Fluid Resuscitation Does Not Improve Renal Oxygenation during Hemorrhagic Shock in Rats

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ABSTRACT

Background: The resuscitation strategy for hemorrhagic shock remains controversial, with the kidney being especially prone to hypoxia.

Methods: The authors used a three-phase hemorrhagic shock model to investigate the effects of fluid resuscitation on renal oxygenation. After a 1-h shock phase, rats were randomized into four groups to receive either normal saline or hypertonic saline targeting a mean arterial pressure (MAP) of either 40 or 80 mmHg. After such resuscitation, rats were transfused with the shed blood. Renal macro- and microcirculation were monitored with cortical and outer-medullary microvascular oxygen pressure, renal oxygen delivery, and renal oxygen consumption measured using oxygen-dependent quenching of phosphorescence.

Results: Hemorrhagic shock was characterized by a drop of aortic blood flow, MAP, renal blood flow, renal oxygen delivery, renal oxygen consumption, and renal microvascular PO_2. During the fluid resuscitation phase, normal saline targeting a MAP = 80 mmHg was the sole strategy able to restore aortic blood flow, renal blood flow, and renal oxygen consumption, although without improving renal oxygen delivery. However, none of the strategies using either normal saline or hypertonic saline or targeting a high MAP could restore the renal microvascular PO_2. Blood transfusion increased microvascular PO_2 but was unable to totally restore renal microvascular oxygenation to baseline values.

Conclusions: This experimental rat study shows that (1) high MAP-directed fluid resuscitation (80 mmHg) does not lead to higher renal microvascular PO_2 compared with fluid resuscitation targeted to MAP (40 mmHg); (2) hypertonic saline is not superior to normal saline regarding renal oxygenation; and (3) decreased renal oxygenation persists after blood transfusion.

What We Already Know about This Topic

- ❖ The kidney is especially susceptible to hypoperfusion during hemorrhagic shock, which can lead to renal failure
- ❖ Hypertonic saline treatment of shock improves perfusion in other organs

What This Article Tells Us That Is New

- ❖ In rats with hemorrhagic shock, correcting mean arterial pressure of 80 mmHg with normal or hypertonic saline did not increase renal oxygenation more than 40 mmHg
- ❖ Rapidly correcting arterial pressure in shock may not guarantee providing sufficient oxygen to the kidneys

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HEMORRHAGIC shock remains a major cause of death among severe trauma patients. Mortality is directly linked to massive blood loss or occurs indirectly due to secondary multiple organ failure.[1] Among these organ failures, acute renal failure is frequent,[2] and it has been shown to be an independent factor associated with mortality in critically ill patients.[3,4] Current guidelines for presurgical treatment of hemorrhagic shock recommend rapid volume resuscitation to restore the intravascular volume.[5] However, this practice is controversial because aggressive restoration of intravascular volume with a rapid increase in blood pressure before controlling the source of hemorrhage can lead to increased mortality.[6] Consequently, "controlled" fluid resuscitation with small volume before stopping the bleeding has been advocated.[6,7] With such a strategy, episodes of hypoperfusion are likely to occur, which may contribute to secondary multiple organ failure.

In this hemorrhagic shock condition, the kidney can suffer the most, as the microvascular PO_2 starts to drop at a much earlier stage than other organs such as the gut or heart.[8] Altogether, these properties put the kidney at risk of ischemia at the very early stage of hemodilution.[9,10] Furthermore, resuscitation with hypertonic saline (HTS) was shown to im-

prove tissue perfusion in other organs,[11,12] but its effects on renal microcirculation have not been studied thus far.

The purpose of this experimental rat study was to investigate the renal microvascular oxygenation in response to hemorrhagic shock and fluid resuscitation. We used a controlled hemorrhagic shock model to test the following hypothesis: (1) whether fluid resuscitation with saline targeting a fixed low or high blood pressure is effective in restoring renal hypoxemia after hemorrhage, (2) whether resuscitation with HTS improves renal microvascular oxygenation at lower or higher low mean arterial blood pressure (MAP) compared with normal saline (NS), and (3) that blood transfusion is superior to fluid resuscitation in restoring renal oxygenation after hemorrhage. To this end, we studied the effect of fluid resuscitation giving normal (0.9% NaCl) or hypertonic (7.5% NaCl) saline targeting either normal (MAP = 80 mmHg) or low (MAP = 40 mmHg) arterial blood pressure in a rat model of controlled hemorrhagic shock. This was followed by blood transfusion to investigate to what extent improvement could be achieved by administration of an oxygen carrying resuscitation fluid.

Materials and Methods

Experimental Procedures

All experiments in this study were approved and reviewed by the Animal Research Committee of the Academic Medical Center at the University of Amsterdam, The Netherlands. Care and handling of the animals were in accordance with the guidelines for Institutional and Animal Care and Use Committees. Experiments were performed on Sprague-Dawley rats (Harlan, Horst, The Netherlands) with a body weight of 334 ± 22 g. The rats were anesthetized with an intraperitoneal injection of a mixture of 100 mg/kg of ketamine (Nimatek®; Eurovet, Bladel, The Netherlands), 1.5 mg/kg of diazepam (Centrafarm, Etten-Leur, The Netherlands), and 3 μg/kg of sufentanil (Janssen-Cilag, Tilburg, The Netherlands). After tracheotomy, the animals were mechanically ventilated with a FiO_2 of 0.4. The body temperature was maintained at 37 ± 0.5°C during the entire experiment by external warming. The ventilator settings were adjusted to maintain end-tidal Pco_2 between 30 and 35 mmHg and arterial PCO_2 between 35 and 40 mmHg. A catheter in the right carotid artery was connected to a pressure transducer to monitor arterial blood pressure and heart rate. The right jugular vein was cannulated for continuous infusion of Ringer lactate (Baxter, Utrecht, The Netherlands) at a rate of 15 ml $\cdot$ kg$^{-1} \cdot$ h^{-1}. The right femoral artery was cannulated for blood shedding and the right femoral vein for fluid resuscitation. The left kidney was exposed, decapsulated, and immobilized in a Lucite kidney cup (K. Effenberger, Pfaffingen, Germany) by an approximately 4-cm incision in the left flank. Renal vessels were carefully separated under preservation of nerves and adrenal gland. A perivascular ultrasonic transient time flow probe was placed around the abdominal aorta (type 2.0 RB; Transonic Systems, Inc., Ithaca, NY) and the left renal artery (type 0.7 RB; Transonic Systems, Inc.) and connected to a dual flow meter (T206; Transonic Systems, Inc.) to continuously measure descending aortic blood flow as a surrogate of cardiac output and renal blood flow (RBF). The left ureter was isolated, ligated, and cannulated with a polyethylene catheter for urine collection. A small piece of aluminum foil was placed on the dorsal site of the renal vein to prevent the contribution of underlying tissue to the phosphorescence signal in the venous Po_2 measurement (described in the "Measurement of Renal Microvascular Oxygenation and Renal Venous Po_2" section).

After the surgical protocol (~60 min), one optical fiber was placed 1 mm above the decapsulated kidney and another optical fiber 1 mm above the renal vein to measure oxygenation by using a phosphorescence lifetime technique.[13] Oxyphor G2 (a two-layer glutamate dendrimer of tetra-(4-carboxy-phenyl) benzoporphyrin; Oxygen Enterprises, Ltd., Philadelphia, PA) was subsequently infused (6 mg/kg) intravenously in 5 min, followed by 30 min of stabilization time.[14,15] A second infusion of Oxyphor G2 was given at t_{120} (2 mg/kg). The surgical field was covered with a humidified gauze compress throughout the entire experiment to prevent drying of the exposed tissue. A short description of the phosphorescence quenching method is given below, and an extensive description of the technology can be found elsewhere.[14]

Arterial blood samples (0.5 ml) were taken from the carotid artery at six time points: (1) baseline (t_0), (2) after hemorrhagic shock (t_{60}), (3) 15 min after starting resuscitation (t_{75}), (4) before blood transfusion (t_{120}), (5) 15 min after starting blood transfusion (t_{135}), and (6) before sacrifice (t_{180}). The blood samples were replaced by the same volume of HES130/0.4 (Voluven®, 6% HES 130/0.4; Fresenius Kabi Nederland B.V., Schelle, Belgium). The samples were allowed for the determination of blood gas parameters (ABL505 blood gas analyzer; Radiometer, Copenhagen, Denmark), which were given as the hemoglobin concentration and hemoglobin oxygen saturation. Renal oxygen delivery was calculated as DO_{2ren} (milliliter per minute) = RBF × arterial oxygen content (1.31 × hemoglobin × SaO_2) + (0.003 × PaO_2), where SaO_2 is arterial oxygen saturation, and PaO_2 is arterial partial pressure of oxygen. Renal oxygen consumption was calculated as VO_{2ren} (milliliter per minute per gram) = RBF × (arterial − renal venous oxygen content difference). Renal venous oxygen content was calculated as (1.31 × hemoglobin × $S_{rv}O_2$) + (0.003 × $P_{rv}O_2$). The renal oxygen extraction ratio was calculated as O_2ER_{ren} (%) = VO_{2ren}/DO_{2ren} × 100. An estimation of the renal vascular resistance was made as renal vascular resistance (dynes sec cm^{-5}) = (MAP/RBF) × 100 × 80. Creatinine clearance (milliliter per minute) was assessed as an index of the glomerular filtration rate. Calculations of the clearance were done using the standard formula: Creatinine clearance = (U_{crea} × V)/P_{crea}, where U_{crea} is the concentration of creatinine in urine, V is the urine volume per unit time, and P_{crea} is the

concentration of creatinine in plasma. All urine samples were analyzed for sodium (Na^+) concentration. The renal energy efficiency for sodium transport (VO_{2ren}/TNa^+) was assessed using the ratio of the total amount of VO_{2ren} over the total amount of sodium reabsorbed (TNa^+, millimolar per minute). The osmolarity of the plasma was determined using the freezing point method by means of an osmotic pressure meter (OSMOSTATION, OM-6050; Arkray Europe, Amstelveen, The Netherlands) from a sample taken at the end of the experiment. Plasma lactate level was measured by the enzymatic colorimetric methods, using the Roche Modular P800 automatic analyzer (Roche Diagnostics, Basel, Switzerland), from samples taken at t_{120} (end of fluid resuscitation) and t_{180} (end of experiment) and centrifuged in sodium fluoride tubes. Interleukin (IL)-6, IL-10, and tumor necrosis factor-α in plasma (t_{180}) were determined using rat single-plex bead kits (Invitrogen, Breda, The Netherlands) and read with a BioRad Bioplex 100 (Biorad, Hercules, CA). The limits of detection for IL-6, IL-10, and tumor necrosis factor-α were 1.0, 6.6, and 1.2 pg/ml, respectively, with coefficients of variation between 5.0 and 6.5%. The limit of detection for lactate was 0.2 mM, and the coefficient of variation was between 2.0 and 4.2%.

Measurement of Renal Microvascular Oxygenation and Renal Venous Po_2

Renal microvascular Po_2 (μPo_2) and renal venous Po_2 ($P_{rv}O_2$) were measured by oxygen-dependent quenching of phosphorescence lifetimes of the systemically infused albumin-targeted (and therefore circulation confined) phosphorescent dye Oxyphor G2.[14–18] Oxyphor G2 (a two-layer glutamate dendrimer of tetra-(4-carboxy-phenyl) benzoporphyrin) has two excitation peaks ($\lambda_{excitation1} = 440$ nm and $\lambda_{excitation2} = 632$ nm) and one emission peak ($\lambda_{emission} = 800$ nm).[18] These optical properties allow (near) for simultaneous lifetime measurements in the microcirculation of the kidney cortex and the outer medulla due to different optical penetration depths of the excitation light. For the measurement of renal venous Po_2 ($P_{rv}O_2$) and $S_{rv}O_2$, a monowavelength frequency-domain phosphorimeter was used. Oxygen measurements based on phosphorescence lifetime techniques rely on the fact that phosphorescence can be quenched by oxygen, resulting in shortening of the lifetime. A linear relationship between reciprocal phosphorescence lifetime and oxygen tension (given by the Stern-Volmer relation) allows for the quantitative measurement of μPo_2.

Experimental Protocol

After stabilization, the animals were bled by the left femoral artery catheter at a rate of 1 ml/min by using a syringe pump (Harvard 33 syringe pump; Harvard Apparatus, South Natick, MA) until reaching an MAP of 30 mmHg. Coagulation of the shed blood was prevented by adding 200 UI of heparin in the syringe. This pressure was maintained for 1 h by reinfusing or withdrawing blood. At the end of this phase, the animals were randomized into four groups for the resus-

citation phase: fluid resuscitation for 60 min with NaCl 0.9% and a targeted MAP = 40 mmHg (group NS40, n = 6), with NaCl 0.9% and a targeted MAP = 80 mmHg (group NS80, n = 6), with NaCl 7.5% and targeted MAP = 40 mmHg (group HS40, n = 6), or with NaCl 7.5% and targeted MAP = 80 mmHg (group HS80, n = 6). At the end of the resuscitation phase (t_{120}), the shed blood was transfused through the right femoral vein catheter at a rate of 0.5 ml/min. The fifth group formed the time-control group (sham-operated rats, n = 3). The experiment was terminated by infusion of 1 ml of 3 M potassium chloride, and the kidney was removed and weighed.

Statistical Analysis

Values are reported as the mean ± SD. The decay curves of phosphorescence intensity were analyzed using a software programmed in Labview 6.1 (National Instruments, Austin, TX). Statistical analysis was performed using GraphPad Prism version 4.0 for Windows (GraphPad Software, San Diego, CA). ANOVA for repeated measurements were used for intergroup and intragroup comparisons, using *post hoc* analyses with the Bonferroni post test when $P < 0.05$. We used one-way ANOVA to compare the renal oxygenation variables at the three different periods separately (namely shock, resuscitation, and transfusion). The P values were two tailed. For all analyses, $P < 0.05$ was considered significant.

Results

Fluid and Electrolyte Balance

The volume of the shed blood during hemorrhage was 9.4 ± 1 ml without differences between groups. The NS40 and NS80 groups required 6.6 ± 3.4 ml and 49 ± 6.9 ml of NaCl 0.9%, respectively ($P < 0.05$), whereas the HS40 and HS80 groups received 2.6 ± 1 ml and 6.9 ± 2.5 ml of NaCl 7.5%, respectively ($P < 0.05$). At the end of the experiment, rats had gained 4.6 ± 1%, 7.8 ± 2%, 1.6 ± 1%, and 0.7 ± 1% of body weight from their preanesthetic weight in the NS40, NS80, HS40, and HS80 groups, respectively ($P < 0.05$). The plasma osmolality at the end of the experiment was 305 ± 4 mOsm/kg in the NS40 group, 297 ± 2 mOsm/kg in the NS80 group, 334 ± 4 mOsm/kg in the HS40 group ($P = 0.009$ *vs.* NS40), 370 ± 13 mOsm/kg in the HS80 group ($P = 0.034$ *vs.* NS80), and 303 ± 10 mOsm/kg in the control group. Plasma sodium concentrations are displayed in figure 1.

Renal Function, Serum Lactate, and Inflammatory Response

The NS80 group had a trend toward higher creatinine clearance at t_{180} (NS). Only the NS40 group had a significantly lower clearance compared with the control group (fig. 2).

After fluid resuscitation (t_{120}), serum lactate level was significantly lower in the NS80 group (fig. 3) compared with other groups ($P < 0.05$ *vs.* HS80, $P < 0.05$ *vs.* NS40 and
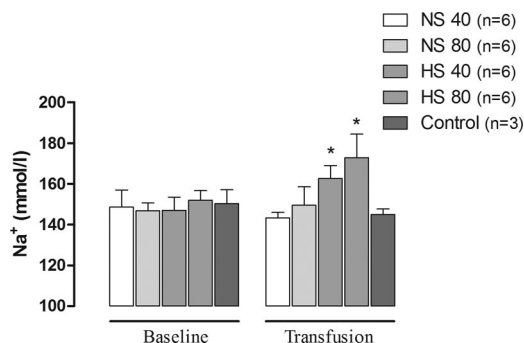
Fig. 1. Sodium concentrations in the five groups at baseline and after blood transfusion. * P < 0.05 *versus* control.

HS40). Blood transfusion was effective in correcting serum lactate levels in all groups. Hemorrhagic shock is known to induce an acute systemic inflammatory response.[19] We assessed plasma IL-6 and tumor necrosis factor-α as indicators of a proinflammatory response and IL-10 for that of an anti-inflammatory response at the end of the blood transfusion period. Compared with the control group, IL-6 increased markedly in all groups undergoing hemorrhagic shock, least in the NS80 group and maximally in the HS40 group ($P < 0.05$; fig. 4). These differences were not due to variations in hemoglobin dilution. Similar findings were obtained for tumor necrosis factor-α, but statistical significance was not reached. There was no significant difference between groups for IL-10.

Systemic and Renal Hemodynamics

Systemic and renal hemodynamic variables are presented in table 1. The baseline values measured in each group were found to be similar. The hemorrhagic shock induced a drop in MAP and aortic blood flow that was similar in all groups. During the fluid resuscitation phase, MAP and aortic blood flow were similar between the NS40 and HS40 groups but were lower in the HS80 group (NaCl 7.5% with targeted MAP = 80 mmHg) compared with the NS80 group (MAP 62 ± 8 mmHg *vs.* 78 ± 1 mmHg at t_{120}, NS). During hemorrhagic shock, RBF dropped to approximately 90% from baseline without significant differences between groups. During fluid resuscitation, RBF was found to be
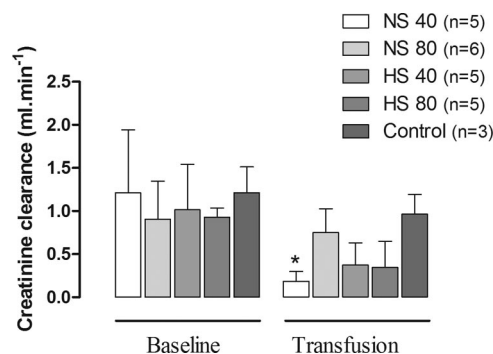

Fig. 2. Evolution of Clear$_{crea}$ (ml/min) between t_0 and t_{180} in the five groups. * P < 0.05 *versus* control.
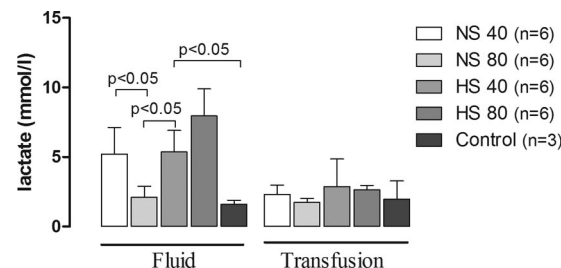

Fig. 3. Evolution of plasma lactate between t_{120} and t_{180} in the five groups.

similar between NS40, HS40, and HS80 groups. The RBF was restored to baseline values in the NS80 group at the end of the resuscitation phase and was significantly higher than in the three other groups ($P < 0.05$). In all groups, RBF was restored to approximately 70% of baseline values after blood transfusion.

Renal Oxygenation

Renal oxygenation variables are presented in figures 5 and 6. DO$_{2ren}$ and VO$_{2ren}$ dropped during the hemorrhagic phase in all groups. During the resuscitation phase, the increase in DO$_{2ren}$ was significantly higher in the NS80 group compared with the three other groups ($P < 0.05$), despite a lower hemoglobin concentration due to hemodilution (fig. 7). After blood transfusion, the DO$_{2ren}$ was found to be similar in all treatment groups, without reaching baseline values. During the fluid resuscitation phase, VO$_{2ren}$ increased significantly more in the NS80 group, when compared with the three other treatment groups ($P < 0.05$). After blood transfusion, VO$_{2ren}$ was found to be higher in the NS80 group, when compared with the HS80 group ($P < 0.05$). O$_2$ER was not found to be significantly different between groups over time. The VO$_2$/TNa$^+$ was found to be significantly higher only in the NS40 group (fig. 8).

Both CμPO$_2$ and MμPO$_2$ decreased during the hemorrhagic phase to approximately 20 and approximately 12 mmHg, respectively. During both the resuscitation and transfusion phases, CμPO$_2$ was found to be similar in all groups (fig. 5). The MμPO$_2$ was found to be significantly higher only in the NS80 group, when compared with the HS80 group ($P < 0.05$) during the resuscitation phase but did not differ between other groups. There was no significant difference in MμPO$_2$ between groups during the transfusion period.

Discussion

The key findings of this study are that (1) saline or hypersaline resuscitation targeting a low or high MAP is highly ineffective in correcting renal microvascular oxygenation in comparison with blood transfusion after hemorrhage and (2) even though blood transfusion after fluid resuscitation was much more effective in correcting renal hypoxemia, it did not fully restore μPO$_2$, despite restoring hemoglobin to baseline values. The current standard strategy in the hypotensive
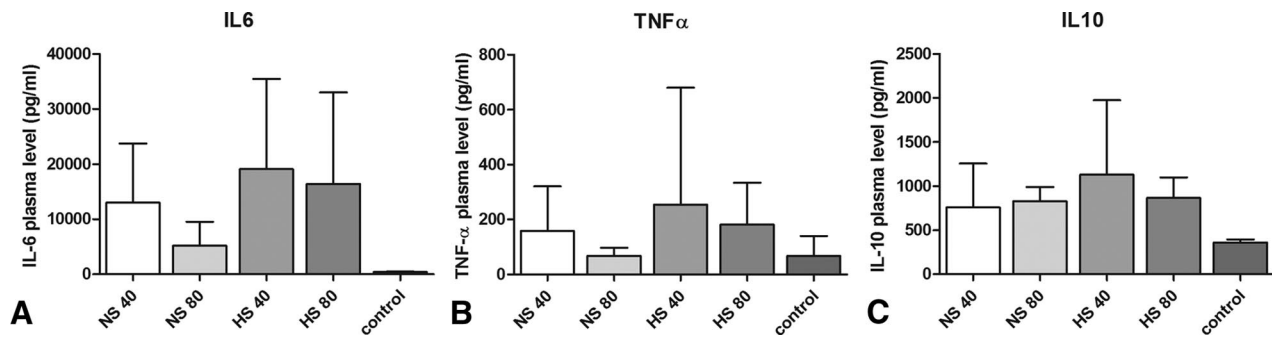
Fig. 4. Interleukin (IL)-6, IL-10, and tumor necrosis factor-α plasma level in the five groups at t_{180}.

trauma patient is to infuse large amounts of fluids as early as possible to restore macrohemodynamics. The primary goal of this treatment is to reestablish organ perfusion in an attempt to prevent secondary organ failure.[6] "Aggressive" resuscitation has raised safety concerns, because it has been proven to enhance bleeding volume,[20] inflammation, organ damage,[21] and mortality[22] before hemostasis has been secured. Therefore, the concept of limited resuscitation has emerged. Infusion of a small amount of fluid may turn out to be adequate in maintaining a sufficient level of organ perfu-

sion necessary to prevent secondary organ dysfunction, if lasting for a short period of time.[6] However, very little information is available concerning this assumption. The kidney is especially exposed to damage if a decrease in oxygen supply results in a decrease in tissue oxygenation to occur.[8] It is now becoming obvious that acute kidney injury increases morbidity and mortality among critically ill patients.[4,23] Furthermore, several animal studies have shown that acute kidney injury has a significant effect on the function of remote organs and may be implicated in the development of multiple

Table 1. Hemodynamic Parameters

Time Point	Baseline	Shock	Fluid	Transfusion
Mean arterial blood pressure (mmHg)				
Control (n = 3)	108 ± 22	108 ± 26	105 ± 16	105 ± 25
NS40 (n = 6)	123 ± 8	32 ± 6*	47 ± 5*	84 ± 23
NS80 (n = 6)	115 ± 4	30 ± 2*	78 ± 2†	77 ± 8*
HS40 (n = 6)	117 ± 17	33 ± 2*	44 ± 2*‡	76 ± 13*
HS80 (n = 6)	125 ± 10	34 ± 6*	62 ± 19*	84 ± 19
Heart rate per minute				
Control (n = 3)	365 ± 35	338 ± 36	358 ± 27	355 ± 45
NS40 (n = 6)	394 ± 56	250 ± 52	286 ± 43	348 ± 32
NS80 (n = 6)	388 ± 45	261 ± 33*	364 ± 41†	376 ± 18
HS40 (n = 6)	385 ± 62	287 ± 48	311 ± 45	355 ± 24
HS80 (n = 6)	398 ± 33	286 ± 54	300 ± 79	395 ± 36
Aortic blood flow (ml/min)				
Control (n = 3)	47 ± 12	44 ± 5	48 ± 10	46 ± 10
NS40 (n = 6)	38 ± 7	8 ± 1*	18 ± 12*	27 ± 8
NS80 (n = 6)	36 ± 10	8 ± 3*	48 ± 15†	33 ± 9
HS40 (n = 6)	40 ± 10	11 ± 6*	19 ± 7*‡	32 ± 8
HS80 (n = 6)	40 ± 10	7 ± 3	27 ± 9‡	32 ± 7
Renal blood flow (ml/min)				
Control (n = 3)	5.5 ± 0.3	5.7 ± 1.0	6.3 ± 0.5	6.2 ± 0.6
NS40 (n = 6)	5.6 ± 1.6	0.4 ± 0.2*	1.5 ± 0.3*	4.2 ± 2.1
NS80 (n = 6)	5.4 ± 1.3	0.4 ± 0.3*	5.3 ± 1.0†	4.8 ± 1.3
HS40 (n = 6)	5.8 ± 1.1	0.5 ± 0.3*	1.7 ± 0.5*‡	4.1 ± 1.0
HS80 (n = 6)	5.6 ± 0.7	0.3 ± 0.2*	1.8 ± 0.8*‡	3.7 ± 1.7
Renal vascular resistances (dynes s cm^{-5})				
Control (n = 3)	1,698 ± 292	1,558 ± 481	1,371 ± 372	1,276 ± 315
NS40 (n = 6)	1,941 ± 331	10,480 ± 8,015	2,633 ± 290	1,914 ± 860
NS80 (n = 6)	1,680 ± 294	14,235 ± 11,259*	1,392 ± 279	1,454 ± 612
HS40 (n = 6)	1,793 ± 3319	91,54 ± 9,887	2,607 ± 705	1,684 ± 559
HS80 (n = 6)	1,844 ± 341	10,813 ± 8,516	2,465 ± 1,026	2,316 ± 1,298

Hemodynamic parameters at four time points t_0, t_{60}, t_{120}, and t_{180} of the five groups. Data expressed in mean ± SD.

* $P < 0.05$ *versus* control. † $P < 0.05$ *versus* NS40, ‡ $P < 0.05$ *versus* NS80.

HTS40 = hypertonic saline targeting a MAP = 40 mmHg; HTS80 = hypertonic saline targeting a MAP = 80 mmHg; NS40 = normal saline targeting a mean arterial pressure (MAP) = 40 mmHg; NS80 = normal saline targeting a MAP = 80 mmHg.
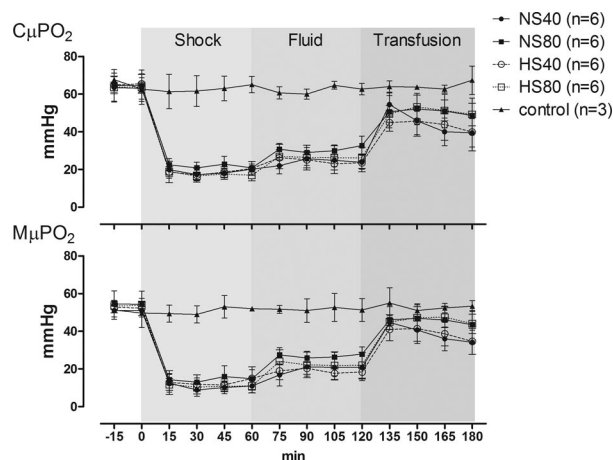
Fig. 5. Evolution of $C\mu PO_2$ (*upper graph*) and $M\mu PO_2$ (*lower graph*) in the five groups throughout the three different phases of the protocol. Hemorrhagic shock from t_0 to t_{60}, fluid resuscitation from t_{60} to t_{120}, and blood transfusion from t_{120} to t_{180}.

Fig. 7. Hemoglobin concentration at the different time points in all groups. * $P < 0.05$ NS80 *versus* HS40, ** $P < 0.05$ NS80 *versus* NS40, # $P < 0.05$ *versus* control.

organ failure.[21,24] Therefore, the purpose of this study was to provide more information regarding the use of "aggressive" *versus* "limited" fluid resuscitation using isotonic or HTS for renal microoxygenation. This study was not designed to assess outcome or bleeding in an uncontrolled hemorrhagic shock model and may not be reflective of the physiology of other organs in the clinical setting.

It can be questioned whether giving a large amount of such fluids may be effective in correcting hemorrhage-induced tissue hypoxemia due to poor oxygen-carrying properties of such fluids. The main finding is that neither fluid resuscitation targeting a high MAP nor a controlled fluid resuscitation targeting a low MAP increases the renal μPO_2 during the fluid resuscitation phase. Some data suggest a direct influence of hemodilution on microvascular flow and renal oxygen supply. The critical hematocrit associated with a decrease in microvascular PO_2 has already been found to be much higher for the kidney than for the heart or intestines.[8] Johannes *et al.*[10] have also found that the renal microvascular PO_2 drops at very early stages of isovolemic hemodilution. This was illustrated by an increased risk of acute kidney
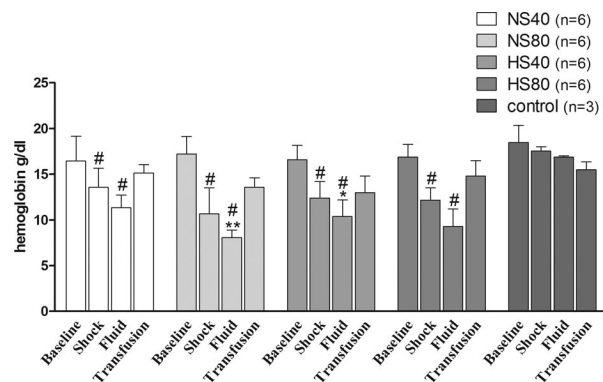
injury with cardiopulmonary bypass-associated hemodilution.[25] The reasons for such a high sensitivity to hemodilution could involve endothelial dysfunction[11] with prothrombotic and proinflammatory phenomenon.[26] Furthermore, there is evidence that hemodilution is likely to increase intrarenal diffusional shunting.[11] Indeed, as hemoglobin concentration drops, a decrease in binding of oxygen molecules occurs, which facilitates diffusional shunting. Moreover, the decrease in oxygen consumption by the vascular wall due to the lower shear stress could increase PO_2 in the outer layer of the arterial wall and enhance renal oxygen shunting by increasing the PO_2 gradient driving shunting force.[27] Finally, the increase in VO_{2ren} associated with fluid resuscitation can also participate in the decrease in microvascular PO_2. Indeed, the VO_{2ren} is highly linked to RBF and renal metabolic activity,[9] with VO_{2ren} increasing yet after the critical DO_{2ren} has been effectively reached.[28]

Fluid resuscitation does not hold any specific oxygen-carrying capacity and is, therefore, not effective in restoring microvascular oxygenation. Only early blood transfusion can expand volume, while maintaining the oxygen-carrying capacity of the circulating blood. Our study showed that providing blood is indeed an adequate means of ensuring oxygen transport to the kidney even after a considerable hypoxemic time. Our study also
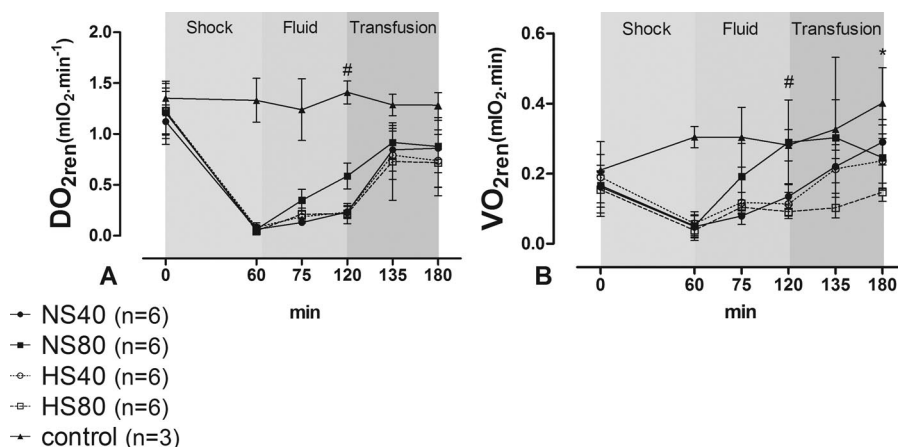

Fig. 6. Evolution of DO_{2ren} and VO_{2ren} (in ml/min) in the five groups during the three periods of the protocol. # $P < 0.05$ NS80 *versus* NS40, HS40, and HS80. * $P < 0.05$ NS80 *versus* HS80.
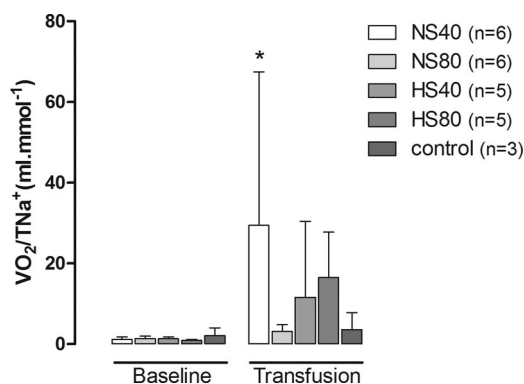
Fig. 8. Evolution of VO_2/TNa^+ between t_0 and t_{180} in the five groups. * $P < 0.05$ NS40 *versus* control and NS80.

showed that correcting arterial blood pressure is no guarantee for achieving the ultimate hemodynamic target of providing sufficient oxygen to the respiring kidney cells.

The renal μPO_2 increased after blood transfusion without returning to baseline values. Raising the hematocrit level is a key factor for increasing renal DO_2. It leads not only to a higher oxygen-carrying capacity because of an augmented hemoglobin concentration but also to an increased microvascular perfusion as a result of restoration of higher viscosity.[29] However, ischemic defects after blood transfusion are a common finding after resuscitation from hemorrhagic shock in other organs.[30] Several authors have reported on the role of reduced red blood cell deformability in the development of microcirculatory dysfunction after hemorrhagic shock.[31,32] Endothelium-leukocyte interactions, vasoconstriction, coagulation activation, interstitial, and endothelial edema are the main factors that are involved in microvascular dysfunction after renal ischemia–reperfusion.[33] Recently, Machiedo *et al.*[32] showed that transfusion of blood from hemorrhagic shock rats to control rats resulted in a decrease in cardiac output and a decrease in organ microcirculatory blood flow.

Kerger *et al.*, using the hamster window chamber model, have highlighted the critical role of microcirculation in the pathophysiology of hemorrhagic shock. They showed that functional capillary density and interstitial PO_2 were two of the main determinants of outcome in hemorrhagic shock.[34] Thus, it seems sensible to develop strategies that could improve microcirculatory blood flow and oxygenation during hemorrhagic shock resuscitation. HTS may hold such proprieties. HTS can improve macrohemodynamics not only by expanding the plasma volume by three to four times with the volume infused through an endogenous fluid redistribution from the interstitial tissue but also by improving cardiac contractility.[35] HTS may also have specific effects at the microcirculatory level. Resuscitation of hemorrhagic shock with HTS has been found to improve intestinal perfusion associated with selective vasodilatation of the A3 arterioles[11] to induce hyperosmolar shrinkage of endothelial cells, decrease capillary resistance[12] and prevent the hemorrhagic shock-induced inflammation.[36] In this study, resuscitation with HTS neither prevented the inflammatory response nor increased the microvascular PO_2 compared with NS.

One could argue that the HS80 group was underresuscitated regarding the lower MAP compared with the NS80 group. However, the volume of HTS being infused was high compared with previous reports, and the plasma osmolarity was well above the physiologic range.[35,37] Two mechanisms may have contributed to worsen systemic and renal hemodynamics in our study when infusing high volumes of HTS. First, infusion of HTS has been shown to induce glomerular afferent arteriole vasoconstriction and to result in a decrease in RBF mediated by the tubuloglomerular feedback.[38] This may account for the decrease of RBF in the groups treated with HTS. Second, the fact that the hemodynamic target was not reached with the MAP being lower than in the NS80 group (62 $\pm$ 8 mmHg *vs.* 78 $\pm$ 1 mmHg at t_{120}) was possibly due to the inability to improve cardiac output. Indeed, in the HS80 group, aortic blood flow was significantly lower compared with the NS80 group. Infusion of HTS and induction of hyperosmolarity have been reported to induce myocardial dysfunction and decrease contractile activity.[38] Therefore, a direct negative effect on cardiac function could participate in the hemodynamic findings. However, the design of the study does not allow us to draw any mechanistic conclusion underlying such systemic and renal effects. In a mouse model of uncontrolled hemorrhage, Amathieu *et al.*[39] found resuscitation with HTS to be associated with renal nephrotoxicity when targeting normal arterial pressure by using nuclear magnetic resonance analysis in urine samples. Even though hazardous osmolarity threshold values are unknown, high levels of osmolarity can activate a bundle of cellular events from damage to proteins and DNA to oxidative stress.[40,41] Finally, the duration and degree of hypotension may participate in discordance between studies.[42]

Although the purpose of this study was to assess kidney oxygenation during resuscitation of hemorrhagic shock, perfusion and function of other organs must, of course, be considered in a clinical perspective. We measured serum lactate as a global perfusion biomarker. After fluid resuscitation (t_{120}), serum lactate level was significantly lower in the NS80 group compared with other groups. However, this difference was not sustained, and the serum lactate level was found to be similar in all groups after blood transfusion (t_{180}).

Our study has several limitations. First, the anesthetic regimen used in our study may have affected the hemodynamic and renal response to hypovolemia. However, the need for deep anesthesia and analgesia during the protocol to prevent any suffering in animals makes anesthesia mandatory. Based on the literature, the ketamine-midazolam-sufentanyl strategy previously used has little effect on hemodynamics and is the regimen of choice in this condition.[43] We chose not to use an α-2 agonist, which can slow down heart rate and affect cardiovascular adaptation to hypovolemia.[44] Second, our study was designed to specifically address the effect of hemorrhage and fluid resuscitation on renal oxygenation. Therefore, the rather short follow-up after induction of hemorrhagic shock and resuscitation does not allow for the drawing of definitive conclusions regarding kidney function and damage. Further, our controlled hemorrhagic shock model is suitable neither for assessing outcome

and bleeding[45] nor for assessing the potential benefit of HTS when traumatic brain injury is present. Sell *et al.*[46] have recently shown better long-term neuronal survival and behavioral recovery in rats undergoing hemorrhagic shock with traumatic brain injury receiving HTS. Third, temperature was carefully controlled during the procedure, a factor that may differ from the clinical scenario in which slight hypothermia often occurs and may affect prognosis.[47] Fourth, we did not measure blood glucose levels. Hirose *et al.*[48] have recently reported that hyperglycemia that occurred during renal ischemia–reperfusion resulted in severe functional injury compared with normoglycemia. Fifth, it must be acknowledged that the use, in the current study, of fresh autologous shed blood transfusion does not fully resemble the clinical scenario of packed red blood cells transfusion, in which time storage can decrease oxygen-carrying capacity.[49,50] Sixth, we acknowledge that the use of arterial-venous oxygen content difference for VO_{2ren} calculation from arterial-venous oxygen content difference could have introduced errors, compared with direct oxygen consumption techniques (*i.e.*, whole body calorimetric techniques or *in vitro* oxygen consumption measurements).[51] Seventh, measuring descending aortic blood flow could not be totally accurate in estimating cardiac output. However, based on the study of Dumans-Nizard *et al.*, the risk of error seems to be minor. Although using a different animal model, the authors have shown that only minor blood flow redistribution exists between supra-aortic and descending aortic territories during hemorrhage.[52] Finally, cytokine levels correlate with the severity of hemorrhagic shock[53] and have been reported to be associated with a poor prognosis in patients with acute kidney injury; further, IL-6 has been linked to the development of remote organ injury in hemorrhagic shock.[54,55] We are aware that the rather small sample size of the groups might have introduced a lack of power in the statistical analysis and prevented us from drawing a conclusion regarding the effect of fluid resuscitation strategies on cytokine levels. Likewise, measuring cytokine levels after blood transfusion may have blunted the effect of fluid resuscitation on inflammatory response. In light of our results and the available literature, our study calls for follow-up work regarding the testing of different resuscitation protocols. The assessment of the renal effect of early initiation of catecholamine infusion to restore arterial blood pressure or the use of blood transfusion earlier in the course of shock on the frontlines is warranted. A strategy based on infusion of catecholamines at the early onset of shock may be found effective in limiting fluid volume and, therefore, hemodilution.[56]

Conclusion

In our hemorrhagic shock model, fluid resuscitation with either normal or HTS targeting a low or high MAP did not result in a correction of shock-induced renal microcirculatory hypoxia. Transfusion of fresh blood was able to improve renal oxygenation but with persistent hypoxic defects. Overall, our results highlight the critical need for developing improved resuscitation strategies in hemorrhagic shock.

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