<Original Article>

Effects of Temporary Blood Administration on Dysoxia and Survival in a Rat Uncontrolled Hemorrhagic Shock Model

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ABSTRACT

Objective: To test whether temporary blood administration improves dysoxia and thereby prolong survival in an ongoing uncontrolled hemorrhagic shock model.

Methods: Light anesthesia was induced with sevoflurane in 18 rats, and spontaneous breathing was maintained. Uncontrolled hemorrhagic shock (UHS) was induced by withdrawal of blood at 2.5 mL / 100 g over a 15-min period, followed by 75 % tail amputation. At 10 min after tail cutting, rats were randomized into three groups (n = 6 each) and received the following resuscitation regimen for 20 min: Group 1 (9 mL shed blood) vs. Group 2 (a mixture of 4.5 mL NS solution and 4.5 mL shed blood) vs. Group 3 (9 mL NS solution). The rats were then monitored for oxygen metabolisms and survival.

Results: The regimen of Group 1 vs. Group 3 prompted a surge in blood pressure and improved oxygen metabolic indices. Four rats in Group 1, three rats in Group 2, and none in Group 3 survived up to 180 min (p = 0.052; Group 1 vs. Group 3). Additional blood loss from the tail stump did not differ significantly among the groups.

Conclusions: In a model of UHS in rats, a temporary resuscitation regimen of whole blood administration compared with NS solution improved tissue dysoxia and possibly survival.

INTRODUCTION

The hallmark of the pathophysiology of hemorrhagic shock (HS) is decreased organ perfusion, leading to the marked and widespread impairment of tissue oxygenation. The standard approach for trauma patients with HS focuses on early volume resuscitation. This includes a bolus of the initial infusion of 1 L of isotonic crystalloid solution to achieve an appropriate response in adult trauma patients [1]. The goal of this treatment is to restore intravascular volume and vital signs as quickly as possible to maintain vital organ perfusion and tissue oxygenation. This approach may be adequate for patients who experience only mild to moderate hemorrhagic insults, but in cases of ongoing uncontrolled life-threatening hemorrhage, attempts to restore blood pressure using crystalloid solution alone may actually increase blood loss, worsen oxygen delivery, and increase mortality [2,3]. Blood administration could provide a better strategy to improve tissue perfusion and oxygenation [4,5]. However, the effects of blood administration on oxygen metabolisms and

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tissue dysoxia in an ongoing uncontrolled life-threatening hemorrhage remain unclear.

We hypothesized that temporary blood administration alone might improve oxygen metabolisms and dysoxia and thereby prolong survival during uncontrolled HS (UHS). To test this hypothesis, a prospective, randomized animal study was conducted using our previously developed, clinically relevant UHS model in rats [5–7].

METHODS

Experiments were started using 18 male albino Sprague–Dawley rats (mean body weight, 393 ± 15 g; range, 365–420 g) after receiving approval for the study protocols by our Institutional Animal Care Committee.

1 Preparations and baseline measurements

Rats were provided with ad libitum access to food and water before the experiment. Each rat was anesthetized with nitrous oxide/oxygen (N_2O:O_2, 50 % : 50 %) plus 5 % sevoflurane in a jar. After weighing, animals were placed in a supine position and allowed to breathe spontaneously via a cone mask under 50 % O_2, 50 % N_2O and sevoflurane anesthesia (2–3%) throughout the preparation. Through an incision at the left groin, a polyethylene catheter (PE 50) was inserted approximately 2 cm into the iliac artery through the femoral artery for pressure monitoring and blood sampling. A similar catheter (PE 60) was inserted into the left femoral vein and advanced 6 cm into the inferior vena cava (IVC) for blood withdrawal. Each catheter was connected to a pressure transducer (MLT0670; ADInstrument, New South Wales, Australia). Electrocardiography, heart rate, arterial blood pressure, central venous pressure, and rectal temperature were continuously recorded using a polygraph (Model Power lab 4/26; ADInstrument). Rectal temperature was maintained at 37.5 ± 0.5 ℃ with a heating pad and lamp during the experiment. The probe (Type UOE-04T; Unique-Medical, Osaka, Japan) for measuring tissue PO_2 (P_TO_2) was inserted 5 mm into the liver via small, sterile abdominal incision in the right hypochondrium, using a 24-gauge angiocatheter sheath as a guide. Tissue temperature was also measured with a thermocoupled microprobe for temperature compensation at 37 ℃ for the PO_2 value. P_TO_2 and temperature were displayed continuously with a dedicated monitor (Type POG-3011; Unique-Medical). These preparative processes were completed within 60 min.

After the above preparation, a period of 10 min was allowed for the animals to reach a steady state, and then baseline data were recorded.

2 UHS and resuscitation

At 5 min after performing the baseline measurements, volume-controlled hemorrhage was initiated by blood withdrawal (2.5 mL / 100 g; approximately 35 % of the circulating blood volume) at a steady rate for 15 min through the vena cava catheter (**Fig. 1**). Sevoflurane levels were titrated at 1–2 % during the volume-controlled hemorrhage and fixed at 2 % thereafter. Removed blood was preserved for the following resuscitation in heparinized syringes (15 U heparin for 9 mL blood) at room temperature. Start of hemorrhage was designated as HS time zero (HST_0). At 5 min after the end of vol-

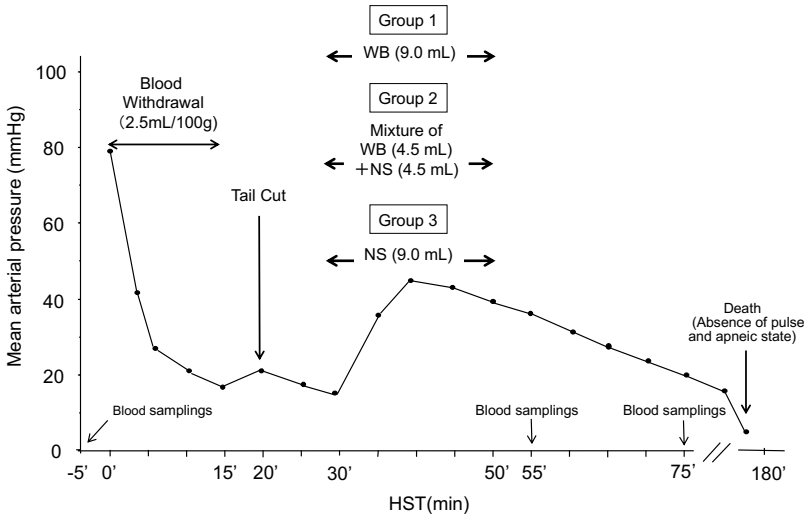

Fig. 1 Experimental protocol. Uncontrolled hemorrhagic shock was induced by blood withdrawal of blood at 2.5 mL/100 g over a 15-min period, followed by tail amputation at hemorrhagic shock time (HST) 20 min. At HST 30 min, rats were randomized into three groups (n = 6 each) and received the following resuscitation regimen until HST 50 min: Group 1 (9 mL whole blood (WB)); Group 2 (a mixture of 4.5 mL normal saline (NS) solution and 4.5 mL WB); Group 3 (9 mL NS solution). All animals were observed until death or for a maximum of 180 min. Blood samplings were performed at HST –5 min, 55 min and 75 min.

ume-controlled hemorrhage (HST_{20}), UHS was initiated by amputation of the tail at 75 % of the tail length (measured from the tip of the tail). All blood shed from the tail was collected, measured, and discarded. At 10 min after the tail cut (HST_{30}), 18 animals were randomly assigned to one of three resuscitation regimens ($n = 6$ each): an infusion of 9 mL shed blood (Group 1) vs. a mixture of 4.5 mL normal saline (NS) solution containing 7.5 U heparin and 4.5 mL shed blood (Group 2) vs. 9 mL NS solution containing 15 U heparin (Group 3). Treatments were started at a rate of 27 mL/h (9 mL for 20 min) by using an infusion pump (Model SPS-1; AS ONE, Osaka, Japan) for all three groups.

All animals were observed until death or for a maximum of 180 min. Survivors at HST_{180} were euthanized by sevoflurane overdose. Death of animals before HST_{180} was defined by absence of pulse and apneic status. Survival time was defined as time from HST_0 to either death or HST_{180}.

3 Blood samplings

Arterial and venous blood samples (0.2 mL each) were drawn to monitor pH, PCO_2, PO_2, base excess, hematocrit and O_2 content (measured using an ABL 80 blood gas analyzer; Radiometer, Copenhagen, Demark) at baseline (HST_{-5}), 5 min (HST_{55}) and 25 min (HST_{75}) after the resuscitation regimen. These values were determined at 37 ℃ without correction for body temperature. The O_2 utilization coefficient, i.e., the O_2 extraction ratio, was calculated as follows: 1 – IVC venous O_2 content / arterial O_2 content. Blood sampling was performed to avoid significant additional effects on mean arterial pressure (MAP) during profound hypotension.

4 Statistical analysis

All data are expressed as mean ± standard deviation (SD). Continuous variables were compared using two-way analysis of variance (ANOVA) for repeated measures among all groups within HST_{90}. If significant differences were identified, ANOVA with Scheffe's procedure for post hoc comparisons was used to evaluate differences at equivalent time points. The primary endpoint was survival rate, which was determined using the Kaplan–Meier method and Mantel–Cox's log-rank test. Values of $p < 0.05$ were considered statistically significant. A group size of 6 rats were allowed detection of a 70 % difference in survival rates between the blood administration group and the NS group with a type I error of 0.05 and a type II error of 0.8.

RESULTS

1 Hemodynamics and Tissue PO_2

Data are shown for Groups 1–3 up to HST_{90} (**Figs. 2–5**). In all rats, MAP decreased in all rats to 30 ± 6 mmHg (range, 25–40 mmHg) at the end of initial volume-controlled hemorrhage (HST_{15}) from the baseline value of 98 ± 17 mmHg (range, 78–130 mmHg) (**Fig. 2**). Group 1 exhibited significant transient increases in MAP after blood administration between HST_{40} and HST_{55} ($p < 0.05$ vs. Group 3 at HST_{40} and HST_{50}, and $p < 0.05$ vs. Groups 2 and 3 at HST_{45} and HST_{55}). Slight increases in MAP were observed in Groups 2 and 3, without significant differences between groups.

Bradycardia usually appeared in the initial volume-control hemorrhage and was preceded by an increase of heart rate in a rat HS model (8). Mean heart rate decreased to 236 ± 78 beats/min (range, 170–386 beats/min) from a baseline of 332 ± 45 beats/min (range, 288–460 beats/min) in all rats during the initial 15-min hemorrhage (**Fig. 3**). The heart rate in all groups then increased steadily until death. Heart rate significantly increased between HST_{65} and HST_{75} in Group 3 compared with Groups 1 and 2 ($p < 0.05$).

Liver PTO_2 decreased from a baseline of 51 ± 26 torr (range, 16–104 torr) to 32 ± 17 torr (range, 14–62 torr) at the end of the initial hemorrhage (HST_{15}) in all groups (**Fig. 4**). Mean PTO_2 tended to increase in Groups 1 and 2 compared with Group 3, but the levels were not significant.

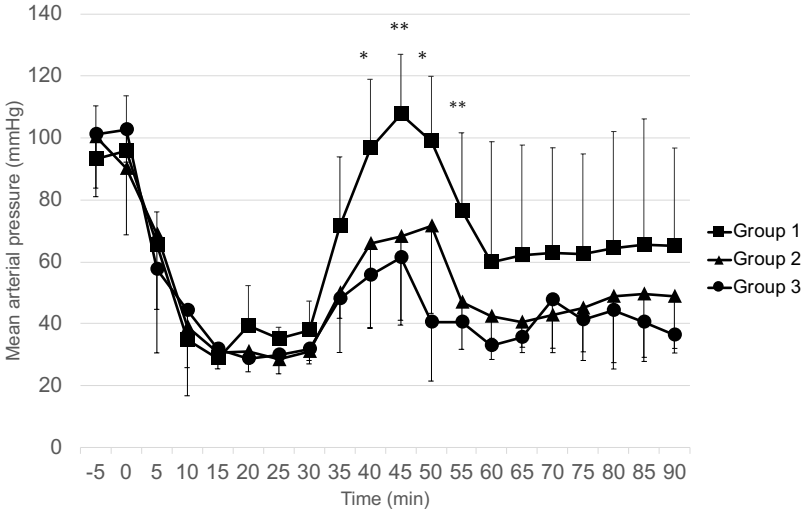

Fig. 2 Changes in mean arterial pressure over time during blood withdrawal of 2.5 ml/100 g over 15 min after tail amputation and uncontrolled hemorrhagic shock. Values represent mean ± standard deviation. Group 1 (squares), whole blood administration; Group 2 (triangles), half-diluted blood administration; Group 3 (circles), normal saline solution administration. *$p < 0.05$ vs. Group 3 and ** $p < 0.05$ vs. Groups 2 and 3.

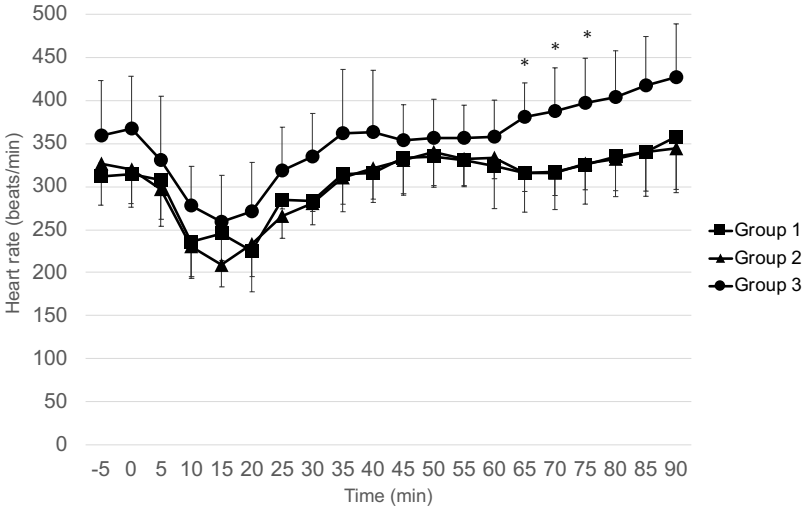

Fig. 3 Changes in heart rates during blood withdrawal of 2.5 ml/100 g over 15 min after tail amputation and uncontrolled hemorrhagic shock. Values represent mean ± standard deviation. Group 1 (squares), whole blood administration; Group 2 (triangles), half-diluted blood administration; Group 3 (circles), normal saline solution administration. * $p < 0.05$ vs. Groups 1 and 2.

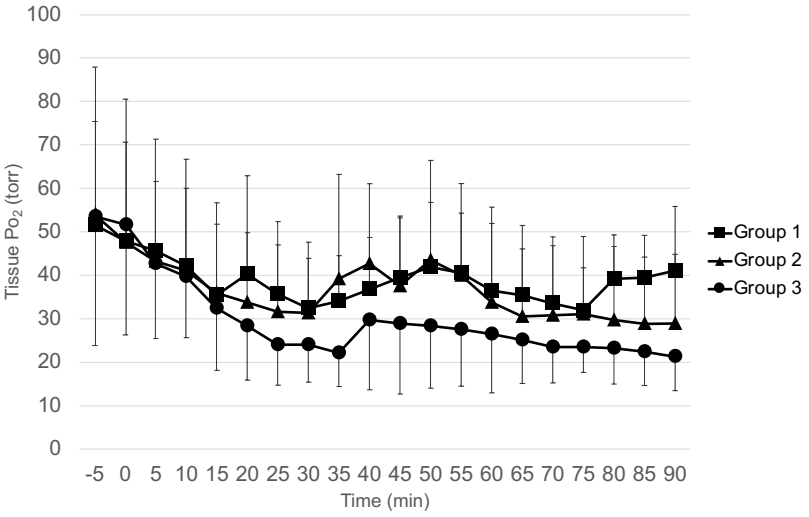

Fig. 4 Changes in liver tissue PO_2 during blood withdrawal of 2.5 ml/100 g over 15 min after tail amputation and uncontrolled hemorrhagic shock. Values represent mean ± standard deviation. Group 1 (squares), whole blood administration; Group 2 (triangles), half-diluted blood administration; Group 3 (circles), normal saline solution administration. No significant difference was seen among groups.

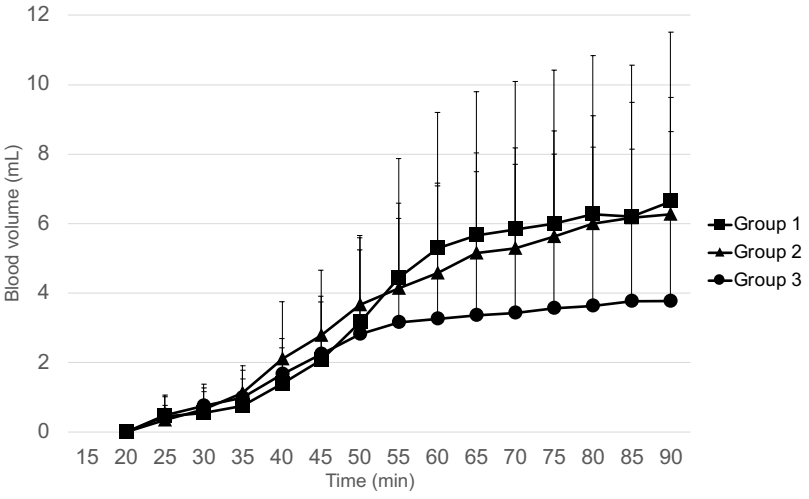

Fig. 5 Changes in blood loss from the tail stump after tail amputation and uncontrolled hemorrhagic shock. Values represent mean ± standard deviation. Group 1 (squares), whole blood administration; Group 2 (triangles), half-diluted blood administration; Group 3 (circles), normal saline solution administration. No significant difference was seen among groups.

2 Blood Loss from The Tail Wound

Additional blood loss from the tail wound steadily increased in all groups after resuscitation and then remained at around 6 mL in Groups 1 and 2 and at around 3.5 mL in Group 3 after HST_{75} (**Fig. 5**). Blood loss tended to be greater in Groups 1and 2 compared with Group 3, but no significant differences were identified among groups.

Table 1 Body weight, arterial blood gases, hematocrit, and O_2 extraction at baseline

	Group 1 (n = 6)	Group 2 (n = 6)	Group 3 (n = 6)
Body weight (g)	382 ± 18	395 ± 8	400 ± 12
PO_2 (torr)	220 ± 41	218 ± 28	186 ± 41
PCO_2 (torr)	34 ± 7	34 ± 4	34 ± 6
pH	7.42 ± 0.03	7.44 ± 0.04	7.43 ± 0.03
Base excess (mmol / L)	–1.9 ± 3.1	–0.3 ± 3.5	–1.4 ± 2.7
Hematocrit (%)	35 ± 2	35 ± 4	35 ± 2
O_2 extraction	0.12 ± 0.07	0.15 ± 0.06	0.23 ± 0.13

Values are expressed as mean ± standard deviation.

Table 2 Arterial blood gases, hematocrit, and O_2 extraction at HST_{55}

	Group 1 (n = 6)	Group 2 (n = 6)	Group 3 (n = 6)
PO_2 (torr)	195 ± 52	189 ± 50	174 ± 58
PCO_2 (torr)	37 ± 8	34 ± 5	27 ± 7
pH	7.35 ± 0.09	7.35 ± 0.07	7.34 ± 0.05
Base excess (mmol / L)	–4.7 ± 3.3	–6.6 ± 2.3	–8.9 ± 1.8*
Hematocrit (%)	30 ± 4	25 ± 4	19 ± 4*
O_2 extraction	0.2 ± 0.8	0.36 ± 1.2	0.49 ± 0.16*

Values are expressed as mean ± standard deviation.
* $p < 0.05$ vs. Group 1

Table 3 Arterial blood gases, hematocrit and additional blood loss from the tail at HST_{75}

	Group 1 (n = 6)	Group 2 (n = 6)	Group 3 (n = 5)
PO_2 (torr)	189 ± 63	226 ± 20	204 ± 20
PCO_2 (torr)	35 ± 4	34 ± 5	29 ± 4
pH	7.35 ± 0.05	7.31 ± 0.10	7.34 ± 0.04
Base excess (mmol / L)	–3.5 ± 1.3	–9.4 ± 4.5	–9.9 ± 1.5
Hematocrit (%)	28 ± 6	23 ± 4	21 ± 3
O_2 extraction	0.38 ± 0.19	0.54 ± 0.14	0.56 ± 0.13

Values are expressed as mean ± standard deviation.

3 Body Weight and Physiological Variables

No significant differences were observed in baseline physiological variables among groups (**Table 1**). In Groups 2 and 3, base excess values gradually decreased and O_2 extraction values increased from baseline toward HST_{55} and HST_{75} (**Tables 2 and 3**). At HST_{55}, Group 3 showed significantly lower base excess and hematocrit and higher O_2 extraction compared with Group 1 ($p < 0.05$) (**Table 2**). No significant differences were seen among groups at HST_{75} (**Table 3**).

4 Survival

No rats in Group 3 survived until HST_{180} (**Fig. 6**). On the other hand, four rats in Group 1 and three in Group 2 survived up to HST_{180}. According to life table analysis (Kaplan–Meier curves), the survival rate in Group 3 tended to be differed from those in Group 1 ($p = 0.052$) and Group 2 ($p = 0.11$).

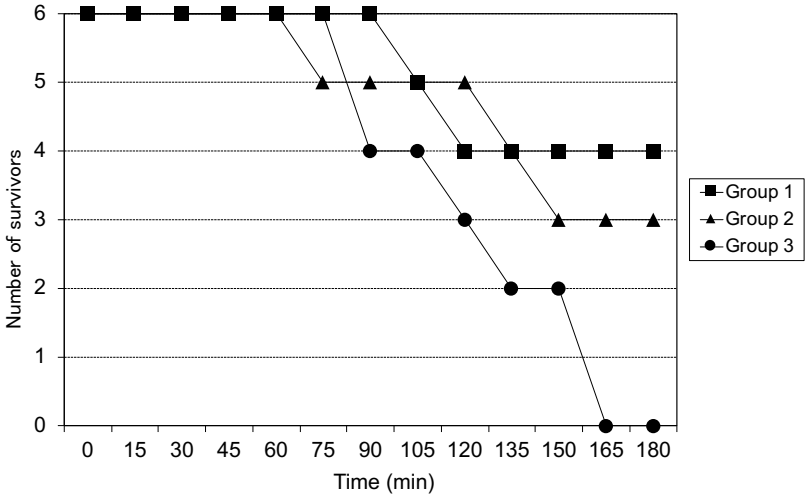

Fig. 6 Life table analysis throughout the experiments. Group 1 (squares), whole blood administration; Group 2 (triangles), half-diluted blood administration; Group 3 (circles), normal saline solution administration. Cumulative survival (Kaplan–Meier) showed a better survival rate in Group 1 than in Group 3 ($p = 0.052$).

DISCUSSION

The goal of resuscitation is to restore organ perfusion and tissue oxygenation, which is accomplished by administering crystalloid solution and blood products to replace lost intravascular volume. This study demonstrated that temporary whole blood administration against the same volume of NS solution possessed a superior ability to improve hypotension, acidosis, O_2 extraction and probably survival during uncontrolled life-threatening HS, but a half-diluted blood product did not show such apparent superiority. Greater amounts of blood transfusion achieved better oxygen delivery, and thereby a trend of better survival in this study. The advantages of blood transfusion for the treatment of HS are well documented. In a rat model of UHS, a resuscitation regimen with crystalloid agent alone resulted in greater deterioration of hemodynamics, metabolic indices, and survival compared with a combination of blood and crystalloid solution [5]. Similarly, in a study of UHS in rats, resuscitation using a combination of blood and crystalloid solution significantly decreased fluid requirement and improved lactic acidosis as compared with crystalloid solution alone [9].

In HS, the body compensates for the reduced blood volume by redistributing blood flow and increasing the inotropic state of the heart, thus preserving cardiac output. As intravascular volume drops further, vasoconstricted tissue reaches the limits of tolerance and worsens tissue perfusion. A prompt increase in MAP, improved base excess, and O_2 extraction, as well as a trend toward improved PTO_2, were seen in Group 1. These events are likely to have been associated with improvements in tissue perfusion.

Deliberate "hypotensive resuscitation" raised from completely limiting fluid resuscitation is recommended until definitive control of bleeding can be achieved because increased blood pressure causes more bleeding and increases mortality [2,10,11]. In a case of continued attempts at blood pressure restoration, increasing blood loss might worsen oxygen delivery and increase mortality; however the surge of MAP was occurred in the limited period and might suppress bleeding in this study. Half-diluted blood administration of Group 2 resulted in similar blood loss in Group 1, but MAP did not surge as much. The balance between increased blood pressure and blood loss might be the key to improve tissue dysoxia and improve survival.

Possible limitations regarding the experimental protocol in this study should be considered. First, whole blood transfusion was given. Whole blood transfusion has not been commonly used in the civilian medical community because component therapy has proven readily available and safe, although military experience suggests a place for whole blood [2,12]. Whole blood transfusion would preserve a better coagulation function, although additional blood loss did not significantly differ among groups. Therefore, the coagulation function during resuscitation should be the subject of a further study. Second, heparin was used, which might have affected bleeding. However, the dose was relatively small and identical across groups. Third, the model used in this study did not include intensive care that might have affected the outcome among the rats. It would be difficult to apply intensive care in the rat a HS model. We did not use a tracheal tube because this would require deeper anesthesia and cause air-way and secretion problems. Fourth, the O_2 utilization coefficient would not mean "systematic", because the IVC vein substituted for the central vein for the O_2 extraction estimation. It was due to a technical difficulty to insert a cannula to the right atrium or pulmonary artery.

We concluded that the temporary resuscitation regimen of whole blood administration improved hemodynamics, oxygen metabolic indices, and seemed to have a better effect on survival compared with NS solution in a rat model of UHS.

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DISCLOSURES

1 Approval of the research protocol

This study was approved by the Animal Ethics Committee of Osaka Medical College, (2019-073, date of approval 2019-6-7) and the experimental procedures followed the Science Council of Japan guidelines for the proper conduct of animal experiments.

2 Animal Studies

All animal experiments were conducted following national guidelines and relevant national laws for the protection of animals.

3 Conflicts of Interest

None

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