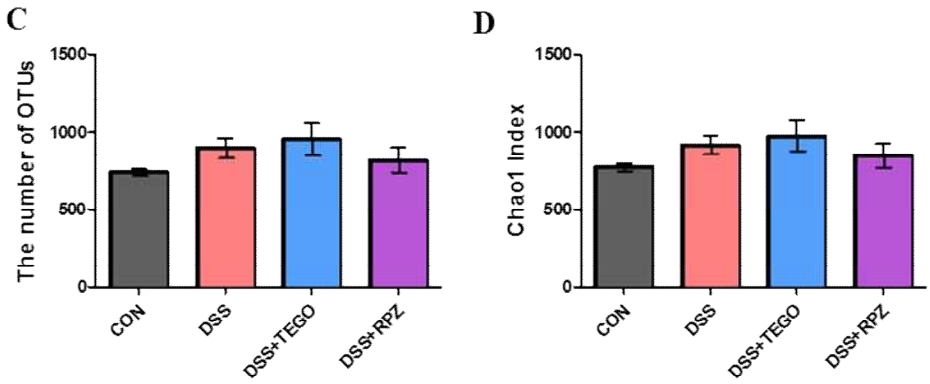
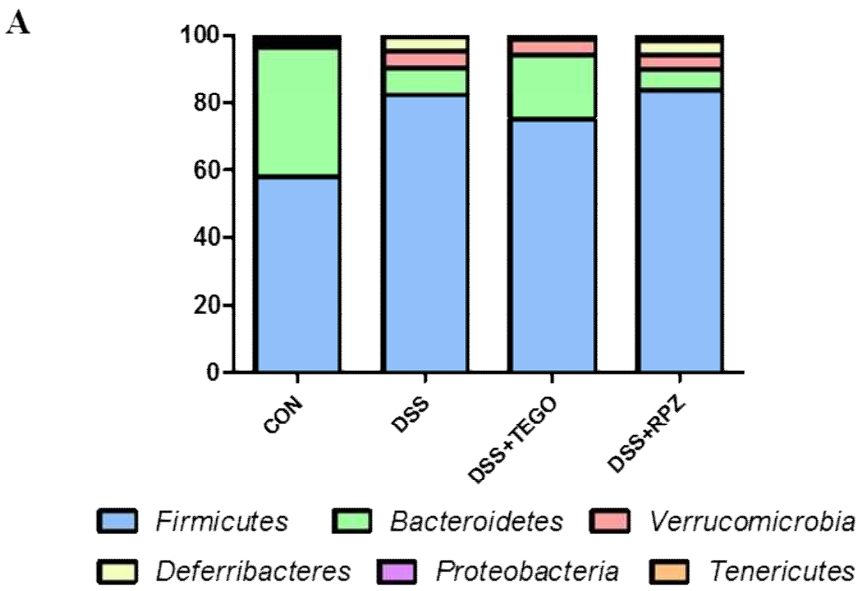
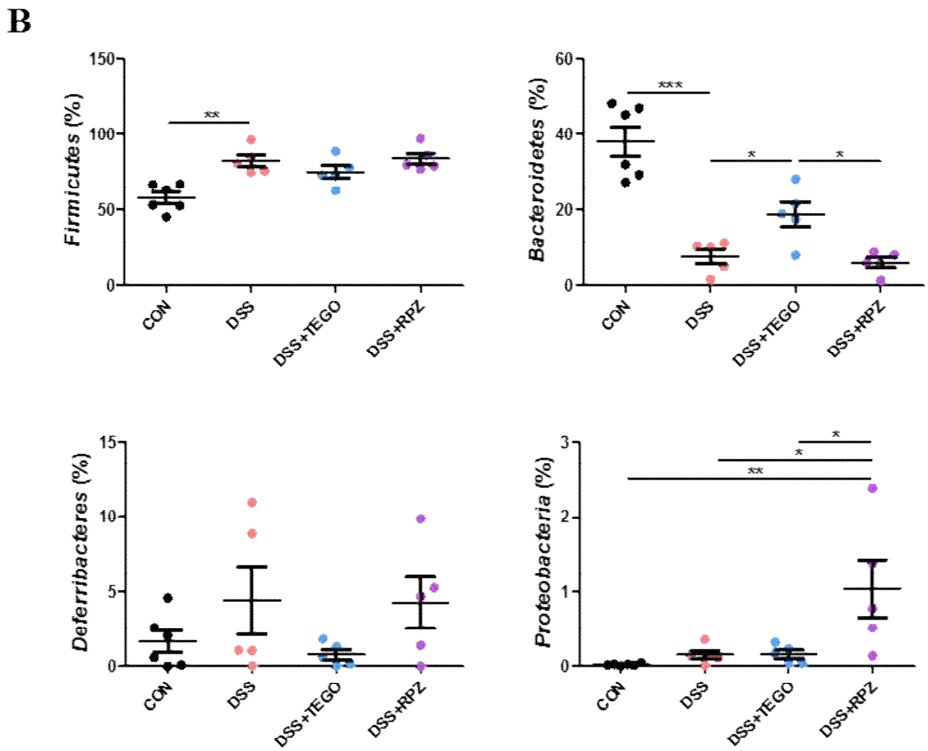


Figure 6. Effect of tegoprazan on alpha and beta diversity indices in DSS-induced colitis. Gut microbiome composition was generated using 16 S rRNA sequencing. (A) Principal coordinate analysis (PCoA). (B) UPGMA dendrogram. (C) The number of observed OUT. (D) Chao 1 index. Data represent the mean ± S.E.M.





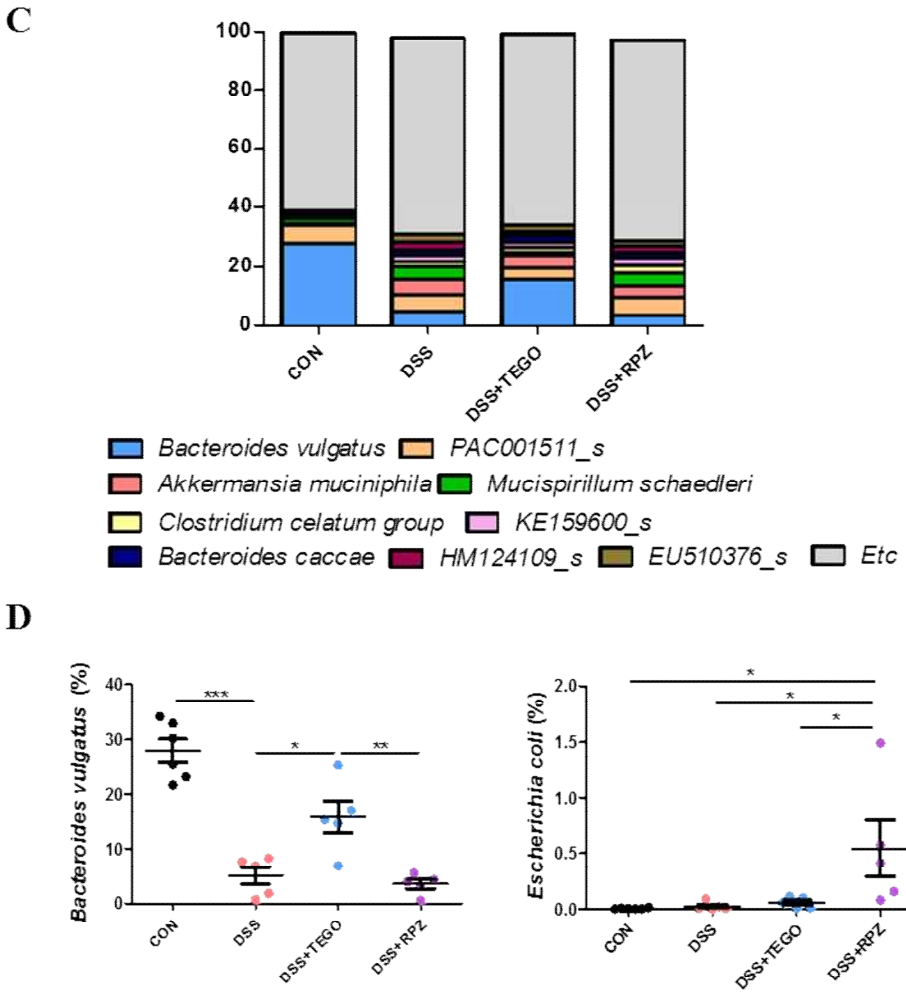


Figure 7. Gut micobial composition in mice after tegoprazan administration. (A) Microbial community bar plot by phylum (B) The abundance ratio of Firmicutes, Bacteroidetes, Defferibacteres and Proteobacteria. (C) Micobial community bar plot by species. (D) The abundance ratio of E.coli and B. vulgatus. Data represent the mean or mean ± S.E.M. Significance is indicated by *p < 0.05, **p < 0.01, and ***p < 0.001 using one-way ANOVA followed by Tukey's test or Dunnett's test.

Table 4. The major gut microbial composition at phylum level (%)

	Control	DSS	DSS+ TEGO	DSS+ RPZ
Firmicutes	58.12 ± 8.99	82.53 ± 8.73 **	75.11 ± 9.45 *	83.91 ± 8.08 ***
Bacteroidetes	38.18 ± 9.57	7.68 ± 4.16 ***	18.83 ± 7.36 *** #	5.97 ± 2.90 *** §
Verrucomicrobia	0.74 ± 1.02	4.91 ± 3.12 *	4.57 ± 1.45 *	4.15 ± 1.73 *
Deferribacteres	1.64 ± 1.77	4.40 ± 5.11	0.78 ± 0.76	4.23 ± 3.84
Tenericutes	1.17 ± 0.79	0.25 ± 0.16	0.50 ± 0.47	0.64 ± 0.72
Proteobacteria	0.03 ± 0.02	0.16 ± 0.12	0.17 ± 0.12	1.04 ± 0.88 ** # §
Actinobacteria	0.13 ± 0.11	0.07 ± 0.02	0.04 ± 0.02	0.05 ± 0.02

Data represent the mean ± SD. Significance is indicated by **p*, #*p and* §$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ using one-way ANOVA followed by Tukey's test or Dunnett's-test (# vs DSS, § vs DSS+TEGO).

Table 5. The top 10 major gut microbial composition at genus level (%)

	Control	DSS	DSS+ TEGO	DSS+ RPZ
Bacteroides	29.22 ± 4.56	7.19 ± 4.00 ***	18.55 ± 7.27 * ##	5.48 ± 2.67 *** §§
PAC001149_g	6.11 ± 8.46	5.73 ± 1.83	3.84 ± 3.69	6.11 ± 3.82
PAC000664_g	1.56 ± 0.57	3.74 ± 0.85 *	7.90 ± 5.61 *	5.58 ± 3.60
Oscillibacter	2.66 ± 0.78	5.58 ± 2.28 *	4.89 ± 1.08	5.53 ± 1.71 *
KE159600_g	0.83 ± 1.31	5.80 ± 3.36	4.17 ± 2.55	4.15 ± 1.73
Akkermansia	0.74 ± 1.03	4.91 ± 3.12 *	2.74 ± 0.87 *	3.42 ± 0.87 *
Pseudoflavonifractor	1.21 ± 0.28	3.02 ± 0.74 **	0.78 ± 0.76 *	4.23 ± 3.84 ***
Mucispirillum	1.64 ± 1.77	4.40 ± 5.11	4.36 ± 4.58	2.47 ± 3.03
PAC001091_g	0.29 ± 0.27	2.59 ± 1.23	2.74 ± 2.75	2.11 ± 1.51
PAC000661_g	2.73 ± 1.05	1.55 ± 1.45	1.97 ± 1.43	2.31 ± 0.67

Data represent the mean ± SD. Significance is indicated by *$p < 0.05$, **p, ##p and §§$p < 0.01$, and ***$p < 0.001$ using one-way ANOVA followed by Tukey's test (# vs. DSS, § vs DSS+TEGO).

Table 6. The top 10 major gut microbial composition at species level (%)

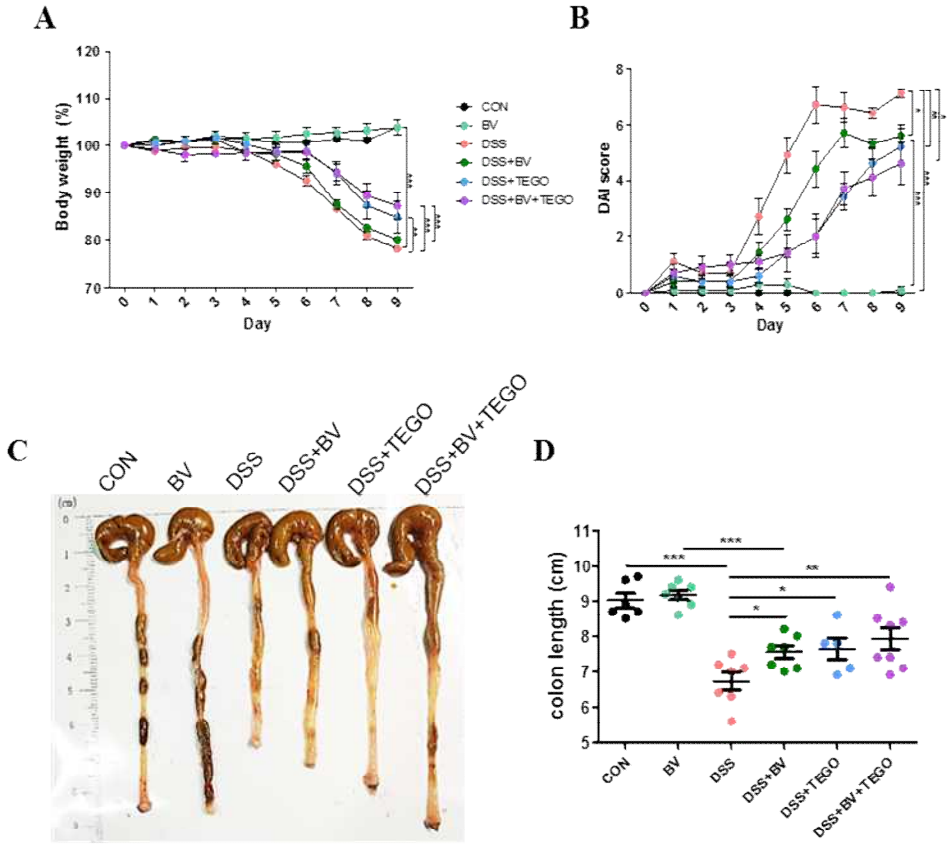
	Control	DSS	DSS+ TEGO	DSS+ RPZ
Bacteroides vulgatus	27.92 ± 5.24	5.08 ± 3.52 ***	15.82 ± 6.54 ** #	3.54 ± 1.86 *** §§
PAC001511_s	6.11 ± 8.46	5.73 ± 1.83	3.84 ± 3.68	6.11 ± 3.82
Akkermansia muciniphila	0.74 ± 1.03	4.91 ± 3.12 *	4.57 ± 1.45 *	4.15 ± 1.73 *
Mucispirillum schaedleri	1.64 ± 1.77	4.40 ± 5.11	0.78 ± 0.76	4.23 ± 3.84
Clostridium celatum group	1.18 ± 1.86	1.77 ± 0.93	1.78 ± 0.77	2.62 ± 0.97
KE159600_s	0.41 ± 0.60	2.09 ± 1.97	1.62 ± 0.74	2.44 ± 1.56
Bacteroides caccae	1.08 ± 0.95	1.88 ± 0.93	2.28 ± 1.17	1.85 ± 1.21
HM124109_s	0.07 ± 0.16	2.78 ± 1.66 **	1.53 ± 1.51	2.25 ± 0.64 *
EU510376_s	0.00 ± 0.00	2.57 ± 4.23	1.91 ± 2.29	1.92 ± 3.45`
AB606350_s	0.27 ± 0.27	2.27 ± 1.85	0.69 ± 0.79	2.82 ± 3.39

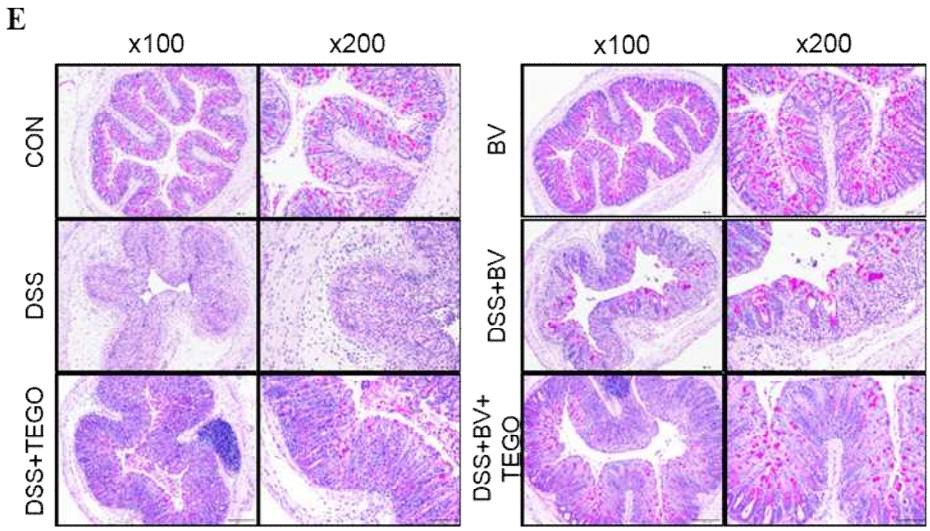
Data represent the mean ± SD. Significance is indicated by *p and #p < 0.05, **p and §§p < 0.01, and ***p < 0.001 using one-way ANOVA followed by Tukey's test (# vs. DSS, § vs DSS+TEGO).

7. Co-administration of *B. vulgatus* and tegoprazan effectively alleviated colitis induced by DSS

To further investigate whether *B. vulgatus* inhibits intestinal inflammation, co-administration of *B. vulgatus* and tegoprazan was performed in DSS-induced colitis model. As shown in Fig. 8, the administration of *B. vulgatus* alone attenuated DSS-induced colitis slightly, and the co-administration of *B. vulgatus* and tegoprazan effectively prevented the symptom of colitis such as weight loss, diarrhea, bleeding, colon shortening, histological damage, and paracellular permeability.

Also, the DSS+BV group reduced plasma levels of Il-2, Il-6, and Tnf slightly compared with the DSS group (Fig. 8A-F). The plasma levels of Il-2, Il-6, and Tnf were lower in the DSS+BV+TEGO group than in the DSS+BV group. These results indicate that *B. vulgatus* contributes to alleviating intestinal inflammation.





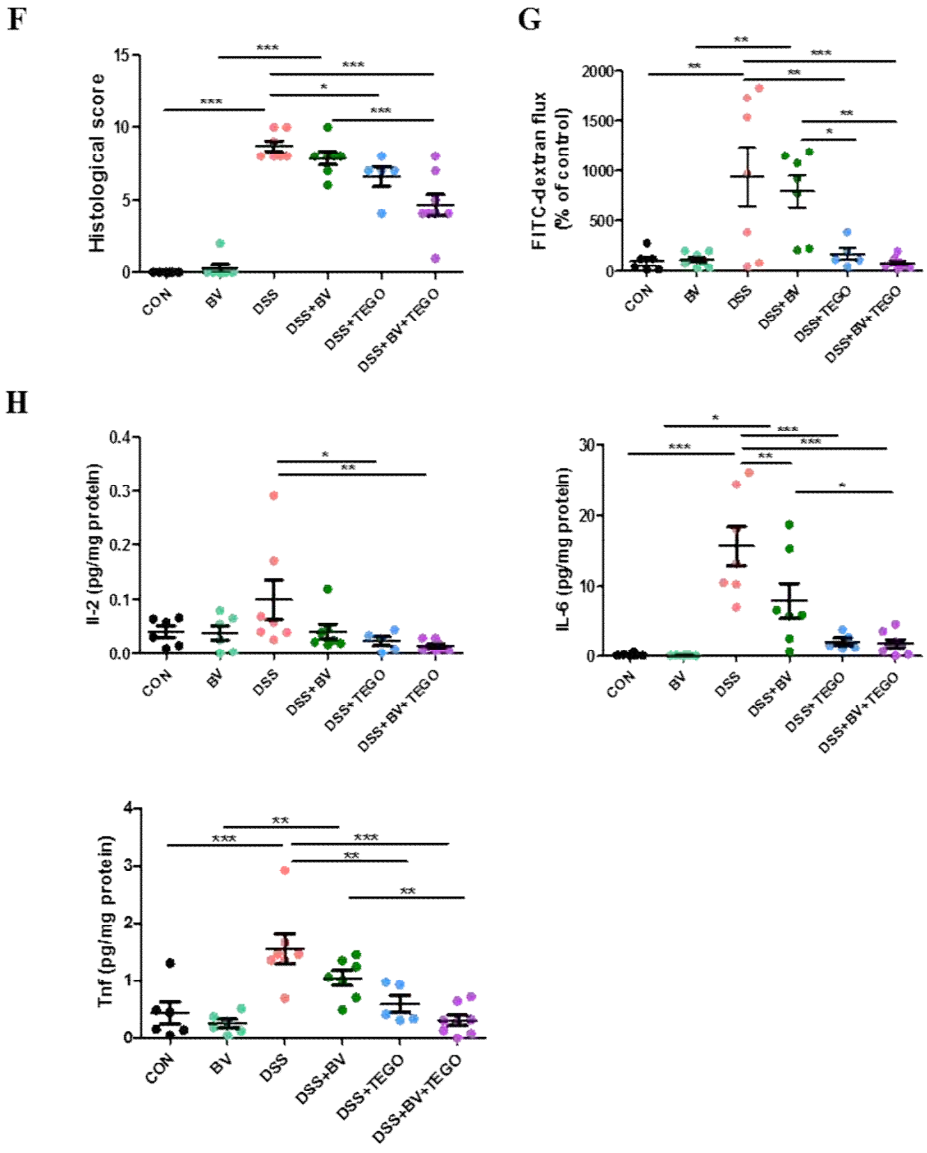


Figure 8. Effect of co-administration of *B. vulgatus* and tegoprazan on the symptom of intestinal inflammation. (A) Body weight change (%). (B) Disease activity index (DAI). (C) Representative images of colons. (D) Colon length. (E) Representative images of periodic acid-Schiff (PAS)-stain. Scale bars = 20 μm for 100× and 100 μm for 200× magnification. (F) Histological score. (G) FITC-dextran flux (arbitrary unit), plotted as percent of control. (H)

Plasma level of Il-2, Il-6 and Tnf. Data represent the mean ± S.E.M. Significance is indicated by *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ using two-way ANOVA and one-way ANOVA followed by Newman-Keuls's test or Dunnett's test.

8. Tegoprazan directly promotes the growth of *B. vulgatus*

To determine whether tegoprazan is directly involved in the growth of B. vulgatus, tegoprazan was treated in *B. vulgatus*. Tegoprazan and rabeprazole were added to the *B. vulgatus* culture medium when the optical density (OD 600) value of *B. vulgatus* was 0.1. After 3 hrs, the samples were serially diluted and plated on GAM agar plates. As a result, tegoprazan promoted *B. vulgatus*, whereas rabeprazole did not promote their growth (Fig. 9A and B). These results suggest that tegoprazan directly enhances the growth of *B. vulgatus*, which helps relieve intestinal inflammation.

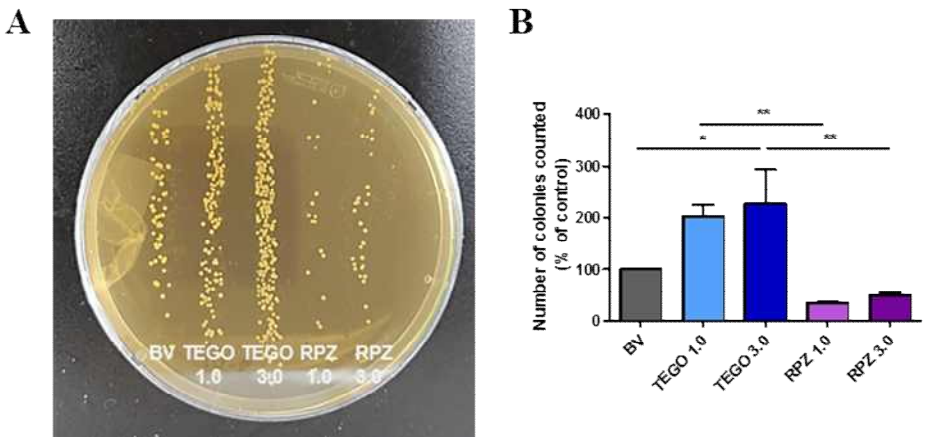


Figure 9. Effect of tegoprazan on the growth of *B. vulgatus*. (A) Representative image of *B. vulgatus* treated with tegoprazan and rabeprazole. (B) Quantification of *B. vulgatus* plated on GAM agar plate, plotted as percent of control. BV, *B. vulgatus* only; TEGO 1.0, *B. vulgatus* treated with tegoprazan 1.0 uM, ; TEGO 3.0, *B. vulgatus* treated with tegoprazan 3.0 uM; ; RPZ 1.0, *B. vulgatus* treated with rabeprazole 1.0 uM; ; RPZ 3.0, *B. vulgatus* treated with rabeprazole 3.0 uM. Data represent the mean ± S.E.M. Significance is indicated by $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ using One-way ANOVA followed by Dunnett's-test.

9. *B. vulgatus* inhibits epithelial adhesion of pathogenic bacteria

Pathogenic bacterial adhesion on host cells plays a critical role in both inflammation and infection.[29] Bacterial adhesion assay was performed to determine whether *B. vulgatus* affects the epithelial adhesion of pathogenic microorganisms. To induce competition between *B. vulgatus* and *S. typhimurium* for adhesion on epithelial cells, two bacteria were mixed with an equal volume and then added onto HT-29 cells. As a result, *B. vulgatus* inhibited the adhesion of *S. typhimurium* to HT-29 cells (Fig. 10A and B). However, tegoprazan did not prevent the adhesion of *S. typhimurium directly*. This result suggests that Tegoprazan impedes epithelial adhesion of pathogenic bacteria through *B. vulgatus*.

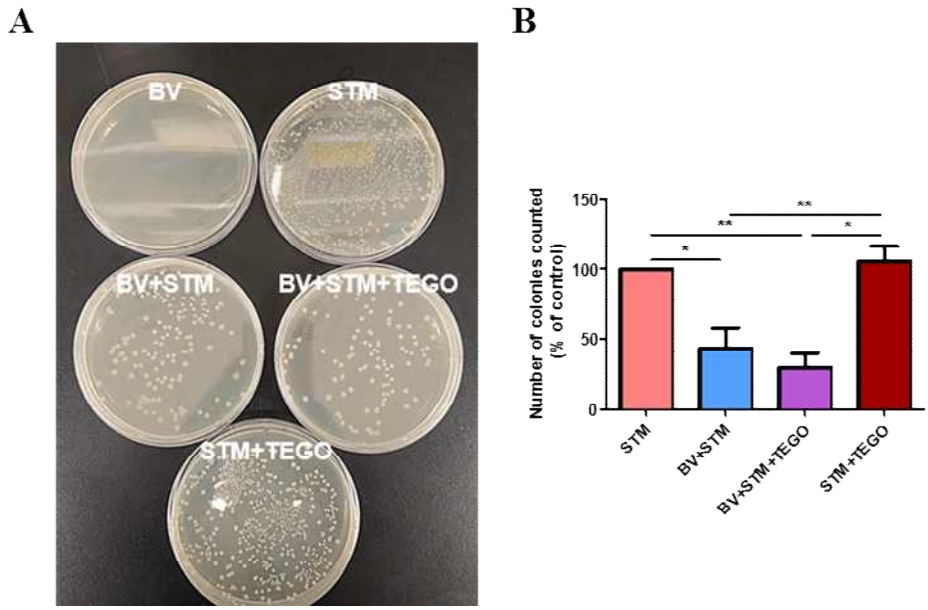


Figure 10. Effect of *B. vulgatus* on pathogenic bacteria. (A) Representative images of adhered *S. typhimurium* colonies. (B) Quantification of *S. typhimurium* plated on Nutrient agar, plotted as percent of control. BV, *B. vulgatus* only; STM, *S. typhimurium only*, BV+STM, *S. typhimurium* treated with *B. vulgatus*; BV+STM+TEGO, *S. typhimurium* treated with *B. vulgatus* and tegoprazan; STM+TEGO, *S. typhimurium* treated with tegoprazan. Data represent the mean ± S.E.M. Significance is indicated by *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ using One-way ANOVA followed by Tukey's test.

10. Tegoprazan alleviates the severity of DNBS-induced colitis

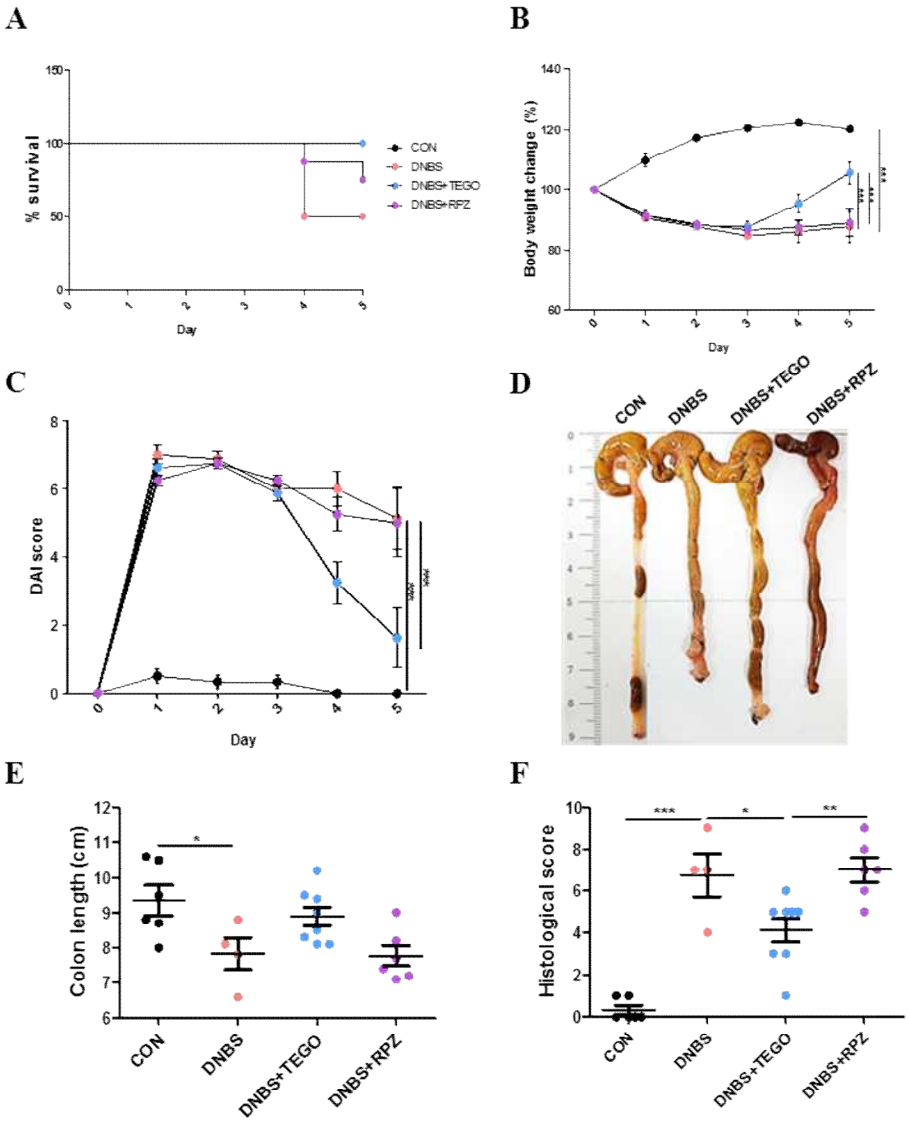
DNBS-induced colitis has a phenotype similar to that of human CD.[30,31] To investigate the anti-inflammatory effects of tegoprazan, colitis was induced in mice by rectal administration of 5 mg DNBS dissolved in 50 % ethanol.

As expected, rectal administration of DNBS quickly triggered severe diarrhea, decreased mobility, and weight loss, resulting in significant mortality (Fig. 11A-C). However, these symptoms improved in mice treated with tegoprazan. Although tegoprazan-treated mice exhibited similar weight loss and DAI scores to the DNBS-treated mice until day 3, these symptoms were significantly relieved compared with DNBS-treated mice. In particular, several mice died in the DNBS-treated groups, excluding the Tegoprazan-treated group, whereas the tegoprazan-treated mice all survived. Also, reduced colon length induced by DNBS was alleviated by tegoprazan (Fig. 11D and E).

In the histological analysis, the DNBS group's colon showed crypt transformation, immune cell infiltration into the mucosa, and perforation (Fig. 11F and G). By contrast, the tegoprazan treatment significantly lessened colonic damage.

Also, the tegoprazan-treated group was reduced mRNA levels of pro-inflammatory cytokines, especially Il17, compared with the DNBS group (Fig. 11H). It is known that IL-17R signaling plays a significant role in developing TNBS-induced colitis.[32]

However, the rabeprazole-treated group did not protect against DNBS-induced colitis. Taken together, these results proved that tegoprazan protects against DNBS-induced colon inflammation, and that may be a promising treatment for Crohn's disease.



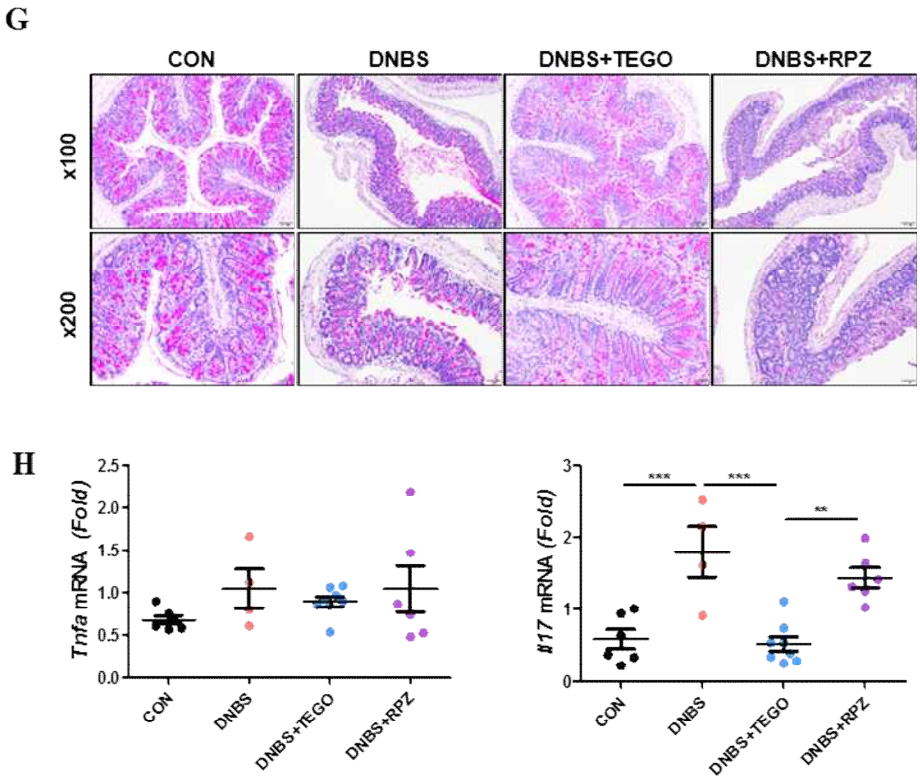


Figure 11. Effect of tegoprazan on the symptom of DNBS-induced colitis. (A) survival rate (%). (B) Body weight change (%). (C) Disease activity index (DAI). (D) Representative images of colons. (E) Colon length. (F) Histological score. (G) Representative images of periodic acid-Schiff (PAS)-stain. Scale bars = 20 μm for 100× and 100 μm for 200× magnification. (F) Histological score. (G) FITC-dextran flux (arbitrary unit), plotted as percent of control. (H) mRNA expression levels of *Tnfa* and *Il17*. Data represent the mean ± S.E.M. Significance is indicated by *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ using two-way ANOVA and one-way ANOVA followed by Tukey's test.

IV. DISCUSSION

Tegoprazan, recently launched, is a new gastric acid inhibitor that overcomes the shortcomings of PPIs. PPIs cause dysbiosis, but it is still unknown whether the dysbiosis is due to the acid-suppressive effect or the specific drug-class effect of PPIs.[14,33] It is well known that dysbiosis of gut microbiota contributes to the pathogenesis of IBD by causing inflammatory responses and disrupting the intestinal mucosa barrier. The disruption of epithelial barrier may result in microbes passages to lamina propria, which could elicit an immune response.

To confirm the anti-colitis effect of tegoprazan, DSS-induced colitis model and DNBS-induced colitis model were used. DSS and DNBS model is widely used in IBD research due to mimic some clinical aspects of UC and CD respectively in humans.[30,31,34] Interestingly, tegoprazan improved colitis symptoms such as body weight loss, diarrhea, colon shortening, and histological damage. Tegoprazan also reduced the expression of inflammatory cytokines such as *Tnf-α*, *Il-6*, and *Il-1*β, which are associated with colonic damage. However, rabeprazole did not show any anti-colitis effect in both DSS-induced mice and DNBS-induced colitis.

Also, the effect of tegoprazan on the intestinal barrier was confirmed via *in vitro* and *in vivo* experiments. The intestinal mucosa barrier plays an important role in IBD because it is the first barrier to protecting against foreign pathogens. Tight junctions located in epithelial and endothelial cell layers play essential roles in formation of the intestinal epithelial barrier. Destruction of the intestinal tight junction barrier increases intestinal permeability, leading to disturbance and inflammation of the mucosal immune system.[35-37] Thus, regulation of tight junctions is important to maintain epithelial barrier integrity in UC. In the DSS-induced colitis model, tegoprazan reduced intestinal permeability and upregulated the expression of tight junction proteins Zo-1 and Occludin. Consistent with the results of in vivo experiments, tegoprazan promoted the expression level of tight junction proteins and reduced gut

permeability in Caco-2 cell monolayer model. On the other hand, rabeprazole did not alleviate barrier damage and showed a tendency to worsen barrier function in a dose-dependent manner. These results suggest that tegoprazan has a direct protective effect on intestinal barrier function.

Recently, numerous studies have demonstrated that gut dysbiosis is induced by inhibition of gastric acid secretion.[38] The long-term use or overuse of PPIs has been exhibited to affect the microbiota's composition and promote bacterial overgrowth.[14] In particular, long-term PPI administration has been reported to exacerbate the severity of IBD patients.

In this study, metagenome analysis was performed to confirm whether tegoprazan causes gut dysbiosis like PPI and the effect of gastric acid suppression by Tegoprazan on the intestinal microbiota composition. As a result of metagenome analysis, Tegoprazan increased the phylum *Bacteroidetes* reduced by DSS. However, rabeprazole did not increase *Bacteroidetes* reduced by DSS and markedly increased *Proteobacteria* at the phylum level. In particular, rabeprazole significantly increased *E.coli* belonging to phylum *Proteobacteria*. In patients with UC, a higher level of Proteobacteria was observed in the severe stage than the mild and moderate and mild stages of inflammation. *Proteobacteria* are often found to be increased in IBD.[39,40] *Tlr-5* KO mice, and *Il-10* KO mice revealed spontaneous colitis, which was associated with an abnormal expansion of *Proteobacteria*.[41,42] Taken together, rabeprazole exacerbates colitis through the abnormal expansion of *Proteobacteria*.

Bacteroides strains are related to the inflammatory response and the immune system. *Bacteroides* surface structures have been shown to exert immunomodulatory effects. *Bacteroides fragilis* produces polysaccharide A (PSA), which can prevent colitis by inhibiting the Th17-related cytokines.[43] In rabbits, the combination of *B. fragilis* and *Bacillus subtilis* promoted GALT (intestinal-associated lymphoid tissue) development.[44] In addition, *Bacteroides thetaiotaomicron* has been reported to alleviate colitis by enhancing barrier function through IL-10 production and reducing pro-inflammatory NF-κB

signaling.[45-47] *B. vulgatus* has been known to be associated with the pathogenesis of intestinal inflammation in animal models of IBD and humans.[48-51] But recently, several studies have been reported that *B. vulgatus* has a protective effect on intestinal inflammation. In *Il-2* KO mice, *B. vulgatus* ameliorated *E.coli*-induced colitis development.[52] Administration of *B. vulgatus* reduced LPS production by gut microbiota and protected against atherosclerosis in coronary artery disease (CAD) mice model.[53] *B. vulgatus* also inhibited intestinal infections by *V.cholerae.*[54] This study revealed that tegoprazan increased the abundance of species *B. vulgatus*. To determine whether *B. vulgatus* relieves colitis, DSS-induced colitis experiment was conducted. Administration of *B. vulgatus* slightly attenuated colitis and co-administration with tegoprazan and *B. vulgatus* more attenuated the symptom of colitis than administration of tegoprazan alone. Furthermore, bacterial growth experiments revealed that tegoprazan directly promotes the growth of *B. vulgatus*. However, rabeprazole did not promote the growth of *B. vulgatus*. Finally, bacterial adhesion assay was conducted to determine how *B. vulgatus* affects other bacteria, especially *Proteobacteria*. Adhesion of pathogenic bacteria to host epithelia is crucial because when bacteria pass through the epithelial cell and enter the lumen leads to bacterial disease such as infection.[55] *B. vulgatus* inhibited the adhesion of *S. typhimurium* to epithelial cells, but tegoprazan did not affect the adhesion of *S. typhimurium* to epithelial cells. These results demonstrated that tegoprazan ameliorates intestinal inflammation by increasing *B. vulgatus*, and increased *B. vulgatus* prevents invasion of the intestine by preventing the adhesion of pathogenic bacteria.

The mouse gut microbiota is similar to that of humans at the phylum level, but the similarity disappears at the lower taxonomic level, as the genus and species organize the predominant phylum *Bacteroidetes* differ quite between mice and humans. Indeed, several studies published before 2000 showed that *B. vulgatus* has been found in IBD patients and plays an important role in IBD's pathogenesis.[56-59] Therefore, further research is needed to clarify if tegoprazan improves intestinal inflammation by inducing *B. vulgatus* in IBD patients.

A previous study has reported that vonoprazan, another potent P-CAB, worsen nonsteroidal anti-inflammatory drug (NSAID)-induced small intestine injury by reducing beneficial bacteria. Analysis of the small intestine microbiota by Y Nadatani et al. showed that *B. vulgatus* was rarely present in the small intestine. One theory is that vonoprazan has alkaline pKa of 9.37,[60] which has relatively weak action in the stomach, but its activity is high in the alkaline small intestine, which might change the intestinal microbiota. On the other hand, tegoprazan has pKa of 5.2, so it is only activated in the stomach and has little effect on the small intestine, so it is thought that it has little effect on the changes of the intestinal microbiota. In addition, the NSAID-induced small intestinal injury model and the DSS and DNBS-induced colitis model have different mechanisms of action,[61] and the composition of microbiota in the small intestine and colon is quite different,[62,63] so that the results may differ. However, the anti-inflammatory effects of other P-CAB drugs are still unknown, further research is required to determine whether the anti-colitic effect of tegoprazan is due to a specific drug class effects of P-CABs. This is the first study to explain how tegoprazan regulates colitis and shows a link between tegoprazan and gut microbiota. Additionally, we showed that *B. vulgatus* was involved in the anti-colitic effect of tegoprazan, suggesting that tegoprazan may be useful in microbiota control and a novel therapeutic agent for IBD containing both UC and CD.

V. CONCLUSION

Potassium competitive acid blockers (P-CABs) suppress gastric acid secretion using a different mechanism than PPIs. Tegoprazan, a novel potassium-competitive acid blocker (P-CAB), has a stronger and longer-lasting effect on gastric acid suppression than traditional PPIs. However, the effect of tegoprazan on intestinal inflammation remains unknown. In particular, long-term PPI administration has been reported to exacerbate the severity of IBD patients. This study demonstrates for the first time that tegoprazan could ameliorate intestinal inflammation by enhancing intestinal epithelial barrier integrity and modulating the composition of the gut microbiota. Tegoprazan could promote the growth of certain gut bacteria, such as *Bacteroides vulgatus*, and maybe a novel therapeutic agent for IBD.

REFERENCES

1. Xavier RJ, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. Nature 2007;448:427-34.
2. Sartor RB. Current concepts of the etiology and pathogenesis of ulcerative colitis and Crohn's disease. Gastroenterol Clin North Am 1995;24:475-507.
3. Thia KT, Loftus EV, Jr., Sandborn WJ, Yang SK. An update on the epidemiology of inflammatory bowel disease in Asia. Am J Gastroenterol 2008;103:3167-82.
4. Ng SC, Tang W, Ching JY, Wong M, Chow CM, Hui AJ, et al. Incidence and phenotype of inflammatory bowel disease based on results from the Asia-pacific Crohn's and colitis epidemiology study. Gastroenterology 2013;145:158-65 e2.
5. Zhang YZ, Li YY. Inflammatory bowel disease: pathogenesis. World J Gastroenterol 2014;20:91-9.
6. Neis EP, Dejong CH, Rensen SS. The role of microbial amino acid metabolism in host metabolism. Nutrients 2015;7:2930-46.
7. Wu HJ, Wu E. The role of gut microbiota in immune homeostasis and autoimmunity. Gut Microbes 2012;3:4-14.
8. Michielan A, D'Inca R. Intestinal Permeability in Inflammatory Bowel Disease: Pathogenesis, Clinical Evaluation, and Therapy of Leaky Gut. Mediators Inflamm 2015;2015:628157.
9. Thursby E, Juge N. Introduction to the human gut microbiota. Biochem J 2017;474:1823-36.
10. Malfertheiner P, Kandulski A, Venerito M. Proton-pump inhibitors: understanding the complications and risks. Nat Rev Gastroenterol Hepatol 2017;14:697-710.
11. Yang YX, Lewis JD, Epstein S, Metz DC. Long-term proton pump inhibitor therapy and risk of hip fracture. JAMA 2006;296:2947-53.

12. de Jager CP, Wever PC, Gemen EF, van Oijen MG, van Gageldonk-Lafeber AB, Siersema PD, et al. Proton pump inhibitor therapy predisposes to community-acquired Streptococcus pneumoniae pneumonia. Aliment Pharmacol Ther 2012;36:941-9.
13. Deshpande A, Pant C, Pasupuleti V, Rolston DD, Jain A, Deshpande N, et al. Association between proton pump inhibitor therapy and Clostridium difficile infection in a meta-analysis. Clin Gastroenterol Hepatol 2012;10:225-33.
14. Imhann F, Bonder MJ, Vich Vila A, Fu J, Mujagic Z, Vork L, et al. Proton pump inhibitors affect the gut microbiome. Gut 2016;65:740-8.
15. Shah R, Richardson P, Yu H, Kramer J, Hou JK. Gastric Acid Suppression Is Associated with an Increased Risk of Adverse Outcomes in Inflammatory Bowel Disease. Digestion 2017;95:188-93.
16. Yang I, Nell S, Suerbaum S. Survival in hostile territory: the microbiota of the stomach. FEMS Microbiol Rev 2013;37:736-61.
17. Andersson K, Carlsson E. Potassium-competitive acid blockade: a new therapeutic strategy in acid-related diseases. Pharmacol Ther 2005;108:294-307.
18. Vakil N. Review article: new pharmacological agents for the treatment of gastro-oesophageal reflux disease. Aliment Pharmacol Ther 2004;19:1041-9.
19. Inatomi N, Matsukawa J, Sakurai Y, Otake K. Potassium-competitive acid blockers: Advanced therapeutic option for acid-related diseases. Pharmacol Ther 2016;168:12-22.
20. Arikawa Y, Nishida H, Kurasawa O, Hasuoka A, Hirase K, Inatomi N, et al. Discovery of a novel pyrrole derivative 1-[5-(2-fluorophenyl)-1-(pyridin-3-ylsulfonyl)-1H-pyrrol-3-yl]-N-meth ylmethanamin e fumarate (TAK-438) as a potassium-competitive acid blocker (P-CAB). J Med Chem 2012;55:4446-56.
21. Kim DK, Lee KH, Kim SJ, Kim SJ, Lee SJ, Park CH, et al. Effects of

Tegoprazan, a Novel Potassium-Competitive Acid Blocker, on Rat Models of Gastric Acid-Related Disease. J Pharmacol Exp Ther 2019;369:318-27.

22. Dieleman LA, Palmen MJ, Akol H, Bloemena E, Pena AS, Meuwissen SG, et al. Chronic experimental colitis induced by dextran sulphate sodium (DSS) is characterized by Th1 and Th2 cytokines. Clin Exp Immunol 1998;114:385-91.
23. Yoon SH, Ha SM, Kwon S, Lim J, Kim Y, Seo H, et al. Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. Int J Syst Evol Microbiol 2017;67:1613-7.
24. Neurath MF. Cytokines in inflammatory bowel disease. Nat Rev Immunol 2014;14:329-42.
25. Pastorelli L, De Salvo C, Mercado JR, Vecchi M, Pizarro TT. Central role of the gut epithelial barrier in the pathogenesis of chronic intestinal inflammation: lessons learned from animal models and human genetics. Front Immunol 2013;4:280.
26. Sambuy Y, De Angelis I, Ranaldi G, Scarino ML, Stammati A, Zucco F. The Caco-2 cell line as a model of the intestinal barrier: influence of cell and culture-related factors on Caco-2 cell functional characteristics. Cell Biol Toxicol 2005;21:1-26.
27. Hooper LV, Littman DR, Macpherson AJ. Interactions between the microbiota and the immune system. Science 2012;336:1268-73.
28. Jandhyala SM, Talukdar R, Subramanyam C, Vuyyuru H, Sasikala M, Nageshwar Reddy D. Role of the normal gut microbiota. World J Gastroenterol 2015;21:8787-803.
29. Ofek I, Hasty DL, Sharon N. Anti-adhesion therapy of bacterial diseases: prospects and problems. FEMS Immunol Med Microbiol 2003;38:181-91.
30. Morampudi V, Bhinder G, Wu X, Dai C, Sham HP, Vallance BA, et al.

DNBS/TNBS colitis models: providing insights into inflammatory bowel disease and effects of dietary fat. J Vis Exp 2014:e51297.

31. Antoniou E, Margonis GA, Angelou A, Pikouli A, Argiri P, Karavokyros I, et al. The TNBS-induced colitis animal model: An overview. Ann Med Surg (Lond) 2016;11:9-15.
32. Zhang Z, Zheng M, Bindas J, Schwarzenberger P, Kolls JK. Critical role of IL-17 receptor signaling in acute TNBS-induced colitis. Inflamm Bowel Dis 2006;12:382-8.
33. Wallace JL, Syer S, Denou E, de Palma G, Vong L, McKnight W, et al. Proton pump inhibitors exacerbate NSAID-induced small intestinal injury by inducing dysbiosis. Gastroenterology 2011;141:1314-22, 22 e1-5.
34. Chassaing B, Aitken JD, Malleshappa M, Vijay-Kumar M. Dextran sulfate sodium (DSS)-induced colitis in mice. Curr Protoc Immunol 2014;104:15 25 1-15 25 14.
35. Tsukita S, Furuse M, Itoh M. Multifunctional strands in tight junctions. Nat Rev Mol Cell Biol 2001;2:285-93.
36. Lee SH. Intestinal permeability regulation by tight junction: implication on inflammatory bowel diseases. Intest Res 2015;13:11-8.
37. Buckley A, Turner JR. Cell Biology of Tight Junction Barrier Regulation and Mucosal Disease. Cold Spring Harb Perspect Biol 2018;10.
38. Martinsen TC, Bergh K, Waldum HL. Gastric juice: a barrier against infectious diseases. Basic Clin Pharmacol Toxicol 2005;96:94-102.
39. Walujkar SA, Dhotre DP, Marathe NP, Lawate PS, Bharadwaj RS, Shouche YS. Characterization of bacterial community shift in human Ulcerative Colitis patients revealed by Illumina based 16S rRNA gene amplicon sequencing. Gut Pathog 2014;6:22.
40. Frank DN, St Amand AL, Feldman RA, Boedeker EC, Harpaz N, Pace NR. Molecular-phylogenetic characterization of microbial community

imbalances in human inflammatory bowel diseases. Proc Natl Acad Sci U S A 2007;104:13780-5.

41. Carvalho FA, Koren O, Goodrich JK, Johansson ME, Nalbantoglu I, Aitken JD, et al. Transient inability to manage proteobacteria promotes chronic gut inflammation in TLR5-deficient mice. Cell Host Microbe 2012;12:139-52.
42. Maharshak N, Packey CD, Ellermann M, Manick S, Siddle JP, Huh EY, et al. Altered enteric microbiota ecology in interleukin 10-deficient mice during development and progression of intestinal inflammation. Gut Microbes 2013;4:316-24.
43. Troy EB, Kasper DL. Beneficial effects of Bacteroides fragilis polysaccharides on the immune system. Front Biosci (Landmark Ed) 2010;15:25-34.
44. Rhee KJ, Sethupathi P, Driks A, Lanning DK, Knight KL. Role of commensal bacteria in development of gut-associated lymphoid tissues and preimmune antibody repertoire. J Immunol 2004;172:1118-24.
45. Gordon JI. Honor thy gut symbionts redux. Science 2012;336:1251-3.
46. Xu J, Gordon JI. Honor thy symbionts. Proc Natl Acad Sci U S A 2003;100:10452-9.
47. Delday M, Mulder I, Logan ET, Grant G. Bacteroides thetaiotaomicron Ameliorates Colon Inflammation in Preclinical Models of Crohn's Disease. Inflamm Bowel Dis 2019;25:85-96.
48. Onderdonk AB, Cisneros RL, Bronson RT. Enhancement of experimental ulcerative colitis by immunization with Bacteroides vulgatus. Infect Immun 1983;42:783-8.
49. Rath HC, Wilson KH, Sartor RB. Differential induction of colitis and gastritis in HLA-B27 transgenic rats selectively colonized with Bacteroides vulgatus or Escherichia coli. Infect Immun 1999;67:2969-74.
50. Bloom SM, Bijanki VN, Nava GM, Sun L, Malvin NP, Donermeyer DL,

et al. Commensal Bacteroides species induce colitis in host-genotype-specific fashion in a mouse model of inflammatory bowel disease. Cell Host Microbe 2011;9:390-403.

51. Hoentjen F, Tonkonogy SL, Qian BF, Liu B, Dieleman LA, Sartor RB. CD4(+) T lymphocytes mediate colitis in HLA-B27 transgenic rats monoassociated with nonpathogenic Bacteroides vulgatus. Inflamm Bowel Dis 2007;13:317-24.
52. Waidmann M, Bechtold O, Frick JS, Lehr HA, Schubert S, Dobrindt U, et al. Bacteroides vulgatus protects against Escherichia coli-induced colitis in gnotobiotic interleukin-2-deficient mice. Gastroenterology 2003;125:162-77.
53. Yoshida N, Emoto T, Yamashita T, Watanabe H, Hayashi T, Tabata T, et al. Bacteroides vulgatus and Bacteroides dorei Reduce Gut Microbial Lipopolysaccharide Production and Inhibit Atherosclerosis. Circulation 2018;138:2486-98.
54. You JS, Yong JH, Kim GH, Moon S, Nam KT, Ryu JH, et al. Commensal-derived metabolites govern Vibrio cholerae pathogenesis in host intestine. Microbiome 2019;7:132.
55. Ribet D, Cossart P. How bacterial pathogens colonize their hosts and invade deeper tissues. Microbes Infect 2015;17:173-83.
56. Tvede M, Bondesen S, Nielsen OH, Rasmussen SN. Serum antibodies to Bacteroides species in chronic inflammatory bowel disease. Scand J Gastroenterol 1983;18:783-9.
57. Matsuda H, Fujiyama Y, Andoh A, Ushijima T, Kajinami T, Bamba T. Characterization of antibody responses against rectal mucosa-associated bacterial flora in patients with ulcerative colitis. J Gastroenterol Hepatol 2000;15:61-8.
58. Bamba T, Matsuda H, Endo M, Fujiyama Y. The pathogenic role of Bacteroides vulgatus in patients with ulcerative colitis. J Gastroenterol 1995;30 Suppl 8:45-7.

59. Onderdonk AB, Franklin ML, Cisneros RL. Production of experimental ulcerative colitis in gnotobiotic guinea pigs with simplified microflora. Infect Immun 1981;32:225-31.
60. Scott DR, Munson KB, Marcus EA, Lambrecht NW, Sachs G. The binding selectivity of vonoprazan (TAK-438) to the gastric H+, K+ -ATPase. Aliment Pharmacol Ther 2015;42:1315-26.
61. Randhawa PK, Singh K, Singh N, Jaggi AS. A review on chemical-induced inflammatory bowel disease models in rodents. Korean J Physiol Pharmacol 2014;18:279-88.
62. Scheithauer TP, Dallinga-Thie GM, de Vos WM, Nieuwdorp M, van Raalte DH. Causality of small and large intestinal microbiota in weight regulation and insulin resistance. Mol Metab 2016;5:759-70.
63. Hillman ET, Lu H, Yao T, Nakatsu CH. Microbial Ecology along the Gastrointestinal Tract. Microbes Environ 2017;32:300-13.

ABSTRACT (IN KOREAN)

염증성 장질환 마우스 모델에서
칼륨 경쟁적 위산분비 차단제인 테고프라잔의 항염증 효과

< 지도교수 천재희 >

연세대학교 대학원 의과학과

손 미 정

염증성 장질환은 장내 비정상적인 염증과 궤양을 반복하는 만성 질환이다. 염증성 장질환의 원인은 아직 불분명하지만, 유전적 요인과 환경적 요인 등 다양한 요인이 복합적으로 작용하는 것으로 알려져 있으며 특히 장내 미생물무리의 조성 변화가 중요한 요인으로 여겨진다. 특히 장내 미생물은 장 상피세포의 분화를 도우며 Tight junction protein 발현 촉진 등을 통해 장벽 기능 강화에 영향을 미친다.

양성자 펌프 억제제(이하 PPI)는 위산 분비 억제제로 전세계적으로 널리 처방되는 약제이지만 PPI를 장기 복용 시 골절, 폐렴, 장내 미생물 불균형과 같은 부작용이 발생할 수 있다는 보고가 있다.

칼륨 경쟁적 위산 분비 차단제(이하 P-CAB)는 PPI와 다른 메커니즘을 사용하여 위산 분비를 억제하며 기존 PPI보다 위산 억제에 대해 더 강력하고 오래 지속되는 효과가 있다. 그러나 장 염증에 대한 테고프라잔의 효과는 아직 알려져 있지 않다.

본 연구에서는 대장염 동물모델을 이용하여, 테고프라잔의 항 대장염 효과를 평가하였다. 테고프라잔은 DSS로 유도한 궤양성 대장염 동물 모델에서 체중 감소, 설사, 출혈, 및 조직 학적 손상과 같은 대장염의 증상을 개선하였고 Tight junction 단백질들의 발현을 상향 조절함으로써 장 상피 장벽 기능을

향상시켰다. 또한, 장내 미생물 유전체 분석을 통해 테고프라잔이 DSS에 의한 장내 미생물의 불균형을 완화시키고 박테로이데스 불가투스를 증가시킴을 확인하였다. 박테로이데스 불가투스는 병원성 세균의 상피 부착을 억제하였고, 대장염 증상 완화에 효과가 있음을 DSS 유도 궤양성 대장염 동물 모델을 통해서 증명하였다. 뿐만 아니라, 테고프라잔은 크론병 모델인 DNBS로 유도한 대장염 모델에서도 대장염의 증상을 개선하는 결과를 보였다. 이러한 결과들을 통해, 테고프라잔이 효과적으로 장 염증을 완화한다는 것을 알 수 있으며, 새로운 염증성 장질환 치료제로서 가능성을 제시할 수 있다.

핵심되는 말: 염증성 장질환, 테고프라잔, 칼륨 경쟁적 위산 분비 차단제, 박테로이데스 불가투스