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Survival and Inflammatory Responses in Experimental Models of Hemorrhage

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Abstract

Background—Alternative experimental models of hemorrhage mimic particular conditions of clinical settings and provide advantages to analyze novel resuscitation treatments. Here, we compared alternative models of hemorrhage and analyzed the effects of resuscitation with Hextend.

Methods—Adult male Sprague-Dawley rats underwent alternative models of hemorrhage: anesthetized without trauma, anesthetized with trauma, or conscious (unanesthetized) hemorrhage. Each model of hemorrhage includes three experimental groups: (C) control without hemorrhage or resuscitation treatment; (NR) animals with hemorrhage but without resuscitation; and (HX) animals with hemorrhage and resuscitation treatment with Hextend.

Results—Conscious animals required the highest hemorrhagic volume, whereas hemorrhage with trauma required the lowest blood volume withdrawal to achieve the same arterial pressure. Conscious hemorrhage exhibited the fastest mortality, but anesthetized animals with or without trauma had similar mortality kinetic. These survival rates did not correlate with blood chemistry, hemodynamic responses, or serum TNF and HMGB1 levels. Hemorrhage in conscious animals or anesthetized animals with trauma increased serum TNF levels by approximately 2-fold compared with hemorrhage in anesthetized animals without trauma. Animals in conscious hemorrhage had similar TNF increases in all the organs, but trauma induced a specific TNF overproduction in the spleen. Resuscitation with Hextend improved survival in all the experimental models, yet its survival benefits were statistically greater in anesthetized animals with trauma. The only two markers similar to the survival benefits of Hextend were the TNF levels in the lung and liver. Hextend significantly improved survival and inhibited pulmonary and hepatic TNF levels in all the experimental models.

Conclusions—The survival benefits of resuscitation with Hextend depended on the experimental models and did not correlate with blood chemistry, hemodynamic, or serum cytokine levels. However, resuscitation with Hextend inhibited TNF levels in the lung and the liver with a pattern that resembled the survival benefits.

Keywords

hemorrhage; resuscitation; cytokines; TNF; inflammation

INTRODUCTION

The prognosis of hemorrhage is determined by hypovolemia and hypotension that limit tissue perfusion and cause oxidative stress and organ injury. After losing over ~45% of the blood volume, the system becomes unable to re-establish tissue perfusion, and hemorrhage causes lethal cardiovascular shock and multiple organ failure. Administration of resuscitation fluid is the basic strategy to re-establish tissue perfusion. The choice of the resuscitation fluid varies depending on personal choices, clinical experience, availability, and cost. The current Advanced Trauma Life Support care guidelines call for an aggressive resuscitation with Ringer's lactate [1]. Ringer's lactate resembles the physiologic composition of the plasma and is the most common resuscitation fluid used to restore circulating volume in critical care. However, recent studies indicate that advanced resuscitation fluids, designed to mimic additional aspects of the blood, provide clinical advantages [2-5]. Among them, the Tactical Combat Casualty Care guidelines recommend Hextend for its logistical benefits [6,7]. Hextend is the novel plasma volume expander containing 6% hetastarch in Ringer's lactate [8]. Hetastarch creates oncotic pressure, which is normally provided by blood proteins and permits retention of intravascular fluid. Resuscitation with Hextend prevents multiple organ injury [9] and improves short-term survival compared with saline solution in classical experimental hemorrhage with anesthesia and without trauma [10-12].

Conventional resuscitation fluids are classically designed to re-establish tissue perfusion. However, inflammatory responses can contribute to organ damage during hemorrhage and resuscitation [13-15]. The moderate production of inflammatory cytokines can be beneficial to promote local coagulation and confine tissue damage. However, overwhelming cytokine production can contribute to lethal cardiovascular shock and multiple organ failure. This effect is particularly dramatic in severe hemorrhage where resuscitation exacerbates the production of inflammatory factors. Tumor necrosis factor (TNF) and high mobility group B protein-1 (HMGB1) are inflammatory and cardio-depressant factors contributing to cardiovascular shock and organ injury in hemorrhage and resuscitation [15-17]. Recombinant TNF is capable of triggering a spectrum of hemodynamic, metabolic, and pathologic symptoms similar to that found in "hemorrhagic shock," and TNF neutralization can prevent cardiovascular shock. On the other hand, HMGB1 represents cellular damage during hemorrhage and resuscitation [18,19], and HMGB1 neutralization prevents liver damage during ischemia and reperfusion [20]. HMGB1 is a constitutive intracellular protein that can be released into the extracellular milieu during cellular damage [18,21]. Extracellular HMGB1 is recognized by the immune system as a danger-associated molecular pattern (DAMP) and activates additional inflammatory responses contributing to organ damage [18,22,23].

Alternative experimental models of hemorrhage can reveal the advantage of specific resuscitation treatments for particular clinical settings. Unlike hemorrhage in many settings of critical care, conventional experimental models of hemorrhage with anesthetized animals are characterized by the extensive use of analgesics and anesthetics that impinge directly upon the physiologic responses. Characteristic examples include phenobarbital increasing extra-alveolar permeability and promoting neutrophil recruitment in the lung [24], whereas morphine also induces immunosuppressive effects mediated by the induction of corticosterone [25]. Thus, therapeutic strategies successful in anesthetized rats might not be beneficial in animals that are awake, or in clinical settings. Likewise, trauma can exacerbate inflammatory responses during hemorrhage and resuscitation and affect the therapeutic potential of particular resuscitation fluids. In agreement with this hypothesis, the use of alternative models of hemorrhage will help to determine the factors limiting the benefits of particular resuscitation fluids and to identify potential biomarkers to determine the prognosis

of the treatment. The objective of this study is to analyze the survival benefits of Hextend in different models of hemorrhage and establish its effects in blood chemistry or cytokine responses. In the present study, we compare the survival, blood chemistry, and cytokine responses in alternative models of hemorrhage, including anesthetized without trauma, anesthetized with trauma, or conscious (unanesthetized) hemorrhage. In addition, we analyze the potential benefits of the resuscitation with Hextend in these experimental models. Blood and organs were analyzed for blood chemistry as well as TNF and HMGB1 responses during resuscitation.

MATERIAL AND METHODS

Animal Experiments

Adult male Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, IN) weighing 420 ± 40 g were acclimated for 7 d at 25°C on a 12 h light/dark cycle. All animal experiments were performed in accordance with the National Institutes of Health Guidelines under protocols approved by the Institutional Animal Care and Use Committee of the North Shore University Hospital and the UMDNJ-New Jersey Medical School. Animals were randomly grouped, and investigators were blinded to the experimental treatment.

Experimental Models of Hemorrhage

All rats were fed *ad libitum* and not fasted the night prior to surgery/hemorrhage. The animals were randomly enrolled in one of the three experimental models of hemorrhage: anesthetized without trauma, anesthetized with trauma, or conscious (unanesthetized) hemorrhage. Each model of hemorrhage included three experimental groups: (C) control without hemorrhage or resuscitation treatment; (NR) non-resuscitation, animals with hemorrhage but without resuscitation fluid, and (HX) Hextend animals with hemorrhage and resuscitation treatment. Control animals were subjected to the same surgical procedures, including anesthesia, catheters implantation, or trauma, but blood was not shed and they did not receive resuscitation fluid. Our study included $n = 8$ /group for the survival studies, and additional $n = 5$ /group for the blood chemistry and cytokine analyses. Those animals with trauma underwent closed femur fracture immediately before the surgical catheter placement as previously described by Bonnarens *et al.* [26]. Briefly, animals were anesthetized by inhalation of isoflurane (5% induction, 2% maintenance; Minrad, Buffalo, NY). With the animal supine, the right femur was placed in abduction and positioned on the center of the support stage. The stage was raised until the blunt guillotine blade was snug with the thigh. The travel of the ramming system was set at 1.5 mm. The 500 g sliding weight was dropped from a height of 25 in. The complete transverse fracture was confirmed by palpation. Hemorrhagic shock, with or without anesthesia was performed similar to that described by Handrigan *et al.* [11] with the modifications of Cai *et al.* [27]. Briefly, all the animals underwent surgical catheters placement (part no. R-FAC; Braintree Scientific, Inc., Braintree, MA) under sterile conditions in the femoral artery and vein by using isoflurane anesthesia (5% induction, 2% maintenance; Minrad, Buffalo, NY) for ~30 min to perform the surgical placement. During the surgical procedures, body temperature was maintained at 37.5°C by using external heating pads. Right femoral artery was cannulated for bleeding. Left femoral artery was used to monitor blood pressure. Left femoral vein was used for the resuscitation treatment. All the animals underwent the same surgical catheters placement, but control animals were not bled or resuscitated, and non-resuscitated animals were bled but received no resuscitation fluid. The catheters were connected to the corresponding fluid reservoir and blood pressure monitor (BPA-400; Micro-Med, Louisville, KY). Then, the conscious animals were allowed to recover for 1 h from the surgery and underwent hemorrhage without anesthesia. These animals were not restrained, and they were allowed free movement in the experimental cage. This strategy diminishes distress, and hemorrhage

itself sedates the animals. This strategy enabled the experiment to be conducted in conscious (unanesthetized) animals by allowing animal free movement after recovery from the surgical preparation, and prevented the animal from biting or twisting the cannulae. The conscious animals received no further anesthesia after the catheter placement. The conscious animals were allowed to recover for approximately 1 h before starting the hemorrhage. Before the hemorrhagic procedure, each enrolled animal was noted to be conscious, alert, free movement, and without evidence of discomfort. Animals in the “anesthetized hemorrhage” with or without trauma were kept anesthetized with isoflurane during the entire hemorrhagic procedure until the resuscitation treatment was finished. Animals in the “anesthetized hemorrhage” with or without trauma were kept anesthetized for ~115 min (~30 min surgical catheter placement, 15 min physiologic baseline, 15 min to reach a MABP of 35–40 mmHg, 15 min keeping that MAP, 40 min for resuscitation treatment). Control animals or animals without resuscitation (NR) did not receive resuscitation fluid but were kept anesthetized for the same time. After the catheter placement, the blood pressure and heart rate were monitored and recorded for 15 min as a physiologic baseline prior to the hemorrhage procedure. Then, hemorrhage was initiated by bleeding the femoral artery for 15 min to reach a mean arterial blood pressure (MABP) of 35–40 mmHg and subsequent maintenance of this blood pressure by continuous blood withdrawal for another 15 min. After the shock phase, the animals to be resuscitated received a total of 15 mL Hextend/kg (equivalent to 1000 mL of Hextend in a 70 kg man) during 40 min at a constant flow administered i.v. into the left femoral vein. Shed blood was considered lost and it was not re-infused. After the surgical procedure, the animals were housed individually in regular cages.

Blood Chemistry and Cytokine Analyses

Additional animals ($n = 5/\text{group}$) were euthanized at 2 h after the hemorrhagic shock for blood analyses. Blood and organ tissues were collected at 2 h after the hemorrhage. Since some animal groups did not receive resuscitation, we referred all the collection times to post-hemorrhage (after finishing hemorrhage). Blood chemistry was analyzed by using the i-Stat blood analyzer (Heska Corporation, Fort Collins, CO) to determine pH, bicarbonate (HCO_3), base excess of extracellular fluid (BEecf), anion gap (AnGap), glucose, hematocrit and hemoglobin, blood gases including total and partial carbon dioxide (TCO_2 , PCO_2), and blood urea nitrogen (BUN). High mobility group B protein-1 (HMGB1) was analyzed by ELISA (IBL, Osceola, WI). Tumor necrosis factor (TNF) was analyzed by ELISA following the manufacturer’s instructions (R&D Systems, Inc., Minneapolis, MN) as we previously described [19]. TNF concentrations in organs were normalized according to the total protein.

Statistical Analyses

Animals that died during the surgical preparation or before completion of the hemorrhagic procedure were excluded from analyses. All data are presented as mean $\pm$ standard deviation (SD). Statistical analyses were performed using the one way ANOVA with the Bonferroni’s correction. Analyses of normality and homogeneity of variance were performed to verify the assumptions of ANOVA. ANOVA was used to compare all treatments and specific pairwise comparisons as stated in the experiments. Student’s *t*-test was used to compare mean values between the two particular experimental groups. Statistical analyses of survival were determined using the log rank test. Kaplan-Meier product-limit estimates of the survival functions were plotted using Prism (version 5; San Diego, CA). Tests resulting in $P < 0.05$ were considered statistically significant.

RESULTS

Survival Responses in Experimental Models of Hemorrhage

Adult male Sprague-Dawley rats with statistically similar body weights and physiologic blood pressures were used to compare the blood volume needed to reach the same mean arterial blood pressure. The animals required withdrawing 21 ± 4.3 mL (anesthesia without trauma), 18.2 ± 3.6 mL (anesthesia with trauma), or 31 ± 5 mL (conscious) blood/kg body weight to reach MABP 35–40 mmHg. The subsequent maintenance of this blood pressure by continuous blood withdrawal for another 15 min required another 8 ± 2.7 mL (anesthesia without trauma), 6 ± 2.5 mL (anesthetized with trauma), or 12 ± 2.4 mL (conscious) blood/kg body weight (Table 1). In total, the animals required $\sim 30 \pm 5$ mL (anesthesia), $\sim 24 \pm 4$ mL (anesthesia with trauma), and $\sim 43 \pm 6$ mL (conscious) of blood/kg body weight, respectively (Fig. 1A). These data represent $\sim 45\%$ and 70% of the estimated blood volume, assuming normal volume of 60 mL/kg of body weight [28,29]. Conscious animals required the highest blood volume withdrawal to achieve similar arterial pressure. The resistance of conscious animals to develop hemorrhagic shock did not correlate with better survival. Conscious animals had the fastest mortality, and the animals with trauma (which required the lowest blood volume withdrawal) had a mortality rate similar to that in anesthetized animals without trauma (anesthesia *versus* anesthesia with trauma; Fig. 1B). All the animals without resuscitation treatment (NR) died within the first 5 h after hemorrhage regardless of the experimental model. Anesthetized animals without resuscitation, with or without trauma had a similar average survival time of 160 ± 26 and 167 ± 27 min, respectively. The shortest average survival time of 87 ± 20 min was found in conscious hemorrhage. Since some animals did not receive resuscitation, we referred all the times to post-hemorrhage (after finishing hemorrhage). Resuscitation with Hextend (HX) provided survival benefits depending on the experimental model of hemorrhage. Resuscitation with Hextend improved survival in 10% of the animals with anesthesia (Fig. 1C), 40% of the animals with anesthesia and trauma (Fig. 1D), and 25% in conscious animals (Fig. 1E). Resuscitation with Hextend improved survival in all the experimental models, yet its survival benefits were statistically greater in anesthetized animals with trauma. However, there is not statistical different between the survival benefits in conscious animals compared with any of the anesthetic groups. In addition to improving survival, Hextend also delayed the onset of death. Those animals that died after the resuscitation with Hextend had an average time of death delayed compared to the animals without resuscitation (Fig. 1F). An exception was found in trauma (anesthesia with trauma); the animals that died after the resuscitation with Hextend had an average time of death similar to those animals without resuscitation. All the animals without resuscitation treatment (NR) failed to re-establish normal blood pressure (Fig. 2), and died within the first 5 h after hemorrhage. All the animals that passed this critical period survived the hemorrhagic shock, and no late deaths were found even when the animals were followed for up to 5 d. Although the previous results suggest that Hextend provides survival benefits, particularly in hemorrhage with anesthesia and trauma, resuscitation with Hextend induced statistically similar hemodynamic responses in all the experimental models. These results suggest that the survival benefits did not correlate with the hemodynamic responses to resuscitation.

Blood Chemistry Analyses During Resuscitation

Characteristic pathologic markers of hemorrhage were assessed by arterial blood chemistry analyses at 2 h after the hemorrhagic shock, which represents the average time of death for conscious animals without resuscitation treatment. All the experimental models of hemorrhage induced a similar azotemia, metabolic acidosis, and hyperglycemia (Fig. 3). All the animals without resuscitation showed similar high levels of blood urea nitrogen (BUN), and although resuscitation with Hextend improved survival, it did not significantly prevent

azotemia in any of the experimental models (Fig. 3A). Hemorrhage also increased the AnGap in all the experimental models, and resuscitation with Hextend only prevented this effect in the conscious (unanesthetized) animals (Fig. 3B). On the other hand, neither hemorrhage nor resuscitation with Hextend affected significantly the bicarbonate levels in anesthetized animals (with or without trauma). However, hemorrhage significantly attenuated serum bicarbonate levels in the conscious animals, and resuscitation with Hextend prevented this effect in the conscious animals in a statistically significant manner (Fig. 3C; $^{\dagger}P < 0.05$ HX *versus* NR conscious). Hemorrhage causes similar hyperglycemic responses in all the animals regardless of the experimental model (Fig. 3D). Resuscitation with Hextend (HX) significantly prevented hyperglycemia in anesthetized animals without trauma, but not in the other models.

Cytokine Responses in Experimental Models of Hemorrhage

Tumor necrosis factor (TNF) and high mobility group B protein-1 (HMGB1) were analyzed at 2 h after hemorrhage, which represents the average time of death for those animals without resuscitation treatment. All experimental models of hemorrhage increased serum TNF and HMGB1 levels but with different patterns (Fig. 4). Serum TNF levels in the animals without resuscitation were statistically lower in anesthetized hemorrhage without trauma than in the other models. On the other hand, the animals without resuscitation in hemorrhage with anesthesia showed significantly higher serum HMGB1 levels compared with the animals in conscious hemorrhage. Serum HMGB1 levels in the anesthetized animals with trauma were not statistically different. The survival benefits induced by resuscitation with Hextend did not correlate with an anti-inflammatory potential to reduce serum TNF levels. Resuscitation with Hextend tends to slightly attenuate serum TNF and HMGB1, particularly in hemorrhage with trauma, yet these effects were not statistically significant. In order to further evaluate these cytokine responses, we also analyzed organspecific responses in the major organs, and TNF concentration was normalized according to total protein concentration. To our knowledge, the present study is the first report comparing the organ-specific inflammatory responses in alternative models of hemorrhage. Previous studies indicated that serum HMGB1 levels are attributable to extracellular release and not to a cellular overexpression [30,31]. Thus, hemorrhage increases serum HMGB1 but not its protein levels in organs. The pattern of TNF production in different organs depended on the experimental model. All experimental models of hemorrhage induced similar pulmonary TNF responses, yet these responses were statistically higher in conscious animals (Fig. 5A). The heart was the only organ mimicking the responses observed in the serum (Fig. 5B), and both trauma and conscious hemorrhage induced significantly higher TNF levels in the heart. Trauma induced a massive TNF production in the spleen (>100 ng TNF/g protein), but lower TNF levels in the liver. In contrast, conscious hemorrhage increased TNF in all the organs, including the spleen and the liver, at a statistically similar rate compared to anesthesia. Resuscitation with Hextend failed to attenuate serum HMGB1 in any of the experimental models described, yet slightly lower HMGB1 levels were found in trauma. The most significant effects that mimic the survival induced by Hextend were the pulmonary and hepatic TNF levels. Resuscitation with Hextend improved survival and attenuated pulmonary and hepatic TNF levels in all the experimental models. In contrast, resuscitation with Hextend did not attenuate cardiac or splenic TNF levels in all the experimental models. Resuscitation with Hextend attenuated cardiac TNF levels in trauma and conscious hemorrhage, but not in hemorrhage with anesthesia and without trauma. Hextend attenuated splenic TNF levels in trauma, but not in the models without trauma. Despite this relationship, our studies cannot establish a statistical correlation between pulmonary or hepatic TNF levels and survival, in part because survival and TNF were analyzed in different groups of animals. In addition, resuscitation with Hextend induces a discrete survival rate, but all the animals without resuscitation died.

DISCUSSION

Many promising strategies in experimental models of hemorrhage failed in clinical trials, in part because classical models of hemorrhage with anesthesia and without trauma miss critical clinical settings [32,33]. Unlike these experimental models, hemorrhage in critical care is normally associated with collateral trauma that impinges directly on the responses to the resuscitation treatment. In addition, classical models of hemorrhage are characterized by the extensive use of analgesics and anesthetics that affect the physiologic inflammatory responses and the prognosis of the resuscitation treatment. Characteristic examples indicate that phenobarbital increases extra-alveolar permeability and promotes neutrophil recruitment in the lung [24]. Morphine potentiates inflammatory responses by disrupting interleukin-1 modulation of the hypothalamic-pituitary-adrenal axis [34]. Depending on the experimental model, morphine can also induce immunosuppressive effects through the induction of corticosterone [25], and can promote macrophage apoptosis *via* p38 MAPK and Fas–Fas ligand interaction [35,36]. These studies suggest that therapeutic strategies successful in anesthetized rats might not be beneficial in unanesthetized animals or nonsurgical clinical settings. Unlike hemorrhage with anesthesia, hemorrhage in conscious animals can provide significant advantages to study novel strategies during hemorrhage and resuscitation. These considerations are akin to that in clinical standards, as different anesthetics used in clinical surgery can affect the therapeutic potential of particular resuscitation fluids. Indeed, the selection of particular resuscitation fluids can be determined by the clinical settings. The current Advanced Trauma Life Support care guidelines call for an aggressive resuscitation with Ringer's lactate [1]. In contrast, the Tactical Combat Casualty Care guidelines recommend Hextend for its logistical benefits[6,7]. These considerations support the need to compare alternative experimental models of hemorrhage mimicking diverse clinical standards.

The present study compared the effects of Hextend in three experimental models of hemorrhage, namely, anesthetized animals without or with trauma, and conscious hemorrhage (without anesthesia). Resuscitation with Hextend provided survival benefits depending on the experimental model. There are three considerations defining the clinical implications of this study. First, our experimental models were based on a fixed-pressure hemorrhage to ensure a similar hemorrhagic shock through the different experimental strategies. Our results show that conscious animals required ~25% additional blood withdrawal to achieve similar hemodynamics. Second, shed blood was considered lost and was not re-infused. Resuscitation included a small volume of 15 mL/kg (equivalent to 1000 mL of Hextend in a 70 kg patient) that represents ~50% (in anesthetized animals with or without trauma) and 33% (in conscious animals) of the shed blood volume. These considerations can have significant implications, as recent studies indicate that high-volume normotensive resuscitation may lead to increased hemorrhage volume and markedly higher mortality in shock associated with vascular injury [37]. Furthermore, high-volume resuscitation minimizes the properties of different resuscitation fluids. These different properties can be more significant in models with a small volume of resuscitation. Studies with limited resuscitation fluid are particularly relevant to clinical settings of rural areas, military operations, or mass casualty events, characterized by limited supplies of resuscitation fluid, long times of evacuation to a medical facility, and hemorrhage associated with severe trauma [12,38]. Third, similar to clinical standards where the patients die quickly or survive long-term, all the control animals either died quickly within the first 7 h or survived over several days. The animals were followed for up to 5 d, and no late deaths were observed. These considerations can provide significant advantages to design novel experimental models for hemorrhage. The conscious animals required the highest blood volume withdrawal to achieve the same arterial pressure. Animals without trauma required a higher blood volume withdrawal than those with trauma to achieve the same arterial

pressure, yet both groups had similar average time of death. However, these differences in shed blood volume did not correlate with the survival or the responses to resuscitation. Likewise, the better ability of the conscious animals to compensate blood loss did not correlate with better survival or hemodynamic responses, and the survival benefits of Hextend were statistically greater in anesthetized animals with trauma.

Inflammatory responses can contribute to organ damage during hemorrhage and resuscitation. A significant number of cytokines, including TNF, HMGB1, IL6, and MIF, have been involved in the inflammatory responses associated with resuscitation. Previous studies indicate that serum TNF and HMGB1 can peak around 2 h after resuscitation, which is similar to the average time of death found in our animals without resuscitation [18-20]. In our studies, the survival benefits of resuscitation with Hextend did not correlate with blood chemistry, hemodynamic responses, or serum TNF and HMGB1 levels. We needed to study organ-specific responses. To our knowledge, the present study is the first report comparing the organ-specific cytokine responses in alternative models of hemorrhage. The only organ that mimicked the pattern of serum TNF was the heart of the animals without resuscitation. There are three considerations indicating that these results do not represent an artifactual measurement of serum TNF in this organ. First, all organs were washed to remove blood. Second, serum TNF levels were found in a range of ~300 pg/mL but cardiac TNF levels were found at the higher concentration of 50–80 ng/g of protein in the organ. Third, cardiac TNF levels of those animals resuscitated with Hextend did not correlate with the serum TNF levels. Hemorrhage increased serum TNF levels in both conscious and trauma animals by ~2-fold compared with anesthetized animals without trauma. Animals in conscious hemorrhage had higher TNF levels in all the analyzed organs, namely heart, lung, spleen, and liver. All these organs increased TNF levels in a statistically similar pattern of ~2-fold, very similar to that found in the serum. These results suggest that the increased inflammatory responses observed in conscious animals were not due to the response of a particular organ but produced by a general cytokine response in all of the organs. In contrast, the higher serum TNF levels found in trauma correlated with a particular TNF overproduction in the spleen. These results may suggest that trauma and anesthetics may impinge on the inflammatory responses through different mechanisms [39]. Nevertheless, although both conscious and anesthesia animals with trauma induced double serum TNF levels, conscious animals had a faster mortality. The animals with trauma had double serum TNF levels but similar mortality as those without trauma. On the other hand, Hextend also improved survival in conscious animals but increased TNF levels in the spleen, which did not correlate with increased serum TNF levels. The only two markers that mimic the survival benefits were the potential of Hextend to reduce TNF levels in the lung and the liver. Resuscitation with Hextend improved survival and attenuated pulmonary and hepatic TNF levels in all the experimental models. In contrast, resuscitation with Hextend did not attenuate cardiac or splenic TNF levels in all the experimental models. Resuscitation with Hextend attenuated cardiac TNF levels in trauma and conscious hemorrhage, but not in hemorrhage with anesthesia and without trauma. Hextend attenuated splenic TNF levels in trauma, but not in the models without trauma. Our results warrant future studies to determine the mechanism of Hextend in those organs.

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FIG. 1.

Survival in experimental models of hemorrhage. Adult male Sprague-Dawley rats underwent hemorrhage with anesthesia but without trauma, hemorrhage with anesthesia and trauma, or conscious (unanesthesia) hemorrhage. (A) All experimental models were standardized to a fixed-pressure hemorrhage to ensure a similar hemorrhagic shock. $*P < 0.05$ of the experimental group *versus* anesthesia (without trauma). Data is presented in mean $\pm$ SD, $n = 8/\text{group}$. (B) All the animals without resuscitation (NR) died within the first 5 h after hemorrhage. On average, the conscious animals died significantly earlier. $^{\dagger}P < 0.05$ of the survival of conscious animals *versus* the other two groups in the survival log rank test. (C)–(E) Resuscitation with Hextend (HX) provided survival benefits in all experimental models of hemorrhage including hemorrhage with anesthesia (C), anesthesia with trauma (D), or conscious (E) hemorrhage. Survival studies include $n = 8/\text{group}$ and $^{\dagger}P < 0.05$ for the survival log rank test. (F) Those animals that died after the resuscitation with Hextend had a statistically higher average time of death in anesthetized (without trauma) and conscious hemorrhage. However, the animals with trauma (anesthesia with trauma) had a similar average time of death whether or not they received Hextend. $*P < 0.01$; NR (conscious) *versus* NR (anesthesia) or NR (anesthesia with trauma). $^{\dagger}P < 0.01$ resuscitation with Hextend (HX) *versus* non-resuscitation (NR) in the same hemorrhagic model.

FIG. 2.

Mean arterial blood pressure (MABP). Arterial blood pressure was recorded during the bleeding and the resuscitation of Hextend solution. All the animals without resuscitation treatment failed to re-establish normal blood pressure. Resuscitation with Hextend allowed the animals to re-establish arterial blood pressure in a statistically similar pattern in all the models, including anesthesia without trauma, anesthesia with trauma, and conscious. Yet, these effects did not correlate with the survival benefits described above. Although conscious animals have a tendency to show higher physiologic MABP, this effect was not statistically significant. All the animals without resuscitation treatment (NR) failed to re-establish normal blood pressure. The figure depicts a representative MABP response of the animals with hemorrhage but without resuscitation subjected to anesthesia without trauma. All the animals with hemorrhage but without resuscitation in the different models of hemorrhage have a statistically similar response, $n = 4/\text{group}$.

FIG. 3.

Blood chemistry analyses. Conscious or anesthetized animals without or with trauma received non-resuscitation treatment (NR) or 15 mL/kg of Hextend (HX). Blood was collected at 2 h after hemorrhage to analyze (A) blood urea nitrogen (BUN), (B) the anion gap (AnGap), (C) bicarbonate (HCO_3), and (D) Glucose. * $P < 0.05$ of nonresuscitation (NR) versus control (sham) group. † $P < 0.05$ resuscitation with Hextend (HX) versus the respective non-resuscitation (NR) group. Data is presented in mean $\pm$ SD, $n = 5$ /group.

FIG. 4.

Serum cytokine levels in the experimental models of hemorrhage. Blood from conscious or anesthetized animals without or with trauma received non-resuscitation treatment (N) or 15 mL/kg of Hextend (HX). Serum TNF (A) or HMGB1 (B) levels were analyzed by ELISA ($n = 5/\text{group}$). Control animals (C) received sham surgery without hemorrhage or resuscitation. Serum cytokine levels did not correlate with the survival benefits of Hextend. $*P < 0.05$ of nonresuscitation (NR) versus the respective control (sham) group. Data is presented in mean $\pm$ SD, $n = 5/\text{group}$. $^{\dagger}P < 0.05$ NR (anesthesia + trauma) or NR (conscious) versus NR (anesthesia).

FIG. 5.

TNF responses in specific organs. Organs from conscious or anesthetized animals without or with trauma received non-resuscitation treatment (NR) or 15 mL/kg of Hextend (HX).

Control animals (C) received sham surgery without hemorrhage or resuscitation. Major organs were harvested at 2 h after the hemorrhage to determine TNF protein concentrations in lung (A), heart (B), spleen (C), and liver (D). TNF concentration was normalized to organ total protein. * $P < 0.05$ NR (conscious) or NR (anesthesia with trauma) versus NR (anesthesia). † $P < 0.01$ Hextend (HX) treatment versus the respective non-resuscitation (NR) group. Data is presented in mean $\pm$ SD, $n = 5$ /group.

TABLE 1**Comparison of Blood Volume Withdrawal**

	Anesthesia	Anesthesia + trauma	Conscious
Hemorrhage	21±4.3 (35%)	18.2±3.6 (~30%)	31±5 (51%)
Shock	8±2.7 (12%)	6±2.5 (10%)	12±4 (20%)
Total	30±5 (~45%)	24±4 (~40%)	43±6 (~72%)

The table represents the blood volume (mL blood/kg body weight) withdrawn in the different model of hemorrhage to achieve MABP 35–40 mm Hg in 15 min (hemorrhage) and subsequent maintenance of this blood pressure by continuous blood withdrawal for another 15 min (shock). These data representing the percent of the estimated blood volume was calculated assuming normal volume of 60 mL/kg of body weight.