Molecular Mechanisms of Transforming Growth Factor-β/Smad7 Signaling Pathway in Ulcerative Colitis

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Abstract

Background and Aims

Abnormal transforming growth factor-β (TGF-β)/Smad7 signaling pathway may be an important mechanism of IBD. Therefore, this study was to investigate whether anti-colitis drugs modulate intestinal epithelial permeability in experimental colitis and to determine its TGF-β/Smad7 signaling pathway.

Methods

A murine colitis model was induced, and then anti-TNF-α and 5-ASA were administered intraperitoneally and orally respectively. Myeloperoxidase (MPO) activity, histological index (HI) of colon and the disease activity index (DAI) scores of mice were detected. Transmission electron microscopy (TEM), immunohistochemical and functional tests which included two methods: one was Evans blue (EB) and the other was FITC-dextran (FD-4), were used to evaluate intestinal mucosal permeability. The expression of epithelial E-cad, Occludin, ZO-1, TGF –β and Smad7 were analyzed. Epithelial MLCK expression and activity were determined.

Results

Anti-TNF-α and 5-ASA both effectively reduced the DAI score and HI, and decreased colonic MPO activity, plasma levels of FD-4 and EB permeation of the intestine. Moreover, anti-TNF-α and 5-ASA downregulated the MLCK expression and activity and the expression of Smad7 in the small intestinal epithelium, and increased the expression of TGF-β (P < 0.050). In colitis mice, TEM revealed partial ileal epithelial injury, intercellular TJ and the expression of E-cadherin, ZO-1 and occludin were decreased, which were alleviated by anti-TNF-α and 5-ASA.

Conclusions

Anti-TNF-α and 5-ASA both showed a significant effect on intestinal epithelial permeability in experimental colitis. The mechanism can be clarified as the increase of TGF-β expression or the decrease of Smad7 expression which could inhibit epithelial MLCK and then reduce the mucosal permeability of ulcerative colitis.

Introduction

The intestinal mucosal barrier dysfunction, characterized by increased intestinal mucosal permeability, has an important effect on the initiation of inflammation in infection and immunity-induced ulcerative colitis (UC)\(^1\-^3\). Intestinal epithelial cells (IECs) form the barrier, which mainly consists of two parts, one is the epithelial tight junction (TJ) and the other is the apical enterocyte membrane. While TJs are located at the top of IECs, and their constituent proteins include occludin, claudin, ZO, JAM-1, etc. Adhesion junction (AJ) is a cell structure adjacent to TJ, and its constituent proteins include E-cadherin, catenin, etc. In transgenic animal model, the absence of E-cadherin can cause the dysfunction of AJs, which is similar to
the pathophysiological process of inflammatory bowel disease (IBD)\textsuperscript{[4]}. Moreover, the enhancement of pro-inflammatory cytokines and the degradation of TJ protein lead to increased permeability of intestinal mucosa in IBD\textsuperscript{[5]}. And the contraction of cytoskeleton involved in actin in IECs could destroy TJs between cells, open the cell space and increase the permeability of intestinal mucosa, while this process requires myosin light chain kinase (MLCK) to phosphorylate myosin light chain (MLC)\textsuperscript{[6-8]}. The abnormality of transforming growth factor-β (TGF-β)/Smad7 signaling pathway may be an important pathogenesis for IBD. Especially the high expression of Smad7 and the imbalance between Smad7 and Smad2, Smad3 can lead to the loss of anti-inflammatory effect of TGF-β, resulting in the sustainability of chronic inflammation in the intestinal tract of UC\textsuperscript{[9-12]}. It has been shown that TGF-β/Smad7 signaling pathway affects the expression of MLCK in vascular smooth muscle cells\textsuperscript{[13,14]}. Furthermore, TGF-β has been shown to affect the expression of MMP-9 in squamous cell carcinoma cell line of human head and neck via Smad/MLCK pathway\textsuperscript{[15]}. And during inflammation of UC patients, MMP-9 could regulate MLCK expression\textsuperscript{[16,17]}. Meanwhile, Su LP et al have found that the increase of intestinal permeability caused by TNF-α is due to activation of MLCK via ERK1/2 signalin enhancment of MMPs and apoptosis\textsuperscript{[18,19]}. Inhibitors targeting MMP has verified desirable anti-inflammatory effect in mice colitis models, but less powerful on humans\textsuperscript{[20]}. Recent studies suggest that mucosal healing is a new goal of UC\textsuperscript{[21]}. Anti-TNF-α has a definite property on promoting mucosal healing, but the specific mechanism is unclear\textsuperscript{[22]}. The effect of 5-ASA on intestinal mucosal healing is still controversial, and there are great differences among individuals. While Salazosulfapyridine and Balsalazide can improve intestinal mucosal permeability\textsuperscript{[23]}. here is no report on whether the efficacious treatment of anti-TNF-α in refractory UC and the effect of promoting mucosal healing are related to the significant improvement in intestinal mucosal permeability and TGF-β/Smad7 signaling pathway. Therefore, anti-TNF-α and 5-ASA were chosen to experiment with dextran sulfate sodium (DSS)-induced colitis to observe their influence on intestinal permeability and further explore possible mechanisms.

### Materials And Methods

#### Animals and Reagents

SLAC Laboratory Animal Co.Ltd, Shanghai, China Offers 8-week-old, weighting between 18g and 22g, Specific pathogen-free (SPF) grade C57BL/6J mice. Under 20±2°C temperature, 50% humidity and light/dark cycles of 12 h, mice were fed with tap water and standard pellet diet. Sigma-Aldrich Co Provided FITC-dextran 4000 (FD-4) and DSS, while the molecular weight of the latter is 8000. And the Nanjing Jiancheng Biotechnology Institute (Nanjing, China) supplied kits for the detection of Evans blue (EB) and Myeloperoxidase (MPO). MLCK ELISA kit was purchased from RB (USA). All the antibodies below, including anti-ZO-1, anti-E-Cadherin, anti-Occludin, anti-MLCK and anti-Smad7 were bought from Abcam (Cambridge, UK). Anti-TGF-β antibody was obtained from Gene Tex (USA). The following instruments were applied in this experiment, including light microscope (Olympus; Japan), Ultraviolet spectrophotometer (752 N; Shanghai, China), transmission electron microscope (TEM; Hitachi; Japan), enzyme-labeling instrument (ELx800; USA), and RT-PCR instrument (LightCycler480; Roche; Switzerland).
**Induction of DSS-Colitis model**

A murine colitis model induced by 5%(w/v) DSS for 7 days of free drinking\[^{24}\].

**Experimental protocols**

Mice were equally-randomly classified into the normal group, the DSS-treated group, the 5-ASA-treated group and anti-TNF-\(\alpha\)-treated group. The latter two groups were set as treated groups. All groups were treated accordingly for 7 days.

**Assessment of Disease Activity Index (DAI)**

Two observers recorded the following daily: body weight, fecal blood and consistency. The average daily DAI score per mouse was calculated according to the standard method\[^{24}\].

**Assessment of Inflammation**

After laparotomy, the first step was to examine the gross mucosal morphology of mouse colon and then two continuous pieces of distal colon were collected. For histological analysis, this study used 10% neutral buffered formalin to immobilize one of the colon to maintain the original morphological structure of the cell, followed by paraffin embedding for sections (4 \(\mu\)m), and finally HE staining. The severity of inflammation was assessed by histological index (HI) \[^{24}\]. The other was homogenized for assessing MPO activity\[^{25}\].

**Assessment of TEM**

A 0.5-cm distal ileal segment within 1cm of the ileocecal junction was fixed in 2.5% glutaraldehyde at 4°C for 6h, and in osmic acid, and then embedded in Epon as the specimen for TEM.

**Assessment of E-cadherin, ZO-1 and occludin Protein Expression**

1cm ileum was fixed in formalin at the above concentrations, and preserved in liquid nitrogen after electron microscopic examination. The expression of E-cadherin, occludin and ZO-1 in ileum epithelial cells was detected by immuno-histochemistry.

**Intestinal Permeability Assay**

According to the previous method, a 6-cm segment of small intestine was used as sac by ligating both ends, and then 1.5 % (w/v) EB in PBS of 0.2 ml was injected into the sac. What’s more, the sac was incubated in 20mL Krebs buffer and removed after 30 min. Then the intestinal lumen was rinsed with physiological saline until the rinse solution was clarified, dried at 37°C for 24h, weighed on the dry weight of the intestinal tissue, and incubated with formamide. The estimated wavelength of dye eluting amount is 655nm. And the permeability of intestine was assessed by EB and the amount of EB was calculated according to the standard curve. FITC was detected in vivo. Ligated the ileum of 6cm after anaesthesia.
and injected into the cavity with 0.2ml FITC solution. The portal vein blood was extracted after 30min to
determine the concentration of FITC in plasma.

Assessment of MLCK Enzymatic Activity

The intestinal mucosa homogenate was prepared at 4 °C by taking out liquid nitrogen frozen intestinal
mucosa and adding a proper amount of extract buffer. MLCK enzymatic activity of small intestine was
detected according to the ELISA kit.

Detection of MLCK via immunohistochemistry

Intestinal mucosa specimens were collected, fixed with 10% formaldehyde solution and embedded in
paraffin. The expression of MLCK protein in ileum epithelial cells was detected by SP method of
immunohistochemistry. The experimental steps were carried out according to the kit instructions. The
control group was treated with phosphate buffered saline (PBS) instead of the primary antibody. Three
visual fields were randomly selected from each slice under light microscope (40 ×), and the distribution of
positive particles in the cells was observed under high power microscope (200 ×). According to the blind
score of two pathologists, brown granules were found to be positive in the cells. The evaluation standard
of cell staining were as follows: ≥75% was +++; 50%<75% was ++, 10%<50% was +, and <10% was
negative. All the experiments were repeated 3 times.

Detection of MLCK, TGF-β and Smad7 via Western blot

The intestinal mucosae specimens were cut into pieces in ice bath and added protein extraction buffer to
prepare homogenate, frozen at-80 °C and thawed for three times to fully release MLCK,TGF-β, Smad7.
Then the homogenate was transferred into Ep tube, and 14000 r/min centrifugation for 15 min to extract
the supernatant. Protein concentration was determined. Specimens were prepared by the following
process: the extract quantified and adjusted for protein concentration was mixed with the sample buffer
for 2 × protein electrophoresis and boiled for 5 min. Polyacrylamide gel electrophoresis (SDS-PAGE)
included 10% separated gel and 5% concentrated gel. And the sample size of each lane was equal.
Protein transfer: electrophoretic bands was transferred to PVDF membranes by electric transfer. Blocking
and antibody binding: blocking non-specific antigen of PVDF membrane, in turn adding primary antibody,
incubating, washing, adding secondary antibody, incubating, exposure after washing.

Assessment of the mRNA contents of TGF-β and Smad7

The contents of TGF-β and Smad7 mRNA in IECs were detected by RT-PCR. RNA was extracted from
samples by Trizol method to establish RT-PCR reaction system and conditions. The samples were well
mixed with Trizol, placed in static condition, and then centrifuged at 12000 r/min for 15 min at 4 °C to
take supernatant. The supernatant was mixed with equal isopropanol well, centrifuged as above to take
the precipitate. And then the precipitate was washed with 75% alcohol, dried, dissolved and frozen at-80
°C. The concentration, purity, quantity and quality of RNA were determined. Reverse transcription cDNA
was prepared by PCR cycle. And PCR reaction system consisted of cDNA 4.00ul, 10 × PCR buffer 1.96ul,
MgCl$_2$ (25Mm) 2.40ul, upstream primer (20pM) 1.00ul, downstream primer (20pM) 1.00ul, dNTP (10mM) 0.36ul, Taq DNA polymerase (5IU/ul) 0.10ul and the deionized water 10.08ul.

**Ethical Considerations**

The local ethics committee approved these experiments and conducted in accordance with laboratory animal management and use guidelines.

**Statistical Analysis**

Using SPSS 20.0 for data statistics, one-way analysis of variance (ANOVA) was used to test overall statistical differences. All results were presented in the form of mean ± standard deviation (SD). P<0.050 was considered statistically significant.

**Results**

**General situation of mice**

In DSS group, the mice showed reduced activity, mental deterioration and weight loss. At the end of the experiment, there were different degrees of blood in feces, and the appearance of feces was soft or thin-shaped. The DAI scores increased gradually with time (Fig. 1). Compared with DSS group, the activity and mental state of mice in treated groups were better while the weight loss was lower and a few mice had slight bloody fecal or OB(+). DAI scores of the treated groups were between DSS group and normal group (Fig. 2).

**Gross observation and pathological examination of colonic tissue**

Colon mucosa was characterized by extensive hyperemia and edema in DSS group. Meanwhile, multiple erosion, bleeding spots and superficial ulcer were observed. But no obvious abnormality was found in normal group. Comfortingly, there were only scattered hyperemia and erosion without obvious bleeding and ulcer in treated groups.

HE pathological examination showed that the colonic mucosa had multiple superficial ulcers, a large number of crypt glands were destroyed and a slew of inflammatory cells infiltrated in DSS group (Fig. 3). While in the normal group, the colonic IECs were intact, the crypt glands were neatly arranged and no inflammatory cell infiltrated (Fig. 4). In treated groups, a few colon mucosa of mice were scattered with superficial ulcer, and the structure of crypt gland decreased and destroyed compared with normal group, but it was significantly less than that in DSS group. At the same time, the degree of infiltration of inflammatory cells in mucosa and submucosa was mild (Fig. 5). Moreover, compared with DSS group, the HI scores of the treated groups were significantly lower (P < 0.050, Fig. 6).

**MPO in colon**
The activity of MPO in colonic homogenate of DSS group was higher, while MPO activity of the treated group decreased than that in DSS group, which suggests that the colon inflammation in DSS group was serious \((P < 0.050, \text{Fig. 7})\) and anti-TNF-\(\alpha\) and 5-ASA could reduce inflammatory injury of colon \((P<0.050, \text{Fig. 8})\).

**Ultrastructure of intestinal mucosal barrier**

TEM was used to observe the ultrastructure of the IECs in the ileum of mice. From the result it can be observed that in the normal group the IECs were intact, the surface microvilli were long and dense, the arrangement was regular, and the cells were closely connected. While the DSS group showed edema or even shedding of IECs, atrophy and sparseness of surface microvilli, enlargement of intercellular space and opening of some TJs. Then the treated groups were mainly characterized by edema of some IECs, reduction of microvilli and opening of TJs, which is much better than DSS group (Fig. 9).

**Intestinal mucosal barrier function**

Compared with these in the normal group, the intestinal EB content and blood FITC level in DSS group increased \((P < 0.050)\), suggesting that the intestinal mucosal barrier in DSS group was damaged and the high molecular weight EB entered the intestinal mucosa through expanded TJs, while FITC was absorbed into the portal vein system. Anti-TNF-\(\alpha\) and 5-ASA could decrease the level of intestinal EB and blood FITC in different degrees \((P < 0.050, \text{Fig. 10, 11})\).

**The protein expression of TJ and AJ**

The expression of Occludin, ZO-1 and E-cadherin in small intestinal mucosal epithelial cells of mice in treated groups was decreased to some extent, but was higher than that of DSS group (Fig. 12 - 14), suggesting that anti-TNF-\(\alpha\) and 5-ASA could improve the destroyed structures of TJ, AJ and other epithelial cell barriers.

**Expression, distribution and activity of MLCK protein**

The expression and activity of MLCK protein in small intestinal epithelial cells were higher in DSS group than those in normal group, but lower in treated group than in DSS group \((P < 0.050, \text{Fig. 15})\). The results of IHC were consistent with those of the Western blot \((P < 0.050)\), suggesting that anti-TNF-\(\alpha\) and 5-ASA intervention can downregulate the MLCK expression and activity in IECs, and thus facilitate the improvement of intestinal mucosal permeability in colitis mice. However, no significant difference was detected between anti-TNF-\(\alpha\) and 5-ASA \((P > 0.050, \text{Fig. 15})\).

**mRNA and protein levels of TGF-\(\beta\) and Smad7**

Compared with the normal group, the mRNA and protein levels of TGF-\(\beta\) and Smad7 were decreased and increased respectively in DSS group. The intervention of anti-TNF-\(\alpha\) and 5-ASA improved the situation above (Fig. 16, 17). These results suggest that anti-TNF-\(\alpha\) and 5-ASA can enhance the expression of TGF-
β and attenuate the expression of Smad7 in IECs of colitis mice, and may regulate the expression of TJ-associated protein through TGF-β/Smad7 signaling pathway, which ultimately regulate the intestinal mucosal permeability in colitis mice.

**Discussion**

UC is a recurrent non-specific inflammatory disease and intestinal mucosal barrier damage may be an important reason for the recurrence of UC\(^{26,27}\). In SAMP1/Yit and IL-10 (-/-) mouse models of colitis, intestinal mucosal permeability increased significantly before intestinal inflammatory changes\(^{28,29}\). Repair of intestinal mucosal barrier, which can block an important part of the pathogenesis of IBD, is beneficial to control or reduce intestinal mucosal inflammation and immune response, control the condition, improve curative effect, promote mucosal healing, and maintain remission, and even play the role of etiological treatment in some cases\(^{30-33}\). Anti-TNF-α could observably decrease neutrophils infiltration in inflammatory mucosa of IBD patients and downregulate the activity of T cells and inflammatory mediators, while inhibiting neutrophils from producing pro-inflammatory mediators including ROS,TNF-α,IL-8 and so on\(^{34,35}\). Above all, through binding to the antibody, TNF-α receptor activation is blocked, leading to reduction of intestinal permeability basically due to the decrease in paracellular permeability across the TJs and apoptosis of IECs. Furthermore, upon blocking lipid rafts, infliximab could repair the colonic barrier of adhesive invasive Escherichia coli in Crohn's disease\(^{36,37}\). In this study, we observed whether anti-TNF-α and 5-ASA could improve intestinal permeability and regulate TGF-β/Smad7 signaling pathway in mice with DSS colitis, which provides a basis for exploring the molecular mechanism of TGF-β/Smad7 signaling pathway in regulating intestinal permeability of UC. And it turned out that anti-TNF-α and 5-ASA could reduce MPO activity, alleviate IECs injury, upregulate TJs and improve intestinal mucosal structure and function, thus reducing clinical symptoms and DAI and HI scores. However, its specific mechanism is not clear.

By blocking the adjacent intestinal epithelial spaces, TJs prevent bacteria, antigens and other substances from entering the intestinal mucosal lamina propria to activate immune cells\(^{6,38,39}\). Clayburgh DR et al found that TJs were significantly damaged, and the permeability of intestinal mucosa was increased in IBD patients\(^{6}\). TJs and AJs are destroyed due to internalization or loss of forming proteins such as occludin (TJs) or E-cadherin (AJs)\(^{40}\). And TJ proteins' recruitment to the apical lateral membrane is critical for closing the paracellular space. However, TJ assembly relies heavily on AJ formation\(^{41}\). 5-ASA could increase intercellular adhesion via membranous restoration of AJ proteins such as β-catenin and E-cadherin, which plays a direct impact on mucosal healing\(^{42,43}\). 5-ASA can also modulate transcriptional regulation of proteins, including junctional adhesion molecules (JAMs), claudins and epithelial cytoskeletal proteins\(^{44}\). TNF-α treatment led to internalization and disruption of junctional proteins like occludin (TJs), E-cadherin (AJs) and desmoglein-2 (desmosomes). With 5-ASA pretreatment, membranous localization of proteins were maintained\(^{44}\).
It has been studied that treated Caco-2 monolayers with TNF-α markedly increased the permeability of cell membrane and decreased claudin-1, occludin, and ZO-1 expression, accompanied by rearrangement of cytoskeletal F-actin\(^{[45-48]}\). Recently, it was found that some IBD-associated loci could regulate the expression of E-cadherin and the stability of AJ, hence, it was confirmed genetically that both of them played a role in intestinal barrier function\(^{[49-52]}\). In our study, we found that anti-TNF-α and 5-ASA increased the expression of the intestinal mucosal TJ protein, including E-cadherin, occludin and ZO-1, which further confirmed that TNF-α can reduce TJ proteins and recombine the cytoskeleton, thus destroying the intestinal epithelial barrier.

Actin-involved cytoskeleton contraction is closely related to MLCK according to the researches. The phosphorylated MLC by MLCK regulates the contraction of smooth and non-smooth muscle to induce cytoskeleton rearrangement, TJ disruption and opening of intercellular space, which eventually promotes the intercellular permeability\(^{[7,8]}\). The degree of phosphorylation of MLC depends on the activity of MLCK\(^{[53]}\). And MLCK could induce occludin endocytosis during A/R injury\(^{[54]}\). TNF-α increases the TJ permeability by an apoptosis-independent way\(^{[55]}\), owing to MLCK-related redistribution of TJ\(^{[60]}\) and NF-κB–related down-regulation of ZO-1. Also TNF-α could upregulate distribution and expression of NF-κB p65\(^{[61-63]}\), and NF-κB could combine with the promoter region of MLCK gene, which increases transcription of MLCK\(^{[63]}\). 5-ASA regulates intestinal epithelial homeostasis by inhibiting ERK1/2, Wnt/β-catenin, NF-κB pathways and inducing cell cycle arrest\(^{[64]}\). Also 5-ASA Pretreatment could alleviate increase of nuclear p65 by TNF-α\(^{[44]}\). Blair SA et al have found that the expression of MLCK in IECs of 26 patients with IBD was increased by immunofluorescence assay\(^{[17]}\). However, intestinal mucosal permeability was not simultaneously measured so that the correlation between MLCK and intestinal mucosal barrier function was not proved. In our study, the expression, distribution and activity of MLCK protein in IECs were also measured. Compared with normal group, the expression of MLCK in DSS group was increased and the activity was enhanced. The intervention of anti-TNF-α and 5-ASA could decrease the expression of MLCK and the activity of MLCK (P < 0.050), which could improve the permeability of intestinal mucosa in colitis mice.

Overall, the present study showed innovative in evaluating the effect of anti-TNF-α and 5-ASA via TGF-β/Smad7 signaling pathway in experimental colitis. And we found that anti-TNF-α and 5-ASA both showed a significant effect on intestinal epithelial permeability in experimental colitis. The mechanism is partly due to the increase of TGF-β expression or the decrease of Smad7 expression which could inhibit epithelial MLCK expression and activity, leading to a reduction of the intestinal mucosal permeability in UC. This study may provide new evidence for treating IBD by upregulating TGF-β expression or downregulating Smad7 expression.

**Declarations**

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Authors’ contributions: Bingqing Bai wrote and carried out most of the study. Huihui Li carried out part of the study and data analysis. Liang Han, Yongyu Mei, and Cui Hu assisted in the completion of animal experiments and some molecular biology experiments. Qiao Mei, Xiaochang Liu designed and guided all experiments. Jianming Xu assisted in guiding experimental design and data analysis.

Ethics approval: The local ethics committee approved these experiments and conducted in accordance with laboratory animal management and use guidelines.

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Figures

![Figure 1](image-url)

**Figure 1**

Score of DAI of the mice in the DSS group
Figure 2

Effect of Anti-TNF-α and 5-ASA on the score of DAI in the DSS-induced colitis mice
Figure 3

Histology of mice colon in the DSS group (H&E×200)
Figure 4

Histology of mice colon in the normal group (H&E×200)
Figure 5

Histology of mice colon in the Anti-TNF-α and 5-ASA group (HE×200)
Figure 6

Effect of Anti-TNF-α and 5-ASA on HI score in the DSS-induced colitis mice $^{*}P<0.050$, vs DSS.
Figure 7

MPO activity of the mice in the DSS group and the normal group *P < 0.050
Figure 8

Effects of Anti-TNF-α and 5-ASA on MPO activity in colonic mucosa in the DSS-induced colitis mice *P < 0.050, vs. DSS
Figure 9

Effects of Anti-TNF-α and 5-ASA on intestinal epithelial structure in the DSS-induced colitis mice (20000×, A: Control group, B: DSS group, C:Anti-TNF-α group, D: 5-ASA group)
Figure 10

Effect of Anti-TNF-α and 5-ASA on the amount of EB permeating into the intestine in DSS-induced colitis mice*P<0.050, vs. DSS
Figure 11

Effect of Anti-TNF-α and 5-ASA on the plasma FITC level in DSS-induced colitis mice. *P<0.050, vs. DSS.
Figure 12

Effects of Anti-TNF-α and 5-ASA on the expression of occludin of intestinal epithelium in the DSS-induced colitis mice
Figure 13

Effects of Anti-TNF-α and 5-ASA on the expression of ZO-1 of intestinal epithelium in the DSS-induced colitis mice
Figure 14

Effects of Anti-TNF-α and 5-ASA on the expression of E-cadherin of intestinal epithelium in the DSS-induced colitis mice
Figure 15

Effect of Anti-TNF-α and 5-ASA on intestinal epithelial MLCK enzymatic activity in DSS-induced colitis mice
Figure 16

Effect of Anti-TNF-α and 5-ASA on intestinal epithelial TGF-β and Smad7 protein expression in DSS-induced colitis mice
Figure 17

Effect of Anti-TNF-α and 5-ASA on intestinal epithelial TGF-β and Smad7 mRNA in DSS-induced colitis mice