Plasmon-activated water enhances gut-barrier function and alleviates inflammation in a mouse model of ulcerative colitis

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Abstract. Ulcerative colitis (UC) is a chronic, relapsing inflammatory bowel disease characterized by disruption of the intestinal epithelial barrier and alterations in mucosal gene expression associated with intestinal integrity. Given the risks associated with UC, novel therapies capable of restoring intestinal barrier function and inhibiting inflammation are needed. Plasmon-activated water (PAW) is a nontoxic form of water with potential in the treatment of inflammatory diseases. The aim of the present study was to evaluate the therapeutic effects of PAW in a murine model of UC. Histological and immunohistochemical analyses were performed on colon tissues from mice with dextran sodium sulfate (DSS)-induced UC treated with either 5-aminosalicylic acid (5-ASA) or PAW. Epithelial cell density was decreased in the DSS model mice compared with that in the normal control mice, whereas treatment with 5-ASA or PAW attenuated this DSS-induced reduction. Microscopy revealed that the DSS/PAW group exhibited significantly reduced epithelial loss, crypt damage and inflammatory cell infiltration compared with that in the DSS group. In addition, immunohistochemical analysis demonstrated that PAW downregulated the DSS-induced expression of tumor necrosis factor-α and keratin 20 in epithelial cells and the lamina propria. Furthermore, PAW also attenuated the DSS-induced loss of expression of three proteins essential for cell adhesion and tight junctions, namely E-cadherin (CDH1), tight junction protein 1 (ZO-1) and occludin, in the colonic epithelium, particularly in intestinal crypts. In addition, mucin 1 (MUC1) expression was decreased and MUC2 expression increased in the mucosal layer of the colons of the DSS/PAW group compared with those in the DSS group. In conclusion, the colonic mucosa is a reliable site for evaluating epithelial damage and inflammatory infiltration. PAW ameliorated DSS-induced UC in mice by modulating the expression of key barrier-associated proteins, including CDH1, occludin, ZO-1, MUC1 and MUC2. These findings highlight the therapeutic potential of PAW in the treatment of colitis.

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Abbreviations: UC, ulcerative colitis; PAW, plasmon-activated water; DSS, dextran sodium sulfate; 5-ASA, 5-aminosalicylic acid; TNF-α, tumor necrosis factor-α; KRT20, keratin 20; CDH1, E-cadherin; ZO-1, tight junction protein 1; MUC1, mucin 1; MUC2, mucin 2; H&E, hematoxylin and eosin

Key words: PAW, UC, 5-ASA, gut barrier, inflammation

Introduction

Ulcerative colitis (UC) is a chronic inflammatory bowel disease (IBD) characterized by frequent relapses and remission phases (1,2). Disruption of the intestinal epithelial barrier makes a crucial contribution to the development of IBD, and the maintenance of appropriate mucosal gene expression is essential for intestinal integrity (3). In the large intestine, preservation of the protective mucosal barrier strengthens the innate defenses of the host (4,5). Given the risk of colorectal cancer (CRC) associated with UC, innovative treatments that are capable of restoring gut-barrier function and suppressing intestinal inflammation are necessary to manage UC and other digestive diseases involving chronic inflammation (6,7).

Following acute or chronic injury, restoration of the mucus layer using intestinal barrier protectants helps to reestablish physiological homeostasis (8). This is important given the continuous turnover of epithelial cells from crypts to villi, which is accompanied by physiological epithelial cell death (9). Maintaining a healthy mucosa promotes long-term clinical remission and improves outcomes in patients with UC (4,10). Therefore, strengthening the intestinal barrier has been indicated to be a viable strategy to achieve long-term therapeutic success in patients with UC (11).

Mesalamine, also known as 5-aminosalicylic acid (5-ASA), is widely used to treat gastrointestinal inflammation (12,13). As a synthetic anti-inflammatory drug, 5-ASA is a first-line treatment for UC (1). However, it does not significantly affect the remission or relapse rates of IBD (14). Combining 5-ASA with additional therapies has been shown to improve clinical outcomes and reduce the inflammatory markers for colorectal sites (15,16). Plasmon-activated water (PAW) is a non-toxic form of water that has shown promise in the treatment of inflammatory diseases, including Alzheimer's disease and chronic kidney disease (17,18). As previously reported (19,20), the presence of hydrogen bonds (HBs) in PAW is diminished due to hot electron transfer from supported gold nanoparticles (AuNPs) under resonant illumination, which disrupts the strong hydrogen bonding network of bulk water. In addition, PAW has an electron-doped structure helps to maintain the stability of the water by preserving its reduced HBs for at least one week. A recent study indicated that PAW is able to alleviate UC symptoms in animal models of IBD (21).

Maintaining the integrity of the gut mucosa ensures resistance to infection, promotes the resolution of inflammation, and supports the restoration of epithelial barrier function (22,23). Although current treatments target gut-barrier restoration, challenges remain. To the best of our knowledge, the present study is the first to examine the effects of PAW on gut barriers in a mouse model of UC.

Materials and methods

Animal study design and housing conditions. A total of 14 male mice (7 weeks old; weight, ~25 g) were purchased from the National Laboratory Animal Center in Taiwan and maintained in the Animal Research Center of Cathay General Hospital (Taipei, Taiwan). The animal experiment was approved by the Institutional Animal Care and Use Committee of Cathay General Hospital (approval no. CGH-IACUC-110-006) and conducted in accordance with the Guide for the Care and Use of Laboratory Animals (8th edition). The AVMA Guidelines for the Euthanasia of Animals: 2020 Edition were adhered to, ensuring that euthanasia was performed with minimal distress. The mice were housed in plastic cages (3-5 mice per cage) under controlled conditions comprising 50±10% humidity, a 12-h light/dark cycle and a temperature of 23±2˚C. The animals had free access to water and were fed a pelleted mouse diet (cat no. D12450H; Research Diets, Inc.). The number of mice per cage was limited to a maximum of five to provide adequate space for movement. In addition, to provide environmental enrichment, wooden blocks and small shelters were provided within the cages.

To induce UC, 3% dextran sodium sulfate (DSS; molecular weight, 36,000-50,000; MP Biomedicals, LLC) was administered to the mice in their drinking water for 7 days, followed by deionized water for the next 7 days. This cycle was repeated once (24). Mice exhibiting any symptoms, including weight changes, fecal consistency and overt bleeding, were kept under close observation. Technicians monitored the activity of all experimental mice daily, replaced drinking water or PAW every 2 days, and measured body weight weekly. The following humane endpoints were established: Body weight loss >15%, infection, severely under-conditioned appearance and abnormal behavior. After 28 days, all mice were euthanized as previously described (21). In brief, the mice were exposed to CO□ at a flow rate of ~50% vol/min. The CO□ flow was maintained for ≥1 min after the cessation of respiration, which was confirmed by observing the absence of breathing and fading of eye color for each mouse.

The experimental timeline for DSS induction, 5-ASA administration and PAW consumption is illustrated in Fig. 1. Following a 7-day quarantine, the mice were randomly divided into four groups based on body weight: Control group (n=2) in which mice were given deionized water every day of the 28-day experimental period; DSS group (n=3) comprising mice with DSS-induced UC; DSS/5-ASA group (n=4) comprising mice with DSS-induced UC receiving 5-ASA via gavage: and DSS/PAW group (n=5) comprising mice with DSS-induced UC who were given PAW as drinking water for the duration of the 28-day study period.

PAW preparation and 5-ASA treatment. PAW was prepared following the procedure described in our previous study (25). Briefly, distilled water was passed through a glass tube containing ceramics with AuNPs adsorbed on their surface. The water was exposed to resonant light emitted by green light-emitting diodes (LEDs), with a wavelength peak centered at 530 nm. PAW (pH 6.96, 23.5˚C) was collected within 2 h in glass sample bottles for subsequent use. The 5-ASA treatment involved the administration of 200 mg/kg 5-ASA once daily by gavage in the week following DSS induction.

Histological and immunohistochemical analyses. Colons were isolated from the mice. After measuring the length of each colon, the colon tissue was fixed with 4% paraformaldehyde in phosphate-buffered saline for 10 min at room temperature and then embedded in paraffin wax. Sections were cut to a thickness of 5 μm and mounted on slides for hematoxylin and eosin (H&E) staining and the immunohistochemical detection

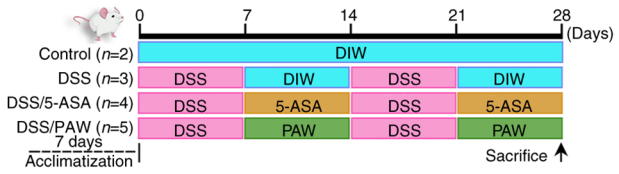

Figure 1. Experimental timeline for DSS induction, 5-ASA administration and PAW consumption. Mice were acclimatized to the environment for 7 days before random assignment into four groups based on body weight: Control, DSS, DSS/5-ASA and DSS/PAW. Each treatment was administered for two one-week periods as shown. The control group drank deionized water; the DSS group was treated with 3% DSS; the DSS/5-ASA group was treated with 3% DSS and 200 mg/kg/day 5-ASA; and the DSS/PAW group was treated with 3% DSS and received PAW to drink. DSS, dextran sodium sulfate; 5-ASA, 5-aminosalicylic acid; PAW, plasmon-activated water; DIW, deionized water.

of tumor necrosis factor α (TNF-α), keratin 20 (KRT20), E-cadherin (CDH1), tight junction protein 1 (ZO-1), occludin, mucin 1 (MUC1) and MUC2. H&E staining was performed at room temperature using a Tissue-Tek DRS™ 2000 Automated Slide Stainer (Sakura Finetek USA, Inc.) according to a standard sequential protocol. Tissue sections were first deparaffinized with two consecutive xylene baths (5 min each), followed by a third xylene bath for 7 min. Rehydration was carried out through a graded ethanol series (100% ethanol for 60 and 90 sec, 95% ethanol for 60 sec and 75% ethanol for 60 sec), followed by rinsing in running water for 3 min. Slides were then stained with hematoxylin for 5 min and briefly differentiated by dipping five times in 1% acid alcohol (1% HCl in 70% ethanol). After rinsing, slides were counterstained with eosin for 3 min at room temperature, dehydrated through graded alcohols and cleared in xylene before mounting. Immunohistochemical staining was performed using a BenchMark GX automated slide stainer (Roche Diagnostics) under closed fixation conditions. The process included deparaffinization with EZ Prep solution (cat. no. 950-102) for 8 min at 75˚C, followed by antigen retrieval with a Cell Conditioning 1 solution (CC1; cat. no. 950-124) or Cell Conditioning 2 solution (CC2; cat. no. 950-123) (all from Roche Tissue Diagnostics; Roche Diagnostics, Ltd.) for 48 min at 95˚C. Primary antibody hybridization was conducted at 37˚C for various durations depending on the antigen. CDH-1, ZO-1, occludin and TNF-α antigens were retrieved with CC1 for 64 min, while MUC1 was retrieved with CC1 for 92 min. Each section was hybridized with specific primary antibodies as follows: Anti-CDH-1 antibody (1:500) for 32 min, anti-ZO-1 antibody (1:500) and anti-occludin antibody (1:200) for 1 h, and anti-TNF-α antibody (1:1,200) and anti-MUC1 antibody (1:50) for 2 h. KRT20 and MUC2 were retrieved using CC2 for 48 min and hybridized with anti-KRT20 antibody (1:400) and anti-MUC2 antibody (1:400) for 2 h. Primary antibodies against TNF-α (cat. no. A0277), CDH1 (cat. no. A20798), ZO-1 (cat. no. A0659) and MUC2 (cat. no. A14659) were purchased from ABclonal Biotech Co., Ltd.; those against MUC1 (cat. no. Ab109185) were purchased from Abcam (Cambridge, UK); and those against KRT20 (cat. no. 17329-1-AP) and occludin (cat. no. 27260-1-AP) were purchased from Proteintech Group, Inc.

All sections were hybridized twice for 12 min at 37˚C with 100 µl Histofine® Simple Stain Mouse MAX PO (R) (cat. no. 414341F; Nichirei Biosciences, Inc.), which comprises anti-rabbit antibody and a universal immunoperoxidase polymer. Visualization was performed with an OptiView DAB IHC detection kit (cat. no. 760-700; Roche Diagnostics), followed by counterstaining with hematoxylin II for 8 min at 25˚C (cat. no. 790-2208; Roche Tissue Diagnostics; Roche Diagnostics, Ltd.) and Bluing Reagent for 4 min at 25˚C (cat. no. 760-2037; Roche Tissue Diagnostics; Roche Diagnostics, Ltd.). After staining, the sections were scanned using a Zeiss Mirax scanner (Carl Zeiss AG) and analyzed independently by a pathologist who was blinded to the experimental groups.

Inflammation characterization of colon tissues and measurement of epithelial cell density. Following euthanasia, the colons of all mice were removed and dissected longitudinally. Inflammation of the colon was assessed based on epithelial loss, crypt damage, goblet cell depletion and inflammatory cell infiltration. The histological evaluation of colonic tissue was performed on the H&E-stained sections in a blinded manner, using a scoring system adapted from that described by Iba *et al* (26). The four parameters were scored as follows: i) Loss of epithelium: 0, no staining; 1, 0-5% (mild); 2, 5-10% (moderate); or 3, >10% (severe); ii) Crypt damage: 0, none; 1, 0-10% (mild); 2, 10-20% (moderate); or 3, >20% (severe), with crypt loss evaluated as the percentage relative to the total mucosal area; ii) Depletion of goblet cells: 0, none; 1, mild; 2, moderate; or 3, severe; and iv) Infiltration of inflammatory cells: 0, none; 1, mild; 2, moderate; or 3, severe. The integrity of the colonic epithelium was evaluated by manually measuring the density of the colonic epithelial cells along the edge of the basement membrane (27).

Statistical analyses. Statistical analysis was conducted using IBM SPSS Statistics (version 27.0; IBM Corp.). One-way analysis of variance followed by Tukey's honestly significant difference post hoc test was employed for comparisons among multiple groups with normally distributed data, and Kruskal-Wallis followed by Dunn's post hoc test was used for comparisons among multiple groups with ordinal data. $P<0.05$ was considered to indicate a statistically significant difference.

Results

5-ASA and PAW increase epithelial cell density in inflamed intestines. During the experiment, one mouse in the DSS/5-ASA group died on day 10, while no mortality was observed in the DSS and DSS/PAW groups. The death was likely associated with the DSS treatment; however, the mortality rate (~8.3%) is lower than that reported in a study by Pan *et al* (24), where mice with 3% DSS-induced with UC using had a mortality rate of ~11.1%. Among the groups with 3% DSS-induced UC, the DSS/PAW group exhibited a significant reduction in body weight and the DSS/5-ASA showed a trend towards reduced body weight on day 14 (Fig. 2A), and a non-significant reduction in colon length was observed in the DSS/5-ASA group (Fig. 2B and C) compared with those in the control group. Epithelial cell density was also assessed to evaluate the integrity of the intracolonic epithelium based on H&E staining (Fig. 2D). The DSS group had the lowest cell

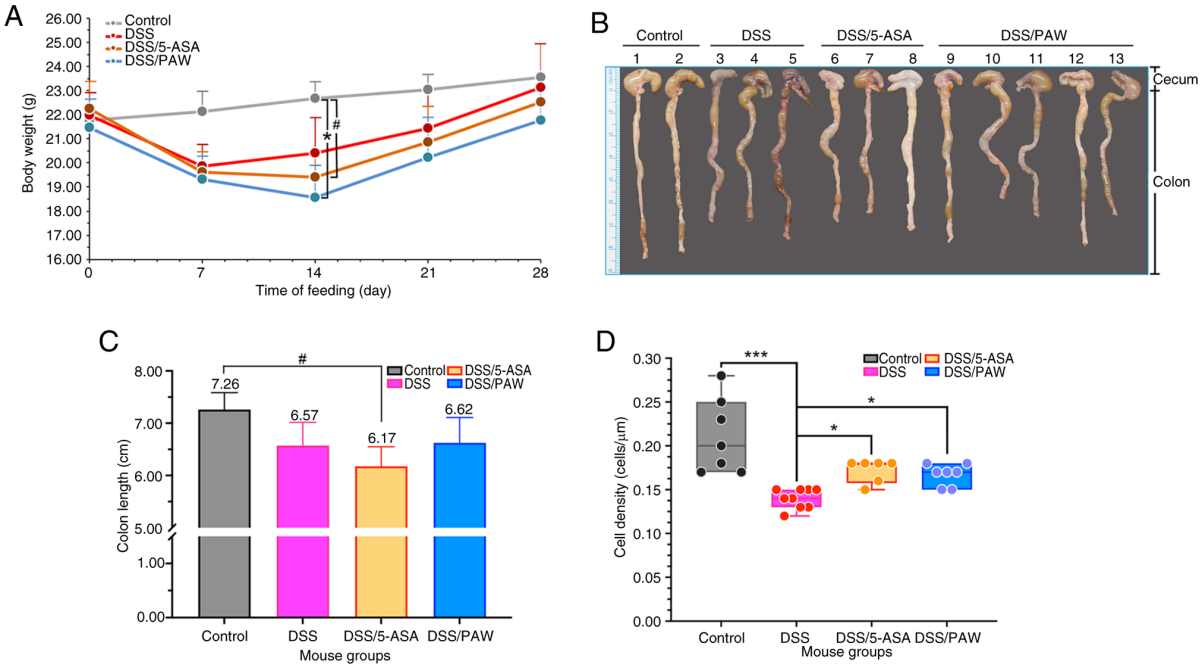

Figure 2. Analysis of body weight and epithelial cell density in the inflamed intestines of mice with DSS-induced ulcerative colitis. (A) Body weights, (B) photographs of the colons, (C) colon lengths and (D) average epithelial cell density in four groups of mice: Control group, deionized water; DSS group, 3% DSS; DSS/5-ASA group, 3% DSS + 200 mg/kg 5-ASA group; DSS/PAW 3% DSS + PAW. Mean values and the positive SD are shown. $^{\#}0.05<P<0.1$, $^{*}P<0.05$, $^{**}P<0.01$ and n.s. as indicated. DSS, dextran sodium sulfate; 5-ASA, 5-aminosalicylic acid; PAW, plasmon-activated water; SD, standard deviation; n.s., not significant.

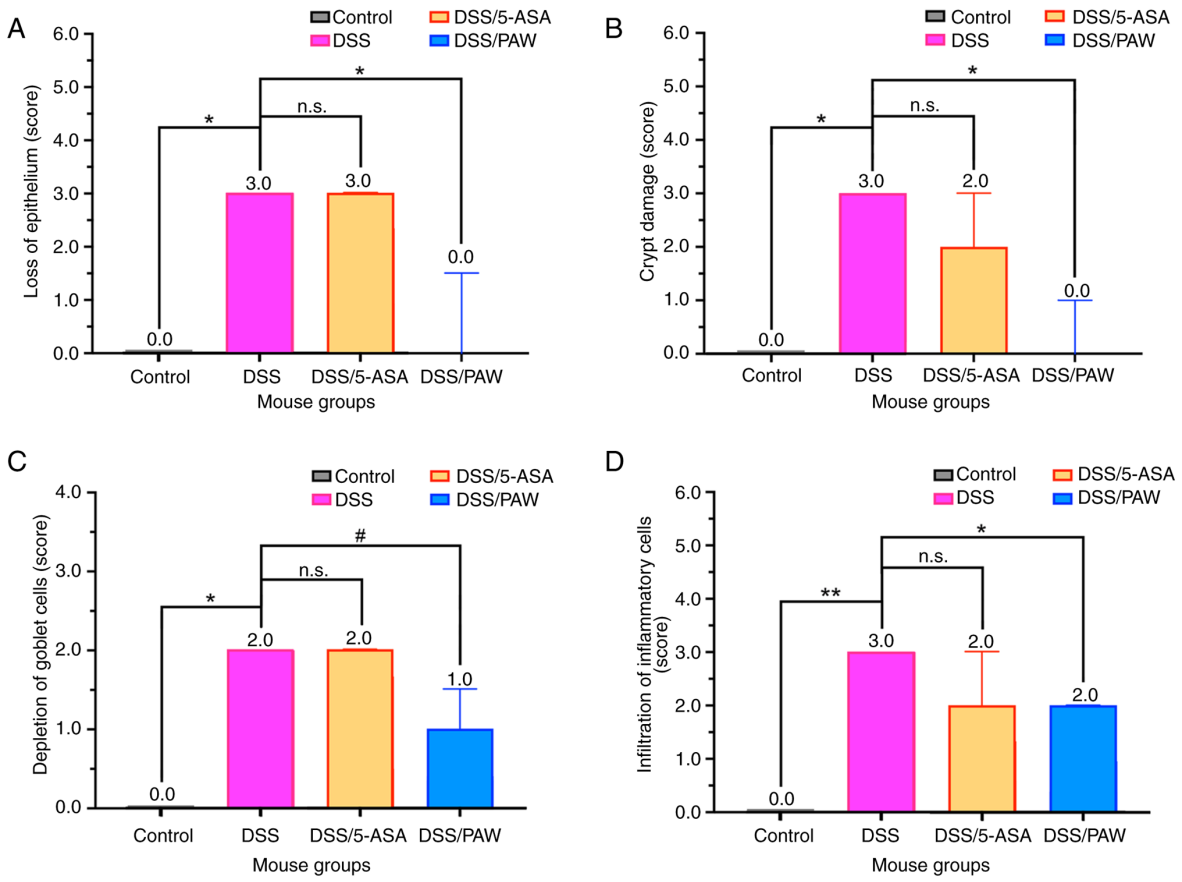

Figure 3. Histological scores for indicators of colonic inflammation in mice with DSS-induced ulcerative colitis. (A) Loss of epithelium, (B) crypt damage, (C) depletion of goblet cells and (D) infiltration of inflammatory cells in the four groups of mice: Control group, deionized water; DSS group, 3% DSS; DSS/5-ASA group, 3% DSS + 200 mg/kg 5-ASA; DSS/PAW group, 3% DSS + PAW. Median values with interquartile range are shown for each group. $^{\#}0.05<P<0.1$, $^{*}P<0.05$, $^{**}P<0.01$ and n.s. as indicated. DSS, dextran sodium sulfate; 5-ASA, 5-aminosalicylic acid; PAW, plasmon-activated water; n.s., not significant.

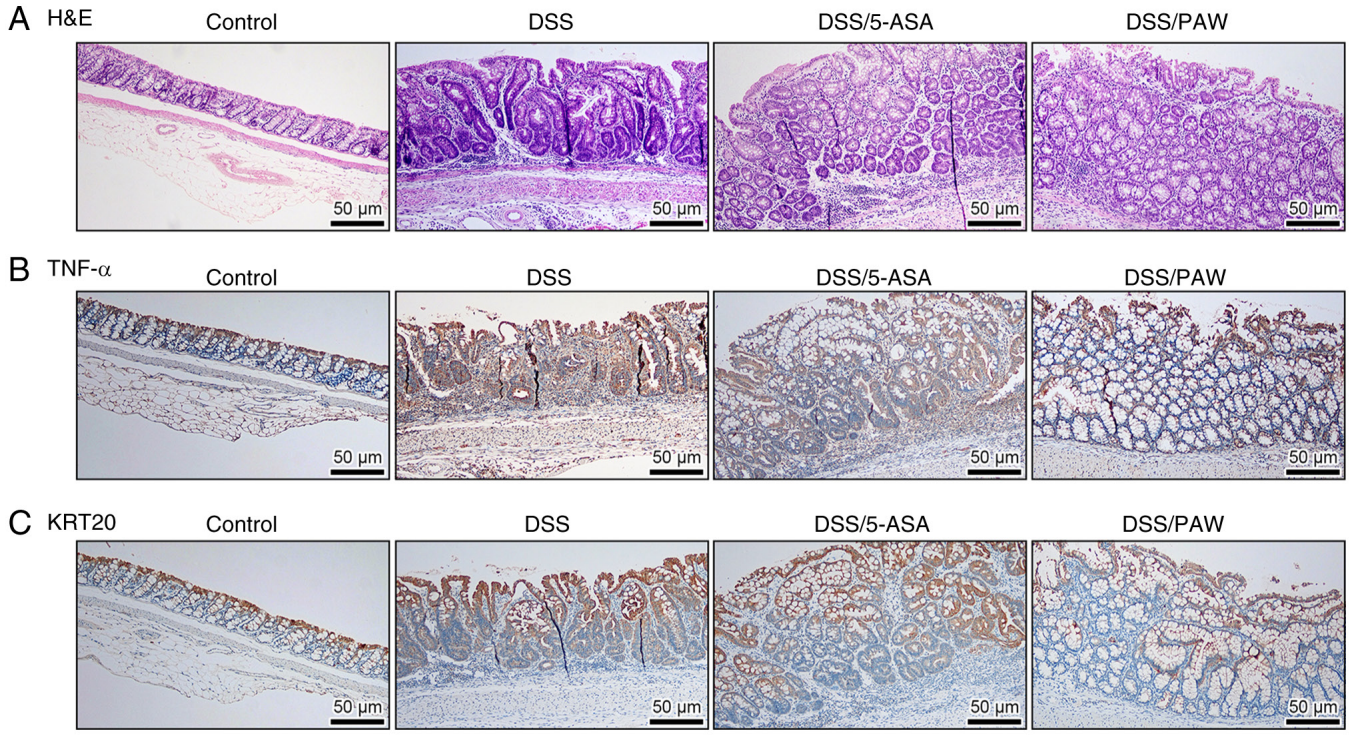

Figure 4. Histological and immunohistochemical analysis of the colonic tissues of mice with DSS-induced ulcerative colitis. (A) Representative H&E staining images and immunostaining of (B) TNF-α and (C) KRT20 in the four groups: Control group, deionized water; DSS group, 3% DSS; DSS/5-ASA group, 3% DSS + 200 mg/kg 5-ASA; and DSS/PAW group, 3% DSS + PAW. Scale bar, 50 μm. H&E, hematoxylin and eosin; DSS, dextran sodium sulfate; TNF-α, tumor necrosis factor-α; KRT20, keratin 20; 5-ASA, 5-aminosalicylic acid; PAW, plasmon-activated water.

density, while the DSS/5-ASA and DSS/PAW groups exhibited significantly increased cell density compared with that in the DSS group.

PAW modulates intestinal inflammatory responses. Three inflammatory indicators, namely epithelial loss, crypt damage and goblet cell depletion, were used to evaluate the extent of colonic epithelial damage (26). The infiltration of inflammatory cells was also assessed. As shown in Fig. 3, comparison of the results in the DSS and DSS/5-ASA groups revealed that 5-ASA did not significantly reduce the scores for these inflammatory indicators, indicating that 5-ASA did not ameliorate the DSS-induced colonic epithelial damage. Compared with the DSS group, the DSS/PAW group showed a significant reduction in epithelial loss (Fig. 3A), crypt damage (Fig. 3B) and inflammatory cell infiltration (Fig. 3D), along with a non-significant decrease in goblet cell depletion (Fig. 3C) compared with that in the DSS group.

Histological examination, as shown in Fig. 4A, revealed that DSS induced structural abnormalities compared with the control group, with dense inflammatory cell infiltration and frequent crypt abscesses. However, both 5-ASA and PAW mitigated these inflammatory responses, with the DSS/PAW group showing the most marked improvement. The DSS-induced inflammatory status was further assessed using two colitis markers, TNF-α and KRT20 (28,29). Immunostaining revealed low expression of TNF-α (Fig. 4B) and KRT20 (Fig. 4C) at the base of the colonic crypts with marked elevation in the superficial epithelium of the normal tissue. DSS treatment upregulated the expression of TNF-α and KRT20 in the mucosal layer. However, treatment with 5-ASA or PAW attenuated the expression of these markers in the epithelial cells and lamina propria. Notably, PAW induced a more pronounced reduction than 5-ASA.

PAW enhances the adhesion and tight junctions of epithelial cells in inflamed intestines. Immunohistochemical analysis was used to analyze the expression of the adhesion molecule CDH1 (Fig. 5A) and the tight junction-associated proteins ZO-1 (Fig. 5B) and occludin (Fig. 5C). These three proteins play key roles in cell proliferation and survival via the regulation of epithelial adhesion and tight junction integrity (30,31). The expression of CDH1, ZO-1 and occludin was increased in the colonic epithelium of the DSS/PAW group, particularly in the intestinal crypts, compared with that in the DSS group. However, the expression of these proteins did not appear to increase in the colons of the DSS/5-ASA group.

PAW influences mucin content in the inflamed intestinal mucosa. Mucins are commonly detected in the colons of patients with UC (32). Among them, MUC1 and MUC2 modulate mucus composition and are implicated in the pathogenesis of UC (33,34). In the present study, MUC2 was evenly distributed in the mucosal epithelial cells of the control group (Fig. 6B), whereas MUC1 expression was minimal (Fig. 6A). In the DSS group, representing the active phase of UC, MUC1 expression was markedly upregulated, whereas MUC2 expression was reduced. Treatment with either 5-ASA or PAW attenuated the change in mucin composition in the inflamed intestinal cells by downregulating MUC1 expression and increasing MUC2 expression. Notably,

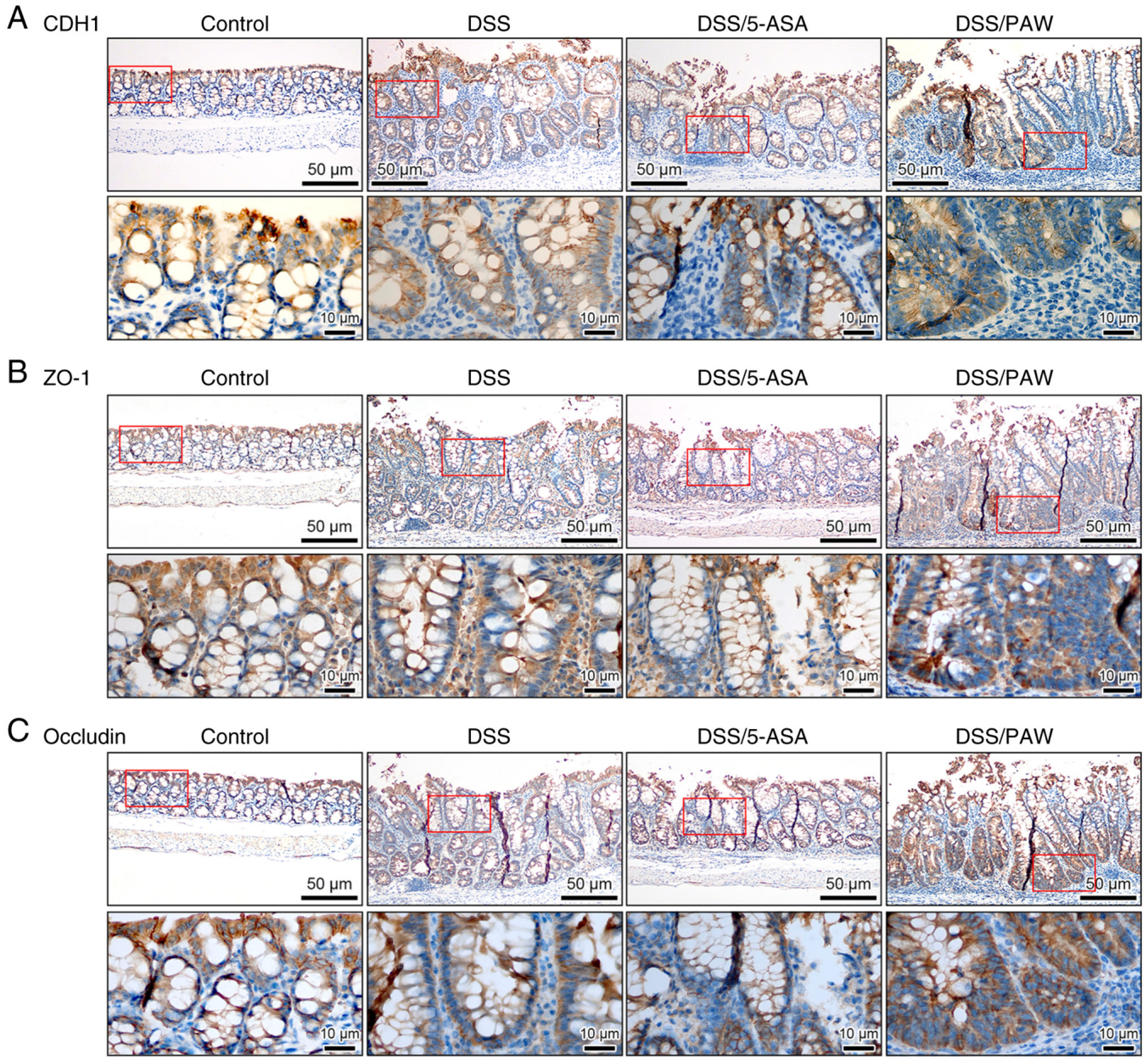

Figure 5. Immunohistochemical analysis of cell adhesion and tight junction molecules in the colonic tissues of mice with DSS-induced ulcerative colitis. (A) CDH1, (B) ZO-1 and (C) occludin expression in the four groups: Control group, deionized water; DSS group, 3% DSS; DSS/5-ASA group, 3% DSS + 200 mg/kg 5-ASA; DSS/PAW group, 3% DSS + PAW. Scale bar, 50 μm. The lower image (scale bar, 10 μm) in each panel represents a closer view of the area in the red box of the upper image. DSS, dextran sodium sulfate; CDH1, E-cadherin; ZO-1, tight junction protein 1; 5-ASA, 5-aminosalicylic acid; PAW, plasmon-activated water.

numerous MUC2-positive cells were detected in the mucosal epithelium of the colons in the DSS/PAW group.

Discussion

PAW is typically synthesized by irradiating AuNP-adsorbed ceramics with resonant light emitted by green LEDs (25,35). As shown in our previous studies (19,20), PAW is a form of pure water characterized by an electron-doped structure and weakened hydrogen bonding. Since PAW is pure water, its concentration is not a relevant parameter in its use. This unique type of water has shown clinical potential and anti-inflammatory effects in colonic epithelial cells (21,36). In the present study, the anti-inflammatory effects of PAW were compared with those of 5-ASA in mice with DSS-induced UC. Notably, cycling treatment previously has been applied in both DSS-induced UC and PAW-treated diseases, serving as an important reference for the design of the present study (24,37).

First-line 5-ASA monotherapy has been reported to have limited efficacy in the treatment of IBD (38), and the results of the animal experiments performed in the present study are consistent with this. As in previous studies, body weight and colon length were measured to assess the extent of colonic inflammation (39,40). The results indicated that 5-ASA did not increase body weight or restore colon length in mice with inflamed intestines, although it did reduce inflammatory responses in the colon. Previous studies have demonstrated that TNF-α and KRT20 are upregulated in chronic DSS-induced

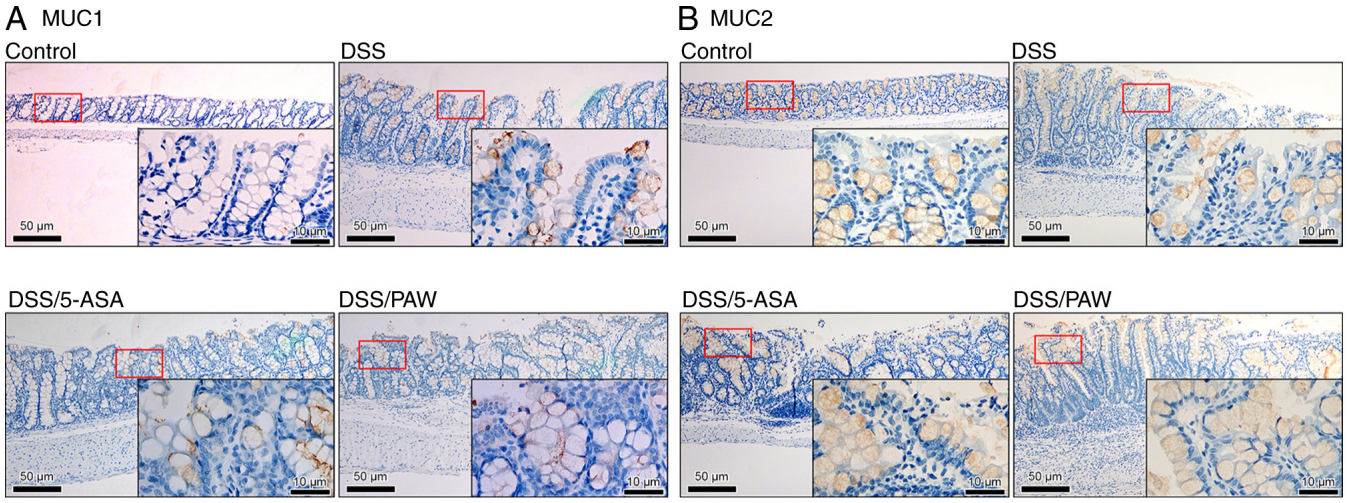

Figure 6. Immunohistochemical analysis of mucins in the colonic tissues of mice with DSS-induced ulcerative colitis. (A) MUC1 and (B) MUC2 expression in the four groups: Control group, deionized water; DSS group, 3% DSS; DSS/5-ASA group, 3% DSS + 200 mg/kg 5-ASA; DSS/PAW group, 3% DSS + PAW. DSS, dextran sodium sulfate; Scale bar, 50 μm. The inset (scale bar, 10 μm) in each panel represents a closer view of the area in the red box. MUC1, mucin 1; MUC2, mucin 2; 5-ASA, 5-aminosalicylic acid; PAW, plasmon-activated water.

UC (28,41), which is consistent with the immunostaining results for these markers in the present study, where elevated levels of TNF-α, an inflammatory cytokine (42), and KRT20, a tumor marker associated with intestinal malignancy (43), were observed. In the present study, 5-ASA treatment reduced the immunohistochemical staining of TNF-α and KRT20 in the inflamed intestines of the UC model mice. Notably, PAW treatment resulted in a more marked reduction in the levels of these two markers. These results suggest that although neither 5-ASA nor PAW completely resolved DSS-induced UC during the study period, both treatments mitigated inflammation in the intestinal microenvironment. Microscopically, PAW was shown to alleviate colonic epithelial damage, further reducing inflammation.

As reported in earlier studies, epithelial injury, crypt damage and goblet cell loss can be used to evaluate the severity of DSS-induced UC (44,45). In the present study, a comparison of these indicators revealed that PAW exerted a significant anti-inflammatory effect while 5-ASA, a drug commonly used in clinical practice to treat UC, did not (46). In addition, while short-term treatment with PAW did not attenuate the DSS-induced loss of body weight or shortening of colon length, it did enhance the microenvironmental immunity of the colon and reduce inflammation. Moreover, PAW was more effective than 5-ASA in suppressing inflammation in mice with DSS-induced UC.

In the present study, PAW did not induce any inflammatory response in the colonic tissues; instead it demonstrated clear anti-inflammatory effects. Previous studies have shown that PAW is safe for long-term consumption, with no observed toxicity; in two extended animal experiments, mice exhibited no abnormalities after consuming PAW for 9 months (25) or 16 months (18). Additionally, results from a human clinical trial (study no. Y800N20A01; sponsored by Taipei Medical University-Shuang Ho Hospital) revealed that PAW caused no adverse effects in humans. These findings are consistent with those of our previous study, which showed that PAW possesses potent antioxidant and anti-inflammatory properties without any

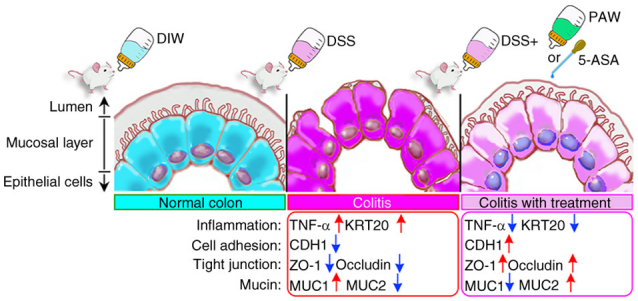

Figure 7. Molecular mechanisms underlying the PAW-mediated enhancement of gut barrier integrity in mice with DSS-induced ulcerative colitis. DSS, dextran sodium sulfate; plasmon-activated water; DIW, deionized water; 5-ASA, 5-aminosalicylic acid; TNF-a, tumor necrosis factor-a; KRT20, keratin 20; CDH1, E-cadherin; ZO-1, tight junction protein 1; MUC1, mucin 1; MUC2, mucin 2.

evident toxicity (18). In the present study, it was also observed that the anti-inflammatory effect of PAW was stronger than that of 5-ASA and effectively mitigated IBD severity. Consequently, it appears that PAW treatment not only ameliorates intestinal inflammation but could also help to avoid the potential effects of 5-ASA, such as autoimmune hepatitis (47). As an alternative to 5-ASA, corticosteroids are commonly used to provide short-term relief; however, they are associated with significant side effects (48). Immunosuppressants and biologics offer improved disease control but pose risks such as infections, and are expensive (49,50). In the present study, PAW outperformed 5-ASA in the restoration of epithelial integrity and modulation of mucin expression, highlighting its potential as a novel treatment strategy for UC.

Maintaining the integrity of the gut mucosa, including the formation of tight epithelial junctions, is essential for enhancing pathogen resistance and supporting gut health (51). The intestinal barrier, protected by a layer of mucus, performs various site-specific protective functions (52). Therefore, the preservation of mucosal integrity helps to modulate the mucosal defense

system and promote anti-inflammatory responses (53). During inflammation, the paracellular space between gut epithelial cells is typically sealed by apical junctional complexes composed of tight and adherens junctions (54,55). CDH1 is an adhesion molecule, while occludin and ZO-1 are tight junction proteins involved in maintaining the mechanical integrity of the intestinal epithelium (31,56,57). Consistent with the findings of de Ponthaud *et al* (58), a reduction in CDH1 levels was observed in the inflamed ileal mucosa in the present study. However, PAW treatment partially restored CDH1 levels, contributing to improved gut health in mice with DSS-induced UC. This recovery may be important, as a loss of CDH1 expression is associated with cancer progression, including the increased proliferation, invasion and metastasis of colorectal neoplasm cells (30). The findings of the present study suggest that PAW may help to restore gut-barrier function by upregulating the expression of tight junction proteins. Barrier integrity and dysfunction are key factors in the maintenance of gut health and related outcomes (57). Consistent with the findings of Guo *et al* (59), the present study indicated that an increase in the expression levels of tight junction proteins, such as occludin and ZO-1, may attenuate the progression of UC.

In addition to adhesion and tight junction molecules, damage to the mucus layer and goblet cells in the intestinal lining is frequently observed during inflammation and plays a key role in disease pathogenesis (52). In the colon, mucus acts as the primary barrier between intestinal microorganisms and the mucosa, contributing to the defense system of the host (60). The efficacy of certain chemotherapeutic agents for CRC has been linked to the differential expression of mucins, particularly MUC1 and MUC2 (61). The present study found that PAW influences the expression of specific genes that may alter the fate of colonic cells. For example, UC leads to long-term changes in goblet cell function (62). Goblet cells are responsible for producing mucins, which are essential for intestinal lubrication, cell signaling, and protecting the epithelium from pathogens, toxins and other irritants (63). Previous studies have shown that mucin expression is dysregulated in intestinal epithelial cells during active inflammation and contributes to the pathological changes observed in IBD (64-66). In inflamed intestinal tissues, a physiological characteristic pattern of high MUC1 and low MUC2 expression levels is associated with UC exacerbation (64,65). MUC2, a secreted mucin produced by goblet cells, forms the primary component of the intestinal mucus barrier, safeguarding epithelial cells from pathogens and maintaining gut homeostasis. Conversely, MUC1 is a membrane-bound mucin expressed on epithelial surfaces; its upregulation during inflammation and infection suggests a role in modulating immune responses and epithelial repair mechanisms. In particular, the downregulation of MUC2 is indicated to play a crucial role in UC pathogenesis (65). In the present study, reduced MUC2 levels were observed within the inflammatory cells of UC model mice; however, MUC2 expression was restored in the DSS/PAW group. These results suggest that PAW treatment increases MUC2 levels, thereby preserving the mucus layer and protecting the colonic epithelium from damage and pathogen infiltration.

The present study has certain limitations. First, the precise mechanism by which PAW improves the gut barrier in UC was not fully elucidated. Second, the sample size used was small; while adhering to the refinement, reduction and replacement principle of animal research, this may impact the generalizability and statistical power of the findings. Third, the unequal group sizes and absence of power calculations represent further limitations of the study. While previous studies have used similar experimental animal numbers (67-69), the small and imbalanced sample sizes may have limited the ability to detect subtle differences between experimental groups, particularly in assays for which the observed changes did not reach statistical significance.

Despite these limitations, the findings are noteworthy and warrant further investigation. While PAW demonstrated stronger anti-inflammatory effects than 5-ASA, future studies should incorporate formal power analyses and use larger, more balanced sample groups. The inclusion of additional experimental groups, such as mice treated with both PAW and 5-ASA, along with increased sample sizes, will enhance statistical robustness and facilitate a deeper understanding of the underlying molecular mechanisms. The molecular effects of PAW on key proteins in the colonic epithelium, including CDH1, ZO-1 and MUC1/2, highlights its potential influence on cell adhesion, tight junction stabilization and mucin regulation. However, further investigation of oxidative signaling (70-72), epigenetic regulation (73,74) and the modulation of tight junctions/mucins (75) may be necessary to clarify the precise molecular mechanisms. In addition, long-term studies involving repeated dosing and varied treatment durations are essential to determine the sustainability and reproducibility of the observed effects. The evaluation of multiple animal models of UC or progression to clinical trials will help establish the broader applicability of PAW and confirm its therapeutic potential in human contexts.

In conclusion, the colonic mucosa serves as a suitable site for the evaluation of epithelial damage and inflammatory cell infiltration (76). As depicted in Fig. 7, both 5-ASA and PAW exhibit anti-inflammatory effects and modulate the expression of mucins, adhesion molecules and tight junction proteins. PAW significantly ameliorated DSS-induced colitis in mice by enhancing gut-barrier integrity and modulating inflammation-related proteins and mucins. Notably, PAW outperformed 5-ASA in restoring epithelial cell density, reducing crypt damage, and increasing the levels of tight junction proteins such as CDH1, ZO-1 and occludin. In addition, PAW restored the balance of MUC1 and MUC2 in the colonic mucosa, a critical factor for maintaining intestinal barrier function. By contrast, while 5-ASA effectively reduced the expression of the inflammation markers TNF-α and KRT20, its effects on epithelial and mucosal repair were less pronounced compared with those of PAW. These findings highlight the therapeutic potential of PAW as a novel intervention for UC and support further investigation into its mechanisms and long-term efficacy.

In summary, the findings of the present study indicate that PAW ameliorates DSS-induced colitis in mice by modulating the expression of CDH1, occludin, ZO-1, MUC1 and MUC2. These results highlight the therapeutic potential of PAW in the treatment of UC.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

CCC, CJC, YCL and CJH took the lead in data curation, contributed equally to formal analysis, investigation and methodology, and took the lead in writing, reviewing and editing the manuscript. CYL and HJY supported data curation, and contributed equally to formal analysis, investigation and methodology. YJL, WCC, CWC, and JLP were involved in formal analysis and investigation, and provided equal supervision. CCC and YCL led conceptualization and supervision, and were responsible for funding acquisition and use, and project administration. CCC, YCL and CJH confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The Institutional Animal Care and Use Committee of Cathay General Hospital approved this study (approval no. CGH-IACUC-110-006).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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