Effects of Intravascular Volume Replacement on Lung and Kidney Function and Damage in Nonseptic Experimental Lung Injury

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ABSTRACT

Background: Intravascular volume replacement is often required in the presence of increased pulmonary capillary leakage, for example in patients with volutrauma with major hemorrhage. In the present study, the effects of Ringer's acetate (RA), gelatin-polysuccinate (GEL), and a modern hydroxyethyl starch (HES, 6% 130/0.42) on lung and kidney function and damage were compared in a two-hit model of acute lung injury. The authors hypothesized that GEL and HES, compared to RA: (1) reduced lung histological damage, (2) impaired kidney morphology and function.

Methods: Acute lung injury was induced in 30 anesthetized pigs by tidal volumes approximately 40 ml/kg, after saline lung lavage. Protective ventilation was initiated and approximately≈25% of estimated blood volume was drawn. Animals were randomly assigned to receive RA, GEL, or HES (n = 10/group) aimed at approximately 90% of intrathoracic blood volume before blood drainage.

What We Already Know about This Topic

- Keeping intravascular volume decreased in acute lung injury improves outcomes but is difficult during major hemorrhage

What This Article Tells Us That Is New

- Intravascular volume expansion with hydroxyethyl starch led to less lung injury compared to Ringer's acetate and less renal damage than gelatin-polysuccinate in experimental acute lung injury after major hemorrhage

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Results: Fluid volumes were higher with RA (2,250 ± 764 ml) than GEL (704 ± 159 ml) and HES (837 ± 82 ml) ($P < 0.05$). Compared to RA, HES reduced diffuse alveolar damage overall, and GEL in nondependent zones only. GEL and HES yielded lower wet-to-dry ratios compared to RA (6.5 ± 0.5 and 6.5 ± 0.6 *vs.* 7.9 ± 0.9, respectively, $P < 0.05$). HES and RA resulted in less kidney damage than GEL, but kidney function did not differ significantly among groups. Compared to GEL, HES yielded lower lung elastance (55 ± 12 *vs.* 45 ± 13 cm H_2O/l, $P < 0.05$) and intra-abdominal pressure (15 ± 5 *vs.* 11 ± 4 cm H_2O, $P < 0.05$).

Conclusions: In this model of acute lung injury, intravascular volume expansion after major hemorrhage with HES yielded less lung damage than RA and less kidney damage than GEL.

LOW tidal volume (V_T) ventilation combined with moderate-to-high levels of positive end-expiratory pressure (PEEP) improves survival in patients with acute lung injury/acute respiratory distress syndrome (ALI/ARDS).[1] Expansion of intravascular volume is often used to improve the hemodynamic instability induced by PEEP, but may exacerbate lung injury.[2] In fact, it has been shown that in ALI/ARDS patients a restrictive intravascular volume expansion approach increased ventilator- and intensive care unit-free days,[3] but the controversy regarding the use of crystalloids *versus* colloids was not assessed.[4]

A recent meta-analysis[5] and different clinical trials[6,7] comparing the use of crystalloids *versus* colloids in critically ill patients found no differences in outcome. However, several beneficial effects of colloids compared to crystalloids have been shown in the lungs, namely reduced alveolar-capillary permeability,[8] less histological damage,[9] and decreased inflammatory cell infiltration.[10,11] Furthermore, colloids may allow faster hemodynamic stabilization compared to crystalloids.[2,12] On the other hand, colloids have been implicated in kidney injury and dysfunction,[13] especially in the presence of sepsis.[14] However, the effects of crystalloids and colloids on lungs and kidneys in nonseptic ALI, for example ventilator-induced lung injury or chest trauma associated with hemorrhage, are not well understood.

The aim of this study was to compare the effects of one commonly used crystalloid (Ringer's acetate [RA]) and two modern colloids (gelatin-polysuccinate [GEL], and hydroxyethyl starch [HES], both in RA) on histological damage, pulmonary edema, lung and kidney function, and inflammatory response in a nonseptic, two-hit model of ALI. Given the differences in oncotic pressure among these fluids, we expected that less colloid than crystalloid would be necessary to maintain hemodynamic stability, resulting in different degrees of pulmonary edema and deterioration of lung mechanics. On the other hand, colloids can induce the death of human proximal tubular cells,[15] which could impair renal function, but such effects seem to be more pronounced with starch than gelatin solutions. The primary hypothesis of the present study was that GEL and HES would lead to less lung histological damage than RA. The secondary hypothesis was that HES would lead to more renal damage and dysfunction than RA and even GEL.

Material and Methods

Figure 1 illustrates the sequence of interventions performed, which have been approved by the local Animal Care Committee (Landesdirektion Dresden, Dresden, Saxony, Germany) and are described in detail in this section.

Anesthesia and Mechanical Ventilation

Animals were premedicated intramuscularly with 10 mg/kg ketamine (Ketamin-ratiopharm; Ratiopharm, Ulm, Germany) and 1 mg/kg midazolam (Midazolam, Ratiopharm), intubated with a cuffed 8.0-mm ID endotracheal tube and mechanically ventilated (EVITA XL, Dräger Medical, Lübeck, Germany). Anesthesia was maintained by means of continuous intravenous infusion

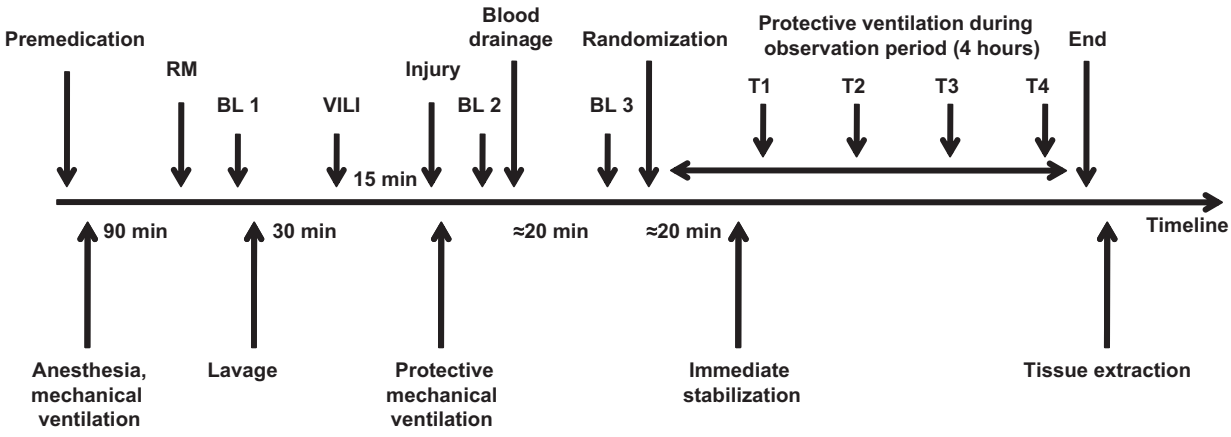

Fig. 1. Time course of interventions. BL = baseline; RM = recruitment maneuver; VILI = ventilator-induced lung injury; T1-T4 = measurement time points during the observation period of 4 h.

of midazolam (1–2 mg kg^{-1} h^{-1}) and ketamine (10–20 mg kg^{-1} h^{-1}). Muscle paralysis was achieved by continuous administration of atracurium (1–2 mg kg^{-1} h^{-1}). Animals were kept in the supine position during the whole experiment. Volume status was maintained with a continuous infusion of RA (Ringer-Acetat-Lösung Bernburg, Serumwerk Bernburg AG, Bernburg, Germany) at 10 ml kg^{-1} h^{-1}. Until induction of ALI, animals were ventilated in volume-controlled mode with the following settings: fraction of inspired oxygen (FIO_2) = 1.0; V_T = 10 ml/kg; PEEP = 5 cm H_2O; inspiratory to expiratory time ratio (I:E) = 1:1; the respiratory rate (RR) was adjusted to achieve a $PaCO_2$ in the range of 35–45 mmHg. After injury, ventilation was changed to pressure-controlled ventilation with FIO_2 = 0.5; V_T = 6 ml/kg, PEEP = 16 cm H_2O and I:E = 1:1. RR was adjusted to ensure an arterial pH > 7.30. Those ventilator settings, especially the PEEP/FIO_2 combination, were adapted from the ARDS Network Assessment of Low Tidal Volume and Elevated End-expiratory Volume to Obviate Lung Injury Trial according to the higher PEEP level arm.[16]

Instrumentation and Induction of ALI

A PiCCO (Pulsion Medical Systems, Munich, Germany) and a pulmonary artery catheter (Opticath, Abbott, Abbott Park, Chicago, IL) were inserted through the right carotid artery and the external jugular vein, respectively. The airflow signal was acquired from the internal flow sensor of the ventilator through a serial interface. The airway pressure was measured at the proximal end of the endotracheal tube with a T-piece connected to a differential pressure transducer (163PC01D48-PCB, Sensortechnics GmbH, Puchheim, Germany). Esophageal pressure was measured with a balloon catheter (Erich Jaeger, Höchberg, Germany) that was advanced into the mid chest and connected to another differential pressure transducer (163PC01D48-PCB, Sensortechnics GmbH). For acquisition of airway flow, as well as airway and esophageal pressures, a LabVIEW-based data acquisition system[17] (National Instruments, Austin, TX) was used, as described elsewhere.[18]

Estimation of intra-abdominal pressure was obtained with a balloon pressure probe (Erich Jäger) filled with 5 ml of distillated water and placed between the bladder and the vesicouterine excavation after a mini-laparotomy, connected to a differential pressure transducer (BD DTXPlus™, Becton Dickinson, NJ), and zeroed at the mid-axillary line. A urinary catheter was inserted into the bladder through the mini-laparotomy.

ALI was induced by means of repeated lung lavage (30 ml/kg) with warm (39°C) 0.9% saline followed by mechanical ventilation with increased V_T (ventilator-induced lung injury) as suggested elsewhere.[19] Lung lavage was performed until PaO_2/FIO_2 stabilized at < 200 mmHg for 30 min,[20] in order to prime lungs for a second insult, namely mechanical ventilation with high V_T. The second hit, or ventilator-induced lung injury, was accomplished by mechanical ventilation with the following settings, which were maintained for 5 min: pressure-controlled mode, driving pressure targeted at $V_T \approx 40$ ml/kg, but not higher than 60 cm H_2O, PEEP = 0 cm H_2O, RR = 10 breaths/min, and FIO_2 = 1.0. Following that, the previous ventilator settings were resumed, resulting in PaO_2/FIO_2 < 100 mmHg.

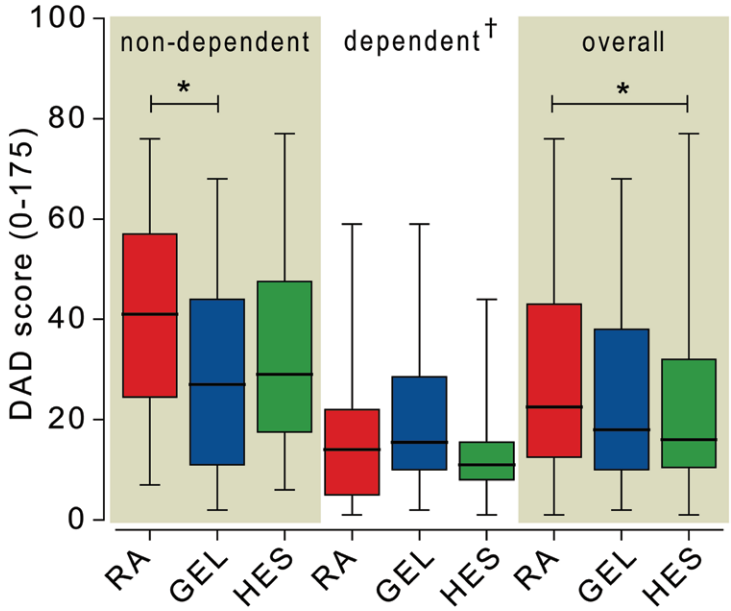

Fig. 2. Diffuse alveolar damage score. Values are shown as box-plots (median, interquartile range, minimum and maximum). Statistical analysis was performed using a mixed linear model with adjustment for repeated measures according to the Tukey Kramer procedure. Statistical significance was accepted at $P < 0.05$; *$P < 0.05$ *versus* RA. † = $P < 0.001$ *versus* nondependent. Dependent = gravitational dependent lung regions (dorsal); nondependent = gravitational nondependent lung regions (ventral). GEL = gelatin-polysuccinate in RA; HES = hydroxyethyl starch in RA; RA = Ringer's acetate.

Table 1. DAD Score

DAD Score	Region	RA	GEL	HES
Intraalveolar edema	Overall	1 (0,4)	1 (0,2)	1 (0,2)*
Interstitial edema	Overall	3 (0,4)	3 (1,6)	2 (1,4)
Hemorrhage	Overall	2 (0,6)	1 (0,2)	1 (0,4)
Inflammatory infiltration	Overall	6 (2,9)	4 (2,8)	4 (2,6)
Epithelial destruction	Overall	4 (1,11)	4 (1,9)	2 (1,6)
Microatelectasis	Overall	4 (1,6)	3 (1,5)	2 (1,6)
Overdistension	Overall	4 (2,6)	2 (1,6)	3 (1,6)
Intraalveolar edema	Nondependent	4 (0,6)	1 (0,4)	2 (0,6)
Interstitial edema	Nondependent	4 (2,6)	3 (1,6)	2 (1,4)
Hemorrhage	Nondependent	5 (2,6)	1 (0,6)*	3 (1,6)
Inflammatory infiltration	Nondependent	8 (5,12)	6 (2,9)*	6 (4,9)
Epithelial destruction	Nondependent	9 (4,15)	4 (1,9)	4 (1,15)
Microatelectasis	Nondependent	5 (4,6)	4 (1,6)	4 (2,6)
Overdistension	Nondependent	6 (5,8)	4 (2,9)	4 (2,6)
Intraalveolar edema	Dependent†	1 (0,2)	1 (0,2)	0 (0,1)
Interstitial edema	Dependent†	1 (1,4)	2 (1,4)	1 (1,3)
Hemorrhage	Dependent†	1 (0,2)	0 (0,2)	0 (0,1)
Inflammatory infiltration	Dependent†	3 (2,6)	3 (2,6)	3 (2,4)
Epithelial destruction	Dependent†	2 (1,6)	4 (1,6)	1 (1,2)‡
Microatelectasis	Dependent†	2 (1,4)	2 (1,4)	1 (1,2)
Overdistension	Dependent†	2 (1,4)	2 (0,4)	1 (0,3)

Values are given as medians and interquartiles. Statistical analysis was performed using the mixed linear model with adjustment after Tukey Kramer procedure. The global significance level was $\alpha = 0.05$ for each model.

* $P < 0.05$ *versus* RA; † $P < 0.001$ *versus* nondependent; ‡ $P < 0.05$ *versus* GEL.

DAD = diffuse alveolar damage; Dependent = gravitational dependent lung regions (dorsal); GEL = gelatin-polysuccinate in RA; HES = hydroxyethyl starch in RA; Nondependent = gravitational nondependent lung regions (ventral); RA = Ringer's acetate.

Blood Gases and Hemodynamics

Arterial and mixed venous blood samples were analyzed using a standard blood gas analyzer (ABL 505; Radiometer, Copenhagen, Denmark). Oxygen saturation and hemoglobin concentration were measured using an OSM 3 Hemoximeter (Radiometer) calibrated for swine blood. Heart rate, mean arterial blood pressure, central venous pressure, pulmonary capillary wedge, and mean pulmonary arterial pressures were measured using a standard monitor (IntelliVue Patient Monitor MP 50 Philips, Böblingen, Germany). Cardiac output from the PiCCO system and pulmonary artery catheter were measured simultaneously as the average of three repeated injections of 10 ml iced saline into the proximal lumen of the pulmonary artery catheter. Extravascular lung water, global end-diastolic volume, and intrathoracic blood volume, were determined using PiCCO algorithms and normalized to the body surface area (extravascular lung water index, global end-diastolic volume index, and intrathoracic blood volume index [ITBVI], respectively), as previously reported.[21] The PiCCO system has been proven reliable for hemodynamic monitoring in pigs with similar weight ranges.[22,23]

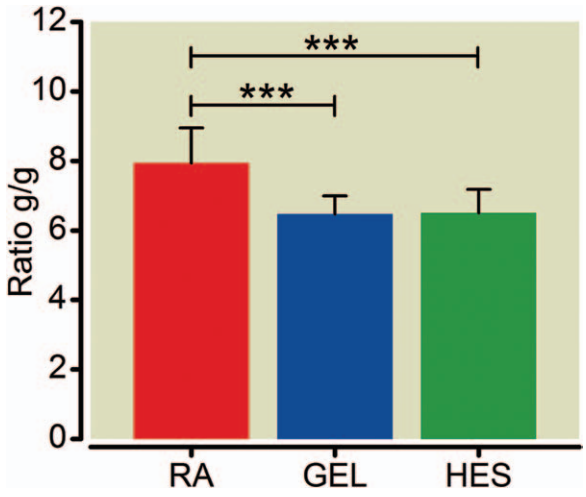

Fig. 3. Wet-to-dry ratio (W/D). Values are shown as mean and standard deviation. Difference among groups was tested using one-way ANOVA. Statistical significance was accepted at $P < 0.05$; *** $P < 0.001$ *versus* RA. GEL = gelatin-polysuccinate in RA; HES = hydroxyethyl starch in RA; RA = Ringer's acetate.

Respiratory System and Lung Mechanics

The elastance and resistance of the respiratory system (E_{RS} and R_{RS}, respectively) and lungs (E_L and R_L, respectively) were calculated offline from continuous recordings (5 min) of airway pressure, esophageal pressure and airway flow using the equation of motion, as described elsewhere.[24]

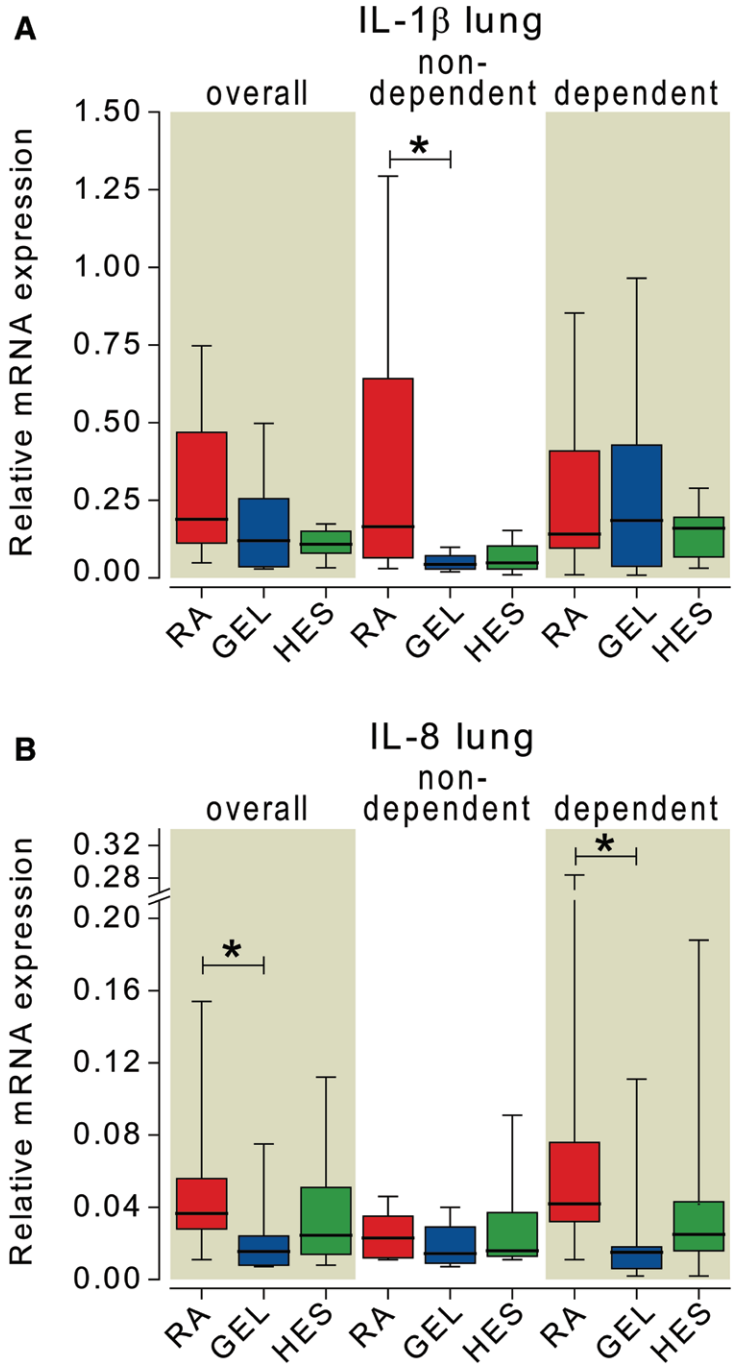

Fig. 4. Relative messenger(m) RNA expression of interleukin(IL)-1β (*A*) and IL-8 (*B*) in lung tissue.Values are given as box plot (median, interquartile range, minimum, and maximum). IL-1β, and IL-8 values were analyzed by Kruskal-Wallis test followed by Dunn's multiple comparison tests. Statistical significance was accepted at $P < 0.05$; *$P < 0.05$ *versus* RA. GEL = gelatin-polysuccinate; HES = hydroxyethyl starch in RA; RA = Ringer's acetate.

Protocol for Measurements

Once instrumentation was completed, a lung recruitment maneuver with an airway pressure of 30 cm H_2O for 30 s was performed. After a stabilization period of 15 min, baseline 1 (BL1) measurements were obtained and ALI was induced. Measurements in injured lungs were then obtained (Injury), and the lung protective ventilatory strategy with high PEEP was initiated. After a further stabilization period of 15 min, measurements were repeated (BL2), and animals were submitted to blood drainage of approximately 25% of the estimated circulatory blood volume[25] through the arterial line, which lasted approximately 20 min. Immediately after the completion of blood drainage, hemodynamic and lung function variables were measured (BL3), and the continuous infusion of RA was reduced to 2 ml/kg/h. The time under hypovolemia was kept as short as possible and animals were then randomly assigned

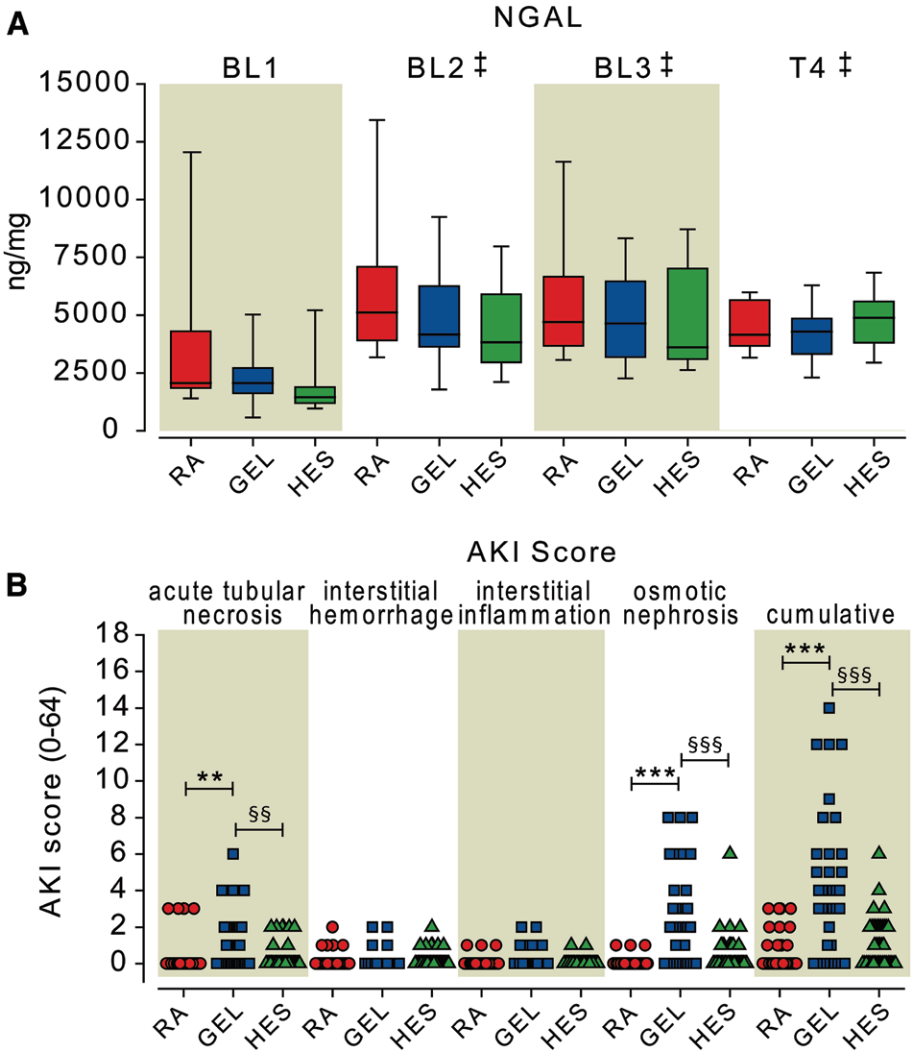

Fig. 5. Time course of neutrophil gelatinase-associated lipocalin (NGAL) in plasma (*A*), and acute kidney injury (AKI) score (*B*). Values of NGAL are given as box-plots (median, interquartile range, minimum, and maximum) in figure *5A*, while scatter values of AKI are shown in figure *5B*. Statistical analysis was performed with Kruskal-Wallis test followed by Dunn's multiple comparison tests (for NGAL), and mixed linear model with adjustment for repeated measures according to the Tukey Kramer procedure (for AKI Score). Statistical significance was accepted at $P < 0.05$; $^{**}P < 0.01$ *versus* RA; $^{***}P < 0.001$ *versus* RA. §§$P < 0.01$ *versus* HES; §§§$P < 0.001$ *versus* HES. BL1 = Baseline 1, at begin of experiments; BL2 = Baseline 2, following induction of lung injury; BL3 = Baseline 3, after blood drainage and preceding randomization; T4 = 4-h observation period. GEL = gelatin-polysuccinate in RA; HES = hydroxyethyl starch in RA; RA = Ringer's acetate.

to one of three groups of intravascular volume replacement (n = 10/group): (1) RA; (2) GEL (4% gelatin-polysuccinate in Ringer's acetate, Gelafusal®, Serumwerk Bernburg AG, Bernburg, Germany); and (3) HES (HES 6% 130/0.42 in Ringer's acetate, Vitafusal® 6%, Serumwerk Bernburg AG). Volume loading was performed to achieve an ITBVI around approximately90% of its BL2 level, in order to avoid hypervolemia, and lasted also approximately 20 min (immediate stabilization). Once the ITBVI target was achieved, RA, GEL, and HES infusion rates were maintained as low as possible to keep the ITBVI approximately constant, while maintaining mean arterial pressure ≥ 60 mmHg. Animals were ventilated for 4 h with unchanged ventilator settings and measurements were obtained every hour (Time 1–4).

Post Mortem Analysis

At the end of the observation period, heparin was administered (1,000 IU/kg i.v.) (Ratiopharm, Ulm, Germany) and animals were killed by i.v. injection of 2 g thiopental (Inresa, Arzneimittel GmbH, Freiburg, Germany) and 50 ml KCl 1 M (Serumwerk Bernburg AG). Lungs and kidneys were removed for further processing. Samples from gravitationally dependent and nondependent areas of the right lower lung lobe, as well as from the upper pole of the right kidney were snap-frozen in liquid nitrogen and stored at −80°C until further analysis.

Bronchoalveolar lavage fluid was then obtained from the right upper lobe by lavage with 50 ml 0.9 % saline solution.

Wet/Dry Ratio

The right middle lobe was weighted (wet weight) and dried in a microwave as described elsewhere (dry weight).[26] The wet-to-dry ratio was then calculated.

Histology

The left lung was perfused with 4% buffered formaldehyde solution while a continuous positive pressure of 16 cm H_2O, that is, equivalent to the PEEP value during the observation period, was maintained at the airways. Lung tissue samples of approximately 8 cm^3 were taken from gravitational dependent (dorsal – lung segment 2 - posterior) and nondependent zones (ventral – segment 3 - anterior) of the right upper lobe. Following perfusion fixation and immersion in 4% buffered formaldehyde solution for 7 days, tissue samples were embedded in paraffin, cut into slices of 5 µm thickness and stained with hematoxylin-eosin for histological analysis.

Photomicrographs at magnifications of × 25, × 100, and × 400 were obtained from four nonoverlapping fields of view per section using a light microscope. Diffuse alveolar damage (DAD) was quantified using a weighted scoring system, as described elsewhere.[17] Briefly, values from 0 to 5 were used to represent the severity of alveolar edema, interstitial edema, hemorrhage, inflammatory infiltration, epithelial destruction, microatelectasis and overdistension, with 0 standing for no effect and 5 for maximum severity. Additionally, the extent of each score characteristic per field of view was determined with values of 0–5, with 0 standing for no appearance and 5 for complete involvement. Scores were calculated as the product of severity and extent of each feature, being situated in the range 0–175.

The left kidney was perfused with Dulbecco's phosphate buffered saline solution and fixed in 4% buffered formaldehyde solution. Following that, tissue samples were taken from the upper pole and evaluated with a scoring system (AKI score), which assessed the severity and involvement of typical histological features as described elsewhere,[27] but slightly modified to include a weighing system for the features investigated. Briefly, in the scoring system values from 0 to 4 represented the severity of the feature, as follows: 0 – normal appearance, 1 – minimum lesion, 2 – weak damage signs, 3 – moderate damage, 4 – strong damage. A further part of our system was used to describe the extent of involvement in each field of view, as follows: 0 – lack of involvement of the feature, 1 – up to 25 %, 2 – 25 to 50 %, 3 – 50 to 75 %, and 4 – 75 to 100 %. For each feature evaluated, severity was multiplied by the extent, leading to values in the range of 0–16.

Scoring of lung and kidney damage was conducted by an anatomy expert (MK), who was blinded to groups.

Inflammatory Mediators and Cell Stress Markers

The messenger RNA (mRNA) expression of tumor necrosis factor-α, interleukin (IL)-1β, IL-6, IL-8, amphiregulin, tenascin-c, intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and E-selectin was quantified in lung tissue samples using quantitative real-time polymerase chain reaction cyclophilin A and ß2-microglobulin served as housekeeping genes. Protein levels of tumor necrosis factor-α, IL-6, and IL-8 were measured in blood, lung tissue as well as bronchoalveolar lavage fluid using commercial enzyme linked immunosorbent assay kits (R&D Systems, Wiesbaden, Germany) according to the manufacturer's instructions.

Markers of Kidney Injury and Apoptosis

Neutrophil gelatinase-associated lipocalin (NGAL) levels in plasma were measured by means of enzyme linked immunosorbent assay (K044, Bio Porto Diagnostics, Gentofte, Denmark) according to the manufacturer's instructions. The mRNA levels of Caspase 3 and Bcl-2 homology domain (BH3) interacting domain death agonist (Bid) were measured in samples of the upper pole of the right kidney by quantitative real-time polymerase chain reaction.

Statistical Analyses

The sample size calculation for testing the primary hypothesis (DAD is reduced after administration of the colloids compared to the crystalloid after hemorrhage in this two-hit model of ALI in pigs) was based on effect estimates obtained from pilot studies, as well as previous data of our group on the impact of mechanical ventilation on DAD in a model of experimental ALI (mean value and dispersion, respectively).[17] Accordingly, we expected that a sample size of 10 animals per group would provide the appropriate power ($1-\beta = 0.8$) to identify significant ($\alpha = 0.05$) differences in DAD among the fluid therapies, taking an effect size $d = 1.6$, equal number of animals per group, two-sided test, and multiple comparisons ($n = 3$) into account ($\alpha^* = 0.0167$, α^* Bonferroni adjusted).

Statistical analysis was performed using SPSS (v. 17.0, Chicago, IL) and SAS (v. 9.2, procedure mixed, SAS Institute, Cary, NC). Each variable was tested for normal distribution using a D'Agostino-Pearson normality test. Data were presented as mean ± SD unless otherwise specified.

To test the primary hypothesis, we used a linear mixed model for repeated measures (compound symmetry, repeated covariance type), including field of view and region (nondependent *vs.* dependent zones) as repeated, independent variables, fluid therapy as fixed, independent variable, as well as their significant interactions, to analyze differences in the dependent variable DAD score. Adjustments for repeated measures were performed according to the Tukey Kramer procedure. Residual plots were used to examine model requirements. Other comparisons were explorative in nature.

For functional variables, comparability of groups at BL1, Injury, BL2, and BL3 was tested with one-way ANOVA followed by a Bonferroni *post-hoc* test, or H-Test (Kruskal-Wallis) followed by a Dunn's *post-hoc* test, as appropriate. Differences in hemodynamics, gas exchange, and respiratory variables

between BL1 and injury, as well as BL2, BL3, and T1 were tested with two-tailed paired *t* tests. *P*-values were adjusted for multiple comparisons according to Bonferroni. Differences among and within groups (time effect T1 to T4) were tested with general linear model statistics using BL2 as the covariate, and adjusted for repeated measurements according to the Bonferroni procedure. BL2 was chosen as the covariate because it was the first time point after the beginning of low V_T with high PEEP, and the last time point preceding the blood drainage. This time point mimicked the beginning of hemorrhage under protective ventilation, where imbalances could affect the time course, and therefore, must be taken into account. The global significance level for all performed tests was $\alpha = 0.05$.

Results

Thirty female juvenile pigs (28.4–42.8 kg) were included in the study. Body weight, total amount of blood drawn and duration of drainage did not differ significantly among groups. The amount of fluid required to achieve and maintain the target ITBVI was higher with RA (2,250 ± 764 ml) than GEL (704 ± 159 ml) and HES (837 ± 82 ml) ($P < 0.05$).

Compared to RA, GEL was associated with a lower DAD score in ventral zones (fig. 2), mainly due to reduced inflammatory infiltrate and hemorrhage (table 1). In addition, HES led to a lower overall DAD score than RA, mainly due to reduced intraalveolar edema (table 1). Volume replacement with both colloids was associated with a lower wet-to-dry ratio than RA (fig. 3). As shown in figure 4, the mRNA expression of IL-1β in ventral zones was lower with GEL than RA. In addition, the overall mRNA expression of IL-8 was lower with GEL compared to RA, mainly in dorsal zones. Gene expression and protein levels of other markers of inflammation and/or cell mechanical stress did not differ significantly among groups.

The overall AKI score was lower with RA and HES than GEL (fig. 5), mainly due to increased acute tubular necrosis and osmotic nephrosis, but the mRNA expression of caspase 3 and Bid in kidney did not differ significantly among groups. Also, urine output and NGAL levels in plasma did not differ significantly among groups throughout the experiments.

Hemodynamic (table 2) and gas exchange (table 3) variables did not differ significantly among groups. As depicted in table 4, HES was associated with decreased E_L compared to GEL, as well as lower intra-abdominal pressure than RA and GEL (table 4).

Discussion

The major finding of this study was that in a nonseptic model of ALI, GEL and HES reduced DAD compared to RA, confirming our primary hypothesis. Furthermore, both colloids decreased wet-to-dry ratios compared to the crystalloid. In kidneys, HES and RA were associated with less acute tubular necrosis and osmotic nephrosis than GEL, challenging our secondary hypothesis.

Relevance of the Acute Lung Injury Model

The combination of saline lung lavage and ventilator-induced lung injury reproduces many histological features seen in ALI/ARDS, especially alveolar hemorrhage, hyaline membrane, neutrophilic infiltration, epithelium and endothelium damage,[28] as well as capillary stress failure.[29] Together with blood drainage, this model may mimic clinical scenarios that are relevant to Anesthesiologists, for example, patients with volutrauma or chest trauma requiring hemodynamic stabilization with fluids due to hemorrhage or intravascular volume shifts, which are seen in operation theatres and intensive care units.

Choice of Fluid Replacement Therapy

Different types of crystalloids and colloids can be used for expanding the circulatory blood volume. Among the crystalloids, Ringer's lactate is frequently chosen since it avoids hyperchloremic acidosis that usually accompanies the use of saline.[30] We opted for RA because acetate has potential advantages compared to lactate, including: (1) faster metabolization; (2) lower respiratory quotient; and (3) lack of effect on gluconeogenesis, with lower blood levels of glucose.[31] On the other hand, acetate-based solutions may promote vasodilatation and hypotension during hemodialysis.[32] Also, acetate can impair the fatty acid metabolism of muscle cells, reducing adenosine triphosphate and the contractile force of the myocardium, but such deleterious effects seem to occur only at relatively high plasma concentrations.[33]

Colloids are able to better expand the circulatory blood volume due to their higher colloid osmotic pressure compared to crystalloids.[34,35] HES and GEL were chosen because of controversies related to immunomodulatory effects[36] and kidney damage.[37] We used these particular colloids also because they are dissolved in acetate containing solutions. A modern HES solution of tetrastarch type has been chosen because it appears to be less associated with kidney injury than a pentastarch one in experimental endotoxemic shock.[38]

Effects of Fluid Replacement on Functional Parameters and Lung Damage

Using a volume-based surrogate of cardiac preload, namely ITBVI, we found that more crystalloids than colloids were necessary to maintain hemodynamic stability. This finding was not unexpected, and is in agreement with previous reports that used other surrogates of cardiac preload to guide fluid therapy.[39,40]

Gas exchange did not differ significantly among groups, but intra-abdominal pressure and E_L were lower in HES than GEL. In a saline lung lavage model of ALI in rabbits, Di Fillipo *et al.*[10] found that intravascular replacement with a modern HES solution resulted in improved oxygenation than a modified gelatin and RA. Differences in severity of

Table 2. Hemodynamics

Variable	Group	BL 1	Injury	BL 2	BL 3	Time 1	Time 2	Time 3	Time 4	BL 1 *versus* Injury	Injury *versus* BL 2	BL 2 *versus* BL 3	BL 3 *versus* Time 1	Time Effect	Group Effect	Time x Group Effect
HR (1/min)										$P = 0.047$	$P = 0.103$	$P < 0.001$	$P = 0.004$	$P = 0.24$	$P = 0.61$	$P = 0.14$
	RA	93 ± 14	89 ± 16	94 ± 17	115 ± 20	102 ± 19	105 ± 21	101 ± 21	97 ± 21							
	GEL	95 ± 11	86 ± 15	87 ± 16	104 ± 23	99 ± 16	103 ± 19	101 ± 20	98 ± 18							
	HES	102 ± 11	97 ± 11	102 ± 16	125 ± 29	112 ± 21	119 ± 25	118 ± 26	117 ± 28							
MAP (mmHg)										$P = 0.076$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.004$	$P = 0.881$	$P = 0.049$
	RA	73 ± 12	78 ± 11	74 ± 12	59 ± 13	72 ± 13	74 ± 12	76 ± 14	72 ± 12							
	GEL	71 ± 7	82 ± 12	73 ± 12	59 ± 9	74 ± 12	73 ± 12	70 ± 10	68 ± 8							
	HES	78 ± 15	87 ± 7	80 ± 9	61 ± 11	81 ± 14	79 ± 14	77 ± 12	77 ± 13							
CI ($l*min^{-1}*m^{-2}$)										$P = 0.414$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.064$	$P = 0.733$	$P = 0.423$
	RA	5.4 ± 1.4	6.0 ± 1.6	4.9 ± 1.2	4.3 ± 1.1	5.0 ± 1.3	4.7 ± 1.3	4.5 ± 1.3	4.4 ± 1.2							
	GEL	6.1 ± 1.5	5.5 ± 1.3	5.1 ± 0.8	4.3 ± 0.5	5.5 ± 0.6	5.1 ± 0.4	4.8 ± 0.6	4.7 ± 0.6							
	HES	6.9 ± 1.2	6.9 ± 1.4	5.2 ± 0.9	4.3 ± 0.8	5.5 ± 0.8	4.9 ± 0.7	4.8 ± 0.7	4.8 ± 0.7							
ITBVI ($ml*m^{-2}$)										$P = 0.006$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.963$	$P = 0.458$	$P = 0.408$
	RA	790 ± 57*	843 ± 98	789 ± 93	662 ± 89	754 ± 94	735 ± 85	722 ± 87	714 ± 86							
	GEL	798 ± 51	869 ± 59	791 ± 57	674 ± 67	777 ± 79	745 ± 55	723 ± 52	733 ± 82							
	HES	873 ± 99	887 ± 99	818 ± 55	697 ± 114	769 ± 58	730 ± 52	702 ± 59	712 ± 63							
GEDVI ($ml*m^{-2}$)										$P = 0.006$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.977$	$P = 0.479$	$P = 0.395$
	RA	633 ± 45*	674 ± 78	631 ± 74	529 ± 71	601 ± 75	589 ± 68	578 ± 70	572 ± 69							
	GEL	638 ± 40	696 ± 47	633 ± 46	539 ± 54	622 ± 64	597 ± 44	579 ± 43	587 ± 66							
	HES	699 ± 79	710 ± 79	654 ± 44	558 ± 91	616 ± 47	585 ± 42	563 ± 47	570 ± 50							
EVLWI ($ml*m^{-2}*kg^{-1}$)										$P < 0.001$	$P = 0.027$	$P = 0.002$	$P < 0.001$	$P < 0.01$ a	$P = 0.779$	$P = 0.768$
	RA	12.5 ± 1.1	20.6 ± 2.1	18.0 ± 3.0	16.2 ± 2.8	15.8 ± 2.8	14.9 ± 3.2	14.7 ± 3.2	14.2 ± 2.5							
	GEL	12.4 ± 0.6	19.5 ± 4.1	16.4 ± 2.8	14.5 ± 2.6	13.7 ± 2.5	13.3 ± 2.5	13.1 ± 2.4	12.9 ± 2.6							
	HES	13.0 ± 1.6	20.4 ± 2.8	16.5 ± 2.5	15.7 ± 3.6	14.6 ± 3.3	14.6 ± 3.3	13.4 ± 2.5	13.3 ± 2.4							

Values are given as mean and standard deviation. Comparability of groups at BL1, Injury, BL2 and BL3 was tested with one-way ANOVA followed by Bonferroni *post-hoc* test, or H-Test (Kruskal-Wallis) followed by Dunn's *post-hoc* test, as appropriate. Effects of Injury, initiation of protective ventilation, blood drainage, and immediate stabilization on variables were tested with paired *t* tests, and adjusted for repeated measurements according to the Bonferroni procedure (BL 1 *vs*. Injury, Injury *vs*. BL 2, BL 2 *vs*. BL 3, BL 3 *vs*. Time 1, respectively). Differences among and within groups (Group and Time Effects, T1-T4, as well as their interaction) were tested with general linear model statistics using BL2 as covariate, and adjusted for repeated measurements according to the Bonferroni procedure; a = linear fitting. Statistical significance was accepted at $P < 0.05$; * $P < 0.05$ *versus* HES.

BL = Baseline; CI = cardiac index; EVLWI = extravascular lung water index; GEDVI = global end-diastolic index; GEL = gelatin-polysuccinate in RA; HES = hydroxyethyl starch in RA; HR = heart rate; Injury = Two-hit Acute Lung Injury (ALI) model; ITBVI = intrathoracic blood volume index; MAP = mean arterial blood pressure; RA = Ringer's acetate.

Table 3. Gas Exchange

Variable	Group	BL 1	Injury	BL 2	BL 3	Time 1	Time 2	Time 3	Time 4	BL 1 *versus* Injury	BL 2 *versus* Injury	BL 2 *versus* BL 3	BL3 *versus* Time 1	Time Effect	Group Effect	Time x Group Effect
PaO_2/FiO_2 (mmHg)										$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.002$a	$P = 0.480$	$P = 0.461$
	RA	537.4±30.9	56.3±19.8	142.6±32.6	172.9±53.4	264.8±83.8	293.3±93.5	320.4±96.1	343.8±104.7							
	GEL	554.1±40.7	58.8±18.6	149.7±59.8	197.5±81.4	265.7±106.5	282.6±112.0	320.3±117.4	344.4±109.8							
	HES	527.1±46.1	67.7±29.5	164.8±41.3	217.1±63.8	306.0±78.2	368.5±85.9	374.3±89.1	405.6±88.1							
Q_{VA}/Q_T (%)										$P < 0.001$	$P < 0.001$	$P < 0.001$	$P = 1.000$	$P = 0.830$	$P = 0.144$	$P = 0.469$
	RA	7.5±2.7	27.5±10.4	9.8±2.8	8.3±3.9	6.9±2.0	6.2±2.0	5.4±2.0	5.3±2.8							
	GEL	6.3±2.6	23.8±6.7	9.8±2.6	6.9±1.7	8.22±4.0	7.1±3.1	5.3±2.4	5.7±2.2							
	HES	9.6±4.4	21.6±2.8	9.8±2.7	6.0±1.0	7.4±2.4	4.1±1.2	4.5±1.5	3.3±0.9							
$PaCO_2$ (mmHg)										$P < 0.001$	$P = 0.249$	$P < 0.001$	$P = 1.00$	$P = 0.008$a	$P = 0.261$	$P = 0.384$
	RA	41.1±1.5	55.1±10.2	55.8±8.5	53.3±7.2	52.2±6.3	52.5±7.5	52.1±7.0	51.4±7.7							
	GEL	39.7±2.7	52.4±7.8	55.6±3.9	52.1±4.0	53.2±4.5	52.8±3.1	53.1±3.7	52.3±3.9							
	HES	41.5±2.5	57.5±10.7	58.7±6.4	56.5±5.7	57.8±7.0	56.8±6.6	57.2±6.8	57.1±7.3							
SvO_2 (%)										$P < 0.001$	$P = 0.001$	$P = 0.003$	$P < 0.001$	$P = 0.001$b	$P = 0.687$	$P = 0.001$c
	RA	80.6±10.8	46.3±18.1	56.2±14.2	52.5±13	57.3±11.3	58.8±11.6	57.9±12.9	56.1±15.1							
	GEL	80.2±6.7	46.2±15.0	54.7±14.3	50.5±8.7	61.3±8.6	60.2±9.0	57.1±8.3	61.8±12.8							
	HES	82.0±10.9	51.0±11.6	58.7±10.1	50.9±7.4	65.3±11.9	59.0±6.2	62.5±8.6	55.8±8.6							
Hb (mM/l)										$P < 0.01$	$P < 0.05$	$P < 0.001$	$P < 0.001$	$P = 0.709$	$P = 0.930$	$P = 0.442$
	RA	6.0±0.4	6.5±0.4	6.6±0.3	6.2±0.5	5.2±0.6	5.6±0.5	5.6±0.7	5.4±0.7							
	GEL	5.9±0.4	6.1±0.5	6.2±0.6	6.0±0.5	5.0±0.5	5.1±0.5	5.2±0.6	5.0±0.6							
	HES	5.8±0.5	6.2±0.8	6.3±0.8	6.0±0.8	4.9±0.7	5.5±0.9	5.3±0.9	5.2±0.9							
pHa										$P < 0.001$	$P = 0.133$	$P < 0.001$	$P = 0.39$	$P = 0.045$a	$P = 0.639$	$P = 0.272$
	RA	7.42±0.04	7.31±0.06	7.30±0.08	7.31±0.07	7.34±0.06	7.35±0.07	7.35±0.07	7.36±0.07							
	GEL	7.45±0.04	7.34±0.05	7.31±0.03	7.34±0.04	7.34±0.06	7.35±0.06	7.35±0.05	7.37±0.04							
	HES	7.45±0.03	7.31±0.07	7.30±0.03	7.32±0.04	7.32±0.06	7.33±0.06	7.33±0.06	7.33±0.07							
HCO_3 (mM/l)										$P < 0.001$	$P = 0.682$	$P = 0.268$	$P < 0.001$	$P = 0.190$	$P = 994$	$P = 0.348$
	RA	26.6±2.4	24.2±2.0	24.0±2.6	24.1±2.3	25.6±2.1	26.2±2.2	26.6±2.1	26.9±1.9							
	GEL	27.6±2.0	24.9±2.0	25.3±2.7	25.5±2.2	26.6±2.7	27.0±3.0	27.1±3.0	28.3±2.9							
	HES	28.0±1.4	25.0±1.9	25.3±1.8	25.6±1.3	26.8±2.0	27.0±1.9	27.2±2.2	27.4±2.4							
BE (mM/l)										$P < 0.001$	$P = 0.784$	$P = 0.958$	$P < 0.001$	$P < 0.001$a	$P = 1.000$	$P = 0.292$
	RA	2.3±2.7	1.0±2.1	0.5±2.6	0.5±2.7	1.8±2.3	2.5±2.3	2.9±2.2	3.2±1.9							
	GEL	3.4±2.3	1.8±2.2	1.9±2.6	1.9±2.3	2.8±2.9	3.3±3.4	3.5±3.3	4.6±3.0							
	HES	3.9±1.5	2.0±1.9	2.1±2.0	2.2±1.5	3.3±2.0	3.5±1.9	3.7±2.2	3.9±2.4							

Values are given as mean and standard deviation. Comparability of groups at BL1, Injury, BL2 and BL3 was tested with one-way ANOVA followed by Bonferroni *post-hoc* test, or H-Test (Kruskal-Wallis) followed by Dunn's *post-hoc* test, as appropriate. Effects of Injury, initiation of protective ventilation, blood drainage, and immediate stabilization on variables were tested with paired *t* tests, and adjusted for repeated measurements according to the Bonferroni procedure (BL 1 *vs*. Injury, Injury *vs*. BL 2, BL 2 *vs*. BL 3, BL 3 *vs*. Time 1, respectively). Differences among and within groups (Group and Time Effects, T1-T4, as well as their interaction) were tested with general linear model statistics using BL2 as covariate, and adjusted for repeated measurements according to the Bonferroni procedure; a = linear, b = quadratic, c = cubic fitting. Statistical significance was accepted at $P < 0.05$.

BE = base excess. BL = baseline; GEL = gelatin-polysuccinate in RA; HES = hydroxyethyl starch in RA; Hb = hemoglobin; HCO_3 = bicarbonate level; Injury = two-hit acute lung injury (ALI) model; PaO_2/FIO_2 = oxygenation index; $PaCO_2$ = partial pressure of CO_2; pHa = arterial pH; Q_{VA}/Q_T = shunt fraction; RA = Ringer's acetate; SvO_2 = mixed venous oxygen saturation.

Table 4. Respiratory Mechanics and Intra-abdominal Pressure

Variable	Group	BL 1	Injury	BL 2	BL 3	Time 1	Time 2	Time 3	Time 4	BL 1 *versus* Injury	BL 2 *versus* Injury	BL 2 *versus* BL 3	BL 3 *versus* Time 1	Time Effect	Group Effect	Time × Group Effect
E_{rs} (cm H_2O*l^{-1})										$P < 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.012$	$P = 0.006$a	$P = 0.091$	$P = 0.002$a
	RA	37.0±7.04	90.6±12.8	77.3±9.2	73.6±9.1	69.9±9.3	69.2±8.7	66.6±10.2	69.3±8.3							
	GEL	36.7±7.3	88.3±19.1	81.2±12.6	77.5±12.7	75.5±12.8	72.9±11.3	69.2±10.4	67.9±11.1							
	HES	36.5±10.8	99.6±23.6	77.9±17.2	73.4±18.1	69.3±17.5	65.1±17.3	61.1±16.3	57.9±14.6							
E_L (cm H_2O*l^{-1})										$P < 0.001$	$P < 0.001$	$P < 0.001$	$P = 1.00$	$P = 0.241$	$P = 0.03$	$P = 0.001$a
	RA	25.6±8.4	77.3±12.6	61.7±9.2	56.0±8.6	55.4±7.8	54.4±8.0	52.0±8.3	54.4±7.1							
	GEL	25.0±7.9	74.1±16.8	64.6±12.4	60.5±13.7	61.1±13.9	59.0±12.7	55.7±11.6	54.9±11.5							
	HES	25.5±10.8	89.5±23.5	64.9±17.6	58.0±16.7	56.7±15.2	52.1±15.7	48.9±15.3	45.1±13.0						*	
R_{rs} (cm H_2O *s*l^{-1})										$P < 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.744$	$P = 0.814$	$P = 0.578$	$P = 0.023$
	RA	8.0±1.2	15.2±6.3	10.0±2.1	9.2±1.6	8.9±1.5	8.5±1.6	8.4±2.0	8.5±1.7							
	GEL	8.6±1.7	15.8±4.2	10.1±1.3	9.0±1.2	9.3±1.5	8.8±1.2	8.7±1.3	8.6±1.5							
	HES	8.8±1.0	18.2±8.2	10.7±3.3	10.3±2.9	9.4±2.6	9.0±2.4	8.5±2.5	8.2±2.3							
R_L (cm H_2O *s*l^{-1})										$P < 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.685$	$P = 0.756$	$P = 0.542$	$P = 0.016$
	RA	6.8±1.3	14.0±6.0	8.9±2.0	8.2±1.5	7.9±1.5	7.6±1.5	7.5±1.8	7.6±1.6							
	GEL	7.0±1.6	14.6±4.0	8.7±1.0	7.9±1.1	8.1±1.1	7.6±0.9	7.6±1.0	7.6±1.4							
	HES	7.2±0.9	17.1±8.3	9.5±3.2	9.1±2.7	8.3±2.4	7.9±2.2	7.5±2.4	7.1±2.3							
IAP (mmHg)										$P = 0.014$	$P = 0.016$	$P = 0.440$	$P < 0.001$	$P = 0.001$a	$P = 0.013$	$P = 0.023$a
	RA	6±3	6±3	7±2	7±2	9±3	11±4	13±3	15±4							
	GEL	4±2	5±2	6±4	7±4	9±3	10±5	12±4	15±5							
	HES	5±2	7±2	8±3	7±3	9±3	9±4	10±4	11±4						*,†	

Values are given as mean and standard deviation. Comparability of groups at BL1, Injury, BL2, and BL3 was tested with one-way ANOVA followed by Bonferroni *post-hoc* test, or H-Test (Kruskal-Wallis) followed by Dunn's *post-hoc* test, as appropriate. Effects of Injury, initiation of protective ventilation, blood drainage, and immediate stabilization on variables were tested with paired *t* tests, and adjusted for repeated measurements according to the Bonferroni procedure (BL 1 *vs*. Injury, Injury *vs*. BL 2, BL 2 *vs*. BL 3, BL 3 *vs*. Time 1, respectively). Differences among and within groups (Group and Time Effects, T1-T4, as well as their interaction) were tested with general linear model statistics using BL2 as covariate, and adjusted for repeated measurements according to the Bonferroni procedure; a = linear fitting effect. Statistical significance was accepted at $P < 0.05$; * $P < 0.05$ *versus* RA, † $P < 0.05$ *versus* GEL.

BL = baseline; E_{rs} = respiratory system elastance; E_L = lung elastance; GEL = gelatin-polysuccinate in RA; HES = hydroxyethyl starch in RA; IAP = intra-abdominal pressure; Injury = two-hit acute lung injury (ALI) model; R_{rs} = respiratory system resistance; R_L = lung resistance; RA = Ringer's acetate.

ALI, animal species and ventilatory settings could possibly explain this discrepancy. Intravascular volume replacement with colloids, compared to crystalloids, was associated with improved respiratory system compliance, but not oxygenation, in patients with ALI of both septic and nonseptic origin,[2] as well as following cardiac and major vascular surgery.[8] The lower intra-abdominal pressure in HES, but not GEL, might be due to different physical-chemical properties,[34] including hydrodynamic particle radius, average molecular weight and osmolarity, likely affecting abdominal leakage.

The increased DAD with RA compared to GEL and HES can be explained by different mechanisms: (1) increased lung edema, and (2) increased transpulmonary pressure. Lung edema, which was more pronounced with the crystalloid in the present study, can induce fragmentation of condroitin sulfate-proteoglycans of the extracellular matrix in the interstitium, leading to loss of elasticity, abnormal interstitial fluid dynamics, impairment of tissue repair and remodeling and triggering of inflammation.[41] On the other hand, increased transpulmonary pressure, as reflected by increased E_L, may result from both lung edema, as well as increased intra-abdominal pressure, and has been identified as a major risk factor for ventilator associated lung injury.[42] The slightly decreased gene expression of IL-1β in nondependent lung zones of IL-8 in dorsal zones during GEL compared to RA seems to suggest a weak anti-inflammatory effect of this colloid. To our knowledge, such an effect of a gelatin solution has not been previously reported.

Impact of Fluid Replacement on Kidney Morphofunction

In our study, there was an increase in NGAL level overtime, independent of the group. GEL was associated with more tubular necrosis and osmotic nephrosis, compared to the other groups, but did not alter plasma NGAL. This morphofunctional dissociation may be related to the time course of NGAL measurement[43] and the intensity of kidney injury, which was relatively low in our model. The most likely mechanism of tubular lesions is the accumulation of proximal tubular lysosomes due to pinocytosis of succinate molecules,[13,44] leading to cell swelling and kidney damage.[45]

The use of HES has been also implicated in kidney dysfunction and damage both in laboratory[27] and clinical studies,[7,46] but data are conflicting.[47] Direct comparisons of the effects of modern HES solutions, especially those of tetrastarch type, and gelatin solutions on kidneys are scarce. In a recent study, gelatin, but also starch solutions, have been implicated in the reduced vitality of human proximal tubular (HK-2) cell patients submitted to abdominal aneurysmectomy.[15] Also in patients with sepsis, gelatin and starch solutions were associated with higher incidences of kidney dysfunction.[48] In the present investigation, HES had no major effect on NGAL levels and AKI scores. This finding is in line with a recent clinical trial showing that HES compared to GEL improved renal function and reduced renal injury during aortic aneurysm surgery.[49] In another study, no difference was observed between HES and GEL with respect to renal function and damage.[50] Possible explanations for these differences between HES and GEL include improved hemorheology and reduced renal arteriolar vasoconstrictor release with modern HES solutions, or a reduction in renal capillary leak.[49] Since intra-abdominal pressure did not differ significantly between RA and GEL, differences in kidney injury could not be ascribed to decreased renal perfusion. Furthermore, there were no significant differences among groups in mRNA expression of apoptosis markers in kidneys.

Possible Clinical Implications

Patients with ALI/ARDS often require expansion of the circulatory blood volume to maintain hemodynamic stability. RA, GEL as well as HES solutions have been widely used to accomplish such a goal.[48,51] Our data suggest that even a restrictive fluid therapy with RA may impair lung function and increase damage compared to colloids. GEL, but not RA or HES, induced tubular necrosis and osmotic nephrosis in the kidneys. Although all types of fluids need to be used cautiously in the presence of risk factors for intra-abdominal hypertension,[52] our results suggest that HES may be less hazardous than RA or GEL in this respect.

Limitations

This study has several limitations. First, a nonseptic, two-hit animal model of ALI was used, which might not fully reproduce the complex features of clinical ALI/ARDS. Accordingly, the model used does not reproduce lung injury as found in patients, and the response to the therapies tested may differ in other models of ALI. Second, the observation time was relatively short. Nevertheless, we were able to detect important differences in lung function, histological damage, and activation of inflammatory response among groups. Third, kidney function was not assessed by urine NGAL, but increases in plasma NGAL have been reported to be highly predictive of kidney failure.[53,54] Fourth, the reduction of circulatory blood volume by approximately 25% was moderate, corresponding to the stage II of hemorrhage according to the Committee on Trauma.[55] This amount was chosen to avoid major hemodynamic instability, especially in the presence of severe hypoxemia. Thus, we cannot rule out that higher exchange rates of intravascular volume yield greater differences among groups. Fifth, fluid therapy was guided by ITBVI, which was measured by a commercial system. When pulmonary perfusion is impaired, ITBVI may be underestimated by the PiCCO system.[56] However, such effect should be comparable in all groups, not affecting our main finding. Sixth, we used a relatively high PEEP (16 cm H_2O), as compared to the recommendation of the ARDS Network protocol.[16] Nevertheless, a recent meta-analysis suggested that higher PEEP in severe ARDS is associated with improved survival.[1] Seventh,

our results cannot be directly extrapolated to other crystalloids or colloids.

Conclusions

In this model of ALI, intravascular volume replacement after hemorrhage with GEL and HES was associated with less lung damage than RA, likely due to lower formation of lung edema and decreased mechanical stress. However, GEL yielded more kidney damage compared to the other fluids. Therefore, taking the impact on lungs and kidneys into account, HES represented a valuable alternative for expansion of the intravascular volume in this nonseptic ALI model.

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