Resuscitation 80 (2009) 253–258

Contents lists available at ScienceDirect

Resuscitation

journal homepage: www.elsevier.com/locate/resuscitation

Experimental paper

Effects of different resuscitation fluids on the rheologic behavior of red blood cells, blood viscosity and plasma viscosity in experimental hemorrhagic shock☆,☆☆

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ARTICLE INFO

Article history:
Received 21 February 2008
Received in revised form 6 October 2008
Accepted 10 October 2008

Keywords:
Hemorrhagic shock
Resuscitation
RBC deformability
Plasma viscosity

ABSTRACT

Background: Hemorrhagic shock is associated with severe rheological abnormalities. We hypothesized that in the setting of hemorrhagic shock, resuscitation can alter hemorheological characteristics dramatically, and different fluids cause different effects. The aim of this study was to investigate whether the type of fluid administered has an impact on hemorheological characteristics at the early stage of resuscitation in a rodent model of hemorrhagic shock.

Methods: Animals were randomized into five groups: (1) sham hemorrhage (SHAM); (2) shock and sham resuscitation (SHOCK); (3) shock and resuscitation with normal saline 32 ml/kg (NS); (4) shock and resuscitation with 7.5% hypertonic saline 4 ml/kg (HS); (5) shock and resuscitation with 7.5% hypertonic saline/6% Dextran 70 4 ml/kg (HSD). Hemorheological characteristics were measured at 60 min after resuscitation.

Results: Results showed that NS resuscitation deteriorated red blood cell (RBC) deformability compared with the SHOCK group. The HS group showed improved RBC deformability compared with the NS group, although the differences were not statistically significant. There were significant improvements of RBC deformability at all shear rates in the HSD group compared with the NS group. Whole blood and plasma viscosities decreased significantly in the SHOCK group compared with the SHAM group. At shear rates of 60 and 150 s^{-1}, the NS group decreased whole blood viscosity compared with the SHOCK group. The HSD group showed elevated plasma viscosity compared with the SHOCK, NS and HS groups.

Conclusion: These results suggested that at the early stage of hemorrhagic shock resuscitation, hypertonic–hyperoncotic resuscitation could improve RBC deformability compared with isotonic crystalloid resuscitation. Dextran 70 could elevate plasma viscosity to nearly baseline level. These effects of hypertonic–hyperoncotic resuscitation could be beneficial to maintain microcirculation.

Introduction

Hemorrhage remains a major cause of death and disability in battlefield injuries, as well as in civilian trauma. Hemorrhagic shock, like other conditions in emergency medicine, is associated with severe rheological abnormalities that play a pathogenetic role and contribute to the persistence or deterioration of the physiological state. The primary defect in all forms of circulatory shock is a critical reduction in blood flow and shear forces. Tissue malperfusion is at least partially caused and perpetuated by severe changes in hemorheology.[1,2] Trauma and hemorrhagic shock cause RBC shape alterations and a significant decrease in RBC deformability, which becomes manifested during the shock period and persists for at least 6 h postshock. Additionally, a direct relationship appears to exist between the magnitude of the physiologic insult and the degree of RBC damage.[3] Tatarishvili et al. found that the hemorheological disorders are among the most significant microcirculatory disturbances in the pathogenesis of both the traumatic and the hemorrhagic shock.[4]

Whole blood viscosity is mainly determined by hematocrit, whereas plasma viscosity mainly depends on the concentration of high-molecular weight proteins.[1] Decreased hematocrit and loss of plasma proteins in hemorrhagic shock cause drops in whole blood and plasma viscosity.

Fluid resuscitation is an essential component of therapy for hemorrhagic shock. Isotonic crystalloids became the standard of

☆ A Spanish translated version of the summary of this article appears as Appendix in the final online version at doi:10.1016/j.resuscitation.2008.10.014.

☆☆ This research was supported by grant Y0905001040131 from the science committee of Beijing in China.

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doi:10.1016/j.resuscitation.2008.10.014

care in the late 1960s and are still used as traditional resuscitation today. Small-volume hypertonic saline was shown to be as effective as large-volume crystalloids in expanding plasma volume and enhancing cardiac output in hemorrhagic shock. Hypertonic saline can increase perfusion of the microcirculation, presumably by decreasing the swelling of red blood cells and the endothelium.[5] Furthermore, the effectiveness of hypertonic saline was found to be enhanced by combination with dextran.[6,7] Crystalloid versus colloid debate in shock resuscitation has existed for many years. The prospective randomized controlled trials comparing crystalloid and colloid resuscitation showed that the use of crystalloids in trauma patients is associated with improved survival. Colloids might reduce the incidence of abdominal compartment syndrome.[8]

Previous works have demonstrated different hemorheologic effects caused by different resuscitation fluids in trauma or surgery. In trauma-hemorrhagic shock, 7.5% hypertonic saline improved red blood cell deformability compared with Ringers lactate.[9] After burn blast combined injury, hypertonic saline solution therapy could significantly improve blood rheology.[10] Clinical use of pentastarch in cardiac surgery decreased hematocrit and plasma viscosity.[11] Based on these previous studies, we made a hypothesis that in the setting of hemorrhagic shock, resuscitation can alter hemorheological characteristics dramatically, and different fluids cause different effects. In the present study, we tested whether the type of fluid – normal saline, 7.5% hypertonic saline and 7.5% hypertonic saline/6% Dextran 70 – has an impact on hemorheological characteristics at the early stage of resuscitation in a rodent model of hemorrhagic shock. The parameters of hemorheology are hematocrit, elongation and aggregation indices of erythrocyte, viscosities of whole blood and plasma.

Methods

All animals were treated according to institutional norms for laboratory animal care. All experiments were approved by the ethics committee of our institute.

Surgical procedures

The rats were anesthetized during the whole experiment with pentobarbital sodium (2%, w/v) (Peking Chemical Agent Co., Peking, China) by intraperitoneal injection of 0.3 ml per 100 g of body weight. Using the aseptic technique, the right carotid artery, the femoral artery and vein were surgically cannulated with polyethylene. The blood pressure, rectal temperature, and heart rate (HR) were monitored continuously with a polygragh (BIOPAC System, Model M100A, Santa Barbara, CA). The heparinized saline used during the surgical procedure consisted of 12.5 IU heparin sodium (Chinese Medicine Group Chemical Agent Co.) per ml of normal saline.

Hemorrhagic shock protocol

Hemorrhage was performed over about 15 min to lower the MAP to 45 mmHg through the femoral arterial catheter. The animals were then subjected to a second slower hemorrhage to maintain the MAP to between 45 and 65 mmHg for 45 min. The total amount of blood withdrawn was recorded.

Resuscitation groups

After vascular cannulation the animals were randomized into the following five groups ($n = 8$/group): Group 1: sham hemorrhage – animals underwent anesthesia and catheter placement only (SHAM); Group 2: shock and sham resuscitation (SHOCK); Group 3: shock and resuscitation with normal saline 32 ml/kg (NS); Group 4: shock and resuscitation with 7.5% hypertonic saline 4 ml/kg (HS); Group 5: shock and resuscitation with 7.5% hypertonic saline/6% Dextran 70 4 ml/kg (HSD). Resuscitation groups have approximate equivalent sodium load.[12,13] Resuscitation was started at the end of hemorrhage.

The normal saline, 7.5% hypertonic saline and 7.5% hypertonic saline/6% Dextran 70 were all prepared in our laboratory and found to be pyrogen free. All the resuscitation fluids were kept and infused at room temperature. The hypertonic fluids were infused at a rate of 0.4 ml/min and normal saline at a rate of 1.6 ml/min.

Blood sampling

1 h after resuscitation, blood samples were collected from the carotid artery and the animals were sacrificed. The blood samples were centrifuged at 2400 × *g* for 10 min to obtain plasma for biochemical analysis. Aliquots were then prepared and stored at −70 °C until the time of biochemical analysis. The blood samples for measurement of hemorheological indices were kept no longer than 2 h.

Measurement of hemorheological indices

All rheological measurements were carried out at 37 °C according to the international guidelines for the measurement of rheological parameters.[14] 7.5 μl blood was suspended in 1 ml 1.5% polyvinylpyrrolidone (PVP) buffer (Molecular Weight [MW] 30 kDa, 61 mM NaCl, 0.8 mM Na_2HPO_4, 0.2 mM KH_2PO_4, pH 7.4, 290 mOsm/kg) and to measure elongation index (EI), a measurement of RBC deformability, at shear rates of 600, 800 and 1000 s^{-1} by using an ektacytometer based on laser diffraction (Model LBY-BX, Precil Company, Beijing, China). After mixing 440 μl blood and 110 μl PVP buffer as above, 500 μl mixture was used to measure the aggregation index with the same ektacytometer.[15]

The hematocrit of blood samples was determined by a semi-automated Microcell Counter F-820 (Sysmex Corporation, Kobe, Japan). Whole blood viscosity was determined at shear rates of 150, 60 and 10 s^{-1} by using a cone-plate viscometer (Model LBY-N6B, Precil Company, Beijing, China). Blood was centrifuged at 3000 rpm for 10 min and the plasma was kept in a tube to be used. Plasma viscosity was measured in a capillary viscometer (Model LBY-N6B, Precil Company, Beijing, China).[15]

Plasma biochemical analysis

Plasma AST and LDH activities were determined spectrophotometrically with kits from Zhongsheng Beikong Bio-technology and Science Inc., Peking, China.

Statistical analysis

Data were demonstrated as mean ± S.D. Diffferences between groups were evaluated using ANOVA with Dunnett test. The statistical differences were considered significant when $P < 0.05$. Statistical analysis was performed using the Statistical Analysis System (SAS).

Results

Animals in different groups had similar weights and volumes of blood loss. This information has been summarized in Table 1.