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ORIGINAL ARTICLE

The effects of etomidate on expression of high mobility group box 1 via the nuclear factor kappa B pathway in rat model of sepsis

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ABSTRACT

Etomidate is an anesthetic agent used in hemodynamically unstable patients, but its use has been controversial in septic patients. The response of high-mobility group box 1 (HMGB1), a late-phase lethal cytokine in sepsis, to etomidate has not been reported. This study investigated the effects of etomidate on the expression and release of HMGB1 and the underlying mechanism using a cecal ligation and puncture (CLP) model. Thirty-six male Sprague–Dawley rats were divided into sham, CLP, and Etomi groups. Sepsis was induced in the CLP and Etomi groups, and intravenous etomidate (4 mg/kg) was infused for 40 min immediately after operation in the Etomi group. Serum creatinine, alanine aminotransferase (ALT), tumor necrosis factor (TNF)-α, interleukin (IL)-6, and HMGB1 levels were measured 6 and 24 hours after surgery. Activation of nuclear factor (NF)-κB and HMGB1 mRNA expression in the liver, lung, kidney, and ileum tissues were measured, and immunohistochemical staining of HMGB1 was implemented. Increases of the TNF-α level 6 h after CLP and ALT and IL-6 levels 24 h after CLP were significantly inhibited by etomidate treatment. Etomidate treatment also significantly attenuated the increase in serum HMGB1 level at 6 and 24 h after CLP and suppressed the NF-κB and HMGB1 mRNA in multiple organs 24 h after CLP. Immunohistochemical staining also revealed that etomidate treatment inhibited HMGB1 expression. Etomidate inhibited the systemic release of HMGB1 and its expression in various organs. The mechanism may be associated with the inhibitory effects of etomidate on pro-inflammatory cytokine release and NF-κB activity.

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Cecal ligation and puncture; etomidate; sepsis; high mobility group box1; nuclear factor kappa B

1. Introduction

Sepsis is an aggravated inflammatory response to infection that can lead to a poor prognosis [1]. Previous studies have attempted to reveal the effects of increased levels of pro-inflammatory cytokines and therapeutic targets to control excessive inflammation [2,3]. High mobility group box 1 (HMGB1) is a non-histone chromosomal protein that promotes transcription and stabilizes nucleosome formation [4]. It has been widely studied as an important inflammatory mediator in sepsis and can be released actively or passively by inflammatory stimuli and cell necrosis [5]. Increased plasma concentration of HMGB1 correlates with a poor prognosis in sepsis, and anti-HMGB1 antibody treatment has protective effects against lethal endotoxemia [6–8].

Etomidate, a potent imidazole hypnotic, has been frequently used as an anesthetic induction agent or sedative for old or critically ill patients owing to its fast onset of therapeutic effect, brief duration of action, and hemodynamic safety [9]. However, for critically ill patients, administration of etomidate may cause mortality and morbidity due to its suppressive effects on adrenal steroidogenesis by inhibition of the 11β-hydroxylase enzyme [10, 11]. Although single-dose etomidate use does not seem to lead to mortality of septic patients [12, 13], available evidence is limited. A few previous studies have revealed that etomidate regulates excessive immune responses by inhibiting pro-inflammatory cytokines such as interleukin (IL)-1β, IL-6, and tumor necrosis factor alpha (TNF-α) and lipopolysaccharide (LPS)-induced nuclear factor kappa B (NF-κB) activation in an acute lung injury model and rat macrophages [14,15]. The reciprocal relationship among acute-phase cytokines, HMGB1, and NF-κB activity has been determined [4], but the effects of etomidate on HMGB1 expression and release have not yet been elucidated in sepsis.

In this study, we hypothesized that etomidate would attenuate the expression and systemic release of HMGB1 and the exaggerated inflammation in vital organs under septic conditions and could be used as a sedative or an anesthesia induction agent in patients with sepsis. We aimed to investigate the effects of etomidate on the release of acute pro-inflammatory cytokines, NF-κB

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activity, and HMGB1 expression using a cecal ligation and puncture (CLP)-induced sepsis model.

2. Materials and methods

2.1. Animals

The Laboratory Animal Ethics Committee of St. Vincent Hospital of the Catholic University of Korea approved all experimental protocols for this study. The care and handling of experimental animals were implemented according to the criteria in the Guide for the Care and Use of Laboratory Animals prepared by the Korean National Institutes of Health.

Male Sprague–Dawley rats, 8 weeks of age and weighing 250–280 g, were acquired from St. Vincent Hospital's Experimental Animal Center. Three rats per cage were housed under regular ambient temperatures and given standard laboratory food and tap water that had been sterilized with ultraviolet light. Etomidate® Lipuro (2 mg/mL; B. Braun, Melsungen, Germany) was purchased from a wholesale pharmacy.

2.2. Experimental protocols

Thirty-six rats were divided into three groups: sham, CLP, and Etomi ($n = 12$ per group). A CLP operation was performed to induce sepsis in the CLP and Etomi groups based on a previous paper [16]. After 8 hours of fasting, anesthesia was induced with 2–4 vol% isoflurane with 50% oxygen in the air by mask inhalation. The tail vein was cannulated with a 24-gauge angiocatheter, and a micro-infusion pump was connected.

The CLP and Etomi groups of rats underwent CLP surgery. After shaving the anterior abdomen, a 2-cm midline incision was made to expose the cecum, which was tied with 6.0-silk suture immediately below the ileocecal junction without bowel obstruction. Then, using an 18-gauge needle, two punctures were made in the cecum, and a small amount of cecal content was forced out of the punctures. After the cecum was replaced, the wounds in the peritoneal wall and skin were closed. Rats in the sham group underwent a similar procedure without puncture or ligation of the cecum.

All rats were administered 3 mL of warm normal saline into the peritoneum. For postoperative pain control, 0.5% bupivacaine was injected into the skin incision site, and 0.1 mg/kg of buprenorphine was injected subcutaneously. Immediately after, 0.5 mL of normal saline was infused for 40 min in the sham and CLP groups, and intravenous etomidate (4 mg/kg) was injected for 40 min in the Etomi group. After recovery from anesthesia at warm temperatures, all rats were returned to the cages.

2.3. Blood sampling for measurement of ALT, creatine, and circulating cytokines

Six rats from each group were sacrificed either 6 h or 24 h after the operation by isoflurane overdose (6–8 vol%) via inhalation. Prior to death, 3 mL of blood samples were harvested from the inferior vena cava, centrifuged with 3000 rpm for 30 min at 4°C, and immediately stored at−80°C. To evaluate the effects of etomidate on organ injury under septic conditions, serum alanine aminotransferase (ALT) and creatinine levels were assessed with an automatic biochemistry analyzer (Hitachi 7180; Hitachi High-Technologies Corp., Tokyo, Japan) and commercially available clinical assay kits. To investigate the effects of etomidate on early-onset pro-inflammatory cytokines, TNF-α, and IL-6 levels were measured with a rat TNF-α Quantikine enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, MN, USA) and a rat IL-6 Quantikine ELISA kit (R&D Systems), respectively. Serum HMGB1 level was analyzed to reveal the effect of etomidate on the release of HMGB1 using a rat HMGB1 ELISA kit (IBL-Hamburg, Hamburg, Germany).

2.4. Preparation of organ tissues

For real-time polymerase chain reaction (RT-PCR) and western blot analysis, tissue specimens from the liver, kidneys, lungs, and ileum (approximately 5 cm from the ileocecal valve) were collected immediately after death and separately homogenized. For histological examination and immunohistochemistry, the left cardiac ventricle of each rat was perfused with 0.9% normal saline, followed by 4% paraformaldehyde. After stiffening, the liver, lungs, and kidneys were obtained and placed in the a matrix. The tissues were sliced to a thickness of 2 mm and soaked in paraformaldehyde overnight. After applying paraffin wax, each tissue was cut into 5-μm-thick slices.

2.5. Hematoxylin and eosin staining for histopathology

A histopathological examination of the liver, lungs, and kidneys was conducted to assess the effects of etomidate on sepsis-induced organ injury. After drying for 45 min, paraffin sections were dewaxed by two changes of xylene for 15 and 20 min, respectively, followed by the completion of an a descending ethanol series, and then rinsed for 2 min in running tap water. Following 5 min of hematoxylin staining, the slides were rinsed with running water, differentiated in 1% acid alcohol, blued in 1% ammonia water, and counterstained with 1% eosin for 1 min. As subsequent steps, the slides were washed in running water, dried using a decreasing ethanol series, cleaned in two changes of xylene for 10 min each, and mounted with neutral

gum. A BX51 light microscope (Olympus Corporation, Tokyo, Japan) was used to analyze and collect images of the slides in order to assess tissue damage and inflammation. Histopathology scores were assessed in each group based on previous references for the semi-quantification of inflammation and organ damage [17]. Alveolar congestions, hemorrhage, alveolar wall thickness, and inflammatory cell aggregation were evaluated for lung tissue. Inflammatory cell infiltration, hepatocyte injury, and hemorrhage were assessed for liver tissue. Injury of renal tissues was estimated by hyperemia of the glomerulus, infiltration of inflammatory cells, and necrotic or sloughed cells in tubular lumens. Each tissue sample was rated with a semi-quantitative score between 0 and 4 by two investigators blinded to group assignment (scores of 0–4 indicate normal, mild, medium, severe, and extremely severe injury). All scores were collected and compared through statistical analysis.

2.6. Western blot analysis to measure NF-κB activation

The impact of etomidate on NF-**κ**B activation was investigated 24 h after surgery to identify the anti-inflammatory mechanism. Using PRO-PREP protein extraction solution (Intron Biotech, Sungnam, Korea), fresh liver, lungs, kidneys, and ileum tissues were homogenized into lysates. After lysates were centrifugated at 13,000 rpm and 4°C for 5 min, the protein concentration of the supernatant was measured using a Bradford assay kit (Pierce Biotechnology, IL, USA). Using sodium dodecyl sulfate – polyacrylamide gel electrophoresis, equal amounts of protein extract were placed onto 10% polyacrylamide gels. Following that, the extracts were transferred using semi-dry trans-blot cells (Bio-Rad Laboratories, Hercules, CA, USA) onto polyvinylidene difluoride membranes. Tris-buffered saline with 0.1% Tween 20 (TBST) and 5% skim milk were used to block the polyvinylidene difluoride membranes containing transferred proteins for 1 h at 25°C. Rabbit anti–NF-κB (p65) and anti–β-actin antibodies (1:1000 each; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) were incubated on the membranes overnight at 4°C following three TBST washes. The membranes were treated with horseradish peroxidase–linked secondary antibodies (1:1000; Cell Signaling Technology, Danvers, MA, USA) for 1 h at 25°C following three more TBST washes. Membranes were also treated with electrochemiluminescence reagents (Amersham, Buckinghamshire, UK) after three final washes in TBST and then digitally exposed to an Image Reader ImageQuant™ LAS 4000 mini (GE Healthcare Europe GmbH, Freiburg, Germany). Using the Multi Gauge version 3.0 software (Fujifilm Life Science, Tokyo, Japan), proteins were quantified for statistical analysis.

2.7. RT-PCR analysis to assess HMGB1 messenger RNA (mRNA) expression

For further investigation of etomidate's effects on transcription of HMGB1 in vital organs, we extracted total RNA from the liver, lungs, kidneys, and ileum 24 h after surgery. Total RNA was extracted using the RNeasy Plus mini kit (QIAGEN GmbH, Hilden, Germany) and reverse transcribed with the PrimeScript RT reagent kit (TaKaRa Bio Inc., Shiga, Japan). Relative mRNA expression levels were assessed using a Roche Diagnostics LightCycler 2.0 real-time PCR system (Roche Diagnostics GmbH, Mannheim, Germany) with a SensiFAST™ SYBR® Hi-ROX kit (Bioline, Luckenwalde, Germany) and gene-specific primers. Primer sequences are presented in Table 1. The following PCR procedure was performed in reaction system liquid with a total volume of 10̇ μL: initial denaturation was implemented for one cycle at 95°C for 30 s and then 41 cycles were performed at 95°C for 5 s and 60°C for 25 s.

2.8. Immunohistochemistry for HMGB1

Immunohistochemistry was performed to determine HMGB1 activity in liver, lung, and kidney tissues. After drying for 45 min, paraffin sections were dewaxed in two changes of xylene for 15 and 20 min, respectively. This step was completed before antigen retrieval in citric acid and a descending ethanol series. The sections were cleared three times with phosphate-buffered saline for 5 min after incubation with 3% hydrogen peroxide for 15 min in a Humidistat box at ambient temperature. The sections were incubated with ImmPRESS anti-rabbit immunoglobulin G secondary antibodies (ready to use; VECTOR Laboratories, CA, USA) at room temperature for 1 h after incubation with monoclonal rabbit anti-rat HMGB1 antibody overnight at 4°C (1:400 for kidneys and lungs, 1:1000 for liver; Cell Signaling Technology). After three 5-min PBS rinses, the slices were developed with 3,3′-diaminobenzidine peroxidase substrates (VECTOR Laboratories), terminated in purified water, and counterstained with hematoxylin for 5 min. After

Table 1. Real-time PCR primer sequences.

Primer	Accession number	Sequence(5′→ 3′)	Size (bp)	Cycle number	Annealing temperature
HMGB1	NM012963	F: GGCGAGCATCCTGGCTTATC R: AGGCAGCAATATCCTTCTCATAC	142	35	60°C
GAPDH	NM0170084	F: GCACAGTCAAGGCTGAGAATG R: ATGGTGGTGAAGACGCCAGTA	142	35	60°C

Note: HMGB1: high mobility group box 1; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

being dried in ethanol at various concentrations and washed in two changes of xylene for 10 min each, the slides were mounted with Canada balsam. Using an Olympus BX51 light microscope (Olympus, Tokyo, Japan) set to × 400, the slices were analyzed and imaged.

2.9. Statistical analysis

The Kolmogorov–Smirnov test was used to examine whether continuous variables followed a normal distribution. For normally distributed data, one-way analysis of variance was performed, followed by Bonferroni or Tukey's post hoc test. Non-normally distributed data were analyzed using the Kruskal–Wallis test with the Mann–Whitney *U* test. For comparison of discrete variables, the Kruskal–Wallis test followed by the Mann–Whitney *U* test was used. All statistical analysis was conducted using SPSS® software for Windows® (version 26.0; IBM Corporation, Armonk, NY, USA). $P < 0.05$ was considered statistically significant.

3. Results

3.1. Serum ALT, creatinine, and histopathologic findings

As shown in Table 2, CLP-induced sepsis resulted in a significant increase in ALT level 6 h after surgery ($P = 0.004$ in the CLP group vs. sham group). However, the ALT level in the Etomi group was not significantly different from that in the sham and CLP groups 6 h after surgery ($P = 0.24$ and 0.93, respectively). At 24 h after surgery, the ALT level was significantly increased in both the CLP and Etomi groups compared to that in the sham group ($P = 0.002$ and 0.002, respectively). Etomidate treatment significantly attenuated the ALT elevation 24 h after surgery ($P = 0.008$ in the Etomi group vs. CLP group). Meanwhile, the creatinine level was significantly increased in the CLP group compared to that in the sham and Etomi groups 6 h after surgery ($P = 0.009$ and 0.009, respectively). The increase in creatinine level seen 24 h after surgery was also mitigated by etomidate treatment ($P = 0.015$ in the Etomi group vs. CLP group).

The histopathological findings are presented in Figure 1. No pathological changes occurred in the sham group during the observation period, whereas severe injuries and infiltration of inflammatory cells in multiple organs were observed in the CLP group 24 h after surgery. In lung tissues from the CLP group, leukocyte infiltration, erythrocyte leakage into the alveolar and interstitial spaces, and edematous changes were observed (black arrows). In the hepatic tissues, the major inflammatory injuries included significant malformation and necrosis, and edema was present in the hepatocytes and interstitial spaces (black arrows). Injuries in the kidney tissues included infiltration of interstitial leukocytes, hyperemia in the renal tubules, endothelial cell swelling, and intercapillary cell proliferation (black arrows). However, these inflammatory changes and tissue injuries were mitigated in the organs of the Etomi group. As shown in Figure 1 B, histopathological scores of the liver, lungs, and kidney tissues were significantly increased in the CLP group than those in the sham group ($P = 0.008$ in all organs). However, etomidate treatment significantly attenuated tissue injury in the liver and lungs ($P = 0.008$ and 0.016 in the liver and lungs, respectively).

3.2. Serum cytokine concentration

Figure 2 shows the changes in serum cytokine concentrations. Six hours after surgery, the serum TNF-α level was significantly increased in both the CLP and Etomi groups compared to that in the sham group ($P = 0.002$ and 0.002, respectively), but significantly less so in the Etomi group compared to the CLP group ($P = 0.008$). Twenty-four hours after surgery, the serum TNF-α level was significantly increased in the CLP group compared to the sham group ($P = 0.002$).

A significant increase in serum IL-6 occurred in the CLP and Etomi groups compared to that the sham group at both 6 h ($P = 0.002$ and 0.004, respectively) and 24 h ($P = 0.002$ and 0.002) after surgery. However, etomidate administration significantly attenuated the IL-6 elevation 24 h after surgery ($P = 0.041$ vs. CLP group).

CLP-induced sepsis increased the serum HMGB1 concentration at both 6 and 24 h after surgery ($P = 0.02$ and 0.002 in the CLP group vs. sham group at 6 h; $P = 0.026$ and 0.002 in the Etomi group vs. sham group at 24 h). However, etomidate treatment inhibited the elevation of serum HMGB1 concentration at

Table 2. Effect of etomidate on changes in serum alanine aminotransferase (ALT) and creatinine in septic rats ($n = 6$ at each timepoints).

		Sham	CLP	Etomidate
ALT(IU/L)	6 h	45.60 ± 5.70†	90.00 ± 31.36*	62.20 ± 21.04
	24 h	45.85 ± 3.56†	173.50 ± 84.20*	82.20 ± 14.19*†
Creatinine (mg/dL)	6 h	0.34 ± 0.04†	0.41 ± 0.02*	0.37 ± 0.02†
	24 h	0.42 ± 0.03	0.51 ± 0.13	0.40 ± 0.03†

Note: Mean ± standard deviation. CLP, the cecal ligation and puncture group; sham, sham operation group; Etomidate, the group undergone cecal ligation and puncture with etomidate treatment; *$P < 0.05$, compared to sham group; †$P < 0.05$, compared to CLP group.

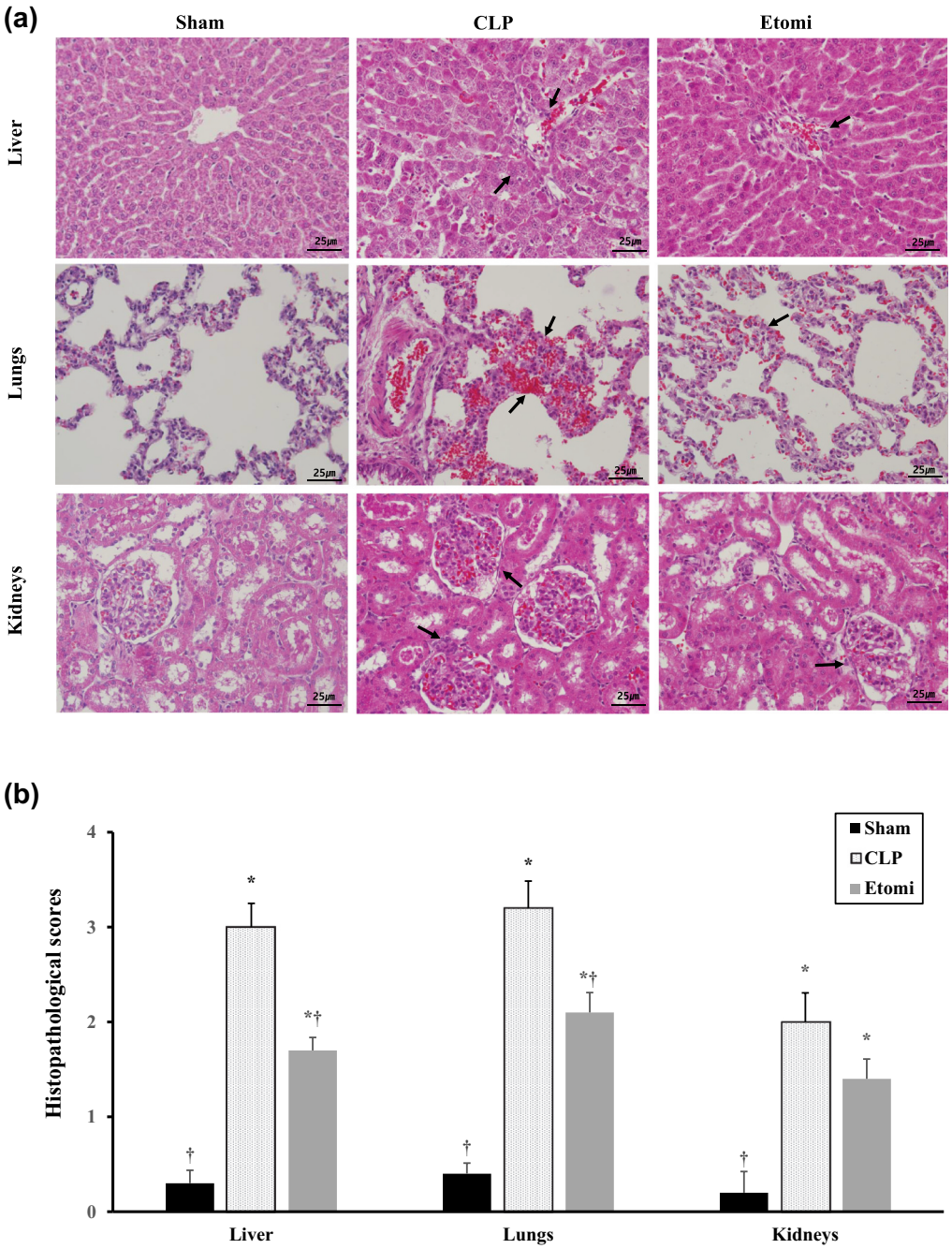

Figure 1. (a) Histopathologic changes in organs of CLP-induced septic rats. Hematoxylin and eosin-stained sections of liver, lungs and kidneys from rats subjected to sham/CLP operation 24 h after surgery (magnification × 400). Etomidate treatment attenuates acute organ injury by CLP-induced sepsis. (b) Quantification of the severity of inflammation in each organ (ranges from 0 to 4). *P<0.05 vs Sham, †P<0.05 vs CLP. Sham: sham operation group, CLP: Cecal ligation and puncture with normal saline group, Etomi: Cecal ligation and puncture with etomidate treatment group.

both 6 and 24 h after surgery (both $P = 0.015$ vs. CLP group).

3.3. Western blot analysis for NF-κB activation

Sepsis significantly increased the activation of NF-κB (Figure 3; $P = 0.017$, $P = 0.004$, $P = 0.002$, and $P = 0.004$ for the liver, lungs, kidneys, and ileum, respectively, in the CLP group vs. sham group). NF-κB activation was significantly increased in the lungs and ileum in the Etomi group compared to that the sham group ($P = 0.009$ for lungs and $P = 0.004$ for ileum) but was significantly suppressed in all four tested organs in the Etomi group compared to the CLP group ($P = 0.041$, $P = 0.004$, $P = 0.002$, and $P = 0.016$ for the liver, lungs, kidneys, and ileum, respectively).

3.4. HMGB1 mRNA expression

Figure 4A shows the results of RT-PCR. CLP-induced sepsis significantly increased HMGB1 mRNA level 24 h after surgery ($P = 0.008$, $P = 0.002$, $P = 0.004$, and $P = 0.004$ for the liver, lungs, kidneys, and ileum, respectively, in the CLP group vs. sham group).

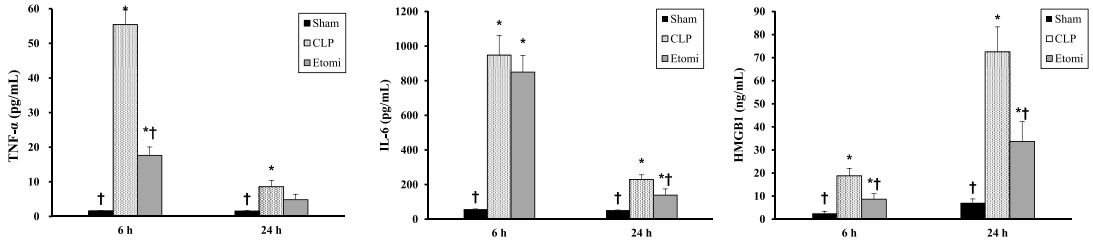

Figure 2. Effects of etomidate treatment in systemic inflammatory cytokines concentration 6 and 24 h after sham/CLP operations. ELISA analysis of serum TNF-α, IL-6 and HMGB1 concentration. Data were presented as mean ± SD ($n = 6$). *P<0.05 vs Sham, †P<0.05 vs CLP. Sham: sham operation group, CLP: Cecal ligation and puncture with normal saline group, Etomi: Cecal ligation and puncture with etomidate treatment group.

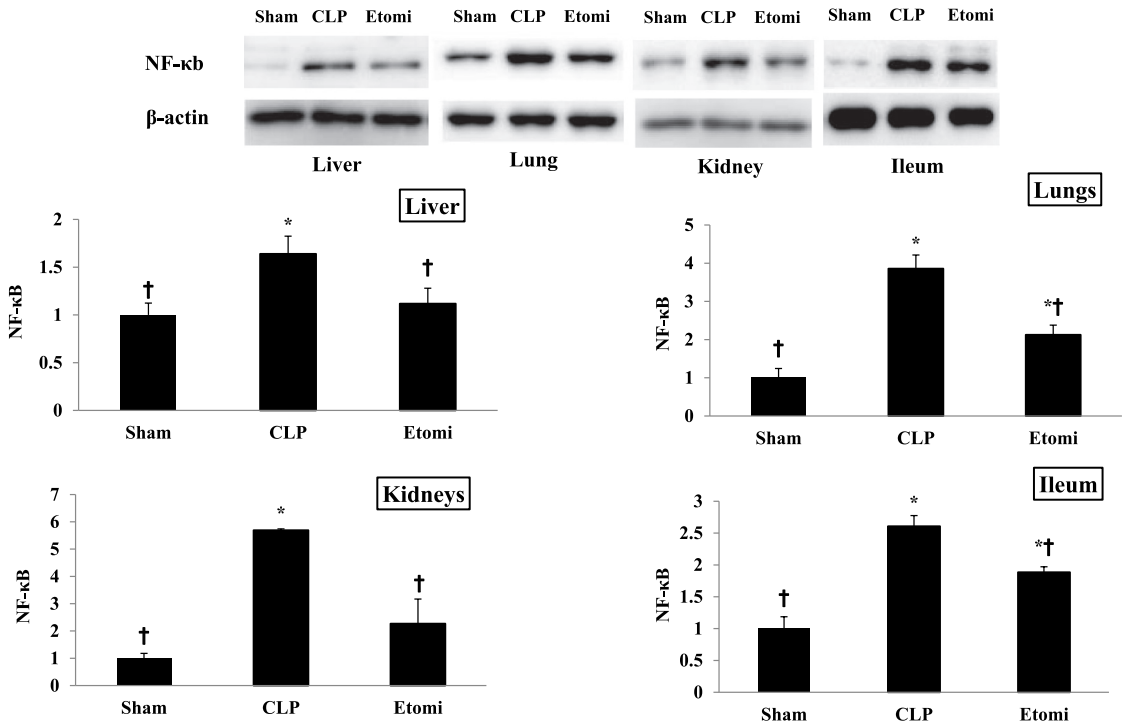

Figure 3. Effect of etomidate on activity of NF-κB in organs of CLP-induced septic rats. Liver, Lungs, kidneys and ileum were collected 24 h after surgery and NF-κB p65 activation was analyzed by Western blot. Etomidate treatment attenuated the increase in NF-κB activity after CLP surgery. *P<0.05 vs Sham, †P<0.05 vs CLP.

Although the HMGB1 mRNA level was significantly increased in the Etomi group over those in the sham group ($P = 0.041$, $P = 0.041$, and $P = 0.009$ for the lungs, kidneys, and ileum, respectively), etomidate treatment attenuated the expression of HMGB1 mRNA in rats with sepsis 24 h after the operation ($P = 0.008$, $P = 0.002$, $P = 0.004$, and $P = 0.004$ for the liver, lungs, kidneys, and ileum, respectively, in the Etomi group vs. CLP group).

3.5. Immunohistochemistry for HMGB1

As presented in Figure 4B, tissues from the sham group showed scarce HMGB1-positive cells. On the other hand, tissues stained for HMGB1 in the CLP group were dark brown in color, and cell nuclei were strongly stained, indicating enhanced HMGB1 expression. However, tissues from rats in the Etomi group displayed lighter staining in the cell nucleus and cytoplasm compared to the CLP group. Etomidate treatment inhibited nuclear translocation and activity of HMGB1 in the liver, lungs, and kidneys.

4. Discussion

This study demonstrated that etomidate pre-treatment attenuated the increase in systemic pro-inflammatory cytokine release and activation of NF-κB in polymicrobial sepsis induced by CLP. HMGB1 expression in various organs and its systemic release were decreased, and organ tissue injury and elevation of serum ALT were attenuated in septic rats treated with etomidate. These

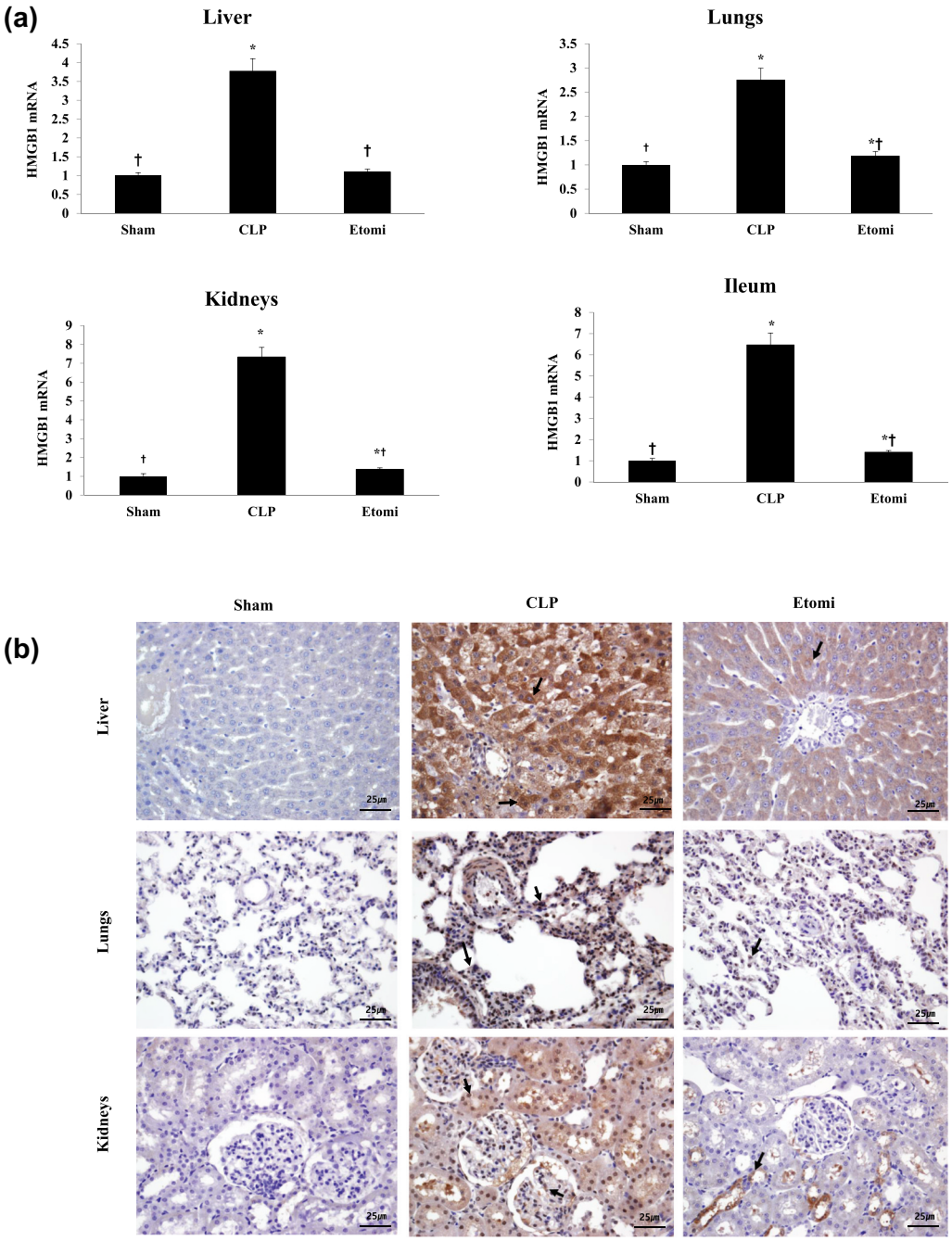

Figure 4. (a) the expressions of HMGB1 mRNA in tissues from rats in each group 24 h after sham/CLP surgeries. Liver, lungs, kidneys and ileum were taken 24 h after surgery. HMGB1 mRNA expression in multiple organs were measured by Real-Time PCR. Etomidate treatment inhibits excessive HMGB1 mRNA expression in various organs. *P<0.05 vs Sham, †P<0.05 vs CLP. (b) Immunohistochemical staining of HMGB1 in liver, lungs, and kidneys from rats 24 h after sham/CLP surgeries (magnification × 400). The expression and translocation of HMGB1 were increased in the septic rats that underwent CLP operation compared with rats that underwent the sham operation (indicated by black arrows), but etomidate treatment attenuated the expression of HMGB1. Sham: sham operation group, CLP: Cecal ligation and puncture with normal saline group, Etomi: Cecal ligation and puncture with etomidate treatment group.

findings suggest that etomidate has protective effects in sepsis.

Excessive inflammatory responses in sepsis that cannot be anticipated by the anti-inflammatory response can result in multiorgan failure [1]. Therefore, several previous studies have tried to reveal the effects of inflammatory cytokines and protective anti-inflammatory strategies for sepsis using in vitro or in vivo models [18,19]. In the early phase of sepsis, a hyperinflammatory response is initiated with dysregulated release of pro-inflammatory cytokines and chemokines [1]. Uncontrolled release of pro-inflammatory cytokines such as TNF-α, IL-1, and IL-6 can trigger organ injuries and is associated with mortality [1]. However, therapeutic interventions targeting such

cytokines are not more beneficial than standard supportive therapy [18,19], and inappropriate suppression of the immune response can result in immunosuppression [20]. Therefore, recent studies have attempted to target HMGB1, which acts as a late-phase pro-inflammatory cytokine. In this study, we performed CLP surgery on rats to mimic polymicrobial sepsis in humans. The findings from rats in our CLP group revealed a similar progression to that of human sepsis with a surge in acute-phase cytokines followed by the late-phase inflammatory cytokine HMGB1.

Nearly all types of cells produce the 30-kDa nuclear and cytosolic protein HMGB1, which facilitates various cellular processes [21]. In inflammatory conditions, inflammatory signaling factors such as IL-1, TNF, or LPS stimulate active release of HMGB1 by innate immune cells. Also, HMGB1 is passively released by necrotic cells to transmit signals to neighboring immune cells and promote inflammation [22]. Compared to the acute release of IL-1, IL-6, and TNF-α, the secretion of HMGB1 is delayed by 12–18 h [23]. HMGB1 also stimulates the release of inflammatory cytokines by macrophages, monocytes, and neutrophils as part of a positive feedback mechanism [23]. Therefore, HMGB1 can act as a pivotal factor in sustained pro-inflammatory reactions. Plasma HMGB1 level is positively correlated with organ damage and mortality in septic patients and experimental animals [7,24]. The results of the present study indicate that increased systemic HMGB1 and HMGB1 mRNA expression levels in the lungs, kidneys, liver, and ileum were associated with histopathologic findings and increased ALT concentration in septic rats. These findings are in line with earlier studies reporting that the serum creatinine level, ALT level, and lung myeloperoxidase activity significantly correlate with HMGB1 mRNA expression [7,25]. Several anesthetics and sedatives, such as propofol, lidocaine, dexmedetomidine, and ketamine, have shown anti-inflammatory effects by attenuation of HMGB1 expression and release [25–28]. Like these agents, etomidate also significantly decreased systemic HMGB1 and HMGB1 mRNA expression levels in multiple organs and therefore mitigated the ALT increase with attenuated organ injury in this study.

After release, HMGB1 binds to Toll-like receptors (TLRs) and receptors for advanced glycation end-products (RAGEs) [4]. This engagement of HMGB1 activates NF-κB and mitogen-activated protein kinase pathways to induce the release of inflammatory cytokines from innate immune cells, upregulation of endothelial adhesion molecules, chemotactic cell movements, leukocyte infiltration, and epithelial cell dysfunction [21]. NF-κB, as a transcription factor regulating target genes, plays important roles in amplification of HMGB1 signaling via a reciprocal functional relationship [4]. RAGE, TLR2, and TLR4 activation by HMGB1 causes NF-κB activation, which increases the expression of RAGEs and TLRs as well as pro-inflammatory and pro-angiogenic genes [4]. This signal amplification of HMGB1 may induce immune dysregulation following the acute hyperinflammatory phase [3]. Accordingly, we identified the effects of etomidate on NF-κB in vital organs to reveal underlying mechanisms of etomidate's inhibition of HMGB1 expression. The findings of this study suggest that etomidate suppresses the tissue mRNA level of HMGB1 via inhibiting the NF-κB signaling pathway under septic conditions.

Clinically, administration of etomidate to critically ill patients remains under debate because of its inhibition of adrenal steroidogenesis [12,29,30]. The relationship between injection of single-dose etomidate and prognosis of septic patients has been studied mostly using retrospective data [12,29], which are not free from confounding factors. Therefore, in vitro or in vivo studies using animals have been performed to reveal the effects of etomidate on inflammation or mortality [15,31]. The anti-inflammatory effects of etomidate have been suggested in several previous studies [15,31,32], but in vivo studies using CLP-induced septic rats have offered inconsistent results regarding mortality [31,33,34]. Zhang et al. reported that etomidate inhibited the serum mRNA expression of TNF-α and IL-6 and NF-κB activation, but the mortality of etomidate-treated septic rats was increased due to lymphocyte apoptosis rather than adrenal suppression [31,34]. However, Wang et al. reported that etomidate treatment resulted in better survival rates by decreasing IL-1 and TNF-α levels and enhancing glucocorticoid receptor expression in septic rats [33]. An in vitro study supports the results of Wang et al., suggesting that etomidate treatment attenuates TNF-α, IL-6, and cluster of differentiation 14 levels; triggers receptors expressed on myeloid cells-1; and inhibits NF-κB activation in LPS-stimulated macrophages [15]. In a human study, continuous infusion of etomidate reduced serum TNF-α, IL-1, and IL-6 levels and postoperative complications in patients with ischemia–reperfusion injury [32]. However, studies assessing the effects of etomidate on the expression and release of HMGB1 related to pro-inflammatory cytokines and NF-κB pathways under septic conditions are limited in number. The results of this study are similar to those of previous studies that suggest protective effects of etomidate on inflammation [32,33]. Because increased HMGB1 concentration correlates with organ injury and poor prognosis in sepsis [6,24], HMGB1 could be a potential target for sepsis treatment [3,5]. This study provides new perspectives on the use of etomidate and could be the basis for new therapeutic strategies for patients with sepsis. In addition, although we did not investigate long-term outcomes, this study can inform further prospective studies for patients with sepsis.

There are several limitations in this study. First, the mortality rate was not measured. A previous study suggested that etomidate pre-treatment decreases the survival rate by 10%–20% in septic rats receiving continuous infusion of etomidate [34]. However, we administered a single large dose of etomidate, and several other anesthetics have shown an increase in survival rates in sepsis models by inhibiting HMGB1 release [25,35,36]. Further studies regarding mortality are still needed. Second, we did not investigate the degree of adrenal insufficiency. Previous studies have suggested that the mortality rate and degree of adrenal injury do not positively correlate in septic rats with etomidate treatment [33,34], and the inhibitory effects of etomidate on steroidogenesis have been revealed in human studies [9]. Third, we assessed the effects of a single fixed dose of etomidate in sepsis. The general anesthesia induction dose of etomidate (0.3 mg/kg) for humans can be converted to 2.7 mg/kg for rats because the body surface area of rats can be converted to about one-ninth that of humans [14]. In previous studies, etomidate was administered to mice at doses ranging from 0.3 to 30 mg/kg [14,37]. We chose to administer a dose of 4 mg/kg for etomidate immediately after surgery in this study because a previous study demonstrated that this dose of etomidate results in better survival than lower (0.6 mg/kg) or higher (10 mg/kg) doses of etomidate in septic rats, and etomidate is most effective when used immediately after modeling [31,33]. Finally, we evaluated septic rats over a relatively short period of 24 h. A prospective, noninterventional study of patients with sepsis found that HMGB1 levels gradually, though not statistically significant, decreased in survivors, whereas they increased between days 1 and 3 in nonsurvivors [6]. If we had performed longer follow-ups of the rats with sepsis and HMGB1 level in this study, we would have been able to evaluate the effects of early decrease of HMGB1 with etomidate treatment on sepsis outcome.

5. Conclusions

The results of this study showed that etomidate attenuated the systemic release of early inflammatory cytokines and NF-κB activation and therefore inhibited the expression and release of HMGB1 and alleviated inflammation in various organs. Further studies on etomidate-related mortality are required.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Data availability statement

The data supporting the findings of the current study are available from the corresponding author on reasonable request.

Ethics approval

This study was approved by the Ethics Committee of the Catholic university of Korea, St Vincent Hospital (CaVin Animal IRB 15–20).

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