Hextend Attenuates Hypercoagulability After Severe Liver Injury in Swine

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Background: Hypercoagulability is a major source of morbidity and mortality after injury. A resuscitation regimen that modulates this coagulopathy may prove beneficial. We sought to evaluate the effects of lactated Ringer’s (LR) solution and Hextend on the resuscitation of uncontrolled hemorrhagic shock.

Methods: Twenty swine underwent invasive line placement, midline celiotomy, and splenectomy. After a 15-minute stabilization period, we recorded a baseline mean arterial pressure and created a grade V liver injury. The animals bled freely for 30 minutes, after which we measured the initial blood loss (that after injury). We blindly randomized the swine to receive LR solution or Hextend to achieve and maintain the baseline mean arterial pressure for 90 minutes postinjury. Laboratory values were obtained at baseline and on completion of the 2-hour study period.

Results: The initial blood loss (before resuscitation) was 22 mL/kg in both treatment groups (p = 0.97). Animals required 119 ± 78 mL/kg of fluid in the LR group and 40 ± 21 mL/kg in the Hextend group (p = 0.01). After resuscitation, the secondary blood loss was 3.7 ± 1.7 mL/kg in the LR group and 4.7 ± 1.1 mL/kg in the Hextend group (p = 0.1). Thrombelastography revealed a hypercoagulable state in all animals after injury. This was less pronounced in those animals resuscitated with Hextend. Routine tests of coagulation did not reveal a hypercoagulable state.

Conclusion: Modulation and restoration of normal coagulation is critical in the management of trauma patients. The patient’s coagulation profile might determine the type of fluid to be used at various times during their course. Thrombelastography is superior to routine coagulation assays for the detection of a hypercoagulable state. Resuscitation with Hextend results in a decreased fluid requirement and attenuation of hypercoagulability after injury without increased blood loss.

Key Words: Coagulopathy, Hemorrhagic shock, Hextend, Lactated Ringer’s (LR), Resuscitation, Thrombelastography (TEG), Trauma.

spleen’s distensibility and the resultant variation in amounts of sequestered blood. We weighed the spleen and infused LR solution to replace three times the spleen weight. The abdomen was then closed with towel clamps.

After a 15-minute stabilization period, we removed the towel clamps and dried the abdomen. Preweighed laparotomy pads were placed in both paracolic gutters and the pelvis to facilitate blood collection. We recorded a baseline MAP and created a standardized grade V liver injury (injury to a central hepatic vein) with a specially designed clamp. The clamp was positioned in the middle of the liver, placing the right hepatic vein, the left hepatic vein, and the portal vein at risk for injury. Figure 1 exhibits a sample injury. This protocol is based on our experience in previous studies of uncontrolled hemorrhagic shock using the grade V liver injury model. The time of injury was considered the start time of the 2-hour study period.

Resuscitation was delayed for 30 minutes to allow the animals to reach their nadir blood pressure. After 30 minutes of uncontrolled hemorrhage, the initial blood loss (before resuscitation), measured by wall suction and the preweighed laparotomy pads, was determined.

We blindly randomized (using a random numbers table) the swine to receive either LR or Hextend resuscitation at 165 mL/min. This rate was chosen because it is approximately one half the rate delivered by the Level I rapid infuser. The resuscitation goal was to achieve and maintain the baseline MAP for 90 minutes postinjury. Six animals received no resuscitation and served as the control group.

On completion of the 2-hour study period, we reopened the abdomen and calculated the secondary blood loss after resuscitation. We removed the liver and determined the specific injury grade to ensure comparable injuries between the study groups. The animals died secondary to exsanguination.

Blood specimens were collected at baseline (before injury) and on completion of the 2-hour study period. Blood assays included lactate level, arterial blood gases, chemistry panel, complete blood count, partial thromboplastin time (PTT), prothrombin time (PT), fibrinogen, D-dimer, and thrombelastography (TEG) (Hemoscope Corporation, Niles, IL). We chose the TEG as a test for overall coagulation because it has been shown to correlate with routine coagulation assays, with the exception that it is superior to routine tests for the detection of a hypercoagulable state.

A sample TEG tracing is seen in Figure 2. The R value, or reaction time, represents the time to onset of clot formation. The normal R value ranges from 3.7 to 8.3 minutes. Thrombelastography R values < 3.7 minutes indicate more rapid clot formation, whereas values greater than 8.3 minutes signify slower clot formation. The α angle measures the rapidity of fibrin build-up and cross-linking (clot strengthening). The normal α angle ranges from 46.8 to 73.6 degrees. Thrombelastography α angles > 73.6 degrees indicate more rapid clot strengthening, whereas values less than 46.8 degrees signify slower clot strengthening. The maximum amplitude (MA) is a direct function of the maximum dynamic properties of fibrin and platelet bonding and represents the ultimate strength of the fibrin clot. The normal MA ranges from 54.5 to 72.5 mm. Thrombelastography MA values greater than 72.5 mm indicate an increased strength of clot, whereas values less than 54.5 mm signify a decreased strength of clot.

This protocol was approved by the Institutional Animal Care and Use Committee at Oregon Health & Science University. This facility adheres to the National Institutes of Health guidelines for the use of laboratory animals.

**Statistical Analysis**

The Student’s t test was used to compare the means of continuous variables. Statistical significance was defined as a value of p < 0.05.

**RESULTS**

All animals survived the 2-hour study period. Mean animal weight, mean injury temperature, and number of vessels injured are shown in Table 1.

The MAP curves of the two treatment groups and the control group are shown in Figure 3. The MAP at the time of injury (and thus the resuscitation endpoint) was 77.7 ± 16.2
mm Hg for the LR swine, 70.5 ± 11.2 mm Hg for the Hextend animals, and 71.5 ± 12.7 mm Hg for the control group. There were no statistically significant differences between these values.

The initial blood loss was 22 mL/kg in both groups ($p = 0.97$). The secondary blood loss after resuscitation was 3.7 ± 1.7 mL/kg in the LR group and 4.7 ± 1.1 mL/kg in the Hextend group ($p = 0.11$). Fluid requirements were 119 ± 78 mL/kg in the LR group and 40 ± 21 mL/kg in the Hextend group ($p = 0.01$). The urine output was 21 ± 13 mL/kg in the LR group and 10 ± 4 mL/kg in the Hextend group ($p = 0.03$). These data are presented in Figure 4.

Laboratory values at study completion are presented in Table 2. Resuscitation with either LR solution or Hextend resulted in a significant hemodilution in comparison with the control group. The standard tests of coagulation (platelets, PTT, PT, and fibrinogen) were similar between the LR and control groups. These values in the LR and control groups were significantly different from those in the Hextend animals.

The TEG values on study completion are presented in Table 3. Figure 5 displays sample TEG values on study completion for both LR and Hextend resuscitation.

### DISCUSSION

Hypercoagulability remains a major source of morbidity and mortality in trauma patients. In 1772, Hewson documented hypercoagulability of an animal’s blood during exsanguination. Nasse (1842), Brucke (1857), and Cohnheim (1877) all confirmed this finding.

The mechanisms by which trauma alters the hemostatic balance to favor hypercoagulability include stasis, vessel wall dysfunction, and alterations in the coagulation cascade. In 1914, Gray and Lunt proposed the liver’s involvement in altering the coagulation cascade. Studies since have revealed multiple factors responsible for these alterations.

Imbalances in the coagulation cascade are the result of procoagulatory pathways and the inhibition of anticoagulation systems. Plasma antithrombin III (ATIII) is a glycoprotein responsible for decreasing the activation of thrombin and factor Xa. Tilsney and Bagge et al. demonstrated decreased ATIII levels in traumatic shock. This finding was further supported by Engelman et al. and Owings et al., who independently showed reductions in ATIII levels in trauma patients with multiple injuries. In that same study, Engelman et al. documented a significantly decreased level of both protein C (PrC) and its activity (FPrC) resulting in hypercoagulability immediately after significant trauma.

In addition to ATIII and PrC alterations, the fibrinolytic system is suppressed in trauma patients. Plasminogen activator inhibitor (PAI)-1 is responsible for this decrease in function. Elevated PAI-1 levels inhibit plasminogen activator and subsequently decrease the production of plasmin. These findings are responsible for the coagulation system alterations seen in the trauma patient with multiple injuries.

Such hypercoagulability during acute trauma, ongoing hemorrhage, and initial resuscitation is beneficial; however, later in the course of trauma patients, it is a major source of morbidity and mortality. The manifestations of these effects are evidenced by the prevalence of deep vein thrombosis and multiple organ failure in this patient population.

Just as Hewson documented the hypercoagulability of an exsanguinating animal’s blood in 1772, so did our current study. At study completion, the TEG R values were significantly decreased in all of the groups, signifying more rapid clot formation. The R values of the LR and control groups were comparable (1.7 ± 0.6 minutes and 1.7 ± 0.8 minutes, respectively; $p = 0.9$), suggesting that resuscitation with LR
solution does not modulate coagulation in the doses given. Resuscitation with Hextend produced a significantly greater R value (2.8 /H11006 1.1 minutes) in comparison with these groups, representing modulation of this more rapid clot formation.

As shown in Table 3, the α angle and the MA were prolonged in the LR and control groups. These values together with their respective R values represent a hypercoagulable state. In the Hextend swine, the α angle and MA were within normal limits.

The standard tests of coagulation (platelets, PTT, PT, and fibrinogen) did not reflect a similar hypercoagulable state on study completion. These results are seen in Table 2. The values are similar for both the control and LR groups. In comparison, Hextend resuscitation produced a reduction in coagulability as measured by the standard tests. However, these parameters remained within normal limits.

These standard measurements of coagulation are insufficient to identify pathologic hypercoagulable states. In 1991, Enderson et al. documented hypercoagulability and suppression of fibrinolysis in 42 adult trauma patients. Despite this fact, standard tests of coagulation (PTT, PT) did not differ significantly between these patients and controls. These findings allude to the need for a more sensitive screening test of coagulation. Engelman et al. identified potential screening tests for the detection of hypercoagulability. These included significantly decreased levels of PrC, ATIII, and FPrC and increased levels of D-dimer, prothrombin fragment 1.2 (PF1.2), and PAI-1.9

We propose the TEG as a more sensitive test of hypercoagulability. The TEG is rapid, inexpensive, and broad in its measurement capabilities. Within 15 minutes, a TEG tracing can provide information on clotting factor activity and plate-
Hextend is an artificial colloid composed of 6% hetastarch in a balanced salt solution (Na⁺, K⁺, Ca²⁺, Mg²⁺, and Cl⁻) with physiologic dextrose and a lactate buffer. In a randomized controlled trial, Gan et al. exhibited no alteration in the TEG R value and a decrease in blood loss in comparing the Hextend group with the control and LR groups.

In the data of Gan et al., Hextend exhibited no increase in blood loss (Fig. 4). The secondary blood loss in both groups was serous fluid and most likely represented third-space resuscitative volume. The secondary blood loss was 3.7 ± 1.7 mL/kg in the LR group and 4.7 ± 1.1 mL/kg in the Hextend group (p = 0.1).

The current study highlights several key points. Hypercoagulability is prevalent immediately after trauma. Acutely, during ongoing hemorrhage and resuscitation, this is beneficial; however, later in the course of trauma patients, modulation of this hypercoagulability and restoration of normal coagulation is critical. The patient’s coagulation profile might determine the type of fluid to be used at various times during their course. The standard tests of coagulation are insufficient for identifying hypercoagulable states. TEG may be a sensitive tool for assessing the coagulation profile and determining the optimal fluid resuscitation. During periods of uncontrolled bleeding and resuscitation, fluids that contribute to a hypocoagulable state should be avoided. Later, in trauma patients manifesting a hypercoagulable state, fluids that modulate hypercoagulability may be preferential.

**References**


**DISCUSSION**

Dr. Stephen M. Cohn (Miami, Florida): Todd and associates have compared the coagulation effects of routine crystalloid resuscitation to colloid resuscitation with Hextend in an established porcine, uncontrolled, hepatic hemorrhage model.

It is a considerably shorter time of resuscitation than identified in Dr. Gurney’s long-term combat casualty resuscitation model. They found that the colloid reduced fluid requirements and attenuated the hypercoagulability that occurred after trauma in resuscitation. As with any animal experiment, one can quibble with the clinical relevance of the model.

Today, most of us involved in trauma or combat casualty animal research strive to replicate the typical patient scenario. I have a few questions regarding the methodology and the potential clinical impact of this study.
First, what was the rationale for the use of a colloid? In other words, is there a need for this product in resuscitation today?

Next, the reliability of the actual laboratory technique employed. When using TEG, it is critical to assure that it’s a reliable and reproducible test. I don’t want anyone to think that this would have clinical relevance, because it’s a very difficult study to do, and the authors can be commended for having performed this study.

Can the authors explain how the samples were temperature-corrected? Was native blood used? Was the sample re-calcified, and was it with or without Kaolin? Did they precisely control the time period after the samples were drawn prior to running them on the TEG?

These details are important in performing the TEG and to establish accurate results. We can all remember all the times we ran platelet-bleeding times, or IV bleeding times, only to find recently that it is totally and completely inaccurate. All the animals were hypercoagulable, regardless of resuscitation fluid. As the authors state, this has been demonstrated clinically by numerous investigators.

Even at the end of the experiment, animals in both groups remained within the range of hypercoagulable. The colloid animals were just less so. Have the authors compared crystalloid solutions to other colloid solutions that require less fluid, such as, say, hypertonic saline? Maybe this effect is related to the dilutional effect of fluid volume on platelet aggregation. Do the authors believe that this is, in fact, some type of survival mechanism and, therefore, beneficial to the organism to be hypercoagulable?

The prothrombin time and PTT values were significantly higher in the Hextend group at the end of the experiment. Were these animals, in fact, hypercoagulable? Did the animals become hypothermic during the experiment, and how does temperature impact on the values of both the TEG and the warmed PT/PTT specimens?

As all the pigs survived and have the same blood pressure and blood loss, how did the apparent TEG difference have any clinical significance? Finally, our anesthesiologists and neurosurgeons have been reluctant to use Hextend for resuscitation of brain-injured patients, even though it is FDA approved.

This reluctance continues despite the demonstration that large volumes, up to six liters, in trauma resuscitation by some clinicians have been used without the apparent development of platelet dysfunction or any added bleeding that has been associated with some of the other starch solutions.

Do the authors have much clinical experience with this product in resuscitation of their patients?

Dr. S. Rob Todd (Houston, Texas): In regard to your first question, the rationale for the use of the colloid can be looked at from two perspectives, a military standpoint and a civilian standpoint. From the military standpoint, obviously, if we can use a lesser amount of fluid, it’s going to be more portable, and it’s going to be more accessible in the field for the military option.

From a civilian and military standpoint, giving these patients less volume also has some of the inherent benefits of that. Hopefully, they’ll become less hypercoagulable, and less loss of temperature and less multiorgan system failure will occur down the road.

Dr. Cohn is exactly correct that in performing a TEG, it is very user-dependant, and we had the luxury of actually having the TEG machine in the lab right beside the animals when we were operating on them. So the blood would be taken from the arterial lining of these animals and placed directly into the cup instantaneously. Because of this, we had no trouble with temperature control or anything of that nature for our TEGs.

In regard to looking at other fluids that might require less volume, that’s a study that is currently in the works at Oregon right now. I do believe that hypercoagulability is probably a survival mechanism. The reason I say that is initially on the trauma, when these patients have ongoing hemorrhage, hypercoagulability is obviously beneficial by helping to establish clot and prevent that hemorrhage from killing the patient, essentially. It is further down the line where it becomes an issue, with the increased risk of DBTs, PEs, et cetera.

As far as the TEDs versus the PTTs, the animals, as I said, were normal thermic throughout, therefore the blood work was also normal thermic throughout. The hypercoagulability seen has been previously documented by Howland and Zuckerman and many other people, as you mentioned.

So, in fact, I do think they were hypercoagulable. I think the appearance of hypocoagulability in the LR animals versus the Hextend animals is probably based off a dilutional effect, and if you look at the Hextend giving a one to one volume expansion versus about a 0.2 to one volume expansion for lactated ringers, I think that is probably what amounted to the difference in the standard tested coagulation.

On how the TED would make a difference in any clinical significance, I think, in order to look at that you would probably have to look at the long-term course of this patient.

Initially, it may not make an obvious difference. But if we could modulate the coagulation profile of the patient throughout their course, I think as far as DBTs down the road, PEs, multiple system organ failure, et cetera, we could probably decrease the amount of all those negative factors.

As far as clinical use goes, I’m currently at UT Houston, and we do use Hextend intermittently, not with any regularity or not with a protocol. But we have not seen any adverse effects from that.