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Human vasoactive hormone adrenomedullin and its binding protein rescue experimental animals from shock

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Abstract

We recently discovered that vascular responsiveness to adrenomedullin (AM), a vasoactive hormone, decreases after hemorrhage, which is markedly improved by the addition of its binding protein AMBP-1. One obstacle hampering the development of AM/AMBP-1 as resuscitation agents in trauma victims is the potential immunogenicity of rat proteins in humans. Although less potent than rat AM, human AM has been shown to increase organ perfusion in rats. We therefore hypothesized that administration of human AM/AMBP-1 improves organ function and survival after severe blood loss in rats. To test this, male Sprague-Dawley rats were bled to and maintained at an MAP of 40 mmHg for 90 min. They were then resuscitated with an equal volume of shed blood in the form of Ringer's lactate (i.e., low-volume resuscitation) over 60 min. At 15 min after the beginning of resuscitation, human AM/AMBP-1 (12/40 or 48/160 $\mu\text{g/kg}$ BW) were administered intravenously over 45 min. Various pathophysiological parameters were measured 4 h after resuscitation. In additional groups of animals, a 12-day survival study was conducted. Our result showed that tissue injury as evidenced by increased levels of transaminases, lactate, and creatinine, was present at 4 h after hemorrhage and resuscitation. Moreover, pro-inflammatory cytokines TNF- α and IL-6 were also significantly elevated. Administration of AM/AMBP-1 markedly attenuated tissue injury, reduced cytokine levels, and improved the survival rate from 29% (vehicle) to 62% (low-dose) or 70% (high-dose). However, neither human AM alone nor human AMBP-1 alone prevented the significant increase in ALT, AST, lactate and creatinine at 4 h after the completion of hemorrhage and resuscitation. Moreover, the half-life of human AM and human AMBP-1 in rats was 35.8 min and 1.68 h, respectively. Thus, administration of human AM/AMBP-1 may be a useful approach for attenuating organ injury, and reducing mortality after hemorrhagic shock.

Keywords

hemorrhage; adrenomedullin; adrenomedullin binding protein; 1; survival

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1. Introduction

Despite advances in trauma management, a large number of such patients die of severe hemorrhagic shock [1,8,23]. Most trauma deaths result from either insufficient tissue perfusion due to excessive blood loss, or the development of inflammation, infection and vital organ damage following resuscitation [1]. Current treatment plans for hemorrhage rely on massive and rapid infusion of crystalloid fluid to maintain blood pressure. Theoretically, replacement of the fluid deficit from hemorrhagic shock should improve cardiac filling, cardiac output, and lessen the need for increasing the peripheral resistance to sustain an effective arterial pressure. However, the majority of victims with severe blood loss do not respond well to fluid restoration. Two separate clinical studies document that despite normalization of blood pressure, heart rate, and urine output, tissue hypoperfusion persists in 80 to 85% of patients, as evidenced by lactic acidemia and decreased mixed venous oxygen saturation [4,31]. As such, the development of effective strategies for the treatment of traumatic blood loss is critical for the improvement of patient outcome following hemorrhagic shock.

Adrenomedullin (AM), a potent vasodilator peptide, was originally isolated from a human pheochromocytoma and reported by Kitamura *et al.* in 1993 [18]. AM acts as a circulating hormone which elicits various biological activities in a paracrine or autocrine manner. Recently, a novel specific AM binding protein (i.e., AMBP-1) in human plasma was identified and the purified protein was reported to be identical to human complement factor H [11,28]. AMBP-1 potentiates AM-induced vascular relaxation in the aorta under normal as well as pathophysiological conditions [42]. Our recent study has shown that vascular responsiveness to AM is depressed after severe blood loss, and AM hyporesponsiveness may play an important role in the transition from reversible hypovolemia to circulatory collapse after severe blood loss [35]. The fact that AMBP-1 improves vascular AM hyporesponsiveness raising the possibility of treating hemorrhagic shock with AM and AMBP-1 [35]. However, the antigenicity of rat proteins in humans prevents the use of this rat complex in humans. Human AM is a 52-amino acid peptide that has a carboxy-terminal amidated residue and a 6-residue ring structure formed by an intramolecular disulfide bridge. Rat AM has 50 amino acid residues, with two amino acid deletions and six substitutions when compared with human AM [29]. Therefore, human proteins must be tested in rats before these findings can be verified in humans. Although less potent than rat AM, human AM has been shown to increase organ perfusion in rats [9]. We therefore hypothesize that administration of human AM/AMBP-1 improves organ function and survival after severe blood loss in rats. The aim of this study was to determine the efficacy of human AM/AMBP-1 on hemorrhage-induced organ injury, inflammation, and mortality in rats.

2. Materials and methods

2.1. Experimental animals

One hundred and ten male Sprague-Dawley rats (275–325g), purchased from Charles River Laboratories (Wilmington, MA), were used in this study. The rats were housed in a temperature-controlled room on a 12-h light/dark cycle and fed on a standard Purina rat chow diet. The rats were fasted for 6 h prior to the procedure. Animal experimentation was carried out in accordance with the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources). This project was approved by the Institutional Animal Care and Use Committee of the Feinstein Institute for Medical Research.

2.2. Animal model of hemorrhage shock

The model of hemorrhage shock used in this experiment was described in detail previously [6,35,36]. Briefly, the rats were anesthetized with isoflurane inhalation. Catheters (PE-50

tubing) were placed in the femoral vein and artery after carefully separating the femoral nerve and blood vessels. The femoral artery on the opposite side was also catheterized. One arterial catheter was used for monitoring the mean arterial pressure (MAP) and heart rate (HR) via a blood pressure analyzer (BPA; Digi-Med, Louisville, Ky), the other was for blood withdrawal and the venous catheter was used for fluid resuscitation. The rats were rapidly bled to an MAP of 40 mmHg within 10 min. This pressure was maintained for 90 min by further withdrawal of small volumes of blood or provision of small volumes of Ringer's lactate. At the end of this hypotensive period, the rats were resuscitated with an equal volume of shed blood in the form of Ringer's lactate (i.e., low-volume resuscitation) over a 60-min period. The shed blood was not used for resuscitation and the animals were not heparinized prior to, during, and following hemorrhage. Sham-operated animals underwent the same surgical procedure but were neither bled nor resuscitated (Sham group).

2.3. Administration of AM and AMBP-1

At 15 min after the beginning of resuscitation in hemorrhaged animals, human AM and AMBP-1 in combination [12/40 $\mu\text{g/kg}$ BW (hemorrhage-low dose AM/AMBP-1 group) or 48/160 $\mu\text{g/kg}$ BW (hemorrhage-high dose AM/AMBP-1 group)], AM alone [48 $\mu\text{g/kg}$ BW (hemorrhage-AM group)], AMBP-1 alone [160 $\mu\text{g/kg}$ BW (hemorrhage-AMBP-1 group)], or vehicle [PBS, 1 ml (Hemorrhage-Vehicle group)] was administered via the femoral venous catheter over a period of 45 min. Blood samples were harvested 4 h post-resuscitation (i.e., 6.5 h from the beginning of hemorrhage) and placed on ice to allow clotting. The samples then were centrifuged at 1200 g for 10 min at 4°C, and the serum samples were stored at -80°C until assayed. Synthetic human AM was purchased from Phoenix Pharmaceuticals (Belmont, CA) with a purity of more than 99% (by HPLC). Human AMBP-1 was purchased from Cortex Biochem (San Leandro, CA) with a purity of more than 98% (by SDS-PAGE). The human AMBP-1 preparation has been tested at the serum/plasma donor level by FDA-approved methods and found negative for HIV I/II, and HCV antibodies and hepatitis B surface antigens.

2.4. Determination of serum levels of transaminases, lactate and creatinine

Serum concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate, and creatinine were determined by using assay kits according to the manufacturer's instructions (Pointe Scientific, Lincoln Park, MI).

2.5. Determination of serum levels of TNF- α and IL-6

The concentrations of TNF- α and IL-6 in the serum were quantified by using commercially obtained enzyme-linked immunosorbent assay (ELISA) kits specific for rat-TNF- α and IL-6 (BD Biosciences, San Diego, CA). The assay was carried out according to the instructions provided by the manufacturer.

2.6. Survival study

In additional groups of animals, human AM/AMBP-1 (12/40 or 48/160 $\mu\text{g/kg}$ BW) or vehicle (PBS, 1 ml) was infused at 15 min after the beginning of low volume resuscitation (an equal volume of shed blood in the form of Ringer's lactate) in hemorrhaged animals for 45 min. The animals then were allowed food and water *ad libitum* and were monitored for 12 days to record survival. At the end of the 12 day survival study, all animals were euthanized using a CO₂ chamber. No blood and tissue samples were collected.

2.7. Determination of the half-life of human AM and human AMBP-1 in rats

The half-life of human AM and human AMBP-1 in rats was determined after a bolus intravenous injection of ¹²⁵I-labeled human AM and biotin-labeled human AMBP-1, respectively. Briefly, the animals were anesthetized with isoflurane inhalation. A steady state

of sedation was maintained with a subsequent intravenous injection of sodium pentobarbital (~30 mg/kg body weight). Polyethylene-50 catheters were placed in the right jugular vein and left femoral artery. For determining the half-life of human AM in the circulation, a bolus injection of ^{125}I -labeled AM (Peninsula Laboratories, San Carlos, CA; 995Ci/mmol; ~500,000 cpm/rat) was administered through the jugular vein catheter. Blood samples (100 μl each) were collected every 3 min for a period of 30 min. In an additional group of animals, biotin-labeled AMBP-1 was administered through the jugular vein catheter to determine the half-life of human AMBP-1 in the circulation. Since we expect that the half-life of human AMBP-1 would be much longer than AM, blood samples were collected every hour for a period of 8 h. After each sample collection, blood volume was replenished with twice the volume of normal saline. The total blood removed was approximately 5% of the total blood volume. For human AM half-life measurement, the radioactivity (cpm) in each sample was measured with a gamma counter. For human AMBP-1 half-life measurement, 0.1- μl of whole blood was fractionated on a 4–12% Bis-Tris gel and then transferred to a 0.45 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 Neutravidin horseradish peroxidase conjugated (Pierce, Rockford, IL) for 1 h at room temperature, and then washed with TBST. 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 levels of biotin-labeled human AMBP-1 in the blood were expressed as the band densities, which were determined using a Bio-Rad image system (Hercules, CA). The half-life of human AM and human AMBP-1 in rats was calculated as we described before [39].

2.8. Statistical analysis

All data are expressed as means $\pm$ SE and compared by one-way analysis of variance (ANOVA) and Student-Newman-Keuls' test. The survival rate was estimated by Kaplan-Meier method and compared by the log-rank test. Differences in values were considered significant if $P < 0.05$.

3. Results

3.1. The amount of withdrawal blood in each group

The amount of withdrawal blood in hemorrhage-vehicle, hemorrhage-low dose AM/AMBP-1, and hemorrhage-high dose AM/AMBP-1 groups was 13.7 ± 0.3 ml, 13.4 ± 0.2 ml, and 13.9 ± 0.3 ml, respectively. There is no statistically significant difference in the amount of withdrawal blood among these groups.

3.2. Effects of human AM/AMBP-1 administration on transaminases after hemorrhagic shock

To determine whether co-administration of human AM/AMBP-1 confers protection against hemorrhagic shock in rats, plasma levels of two liver enzymes ALT and AST were determined. As an indicator of hepatic injury, the circulating levels of ALT and AST were increased by 71–75% after hemorrhagic shock (Figs. 1A–B). Administration of human AM/AMBP-1 during fluid resuscitation significantly attenuated the ALT and AST levels (Figs. 1A–B), indicating a protective role of AM/AMBP-1 in hemorrhagic shock.

3.3. Effects of human AM/AMBP-1 administration on lactate after hemorrhagic shock

As shown in Figure 2, serum levels of lactate, a marker for systemic hypoxia, were increased by 120% 4 h after the completion of hemorrhage and resuscitation. Administration of human AM/AMBP-1 significantly decreased lactate levels by 30% ($P < 0.05$), although lactate levels

in AM/AMBP-1 treated hemorrhage animals were still higher than those in sham operated animals (Fig. 2).

3.4. Effects of human AM/AMBP-1 administration on creatinine after hemorrhagic shock

To evaluate the renal function, serum creatinine levels were also determined. As indicated in Figure 3, serum creatinine levels increased significantly after hemorrhage and resuscitation. Human AM/AMBP-1 administration completely abrogated hemorrhagic shock-induced increase of serum creatinine levels under such conditions (Fig. 3).

3.5. Effects of human AM/AMBP-1 administration on pro-inflammatory cytokines after hemorrhagic shock

To explore the mechanisms underlying human AM/AMBP-1-mediated protection against tissue injury, the effect of human AM/AMBP-1 administration on systemic accumulation of pro-inflammatory cytokines was assessed. Consistent with an earlier observation [2], serum TNF- α levels increased by >10 fold at 4 h after the completion of hemorrhage and resuscitation (Fig. 4A). TNF- α levels were reduced by 45% after low dose human AM/AMBP-1 treatment. High dose human AM/AMBP-1 treatment reduced serum TNF- α levels by 83% ($P<0.05$, Fig. 4A). The changes in serum levels of IL-6 after hemorrhage were similar to that of TNF- α . Serum IL-6 levels were significantly increased after hemorrhage (by 207%, $P<0.05$) (Fig. 4B). Administration of either low dose or high dose human AM/AMBP-1 reduced circulating IL-6 to sham control levels (Fig. 4B).

3.6. Effects of human AM/AMBP-1 administration on survival after hemorrhagic shock

To determine whether administration of human AM plus human AMBP-1 improves survival after hemorrhage, a 12-day survival study was conducted. Our results indicate that the survival rate was significantly improved from 29% in vehicle-treated animals to 62% in the low-dose and 70% in high-dose human AM/AMBP-1-treated animals ($P<0.05$, Fig. 5).

3.7. Effects of human AM alone or human AMBP-1 alone on serum levels of liver enzymes, lactate, and creatinine

As shown in Table 1, neither human AM alone nor human AMBP-1 alone prevented the significant increase in ALT, AST, lactate and creatinine at 4 h after the completion of hemorrhage and resuscitation. Although AST, lactate and creatinine levels were slightly reduced after human AM or human AMBP-1 treatment, the decrease is not statistically significant from vehicle-treated animals. Thus, administration of human AM alone or human AMBP-1 alone after hemorrhage does not produce significant protection.

3.8. The half-life of human AM and human AMBP-1 in rats

To determine the half-life of human AM or human AMBP-1 in rats, ^{125}I -labeled human AM or biotin-labeled human AMBP-1 was administered to normal animals through a jugular vein catheter, respectively. Our results showed that the half-life of human AM and human AMBP-1 in rats was 35.8 min and 1.68 h, respectively.

4. Discussion

Traumatic injury with severe blood loss is a major public health problem in the US across age, race, gender, and economic boundaries. Activation of the systemic pro-inflammatory response and tissue injury are important pathophysiologic components of hemorrhagic shock [27]. Conventional resuscitation fluids were designed to increase the circulating blood volume in order to re-establish tissue perfusion during shock and to compensate for blood loss during hemorrhage. However, an inappropriate resuscitation treatment can exacerbate inflammatory

responses, which can be more dangerous than the original shock and produce a lethal multiple organ failure. An ideal resuscitation fluid should provide a therapeutic potential to improve organ perfusion and mitigate the inflammatory response, which are the two most relevant processes mediating lethal organ injury during hemorrhagic shock.

AM was originally isolated from a human pheochromocytoma based on its ability to raise cyclic adenosine 3'-5'-monophosphate (cAMP) levels in platelets and to cause strong hypotension [18]. Since then, it has attracted the interest of investigators in the cardiovascular field because of AM's potent and long-lasting vasoactive properties. Infusion of AM causes vasodilatation, diuresis, and natriuresis and inhibits aldosterone secretion in normal animals [30]. In addition to its well-known vasodilatory effects, AM also modulates production of inflammatory cytokines. Studies have shown that AM suppresses pro-inflammatory cytokines expression in various cell types [16,19]. Mice heterozygous for adrenomedullin exhibit a more extreme inflammatory response to endotoxin-induced septic shock [7]. Thus, AM may be an excellent candidate for the resuscitation of trauma victims.

Various studies have demonstrated that circulating levels of AM increase in patients with hemorrhagic and cardiogenic shock [10,17], ischemia-reperfusion injury [22,25], systemic inflammatory response syndrome [26,34], and following major surgery [14] or hypoxia [5, 24]. Fujioka *et al.* showed that plasma levels of AM are increased in a dog model of hemorrhagic shock [15]. Studies from our laboratory also showed that AM gene expression in the small intestine increases significantly after hemorrhagic shock [35]. The increased levels of AM may be a protective mechanism under such conditions. However, the current study as well as our previous publication clearly demonstrates that administration of AM alone is insufficient to prevent organ injury after hemorrhage and resuscitation [36].

Recent studies by Elsasser *et al.* have demonstrated the presence of a specific AM binding protein in mammalian blood [11]. It was discovered in part by the specific binding of ¹²⁵I-AM to a 120 kDa band on a blot obtained from a nonreducing, electrophoretic gel separation of serum proteins from several species including humans. Pío *et al.* purified this binding protein, named it AMBP-1, and discovered that AMBP-1 is identical to complement factor H [28]. The binding of AM to AMBP-1 is strong and not interrupted *in vitro* by acidic conditions or high salt concentration. The primary site of AMBP-1 biosynthesis is the liver [13,20,32], although it is also synthesized by extrahepatic cells, such as mononuclear phagocytes, fibroblasts, endothelial cells, mesangial cells, astrocytes, oligodendrocytes, and neurons [12]. The presence of AMBP-1 in tissues may affect the autocrine/paracrine actions of AM. In this regard, we have measured AMBP-1's levels after hemorrhage and resuscitation [35]. Our results showed that AMBP-1 gene expression in the liver and gut is significantly downregulated at 1.5 h after hemorrhage and resuscitation. Similarly, AMBP-1 protein levels in the gut and plasma decrease markedly at 1.5 h after resuscitation. Therefore, there is an AMBP-1 deficiency in hemorrhagic shock. However, the current study as well as our previous publication indicates that similar to administration of AM alone, administration of AMBP-1 alone is insufficient to prevent organ injury after hemorrhage and resuscitation [6,36]. The present study clearly demonstrates that administration of human AM in combination with human AMBP-1, even with low volume crystalloid resuscitation, attenuated tissue injury, downregulated inflammatory cytokines, and reduced mortality in a rat model of hemorrhagic shock. Therefore, the combination of AM/AMBP-1 is superior to AM or AMBP-1 alone in the treatment of hemorrhagic shock.

Circulating AMBP-1 affects the bioactivity of AM under normal and pathologic conditions. Our previous study has shown that the expression of AM receptors is not altered after hemorrhage and resuscitation. The decreased AMBP-1 expression and release after hemorrhage rather than alterations in AM receptors are responsible for the reduced microvascular responsiveness to AM [35]. Binding to AMBP-1 increases receptor-mediated

effects of AM but suppresses its receptor-independent antimicrobial activity [3]. AMBP-1 does not change the affinity of AM receptors for AM, but has sequences which may bind to cell surface adhesion molecules and could therefore bring AM near its receptors and raise the effective concentration of AM [28]. AM binding with AMBP-1 may also create a locally contained reservoir of AM at high concentrations. In this regard, AMBP-1 may increase the AM effectiveness without modifying the affinity of its receptor. Thus, it is highly possible that the protection of human AM/AMBP-1 after hemorrhagic shock is associated with enhanced AM receptor-mediated effect of AM.

AMBP-1 appears to play an important role in AM-induced vascular relaxation [42]. 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 sham-operated animals [42]. AMBP-1 alone is associated with only minimal vascular relaxation [42]. Moreover, AMBP-1 potentiates AM's downregulatory effect on LPS-induced cytokine production [40]. In the Kupffer cells primary culture, AM or AMBP-1 alone inhibited LPS-induced TNF- α production by 52% and 44%, respectively. However, co-culture with AM in combination with AMBP-1 reduced TNF- α production by 90% [40]. A recent study from our laboratory has shown that 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) [21]. A general approach reducing the pro-inflammatory cytokine response has been shown to be beneficial in hemorrhage [41]. Moreover, AMBP-1, i.e., complement factor H, inhibits activation of the alternative pathway of the complement system. AM influences the complement regulatory function of factor H by enhancing the cleavage of C3b via factor I [28]. Activation of the complement cascade is known to play a key role in the adverse immune consequences of hemorrhagic trauma with subsequent shock and resuscitation [33]. Therefore, in addition to the vasoactive function and anti-inflammatory property, inhibition of the alternative complement pathway may be another mechanism for AM/AMBP-1's beneficial effect after hemorrhagic shock. The binding of AM to AMBP-1 is strong. It is possible that AMBP-1 inhibits the degradation of the biologically active AM. Thus, the infused human AM/AMBP-1 may circulate in the plasma for an extended period during the experimental protocol.

Our ultimate goal is to develop the clinical utilization of human AM/AMBP-1 as a safe and effective resuscitation approach for the trauma victim with severe blood loss. Although immunogenic issues of human proteins in rats are not the focus of this study, our results have shown that there are no adverse effects of human AM/AMBP-1 in rats even after hemorrhage. Since we are not going to use rat AM/AMBP-1 to treat trauma victims, we did not propose to compare potency of rat AM vs. human AM in rats. However, after comparing with our published studies [6,36], human AM/human AMBP-1 is as effective as rat AM/human AMBP-1 after hemorrhage and resuscitation in rats. The doses and ratio of human AM and human AMBP-1 used in this study were chosen based on our previous studies using rat AM and human AMBP-1 [6,35–38]. We will conduct additional efficacy studies in the future to determine the optimal protective dosage and ratio of human AM/AMBP-1 after hemorrhage and resuscitation.

In summary, tissue injury as evidenced by increased levels of transaminases, lactate, and creatinine was present at 4 h after hemorrhage and resuscitation. Moreover, pro-inflammatory cytokines TNF- α and IL-6 were also significantly elevated. Administration of human AM in combination with human AMBP-1 markedly attenuated tissue injury, reduced cytokine levels, and improved survival. However, neither human AM alone nor human AMBP-1 alone prevented the significant increase in ALT, AST, lactate and creatinine at 4 h after the completion of hemorrhage and resuscitation. Moreover, the half-life of human AM and human AMBP-1 was 35.8 min and 1.68 h, respectively. Thus, administration of human AM/AMBP-1

may be a useful approach for attenuating organ injury, and reducing mortality after hemorrhagic shock.

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References

1. Anderson RN, Smith BL. Deaths: leading causes for 2001. *Natl Vital Stat Rep* 2003;52:1–85.
2. Ayala A, Wang P, Ba ZF, Perrin MM, Ertel W, Chaudry IH. Differential alterations in plasma IL-6 and TNF levels following trauma and hemorrhage. *Am J Physiol* 1991;260:R167–R171. [PubMed: 1992817]
3. Beltowski J, Jamroz A. Adrenomedullin - what we know 10 years since its discovery? *Pol J Pharmacol* 2004;56:5–27. [PubMed: 15047974]
4. bou-Khalil B, Scalea TM, Trooskin SZ, Henry SM, Hitchcock R. Hemodynamic responses to shock in young trauma patients: need for invasive monitoring. *Crit Care Med* 1994;22:633–9. [PubMed: 8143473]
5. Cejudo-Martin P, Morales-Ruiz M, Ros J, Navasa M, Fernandez-Varo G, Fuster J, et al. Hypoxia is an inducer of vasodilator agents in peritoneal macrophages of cirrhotic patients. *Hepatology* 2002;36:1172–9. [PubMed: 12395327]
6. Cui X, Wu R, Zhou M, Dong W, Ulloa L, Yang H, et al. Adrenomedullin and its binding protein attenuate the proinflammatory response after hemorrhage. *Crit Care Med* 2005;33:391–8. [PubMed: 15699844]
7. Dackor R, Caron K. Mice heterozygous for adrenomedullin exhibit a more extreme inflammatory response to endotoxin-induced septic shock. *Peptides* 2007;28:2164–70. [PubMed: 17889965]
8. Deitch EA, Dayal SD. Intensive care unit management of the trauma patient. *Crit Care Med* 2006;34:2294–301. [PubMed: 16878037]
9. Dogan A, Suzuki Y, Koketsu N, Osuka K, Saito K, Takayasu M, et al. Intravenous infusion of adrenomedullin and increase in regional cerebral blood flow and prevention of ischemic brain injury after middle cerebral artery occlusion in rats. *J Cereb Blood Flow Metab* 1997;17:19–25. [PubMed: 8978383]
10. Ehlenz K, Koch B, Preuss P, Simon B, Koop I, Lang RE. High levels of circulating adrenomedullin in severe illness: Correlation with C-reactive protein and evidence against the adrenal medulla as site of origin. *Exp Clin Endocrinol Diabetes* 1997;105:156–62. [PubMed: 9228512]
11. Elsasser TH, Kahl S, Martinez A, Montuenga LM, Pio R, Cuttitta F. Adrenomedullin binding protein in the plasma of multiple species: characterization by radioligand blotting. *Endocrinol* 1999;140:4908–11.
12. Friese MA, Hellwage J, Jokiranta TS, Meri S, Muller-Quernheim HJ, Peter HH, et al. Different regulation of factor H and FHL-1/reconnectin by inflammatory mediators and expression of the two proteins in rheumatoid arthritis (RA). *Clin Exp Immunol* 2000;121:406–15. [PubMed: 10931160]
13. Friese MA, Hellwage J, Jokiranta TS, Meri S, Peter HH, Eibel H, et al. FHL-1/reconnectin and factor H: two human complement regulators which are encoded by the same gene are differently expressed and regulated. *Mol Immunol* 1999;36:809–18. [PubMed: 10698334]
14. Fujioka S. Increased plasma concentration of adrenomedullin during and after major surgery. *Surg Today* 2001;31:575–9. [PubMed: 11495150]
15. Fujioka S, Ono Y, Kangawa K, Okada K. Plasma concentration of adrenomedullin is increased in hemorrhagic shock in dogs. *Anesth Analg* 1999;88:326–8. [PubMed: 9972749]
16. Isumi Y, Kubo A, Katafuchi T, Kangawa K, Minamino N. Adrenomedullin suppresses interleukin-1 β -induced tumor necrosis factor- α production in Swiss 3T3 cells. *FEBS Lett* 1999;463:110–4. [PubMed: 10601648]
17. Kikumoto K, Kubo A, Hayashi Y, Minamino N, Inoue S, Dohi K, et al. Increased plasma concentration of adrenomedullin in patients with subarachnoid hemorrhage. *Anesth Analg* 1998;87:859–63. [PubMed: 9768783]

18. Kitamura K, Kangawa K, Kawamoto M, Ichiki Y, Nakamura S, Matsuo H, et al. Adrenomedullin: a novel hypotensive peptide isolated from human pheochromocytoma. *Biochem Biophys Res Commun* 1993;192:553–60. [PubMed: 8387282]
19. Kubo A, Minamino N, Isumi Y, Katafuchi T, Kangawa K, Dohi K, et al. Production of adrenomedullin in macrophage cell line and peritoneal macrophage. *J Biol Chem* 1998;273:16730–8. [PubMed: 9642228]
20. Luo W, Vik DP. Regulation of complement factor H in a human liver cell line by interferon-gamma. *Scand J Immunol* 1999;49:487–94. [PubMed: 10320641]
21. Miksa M, Wu R, Cui X, Dong W, Das P, Simms HH, et al. Vasoactive hormone adrenomedullin and its binding protein: anti-inflammatory effects by up-regulating peroxisome proliferator-activated receptor-gamma. *J Immunol* 2007;179:6263–72. [PubMed: 17947702]
22. Miyao Y, Nishikimi T, Goto Y, Miyazaki S, Daikoku S, Morii I, et al. Increased plasma adrenomedullin levels in patients with acute myocardial infarction in proportion to the clinical severity. *Heart* 1998;79:39–44. [PubMed: 9505917]
23. Moore FA, McKinley BA, Moore EE. The next generation in shock resuscitation. *The Lancet* 2004;363:1988–96.
24. Nagata D, Hirata Y, Suzuki E, Kakoki M, Hayakawa H, Goto A, et al. Hypoxia-induced adrenomedullin production in the kidney. *Kidney Int* 1999;55:1259–67. [PubMed: 10200989]
25. Nagaya N, Nishikimi T, Uematsu M, Yoshitomi Y, Miyao Y, Miyazaki S, et al. Plasma adrenomedullin as an indicator of prognosis after acute myocardial infarction. *Heart* 1999;81:483–7. [PubMed: 10212165]
26. Nishio K, Akai Y, Murao Y, Doi N, Ueda S, Tabuse H, et al. Increased plasma concentrations of adrenomedullin correlate with relaxation of vascular tone in patients with septic shock. *Crit Care Med* 1997;25:953–7. [PubMed: 9201046]
27. Peitzman AB, Billiar TR, Harbrecht BG, Kelly E, Udekwu AO, Simmons RC. Hemorrhagic shock. *Curr Probl Surg* 1995;32:925–1002. [PubMed: 7587344]
28. Pio R, Martinez A, Unsworth EJ, Kowalak JA, Bengoechea JA, Zipfel PF, et al. Complement factor H is a serum binding protein for adrenomedullin. The resulting complex modulates the bioactivities of both partners. *J Biol Chem* 2001;276:12292–300. [PubMed: 11116141]
29. Sakata J, Shimokubo T, Kitamura K, Nakamura S, Kangawa K, Matsuo H, et al. Molecular cloning and biological activities of rat adrenomedullin, a hypotensive peptide. *Biochem Biophys Res Commun* 1993;195:921–7. [PubMed: 7690563]
30. Samson WK. Adrenomedullin and the control of fluid and electrolyte homeostasis. *Annu Rev Physiol* 1999;61:363–89. [PubMed: 10099693]
31. Scalea TM, Maltz S, Yelon J, Trooskin SZ, Duncan AO, Sclafani SJ. Resuscitation of multiple trauma and head injury: role of crystalloid fluids and inotropes. *Crit Care Med* 1994;22:1610–5. [PubMed: 7924373]
32. Schwaebler W, Zwirner J, Schulz TF, Linke RP, Dierich MP, Weiss EH. Human complement factor H: expression of an additional truncated gene product of 43 kDa in human liver. *Eur J Immunol* 1987;17:1485–9. [PubMed: 2445583]
33. Szebeni J, Baranyi L, Savay S, Gotze O, Alving CR, Bunger R, et al. Complement activation during hemorrhagic shock and resuscitation in swine. *Shock* 2003;20:347–55. [PubMed: 14501949]
34. Ueda S, Nishio K, Minamino N, Kubo A, Akai Y, Kangawa K, et al. Increased plasma levels of adrenomedullin in patients with systemic inflammatory response syndrome. *Am J Respir Crit Care Med* 1999;160:132–6. [PubMed: 10390390]
35. Wu R, Cui X, Dong W, Zhou M, Simms HH, Wang P. Mechanisms responsible for vascular hyporesponsiveness to adrenomedullin after hemorrhage: the central role of adrenomedullin binding protein-1. *Ann Surg* 2005;242:115–23. [PubMed: 15973109]
36. Wu R, Dong W, Zhou M, Cui X, Simms HH, Wang P. A novel approach to maintaining cardiovascular stability after hemorrhagic shock: Beneficial effects of adrenomedullin and its binding protein. *Surgery* 2005;137:200–8. [PubMed: 15674202]
37. Wu R, Dong W, Zhou M, Simms HH, Marini CP, Ravikumar TS, et al. Adrenomedullin and adrenomedullin binding protein-1 prevent metabolic acidosis after uncontrolled hemorrhage in rats. *Crit Care Med* 2007;35:912–8. [PubMed: 17255858]

38. Wu R, Wang P. Preclinical studies with adrenomedullin and its binding protein as cardiovascular protective agents for hemorrhagic shock. *Cardiovasc Drug Rev* 2006;24:204–13. [PubMed: 17214597]
39. Wu R, Zhou M, Cui X, Simms HH, Wang P. Ghrelin clearance is reduced at the late stage of polymicrobial sepsis. *Int J Mol Med* 2003;12:777–81. [PubMed: 14533009]
40. Wu R, Zhou M, Wang P. Adrenomedullin and adrenomedullin binding protein-1 downregulate TNF- α in macrophage cell line and rat Kupffer cells. *Regul Pept* 2003;112:19–26. [PubMed: 12667621]
41. Yang S, Hu S, Choudhry MA, Rue LW III, Bland KI, Chaudry IH. Anti-rat soluble IL-6 receptor antibody down-regulates cardiac IL-6 and improves cardiac function following trauma-hemorrhage. *J Mol Cell Cardiol* 2007;42:620–30. [PubMed: 17313958]
42. Zhou M, Ba ZF, Chaudry IH, Wang P. Adrenomedullin binding protein-1 modulates vascular responsiveness to adrenomedullin in late sepsis. *Am J Physiol Regul Integr Comp Physiol* 2002;283:R553–R560. [PubMed: 12184987]

Figure 1.

Alterations in circulating levels of (A) alanine aminotransferase (ALT) and (B) aspartate aminotransferase (AST) at 4 h after the completion of hemorrhagic shock and fluid resuscitation treated with vehicle (Hemorrhage-Vehicle) or human adrenomedullin (AM)/human adrenomedullin binding protein (AMBP)-1 (Hemorrhage-Low dose AM/AMBP-1: 12/40 $\mu\text{g/kg}$ BW; Hemorrhage-High dose AM/AMBP-1: 48/160 $\mu\text{g/kg}$ BW). Data are presented as means $\pm$ SE ($n = 6-8/\text{group}$) 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 2.

Alterations in circulating levels of lactate at 4 h after the completion of hemorrhagic shock and fluid resuscitation treated with vehicle (Hemorrhage-Vehicle) or human adrenomedullin (AM)/human adrenomedullin binding protein (AMBP)-1 (Hemorrhage-Low dose AM/AMBP-1: 12/40 $\mu\text{g/kg}$ BW; Hemorrhage-High dose AM/AMBP-1: 48/160 $\mu\text{g/kg}$ BW). Data are presented as means $\pm$ SE ($n = 6-8/\text{group}$) 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 circulating levels of creatinine at 4 h after the completion of hemorrhagic shock and fluid resuscitation treated with vehicle (Hemorrhage-Vehicle) or human adrenomedullin (AM)/human adrenomedullin binding protein (AMBP)-1 (Hemorrhage-Low dose AM/AMBP-1: 12/40 μg/kg BW; Hemorrhage-High dose AM/AMBP-1: 48/160 μg/kg BW). Data are presented as means ± 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 4.

Alterations in circulating levels of (A) TNF- α and (B) IL-6 at 4 h after the completion of hemorrhagic shock and fluid resuscitation treated with vehicle (Hemorrhage-Vehicle) or human adrenomedullin (AM)/human adrenomedullin binding protein (AMBP)-1 (Hemorrhage-Low dose AM/AMBP-1: 12/40 μ g/kg BW; Hemorrhage-High dose AM/AMBP-1: 48/160 μ g/kg BW). 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 5.

Alterations in the survival rate at 12 days after hemorrhage and resuscitation treated with vehicle (Vehicle) or human adrenomedullin (AM)/human adrenomedullin binding protein (AMBP)-1 (Low dose AM/AMBP-1: 12/40 $\mu\text{g/kg}$ BW; High dose AM/AMBP-1: 48/160 $\mu\text{g/kg}$ BW). The survival rate was estimated by the Kaplan-Meier method and compared by using the log-rank test. * $P < 0.05$ vs. vehicle group.

Table 1
Effects of human AM (48 µg/kg BW) alone or human AMBP-1 (160 µg/kg BW) alone on serum levels of liver enzymes, lactate, and creatinine

	Sham	Hemorrhage-Vehicle	Hemorrhage-AM	Hemorrhage-AMBP-1
ALT (IU/L)	14±0.7	62±6.5 [*]	61±16.7 [*]	63±10.1 [*]
AST (IU/L)	30±5.2	116±4.7 [*]	94±19.1 [*]	106±13.6 [*]
Lactate (mg/dL)	12±1.2	38±5.0 [*]	36±5.7 [*]	34±7.7 [*]
Creatinine (µmol/L)	83±11.4	249±14.9 [*]	222±34.0 [*]	214±59.1 [*]

^{*} P <0.05 versus sham-operated animals. Values are presented as means ± SE (n = 5/group) and compared by one-way ANOVA and Student-Newman-Keuls test.