

Fluid Resuscitation Aggravates the Cellular Injury in a Hemorrhagic Shock Model

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Keywords

0.9% NaCl · Hydroxyethyl starch · Kidney · Hemorrhagic shock · Resuscitation

Abstract

Background: Resuscitation is the initial step for hemorrhagic shock. However, there is still controversy as to which fluid achieves the best results clinically and experimentally. **Aim:** It was aimed to investigate the effects of 0.9% NaCl (sodium chloride) and 6% HES (hydroxyethyl starch) on the kidney and blood environment. **Methods:** Twenty-four male Wistar rats were assigned as control, shock, and resuscitated (colloid: 6% HES and crystalloid: 0.9% NaCl) groups. Besides hemodynamics (mean arterial pressure and shock index) monitoring and kidney function evaluation, hemolysis, oxidative stress, inflammation, and glycocalyx degradation were evaluated in the plasma and kidney. **Results:** (1) Macrohemodynamics were successfully restored by both fluids. (2) Although 3 times more crystalloid volume was applied compared to the colloid resuscitation, similar hematocrit levels were found in both resuscitation strategies (32.8 ± 2.3 vs. 33.3 ± 1.0). (3) NaCl resuscitation led to increases in the hemolytic index, catalytic iron, and sialic acid compared to control, while HES administration increased the levels of malondialdehyde, ischemia-modified albumin, and sialic acid. (4) However, both fluid resuscitation strategies could inhibit inflammation and oxidative stress in the kidney and restore

kidney function parameters. **Conclusion:** Although both NaCl and HES resuscitation showed protection of the kidney function against oxidative stress and inflammation, these fluids initiated the injury process.

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Introduction

Hemorrhagic shock is a clinical condition characterized by tissue and organ ischemia as a result of a decrease in blood volume due to bleeding [1]. The first treatment approach against bleeding is fast and insistent fluid administration to increase blood pressure and hence optimize tissue perfusion. The volume and type of fluid used are the factors that directly affect oxygenation [2].

Crystalloids and colloid derivatives are used as resuscitation fluids. Experimental and clinical studies on colloids have also shown that hydroxyethyl starch (HES) molecules induce necrosis in the proximal tubules and cause coagulopathy as well [3]. On the other hand, crystalloids are much smaller than colloids and thus quickly pass to the extracellular space. Hence, previous animal experiments have already shown that there is a need for 3-fold more volume for crystalloids to achieve the hemodynamic target [4]. Blood begins to be diluted during fluid administration, and hemolysis occurs in the ongoing process [5].

Dilutional hemolysis liberates free iron moieties from red blood cells. It is known that catalytic iron, which is a part of free hemoglobin molecules and formed as a result of hemolysis, plays a role in the production of reactive oxygen species (ROS) by 2 reactions known as Fenton and Haber-Weis and may initiate oxidative stress [6, 7]. Mediators contributing to oxidative stress may cause damage to the glycocalyx which is the luminal surface cover of the vessels [8]. Moreover, recent studies have shown that there is a high correlation between glycocalyx components in blood and renal function [9].

We hypothesized that resuscitation approaches in the early period would be better for the understanding of the injury. We evaluated the acute effects of NaCl and HES fluids on the kidney tissue redox homeostasis inflammation and blood environment in a rat model of hemorrhagic shock. To understand the contribution of the hemolytic process, catalytic iron levels and hemolysis index were measured. Serum levels of sialic acid and syndecan-1 were determined for glycocalyx damage assessment. Tumor necrosis factor-alpha (TNF- α) levels were used for the evaluation of inflammation. As a biochemical routine analysis, serum lactate, creatinine, and urea levels were measured.

Materials and Methods

Animals

Twenty-four male Wistar rats (350 \pm 50 g) used in this study were provided by the Aziz Sancar Experimental Medical Research Institute.

Experimental Preparation

All rats were anesthetized with sodium pentothal (80 mg/kg i.p.). After anesthesia induction, tracheotomy was performed for spontaneous ventilation. At 37°C, body temperature was maintained using external heating and a heating pad connected to a rectal probe. Femoral artery, carotid artery, and femoral vein were cannulated with polyethylene catheters (0.9 mm), followed by a 20-min hemodynamic stabilization period. The cannula in the carotid artery was used to measure mean arterial pressure (MAP) and heart rate (HR) via a data acquisition system (Biopac, MP45). The cannula in the femoral artery was used to perform the shock model and blood samplings. The femoral vein was for fluid administrations.

Experimental Protocol

After the stabilization period (20 min), 4 groups ($n = 6$) were established as follows: the control group was designed without hemorrhage or resuscitation processes. For the shock group, rats were bled as described below for 60 min. The first resuscitation group was designed as the colloid group which includes a hemorrhagic process for 60 min and a resuscitation process for 60 min with 6% HES (130/0.4) solution. The second resuscitation group

was designed as the crystalloid group. Sixty-minute hemorrhagic process and 60-min resuscitation process with 0.9% NaCl solution were performed for the crystalloid group.

Shock and Resuscitation Protocol

Blood was withdrawn from the femoral artery until a target MAP of around 40 mm Hg was achieved in the shock, colloid, and crystalloid groups. Bleeding was provided by using a syringe pump (1 mL/min). The level of MAP as 40 mm Hg was maintained throughout 60 min. At the end of the hemorrhage process, the rats were resuscitated with 0.9% NaCl or 6% HES according to the respective group until the MAP reaches 80 mm Hg throughout 60 min.

Hemodynamic Measurements and Blood Samplings

Throughout the experimentation, MAP and HR were continuously measured. Additionally, the shock index (SI) was calculated as SI (mm Hg min) = HR/SP, where HR is the heart rate and SP is the systolic pressure [10]. For representative demonstration of biochemical and hemodynamic measurements, 3 time points were determined: baseline ($t = 0$ min), shock ($t = 60$ min), and 60 min after starting fluid administration ($t = 120$ min).

Biochemical Measurements

Blood samples were centrifuged (3,000 g, 5 min). The obtained serum and tissue samples were frozen at -80°C until the measurement day. For tissue samples, biochemical tests were performed after homogenization by using a Teflon homogenizer with a buffer solution of phosphate buffer.

Determination of Protein Content

Protein contents were measured in serum and homogenate samples by using Coomassie brilliant blue according to Bradford assay [11]. In brief, samples were diluted with Bradford reagent and measured at 595 nm. After that, calculation was performed according to the standard curve.

Routine Biochemical Measurements

Lactate, creatinine, and urea levels were measured in serum samples using an autoanalyzer such as Cobas C501 and 8000 C502 module. The test principle of creatinine was Jaffe assay as a kinetic colorimetric method. In brief, potassium hydroxide (900 mmol/L), phosphate (135 mmol/L), and picric acid (38 mmol/L) reagents were used for the Jaffe test. Urea levels were measured kinetically using the urease method. Lactate levels were measured by the colorimetric test. UREAL, CREJ2, and LACT2 kits were the commercial kits used for these assays. The internal and external quality control of the autoanalyzer was within the specified limits of the procedure.

Measurements Related to Hemolytic Process

Free hemoglobin levels, hemolysis index, and catalytic iron levels were measured to assess the hemolytic process in serum. Free hemoglobin levels were determined using a spectrophotometric assay. In brief, serum samples were diluted 11 times using sodium bicarbonate (0.942 M) and the absorbance read at different wavelengths (415, 380, and 470 nm) [12]. The hemolysis index was calculated by proportioning the free hemoglobin levels at baseline and resuscitation time points [13]. The formula used for hemolysis index determination is baseline free hemoglobin concentration/resuscitation free hemoglobin concentration. Collected blood samples just after the colloid or crystalloid treatment time points

Table 1. MAP and SI values on baseline, shock, and resuscitation time points

	Baseline	Shock	Resuscitation
MAP, mean ± SD, mm Hg			
Control	103.5±7.8	97.7±8.7	101±5.4
Shock	109.4±6.0	39.5±1.5 ^{aaa}	31.8±0.7 ^{aaa}
Colloid	99.0±7.1	45±2.6 ^{aaa}	83.2±1.2 ^{bbb}
Crystalloid	117.5±5.2	42.1±2.3 ^{aaa}	78.2±3.1 ^{bbb}
SI, mean ± SD, mm Hg min			
Control	3.1±0.2	3.2±0.2	3.2±0.3
Shock	3.0±0.2	6.3±0.6 ^{aaa}	7.9±0.5 ^{aaa}
Colloid	2.9±0.1	5.7±0.4 ^{aaa}	3.6±0.1 ^{bbb}
Crystalloid	2.9±0.2	6.6±0.4 ^{aaa}	4.2±0.2 ^{bbb}

MAP, mean arterial pressure; SI, shock index. ^{aaa} $p < 0.001$ versus control. ^{bbb} $p < 0.001$ versus shock.

were used for the measurements. The preanalytical sources of error of in vitro hemolysis were excluded in control and case groups.

For catalytic iron measurement, all reagents used in this measurement were treated with Chelex except for samples for eliminating iron contamination. After that, deoxyribonucleic acid (DNA: 0.5 mL, 1 mg/mL), bleomycin sulfate (0.05 mL, 1 mg/mL), magnesium chloride (MgCl₂: 0.1 mL, 50 mM), sample (0.1 mL), hydrochloric acid (HCl: 0.05 mL, 10 mM), distilled water (0.1 mL), and ascorbic acid (0.1 mL) were mixed and incubated at 37°C throughout for 120 min, and the reaction was stopped by using ethylenediaminetetraacetic acid (1 mL, 0.1 M) administration. Afterward, 1 mL (in 1% 50 mM sodium hydroxide [NaOH]) thiobarbituric acid was added to HCl (1 mL, 25%) and incubated in a water bath (100°C, 15 min). Catalytic iron levels were measured at 532 nm. All these procedures were also made for standard iron solutions, and a standard curve was drawn [14].

Measurements Related to Oxidative Stress and Inflammation

Hydroxyl (OH⁻), malondialdehyde (MDA), and ischemia-modified albumin (IMA) levels were measured to evaluate oxidative stress and ischemia. Moreover, TNF- α levels were measured for the assessment of inflammation. For OH⁻ measurement, sodium biphosphate (NaH₂PO₄: 0.1 mL, 1 M), sodium azide (NaN₃: 0.05 mL, 10 mM), dimethyl sulfoxide (DMSO: 0.05 mL, 4 mM), nicotinamide adenine dinucleotide phosphate (NADPH: 0.05 mL, 4 mM), and 0.1 M 0.05 mL stock solutions were mixed and slightly shaken. After that, this mixture was incubated (37°C, 10 min). Then, the mixture was treated with 0.15 mL 10% TCA (in 0.25 HCl) and incubated in a water bath throughout 30 min. Absorbance was measured at 570 nm, and concentration was calculated using the Lambert-Beer formula [15]: ($A = \epsilon c l, 6.22 \text{ L} \times [\text{mol cm}^{-1}]$). For MDA measurement, 250 μL serum was treated with 1,000 μL stock solution and waited throughout 15 min. After that, the solution was incubated at 100°C in a water bath for 10 min. Subsequently, the supernatant was collected which was centrifuged at 1,000 g for 5 min and was measured at 535 nm. The Lambert-Beer formula was used to calculate concentration from the absorbance [16]: ($A = \epsilon c l, \epsilon_{\text{MDA}} = 1.56 \times 10^6 \text{ L} \times [\text{mol cm}^{-1}]$). For IMA measurement, the serum sample (40 μL) was treated with cobalt chloride (CoCl₂: 10 μL , 0.1%) and incubated

Table 2. Serum lactate, creatinine, and urea levels

	Control	Shock	Colloid	Crystalloid
Lactate, mean ± SD, mmol/L	4.5±3.9	11.8±9.6 ^a	5.9±2.3 ^b	3.3±1.5 ^b
Creatinine, mean ± SD, mg/dL	0.3±0.1	0.9±0.2 ^a	0.4±0.2	0.4±0.3
Urea, mean ± SD, mg/dL	53.8±4.1	55.5±18.6	66.2±7.7	61.6±23.5

^a $p < 0.05$ versus control. ^b $p < 0.05$ versus shock.

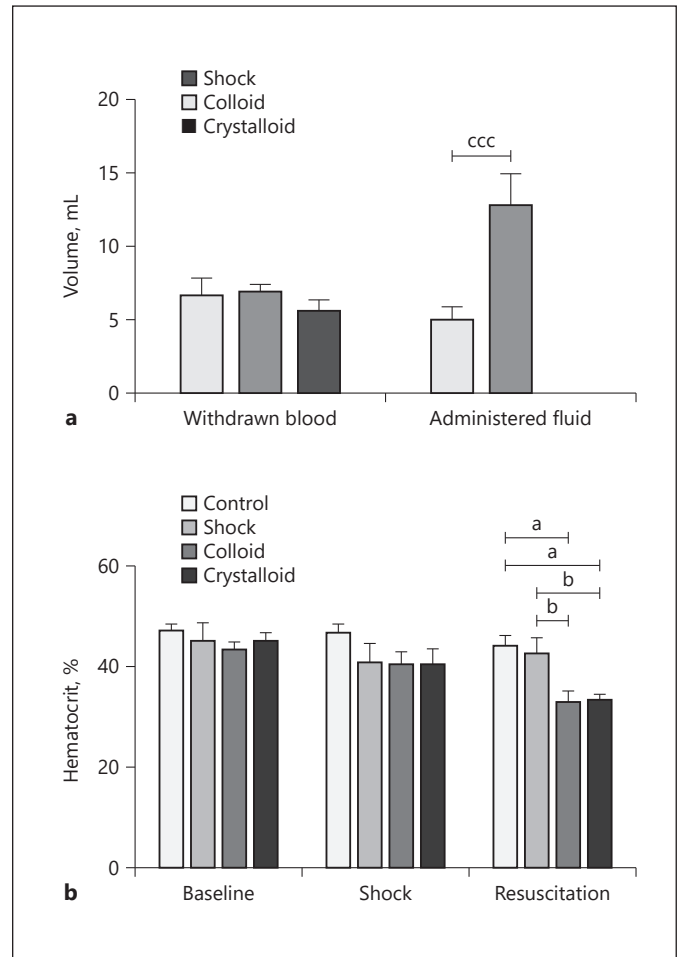


Fig. 1. Withdrawn blood volume and administered fluid volume (a) and hematocrit levels (b) (^a $p < 0.05$, ^b $p < 0.05$, and ^{ccc} $p < 0.001$).

throughout 10 min. After that, dithiothreitol (DTT: 10 μL) was added to the solution. The color change was seen after 2 min, and sodium chloride (NaCl: 200 μL , 0.9%) was added to the mixture. Finally, the absorbance of the solution was measured at 470 nm. Blanks were prepared without DTT addition, and distilled water was added instead of DTT. The calculation was made by subtracting the samples and blanks [17]. For TNF- α measurement, a commercially available ELISA kit was used (Ebioscience).

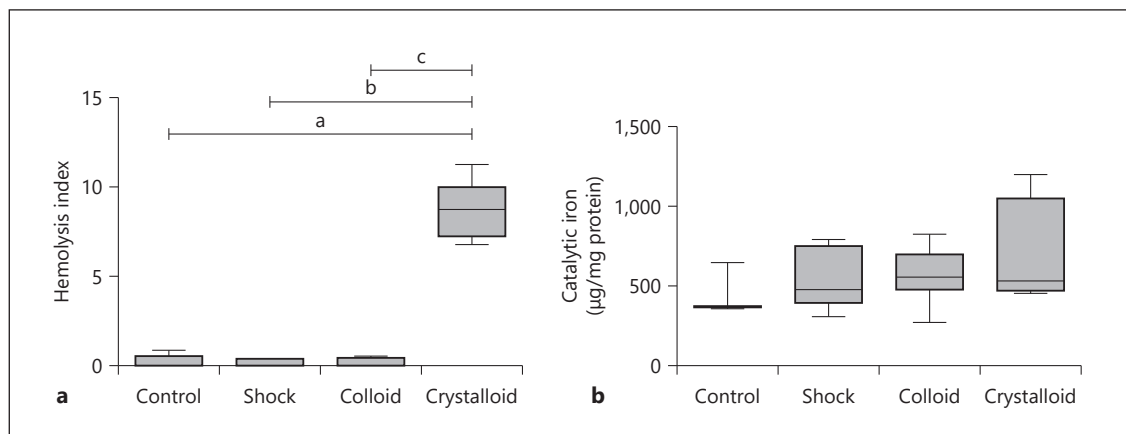


Fig. 2. Hemolytic index (a) and catalytic iron (b) (^a $p < 0.05$, ^b $p < 0.05$, and ^c $p < 0.05$).

Measurements Related to Glycocalyx Damage

Serum sialic acid and syndecan-1 levels were measured to evaluate glycocalyx degradation. For sialic acid measurement, serum samples (0.2 mL) were mixed with perchloric acid (1.5 mL, 5%). After that, the solutions were incubated in a water bath (100°C, 5 min). Five minutes later, the solutions were centrifuged (2,500 g, 4 min). The obtained supernatants were mixed with Ehrlich solution (0.2 mL). The mixtures were incubated in a water bath (100°C, 15 min). Last, distilled water (1 mL) was added, and the absorbance of the solution was measured at 525 nm. The concentration of sialic acid was calculated using the standard curve [18]. For syndecan-1 level measurement, a commercially available ELISA kit was used (Elabscience).

Statistical Analysis

First, all data sets were tested whether appropriate to gauss distribution. Withdrawn blood, administered fluid volume, hematocrit, MAP, SI, lactate, creatinine, and urea data sets were normally distributed while hemolytic index, catalytic iron, OH⁻, MDA, IMA, TNF- α , sialic acid, and syndecan-1 data sets were nonnormally distributed. Normally distributed sets were presented as mean \pm SD while others as median (min–max). One-way ANOVA following the Tukey test as post hoc analysis was used for normally distributed sets. Nonnormally distributed sets were analyzed by using the Dunn's multiple comparison test as post hoc analysis to find out significant differences between groups after the Kruskal-Wallis test. A p value of <0.05 was considered statistically significant. Statistical analysis was performed using GraphPad Prism v 5.0 (GraphPad Software, San Diego, CA, USA).

Results

Table 1 shows systemic hemodynamic data sets, and Table 2 shows routine biochemical parameters. Figure 1 demonstrates the amount of withdrawn blood and administered fluid volume. Figure 2 exhibits the hemolytic index, catalytic iron, and hematocrit levels. Figures 3–5

represent oxidative stress markers. Figure 6 shows the inflammation parameter, and Figure 7 displays glycocalyx degradation parameters.

Systemic Hemodynamics

The initial values of the 4 groups were similar to each other. In both shock and resuscitation groups, blood withdrawn led to a significant effect on blood pressure, and the target pressure of 40 mm Hg was attained. MAP values that decreased in the shock group were increased by both resuscitation protocols (shock: 31.8 ± 0.7 mm Hg, $p < 0.001$, vs. colloid: 83.2 ± 1.2 mm Hg, crystalloid: 78.2 ± 3.1 mm Hg), and the target MAP of 80 mm Hg was successfully attained and kept at target pressure throughout resuscitation in both groups. SI levels were also normalized by 2 fluid treatment protocols (colloid: 3.6 ± 0.1 mm Hg min, $p < 0.001$, vs. shock; crystalloid: 4.2 ± 0.2 mm Hg min, $p < 0.001$, vs. shock) (Table 1).

Withdrawn Blood, Administered Fluid Volume, and Hematocrit Levels

The withdrawn blood volume was not significantly different among the groups; however, the amount of the administered fluid as crystalloid was significantly higher than that in the colloid group (colloid: 5.0 ± 2.0 mL, $p < 0.001$, vs. crystalloid: 12.8 ± 5.2 mL). Hematocrit values were similarly decreased in both fluid treatment groups compared to the control group (control: $46 \pm 1.6\%$ vs. colloid: $38.8 \pm 5.4\%$, crystalloid: $39.6 \pm 5.8\%$) (Fig. 1a, b).

Routine Biochemical Findings

Hemorrhagic shock led to a significantly increased lactate level (shock: 11.8 ± 9.6 mmol/L vs. control: 4.5 ± 3.9

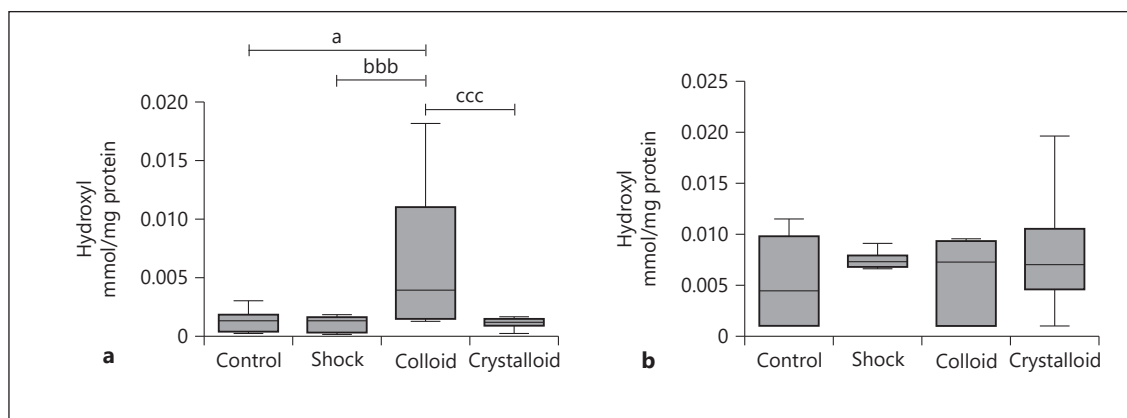


Fig. 3. Serum hydroxyl (a) and kidney tissue hydroxyl (b) levels, (^a $p < 0.05$, ^b $p < 0.05$, ^{aa} $p < 0.01$, ^{bbb} $p < 0.001$, and ^{ccc} $p < 0.001$).

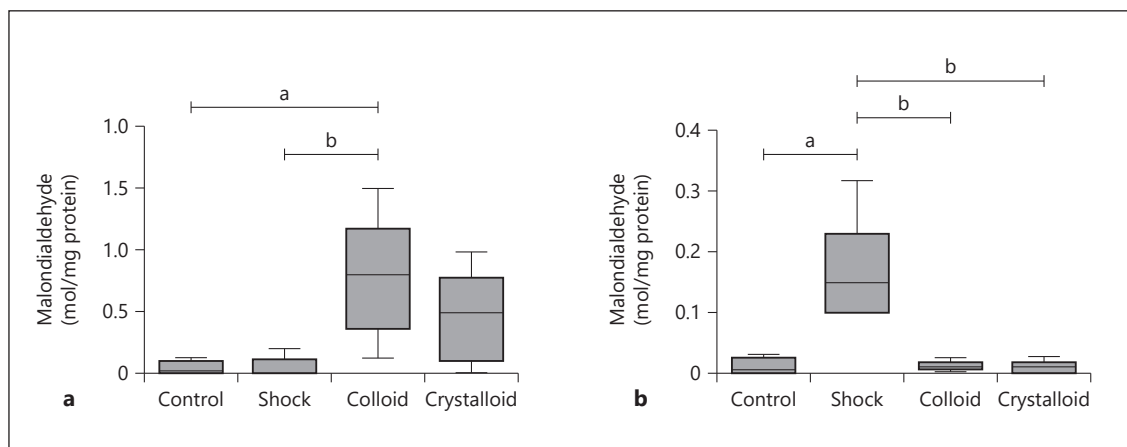


Fig. 4. Serum malondialdehyde (a) and kidney tissue malondialdehyde (b) levels (^a $p < 0.05$ and ^b $p < 0.05$).

mmol/L; $p < 0.05$), and both fluid treatments decreased lactate levels compared to the shock group (shock: 11.8 ± 9.6 mmol/L vs. colloid: 5.9 ± 2.3 mmol/L, crystalloid: 3.3 ± 1.5 mmol/L; $p < 0.05$). Creatinine levels were also increased in the shock group (shock: 0.9 ± 0.2 mg/dL vs. control: 0.3 ± 0.1 mg/dL; $p < 0.05$), and the mean levels in both fluid administered groups were comparable to the control group. However, urea levels were similar in all groups (Table 2).

Hemolytic Index and Catalytic Iron Levels

The hemolytic index was found to be significantly higher in the crystalloid group than in all other groups (crystalloid: $8.7 [6.7-11.3]$ vs. control: $0.1 [0.03-0.8]$, shock: $0.3 [0.09-0.4]$, and colloid: $0.4 [0.3-0.5]$; $p < 0.05$). The highest level of catalytic iron was found to be in the crystalloid group without no significance (crystalloid: $532 [456-1,200]$) (Fig. 2).

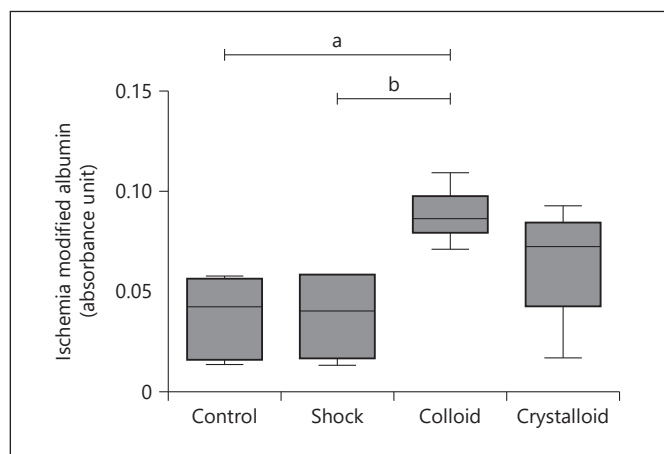


Fig. 5. Serum ischemia-modified albumin levels (^a $p < 0.05$ and ^b $p < 0.05$).

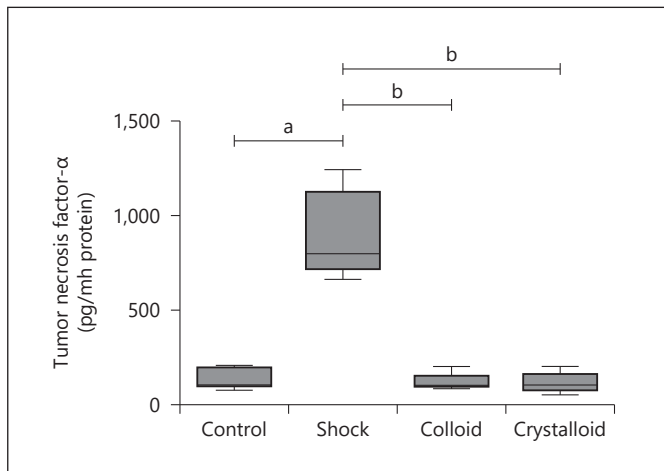


Fig. 6. Kidney tissue tumor necrosis factor- α levels (^a $p < 0.05$ and ^b $p < 0.05$).

Oxidative Stress and Inflammation Levels

Serum OH^- levels were significantly increased in the colloid group compared to all other groups (colloid: 0.003 [0.001–0.01] mmol/mg protein vs. control: 0.001 [0.0003–0.003], shock: 0.001 [0.0002–0.001], and crystalloid: 0.001 [0.0003–0.002], $p < 0.01$). However, no significant differences in OH^- levels of kidney tissues were found among groups. While serum MDA levels showed a significant increase in the colloid group compared to the control and shock groups (colloid: 0.8 [0.1–1.5] mol/mg protein vs. control: 0.03 [0.01–0.1] mol/mg protein, shock: 0.006 [0.001–0.2] mol/mg protein; $p < 0.05$), renal tissue MDA levels in the shock group were increased compared to all other groups, and both fluid types restored the serum MDA levels (shock: 0.15 [0.1–0.03] mol/mg protein, $p < 0.05$, vs. control: 0.007 [0.003–0.03] mol/mg protein, colloid: 0.01 [0.003–0.03] mol/mg protein, and crystalloid: 0.01 [0.003–0.03]). IMA values were significantly increased in the colloid group compared to the control and shock groups (colloid: 0.08 [0.07–0.1] absorbance unit vs. control: 0.04 [0.01–0.06] absorbance unit, shock: 0.04 [0.01–0.06] absorbance unit; $p < 0.05$) (Fig. 3–5). Renal tissue TNF- α levels showed an increase in the shock group compared to the control and resuscitated groups (shock: 800 [665–1,244] pg/mg protein vs. control: 100 [80–200], colloid: 100 [90–200] pg/mg protein, and crystalloid: 100 [50–200] pg/mg protein; $p < 0.05$) and decreased in the fluid treatment groups irrespective of the fluid type compared to the shock group (shock: 800 [665–1,244] pg/mg protein vs. colloid: 100 [90–200] pg/mg protein, crystalloid: 100 [50–200] pg/mg protein; $p < 0.05$) (Fig. 6).

Glycocalyx Degradation Product Levels

Serum sialic acid levels were increased in both fluid treatment groups compared to control (control: 0.02 [0.001–0.05] mol/mg protein vs. colloid: 0.09 [0.08–0.1] mol/mg protein, crystalloid: 0.08 [0.07–0.1] mol/mg protein; $p < 0.05$), and the highest level of syndecan-1 without no significance was found to be in the shock group (0.6 [0.1–1.2] ng/mg protein) (Fig. 7a, b).

Discussion

The aim of this study was to evaluate the effects of different types of fluids used to improve the macrohemodynamics in a rat model of hemorrhagic shock. The key findings were as follows: (1) fluid treatments – regardless of the type of fluid – improved macrohemodynamics and renal function in the early period of hemorrhagic shock. (2) However, fluid resuscitation may cause problems that are not present in the early period of shock, such as glycocalyx damage and oxidative stress. (3) Sialic acid, which is a glycocalyx component, may not be decisive for kidney function.

Hemorrhagic shock is characterized by low blood pressure and is one of the main causes of renal dysfunction. Renal dysfunction is also an independent risk factor for death among hemorrhage patients. Hence, kidney-targeted approaches could improve patients' outcomes. The current treatment approach is based on restoration of the macrohemodynamics such as blood pressure and heart rate during hemorrhagic shock [19, 20]. Fluid treatments are used as the first step for normalization deteriorated macrohemodynamics. However, there is still no consensus on which type of fluid to use for best renal or biochemical outcome [20].

Crystalloid solutions are known to be poorly capable of plasma volume expanding due to small molecule structures of dissolved electrolytes and tend to escape into the extravascular space [4]. Hence, there is a need for high-volume crystalloid to achieve target hemodynamics. However, large-volume crystalloid administration results in pulmonary edema and/or acidosis and oxidative stress due to hemolysis [2]. As another alternative fluid, synthetic colloid (HES) solutions have high-molecular-weight particles and hold the plasma within the intravascular space, effecting plasma colloid oncotic pressure. However, the resultant effects of HES infusion on the kidney are still in a black box. For instance, HES administration is known to develop kidney injury [21]. In a study conducted by Shaw and Kelum [22], colloids were not shown to be superior to crystal-

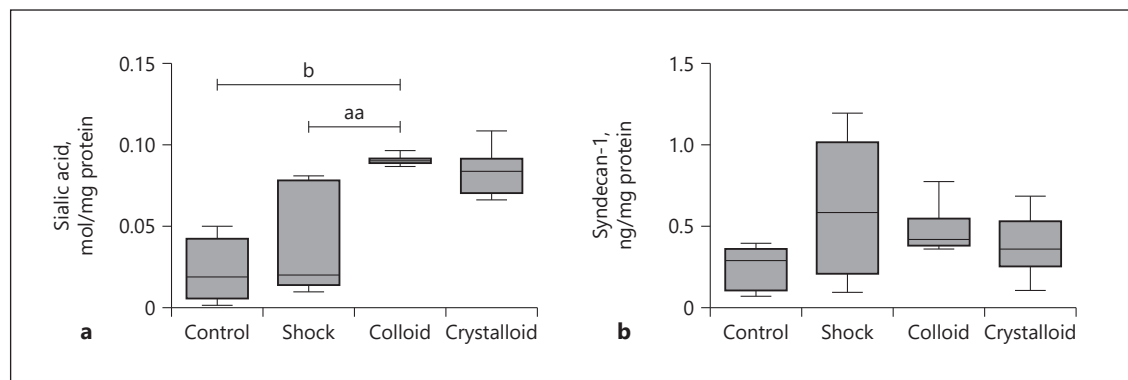


Fig. 7. Serum sialic acid (a) and syndecan-1 (b) levels (^{aa} $p < 0.01$ and ^b $p < 0.05$).

loids. Even in a meta-analysis, it has been reported that the mortality rate was found to be higher in colloid treatment [23]. On the other hand, in a study by László et al. [4], colloids have been shown to be more advantageous to achieve hemodynamic stability than crystalloids. This darkness may have been caused by variations in different patient groups or different volume amounts. Standardized animal experiments are therefore required.

Despite the aforementioned side effects, both types of fluid treatments were proven to improve macrohemodynamics in shock [24]. Hence, the use of resuscitation fluids would be better than not being used. In our study, macrohemodynamic parameters were also restored by both fluids, and the achievement of the hemodynamic target of both fluids was found to be the same. Moreover, fluid applications also – apparently – improved the shock state reflecting as the shock index [25].

Results from both macrohemodynamics and routine biochemistry (lactate, urea, and creatinine) can provide information on the success of fluid administration. An increase in lactate level due to anaerobic cellular process is used to indicate the metabolism shifting through the anaerobic side. In our study, the administration of both fluids could decrease the lactate levels compared to shock. Additionally, both fluids could restore parameters related to renal function. These results confirm the conventional approaches to fluid treatments. However, given the mortality and morbidity rates of shock patients, it raises doubts about the adequacy of the relevant parameters. Another important point is that the relevant parameters do not provide information about the whole-body system [19]. Hence, our study was designed to give a cellular perspective to the fluid resuscitation concept.

In our study, as a requirement of goal-directed therapy, 3 times more crystalloid volume was applied com-

pared to the colloid resuscitation. This 3-fold difference was due to the unbalance character of the fluids used in the present study [26], and despite this 3-fold difference, equal hematocrit levels were found in both resuscitation strategies. This result was previously described as general characteristics of crystalloid-based solutions due to rapidly shifting to the extravascular area [4]. Although both hematocrit levels were similar in both replacement strategies, a large volume of crystalloid in the vasculature could trigger several processes. Our study attempted to establish the cellular processes related to excessive stress in the vascular bed.

Dilutional hemolysis occurs in fluid treatments due to the failure of erythrocyte membrane resistance to intravascular osmotic stress [5]. In our current study, hemolysis accompanied by an increase in catalytic iron level was also observed in 0.9% NaCl resuscitation as a result of 3-fold excess volume administration. Catalytic iron formation after hemolysis can cause free radical formation through Fenton reactions [6, 7, 27]. Catalytic iron is an important mediator that contributes to the process of organ damage since it is directly associated with oxidative stress [27]. However, in our study, increased catalytic iron levels were followed by a restored renal function in crystalloid resuscitation. This suggests that catalytic iron levels in this model were not sufficient to disrupt renal function. Although no deterioration in kidney function was found in our model, the use of 0.9% NaCl in the long term should be evaluated. However, when the innocence of fluids is questioned, the high levels of serum OH^- were found to be in colloid resuscitation but not in crystalloid. This apparent contradiction between catalytic iron and OH^- levels suggests that colloid resuscitation might trigger Haber-Weiss reaction instead of Fenton reaction resulting in ROS formation in colloid resuscitation. Fenton

and Haber-Weiss reactions are 2 different pathways that can occur following each other and used in ROS formation. However, while Fenton starts with catalytic iron mediated, Haber-Weiss – known as the net reaction – forms OH^- directly by the reaction of superoxide and hydrogen peroxide and is faster than Fenton reactions [28]. When hemolysis and thereby catalytic iron levels were taken into consideration, the damage expected in crystalloids was observed in colloids due to the net and rapid reactions of Haber-Weiss. In this case, the use of iron chelates in crystalloids may further reduce oxidative damage. However, the measurement of reactive oxygen species other than hydroxyl in both kidney and serum is certain to strengthen this hypothesis.

The vascular network penetrates all organ systems. Therefore, damage to any organ may cause some parts to break out of the cell and into the blood. If a specific marker is measured in the blood, it may be possible to comment on that organ. In our study, MDA and IMA parameters related to oxidative stress were measured. MDA is a lipid peroxidation product, and IMA is a molecule that is formed by a change in the three-dimensional structure of albumin in cases of oxidative stress. Therefore, these molecules can be used to evaluate the oxidative process [17, 29]. In the results of MDA and IMA levels, the application of both fluids caused stress that was not in shock and also showed similar effects on these 2 parameters. When all the oxidative stress-related processes that we claim in our study are considered, certainly the measurement of reactive oxygen species other than hydroxyl in both kidney and serum will strengthen the explanation of these processes.

Glycocalyx – which is a rich layer of carbohydrate – covers the luminal surface of the vascular endothelial cells. Sialic acid and syndecan-1 which we measured in our study are closely related components. It is an important structure with basic functions such as vascular reactivity, leukocyte adhesion, migration, and cell-cell recognition [30]. Glycocalyx is damaged in the cases of oxidative stress and inflammation and plays a critical role in acute kidney injury, and resuscitation fluids may contribute to the degradation process [31, 32]. In our study, sialic acid is poured in both fluid therapies while another component, syndecan-1, showed relative improvement in fluid therapy. Sialic acid is placed at the terminal of the cell surface layer and syndecan-1 is more internally and has 5 bond regions. Hence, the position of the syndecan-1 molecule in the glycocalyx layer and the number of ligands can be attributed to this controversy [33]. This result has been achieved when renal function and syndecan-1 levels are evaluated together; protection of glyco-

calyx components other than sialic acid – such as syndecan-1 – with early period intervention is effective in protecting renal function. Therefore, these results suggest that although fluid administration has a positive effect on the kidneys in terms of oxidative stress and inflammation, it can induce damage in other organs or in the blood itself. Glycocalyx components should be measured in the kidney tissue and other organs to obtain a definite conclusion about this subject. In our study, glycocalyx components were evaluated in serum content.

Additionally, in a long-term study in which the shock duration is long and made by Ergin et al. [34], it was shown that peroxidation products cannot be suppressed by fluid therapies in kidney tissues, and tissue damage in kidneys has been shown to be suppressed by fluid therapies in our short-term shock model. In another study, it was also shown that HES has a protective property in a long-period shock model setting [35]. Similarly, in a previous study – which made long-term fluid therapies – the renal inflammation values could not be suppressed. However, these short-term model inflammation biomarkers were improved on the kidney [24]. This result highlighted the importance of both the early period and the short-term intervention in reducing the destructive effect of fluids' own. In a study done by Boyd et al. [36], as they mentioned, studies on time-dependent HES treatment should continue. This study bears considerable importance as it is the first study to evaluate the effects of different fluid types on the kidney both in terms of early period intervention and short-term fluid at the same time.

Conclusion

Our study demonstrated that hemorrhage-induced kidney dysfunction is in parallel with oxidative stress and inflammation. This injury process in the kidney and macrohemodynamic failure could be restored by fluid resuscitation. However, fluid resuscitation may cause additional injury that is not present in the early period of shock, such as glycocalyx damage and oxidative stress. Hence, there is a need for new-generation fluids which can reduce oxidative inflammatory processes in all tissues and organs.

Statement of Ethics

Ethics permission was obtained from the Istanbul University Animal Experiments Local Ethics Committee (105706), and care and handling of the animals were performed according to the ARRIVE guidelines, the UK animals Act 1986, and EU directive 2010/63/EU.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

U.A. contributed to conceptualization; K.V. and K.C. contributed to methodology; K.V. contributed to formal analysis; K.V. and U.A. contributed to writing – original draft preparation; K.V. and U.A. contributed to writing – review and editing; U.A. contributed to supervision; K.V. and U.A. contributed to interpretation of data for the work; U.A. contributed to revising it critically for important intellectual content.

Data Availability Statement

Data are not available publicly due to security and privacy reasons. However, the data generated or analyzed during this study can be provided upon request from the correspondence author.

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