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Ghrelin ameliorates intestinal barrier dysfunction in experimental colitis by inhibiting the activation of nuclear factor-kappa B

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ABSTRACT

Aim: This study aimed to investigate the effect and underlying mechanism of ghrelin on intestinal barrier dysfunction in dextran sulfate sodium (DSS)-induced colitis.
Methods and results: Acute colitis was induced in C57BL/6J mice by administering 2.5% DSS. Saline or 25, 125, 250 μg/kg ghrelin was administrated intraperitoneally (IP) to mice 1 day before colitis induction and on days 4, 5, and 6 after DSS administration. IP injection of a ghrelin receptor antagonist, [D-lys^3]-GHRP-6, was performed immediately prior to ghrelin injection. Ghrelin (125 or 250 μg/kg) could reduce the disease activity index, histological score, and myeloperoxidase activities in experimental colitis, and also prevented shortening of the colon. Ghrelin could prevent the reduction of transepithelial electrical resistance and tight junction expression, and bolstered tight junction structural integrity and regulated cytokine secretion. Ultimately, ghrelin inhibited nuclear factor kappa B (NF-κB), inhibitory κB-α, myosin light chain kinase, and phosphorylated myosin light chain 2 activation.
Conclusions: Ghrelin prevented the breakdown of intestinal barrier function in DSS-induced colitis. The protective effects of ghrelin on intestinal barrier function were mediated by its receptor GHSR-1a. The inhibition of NF-κB activation might be part of the mechanism underlying the effects of ghrelin that protect against barrier dysfunction.

1. Introduction

The gastrointestinal mucosa is coated by a single layer of epithelial cells that act as a 'barrier' to prevent the uncontrolled entry of micro-organisms and antigens. Intestinal barrier function is maintained by tight junction (TJ) proteins, adherence junction proteins, and other molecular complexes [1]. TJs, which include claudins, occludin, and zonula occludens-1 (ZO-1), are highly dynamic complexes that seal the space among adjacent epithelial cells and are involved in regulating ion-selective, electrolyte secretion and absorption. Defects in TJ integrity might be a crucial cause of increased permeability, which eventually leads to intestinal barrier dysfunction [2].

Inflammatory bowel disease (IBD), an entity that includes Crohn's disease and ulcerative colitis, is an inflammatory disorder of intestinal tract. IBD is characterized by intestinal barrier dysfunction, tissue disruption with relapsing diarrhea, and inflammatory cell infiltration [3]. Although the precise molecular mechanism of IBD remains poorly understood, accumulating evidence suggests that barrier dysfunction and a susceptible intestinal immune system play crucial roles in the early stages of IBD pathogenesis [4]. There are no specific therapeutic methods for treating IBD. Some strategies and commonly prescribed drugs, such as immunosuppressive agents and corticosteroids, are used to attenuate clinical symptoms rather than to achieve a cure [5].

Ghrelin is a 28 amino acid polypeptide that is predominantly secreted by X/A-like endocrine cells of stomach. It was initially identified as an appetite-regulating hormone that acts at the level of the pituitary [6]. Many studies have reported that ghrelin and its G-protein-coupled receptor, also known as growth hormone secretagogue receptor type 1a (GHSR-1a), are widely expressed throughout the body [7]. These findings indicated that ghrelin might play a physiological role in many tissues and exert a number of biological effects, including anti-inflammatory and immunoregulatory activities. Contradictory studies exist regarding

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whether ghrelin has beneficial effects on the treatment of experimental IBD. One study showed ghrelin administration resulted in the significant amelioration of colitis in a murine experimental IBD model [8], while another study found that treatment with ghrelin could exacerbate clinical outcomes [9]. Interestingly, increased levels of ghrelin were observed by clinical investigators in the active-phase of IBD patients, whereas an opposite trend was observed during the disease remission phase [10], which indicated the potential effects of ghrelin in IBD. To our knowledge, the effects of ghrelin on barrier dysfunction in IBD have not yet been reported. Therefore, this study was designed to explore whether ghrelin functions in regulating barrier dysfunction in dextran sulfate sodium (DSS)-induced colitis and to characterize potential mechanism(s).

2. Material and methods

2.1. Animals

Male C57BL/6J mice (8 weeks old) were purchased from SLAC Laboratory Animal Co., Ltd. (Shanghai, China). Mice were housed under a 12 h dark/light cycle at controlled room temperature (22 °C) and a relative humidity of 50%, provided with tap water *ad libitum* and standard laboratory chow. Before the experimental procedures, animals were acclimatized for five days. All animal-related experimental protocols were in accordance with the Animal Care and Use Committee of Shanghai Tenth People's Hospital (2011-RES1).

2.2. Induction of colitis and treatment

Sixty mice were randomly divided into six groups : normal control (NC) group, DSS + saline treated group, DSS + ghrelin treated group (25, 125, 250 μg/kg), DSS + ghrelin (250 μg/kg)+[D-lys^3]-GHRP-6 group (9.4 mg/kg). For induction of acute colitis, 2.5% w/v DSS (MP, USA) was dissolved in the drinking water and administered to mice for 7 days, followed by fresh water for 3 days. Saline or ghrelin (ProSpec, Isreal) were administered intraperitoneally (IP) to mice 1 day before the colitis induction and on days 4, 5 and 6 after DSS administration. IP injection of [D-lys^3]-GHRP-6 (Tocris, UK) was immediately performed before ghrelin treatment.

Mice were sacrificed on day 10 and colon tissues were rapidly removed and measured for length. After being washed out the feces in the colonic lumen by phosphate-buffered saline (PBS), the colons were fixed in 4% neutral paraformaldehyde solution for morphologic evaluation. The rest portions were stored in liquid nitrogen for further investigation.

2.3. Evaluation of disease activity index (DAI)

Throughout the experiment, we recorded the following parameters of each mouse daily to determine the DAI according to Cooper [11]: loss of body weight, stool consistency, presence of occult or gross bleeding in feces. The detailed information was listed in Table 1.

Table 1
Disease activity index parameters.

Score	Weight loss (%)	Stool consistency,	Occult/gross bleeding
0	None	Normal	Normal
1	1–5	–	–
2	5–10	Loose stool	Hemoccult +
3	10–20	–	–
4	>20	Diarrhea	Gross bleeding

DAI score = (combined score of weight loss, stool consistency and bleeding)/3.

2.4. Evaluation of histological score

The colons were fixed in 4% phosphate-buffered paraformaldehyde solution for 24 h, dehydrated via graduated ethanol series and embedded in paraffin blocks. Colon sections (5 μm) were cut and stained with hematoxylin and eosin (H&E). Morphological changes in colons were evaluated by two pathologists who did not know the experiment design. The assessment criteria and the score calculating method were chosen according to the standard scoring system [12].

2.5. Myeloperoxidase (MPO) assay

The measurement of MPO activity in colon was performed by the instructions of MPO assay kit (Jiancheng Bio, China). The absorbance was detected at 450 nm for 5 min by using the spectrophotometer (Beckman, USA). One unit of MPO activity was defined as that degrading peroxide (1 mM) per minute at 25 °C. The result was expressed as U/g of colon tissues.

2.6. Transepithelial electrical resistance (TER) measurement

After the mice were sacrificed, fresh colon mucosae were quickly isolated from the muscular layer of tissues and mounted in Ussing chamber (Physiologic Instrument, USA) for measuring TER. The mucosae (exposed area was 0.5 cm^2)were maintained in both sides of chamber which were filled with 5 ml oxygenated Kreb's solution and circulated with 5% CO_2 and 95% O_2 at 37 °C. The spontaneous potential difference (PD) was maintained at 0 mV by using an automatic voltage clamping device. Then 1 mV pulse was introduced for 1 s at 60 s intervals to detect the short-circuit current (ΔIsc). The TER was calculated by Ohm's Law according to the formula TER ($\Omega \cdot cm^2$) = PD/ΔIsc.

2.7. Transmission electron microscopy (TEM)

Colons were cut into 10 mm^2 and fixed in 2.5% glutaraldehyde for 5 h at 4 °C. After washed by ice-cold PBS, the segments were fixed in 1% osmic acid for 2 h, then washed by PBS again and dehydrated in graded ethanol, and finally embedded in epoxy resin. Ultrathin sections (70 nm) were cut and then examined by TEM (JEM 1230, Japan).

2.8. Western blot analysis

Colons were grounded rapidly in liquid nitrogen. Cytoplasmic and nuclear proteins were extracted following the manufacturer's instructions of nuclear extract kit (Pierce, USA). Total proteins were extracted by radio-immunoprecipitation assay. Protein concentrations were then determined by BCA method (Beyotime, China). Equal amounts of samples were added to sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS-PAGE Bio-Rad, USA), and transferred to polyvinyl difluoride membranes following the standard method. The membranes were blocked with 5% non-fat milk in Tris-buffered saline/0.1% Tween-20 at room temperature for 1 h, then incubated with primary antibodies at 4 °C over night and specific secondary antibodies for 1 h at room temperature. Finally, membranes were detected by Odyssey two-color infrared laser imaging system (Li-Cor, USA).

2.9. Quantitative real-time PCR

Total RNA was extracted by TRIzol (Invitrogen, USA) and 2 μg of that was reverse-transcribed by a RT regent kit (Takara, Japan) with oligo(dT) primer. Then the cDNA was used as the template in real-time PCR reaction by ABI Prism 7900 HT Sequence Detection

System. Primer sequences were listed in Table 2. The relative mRNA levels were normalized to those of GAPDH mRNA, and the fold change of each mRNA level was calculated using the Comparative CT ($2^{-\Delta\Delta CT}$) method.

2.10. Determination of cytokines in colon mucosa

Colon tissues were homogenized by ice-cold lysis buffer containing 1% phosphatase inhibitor and 1% protease inhibitor cocktail. Then the lysates were centrifuged at 10,000g for 20 min at 4 °C. The supernatant was stored at −80 °C for further investigation. Levels of colonic TNF-α, IFN-γ IL-1β, IL-6, IL-10 and IL-17 were measured by mouse enzyme-linked immunosorbent assay (*ELISA*) kits (Elabscience, China) followed by the manufacturer's guidelines.

2.11. Immunochemistry

The paraffin-embedded tissues were cut into 5 μm sections and moved to slides. The sections were deparaffinized and rehydrated by using graded ethanol. Then the sections were boiled in EDTA buffer (pH 9) for 20 min for antigen retrieval. After blocked with peroxidase blocking solutions and goat serum for 30 min respectively, the sections were incubated antibody against NF-κB p65 (1:100) at 4 °C over night. The antibody binding was detected by using an Envision Detection Kit, Peroxidase/DAB, Rabbit/Mouse (Gene Tech, China). Hematoxylin was used to counterstain the nucleus. The sections were observed under a light microscope (Leica, Germany) at a magnification of ×400.

2.12. Statistical analysis

The statistical analysis was carried out by SPSS19.0 software and data were performed by a two-tailed unpaired Student's t-test or one way ANOVA. Results were expressed as means ± standard deviation (SD). $P < 0.05$ was considered statistically significant.

3. Results

3.1. Ghrelin attenuated disease activity in DSS-induced colitis

All animals survived the experiment. Protein expression level of GHSR-1a was detected in mouse colons (Fig. 1A). The DAI of mice subjected to DSS administration increased from day 3 and reached a peak on day 10 when compared to the NC group (Fig. 1B). Mice in the DSS group experienced weight loss, diarrhea, and bloody stools. The ghrelin-treated group exhibited different therapeutic effects. There was no marked difference in clinical parameters between the DSS and low-dose ghrelin-treated (25 μg/kg) groups throughout the experiment ($P > 0.05$). However, the DAI decreased from day 3 when mice were treated with moderate (125 μg/kg) or high-dose (250 μg/kg) ghrelin ($P < 0.01$). The GHSR-1a antagonist [D-lys^3]-GHRP-6 could reverse the therapeutic effects of high-dose ghrelin ($P < 0.01$).

Table 2
Real-time PCR primer sequences.

Gene		Primer sequences (5′ → 3′)
Claudin-1	Forward	GGCTGTCATTGGGGGTGCGATATTT
	Reverse	CCAAATTCGTACCTGGCATTGACTG
Occludin	Forward	ACAAGAGGAATTTTGACACTGGCCT
	Reverse	CAGCAGCAGCCATGTACTCTTCACT
ZO-1	Forward	CTCCAAAAACTCTTGTGAAATCGCA
	Reverse	CATCTTCATCCTCTTCCACAGCTGA
GAPDH	Forward	ACATCATCCCTGCATCCACT
	Reverse	GTCCTCAGTGTAGCCCAAG

Colon lengths were measured after mice were sacrificed (Fig. 1C), and were shorter in the DSS group than those in the NC group ($P < 0.01$). The colons of all three ghrelin-treated groups did not become shorter than those of the DSS group ($P < 0.01$). The administration of [D-lys^3]-GHRP-6 neutralized this effect ($P < 0.01$ *vs.* 250 μg/kg ghrelin group). Histological findings were also evaluated at the end of experiment (Fig. 1D and E), and the DSS group showed severe mucosal ulceration, leukocyte infiltration, and massive depletion of crypts, goblet cells, and epithelial cells. The histological score of DSS group were markedly higher than those of the NC group ($P < 0.01$). The DSS-induced mucosal lesions were partially attenuated by moderate or high-dose ghrelin as assessed by microscopy, and the histological scores in both groups were dramatically reduced ($P < 0.01$). Administration of [D-lys^3]-GHRP-6 showed significant counteractive activities when compared to the high-dose ghrelin group ($P < 0.01$).

Neutrophil infiltration in colons was quantified by measuring MPO activity (Fig. 1F). MPO activity in the DSS group was increased by approximately sixfold compared with the NC group, and administration of ghrelin (125 or 250 μg/kg) could reduce MPO activity and neutrophil infiltration ($P < 0.01$). Furthermore, high-dose ghrelin exerted a more significant role in reducing MPO activity in contrast to the moderate-dose group ($P < 0.01$). In the [D-lys^3]-GHRP-6 group, levels of MPO were increased markedly compared with the high-dose ghrelin-treated group ($P < 0.01$).

3.2. Ghrelin supported the integrity of intestinal barrier function in DSS-induced colitis

Intestinal barrier function was assessed by investigating TER (Fig. 2A). The reduced TER indicated increased intestinal permeability. In the DSS group, TER was reduced significantly in contrast to the NC group ($P < 0.01$). The injection of 125 or 250 μg/kg ghrelin could increase TER ($P < 0.01$), and the high-dose group showed a more significant effect ($P < 0.01$ *vs.* 125 μg/kg ghrelin group). Neutralization with [D-lys^3]-GHRP-6 could significantly reduce TER ($P < 0.01$ *vs.* 250 μg/kg ghrelin group).

Ultra-structural changes in colon tissues were investigated using TEM (Fig. 2B). Intestinal transepithelial permeability is normally maintained by epithelial cells and TJs. The DSS group exhibited some abnormal changes, such as a significant increase in intercellular space, the partial depletion of TJs and microvilli, and the vacuolization within cells. Administration of ghrelin partially ameliorated these changes, mitigating the disruption of TJs and reducing injury to the epithelium. In the [D-lys^3]-GHRP-6 group, the ultra-structure of the colon was not ameliorated.

We next examined the protein and mRNA expression levels of claudin-1, occludin, and ZO-1 in the colon (Fig. 2C and D). Western blotting and quantitative real-time PCR results showed that the total protein or mRNA expression levels of the three TJ components were markedly decreased in the DSS group compared to the NC group. However, injection with ghrelin (125 or 250 μg/kg) could inhibit the reduction of TJ components at both the protein and gene expression levels ($P < 0.05$ or $P < 0.01$). Interestingly, low-dose ghrelin could increase the mRNA expression levels of occludin and ZO-1 ($P < 0.01$), whereas a limited effect on claudin-1 mRNA expression was observed ($P > 0.05$). Treatment with [D-lys^3]-GHRP-6 caused an opposite effect on the expression of TJs ($P < 0.01$ *vs.* 250 μg/kg ghrelin group).

3.3. Ghrelin regulated the levels of cytokines in DSS-induced colitis

The levels of cytokines in the colon were measured by *ELISA* (Fig. 3). In the DSS group, levels of TNF-α, IFN-γ IL-1β, IL-6, and IL-17 were higher than those in the NC group ($P < 0.01$). Low-dose ghrelin

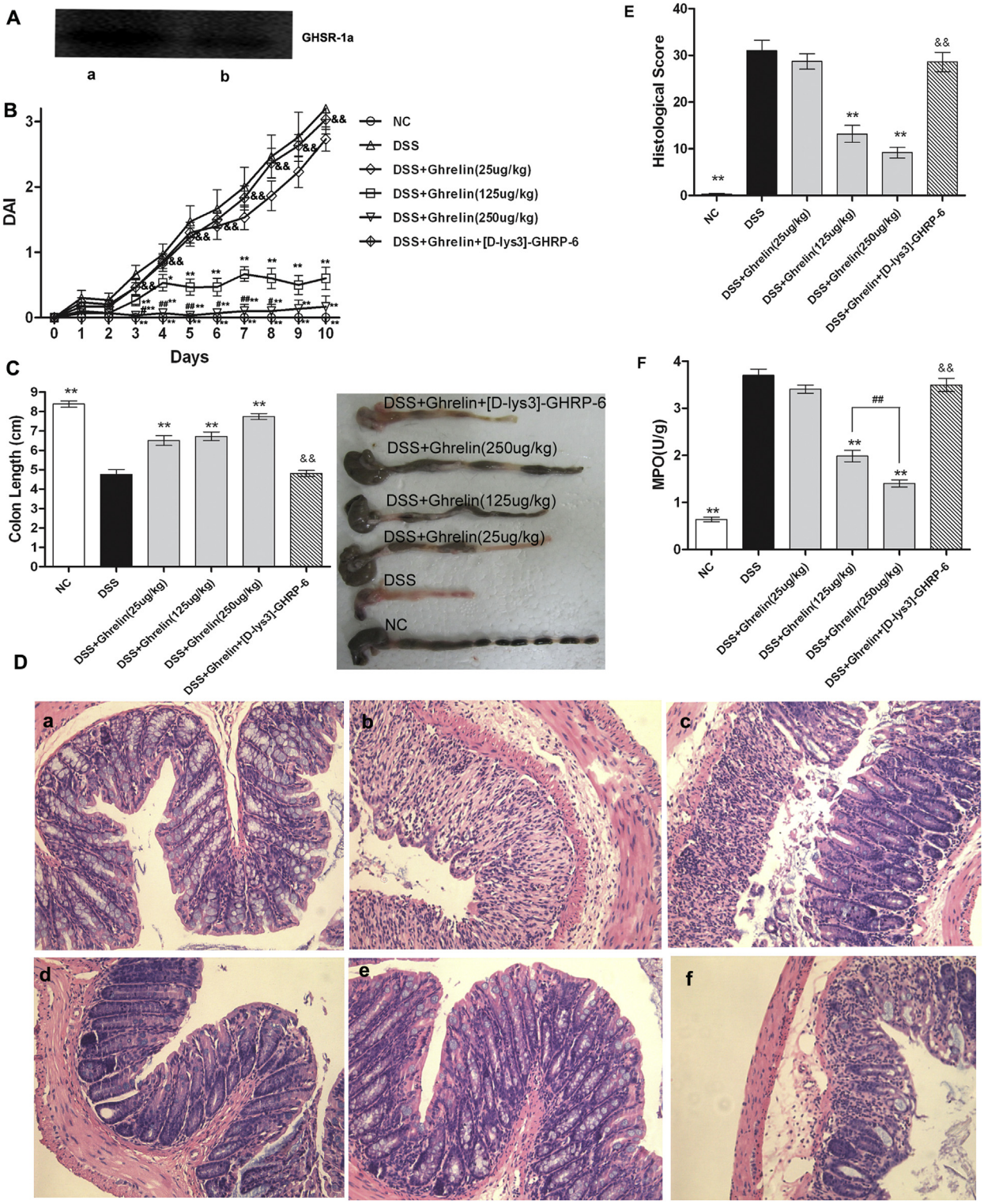

Fig. 1. Effects of ghrelin on clinical parameters in DSS-induced colitis. Levels of GHSR-1a expression in mouse colons were detected by immunoblotting (A; a. hypothalamus, b. colon). Colitis was induced by DSS (n = 10). Saline or ghrelin (25, 125, 250 μg/kg) were administered intraperitoneally 1 day before and on days 4, 5, and 6 after DSS administration. [D-lys^3]-GHRP-6 was administered immediately before the injection of ghrelin. Disease activity index (B) was assessed daily. At the end of the study, colon length (C), the histological score (E), and myeloperoxidase activity (F) were evaluated. Colon H&E staining was also shown (D; 200×, a. NC, b. DSS c–e. DSS + ghrelin 25 (c), 125 (d), 250 (e) μg/kg, f. DSS + ghrelin + [D-lys^3]-GHRP-6); $^{*}P < 0.05$, $^{**}P < 0.01$ vs. DSS; && $P < 0.01$ vs. ghrelin (250 μg/kg); $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. ghrelin (125 μg/kg).

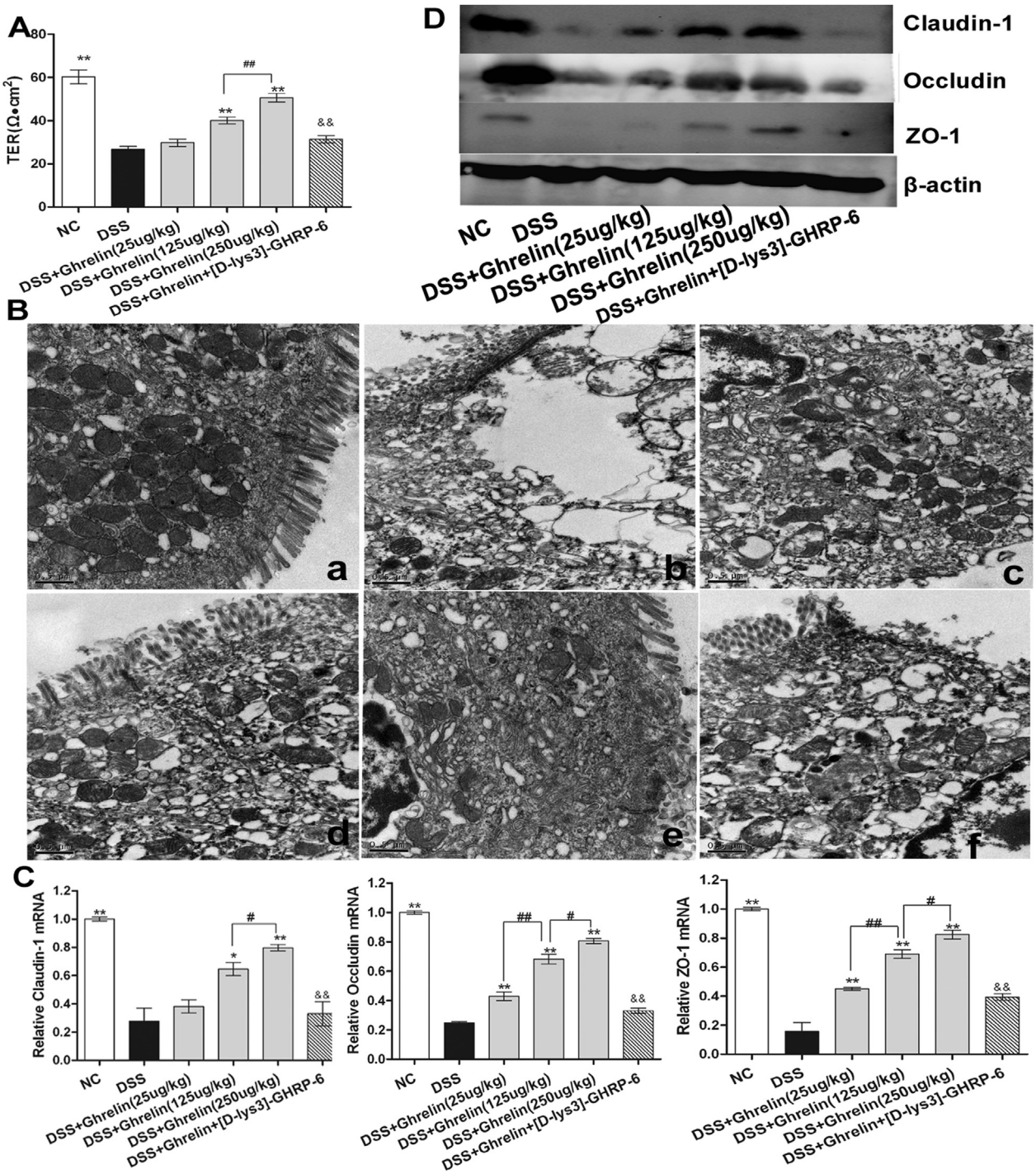

Fig. 2. Effects of ghrelin on intestinal barrier function in DSS-induced colitis. Transepithelial electrical resistance was measured with an Ussing chamber (A). The ultrastructure of the colon was examined by transmission electron microscopy (B; 20,000×, a. NC, b. DSS c–e. DSS + ghrelin 25 (c), 125 (d), 250 (e) μg/kg, f. DSS + ghrelin + [D-lys^3]-GHRP-6). Levels of claudin-1, occludin, and ZO-1 expression were investigated using quantitative real-time PCR (C) and immunoblotting (D); $^{*}P < 0.05$, $^{**}P < 0.01$ *vs.* DSS; && $P < 0.01$ *vs.* ghrelin (250 μg/kg); $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ *vs.* ghrelin (125 μg/kg).

did not show an effect in regulating the secretion of those pro-inflammatory cytokines ($P > 0.05$), whereas moderate or high-dose ghrelin administration exerted anti-inflammatory effects and markedly reduced the levels of these cytokines ($P < 0.01$). Furthermore, the tissue levels of IL-10 showed no significant difference in either the NC or DSS groups ($P > 0.05$). Administration of ghrelin (125 or 250 μg/kg) increased IL-10 secretion ($P < 0.01$). Blockade of GHSR-1a could weaken the effects of high-dose ghrelin ($P < 0.01$).

3.4. Ghrelin inhibited the activation of NF-κB in DSS-induced colitis

To determine the effect of ghrelin on NF-κB activation, we investigated NF-κB nuclear translocation by immunochemistry (Fig. 4A). We also measured the protein expression levels of cytoplasmic inhibitory κB (IκB)-α and nuclear NF-κB p65 (Fig. 4B). We detected very limited immunoreactivity for NF-κB p65 in the nucleus of healthy animal colons. However, strongly positive

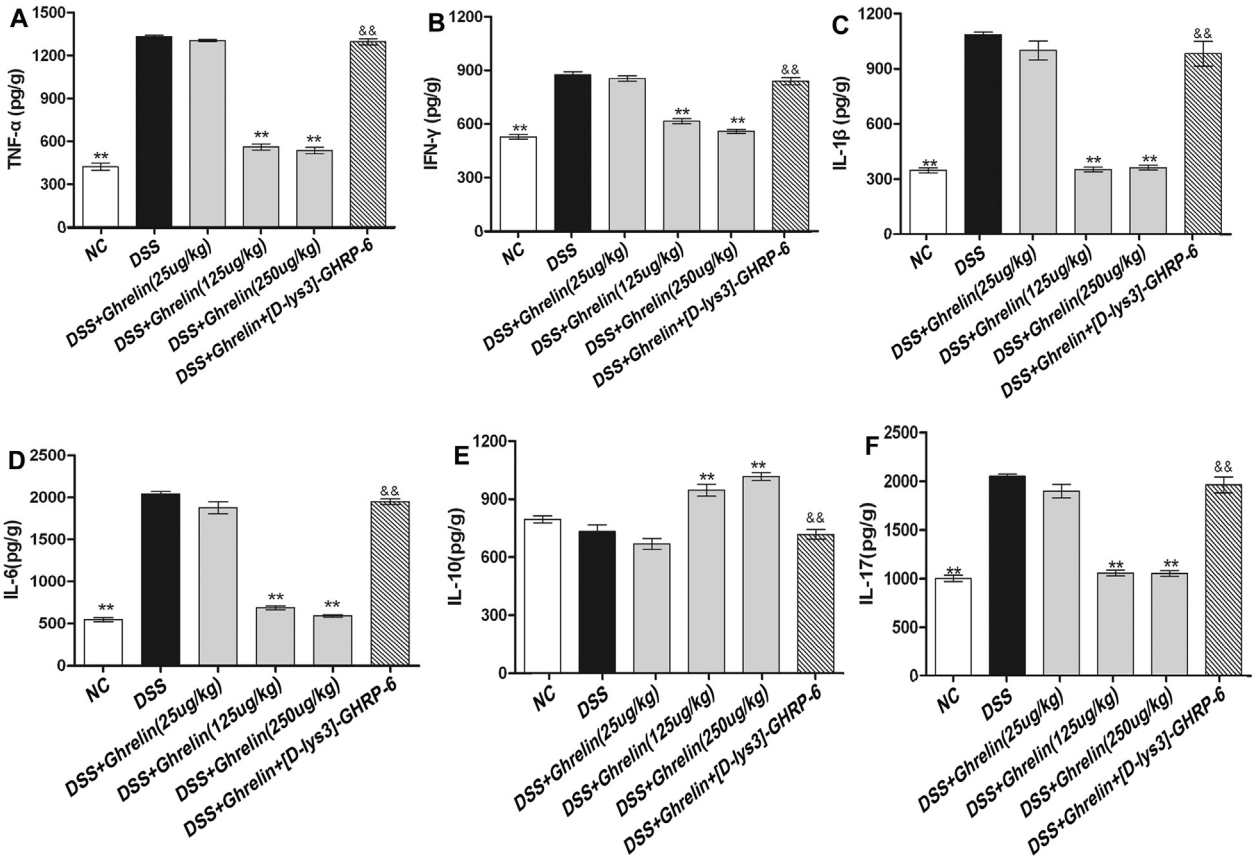

Fig. 3. Effects of ghrelin on cytokine expression levels. Levels of TNF-α (A), IFN-γ (B), IL-1β (C), IL-6 (D), IL-10 (E), and IL-17 (F) protein were measured by *ELISA*; $^{**}P < 0.01$ *vs*. DSS; && $P < 0.01$ *vs*. ghrelin (250 μg/kg).

expressions of NF-κB p65 in the nucleus of the DSS-administered mice were detected. Treatment with ghrelin (125 or 250 μg/kg) markedly reduced the nuclear translocation of NF-κB p65, whereas a GHSR-1a antagonist could reverse these effects. Based on the immunoblotting results, ghrelin(125 or 250 μg/kg) enhanced cytoplasmic IκB-α expression, and reduced the expression of NF-κB p65 in the nucleus compared with the DSS group. An opposite result was observed in the [D-lys^3]-GHRP-6 group.

Myosin light chain kinase (MLCK) and phosphorylated myosin light chain 2 (pMLC) play crucial roles in the dysregulation of TJs and further affect the permeability of the intestinal epithelium. Therefore, we investigated the expression of MLCK and pMLC at the protein level (Fig. 4C). Ghrelin reduced the expression of MLCK and pMLC compared with the DSS group. The high expression levels of these proteins were not altered in the [D-lys^3]-GHRP-6 group.

4. Discussion

The acute colitis induced by DSS features disruption of enterocytes, mucosal inflammation with macrophages and neutrophils infiltration, and massive production of pro-inflammatory cytokines. Herein, we first evaluated the general effects of different concentrations of ghrelin on the clinical symptoms of experimental colitis. We found that moderate and high-dose ghrelin showed obviously beneficial effects on colitis, whereas low-dose ghrelin could scarcely prevent the progression of disease. An effective dose of ghrelin not only prevented weight loss, diarrhea, and bloody stools at a macroscopic level, but also reduced the histological score at a microscopic level. These findings suggested a potentially protective effect on acute colitis.

Next, we investigated whether ghrelin could prevent epithelial barrier dysfunction in the colon. Our tests revealed that reduced TER and disrupted TJs occurred in DSS-induced colitis. An effective dose of ghrelin could significantly reduce epithelial permeability and prevent the damage and loss of TJs. As we know, intercellular TJs are critically important components of the epithelial structures that form the intestinal barrier. This barrier normally prevents the penetration of toxic substances (bacteria and antigens) into the intestinal lumen [1]. In IBD patients, it has been reported that barrier dysfunction usually occurs and is considered to be a primary etiological factor of IBD [13]. To date, the precise mechanism of altered epithelial permeability remains unclear. Some inflammatory cytokines (such as TNF-α and IFN-γ) that are massively secreted in IBD, might be major causes of the disrupted TJs. Accumulating evidence suggests that TJs are partially regulated by inflammatory cytokines (mainly TNF-α) through NF-κB p65-mediated activation of the MLCK-pMLC pathway [14].

NF-κB is a pleiotropic transcription factor that plays an important role in intestinal immunity. It controls the transcriptional activities of some promoters for pro-inflammatory cytokines and transcription factors that are involved in intestinal inflammation. Inactive NF-κB is sequestered by IκB in the cytoplasm. Translocation of NF-κB dimers from the cytoplasm to the nucleus occurs after IκB degradation. Activated NF-κB can regulate the expression of target genes. Because a κB binding site is located in the MLCK promoter region, the nuclear translocation of NF-κB p65 can lead to the activation of MLCK, and subsequently lead to the enzyme-mediated phosphorylation of MLC. Then, pMLC activates the Mg^{2+}-myosin ATPase, which provides the energy needed for the contraction of TJs, eventually leading to increased intestinal epithelial permeability [15]. Our

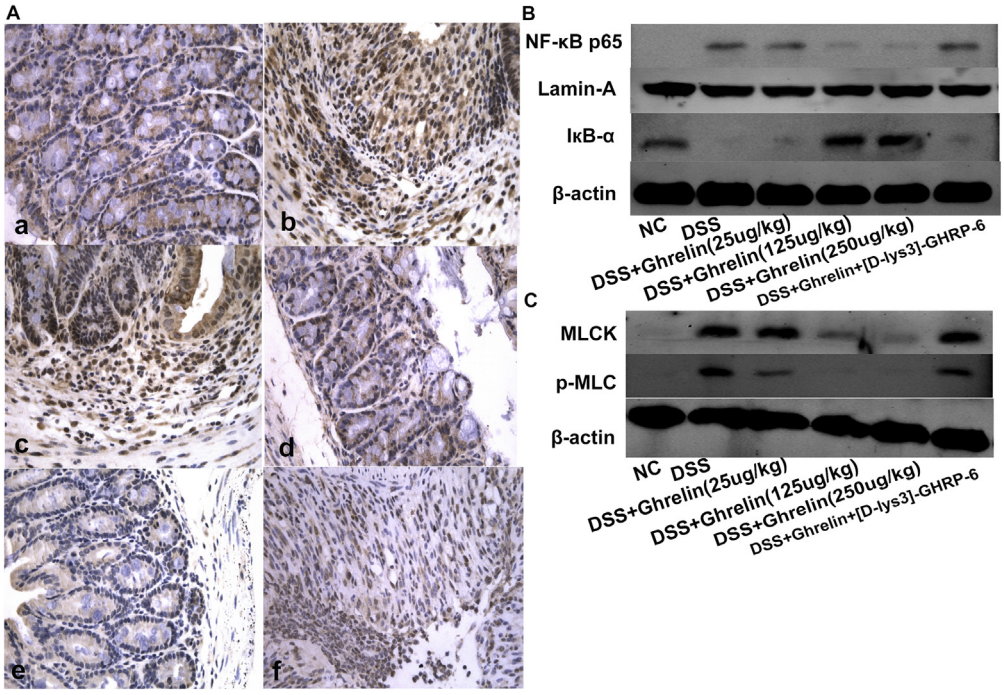

Fig. 4. Ghrelin inhibited the activation of NF-κB and MLCK. The translocation of NF-κB p65 was examined by immunochemistry (A; 400×, a. NC, b. DSS, c–e. DSS + ghrelin 25 (c), 125 (d), and 250 (e) g/kg, f. DSS + ghrelin + [D-lys3]-GHRP-6). The protein expression levels of nuclear NF-κB p65, cytoplasmic IκB-α (B), and total MLCK and pMLC (C) were assessed by immunoblotting.

findings demonstrated that ghrelin could supress IκB-α degradation and decrease the nuclear expression of NF-κB p65, and subsequently inhibit the activation of MLCK and pMLC. These activities ultimately prevent the loss of TJs at both protein and gene levels.

NF-κB activation also plays an essential role in regulating the secretion of pro-inflammatory cytokines. An imbalance between anti- and pro-inflammatory cytokines usually leads to inflammation [16]. In IBD, levels of pro-inflammatory cytokines, such as TNF-α, IFN-γ, IL-1β, IL-6, and IL-17, are increased. These cytokines play roles in the inflammatory response via several mechanisms, such as causing local ischemia, increasing membrane permeability, and impairing function of the endothelium. Recent studies have indicated that the interaction between pro-inflammatory cytokines and the intestinal mucosal immune system can lead to the disruption of TJs both in vivo and in vitro [17–19]. IL-10 is usually considered to be an anti-inflammatory cytokine, and treatment with IL-10 in experimental colitis can reduce damage to the intestinal mucosa [20]. In our present study, we found that ghrelin could down-regulate secretion of pro-inflammatory cytokines and increase levels of IL-10. These findings indicated the anti-inflammatory activities of ghrelin in experimental colitis. Moreover, we can speculate that the protective effects of ghrelin on the intestinal barrier can be partially attributed to its anti-inflammatory activities.

There are two types of ghrelin receptors, GHSR-1a and GHSR-1b. To the best of our knowledge, GHSR-1a mediates the endocrine actions of ghrelin, whereas the function of GHSR-1b is not yet known. In the colon, GHSR-1a is mainly expressed in mast cells, lymphocytes, and endocrine cells [21]. We can speculate that the effects of ghrelin on the intestinal tract might be mediated by GHSR-1a. To test this hypothesis, we used a selective GHSR antagonist [D-lys^3]-GHRP-6 to abolish the actions of ghrelin. We adopted a 37.5-fold dose of ghrelin administered which was shown to effectively blockage the GHSR-1a [22]. In our study, [D-lys^3]-GHRP-6 disabled the effects of ghrelin, indicating that ghrelin exerted a protective effect on barrier dysfunction through the GHSR-1a pathway.

In conclusion, we showed that ghrelin could prevent the breakdown of intestinal barrier function in a DSS-induced colitis model. The effects of ghrelin were mediated by GHSR-1a. Additionally, inhibition of NF-κB activation might be part of the mechanism that underlies the protective effects of ghrelin against barrier dysfunction.

Conflict of interest

The authors declared no conflict of interest.

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Transparency document

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