



Berberine Prevents NSAID-Induced Small Intestinal Injury by Protecting Intestinal Barrier and Inhibiting Inflammasome-Associated Activation

Mikako Ishiguro¹ · Masahiro Takahara¹ · Akinobu Takaki¹ · Sakiko Hiraoka¹ · Jyunki Toyosawa¹ · Yuki Aoyama¹ · Shoko Igawa¹ · Yasushi Yamasaki¹ · Toshihiro Inokuchi¹ · Hideaki Kinugasa¹ · Motoyuki Otsuka¹

Received: 5 February 2025 / Accepted: 17 July 2025 / Published online: 2 August 2025

© The Author(s) 2025

Abstract

Background Nonsteroidal anti-inflammatory drugs (NSAID), which are commonly used to manage pain and inflammation, often cause gastrointestinal injuries, including small intestinal damage. Berberine (BBR) is a traditional Chinese medicine that protects against these injuries. However, the mechanism of action is not fully understood.

Aims This study aimed to evaluate the protective effects of BBR against NSAID-induced intestinal injury and elucidate the underlying molecular mechanisms.

Methods We evaluated the effects of BBR on NSAID-induced intestinal injury using a combination of mouse models and human gut organoids. Mice were treated with indomethacin with or without BBR to induce small intestinal injury. Human gut organoids were exposed to NSAID, with or without BBR, to assess their direct epithelial effects. Histological analyses, cytokine measurements, and Western blotting were performed to evaluate intestinal damage, tight junction integrity, and inflammasome-associated activation.

Results In NSAID-treated mice, BBR markedly reduced ulcers and adhesions and preserved ileal Claudin-1, Occludin, and Zonula Occludens-1 (ZO-1) levels. BBR inhibited both NOD-like receptor family pyrin domain-containing 6 and NOD-like receptor family caspase recruitment domain-containing protein 4 inflammasome activation, reducing Caspase-1 maturation and downstream interleukin-1 β and tumor necrosis factor- α release. In human gut organoids, BBR demonstrated comparable protective effects by directly mitigating NSAID-induced epithelial barrier disruption caused by Claudin-1 and Occludin downregulation, although it did not restore ZO-1 expression.

Conclusions BBR effectively prevented NSAID-induced small intestinal injury by maintaining tight junction integrity and inhibiting inflammasome-associated activation, indicating its potential as a therapeutic agent against such damage.

Keywords Nonsteroidal anti-inflammatory drugs-induced small intestinal injury · Berberine · Tight junction protein · Inflammasomes

Introduction

Nonsteroidal anti-inflammatory drugs (NSAID) are widely used as antipyretic and analgesic agents for the treatment of cold, arthritis, and joint pain. Despite their efficacy as antipyretic and analgesic agents, adverse events affecting

the digestive system, such as gastric and duodenal ulcers, occur with NSAID administration [1, 2]. However, with the recent advent of capsule- and balloon-assisted endoscopy, NSAID have frequently been shown to cause small intestinal mucosal injuries [3–7]. Small intestinal injuries induced by NSAID manifest in various forms, including erythema, erosion, ulcers, and membranous strictures, which frequently occur throughout the small intestine with bleeding or perforation. When NSAID are used, it is necessary to prevent mucosal injury to the upper gastrointestinal tract and small intestine.

The efficacy of widely used proton-pump inhibitors [8, 9] against NSAID-induced small intestinal injuries remains

✉ Masahiro Takahara
mtakahara@cc.okayama-u.ac.jp

¹ Department of Gastroenterology and Hepatology, Dentistry and Pharmaceutical Sciences, Okayama University Graduate School of Medicine, 2-5-1 Shikata-Cho, Kita-Ku, Okayama 700-8558, Japan

unclear. Previous studies have reported that proton-pump inhibitors can aggravate these injuries [10, 11].

Upper gastrointestinal injuries caused by NSAID are primarily mediated by the inhibition of cyclooxygenase (COX), an enzyme responsible for prostaglandin synthesis that plays a central role in mucosal defense mechanisms. COX-1 protects the gastric mucosa, whereas COX-2 is involved in inflammatory and pain responses. Consequently, the use of selective COX-2 inhibitors is a potential strategy for preventing mucosal damage [8, 9]. However, previous studies reported a high incidence of small intestinal injury despite the use of selective COX-2 inhibitors [4, 6, 7]. Additional studies have suggested that the underlying mechanism of NSAID-induced small intestinal injuries involves invasion of the intestinal microbiota owing to the direct NSAID-induced disruption of the small intestinal epithelial barrier function through tight junctions [1, 12–14].

Berberine (BBR), a traditional Chinese medicine, has been investigated for its potential effects on various signaling pathways and molecular targets involved in the microbiota, inflammation, and immune responses to multiple diseases [15–17]. Some studies have suggested that BBR effectively prevents NSAID-induced small intestinal injury [18–20]; however, the mechanisms of action remain undefined. This study examined the mechanism of action of BBR against NSAID-induced small intestinal injury using murine and human gut organoid models.

Methods

Mice

Seven-week-old BALB/c mice were obtained from CLEA Japan, Inc. (Tokyo, Japan) and maintained under specific pathogen-free conditions at the Animal Care Facility of Okayama University. All experimental protocols and procedures adhered to the guidelines of the Okayama University Committee for the Care and Use of Laboratory Animals. The Animal Experiments Ethics Committee of Okayama University approved the study protocol (protocol number: OKU-2023346). We minimized the number of animals used while maintaining statistical robustness. All methods were performed following the relevant guidelines and regulations, including the ARRIVE guidelines for reporting animal research, with anesthesia and euthanasia procedures adhering to the American Veterinary Medical Association Guidelines for the Euthanasia of Animals (2020) to ensure ethical treatment [21].

Chemicals

BBR (chloride form; 98% purity) was purchased from Sigma-Aldrich (St. Louis, MO, USA). The BBR dose was selected based on preliminary experiments conducted in our laboratory, which demonstrated its efficacy in reducing NSAID-induced intestinal damage without any observable toxicity. For the *in vitro* experiments, 100 μ M BBR was used. For the *in vivo* experiments, BBR was dissolved in drinking water at a concentration of 4 mg/mL. For *in vitro* experiments, indomethacin (IND) (purity, 99%; Sigma-Aldrich) was used at a concentration of 1 mM. For *in vivo* studies, IND was incorporated into a normal diet at a concentration of 0.005%.

Cell Culture and Treatments

Human iPSC-derived apical gut organoids were purchased from Dai Nippon Print (Tokyo, Japan). The organoids were cultured in a xenogeneic-free medium consisting of 85% Knockout Dulbecco's modified Eagle medium and 15% CTS Knockout SR XenoFree Medium (both from Life Technologies, Carlsbad, CA, USA) supplemented with 2 mmol/L GlutaMAX (Life Technologies), 0.1 mmol/L MEM Non-Essential Amino Acids (Life Technologies), 1% penicillin–streptomycin (Sigma–Aldrich), 50 μ g/mL L-ascorbic acid (Fujifilm Wako Pure Chemical; Osaka, Japan), 10 ng/mL heregulin- β 1 (Fujifilm Wako Pure Chemical), 200 ng/mL recombinant human insulin-like growth factor-1 (Fujifilm Wako Pure Chemical), and 10 ng/mL fibroblast growth factor 2 (Fujifilm Wako Pure Chemical). The organoids were treated with 100 μ M BBR and 1 mM IND for three days. After treatment, the organoids were either fixed in 10% formalin for hematoxylin and eosin (H&E) staining or freshly frozen in liquid nitrogen and stored at -80°C for Western blotting. Twelve organoids were analyzed, including those used in the preliminary experiments. For morphological quantification, three representative organoids per group were selected based on their structural preservation and imaging quality. Western blotting was performed with independently prepared samples, and each blot was repeated at least three times. Densitometric analysis was based on the average of three biological replicates.

Each experiment included a control group treated with equal concentrations of dimethyl sulfoxide.

In vivo Experimental Design

An IND-induced intestinal ulceration mouse model was used to assess the effects of BBR. Seven-week-old female mice were divided into three groups: normal control diet (Con

group), diet containing IND (N group), and diet containing IND with BBR (BBR group). The mice were weighed daily and sacrificed on day 5 under deep anesthesia with 4% isoflurane.

The damaged areas were delineated using 1% Evans blue (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) administered intraperitoneally one day before sacrifice. To avoid potential interference from Evans blue in serum analysis, experiments for gross lesion evaluation and specimen sampling (e.g., serum cytokines) were conducted separately. During autopsy, the mice were examined for ascites and adhesions in the small intestine, which are indicative of perforation. Blood samples were collected from the vena cava of the mice. The small intestine was spread onto paper, measured in length, and opened on the antimesenteric side. Intestinal weight was not assessed in this study. The total number of ulcerative lesions was recorded. The terminal ileum was excised, fixed in 10% formalin for H&E staining and immunofluorescence staining, fresh-frozen in liquid nitrogen, and stored at -80 °C for Western blotting.

Fluorescein Isothiocyanate-Dextran Intestinal Permeability Assay

Intestinal permeability was assessed using fluorescein isothiocyanate-labeled dextran (Chondrex, Inc. Woodinville, WA, USA). Seven-week-old female mice were divided into three groups: regular control diet (Con group), diet containing IND (N group), and diet containing IND with BBR (BBR group). After a 4 h fast, mice were orally gavaged with FITC-dextran (4 kDa; 80 mg/mL in PBS) at a dose of 600 mg/kg body weight. Four hours later, blood was collected, plasma was isolated by centrifugation (2000 × g, 10 min, 4 °C), and fluorescence was measured using a Glo-Max® Explorer Multimode Microplate Reader (Promega Corporation, Madison, WI, USA) at excitation and emission wavelengths of 485 and 528 nm, respectively.

Western Blotting

Total protein was extracted from the small intestine using a Minute Protein Extraction Kit (Invent Biotechnologies, Inc., Plymouth, MN, USA). Protein concentrations were quantified using the bicinchoninic acid method (BCA Protein Assay Kit; Takara Bio Inc., Otsu, Japan) and adjusted to ensure equal loading across samples. Equivalent amounts of protein from each group were separated on sodium dodecyl sulfate–polyacrylamide gels and transferred onto polyvinylidene fluoride membranes. The membranes were then incubated with primary antibodies against Nucleotide-binding oligomerization domain-like receptor family pyrin domain-containing protein 3 (NLRP3) (rabbit monoclonal, D4D8T, 1:1000), NOD-like

receptor family pyrin domain-containing 6 (NLRP6) (rabbit monoclonal, F8J8R, 1:1000), Occludin (rabbit monoclonal, E6B4R, 1:1000), Caspase-1 (rabbit monoclonal, E9R2D, 1:1000), Cleaved caspase-1 (Asp296) (rabbit monoclonal, E2G2I, 1:1000), Zonula Occludens—2 (ZO-2) (rabbit polyclonal, 2847 s, 1:1000) (Cell Signaling Technology, Inc., Beverly, MA, USA), Zonula Occludens -1 (ZO-1) (rabbit monoclonal, BLR092G, 1:1000), Claudin-1 (rabbit monoclonal, EPR25359-48, 1:1000), and NOD-like receptor family caspase recruitment domain-containing protein 4 (NLRC4) (rabbit monoclonal, EPR19733, 1:1000) (Abcam, Cambridge, MA, USA). Protein bands were detected by chemiluminescence following incubation with the appropriate secondary antibodies. β -actin was used as a loading control. Quantitative analysis of band intensity was performed using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Immunofluorescence Staining

Paraffin-embedded sections of human iPSC-derived intestinal organoids and BALB/c mouse ileum were deparaffinized in xylene, rehydrated using a graded ethanol series, and subjected to antigen retrieval in 10 mM citrate buffer (pH 6.0) at 95 °C for 20 min. After cooling, sections were blocked with 5% goat serum for 30 min at 20 °C, then incubated overnight at 4 °C with primary antibodies against ZO-1 (mouse monoclonal, ZO-1-1A12, 1:1000) (Invitrogen, Carlsbad, CA, USA), Claudin-1 (rabbit polyclonal, 28,674–1-AP, 1:200) (Proteintech, Rosemont, IL, USA), and Occludin (mouse monoclonal, OC-3F10, 1:400) (Invitrogen). The following day, the sections were washed thrice in PBS and incubated for 1 h at room temperature with Alexa Fluor 488 goat anti-rabbit IgG (Molecular Probes, Eugene, OR, USA, 1:300) and Alexa Fluor 594 goat anti-mouse IgG (Molecular Probes, Eugene, OR, USA, 1:300). After three PBS washes, the nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI; #H-1200; Vector Laboratories, Burlingame, CA, USA), mounted with antifade medium, and imaged using Olympus cellSens imaging software (Olympus, Tokyo, Japan).

Multiple Cytokine Assays

Cytokine levels were measured using a BioPlex 200 System (BioPlex Pro Mouse Cytokine Standard GI 8-PlexA; Bio-Rad, Hercules, CA, USA). Each sample was tested in duplicate, and the median values were recorded. A value of zero was assigned to values below the measurement sensitivity.

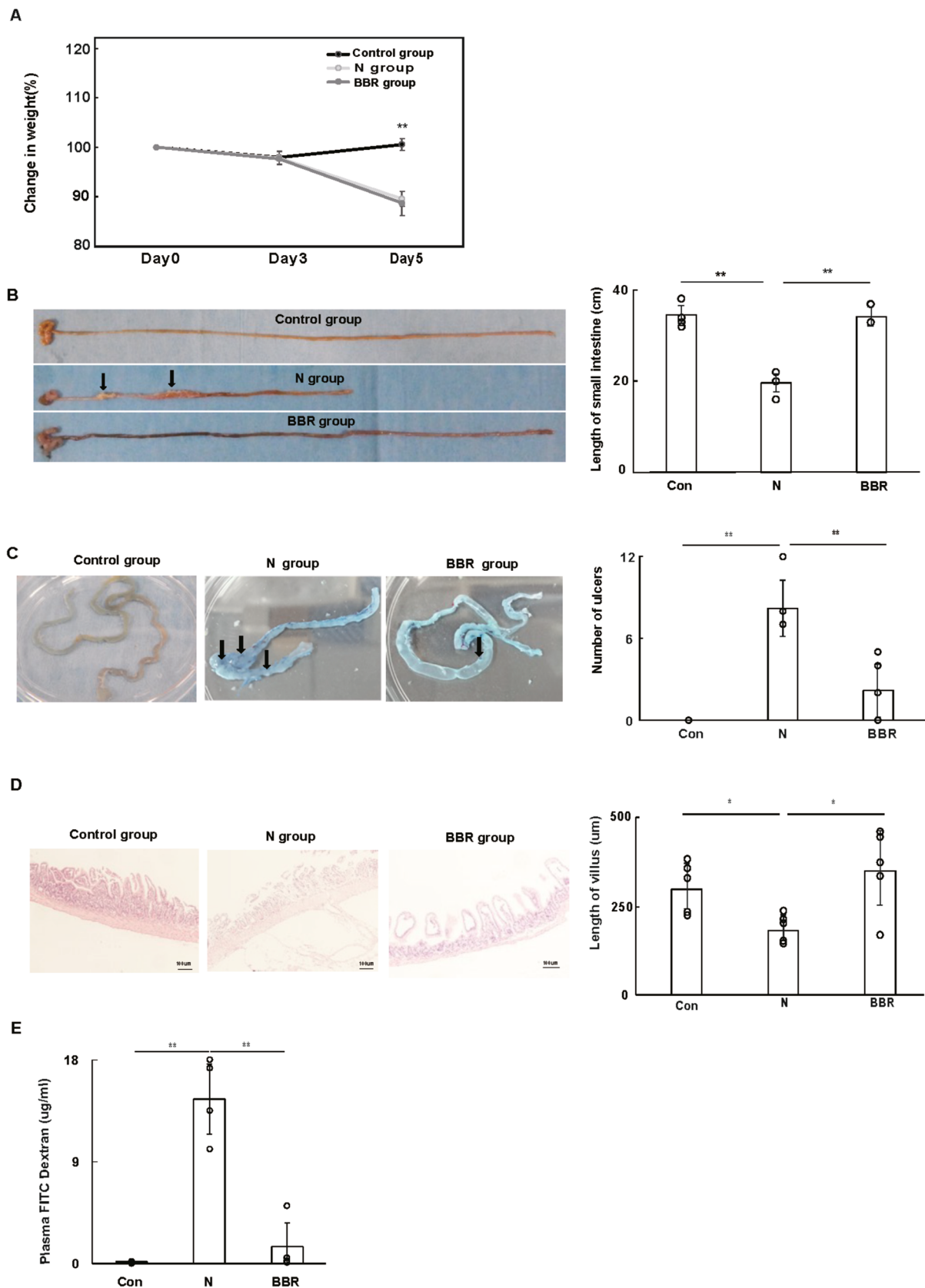


Fig. 1 BBR protected against NSAID-induced small intestinal injury. Seven-week-old BALB/c mice were divided into three groups: normal diet (control group [Con]), diet containing IND (N group [N]), and diet containing IND with BBR (BBR group [BBR]). The mice were monitored for up to five days, sacrificed, and their tissues were analyzed. **a** Body weight changes in the experimental group. **b** Representative gross appearance of the small intestine of mice in each group (left panel). Arrows indicate ulcerative lesions. The mean length of the small intestine of five mice from each experimental group is shown in the right panel. **c** Ulcer lesions stained with Evans blue in water (left panel). Arrows indicate ulcerative lesions. The mean number of ulcer lesions in five mice from each group is shown in the right panel. **d** Representative histological images of the terminal ileum of mice in each group (left panel). Scale bar, 100 μ m. The mean lengths of the small intestinal villi of five mice from each experimental group are shown in the right panel. **e** Intestinal permeability was evaluated using the FITC-dextran (4 kDa) assay. Four mice were used for each group. All data are shown as mean $\pm$ SD. * $P < 0.05$, ** $P < 0.01$. BBR, berberine; IND, indomethacin; NSAID, nonsteroidal anti-inflammatory drug; FITC, fluorescein isothiocyanate; SD, standard deviation

Statistical Analyses

Data are presented as mean $\pm$ standard deviation for parametric data and median for non-parametric data. Parametric data were analyzed using Student's *t*-test, whereas non-parametric data were evaluated using the Mann–Whitney U test. Statistical significance was set at $P < 0.05$. All analyses were conducted using JMP software (version 15; SAS Institute, Cary, NC, USA).

Results

BBR Prevented NSAID-Induced Small Intestinal Injury

We determined the effects of BBR in a murine model of NSAID-induced small intestinal injury. No significant differences were observed in weight loss after the induction of small intestinal injury by NSAID between mice treated with and without BBR (Fig. 1a); however, mice treated with BBR had significantly longer intestinal tracts (Fig. 1b). BBR-treated mice exhibited no abnormal behavior or clinical signs of toxicity. Mice not treated with BBR had several adhesive lesions in the small intestinal tract, which could be owing to NSAID-induced ulcers; these lesions were not observed in mice treated with BBR (Fig. 1b). Moreover, the number of ulcers induced in the small intestine was notably reduced in BBR-treated mice (Fig. 1c). Histologically, the terminal ileal villi were substantially longer in mice treated with BBR, suggesting that BBR prevented severe NSAID-induced epithelial damage in the intestine (Fig. 1d). Furthermore, FITC-dextran intestinal permeability assays revealed that plasma FITC

concentrations were significantly elevated in the NSAID-treated group, indicating intestinal barrier dysfunction. In contrast, this increase was suppressed in the BBR-treated group (Fig. 1e), suggesting that BBR administration may prevent NSAID-induced small intestinal injury.

BBR Protected Against NSAID-Induced Intestinal Epithelial Damage in Human Intestinal Organoid Models

Intestinal epithelial cells are frontline cells exposed to NSAID. Therefore, human-induced pluripotent stem cell-derived apical gut organoids were used to determine the direct effects of NSAID on human intestinal epithelial cells with or without BBR in vitro. Unlike conventional intestinal organoids, which are apical-out, the direct impact of NSAID on intestinal epithelial cells was assessed by the simple addition of NSAID to the culture media. Three days later, the intestinal epithelial cells peeled off, indicating that NSAID directly induced damage (Fig. 2a). However, concomitant BBR administration significantly reduced intestinal epithelial damage (Fig. 2a). These organoid models do not contain immune cells; thus, the damage induced by NSAID and their protective effects are attributed to mechanisms other than the immune system.

To determine the molecular mechanisms underlying the protective effects of BBR against NSAID-induced intestinal epithelial injury, we examined the status of the tight junction system because BBR reportedly reduces the impairment of tight junctions in the intestinal epithelia under inflammatory and noninflammatory conditions [22–24]. We focused on Claudin-1, Occludin, and ZO-1 because they represent key transmembrane and scaffolding components critical for maintaining epithelial barrier integrity [25]. We performed immunofluorescence for the tight junction proteins Claudin-1, Occludin, and ZO-1 in human gut organoids treated with NSAID, with or without BBR. Fluorescent staining of these tight junction proteins revealed that in organoids treated with NSAID alone, the marginal localization of Occludin, Claudin-1, and ZO-1 was fragmented and weakened. In contrast, in the BBR-co-treated group, continuous belt-like localization along the cell boundaries was maintained (Fig. 2b). Western blot analysis revealed that the expression levels of Occludin and Claudin-1 were significantly reduced in the NSAID-treated group, whereas no significant differences were observed in ZO-1 expression between the groups (Fig. 2c). We also assessed ZO-2 expression and found no significant changes (Supplementary Fig. 1). These results indicate that BBR may protect against NSAID-induced damage to intercellular tight junctions in the intestinal epithelia.

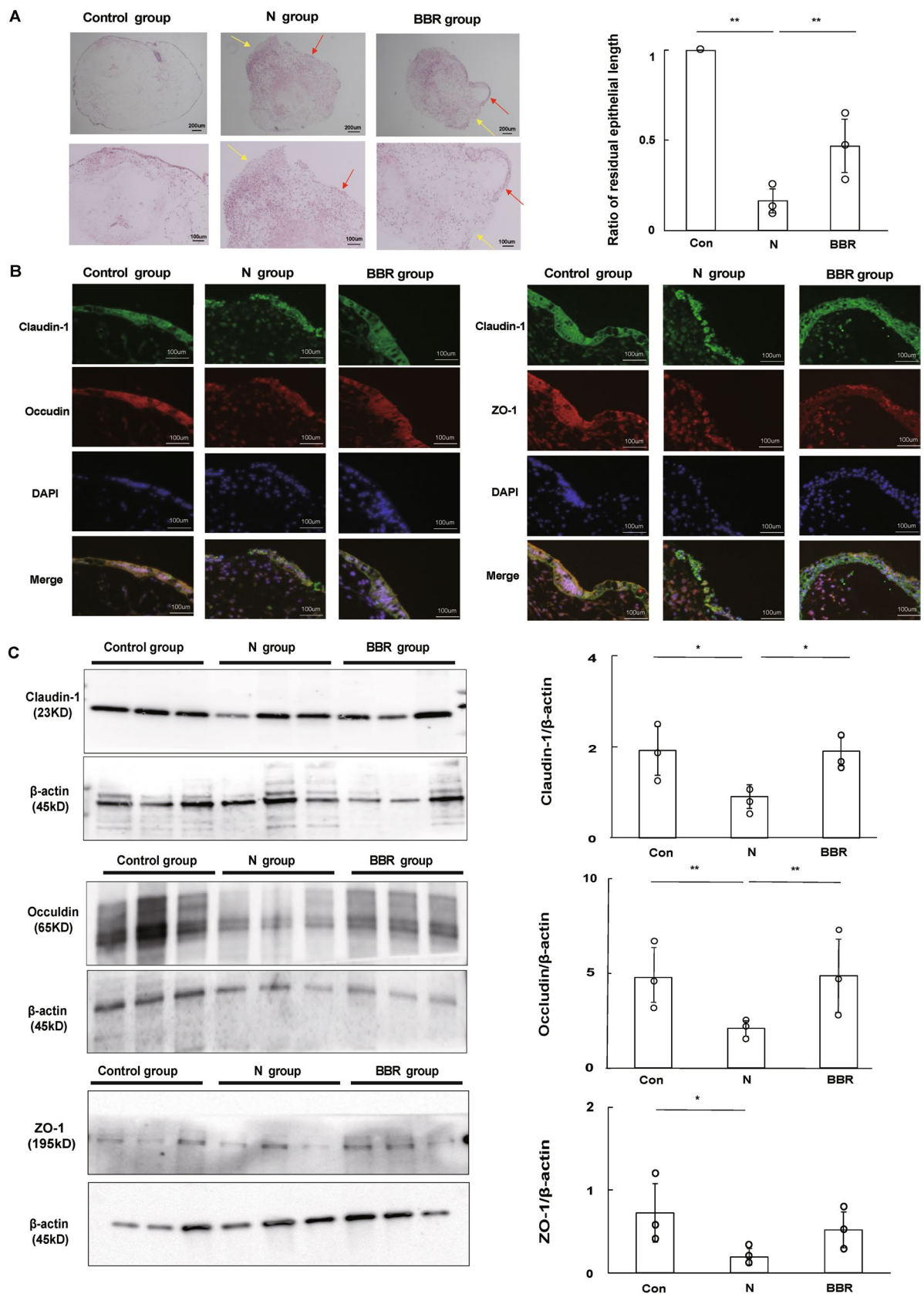


Fig. 2 BBR protected against NSAID-induced intestinal epithelial damage in the human intestinal organoid model. Human-induced pluripotent stem cell-derived apical gut organoids were cultured with 1 mM IND for three days, with or without 100 mM BBR (BBR and N groups, respectively). Control organoids were cultured without IND (control group [Con]). **a** Representative histological images of organoids are shown in the left panel. Red arrows indicate normal epithelial cells and yellow arrows indicate damaged epithelial cells. The ratio of the residual epithelial length was calculated by dividing the horizontal length of the epithelial cells by the horizontal length of the circumference of the corresponding organoids (right panel). Data are shown as the mean $\pm$ SD of three experiments for each group; $*P < 0.05$. **b** Representative immunofluorescence staining of terminal ileum sections. The left panels show Claudin-1 (green) and Occludin (red), and the right panels show Claudin-1 (green) and ZO-1 (red). Nuclei were counterstained with DAPI (blue). **c** Representative Western blots of Claudin-1, Occludin, and ZO-1 expression levels in organoids from each group (left panels). The bar graph shows the relative expression of each protein normalized to that of β -actin (right panel). Data are shown as the mean $\pm$ SD of three experiments per group. $*P < 0.05$. BBR, berberine; IND, indomethacin; NSAID, nonsteroidal anti-inflammatory drug; SD, standard deviation; ZO-1, Zonula Occludens-1; DAPI, 4',6-diamidino-2-phenylindole

BBR Reduced Serum Inflammatory Cytokine Levels in Mice

We previously reported that serum levels of inflammatory cytokines were notably elevated in mice with NSAID-induced small intestinal injury [6] and examined cytokine levels in the sera of NSAID-treated mice with or without concomitant BBR treatment. Interleukin (IL)-1 β and tumor necrosis factor (TNF)- α levels considerably decreased in BBR-treated mice (Fig. 3). These findings suggest that BBR decreased cytokine levels that were elevated by NSAID-induced intestinal injury, and that this effect could be mediated by protecting tight junctions against damage. These

cytokine-mediated effects represent functions of the innate immune system.

BBR Preserved Ileal Tight Junctions and Inhibited Inflammasome Activation in Mice

Consistent with the in vitro findings obtained using human organoids, immunofluorescence for tight junction proteins revealed that vehicle-treated mice displayed a continuous Claudin-1 green belt accompanied by densely packed red Occludin/ZO-1 puncta along the apical junction. NSAID administration disrupted this organization: the Claudin-1 belt was fragmented, and Occludin/ZO-1 puncta became sparse. BBR treatment suppressed this disruption, maintained belt continuity, and preserved puncta density (Fig. 4a). Western blot analysis confirmed that BBR suppressed NSAID-induced downregulation of Claudin-1, Occludin, and ZO-1 (Fig. 4b). NLRP6 inflammasome is associated with increased intestinal permeability and inflammation [26, 27]. The ileal terminal tissues of mice with NSAID-induced intestinal injury were examined to determine whether BBR reduced NLRP6 inflammasome activation. The expression levels of NLRP6 were notably reduced in BBR-treated mice (Fig. 5a). We also examined NLRC4 and NLRP3 because NLRC4 has been implicated in epithelial inflammasome-mediated injury and NLRP3 is a prototypical inflammasome activated under various stress conditions [13, 28]. Another inflammasome, NLRC4, showed a similar trend (Fig. 5b); however, NLRP3 expression levels remained unchanged across all treatment groups (Supplementary Fig. 2). Consistently, the expression levels of Caspase-1 and Cleaved caspase-1 were significantly reduced in BBR-treated mice (Figs. 5c and d). These findings suggest that BBR prevents

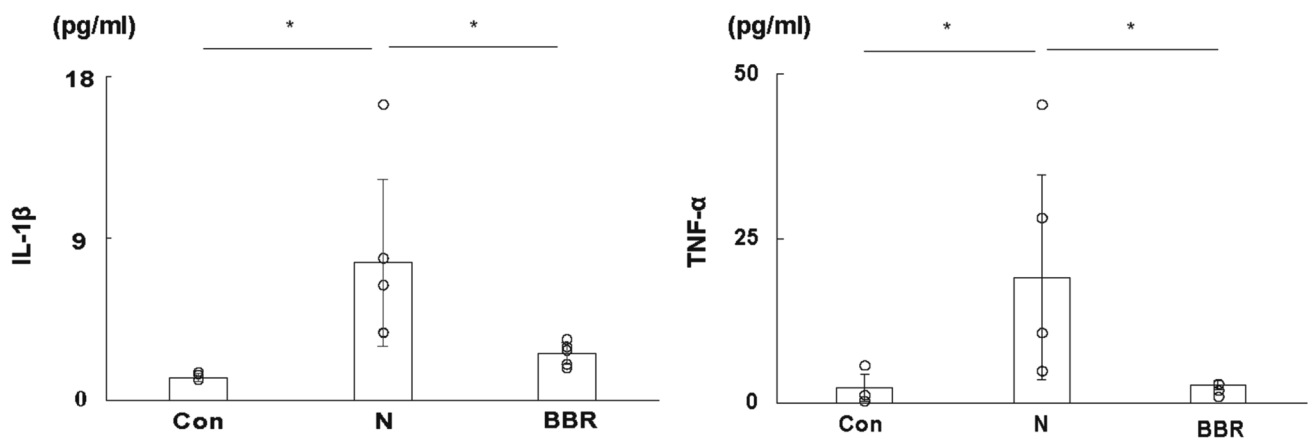


Fig. 3 BBR decreased the serum inflammatory cytokine levels in mice. The mice were treated as described in the legend of Fig. 1. Serum IL-1 β (left) and TNF- α (right) levels determined on day 5 for each mouse group are shown. Con, control group; N, N group; BBR,

BBR group. Data are shown as the mean $\pm$ SD of five mice per group. $*P < 0.05$. BBR, berberine; IL, interleukin; TNF, tumor necrosis factor; SD, standard deviation

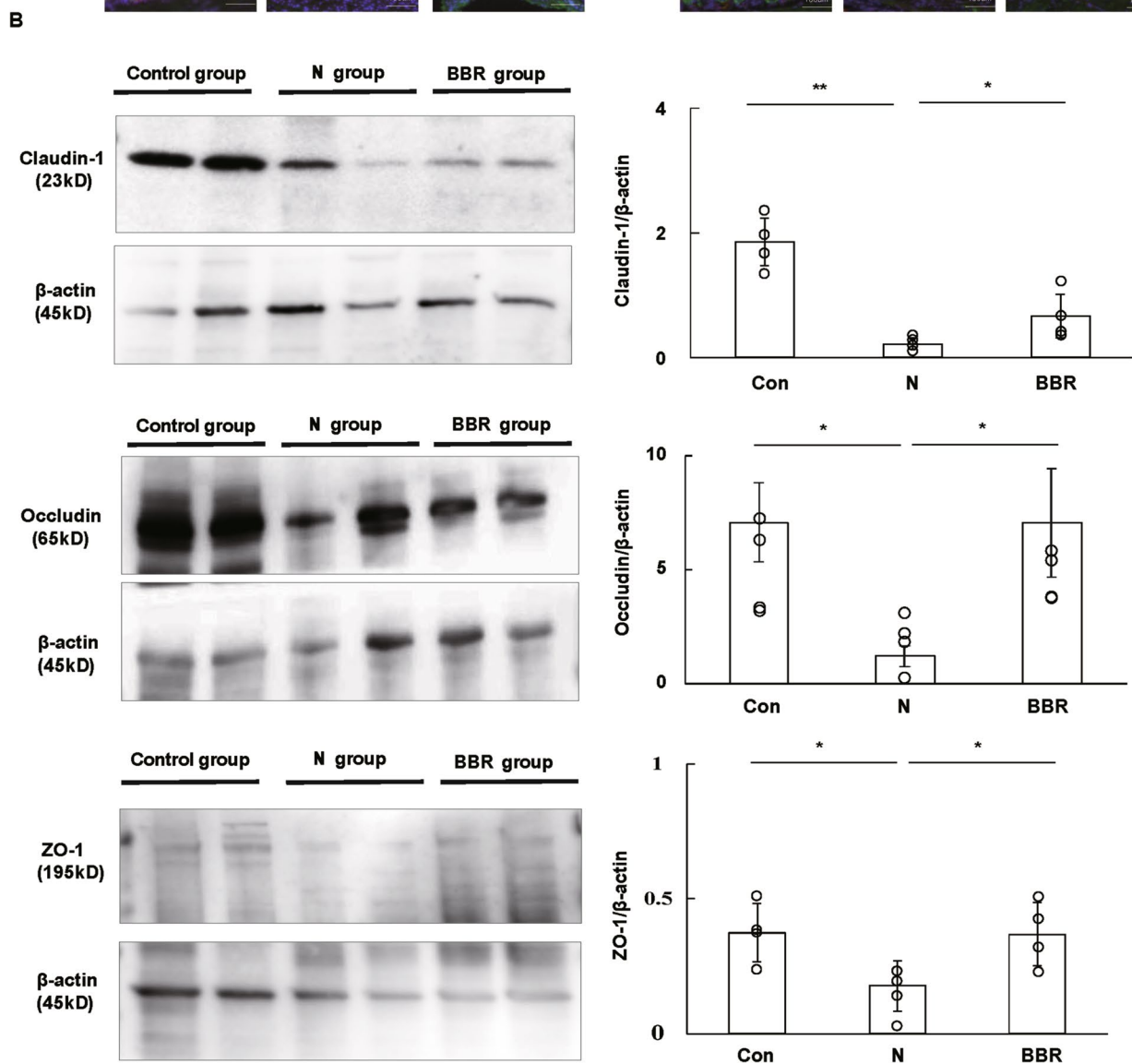
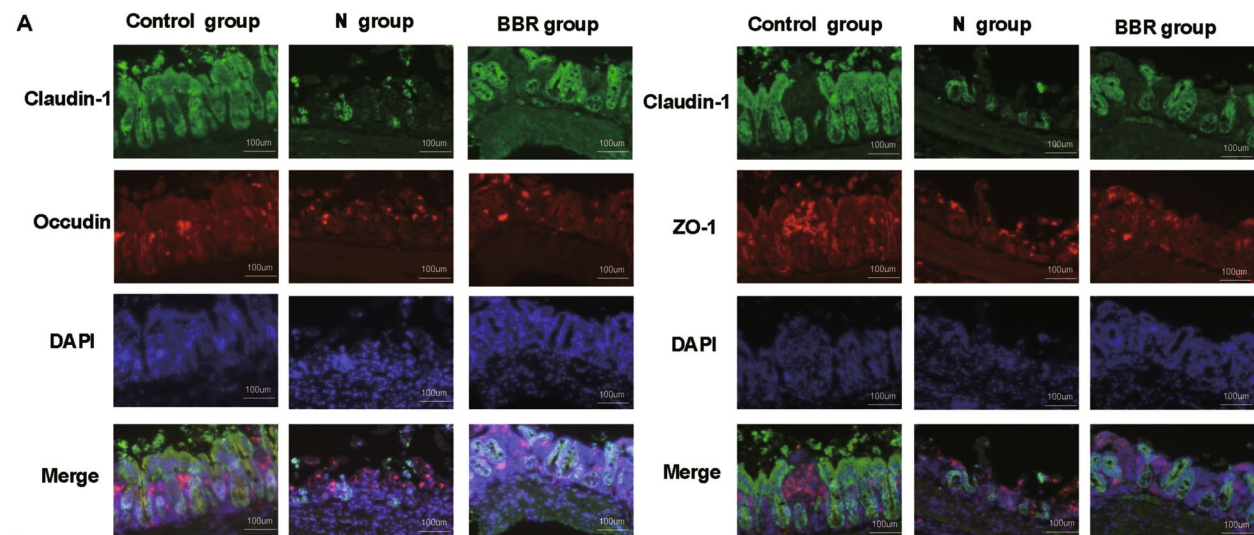


Fig. 4 BBR protects against ileal tight junction damage. The expression levels and localization of tight junction proteins in the terminal ileum tissues of each mouse group were assessed using immunofluorescence staining and Western blotting. Mice were grouped as described in the legend of Fig. 1. **a** Representative immunofluorescence staining of the terminal ileum tissue in each group. Left panels show Claudin-1 (green) and Occludin (red), and the right panels show Claudin-1 (green) and ZO-1 (red). Nuclei were counterstained with DAPI (blue). **b** Representative Western blots of Claudin-1, Occludin, and ZO-1 expression levels in the terminal ileum tissues of each group. The bar graph shows the ratio of the expression levels of each protein to that of β -actin. Con, control group; N, N group; BBR, BBR group. Data are shown as mean $\pm$ SD of four mice in each group. * $P < 0.05$, ** $P < 0.01$. BBR, berberine; ZO-1, zonula occludens 1; DAPI, 4',6-diamidino-2-phenylindole; SD, standard deviation

the increased expression of inflammatory cytokines by inhibiting inflammasome activation and concurrently preserving tight junction integrity, leading to reduced NSAID-induced damage to the intestinal epithelium.

Discussion

The side effects of NSAID on the digestive system include gastric ulcers and other upper gastrointestinal disorders, with a frequent incidence of small bowel injury [3–6]. Using murine and in vitro human organoid models, we found that BBR protected against NSAID-induced intestinal injury by enhancing tight junction protein expression and reducing inflammasome-associated activation and inflammatory cytokine levels.

BBR is a natural product that is widely used in traditional Chinese medicine. In addition to its various bioactive actions, mucosal protection and anti-inflammatory effects of BBR have been reported [15–17]. Therefore, we hypothesized that BBR affects NSAID-induced small bowel injury. Consistent with several previous studies investigating the favorable effects of BBR on NSAID-induced small bowel injury [18–20], the protective effects of BBR against NSAID-induced intestinal injury were confirmed in murine and human gut organoid models. Despite mild NSAID-induced intestinal injury, weight loss was observed in the BBR group. BBR reportedly has metabolic effects [29]. Therefore, we hypothesized that the observed weight loss was because of its bioactive actions. Although a decrease in adenosine deaminase mRNA levels or enteric nervous system repair has been reported as the mechanism underlying the protective effects of BBR against NSAID-induced intestinal injury [18–20], we identified more direct effects on the intestinal epithelium through an enhanced tight junction system and reduced inflammasome-associated

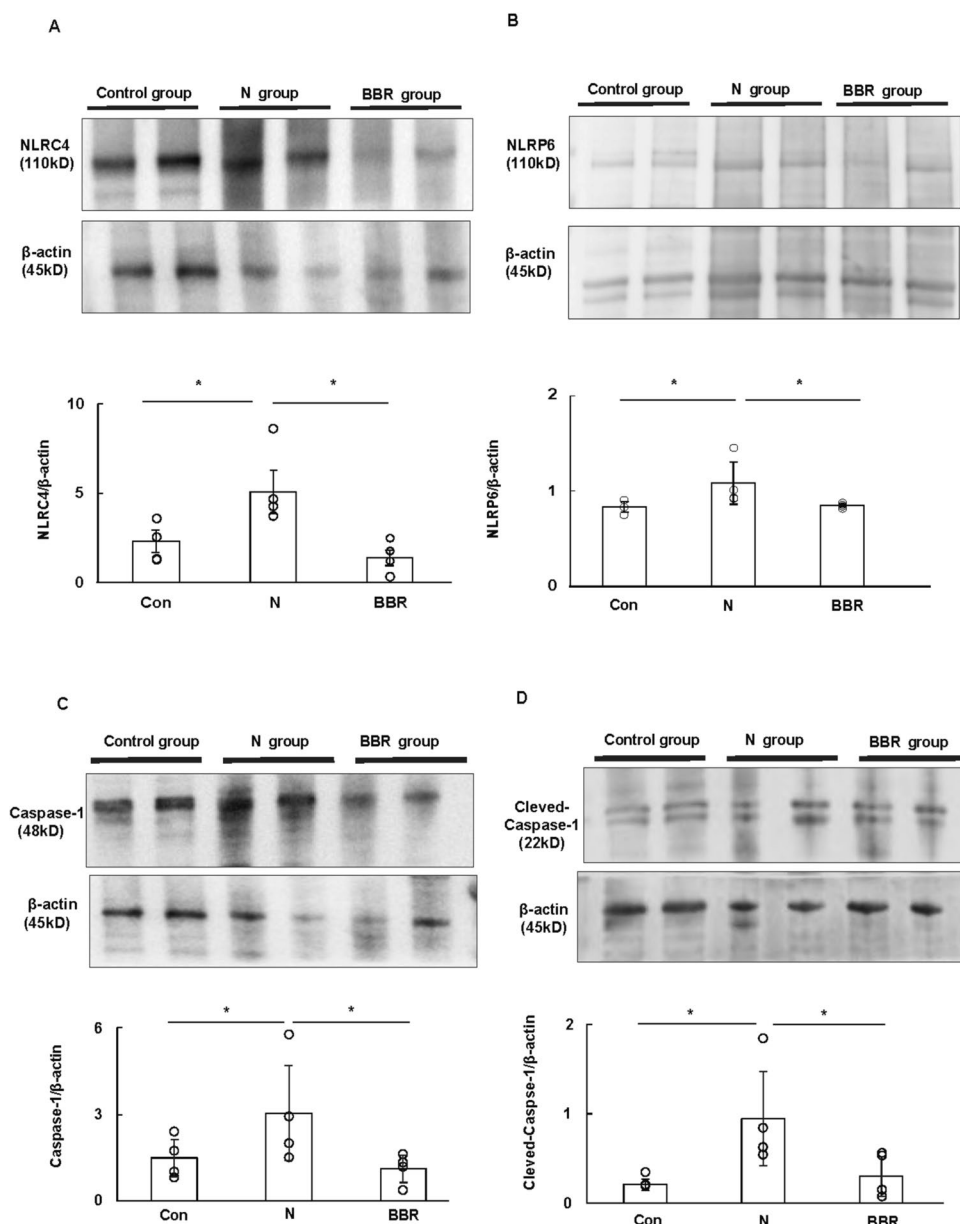
activation. Taken together, these favorable molecular effects protect against NSAID-induced intestinal injury. Further clinical trials should be conducted to confirm the protective effects of BBR against NSAID-induced intestinal injuries.

Tight junctions are crucial for the intestinal physical barrier and play a critical role in maintaining intestinal permeability and preventing the invasion of exogenous bacteria-related pathogens [30]. Here, we observed a protective effect of BBR on tight junctions in murine and human organoid models in which intestinal injury was directly induced by NSAID, probably through the enhanced expression of Claudin-1 and Occludin. In contrast, the ZO-1 levels in organoids remained unchanged. Consistent with our results, BBR reportedly has a protective effect on tight junctions in the intestinal epithelium in other murine models of intestinal damage [13, 23, 31]. Therefore, the mucosal protective effects of BBR may be partially mediated by the enhanced effects of key tight junctions. The absence of ZO-1 modulation in organoids may reflect the requirement for stromal or immune cell-derived signals to regulate scaffold protein dynamics, which are lacking in epithelial-only cultures [32]. Further investigation is required to elucidate these regulatory mechanisms.

Once tight junctions are damaged, intestinal pathogens invade the submucosal layer, triggering an inflammatory response. Inflammation is also involved in NSAID-induced intestinal damage [1, 12–14]. Inflammasome-associated activation is reportedly involved in NSAID-induced small intestinal injury [1, 13, 33]. Although the NLRP3 inflammasome is allegedly involved in NSAID-induced small intestinal injury [13, 28], no notable changes in NLRP3 activation were observed in this study. BBR markedly suppresses NLRC4 and NLRP6 inflammasome components in the mouse ileum pathways that drive Caspase-1-mediated cytokine release and epithelial permeability [26–28]. Reduced Caspase-1 cleavage may also limit pyroptotic/apoptotic cell death, further preserving the barrier integrity [34]. Although we did not assess epithelial apoptosis or use NLRC4/NLRP6 knockout models in this study, our findings highlight the potential of BBR as a dual inflammasome inhibitor, and warrant further functional validation.

Infliximab, a TNF- α inhibitor, prevents NSAID-induced small intestinal injury enteropathy [35]. We observed that BBR reduced the levels of TNF- α , IFN- γ , IL-2, and IL-4 (Supplementary Fig. 3). These cytokines destabilize tight junctions by modulating Claudin-1, Occludin, and ZO-1 [36, 37]. In BBR-treated mice, parallel suppression of these mediators and preservation of junctional integrity provided a coherent mechanistic link between immune modulation and barrier protection. Given the pleiotropy of BBR, future studies should dissect individual cytokine–junction pathways to refine its therapeutic applications.

Fig. 5 BBR inhibits inflammasome activation in the terminal ileum. The expression levels of inflammasome-associated proteins in the terminal ileum tissues of each mouse group were assessed by Western blotting. Mice were grouped as described in the legend of Fig. 1. **a–d** Representative images of NLRC4 (**a**), NLRP6 (**b**), Caspase-1 (**c**), Cleaved caspase-1 (**d**) expression levels. The bar graph shows the ratio of the expression levels of each protein to the expression levels of β -actin. Con, control group; N, N group; BBR, BBR group. Data are shown as mean $\pm$ SD of four mice in each group. * $P < 0.05$. BBR, berberine; NLRC4, NOD-like receptor family caspase recruitment domain-containing protein 4; NLRP6, NOD-like receptor family pyrin domain-containing 6; SD, standard deviation



Conclusion

BBR demonstrated robust protective effects against NSAID-induced small intestinal injury by preserving tight junction integrity (via Claudin-1 and Occludin stabilization; ZO-1 was unchanged in organoids) and inhibiting inflammasome-associated activation (including the NLRC4 and NLRP6 pathways). These findings suggest that BBR mitigates intestinal barrier dysfunction and excessive inflammatory responses, making it a promising therapeutic candidate for preventing NSAID-induced intestinal injury. Further clinical studies are warranted to establish its efficacy and potential application in a broader context.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10620-025-09276-5>.

Acknowledgments We would like to thank Editage (www.editage.jp) for English language editing.

Author Contributions M.I. and M.T. conceived and designed the study, performed the experiments, analyzed the data, and drafted the manuscript. A.T. assisted in the study design, analyzed the data, and drafted the manuscript. S.H., J.T., Y.A., S.I., Y.Y., T.I., and H. K. assisted in the study design. M.O. supervised the study.

Funding Open Access funding provided by Okayama University. Grants-in-Aid from the Ministry of Education, Culture, Sports, Science, and Technology, Japan, JP20K16957, Mitsubishi Foundation, 202310009.

Data Availability The data supporting the findings of this study are available from the corresponding author (M.T.) upon reasonable request.

Declarations

Competing interests The authors have no competing interests, as defined by *Nature Research* or any other interests that might be perceived to influence the results and/or discussion reported in this paper.

Open Access This article is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License, which permits any non-commercial use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by-nc/4.0/>.

References

- Watanabe T, Fujiwara Y, Chan FKL. Current knowledge on non-steroidal anti-inflammatory drug-induced small-bowel damage: a comprehensive review. *J Gastroenterol* 2020;55:481–495.
- Huang JQ, Sridhar S, Hunt RH. Role of *Helicobacter pylori* infection and non-steroidal anti-inflammatory drugs in peptic-ulcer disease: a meta-analysis. *Lancet* 2002;359:14–22.
- Maiden L, Thjodleifsson B, Theodors A, Gonzalez J, Bjarnason I. A quantitative analysis of NSAID-induced small bowel pathology by capsule enteroscopy. *Gastroenterology* 2005;128:1172–1178.
- Sugimori S, Watanabe T, Tabuchi M et al. Evaluation of small bowel injury in patients with rheumatoid arthritis by capsule endoscopy: effects of anti-rheumatoid arthritis drugs. *Digestion* 2008;78:208–213.
- Fukumoto A, Tanaka S, Shishido T, Takemura Y, Oka S, Chayama K. Comparison of detectability of small-bowel lesions between capsule endoscopy and double-balloon endoscopy for patients with suspected small-bowel disease. *Gastrointest Endosc* 2009;69:857–865.
- Kono Y, Kawano S, Takaki A et al. Oxidative stress controlling agents are effective for small intestinal injuries induced by non-steroidal anti-inflammatory drugs. *J Gastroenterol Hepatol* 2017;32:136–145.
- Graham DY, Opekun AR, Willingham FF, Qureshi WA. Visible small-intestinal mucosal injury in chronic NSAID users. *Clin Gastroenterol Hepatol* 2005;3:55–59.
- Lanas A, García-Rodríguez LA, Arroyo MT et al. Effect of antisecretory drugs and nitrates on the risk of ulcer bleeding associated with nonsteroidal anti-inflammatory drugs, antiplatelet agents, and anticoagulants. *Am J Gastroenterol* 2007;102:507–515.
- Lanza FL, Chan FK, Quigley EM. Guidelines for prevention of NSAID-related ulcer complications. *Am J Gastroenterol* 2009;104:728–738.
- Wallace JL, Syer S, Denou E et al. Proton pump inhibitors exacerbate NSAID-induced small intestinal injury by inducing dysbiosis. *Gastroenterology* 2011;141:1314–1322.e1-5.
- Washio E, Esaki M, Maehata Y et al. Proton pump inhibitors increase incidence of nonsteroidal anti-inflammatory drug-induced small bowel injury: a randomized, placebo-controlled trial. *Clin Gastroenterol Hepatol* 2016;14:809–815.e1.
- Takeuchi K, Satoh H. NSAID-induced small intestinal damage—roles of various pathogenic factors. *Digestion* 2015;91:218–232.
- Otani K, Watanabe T, Shimada S et al. Colchicine prevents NSAID-induced small intestinal injury by inhibiting activation of the NLRP3 inflammasome. *Sci Rep* 2016;6:32587.
- Rekatsina M, Paladini A, Cifone MG, Lombardi F, Pergolizzi JV, Varrassi G. Influence of microbiota on NSAID enteropathy: A systematic review of current knowledge and the role of probiotics. *Adv Ther* 2020;37:1933–1945.
- Kong WJ, Zhang H, Song DQ et al. Berberine reduces insulin resistance through protein kinase C-dependent up-regulation of insulin receptor expression. *Metabolism* 2009;58:109–119.
- Takahara M, Takaki A, Hiraoka S et al. Berberine improved experimental chronic colitis by regulating interferon- γ - and IL-17A-producing lamina propria CD4(+) T cells through AMPK activation. *Sci Rep* 2019;9:11934.
- Zhu C, Li K, Peng XX et al. Berberine a traditional Chinese drug repurposing: Its actions in inflammation-associated ulcerative colitis and cancer therapy. *Front Immunol* 2022;13:1083788.
- Watanabe-Fukuda Y, Yamamoto M, Miura N et al. Orengedokuto and berberine improve indomethacin-induced small intestinal injury via adenosine. *J Gastroenterol* 2009;44:380–389.
- Chao G, Ye F, Yuan Y, Zhang S. Berberine ameliorates non-steroidal anti-inflammatory drugs-induced intestinal injury by the repair of enteric nervous system. *Fundam Clin Pharmacol* 2020;34:238–248.
- Chao G, Wang Q, Ye F, Zhang S. Gene expression analysis in NSAID-induced rat small intestinal disease model with the intervention of berberine by the liquid chip technology. *Genes Environ* 2021;43:32.
- Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. *PLoS Biol* 2010;8:e1000412.
- Gu L, Li N, Li Q et al. The effect of berberine in vitro on tight junctions in human Caco-2 intestinal epithelial cells. *Fitoterapia* 2009;80:241–248.
- Gu L, Li N, Gong J, Li Q, Zhu W, Li J. Berberine ameliorates intestinal epithelial tight-junction damage and down-regulates myosin light chain kinase pathways in a mouse model of endotoxemia. *J Infect Dis* 2011;203:1602–1612.
- Cao M, Wang P, Sun C, He W, Wang F. Amelioration of IFN- γ and TNF- α -induced intestinal epithelial barrier dysfunction by berberine via suppression of MLCK-MLC phosphorylation signaling pathway. *PLoS One* 2013;8:e61944.
- Chelakkot C, Ghim J, Ryu SH. Mechanisms regulating intestinal barrier integrity and its pathological implications. *Exp Mol Med* 2018;50:1–9.
- Seregin SS, Golovchenko N, Schaf B, Chen J, Eaton KA, Chen GY. NLRP6 function in inflammatory monocytes reduces susceptibility to chemically induced intestinal injury. *Mucosal Immunol* 2017;10:434–445.
- Frühbeck G, Gómez-Ambrosi J, Ramírez B et al. Decreased expression of the NLRP6 inflammasome is associated with increased intestinal permeability and inflammation in obesity with type 2 diabetes. *Cell Mol Life Sci* 2024;81:77.
- Zmora N, Levy M, Pevsner-Fischer M, Elinav E. Inflammasomes and intestinal inflammation. *Mucosal Immunol* 2017;10:865–883.
- Kong WJ, Vernieri C, Foiani M, Jiang JD. Berberine in the treatment of metabolism-related chronic diseases: A drug cloud (dCloud) effect to target multifactorial disorders. *Pharmacol Ther* 2020;209:107496.

30. Horowitz A, Chanez-Paredes SD, Haest X, Turner JR. Paracellular permeability and tight junction regulation in gut health and disease. *Nat Rev Gastroenterol Hepatol* 2023;20:417–432.
31. Hou Q, Zhu S, Zhang C et al. Berberine improves intestinal epithelial tight junctions by upregulating A20 expression in IBS-D mice. *Biomed Pharmacother* 2019;118:109206.
32. Ren J, Huang S. Intestinal organoids in inflammatory bowel disease: advances, applications, and future directions. *Front Cell Dev Biol* 2025;13:1517121.
33. Higashimori A, Watanabe T, Nadatani Y et al. Mechanisms of NLRP3 inflammasome activation and its role in NSAID-induced enteropathy. *Mucosal Immunol* 2016;9:659–668.
34. Scalavino V, Piccinno E, Giannelli G, Serino G. Inflammasomes in intestinal disease: mechanisms of activation and therapeutic strategies. *Int J Mol Sci* 2024;25:13058.
35. Watanabe T, Tanigawa T, Shiba M et al. Anti-tumour necrosis factor agents reduce non-steroidal anti-inflammatory drug-induced small bowel injury in rheumatoid arthritis patients. *Gut* 2014;63:409–414.
36. Turner JR. Intestinal mucosal barrier function in health and disease. *Nat Rev Immunol* 2009;9:799–809.
37. Kaminsky LW, Al-Sadi R, Ma TY. IL-1 β and the intestinal epithelial tight junction barrier. *Front Immunol* 2021;12:767456.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.