Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.JournalofSurgicalResearch.com

Rapid valproic acid-induced modulation of the traumatic proteome in a porcine model of traumatic brain injury and hemorrhagic shock

Michael Weykamp, BS,[a] *Vahagn C. Nikolian, MD,*[a] *Isabel S. Dennahy, MD,*[a] *Gerald A. Higgins, MD, PhD,*[b] *Patrick E. Georgoff, MD,*[a] *Henriette Remmer, PhD,*[c] *Mohamed H. Ghandour, BS,*[a] *and Hasan B. Alam, MD*[a,*]

[a] *Department of Surgery, University of Michigan Medical School, Ann Arbor, Michigan*
[b] *Department of Computational Medicine and Bioinformatics, University of Michigan Medical School, Ann Arbor, Michigan*
[c] *Department of Biological Chemistry, University of Michigan Medical School, Ann Arbor, Michigan*

ARTICLE INFO

Article history:
Received 19 September 2017
Received in revised form
10 January 2018
Accepted 22 February 2018
Available online 25 April 2018

Keywords:
Valproic acid
Histone deacetylase inhibitor
Proteomics
Posttranslational modification
Hemorrhage
Traumatic brain injury

ABSTRACT

Background: Histone deacetylase inhibitors such as valproic acid (VPA) improve survival in lethal models of hemorrhagic shock and polytrauma. Although VPA is known to modulate transcription, its ability to reduce mortality within minutes of administration suggests involvement of a rapid, posttranslational mechanism. We hypothesized that VPA treatment would cause proteomic changes within minutes of treatment including quantitative and/or posttranslational differences in structural and/or effector proteins.

Materials and methods: We used a porcine model of traumatic brain injury (computer-controlled cortical impact, 12 mm depth) and hemorrhagic shock (40% hemorrhage). Animals were kept in shock for 2 h and randomized to two groups ($n = 3$): normal saline (volume = 3:1 hemorrhage volume) or normal saline + VPA (150 mg/kg, single dose). Peripheral blood mononuclear cells were collected at baseline, postshock, and post-resuscitation. Intracellular protein profiles were assessed using 1 dimensional gel electrophoresis, liquid chromatography, mass spectrometry, and analyzed with Ingenuity Pathway Analysis software.

Results: Animals treated with VPA demonstrated significant proteomic changes. Quantitative differences were found in over 200 proteins including effector, regulatory, and structural proteins in critical cell signaling pathways. Posttranslational modification analysis demonstrated differential VPA-induced acetylation of lysine residues in histone and nonhistone proteins. Pathway analysis correlated these changes with significant increases in numerous prosurvival and cytoskeletal intracellular pathways, including Rho GTPase signaling ($P = 1.66E-11$), integrin signaling ($P = 4.19E-21$), and a decrease in Rho guanosine nucleotide dissociation inhibitor signaling ($P = 4.83E-12$).

Author contributions: M.W., H.B.A helped in conception and design. M.W., V.C.N., I.S.D., P.E.G., M.H.G. helped in data acquisition; M.W., G.A.H., H.R., H.B.A. helped in data interpretation; M.W., V.C.N., I.S.D., G.A.H., H.R. helped in article preparation; all authors helped in critical revisions. H.B.A. provided grant funding.

This study was presented at the Annual Clinical Congress of the American College of Surgeons (San Diego, CA, October 2017).

* *Corresponding author*. Norman Thompson Professor of Surgery, and Head of General Surgery, University of Michigan Hospital, 2920 Taubman Center/5331, 1500 E. Medical Center Drive, Ann Arbor, MI 48109-5331. Tel.: +1 734 936 5823; fax: +1 734 936 5830.
E-mail address: alamh@med.umich.edu (H.B. Alam).

0022-4804/$ – see front matter
https://doi.org/10.1016/j.jss.2018.02.046

Conclusions: In a porcine model of severe injuries, a single dose of VPA is associated with protective changes in the proteome that are measurable within minutes of treatment.

Introduction

Valproic acid (VPA) has been studied extensively in the last decade as an adjunctive treatment in trauma and has been shown to improve survival and other clinically relevant outcomes in animal models of severe injuries.[1-3] VPA is best known as an antiepileptic drug, which works by modulating gamma-aminobutyric acid channel activity.[4] When administered in higher doses, however, VPA has also been shown to have activity as a histone deacetylase inhibitor.[5] This is significant because hemorrhagic shock has been shown to result in an imbalance in histone acetyltransferase and histone deacetylase (HDAC) activity, resulting in a state of global hypoacetylation.[6] VPA is particularly attractive for use in trauma as a "bridge" to conventional treatments because of its logistical advantages. It is lightweight, stable at room temperature, and easy to reconstitute quickly, which makes it practical for use in the prehospital environment where most hemorrhagic shock-related deaths occur.[7]

Consistently positive results from rodent and porcine experiments have resulted in VPA being approved for human testing in the form of a phase I clinical trial in hemorrhagic shock (NCT01951560). Despite this encouraging progression through preclinical animal models, the exact mechanism by which VPA derives its acute therapeutic benefit in traumatic injury remains incompletely understood. Knowledge of VPA's action as a histone deacetylase inhibitor has focused much of our prior mechanistic work on assessing its effect on transcription; changing the acetylation profile of nuclear histone proteins, treatment with VPA subsequently leads to improved accessibility of DNA to transcriptional machinery.[8-11] Our group and others have established that VPA treatment induces changes in protein expression that are associated with improved cell survival.[9,12-14]

The vast majority of these studies have been conducted using animal tissues collected hours after VPA administration to allow for these drug-induced transcriptional changes to occur and be translated downstream.[6,8,15] However, data from animal models of hemorrhagic shock and polytrauma suggest that the vast majority of the VPA-related survival advantage actually occurs within minutes of VPA administration.[9] This rapid benefit is unlikely to be due to changes at the level of gene transcription (and new protein production), which is a slow process, but instead through quick alterations in the functional status of existing proteins. As such, the aim of this study was to assess the early effects of VPA, which likely underlie its immediate survival benefit. Specifically, it was designed to test the hypothesis that VPA administration would significantly alter the quantity and posttranslational state of intracellular proteins within minutes of treatment.

Methods

This protocol was reviewed and approved by the University of Michigan institutional animal care and use committee, and all experiments were performed in compliance with animal welfare and research regulations. Female Yorkshire swine (39-44 kg; Michigan State University, East Lansing, MI) were used in this study. Female sex was chosen for their simpler urethral anatomy and lower risk for injury as it pertains to placing our suprapubic catheters. Animals underwent a standard acclimatization process that has been described in previous publications. Please refer to previous publications by Imam *et al.*[16] and Jin *et al.*[17] for details of the model. Brief descriptions are provided in the following.

Porcine model of hemorrhagic shock and traumatic brain injury

We elected to study VPA's effects on the posttraumatic proteome in swine instead of using samples from human trauma patients from our current clinical trial to allow for tight experimental control that would not be possible in humans. This eliminates significant population heterogeneity as well as variation in injury phenotype with respect to time from injury to presentation, the nature of injuries sustained, concurrent medical management, patient comorbidities, etc.

Anesthesia and perioperative drug administration

Anesthesia was induced with a combination of Telazol (0.5 mg/kg intramuscular injection; Pfizer, New York, NY) and inhaled isoflurane (1%-3%) and maintained using inhaled isoflurane (1%-2%) for the duration of the operation.

Instrumentation and technique

A pulmonary artery catheter was inserted via the right external jugular vein using percutaneous ultrasound-guided technique. Bilateral femoral artery cannulae were placed using cutdown technique and used for intraarterial blood pressure monitoring, hemorrhage, and laboratory blood draws. Unilateral femoral venous access was obtained for administration of drugs and fluids. A mini-laparotomy allowed for placement of a suprapubic urinary catheter. Animals were then placed in a prone position, and the head was secured in a stereotactic frame.

Traumatic brain injury and hemorrhage

A scalp incision was made to expose the skull, and a small burr hole was created 10 mm anterior and 10 mm lateral to the bregma on the left side. A dual lumen catheter was placed into