

Preventive administration of verapamil attenuates dextran sulfate sodium-induced colitis in mice

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Abstract. Inflammatory bowel disease, including ulcerative colitis (UC) and Crohn's disease, is a chronic immune-mediated disorder characterized by relapsing gastrointestinal inflammation. UC is an idiopathic chronic relapsing-remitting inflammatory disorder affecting the colon, characterized by diarrhea and rectal bleeding. Verapamil, a phenylalkylamine-class L-type calcium channel blocker, has long been used to treat cardiovascular diseases such as hypertension, angina and arrhythmia. The present study aimed to investigate whether verapamil ameliorates UC symptoms in a murine model. In addition, the effects of verapamil on i) inflammatory cytokines, ii) the intestinal microbiota and iii) the intestinal mucosal barrier, all of which are implicated in UC pathogenesis, were investigated. UC was induced in mice by administering 2.5% dextran sulfate sodium (DSS) in the drinking water. Preventive oral administration of verapamil (10 mg/kg/day) initiated 7 days before DSS exposure attenuated body weight loss, diarrhea, and bloody stools in mice with DSS-induced colitis. Verapamil also reduced colonic inflammatory cytokine levels, partially reversed DSS-associated alterations in the intestinal microbiota, and preserved the expression of selected tight junction-related proteins and colonic glutathione levels. These findings suggested that verapamil may exert protective effects in this experimental model of colitis; however, further studies are needed to determine its potential clinical relevance in UC.

Introduction

Inflammatory bowel disease (IBD) is a chronic immune-mediated disorder characterized by relapsing gastrointestinal

inflammation that includes ulcerative colitis (UC) and Crohn's disease (1-3). In 2017, IBD was estimated to account for 6.8 million cases, 0.83 million years of life lost, and 1.02 million years lived with disability worldwide (4). Patients with IBD have a 2.7-fold higher risk of developing colorectal cancer than the general population (5). UC is a chronic inflammatory disorder primarily affecting the colonic and rectal mucosa. Although its etiology remains unclear, accumulating evidence suggests that dysregulated immune responses, oxidative stress, intestinal epithelial barrier dysfunction, and impaired cellular homeostasis contribute to its pathogenesis (6,7). Despite substantial advances in UC management, current therapies remain inadequate for many patients. Therefore, novel therapeutic strategies are needed to suppress disease progression and prevent colitis-associated carcinogenesis.

Verapamil, a phenylalkylamine L-type calcium channel blocker, has long been used to treat cardiovascular diseases such as hypertension, angina, and arrhythmia. Emerging evidence suggests that verapamil may also benefit diabetes, liver disease, and inflammatory neurological disorders (8). Both *in vitro* and *in vivo* studies have demonstrated its anti-inflammatory effects. Verapamil attenuates acute liver injury and suppresses pro-inflammatory cytokine production *in vivo* (9,10), while *in vitro* studies have shown that it reduces pro-inflammatory cytokine production (11,12). However, its effects on UC remain unclear.

UC is characterized by recurrent episodes of relapse and remission. Therefore, interventions that reduce susceptibility to subsequent intestinal inflammation may help prevent relapse. In this study, verapamil was administered prophylactically before dextran sulfate sodium (DSS) exposure. We investigated whether verapamil protects against DSS-induced colitis and evaluated its effects on inflammatory cytokine production, intestinal microbiota composition, selected tight junction-related proteins, and colonic GSH levels.

Materials and methods

Animal experiments. Specific pathogen-free 6-week-old male ddY mice (25-30 g) were purchased from Sankyo Labo Service Corporation (Tokyo, Japan). The mice were acclimatized under the following controlled conditions: temperature (23±2°C), humidity (55±5%), and light (a 12 h light/dark cycle). Thereafter, the mice were divided into groups (six mice

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per group) such that the mean body weight was comparable among groups. All animal experiments were approved by the Institutional Animal Care and Use Committee of Hokkaido University of Science (approval no. 2025-005; Sapporo, Japan) and conducted in accordance with the relevant guidelines and regulations. At the culmination of the experiment, mice were euthanized via CO₂ asphyxiation using a gradual-fill method with a displacement rate of 30% of the chamber volume per minute. Mice were monitored at least once daily throughout the experiment. During DSS administration, the same six mice in each group were weighed daily, and their stool consistency, rectal bleeding, and general appearance were assessed daily. Humane endpoints were defined as loss of more than 20% of the initial body weight, severe or persistent diarrhea or rectal bleeding, marked lethargy, hunching, dehydration, inability to access food or water, or a moribund condition. None of the mice reached the humane endpoints before the scheduled endpoint of the experiment.

UC model mice. Colitis was induced by administering 2.5% DSS in drinking water for 7 days. Verapamil (10 mg/kg/day) was administered orally beginning 7 days before DSS exposure. This preventive protocol was designed to evaluate protection against DSS-induced epithelial injury and inflammatory responses rather than treatment of established colitis. It may also partly reflect the clinical strategy of preventing relapse during UC remission.

The verapamil dose was selected based on previous *in vivo* mouse studies reporting anti-inflammatory effects at a comparable dose (10 mg/kg) (13,14). Because only a single dose was evaluated, this study was not designed to determine the optimal dose or characterize a dose-response relationship. Mice were randomly assigned to four groups (n=6/group): control, DSS, verapamil, and DSS + verapamil.

Assessment of disease activity index (DAI). To assess UC severity, we recorded and calculated body weight, stool consistency, and gross rectal bleeding according to a previously published grading system (15). Briefly, weight loss was scored as follows: 0-5%, score 0; 5-10%, score 1; 10-15%, score 2; 15-20%, score 3; >20%, score 4. Stool consistency: normal, score 0; loose stools, score 2; watery diarrhea, score 4. Bloody stool: none, score 0; presence of bleeding, score 4.

Histological analysis. Colon tissue samples were fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned at 4 μ m, and stained with hematoxylin and eosin (H&E). Histological examination was performed using a ZEISS Axio Vert.A1 microscope (Carl Zeiss, Jena, Germany).

16S rRNA gene sequencing. Fecal samples were collected, and bacterial genomic DNA was extracted using the FastGene Gel/PCR Extraction Kit (Nippon Genetics Co., Ltd., Tokyo, Japan; Cat. #FG-91202). The V3-V4 region of the bacterial 16S rRNA gene was amplified using locus-specific primers 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-GACTAC HVGGGTATCTAATCC-3') (16). For Illumina library preparation, Illumina overhang adapter sequences were incorporated into these locus-specific primers; the sequences of the resulting primers were as follows: forward primer, 5'-TCGTCGGCAGCG

TCAGATGTGTATAAGAGACAG-CCTACGGGNGGCWGCAG-3'; reverse primer, 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-GACTACHVGGGTATCTAATCC-3'. PCR amplification was performed using KAPA HiFi HotStart ReadyMix (Nippon Genetics Co., Ltd.; Cat. #KK2601) under the following conditions: initial denaturation at 95°C for 3 min; 25 cycles of denaturation at 95°C for 30 sec, annealing at 55°C for 30 sec, and extension at 72°C for 30 sec; and a final extension at 72°C for 5 min. PCR products were purified using AMPure XP beads (Beckman Coulter, Indianapolis, IN, USA; Cat. #A63881) and indexed using the Nextera XT Index Kit (Illumina, San Diego, CA, USA). Libraries were further purified and quantified before sequencing. Paired-end sequencing (2x300 bp) was performed on an Illumina MiSeq platform. Raw sequencing data were processed using QIIME 2 (version 2022.2). Demultiplexed sequences underwent quality filtering and were denoised using the DADA2 plugin with default parameters, including chimera removal. Amplicon sequence variants (ASVs) were generated, and taxonomic assignment was performed using a Naïve Bayes classifier trained on the SILVA 138 reference database. Alpha diversity indices (observed ASVs, Shannon, Simpson, Chao1, and ACE) were calculated using QIIME 2. The sequencing data were deposited in the DDBJ Sequence Read Archive under accession number PRJDB40750 (DRR932518-DRR932529).

Enzyme-linked immunosorbent assay (ELISA). Colon tissues from each group were homogenized in lysis buffer to extract total protein. Total protein concentration was measured using the BCA Protein Assay Kit (Takara Bio Inc., Shiga, Japan; Cat. #T9300A). The following Mouse DuoSet ELISA Kits were used according to the manufacturer's instructions: IL-1 β (Cat. #DY401-05), IL-6 (Cat. #DY406-05), and TNF- α (Cat. #DY410) (all from R&D Systems, Minneapolis, MN, USA). The DuoSet Ancillary Reagent Kit 2 (Cat. #DY008B; R&D Systems) was used as a supplementary reagent.

Western blotting. Colon tissues from each group were homogenized in lysis buffer [50 mM HEPES (pH 7.4), 5 mM ethylenediaminetetraacetic acid (EDTA), 120 mM NaCl, 1% Triton X-100, protease inhibitors (10 μ g/ml aprotinin, 1 mM phenylmethylsulfonyl fluoride, and 10 μ g/ml leupeptin), and phosphatase inhibitors (50 mM sodium fluoride, 1 mM sodium orthovanadate, and 10 mM sodium pyrophosphate)] to extract total protein. Total protein concentration was determined using the BCA Protein Assay Kit (Takara Bio Inc.; Cat. #T9300A). Claudin-1 and claudin-4 protein levels were analyzed by western blotting. Lysates were centrifuged at 10,000 x g for 15 min, and 20 μ g of protein from the supernatant was resolved by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The separated proteins were transferred to polyvinylidene difluoride (PVDF) membranes. Membranes were blocked with 5% non-fat dry milk in Tris-buffered saline containing 0.1% Tween-20 (TBST) for 1 h at room temperature. Membranes were incubated with the corresponding primary antibodies: anti-claudin-1 rabbit polyclonal antibody (Proteintech, Rosemont, IL, USA; Cat. #13050-1-AP; dilution 1:1,000), anti-claudin-4 rabbit polyclonal antibody (Proteintech; Cat. #16195-1-AP; dilution 1:1,000), and anti- β -actin mouse monoclonal antibody (Sigma-Aldrich, St. Louis, MO, USA; Cat. #A2228; dilution 1:5,000). Following incubation with the primary antibodies, membranes were incubated

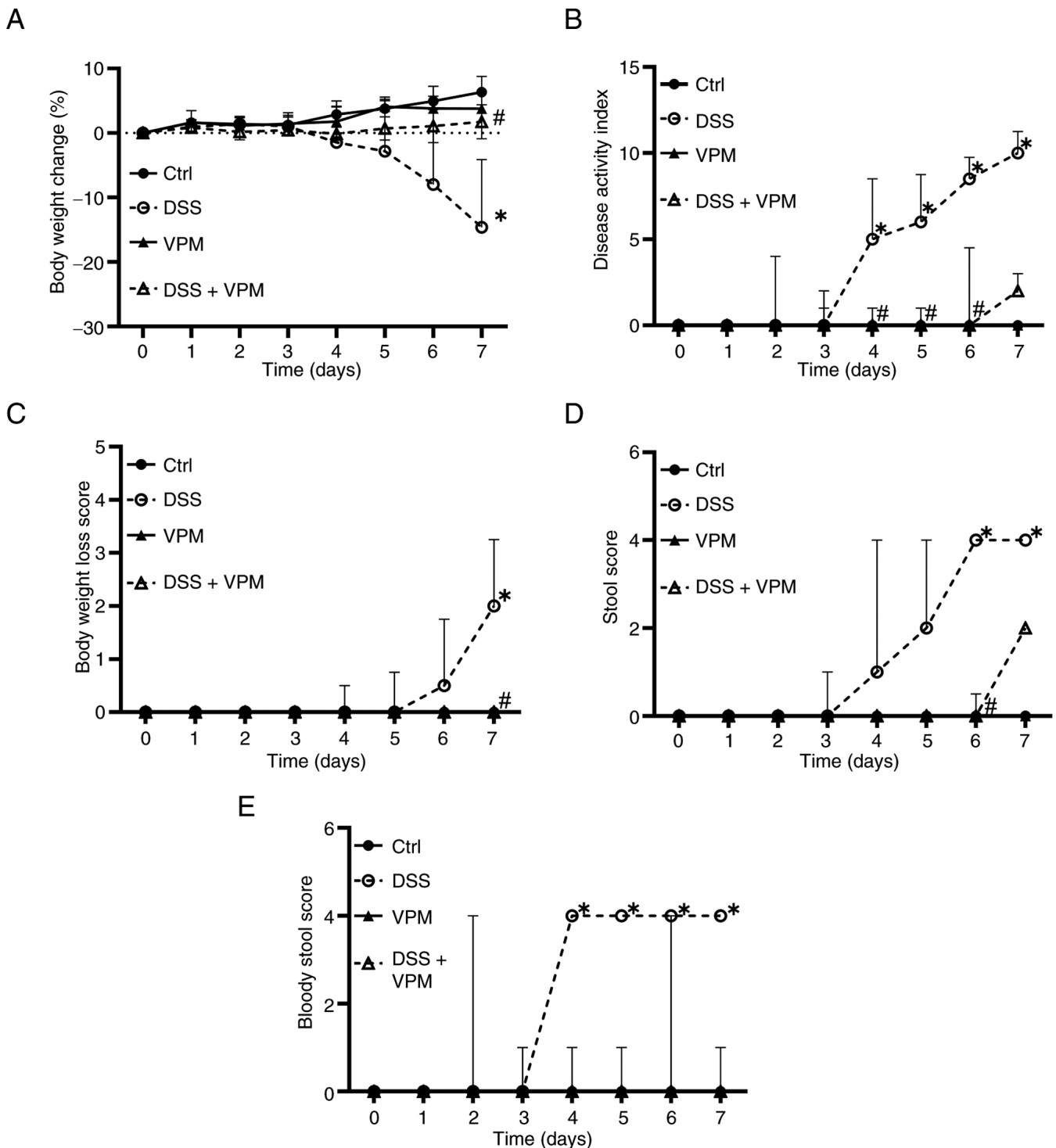


Figure 1. Effects of VPM on pathology in the DSS-induced colitis model. ddY mice were orally administered water or VPM (10 mg/kg body weight) for 7 days, followed by 2.5% DSS-containing drinking water or tap water for an additional 7 days. (A) Body weight was measured daily in the same six mice in each group. (B) To assess the disease activity index of colitis, body weight, stool consistency, and gross rectal bleeding were recorded and scored. Scoring criteria were as follows: (C) Body weight loss score (0-5%, 0; 5-10%, 1; 10-15%, 2; 15-20%, 3; >20%, 4); (D) stool consistency score (normal, 0; soft stool, 2; watery diarrhea, 4); and (E) bloody stool score (none, 0; presence of bleeding, 4). Data in panel A are presented as mean $\pm$ SD. Data in panels B-E are presented as median with interquartile range. * $P < 0.05$ vs. Ctrl; # $P < 0.05$ vs. DSS. Ctrl, control; DSS, dextran sulfate sodium; VPM, verapamil.

with horseradish peroxidase-conjugated secondary antibodies: anti-rabbit (Cell Signaling Technology, MA, USA; Cat. #7074; dilution 1:2,000) or anti-mouse (Cell Signaling Technology; Cat. #7076; dilution 1:2,000). Chemiluminescence was detected using Immobilon (Merck, Darmstadt, Germany). All western blotting experiments were performed in triplicate.

Measurement of glutathione (GSH) levels. To measure GSH levels, colon samples were mixed with 0.6 mM 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB; Cat. #D029, Dojindo Laboratories, Kumamoto, Japan), 0.2 mM reduced nicotinamide adenine dinucleotide phosphate (Cat. #44332000, Sigma-Aldrich, St. Louis, MO, USA), and 5 mM EDTA in

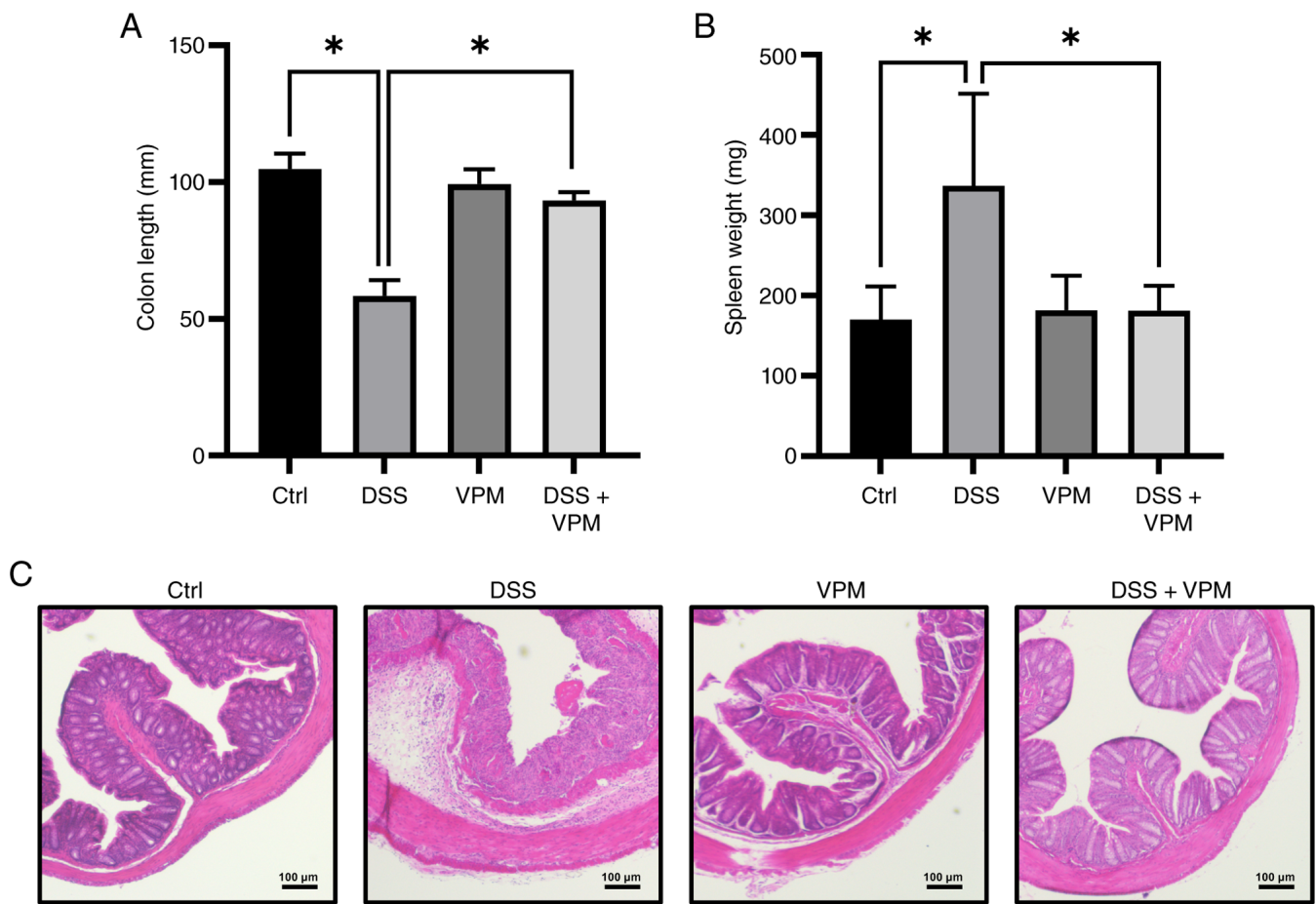


Figure 2. Effects of verapamil on macroscopic and histological changes in DSS-induced colitis. Mice were orally administered water or verapamil (10 mg/kg body weight) for 7 days, followed by 2.5% DSS-containing drinking water or tap water for an additional 7 days. The colon and spleen were excised to measure colon length (A) and spleen weight (B). Colon sections were stained with H&E and examined histologically (C). Scale bar, 100 μ m. Data are presented as mean $\pm$ SD (six mice per group). * P <0.05. Ctrl, control; DSS, dextran sulfate sodium; VPM, verapamil.

0.1 M sodium phosphate buffer (pH 7.5). The reaction was initiated by adding GSH reductase (Cat. #46541005, Oriental Yeast Co., Ltd., Tokyo, Japan) (17). Absorbance was measured at 412 nm using a spectrophotometer (UV-1800, Shimadzu Corporation, Kyoto, Japan).

Statistical analyses. Data are expressed as the mean $\pm$ standard deviation (SD) for continuous variables and as the median with interquartile range (IQR) for ordinal scoring data. Six mice per group were used in the animal experiments. The sample size ($n=6$ per group) was determined with reference to previous studies using DSS-induced colitis models, in which similar sample sizes were sufficient to detect statistically significant differences (18). In addition, the number of animals was minimized in accordance with ethical guidelines for animal experimentation. Statistical analyses were performed using GraphPad Prism (version 10.0; GraphPad Software, San Diego, CA, USA). Body weight changes over time were analyzed using two-way mixed-design ANOVA, with treatment group as the between-subject factor and time as the within-subject repeated-measures factor. As sphericity was not assumed, the Geisser-Greenhouse correction was applied to effects involving the repeated-measures factor. Tukey's multiple-comparisons test was subsequently used for

post hoc comparisons. Continuous data measured at a single endpoint and compared among the four treatment groups, including colon length, spleen weight, inflammatory cytokine levels, claudin expression levels and GSH levels, alpha diversity indices, and the relative abundance of *Desulfobacterota* G 459543, were analyzed using one-way ANOVA followed by Tukey's multiple-comparison test. Ordinal scoring data, including DAI, body weight loss score, stool consistency score and bloody stool score, were analyzed separately at each time point using the Kruskal-Wallis test followed by Dunn's multiple-comparisons test. PERMANOVA was used to analyze beta diversity. P <0.05 was considered to indicate a statistically significant difference.

Results

Effects of verapamil on pathology in DSS-induced colitis. Oral administration of verapamil (10 mg/kg/day) initiated 7 days before DSS exposure significantly attenuated DSS-induced weight loss (day 7: $P=0.041$ vs. DSS) (Fig. 1A). The DAI, which reflects body weight loss, stool consistency, and bloody stool, markedly increased in DSS-treated mice. Verapamil significantly reduced DAI scores in mice with DSS-induced colitis (day 4: $P=0.0367$; day 5: $P=0.0366$; day 6: $P=0.0384$)

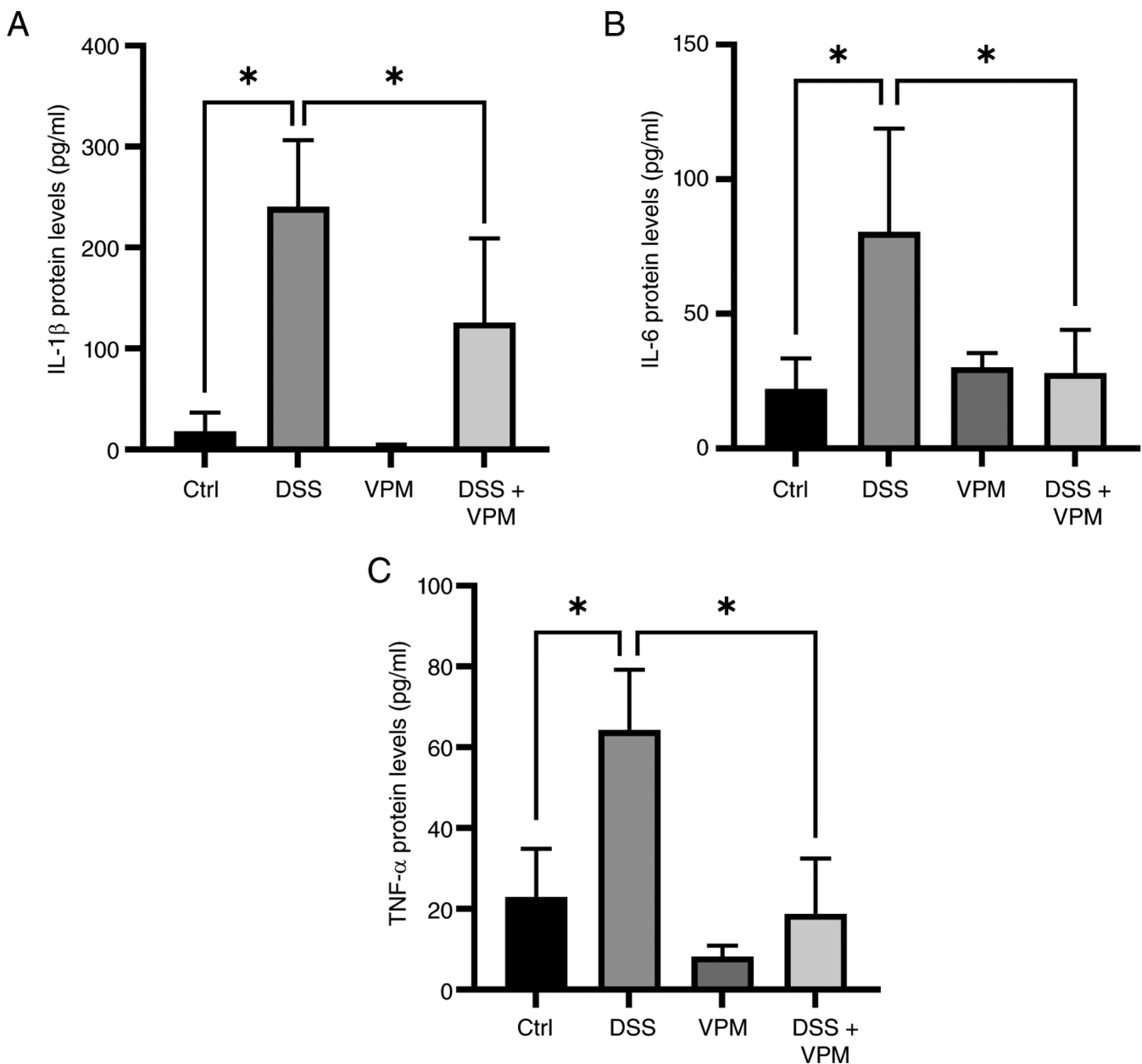


Figure 3. Effects of VPM on inflammatory cytokine levels in DSS-induced colitis. Mice were orally administered water or VPM (10 mg/kg body weight) for 7 days, followed by 2.5% DSS-containing drinking water or tap water for an additional 7 days. Mice were then euthanized, and colon tissues were harvested. Protein levels of (A) IL-1 β , (B) IL-6 and (C) TNF- α in the colon were measured by ELISA. Data are presented as mean $\pm$ SD from three independent experiments. * P <0.05. Ctrl, control; DSS, dextran sulfate sodium; VPM, verapamil.

(Fig. 1B-E). Verapamil alone did not cause obvious changes in body weight or DAI score compared with the control group under the present experimental conditions.

Effects of verapamil on the macroscopic and histological assessment of colitis in DSS-induced colitis. To evaluate the macroscopic and histological effects of verapamil on colitis, we measured colon length and spleen weight and performed H&E staining. DSS administration resulted in colon shortening and spleen enlargement compared with the control group (colon: P <0.0001 vs. Ctrl group; spleen: P =0.0005 vs. Ctrl group). Verapamil significantly attenuated DSS-induced colon shortening and spleen enlargement (colon: P <0.0001 vs. DSS group; spleen: P =0.0018 vs. DSS group) (Fig. 2A and B).

Histological examination revealed severe epithelial disruption, crypt distortion, and inflammatory cell infiltration in DSS-treated mice. By contrast, verapamil-treated mice exhibited reduced epithelial damage and inflammatory infiltration (Fig. 2C). No obvious macroscopic or histological abnormalities were observed in mice treated with verapamil alone.

Effects of verapamil on inflammatory cytokines in DSS-induced colitis. Excessive production of inflammatory cytokines (IL-1 β , IL-6, and TNF- α) is known to play a crucial role in the progression of colitis pathology (19). We investigated the effect of verapamil on inflammatory cytokine levels in mice with DSS-induced colitis. Colonic levels of IL-1 β , IL-6, and TNF- α were significantly elevated in DSS-treated mice

Table I. Effect of verapamil on alpha diversity in DSS-induced colitis.

Group	ASVs	Chao1 index	ACE index	Shannon index	Simpson index
Ctrl	281±13	281±13	281±13	6.597±0.041	0.981±0.002
DSS	248±8	248±8	248±8	6.023±0.070	0.964±0.003
VPM	246±13	247±13	247±13	6.273±0.110	0.974±0.002
DSS + VPM	223±30	223±30	223±30	5.792±0.202	0.958±0.006

Mice were orally administered water or VPM (10 mg/kg body weight) for 7 days, followed by 2.5% DSS-containing drinking water or tap water for an additional 7 days. Alpha diversity indices (observed ASVs, Chao1, ACE, Shannon, Simpson) were calculated based on microbial community analysis. Data are expressed as mean ± SD (three mice per group). ASV, amplicon sequence variant; ACE, abundance-based coverage estimator; Ctrl, control; DSS, dextran sulfate sodium; VPM, verapamil.

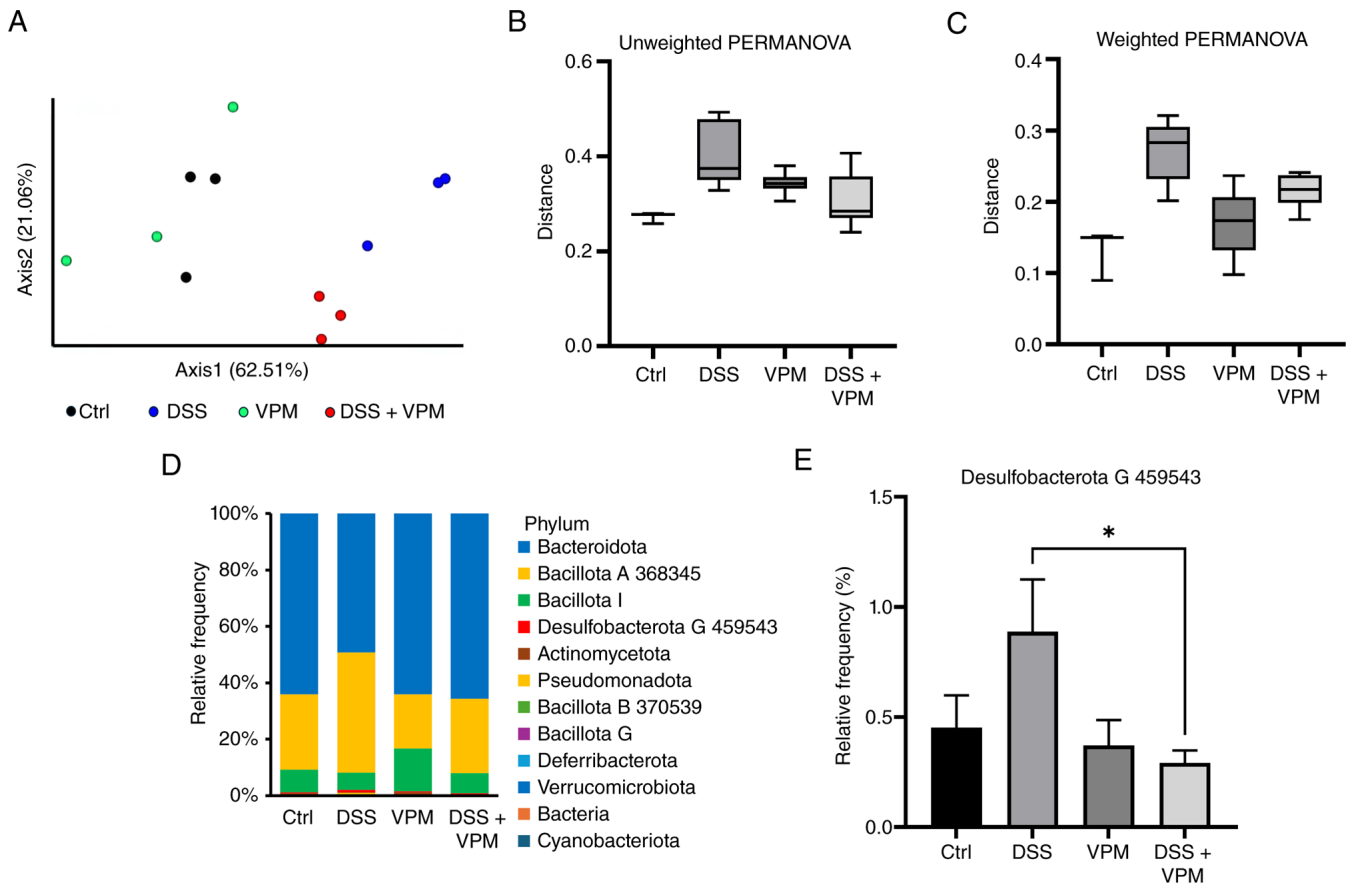


Figure 4. Effects of VPM on intestinal microbiota composition in DSS-induced colitis. Mice were orally administered water or VPM (10 mg/kg body weight) for 7 days, followed by 2.5% DSS-containing drinking water or tap water for an additional 7 days. (A) Principal coordinate analysis based on beta diversity analysis. (B) Unweighted PERMANOVA analysis. (C) Weighted PERMANOVA analysis. (D) Relative abundance of bacterial taxa at the phylum level. Relative abundance of (E) *Desulfobacterota* G 459543. * $P < 0.05$. Ctrl, control; DSS, dextran sulfate sodium; VPM, verapamil.

compared with controls (IL-1 β : $P = 0.0004$ vs. Ctrl group; IL-6: $P = 0.0118$ vs. Ctrl group; TNF- α : $P = 0.0016$ vs. Ctrl group). Verapamil significantly reduced the DSS-induced increase in these pro-inflammatory cytokines (IL-1 β : $P = 0.0462$ vs. DSS group; IL-6: $P = 0.0233$ vs. DSS group; TNF- α : $P = 0.0007$ vs. DSS group) (Fig. 3).

Effects of verapamil on intestinal microbiota in DSS-induced colitis. We evaluated alpha and beta diversity to examine the intestinal microbiota. The alpha diversity

indices, including amplicon sequence variants and the Chao1, ACE, Shannon, and Simpson indices, did not differ significantly among groups (Table I). Beta diversity analysis using principal coordinate analysis based on UniFrac distances demonstrated partial clustering among groups (Fig. 4A). PERMANOVA revealed significant overall differences among groups for both weighted ($P = 0.0002$) and unweighted ($P = 0.0001$) UniFrac distances (Fig. 4B and C); however, pairwise comparisons between individual groups did not reach statistical significance. Fig. 4D shows the

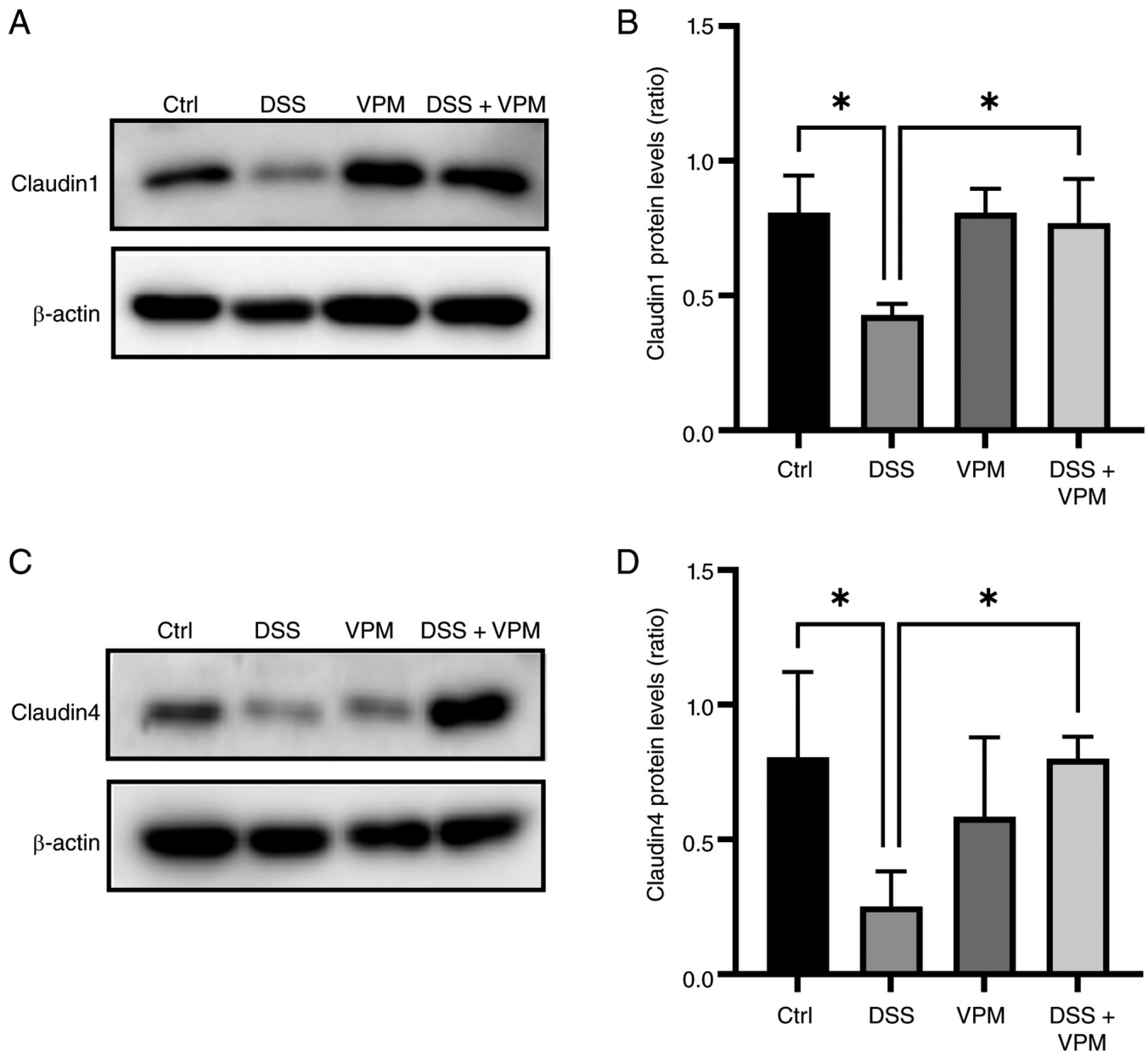


Figure 5. Effects of VPM on selected tight junction-related proteins in DSS-induced colitis. Mice were orally administered water or VPM (10 mg/kg body weight) for 7 days, followed by 2.5% DSS-containing drinking water or tap water for an additional 7 days. Colon tissues were harvested after euthanasia. (A) Representative western blotting image of claudin-1 expression. (B) Densitometric quantification of claudin-1 expression normalized to β -actin. (C) Representative western blotting image of claudin-4 expression. (D) Densitometric quantification of claudin-4 expression normalized to β -actin. Data are presented as mean $\pm$ SD (six mice per group). * $P < 0.05$. Ctrl, control; DSS, dextran sulfate sodium; VPM, verapamil.

phylum-level classification of the intestinal microbiota. The relative abundance of Desulfobacterota G 459543 was significantly lower in the DSS + verapamil group than in the DSS group ($P = 0.0062$) (Fig. 4E). In addition, verapamil alone did not cause obvious changes in alpha diversity indices or phylum-level microbiota composition compared with the control group under the present experimental conditions (Table I and Fig. 4D). These findings indicate that verapamil modulates the abundance of a specific bacterial taxon altered by DSS treatment.

Effects of verapamil on selected tight junction-related proteins in DSS-induced colitis. Claudins are key regulators of tight junction structure and epithelial barrier

integrity (20). Therefore, claudin-1 and claudin-4 were examined as representative tight junction-related proteins. Their expression was significantly reduced in DSS-treated mice (claudin-1: $P = 0.0057$ vs. Ctrl; claudin-4: $P = 0.0470$ vs. Ctrl). Verapamil significantly attenuated these reductions (claudin-1: $P = 0.0098$ vs. DSS; claudin-4: $P = 0.0382$ vs. DSS) (Fig. 5).

Effect of verapamil on GSH levels in DSS-induced colitis. Colonic GSH levels, measured as an indicator of antioxidant capacity, were significantly decreased in DSS-treated mice ($P = 0.0012$ vs. control). Verapamil significantly suppressed the DSS-induced reduction in GSH levels ($P = 0.007$ vs. DSS group) (Fig. 6).

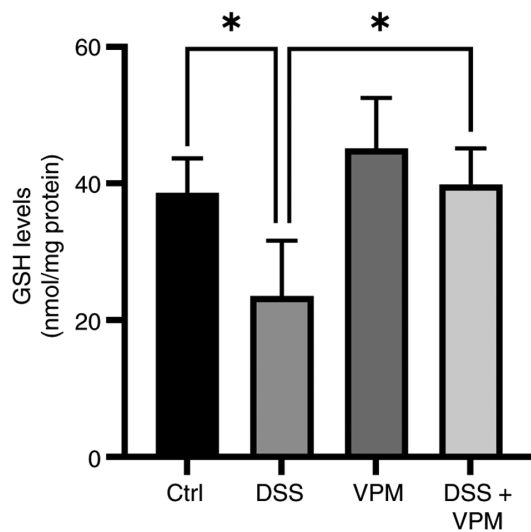


Figure 6. Effect of VPM on GSH levels in DSS-induced colitis. Mice were orally administered water or VPM (10 mg/kg body weight) for 7 days, followed by 2.5% DSS-containing drinking water or tap water for an additional 7 days. Colon tissues were harvested after euthanasia. GSH concentrations in colon tissues were measured. Data are presented as mean $\pm$ SD (six mice per group). * $P < 0.05$. Ctrl, control; DSS, dextran sulfate sodium; GSH, glutathione; VPM, verapamil.

Discussion

This study showed that preventive administration of verapamil attenuated the clinical and pathological features of DSS-induced colitis. It reduced colonic inflammatory cytokine levels, partially reversed DSS-associated alterations in the intestinal microbiota, preserved claudin-1 and claudin-4 expression, and maintained colonic GSH levels. These findings suggest that verapamil exerts protective effects in this experimental model, although its therapeutic efficacy after disease onset was not evaluated.

Although the etiology of UC remains unclear, excessive inflammatory cytokine production, microbial dysbiosis, and intestinal epithelial barrier dysfunction are widely recognized as key features of its pathogenesis (21–23). In this study, verapamil significantly suppressed DSS-induced increases in IL-1 β , IL-6, and TNF- α . Previous studies have shown that verapamil suppresses activation of the NLRP3 inflammasome, reduces pro-inflammatory cytokines such as IL-1 β (24,25), and attenuates acute liver injury by downregulating inflammatory cytokines (9). Our findings are consistent with these reports and further support the anti-inflammatory effects of verapamil. However, the upstream signaling mechanisms underlying these effects were not investigated in colon tissues. NF- κ B, MAPK, and NLRP3 inflammasome-related signaling pathways are key regulators of inflammatory cytokine production in experimental inflammation (26–28). Therefore, although verapamil reduced inflammatory cytokine levels, the molecular mechanisms responsible remain unclear. Further studies are needed to determine whether verapamil directly modulates these signaling pathways in DSS-induced colitis.

Alterations in the gut microbiota are increasingly implicated in UC pathogenesis. In this study, overall beta diversity analysis revealed significant differences among groups,

although pairwise comparisons did not reach statistical significance. In addition, the abundance of *Desulfobacterota* G 459543 was significantly lower in the DSS + verapamil group than in DSS-treated mice. These findings suggest that verapamil partially modulates DSS-induced microbial dysbiosis. Drugs used to treat cardiovascular disease, including calcium channel blockers, affect the intestinal microbiota, suggesting that these drugs may regulate gut microbial composition (29).

Disruption of the intestinal epithelial barrier is a hallmark of UC. Tight junction proteins help regulate paracellular permeability and epithelial integrity (7,20). In this study, DSS reduced claudin-1 and claudin-4 expression, and verapamil attenuated these reductions, suggesting that it may preserve selected tight junction-related proteins in DSS-induced colitis. However, assessment of claudin-1 and claudin-4 alone is insufficient to characterize intestinal barrier function. Additional barrier-associated molecules, including the tight junction proteins ZO-1 and occludin, the adherens junction protein E-cadherin, and the mucin MUC2, together with functional permeability assays, should be evaluated in future studies. Collectively, these results suggest that verapamil attenuates DSS-induced colitis through mechanisms associated with reduced inflammatory cytokine production, alterations in selected bacterial taxa, and preservation of selected tight junction-related proteins. The relative contribution of these mechanisms remains to be determined.

Oxidative stress is closely linked to inflammation, gut microbial dysbiosis, and epithelial barrier dysfunction in IBD (30–32). Reduced GSH is a major intracellular antioxidant that maintains cellular redox homeostasis (33,34). In this study, DSS reduced colonic GSH levels, whereas verapamil attenuated this reduction, suggesting that preservation of GSH may contribute to its protective effects. However, GSH alone is insufficient to characterize oxidative stress or establish an antioxidant mechanism. Classical markers of oxidative stress, antioxidant defense, and neutrophil-associated inflammation—such as malondialdehyde, 4-hydroxynonenal, reactive oxygen species, superoxide dismutase, catalase, GSH peroxidase, and myeloperoxidase—were not evaluated. Therefore, further studies are needed to determine whether verapamil directly modulates oxidative stress and antioxidant defenses in DSS-induced colitis.

Systematic drug repurposing (also referred to as drug repositioning or drug reprofiling) involves evaluating approved drugs for new therapeutic indications (35). Verapamil is currently approved to treat cardiovascular diseases, including angina, hypertension, and supraventricular tachycardia (36). The present findings support further investigation of verapamil in experimental models of colitis. However, this study did not compare verapamil with established UC therapies, such as 5-aminosalicylic acid preparations, immunomodulators, or biologics. Additionally, verapamil was not evaluated in human cells, clinical samples, or clinical trials. Therefore, these findings should not be interpreted as evidence of clinical effectiveness in UC.

This study had several limitations. First, only a single dose of verapamil was evaluated. Thus, the optimal dose, dose-response relationship, and therapeutic window remain unknown. Second, verapamil was administered prophylactically before DSS exposure, precluding assessment of therapeutic

efficacy after disease onset. Third, the DSS-induced murine model does not fully reproduce the complexity of human UC. Fourth, intestinal barrier assessment was limited to claudin-1 and claudin-4 and additional barrier-related molecules and functional permeability assays should be included in future studies. Fifth, although colonic IL-1 β , IL-6, and TNF- α levels were measured, upstream inflammatory signaling pathways were not investigated. Sixth, GSH was the only oxidative stress-related marker evaluated; additional markers of oxidative stress and antioxidant defense should also be assessed. Finally, verapamil was neither compared with established UC therapies nor evaluated in human cells, clinical samples, or clinical trials. These limitations should be addressed before the clinical relevance of verapamil for UC can be established.

In conclusion, preventive administration of verapamil attenuated DSS-induced colitis in mice. This protection was associated with reduced inflammatory cytokine production, alterations in selected bacterial taxa, preservation of claudin-1 and claudin-4 expression, and maintenance of colonic GSH levels. These findings suggest that verapamil may exert protective effects in this experimental model of colitis.

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Availability of data and materials

The 16S rRNA gene sequencing data generated in the present study have been deposited in the DDBJ Sequence Read Archive under BioProject accession number PRJDB40750 (DRR932518-DRR932529) and are available at the following URL: <https://ddbj.nig.ac.jp/search/entry/bioproject/PRJDB40750>. The remaining data generated in the present study may be requested from the corresponding author.

Authors' contributions

NO performed the experiments, analyzed the data and drafted the manuscript. KS contributed to the conception and design of the study, supervised the experiments, interpreted the data and revised the manuscript. RT contributed to the analysis and interpretation of data, and critically revised the manuscript for important intellectual content. KS and RT confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All animal experiments were approved by the Institutional Animal Care and Use Committee of Hokkaido University of Science (approval no. 2025-005) and conducted in accordance with the relevant guidelines and regulations.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no known competing interests.

Use of artificial intelligence tools

During the preparation of this work, AI tools [ChatGPT (OpenAI)] were used to improve the readability and language of the manuscript, and subsequently, the authors revised and edited the content produced by the AI tools as necessary, taking full responsibility for the ultimate content of the present manuscript.

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