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Original Research Article

Effectiveness of different resuscitation methods for severe uncontrolled hemorrhagic shock in a dog model

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Abstract

Purpose: *To compare the effects of pituitrin resuscitation, hypertonic saline resuscitation and limited fluid resuscitation on a novel dog model of severe uncontrolled hemorrhagic shock (UCHS).*
Methods: *Severe UCHS was produced in healthy Chinese dogs (n = 24) using a standard method. The hemorrhaged dogs were randomly divided into three groups of 8 dogs each: vasopressin group (group A), hypertonic saline group (group B), and low-volume fluid resuscitation group (group C). Group A received pituitrin at an initial dose of 0.1U/kg intravenously, which was thereafter reduced to 0.04 U/kg/min. Group B dogs received 7.5 % hypertonic saline (6 mL/kg), while dogs in group C were treated with 6 % hydroxyethyl starch (HES) 200/0.5. Mean arterial pressure (MAP) of the dogs was maintained between 50 and 55 mmHg, and after 1 h, full-volume resuscitation was administered. Hemodynamic parameters, blood gas, levels of inflammatory factors and blood loss were assessed at different time points.*
Results: *Compared with group A, hemodynamic parameters in group B were higher; hematocrit of group B was lower; IL-10 of groups B and C were reduced, but TNF-α, TNF-α/IL-10 and ACTH were elevated ($p < 0.05$). Relative to group C, base deficits in groups A and B were low. During uncontrolled hemorrhage phase, blood loss in group B was higher than that in other groups ($p < 0.05$).*
Conclusion: *The results obtained in this study suggest that pituitrin resuscitation produces relatively optimal effect through effective maintenance of coronary perfusion pressure (CPP) and reduction of inflammatory responses.*

Keywords: *Uncontrolled hemorrhagic shock, Pituitrin resuscitation, Limited fluid resuscitation, Hypertonic saline resuscitation, Heart rate*

INTRODUCTION

Traumatic hemorrhagic shock (THS) is a clinical emergency. Due to the fact that pre-hospital interventions cannot ensure hemostasis, many patients with hemorrhage usually suffer from UCHS before surgery. For this reason, studies have focused on the timing and management strategy during the early stage of UCHS resuscitation immediately after trauma. Traditional fluid resuscitation results in dilution of red blood cells (RBCs) and decreased blood oxygen-carrying capacity. Large amounts of fluid infusion also cause hypothermia and coagulopathy, which can lead to imbalance between organ perfusion and hemostasis in patients with UCHS, and trauma-associated mortality [1].

Studies have shown that strategies aimed at reducing bleeding and hemodilution via the use of vasoconstrictors or low-volume fluid resuscitation perform better than traditional fluid resuscitation [2-6]. Only a few studies have directly compared these methods under the same conditions. Hence, it has become necessary to study and compare the effects of different resuscitation methods. The use of vasoactive drugs such as vasopressin and terlipressin, and Ringer's lactate (RL) solution for resuscitation in rat model of UCHS support their early optimization in hemodynamics and anti-inflammatory response [7]. In addition, the role of hypertonic saline in early phases of resuscitation has not been fully elucidated. This may involve recovery and release of stress-related pro-inflammatory cytokines. The present study compared the priority of pituitrin resuscitation, hypertonic saline and limited fluid resuscitation in a dog model of severe UCHS.

EXPERIMENTAL

Materials

Automated blood gas analyzer Premie 3000 was obtained from GE (USA). Double antibody sandwich ABC-ELISA kit was purchased from Changchun Huili Biotechnology Co. Ltd (China), while SPSS statistical software was product of IBM (USA). Datex-Ohmeda multi-function monitor was obtained from Thermo Fischer Scientific Co. Ltd (USA). This research was approved by the Animal Ethical Committee of Department of Anesthesiology, Nanjing Chest Hospital Affiliated to Southeast University School of Medicine, Nanjing (approval no. = 2018249), and was conducted according to "Principles of Laboratory Animal Care" (NIH publication no. 85-23, revised 1985) [8].

Experimental dogs and establishment of severe UCHS model

Twenty-four (24) Chinese rural dogs were provided by the Nanjing Military Region Animal Experimental Center (no. SCXK (S) 2011 - 0022), and were housed in metal cages under standard laboratory conditions for 3 - 7 days. The dogs were fasted for 12 h prior to commencement of the study. They were weighed and anesthetized with 5 % pentobarbital sodium injection intramuscularly at a dose of 3 mL/kg. Then, the right femoral artery and external jugular vein were punctured with catheters and connected to the Datex-Ohmeda multi-function monitor for invasive measurement of SBP, DBP, MAP, HR, and CVP.

A 10-cm incision was made through the midline of the abdomen. Then, a branch of the mesenteric artery (diameter of 3 - 4 mm) was selected and cut open to bleed. Blood (200 mL) was removed through the femoral artery to reduce MAP to 50 mmHg in order to successfully establish severe UCHS. The blood was anticoagulated with heparin and transfused back to the dog during volume resuscitation; the volume of blood was approximately one-third the volume of hemorrhage.

Experimental design

Dogs with UCHS were randomly allocated to three groups (n=8): vasopressin group (group A), hypertonic saline group (group B), and low-volume fluid resuscitation group (group C). Dogs in group A received pituitrin intravenously at a loading dose of 0.1 U/kg immediately after MAP decreased to 50 mmHg, followed by 0.04 U/kg/min intravenous infusion intermittently to make MAP ≥ 50 mmHg. Group B received rapid intravenous infusion of 7.5 % hypertonic saline chloride at a dose of 6 mL/kg bwt when MAP decreased to 50 mmHg, in order to sustain MAP ≥ 50 mmHg. Group C dogs were treated with intravenous infusion of 6 % HES 200/0.5 immediately after MAP decreased to 50 mmHg. Infusion lasted 1 h, but the speed and volume were controlled to make MAP ≥ 50 mmHg. The entire procedure was carried out at 22 °C, at humidity of 60 - 65 %. One hour after initiation of resuscitation in each group (T_2), the branch of the mesenteric artery was closed back to ensure complete hemostasis. Volume resuscitation was considered adequate when the transfusion of autologous blood, or the infusion of HES or RL solution was one-third the total blood loss. The catheters were then removed after observing the dogs for another 2 h (T_3). Anesthesia was finally withdrawn. The dogs were allowed free access to water and feed. Dog behavior, water and feed intake, and signs of active hemorrhage were monitored for 72 h (T_4) after removal of catheter. Penicillin sodium was administered at 800 KU, twice a day throughout the period of observation.

Measurement of outcomes

During the period from T_o to T_3, hemodynamic parameters were recorded at intervals of 5 min until recovery was stabilized. Arterial blood samples were collected before establishment of UCHS model (T_o), after model preparation (T_1);

and at 15 min (T_{1-15}), 30 min (T_{1-30}), 45 min (T_{1-45}), 60 min (T_2) after resuscitation; and 2 h after ligation of mesenteric artery (T_3). Hematocrit (HCT), lactic acid, blood glucose, base deficit (BD), arterial carbon dioxide ($paCO_2$), arterial pressure of oxygen (pO_2) and pH were monitored using an automated blood gas analyzer. In addition, blood samples were collected at T_o, T_2, T_3 and 72 h after T_2 (T_4). The blood was stored at -80 °C. Serum levels of IL-10, TNF-α, and ACTH were estimated using enzyme-linked immunosorbent assay (ELISA). The TNF-α/IL-10 ratio was also calculated. Dog survival (S) was calculated at T_1, T_2, T_3, and T_4 as shown in Eq 1.

S (%) = (n/8)100 ……… (1)

where *n* is the number of dogs that survived at a particular time *t*. The volume of blood loss was measured during UCHS model preparation (period of acute traumatic blood loss), and during resuscitation (period of recovery from uncontrolled bleeding).

Statistical analysis

Data are expressed as mean ± SEM, and SPSS was used for statistical analysis (version 19.0). Groups were compared using Chi-squared test. Values of $p < 0.05$ were considered statistically significant.

RESULTS

Table 1 shows comparison of baseline characteristics among three groups. Hemodynamic variables were comparable among three groups at T_0 and T_1 time points ($p > 0.05$). Group B had greater SBP, DBP, and MAP at T_{1-15}, T_{1-30}, T_{1-45}, and T_2 than group A ($p < 0.05$); elevated HR at T_{1-30}, T_{1-45}, and T_2 ($p < 0.05$), and higher CVP at T_{1-30} and T_{1-45} ($p < 0.05$). Group A and group B had higher SBP at T_{1-15} than group C ($p < 0.05$ and 0.01, respectively). Group A had decreased SBP and HR at T_2, when compared to group C ($p < 0.05$). Group B had increased DBP and MAP at T_{1-15}, T_{1-30}, and T_{1-45} ($p < 0.05$), and elevated CVP and HR at T_{1-30} relative to group C ($p < 0.05$) (Figure 1).

There were no significant differences in blood gas measurements (Hct, Lac and BD) and blood glucose levels among three groups at T_0 ($p > 0.05$). Group B had lower Hct at T_2 than group A ($p < 0.05$). Group A and group B had lower Lac values at T_2 than group C ($p < 0.05$) (Figure 2). There were no significant differences in IL-10, TNF-α, ACTH levels, and TNF-α/IL-10 ratio among three groups at T_0 ($p < 0.05$). Groups B and C had lower IL-10 levels at T_2, T_3, and T_4 than group A ($p < 0.01$), and higher TNF-α, TNF-α/IL-10 and ACTH at T_2, T_3, and T4 ($p < 0.01$). Group C had lower IL-10 ($p < 0.05$) and higher TNF-α, TNF-α/ IL-10 ($p < 0.01$) and ACTH ($p < 0.01$) than group B at T_2, T_3, and T_4 (Figure 3).

All dogs survived through to the end of resuscitation (T_3). One dog in group A died less than 72 h after resuscitation. Both group B and group C had one dead dog within 48 h after resuscitation. There were no statistically significant differences in the survival among these groups at any point in time ($p > 0.05$). During the acute blood loss period, there was no difference in the volume of the blood loss among three groups. During the uncontrolled blood loss period, the volume of the blood loss in group B dogs was significantly greater, when compared with the other groups ($p < 0.05$, Figure 4).

DISCUSSION

Establishment of an animal model of UCHS is a difficult task. Different animal models have been developed based on different methods and study purposes. Unlike the traditional Wiggers model of controlled hemorrhagic shock which is stable and replicable, it is usually difficult to simulate UCHS that mimic human hemorrhage, whether as penetrating injury to the liver or spleen, or as a rupture of visceral artery [9].

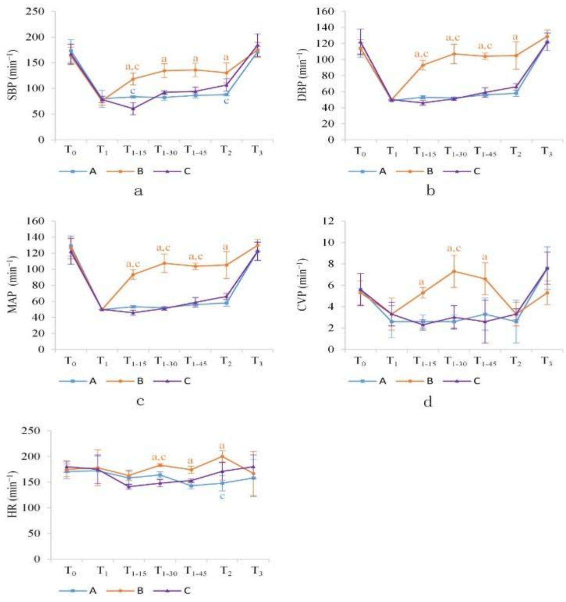

Figure 1: Comparison of three resuscitations on hemodynamics in experimental groups. Group A: pituitrin resuscitation; Group B: hypertonic saline resuscitation; Group C: limited fluid resuscitation; $^ap < 0.05$ relative to group A; $^cp < 0.05$ relative to group C

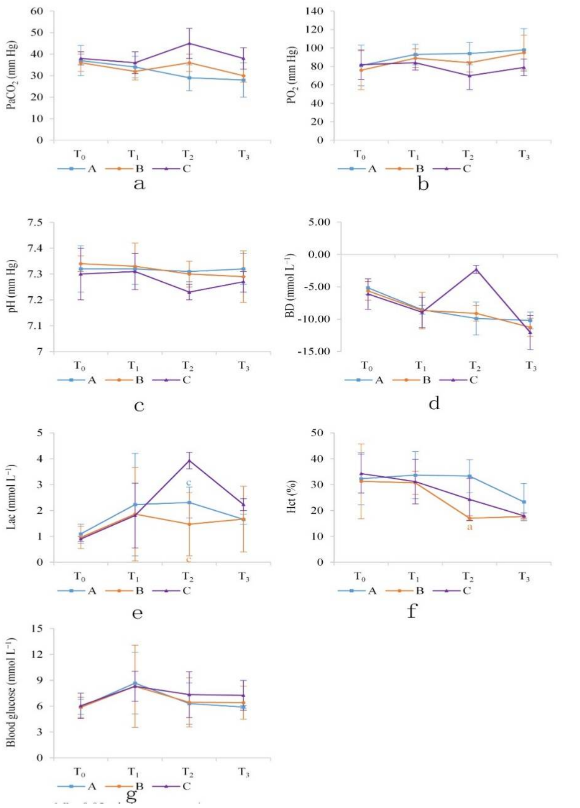

Figure 2: Comparison of blood gas, Lac, Hct and blood glucose levels in experimental groups. Group A, pituitrin resuscitation; Group B, hypertonic saline resuscitation; Group C, limited fluid resuscitation; $^{a}p < 0.05$ relative to group A, $^{c}p < 0.05$ relative to group C

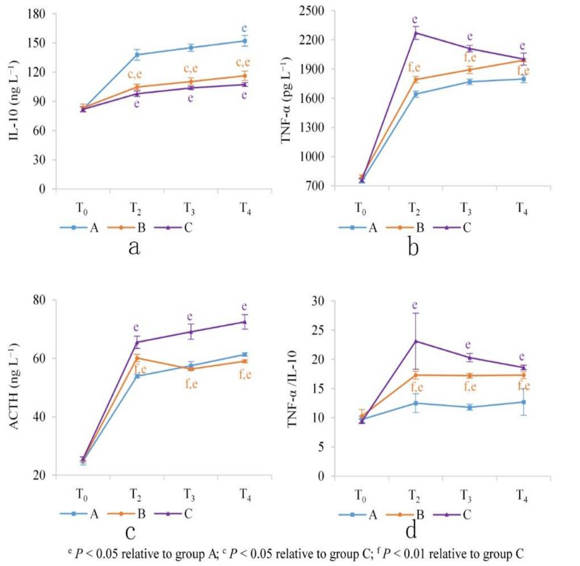

Figure 3: Comparison of IL-10, TNF-α, and ACTH at different times in experimental groups. Group A: pituitrin resuscitation; Group B: hypertonic saline resuscitation; Group C: limited fluid resuscitation; $^{e}p < 0.05$ relative to group A; $^{c}p < 0.05$ relative to group C; $^{f}p < 0.01$ relative to group C

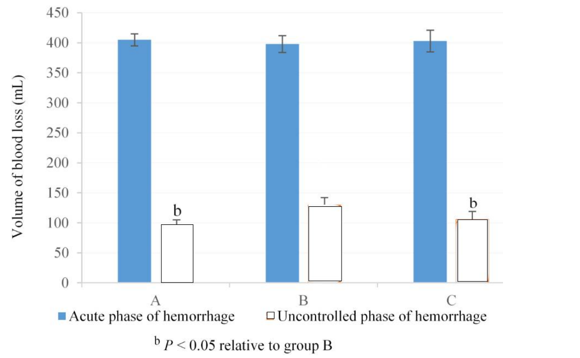

Figure 4: Comparison of blood loss in experimental groups. Group A: pituitrin resuscitation; Group B: hypertonic saline resuscitation; Group C: limited fluid resuscitation

The animal model of UCHS used in this study was based on the method described by Wang [10], in which the uterine artery was cut to produce intra-abdominal hemorrhage, and the carotid artery was punctured for bleeding and self-transfusion. First, a 10 cm incision was made in the abdomen of a Chinese rural dog, and a mesenteric artery branch with a diameter of 3 - 4 mm was cut to induce intra-abdominal hemorrhage.

The femoral artery was also severed and allowed to bleed continuously up to 200 mL. The blood from the femoral artery was collected and anticoagulated with heparin for self-transfusion during resuscitation. This model simulated pathophysiological changes in vascular contractions and coagulation after injury, which may be used to compare early recovery outcomes among three commonly used methods of resuscitation (vasopressin, hypertonic saline and low-pressure fluid resuscitations) in severe UCHS. Vasopressin and low-volume fluid resuscitations are aimed at effectively maintaining CPP. In this study, 50 mmHg was used as the target blood pressure, since a pilot study showed that dogs would fall into severe decompensated state if MAP is reduced to 40 mmHg (less than one-third the baseline), and may die if the blood pressure decreases further. Studies have shown that small volume of hypertonic saline significantly improves hemodynamic measurements in a shock state, thereby reducing mortality [11,12]. Hence, 6 mL/kg bwt of 7.5 % hypertonic sodium chloride was used in this study.

The results showed that all the dogs survived to the end of resuscitations (T_3). Survival was comparable among the groups. During the period of acute blood loss, there were no significant differences in the volume of blood loss among the groups. However, during the period of uncontrolled blood loss, the volume of blood loss in group B was significantly higher than those in groups A and C. These results suggest that the three resuscitation methods may be effective against severe UCHS, and are in agreement with those of previous reports [5-7].

Hemodynamic parameters were comparable among the three groups at T_o and T_1. SBP, DBP; and MAP were significantly higher in group B at T_{1-15}, T_{1-30}, T_{1-45}, and T_2 than in group A at the corresponding time points. In group A, HR was significantly higher at T_{1-30}, T_{1-45}, and T_2, while CVP was markedly higher at T_{1-30} and $T_{1-45,}$ when compared with group B. The SBP at T_{1-15} was significantly higher in groups A and B than in group C. However, SBP and HR at T_2 were significantly reduced in group A, when compared with group C. Similarly, DBP and MAP at T_{1-15}, T_{1-30}, and T_{1-45}, and CVP and HR at T_{1-30} were significantly increased in group B, relative to group C. There were no significant differences in blood gas measurements among the three groups at T_o. However, the HCT of group B was significantly lower at T_2 than that of group A.

In addition, the level of lactic acid was markedly lower in groups A and B at T_2 than in group C. These results suggest that hypertonic saline effectively maintains circulation and blood pressure in dogs, and that pituitrin may be more reliable in maintaining stable hemodynamics than the other two treatments. The volume expansion effect of hypertonic saline in group B may have diluted the blood. Tissue perfusion during low-pressure fluid resuscitation appears to be inferior, relative to the other two groups.

There were no significant differences in IL-10, TNF-α, and ACTH levels, and TNF-α/IL-10 among the groups at T_o. The levels of IL-10 at T_2, T_3, and T_4 were markedly lower in groups B and C than in group A. However, group C had significantly lower level of IL-10 and higher levels of ACTH, TNF-α and TNF-α/ IL-10 than group B at T_2, T_3, and $T_{4.}$ These results suggest that stress and inflammatory responses in group A may not be as extensive as those of groups B and C. Dogs in group B may be more experienced and less stressed with reduced inflammation, when compared with group C dogs.

CONCLUSION

These results suggest that pituitrin resuscitation produces relatively optimal effect via effective maintenance of coronary perfusion pressure (CPP) and reduction of inflammatory responses to drugs.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. All authors read and approved the manuscript for publication. Yang Sun conceived and designed the study. Shan Jiang, Shaoming He, Yang Sun collected and analyzed the data, while Shan Jiang wrote the manuscript.

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