Low dose of Esmolol attenuates sepsis-induced immunosuppression via modulating T-lymphocyte apoptosis and differentiation

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Abstract

Background

Persistent immunosuppression characterized by immune cells apoptosis and T- lymphocyte subsets imbalance, occurs in septic patients with poor outcomes. Recent studies reported that Esmolol improved survival in septic shock via modulating immune responses. We hypothesized Esmolol alleviate sepsis-induced immunosuppression by regulating immune cell apoptosis and differentiation and sought to investigate the potential mechanisms and optimal dose.

Methods

Four hours after cecal ligation and puncture (CLP), Wistar rats were randomized into CLP, CLP + E-5 (Esmolol:5mg.kg^{-1}.h^{-1}) and CLP + E-18 (Esmolol:18mg.kg^{-1}.h^{-1}) group. Another 8 rats were under sham operation. 18 hours after CLP, hemodynamics and organ histological injuries were evaluated, peripheral blood mononuclear cells apoptosis and circulating T-lymphocyte subsets counts were determined by flow cytometry, protein expressions of p-Akt, Bcl-2, cleaved Caspase-3 and p-Erk1/2 were determined by western blot and immunohistochemistry. mRNA and protein expressions of β1-adrenoreceptors were evaluated by quantitative real-time PCR and immunohistochemistry.

Results

CLP induced tachycardia, hypotension, hyperlactatemia and multiple organ injuries. Infusion of Esmolol at 5mg.kg^{-1}.h^{-1} didn't reduce heart rate, but improved mean arterial pressure, lactatemia and multiple organ injuries, decreased circulating T-lymphocyte apoptosis and the ratio of T help 2 cells and T help 1 cells, unregulated p-Akt and Bcl-2 expression and down-regulated cleaved Caspase-3 and p-Erk1/2 expression.

Conclusions

Esmolol without heart rate reduction attenuated sepsis-induced immunosuppression by preventing T-lymphocytes from apoptosis through regulating Akt/Bcl-2/Caspase-3 pathway and reducing naive CD4^{+} T cells differentiation to T help 2 cell through inhibiting Erk1/2 activation. Esmolol may be a potential immune regulator in septic shock.

Introduction

Septic shock developed as a dysregulated host inflammatory response to infection, resulting in multiple organ dysfunction associated with high mortality worldwide[1]. After the onset of sepsis, both pro-and anti-inflammatory immune responses occur. If sepsis persists, patients will enter a marked immunosuppressive state[2]. Immunosuppressed septic patients are at high risk of secondary nosocomial infection resulting in an increasing 13% mortality of septic patients[3]. Cellular apoptosis, such as monocytes, B-lymphocytes and T-

lymphocytes, and up-regulated ratio of T helper 2 cells (Th2) and T helper 1 cells (Th1) are the most reported mechanisms contributing to immunosuppression during septic shock[4, 5].

In experimental and clinical studies, Esmolol, a highly selective ultra-short-acting β1-adrenoreceptor blocker, was recently reported to improve cardiovascular function and survival in septic shock[6–8]. The beneficial effects of Esmolol in septic shock were previously considered due to its hemodynamic effects[9]. However, recent evidence showed that the beneficial effects of Esmolol in septic shock were also associated with immunomodulation[10, 11]. Whereas, how Esmolol influencing immune response in septic shock and the optimal dose are unclear. Our study was aimed to determine whether Esmolol could attenuate peripheral blood mononuclear cells (PBMCs) apoptosis and reduce the ratio of Th2 and Th1 to alleviate immunosuppression in septic shock as well as the best dose.

As reported, lymphocytes and monocytes expressed β1-adrenoreceptors[12], which belong to the G-protein-coupled receptor(GPCR) super-family. GPCRs pass on their signaling via G-protein. A large amount of molecules, such as protein kinase B (Akt) and extracellular-signal-regulated kinase (Erk), are involved in G-protein signaling cascades, which trigger a series of cellular activities. The Akt pathway participates in sepsis-induced apoptosis[13]. Qi *et al.* reported that phosphorylation of Akt, which induced up-regulation of B-cell leukemia/lymphoma 2 (Bcl-2) and down-regulation of cleaved Caspase-3, decreased sepsis-induced cardiomyocyte apoptosis both in vitro and in vivo[14]. Thus, we aimed to determine whether Esmolol reduces sepsis-induced immune cells apoptosis by modulating Akt/Bcl-2/Caspase-3 pathway. Furthermore, cytokines, such as IL-4 and IL-10, promoted naive $CD4^+$ T cells (Th0) differentiation to Th2 through activating Stat6[15], which was regulated by the Erk1/2 pathway. Inhibition of Erk1/2 suppressed Th0 differentiation to Th2[16]. Hence, we also aimed to determine whether Esmolol reduces Th0 differentiation to Th2 by inhibiting Erk1/2 activation.

Materials And Methods

Animals

Adult male Wistar rats weighing 300–400g were obtained from the Center for Animal Experiments of Wuhan University. All animal experiments were approved by the Institutional Animal Care and Use Committee of the Animal Experiment Center of Wuhan University (E2020072901) and followed the institutional and national guidelines.

Study design

The cecal ligation and puncture (CLP) model was used to develop a septic shock model as previously described [17]. Four hours after CLP, all rats were randomized into 3 groups (CLP, CLP + E-5, CLP + E-18). Another 8 rats were under sham operation. Four hours after surgery, all rats received fluid resuscitation (Saline $10ml.kg^{-1}.h^{-1}$), antibiotic (Meropenem $10mg.kg^{-1}$) and analgesic (Nalbuphine $0.2mg.kg^{-1}.h^{-1}$). The rats in CLP + E-5 and CLP + E-18 groups received infusion of Esmolol at $5mg.kg^{-1}.h^{-1}$ and $18\ mg.kg^{-1}.h^{-1}$ respectively. Assessments were performed 18 hours after CLP or sham operation.

Hemodynamics and organ injuries measurement

The pressure transducer catheter was inserted into the right carotid artery of anesthetized rats and connected with the BL-420N biological signal recorder (Taimeng, Chengdu, China) to measure the HR and MAP. Arterial blood was collected for lactate detection using an ABL800 FLEX blood-gas Analyser™ (Radiometer, Denmark). Heart, lung and spleen tissue sections were stained with hematoxylin and eosin (H&E) and observed morphological changes under the optical microscope (Olympus, Tokyo, Japan) at 200×magnification. The histopathological lesions were quantified using five randomly selected fields per slide[18].

Flow cytometry

The PBMCs of all rats were isolated by Ficoll-Paque (Cytiva, Sweden). Cell surface markers are used to identify circulating monocytes ($CD11a^+CD11b^+$), B-lymphocytes ($CD45RA^+$), T-lymphocytes ($CD3^+$) and CD4 T-lymphocytes ($CD3^+CD4^+$) in PBMCs. According to the manufacturer's protocol, apoptosis detection was performed using a FITC Annexin V Apoptosis Detection Kit with 7-amino-actinomycin D (7-AAD). After being stimulated with leukocyte activation cocktail, CD4 T-lymphocytes were stained with interferon (INF)-γ and interleukin (IL)-4 to identify Th1 and Th2 counts respectively. Antibody and gating information were shown in supplemental Table S1 and supplemental Figure S1.

Western blot

Due to the limited numbers of circulating T-lymphocytes, splenic $CD4^+$ T-lymphocytes were used. Splenic $CD4^+$ T-lymphocytes were purified using Rat CD4 microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany) following the manufacturer's instructions. The purity of splenic $CD4^+$ T-lymphocytes was identified by flow cytometry (Beckman Coulter, Indianapolis IN, USA) using PE/CY7-labeled anti-CD4 antibody (supplemental Table S1 and supplemental Figure S2).

The protein was extracted from isolated splenic $CD4^+$ T-lymphocytes of all rats. After separation on an SDS-PAGE gel, the protein samples were transferred to a PVDF membrane (Millipore, USA) and incubated with the following antibodies: anti-phosphorylated-Akt (p-Akt), anti-Akt, anti-phosphorylated-Erk1/2 (p-Erk1/2), anti-Erk1/2, anti-Bcl-2, anti-cleaved Caspase-3, and anti-Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), followed incubation with horseradish peroxidase (HRP)-conjugated secondary antibody (supplemental Table S1). The blots were stained with an ECL Plus kit (Beyotime, China) and visualized using the ECL Imaging System (Tanon, Shanghai, China). Finally, blots were normalized to GAPDH and quantitatively analyzed using Image J Software (NIH, Bethesda, MD, USA).

Immunohistochemistry

The spleen tissue sections of 4 rats in each group were incubated with the following antibodies: p-Akt, p-Erk1/2, Bcl-2, cleaved Caspase-3 and β1 adrenoreceptors(supplemental Table S1), followed by counterstaining with hematoxylin. Five randomly chosen fields of view at 400 magnification were quantified for each section. As previously described[19], the staining intensity was graded semi-quantitatively (0: undetectable; 1+: weak; 2+: moderate; 3+: strong).

Immunofluorescence

The spleen tissue section of a healthy rat was incubated with rabbit anti-CD4 antibody and anti-β1 adrenoreceptor antibody, followed by staining with fluorochrome-conjugated secondary antibodies

(supplemental Table S1). The section was stained with 4’-6-diamidino-2-phenylindole (DAPI) and then observed under a fluorescence microscope (Olympus, Tokyo, Japan).

Quantitative real-time PCR (qRT-PCR)

Total RNA of splenic $CD4^+$ T-lymphocytes of 6 rats in each group was extracted. According to the manufacturer's manual, the purified mRNA from each sample was reverse transcribed into complementary deoxyribonucleic acid (cDNA) using PrimeScript RT Master Mix (Vazyme, Nanjing, China)(supplemental Table S2). qRT-PCRs were performed using the UltraSYBR Mixture (CWbio, Beijing, China). The relative mRNA expression level of Stat6 and β1-adrenoreceptor in splenic $CD4^+$ T-lymphocytes were calculated using the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

Data are expressed as median with interquartile range (IQR) in main text and tables and as median with upper edges of error bars representing the 75th percentile in figures. The Mann-Whitney test was performed to evaluate the significance of differences between the sham and CLP group. Kruskal-Wallis test was performed between CLP, CLP + E-5 and CLP + E-18 groups. When the Kruskal-Wallis test was significant at the 5% level, Dunnett's multiple post-hoc comparisons were performed. The data were plotted using GraphPad Prism 7.0 software (GraphPad Software, San Diego, CA) and analyzed using IBM-SPSS Statistics 23.0 (IBM Corp., NY, USA).

Results

Model characterization

Compared to rats in sham group, CLP induced arterial hypotension (sham 131mmHg (123-136mmHg); CLP 77mmHg (71-86mmHg), $p = 0.001$), tachycardia (sham 335bpm(319-352bpm); CLP 371bpm (363-380bpm), $p = 0.005$) and elevated lactatemia (sham 0.9mmol.l^{-1} (0.4-1.2mmol.l^{-1}); CLP 2.4mmol.l^{-1}(1.6-5.4mmol.l^{-1}), $p = 0.001$)(Table 1). CLP induced 1) cardiac muscle fibers destruction, congestion and inflammatory infiltration(Fig. 1a); 2) apparent inflammatory cells aggregation, intra-alveolar capillary hemorrhages and thickening of the alveolar walls in lung tissues(Fig. 1b) and 3) depletion of reticuloendothelial cells and lymphocytes in spleen tissues(Fig. 1c). The heart ($p = 0.002$), lung ($p = 0.002$) and spleen ($p = 0.002$) injury scores were increased in the CLP group (Table 1).

Effects of different doses of Esmolol on hemodynamics and organ injuries

Compared to rats in CLP groups, Esmolol infused at 5mg.kg^{-1}.h^{-1} didn't reduce HR (CLP 371bpm (363-380bpm); CLP + E-5 368bpm (356-389bpm), $p = 1.000$), but restored MAP (CLP 77mmHg (71–86 mmHg); CLP + E-5 101mmHg (98-105mmHg), $p = 0.006$) and decreased the circulating level of lactate (CLP 2.4mmol.l^{-1}(1.6-5.4mmol.l^{-1}); CLP + E-5 1.4mmol.l^{-1}(1.2-1.7mmol.l^{-1}), $p = 0.046$)(Table 1). Compared to rats in CLP groups, Esmolol infused at 18mg.kg^{-1}.h^{-1} reduced HR (CLP 371bpm (363-380bpm); CLP + E-18 245bpm (236-267bpm), $p = 0.001$), but didn't worsen MAP (CLP 77mmHg (71-86mmHg); CLP + E-18 92mmHg (80-104mmHg), $p = 0.529$), and had no effects on circulating level of lactate (CLP 2.4mmol.l^{-1}(1.6-5.4mmol.l^{-1}); CLP + E-18

2.4mmol.l^{-1}(2.1-2.6mmol.l^{-1}), $p = 0.785$) (Table 1). Infusion of Esmolol at 5mg.kg^{-1}.h^{-1} improved CLP-induced 1) cardiac muscle fibers destruction, congestion and inflammatory infiltration (Fig. 1a); 2) inflammatory cells aggregation, intra-alveolar capillary hemorrhages and thickening of the alveolar walls in lung tissues (Fig. 1b) and 3) depletion of reticuloendothelial cells and lymphocytes in spleen tissues(Fig. 1c). Compared to CLP group, the heart ($p = 0.024$), lung ($p = 0.012$) and spleen ($p = 0.043$) injury scores were reduced in CLP + E-5 group(Table 1). Infusion of Esmolol at 18mg.kg^{-1}.h^{-1} trended to improved CLP induced heart, lung and spleen injuries, but didn't reach a statistic significance (heart: $p = 0.516$; lung: $p = 0.117$; spleen: $p = 0.086$)(Table 1).

Table 1 Comparison of hemodynamics and organ injury score 18 hours after operation in different groups

Variables	Sham	IQR	CLP	IQR	CLP+E-5	IQR	CLP+E-18	IQR	*p*-value
	Median		Median		Median		Median		
Heart rate(min^{-1})	335	319-352	371	363-380	368	356-389	245	236-267	0.005 CLP *vs.* Sham
									1.000 CLP *vs.*CLP+E-5
Mean arterial pressure (mmHg)	131	123-136	77	71-86	101	98-105	92	80-104	0.001 CLP *vs.* CLP+E-18
Lactatemia ($mmol.L^{-1}$)	0.9	1.0-1.1	2.4	2.1-3.2	1.4	1.2-1.7	2.4	1.4-3.7	0.001 CLP *vs.* Sham
									0.006 CLP *vs.*CLP+E-5
heart injury score	0.3	0.20-0.55	2.1	2.0-2.2	1.5	1.25-1.75	1.9	1.5-2.0	0.529 CLP *vs.* CLP+E-18
									0.001 CLP *vs.* Sham
lung injury score	0.4	0.40-0.55	2.3	2.2-2.4	1.6	1.45-1.75	1.9	1.8-2.0	0.046 CLP *vs.*CLP+E-5
									0.785 CLP *vs.* CLP+E-18
spleen injury score	0.3	0.05-0.40	2	2.0-2.15	1.8	1.5-1.8	1.8	1.65-1.95	0.002 CLP *vs.* Sham
									0.024 CLP *vs.*CLP+E-5
									0.516 CLP *vs.* CLP+E-18
									0.002 CLP *vs.* Sham
									0.012 CLP *vs.*CLP+E-5
									0.117 CLP *vs.* CLP+E-18
									0.002 CLP *vs.* Sham
									0.043 CLP *vs.*CLP+E-5
									0.086 CLP *vs.* CLP+E-18

CLP: cecal ligation and puncture; IQR: interquartile range; CLP+E-5: CLP with Esmolol infused at 5 $mg.kg^{-1}.h^{-1}$; CLP+E-18: CLP with Esmolol infused at 18 $mg.kg^{-1}.h^{-1}$

Effects of different doses of Esmolol on PBMCs apoptosis

Compared to sham group, CLP increased apoptosis of monocytes ($p = 0.001$), B-lymphocytes ($p = 0.001$) and T-lymphocytes ($p = 0.001$)(Fig. 2). Infusion of Esmolol at both 5 and 18$mg.kg^{-1}.h^{-1}$ didn't reduce CLP-induced apoptosis of monocytes (CLP; *vs.* CLP + E-5, $p = 0.993$; *vs.* CLP + E-18, $p = 0.934$)(Fig. 2a) and B-lymphocytes (CLP; *vs.* CLP + E-5, $p = 0.989$; *vs.* CLP + E-18, $p = 0.992$)(Fig. 2b). Infusion of Esmolol at 5$mg.kg^{-1}.h^{-1}$ reduced significantly apoptosis of T-lymphocytes ($p = 0.019$)(Fig. 2c). However, infusion of Esmolol at 18$mg.kg^{-1}.h^{-1}$ didn't significantly reduced apoptosis of T-lymphocytes ($p = 0.999$)(Fig. 2c).

Effects of different doses of Esmolol on Th0 differentiation

CLP induced an increase of both Th1($p = 0.005$) and Th2 ($p = 0.001$) compared to the sham group (Fig. 3b and c). Infusion of Esmolol at both 5 and 18 $mg.kg^{-1}.h^{-1}$ decreased CLP-induced increase of Th2 (CLP; *vs.* CLP + E-5, $p = 0.042$; *vs.* CLP + E-18, $p = 0.029$), only at 18$mg.kg^{-1}.h^{-1}$ attenuated CLP-induced increase of Th1 (CLP; *vs.* CLP + E-5, $p = 0.232$; *vs.* CLP + E-18, $p = 0.049$). Thus, infusion of Esmolol at 5$mg.kg^{-1}.h^{-1}$ rather than 18$mg.kg^{-1}.h^{-1}$ lowered the ratio of Th2 and Th1 (CLP; *vs.* CLP + E-5, $p = 0.049$; *vs.* CLP + E-18, $p = 0.992$)(Fig. 3d).

Effects of different doses of Esmolol on apoptosis-associated signaling proteins

Tested by western blot, CLP decreased expression of p-Akt ($p = 0.010$) and Bcl-2 ($p = 0.049$) and increased cleaved Caspase-3 ($p = 0.001$) compared to the Sham group (Fig. 4a1, b1 and c1). Infusion of Esmolol at 5$mg.kg^{-1}.h^{-1}$ increased expression of p-Akt ($p = 0.048$) and Bcl-2 ($p = 0.032$) and decreased cleaved Caspase-3 ($p = 0.048$) compared to CLP group(Fig. 4a1, b1 and c1). However, infusion of Esmolol at 18$mg.kg^{-1}.h^{-1}$ just had a tendency to increase expression of p-Akt ($p = 0.250$) and Bcl-2 ($p = 0.256$) and decrease cleaved Caspase-3 ($p = 0.054$) compared to CLP group but didn't reach a statistic significance(Fig. 4a1, b1 and c1). Then, immunohistochemistry confirmed the results of western blot(Sham *vs.* CLP: p-Akt, $p = 0.029$; Bcl-2, $p = 0.029$; cleaved Caspase-3, $p = 0.029$; CLP *vs.* CLP + E-5: p-Akt, $p = 0.009$; Bcl-2, $p = 0.033$; cleaved Caspase-3, $p = 0.027$; CLP *vs.*CLP + E-18: p-Akt, $p = 0.698$; Bcl-2, $p = 0.628$; cleaved Caspase-3, $p = 0.996$) (Fig. 4a2, b2 and c2 and supplemental Figure S3a, 3b and 3c).

Effects of different doses of Esmolol on Th0 differentiation-associated signaling proteins

Compared to the sham group, CLP increased Erk1/2 phosphorylation analyzed by western blot ($p = 0.015$) (Fig. 5a1). Infusion of Esmolol at 5$mg.kg^{-1}.h^{-1}$ reduced CLP-induced increased Erk1/2 phosphorylation ($p = 0.009$)(Fig. 5a1). However, infusion of Esmolol at 18$mg.kg^{-1}.h^{-1}$ didn't significantly decrease CLP-induced increased Erk1/2 phosphorylation ($p = 0.992$)(Fig. 5a1). The results were confirmed by immunohistochemistry (CLP *vs.* sham, $p = 0.029$; *vs.* CLP + E-5, $p = 0.041$; *vs.* CLP + E-18, $p = 0.223$)(Fig. 5a2 and supplemental Figure

S3d). Due to the unspecific Stat6 phosphorylation antibody, we failed to quantify Stat6 activation form. We tested Stat6 mRNA expression by qRT-PCR. CLP increased Stat6 mRNA expression compared to the sham group ($p = 0.004$)(Fig. 5b). Infusion of Esmolol at both 5 and 18mg.kg^{-1}.h^{-1} had a tendency to decrease mRNA expression of Stat6 but had no statistical significance (CLP *vs.* CLP + E-5, $p = 0.550$; *vs.* CLP + E-18, $p = 0.985$) (Fig. 5b).

Effects of different doses of Esmolol on the β1-adrenoreceptor expression on T- lymphocytes

Immunofluorescence confirmed β1-adrenoreceptors were expressed on the surface of splenic CD4$^+$ T-lymphocytes (supplemental Figure S4). Compared to the sham group, CLP decreased mRNA ($p = 0.002$) and protein ($p = 0.029$) expression of β1-adrenoreceptor in splenic CD4$^+$ T-lymphocytes tested respectively by qRT-PCR and immunohistochemistry (supplemental Figure S5). Infusion of Esmolol at both 5 and 18 mg.kg^{-1}.h^{-1} didn't modulate the mRNA (CLP *vs.* CLP + E-5, $p = 0.669$; *vs.* CLP + E-18, $p = 0.869$)(supplemental Figure S5a) and protein (CLP *vs.* CLP + E-5, $p = 0.352$; *vs.* CLP + E-18, $p = 0.824$)(supplemental Figure S5b) expression of β1-adrenoreceptor in splenic CD4$^+$ T lymphocytes.

Discussion

Recently, accumulating evidence suggested that immunosuppression occurs after the onset of septic shock[20]. Apoptosis of immune cells and imbalance of Th1 and Th2 are the most reported mechanisms contributing to immunosuppression[4, 5]. Our results demonstrated that Esmolol without HR reduction decreased T-lymphocytes apoptosis and drifted a shift from Th2 to Th1 in septic shock models, which attenuated sepsis-induced immunosuppression. Esmolol might alleviate T-lymphocyte apoptosis by regulating Akt/Bcl-2/Caspase-3 pathway and reduced Th0 differentiation to Th2 by inhibiting Erk1/2 activation in septic shock models. As a result, Esmolol without HR reduction restored MAP, decreased lactatemia and alleviated multiple organ injuries in our study.

Previous studies reported that immunosuppression are predominately resulted from apoptosis of monocytes, B-lymphocytes and T-lymphocytes in septic shock patients[21, 22]. In our study, CLP induced apoptosis of circulating monocytes, B-lymphocytes and T-lymphocytes, which was similar to clinical settings. The mechanism of triggering cellular apoptosis was not well studied. During septic shock, the sympathetic nervous system is over-activated and a large number of catecholamines are secreted[23]. Lymphocytes and monocytes express adrenergic receptors. Overstimulating these cells by catecholamines through adrenergic receptors contributes to apoptosis[24]. Our results showed that blocking β1-adrenergic receptors by Esmolol at low dose significantly reduced T-lymphocyte apoptosis.

The mechanisms of Esmolol reducing T-lymphocytes apoptosis are explored in our study. Previous studies reported that over-secretion of norepinephrine promoted cardiomyocyte apoptosis via activating β-adrenoreceptor in septic shock mice[25, 26], which could be inhibited by blockade of β1-adrenoceptor through decreasing Caspase-3 activation[26]. An *et al.* reported that myocardial apoptosis was alleviated through Akt/Bcl-2 pathway activation in sepsis[27]. Zhang *et al.* observed that activation of the Akt pathway reduced the expression of cleaved Caspase-3, subsequently ameliorating sepsis-induced acute lung injury[28]. Our results revealed that Esmolol at low dose reversed CLP-induced decrease of Akt phosphorylation and Bcl-2 expression

and increase of cleaved Caspase-3 in animal models. Therefore, Esmolol might inhibit apoptosis of T-lymphocytes by modulating the Akt/Bcl-2/Caspase-3 pathway.

Previous studies showed the imbalance of T-lymphocyte sub-populations in septic patients, such as an increase in Th2 counts and an augmented ratio of Th2 and Th1[5, 29, 30]. Our results revealed that both Th1 and Th2 counts were up-regulated in the septic shock models. Whereas, Th2 increased more than Th1, thus the ratio of Th2 and Th1 was increased in septic shock models. Esmolol at low dose significantly reduced Th2 counts but not Th1 counts, thus restored the ratio of Th2 and Th1.

Cytokines primarily influenced Th0 differentiation. Cytokines, such as IL-4 and IL-10, promote Th0 differentiation to Th2, mainly dependent on Stat6 activation[31]. Erk1/2 was involved in the regulation of Stat6 activity. Previous studies have reported that inhibition of Erk1/2 activation suppressed IL-4-induced Stat6 activity[16]. In our research, CLP increased the phosphorylation of Erk1/2. Infusion of the low dose of Esmolol reduced Erk1/2 phosphorylation. The results indicated that Esmolol might influence Th0 differentiation by inhibiting Erk1/2 activation. We further observed Stat6 expression. Infusion of Esmolol trended to decrease CLP-induced increased mRNA expression of Stat6 but didn't reach statistical significance. Indeed, mRNA expression could not reflect total protein activation. Observation of Stat6 activation is required in future work. Besides, Esmolol may modulate Th0 differentiation through Erk1/2 by other mechanisms, such as influencing cytokines secretion. As previously reported[32, 33], Erk1/2 activation up-regulated IL-4 and IL-10 production so that promote Th0 differentiation to Th2. In fact, our previous study has shown that Esmolol decreased the level of IL-10[10]. Thus, Esmolol might modulate Th0 differentiation by decreasing relative cytokines production by inhibiting Erk1/2 activation. More studies are needed to clarify the mechanisms of Esmolol on Th0 differentiation.

In septic shock, excessive stimulation by catecholamine resulted in the reduction of β1-adrenoreceptor density on cardiomyocytes[6]. Our results demonstrated that the mRNA and protein expression of β1-adrenoreceptor in T lymphocytes decreased in septic shock rats. Esmolol infusion at different doses didn't restore mRNA or protein expression of β1-adrenoreceptor on T-lymphocytes in septic shock rats. Thus, it seems that Esmolol exerted its effects on T-lymphocytes through downstream pathways rather than regulating the expression of its receptor.

In addition, the optimal dose of Esmolol for immunoregulation in septic shock remains unclear. Our results showed that Esmolol at high dose with HR reduction didn’t significantly decreased T-lymphocytes apoptosis and the ratio of Th2 and Th1 in septic shock models, which are not as effective as that of at low dose without HR reduction. More studies about dose-effect relationship are needed to confirm our findings.

Conclusions

In conclusion, in experimental septic shock, a low dose of Esmolol without HR reduction attenuated sepsis-induced T-lymphocyte apoptosis by regulating Akt/Bcl-2/Caspase-3 pathway and reduced Th0 differentiation to Th2 by inhibiting Erk1/2 activation. Esmolol with immune regulation effects may be a potential treatment in septic shock.

Abbreviations

Akt: protein kinase B;

Bcl-2: B-cell leukemia/lymphoma 2;

CLP: cecal ligation and puncture;

Erk: extracellular-signal-regulated kinase;

HR heart rate

IL-10: interleukin 10;

IL-4:interleukin 4;

INF-γ: interferon-γ;

IQR: interquartile range

MAP: mean arterial pressure

mRNA: messenger ribonucleic acid;

p-Akt: phosphorylated protein kinase B;

PBMCs: peripheral blood mononuclear cells;

p-Erk1/2: phosphorylated-Erk1/2;

Th1: T helper 1 cells;

Th2: T helper 2 cells;

Th0: naive $CD4^+$ T cells

Declarations

Ethics approval and consent to participate

All animal experiments were approved by the Institutional Animal Care and Use Committee of the Animal Experiment Center of Wuhan University.

Consent for publication

All authors read and approved the final manuscript.

Availability of data and materials

All data used for the analysis can be obtained from the statistician.

Competing interests

All authors have disclosed that they do not have any potential conflicts of interest.

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Authors' contributions

Drs. Wei and Yang take responsibility for content of the manuscripts. Drs. Ma, Cheng, and Zheng contribute equally to the study and share the first authorship. Drs. Wei and Yang contribute equally to the study. Drs. Wei and Ma contributed to the study concept and design. Drs. Ma, Zheng, He, and Zhou were involved in experiments. Dr. Cheng contributed to the statistical analysis. Drs. Wei, Yang, Ma, Wang, and He contributed to the drafting of the article. All authors read and approved the final manuscript.

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Figures

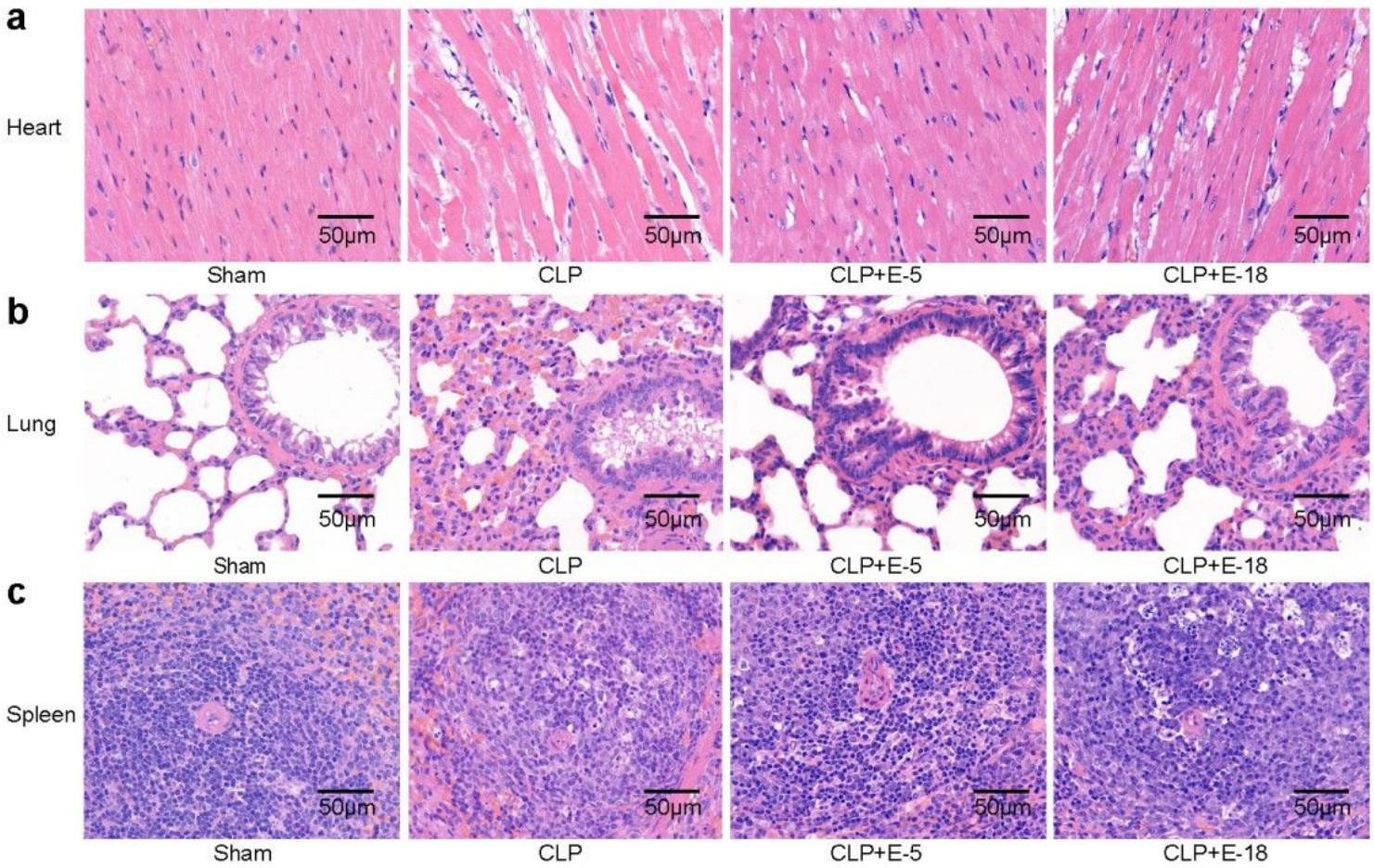

Figure 1

Effects of different doses of Esmolol on CLP-induced heart, lung and spleen injuries Heart (a), lung (b) and spleen (c) tissue were stained with hematoxylin-eosin (H&E). The representative sections are shown at×200 original magnification and scale bars are 50 mm.

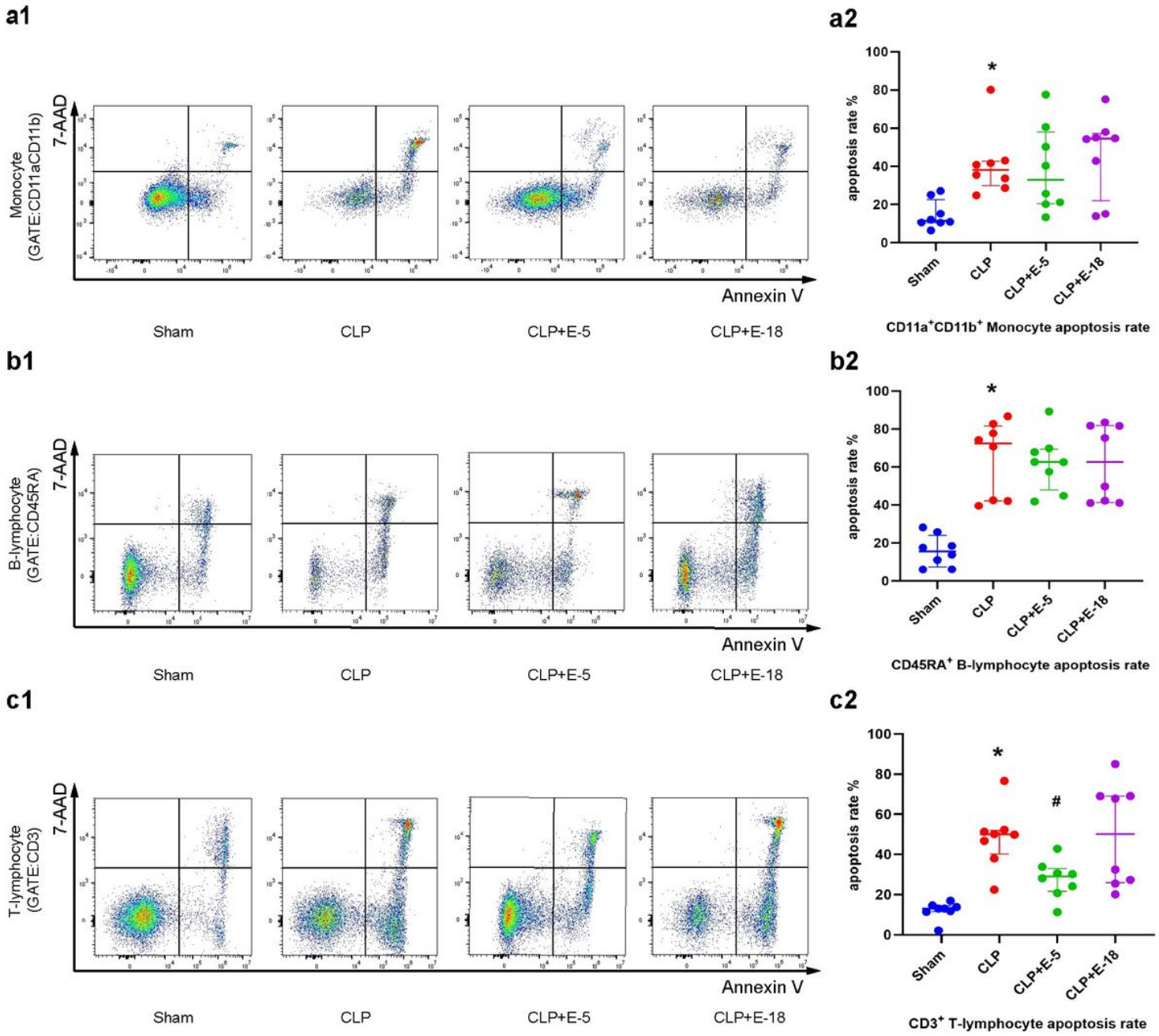

Figure 2

Effects of different doses of Esmolol on apoptosis of PBMCs by flow cytometry Density plots of monocytes (a1), B lymphocytes (b1) and T lymphocytes (c1) apoptosis for one representative rat per group are shown in the panel. Apoptosis rates of circulating monocytes (a2), B-lymphocytes (b2) and T-lymphocytes (c2) in different groups were shown in the histogram. Data are expressed as median ± interquartile range, n=8. The upper edges of error bars represent the 75th percentile in each group. *$p < 0.05$: CLP group *vs.* Sham group; #$p < 0.05$: CLP+E-5 *vs.* CLP group. CLP: cecal ligation and puncture; CLP+E-5: CLP with Esmolol infused at 5 $mg.kg^{-1}.h^{-1}$; CLP+E-18: CLP with Esmolol infused at $18mg.kg^{-1}.h^{-1}$.

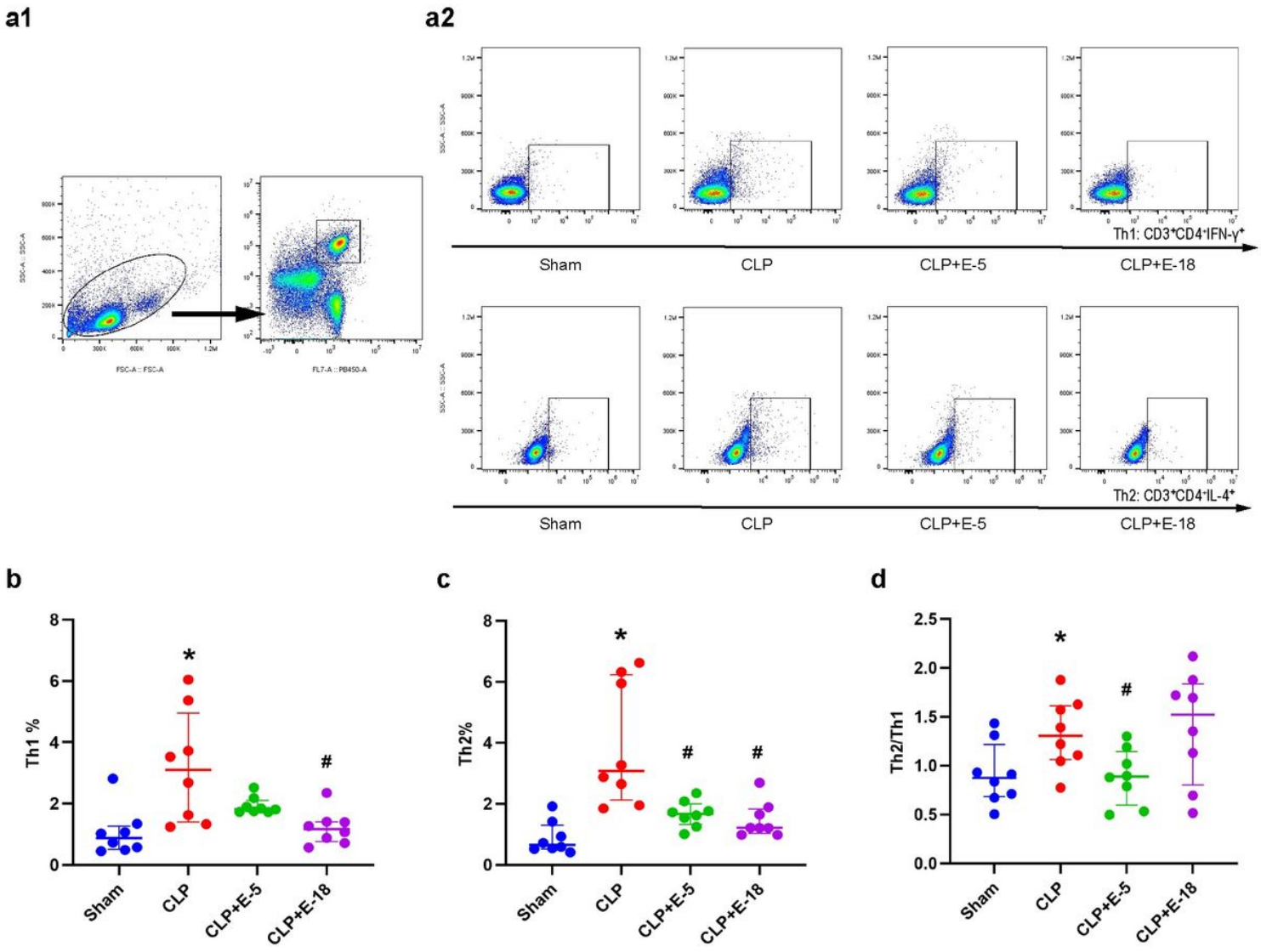

Figure 3

Effects of different doses of Esmolol on T-lymphocyte subsets by flow cytometry $CD3^+CD4^+$ cells were analyzed and further specified T helper cells (a1). Th1 cells are defined as $INF\text{-}\gamma^+$ cells in $CD3^+CD4^+$cells. Th2 cells are defined $IL\text{-}4^+$ cells in $CD3^+CD4^+$ cells. Density plots of T helper 1 cells (Th1) and T helper 2 cells (Th2) for one representative rat per group are shown in panel (a2). The percentages of Th1 (b) and Th2 (c) in T help cells (Th) and the ratio of Th2/Th1 (d) were shown in the histogram. Data are expressed as median ± interquartile range, n=8. The upper edges of error bars represent the 75th percentile in each group. *$p < 0.05$: CLP group *vs.* Sham group; #$p < 0.05$: CLP+E-5 *vs.* CLP group. CLP: cecal ligation and puncture; CLP+E-5: CLP with Esmolol infused at 5 $mg.kg^{-1}.h^{-1}$; CLP+E-18: CLP with Esmolol infused at 18$mg.kg^{-1}.h^{-1}$.

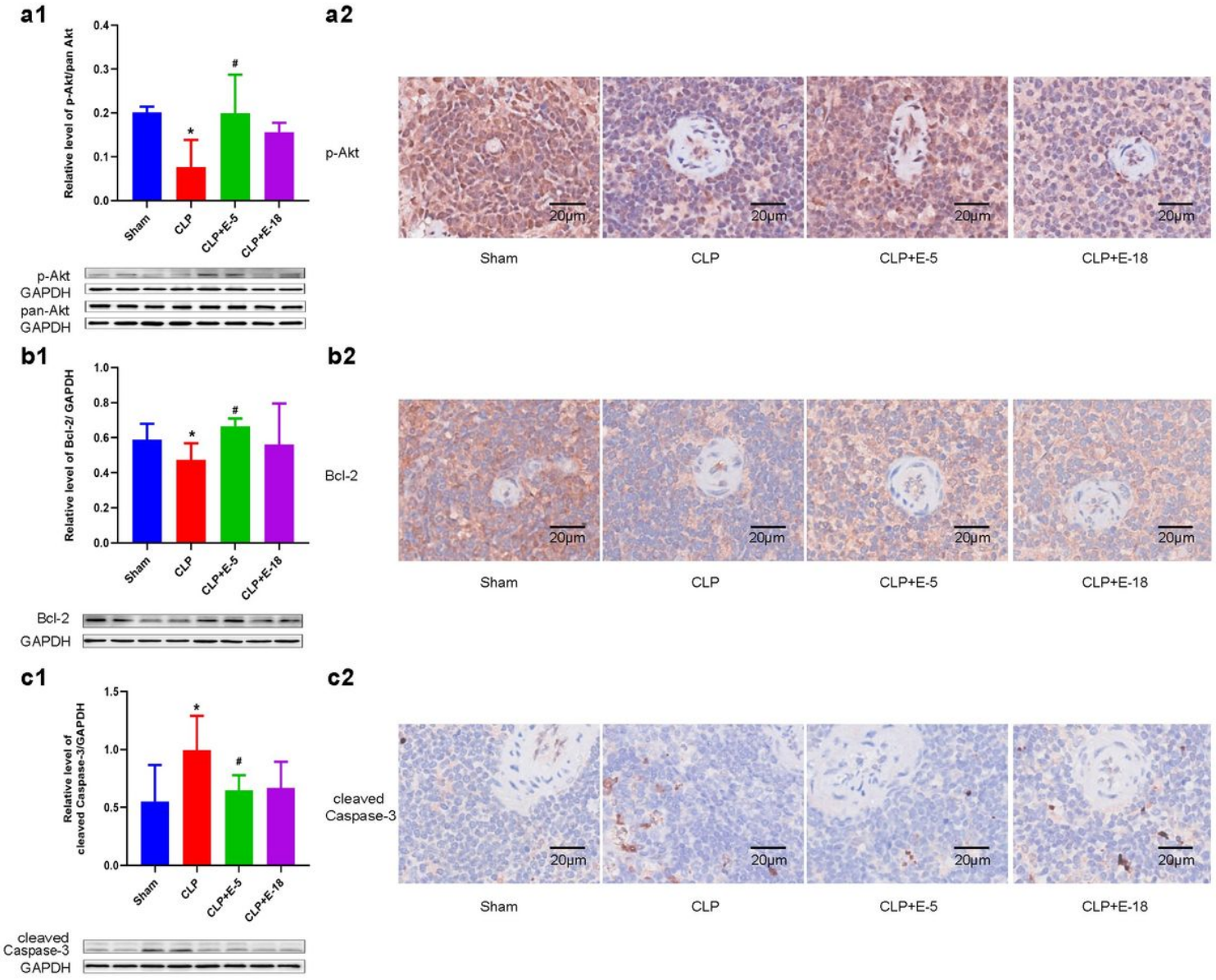

Figure 4

Effects of different doses of Esmolol on apoptosis-associated signaling proteins Western blots revealed phosphorylated Akt (p-Akt)(a1), Bcl-2(b1) and cleaved Caspase-3(c1). Proteins were obtained from splenic $CD4^+$ T-lymphocytes lysates (n=8) prepared from all experimental rat groups. Two typical western blots are shown below each histogram. Densitometric analysis was used to calculate the normalized protein ratio. Immunohistochemistry revealed phosphorylated Akt (p-Akt)(a2), Bcl-2(b2) and cleaved Caspase-3(c2) in the splenic tissues for one representative rat per group (n=4). Data are expressed as median ± interquartile range. The upper edges of error bars represent the 75th percentile in each group. *$p < 0.05$: CLP group *vs.* Sham group; #$p < 0.05$: CLP+E-5 *vs.* CLP group. CLP: cecal ligation and puncture; CLP+E-5: CLP with Esmolol infused at 5 $mg.kg^{-1}.h^{-1}$; CLP+E-18: CLP with Esmolol infused at $18mg.kg^{-1}.h^{-1}$.

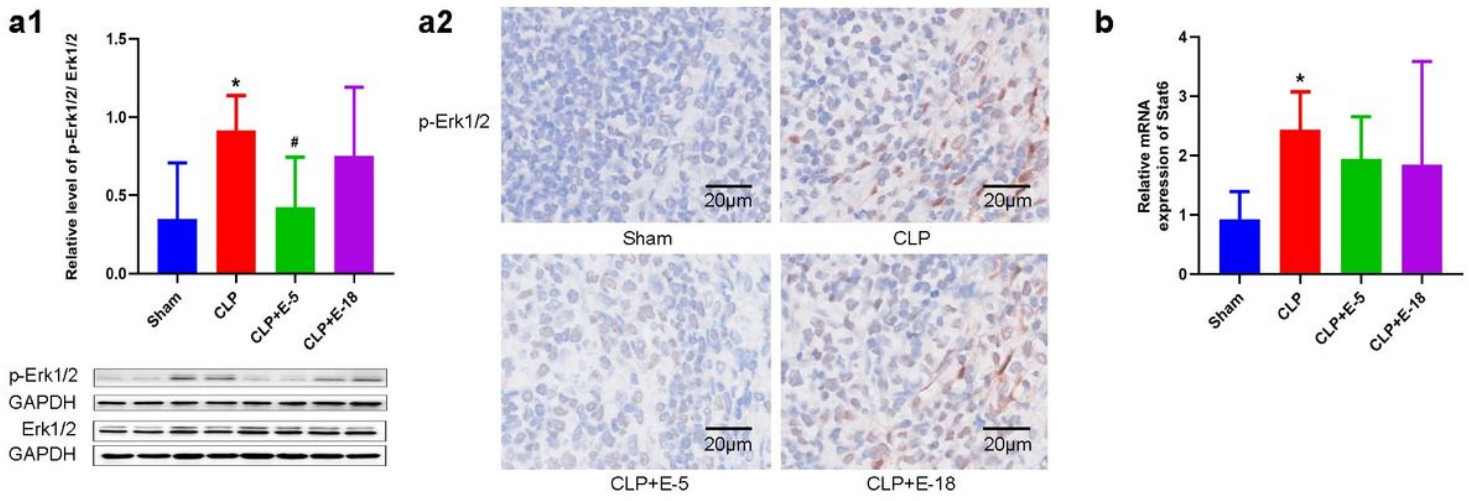

Figure 5

Effects of different doses of Esmolol on naive CD4$^+$ T cells (Th0) differentiation-associated signaling protein Western blots revealed phosphorylated Erk1/2 (p-Erk1/2)(a1). Proteins were obtained from splenic CD4$^+$ T-lymphocytes lysates (n=8) prepared from all experimental rat groups. Two typical western blots are shown below each histogram. Densitometric analysis was used to calculate the normalized protein ratio. Immunohistochemistry revealed phosphorylated Erk1/2(p-Erk1/2) in the splenic tissues for one representative rat per group (n=4)(a2). The representative sections are shown at×400 original magnification, and scale bars are 20 mm. (b) Quantitative mRNA expression levels of Stat6 were evaluated by real-time PCR (qRT-PCR). The results were first normalized to one housekeeping gene and thereafter to sham expression, which was set at 1. Data are expressed as median ± interquartile range (n=6). The upper edges of error bars represent the 75th percentile in each group. *$p < 0.05$: CLP group *vs.* Sham group; #$p < 0.05$: CLP+E-5 *vs.* CLP group. CLP: cecal ligation and puncture; CLP+E-5: CLP with Esmolol infused at 5 mg.kg^{-1}.h^{-1}; CLP+E-18: CLP with Esmolol infused at 18mg.kg^{-1}.h^{-1}.

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