Esmolol inhibits inflammation and apoptosis in the intestinal tissue via the overexpression of NF-κB-p65 in the early stage sepsis rats

Chang-An Guo[1,2,3], Li Ma[4]*, Xiao-Lu Su[5], Ying-Zhen Wang[4], Ling-Ling Zhen[4], Bei Zhang[4], Hong An[4], Hong-Bin Liu[3]**

[1]Second Clinical Medical College, Lanzhou University, Gansu Province, China
[2]First Aid Center, Lanzhou University Second Hospital, Gansu Province, China
[3]Department of General Surgery, The 940th Hospital of Joint Logistics Support Force of Chinese People's Liberation Army, China
[4]Intensive Care Unit, Lanzhou University Second Hospital, Gansu Province, China
[5]Department of Pathology, Lanzhou University Second Hospital, Gansu Province, China

Cite this article as: Guo CA, Ma L, Su XL, et al. Esmolol inhibits inflammation and apoptosis in the intestinal tissue via the overexpression of NF-κB-p65 in the early stage sepsis rats. Turk J Gastroenterol 2020; 31(4): 331-41.

ABSTRACT

Background/Aims: Accumulating evidence reveals esmolol could protect the gut mucosa through the regulation of immune response and inflammation in
patients with sepsis. However, its underlying mechanism is not fully understood.

Materials and Methods: Diamine oxidase (DAO), intestinal fatty acid-binding protein (I-FABP), interleukin (IL)-6, and IL-10 in the plasma of rats were detected by ELISA assay. Western blotting was utilized to measure the expression levels of NF-kappa B-p65, Bcl-2, and cleaved caspase-3 in the intestinal tissues. The survival analysis was performed in each group.

Results: The plasma levels of DAO and IL-10 levels were increased, whereas that of I-FABP and IL-6 were decreased in the sepsis rats after esmolol treatment, indicating that after the esmolol treatment, the intestinal inflammation and damages were remarkably reduced as compared to those in the normal saline treated sepsis rats. NF-κB-p65 and Bcl-2 were highly expressed, but cleaved caspase-3 showed lower expression in the esmolol treated groups. However, at the same time, we observed contrasting results in the normal saline treated group. Western blotting data indicated that the esmolol treatment inhibited the inflammation and apoptosis in the intestinal tissue due to the overexpression of NF-κB-p65 in the celiac sepsis rats. The survival analysis results indicate that the esmolol infusion should be used in the early stages sepsis rats.

Conclusion: Esmolol can suppress inflammation and apoptosis in the intestinal tissue via the overexpression of NF-kappa B-p65 in the early stage sepsis rats. kappa BEarly-stage use of esmolol might be an ideal treatment method for sepsis.

Keywords: Esmolol, intestine, sepsis, apoptosis, NF-kappa B

INTRODUCTION

Serious bacterial infection can lead to sepsis, multiple organ dysfunction syndrome (MODS), and septic shock (1, 2). Although we have made delectable progress in the treatment of sepsis, septic shock accounts for 10% of the patients in the Intensive Care Unit (ICU), and 20% - 30% of all mortality in the ICU patients (3, 4).

The intra-abdominal infections (IAIs) related mortality is the second most common event in the ICU (5). The intestine is the first organ to be affected by the development and deterioration of IAIs. The intestinal mucosa of all mammals is an important physical barrier to separate the gut content and bacteria from the internal environment. Its integrity or incompletion plays a crucial role in the onset of sepsis, and its deterioration and recovery (6). Sepsis induces multiple dysfunctions in the intestinal mucosal epithelium, such as the increase in cytokine production (7) and epithelial cell apoptosis (8, 9). Recent studies have reported that preventing sepsis-induced apoptosis of the intestinal mucosal cells can dramatically improve the survival rate of the patients with sepsis (10).

The autonomic nervous system and host immune system play a key role in the resistance to infection, but this resistance is commonly excessive and detrimental. The sympathetic overstimulation and the overactivation of catecholamine and the inflammatory factors released can drive a positive feedback circulation of the cardiovascular and other organ dysfunction (11). The previous study has indicated that the lower sympathetic level or/and higher

Corresponding Author: Li Ma, Hong-Bin Liu; mali1105@126.com; liuhongbin999@163.com
Received: May 15, 2019 Accepted: July 9, 2019

DOI: 10.5152/tjg.2020.19341

parasympathetic level can remarkably increase the survival rate in the patients with sepsis (12).

Beta-blockade can effectively reduce the pro-inflammatory factors in the body, improve the cardiac function, and enhance the survival rate (13, 14). Esmolol, one of the β1-adrenergic receptor blockers, has been used by the researchers in animal studies on sepsis (15, 16), which demonstrate promising effects in experimental models. However, the exact mechanisms underlying the favorable effects are not fully understood. In this study, a rat sepsis model was established to investigate whether esmolol can alleviate the dysfunctions of the gastrointestinal tract and to elucidate the underlying mechanism of beta-1-blockers in the treatment of the celiac infection.

MATERIALS AND METHODS

Animals

Two hundred and ninety-four SD rats (male, 250-300 g) were purchased from the laboratory animal center in the Basic Medical College of the Lanzhou University. In the present study, we selected 54 rats and randomly divided them into three groups (each group containing 18 rats) as follows: SNS (Sham operation + normal saline (NS) treated); CNS (Cecal ligation and puncture + NS treated), and CE (Cecal ligation and puncture + Esmolol treated). Then, each group was randomly divided into three subgroups (n=6), which included 6hS, 12hS, and 24hS (6, 12, and 24 h subgroups, respectively), according to the esmolol or NS infusion time after the operation. Subsequently, 240 rats were randomly separated into three groups for 7-day survival rates analyses: SNS (n=60), CNS (n=90), and CE (n=90). Then, the rats in each group were randomly divided into three subgroups (6hS, 12hS, and 24hS), as mentioned above.

This study was approved by the experimental animal ethics committee of the Lanzhou University Second Hospital (License number: SCXK 2013-0002). Esmolol (Qilu Pharmaceutical Co., Ltd. Shandong Province, China) and NS (Kelun Pharmaceutical Co., Ltd. Sichuan Province, China) were kindly donated by the pharmacy at the Lanzhou University Second Hospital.

MAIN POINTS

- *Esmolol can suppress inflammation and apoptosis in the intestinal tissue.*
- *Esmolol promote NF-kappa B-p65 and Bcl-2 expression, but inhibit cleaved caspase-3 expression.*
- *Esmolol can suppress inflammation and apoptosis via the overexpression of NF-kappa B-p65.*

Establishment of the animal model

After inducing general anesthesia under isoflurane, the sepsis model of the rats was established via the cecal ligation and puncture (CLP) method, which was performed in the CNS and CE groups, as described previously (17). In the SNS group, we just separated the cecum and mesentery, but the intestinal gut was not ligated and punctured. We settled the infusion tube in the internal jugular vein by catheterization. Subsequently, the rats received an immediate subcutaneous injection of 2 mL NS to compensate for the insensible fluid losses that occurred during the surgery. The esmolol solution (esmolol injection was diluted to 5 mg/mL with normal saline) was continuously injected into the internal jugular vein of the rats at a flow velocity of 1 mL/h in the 6hS, 12hS, and 24hS subgroups in the CE group. NS was continuously injected at a flow velocity of 1 mL/h in the other groups. The fluid was pumped at a flow velocity, which was determined according to the water intake and urine volume of the rat, as described previously (18). To maintain the liquid input even, we used an infusion pump (Zhejiang Smith Medical instrument Co., Ltd., China) for continuous pumping in this experiment. After the liquid was infused, the neck incision of the rats was sutured, and fasting and water insufflation were performed. Except for the 7-day survival analyses rats (240 rats), all the other rats (54 rats) were sacrificed at 24 h from the starting point of the procedure.

Enzyme-linked immunosorbent assay

Each sacrificed rat was used to collect the blood samples (4 mL), which were incubated at room temperature for 2 h and then centrifuged to obtain the plasma. The enzyme-linked immunosorbent assay (ELISA) kits were used to detect the rat plasma levels of IL-6, IL-10, Diamine oxidase (DAO), and intestinal fatty acid-binding protein (I-FABP). The specific procedure was carried out strictly according to the manufacturer's instructions, and the absorbance of all the objects was measured at 450 nm using a microplate reader (Thermo Fisher, China).

Western blotting

The intestinal tissues, which were obtained from each of the 24 h subgroups, were ground into tissue homogenates and lysed for 30 min on ice in the radioimmunoprecipitation assay (RIPA) buffer. Then, the supernatants were used for the western blot analysis of NF-kappa B-p65, Bcl-2, and cleaved caspase-3 proteins. The samples were

subjected to 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to the nitrocellulose membranes. Then, the blots were detected using the primary antibodies (Wuhan Fine Biotech Co., Ltd. Hubei Province, China). After washing 4 times with PBS-Tween 20, horseradish peroxidase-conjugated secondary antibodies were added. The procedure of Western blotting was performed as previously described (19). The expression of β-actin was used to show equal protein loading. The blots were detected using the improved chemiluminescence reaction. Gray quantification of the protein bands was measured by the ImageJ software. All the experiments were carried out at least 3 times.

Hematoxylin Eosin (HE)staining

To observe the morphological and inflammatory changes under the binocular optical microscope, the fresh intestinal tissues of the rats were washed with NS 3 times, fixed with 10% neutral formaldehyde buffer, dehydrated, treated with paraffin, sliced with microtome, roasted, dewaxed, and stained with HE. The lengths (μm) of the small intestine villus (crypt neck to the villus tip) in 24 h group were measured.

Statistical analysis

The data are shown as mean±SD. The data analysis was performed using the Statistical Packages for the Social Sciences (SPSS) version 23.0 software (IBM Corp.; Armonk, NY, USA). The statistical significance of the differences in the continuous variable data between multiple groups was evaluated using a one-way analysis of variance (ANOVA) LSD comparison test. The survival analysis of the rats was estimated by the Kaplan-Meier method and compared by the log-rank test. The rates were compared using the Chi-square test. $p<0.05$ was considered statistically significant.

RESULTS

Esmolol decreases levels of I-FABP but increases the activity levels of DAO in plasma

The ELISA data showed that the concentration of DAO in the 6hS, 12hS, and 24hS subgroups in the CNS and CE groups were significantly lower than corresponding those in the SNS group. However, the concentrations of DAO in the 6hS, 12hS, and 24hS subgroups in the CE group were higher than corresponding those in the CNS group in a treatment time-dependent manner (CE6hS vs CE12hS, $p>0.05$; CE6hS vs CE24hS, $p<0.05$; CE12hS vs CE24hS, $p<0.05$) (Figure 1a). The concentration of I-FABP in the 6hS, 12hS, and 24hS in the CNS and CE groups were significantly higher than those in the SNS group. However, the concentration of I-FABP in the 6hS, 12hS, and 24hS subgroups in the CE group were lower than corresponding those in the CNS group in a treatment time-dependent manner (CE6hS vs CE12hS, $p>0.05$; CE6hS vs CE-24hS, $p<0.05$; CE12hS vs CE24hS, $p<0.05$) (Figure 1b). The test results for I-FABP and DAO were as per the morphological changes in the intestine (HE stain), indicating that the rats treated with esmolol had relatively few lesions than those treated with NS, including the epithelial cell swelling and disappearing, villus shortening, and gland structure destruction (Figures 2, 3).

Esmolol reduces the concentration levels of IL-6 and enhances the levels of IL-10

The concentrations of IL-6 and IL-10 in the 6hS, 12hS, and 24hS subgroups in the CNS and CE groups were significantly higher than those in the SNS group. The concentration of IL-6 in the 6hS, 12hS, and 24hS in the CE group were lower than corresponding those in the CNS group (Figure 1c). The concentration of IL-10 in the 6hS, 12hS, and 24hS subgroups in the CE group were higher than corresponding those in the CNS group (Figure 1d). The IL-6 levels were reduced but the IL-10 levels were elevated in a treatment time-dependent manner (6hS, 12hS, and 24hS) in the CE group (CE6hS vs CE-12hS, $p<0.05$; CE6hS vs CE24hS, $p<0.05$; CE12hS vs CE-24hS, $p<0.05$). These results are as per the morphological changes observed in the intestine (HE stain), which showed that the rats treated with esmolol had relatively less inflammatory cells infiltrating than that in the rats treated with NS (Figure 2).

Statistical analysis

The Kaplan-Meier analysis (log-rank test) was used to analyze the 7-day survival rates after surgery. The 6hS, 12hS, and 24hS subgroups in the CE groups had higher 7-day survival rates than those in the corresponding subgroups of the CNS group. The 12hS and 24hS subgroups had higher 7-day survival rates than the 6hS subgroup in the CE group. There was no difference in the survival rate between the 12hS and 24hS subgroups in the CE group (Figure 4).

Esmolol inhibits apoptosis via the overexpression of NF-kappa B-p65

As compared to the 24hS subgroup of the SNS group, there was no significant difference in the protein expression levels of NF-kappa B-p65 and Bcl-2 in the 24hS subgroup of the CNS group, but the expression levels of cleaved caspase-3 elevated significantly in the 24hS

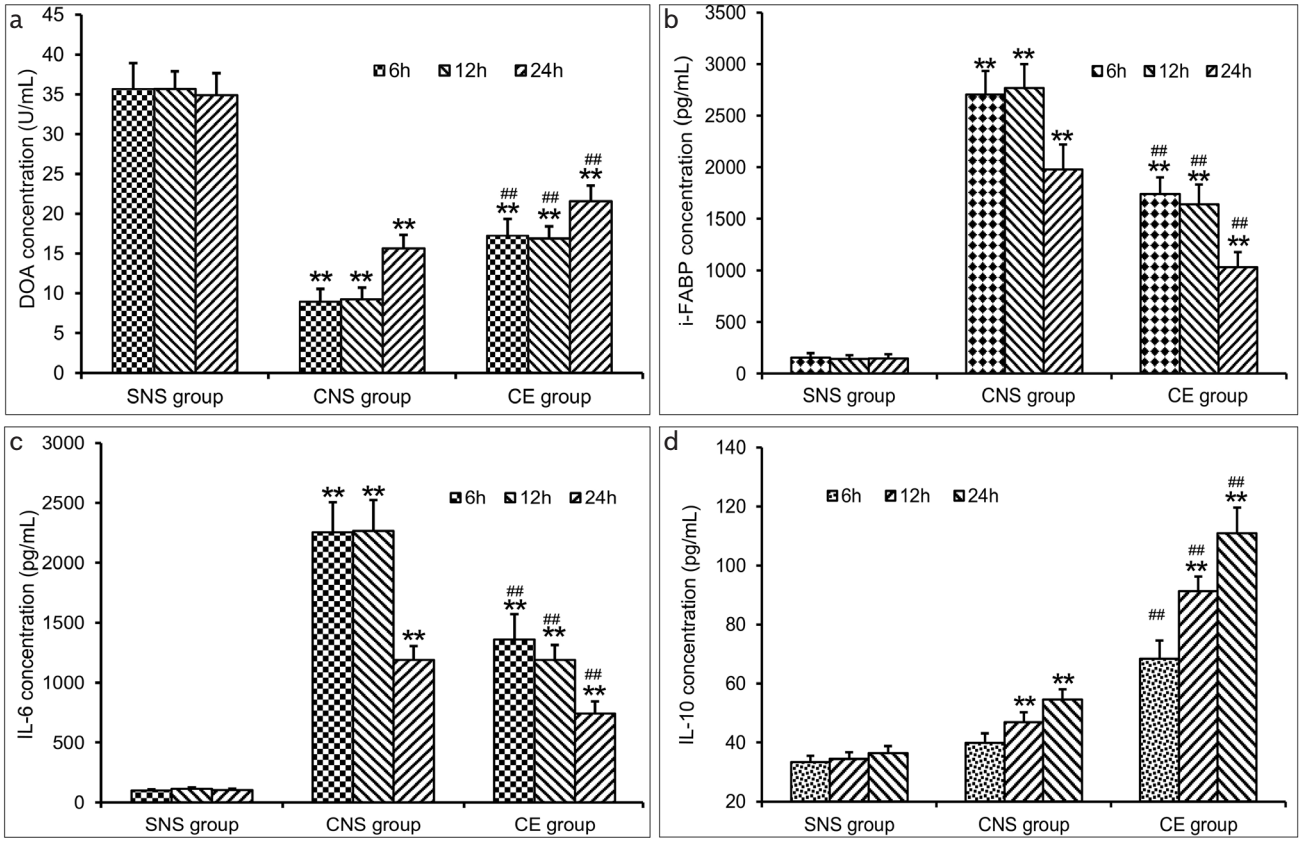

Figure 1. a-d. ELISA assay shows that esmolol increases DAO and IL-10 concentration and decrease i-FABP and IL-6 concentration in sepsis rat plasmas. a, d) For the DAO concentrations, which in corresponding subgroups of CNS and CE group lower than those in SNS group, but which in CE group higher than those in CNS group. b, c) For the I-FABP or IL-6 concentrations, which in corresponding subgroups of CNS and CE group higher than those in SNS group, but which in CE group lower than those in CNS group. D. For the IL-10 concentrations, which in corresponding subgroups of CNS and CE group higher than those in SNS group, but which in CE group higher than those in CNS group. The asterisk (*) means corresponding subgroups of SNS group compared with those in CNS group and CE group * $p<0.05$, ** $p<0.01$. The well number (#) means corresponding subgroups of CNS group compared with those in CE group # $p<0.05$, ## $p<0.01$.

subgroup of the CNS group ($p<0.01$). The treatment with esmolol for 24 h significantly elevated the protein expression levels of NF-kappa B-p65 and Bcl-2 in the CE group as compared to that in the corresponding SNS and CNS groups, but the protein expression levels of cleaved caspase-3 in the CE group were lower than that in the CNS group ($p<0.01$) (Figure 5).

DISCUSSION

Serious bacterial infection can lead to sepsis syndrome, in which the cytokines are overactivated to resist the pathogen, leading to immune disorder (20). According to the 2012 "Surviving Sepsis Campaign Guidelines", sepsis can be classified into simple sepsis, severe sepsis, and septic shock in terms of increasing severity (21). Simple sepsis is early state sepsis, which is usually defined as a systemic inflammatory response syndrome (SIRS), commonly caused by the newly established infection; it can progress to severe sepsis or septic shock and may not be diagnosed accurately and on time (22). Sepsis that is complicated by multiple organ dysfunction is termed as severe sepsis (3, 21). Septic shock is defined as the most severe result of sepsis, in which the abnormalities related to multiple organ dysfunction are associated with higher mortality than the early-stage sepsis alone (4). In summary, as with the treatment of early oncological diseases, the treatment of early-stage sepsis is crucial and can stop the disease from developing, preventing it from progressing further and becoming a life-threatening condition.

The intestine plays a key role in the occurrence and development of sepsis and is commonly characterized as

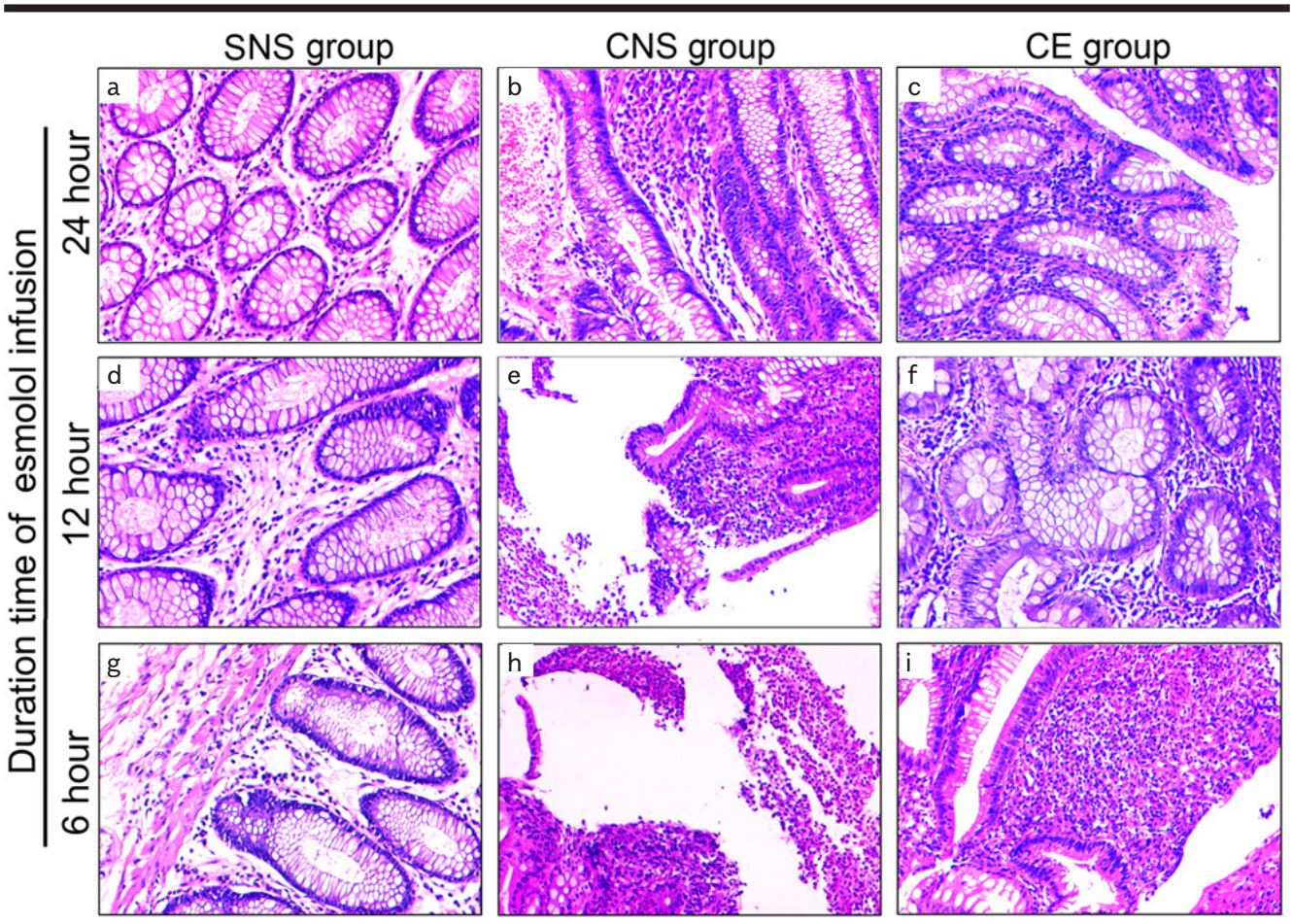

Figure 2. a-i. Intestinal tissue pathological changes of sepsis rats. Rats were treated with esmolol for 6h, 12h and 24h, intestinal tissues from SNS, CNS and CE group were stained with HE, inflammatory changes were observed under microscope. No significant pathological changes were found in Sham group (a, d, g). Significant pathological changes of tissue were found in each subgroups of CNS group, which include epithelial cells swell and destroyed, tissue necrogenesis, inflammatory cells infiltrating (b, e, h): b) epithelial cells appear swell, relative less inflammatory cells infiltrating. e, h) epithelial cells appear swelling and destroyed, tissue necrogenesis, inflammatory cells infiltrating. These tissue destroy and inflamatic changes were reduced in each corresponding subgroups of CE group (c, f, i). HE staining 200 ×.

the "motor" of MODS (23). The intestinal mucosal barrier gets destroyed by the endogenous cytokines, which results in the leakage of toxic antigens and the translocation of the bacteria from the gut into the tissues and circulatory system, finally leading to MODS (24).

The homeostatic regulation, cardiovascular system, immune system, and other organs are regulated by the adrenergic system. A lot of lymphoid organs and cells express the β-adrenergic receptors on their surfaces. Sympathetic activation is a well-known feature of sepsis, which may regulate the immune and inflammation state. In the early stages of sepsis, the plasma levels of catecholamine are increased and the β-adrenoceptors are stimulated excessively, which leads to immunosuppression and the downregulation of the receptors (25). In severe septic shock, the catecholamines may lead to an imbalance between the vasoconstriction and vasodilation due to which the splanchnic (except heart and brain) and skin blood volume may reduce in a relatively short time, which may be pernicious over a long time. The stress-related gastrointestinal mucosal injury that is caused by sepsis is one of the severe complications in sepsis (26). A recent study has proposed that the β-blockers might have remarkable therapeutic potential in the treatment of lethal sepsis by attenuating the sympathetic activation and excessive inflammatory responses (27). Esmolol is a safe medicine that has a rapid onset of action and is elim-

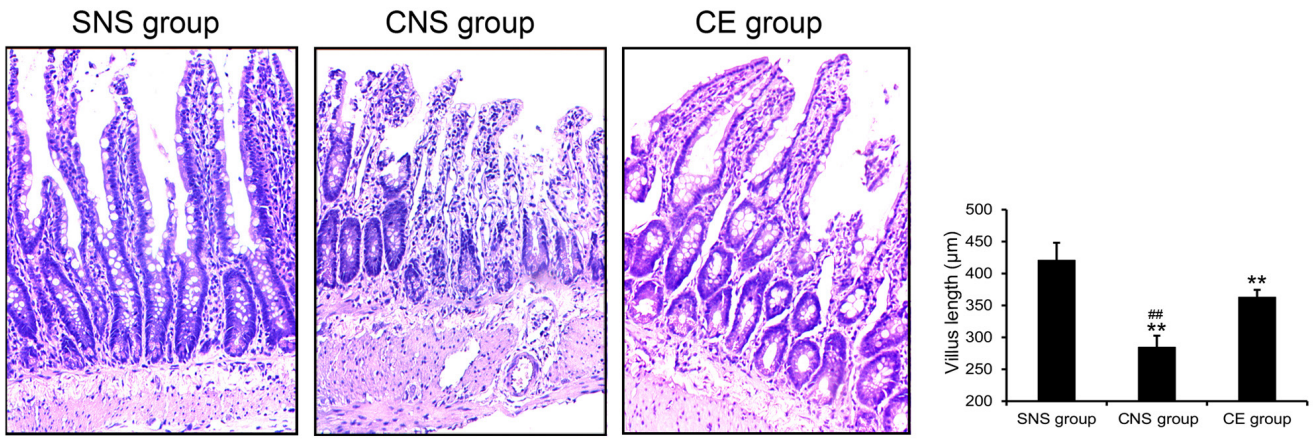

Figure 3. Villus length was quantified in sections of small intestinal. Sepsis-induced villus atrophy is exacerbated in CNS group (HE stain). CNS and CE group rats had markedly shorter villi than SNS group. CNS group had significantly more serious villus atrophy and shorter villi than CE group. Magnification 100x. Villus length was quantified in sections of small intestinal. n=6/group. The asterisk (*) means the villus length in CNS and CE group compared with those in SNS group. ** p<0.01. The well number (#) means subgroups of CNS group compared with corresponding those in. ## p<0.01.

inated in the body in a relatively short time; it not only has some potential treatment effects but can also protect the gut mucosa in sepsis (27). However, the therapeutic effects and underlying mechanism of action of esmolol in sepsis are not fully understood.

I-FABP expresses in the mucosal epithelial cells in all of the small intestine and partially in the colon tissues (28). Once the intestinal epithelial cells and cell junctions are injured, I-FABP leaks into the intercellular space from the enterocytes and permeates into the blood plasma. The plasma concentration of I-FABP was strongly associated with the severity of intestinal inflammation and injury (29). Our data shows that the plasma levels of I-FABP in the 6hS, 12hS, and 24hS subgroups of the CNS and CE groups were higher than those in the SNS group, which is in accordance with the previous studies that showed that the intestine injured by the damage factors has a significant elevation in the plasma levels of I-FABP (30). The rats were treated with esmolol for 6 h, 12 h, and 24 h separately, and the plasma concentrations of I-FABP in each subgroup showed lower values than those in the CNS group in a treatment time-dependent manner (CE6hS vs CE12hS, $p>0.05$; CE6hS vs CE24hS, $p<0.05$; CE12hS vs CE24hS, $p<0.05$). This result indicates that the esmolol treatment for 6 h, 12 h, and 24 h can remarkably reduce the damages to the intestinal mucosa in a treatment time-dependent manner as compared to those in the CNS group; this is because the plasma concentration of I-FABP was strongly associated with the severity of the intestinal inflammation and injury.

DAO, which can inhibit intestinal allergy, inflammation, and injury by eliminating histamine and its analogues, is a highly active enzyme that mainly localizes in the intracellular intestinal mucosa of the mammalian species, including humans (31). Previous studies have suggested that the intestinal DAO activity positively correlates with the plasma levels of DAO in the maturing rats (32). Any adverse effects on the intestine, which include inflammation, injury or ischemia, can cause marked reduction in the plasma activity levels of DAO and elevation in the levels of histamine. In our study, the plasma activity levels of DAO in the CNS and CE groups were lower than those in the SNS group. This result is consistent with the previous study, which showed that the intestine injured by the adverse factors has low activity levels of DAO, indicating that the celiac disease-related sepsis can cause injury to the intestinal mucosa, leading to a decrease in the activity levels of DAO (31). The excessive accumulation of histamines caused by the decline in the activity levels of DAO can cause injuries in the intestinal mucosa, triggering a vicious cycle that leads to a reduction in the activity levels of DAO in return. The sepsis model rats were treated with esmolol for 6 h, 12 h, and 24 h, and the plasma concentrations of DAO were found to be significantly higher than those in the CNS group in a treatment time-dependent manner (CE6hS vs CE12hS, $p>0.05$; CE6hS vs CE24hS, $p<0.05$; CE12hS vs CE24hS, $p<0.05$). This result indirectly

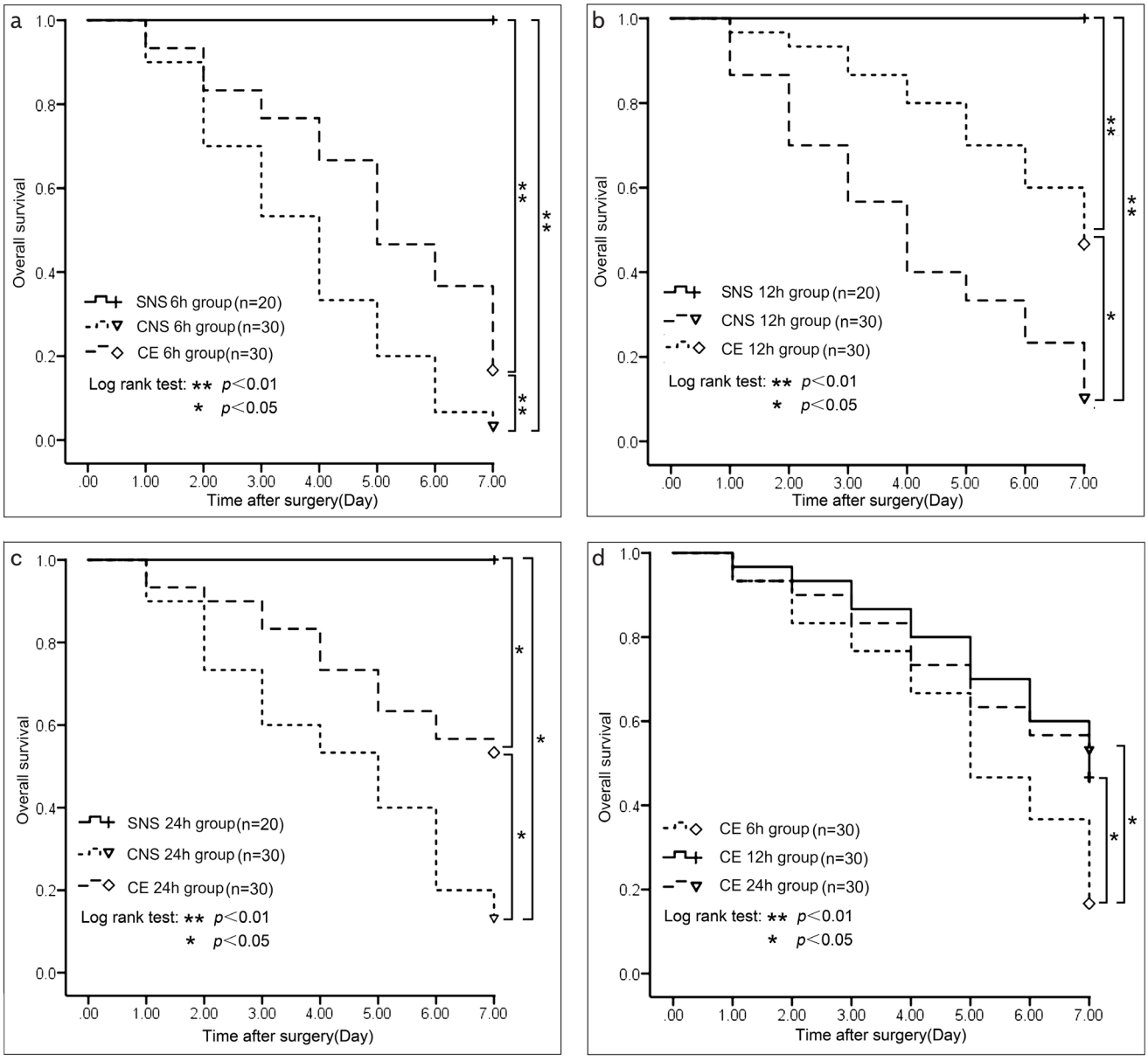

Figure 4. a-d. Survival analysis of rats in SNS, CNS and CE groups. The 6hS, 12hS and 24hS mortalities in CEgroup lower than corresponding those in CNS group. The 12hS and 24hS have higher survival rates than 6hS in CE group, but there is no survival rate difference between 12hS and 24hS in CE group (* p<0.05, ** p<0.01). All sham animals survived.

indicates that the esmolol treatment for 6 h, 12 h, and 24 h remarkably reduced the damages to the intestinal mucosa in a treatment time-dependent manner as compared to those in the CNS group; this is because the plasma levels of DAO strongly associated with the severity of the intestinal inflammation and injury (31). The protective effect of esmolol was also reflected in the morphological changes observed in the corresponding intestinal tissue.

IL-6 is one of the important pro-inflammatory cytokines, which is mainly released by the monocytes or macrophages (33). IL-10 is a critical suppressive cytokine of the inflammatory and immune response, which plays an important role in the pathophysiology of infection (34). In this study, the plasma levels of IL-6 and IL-10 were selected as the inflammatory markers to evaluate the severity of the intestinal inflammation and injury in the

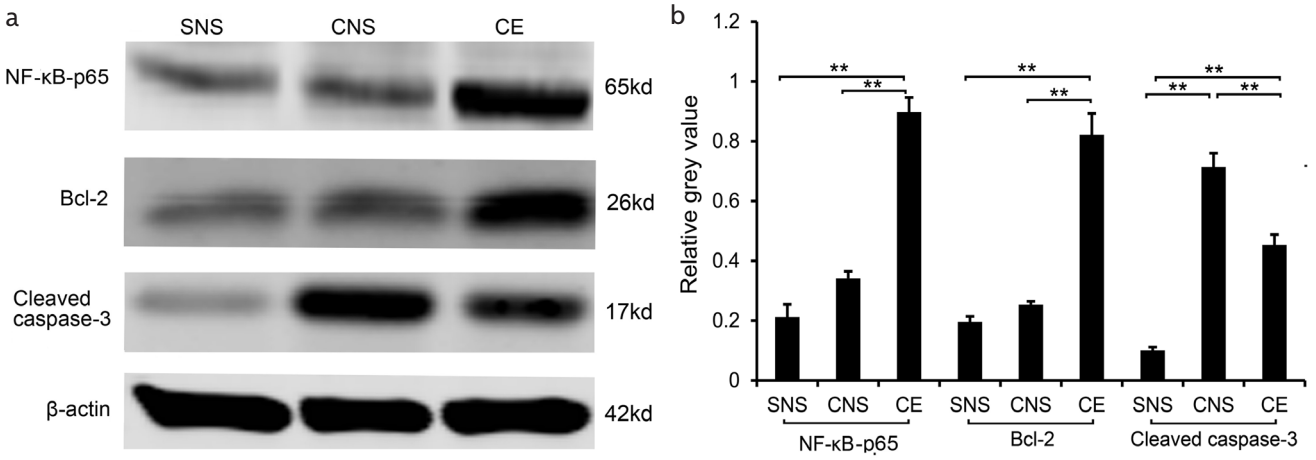

Figure 5. a, b. Esmolol inhibits apoptosis via NF-kappa B-p65 overexpression. For 24 hours treated subgroups, there is no significant difference of NF-kappa B-p65 and Bcl-2 between CNS and SNS group, but those in CE group has a significant higher expression than SNS group. The level of cleaved caspase-3 has a significantly higher level in CNS group than SNS and CE group (**p<0.01).

celiac disease-related sepsis, as shown in the previous studies (35). We observed that the plasma levels of IL-6 and IL-10 in the CNS and CE groups were significantly higher than those in the SNS group, which is consistent with the previous studies that showed that the inflammation was activated by celiac sepsis and the production of pro-inflammatory factor IL-6 and anti-inflammatory factor IL-10 were increased in sepsis (36). Also, as compared with the levels of IL-6 and IL-10 in the 6hS, 12hS, and 24hS subgroups of the CNS group, the corresponding subgroups in the CE group had higher IL-10 but lower IL-6 levels. The IL-10 levels were elevated but the IL-6 levels were reduced in a treatment time-dependent manner (6hS, 12hS, and 24hS) in the CE group (CE6hS vs CE12hS, $p<0.05$; CE6hS vs CE24hS, $p<0.05$; CE12hS vs CE24hS, $p<0.05$).

Given the inflammation-promoting effects of IL-6 and the inflammation inhibitory effects of IL-10 (33, 34), the time-dependent changes in the plasma concentrations of IL-6 and IL-10 in the CE group directly reflect the inflammation inhibitory effects of esmolol. Apoptosis, mediated by several anti-apoptotic and pro-apoptotic proteins, is a tightly regulated cell death process that plays an important role in inflammation (37, 38). Bcl-2 and caspase-3 proteins are the two important agents that suppress and promote cell apoptosis (38, 39). The morphological changes (HE staining) observed in the intestine of the esmolol treated group had relatively less cell apoptosis lesion than those in the NS treated group. This result is per that of the CNS group, which showed lower levels of Bcl-2 but higher levels of cleaved caspase-3 than the CE group. Therefore, it can be speculated that esmolol can partially inhibit the sepsis-induced cell apoptosis and necrosis in the intestinal mucosa by enhancing the levels of Bcl-2 protein and reducing the levels of cleaved caspase-3 protein.

NF-kappa B-p65 is one of a NF-kappa Bkappa B family proteins that include 5 family members, p50, p52, p65, RelB, and cRel, which commonly exist as inactive dimers in the cytoplasm in combination with inhibition kappa B (Ikappa B). In this study, we selected NF-kappa B-p65 as a marker to investigate the effects of esmolol on the treatment of sepsis. The activated NF-kappa B binds with the target genes and exerts inhibitory or promoting effects on inflammation and apoptosis (40). NF-kappa B can increase the plasma and tissue levels of the pro-inflammatory cytokines (IL-6 and TNF-α), which can amplify the inflammation response and prolong the duration of inflammation (41). However, the pro-inflammatory cytokines directly or indirectly activate the NF-kappa B pathway, establishing a positive feedback loop that can strengthen the inflammatory response. Therefore, inhibiting the NF-kappa B activity is a therapeutic method in the treatment of inflammation (42). However, NF-kappa B sometimes works as an anti-inflammatory factor and influences the production of IL-10 (43, 44). Many studies have indicated that increased levels of NF-kappa B can play both the beneficial and adverse roles in sepsis (45, 46).

As the enhanced activity of NF-kappa B can lead to the alleviation of inflammation and apoptosis in the intesti-

nal tissues of murine models (47), but a reduction in the NF-kappa B activity can lead to exacerbation of inflammation and apoptosis in the intestinal tissue (48, 49). The previous study has also reported that the decreased levels of NF-kappa B can lead to exacerbation of inflammation and apoptosis in the intestinal tissue in the CLP model (50). Interestingly, the western blot analysis shows that those in the CE group had higher levels of NF-kappa B-p65 than those in the CNS group. In this case, we speculate that the relevant molecular mechanism of anti-apoptosis and anti-disruption effects of esmolol may be mediated through the enhanced levels of NF-kappa B in the intestine with sepsis.

Although NF-kappa B plays an advantageous role in the intestinal tissue with sepsis, low NF-kappa B activity induces a significant increase in apoptosis and a remarkable decrease in inflammation in ischemia and reperfusion experiments (51). In this case, the treatment method of NF-kappa B inhibition in sepsis should be evaluated deliberately.

The rats in the CE group had a higher 7-day survival rate than those in the CNS group. The rats in the 12hS and 24hS subgroups had higher survival rates than those in the 6hS subgroup, but there was no significant difference between the 12hS and 24hS subgroups. These results indicate that esmolol can protect the intestine from sepsis, but the protective effect is mainly exhibited in the early stages of the esmolol infusion. This study highlights that it might be a better approach to infuse esmolol in early-stage patients with sepsis.

In this *in vivo* study of rats, we have shown that esmolol could suppress the inflammation and reduce the sepsis caused by the damages to the intestinal tissue, which is reflected in the levels of IL-6, IL-10, I-FABP, and DAO. The results in this study indicate the underlying mechanism by which esmolol can suppress inflammation and sepsis caused damages and apoptosis via the overexpression of NF-kappa B-p65 in the early stage sepsis rats. The NF-kappa B-p65 inhibition method might not be beneficial to the sepsis patients, but the early-stage use of esmolol might be an ideal treatment method for sepsis.

Ethics Committee Approval: Ethics committee approval was received for this study from the experimental animal ethics committee of the Lanzhou University Second Hospital (SCXK 2013-0002).

Informed Consent: N/A.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - C.A.G., L.M.; Design - H.B.L., L.M.; Supervision - Li Ma.; Resource - L.M., H.B.L.; Materials - X.L.S., Y.Z.W.; Data Collection and/or Processing - X.L.S., Y.Z.W., L.L.Z., B.Z., H.A.; Analysis and/or Interpretation - C.A.G.; Literature Search - C.A.G.; Writing - C.A.G.; Critical Reviews - H.B.L., L.M.

Acknowledgements: The authors are grateful to laboratory staff of Lanzhou University Second Hospital.

Conflict of Interest: The authors declared that this study has received no financial support.

Financial Disclosure: This study was supported by the Rui Yi Special Fund for Emergency Medical Research of the Peking Union Medical College Foundation (R2015003), the Hospital Doctor Fund of the Lanzhou University Second Hospital (ynbskyjj2015-2-2), and General Project of Chinese People's Liberation Army (CLZ14J005).

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