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# Treatment with a histone deacetylase inhibitor, valproic acid, is associated with increased platelet activation in a large animal model of traumatic brain injury and hemorrhagic shock

*Simone E. Dekker, BSc,[a,b] Martin Sillesen, MD,[c,d] Ted Bambakidis, MSc,[a] Anuska V. Andjelkovic, MD, PhD,[e,f] Guang Jin, MD, PhD,[a] Baoling Liu, MD,[a] Christa Boer, PhD,[b] Pär I. Johansson, MD, Dmsc, MPA,[g,h] Durk Linzel, MD,[a,i] Ihab Halaweish, MD,[a] and Hasan B. Alam, MD[a,*]*

[a] *Department of Surgery, University of Michigan Hospital, Ann Arbor, Michigan*
[b] *Department of Anesthesiology, VU University Medical Center, Institute for Cardiovascular Research, Amsterdam, the Netherlands*
[c] *Division of Trauma, Emergency Surgery And Surgical Critical Care, Department of Surgery, Massachusetts General Hospital/Harvard Medical School, Boston, Massachusetts*
[d] *Department of Surgery, Copenhagen University Hospital, Hillerød, Denmark*
[e] *Department of Pathology, University of Michigan Medical School, Ann Arbor, Michigan*
[f] *Department of Neurosurgery, University of Michigan Medical School, Ann Arbor, Michigan*
[g] *Capital Region Blood Bank, University of Copenhagen, Rigshospitalet, Denmark*
[h] *Department of Surgery, University of Texas Medical School, Centre for Translational Injury Research (CeTIR), Houston, Texas*
[i] *Department of Emergency Medicine, VU University Medical Center, Amsterdam, the Netherlands*

ARTICLE INFO

*Article history:*
Received 15 January 2014
Received in revised form
15 January 2014
Accepted 25 February 2014
Available online 1 March 2014

*Keywords:*
Traumatic brain injury
Hemorrhagic shock
Histone deacetylase inhibitor
Fresh frozen plasma

ABSTRACT

*Background:* We have previously shown that resuscitation with fresh frozen plasma (FFP) in a large animal model of traumatic brain injury (TBI) and hemorrhagic shock (HS) decreases the size of the brain lesion, and that addition of a histone deacetylase inhibitor, valproic acid (VPA), provides synergistic benefits. In this study, we hypothesized that VPA administration would be associated with a conservation of platelet function as measured by increased platelet activation after resuscitation.

*Materials and methods:* Ten swine (42-50 kg) were subjected to TBI and HS (40% blood loss). Animals were left in shock for 2 h before resuscitation with either FFP or FFP + VPA (300 mg/kg). Serum levels of platelet activation markers transforming growth factor beta, CD40 L, P-selectin, and platelet endothelial cell adhesion molecule (PECAM) 1 were measured at baseline, postresuscitation, and after a 6-h observation period. Platelet activation markers were also measured in the brain whole cell lysates and immunohistochemistry.

This article was presented at the ninth Annual Academic Surgical Congress, San Diego, California, February 4–6, 2014.
* *Corresponding author*. Department of Surgery, 2920 Taubman Center, University of Michigan Hospital, 1500 E. Medical Center Drive, Ann Arbor, MI 48109. Tel.: +1 734 936 5823; fax: +1 734 936 6927.
E-mail address: alamh@med.umich.edu (H.B. Alam).

http://dx.doi.org/10.1016/j.jss.2014.02.049

Neuroprotection
Platelet activation
Resuscitation
Swine

*Results:* Circulating P-selectin levels were significantly higher in the FFP + VPA group compared with the FFP alone group (70.85 ± 4.70 *versus* 48.44 ± 7.28 ng/mL; $P < 0.01$). Likewise, immunohistochemistry data showed elevated P-selectin in the VPA treatment group (22.30 ± 10.39% *versus* 8.125 ± 3.94%, $P < 0.01$). Serum sCD40L levels were also higher in the FFP + VPA group (3.21 ± 0.124 *versus* 2.38 ± 0.124 ng/mL; $P < 0.01$), as was brain sCD40L levels (1.41 ± 0.15 *versus* 1.22 ± 0.12 ng/mL; $P = 0.05$). Circulating transforming growth factor beta levels were elevated in the FFP + VPA group, but this did not reach statistical significance (11.20 ± 1.46 *versus* 8.09 ± 1.41 ng/mL; $P = 0.17$). Brain platelet endothelial cell adhesion molecule 1 levels were significantly lower in the FFP + VPA group compared with the FFP group (5.22 ± 2.00 pg/mL *versus* 7.99 ± 1.13 pg/mL; $P = 0.03$).
*Conclusions:* In this clinically relevant large animal model of combined TBI + HS, the addition of VPA to FFP resuscitation results in an early upregulation of platelet activation in the circulation and the brain. The previously observed neuroprotective effects of VPA may be due to a conservation of platelet function as measured by a higher platelet activation response after resuscitation.



## 1. Introduction

Trauma is the leading cause of death among young people, accounting for nearly 6 million deaths worldwide each year [1]. Hemorrhagic shock (HS) and traumatic brain injury (TBI) frequently co-occur and account for most trauma-related mortalities [2]. Approximately one-third of TBI patients [3] and one-quarter of general trauma patients [4] exhibit abnormal coagulation tests on hospital admission. Although the frequency and severity of coagulation disturbances is well documented [4,5], the role of platelets in these disturbances is poorly understood. Platelet count has been shown to predict lesion progression and mortality in TBI patients [6]. In addition, we recently found that platelet function decreases after TBI + HS [7], and dysfunction is associated with increased mortality [8,9] and bleeding complications [10] in general trauma and TBI patients. However, relatively little is known about platelet activation and function after the initial TBI insult as well as the importance of these processes in the initiation or attenuation of secondary brain injury. The method of resuscitation following TBI merits particular attention, as the choice of fluid may play a critical role in the development of platelet dysfunction. Data from our lab have indicated that fresh frozen plasma (FFP) resuscitation increases platelet function in the first hours after resuscitation [11]. We have also shown that valproic acid (VPA), a commonly prescribed anti-epileptic drug, can improve platelet functions. VPA when given in large doses acts as a histone deacetylation inhibitor to improve outcomes in large animal models of lethal insults [12]. Addition of VPA to hetastarch resuscitation was found to be neuroprotective in a large animal model of TBI + HS [13]. Similarly, early administration of VPA to the FFP-resuscitated animals was associated with smaller lesion size and decreased brain swelling when compared with FFP infusion alone [14]. In the present study, we investigated whether these differences in lesion size and brain swelling are associated with differential effects of FFP and FFP + VPA on *in vivo* platelet activation. We hypothesized that resuscitation with FFP + VPA would preserve platelet functions, as measured by increased platelet activation markers in brain tissue and serum, compared with FFP alone.

## 2. Materials and methods

All experiments were conducted in accordance with the Animal Welfare Act and other federal statutes and regulations related to animal research. The study complied with the Guide for the Care and Use of Laboratory Animals, Institute for Laboratory Animal Research (1996) and was approved by the institutional animal care and use committee. All experiments were performed under the supervision of a veterinarian.

### 2.1. *Animal preparation and monitoring*

Ten female Yorkshire swine (42–50 kg; Tufts Veterinary School, Grafton, MA) were allowed to acclimate for 3 d and examined by a veterinarian to ensure good health. Animals were anesthetized and prepared as previously described [14]. To summarize, animals were sedated, intubated, and supported on a mechanical ventilator with inhaled isoflurane maintained at 1%–3% for the duration of the experiment. Invasive hemodynamic monitoring was accomplished by cannulating the left femoral artery, left femoral vein, right femoral artery, and right external jugular vein. The animal was moved to a sternal position, and the head was fixed in a custom-made stereotactic frame to prevent movement. A craniotomy was performed to provide access for brain oxygenation and intracranial pressure monitoring, and also for the TBI insult.

### 2.2. *TBI, hemorrhage, and resuscitation protocol*

TBI + HS insults and resuscitation were conducted as previously published [14]. Briefly, a computer-controlled cortical impact device was used to deliver a precise and reproducible TBI [15]. Volume-controlled hemorrhage commenced concurrent with TBI. The total blood volume was estimated, and 40%–45% was withdrawn through the femoral artery at a rate of 3.15% total blood volume per minute. Animals were left in shock (Mean arterial pressure maintained between 30–35 mmHg) for 120 min after hemorrhage. After 2 h of shock, animals were randomly resuscitated with either (i) FFP at 50 mL/min or (ii) FFP at 50 mL/min plus VPA 300 mg/kg (EMD