Published in final edited form as:

Peptides. 2010 May ; 31(5): 872–877. doi:10.1016/j.peptides.2010.01.010.

HUMAN ADRENOMEDULLIN AND ITS BINDING PROTEIN AMELIORATE SEPSIS-INDUCED ORGAN INJURY AND MORTALITY IN JAUNDICED RATS

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Abstract

Sepsis is a serious complication for patients with obstructive jaundice. Although administration of adrenomedullin (AM) in combination with its binding protein (AMBP-1) is protective after injury, it remains unknown whether AM/AMBP-1 ameliorates sepsis-induced organ injury and mortality in the setting of biliary obstruction. The aim of this study is, therefore, to test the efficacy of human AM/AMBP-1 in a rat model of obstructive jaundice and polymicrobial sepsis. To study this, obstructive jaundice was induced in male adult rats (275–325g) by common bile duct ligation (BDL). One week after BDL, the rats were subjected to sepsis by cecal ligation and puncture (CLP). Plasma levels of AM and AMBP-1 were measured at 20 h after CLP. In additional groups of BDL+CLP rats, human AM/AMBP-1 (24/80 μ g/kg BW) or vehicle (i.e., human albumin) was administered intravenously at 5 h after CLP. Blood and tissue samples were collected at 20 h after CLP for various measurements. To determine the long-term effect of human AM/AMBP-1 after BDL+CLP, the gangrenous cecum was removed at 20 h after CLP and 7-day survival was recorded. Our results showed that plasma levels of AM were significantly increased while AMBP-1 levels were markedly decreased after BDL+CLP ($n=8$, $P<0.05$). Administration of human AM/AMBP-1 attenuated tissue injury and inflammatory responses after BDL+CLP. Moreover, human AM/AMBP-1 significantly increased the survival rate from 21% ($n=14$) to 53% ($n=15$). Thus, human AM/AMBP-1 ameliorates sepsis-induced organ injury and mortality in jaundiced rats. Human AM/AMBP-1 can be further developed as a novel treatment for sepsis in jaundiced patients.

Keywords

Sepsis; jaundice; vasoactive peptide; tissue injury; survival

1. INTRODUCTION

Obstructive jaundice occurs in a variety of clinical settings, such as gallstone impaction, biliary atresia, and tumor compression. Despite advances in preoperative evaluation, operative techniques, postoperative care, and the utilization of powerful broad-spectrum antibiotics, sepsis and subsequent multiple organ failure remain a leading cause of perioperative morbidity and mortality in obstructive jaundice [27,36,38,42]. As a result, numerous agents and interventions have been studied to treat sepsis in jaundiced patients, however, none has so far

been entirely successful [10,21]. It remains important, therefore, to find an effective therapeutic agent which ameliorates sepsis-induced organ injury and mortality in jaundiced patients.

Adrenomedullin (AM) is a ubiquitously expressed, pluripotent peptide initially isolated by Kitamura *et al.* in 1993 from a human pheochromocytoma [19]. Human AM is a 52-amino acid peptide. Rat AM has 50 amino acids, with 2 deletions and 6 substitutions compared with the human peptide. AM has been shown to have a remarkable range of actions, from vasodilation, through modulating hormone secretion, to anti-inflammation [11,15,17,20,34]. Various studies have demonstrated that circulating levels of AM increase in patients with sepsis [31], which may play a major role in initiating the hyperdynamic response during the early stage of sepsis [47,49]. In 1999, a specific AM binding protein (i.e., AMBP-1) was discovered in mammalian blood [5,32]. AMBP-1 potentiates AM biological activities under normal as well as pathophysiologic conditions [5,32,49]. A deficiency of AMBP-1 in humans is associated with higher susceptibility to recurrent infections [30]. Our previous studies have shown that AMBP-1 levels decrease significantly at the late stage of sepsis, which appears to be responsible for the transition from the hyperdynamic phase to the hypodynamic phase during the progression of polymicrobial sepsis [47,49]. The primary site of AMBP-1 biosynthesis is the liver [8,22,35]. To the best of our knowledge, however, alterations in AMBP-1 levels in obstructive jaundice have not been reported previously.

Using a rat model of polymicrobial sepsis induced by cecal ligation and puncture (CLP), we have recently demonstrated that administration of AM in combination with AMBP-1 attenuates tissue injury, inhibits inflammatory responses, and reduces mortality in sepsis [47,48]. However, it remains unknown whether AM/AMBP-1 ameliorates sepsis-induced organ injury and mortality in the setting of biliary obstruction. The aim of this study is, therefore, to test the efficacy of human AM/AMBP-1 in a rat model of obstructive jaundice and polymicrobial sepsis.

2. MATERIALS AND METHODS

2.1. Experimental animals

Male Sprague-Dawley rats (275–325g) purchased from Charles River Laboratories (Wilmington, MA) were housed in light-controlled room with a 12-h light/dark cycle and allowed free access to water and standard rat chow. Rats were acclimatized for at least 1 week before experimentation. All animals received humane care in compliance with the National Research Council's Guide for the Care and Use of Laboratory Animals. The animal protocol was approved by the Institutional Animal Care and Use Committee of The Feinstein Institute for Medical Research.

2.2. Animal model of obstructive jaundice and polymicrobial sepsis

Obstructive jaundice was induced by common bile duct ligation (BDL). Briefly, the rats were anesthetized with isoflurane inhalation and a 2-cm midline abdominal incision was made. The bile duct was then exposed, doubly ligated with a 2-0 silk suture, and excised between the two ligatures. The abdominal incision was closed in layers and the rats received 3 ml/100g BW saline subcutaneously. After recovering, rats were returned to cages and were provided food and water. One week after BDL, polymicrobial sepsis was induced by cecal ligation and puncture (CLP) as we previously described [44]. Briefly, the rats were again anesthetized with isoflurane. The midline abdominal incision was reopened. The cecum was then exposed, ligated just distal to the ileocecal valve to avoid intestinal obstruction, punctured twice with an 18-gauge needle, and returned to the abdominal cavity. The incision was then closed in layers. Sham-operated animals (i.e., control animals) underwent the same procedure with the exception that neither the common bile duct nor the cecum was ligated and/or punctured. The

animals were resuscitated with 3 ml/100g body weight (BW) normal saline subcutaneously immediately after surgery.

2.3. Measurement of plasma AM levels

Plasma levels of AM were measured using a rat AM radioimmunoassay kit from Peninsula Labs (Belmont, CA), according to the manufacturer's instructions. All samples were tested in duplicate.

2.4. Western blotting analysis of AMBP-1

Polyclonal antibodies against human AMBP-1 were obtained from Accurate Chemical (Westbury, NY). Our previous study indicates that the anti-human AMBP-1 antibodies recognize rat AMBP-1 [2]. We used such antibodies, since anti-rat AMBP-1 antibodies are not commercially available. 10 μ l plasma was fractionated on 4–12% Bis-Tris gel and transferred to 0.2 μ m-nitrocellulose membrane. Blots were then hybridized with anti-human AMBP-1 polyclonal antibodies. After incubation with the horseradish peroxidase-linked anti-rabbit IgG (Cell Signaling Technology, Beverly, MA), the signal was detected with the chemiluminescent peroxidase substrate (ECL, Amersham Biosciences, Piscataway, NJ). The relative band intensity was quantified using a Bio-Rad image system (Hercules, CA).

2.5. Administration of human AM and human AMBP-1

At 5 h after the onset of sepsis in jaundiced rats, the left femoral vein was cannulated with a polyethylene-50 tubing under isoflurane anesthesia. Human AM (24 μ g/kg BW, Phoenix Pharmaceuticals, Belmont, CA) in combination with human AMBP-1 (80 μ g/kg BW, Cortex Biochem, San Leandro, CA), or vehicle (i.e., human albumin, 104 μ g/kg BW) were administered via the femoral vein catheter over 30 min at a constant infusion rate. Blood and tissue samples were collected at 20 h after CLP for various measurements.

2.6. Determination of total bilirubin, direct bilirubin, transaminases, lactate and creatinine

Blood samples were centrifuged at 3000 *g* for 10 min at 4°C, and the serum samples were stored at –80°C until assayed. Commercially available assay kits were used for the determination of serum concentrations of total and direct bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate, and creatinine according to the manufacturer's instructions (Pointe Scientific, Canton, MI). All samples were tested in duplicate.

2.7. Determination of TNF- α and IL-6

Serum levels of TNF- α and IL-6 was measured using specific enzyme linked immunosorbent assay (ELISA) kits (BioSource International, Camarillo, CA) according to the manufacturer's instructions. All samples were tested in duplicate.

2.8. Measurement of myeloperoxidase (MPO) activity

MPO activity in the lungs, liver and small intestine (i.e., jejunum) was determined by using the peroxidase-catalyzed reaction as we described previously [43]. All samples were tested in duplicate.

2.9. Survival study

In additional BDL+CLP animals, human AM/AMBP-1 or vehicle was administered intravenously as described above at 5 h after CLP. At 20 h after CLP, the necrotic cecum was excised and the abdominal cavity was washed twice with using 40 ml of warm, sterilized normal saline solution. The abdominal incision was closed in layers. The animals then were allowed food and water ad libitum. All surviving animals were sacrificed on Day 7.

2.10. Statistical analysis

All data are expressed as means $\pm$ SE and compared by one-way analysis of variance (ANOVA). When the ANOVA was significant, post-hoc testing of differences between groups was performed using Student-Newman-Keuls method. The survival rate was estimated by Kaplan-Meier method and compared by the log-rank test. A P value < 0.05 was considered statistically significant.

3. RESULTS

3.1. Obstructive jaundice exacerbates sepsis-induced organ injury and inflammatory responses in rats

As shown in Table 1, CLP alone had no major effects on plasma levels of total and direct bilirubin at 20 h after the onset of sepsis as compared to sham-operation. 7-day BDL increased plasma levels of total and direct bilirubin by 52 and 123 fold, respectively ($P<0.05$). No statistically significant differences in plasma bilirubin levels were found between BDL alone and BDL+CLP groups. As an indicator of hepatic injury, plasma levels of ALT and AST were increased significantly at 20 h after CLP in non-jaundiced rats ($P<0.05$). Further increases in plasma levels of ALT and AST were found at 20 h after CLP in jaundiced rats as compared to CLP alone rats ($P<0.05$). Plasma levels of lactate, a marker for systemic hypoxia, were increased by 89% at 20 h after CLP in non-jaundiced rats ($P<0.05$). A 41% further increase in plasma levels of lactate was found at 20 h after CLP in jaundiced rats as compared to CLP alone rats ($P<0.05$). Similarly, plasma levels of creatinine, an indicator of renal injury, were increased by 59% at 20 h after CLP in non-jaundiced rats ($P<0.05$). An 80% further increase in plasma levels of creatinine was found at 20 h after CLP in jaundiced rats as compared to CLP alone rats ($P<0.05$). Plasma levels of pro-inflammatory cytokine TNF- α and IL-6 were increased significantly at 20 h after CLP in non-jaundiced rats ($P<0.05$). BDL further increased plasma levels of TNF- α and IL-6 by 89% and 67%, respectively, at 20 h after CLP ($P<0.05$). Similarly, MPO activities in the lungs, liver and gut, an indicator of neutrophil infiltration, were increased markedly at 20 h after CLP in non-jaundiced rats ($P<0.05$). BDL further increased pulmonary, hepatic and intestinal MPO activities at 20 h after CLP ($P<0.05$). These results clearly indicate that obstructive jaundice exacerbates sepsis-induced organ injury and inflammatory responses in rats.

3.2. Alterations in plasma levels of AM and AMBP-1 after CLP, BDL and BDL+CLP

As shown in Figure 1A, plasma levels of AM were increased significantly in CLP alone, BDL alone and BDL+CLP rats as compared to those in sham-operated rats ($P<0.05$). Unlike AM, plasma levels of AMBP-1 were decreased markedly in CLP alone, BDL alone and BDL+CLP rats as compared to those in sham-operated rats ($P<0.05$, Fig. 1B). However, there is no statistically significant difference in circulating levels of AM (Fig. 1A) and AMBP-1 (Fig. 1B) among CLP alone, BDL alone and BDL+CLP rats.

3.3. Human AM/AMBP-1 attenuates organ injury after BDL+CLP

As indicated in Figures 2A–B, administration of human AM/AMBP-1 decreased serum levels of ALT and AST significantly at 20 h after CLP in jaundiced rats ($P<0.05$). Similarly, significantly increased serum levels of lactate at 20 h after CLP in jaundiced rats were also markedly decreased by human AM/AMBP-1 treatment ($P<0.05$, Fig. 2C). As shown in Figure 2D, serum levels of creatinine were markedly lower in human AM/AMBP-1 treated rats than those in vehicle-treated rats at 20 h after BDL+CLP.

3.4. Human AM/AMBP-1 downregulates inflammatory responses after BDL+CLP

As demonstrated in Figures 3A–B, serum levels of TNF- α and IL-6 were 8 and 6 fold higher in vehicle treated BDL+CLP rats than those in sham control rats. Human AM/AMBP-1 administration significantly downregulated serum levels of TNF- α and IL-6 at 20 h after BDL+CLP. Similarly, pulmonary, hepatic and intestinal MPO activities increased dramatically at 20 h after CLP in jaundiced rats ($P<0.05$, Figs. 4A–C). Treatment with human AM/AMBP-1 at 5 h after the onset of sepsis in jaundiced rats significantly reduced pulmonary, hepatic and intestinal MPO activities.

3.5. Human AM/AMBP-1 improves survival after BDL+CLP

As shown in Figure 5, the survival rate after CLP and cecal excision in jaundiced rats with vehicle administration was 50% at day 2 and decreased to 21% at days 5–7. Treatment with human AM/AMBP-1, however, improved the survival rate to 87% at day 2 and 53% at days 6–7, which was significantly higher than the BDL-CLP vehicle treated group ($P<0.05$, Fig. 5).

4. DISCUSSION

The failure of many prior clinical trials for sepsis has been attributed in part to the heterogeneity within the study population [6]. In this regard, we tested the efficacy of a novel treatment for sepsis (i.e., human AM/AMBP-1) in a rat model of polymicrobial sepsis following biliary obstruction in this study. Our results clearly showed that administration of human AM/AMBP-1 at 5 h after the onset of sepsis (i.e., post treatment) inhibits the inflammatory response, ameliorates organ injury and improves survival in jaundiced rats.

AM circulates in the plasma in the low picomolar range and can exert a multitude of biological effects through an autocrine/paracrine mode of action. The best-characterized function of AM is as a potent vasodilator. Infusion of AM in humans and in several species of experimental animals causes a prolonged, dose-dependant vasorelaxation and hypotension [13,14,19]. Soon after the discovery of AM, clinician scientists began measuring the plasma levels of this peptide in a multitude of different human diseases and conditions. Elevated plasma AM levels were reported in patients with sepsis [31], systemic inflammatory response syndrome [31,41], and following a major surgery [9], hypoxia [1,12,28,40], hemorrhagic and cardiogenic shock [4, 18], or ischemia-reperfusion injury [26,29]. The inflammatory cytokines TNF- α , IL-1 β , and bacterial endotoxin strongly stimulate the synthesis and secretion of AM [16,39]. It is speculated that the elevation in AM levels may be a protective mechanism to counteract cardiovascular disorders under such conditions [37]. In the current study, we also show that the upregulation of AM occurs after sepsis, jaundice as well as jaundice plus sepsis in rats. Although the levels of AM are higher in the jaundice alone group than the jaundice plus sepsis group, that difference is not statistically significant. Therefore, such a difference may simply reflect experimental variations. The increased levels of AM appear to be a protective mechanism under such conditions. However, AM may be primarily acting in the early hyperdynamic phase of sepsis, because transition to the later hypodynamic phase is associated with marked reduction in vascular responsiveness to AM and administration of low dose of exogenous AM fails to maintain cardiovascular stability in the CLP model of sepsis in the rat [47].

Recently, the understanding of the regulation of AM activity in sepsis has been greatly expanded after the discovery of a specific AM binding protein in mammalian blood [5,7,47]. Nonradioactive ligand-blotting assay together with high-pressure liquid chromatography/sodium dodecyl sulfate-polyacrylamide gel electrophoresis purification techniques permitted the isolation of AMBP-1, which by database comparison, was subsequently identified as complement factor H, an inhibitor of the classic and alternative pathways of the complement

cascade [32]. Circulating AMBP-1 affects the bioactivity of AM under normal and pathologic conditions [32]. AMBP-1 enhances AM-induced vascular relaxation [49]. Our previous studies have shown that AMBP-1 levels are significantly reduced in the hypodynamic phase of sepsis, which appears to be responsible for the reduced vascular responsiveness to AM under such a condition [3]. In this regard, additional AMBP-1 is needed to restore vascular responsiveness to AM in order to maintain tissue perfusion in septic shock. However, administration of AMBP-1 alone is insufficient in preventing organ injury in sepsis [47]. Therefore, a combination of AM and AMBP-1 is required for their beneficial effects under such conditions [47,48].

As demonstrated in this study, human AM and AMBP-1 in combination ameliorated sepsis-induced organ injury and mortality in the setting of biliary obstruction in rats. These beneficial effects may be related with their vasoactive [47] and anti-inflammatory [48] properties. A study from our laboratory has shown that AMBP-1 in an organ bath at concentrations of 2 and 5 nM is able to enhance AM-induced relaxation of aortic rings taken from normal rats [49]. AMBP-1 alone is associated with only minimal vascular relaxation [49]. AMBP-1 also potentiates AM's downregulatory effect on LPS-induced cytokine production [46]. Our previous studies have shown that AM or AMBP-1 alone only moderately reduces LPS-induced TNF- α production in Kupffer cells, while AM and AMBP-1 in combination dramatically downregulate TNF- α production [46]. The direct anti-inflammatory effect of AM/AMBP-1 is mediated through both the cAMP-dependent pathway and proline-rich tyrosine kinase-2 (Pyk-2)-ERK1/2-dependent induction of peroxisome proliferator-activated receptor- γ (PPAR- γ) [23]. Moreover, AM has been shown to downregulate chemokine levels both *in vivo* and *in vitro* [11,17]. It can inhibit neutrophil activation and migration to inflammatory sites like the liver by suppressing upregulation of the adhesion molecule CD11 [33]. Thus, AM and AMBP-1 targets both cardiovascular and inflammatory responses at the same time. However, the precise mechanism responsible for these beneficial effects warrants further investigation.

Development of bacteremia, sepsis, and an overwhelming systemic inflammatory response are major causes of morbidity and mortality in patients with cholestatic liver disease [36,42]. Consistent with earlier observations [10,24,25], our current study demonstrates a profound proinflammatory response, characterized by an unregulated production of proinflammatory cytokines and increased neutrophil infiltration, presents in animals with biliary obstruction following sepsis. This proinflammatory milieu is associated with elevated markers of organ injury. However, the mechanism underlying this phenomenon remained largely unknown. Since a deficiency of AMBP-1 in humans has been shown to be associated with higher susceptibility to recurrent infections [30], AMBP-1 deficiency may play a major role in the development of infection/sepsis in jaundiced patients.

Our ultimate goal is to develop the clinical utilization of AM/AMBP-1 as a safe and effective treatment for sepsis patients. However, the antigenicity of animal proteins in humans prevents the use of rat AM/AMBP-1 in patients. Therefore, human proteins must be tested in animal models before these findings can be verified in humans. In this regard, human AM and AMBP-1, not rat AM and AMBP-1, were used in the current study. The dosages of human AM and AMBP-1 (i.e., 24/80 μ g/kg BW) used here were chosen based on our previous study, which shows that these doses of human AM and AMBP-1 provide the best protection in a rat model of CLP alone [45]. Since there is no statistically significant difference in circulating levels of AM and AMBP-1 between CLP and BLD+CLP groups, the same doses of AM and AMBP-1 were used in this study. However, human AM/AMBP-1 at the dose of 24/80 μ g/kg BW only provided partial protection in this rat model of obstructive jaundice and polymicrobial sepsis. Thus, the optimal dose(s) of human AM/AMBP-1 after sepsis in jaundiced rats remains to be determined. We will address this issue by determining the dose-response effect of human AM/AMBP-1 in this rat model of obstructive jaundice and polymicrobial sepsis in the near

future. Moreover, our previous study has clearly shown that AM or AMBP-1 alone has no measureable beneficial effects in the rat model of CLP alone [47]. It is unlikely that AM or AMBP-1 alone will be protective in the rat model of BDL+CLP. Therefore, we did not repeat the AM or AMBP-1 alone study in the double hit model (i.e., BDL+CLP). In addition, neither AM alone nor AMBP-1 alone can be further developed as a promising drug candidate for the treatment of sepsis patients with bile duct diseases.

In summary, obstructive jaundice potentiates sepsis-induced proinflammatory responses and organ injury in rats. Administration of human AM in combination with human AMBP-1 after the onset of sepsis markedly attenuated tissue injury, downregulated proinflammatory cytokine levels, inhibited neutrophil infiltration, and improved survival in jaundiced rats. Thus, administration of human AM/AMBP-1 may provide a novel approach to the treatment of sepsis in patients with pre-existing liver diseases such as obstructive jaundice. We believe that the current study is an important step towards commercial utilization of human AM and AMBP-1 as a safe and effective treatment for patients with sepsis and septic shock.

Acknowledgments

This study was supported by National Institutes of Health grants.

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Figure 1.

Alterations in plasma levels of (A) AM and (B) AMBP-1 in sham-operated animals (Sham), cecal ligation and puncture (CLP) animals at 20 h after CLP, common bile duct ligation (BDL) animals at 7 days after BDL, and BDL+CLP animals at 20 h after CLP. Data are presented as means $\pm$ SE ($n = 4-8$ /group) and compared by one-way analysis of variance and Student-Newman-Keuls' test: * $P < 0.05$ vs. sham group.

Figure 2.

Alterations in serum levels of (A) alanine aminotransferase (ALT), (B) aspartate aminotransferase (AST), (C) lactate and (D) creatinine in sham-operated animals (Sham) and common bile duct ligation (BDL) plus cecal ligation and puncture (CLP) animals (BDL+CLP) treated with normal saline (Vehicle) or human AM/AMBP-1 (24/80 μ g/kg BW) at 20 h after CLP. Data are presented as means $\pm$ SE ($n = 6-8$ /group) and compared by one-way analysis of variance and Student-Newman-Keuls' test: * $P < 0.05$ vs. sham group; # $P < 0.05$ vs. vehicle group.

Figure 3.

Alterations in serum levels of (A) TNF- α and (B) IL-6 in sham-operated animals (Sham) and common bile duct ligation (BDL) plus cecal ligation and puncture (CLP) animals (BDL+CLP) treated with normal saline (Vehicle) or human AM/AMBP-1 (24/80 μ g/kg BW) at 20 h after CLP. Data are presented as means $\pm$ SE (n = 8/group) and compared by one-way analysis of variance and Student-Newman-Keuls' test: * P < 0.05 vs. sham group; # P < 0.05 vs. vehicle group.

Figure 4.

Alterations in MPO activity in the lung (A), liver (B) and gut (C) in sham-operated animals (Sham) and common bile duct ligation (BDL) plus cecal ligation and puncture (CLP) animals (BDL+CLP) treated with normal saline (Vehicle) or human AM/AMBP-1 (24/80 μ g/kg BW) at 20 h after CLP. Data are presented as means $\pm$ SE (n = 8/group) and compared by one-way analysis of variance and Student-Newman-Keuls' test: * P < 0.05 vs. sham group; # P < 0.05 vs. vehicle group.

Figure 5.

Alterations in the survival rate at 7 days after common bile duct ligation (BDL) plus cecal ligation and puncture (CLP) and cecal excision with normal saline treatment (Vehicle) or human AM/AMBP-1 (24/80 μ g/kg BW) treatment. There were 14 rats in vehicle-treated group and 15 rats in AM/AMBP-1-treated group. The survival rates were estimated by the Kaplan-Meier method and compared by using the log-rank test. * $P < 0.05$ vs. vehicle group.

Table 1

Effects of bile duct ligation (BDL) on cecal ligation and puncture (CLP)-induced organ injury and inflammatory responses in rats.

	Sham	CLP	BDL	BDL+CLP
Total bilirubin (mg/dL)	0.17±0.03	0.23±0.05	9.07±0.37 [*]	8.52±0.68 [*]
Direct bilirubin (mg/dL)	0.06±0.01	0.09±0.03	6.89±0.21 [*]	6.51±0.51 [*]
ALT (IU/L)	37±7.2	87±4.1 [*]	157±9.8 [*]	197±12.4 ^{*#}
AST (IU/L)	35±1.4	119±6.6 [*]	232±10.1 [*]	281±13.8 ^{*#}
Lactate (mmol/L)	2.1±0.11	4.0±0.25 [*]	3.2±0.38 [*]	5.6±0.36 ^{*#}
Creatinine (mmol/L)	0.5±0.06	0.8±0.07 [*]	0.9±0.11 [*]	1.3±0.10 ^{*#}
TNF-α (pg/ml)	6.7±1.19	31.9±3.11 [*]	22.4±2.12 [*]	60.4±4.35 ^{*#}
IL-6 (pg/ml)	107.1±5.98	466.6±39.67 [*]	169.3±16.44	779.6±61.52 ^{*#}
Pulmonary MPO (U/g)	9.52±0.60	47.9±8.19 [*]	31.5±2.59 [*]	72.1±10.51 ^{*#}
Hepatic MPO (U/g)	0.29±0.06	1.08±0.15 [*]	1.47±0.40 [*]	2.44±0.40 ^{*#}
Intestinal MPO (U/g)	0.07±0.02	4.08±0.96 [*]	0.07±0.02 [*]	6.81±1.28 ^{*#}

Data are expressed as means ± SE (n=6–8/group) and compared by one-way analysis of variance (ANOVA) and Student-Newman-Keuls method:

^{*} $P < 0.05$ versus sham-operated animals;

[#] $P < 0.05$ versus CLP alone animals.