Original article

Protective effects of hemin pretreatment combined with ulinastatin on septic shock in rats

YU Jian-bo and YAO Shang-long

Keywords: hemin; ulinastatin; septic shock; heme oxygenase; carbon oxide

Background Urinary trypsin inhibitor inhibits the enhanced production of pro-inflammatory molecules. Hemeoxygenase-1 induction protects against ischemia/reperfusion injury, oxidative stress, inflammation, transplant rejection, apoptosis, and other conditions. However, it is unknown if a combined hemin and ulinastatin pretreatment could result in protective effects for septic shock. In this study, we investigated the role of heme pretreatment combined with ulinastatin on septic shock in rats.

Methods Eighty healthy, male Sprague-Dawley rats were randomly divided into four groups: group S, group H, group U and group HU. Groups S and U received 1 ml normal saline intraperitoneally, while groups H and HU both received 1 ml (100 mg /kg) hemin. Twenty-four hours later, 0.5 ml (10 mg/kg) E. coli lipopolysaccharide was injected intravenously to replicate the experimental model of septic shock. After an initial 25% decrease in the mean arterial pressure, corresponding to time point 0, groups HU and U received 0.5 ml 10 000 U/kg ulinastatin intravenously, and the others received 0.5 ml normal saline.

Results The number of deaths in groups H and U was lower than that in the group S (P<0.05), and was higher than that in group H (all P<0.05). The mean arterial pressure (MAP) in the group S was significantly greater than that in group H (P<0.05), and was lower than that in group HU and group U (P<0.05). The plasma levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine (Cr) and blood urea nitrogen (BUN), the malondialdehyde (MDA) of liver, kidney and lung, and the lung Evans blue (EB) contents in groups H and U, were greater than that in group HU (all P<0.05), and were lower than that in group S (all P<0.05). In contrast, the plasma levels of CO in groups H and HU were higher than that in groups S and U (all P<0.05), and SOD of liver, kidney and lung in groups H and U were higher than that in group S, and were lower than that in group HU (all P<0.05). The levels of TNF-α, IL-6, IL-8 and β-glucuronidase (GCD) activity of plasma in groups U and HU were lower than those in groups H and S, all having a P<0.05, while there were no significant differences between group H and group S, or between group HU and group U (all P>0.05). The HO-1 mRNA and HO-1 protein levels from hepatic, renal, and pulmonary tissue in groups S and U were lower than those in groups H and HU (all P<0.05), but there were no significant differences between groups S and U, or between groups H and HU (all P>0.05). The HO-2 mRNA and HO-2 protein were not significantly different among the four groups (all P>0.05).

Conclusions Combined pretreatment with hemin and ulinastatin in septic shock rats results in an improved response by the upregulation of HO-1 protein followed by increasing CO with resistance to increased oxidative stress, restraining the release of inflammatory mediators, and inhibiting β-GCD activity.

S eptic shock is a disease process caused by a severe underlying infection. In the case of Gram-negative bacterial infections, the release of endotoxin or lipopolysaccharide (LPS) from the bacterial cell wall causes a dramatic stimulation of LPS-responsive immune cells in the host. Macrophages, which are the cells most sensitive to LPS stimulation, become activated and release a battery of endogenous mediators and defense molecules, including pro-inflammatory cytokines such as interleukin (IL)-1. Although pro-inflammatory cytokines can protect the host against infection, if the infection is left unchecked this process may progress to refractory hypotension, multiple organ system failure, and death. The mortality resulting from septic shock, the most common cause of death in the critical care unit has not decreased in the past decade.

Although the pathogenesis of septic shock is extraordinarily complex, nitrous oxide (NO), which is generated through the iNOS pathway, is an important mediator of the hypotension and death associated with it. Pro-inflammatory cytokines such as IL-1 and tumor necrosis factor alpha (TNF-α) induce iNOS and increase NO production in cultured vascular smooth muscle cells. Aiming at overproduction of NO, inhibitors of nitric oxide synthase (NOS) have been reported to increase mean arterial pressure, reduce oxygenation and perfusion of vital organs and increase the mortality rate.

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synchronously. Several studies indicated that methylene blue can reverse the refractory hypotension by inhibiting soluble guanylate cyclase and NOS, but application of this novel therapeutic approach for the treatment of septic shock needs further investigation, especially for a patient’s prognosis. Many anti-inflammatory agents, ranging from intravenous ibuprofen to an inhibitor of the pro-inflammatory cytokine TNF-α, have been evaluated for the treatment of septic shock, but none have proved to be successful therapeutic interventions to date.

Urinary trypsin inhibitor (UTI) is a multivalent Kunitz-type serine protease inhibitor, found in human urine and blood, and recognized to be degenerated from pre-α-inter-α-trypsin inhibitors induced by neutrophil elastase during inflammation. UTI principally inhibits inflammatory proteases, including trypsin, α-chymotrypsin, plasmin, cathepsin G, and leukocyte elastase as well as the proteases of the coagulation cascade. UTI has anti-inflammatory properties apart from the blocking of protease pathway in vitro as found with other serine type protease inhibitors. UTI inhibits the LPS-induced increased production of pro-inflammatory molecules such as prostaglandin H2 synthase-2, thromboxane B2, interleukin (IL)-8, and tumor necrosis factor (TNF)-α. Endogenous carbon monoxide (CO) shares many similarities with NO such as CO binding to the heme moiety of cytosolic guanylyl cyclase resulting in the formation of cGMP. Heme oxygenase (HO) is an enzyme catalyzing the degradation of heme to generate CO. Two distinct forms of HO have been identified. HO-1, an inducible isozyme, and HO-2, a non-inducible isozyme. HO-1 induction protects against ischemia/reperfusion injury, oxidative stress, inflammation, transplant rejection, apoptosis, and other conditions. However, whether hemin pretreatment combined with ulinastatin could cause protective effects on septic shock is largely unknown.

Therefore, the present study was designed to learn if hemin pretreatment combined with ulinastatin confers protection against septic shock in rats.

METHODS

Animals

Two-month male Sprague-Dawley rats (160–185 g) were purchased from the Laboratory Animal Center of Tongji Medical College. Only five animals were housed in the same cage, acclimatized to a 12-hour light-dark cycle and allowed free access to food and water for a 5-day period prior to the experimental procedure. During the experiments, the environmental temperature was maintained between 23°C and 25°C. The experimental protocol was approved by the local animal care and use committee, and all animals received humane care according to the criteria outlined in the “Guide for the Care and Use of Laboratory Animals” prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH publication 86-23, revised 1985).

Rats were fasted for 12 hours but allowed free access to water before the induction of anesthesia. The rats received an intraperitoneal injection of 20% urethane (1.5 g/kg) that kept them anesthetized throughout the experiment. The right carotid artery and the intra-jugular vein were cannulated with PE-50 tubing to monitor the mean arterial pressure (MAP) using Hellige monitor instruments (Germany), and with a 24-g cannula for intravenous injections. Subsequently, the animals were heparinized with 500 U/kg heparin.

Eighty rats were randomly divided into four groups: group S (n=20), group H (n=20), group U (n=20) and group HU (n=20). Twenty-four hours before producing the experimental model of septic shock, groups S and U received 1 ml normal saline, while groups H and HU received 1 ml of 100 mg/kg body weight hemin (Sigma, USA) intraperitoneally. The injection of 0.5 ml of 10 mg/kg LPS (O111B4, Sigma, USA) intravenously produced the experimental model of septic shock. After an initial 25% decrease in MAP, corresponding to time point 0 of the experiment, groups U and HU received 0.5 ml of 10 000 U/kg ulinastatin (Guangdong Techpool Biochem, China; which was reconstituted with normal saline) intravenously, and the other 2 groups received 0.5 ml normal saline. The rats received 0.5 ml of 30 mg/kg Evans blue (EB, Serva, Germany) intravenously 5 minutes before administering the LPS. MAP was monitored continuously, and if the MAP did not decrease within 2 hours, the animal was excluded from the study.

Monitoring and recording indexes

MAP and ECG were monitored continuously throughout the experiment. The MAP was recorded after the induction of the septic shock at 30 minutes, 60 minutes, 90 minutes and 120 minutes. And the deaths within 7 hours were recorded.

Sampling and storage

The blood samples were collected 7 hours after LPS administration in a living rat or in a dying rat having an ECG displaying HR ≤ 20 beats/min, or a ventricular flutter, or a ventricular fibrillation, and centrifuged at 4000 r/min for 10 minutes at 4°C. The rats were sacrificed, and their liver, lung and kidney were removed, and quickly perfused with phosphate-buffered saline to remove the blood. The blood samples and the remaining tissues after perfusion and rinsing were frozen in liquid nitrogen and stored at −70°C until assayed.

Biochemical measurements

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine (Cr) and blood urea nitrogen (BUN) levels in the plasma were measured with a Hitachi7060 Fully Automated Biochemistry Analyzer (Japan). Hepatic, renal and pulmonary superoxide dismutase, SOD activity and malondialdehyde, MDA contents were determined by spectrophotometry and by the method of Ohkawa et al.
respectively. SOD activity and MDA contents were expressed as per unit of protein determined by the Lowry method.13

Measurement of Evans blue (EB) contents
The right lung was weighed and then incubated in formamide for 18 hours at 37°C to extract the EB dye. The left lung was weighed and then dried in an oven at 55°C, and the wet-to-dry weight ratio from that lung was used to estimate the dry weight of the right lung. The extracted dye was quantitated in a spectrophotometer by measuring the absorbance at 620 nm and comparing to standards of Evans blue dye dissolved in formamide. The Evans blue dye extravasation was expressed as nanograms of Evans blue dye per milligram of dry tissue.

β-Glucuronidase assay
β-Glucuronidase activity in rat plasma was assayed using the FluorAce β glucuronidase reporter assay kit (Bio-Rad, Hercules, CA, USA).

Measurement of CO in plasma
CO level in plasma was determined by the method of Chalmers,14 and was expressed as milligram of CO per liter of fresh blood.

Measurements of IL-6, IL-8 and TNF-α in plasma
IL-6, IL-8 and TNF-α levels present in serum were determined using commercially available murine-specific sandwich enzyme-linked immunosorbent assay (ELISA) kits supplied by Santa Cruz (USA).

RNA extraction and reverse transcription (RT)-PCR
Total RNA was extracted from the liver, kidney and lung by employing a Total Quick RNA kit (TA200TQR, Talent, Italy). The ethanol-precipitated RNA fraction (1 µg) was reverse transcribed using a Revert Aid™ First Strand cDNA Synthesis kit (MetaBios Inc, Canada), according to the manufacturer’s protocol, and 50 µl of first-strand cDNA solution was obtained. The primers used in this study were 5’-CGACAGCATGTCCCAGGATT-3’ for rat HO-1, 623 bp; 5’-TTCTACCTGTTCGAGCATGTGG-3’ and 5’-TGTTAGCATGGAGCCAGCCT-3’ for rat HO-2, 180 bp; 5’-TTCTACCTGTTCGAGCATGTGG-3’ and 5’-TCGCTCTATCTCCTCTTCCAGG-3’ for rat HO-1, 160 bp; 5’-TTCTACCTGTTCGAGCATGTGG-3’ and 5’-TGTTAGCATGGAGCCAGCCT-3’ for rat HO-2, 180 bp; 5’-TTCTACCTGTTCGAGCATGTGG-3’ and 5’-TGTTAGCATGGAGCCAGCCT-3’ for rat β-actin, 623 bp. RT-PCR was performed as described by Suzuki et al.15 The integral optical density (IOD) of the DNA bands was measured with a Shanghai Sixing Image Analysis System. The IOD ratio of HO-1, or HO-2, and β-actin was calculated, and used as an expression of HO-1 mRNA or HO-2 mRNA.

Western blot analysis
Tissue samples were homogenized in 13.2 mmol/L Tris-HCl, 5.5% glycerol, 0.44% SDS, and 10% β-mercaptoethanol. An equal amount of extracted soluble protein (50 µg) was fractionated by Tris-glycine-SDS-polyacrylamide gel (12%) electrophoresis, and Western blotting was performed as described16 with use of a polyclonal rabbit antibody to recombinant rat HO-1 or HO-2 protein (1:1000, Santa Cruz). The IOD of protein bands were measured with a LEICA-550IW Image Analysis System. The product of IOD and the area was as an expression of HO-1 protein (or HO-2 protein).

Statistical analysis
All data were expressed as mean ± standard deviation (SD). Comparisons of mortality between groups were made by the χ2 goodness of fit test, and intergroup comparisons among the four groups were determined by one-way ANOVA. When the F value was significant (P<0.05), a post hoc analysis was performed with the Duncan new multiple range test in order to test the difference between means. P<0.05 was considered statistically significant.

RESULTS

Deaths
Seven animals died in the group H, six animals died in the group U and one animal died in group HU compared to fourteen animals in group S (all P<0.05). There was no significant difference in death rate between group H and group U (P>0.05).

Comparisons of MAP
The changes of MAP among the four groups are shown in Table 1. After 60-minute septic shock, the MAP in the group S was significantly higher than that in the group H (all P<0.05), and lower than that in groups HU and U (all P<0.05). There was no significant difference between the group HU and the group U (P>0.05).

Comparisons of IL-6, IL-8, TNF-α, CO and β-GCD activity
The comparisons of serum IL-6, IL-8, TNF-α, CO, and β-GCD activity among the four groups are shown in Table 2. The plasma levels of TNF-α, IL-6, IL-8 and β-GCD activity in groups HU and U were dramatically lower than that in groups H and S (all P<0.05), while there were no significant difference between the group H and the group S or between the group HU and the group U (all P>0.05). The concentration of CO in groups S and U were lower than in groups H and HU (all P<0.05). There were no significant differences between the group H and the group HU or between the group S and the group U (all P>0.05).

Comparisons of Cr and BUN concentrations, ALT and AST activities, and EB contents
The comparisons of the Cr and BUN concentrations, ALT and AST activities, and EB contents among four groups are shown in Table 3. These five indices in groups H and U were significantly lower than that in group S (all P<0.05), and greater than in group HU respectively (all P<0.05). There were no significant differences in five indices between the group H and the group U (all P>0.05).
Table 1. The changes of MAP among four groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Before treatment MAP (mmHg)</th>
<th>30-minute</th>
<th>60-minute</th>
<th>90-minute</th>
<th>120-minute</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>72.2±9.3</td>
<td>70.1±9.2</td>
<td>67.1±7.4</td>
<td>65.1±7.3</td>
<td>63.4±6.8</td>
</tr>
<tr>
<td>H</td>
<td>72.8±9.0</td>
<td>69.5±7.5</td>
<td>62.0±8.5</td>
<td>60.0±7.4</td>
<td>58.6±6.5*</td>
</tr>
<tr>
<td>U</td>
<td>72.4±9.2</td>
<td>71.1±8.7</td>
<td>72.4±7.9*</td>
<td>74.6±6.0*</td>
<td>78.9±6.8*</td>
</tr>
<tr>
<td>HU</td>
<td>72.5±9.7</td>
<td>71.6±8.5</td>
<td>73.6±8.4</td>
<td>75.9±6.5</td>
<td>79.6±6.6*</td>
</tr>
</tbody>
</table>

*Indicates test group significantly different from group S (one-way ANOVA, P<0.05).

Table 2. Comparisons of the concentration of serum TNF-α, IL-6, IL-8 and CO and the activity of β-GCD among the four groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>TNF-α (ng/ml)</th>
<th>IL-6 (ng/ml)</th>
<th>IL-8 (ng/ml)</th>
<th>β-GCD (10⁻⁹ μmol·h⁻¹·ml⁻¹)</th>
<th>CO (ng/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>8.59±2.47</td>
<td>0.54±0.17</td>
<td>1.04±0.29</td>
<td>54.2±13.1</td>
<td>0.84±0.14</td>
</tr>
<tr>
<td>H</td>
<td>8.48±2.33</td>
<td>0.51±0.15</td>
<td>1.01±0.27</td>
<td>46.3±10.5</td>
<td>0.99±0.18</td>
</tr>
<tr>
<td>U</td>
<td>7.15±2.28*</td>
<td>0.37±0.14*</td>
<td>0.81±0.20*</td>
<td>40.7±9.78*</td>
<td>0.83±0.15</td>
</tr>
<tr>
<td>HU</td>
<td>7.07±1.90*</td>
<td>0.38±0.12*</td>
<td>0.79±0.22*</td>
<td>39.2±9.55*</td>
<td>1.01±0.19*</td>
</tr>
</tbody>
</table>

*Indicates test group significantly different from group S (one-way ANOVA, P<0.05).

Table 3. Comparisons of the Cr and BUN concentrations, ALT and AST activities, and EB contents among four groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cr (µmol/L)</th>
<th>BUN (mmol/L)</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>EB (ng/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>120.8±19.1</td>
<td>11.5±2.6</td>
<td>66.9±12.0</td>
<td>105.3±12.2</td>
<td>230.4±44.8</td>
</tr>
<tr>
<td>H</td>
<td>108.9±14.7</td>
<td>9.8±2.6</td>
<td>59.2±10.8</td>
<td>96.6±12.1</td>
<td>201.1±40.5</td>
</tr>
<tr>
<td>U</td>
<td>106.8±14.8</td>
<td>9.3±2.8</td>
<td>58.1±11.2</td>
<td>95.5±12.6</td>
<td>197.8±41.8</td>
</tr>
<tr>
<td>HU</td>
<td>97.7±14.1*</td>
<td>7.1±2.4*</td>
<td>51.6±11.0*</td>
<td>86.5±14.4*</td>
<td>174.8±36.7*</td>
</tr>
</tbody>
</table>

*Indicates test group significantly different from group H (one-way ANOVA, P<0.05).

Table 4. Comparisons of the MDA contents and SOD activity in kidney, lung and liver among four groups

<table>
<thead>
<tr>
<th>Kidney</th>
<th>Lung</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
<td>MDA (nmol/mg protein)</td>
<td>SOD (NU/mg protein)</td>
</tr>
<tr>
<td>S</td>
<td>2.63±0.66*</td>
<td>14.4±1.89*</td>
</tr>
<tr>
<td>H</td>
<td>2.09±0.52</td>
<td>17.7±2.78</td>
</tr>
<tr>
<td>U</td>
<td>2.06±0.55</td>
<td>16.9±3.01</td>
</tr>
<tr>
<td>HU</td>
<td>1.57±0.47*</td>
<td>19.9±3.77*</td>
</tr>
</tbody>
</table>

*Indicates test group significantly different from group H (one-way ANOVA, P<0.05).

Comparisons of SOD activity and MDA contents

The comparisons of the MDA contents and SOD activity in kidney, lung and liver among four groups are shown in Table 4. The nephric, pulmonary and hepatic MDA contents in groups H and U were significantly lower than that in the group S (all P<0.05), and greater in the group HU (all P<0.05). The SOD activity was reverse in the four groups respectively. There were no significant differences in MDA contents and SOD activity between groups H and U (all P>0.05).

Comparisons of HO-1 mRNA, HO-2 mRNA, HO-1 protein and HO-2 protein expression

The comparisons of HO-1 mRNA, HO-2 mRNA, HO-1 protein and HO-2 protein in nephric, pulmonary and hepatic tissue are shown in Figures 1 to 6. The HO-1 mRNA and HO-1 protein of nephric, pulmonary and hepatic tissue in groups S and U were lower than groups H and HU (all P<0.05). There were no significant differences between groups S and U, or between groups H and HU (all P>0.05). The HO-2 mRNA and HO-2 protein among four groups were not significantly different (all P>0.05).

DISCUSSION

In the present study, the role of hemin pretreatment combined with ulinastatin on septic shock in rats was investigated. One of the principal finding of this study was that despite inducing HO-1 in vital organs and increasing the concentration of CO in plasma, hemin pretreatment did not effect the concentrations of IL-6, IL-8 and TNF-α, and β-GCD activity in plasma. Second, ulinastatin reduced the plasma TNF-α, IL-6 and IL-8

![Figure 1](https://example.com/figure1.png)
It is well known that HO-1, also known as heat shock protein 32 (HSP32), is a potentially important stress response protein. Indeed, HO-1 is induced not only by the substrate heme but also by a variety of nonheme substance such as heavy metals, endotoxin, heat shock, cytokines, and prostaglandins. In addition to its role in heme degradation, the induction of HO-1 by a wide variety of factors may function as a defense against oxidative stress in situations such as inflammation, irradiation, and light.10

Upregulation of HO-1 protein by hemin pretreatment in septic shock rats was found in the liver, lung and kidney resulting in a reduction of the end-organs dysfunctions, and increases of CO concentration and SOD, and MDA contents and decreased pulmonary vascular leakage. Masini and colleagues17 demonstrated that induction of HO-1 by hemin significantly decreased infarct size, incidence of reperfusion arrhythmias, MDA generation,
and calcium overload induced by cardiac ischemia-reperfusion in rat. Endotoxin treated rats pretreated with hemin had the diaphragmatic dysfunction ameliorated and decreasing MDA contents limited by increasing HO-1 protein expression and HO-1 activity.\textsuperscript{18} The protective mechanisms of HO-1 may be its augmentation of iron eflux, reflecting a role for HO-1 in modulating intracellular iron levels and regulating cell viability.\textsuperscript{10} Bilirubin and biliverdin which are two metabolites of heme degradation, act as scavengers of toxic oxygen radicals. Bilirubin is a strong antioxidant in mammnals.\textsuperscript{19} CO, a metabolite of heme degradation, can negatively feed back NO generation,\textsuperscript{10} which can be cytotoxic because of its strong oxidation potential. It was observed that the serum IL-6, IL-8, TNF-\(\alpha\) levels and \(\beta\)-GCD activity of hemin pretreated septic shock rats were not statistically significant, from saline controls. However, Tamion et al\textsuperscript{20} reported that the induction of HO-1 by hemoglobin pretreatment in hemorrhagic shock rats markedly decreases the level of TNF-\(\alpha\) mRNA expression in peritoneal macrophages.

Ulinastatin is widely used in critical illness. UTI mainly inhibits inflammatory proteases, including trypsin, \(\alpha\)-chymotrypsin, plasmin, cathepsin \(G\), and leukocyte elastase, as well as proteases of the coagulation cascade. As with other serine type protease inhibitors, UTI has anti-inflammatory properties apart from blocking of proteases. Sato et al\textsuperscript{21} suggested that high-dose ulinastatin administration to maintain a sufficient concentration of circulating protease inhibitors might suppress over-induction of IL-6, IL-8 and polymorphonuclear elastase in open-heart surgery. Furthermore, ulinastatin decreases the TNF-\(\alpha\) production of LPS-stimulated monocytes,\textsuperscript{22} MDA contents in the transplanted small intestine\textsuperscript{23} and \(\beta\)-GCD activity during cardiopulmonary bypass.\textsuperscript{24} In addition, Huo et al\textsuperscript{25} suggested that the pro-inflammatory mediators in the serum of the rats given high tidal volume ventilation increased endothelial permeability by reorganizing actin cytoskeleton. Pretreatment with ulinastatin lessened the permeability by inhibition of pro-inflammatory mediators. In agreement with these results, it was found that ulinastatin administration to septic shock rats inhibits the increases of IL-6, IL-8, TNF-\(\alpha\) levels and \(\beta\)-GCD activity, and enhances SOD activity. In addition it was observed that ulinastatin does not induce HO-1 expression in vital organs.

In this study, we found hemin pretreatment combined with ulinastatin in septic shock rats could decrease the deaths of the experimental animals. The levels of plasma ALT, AST, Cr and BUN, MDA of liver, kidney and lung, the lung EB contents, and the levels of TNF-\(\alpha\), IL-6, IL-8 and \(\beta\)-GCD activity of plasma were significantly reduced, while the SOD activity of liver, kidney and lung and COHb of plasma were increased. These results indicated that hemin pretreatment combined with ulinastatin can improve the function of liver, kidney and lung by restricting the releasing of TNF-\(\alpha\), IL-6, IL-8 and \(\beta\)-GCD activity. It was observed that up-regulation of HO-1 protein by hemin pretreatment in septic shock rats in the liver, lung and kidney, did not limit the release of TNF-\(\alpha\), IL-6, IL-8 and \(\beta\)-GCD activity. The ulinastatin restricted the release of TNF-\(\alpha\), IL-6, IL-8 and \(\beta\)-GCD activity but not the upregulating of HO-1 protein.

In conclusion, hemin pretreatment combined with ulinastatin in septic shock rats can result in a more satisfactory therapy by the upregulation of HO-1 protein; CO increase; restricting increased oxidative stress; stabilizing the lysosomal membrane by preventing \(\beta\)-GCD release; limiting the releasing of inflammation mediators. This therapeutic approach may become a basis for a novel strategy for treatment of septic shock.

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