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Hemoglobin-based oxygen carriers promote systemic hyperfibrinolysis that is both dependent and independent of plasmin

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ABSTRACT

Background: Hyperfibrinolysis plays an integral role in the genesis of trauma-induced coagulopathy. Recent data demonstrate that red blood cell lysis promotes fibrinolysis; however, the mechanism is unclear. Hemoglobin-based oxygen carriers (HBOCs) have been developed for resuscitation and have been associated with coagulopathy. We hypothesize that replacement of whole blood (WB) using an HBOC results in a coagulopathy because of the presence of free hemoglobin.

Materials and Methods: WB was sampled from healthy donors ($n = 6$). The clotting profile of each citrated sample was evaluated using native thromboelastography. Serial titrations were performed using both HBOC (PolyHeme) and normal saline (NS; 5%, 25%, and 50%) and evaluated both with and without a 75-ng/mL tissue plasminogen activator (tPA) challenge. Tranexamic acid (TXA) was added to inhibit plasmin-dependent fibrinolysis. Fibrinolysis was measured and recorded as lysis at 30 min (LY30), the percentage of clot LY30 after maximal clot strength. Dilution of WB with NS or HBOC was correlated using LY30 via Spearman rho coefficients. Groups were also compared using a Friedman test and *post hoc* analysis with a Bonferroni adjustment.

Results: tPA-provoked fibrinolysis was enhanced by both HBOC (median LY30 at 5%, 25%, and 50% titrations: 11%, 21%, and 44%, respectively; Spearman = 0.94; $P < 0.001$) and NS (11%, 28%, and 58%, respectively; Spearman = 0.790; $P < 0.001$). However, HBOC also enhanced fibrinolysis without the addition of tPA (1%, 4%, 5%; Spearman = 0.735; $P = 0.001$) and NS did not (1%, 2%, 1%; $r = 0.300$; $P = 0.186$. Moreover, addition of TXA did not alter or inhibit this fibrinolysis (WB *versus* 50% HBOC: 1.8% *versus* 5.7%, $P = 0.04$). There was no significant difference in fibrinolysis of HBOC with or without TXA (50% HBOC *versus* 50% HBOC + TXA: 5.6% *versus* 5.7%, $P = 0.92$). In addition, the increased fibrinolysis seen with NS was reversed when TXA was present (WB *versus* 50% NS: 1.8% *versus* 1.7%, $P = 1.0$).

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Conclusions: HBOCs enhance fibrinolysis both with and without addition of tPA; moreover, this mechanism is independent of plasmin as the phenomenon persists in the presence of TXA. Our findings indicate the hemoglobin molecule or its components stimulate fibrinolysis by both tPA-dependent and innate mechanisms.

1. Introduction

Vascular patency is maintained through fibrinolysis, the physiological counterbalance to coagulation [1]. Although descriptions of coagulopathic bleeding in trauma date back to >200 y ago [2], our knowledge of how the fibrinolytic system is regulated and how its dysfunction contributes to the development of refractory bleeding remains incomplete. Starzl *et al.* [3] appreciated the physiological role of systemic fibrinolysis in the 1960s during liver transplantation, using thromboelastography (TEG). They noted that some patients developed excessive bleeding particularly in the early phase of transplant, and initially recommended antifibrinolytics to prevent systemic fibrinolysis. However, this practice was terminated several years later when they noted an increase in venous thromboembolism and mortality [4]. Although relatively uncommon, excessive systemic fibrinolysis (hyperfibrinolysis) is associated with uncontrollable bleeding and increased mortality [5]. Percent clot lysis at 30 min (LY30) at the time of admission >15% has been associated with a mortality rate of 70% in some trauma reports [5–7]. Conversely, excessive inhibition of the fibrinolytic system (shutdown) is associated with venous thromboembolism, multiple organ failure, prolonged intensive care unit stay, and significant morbidity [8]. Clearly, tight regulation of fibrinolysis during hemorrhage is key in providing optimal levels of coagulation and fibrinolysis to control bleeding while maintaining systemic vascular patency.

Hemoglobin-based oxygen carriers (HBOCs) have been developed in an attempt to provide oxygen delivery when blood products are not available [9]. Newer generation agents include purified, polymerized hemoglobin molecules. Several of these products have been tested for human use; however, FDA approval remains elusive because of potential adverse events including coagulopathy, nitric oxide scavenging leading to vasoconstriction, and myocardial ischemia [10]. These purified solutions provide an excellent tool for the preliminary evaluation of hemoglobin as a mediator of fibrinolytic dysfunction using TEG.

The addition of red blood cell and platelet lysates to healthy volunteer whole blood (WB) results in hyperfibrinolysis and fibrinolysis shutdown, respectively [11]. Recent proteomic evaluation of human trauma patients in hemorrhagic shock has shown elevated plasma levels of hemoglobin and its subunits [12]. Therefore, we hypothesize that hemoglobin may be a mediator of postinjury hyperfibrinolysis.

2. Materials and methods

2.1. Subjects

Assays were performed on citrated WB samples collected from healthy volunteers after obtaining informed consent. No one in the study had a known coagulation disorder, nor were they taking any medications known to affect coagulation or fibrinolysis. Each group contained six male subjects with a median age of 30 y.

2.2. Tissue plasminogen activator

Human tissue plasminogen activator (tPA) from Molecular Innovation (Novi, MI) was diluted in 5% bovine serum albumin in phosphate-buffered saline and aliquoted with a concentration of 10 μg/mL. Aliquots were stored in liquid nitrogen and thawed immediately before each experiment. The dose of tPA administered equated to a final tPA concentration of 75 ng/mL in each sample. This concentration has previously been found to be an inflection point for which a citrated native TEG increased fibrinolytic activity compared with a non–tPA-challenged WB sample in healthy volunteers [13].

2.3. Hemoglobin-based oxygen carrier

The HBOC in this study was PolyHeme (Northfield Laboratories, IL), a polymer of hemoglobin molecules [14]. PolyHeme was stored at 4ºC and accessed from its storage container using a sterile coupler and large bore needle.

2.4. Tranexamic Acid

Tranexamic acid (TXA) was used to block the action of plasmin. TXA is a synthetic lysine analog that reversibly binds to plasminogen, preventing its interaction with fibrin and conversion to plasmin [15]. TEG cups (Haemonetics, Niles, IL) containing air-dried TXA at a concentration of 75 ng/mL were stored at 4ºC per manufacturer instruction and used to perform assays as described in the following section.

2.5. Thromboelastography

Blood was collected in 3.5-mL citrated blood tubes via venipuncture and assayed at room temperature between 20 min and 2 h after blood draw, as recommended by the manufacturer. WB was mixed with an HBOC (PolyHeme; Northfield) or normal saline (NS) to reach a total volume of 500 μL in individual Eppendorf tubes (Hamburg, Germany). The fraction of WB replaced with HBOC or NS ranged from 5–50%. Assays were performed with and without the addition of tPA (added within 5 min of assay start time). tPA assays were also performed in the presence of TXA. Citrated native samples were recalcified, and thromboelastography assays were performed using the TEG 5000 Thrombelastograph Hemostasis Analyzer (Haemonetics) per manufacturer instructions. Fibrinolysis was measured using the LY30 after MA (%). These