

Hydroxyethyl Starch 6% (130/0.4) Ameliorates Acute Lung Injury in Swine Hemorrhagic Shock

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ABSTRACT

Background: Traumatic hemorrhage induces acute lung injury. The aim of this study was to assess whether lactated Ringer's solution or 6% hydroxyethyl starch 130/0.4 would have different effects on acute lung injury following hemorrhagic shock.

Methods: Twenty healthy pigs (19 ± 2 kg) were subjected to hemorrhage and were randomly allocated to two groups: Group A (10 pigs) who received lactated Ringer's solution and Group B (10 pigs) who received hydroxyethyl starch 130/0.4. Hemodynamic response and serum lactate were measured at predetermined phases. Four hours after fluid resuscitation animals were euthanized. Lungs were harvested, and tissue samples were collected. Focal thickening of the alveolar membranes, vascular congestion, number of activated neutrophils, alveolar edema, interstitial neutrophil infiltration, intraalveolar infiltration, and alveolar hemorrhage were assessed. Each feature was given a score from 0 to 3 (0 = absence, 3 = severe). The wet/dry ratio was also calculated, and with the use of Evans blue dye extravasation method, capillary permeability was assessed.

Results: The total histology score of Group A differed significantly from that of Group B, being significantly lower in Group B animals $P = 0.048$. The wet/dry weight ratio was significantly higher in the lactated Ringer's group (median [range]) (Group A, 5.1 [0.5]; Group B, 4.9

[0.3]; $P = 0.009$). The Evans blue dye extravasation method was utilized to study the lung capillary permeability. The animals in Group B showed a marked reduction in microvascular capillary permeability compared with the animals in Group A (Group A, 58.5 [21] mg/g; Group B, 51.5 [14] mg/g; $P = 0.017$).

Conclusions: Our study indicates that resuscitation after hemorrhagic shock with hydroxyethyl starch 130/0.4 led to less lung edema and less microvascular permeability in this swine model.

What We Already Know about This Topic

- ❖ Acute lung injury after trauma has multiple potential causes, including volume overload from transfused blood products and fluids

What This Article Tells Us That Is New

- ❖ In hypovolemic, anemic swine, administration of 6% hydroxyethyl starch 130/0.4 led to significantly less pulmonary edema and alveolar pathology than lactated Ringer's solution

TRAUMA represents 9% of worldwide deaths (more than five million deaths worldwide).¹ It has been well demonstrated that traumatic hemorrhage induces acute lung injury (ALI), which is associated with pulmonary edema due to an increase in capillary permeability and infiltration of inflammatory cells into the interstitium and airspaces. The mechanisms involved in hemorrhage-induced ALI include pulmonary inflammatory response after ischemia/reperfusion and some degree of oxidative stress to lung cells.² The incidence of ALI in severely injured trauma patients remains between 30% and 50%. The mortality rate of patients who develop ALI has been 10%, depending on the severity of their pulmonary dysfunction.³ Treatment of ALI should be aimed at reversing the underlying diseases associated with ischemia/reperfusion and modifying abnormalities in vascular permeability and inflammation.⁴

Several investigators have stated that the use of different resuscitation regimens may have an impact on the development of ALI after hemorrhagic shock, by affecting endothelial permeability and capillary leakage.^{3,5,6} Because hydroxyethyl starch 130/0.4 (HES 130/0.4) has been shown to have

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antiinflammatory properties after septic shock,^{7–11} we hypothesized that 6% HES 130/0.4 would also alleviate ALI after hemorrhagic shock. We therefore conducted a study designed to mimic the clinical situation of a patient with hemorrhage followed by fluid resuscitation.

Materials and Methods

Animal Preparation

The study was approved by the General Directorate of Veterinary Services (Athens, Greece) (permit no. K/8052/25-10-2007), according to the Greek legislation regarding ethical and experimental procedures. Twenty male Landrace/ Large-White piglets, aged 10–15 weeks and weighing 19 ± 2 kg, of conventional microbiologic status, all from the same breeder (Validakis, Athens, Greece), were included in the study. All animals were housed in single environmentally controlled cages. Animals were acclimatized to the laboratory conditions for 1 week before the experimentation and had free access to a commercially prepared food and water. The animals were fasted overnight before the experiment but had free access to water.

Sedation in each animal was achieved with intramuscular ketamine hydrochloride (Merial, Lyon, France) 10 mg/kg, midazolam (Roche, Athens, Greece) 0.5 mg/kg, and atropine sulfate (Demo, Athens, Greece) 0.05 mg/kg. Afterward, animals were transported to the operating room where intravenous access was achieved *via* catheterization of the marginal auricular vein. Anesthesia was induced with an intravenous bolus dose of propofol (Diprivan 1% w/v; Astra Zeneca, Luton, United Kingdom) (2.0 mg/kg) and fentanyl (Janssen, Pharmaceutica, Beerse, Belgium) (2 μ g/kg). While spontaneously breathing but anesthetized, the pigs were intubated with a 5.0 mm endotracheal tube (MLT™ 5.0 Oral 27 mm; Mallinckrodt, Pleasanton, CA). Auscultation and inflation of both lungs confirmed correct placement of the endotracheal tube. After securing the endotracheal tube on the upper jaw; hair was clipped from the ventral thorax to facilitate the use of self-adhesive electrodes.

The animals were then immobilized in a supine posture on the operating table. To ascertain synchrony with the ventilator, additional propofol 1 mg/kg, cis-atracurium (Nimbex 2 mg/ml; GlaxoSmithKline, Athens, Greece) 0.15 mg/kg, and fentanyl 0.01 mg/kg were administered. Then animals were mechanically ventilated (ventiPac; Sims Pneu-pac Ltd., Luton, United Kingdom) with 21% inspired oxygen. Anesthesia was maintained with infusion of propofol 150 μ g/kg per min. Normocapnia was achieved using continuous monitoring of end-tidal CO₂ (ETCO₂) (Nihon Kohden Corp., Bergamo, Italy), and the respiratory frequency was adjusted to maintain ETCO₂ 35–40 mmHg. Pulse oximetry (SpO₂) (Vet/Ox Plus 4700; Heska Corp., Fort Collins, CO) was also utilized as a means of peripheral tissue oxygenation and was monitored throughout the experiment. The pulse oximeter sensor was placed on the tongue of the anesthetized and intubated animal.

Five adhesive electrodes were attached to the ventral thorax to accommodate electrocardiographic monitoring (Mennen Medical, Envoy, Papapostolou, Athens, Greece) using leads I, II, III, aVR, aVL, and aVF while the heart rate was determined by the electrocardiographic signal. For measurement of the aortic pressure, a normal saline-filled (model 6523, USCI CR; Bart, Inc., Papapostolou, Athens, Greece) arterial catheter was inserted and forwarded into the descending aorta after surgical preparation of the right internal carotid artery. The systolic and diastolic pressures were recorded while mean aortic pressure (MAP) was determined by the electronic integration of the aortic blood pressure waveform.

The left internal jugular vein was surgically prepared, and a catheter was inserted (Opticath 5.5 F, 75 cm; Abbott, Athens, Greece) for exsanguination and fluid loading. The right internal jugular vein was also cannulated, and a catheter was inserted to measure central venous pressure. Intravascular catheters were attached to pressure transducers that were aligned to the level of the right atrium. This allowed the recording of central venous pressure and systolic and diastolic pressures of the aorta. Body temperature was monitored by a rectal temperature probe and was maintained between 38.5°C and 39.5°C with a heating blanket. Arterial blood gases were obtained using heparinized syringe for analyzing pH, hemoglobin, PO₂, PCO₂, base deficit, lactate, hematocrit, and electrolytes were measured using a blood-gas analyzer (Stat profile Critical Care Xpress; Nova Biomedical, Waltham, MA).

Experimental Protocol

The duration of the protocol was 8 h and was divided into five distinct phases.

Stabilization Phase (T1). After induction of anesthesia and instrumentation for hemodynamic variables, the animals were allowed to stabilize for 45 min. Once a steady state was achieved, baseline measurements including all the aforementioned variables were obtained.

Hemorrhagic Phase (T2). After data collection, infusion of propofol was reduced to 75 μ g \cdot kg⁻¹ \cdot min⁻¹. Acute hemorrhage was induced by blood withdrawal of 50% of the estimated total blood volume by stepwise volumes in 5-min intervals from the internal jugular vein. Total blood volume was estimated as 7% of total body weight. The procedure lasted approximately 20 min and was terminated when MAP decreased to 40–45 mmHg. All data were obtained after completion of blood withdrawal.

Maintenance Phase (T3). The animals were left in shock for an additional 90-min period during which no fluid was administered, but propofol infusion was maintained at 75 μ g \cdot kg⁻¹ \cdot min⁻¹. This 90-min delay before initiation of resuscitation allowed the animals to reach their nadir blood pressure and replicates the civilian trauma scenario.²

Infusion Phase (T4). At the end of the 90-min shock period and after obtaining hemodynamic parameters and arterial blood samples, animals were randomly allocated using sealed

envelopes into two groups, based on the type of infused fluid: Group A was resuscitated with lactated Ringer's solution in 20 min until MAP reached 90% of the baseline value. Group B received 6% HES 130/0.4 in 20 min, and infusion was stopped when MAP had reached 90% of the baseline value. To ensure blinding, the principal investigator covered the bottles of the resuscitation fluids with a nontransparent foil to ensure that the investigators involved in data collection were unaware as to the allocation of each animal.

Observation Phase (T5). During the ensuing 4 h the animals were left for observation, and no more resuscitation attempts were performed. MAP, central venous pressure, heart rate, and body temperature were monitored continuously throughout the five distinct time phases of the experiment. At the end of the observation phase, all animals were euthanized using intravenous solution of thiopental (2 g).

After the 4-h interval lung tissue samples were taken from dependent and nondependent parts of both lower and upper lobes. They were immersed in 10% paraformaldehyde for at least 72 h, dehydrated with graded alcohol, embedded in paraffin, and cut in a series of 0.4- μ m-thick slices that were stained with hematoxylin and eosin. Histologic analyses of tissue samples were performed in a blinded fashion by a pathologist according to a previously described scoring system.⁷ Features were focal thickening of the alveolar membranes, vascular congestion, number of activated neutrophils, alveolar edema, interstitial neutrophil infiltration, intraalveolar neutrophil infiltration, and alveolar hemorrhage. Each feature was assigned a score from 0 to 3 based on its absence (0) or presence to a mild (1), moderate (2), or severe (3) degree, and a total cumulative histology score was determined. To eliminate observer bias, the samples were evaluated simultaneously by two independent pathologists, blinded to the allocation of each animal.

To assess the lung wet/dry ratio, which represents the percentage of tissue water and is an index of tissue microvascular permeability, lung samples were also collected after the swine were euthanized, as described previously.⁹ Excess fluid was blotted from the samples, and wet weights were measured in the operating room. Dry weights were measured after drying samples at 80°C for 72 h.

Pulmonary capillary leakage was determined with the Evans blue dye extravasation method, as described previously.⁹ Two percent Evans blue dye (20 mg/kg; Sigma Chemical, St. Louis, MO) was injected *via* the jugular vein 15 min before swine euthanasia. The lung tissues were excised and weighed. To each sample of tissues, 4.0 ml formamide was added and incubated at 37°C for 24 h. If necessary, the incubation time was prolonged until the blue color of the samples completely disappeared. After filtration with a glass filter, the absorbance of the filtrate was measured at 620 nm in a Beckman spectrophotometer (Beckman Coulter, Inc., Brea, CA). The total amount of dye can be calculated by means of a standard calibration curve. Microvascular permeability in the lungs was shown as the micrograms (μ g) of Evans blue dye in every milligram (mg) of tissue. The pathol-

Table 1. Baseline Values of Each Variable in Both Groups

Variable	Group A	Group B	P Value
SAP (mmHg)	105.3 \pm 12.5	103.1 \pm 11	0.81
DAP (mmHg)	80.3 \pm 12.5	75.9 \pm 13.7	0.462
MAP (mmHg)	88.6 \pm 12.1	84.9 \pm 12.3	0.509
HR (beats/min)	116.5 \pm 13.8	107.9 \pm 18	0.246
CVP (mmHg)	8.2 \pm 1.9	7.4 \pm 1.9	0.346
Lactate (mM)	1.45 \pm 0.46	1.42 \pm 0.33	0.87

Data are presented as mean \pm SD.

CVP = central venous pressure; DAP = diastolic aortic pressure; HR = heart rate; MAP = mean aortic pressure; SAP = systolic aortic pressure.

ogists who assessed microvascular permeability were also blinded to the allocation of each animal.

Statistical Analysis

Statistical analyses were performed using SPSS for Mac, version 16.0 (SPSS, Chicago, IL). Results are presented as mean \pm SD for normally distributed variables; otherwise, they are presented as median (range). Student *t* test was applied for normally distributed variables; Mann-Whitney U test was applied for analysis of wet/dry ratio, lung capillary permeability (continuous variables), and histology scores (ordinal). Two-way ANOVA for repeated measures was used. To study the effect of time and group allocation on each of the variables, a 2 \times 5 mixed ANOVA model was used with group (two levels) as a between subjects factor and time (five levels) as a repeated measures factor. A Bonferroni correction was used for *post hoc* analysis. A *P* value < 0.05 (by two-tailed testing) was considered to indicate statistical significance. We conducted a pilot study to provide a database estimate of the current study. The population parameter for the effect size underlying the power analysis was estimated from a pilot study. In that pilot study, the means (for wet/dry ratio) in Groups A and B were 5.05 and 4.57, respectively, and the standard deviations were 0.31 and 0.36, respectively, such that the sample effect size was 1.39. Given this effect size and $\alpha = 0.05$, power analysis indicated that an allocation of 10 animals to each Group ($n_1 = n_2 = 10$) would permit detection of a wet/dry weight ratio for at least a power of 80% (actual power 82.2%). There were no missing data for any of the variables included in the analysis.

Results

There were no statistically significant differences between the two groups during T1 regarding hemodynamic variables and lactate concentration (table 1). Animals in Group A received (mean \pm SD) 785 \pm 32 ml lactated Ringer's solution, whereas animals in Group B received 310 \pm 42 ml HES 130/0.4, 6%. The hemodynamic response did not differ between the two groups (fig. 1). Blood lactate was increased in all animals after the hemorrhagic phase. However, this increase was more pronounced in Group A, reaching statistical significance only at the last phase (T5) of the experiment

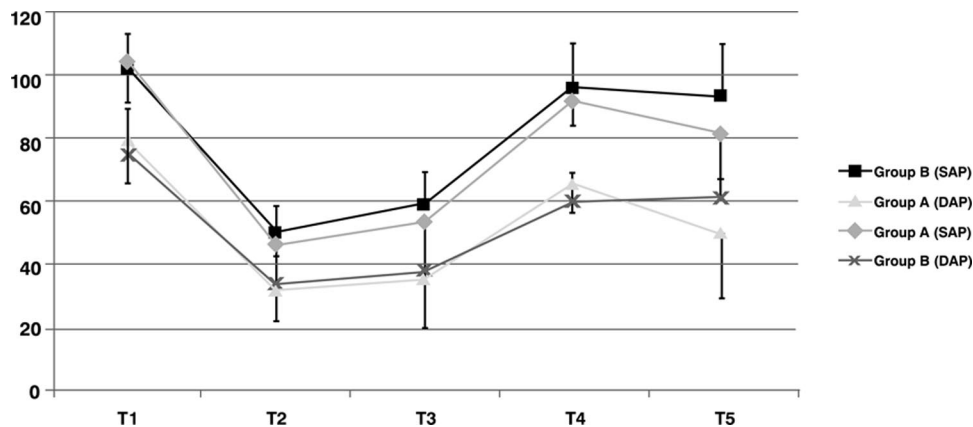


Fig. 1. Mean values of the hemodynamic parameters in mmHg during the different phases of the experiment. Bars = 1 SD. DAP = diastolic aortic pressure; SAP = systolic aortic pressure; T1 = stabilization phase; T2 = hemorrhagic phase; T3 = maintenance phase; T4 = infusion phase; T5 = observation phase.

(lactate level, 2.42 ± 1.77 mM for 6% HES group and 5.59 ± 0.94 mM for Group A ($P < 0.001$)) (fig. 2).

The total histology score of Group A differed significantly from that of Group B, being significantly lower in Group B animals, $P = 0.048$ (fig. 3). The degree of pulmonary neutrophil infiltration ($P = 0.044$) and intraalveolar edema was significantly increased in Group A animals. The typical histologic morphology of the two groups is depicted in figure 4.

The wet/dry weight ratio was significantly higher in the lactated Ringer's group (median [range]) (Group A, 5.1 [0.5]; Group B, 4.9 [0.3] $P = 0.009$). The Evans blue dye extravasation method was utilized to study the lung capillary permeability. Animals in Group B showed a marked reduction in microvascular capillary permeability compared with animals in Group A (fig. 5) (Group A, 58.5 [21] mg/g; Group B, 51.5 [14] mg/g; $P = 0.017$).

Discussion

The main goals of resuscitation after hemorrhagic shock are to stop the source of the hemorrhage and to restore circulating blood volume.¹¹ Actively bleeding patients should have

their intravascular fluid replaced because tissue oxygenation will not be compromised, even at low hemoglobin concentrations, as long as circulating volume is maintained.¹² The optimal type of fluid for intravascular volume resuscitation in critically ill patients has remained a matter of debate for several decades. On one hand with their higher molecular weight, colloids remain in the intravascular space longer and, therefore, provide more rapid hemodynamic stabilization than crystalloids, which on the other hand extravasate to a greater degree so that more fluids are required to achieve the same endpoints.¹³

The prototypical colloid is albumin, which is synthesized in the liver and is responsible for 80% of the oncotic pressure of the plasma. Frequently mentioned disadvantages of albumin include its critical short supply, the fact that it is a blood product, the theoretical risk of transmitting unknown infectious particles (such as prions), and, like all other colloids, its cost.^{14,15} Of the synthetic colloids, dextrans and gelatins are rarely considered for the resuscitation of trauma patients because of the anticoagulant properties of the first and the modest and short-lived effectiveness in expanding plasma

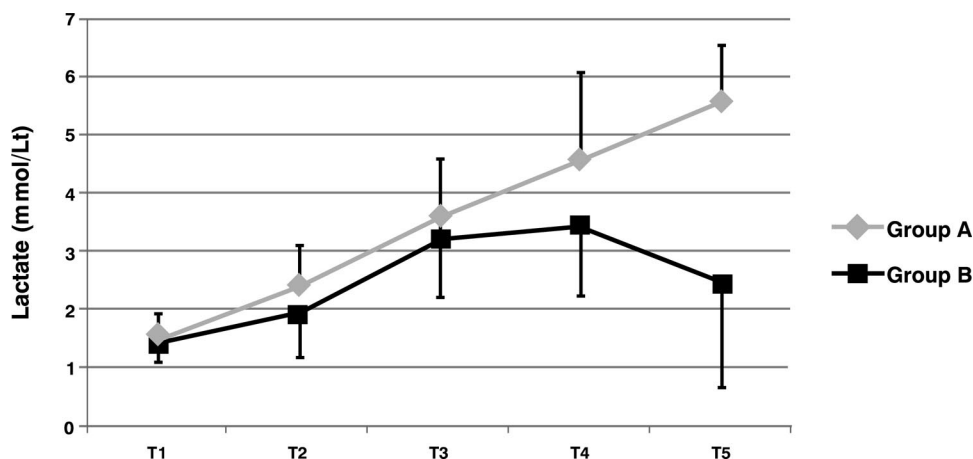


Fig. 2. Mean value of lactate fluctuating during the different phases of the experiment. Bars = 1 SD. T1 = stabilization phase; T2 = hemorrhagic phase; T3 = maintenance phase; T4 = infusion phase; T5 = observation phase.

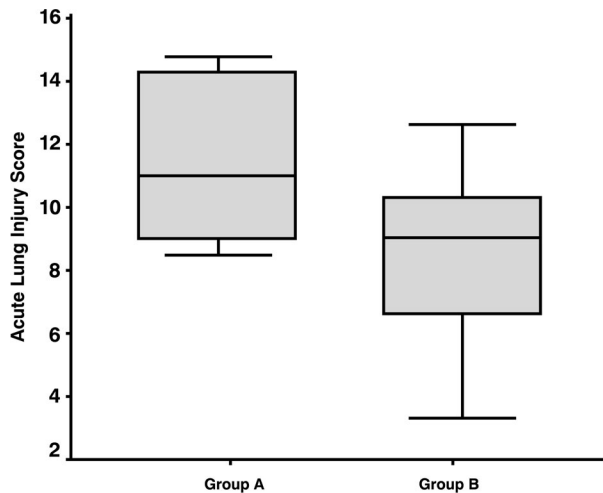


Fig. 3. Acute lung injury score in both groups. The *top* of the box represents the 75th percentile, the *bottom* of the box represents the 25th percentile, and the *line* in the *middle* represents the 50th percentile (median). The *lines* that extend out of the *top* and *bottom* of the box represent the highest and lowest values that are not outliers or extreme values.

volume of the latter.¹⁵ One of the most frequently used colloids is the group of HES solutions with lesser degrees of substitution with fewer negative effects.¹⁶ Besides their effectiveness in expanding plasma volume, they are readily available, carry no risk of transmitting infectious diseases, have minimal side effects, and are less expensive than albumin.¹⁵ Importantly, in addition to their effects as intravascular volume expanders, HES solutions may have other properties.¹⁷

There is increasing evidence that certain initial resuscitative strategies may be more effective than standard crystalloid resuscitation in protecting against the acute deleterious changes in organ and cellular function observed after trauma and major hemorrhage. HES has been shown to reduce the inflammatory response and interstitial edema in the lung caused by increased capillary permeability in various experimental studies.^{6,8,9,18} In a recent study, Di Filippo *et al.* reported that when compared with a crystalloid and a gelatin solution, the administration of HES reduced the inflammatory response and oxidative stress in the lung during experimentally induced ALI in rabbits.¹⁹ Furthermore, it has been postulated that HES may have anti-inflammatory properties for which a neutrophil-dependent mechanism is responsible.²⁰

ALI is characterized by increased alveolar-capillary permeability and hypoxemia.²¹ As the syndrome develops, an acute inflammatory response in the lung progresses to diffuse injury.²² This condition is associated with pulmonary edema due to an increase in capillary permeability and infiltration of inflammatory cells into the interstitium and airspaces and hypoxia secondary to the injury at the level of the endothelial basement membrane.²¹ Neutrophils play a central role in the development acute lung leak. Early in the course of ALI large numbers of neutrophils are sequestered in the lung microvasculature and migrate from the pulmonary capillaries. How-

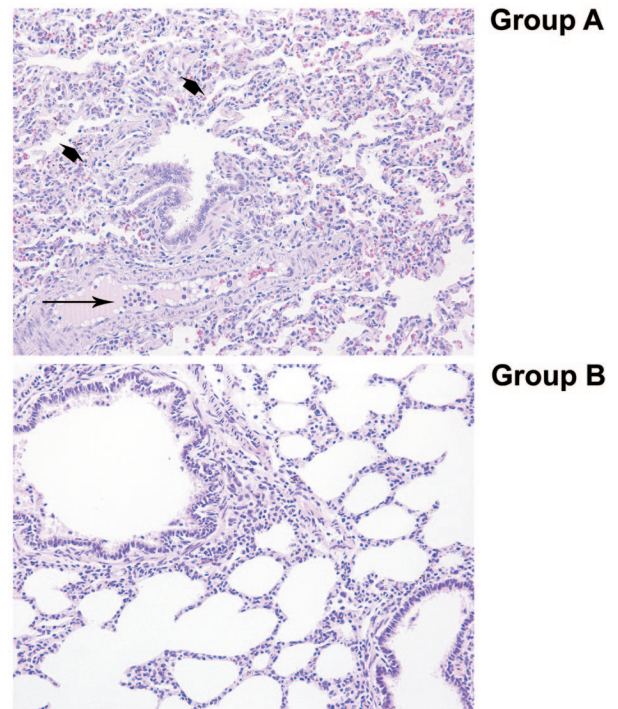


Fig. 4. Representative figures from Group A and Group B, hematoxylin and eosin stain; original magnification, $\times 100$. Group A: congested lung, with signs of recent hemorrhage (erythrocytes in the alveolar spaces) and infiltration of acute and chronic inflammatory cells, both within vessels (*arrow*) and in the interalveolar diaphragms (*arrowheads*). Group B: section demonstrating normal parenchymal architecture, with sparse inflammatory infiltrates. Two peripheral bronchial parts are also included, without any evidence of congestion or edema.

ever, it is important to note that sequestration alone is not sufficient for the development of lung injury.²² Adhesion and neutrophil activation are also required. Neutrophil adherence to endothelial cells is mediated predominantly through interactions between adhesion molecules on the endothelial surface and integrins on the leukocyte surface. Activated neutrophils migrate across the endothelial barrier, accumulate in surrounding tissue, and then release several toxic substances, including reactive oxygen species and proteolytic enzymes that cause capillary leak and lung injury. Histologically, the hallmark of ALI is the accumulation of neutrophils in the microvasculature of the lung.^{9,22}

To our knowledge, this is the first study to examine the role of 6% HES 130/0.4 in the development of acute lung injury after hypovolemia in a pig model of hemorrhagic shock. In the present study we chose a model of hemorrhage and resuscitation which is able to mimic the clinical situation, frequently encountered in the emergency and operating rooms. The results of the present study showed that resuscitation after hemorrhagic shock with 6% HES 130/0.4 resulted in less edema. The mechanism for this effect was not explored.

Recently, several studies have investigated the effects of HES on capillary permeability in acute endotoxemia and

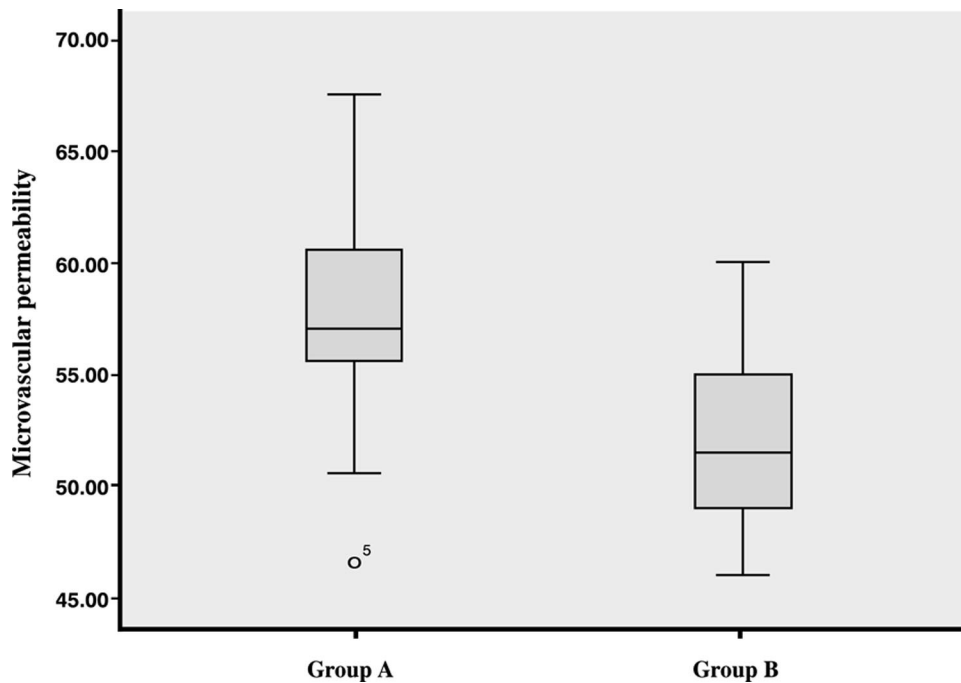


Fig. 5. Microvascular permeability in both groups (milligrams per gram). The *top* of the box represents the 75th percentile, the *bottom* of the box represents the 25th percentile, and the *line* in the *middle* represents the 50th percentile (median). The *lines* that extend out of the *top* and *bottom* of the box represent the highest and lowest values that are not outliers or extreme values. *Circle* shows an extreme value in Group A.

showed that its use may attenuate pulmonary capillary leakage.^{6,8,9} It is suggested that HES macromolecules act by physically sealing the barrier defects created by the injury. This hypothesis was formed on the basis that increased transport is associated with a widening of the interendothelial cleft in postcapillary venules.²³ Furthermore, it has been postulated that HES may attenuate capillary leakage by modulating inflammatory response, in a rat sepsis model induced by cecal ligation and perforation, by inhibiting inflammatory mediators and neutrophil recruitment and infiltration.^{8,9,24} Oz *et al.* demonstrated that HES might affect microvascular dysfunction by influencing neutrophil binding to stimulated endothelial cells.²⁵

It is already known that lactate values are a very reliable predictor of outcome in fluid resuscitation, a marker of inadequate perfusion, and a prognosis determinant in hypovolemic shock.²⁶ Consequently, the return of tissue perfusion results in rapid clearance of lactate in the liver. Hypoperfusion is the commonly proposed mechanism of lactic acidosis, but other mechanisms may be more important. A growing body of literature supports inflammation as the cause of increased lactate in several clinical situations (*e.g.*, sepsis, burns, trauma).²⁷ One likely explanation concerning the lower lactate levels measured after 6% HES 130/0.4 infusion may be the lower whole blood viscosity after administration of the particular solution compared with older starch preparations.²⁸

Our study has several limitations: first our model of controlled hemorrhage does not take into account the associated traumatic injuries. Second the hemodynamic response to

hemorrhage is affected by the general anesthesia and especially by propofol. It is well known that hemorrhagic shock alters the pharmacokinetics and pharmacodynamics of propofol by modifying its intercompartmental clearance. As a result, less propofol is required to achieve a desired drug effect during hemorrhagic shock.²⁹ Another significant limitation of this study is that no sham group was studied. However, the General Directorate of Veterinary Services would not grant us approval for such a group, as the expected mortality of the animals during the hemorrhagic phase would be extremely high. Thus, following the universally accepted three Rs (reduction, refinement, replacement) in experimental research, the smallest number of animals was sought to test our hypothesis. The rationale behind this was that a sham group would add little or no information to the main hypothesis of this study, but it would probably augment the number of used animals by 10. Because reduction of used animals in biomedical research is a primary goal, 10 more animals in a sham group would abolish the first R. The most significant limitation in this study was that lung injury was evaluated early after initiation of hemorrhagic shock. The observation phase in different studies varies from 3 to 24 h.^{7,30} Because in our study we observed significant differences between the two groups when evaluated 4 h after fluid infusion, this delay was sufficient to detect any differences, should these differences exist in the first place. We are unable to comment whether the detected differences would have been attenuated or remained the same if the animals had been kept alive longer. Furthermore, because this is a swine study, its data should not be extrapolated to humans.

Within these limitations, we conclude that the current experimental data from our study indicate that resuscitation after hemorrhagic shock with HES led to less pulmonary edema and less microvascular permeability in this swine model. However, further experimental research is needed to clarify the precise underlying mechanisms and potential benefits of 6% HES 130/0.4. Furthermore, these resuscitation strategies must prove their efficacy in the clinical arena, and therefore further randomized trials will be needed. The results of these studies will hopefully advance and improve the early care of severely injured patients.

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