European Review for Medical and Pharmacological Sciences 2019; 23 (3 Suppl): 184-191

Glutamine has a protective role on intestinal tissues *via* targeting NF-κB pathway in rats with sepsis

T.-Y. WU, H.-B. ZHANG

Department of Critical Care Medicine, Haijiya Hospital, Shanxian County, Heze, China

Abstract. – OBJECTIVE: To study the protective effect of glutamine on the intestinal tissues of septic rats by regulating the nuclear factor-κB (NF-κB) pathway.

MATERIALS AND METHODS: A total of 30 rats were divided into the Sham group, Model group, and Glutamine group using a random number table. The changes in the intestinal tissues in rats were observed *via* hematoxylin-eosin (HE) staining, and the difference in the content of serum inflammatory factor tumor necrosis factor-α (TNF-α) was detected *via* enzyme-linked immunosorbent assay (ELISA). Moreover, the apoptosis of the intestinal tissues was detected *via* terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay, and the protein expression of NF-κB in intestinal tissues was detected *via* Western blotting.

RESULTS: In the Sham group, the rats had normal activity and good mental state, and there were no evident lesions in the abdominal cavity. Compared with the rats in the Sham group, the rats in the Model group had very poor mental state and erected hair, and they trembled and barely moved. After the abdomen was opened, there were bad smell and evident bleeding in the abdominal cavity, and the cecum became black with adhesion and swelling. In the Glutamine group, the symptoms were significantly alleviated compared with the Model group. The morphological observation of the intestinal tissues revealed that in the Sham group, the intestinal villi were regularly and clearly arranged, and there was no congestion in the capillaries. Compared with the Sham group, the intestinal villi were disorderly arranged with rupture in the Model group, and the severe capillary congestion was clearly visible and accompanied by ulcer. In the Glutamine group, the intestinal villi had normal morphology and regular arrangement after treatment, the subepithelial space was significantly dilated, the capillary dilation and the congestion could be seen and the lamina propria was intact. In the Sham group, the pathological score was 0 point, and the intestinal mucosa and villi had normal structure. Compared with that in the Sham group, the pathological score of the intestinal tissues were significantly increased in the Model group ($p<0.05$). In the Glutamine group, the pathological score significantly declined after treatment compared with that in the Model group ($p<0.05$). Besides, the content of the inflammatory factor TNF-α in the intestinal tissues was the highest in the Model group ($p<0.05$), and it was lower in the Glutamine group than that in the Sham group ($p<0.05$), indicating that glutamine can effectively reduce the content of the inflammatory factor TNF-α, exerting a certain protective effect on the intestinal tissues. The number of apoptotic intestinal epithelial cells was remarkably increased in the Model group compared with that in the Sham group ($p<0.05$), and it was remarkably decreased in the Glutamine group compared with that in the Model group ($p<0.05$). The Model group had a significantly higher protein expression of NF-κB in intestinal tissues than in the Sham group and Glutamine group ($p<0.05$), Sham group had the lowest protein expression of NF-κB in intestinal tissues ($p<0.05$), and the Glutamine group had a significantly lower protein expression of NF-κB in intestinal tissues than the Model group ($p<0.05$).

CONCLUSIONS: Glutamine inhibits the protein expression of NF-κB, thereby exerting a protective effect on intestinal tissues of sepsis rats.

Key Words:
Glutamine, NF-κB signaling pathway, Septic rats, Intestinal tissues.

Introduction

The inflammatory factors can lead to sepsis. In sepsis, homeostasis cannot be restored due to the immune response caused by invading pathogens, ultimately leading to the pathological syndrome characterized by persistent excessive inflamma-

Corresponding Author: Haibin Zhang, BM; e-mail: 155233475@qq.com

tion and immunosuppression. The common complications of sepsis include burns, severe infection, and shock. The further development of sepsis can result in circulatory system failure, multiple organ dysfunction syndromes, multiple organ failure, and even death[1-3]. As the power organ in the pathogenesis of sepsis, the intestinal tract is damaged, so that the intestinal epithelial cells are damaged and the barrier function is destroyed, leading to the persistent sepsis. Therefore, protecting the intestinal barrier function has an important clinical significance in the treatment and prognosis of sepsis[4,5]. Zhou et al[6] have shown that the nuclear factor-κB (NF-κB) signaling pathway is activated in septic patients, and various drugs that inhibit the activation of the NF-κB signaling pathway can effectively protect multiple organ functions and increase the survival rate of septic patients. Moreover, it has been found in animal experiments that a large amount of NF-κB can be detected in the organs in the sepsis model. In the vital organs in sepsis, such as intestinal tract, the activity of NF-κB is enhanced, stimulating the occurrence and development of sepsis. NF-κB can dramatically increase the expressions of the inflammatory factors, aggravating the disease[7,8]. Glutamine is closely related to intestinal immunity, and also an indispensable part constructing the intestinal mucosal barrier function. Septic patients have a huge demand for glutamine, but the significantly enhanced intestinal metabolism makes the glutamine in the body insufficient. Many investigations[9] have found that the rational use of glutamine based on enteral and parenteral nutrition can well regulate the body's immune function and inflammatory responses. In this paper, the protective effect of glutamine on the intestinal tissues in septic rats by regulating the activity of the NF-κB pathway was explored.

Materials and Methods

Laboratory Animals

A total of 30 healthy adult female Sprague-Dawley rats weighing 180-220 g and aged 6-8 weeks old were provided by Shandong Laboratory Animal Center. They were fed in separate cages in the specific pathogen-free animal room under the room temperature of (22 ± 2)°C, the humidity of 50-60% and a 12/12 h light/dark cycle, and had free access to food and water. This study was approved by the Animal Ethics Committee of Haijiya Hospital, Shanxian County Animal Center.

Reagents and Instruments

The reagents and instruments used in this experiment included the refrigerated centrifuge 3K15 and biosafety cabinet TYPE B2 (Sigma, Louis, MO, USA), BH2 microscope (Olympus, Tokyo, Japan), rat NF-κB enzyme-linked immunosorbent assay (ELISA) kit (USCNLIFE™, Wuhan, China), tumor necrosis factor-α (TNF-α), ELISA kit (ImmunoWay, Hudson, WI, USA), glutamine standard (Boster, Wuhan, China), micro high-speed refrigerated centrifuge (Thermo, Waltham, MA, Germany), *in situ* cell death assay tool (Roche, Basel, Switzerland), and microanalytical balance (Mettler-Toledo, Columbus, OH, USA).

Animal Modeling and Grouping

The 30 rats were divided into the Sham group, Model group, and Glutamine group. The mouse model of cecal ligation and puncture (CLP) is the most widely used model for studying sepsis and septic shock. In the present study, the rat model of sepsis was established *via* CLP, as follows: (1) the rats were first anesthetized *via* intraperitoneal injection of 0.1% pentobarbital sodium (4 mL/kg); (2) the rats were fixed on the experimental table in a supine position, an about 1.5 cm-long incision was made along the midline of abdomen, and then the cecum was ligated at the root; (3) the cecum was thoroughly acupunctured for 3 times to form the cecal fistula, and then the cecum was placed back in the abdominal cavity. Finally, the incision was sutured. In the Sham group, the abdominal cavity was cut open and only closed, and the cecum was not ligated and punctured. After the operation, all rats were immediately placed on the warm bed and then placed back in the cage after the normal activity was recovered. In the Glutamine group, the rats were gavaged with glutamine (12 g/kg) at 1 week before the operation. In the Sham group and Model group, the rats were gavaged with an equal volume of normal saline at 1 d before the operation. After modeling, the fever occurred, the heart rate was increased by 50%, the respiratory rate doubled, and there were piloerection and fatigue in the Model group, indicating the successful modeling.

Collection and Observation of Specimens

At 1 d after the operation, the blood was drawn from the abdominal aorta in each group and centrifuged at 1000 rpm using the high-speed

refrigerated centrifuge. The serum separated was taken, stored in a refrigerator at -80°C, and then used for ELISA.

The cecum was taken as follows: after the rats were sacrificed, the abdominal cavity was immediately cut open to expose the small intestine, and the small intestine was carefully peeled off and placed in 10% formaldehyde for preparation of pathological sections.

Observation of General State of Rats

After CLP in the three groups, the general state of rats was observed, and the abdominal cavity was cut open to observe the changes inside according to the references[10].

Morphological Observation of Intestinal Tissues

The tissue blocks (generally 0.5 cm in thickness at most) were placed in the fixing solution and already prepared (10% formalin, Bouin's fixative, etc.) to denature and coagulate the proteins in the tissues and cells. Then, the blocks were dehydrated with alcohol at different concentrations and with 70%, 95%, and 100% ethanol for 3 times. The transparent tissue blocks were placed in the melted paraffin and placed in the paraffin oven till the blocks were completely embedded into paraffin. After cooling and coagulation, the paraffin-embedded blocks were sliced into 8 μm-thick sections using the microtome, baked in an oven at 60°C for 30 min, deparaffinized with xylene (5 min×3 times), and dehydrated with 100%, 95%, and 70% ethanol for 3 times. The transparent sections were stained with hematoxylin dye for 5 min, sealed with gum, and covered with the cover glass. Finally, five fields of view were randomly selected in each section and photographed under a light microscope (200× and 400×).

Observation and Scoring of Pathological Changes in Intestinal Tissues

The pathological changes in the intestinal tissues were observed under the light microscope, and the pathological injury of the intestinal tissues was scored according to the reference[11].

Determination of Serum Inflammatory Factor TNF-α in Rats

The level of the pro-inflammatory cytokine TNF-α in the intestinal tissues was detected *via* ELISA, as follows: 100 μL of specimens to be detected were added into each well of ELISA plate and incubated at 37°C for 60 min. Then, the liquid was discarded, and the plate was repeatedly patted dry without washing. 100 μL of freshly diluted biotin-labeled antibodies were added into each well for incubation at 37°C for 60 min, followed by washing for 3 times. Then, 100 μL of horseradish peroxidase (HRP)-streptavidin-labeled antibodies were added into each well for further incubation at 37°C for 60 min, followed by washing for 3 times. 100 μL of tetramethylbenzidine (TMB) a developing solution (Thermo Fisher Scientific, Waltham, MA, USA) freshly prepared was added into each well, followed by reaction in a dark place at 37°C for 30 min. Finally, the stop buffer was added into each well, and the liquid turning from blue to yellow indicated the successful assay. The assay should be completed within 20 min.

Detection of Apoptosis of Intestinal Tissues via TUNEL

The paraffin-embedded sections of the intestinal tissues were prepared and stained according to the instructions of TUNEL (Roche, Basel, Switzerland) kit. Then, the apoptosis of the intestinal tissues in each group was observed and photographed under the light microscope. The apoptotic cells were yellow brown. Five fields of view were selected, and the color was observed, based on which the number of apoptotic cells was calculated, and the average was taken in five fields.

Detection of NF-κB Protein Expression in Intestinal Tissues Via Western Blotting

The cells were added with an appropriate amount of radioimmunoprecipitation assay (RIPA) lysis buffer and the protease inhibitor phenylmethylsulfonyl fluoride (PMSF; RIPA: PMSF = 100: 1) (Beyotime, Shanghai, China). The intestinal tissues were cut into pieces, added with lysis buffer (10:1), homogenized using the homogenizer, and transferred into an Eppendorf (EP) tube, followed by centrifugation at 14,000 rpm at 4°C for 30 min using the high-speed refrigerated centrifuge. Then, the protein supernatant was collected and subjected to a heating bath at 95°C for 10 min for protein denaturation. The protein sample prepared was placed in the refrigerator at -80°C for later use, and the protein was quantified using the bicinchoninic acid (BCA) kit (Pierce, Rockford, IL, USA). After that, the dodecyl sulfate, sodium salt-polyacrylamide

gel electrophoresis (SDS-PAGE) gel was prepared, and the protein sample was loaded into the gel loading well for electrophoresis under the constant pressure of 80 V for 2.5 h. Then, the protein was transferred onto a polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA) using a semi-dry transfer method. The PVDF membranes were immersed in Tris-Buffered Saline Tween (TBST) containing 5% skim milk powder and shaken slowly for 1 h on a shaking table to be sealed. Then, the protein was incubated with the primary antibody diluted with 5% skim milk powder, rinsed with TBST for 3 times (10 min/time), incubated again with the secondary antibody at room temperature for 2 h, and rinsed again with TBST twice and with TBS once (10 min/time). Finally, the protein was detected using the enhanced chemiluminescence (ECL) reagent, followed by exposure in a dark room. The relative expression of the protein was analyzed using Image-Pro Plus v6 (Media Cybernetics, Silver Spring, MD, USA).

Statistical Analysis

The Statistical Product and Service Solutions (SPSS) 13.0 (SPSS Inc., Chicago, IL, USA) software was used for analysis. The Chi-square test was performed for the comparison of tissue morphology, the number of apoptotic cells, and NF-κB protein among the three groups, and the *t*-test was performed for the data analysis among groups. The calculation results were expressed as mean ± standard deviation ($\bar{x} \pm s$) and percentage. $p<0.05$ suggested that the results had statistically significant differences.

Results

Observation of General State

In the Sham group, the rats had normal activity and good mental state, and there were no evident lesions in the abdominal cavity. Compared with the rats in the Sham group, the rats in the Model group had very poor mental state and erected hair. Moreover, they trembled and barely moved. After the abdomen was opened, there were bad smell and evident bleeding in the abdominal cavity, and the cecum became black with adhesion and swelling. In the Glutamine group, the symptoms were significantly alleviated compared with those in the Model group.

Morphology of Intestinal Tissues

In the Sham group, the intestinal villi were regularly and clearly arranged, and there was no congestion in the capillaries. Compared with those in the Sham group, the intestinal villi were disorderly arranged with rupture, and the severe capillary congestion was clearly visible accompanied by ulcer in the Model group. In the Glutamine group, the intestinal villi had normal morphology and regular arrangement after treatment, the subepithelial space was significantly dilated, the capillary dilation and congestion could be seen, and the *lamina propria* was intact (Figure 1).

Pathological Score of Intestinal Tissues

In the Sham group, the pathological score was 0 point, and the intestinal mucosa and villi had normal structure. Compared with that in the Sham group, the pathological score of the intestinal tissues was significantly increased in the

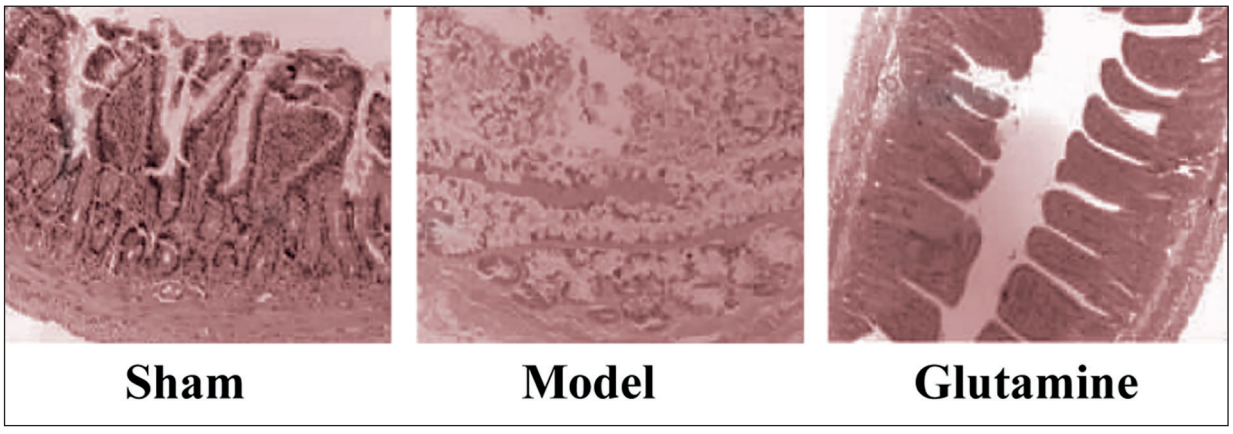

Figure 1. Morphology of intestinal tissues in the three groups (×100).

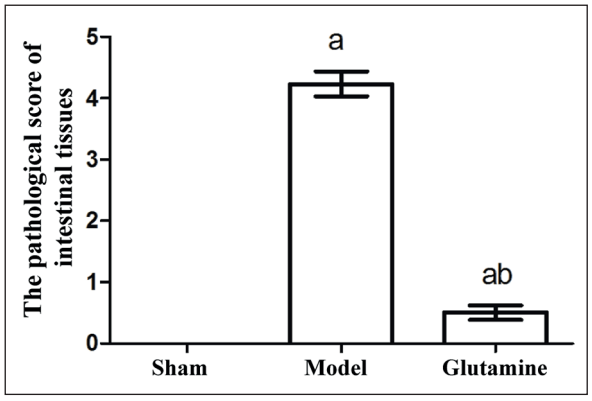

Figure 2. Pathological score of intestinal tissues in the three groups. Note: [a]*vs.* Sham group (p<0.05), [b]*vs.* Model group (p<0.05).

Model group (p<0.05). In the Glutamine group, the pathological score significantly declined after treatment compared with that in the Model group (p<0.05) (Figure 2).

Comparison of Serum TNF-α Content

The content of inflammatory factor TNF-α in the intestinal tissues was the highest in the Model group (p<0.05), and it was lower in the Glutamine group than that in the Sham group (p<0.05), indicating that glutamine can effectively reduce the content of the inflammatory factor TNF-α, exerting a certain protective effect on intestinal tissues (Table I).

Apoptosis Assay

The apoptotic cells were brown. The number of apoptotic intestinal epithelial cells was remarkably increased in the Model group compared with that in the Sham group (p<0.05), and it was remarkably decreased in the Glutamine group compared with that in the Model group (p<0.05) (Model group>Glutamine group>Sham group) (Figure 3).

Table I. Serum TNF-α content in the three groups.

Group	TNF-α content/pg·mL^{-1}
Sham group	120.53 ± 6.08
Model group	139.62 ± 9.17[a]
Glutamine group	125.82 ± 10.37[a,b]

Note: a *vs.* Sham group (p < 0.05), b *vs.* Model group (p < 0.05).

Protein Expression of NF-κB in Intestinal Tissues Detected via Western Blotting

The Model group had a significantly higher protein expression of NF-κB in intestinal tissues than the Sham group and Glutamine group (p<0.05), the Sham group had the lowest protein expression of NF-κB in the intestinal tissues (p<0.05), while the Glutamine group had a significantly lower protein expression of NF-κB in intestinal tissues than in the Model group (p<0.05) (Figure 4).

Discussion

Due to the complex pathogenesis of sepsis, the timeliness of clinical intervention treatment, and the diversity of the body's response to sepsis and clinical treatment[12], the clinical research on the pathophysiology, prevention, and treatment of sepsis is greatly limited. Therefore, the clinical treatment of sepsis is still dominated by symptomatic and supportive treatment[13,14]. The mortality and morbidity rates of sepsis remain high, and sepsis also induces secondary diseases,

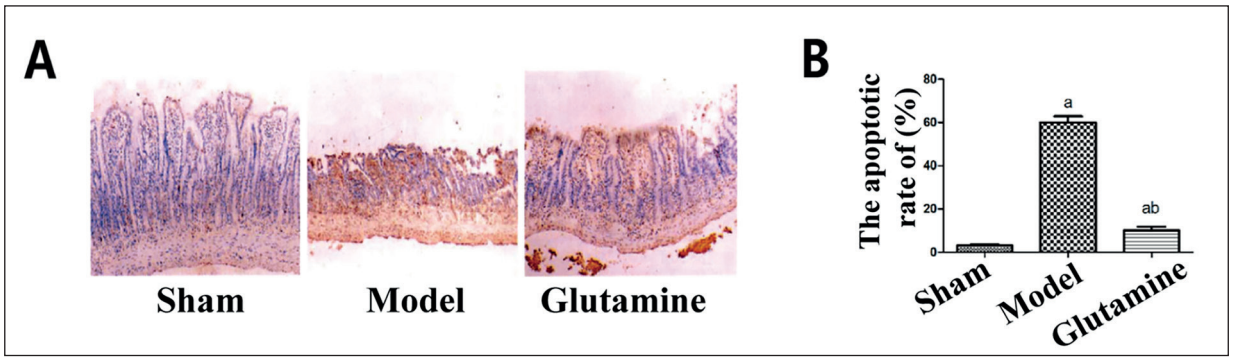

Figure 3. Apoptosis of intestinal epithelial cells in the three groups (×400). ***A,*** Staining of apoptosis of intestinal epithelial cells, ***B,*** comparison of apoptosis rate in rats. [a]*vs.* Sham group (p<0.05), [b]*vs.* Model group (p<0.05).

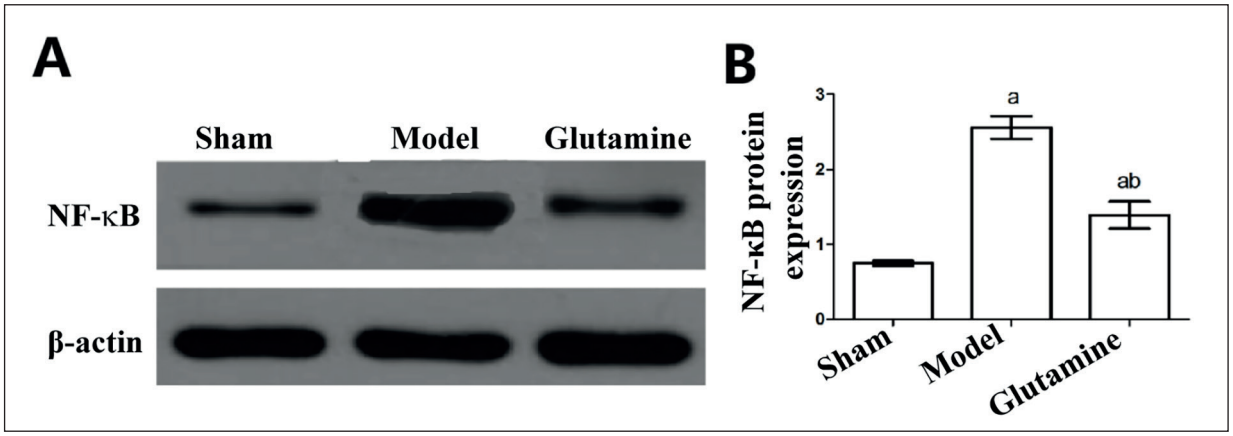

Figure 4. NF-κB protein expression in intestinal tissues. ***A,*** NF-κB protein expression in intestinal tissues in the three groups, ***B,*** comparison of NF-κB protein in intestinal tissues. [a]*vs.* Sham group ($p<0.05$), [b]*vs.* Model group ($p<0.05$).

such as septic shock and multiple organ failure. At present, the understanding of the pathogenesis of human sepsis, at least in part, depends on the laboratory animal models, especially the CLP model. Therefore, the animal model of sepsis was established in this paper to explore the protective effect of glutamine on the intestinal tract of septic rats by inhibiting the NF-κB pathway.

In the Sham group, the rats had normal activity and good mental state, and there were no evident lesions in the abdominal cavity. Compared with the Sham group, the rats in the Model group had very poor mental state and erected hair, and they trembled, and barely moved. After the abdomen was opened, there were bad smell and evident bleeding in the abdominal cavity, and the cecum became black with adhesion and swelling. In the Glutamine group, the symptoms were significantly alleviated compared with the Model group. The morphological observation of the intestinal tissues revealed that in the Sham group, the intestinal villi were regularly and clearly arranged, and there was no congestion in the capillaries. Compared with those in the Sham group, the intestinal villi were disorderly arranged with rupture, and the severe capillary congestion was clearly visible accompanied by ulcer in the Model group. In the Glutamine group, the intestinal villi had normal morphology and regular arrangement after treatment, the subepithelial space was significantly dilated, the capillary dilation and congestion could be seen, and the *lamina propria* was intact. In the Sham group, the pathological score was 0 point, and the intestinal mucosa and villi had normal structure. Compared with that in the Sham group, the pathological score of the intestinal tissues was significantly increased in the Model group ($p<0.05$). In the Glutamine group, the pathological score significantly declined after treatment compared with that in the Model group ($p<0.05$). According to Emadi et al[15], glutamine provides nutrients for intestinal mucosal cell metabolism, which can effectively maintain the integrity of intestinal epithelial cell structure[16]. In the case of intestinal injury, such as sepsis, the content of glutamine in intestinal epithelial cells will significantly decline, the intestinal villi will become scarce and short, and the mucosal atrophy and even shedding will occur, causing certain damage to the intestinal immune function. Moreover, it has also been confirmed in some clinical practices that the administration of glutamine *in vitro* can also effectively prevent intestinal injury, maintain the normal function of intestinal tissues, keep the weight and structure of intestinal mucosa intact, and enhance the cell viability, effectively reducing intestinal diseases caused by inflammatory factors[17].

The detection results showed that the content of the inflammatory factor TNF-α in intestinal tissues was the highest in the Model group ($p<0.05$), and it was lower in the Glutamine group than that in the Sham group ($p<0.05$), indicating that glutamine can effectively reduce the content of the inflammatory factor TNF-α, exerting a certain protective effect on the intestinal tissues. Sepsis is a syndrome caused by a systemic inflammatory response, and TNF-α is an inflammatory factor that promotes sepsis, which is involved in the activation of multiple signal transduction pathways and various kinases and transcriptional regulation, exerting a wide range

of biological effects and ultimately leading to the occurrence and development of sepsis[18]. Cai et al[19] have found that NF-κB can regulate the activation of TNF-α, thereby aggravating sepsis.

The number of apoptotic intestinal epithelial cells was remarkably increased in the Model group compared with that in the Sham group (p<0.05), and it was remarkably decreased in the Glutamine group compared with that in the Model group (p<0.05). There are reports[20] indicating that the immune dysfunction in sepsis is associated with apoptosis of many intestinal cells and immunosuppression, manifested as the decline in the proliferation of intestinal cells. Some studies have also found that apoptosis of the intestinal cells reduces the number of intestinal cells, which will further affect the prognosis of sepsis patients. In addition, the Model group had a significantly higher protein expression of NF-κB in intestinal tissues than in the Sham group and Glutamine group (p<0.05), the Sham group had the lowest protein expression of NF-κB in intestinal tissues (p<0.05), and the Glutamine group had a significantly lower protein expression of NF-κB in intestinal tissues than the Model group (p<0.05). The above findings showed that glutamine treatment could inhibit the expression of NF-κB in intestinal tissues of rats, demonstrating that the anti-inflammatory effect of glutamine is related to the fact that it inhibits the TNF-α expression to suppress NF-κB activity and reduce the inflammatory mediators[21].

Conclusions

We showed that glutamine downregulates the NF-κB signaling pathway, thereby exerting a protective effect on intestinal tissues of sepsis rats.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Bellomo R, Kellum JA, Ronco C, Wald R, Martensson J, Maiden M, Bagshaw SM, Glassford NJ, Lankadeva Y, Vaara ST, Schneider A. Acute kidney injury in sepsis. Intensive Care Med 2017; 43: 816-828.
2) Li JM, Zhang H, Zuo YJ. MicroRNA-218 alleviates sepsis inflammation by negatively regulating VOPP1 via JAK/STAT pathway. Eur Rev Med Pharmacol Sci 2018; 22: 5620-5626.
3) Rhodes A, Evans LE, Alhazzani W, Levy MM, Antonelli M, Ferrer R, Kumar A, Sevransky JE, Sprung CL, Nunnally ME, Rochwerg B, Rubenfeld GD, Angus DC, Annane D, Beale RJ, Bellinghan GJ, Bernard GR, Chiche JD, Coopersmith C, De Backer DP, French CJ, Fujishima S, Gerlach H, Hidalgo JL, Hollenberg SM, Jones AE, Karnad DR, Kleinpell RM, Koh Y, Lisboa TC, Machado FR, Marini JJ, Marshall JC, Mazuski JE, McIntyre LA, McLean AS, Mehta S, Moreno RP, Myburgh J, Navalesi P, Nishida O, Osborn TM, Perner A, Plunkett CM, Ranieri M, Schorr CA, Seckel MA, Seymour CW, Shieh L, Shukri KA, Simpson SQ, Singer M, Thompson BT, Townsend SR, Van der Poll T, Vincent JL, Wiersinga WJ, Zimmerman JL, Dellinger RP. Surviving sepsis campaign: international guidelines for management of sepsis and septic shock: 2016. Crit Care Med 2017; 45: 486-552.
4) Smith JM, Skeans MA, Horslen SP, Edwards EB, Harper AM, Snyder JJ, Israni AK, Kasiske BL. OPTN/SRTR 2015 annual data report: intestine. Am J Transplant 2017; 17 Suppl 1: 252-285.
5) Moor K, Diard M, Sellin ME, Felmy B, Wotzka SY, Toska A, Bakkeren E, Arnoldini M, Bansept F, Co AD, Voller T, Minola A, Fernandez-Rodriguez B, Agatic G, Barbieri S, Piccoli L, Casiraghi C, Corti D, Lanzavecchia A, Regoes RR, Loverdo C, Stocker R, Brumley DR, Hardt WD, Slack E. High-avidity IgA protects the intestine by enchaining growing bacteria. Nature 2017; 544: 498-502.
6) Zhou L, Hu Y, Li C, Yan Y, Ao L, Yu B, Fang W, Liu J, Li Y. Levo-corydalmine alleviates vincristine-induced neuropathic pain in mice by inhibiting an NF-kappa B-dependent CXCL1/CXCR2 signaling pathway. Neuropharmacology 2018; 135: 34-47.
7) Shi C, Wang Y, Guo Y, Chen Y, Liu N. Cooperative down-regulation of ribosomal protein L10 and NF-kappaB signaling pathway is responsible for the anti-proliferative effects by DMAPT in pancreatic cancer cells. Oncotarget 2017; 8: 35009-35018.
8) Yang Y, Wang X, Zhang Y, Yuan W. Hepatitis B virus X protein and proinflammatory cytokines synergize to enhance TRAIL-induced apoptosis of renal tubular cells by upregulation of DR4. Int J Biochem Cell Biol 2018; 97: 62-72.
9) Cluntun AA, Lukey MJ, Cerione RA, Locasale JW. Glutamine metabolism in cancer: understanding the heterogeneity. Trends Cancer 2017; 3: 169-180.
10) Danielski LG, Giustina AD, Goldim MP, Florentino D, Mathias K, Garbossa L, de Bona SR, Laurentino A, Goulart M, Michels M, de Queiroz KB, Kohlhof M, Rezin GT, Fortunato JJ, Quevedo J, Barichello T, Dal-Pizzol F, Coimbra RS, Petronilho F. Vitamin B6 reduces neurochemical and long-term cognitive alterations after polymicrobial sepsis: involvement of the kynurenine pathway modulation. Mol Neurobiol 2018; 55: 5255-5268.
11) Párraga Ros E, Correa-Martín L, Sánchez-Margallo FM, Candanosa-Aranda IE, Malbrain MLNG, Wise R, Latorre R, López Albors O, Castellanos G. Intestinal histopathological changes in a porcine model of

pneumoperitoneum-induced intra-abdominal hypertension. Surgical Endoscopy 2018; 32: 3989-4002.

12) Jensen JS, Peters L, Itenov TS, Bestle M, Thormar KM, Mohr TT, Lundgren B, Grarup J, Lundgren JD; Procalcitonin And Survival Study (PASS) Group. Biomarker-assisted identification of sepsis-related acute liver impairment: a frequent and deadly condition in critically ill patients. Clin Chem Lab Med 2019. pii: /j/cclm.ahead-of-print/cclm-2018-1350/cclm-2018-1350.xml. doi: 10.1515/cclm-2018-1350. [Epub ahead of print].

13) Tai LH, Ananth AA, Seth R, Alkayyal A, Zhang J, de Souza CT, Staibano P, Kennedy MA, Auer RC. Sepsis increases perioperative metastases in a murine model. BMC Cancer 2018; 18: 437.

14) Vallabhajosyula S, Jentzer JC, Geske JB, Kumar M, Sakhuja A, Singhal A, Poterucha JT, Kashani K, Murphy JG, Gajic O, Kashyap R. New-onset heart failure and mortality in hospital survivors of sepsis-related left ventricular dysfunction. Shock 2018; 49: 144-149.

15) Emadi A, Law JY, Strovel ET, Lapidus RG, Jeng L, Lee M, Blitzer MG, Carter-Cooper BA, Sewell D, Van Der Merwe I, Philip S, Imran M, Yu SL, Li H, Amrein PC, Duong VH, Sausville EA, Baer MR, Fathi AT, Singh Z, Bentzen SM. Asparaginase Erwinia chrysanthemi effectively depletes plasma glutamine in adult patients with relapsed/refractory acute myeloid leukemia. Cancer Chemother Pharmacol 2018; 81: 217-222.

16) Cormerais Y, Massard PA, Vucetic M, Giuliano S, Tambutte E, Durivault J, Vial V, Endou H, Wempe MF, Parks SK, Pouyssegur J. The glutamine transporter ASCT2 (SLC1A5) promotes tumor growth independently of the amino acid transporter LAT1 (SLC7A5). J Biol Chem 2018; 293: 2877-2887.

17) Tian L, Zhou XQ, Jiang WD, Liu Y, Wu P, Jiang J, Kuang SY, Tang L, Tang WN, Zhang YA, Xie F, Feng L. Sodium butyrate improved intestinal immune function associated with NF-kappaB and p38MAPK signalling pathways in young grass carp (Ctenopharyngodon idella). Fish Shellfish Immunol 2017; 66: 548-563.

18) Galbraith GM, Steed RB, Sanders JJ, Pandey JP. Tumor necrosis factor alpha production by oral leukocytes: influence of tumor necrosis factor genotype. J Periodontol 1998; 69: 428-433.

19) Cai X, Cao C, Li J, Chen F, Zhang S, Liu B, Zhang W, Zhang X, Ye L. Inflammatory factor TNF-α promotes the growth of breast cancer via the positive feedback loop of TNFR1/NF-κB (and/or p38)/p-STAT3/HBXIP/TNFR1. Oncotarget 2017; 8: 58338-58352.

20) Kawakami K, Minami N, Matsuura M, Iida T, Toyonaga T, Nagaishi K, Arimura Y, Fujimiya M, Uede T, Nakase H. Osteopontin attenuates acute gastrointestinal graft-versus-host disease by preventing apoptosis of intestinal epithelial cells. Biochem Biophys Res Commun 2017; 485: 468-475.

21) El-Ansari R, Craze ML, Alfarsi L, Soria D, Diez-Rodriguez M, Nolan CC, Ellis IO, Rakha EA, Green AR. The combined expression of solute carriers is associated with a poor prognosis in highly proliferative ER+ breast cancer. Breast Cancer Res Treat 2019: 175: 27-38.