Ghrelin improves tissue perfusion in severe sepsis via downregulation of endothelin-1

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Abstract

Objectives: Severe sepsis is associated with increased total peripheral resistance (TPR) and decreased organ blood flow, in which endothelin-1 (ET-1) plays an important role. Plasma levels of ghrelin, a newly-identified endogenous ligand for growth hormone secretagogue receptor and a potent vasodilatory peptide, are significantly reduced in sepsis. Ghrelin downregulation heralds the hypodynamic response in severe sepsis. Therefore, we hypothesized that the administration of exogenous ghrelin improves organ blood flow by downregulation of ET-1 under such conditions.

Methods: Male adult Sprague–Dawley rats were subjected to sepsis by cecal ligation and puncture (CLP). At 5 h post-CLP, a bolus intravenous injection of 2 nmol ghrelin was followed by a continuous infusion of 12 nmol ghrelin via a primed mini-pump over 15 h. At 20 h post-CLP (i.e., severe sepsis), cardiac output (CO), stroke volume (SV), TPR, and organ blood flow were measured using 141Ce-microspheres. Plasma ET-1 levels and preproET-1 gene expression in the liver, small intestine, and kidneys were measured by ELISA and RT-PCR, respectively. The direct effect of ghrelin on ET-1 production was studied using cultured human umbilical vein endothelial cells (HUVECs) treated with tumor necrosis factor-α (TNF-α).

Results: Ghrelin administration reduced TPR, increased CO, SV, and organ blood flow, downregulated preproET-1 gene expression, and decreased plasma levels of ET-1 in sepsis. Ghrelin inhibited TNF-α-induced ET-1 release from HUVECs in a dose-dependent manner. Moreover, ghrelin inhibited TNF-α-induced activation of nuclear factor-κB (NF-κB) in HUVECs.

Conclusions: The improvement of tissue perfusion by ghrelin in severe sepsis appears to be mediated by downregulation of ET-1 involving a NF-κB-dependent pathway.

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Keywords: Peptide hormones; Sepsis; Blood flow; Endothelin-1; Nuclear factor-κB

1. Introduction

Severe sepsis, a lethal syndrome that develops in response to infection and other systemic inflammations, continues to be one of the leading causes of death in intensive care units. A large number of septic patients die due to the ensuing septic shock and multiple organ failure [1–4]. The cardiovascular response to polymicrobial sepsis is characterized by an early, hyperdynamic phase followed by a late, hypodynamic phase [5]. The hyperdynamic phase features increased cardiac output and tissue perfusion, increased oxygen delivery and consumption, and decreased total peripheral resistance. The hypodynamic phase is evidenced by reduced cardiac output and microvascular blood flow, decreased oxygen delivery, increased total peripheral resistance, and lactic acidosis [6]. One difficulty in the management of septic patients is to prevent the
transition from the early, hyperdynamic to the late, hypodynamic phase.

Ghrelin, a novel endogenous ligand for the growth hormone secretagogue receptor (GHSR), is a 28 amino-acid acylated peptide predominantly produced by the stomach [7]. In addition to growth hormone-releasing properties [8], ghrelin possesses other endocrine and non-endocrine characteristics as indicated by central and peripheral GHSR distribution [9,10]. Our recent studies have shown that although ghrelin levels decrease at both early and late stages of sepsis, its receptor gene expression and protein levels are markedly elevated in early sepsis [11]. Ghrelin-induced relaxation in resistance blood vessels of the small intestine increases significantly during the early stage of sepsis, but is not altered during the late stage of sepsis. This suggests that the increased cardiovascular responsiveness to ghrelin may cause the hyperdynamic response of early sepsis, and that decreased ghrelin production may herald the hypodynamic response of late sepsis [11]. Moreover, treatment with ghrelin significantly downregulates circulating inflammatory cytokines in a rat model of polymicrobial sepsis [12].

Endothelin (ET)-1 is the most potent known vasoconstrictor released by vascular endothelial cells [13]. Numerous studies have shown that the significantly increased plasma ET-1 levels in sepsis may be associated with the increased vascular resistance and decreased organ blood flow under such conditions [14,15]. Li et al. reported that ghrelin mitigates proinflammatory cytokine production and mononuclear cell binding through inhibition of nuclear factor-kB (NF-κB) activation in human endothelial cells [16]. Since NF-κB activity is also linked with ET-1 expression [17], ghrelin may have direct effects on ET-1 expression in endothelial cells. We, therefore, hypothesized that the administration of exogenous ghrelin improves organ blood flow by downregulation of ET-1 in severe sepsis. The primary aim of this study was to determine whether ghrelin prevents the hypodynamic response and organ injury in sepsis and, if so, whether downregulation of ET-1 by ghrelin is involved in such a process.

2. Materials and methods

2.1. Animal model of sepsis

Male Sprague–Dawley rats (275–325g) were housed in a temperature-controlled room on a 12-h light/dark cycle and fed a standard Purina rat chow diet. Prior to the induction of sepsis, rats were fasted overnight but allowed water ad libitum. Rats were anesthetized using isoflurane inhalation, and the ventral neck, abdomen and groin were shaved and washed with 10% povidone iodine. Cecal ligation and puncture (CLP) was performed as we previously described [18], and the animals were randomly assigned to various groups. Sham-operated animals (i.e., control animals) underwent the same procedure with the exception that the cecum was neither ligated nor punctured. The animals were resuscitated with 3 ml/100g BW normal saline subcutaneously immediately after surgery. Animals were then anesthetized at 20 h after CLP (i.e., severe sepsis) or sham operation for various measurements or collection of tissue/blood samples. All experiments were performed in accordance with the National Institutes of Health guidelines for the use of experimental animals. This project was approved by the Institutional Animal Care and Use Committee of the Institute for Medical Research at North Shore-LIJ.

2.2. Administration of ghrelin

Rat ghrelin (Phoenix Pharmaceuticals, Belmont, CA) was dissolved in normal saline to a final concentration of 100 μM. 200-μl mini-pumps (Alzet, infusion rate 8 μl/h) were primed with ghrelin solution or vehicle (normal saline) for 3 h prior to implantation. At 5 h after CLP or sham operation, the animals were anaesthetized again by isoflurane inhalation. After a slow intravenous bolus injection of 2 nmol ghrelin or 200 μl vehicle, the minipump was then connected to a jugular venous catheter and implanted subcutaneously. Twenty hours after CLP (i.e., 15 h after implantation of the mini-pump) the rats were sacrificed, and blood and tissue samples were collected. The total dose of ghrelin each rat received was approximately 45 nmol/kg BW. In an additional group of animals, blood samples were withdrawn before the injection (5 h after CLP), 15 min after the bolus ghrelin injection, 5 h after the bolus ghrelin injection (10 h after CLP) and 15 h after the bolus ghrelin injection (20 h after CLP). Plasma levels of ghrelin were measured by ELISA according to the manufacturer’s instructions (Linco Research, Inc, St. Charles, MO).

2.3. Determination of cardiac output, total peripheral resistance, and organ blood flow

At 20 h after CLP or sham operation, the animals were anaesthetized again using isoflurane inhalation. Both the right femoral artery and vein were cannulated with polyethylene-50 tubing. An additional polyethylene-50 catheter was inserted into the left ventricle via the right carotid artery. Cerium-141-labeled microspheres (DuPont/NEN, Boston, MA) were suspended in 15% dextran, containing 0.05% Tween-80 surfactant to prevent aggregation. The microspheres were dispensed with a vortex shaker for 3 min and a 0.2- to 0.25-ml suspension of microspheres with an activity of ~4 μCi/rat was infused into the left ventricle over a period of 20 s at a constant rate. An estimated 150,000 microspheres were injected into each rat. The reference blood sample was withdrawn from the femoral arterial catheter beginning 20 s before microsphere infusion and continuing for 80 s at a rate of 0.7 ml/min. Normal saline
(0.8 ml) was administered through the left ventricular catheter immediately after microsphere infusion over a period of 40 s. Mean arterial pressure and heart rate were monitored before the injection of radioactive microspheres by using a blood pressure analyzer (Digi-Med, Louisville, Ky). At the end of the experiment, the rat was euthanized by an overdose of sodium pentobarbital. Various organs were harvested, washed with normal saline, and gently blotted on filter paper. The liver, spleen, pancreas, mesentery, stomach, small intestine, cecum, large intestine (including the colon and upper part of the rectum, for calculating portal blood flow), and kidneys were weighted and placed in a counting tube, and the radioactivity was counted in a gamma counter (Minaxi Auto-gamma Counter, 500 Series, Packard Instrument Co). The reference blood sample was transferred into a counting tube and counted. The remaining microspheres, which were left in the syringe after injection, were also counted. Blood flow in various organs, cardiac output, stroke volume, and total peripheral resistance were calculated as we previously described [19].

2.4. Determination of lactate and creatinine

Serum concentrations of lactate and creatinine were determined by using assay kits according to the manufacturer’s instructions (Pointe Scientific, Lincoln Park, MI).

2.5. RNA extraction and determination of preproendothelin-1 (preproET-1) genes

Total RNA was extracted from the liver, small gut and kidneys by Tri-Reagent (Molecular Research Center, Cincinnati, OH). Five µg of RNA from each tissue were reverse-transcribed in a 20 µl reaction volume containing 50 mM KCl, 10 mM Tris–HCl, 5 mM MgCl2, 1 mM dNTP, 20 U RNase inhibitor, 2.5 mM oligo d(T)16 primer and 50 U reverse transcriptase. The reverse transcription reaction solution was incubated at 42 °C for 1 h, followed by heating at 95 °C for 5 min. One µl cDNA was amplified with 0.15 µM each of 3’ and 5’ primers, specific for rat preproET-1 (248 bp) (5’TGC TCG TCC CTG ATG GAT A 3’, 5’TTC TCC ATA ATG TCT TCA GCC 3’) (accession No. Y00749), rat glyceraldehyde-3-phosphate-dehydrogenase (G3PDH) [20] (983 bp) (5’TGA AGG TCG TGT TCA ACG GAT TTG GC 3’, 5’CAT GTA GGC CAT GAG GTC CAC CAC 3’) in 25 µl of PCR mixture containing 50 mM KCl, 10 mM Tris–HCl, 2 mM MgCl2, 0.2 mM dNTP and 0.7 U AmpliTaq DNA polymerase. PCR was carried out in a Bio-Rad thermal cycler. The amplification cycle (denaturation at 95 °C for 45 s, annealing at 60 °C for 45 s, and extension 72 °C for 90 s) was repeated for 28 cycles. Following RT-PCR, 5 µl of the reaction mixture was electrophoresed in 1.2% TBE-agarose gel containing 0.22 µg/ml ethidium bromide. The gel was then developed and band intensities were normalized by G3PDH using the Bio-Rad image system (Hercules, CA).

2.6. Measurement of Plasma ET-1

Plasma samples were assayed with an ET-1 enzyme-linked immunosorbent assay (R and D Systems) after extraction according to the instructions provided by the manufacturer. The assay has less than 1% cross-reactivity with big ET-1 and 45% and 14% cross-reactivity with ET-2 and ET-3, respectively. All ET-1 values were corrected for recovery and expressed as picograms per milliliter plasma (pg/ml). The plasma levels of ET-1 in the sham animals in our study are similar to the normal values as provided by the manufacturer.

2.7. Administration of PD 142893

PD 142893 (Sigma-Aldrich; St. Louis, MO), a potent ET(A)- and ET(B)-receptor antagonist [21], was dissolved in normal saline to a final concentration of 0.5 mg/ml. 200-µl mini-pumps (Alzet, infusion rate 8 µl/h) were primed with PD 142893 solution for 3 h prior to implantation. At 5 h after CLP, the animals were anaesthetized again by isoflurane inhalation. After a slow intravenous bolus injection of 0.015 mg PD 142893, the mini-pump was then connected to a jugular venous catheter and implanted subcutaneously. Cardiac output and organ blood flow were measured at 20 h after CLP as described above. The total dose of PD 142893 each rat received was approximately 0.25 mg/kg BW.

2.8. Cell line and cell culture

Primary cultures of human umbilical vein endothelial cells (HUVECs) were obtained from American Type Culture Collection (ATCC; Manassas, VA). Cells were cultured in Ham’s F12K medium (ATCC; Manassas, VA) with 2 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate and supplemented with 0.1 mg/ml heparin and 0.05 mg/ml endothelial cell growth supplement (Sigma-Aldrich; St. Louis, MO), containing 10% fetal bovine serum at 37 °C in a humidified atmosphere containing 5% CO2. Confluent endothelial monolayers were G0–G1 synchronized by leaving the cells in basal medium containing 2% fetal bovine serum for 24 h. Experiments were initiated using synchronized confluent cells (passages 4–8). Ghrelin and TNF-α were added as described in the figure captions. Supernatant levels of ET-1 were measured by ELISA as described above.

2.9. Western blotting analysis of NF-κB nuclear translocation

G0–G1 synchronized cells were treated with ghrelin or left untreated for 1 h before the addition of TNF-α. After 20 min, nuclear and cytoplasmic extracts were prepared for Western blot analysis of NF-κB p65 (nuclear) and IκBα (cytoplasmic) expression. Equal amounts of cell lysates (20
μg/lane) were fractionated on 4–12% Bis–Tris gel and transferred to 0.2 μm-nitrocellulose membrane. Nitrocellulose blots were blocked by incubation in TBST (10 mM Tris–HCl, pH 7.5, 150 mM NaCl, 0.1% Tween 20) containing 5% milk for 1 h. Blots were incubated with rabbit NF-κB p65 and IκBα polyclonal antibodies (1:500, Santa Cruz Biotechnology; Santa Cruz, CA) overnight at 4 °C. The blots were then washed in TBST 5 times for 10 min. Blots were incubated with horseradish peroxidase-linked anti-rabbit IgG (Cell Signaling Technology, Beverly, MA) for 1 h at room temperature, and then washed 5 times in TBST for 10 min. A chemiluminescent peroxidase substrate (ECL, Amersham Biosciences, Piscataway, NJ) was applied according to the manufacturer’s instructions, and the membranes were exposed briefly to X-ray film. The band densities were determined using a Bio-Rad image system (Hercules, CA).

2.10. Statistical analysis

All data are expressed as mean±SE and compared by one-way analysis of variance (ANOVA) and the Student–Newman–Keuls method. Differences in values were considered significant if p < 0.05.

3. Results

3.1. Plasma levels of ghrelin after treatment

As shown in Table 1, plasma levels of ghrelin are 47 ng/ml at 15 min after the bolus injection and maintain above 1 ng/ml even at 15 h after the bolus injection, which is more than 25 fold higher than non-treated animals.

3.2. Effects of ghrelin administration on systemic hemodynamic parameters and organ blood flow

As indicated in Fig. 1A, no significant changes in heart rate (HR) were found at 20 h after the onset of sepsis in vehicle-treated as well as ghrelin-treated animals when compared with that in sham-operated animals. However, mean arterial pressure (MAP) in CLP vehicle-treated
animals was 14% lower than that in sham-operated animals at 20 h after CLP. Treatment with ghrelin significantly elevated MAP to near sham levels ($p<0.05$, Fig. 1B). However, ghrelin did not have any significant effects on HR and MAP in sham animals (Fig. 1A–B). As shown in Fig. 2A–B, CO and SV markedly decreased at 20 h after the onset of sepsis in vehicle-treated animals ($p<0.05$). Administration of ghrelin significantly improved the depressed CO and SV to levels which were similar to that of sham animals. Conversely, TPR increased by 28% at 20 h after CLP in vehicle treated animals as compared with that in sham-operated animals ($p<0.05$, Fig. 2C). Ghrelin treatment markedly reduced TPR levels by 20% ($p<0.05$), and there was no significant difference in TPR levels between sham-operated and CLP ghrelin-treated animals (Fig. 2C). In addition, total hepatic blood flow (Fig. 3A), hepatic arterial blood flow (Fig. 3B), portal blood flow (Fig. 3C), intestinal blood flow (Fig. 3D), and renal blood flow (Fig. 3E) decreased markedly in vehicle-treated septic animals ($p<0.05$). Ghrelin treatment significantly improved blood flow in all the tested organs ($p<0.05$, Figs. 3A–E).

3.3. Effects of ghrelin administration on serum levels of lactate and creatinine

As shown in Fig. 4A–B, rats subjected to sepsis had 1.4–2 fold increase in circulating levels of lactate and creatinine compared to sham-operated animals ($p<0.05$). When septic animals were treated with ghrelin, the levels of lactate and creatinine were markedly reduced, and creatinine levels were not significantly different from those of sham-operated animals.

3.4. Effects of ghrelin administration on preproET-1 expression and release

PreproET-1 gene expression in the liver (Fig. 5A), small gut (Fig. 5B) and kidneys (Fig. 5C) increased by
85%, 35% and 47%, respectively, and circulating levels of ET-1 (Fig. 5D) were more than doubled at 20 h after the onset of sepsis. However, the increases in preproET-1 gene expression and plasma levels of ET-1 were significantly blunted by ghrelin treatment \( (p<0.05, \text{Fig. 5A–D}). \) There was no difference noted when ghrelin-treated septic animals were compared to sham-operated animals (Fig. 5A–D).

### 3.5. Effects of PD 142893 on cardiac output and major organ blood flow

As indicated in Table 2, markedly decreased CO at 20 h after the onset of sepsis was significantly elevated by PD 142893 treatment \( (p<0.05). \) Similarly, significantly reduced total hepatic, intestinal, and renal blood flow at 20 h after CLP were improved by PD 142893 treatment \( (p<0.05). \)

### 3.6. Ghrelin inhibits TNF-α-induced ET-1 secretion in HUVECs

As indicated in Fig. 6, when cells were incubated with TNF-α alone, supernatant levels of ET-1 increased by 24% at 4 h after stimulation. However, in the presence of increasing concentrations of ghrelin, there was a significant reduction of ET-1 levels in the supernatant. When cells were pre-treated with 1000 ng/ml ghrelin for 1 h, the supernatant levels of ET-1 were lower than those in the medium group at 4 h after TNF-α stimulation.

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**Table 2**

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>CLP + Vehicle</th>
<th>CLP + PD 142893</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac output (ml/min/100 g BW)</td>
<td>27.8 ± 1.0</td>
<td>18.8 ± 0.7*</td>
<td>28.8 ± 2.3*</td>
</tr>
<tr>
<td>Total hepatic BF (ml/min/100 g BW)</td>
<td>185.9 ± 4.5</td>
<td>124.9 ± 9.6*</td>
<td>160.5 ± 13.4*</td>
</tr>
<tr>
<td>Intestinal BF (ml/min/100 g BW)</td>
<td>175.4 ± 7.3</td>
<td>112.4 ± 11.3*</td>
<td>149.6 ± 12.7*</td>
</tr>
<tr>
<td>Renal BF (ml/min/100 g BW)</td>
<td>742.8 ± 33.9</td>
<td>401.4 ± 80.5*</td>
<td>621.5 ± 42.6*</td>
</tr>
</tbody>
</table>

BF, blood flow; BW, body weight; * \( p<0.05 \) vs. sham group; # \( p<0.05 \) vs. CLP – Vehicle. Values are presented as means ± SE \( (n=6–7)/\text{group}) \) and compared by one-way ANOVA and Student–Newman–Keuls test. Values in sham and CLP+ Vehicle treated animals are derived from Figs. 2, 3.
3.7. Ghrelin inhibits TNF-α-induced NF-κB translocation in HUVECs

To study whether ghrelin has any direct effect on NF-κB translocation in HUVECs, only the most effective concentration of ghrelin (i.e., 1000 ng/ml) from the above experiment was used to pre- or simultaneously treat the cells with TNF-α. As demonstrated in Fig. 7, TNF-α induced a clear NF-κB nuclear translocation in an IκBα-dependent manner in HUVECs. Pre- or simultaneous treatment with ghrelin, however, inhibited the degradation of IκBα, and prevented the translocation of NF-κB into the nucleus.

4. Discussion

Ghrelin is a novel peptide recently identified as an endogenous ligand for the growth hormone secretagogue receptor [7]. It is produced predominantly by the stomach, with substantially lower amounts derived from other central and peripheral tissues [22,23]. Human ghrelin is a 28-amino acid peptide and has an n-octanoyl group at Ser3, a modification essential for its activity and not previously reported in a human peptide [24,25]. Rat ghrelin differs from human ghrelin by only two amino acids [25]. The biological effects of ghrelin are mediated through the ghrelin receptor GHSR. Although a group of synthetic molecules featuring growth hormone secretagogue can bind to GHSR, ghrelin is the only identified endogenous ligand for this receptor. GHSR is found in the pituitary, hypothalamus, stomach, heart, blood vessels, lungs, pancreas, intestines, kidneys, adipose tissue, and immune system (B and T cells, neutrophils) [26–28]. The wide distribution of the ghrelin receptor may indicate multiple paracrine, autocrine and endocrine roles for ghrelin. The presence of appreciable amounts of GHS binding sites and mRNA in the cardiovascular system suggests that ghrelin may have direct cardiovascular effects through growth hormone-independent mechanisms [29,30]. Wiley and Davenport showed that in addition to its effects on growth hormone release and energy homeostasis, ghrelin is also a potent vasodilatory peptide [31].

We recently demonstrated that although ghrelin levels decrease at both early and late stages of sepsis, its receptor is markedly upregulated in early sepsis [11]. Moreover, ghrelin-induced relaxation of resistance blood vessels in the isolated small intestine increases significantly at the early stage of sepsis, but was not altered at the late stage of sepsis [11]. This would suggest that the decreased ghrelin production heralds the hypodynamic response in late sepsis. Since ghrelin receptor and vascular responsiveness to ghrelin are not reduced at the late stage of sepsis, administration of ghrelin may be a useful adjunct in the treatment of clinical sepsis. However, ghrelin is a small peptide with a relatively short half-life. Our recent study has indicated that ghrelin’s half-life is 11 min in the normal rat and 17 min at late sepsis [32]. Therefore, a continuous infusion of ghrelin is required to maintain an effective concentration in the circulation. In this study, we used an osmotic mini-pump to infuse ghrelin intravenously after a bolus injection, and maintained a relatively high level of ghrelin in the circulation even at the end of the experiment. Our results indicate that ghrelin administration improved CO and SV, reduced TPR, and increased organ blood flow. The cardiovascular response to ghrelin appears to be similar to the hyperdynamic response observed in the early stage of sepsis. In healthy human volunteers, intravenous administration of exogenous ghrelin significantly reduced peripheral vascular resistance and increased cardiac output without a significant change in heart rate [33]. Therefore, ghrelin’s effects in preventing the transition from the hyperdynamic phase to the hypodynamic phase in sepsis appears to be related with its effects on the cardiovascular system. However, the underlying mechanism remains unknown.

Various vasoconstrictors are elevated in sepsis. One of the most potent vasoconstrictors elevated under such conditions is ET-1, a 21-amino acid peptide [14]. It was first isolated from porcine aorta endothelial cells in 1988 by Yanagisawa et al. [13]. Shortly thereafter, ET-2 and ET-3 were sequenced [34]. Although ET isopeptides possess a highly conserved primary structure, they differ in their receptor selectivity and biological activities [35–37]. ET-1’s affinity for ETA receptors is 8 times stronger than ET-2’s, and 1000 times stronger than ET-3’s. Since ETA receptors primarily mediate vasoconstriction, ET-1 is the predominant vasoconstrictive isopeptide. As such, ET-1 is thought to play a key role in regulating hemodynamic responses that take place during various disease states including sepsis. ETA receptor blockade attenuates the increased total peripheral resistance and decreased cardiac output seen during hypodynamic sepsis, suggesting that ET-1 plays a role in producing the hypodynamic phase [15]. In this study, we found that ghrelin administration significantly decreased plasma ET-1 levels in sepsis. However, the major actions of ET-1 appear to be a local autocrine and paracrine factor, rather than a circulating hormone; plasma concentrations may not reflect local tissue concentrations [38]. We therefore measured tissue expression of mRNA encoding preproendothelin-1 by RT-PCR, as an indicator of local production of ET-1 in this rat model of polymicrobial sepsis.
Our results showed that ghrelin also downregulated ET-1 gene expression in the liver, small gut, and kidneys in sepsis. Moreover, administration of a non-selective endothelin receptor antagonist, PD 142893, elevated cardiac output and improved major organ perfusion after CLP. This result further confirms that ghrelin’s beneficial effect in sepsis is indeed mediated through downregulation of ET-1 under such conditions.

ET-1 gene is constitutively expressed in cultured endothelial cells. The release of ET-1 from endothelial cells can be stimulated by several factors such as TNF-α, interleukin-1, thrombin, angiotensin II, and oxygen radicals [17,39–41]. Our result clearly shows that ghrelin inhibited TNF-α-induced ET-1 release from HUVECs in a dose-dependent manner. NF-κB is a critical signaling molecule in ET-1 transcription. Overexpression of the cytoplasmic inhibitor of NF-κB or deletion of the NF-κB binding site reduced ET-1 induction, whereas overexpression of NF-κB p65 induced ET-1 even in the absence of other stimuli [17]. Li et al. reported that ghrelin mitigates proinflammatory cytokine production and mononuclear cell binding through inhibition of NF-κB activation in cultured human endothelial cells [16]. Our result also indicates that ghrelin inhibits the degradation of IκBα, and prevents the translocation of NF-κB into the nucleus. Therefore, a potential mechanism by which ghrelin modulates ET-1 expression is to block activation of the transcription factor NF-κB.

The lowest effective concentration of ghrelin to inhibit ET-1 release under in vitro conditions is between 10–100 ng/ml. Plasma levels of ghrelin are 47 ng/ml at 15 min after the bolus injection and maintain above 1 ng/ml even at 15 h after the bolus injection. Given the fact that ghrelin’s half life is about 11 to 17 min [32], plasma levels of ghrelin would certainly reach approximately 100 ng/ml shortly after its bolus injection. Although the concentration of ghrelin at the middle and end of the protocol is lower than the effective concentration, it has initially reached the effective concentration we used in vitro (i.e., 10–100 ng/ml). Since rat ghrelin was used to treat septic rats and human ghrelin was used to incubate with HUVECs, a species difference in ghrelin may be the potential reason responsible for the observation that higher dosages of ghrelin are needed under in vitro conditions. Moreover, although HUVECs are widely used to study endothelial functions, their sensitivity to ghrelin may be lower than that of other endothelial cells. Therefore, more ghrelin may be needed to produce similar effects in HUVECs than in endothelial cells of septic rats.

In summary, cardiac output and organ blood flow were markedly decreased, whereas total peripheral resistance was significantly increased in late sepsis. Treatment with ghrelin, however, elevated cardiac output and stroke volume, reduced total peripheral resistance, increased organ blood flow, downregulated preproET-1 gene expression, and decreased plasma levels of ET-1. The beneficial effect of ghrelin in severe sepsis appears to be mediated by downregulation of ET-1 involving a NF-κB dependent pathway. Thus, ghrelin may provide a novel approach for anti-sepsis therapy.

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