ORIGINAL ARTICLE

Modulation of inflammation during acute normovolemic anemia with different fluid replacement

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ABSTRACT

Background. Acute normovolaemic anemia (ANA) frequently occurs during cardiopulmonary bypass (CPB) and major surgeries. We investigated whether fluids (with different compositions) used to replace blood elicit any degree of systemic or lung inflammatory response.

Methods. We evaluated systemic and pulmonary inflammatory responses in a swine model of acute normovolemic anemia induced by 6% hydroxyethyl starch 130/0.4 (HES, N.=7), 0.9% saline solution (SS, N.=7), and gelatine (GEL, N.=7). Cytokine levels and neutrophil oxidative burst were analysed in the blood at baseline, 0, 60, and 120 min after hemodilution (TBL, TA, T60A, and T120A, respectively) as well as 60 (T60BI) and 120 min (T120BI) after autologous blood reinfusion. Lung histology and expression of cyclooxygenase-2 (COX-2) and E-selectin were analysed at T120BI.

Results. TNF-α, IL-6, and IL-10 levels at T60A were significantly higher in the GEL ($P<0.05$) and SS ($P<0.05$) groups than in the Control group. IL-1β was increased significantly in the GEL group ($P<0.05$) at T60H. Stimulated neutrophil oxidative burst in the blood was increased significantly only in the GEL group at TA ($P<0.05$). The GEL group presented higher COX-2 and E-selectin expression, followed by the saline and starch groups. The presence of inflammatory cell infiltration, oedema, congestion, and alveoli collapse was increased in the SS and GEL groups.

Conclusion. In this animal model of acute normovolemic hemodilution, fluid solutions of hydroxyethyl starch, normal saline, and modified fluid gelatine were shown to be effective in replacing blood during ANA. However, compared to HES, GEL and NS elicited a more intense systemic and lung inflammatory response. *(Minerva Anestesiol 2013;79:1113-25)*

Key words: Anemia - Inflammation - Cardiopulmonary bypass.

Acute normovolemic anemia (ANA) was introduced into surgical practice some years ago, but until recently, cardiopulmonary bypass (CPB) represented the bulk of its utilisation.[1, 2] With growing evidence of negative allogeneic blood transfusion results, primarily following surgical procedures,[3-6] lower perioperative transfusion acceptance triggers have become increasingly incorporated into clinical practice.[7] In all these situations, acellular fluids, such as crystalloids, colloids, and a combination of both, are mandatory to ensure adequate volemia. In previous studies, we investigated the pulmonary and cardiac effects of fluids with distinct compositions during the ANA process. Colloids, herein represented by

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starch, proved to be superior to crystalloids.[8-10] However, the role of the inflammatory response induced by these fluids needs to be better clarified.

Fluids can have widely divergent impacts on the immune response and neutrophil activation.[11-14] Several studies have shown controversial results because colloids can have either pro-inflammatory [11-13] or anti-inflammatory actions.[15-18]

Because the literature related to ANA and inflammation is scarce, we hypothesised that colloids and crystalloids may promote a diverse systemic inflammatory response in a non-hypovolemic or shock state. We aimed to investigate the effects of 6% hydroxyethyl starch 130/0.4, gelatine, and a 0.9% saline solution on lung inflammatory processes in an animal model of ANA. Therefore, we evaluated serum cytokines, neutrophil and macrophage oxidative burst, and lung cyclooxygenase-2 (COX-2) and E-selectin expression levels.

Materials and methods

Experimental preparation

After Institutional Ethical Committee approval (nº 846/05), 28 Landrace X Large White crossbreed pigs (30.2±3.1 kg) were anesthetised, and pulmonary artery catheters (PACs) and femoral arterial and venous catheters were placed for hemodynamic monitoring and blood withdrawal and replacement.

The animals underwent a 12-h fast with free access to water and were pre-medicated with intramuscular ketamine 5 mg/kg^{-1} and midazolam 0.25 mg/kg^{-1}. Anesthesia was induced with propofol 5 mg/kg^{-1} and was maintained with 1 MAC isoflurane (1.4V%) [19] in 40% oxygen after orotracheal intubation. Pancuronium bromide 0.3 $mg/kg \cdot h^{-1}$ was administered to achieve muscular relaxation. The animals were mechanically ventilated with volume-controlled ventilation at a tidal volume of 8 mL/kg and PEEP of 5 cmH_2O, and the respiratory rate was adjusted to maintain an end-tidal CO_2 of 40±5 mmHg (Cícero; Dräger, Lübeck, Germany). Saline solution was administered at a rate of 5 mL/kg/h during the entire procedure.

Study design

After instrumentation, the animals were stabilised for 30 min and randomised into four groups: Control (N.=7), anesthesia only; SS (N.=7), saline solution (0.9%) (Baxter Healthcare Corporation, Deerfield, IL, USA); HES (N.=7), 6% hydroxyethyl starch 130/0.4 (Fresenius AG, Germany); and GEL (N.=7), 4% modified fluid gelatine (B. Braun, Crissier, Switzerland). Blood was removed within 30 min and simultaneously replaced with SS, GEL, or HES, depending on the group. Blood replacement was performed at a 1:1 ratio in the colloid groups and at a 3:1 ratio in the crystalloid group.

The volume of blood removed was calculated according to the formula[20]: V = EBV X (Hi-Ht)/Ht average, where EBV=estimated blood volume (70 mL/kg for swine), Hi=initial hematocrit, Ht=target hematocrit (15%), and Ht average = average hematocrit. Blood was maintained at room temperature (22-25 °C) in a CPDA package until reinfusion, which was performed 120 min after ANA to simulate a clinical situation of blood transfusion.

Hemodynamic measurements and blood samples for biochemistry and arterial and mixed venous blood gas values (ABL 555, Radiometer, Copenhagen, Denmark) were performed as follows: 30 min after instrumentation (TINST); immediately (TA), 60 min (T60A), and 120 min (T120A) after the end of acute normovolemic anemia; and 60 min (T60BI) and 120 min (T120BI) after the autologous blood reinfusion. Blood samples for inflammatory studies were collected at the same time points and 20 min after premedication (BASELINE). Bronchoalveolar lavage fluid (BALF) was collected after T120BI from the right lung through bronchoscopy to measure oxidative burst in leukocytes. At the end of the study, the animals were euthanised with an additional bolus of anesthetic and potassium chloride.

Oxidative burst assay

Spontaneous and phorbol myristate acetate (PMA)-induced oxidative burst assays in leukocytes from peripheral blood and BALF were per-

formed with a flow cytometer (FACSCalibur, BD Biosciences, San Jose, CA, USA). Oxidative burst was measured using a non-fluorescent probe, 2',7'-dichlorofluorescein-diacetate (DCFH-DA), which can penetrate into the intracellular matrix of cells, where it is oxidised by reactive oxygen species into fluorescent dichlorofluorescein (DCF).[21] Heparinised whole blood and BALF ($2x10^5$ cells) were incubated in a 37°C shaking water bath for 30 min with 200 µl of DCFH-DA (0.3 µM, Molecular Probes, Invitrogen, Carlsbad, CA, USA), 200 µl of DCFH-DA, and 100 µL of PMA (1 ng/µL, Calbiochem, Gibbstown, NJ, USA). Immunofluorescence measurements were performed in an optical flow cytometer [22] connected to a computer (Macintosh Apple, Cupertino, CA, USA). The events (10,000) were acquired and analysed using CellQuest software (Becton Dickinson Immunocytometry Systems, San Jose, CA, USA). The neutrophils in blood and the neutrophils and macrophages in BALF were gated according size (forward light scatter [FSC]) and granularity (90-degree side light scatter [SCC]), excluding the lymphocyte populations using gate analyses. The fluorescence results were recorded with a logarithmic scale, and DCF fluorescence was filtered and measured using the photomultiplier in the green fluorescence channel (FL-1 detector). The results were expressed as the geometric mean fluorescence intensity (GMFI).

Cytokine levels

Blood samples were collected and centrifuged to determine the serum levels of interleukin 1β (IL-1β), IL-6, tumour necrosis factor (TNF-α), and IL-10. The cytokines were measured by a commercially available enzyme-linked immunosorbent assay (ELISA) kit with specific monoclonal antibodies according to the manufacturer's instructions (R&D Systems, Minneapolis, MN, USA).

Lung histopathology

At the end of the experiment, with the animals still alive, the lungs were inflated with 20 cmH_2O of pressure prior to sectioning for the histological examination. After opening the chest wall, fragments from the right apical, left apical, right diaphragmatic, and left diaphragmatic lung were removed, fixed in a 4% formaldehyde solution, and embedded in paraffin. Sections (5 µm in diameter) were stained with hematoxylin and eosin (H&E) and analysed by a veterinary pathologist blinded to the experimental protocol who described the analysis according to a modified ventilator-induced lung injury (VILI) histopathology scoring system, as previously described.[23] Lung histopathology was scored according to the following four items: alveolar congestion, hemorrhage, infiltration or aggregation of leukocytes in the airspace or vessel wall, and thickness of the alveolar wall/hyaline membrane formation. Each of the items was scored from 0 to 4 according to the extent of injury. A score of 0 represented no injury scored; 1 represented mild, less than 25% lung involvement; 2 represented moderate, 25% to 50% lung involvement; 3 represented severe, 50% to 75% lung involvement; and 4 represented very severe, more than 75% lung involvement. An overall VILI score was obtained based on the summation of all the scores in each animal (N.=7 per group).

Lung immunohistochemistry

Sections (3-µm-thick) from paraffin blocks were fixed on adhesive-coated slides. The slides were incubated at 4 °C for 16 h with anti-COX-2 (mouse pAb anti-human COX-2, Santa Cruz Biotechnology, CA, USA) and anti-E-selectin (murine mAb anti-human E-selectin, Santa Cruz Biotechnology) antibodies at 1:50 and 1:100 dilutions, respectively. The slides were rinsed with phosphate buffer solution (PBS) for 30 min, incubated with peroxidase-conjugated streptavidin (streptABC kit, Dako, Glostrup, Denmark) at a 1:400 dilution in PBS for 45 min at room temperature, and then rinsed again with PBS for 30 min. The colour was developed by incubating the slides in 0.06% diaminobenzidine (DAB) in PBS for 15 min, and the slides were then rinsed in tap water, counterstained with Harris hematoxylin, dehydrated, coverslipped, and reviewed under an optical microscope.

The expression of COX-2 and E-selectin was analysed based on a modified method, as previously described.[24] Briefly, using ImageJ software version 1.44p (NIH free software, USA), four images from each lobe of all animals were changed to black and white pixels, with black representing the deposits of the target proteins and white representing the non-stained areas of the image. Using the image Adjust Threshold command, the image was then changed to red and white, with the colour of deposits being brown. The image was analysed to calculate the total red area in pixels squared. For each animal, the total area values were obtained from the sum of the mean from each lung lobe (right apical, left apical, right diaphragmatic, and left diaphragmatic lobes).

Statistical analysis

The data were tested for normality by the Kolmogorov-Smirnov test. Parametric data were compared within groups and between groups by an analysis of variance (ANOVA) for repeated measures with the Tukey-Kramer post-hoc multiple comparison test. Data without a normal distribution were analysed within groups using the Friedman test and between groups using the Kruskal-Wallis test. When appropriate, a post-hoc multiple comparison analysis was performed using Dunn's test (SigmaStat 3.11, Systat Software Inc., San Jose, CA, USA). Weight, diuresis, blood withdrawn, and infused fluids were compared by one-way analysis of variance (ANOVA). For the immunohistochemical and histopathological scores, a non-parametric Kruskal-Wallis followed by a post-hoc Dunn´s test was used. $P<0.05$ was considered statistically significant. The data are expressed as the means ± standard deviation (SD) unless otherwise indicated.

Results

Body weight did not vary among the groups. The amount of blood withdrawn was similar among the three study groups, whereas the infused volume (HES: 1539±103 mL; SS: 4971±1186 mL; GEL: 1629±170 mL; $P<0.001$) and urine output (Control: 350±129 mL; HES: 806±539 mL; SS: 1946±1268 mL; GEL: 514±196 $P<0.01$) were significantly higher in the group treated with saline solution compared to the other groups.

Hemodynamic and metabolic data

During the observation period, no changes were observed in the Control group. Hemodilution elicited a compensatory response in HR and CI with no significant difference among treated groups, except for the Ht and PaO_2/FiO_2 ratio. The Ht was significantly higher in the SS group compared to the HES and GEL groups at T60H (18±1%, 15±1%, and 15±0% in the SS, HES, and GEL groups, respectively) and T120H (19±1%, 16±1%, and 16±0% in the SS, HES, and GEL groups, respectively). The PaO_2/FiO_2 was significantly lower in the SS group compared to the HES and GEL groups at TH (303±31, 449±25, and 414±14 in the SS, HES, and GEL groups, respectively) and T60BI (255±27, 346±25, and 341±25 in the SS, HES, and GEL groups, respectively). At T120BI, the SS group was different only compared to the HES group (264±37 and 350±30, SS and HES, respectively).

Inflammatory response

Oxidative burst

All groups submitted to ANA showed a significant increase in the peripheral blood spontaneous neutrophil oxidative burst response compared with the control group and their respective baseline levels, with no differences among treated groups. The stimulated neutrophil oxidative burst increased significantly only in the GEL group after ANA (Table I). Spontaneous monocyte activity significantly increased in the GEL group. PMA-stimulated monocyte activity was not measured because the location of these cells changed in the gate analysis following stimulation with PMA.

Regarding the BALF cells, the GEL group presented increased burst activity in neutrophils and macrophages in both the spontaneous and

Table I.—*Neutrophil and macrophage oxidative burst activation. Data are presented as the mean±SEM.*

	Group	Baseline	TINST	TA	T60A	T120A	T60BI	T120BI
Neutrophils								
Spontaneous	Control	26.6±4.5	27.1±8.1	25.3±7.6	33.7±7.7	29.7±8.8	25.3±7.6	27.7±8.6
(GMFI)	HES	29.9±4.2	32.2±5.9	60.6±10.5*†	49.8±5.2*†	45.2±5.8*†	38.6±7.9†	34.7±4.9
	SS	29.0±1.7	30.2±7.0	55.9±10.4*†	45.9±8.1*†	46.2±9.4*†	35.5±7.0†	30.4±10.1
	GEL	28.1±2.7	26.3±8.0	67.5±23.7*†	50.8±13.9†	46.5±11.6*†	36.5±5.4†	33.6±5.4
PMA	Control	1152±574	1149±678	1143±660	1272±671	1125±598	1198±702	1290±776
(GMFI)	HES	1149±529	1341±623	1927±881	1542±719	1622±650	1376±584	1342±571
	SS	1198±337	1440±511	1963±894	1808±984	1879±980	1786±827	1620±894
	GEL	1183±469	1076±368	2292±937*†	1782±444	2031±910	1745±736	1747±953
Monocytes								
Spontaneous	Control	11.2±4.6	13.8±4.9	10.6±4.3	11.2±3.2	11.2±4.1	11.4±5.2	11.4±5.0
(GMFI)	HES	11.0±6.3	12.8±7.8	19.3±7.5	19.2±11.2	14.9±10.1	12.4±5.0	11.1±5.7
	SS	9.8±3.7	11.2±5.5	19.1±9.2	15.6±8.1	14.9±7.7	11.4±6.9	10.3±6.3
	GEL	10.3±3.9	8.3±3.1	21.8±7.7*†	20.9±15.5†	14.4±3.8	11.6±3.9	11.0±3.4

PMA: PMA stimulated activity; GMFI: geometric mean fluorescence intensity. HES: hydroxyethyl starch group; SS: normal saline group; GEL: gelatin group; Baseline: before the induction of anesthesia; TINST: 30 min after instrumentation; TA: immediately after ANA; T60A: 60 min after ANA; T120A: 120 min after ANA; T60BI: 60 min after blood infusion; T120BI: 120 min after blood infusion. * P<0.05 from Baseline; † p<0.05 from Control group.

stimulated oxidative burst responses. In contrast, the HES group presented significant differences in the stimulated neutrophil activity compared with the control group (Figure 1).

Cytokines

Serum TNF-α was significantly increased in the GEL group at TA, T60A, T120A, and T120BI. In the HES and SS groups, an increase in serum TNF-α was observed only at T60A (Figure 2). IL-1β presented a significant increase in the GEL group at T60A compared with the Control and HES groups. IL-6 showed a significant increase in the GEL and SS groups at T60 compared with the control group and at T60BI compared with baseline for the GEL group. IL-10 was increased significantly in the SS (T60A)

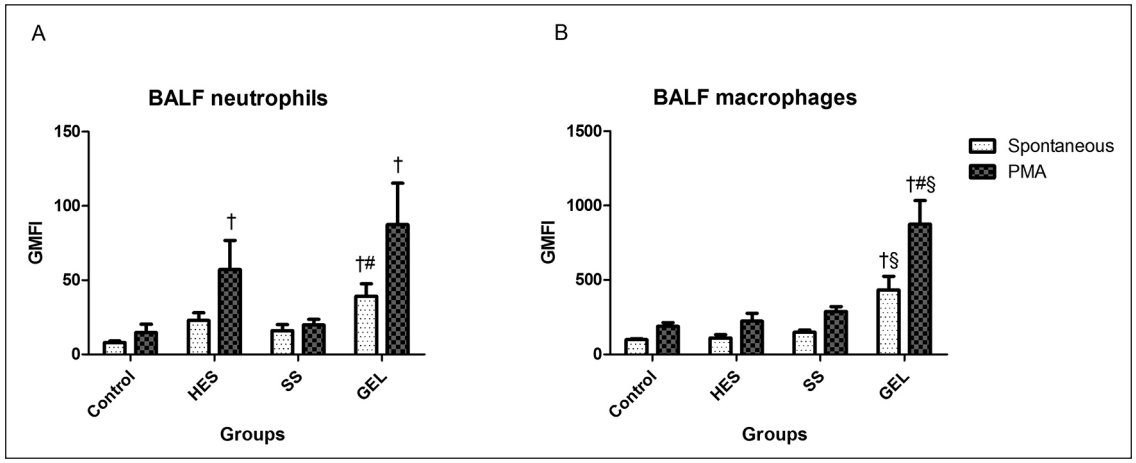

Figure 1.—BALF neutrophil and macrophage spontaneous (white bars) and PMA- induced (dark bars) burst activity after hemodilution in the Control, hydroxyethyl starch (HES), 0.9% saline solution (SS), and gelatine (GEL) groups. The data are presented as the group mean±SD. A) Neutrophil burst activity; B) macrophage burst activity. †P<0.05 compared to the control group; §, P<0.05 compared to the HES group; #P<0.05 compared to the SS group.

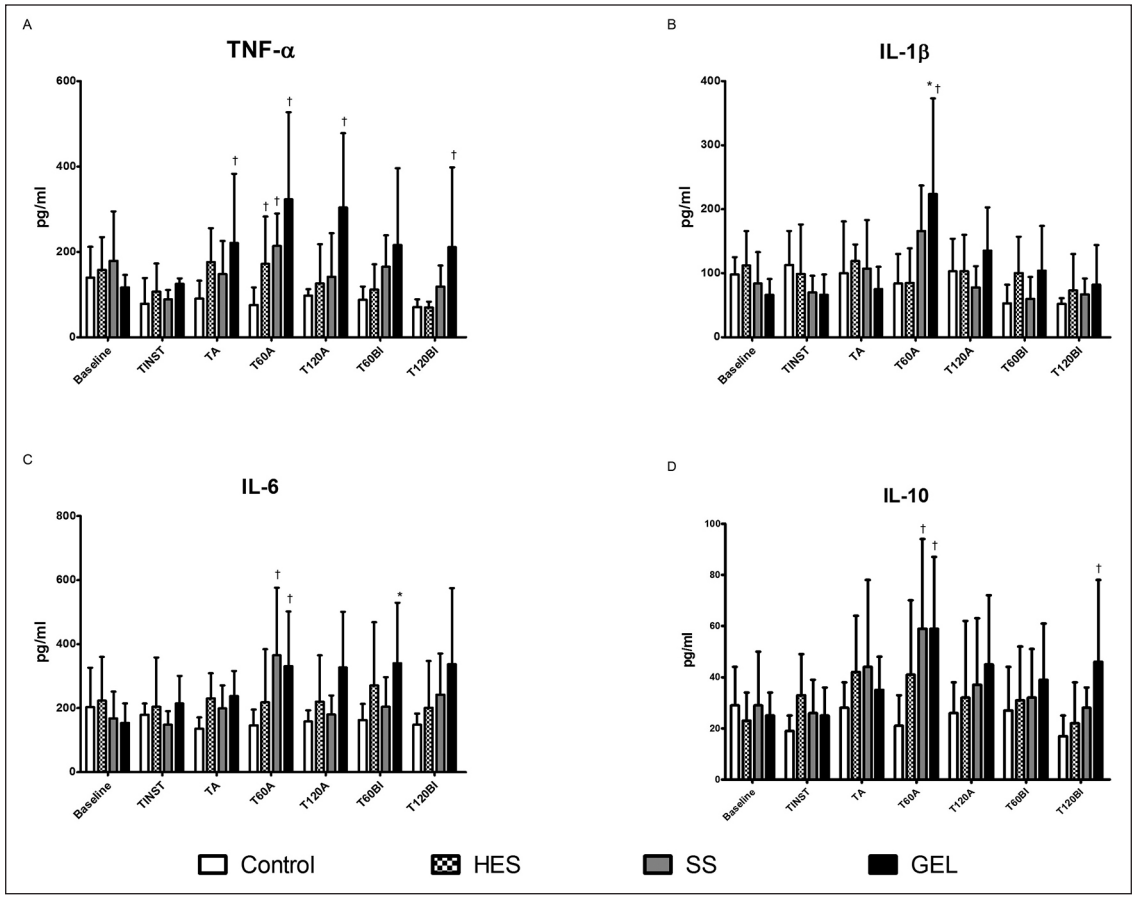

Figure 2.—Serum cytokines measured by ELISA. The values are expressed in pg/ml. The data are presented as the group mean±SD. A) Tumour necrosis factor (TNF-α); B) interleukin IL-1β; C) interleukin IL-6. D) interleukin IL-10. HES, hydroxyethyl starch; SALINE, 0.9% saline solution; GEL, gelatine; Baseline, before the induction of anesthesia; TINST, 30 min after instrumentation; TA, immediately after ANA; T60A, 60 min after ANA; T120A, 120 min after ANA; T60BI, 60 min after blood infusion; T120BI, 120 min after blood infusion.
*P<0.05 compared to the BASELINE time point for each group; †P<0.05 compared to the control group.

and GEL groups (T60A and T120 BI) compared with the control group.

Lung histopathology scores

Photomicrographs of the H&E-stained lung sections and the lung histopathology scores are depicted in Figure 3. For the animals treated with GEL, the overall lung histopathology score was higher than the Control, HES, and SS groups (P<0.01).

The GEL group presented a significant increase in cellularity, predominantly due to the presence of neutrophils in the pulmonary parenchyma and the adjacent bronchial and bronchiolar regions (2, 1-3, median and interquartile range, respectively, P<0.05), compared with the control (0, 0-0.5), HES (0, 0-1), and SS (1, 0-1) groups. The GEL group also presented moderate vascular congestion (2, 1-3), although it was not significantly higher compared to the control (0, 0-0.5), HES (0, 0-1), or SS (1, 0-1) group. The SS group demonstrated a moderate thickness of the alveolar wall/hyaline membrane (1, 0-2, P<0.05) compared with the control (0, 0-0), HES (0, 0-1), and GEL (0, 0-1) groups.

Minimal alveolar hemorrhaging was observed in the HES (0, 0-1), SS (0, 0-1), and GEL (0, 0-1) groups.

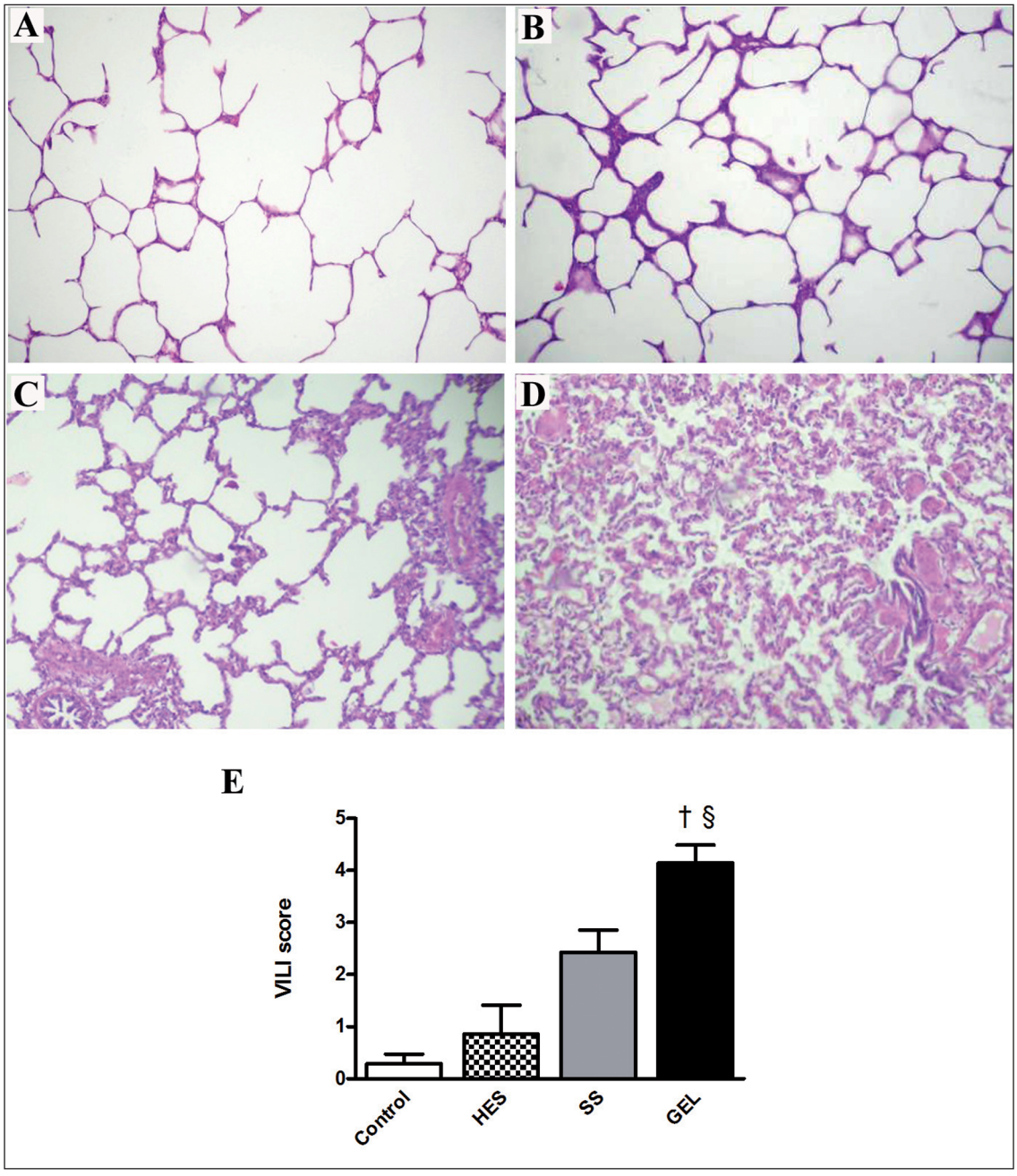

Figure 3.—Representative photomicrographs with H&E staining (x200) of the lungs of pigs submitted to hemodilution. A) Control group; B) HES group; C) SS group with moderate thickening of the alveolar wall/hyaline membrane; D) GEL group with moderate vascular congestion and increased neutrophil infiltration; E) overall lung histopathology score.
†P<0.05 compared to the control group; §P<0.05 compared to the HES group.

Immunohistochemical analysis

Representative photomicrographs of the lung immunohistochemistry are shown in Figures 4 and 5. The GEL group presented more intense intravascular leukocyte COX-2 expression compared with the control, HES, and SS groups (P<0.001); in the SS and HES groups, this expression was

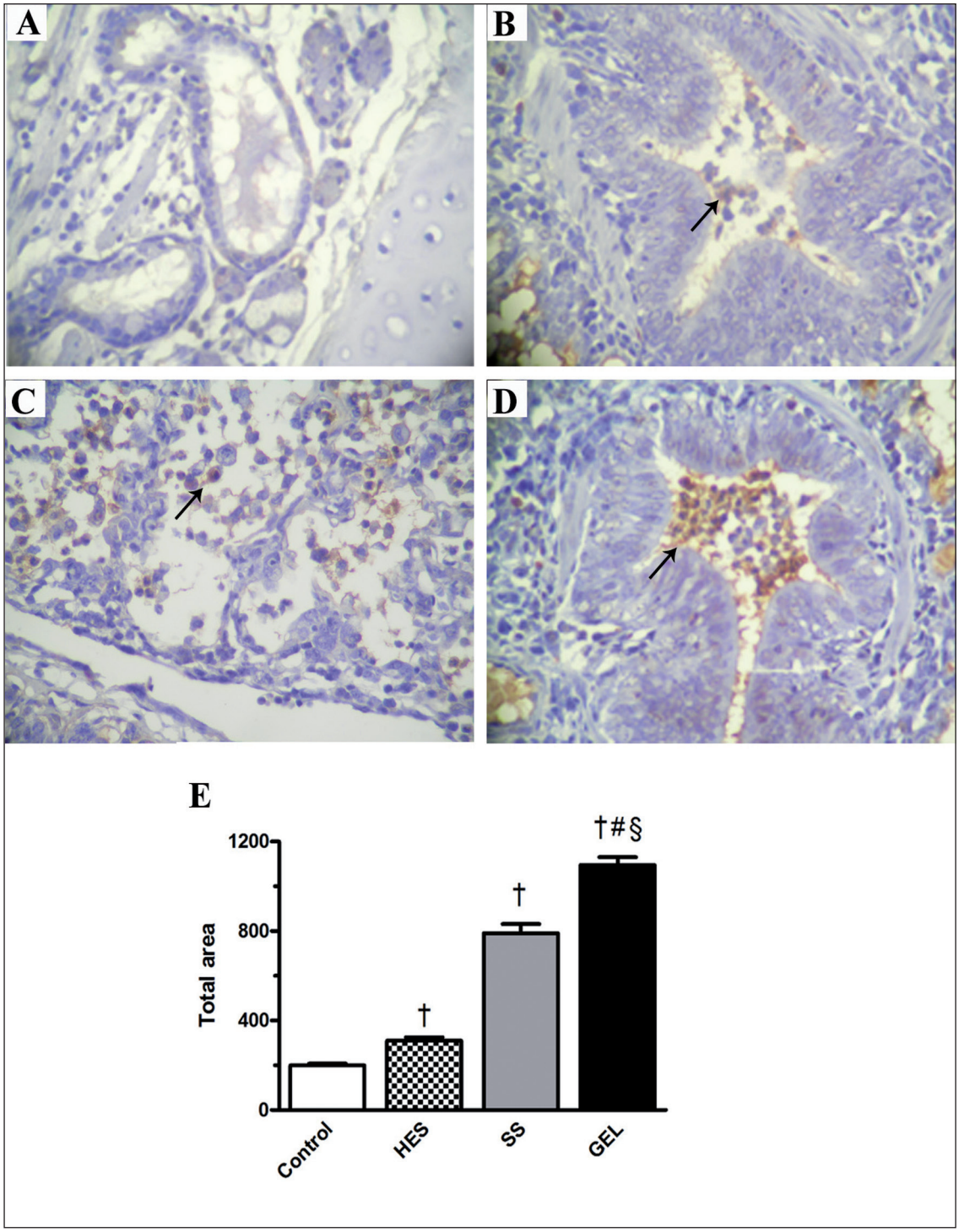

Figure 4.—Representative photomicrographs (x400) with COX-2-positive immunoreactivity of the lungs of pigs submitted to hemodilution. A) Control group with a negative COX-2 staining reaction; B) HES group with a weak intracellular COX-2 staining reaction; C) SS group with a moderate intracellular COX-2 staining reaction; D) GEL group with strong positive intracellular expression; E) total area of COX-2 staining. Arrows indicate positive immunoreactivity in the cell cytoplasm.
†P<0.05 compared to the control group; §P<0.05 compared to the HES group; #P<0.05 compared to the SS group.

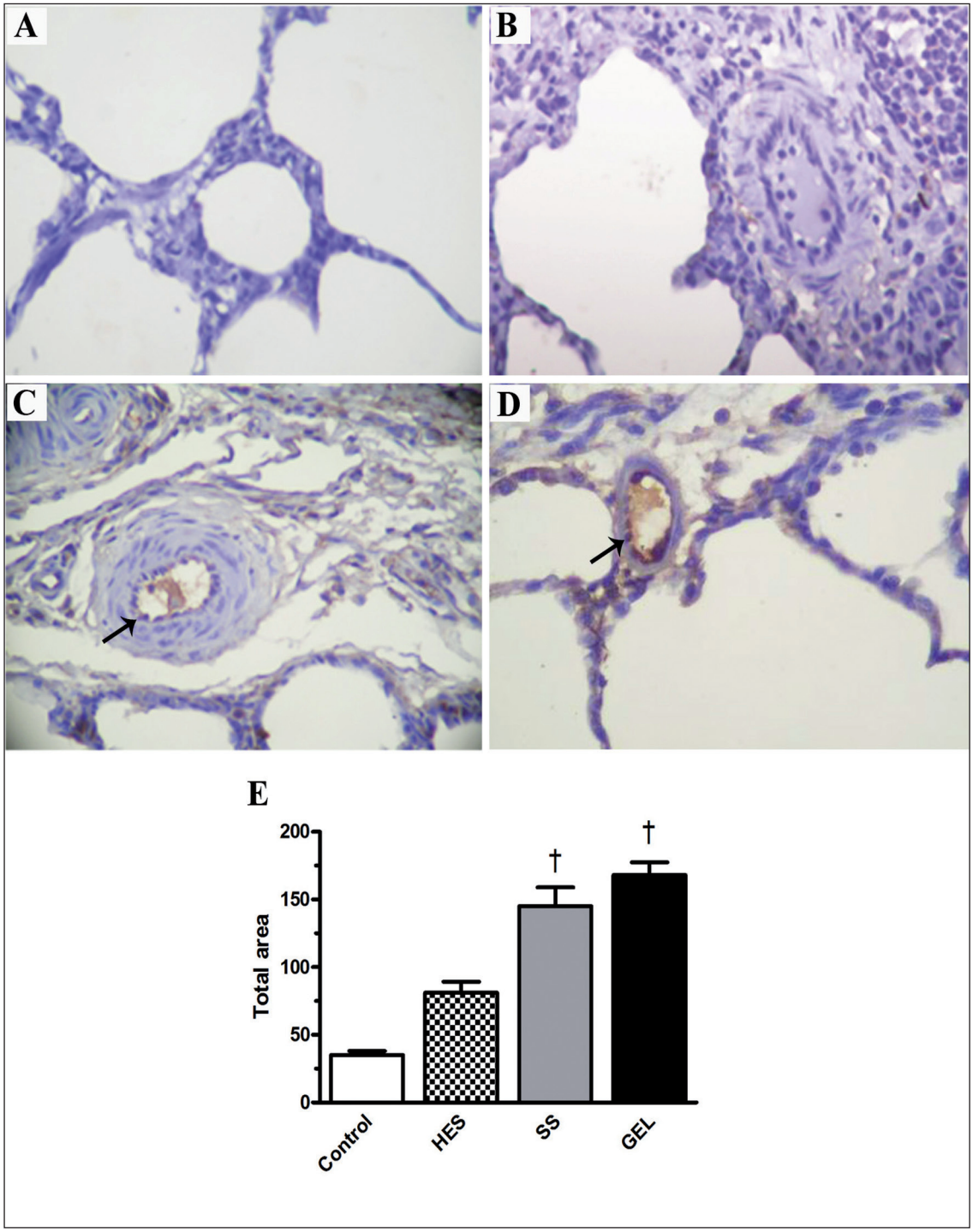

Figure 5.—Representative photomicrographs (x400) with E-selectin-positive immunoreactivity of the lungs of pigs submitted to hemodilution. A) Control group presenting endothelial cells with negative E-selectin staining; B) HES group presenting endothelial cells with weak positive E-selectin staining; C) SS group with moderate E-selectin staining; D) GEL group with strong positive staining of the endothelial cells; E) total area of E-selectin staining. Arrows demonstrate positive immunoreactivity in the membranes of the endothelial cells.
†P<0.05 compared to the control group.

moderate compared with the control group (P<0.05). The total area of E-selectin staining was significantly higher (P<0.001) in the GEL and SS groups compared to the control group (P<0.05).

Discussion

Fluid reposition plays a pivotal role in the management of patients in a range of clinical settings. However, the choice of the best fluid is still controversial. The main findings of this study demonstrate that the fluids utilised to replace blood during normovolemic acute anemia may influence the inflammatory response, altering cytokine release, modulating the leukocyte oxidative burst response, and promoting a wide range of histopathological alterations in the lung.

Polymorphonuclear neutrophils (PMNs) are responsible for the first defensive response to infection and inflammation, responding to chemotactic factors. The release of reactive oxygen species is the key response of neutrophil activation, with the extent of this action indicating the degree of stimulation.[25] The more intense presence of neutrophils in the pulmonary parenchyma and adjacent bronchial and bronchiolar regions shown in the GEL group clearly demonstrated that lung inflammation was more critical within this group and that it cannot be underestimated. Interestingly, fluid replacement during ANA was shown to activate oxidative pathways, as measured by the oxidative burst assay of circulating neutrophils in a similar pattern in all treated groups. However, in the BALF cells, the GEL group presented a greater response compared to the HES and SS groups. In vitro studies have demonstrated different results regarding the effects of fluids on leukocyte oxidative burst. Jeger *et al.*[18] observed alterations in neutrophil respiratory burst activity only with low molecular HES (70000 kDa/0.5), whereas Welter *et al.*. [26] reported increased neutrophil functioning after incubation with gelatine. In addition, Rhee *et al.* demonstrated that colloids and crystalloids could have different dose-related effects on neutrophil oxidative burst activity, with the exception of albumin and hypertonic saline solution.[13]

This hemodilution model promoted a significant increase in systemic cytokines, although at a lower scale compared with other stimuli (*e.g.*, sepsis and hemorrhagic shock). IL-6 and TNF-α showed a marked and sustained increase in the GEL group compared with the control group; in the SS and HES groups, the increase in these cytokines occurred at only one time point. With regard to TNF-α, the GEL group presented the highest value (323 pg/mL). In a study on hemorrhagic shock,[11] TNF-α reached 500 pg/ml in the gelatine-resuscitated group and 30000 pg/ml in a model of endotoxic shock.[27] With regard to IL-1β, only the GEL group presented a significant increase, reaching a value of 224±56 pg/ml. However, no differences were observed among the groups for any of the evaluated systemic cytokines. Using animals treated with different volumes of HES (130/0.4), Feng *et al.*[28] demonstrated that this solution attenuated cytokine increase, which is in accordance with our results in which the HES group showed the lowest response. The significant increases in TNF-α, IL-6, and IL-1β observed in the GEL group corroborate the higher COX-2 expression observed in this group. COX-2 expression may also be the result of cytokines produced in response to different stimuli.[29] The up-regulation of COX-2 in the liver, kidney,[30] and lung [31] was verified in animal models of hemorrhage. Additionally, the LPS-induced stimulation of inflammatory cytokines is associated with an increase in COX-2 expression in isolated alveolar macrophages and neutrophils.[32] According to Rajnik *et al.*,[31] genes that encode COX-2 have been implicated in a number of inflammatory responses. Because pro-inflammatory cytokines such as TNF-α and IL-1 trigger the up-regulation of COX-2 by inflammatory cells, the increase in COX-2 expression in the lung cells of the GEL group may be related to this process.

The more pronounced pulmonary inflammatory cell infiltration in the GEL group confirms the cytokine and COX-2 expression results. The HES group presented minor changes in lung histology and discrete mononuclear infiltration, whereas the GEL group showed a greater infiltration of leukocytes. These findings are in agreement with those of Feng *et al.*,[28] who observed that the HES group had less alveolar septal thickening, neutrophil infiltration, oedema,

and alveolar congestion compared with the gelatine group. The SS group showed the worst grade of alveolar wall/hyaline membrane thickening, which was related to capillary leakage and the more pronounced increase in hematocrit observed in this group compared to the HES and GEL groups at 60 and 120 minutes after hemodilution. Balkamou *et al.*[15] demonstrated less pulmonary oedema after hemorrhagic shock with HES compared with lactate Ringer´s in a swine model. The detected alterations, which were not homogeneous in the different examined fragments, may explain the relative maintenance of systemic oxygenation in the GEL group and the significantly lower PaO_2/FIO_2 ratio verified in the SS group. The animals submitted to ANA with saline and gelatine presented higher pulmonary artery pressure, particularly at the end of the experiment, compared with the HES and control groups. The lung alterations observed in the SS group were most likely intensified by an increased pre-alveolar microvascular pressure provoked by the increased hydrostatic pressure accompanied by low oncotic pressure.[33] Among all treatment groups, HES presented the lowest lung injury score compared with SS and GEL. Silva *et al.*[34] verified that both colloids had a better response compared with acetated Ringer´s solution.

We speculate that the GEL molecules, which are derived from animal tissue, carry a higher allergenic content compared with the HES molecules [11] and that in swine, this difference has a different impact, triggering a more pronounced inflammatory response. Clinical and experimental studies have demonstrated higher pulmonary function impairment and a more pronounced proinflammatory response with gelatine compared with other colloids and crystalloids.[11, 35] However, a recent study in humans undergoing CPB using gelatine or LR to prime the pump suggested that gelatine is not associated with an increased inflammatory response when IL-6, IL-8, and TNF-α were studied.[36,]

In addition to the attenuation of cytokine release,[28] HES has been demonstrated to have other beneficial effects, e.g., reducing capillary permeability and oedema formation,[37] acting as a sealant for leaky capillary pores,[38] protecting cellular morphology and reducing myocardial water and injury.[10, 39] All these properties may contribute to the less intense inflammatory responses verified in the HES group in this study.

One of the main limitations of this study may be attributed to the half-lives of the cytokines because we may have failed to detect the major peak cytokine levels due to our sampling time points. Additionally, the extreme variability of inflammatory responses observed in different lung sites suggests that the observation time was too short. If this type of lung injury displays a temporal evolution, worsening with time and strongly affecting the gas exchange capacity, answering such questions with the present model is impossible. We must be very cautious when extrapolating these results to clinical practice; the initial lung injury, as shown in this study, may explain some of the undesirable pulmonary effects related to more intense fluid therapy in different clinical scenarios.

Another limitation of this study was the absence of more appropriate methods to evaluate the adequacy of volume replacement after ANA, such as dynamic (stroke volume variation or pulse pressure variation) and volumetric variables (extravascular lung water or global end-diastolic volume using the PiCCO system). However, we can infer that all groups were comparable from a hemodynamic point of view because the PAM, CI, and PVC were quite similar. MPAP and PWCP were higher in the GEL and SS groups but remained near physiological levels.

Different clinical conditions such as trauma, hemorrhage, and sepsis are associated with lung neutrophil infiltration. Our results suggest that fluid replacement may alter the inflammatory response, which could potentiate the lung injury in these situations.

Conclusions

In this model of ANA, there was no systemic difference elicited by fluid replacement with HES, SS, or GEL in cytokine levels or oxidative burst. Pulmonary changes were more severe with GEL and SS, with greater leukocyte infiltration and higher COX-2 expression in the GEL group and lung oedema in the SS group. These results

showed that HES could be an option in this type of situation because it promoted the less intense overall inflammatory response. However, we cannot confirm that these responses influence the clinical evolution, which reinforces the need for a more profound analysis of the use of intravascular replacement agents in experimental and clinical scenarios.

Key messages

— Fluids can elicit an inflammatory response.

— Crystalloids are more associated with lung oedema.

— Gelatine yielded a more intense inflammatory response in lung tissue.

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