Plasma Is the Physiologic Buffer of Tissue Plasminogen Activator-Mediated Fibrinolysis: Rationale for Plasma-First Resuscitation after Life-Threatening Hemorrhage

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BACKGROUND: Prehospital resuscitation with crystalloid exacerbates fibrinolysis, which is associated with high mortality. We hypothesized that plasma compared with crystalloid resuscitation prevents hyperfibrinolysis in a tissue plasminogen activator (tPA)-rich environment via preservation of proteins essential for regulation of fibrinolysis.

STUDY DESIGN: Healthy individuals donated blood, which was assayed using a native (nonactivated) thrombelastography (TEG). Whole-blood was mixed with normal saline (NS) or platelet poor plasma (PPP) at progressive dilutions. Tissue plasminogen activator was added to promote a fibrinolytic environment. In a separate experiment, PPP was run through a 100 kDa filter and liquid remaining on top of the filter (TFP) and below the filter (BFP) was obtained. Whole blood was diluted by 50% with TFP, BFP, and NS and assayed with a tPA TEG challenge. The TFP and BFP were assayed for protein concentration and protein composition.

RESULTS: Normal saline and PPP dilution of whole blood without tPA did not affect clot lysis at 30 minutes (LY30) (NS Spearman's rho 0.300, $p = 0.186$ and PPP 0.294, $p = 0.288$). When tPA was added, NS dilution of whole blood increased LY30 in a percentage-dependent manner (0.844, $p < 0.001$), but did not significantly increase with PPP dilution (0.270, $p = 0.202$). The difference in LY30 from whole blood to diluted whole blood with PPP (mean change, −1.05, 95% CI, −9.42 to 7.33) was similar with TFP (1.23, 95% CI, −5.20 to 7.66, $p = 0.992$). However, both BFP (37.65, 95% CI 24.47 to 50.82, $p = 0.001$) and NS (47.36, 95% CI 34.3 to 60.45, $p < 0.001$) showed large increases in fibrinolysis compared with PPP.

CONCLUSIONS: Crystalloid and plasma dilution of whole blood does not increase fibrinolysis. However, NS dilution of whole blood increases susceptibility to tPA-mediated fibrinolysis. Plasma resuscitation, simulated by plasma dilution of whole blood, attenuates increased susceptibility to tPA-mediated fibrinolysis. The benefits of plasma resuscitation are mediated through preservation of plasma proteins. (J Am Coll Surg 2015;220:872−879. © 2015 by the American College of Surgeons)

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Abbreviations and Acronyms
BFP = liquid below the filter
IQR = interquartile range
LY30 = clot lysis at 30 minutes
NS = normal saline
PPP = platelet poor plasma
TEG = thromboelastography
TFP = liquid on top of the filter
TIC = trauma-induced coagulopathy
tPA = tissue plasminogen activator

The optimal pre-hospital resuscitation strategy in trauma patients experiencing hemorrhagic shock remains unclear. Historically, crystalloids were administered to normalize blood pressure in all trauma patients, which was prompted by the Advanced Trauma Life Support (ATLS) guidelines.[1] In the early 1990s, a clinical trial suggested that pre-hospital saline increased mortality in patients who were actively bleeding.[2] Subsequently, the concept of permissive hypotension was adopted by many centers.[3] The perceived beneficial mechanisms of limited pre-hospital crystalloid resuscitation were: limiting the rise in blood pressure would prevent "popping" off blood clots on hemostatic injuries and minimizing hemodilution of coagulation factors. These concepts date back to the 1960s.[4] Although permissive hypotension may benefit some trauma patients to a degree, a more recent analysis indicated that pre-hospital resuscitation to correct severe shock is life-saving.[5] On the other hand, normal saline (NS) infusion in humans not in shock has been associated with improved hemostasis measured by thrombelastography (TEG),[6] which brings into question the mechanism of saline dilution driving trauma-induced coagulopathy (TIC).

Trauma-induced coagulopathy impairs clot formation and promotes fibrinolysis,[7] and the mechanisms driving these 2 aspects of coagulation appear to be separate.[8,9] With the widespread use of TEG during trauma resuscitation, correlation has been observed between the volume of pre-hospital crystalloid and increased levels of systemic fibrinolysis.[10] Fibrinolysis is part of normal clot remodeling and is important physiologically to keep small vessels patent in a contained vascular bed.[11] However, systemic overactivation of fibrinolysis (hyperfibrinolysis) in trauma has mortality rates ranging from 40% to 90%.[12-15] In our ongoing experience with pre-hospital plasma resuscitation, we observed an apparent reversal of hyperfibrinolysis. With mounting evidence that tPA elevation is the driver of postinjury hyperfibrinolysis,[16] the relationship of saline infusion and the development of hyperfibrinolysis needs to be established. We hypothesized that saline dilution of whole blood does not increase fibrinolysis, but in the presence of tPA, whole blood diluted by saline enhances fibrinolysis predominantly by decreasing the concentration of plasma proteins that contribute to regulation of the fibrinolytic system.

METHODS

Subjects

After obtaining informed consent under an institutional review board-approved protocol (COMIRB # 14-0366), blood samples were obtained from healthy volunteers with no known abnormalities in the coagulation or fibrinolytic systems; none of these individuals had taken salicylic acid or other nonsteroidal anti-inflammatory medications within 120 hours of the experiment. Six subjects were recruited for each experiment. There were 4 men and 2 women, with a median age of 30 years, range 26 to 67 years. The women were not taking oral contraceptive medications and were not pregnant.

Tissue plasminogen activator

Human single chain tPA (Molecular Innovation) was diluted in 5% bovine serum albumin in phosphate buffered saline to a final concentration of 10 μg/μL. Individual aliquots of tPA (prepared for a final concentration of 75 ng/mL) were stored at −80°C and thawed immediately before use. The 75 ng/mL concentration of of tPA was previously found to be an inflection point for which a citrated native TEG increased fibrinolytic activity compared with a non- tPA−challenged whole blood sample in healthy volunteers (data not shown).

Platelet poor plasma

Platelet poor plasma was used to eliminate the known inhibitory effects of platelets on fibrinolysis. A 3.3-mL aliquot of blood was collected in 3% citrated tubes from healthy volunteers and was centrifuged at 6,000 *g* for 10 minutes at 4°C. Plasma was removed and spun at 12,500 *g* for 10 minutes at the same temperature to remove contaminating platelets and acellular debris. Platelet poor plasma was stored on ice (<2 hours), and an aliquot of PPP was flash frozen and stored at −80°C for later proteomic analysis.

Platelet poor plasma filtration

One hundred kDa molecular weight filters were purchased from Sigma Adlrich (Product # Z677906) and used to filter 500 μL of PPP. A 100 kDa cutoff was used because the majority of SERPINs (serine protease inhibitors) known to affect fibrinolysis are below 100 kDa. The PPP was centrifuged at 15,000 *g* for 45 minutes at 4°C. The liquid that passed through the filter was