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Hemodilution is Not Critical in the Pathogenesis of the Acute Coagulopathy of Trauma

Max Valentin Wohlauer, M.D.,* Ernest E. Moore, M.D.,*,†2 Nathan M. Droz, B.S.,∥ Jeffrey Harr, M.D.,* Eduardo Gonzalez, M.D.,* Miguel Fragoso, D.V.M.,*, and Christopher C. Silliman, M.D., Ph.D.‡,§

*Department of Surgery, University of Colorado Denver, Denver, Colorado; †Department of Surgery, Denver Health Medical Center, Denver, Colorado; ‡Department of Pediatrics, University of Colorado Denver, Denver, Colorado; §Research Department, Bonfils Blood Center, Denver, Colorado; and ∥Creighton University Medical Center, Omaha, Nebraska

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Background. The acute coagulopathy of trauma is multifactorial, but generally believed to be aggravated by coexisting acidosis, hypothermia, and hemodilution. While acidosis and hypothermia have been extensively evaluated, there is a paucity of data on the independent role of hemodilution in this scenario. We therefore hypothesized that hemodilution will impair coagulation following experimental trauma and hemorrhagic shock.

Methods. Adult male Spraque-Dawley rats underwent trauma and hemorrhagic shock, followed by resuscitation with 2 × SBV using normal saline (NS). Thrombelastography (TEG) was performed before and after shock.

Results. In this trauma model, resuscitation resulted in a hemodilution of 50% (43% ± 4.05% versus 19.8% ± 3.96% Hct pre-shock versus post-shock, P < 0.0001). Despite the substantial hemodilution, there was no significant change in clot strength (12.96 ± 2.84 versus 11.79 ± 1.28 dynes/cm² G pre-shock versus post-shock, P = 0.13). Similarly, the onset of coagulation (R time) was not impaired (1.68 ± 1.74 s versus 1.75 ± 0.63 s R time pre-shock versus post-shock, P = 0.45).

Conclusion. In the absence of hypothermia and acidosis, hemodilution (≤50%) has a trivial effect on coagulation following trauma and hemorrhagic shock. These data call to question the commonly held belief that hemodilution per se is critical in the development of post-injury coagulopathy. © 2012 Elsevier Inc. All rights reserved.

Key Words: blood coagulation disorders; coagulation; coagulopathy; acute coagulopathy of trauma; hemorrhagic shock; transfusion; massive transfusion; hemodilution; dilutional coagulopathy; resuscitation; critical care; crystalloid.

INTRODUCTION

Beginning with the discovery that post-injury shock was caused by acute blood loss [1] research has continued in its endeavor to optimize resuscitation strategies. In World War II, whole blood transfusion was finally accepted as beneficial for resuscitation of traumatic shock [2, 3]. With the discovery of ABO blood groups [4], blood component separation [5], and storage [6], initial investigation focused on replacement of these components following massive transfusion [7]. As a result, much of the emphasis on the coagulopathy following life threatening injury was attributed to the dilution of clotting factors as a result of component therapy [8]. In fact, avoiding hemodilution formed the basis for hypotensive resuscitation, i.e., the dual benefit of avoiding blood pressure dislodgement of a hemostatic plug and hemodilution that would provoke a coagulopathy [9]. For the past 30 y, contributions of physiologic derangements, beyond plasma clotting factors, added a further dimension to the investigative process, notably the bloody vicious cycle emphasizing the role of coexisting hypothermia and acidosis [10, 11]. It is believed that the acute coagulopathy of trauma is multifactorial; aggravated by coexisting acidosis, hypothermia, and hemodilution. While the independent contributions of acidosis and hypothermia have been extensively evaluated, there is a paucity of data on...
the independent role of hemodilution in this scenario. Traditionally, evaluation and treatment of post-injury coagulopathy has been based largely on the platelet-poor plasma assessment of the extrinsic pathway (prothrombin time, PT) and the intrinsic contact pathway (activated partial thromboplastin time, aPTT). These tests, however, reflect <5% of the thrombin generated during clot formation [12]. Previous clinical studies have shown that up to 35% of the variability in the INR and 20% of the variability of the PTT are unrelated to underlying clotting factor levels [7]. For the past decade, coagulation research has been redirected based on the cell-model of hemostasis, recognizing the central role of platelets, the endothelium, and their interaction on the cell-model of hemostasis [13]. Thrombelastography (TEG), employed in liver transplant [14] and cardiac surgery [15] for half a century, has recently been applied to the trauma setting, facilitated by the modification of provoking coagulation with tissue factor, i.e., the rapid TEG. Additionally, TEG has been shown to be superior to PT and INR in detecting clinically relevant clotting abnormalities following experimental trauma and hemorrhagic shock [16]. We therefore hypothesized that hemodilution has an independent effect on the acute coagulopathy of trauma, using TEG as a comprehensive assessment of clot integrity.

MATeRIALS AND METHODS

All reagents were purchased from Sigma-Aldrich Corp. (St. Louis, MO) unless otherwise specified. Thrombelastography equipment and supplies were obtained from Haemonetics Corporation (Niles, IL); 0.9% injection grade normal saline (NS) was purchased from Baxter Healthcare (Deerfield, IL); iSTAT equipment and supplies were purchased from Abbott Laboratories (Abbott Point of Care, Princeton, NJ). Polyethylene tubing was acquired from Fisher Scientific (Pittsburgh, PA). No heparin was given in this study.

Trauma Model

Adult male Sprague-Dawley rats (Harlan Laboratories, Indianapolis, IN) weighing 350–450 g were housed under barrier-sustained conditions and allowed free access to food and water before use. All animals were maintained in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals, and this study was approved by the University of Colorado Health Sciences Center Animal Care and Use Committee. Animals (n = 10) were anesthetized with inhaled isoflurane, and a cardiac puncture was performed to collect a blood sample for a pre-shock TEG. The femoral artery and vein were then cannulated with polyethylene (PE-50) tubing for continuous invasive pressure monitoring and to establish venous access for resuscitation. A tracheotomy was performed, at which point the animal was placed on 40% O2 using an air-oxygen mixer (Sechrist, Anaheim, CA) at a flow rate of 2 L per min. After a 45-min observation period, a laparotomy was performed to simulate tissue injury, and hemorrhagic shock was induced over a period of 10-min through the arterial catheter to maintain a mean arterial pressure (MAP) of 35 mmHg for 30 min. NS was infused at a rate of 0.4 mL/h to maintain patency of the femoral arterial line during shock. At the end of shock, animals were resuscitated with 2 × shed blood volume in NS over a 30-min period. Animals were then observed for 1.5 h following resuscitation. TEG was performed at baseline, and at the completion of the experiment, 120 min post-shock. Euthermia was maintained throughout the experiment with the use of a heat lamp. Pre-shock and post-shock arterial blood gas measurements were performed to confirm the absence of metabolic acidosis, as well as pre-shock and post-shock hemocrit levels to confirm that 50% hemodilution was achieved (Abbott Point of Care, Princeton, NJ) [17, 18].

Thrombelastography

Thrombelastography (TEG) was performed with blood collected from cardiac puncture at preshock and at the end of the experiment. One mL blood was anticoagulated with 100 µL 3.2% sodium citrate and vortexed briefly to avoid platelet clumping [19]. The citrated whole blood was allowed to sit for 30 min according to the manufacturer’s instructions. Kaolin was used as an activator, and 340 µL of the blood was added to 20 µL of 0.2 M calcium chloride in a disposable plastic cup, with the assay performed on a TEG 5000 thrombelastograph hemostasis analyzer (Haemonetics, Niles, IL) at 37 °C within 2 h of blood collection. All TEG parameters were recorded from standard tracings: reaction time (R, min), coagulation time (K, min), angle (α, degrees), maximum amplitude (MA, mm), clot strength (G, dynes/cm²), and estimated percentage lysis (LY30, Lysis at 30 min, %). The various components of the TEG tracing are depicted in Figure 1 [12]. The SP is a measure of the time to initial clot formation, interpreted from the earliest resistance detected by the TEG analyzer causing the tracing to split; this is the terminus of all other platelet-poor plasma clotting assays (e.g., PT and aPTT). The R value, the time elapsed from start of the test until the developing clot provides enough resistance to produce a 2 mm amplitude reading on the TEG tracing, represents the initiation phase of enzymatic clotting factors. K measures the time from clotting factor initiation (R) until clot formation reaches amplitude of 20 mm. The angle (α) is formed by the slope of a tangent line traced from the R to the K time measured in degrees. K time and angle (α) denote the rate at which the clot strengthens and is most representative of thrombin cleavage of the fibrinogen into fibrin. The MA indicates the point at which clot strength reaches its maximum amplitude in mm on the TEG tracing, and reflects the end result of platelet-fibrin interaction via the GPIIb-IIIa receptors. G is a calculated measure of total clot strength derived from amplitude (A, mm) G = (5000 – A)/100 A. The process of fibrinolysis leads to clot dissolution, leading to a decrease in clot strength; estimated percent lysis (LY30) measures the degree of fibrinolysis 30 min after MA is reached.

![FIG. 1. TEG diagram. The following TEG parameters were recorded from standard tracings: reaction time (R, min), coagulation time (K, min), angle (α, degrees), maximum amplitude (MA, mm), clot strength (G, dynes/cm²), and estimated percentage lysis (LY30, lysis at 30 min, %).](image-url)
RESULTS

In the absence of hypothermia and acidosis, hemodilution does not have a significant impact on coagulation. In this trauma model, rats were resuscitated from hemorrhagic shock, resulting in a hemodilution of 50% (43% ± 4.05% versus 19% ± 3.96% Hct pre-shock versus post-shock, P < 0.0001). Temperature was monitored throughout the experiment and eutermia maintained with the use of a heat lamp (97.4 ± 0.39°F versus 97.2 ± 0.39°F pre-shock versus post-shock, P = 0.37). Additionally, blood pH was measured and unchanged (7.41 ± 0.02 versus 7.40 ± 0.02 pre-shock versus post-shock, P = 0.33). (Results are summarized in Table 1).

Clot integrity is maintained following hemodilution. Despite the substantial hemodilution, there was no statistically significant change in clot strength following fluid resuscitation (12.96 ± 2.84 versus 11.79 ± 1.28 dynes/cm² G pre-shock versus post-shock, P = 0.13). The maximum amplitude (MA) of the clot was not impaired by hemodilution (71.37 ± 5.65 mm versus 70.07 ± 2.39 mm MA pre-shock versus post-shock, P = 0.26).

Enzymatic activity is preserved following hemodilution. Similarly, the onset of coagulation (R time) was not impaired (1.68 ± 1.74 min versus 1.75 ± 0.63 min R time pre-shock versus post-shock, P = 0.45). Neither the K time (0.91 ± 0.24 min versus 0.8 ± 0.00 min K time pre-shock versus post-shock, P = 0.09) nor the angle (α) (81.55 ± 4.99 degrees versus 82.04 ± 1.21 degrees angle (α pre-shock versus post-shock, P = 0.40) were altered by hemodilution.

Hemodilution does not induce fibrinolysis. In addition to the preservation of clot strength and clotting factor activity, hemodilution did not influence fibrinolysis (0.28 ± 0.43% versus 1.44% ± 3.33% LY30, pre-shock versus post-shock, P = 0.16).

DISCUSSION

This in vivo model simulating trauma and hemorrhagic shock does not indicate an independent adverse effect of hemodilution. Our model used crystalloid, not stored blood, as the resuscitation fluid in order to directly investigate the role of dilution of clotting factors in the development of microvascular bleeding, and found that neither the clot integrity (G) nor the onset of coagulation (R time) was impaired by hemodilution. In contrast to the role of hemodilution, the adverse effects of acidosis and hypothermia on post-injury coagulopathy have been evaluated in vitro [20] in animal experiments [21] and in clinical studies [10, 11].

Even though dilution of clotting factors contributing to post-injury coagulopathy has been widely accepted in clinical practice, the literature is divided on the subject. Simmons et al. concluded in their landmark Vietnam War study that simple dilution effects did not explain the major clinical bleeding they were observing in the wounded soldiers [7]. Miller et al., however, attributed microvascular bleeding to dilutional thrombocytopenia in their cohort of massively transfused battle casualties [22]. In a civilian study, Counts et al. in 1979 concluded that microvascular bleeding was due to dilutional thrombocytopenia in massively transfused patients, which became the rationale for a randomized prospective trial evaluating preemptive platelet transfusion [23]. After their study showed that prophylactic platelet or FFP transfusion did not improve hemostasis or mortality [24], and noting that clotting factors declined suddenly and out of proportion to the expected decline based on dilution alone, the authors ultimately concluded that hemodilution played an inconsequential role in the development of microvascular bleeding [25]. A similar trial failed to show benefit for prophylactic platelet transfusion during cardiac surgery [26]. The results imply that platelets are released into the circulation to counteract the effects of hemodilution.

Various clinical studies have shown that INR and PTT fail to correlate with clinical bleeding in trauma patients [7, 24]. Martini et al. have shown that the INR and PTT were not sensitive in detecting these coagulation defects, whereas TEG differentiated the mechanism related to clotting abnormalities in experimental trauma and hemorrhagic shock [16]. Due to the limitations of INR and PTT in predicting microvascular bleeding unless greatly prolonged, thrombelastography was used as a global assessment of coagulation integrity in this study.

**TABLE 1**

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<tr>
<th>Clotting Capacity Remains Intact Despite Hemodilution</th>
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<td><strong>Pre-shock</strong></td>
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A potential limitation of our study is that fibrinogen and platelet levels were not measured directly. The TEG parameters that reflect these parameters, fibrinogen activity (K time, angle), fibrin clot stability (LY30), and platelet integrity (MA), were unaltered. Also, the study is limited by variations in coagulation status between animals in this study and other studies. Problems with variability in coagulation-based assays have been demonstrated clinically as well [27], leading the authors to search for reasons to account for these variations. A previous study by Kim et al., for example, evaluating the effect of hemodilution on coagulation in Sprague Dawley rats, employed twice the recommended concentration of citrate as an anticoagulant, which could explain the relative hypocoagulability in their results. Furthermore, the Kim study corroborated our findings, that coagulopathy was not observed at mild (25%) or moderate (50%) hemodilution, highlighting the important functional reserve of coagulation factors in the setting of trauma and hemorrhagic shock [28].

While our focus was specifically on the role of clotting factor dilution on coagulation integrity, our results showed that resuscitating with a crystalloid equivalent to the rat’s blood volume did not contribute to the development of microvascular bleeding; however, the direct contribution of stored blood components, specifically pRBCs on coagulation integrity, was not investigated in this study. Hemodilution of 50% was chosen in order to avoid critical anemia (<7 g/dL hemoglobin). A previous study of experimental hemorrhagic shock implicated hemodilution in the uniform lethality that ensued; however, this study was confounded by the fact that rats were resuscitated with crystalloid to a hematocrit of 5%. Alternatively, a model of hemorrhagic shock in which critical anemia was avoided demonstrated that hemodilution had no effect on mortality [29]. Therefore, in the presence of critical anemia, it is conceivable that irreversible hemorrhagic shock leads to lethality due to profoundly impaired oxygen carrying capacity rather than dilution of clotting factors.

Ruttman et al. investigated the role of dilution of clotting factors on clot formation using TEG [30]. Unexpectedly, administration of a 1 L NS bolus to healthy subjects induced a transient hypercoagulable state associated with a reduction in antithrombin III (ATIII) levels, leading to a decrease in the onset of coagulation (R) without difference in K, angle, or MA [31]. ATIII exhibits second-order kinetics, making it exceptionally sensitive to a reduction in concentration compared to other clotting factors [32].

In summary, there was no change in mean post-shock coagulation values reported in our study and, in spite of the variation between the rodents, none of the animals in the study developed clinically detectable microvascular bleeding.

Hemodilution (≤50%) had an inconsequential effect on coagulation following trauma and hemorrhagic shock as an independent factor. These data call to question the commonly held belief that hemodilution is critical in the development of post-injury coagulopathy. The highly variable clinical response to hemorrhagic shock, compounded by the effects of massive transfusion of stored blood components, disseminated intravascular coagulation, and qualitative platelet defects are more likely to contribute to the complex milieu in which the development of post-injury coagulopathy occurs, rather than hemodilution by itself.

ACKNOWLEDGMENTS

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REFERENCES

16. Martini WZ, Cortez DS, Dubick MA, et al. Thrombelastography is better than PT, aPTT, and activated clotting time in detecting clinically relevant clotting abnormalities after hypothermia.


