

Dexmedetomidine protects against burn-induced intestinal barrier injury via the MLCK/p-MLC signalling pathway

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Research

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Abstract

Background Dexmedetomidine, a potent α_2 -adrenoceptor agonist with analgesic, sedative, anti-inflammatory, and anti-apoptotic effects, is commonly used in patients with critical illness in intensive care units. Accumulating evidence indicates that dexmedetomidine can protect against intestinal dysfunction. However, the specific mechanisms of its protective effects against burn-induced intestinal barrier injury remain unclear. Here, we aimed to explore the possible positive effects of dexmedetomidine on burn-induced intestinal barrier injury and the role of the myosin light chain kinase (MLCK)/phosphorylated myosin light chain (*p*-MLC) signalling pathway in an experimental model of burn injury.

Methods In this study, the intestinal permeability of burn-induced intestinal barrier damage was assessed by estimating the plasma concentration of 4.4 kDa fluorescein isothiocyanate-labelled dextran (FITC-dextran). Histological changes were evaluated using haematoxylin and eosin (HE) staining. Tight junction proteins were evaluated by western blot and immunofluorescence analyses to assess the structural integrity of intestinal tight junctions. The level of inflammation was determined by enzyme-linked immunosorbent assay (ELISA).

Results Our findings demonstrated that the increase in intestinal permeability caused by burn injury is accompanied by histological damage to the intestine, decreases in the expression of the tight junction proteins Zonula Occludens-1 (ZO-1) and Occludin, increases in inflammatory cytokine levels and elevation of both MLCK protein expression and MLC phosphorylation. After dexmedetomidine treatment, the burn-induced changes were ameliorated.

Conclusions In conclusion, dexmedetomidine exerted an anti-inflammatory effect and protected tight junction complexes against burn-induced intestinal barrier damage by inhibiting the MLCK/*p*-MLC signalling pathways, suggesting that it may be an effective drug in the treatment of burn-induced intestinal injury.

Trial registration Not applicable.

Background

Massive burn injuries are a type of severe trauma. According to the statistics of the World Health Organization, the mortality rate is extremely high, the main cause of death is multiple organ failure, and the current treatment measures lack effectiveness and targets(1). The intestinal tract is the largest bacterial reservoir and “endotoxin bank” in the human body. Bacteria and endotoxins can invade through the intestinal tract and spread throughout the body, a process called enterogenous infection(2). Research has confirmed that intestinal barrier dysfunction is the initiating factor for multiple organ failure after severe burns(3). Therefore, protection of intestinal barrier function is a key measure to improve the survival rate of patients with burns. Although the strategies for the prevention and treatment of intestinal barrier dysfunction after burns, such as early enteral nutrition, establishment of a fast and effective

comprehensive visceral resuscitation treatment plan, prevention and treatment of inflammatory mediators against secondary damage, and supplementation of essential amino acids, are becoming increasingly ideal, the specific mechanism underlying the protection of intestinal barrier function is not yet clear.

Some scholars have found that myosin light chain kinase (MLCK) can mediate intestinal barrier dysfunction after burns, increasing the permeability of the tight connections in the intestinal epithelium(4). In addition, studies have confirmed that the inflammatory factor tumor necrosis factor (TNF)- α can cooperate with other cytokines to induce the apoptosis of intestinal epithelial cells after burns and change the composition of cell membrane lipids. Through activation of MLCK, induction of MLCK protein expression and triggering of myosin light chain (MLC) phosphorylation, inhibition of tight junction protein expression and redistribution of these proteins leads to intestinal barrier dysfunction after burns(5).

Dexmedetomidine is a highly selective α_2 receptor agonist with sedative, anti-anxiety, hypnotic, analgesic and sympathetic nerve block effects. In 2012, scholars confirmed that dexmedetomidine can reduce intestinal barrier dysfunction induced by intestinal ischaemia-reperfusion injury in rats; its protective effect can be directly observed by haematoxylin and eosin (HE) staining of intestinal tissue, and it can reduce the concentration of the inflammatory factors TNF- α and IL-6 in intestinal mucosal tissues, but the specific mechanism has not been clarified(6).

We hypothesized that dexmedetomidine can improve burn-induced intestinal barrier damage by modulating the MLCK/phosphorylated MLC (*p*-MLC) pathway. Thus, this study aimed to explore the role of dexmedetomidine in protecting intestinal barrier function from burn injury-induced damage and to explore whether its mechanism is related to the MLCK/*p*-MLC pathway.

Methods

Animals

Healthy adult male Sprague-Dawley rats (240 ~ 260 g) were purchased from Huafukang Bioscience Company (licence number SCXK 2019-0008; Beijing, China). Rats were housed in cages under specific pathogen-free conditions, allowed access to food and water *ad libitum* and maintained on a 12 h dark/light cycle (room temperature 20–24 °C). All animal protocols were reviewed and approved by the Institutional Animal Care and Use Committee of Tianjin Medical University General Hospital (Tianjin, China).

Burn model

Twenty-four hours before establishment of the burn model, 80 g/L sodium sulfide was applied to the depilated dorsal surface of rats. Food and water were removed 12 h before the experiment. Animals were anaesthetized via injection of sodium pentobarbital (30 mg/kg) into the abdominal cavity. Then, a 40%

total body surface area (TBSA) model was established using a desktop super temperature-controlled scald instrument (ZS-YLS-5Q, Beijing ZhongShiDiChuang Technology Development Company, Beijing, China). Rats were scalded at 92 °C for 18 s in a 4 cm × 4 cm area on the dorsal surface. Subsequently, rats were subcutaneously injected in the neck with 0.9% physiological saline (0.05 ml/g) as a resuscitation fluid.

Grouping and dexmedetomidine treatment

Animals were randomly divided into 4 groups: sham operation group (Sham group), sham operation plus dexmedetomidine group (Sham + Dex group), severe burn group (Burn group), and severe burn plus dexmedetomidine group (Burn + Dex group). Rats in the Sham group received approximately the same treatment as those in the Burn group without burning. A 24 G venous needle was used to puncture the tissue and place the catheter. Rats in all groups were administered 0.9% normal saline (2 ml/kg/h) for 3 h. Subsequently, rats in the Sham + Dex group and the Burn + Dex group were injected with dexmedetomidine (5 µg/kg/h) for 4 h, and rats in the Sham group and the Burn group were administered the identical dose of 0.9% normal saline by continuous infusion. The experimental design is shown in Fig. 1.

Measurement of intestinal permeability

Intestinal permeability was determined by estimating the serum concentration of a 4.4 kDa fluorescein isothiocyanate-labelled dextran (FITC-dextran; Sigma, USA) marker. A 15 cm segment of the intestine in the proximal 5 cm of the caecum was separated and ligated, and damage to mesenteric vessels was avoided during separation and ligation. Each bilateral end of the separated intestinal segment was ligated with silk sutures to prevent FITC-dextran leakage. FITC-dextran (1 ml, 25 mg/ml) was injected into the lumen of the ligated segment, which was then returned to the intestine and closed to the abdomen. After 30 min, a blood sample was obtained by puncturing the inferior vena cava of the rat. The sample was centrifuged at 4 °C and 3000 × *g* for 15 min, and the plasma was separated to analyse the concentration of FITC-dextran. PBS was added to the plasma to a final ratio of 1:10, and the FITC-dextran concentration in the plasma samples was then analysed using a fluorometer (EnSpire, PerkinElmer, USA) at an excitation wavelength of 480 nm and an emission wavelength of 520 nm. A standard curve generated by dilution of FITC-dextran with PBS was used to calculate the concentration of FITC-dextran in the plasma samples.

HE staining

Six hours after model establishment, 6 rats in each group were intraperitoneally injected with sodium pentobarbital (30 mg/kg). Under deep anaesthesia, the rats were perfused with normal saline solution via the aorta. The intestinal tissues were collected and promptly rinsed with cold PBS and immediately fixed with 4% paraformaldehyde for 24 h. The paraformaldehyde-fixed samples were embedded in paraffin, and a microtome was used to section the samples. The samples were stained with HE after deparaffinization and dehydration. Histological changes in the intestinal mucosa were observed with a

BX51 microscope (OLYMPUS, Japan). The pathological structural damage score of the intestinal lesion was calculated according to the Appleyard method(7).

Enzyme-linked immunosorbent assay (ELISA)

Six hours after model establishment, 6 rats in each group were sacrificed under deep anaesthesia. Intestinal tissues were excised and weighed, and PBS buffer was added. Tissue was lysed and homogenized using an ultrasonic pulverizer (Ningbo Xinzhi Biotechnology Co., Ltd). After centrifugation at 4 °C and 14000 × *g* for 20 min, the supernatants were transferred into new tubes. The plasma taken from the inferior vena cava was centrifuged at 4 °C and 15000 × *g* for 10 min to obtain serum. Mouse ELISA kits were used to detect the cytokines TNF- α (ARG80120, Arigo, Taiwan, China), IL-6 (ARG80233, Arigo, Taiwan, China) and IL-10 (ARG80234, Arigo, Taiwan, China). All experimental procedures strictly followed the manufacturer's instructions as previously described.

Western blotting

Six and twelve hours after model establishment, 6 rats in each group were sacrificed under deep anaesthesia. Intestinal tissues were excised and weighed, and PBS buffer and a protease inhibitor were added. Tissue was lysed and homogenized using an ultrasonic pulverizer. Next, the homogenates were centrifuged at 4 °C and 15000 × *g* for 15 min, and the supernatants were transferred into new tubes to estimate the protein concentration using a BCA protein assay kit (Thermo Fisher Scientific, USA). Proteins were separated by 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and were then electrotransferred to a polyvinylidene difluoride (PVDF) membrane. The membrane was blocked with 5% nonfat milk dissolved in TBST buffer for 1 h and was then subsequently incubated with primary antibodies specific for Zonula Occludens-1 (ZO-1; 1:1000, Abcam, UK), Occludin (1:1000, Abcam, UK), MLCK (1:1000, CST, USA), MLC (1:1500, CST, USA), *p*-MLC (1:1000, CST, USA), and β -actin (1:1500, CST, USA) overnight at 4 °C. The membrane was then washed 5 times with 1 × TBST and incubated with the corresponding peroxidase-conjugated secondary antibodies (1:5000, KPL, USA) at room temperature for 1 h. The membrane was soaked in Chemiluminescence Plus reagent, and the protein bands were visualized with an enhanced chemiluminescence (ECL) reagent. The intensity of each band was determined with GeneTools software (Syngene, Cambridge, UK). The relative levels of ZO-1, Occludin, MLCK, MLC and *p*-MLC were normalized to that of β -actin.

Immunofluorescence analysis

Paraffin sections of rat intestinal tissue were obtained and rinsed with 0.01 M PBS 3 times for 5 min each. Then, 10% normal goat serum was added dropwise and blocked at 37 °C for 45 min. The excess liquid was aspirated, and the anti-ZO-1 (dilution 1:800, Abcam) and anti-Occludin (dilution 1:300, Abcam) antibodies were added. The sections were incubated at 37 °C for 1 h and were then placed (in a wet box) in a refrigerator at 4 °C overnight. The sections were washed with 0.01 M PBS 3 times for 5 min each. Goat anti-rabbit IgG-FITC (1:200) was added in the dark and incubated at 37 °C for 45 min. The secondary antibody solution was aspirated and discarded in the dark (without washing past this step), and DAPI staining solution (2.5 μ g/ml) was added and incubated at room temperature for 20 min. The

sections were rinsed with 0.01 M PBS 6 times for 5 min each in the dark. The sections were mounted with a fluorescence quencher in the dark, observed under a fluorescence microscope with the appropriate excitation wavelength, and images were acquired to record the experimental results. The fluorescence intensity was evaluated using a Zeiss Axioskop 2 (Carl Zeiss MicroImaging, Inc. Weimar, Germany).

Statistical analysis

Data are presented as the mean \pm standard deviation (SD) values and were analysed using SPSS 21.0 (SPSS, Inc.). The significance of differences was assessed by analysis of variance (ANOVA) or a t-test. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Dexmedetomidine ameliorates the burn-induced increase in intestinal permeability

Intestinal barrier permeability was assessed by injecting rats with a 4.4 kDa FITC-dextran marker within the ligated segment of the intestine. We measured the plasma FITC concentration at 1 h, 3 h, 6 h and 12 h after completion of dexmedetomidine infusion. The FITC concentration was significantly increased at each time point after the burn operation compared to that in the Sham group ($P < 0.05$). Six hours after model establishment, the concentration of dexmedetomidine in the treatment group was drastically decreased compared with that in the Burn group ($P < 0.05$) (Fig. 2). Therefore, based on the above results, we selected a time point later than 6 h for subsequent experiments.

Dexmedetomidine alleviates histological damage to the intestine induced by burn injury

Intestinal tissue was extracted 6 h after dexmedetomidine infusion. As shown in Fig. 3A and 3B, histological examination of intestinal tissue showed normal-appearing villi in Sham group and Sham + Dex group rats. The intestinal tissue was severely damaged in Burn group rats, as indicated by partial destruction of the intestinal structure, partial loss of epithelial cells, exposure of the lamina propria, degeneration, necrosis, and signs of inflammatory cell infiltration (Fig. 3C). Compared with that of Burn group rats, the intestinal tissue of Burn + Dex group rats exhibited relatively decreased amounts of inflammatory cell infiltration, and the intestinal structure was less severely damaged (Fig. 3D). The pathological structural damage score of the intestinal lesion was calculated according to the Appleyard method(7). As shown in Fig. 3E, the intestinal tissue pathological scores of Burn group and Burn + Dex group rats were dramatically higher than those of Sham group rats ($P < 0.05$). In addition, the pathological scores of Burn + Dex group rats were significantly reduced compared with those of Burn group rats ($P < 0.05$).

Dexmedetomidine attenuates burn-induced changes in the expression and distribution of the tight junction proteins ZO-1 and Occludin

We aimed to evaluate the effect of dexmedetomidine on the tight junction structure of the intestinal barrier. Total protein was extracted 6 h and 12 h after model establishment, and the levels of ZO-1 and Occludin were estimated by western blot analysis. The expression levels of these tight junction proteins were dramatically decreased after burn treatment at both 6 h and 12 h, suggesting that burn-induced alterations in tight junction protein expression may be responsible for the increased intestinal permeability. However, dexmedetomidine treatment greatly alleviated these decreases ($P < 0.05$). Dexmedetomidine treatment alone had no effect on ZO-1 or Occludin levels compared with those in the Sham group, ($P > 0.05$) (Fig. 4).

Twelve hours after model establishment, intestinal tissues were collected, and the distribution of ZO-1 and Occludin was evaluated by immunofluorescence. The distribution extent of the tight junction proteins ZO-1 and Occludin in intestinal tissue was decreased 12 h after burn treatment, suggesting that the burn-induced change in the distribution of tight junction proteins in the intestinal tissue may cause the increase in intestinal permeability. Dexmedetomidine treatment significantly alleviated this change ($P > 0.05$). Dexmedetomidine treatment alone had no effect on the distribution of ZO-1 and Occludin in intestinal tissue compared with that in the Sham group ($P > 0.05$) (Fig. 5).

Dexmedetomidine reduces inflammation caused by burns

To investigate the effects of burn injury and dexmedetomidine treatment on the inflammatory response in rats, we measured the levels of the pro-inflammatory factors TNF- α and IL-6 and the anti-inflammatory factor IL-10 in intestinal tissues and serum extracted 6 h after completion of dexmedetomidine infusion. The expression levels of TNF- α , IL-6 and IL-10 were evidently enhanced in both burn groups compared to the Sham and Sham + Dex groups ($P < 0.05$). After dexmedetomidine treatment, the levels of pro-inflammatory cytokines, such as TNF- α and IL-6, were drastically decreased in the Burn + Dex group compared with the Burn group, yet the level of the anti-inflammatory cytokine IL-10 was increased compared to that in the Burn group ($P < 0.05$) (Fig. 6).

Dexmedetomidine reduces MLCK-mediated MLC phosphorylation after burn injury

Numerous documents have reported that MLCK-dependent MLC phosphorylation is vital to intestinal barrier disruption(8). Therefore, our group considered that the mechanism by which dexmedetomidine reduces burn-induced intestinal barrier injury may be related to MLCK-dependent MLC phosphorylation. As shown in Fig. 7, the level of *p*-MLC in intestinal tissue was significantly increased in the Burn and Burn + Dex groups compared with that in the Sham group ($P < 0.05$), while dexmedetomidine injection greatly suppressed the burn-induced increase in the *p*-MLC level ($P < 0.05$).

MLCK is a key kinase for MLC phosphorylation, and its expression was significantly enhanced in the Burn and Burn + Dex groups compared to the Sham group ($P < 0.05$). Compared with that in the Burn group, the expression of MLCK was significantly reduced after Dex treatment in the Burn + Dex group ($P < 0.05$) (Fig. 7).

Discussion

Extensive burn injury is a type of severe trauma. As early as 1986, scholars proposed the famous argument that the gastrointestinal tract is the primary organ of multiple organ dysfunction syndrome (MODS)(9). MODS is the root cause of death in severe burn patients(10, 11). Severe burns can induce destruction of the intestinal mucosal barrier and changes in permeability and are closely related to secondary MODS(12). A study showed that intestinal mucosal permeability was significantly increased in mice 1 h after infliction of a 30% TBSA burn, and the morphological analysis of intestinal tissue by HE staining showed that the intestinal barrier was disrupted(13). In this study, healthy and pathogen-free adult male SD rats were selected as the research object. Using a desktop super temperature-controlled scald instrument, burn injury was inflicted by creation of a 4 cm² scald wound on the dorsal surface at 92 °C for 18 s, establishing a 40% TBSA 3rd degree scald model. The pre-experiment satisfied the skin morphology criteria. The HE staining results proved that the degree of the burns was consistent with severe burns, indicating that the model was successfully established.

Intestinal permeability is one of the most important indicators for evaluating intestinal function(14, 15). In the present study, the burn-induced increase in intestinal permeability to 4.4-kDa FITC-dextran was notably eliminated 6 h after dexmedetomidine treatment, proving the action of repair mechanisms after dexmedetomidine infusion. Therefore, we selected a time point of later than 6 h for subsequent experiments. Intestinal permeability caused by burn injury was accompanied by histological changes in the intestine, decreases in the expression and distribution of the tight junction proteins ZO-1 and Occludin, and increases in inflammatory cytokine levels, in addition to elevation of both MLCK protein expression and MLC phosphorylation. After dexmedetomidine treatment, those burn-induced changes were improved.

The intestinal barrier comprises mechanical, immunological, biological, and chemical barriers(16). The mechanical barrier is a pivotal part of the intestinal barrier and is maintained through intestinal epithelial cells and intercellular junctions(17). Tight junctions consist of multiple proteins such as ZO, occludins and claudins(18). The intracellular C-terminus of occludin is linked with ZO(19). Those tight junction complexes are vital for maintaining intestinal barrier integrity after injury(20). Reports have indicated that burn-induced intestinal damage leads to reduced expression of tight junction proteins(21), consistent with our results. In addition, we revealed that the expression levels of the classical tight junction regulatory proteins ZO-1 and Occludin were evidently elevated by dexmedetomidine treatment during recovery from burn injury. Their distribution in intestinal tissue was also significantly increased. These findings prove that dexmedetomidine can enhance the expression and distribution of the tight junction proteins ZO-1 and Occludin, thereby ameliorating intestinal barrier damage.

Previous studies have confirmed that inflammatory cytokine-mediated intestinal epithelial barrier dysfunction promotes multiple enteropathies, including IBD(22, 23). These and other inflammatory cytokines disrupt the integrity of tight junction complexes and enhance intestinal barrier permeability. Numerous studies have shown that the levels of some pro-inflammatory cytokines, such as TNF- α , IFN- γ , IL-6 and IL-1 β , are notably elevated in mice, rats and human patients with severe burns(24–26). These alterations in pro-inflammatory and anti-inflammatory cytokines may promote burn-induced intestinal barrier injury. In our research, we measured the levels of the pro-inflammatory cytokines TNF- α and IL-6 and the anti-inflammatory cytokine IL-10. TNF- α is an early pro-inflammatory factor that is released mainly by macrophages and monocytes and participates in inflammatory and immune responses. In addition, TNF- α can disrupt the intestinal barrier by decreasing tight junction protein levels and reorganizing the cytoskeletal structure(27). IL-6 is an early inflammatory factor that is rapidly induced in the setting of infections, burns, tumours, etc., and its concentration is increased as a better predictor of disease. IL-10 is a potent cytokine inhibitor of macrophages that moderates inflammation. Our findings indicated that dexmedetomidine can downregulate the expression of the pro-inflammatory factors TNF- α and IL-6 and promote the expression of the anti-inflammatory factor IL-10. These conclusions are consistent with the results found by Chen et al(4). Our outcomes verified that inflammatory cytokines can damage the intestinal epithelial barrier by decreasing tight junction protein levels, while dexmedetomidine treatment can reverse this trend.

Tight junctions are in a state of relatively stable dynamic remodelling and are regulated by various factors, such as mitogen-activated protein kinases (MAPKs), Ca²⁺-induced E-cadherin, phospholipase C, protein kinase A, Rho GTPase, tyrosine kinase, and MLCK(8). Among these factors, MLCK plays a vital role in the regulation of tight junction dynamics(28). To explore the causes of the structural damage to tight junctions during burn-induced intestinal injury, we considered the MLCK/p-MLC pathway, which has been illustrated to induce disruption of tight junctions after MLC and MLCK phosphorylation. MLC phosphorylation alone has been proven to be adequate to induce tight junction regulation and to enhance tight junction permeability, which is closely related to morphological and biochemical changes in the tight junction proteins ZO-1 and Occludin(29). Our research showed that burn-induced intestinal barrier damage was accompanied by enhancement of MLCK protein expression and MLC phosphorylation. However, dexmedetomidine not only suppressed the burn-induced increases in MLCK expression and MLC phosphorylation but also reduced intestinal barrier permeability, ameliorated intestinal pathological damage, and increased the expression and distribution of the tight junction proteins ZO-1 and Occludin. Therefore, these experiments demonstrated that the MLCK-dependent MLC phosphorylation signalling pathway may be related to burn-induced intestinal barrier injury.

Conclusions

In conclusion, dexmedetomidine can relieve burn-induced intestinal epithelial hyperpermeability by reducing inflammation and enhancing the expression and distribution of the tight junction proteins ZO-1 and Occludin. These effects may be exerted via inhibition of MLCK/p-MLC pathway activation.

List Of Abbreviations

Complete spelling	Abbreviation
myosin light chain kinase	MLCK
phosphorylated myosin light chain	p-MLC
fluorescein isothiocyanate-labelled dextran	FITC-dextran
haematoxylin and eosin	HE
enzyme-linked immunosorbent assay	ELISA
Zonula Occludens-1	ZO-1
myosin light chain kinase	MLCK
tumor necrosis factor	TNF
myosin light chain	MLC
total body surface area	TBSA
sodium dodecyl sulfate–polyacrylamide gel electrophoresis	SDS-PAGE
polyvinylidene difluoride	PVDF
enhanced chemiluminescence ECL	ECL
standard deviation	SD
analysis of variance	ANOVA
multiple organ dysfunction syndrome	MODS
mitogen-activated protein kinases	MAPKs

Declarations

Ethics approval and consent to participate

All animal protocols were reviewed and approved by the Institutional Animal Care and Use Committee of Tianjin Medical University General Hospital (Tianjin, China).

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and analysed in the present research are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

QC and JY made substantial contributions to the conception and design of the research. CX and BYX and WYQ conducted all data acquisition, analysis and interpretation. QC, JY and CX drafted the manuscript and critically revised the manuscript for important intellectual content. XKL and YYH were responsible for reviewing the manuscript. All authors read and approved the final manuscript. **Acknowledgements**

Not applicable.

Patient consent for publication

Not applicable.

References

1. Xiao XG, Zu HG, Li QG, Huang P. Sivelestat sodium hydrate attenuates acute lung injury by decreasing systemic inflammation in a rat model of severe burns. *Eur Rev Med Pharmacol Sci.* 2016;20(3):528–36.
2. Haines RJ, Wang CY, Yang CGY, Eitnier RA, Wang F, Wu MH. Targeting palmitoyl acyltransferase ZDHHC21 improves gut epithelial barrier dysfunction resulting from burn-induced systemic inflammation. *Am J Physiol Gastrointest Liver Physiol.* 2017;313(6):G549-G57.
3. Osuka A, Kusuki H, Matsuura H, Shimizu K, Ogura H, Ueyama M. Acute intestinal damage following severe burn correlates with the development of multiple organ dysfunction syndrome: A prospective cohort study. *Burns.* 2017;43(4):824–9.
4. Chen C, Wang P, Su Q, Wang S, Wang F. Myosin light chain kinase mediates intestinal barrier disruption following burn injury. *PLoS One.* 2012;7(4):e34946.
5. Zahs A, Bird MD, Ramirez L, Turner JR, Choudhry MA, Kovacs EJ. Inhibition of long myosin light-chain kinase activation alleviates intestinal damage after binge ethanol exposure and burn injury. *Am J Physiol Gastrointest Liver Physiol.* 2012;303(6):G705-12.

6. Zhang XY, Liu ZM, Wen SH, Li YS, Li Y, Yao X, et al. Dexmedetomidine administration before, but not after, ischemia attenuates intestinal injury induced by intestinal ischemia-reperfusion in rats. *Anesthesiology*. 2012;116(5):1035–46.
7. Appleyard CB, Wallace JL. Reactivation of hapten-induced colitis and its prevention by anti-inflammatory drugs. *Am J Physiol*. 1995;269(1 Pt 1):G119-25.
8. He W, Wang Y, Wang P, Wang F. Intestinal barrier dysfunction in severe burn injury. *Burns Trauma*. 2019;7:24.
9. Carrico CJ, Meakins JL, Marshall JC, Fry D, Maier RV. Multiple-organ-failure syndrome. *Arch Surg*. 1986;121(2):196–208.
10. Pompermaier L, Steinvall I, Elmasry M, Thorfinn J, Sjoberg F. Burned patients who die from causes other than the burn affect the model used to predict mortality: a national exploratory study. *Burns*. 2018;44(2):280–7.
11. Greenhalgh DG. Sepsis in the burn patient: a different problem than sepsis in the general population. *Burns Trauma*. 2017;5:23.
12. Ikeda M, Shimizu K, Ogura H, Kurakawa T, Umemoto E, Motooka D, et al. Hydrogen-Rich Saline Regulates Intestinal Barrier Dysfunction, Dysbiosis, and Bacterial Translocation in a Murine Model of Sepsis. *Shock*. 2018;50(6):640–7.
13. Huang Y, Feng Y, Wang Y, Wang P, Wang F, Ren H. Severe Burn-Induced Intestinal Epithelial Barrier Dysfunction Is Associated With Endoplasmic Reticulum Stress and Autophagy in Mice. *Front Physiol*. 2018;9:441.
14. Deitch EA. Intestinal permeability is increased in burn patients shortly after injury. *Surgery*. 1990;107(4):411–6.
15. Epstein MD, Tchervenkov JI, Alexander JW, Johnson JR, Vester JW. Increased gut permeability following burn trauma. *Arch Surg*. 1991;126(2):198–200.
16. Turner JR. Intestinal mucosal barrier function in health and disease. *Nat Rev Immunol*. 2009;9(11):799–809.
17. Marchiando AM, Graham WV, Turner JR. Epithelial barriers in homeostasis and disease. *Annu Rev Pathol*. 2010;5:119–44.
18. Shen L, Weber CR, Raleigh DR, Yu D, Turner JR. Tight junction pore and leak pathways: a dynamic duo. *Annu Rev Physiol*. 2011;73:283–309.
19. Buschmann MM, Shen L, Rajapakse H, Raleigh DR, Wang Y, Wang Y, et al. Occludin OCEL-domain interactions are required for maintenance and regulation of the tight junction barrier to macromolecular flux. *Mol Biol Cell*. 2013;24(19):3056–68.
20. Liu Z, Li N, Neu J. Tight junctions, leaky intestines, and pediatric diseases. *Acta Paediatr*. 2005;94(4):386–93.
21. Wang FJ. [Research on intestinal tight junction barrier dysfunction should be emphasized in burn injury]. *Zhonghua Shao Shang Za Zhi*. 2010;26(5):331–3.

22. Blander JM. Death in the intestinal epithelium-basic biology and implications for inflammatory bowel disease. *FEBS J.* 2016;283(14):2720–30.
23. Xiao YT, Yan WH, Cao Y, Yan JK, Cai W. Neutralization of IL-6 and TNF-alpha ameliorates intestinal permeability in DSS-induced colitis. *Cytokine.* 2016;83:189–92.
24. Finnerty CC, Przkora R, Herndon DN, Jeschke MG. Cytokine expression profile over time in burned mice. *Cytokine.* 2009;45(1):20–5.
25. Orman MA, Nguyen TT, Ierapetritou MG, Berthiaume F, Androulakis IP. Comparison of the cytokine and chemokine dynamics of the early inflammatory response in models of burn injury and infection. *Cytokine.* 2011;55(3):362–71.
26. Finnerty CC, Herndon DN, Chinkes DL, Jeschke MG. Serum cytokine differences in severely burned children with and without sepsis. *Shock.* 2007;27(1):4–9.
27. Ye X, Sun M. AGR2 ameliorates tumor necrosis factor-alpha-induced epithelial barrier dysfunction via suppression of NF-kappaB p65-mediated MLCK/p-MLC pathway activation. *Int J Mol Med.* 2017;39(5):1206–14.
28. Shen L, Black ED, Witkowski ED, Lencer WI, Guerriero V, Schneeberger EE, et al. Myosin light chain phosphorylation regulates barrier function by remodeling tight junction structure. *J Cell Sci.* 2006;119(Pt 10):2095–106.
29. Wu CC, Lu YZ, Wu LL, Yu LC. Role of myosin light chain kinase in intestinal epithelial barrier defects in a rat model of bowel obstruction. *BMC Gastroenterol.* 2010;10:39.

Figures

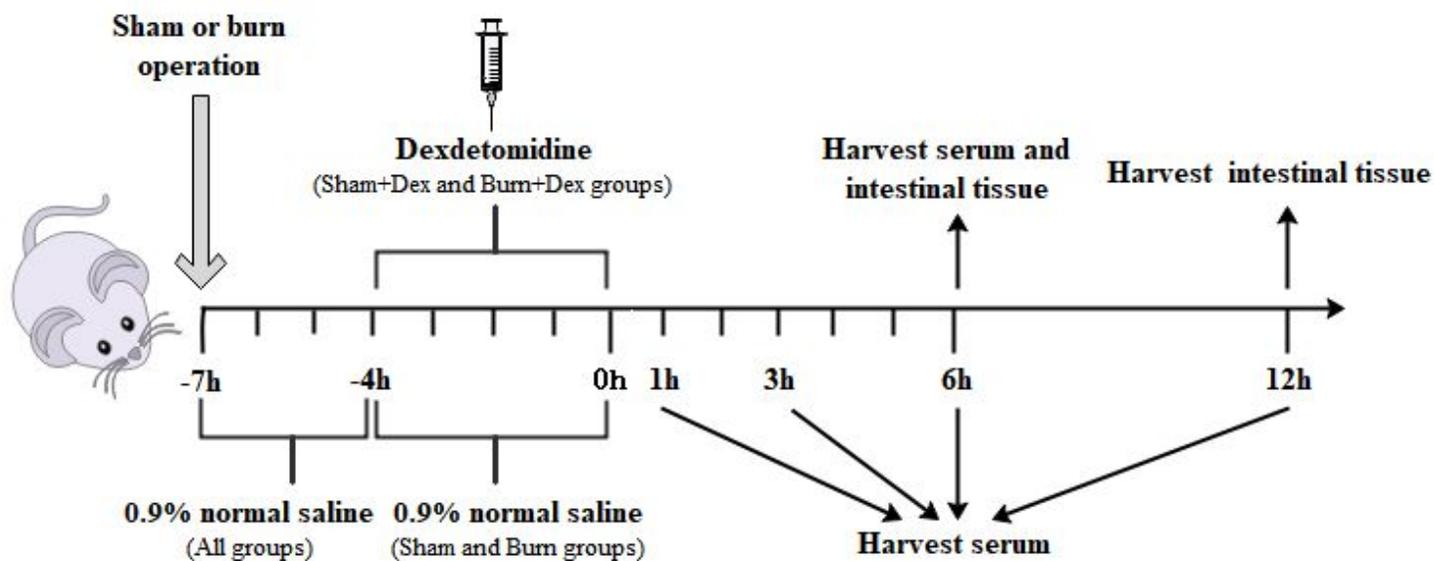


Figure 1

Experimental design. Rats were subjected to sham or burn operation. Rats in all groups were administered 0.9% normal saline (2 ml/kg/h) for 3 h. Then, rats in groups A and B were administered

dexmedetomidine (5 µg/kg/h), and rats in groups C and D were administered the same amount of 0.9% normal saline for 4 h. Serum was separated from venous blood, which was harvested at 1 h, 3 h, 6 h, and 12 h, and 4.4 kDa FITC-dextran was injected into the ligated intestinal segment 30 min before each of these time points. At 6 h and 12 h, intestinal tissue and serum were obtained from rats in each group to measure the levels of inflammatory cytokines and proteins and for HE staining.

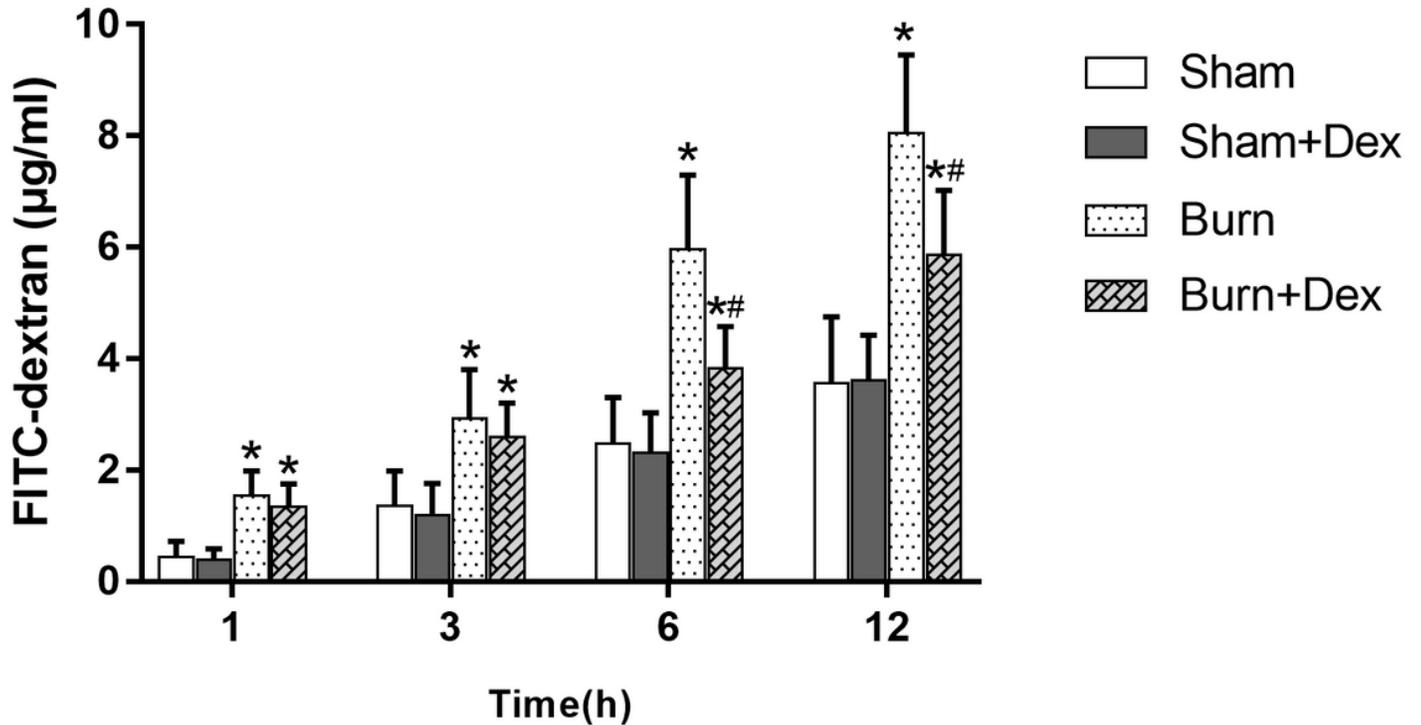


Figure 2

Infusion of dexmedetomidine after burn injury can improve intestinal barrier permeability. Plasma concentrations of FITC-dextran in each group were measured at 1, 3, 6, and 12 h after completion of dexmedetomidine infusion (n=4 rats per group). *P<0.05 vs the Sham group; #P<0.05 vs the Burn group.

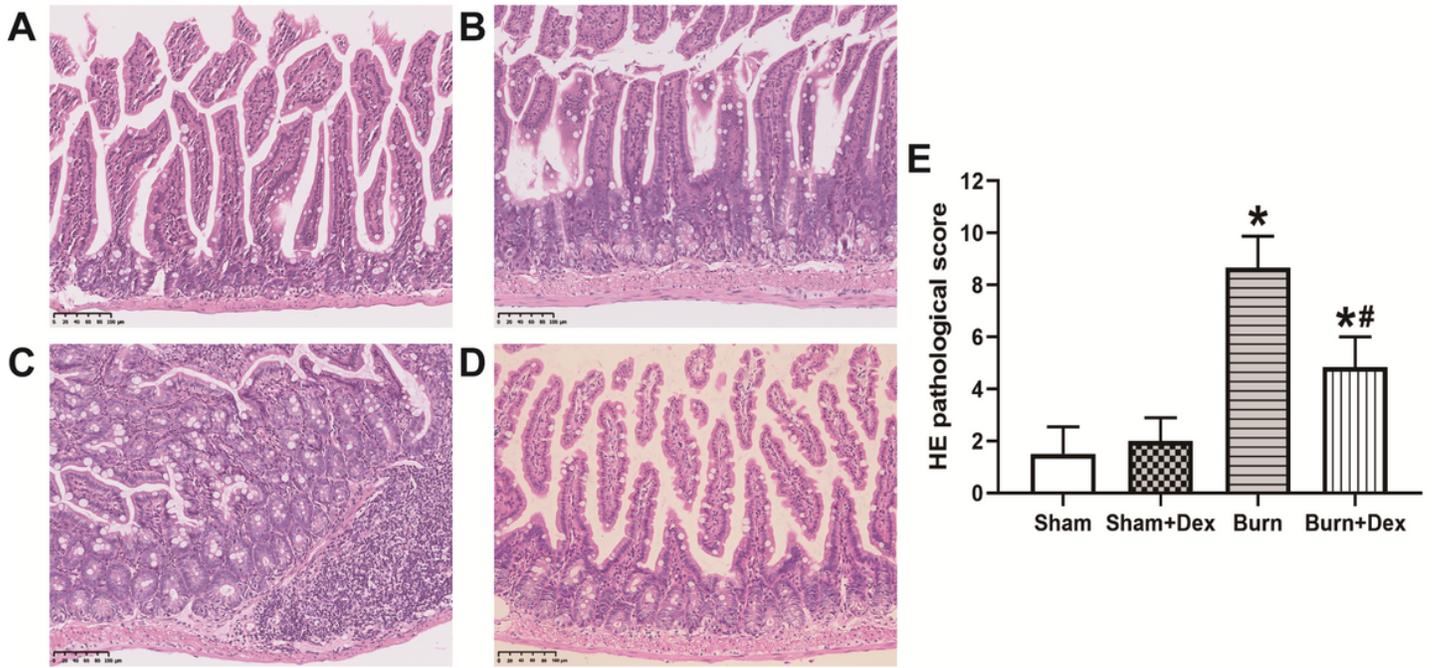


Figure 3

Effects of burns and dexmedetomidine on intestinal histopathology. Mice were sacrificed 6 h after sham or burn operation, and intestinal tissues were harvested for HE staining (A: Sham group, B: Sham+Dex group, C: Burn group, D: Burn+Dex group). Intestinal injury was scored by visualizing the morphological structure (E). * $P < 0.05$ vs the Sham group; # $P < 0.05$ vs the Burn group.

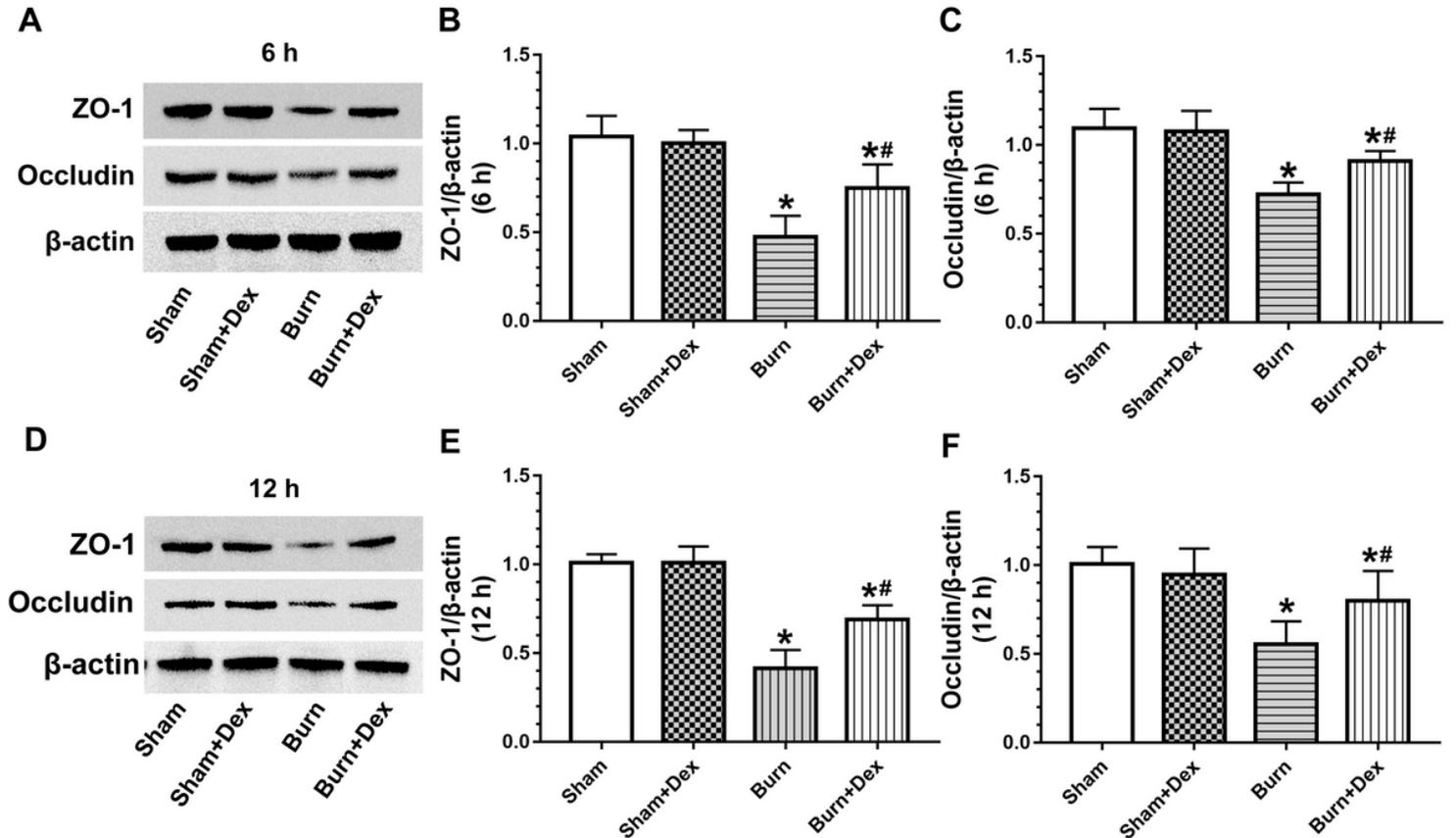


Figure 4

Dexmedetomidine enhances the expression of tight junction proteins in the intestinal barrier. The expression of ZO-1 and Occludin proteins was detected by western blotting at 6 h and 12 h (A, E). Quantitative analysis of ZO-1 (B, E) and Occludin (C, F) levels, shown as the ratio of each protein band density to that of β -actin (n=6 rats per group). *P<0.05 vs the Sham group; #P<0.05 vs the Burn group.

A

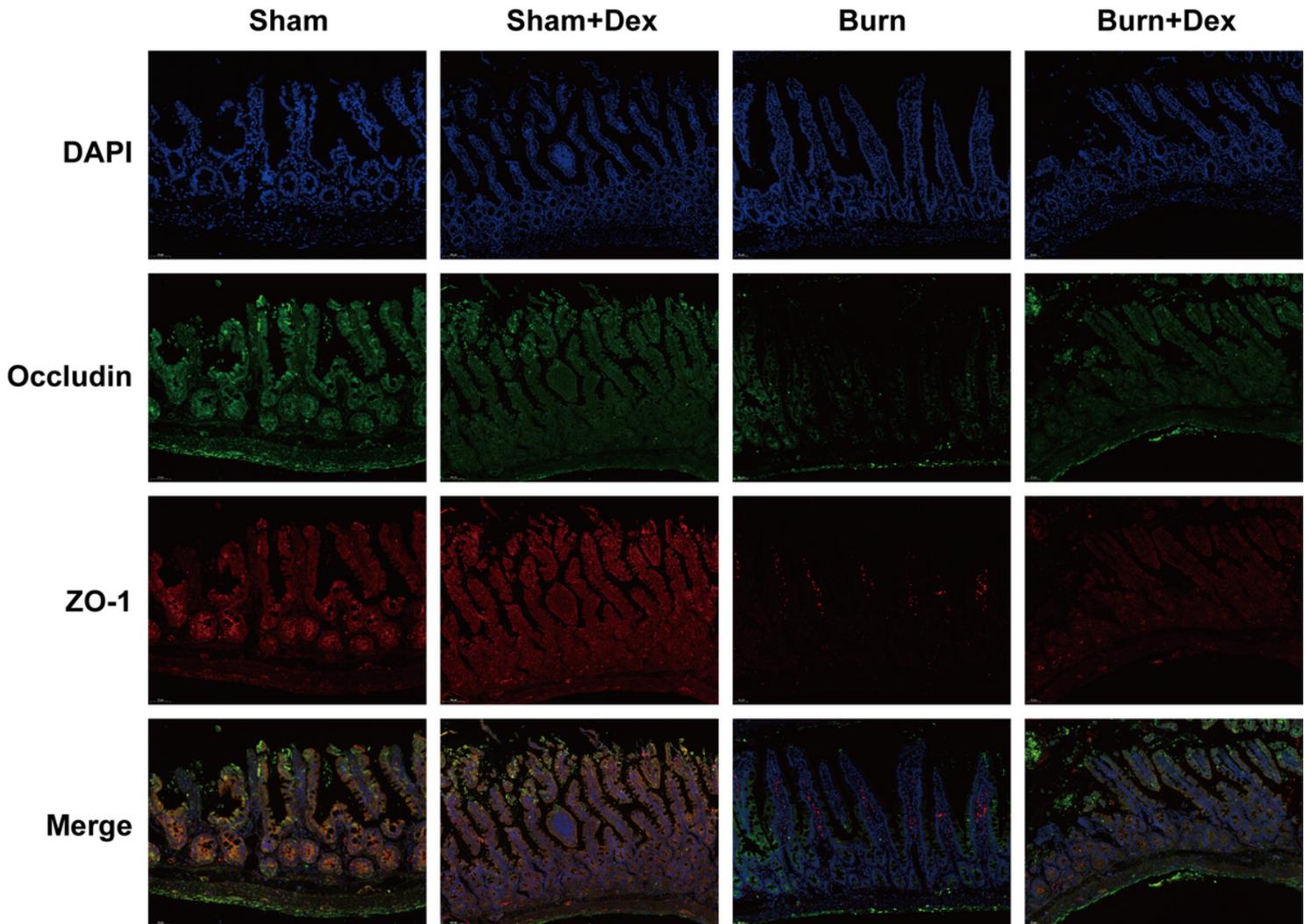


Figure 5

Dexmedetomidine enhances the distribution of tight junction proteins in the intestinal barrier. The distribution of ZO-1 and Occludin proteins was detected by immunofluorescence at 12 h.

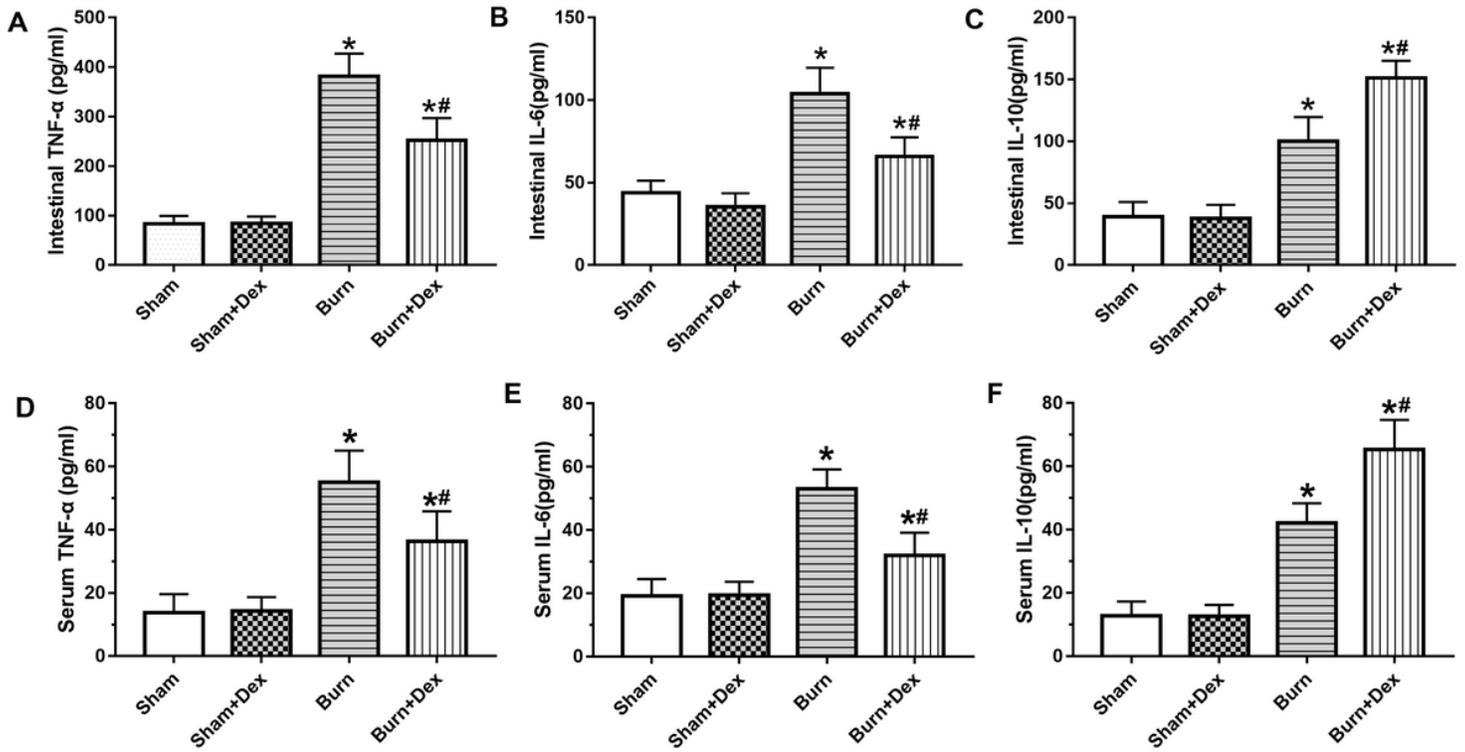


Figure 6

Effects of dexmedetomidine infusion after burn injury on the inflammatory response in rat intestinal tissues and serum. The levels of the pro-inflammatory cytokines TNF- α (A, D) and IL-6 (B, E) and the anti-inflammatory cytokine IL-10 (C, F) in intestinal tissue and serum from rats in each group were measured with ELISA kits (n = 6 rats per group). *P<0.05 vs the Sham group; #P<0.05 vs the Burn group.

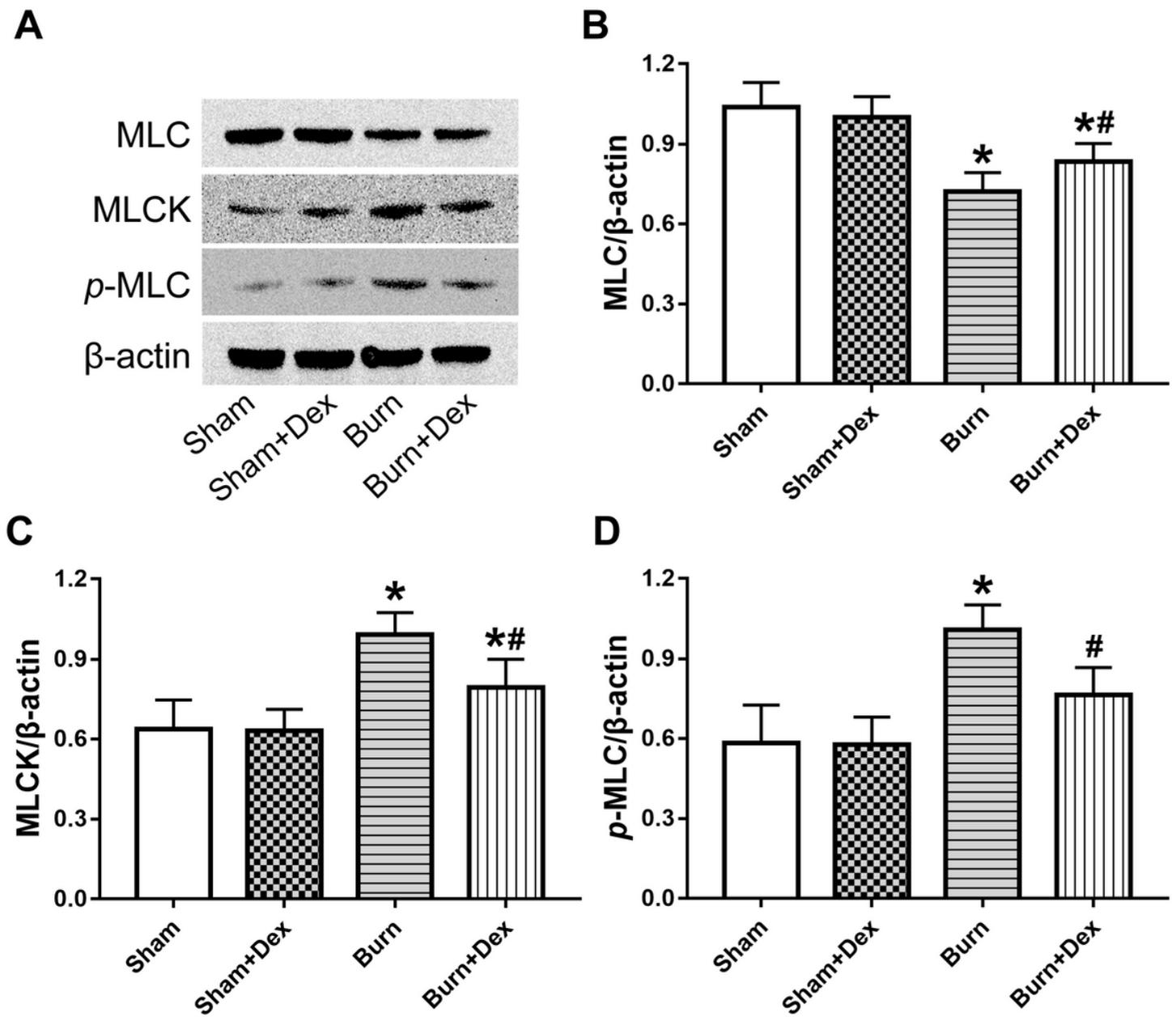


Figure 7

Dexmedetomidine can reduce the phosphorylation of MLC after burn injury. The expression of MLC and MLCK and the phosphorylation of MLC were evaluated by western blotting at 6 h (A). Quantitative analysis of MLC (B), MLCK (C) and p-MLC (D) levels, shown as the ratios of the corresponding band densities to that of β -actin (n=6 rats per group). *P<0.05 vs the Sham group; #P<0.05 vs the Burn group.