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Impact of blood products on platelet function in patients with traumatic injuries: a translation study

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ARTICLE INFO

Article history:
Received 11 October 2016
Received in revised form
22 December 2016
Accepted 16 February 2017
Available online xxx

Keywords:
Blood
Platelet
Patients
Trauma
Translation
Study

ABSTRACT

Background: Reductions in platelet (PLT) count and function are associated with poor outcomes in trauma patients. We proposed to determine if patients expected to receive blood products have a decrease in PLT function higher than expected based on the reduction in PLT count, and if the reduction in function could be associated with the donor plasma/supernatant received.

Methods: PLT count and function were measured on admission to the emergency department and intensive care unit in severely injured patients expected to receive a transfusion. PLT function was measured by Multiplate aggregometry in response to five agonists. Function was corrected for alterations in count. *In vitro* studies were conducted in the blood of normal subjects to assess the effect of dilutions with AB donor plasma on PLT function.

Results: Forty-six patients were enrolled, with 87% requiring a transfusion. Median Injury Severity Score was 23 (13.29) and mortality 15%. PLT count and function were decreased from emergency department to intensive care unit admission by 25% and 58%, respectively. Decreases in function persisted after adjustment for count. Patients requiring large volumes of blood products had reductions in function that were disproportionately greater. Reductions in PLT function were greatest after transfusion of PLTs. In *in vitro* studies with a 30% dilution by autologous plasma caused a relational reduction in function, whereas allogenic plasma resulted in greater decreases that were highly variable between donors.

Conclusions: Within hours of injury a decrease in both PLT count and function occurs, that is aggravated with the administration of blood products, with transfusion of PLTs showing

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0022-4804/$ – see front matter
http://dx.doi.org/10.1016/j.jss.2017.02.037

the greatest effect. The effect on PLT function of allogenic transfused plasma appears to be highly donor related.

Introduction

Platelet (PLT) count and function are highly associated with morbidity and mortality of the patient with traumatic injuries.[1-5] A low PLT count at admission has been associated with as a decreased rate of survival and a reduction in PLT function. Decreases in PLT function are associated with higher incidence of acute coagulopathy of trauma higher ventilation requirements, inflammation/infection, traumatic brain injury, and mortality.[1-5] In addition, a high degree of variability exists in PLT function adjusted for PLT count in patients with severe traumatic injuries who have been transfused large volumes of blood products.

In vitro, the use of blood products is suggested to decrease PLT function and to vary as to how long the red cells and PLTs were stored.[6] In addition, the supernate (plasma) from stored red blood cells (RBCs) inhibits PLT function.[7] Evaluation of *in vitro* PLT function in reconstituted whole blood variants suggest that additives or components in packed RBCs or fresh frozen plasma (FFP) depress PLT function. Thus, the age of blood products and constituents of the products appear to affect PLT function.

For these reasons, we developed this study to test the hypothesis that patients expected to receive large amounts of blood products would have a higher than expected decrease in PLT function based on PLT count. A second hypothesis is that the reduction in PLT function is associated with the donor plasma/supernatant received in stored blood products.

Methods

Human subjects

This prospective observational study was conducted at Memorial Hermann Hospital Texas Medical Center, a Level I trauma center, and The University of Texas Health Science Center at Houston (UTHealth). Prior approval was obtained from the UTHealth Institutional Review Board (HSC-GEN-12-0059). Adult admitted patients were eligible for inclusion in this study if they met the hospital criteria for the highest level of trauma team activation. This was a sampling of convenience based on when research laboratory staff were available to obtain and process samples. Patients were excluded if they were aged <16 y, pregnant, prisoners, enrolled in other studies, or declined to give consent. There were 813 patients screened for enrollment in studies over the 8-mo period. The majority were excluded as they were not expected to receive a transfusion. Many of those expected by staff to receive a transfusion of blood products were enrolled in ongoing randomized trials.[8] Patients from whom an initial blood draw could not be obtained were also excluded from enrollment. Consent was obtained from the patient or a legally authorized representative within 72 h of admission. A waiver of consent was obtained from the Institutional Review Board for those patients discharged or who died within 24 h. In the remaining cases in which consent could not be obtained, the patient was excluded from the study, and their blood samples were destroyed. Blood samples were also collected from consented healthy subjects to serve as controls and for the *in vitro* experiments under a separate protocol (HSC-MS-09-0314).

Clinical studies

On hospital admission, 20 mL of blood was obtained. Blood was transferred into vacutainer tubes containing 3.2% citrate and inverted to ensure proper anticoagulation. If the patient was expected by the research staff to receive a transfusion of blood products in the course of their initial care, a second sample was obtained on admission to the intensive care unit (ICU). Patient demographics, vital signs, standard laboratory values, mechanisms, and severity of injuries were collected at the time of admission. Blood products transfused were identified as RBCs, FFP, and PLTs. A unit of PLTs was defined as six packs. The age of PLTs transfused was also recorded.

In vitro *studies*

Twenty milliliters of blood were obtained from control subjects who were not on PLT inhibitors or had an infection. Serial dilutions with saline and autologous plasma were performed to assess the effect of the dilution on PLT function in blood. Subject autologous FFP was prepared by snap freezing a plasma sample in liquid nitrogen then thawing it in a 37° water bath. We have previously demonstrated this procedure to decrease the hemostatic function of plasma.[9,10] Additional dilution studies with AB plasma purchased from the local blood bank were also performed. The donor FFP was collected in citrate phosphate dextrose anticoagulant and frozen before using following standard blood banking procedures.

PLT function

Investigations on whole blood PLTs function were performed 30 min after blood draw by trained personnel on-call. The analyses were made on Multiplate (Verum Diagnostica GmbH, Munich, Germany) using the multiple electrode aggregometry technology that has a turnaround time of 10 min per test period.[11-13] Multiplate has five different test cells that allow simultaneous measurement of five different agonists to interrogate specific functional pathways[11-13]; each cell has two sets of 3-mm silver-coated copper electrode, a Teflon-coated stirring magnet, and requires 300 μL of whole citrate blood. PLTs were activated through five different agonist: adenosine diphosphate (ADP; 20 μL 6.5 μM, receptor pathway P2Y12), collagen (COL; 20 μL 3.2 μg/mL, GPIa/IIa, and GPVI receptors pathways), thrombin receptor-activating peptide-6 (TRAP; 20 μL 32 μM, PAR receptor pathway), arachidonic acid