Published in final edited form as:

J Am Coll Surg. 2019 March ; 228(3): 265–275. doi:10.1016/j.jamcollsurg.2018.12.026.

Valproic Acid Attenuates Neural Apoptosis, Inflammation, and Degeneration 30 Days after Traumatic Brain Injury, Hemorrhagic Shock, and Polytrauma in a Swine Model

Panpan Chang, MD, PhD^{*,1,2}, Aaron M. Williams, MD^{*,2}, Umar F. Bhatti, MD², Ben E. Biesterveld, MD², Baoling Liu, MD², Vahagn C. Nikolian, MD², Isabel Dennahy, MD², Jessica Lee, Yongqing Li, MD, PhD², and Hasan B. Alam, MD²

¹Trauma Medicine Center, Peking University People's Hospital, Beijing, China.

²Department of Surgery, University of Michigan Health System, Ann Arbor, Michigan, USA

Abstract

Introduction: A single-dose (150mg/kg) of valproic acid (VPA) has been shown to decrease brain lesion size and improve neurologic recovery in pre-clinical models of traumatic brain injury (TBI). However, the long-term impact of single-dose VPA following TBI has not been well evaluated.

Methods: Yorkshire swine were subjected to TBI (cortical impact), hemorrhagic shock, and polytrauma. Animals remained in hypovolemic shock for two hours prior to resuscitation with normal saline (NS, volume = 3x hemorrhaged volume) or NS + VPA (150 mg/kg) (n=5/cohort). Brain samples were harvested 30 days following injuries. The cerebral cortex adjacent to the site of cortical impact was evaluated using TUNEL assay, immunohistochemistry, and Western blot analysis.

Results: VPA treatment significantly decreased the number of TUNEL (+) cells and expression of cleaved-caspase 3. Expression of ionized calcium binding adaptor molecule-1 (Iba1), glial fibrillary acid protein (GFAP), amyloid beta (A β), and phosphorylated-Tau (p-Tau) were significantly attenuated ($p < 0.05$) in the VPA-treated animals compared to the NS group. VPA treatment also significantly increased ($p < 0.05$) expression of brain-derived neurotrophic factor (BDNF) compared to the NS group. Lastly, nuclear NF- κ B was significantly decreased ($p < 0.05$), while cytosolic I κ B- α expression was significantly increased ($p < 0.05$) following treatment with VPA compared to the NS group.

Conclusions: Following a single-dose, VPA treatment can decrease neural apoptosis, inflammation, and degenerative changes, and promote neural plasticity in a long-term model of TBI. Furthermore, VPA acts, in part, via regulation of NF- κ B and I κ B- α pathways.

Addresses for correspondence: Hasan B. Alam, MD, Norman Thompson Professor of Surgery, and Head of General Surgery, University of Michigan Hospital, 2920 Taubman Center/5331, University of Michigan Hospital, 1500 E. Medical Center Drive, Ann Arbor, MI 48109-5331, alamh@med.umich.edu.

*designates co-first authors

Level of Evidence: pre-clinical study.

Conflict of Interest: none to report.

Keywords

neural apoptosis; neuroinflammation; neurodegeneration; valproic acid; traumatic brain injury

Introduction

Traumatic brain injury (TBI) remains a leading cause of preventable death in trauma.(1, 2) In the United States (U.S.), TBI affects nearly 2 million people each year and contributes to permanent disability and death.(3) Although many health care providers consider TBI an “event,” TBI is, in fact, a chronic disease process.(4) According to the World Health Organization, TBI is considered to be a permanent process, resulting in irreversible alterations to the brain; this often requires long-term observation or care.(5) Furthermore, the long-term impact of TBI has been associated with an increased incidence of neurodegenerative diseases, including Chronic Traumatic Encephalopathy (CTE) and Alzheimer’s Disease (AD).(1, 6–10) Despite improvements in medical care for TBI and its long-term complications, the need remains for better pharmacologic agents that can decrease morbidity and improve long-term neurologic outcomes following TBI.

In recent years, valproic acid (VPA) has emerged as a promising pharmacologic agent for the treatment of TBI. VPA, a histone deacetylase inhibitor (HDACI), acts through epigenetic modulation to induce post-translational modification of histone and non-histone proteins. In large animal models of TBI and hemorrhagic shock (HS), with and without polytrauma, single-dose VPA (150 mg/kg) administration decreases brain lesion size, improves neurologic outcomes, and promotes faster neurocognitive recovery compared to controls.(11, 12) In the short-term (9 hours following injury), these neuroprotective effects have been attributed to increased neurogenesis and decreased neuroinflammation in the injured brain.(13) At 24 hours following TBI, these protective effects appear sustained in peripheral blood mononuclear cells (PBMCs), which serve as a ‘window’ into brain alterations following TBI.(14) Despite increasing knowledge of VPA’s therapeutic effects in short-term settings, the long-term impact of single-dose VPA administration, however, remains unknown.

The aim of this study was to investigate the long-term impact of single-dose VPA (150 mg/kg) treatment in a large animal trauma model of TBI. We hypothesized that single-dose VPA administration would attenuate neural apoptosis, inflammation, and degeneration and promote neural plasticity in a long-term swine model of TBI, HS, and polytrauma.

Methods

This protocol was reviewed by the Institutional Animal Care and Use Committee at the University of Michigan. Experiments were conducted in compliance with all regulations regarding research and animal welfare.

Injury Model: Traumatic Brain Injury, Hemorrhage, and Polytrauma

This 30-day survival study has been described in-depth previously.(12) Briefly, female Yorkshire swine (37–50 kg; Michigan State University, East Lansing, MI) were used for the study. Animals were anesthetized, mechanically ventilated, and instrumented for observation

of hemodynamic and intracranial parameters. A 21-mm burr hole was made anterior and lateral to the bregma on the right side of the skull. A computer-controlled cortical impact-induced TBI with a 20-mm cylindrical impactor was performed (4 m/s velocity, 100-millisecond dwell time, and 8-mm depth of penetration). Animals were hemorrhaged 40% of their total blood volume and had the following injuries inflicted: rectus abdominis crush, rib fracture, liver injury and spleen injury. A sham group underwent instrumentation and monitoring, but no injuries (n=5).

Shock, Resuscitation, and Treatment

Following completion of hemorrhage, animals were kept in shock (mean arterial pressure [MAP] of 30–35 mmHg via titration of isoflurane) for 2 hours. This simulated prolonged time to treatment in an austere environment. Animals were then randomized to one of two groups: normal saline (NS) resuscitation or NS resuscitation + VPA treatment (150 mg/kg over 3 hours) (n=5 cohort). Animals randomized to VPA treatment received a single 150mg/kg dose of intravenous VPA given over 3 hours, starting 1 hour after initiation of shock. This simulated early medic response time. At the end of the shock period (2 hours following injuries), all animals received resuscitation with NS (3x the volume of blood lost) over 1 hour.

Observation, Recovery, Neurologic Evaluation, and Imaging

Animals were observed for 2 hours after resuscitation and then received autologous packed red blood cell transfusion to simulate transfer to place of definitive care. After transfusion was complete, invasive monitoring was discontinued and animals were weaned from mechanical ventilation. Animals were then monitored for a total of 30 days. They were assessed daily using a validated tool for assessing both the severity of neurologic injury and recovery trajectory.(11, 15) Once animals were able to participate, neurocognitive testing (modified operant conditioning model) was initiated.(16) Animals also underwent T2-weighted magnetic resonance imaging under anesthesia on post-injury days (PID) 3 and 10.

Euthanasia

After 30 days of observation, animals were euthanized and brains were harvested. Using a titanium sectioning block (University of Michigan Medical Innovation Center; Ann Arbor, MI), brains were sliced into 5mm coronal sections. Brain sections then underwent formalin fixation for 48–72 hours and were then transferred to 70% ethanol for preservation. Brain tissue from the cortex adjacent to the most injured site was used for all subsequent analyses listed below.

Terminal Deoxynucleotidyl Transferase dUTP nick End Labeling (TUNEL) Assay

A TACS® 2 TdT-Fluor In Situ Apoptosis Detection Kit (Trevigen, 4812–30-K, Gaithersburg, MD) was used according to manufacturer guidelines to detect neural apoptosis following injury. Briefly, sections were incubated with Proteinase K for 30 minutes. After washing three times, slides were incubated with the labeled reactant for 1 hour. Stop buffer was added followed by staining with Trep-Fluor solution and DAPI. Imaging was obtained using a fluorescence microscope (Olympus, BX53, Tokyo, Japan).

Immunohistochemistry Analysis

Immunohistochemistry (IHC) was used to assess the expression levels of ionized calcium binding activated molecule-1 (Iba1), glial fibrillary acidic protein (GFAP), amyloid-beta (A β), and phosphorylated-Tau protein (p-Tau). Cortical sections (40 μ m) adjacent to the injury were first incubated in 0.3% hydrogen peroxide (H₂O₂) solution followed by 30 minutes blocking in Tris-HCl Buffered Saline (TBS) supplemented with 0.05% Triton-100 and 4% normal goat serum. Sections were incubated with antibodies in TBS supplemented with 0.05% Triton-100 and 4% normal goat serum overnight in 4°. After washing 3 times, sections were then incubated with biotinylated secondary antibody (1:1000; Vector Laboratories, Burlingame, CA) in TBS supplemented with 0.05% Triton-100 and 4% normal goat serum for 1 hour. Sections were then incubated for 1 hour in avidin-biotin substrate (ABC kit, Vector Laboratories, Burlingame, CA). All sections then incubated in 3,3'-diaminobenzidine (DAB) solution (Vector Laboratories) until desired stain intensity develops. Finally, sections were dehydrated in ethanol and xylene, and then cover-slipped using a mounting medium. Both positive and negative controls to each antibody were performed to confirm specificity.

Western Blot Analyses

Western Blot was used to confirm findings from TUNEL assay and IHC. Tissue was lysed in radioimmunoprecipitation assay (RIPA) buffer or mitochondria/cytosol fractions were isolated using a Abcam's Mitochondria/Cytosol Fractionation Kit (Abcam, ab65320, Cambridge, MA) according to the manufacturer's instructions. Primary antibodies used and their respective dilutions were as follows: rabbit anti-cleaved caspase 3 (c-cas 3, 1:1000) from Cell Signaling Technology (Danvers, MA); rabbit anti-Iba1 (1:1000), rabbit anti-GFAP (1:1000); rabbit anti-A β (1:1000); rabbit anti-Tau (1:1000), rabbit anti-p-Tau (1:1000), rabbit anti-brain-derived neurotrophic factor (BDNF, 1:1000), rabbit anti-NF- κ B (1:1000), rabbit anti-IkB α (1:1000), rabbit anti-histone 3 (H3, 1:1000), mouse anti- β -actin (1:3000) from Abcam.

Statistical Analyses

All analyses were performed using GraphPad Prism version 6.00 (GraphPad Software; San Diego CA). One-way analysis of variance (ANOVA) with Bonferroni post-hoc testing was used to detect differences among three or more groups. Data are presented as mean $\pm$ standard error of the mean (SEM). For all tests, a $p < 0.05$ was considered statistically significant.

Results

VPA Decreases Brain Lesions Size and Improves Neurologic Outcomes

As previously reported,⁽¹⁷⁾ VPA-treated animals had significantly lower ($p < 0.05$) neurologic severity scores during the first five days of recovery. Time until complete neurologic recovery was also significantly shorter for VPA-treated animals (days to recovery: NS = 9.4 ± 3.4 ; NS + VPA = 5.2 ± 1.8 ; $p = 0.04$). VPA-treated animals were able to begin neurocognitive testing earlier (days to initiation: NS = 6.2 ± 1.6 ; NS + VPA = 3.6

± 1.5 ; $p = 0.002$) and required significantly less sessions for task mastery (days to mastery: NS = 7.0 ± 1.0 ; NS + VPA = 4.8 ± 0.5 ; $p = 0.03$). Brain lesion size on PID3 was also significantly decreased for VPA-treated animals compared to the NS group (mean lesion size, mm^3 : NS = 4956 ± 1511 ; NS + VPA = 828 ± 279 ; $p = 0.04$).

VPA Attenuates Long-Term Neural Apoptosis

TUNEL assay and expression of c-caspase 3 were used to evaluate neural apoptosis at the end of the 30-day observation period. TUNEL-positive cells were not expressed in the Sham group (Figure 1A). In the NS group, there was an increase in TUNEL-positive cells compared to Sham. However, VPA-treated animals had a decreased number of TUNEL-positive cells compared to the NS group.

Western Blot analysis was used for confirmation of these findings. In the NS group, there was a significant increase ($p < 0.01$) in expression of c-caspase 3 compared to Sham (Figure 1B). However, VPA treatment significantly ($p < 0.01$) attenuated these changes to a level similar to Sham.

VPA Attenuates Long-Term Neuroinflammation

IHC and Western Blot were used to evaluate Iba1 and GFAP, respectively. IHC revealed that Iba1 and GFAP expression were increased in the NS group compared to Sham (Figure 2A). However, VPA-treated animals had decreased expression of Iba1 and GFAP relative to the NS group.

Western Blot confirmed IHC findings. Iba1 and GFAP expression were significantly increased ($p < 0.001$) in the NS compared to the Sham group (Figure 2B and C). However, VPA administration significantly attenuated ($p < 0.001$ and $p < 0.01$, respectively) Iba1 and GFAP expression similar to the Sham group.

VPA Attenuates Long-Term Neurodegeneration

IHC and Western Blot were used to detect A β and p-Tau, well-known markers of neurodegenerative disease. IHC revealed that there was a significant increase in A β and p-Tau in the NS group compared to Sham (Figure 3A). However, VPA treatment decreased expression of A β and p-Tau compared to NS group.

Western Blot analysis confirmed IHC findings. In the NS group, A β and p-Tau were significantly increased ($p < 0.001$ and $p < 0.01$, respectively) compared to Sham (Figure 3B and C). However, this increased expression was significantly attenuated ($p < 0.001$ and $p < 0.05$) in the VPA treatment group.

VPA Promotes Long-Term Neuroplasticity

Using Western Blot analysis, BDNF expression was evaluated. BDNF is key player in promoting neural plasticity and survival in the central nervous system (CNS). In the NS group, BDNF expression was noted to be significantly decreased ($p < 0.01$) compared to Sham (Figure 4). However, VPA administration significantly increased ($p < 0.05$) BDNF

expression compared to the NS group and was not statistically different from the Sham group.

VPA Regulates NF- κ B and I κ B- α Pathways

Translocation of nuclear NF- κ B and degradation of cytosolic I κ B- α were assessed using Western Blot analysis. NF- κ B is a key transcription factor that regulates the neuroinflammation and apoptosis, while I κ B- α is an inhibitor of NF- κ B. In the NS group, there was a significant increase ($p < 0.001$) in nuclear NF- κ B and decrease ($p < 0.05$) in cytosolic I κ B- α compared to the Sham group (Figure 5). However, VPA treatment significantly decreased ($p < 0.001$) nuclear NF- κ B expression and increased ($p < 0.05$) cytosolic I κ B- α expression to levels similar to Sham.

Discussion

In this study, we found that single-dose VPA (150mg/kg) administration following TBI confers long-term beneficial effects on the injured brain. Single-dose VPA achieves these benefits by promoting a decrease in brain apoptosis, inflammation, and degeneration, and by increasing neural plasticity following TBI. VPA acts, in part, by regulation of pathways related to NF- κ B and I κ B- α . As such, single-dose VPA administration following TBI confers both neuroprotection and decreases the risk of long-term sequelae and complications, including neurodegeneration, associated with TBI.

TBI remains a leading cause of morbidity and death in trauma.(2, 3) Although TBI has direct initial consequences, it can lead to long-term alterations of the brain that can persist for months and even years after injury.(6) These long-term consequences, which include apoptosis, inflammation, degeneration, and others, place patients at increased risk for development of neurologic disorders (seizures, sleep disorders, neuroendocrine dysregulation), psychiatric problems, and even neurodegenerative disorders, including AD and CTE.(6) These sub-acute and chronic alterations can ultimately increase long-term mortality, reduce life expectations, and drastically affect quality of life.(6) At present, there are limited pharmacologic strategies to improve early neurologic outcomes and attenuate long-term complications following TBI.

Here we demonstrate that apoptosis is a highly important mechanism for programmed cell death even at a month following TBI. After injury, neural programmed cell death can be mediated by activation of intrinsic and extrinsic apoptotic pathways.(18, 19) In contrast to neural necrosis, apoptosis appears to be delayed and prolonged, contributing to the progressive nature of TBI. (20–22) Several human studies have demonstrated that pro-apoptotic proteins are upregulated in plasma and CSF following TBI,(23, 24) while apoptosis in brain can continue to occur weeks to months following injury.(21) As such, delayed apoptotic death may underlie some of the long-term consequences of TBI and is an attractive therapeutic target. In this study, we demonstrate that VPA decreases neural apoptosis at the 30-day time point following TBI.

Neural apoptosis is significant contributor to long-term morbidity after TBI, though other pathologic mechanisms play a role. Neuroinflammatory processes are also considered to be

an important contributor to secondary injury following TBI.(25) In both experimental and clinical tissue, TBI promotes activation of microglia and recruitment of circulatory inflammatory cells, including macrophages, to the area of brain injury.(26–28) In this study, we assessed Iba-1 expression, a well-known marker of microglial activation that plays a key role in cytoskeletal reorganization and configuration of the plasmalemma during phagocytosis.(29) We found that VPA administration decreased expression of Iba1, 30 days following TBI compared to controls. As these inflammatory cells are involved in expression of pro-inflammatory chemokines and cytokines (interleukin [IL]-1B, tumor necrosis factor- α [TNF- α], and IL-6),(30, 31) which can be neurotoxic, VPA may help to ultimately decrease long-term neuroinflammation in injured brain. Furthermore, astrocytes, which play a key role in the blood brain barrier, neural signaling, and scar border formation, often become activated following trauma.(32) In recent years, GFAP, a marker of astrocyte activation, has been shown to be a marker of severe TBI.(33) We also found that VPA treatment significantly attenuated GFAP expression following TBI, reflecting VPA's ability to decrease inappropriate astrocyte activation leading to neuroinflammation. These findings correlate with our prior work demonstrating that VPA attenuates plasma GFAP levels following TBI.(34)

In recent years TBI has been highlighted to be an important risk factor for the development of progressive neurodegenerative diseases, including AD, CTE, and Parkinson's disease (PD).(35) There is strong clinical evidence demonstrating that early TBI can increase the incidence of neurodegeneration.(36, 37) Although this may be multifactorial (environment, genetics, etc), it appears that prolonged inflammation and focal protein aggregation may be involved. In experimental and clinical models, expression of A β and p-Tau proteins, which accumulate in both grey and white matter, may play a key role in mediating neurodegeneration.(38–41) In this study, we were able to demonstrate that single-dose VPA treatment decreased expression of both A β and p-Tau, reflecting a decrease in AD-like pathology. We are unsure to what extent these alterations would affect disease onset or clinical manifestations; however, we plan to conduct additional studies for a more comprehensive analysis. Other neurodegenerative diseases (PD, ALS, HD) are at increased risk of development following TBI; however, this was not assessed.

Achieving neuroplasticity following TBI has also been a significant area of interest. Neural plasticity is linked to cellular responsiveness reflecting the neuronal capability to adapt and survive following TBI. In recent years, BDNF has emerged as a critical mediator of neuronal plasticity.(42, 43) Numerous studies indicate the beneficial effects of BDNF in promoting neurogenesis.(44–46) Following TBI, the significant increase in pro-inflammatory cytokines causes a significant reduction in BDNF gene expression.(47–49) This decrease has even been demonstrated following systemic inflammation (IL-1B or LPS administration).(50–52) In this study, we found that concurrent HS and TBI, promoting systemic and local inflammation, decreased BDNF expression in the NS group; however, treatment with VPA significantly increased BDNF expression to a level similar to Sham. As decreased BDNF expression may ultimately compromise hippocampal-learning and spatial memory and increase apoptosis contributing to neuropsychiatric diseases (53–55), VPA administration may be able to improve these clinical alterations.

We also sought to assess potential pathways related to attenuation of long-term neural apoptosis, inflammation, and degeneration. In the central nervous system, NF- κ B transcription factors are key players in promoting neurotoxicity following TBI.(56) Although NF- κ B is present in latent glia, several studies demonstrate that glial responses to trauma are mediated through nuclear translocation of NF- κ B. Following microglial activation of NF- κ B, reactive oxygen species and proinflammatory cytokines are released, contributing to secondary neurotoxicity. (57, 58) Furthermore, NF- κ B can also regulate inflammatory processes that worsen inflammation-induced neurodegeneration. In this study, VPA administration attenuated nuclear translocation and activation NF- κ B following TBI. Numerous other pathways (toll-like receptor signaling, cyclooxygenase-2, complement signaling, cytokines) also mediate neural inflammation and degeneration, which may also be affected following VPA administration;(59) however, we only chose to study NF- κ B activation and its inhibitor, *I κ B- α* , as we suspected it was the pathway most likely regulated by VPA.

Overall, these findings contribute to the literature evaluating the neuroprotective mechanisms of VPA following TBI. Prior pre-clinical studies have only been able to demonstrate sustained effects of up to 24 hours. Nine hours following TBI, VPA has been shown to increase neurogenesis and inhibit neuroinflammation in the injured brain.(13) PBMCs have also demonstrated sustained effects over the first 24 hours following TBI.(14) However, this is the first study to demonstrate that the beneficial effects of single-dose VPA following TBI are sustained over a 30-day period. As VPA continues to move further toward human translation,(60) this appears to be an additional long-term benefit, especially with limited pharmacologic agents for long-term brain alterations and complications of TBI.

There are several limitations to this study. First, sample size study was limited by ethical considerations and costs and therefore the results may be prone to type II error. Second, although swine are commonly used for human translation, they serve as imperfect surrogates for human subjects. Third, cortical impact TBI was used for this study. However, this is not representative of all types of TBI and additional work is required to determine if these beneficial effects remain in other types, such as blast injuries. Fourth, we evaluated the impact of VPA treatment on brain alterations at 30 days following injuries. However, we realize that longer-term evaluations are required over the course of years to fully assess VPA protective effects. Fifth, we only assessed the cortex adjacent to the site of injury; therefore, we did not evaluate VPA's global effects on the brain induced by TBI. Lastly, we recognize that we chose to assess a select number of markers and pathways in chronic brain alterations. A more comprehensive analysis involving additional markers, regulatory pathways, and underlying mechanisms is required for more definitive conclusions.

In conclusion, this study highlights the long-term impact of single-dose VPA administration following TBI. In addition to decreasing brain lesion size and improving neurologic recovery,(11, 12) single-dose VPA following TBI appears to have long-term protective effects. Not only does single-dose VPA decrease neural apoptosis, inflammation, and degeneration, but it also promotes neural plasticity and neuronal survival. It acts, in part, by regulation of NF- κ B-related pathways. Although additional work is required to evaluate its

long-term impact, single-dose VPA for TBI confers early neuroprotection and decreases risk of long-term sequelae and brain alterations associated with TBI.

Acknowledgments

Funding: This work was supported by a grant from the US Army Medical Research and Materiel Command, Contract W81XWH-09-1-0520 (PI: Hasan B. Alam).

Meeting Presentation: This manuscript was presented at the 2018 American College of Surgeons Meeting in Boston, MA and received the “Best e-Poster Award” at the scientific forum.

References

1. Chauhan NB. Chronic neurodegenerative consequences of traumatic brain injury. *Restorative neurology and neuroscience*. 2014;32(2):337–65. [PubMed: 24398724]
2. Menon DK, Schwab K, Wright DW, Maas AI. Position statement: definition of traumatic brain injury. *Archives of physical medicine and rehabilitation*. 2010;91(11):1637–40. [PubMed: 21044706]
3. Taylor CA, Bell JM, Breiding MJ, Xu L. Traumatic Brain Injury-Related Emergency Department Visits, Hospitalizations, and Deaths - United States, 2007 and 2013. *Morbidity and mortality weekly report Surveillance summaries* (Washington, DC : 2002). 2017;66(9):1–16.
4. Masek BE, DeWitt DS. Traumatic brain injury: a disease process, not an event. *Journal of neurotrauma*. 2010;27(8):1529–40. [PubMed: 20504161]
5. Corrigan JD, Hammond FM. Traumatic brain injury as a chronic health condition. *Archives of physical medicine and rehabilitation*. 2013;94(6):1199–201. [PubMed: 23402722]
6. Bramlett HM, Dietrich WD. Long-Term Consequences of Traumatic Brain Injury: Current Status of Potential Mechanisms of Injury and Neurological Outcomes. *Journal of neurotrauma*. 2015;32(23):1834–48. [PubMed: 25158206]
7. DeKosky ST, Blennow K, Ikonovic MD, Gandy S. Acute and chronic traumatic encephalopathies: pathogenesis and biomarkers. *Nature reviews Neurology*. 2013;9(4):192–200. [PubMed: 23558985]
8. Goldman SM, Tanner CM, Oakes D, Bhudhikanok GS, Gupta A, Langston JW. Head injury and Parkinson's disease risk in twins. *Annals of neurology*. 2006;60(1):65–72. [PubMed: 16718702]
9. Lye TC, Shores EA. Traumatic brain injury as a risk factor for Alzheimer's disease: a review. *Neuropsychology review*. 2000;10(2):115–29. [PubMed: 10937919]
10. Wilson L, Stewart W, Dams-O'Connor K, Diaz-Arrastia R, Horton L, Menon DK, et al. The chronic and evolving neurological consequences of traumatic brain injury. *The Lancet Neurology*. 2017;16(10):813–25. [PubMed: 28920887]
11. Halaweish I, Bambakidis T, Chang Z, Wei H, Liu B, Li Y, et al. Addition of low-dose valproic acid to saline resuscitation provides neuroprotection and improves long-term outcomes in a large animal model of combined traumatic brain injury and hemorrhagic shock. *The journal of trauma and acute care surgery*. 2015;79(6):911–9; discussion 9. [PubMed: 26680134]
12. Nikolian VC, Georgoff PE, Pai MP, Dehaney IS, Chtraklin K, Eidy H, et al. Valproic acid decreases brain lesion size and improves neurologic recovery in swine subjected to traumatic brain injury, hemorrhagic shock, and polytrauma. *The journal of trauma and acute care surgery*. 2017.
13. Nikolian VC, Denny IS, Higgins GA, Williams AM, Weykamp M, Georgoff PE, et al. Transcriptomic changes following valproic acid treatment promote neurogenesis and minimize secondary brain injury. *The journal of trauma and acute care surgery*. 2018;84(3):459–65. [PubMed: 29251707]
14. Georgoff PE, Nikolian VC, Higgins G, Chtraklin K, Eidy H, Ghandour MH, et al. Valproic acid induces prosurvival transcriptomic changes in swine subjected to traumatic injury and hemorrhagic shock. *The journal of trauma and acute care surgery*. 2018;84(4):642–9. [PubMed: 29251706]

15. Rhee P, Talon E, Eifert S, Anderson D, Stanton K, Koustova E, et al. Induced hypothermia during emergency department thoracotomy: an animal model. *The Journal of trauma*. 2000;48(3):439–47; discussion 47–50. [PubMed: 10744281]
16. Alam HB, Bowyer MW, Koustova E, Gushchin V, Anderson D, Stanton K, et al. Learning and memory is preserved after induced asanguineous hyperkalemic hypothermic arrest in a swine model of traumatic exsanguination. *Surgery*. 2002;132(2):278–88. [PubMed: 12219024]
17. Nikolian VC, Georgoff PE, Pai MP, Dennahy IS, Chtraklin K, Eidy H, et al. Valproic acid decreases brain lesion size and improves neurologic recovery in swine subjected to traumatic brain injury, hemorrhagic shock, and polytrauma. *The journal of trauma and acute care surgery*. 2017;83(6):1066–73. [PubMed: 28697014]
18. Keane RW, Kraydieh S, Lotocki G, Alonso OF, Aldana P, Dietrich WD. Apoptotic and antiapoptotic mechanisms after traumatic brain injury. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism*. 2001;21(10):1189–98.
19. Eldadah BA, Faden AI. Caspase pathways, neuronal apoptosis, and CNS injury. *Journal of neurotrauma*. 2000;17(10):811–29. [PubMed: 11063050]
20. Flygt J, Djupsjo A, Lenne F, Marklund N. Myelin loss and oligodendrocyte pathology in white matter tracts following traumatic brain injury in the rat. *The European journal of neuroscience*. 2013;38(1):2153–65. [PubMed: 23458840]
21. Lotocki G, de Rivero Vaccari JP, Alonso O, Molano JS, Nixon R, Safavi P, et al. Oligodendrocyte vulnerability following traumatic brain injury in rats. *Neuroscience letters*. 2011;499(3):143–8. [PubMed: 21669255]
22. Raghupathi R. Cell death mechanisms following traumatic brain injury. *Brain pathology (Zurich, Switzerland)*. 2004;14(2):215–22.
23. Uzan M, Erman H, Tanriverdi T, Sanus GZ, Kafadar A, Uzun H. Evaluation of apoptosis in cerebrospinal fluid of patients with severe head injury. *Acta neurochirurgica*. 2006;148(11):1157–64; discussion. [PubMed: 16964558]
24. Wagner AK, Amin KB, Niyonkuru C, Postal BA, McCullough EH, Ozawa H, et al. CSF Bcl-2 and cytochrome C temporal profiles in outcome prediction for adults with severe TBI. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism*. 2011;31(9):1886–96.
25. Das M, Mohapatra S, Mohapatra SS. New perspectives on central and peripheral immune responses to acute traumatic brain injury. *Journal of neuroinflammation*. 2012;9:236. [PubMed: 23061919]
26. Acosta SA, Tajiri N, Shinozuka K, Ishikawa H, Grimmig B, Diamond DM, et al. Long-term upregulation of inflammation and suppression of cell proliferation in the brain of adult rats exposed to traumatic brain injury using the controlled cortical impact model. *PloS one*. 2013;8(1):e53376.
27. Gentleman SM, Leclercq PD, Moyes L, Graham DI, Smith C, Griffin WS, et al. Long-term intracerebral inflammatory response after traumatic brain injury. *Forensic science international*. 2004;146(2–3):97–104. [PubMed: 15542269]
28. Loane DJ, Kumar A, Stoica BA, Cabatbat R, Faden AI. Progressive neurodegeneration after experimental brain trauma: association with chronic microglial activation. *Journal of neuropathology and experimental neurology*. 2014;73(1):14–29. [PubMed: 24335533]
29. Hoogland IC, Houbolt C, van Westerloo DJ, van Gool WA, van de Beek D. Systemic inflammation and microglial activation: systematic review of animal experiments. *Journal of neuroinflammation*. 2015;12:114. [PubMed: 26048578]
30. Morganti-Kossmann MC, Rancan M, Stahel PF, Kossmann T. Inflammatory response in acute traumatic brain injury: a double-edged sword. *Current opinion in critical care*. 2002;8(2):101–5. [PubMed: 12386508]
31. Ziebell JM, Morganti-Kossmann MC. Involvement of pro- and anti-inflammatory cytokines and chemokines in the pathophysiology of traumatic brain injury. *Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics*. 2010;7(1):22–30. [PubMed: 20129494]

32. Hansson E, Werner T, Bjorklund U, Skioldebrand E. Therapeutic innovation: Inflammatory-reactive astrocytes as targets of inflammation. *IBRO reports*. 2016;1:1–9. [PubMed: 30135924]
33. Lei J, Gao G, Feng J, Jin Y, Wang C, Mao Q, et al. Glial fibrillary acidic protein as a biomarker in severe traumatic brain injury patients: a prospective cohort study. *Critical care (London, England)*. 2015;19:362. [PubMed: 26455520]
34. Korley FK, Nikolian VC, Williams AM, Dannahy IS, Weykamp M, Alam HB. Valproic Acid Treatment Decreases Serum Glial Fibrillary Acidic Protein and Neurofilament Light Chain Levels in Swine Subjected to Traumatic Brain Injury. *Journal of neurotrauma*. 2018;35(10):1185–91. [PubMed: 29415612]
35. Daneshvar DH, Riley DO, Nowinski CJ, McKee AC, Stern RA, Cantu RC. Long-term consequences: effects on normal development profile after concussion. *Physical medicine and rehabilitation clinics of North America*. 2011;22(4):683–700. ix. [PubMed: 22050943]
36. Jellinger KA, Paulus W, Wrocklage C, Litvan I. Traumatic brain injury as a risk factor for Alzheimer disease. Comparison of two retrospective autopsy cohorts with evaluation of ApoE genotype. *BMC neurology*. 2001;1:3. [PubMed: 11504565]
37. Plassman BL, Havlik RJ, Steffens DC, Helms MJ, Newman TN, Drosdick D, et al. Documented head injury in early adulthood and risk of Alzheimer's disease and other dementias. *Neurology*. 2000;55(8):1158–66. [PubMed: 11071494]
38. Bramlett HM, Kraydieh S, Green EJ, Dietrich WD. Temporal and regional patterns of axonal damage following traumatic brain injury: a beta-amyloid precursor protein immunocytochemical study in rats. *Journal of neuropathology and experimental neurology*. 1997;56(10):1132–41. [PubMed: 9329457]
39. Graham DI, Gentleman SM, Lynch A, Roberts GW. Distribution of beta-amyloid protein in the brain following severe head injury. *Neuropathology and applied neurobiology*. 1995;21(1):27–34. [PubMed: 7770117]
40. Irving EA, Nicoll J, Graham DI, Dewar D. Increased tau immunoreactivity in oligodendrocytes following human stroke and head injury. *Neuroscience letters*. 1996;213(3):189–92. [PubMed: 8873146]
41. Pierce JE, Trojanowski JQ, Graham DI, Smith DH, McIntosh TK. Immunohistochemical characterization of alterations in the distribution of amyloid precursor proteins and beta-amyloid peptide after experimental brain injury in the rat. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 1996;16(3):1083–90. [PubMed: 8558237]
42. Calabrese F, Rossetti AC, Racagni G, Gass P, Riva MA, Molteni R. Brain-derived neurotrophic factor: a bridge between inflammation and neuroplasticity. *Frontiers in cellular neuroscience*. 2014;8:430. [PubMed: 25565964]
43. Bramham CR, Messaoudi E. BDNF function in adult synaptic plasticity: the synaptic consolidation hypothesis. *Progress in neurobiology*. 2005;76(2):99–125. [PubMed: 16099088]
44. Chan JP, Cordeira J, Calderon GA, Iyer LK, Rios M. Depletion of central BDNF in mice impedes terminal differentiation of new granule neurons in the adult hippocampus. *Molecular and cellular neurosciences*. 2008;39(3):372–83. [PubMed: 18718867]
45. Sairanen M, Lucas G, Ernfors P, Castren M, Castren E. Brain-derived neurotrophic factor and antidepressant drugs have different but coordinated effects on neuronal turnover, proliferation, and survival in the adult dentate gyrus. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2005;25(5):1089–94. [PubMed: 15689544]
46. Waterhouse EG, An JJ, Orefice LL, Baydyuk M, Liao GY, Zheng K, et al. BDNF promotes differentiation and maturation of adult-born neurons through GABAergic transmission. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2012;32(41):14318–30. [PubMed: 23055503]
47. Raetz CR, Whitfield C. Lipopolysaccharide endotoxins. *Annual review of biochemistry*. 2002;71:635–700.
48. Zhao B, Tang HL, Dan QQ, Zhao N, Liu J. [Changes of BDNF expression in neurons in traumatic brain injury rats]. *Sichuan da xue xue bao Yi xue ban = Journal of Sichuan University Medical science edition*. 2012;43(2):236–9, 49. [PubMed: 22650039]

49. Schober ME, Block B, Requena DF, Hale MA, Lane RH. Developmental traumatic brain injury decreased brain derived neurotrophic factor expression late after injury. *Metabolic brain disease*. 2012;27(2):167–73. [PubMed: 22527999]
50. Guan Z, Fang J. Peripheral immune activation by lipopolysaccharide decreases neurotrophins in the cortex and hippocampus in rats. *Brain, behavior, and immunity*. 2006;20(1):64–71.
51. Lapchak PA, Araujo DM, Hefti F. Systemic interleukin-1 beta decreases brain-derived neurotrophic factor messenger RNA expression in the rat hippocampal formation. *Neuroscience*. 1993;53(2):297–301. [PubMed: 8492907]
52. Schnydrig S, Korner L, Landweer S, Ernst B, Walker G, Otten U, et al. Peripheral lipopolysaccharide administration transiently affects expression of brain-derived neurotrophic factor, corticotropin and proopiomelanocortin in mouse brain. *Neuroscience letters*. 2007;429(1):69–73. [PubMed: 17976910]
53. Arai K, Matsuki N, Ikegaya Y, Nishiyama N. Deterioration of spatial learning performances in lipopolysaccharide-treated mice. *Japanese journal of pharmacology*. 2001;87(3):195–201. [PubMed: 11885968]
54. Nolan Y, Vereker E, Lynch AM, Lynch MA. Evidence that lipopolysaccharide-induced cell death is mediated by accumulation of reactive oxygen species and activation of p38 in rat cortex and hippocampus. *Experimental neurology*. 2003;184(2):794–804. [PubMed: 14769372]
55. Pugh CR, Kumagawa K, Fleshner M, Watkins LR, Maier SF, Rudy JW. Selective effects of peripheral lipopolysaccharide administration on contextual and auditory-cue fear conditioning. *Brain, behavior, and immunity*. 1998;12(3):212–29.
56. Shih RH, Wang CY, Yang CM. NF-kappaB Signaling Pathways in Neurological Inflammation: A Mini Review. *Frontiers in molecular neuroscience*. 2015;8:77. [PubMed: 26733801]
57. Kaltschmidt C, Kaltschmidt B, Baeuerle PA. Brain synapses contain inducible forms of the transcription factor NF-kappa B. *Mechanisms of development*. 1993;43(2–3):135–47. [PubMed: 8297787]
58. Block ML, Zecca L, Hong JS. Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. *Nature reviews Neuroscience*. 2007;8(1):57–69. [PubMed: 17180163]
59. Sochocka M, Diniz BS, Leszek J. Inflammatory Response in the CNS: Friend or Foe? *Molecular neurobiology*. 2017;54(10):8071–89. [PubMed: 27889895]
60. Georgoff PE, Nikolian VC, Bonham T, Pai MP, Tafatia C, Halaweish I, et al. Safety and Tolerability of Intravenous Valproic Acid in Healthy Subjects: A Phase I Dose-Escalation Trial. *Clin Pharmacokinet*. 2018;57(2):209–19. [PubMed: 28497259]

Figure 1. VPA Attenuates Neural Apoptosis at 30 Days Following TBI.

TUNEL assay and expression of cleaved caspase 3 using Western Blot were used. **(A)** In the NS group, there was an increase in TUNEL-positive cells compared to Sham. However, VPA treatment attenuated the number of TUNEL-positive cells. **(B)** Using Western Blot, expression of cleaved-caspase 3 was significantly increased ($p < 0.01$) in the NS group compared to Sham. However, treatment with VPA significantly attenuated ($p < 0.01$) these alterations to a level similar to Sham. $p < 0.05$ was considered statistically significant. Images shown at 200 μm . β -actin was used as an internal control. NS, normal saline; VPA, valproic acid; *c-caspase 3*, cleaved caspase 3.

Figure 2. VPA Attenuates Neuroinflammation at 30 Days Following TBI.

Immunohistochemistry and Western Blot analyses were used to assess Iba1 and GFAP, markers of microglia and astrocytes. **(A)** Using immunohistochemistry, there was an increase in Iba1 and GFAP-positive cells in the NS group compared to Sham. However, VPA treatment decreased the number of Iba1 and GFAP-positive cells. **(B)** Western Blot was used for confirmation. In the NS group, there a significant increase ($p < 0.001$) in Iba1 and GFAP expression compared to Sham. However, VPA administration significantly attenuated ($p < 0.01$ and $p < 0.001$, respectively) expression of Iba1 and GFAP. Images shown at 200 μ m. β -actin was used as an internal control. NS, normal saline; VPA, valproic acid; Iba1, ionized calcium binding adaptor molecule-1; GFAP, glial fibrillary acidic protein.

Figure 3. VPA Attenuates Neural Degeneration at 30 Days Following TBI.

Immunohistochemistry and Western Blot analyses were used to assess Aβ and p-Tau, markers of neurodegenerative diseases. **(A)** Using immunohistochemistry, there was an increase in Aβ and p-Tau in the NS group compared to Sham. However, VPA treatment decreased these alterations. **(B and C)** Western Blot was used for confirmation. In the NS group, there was significant increase ($p < 0.001$ and $p < 0.01$) in Aβ and p-Tau expression compared to Sham. However, VPA administration significantly attenuated ($p < 0.001$ and $p < 0.05$, respectively) expression of Aβ and p-Tau. Images shown at 200 μm. β-actin was used as an internal control. *NS*, normal saline; *VPA*, valproic acid; *Aβ*, amyloid beta; *p-Tau*, phosphorylated Tau.

A **B**

Figure 4. VPA Promotes Neuroplasticity 30 Days Following TBI.

Western Blot analysis was used to assess BDNF expression. In the NS group, there was a significant decrease ($p < 0.01$) in BDNF expression compared to Sham. However, VPA administration significantly attenuated ($p < 0.05$) the decrease in BDNF. β -actin was used as an internal control. *NS*, normal saline; *VPA*, valproic acid; *BDNF*, brain-derived neurotrophic factor.

Figure 5. VPA Promotes Activation of Nuclear NF- κ B and Degradation of cytosolic I κ B. Western Blot analysis was used. (A) In the NS group, there was a significant increase ($p < 0.001$) in nuclear NF- κ B compared to Sham. However, treatment with VPA significantly decreased ($p < 0.001$) nuclear NF- κ B expression similar to Sham. (B) In contrast, there was a significant decrease ($p < 0.05$) in cytosolic I κ B- α in the NS group compared to Sham. However, VPA treatment significantly increased ($p < 0.05$) cytosolic I κ B- α expression

similar to Sham. β -actin was used as an internal control. *NS*, normal saline; *VPA*, valproic acid; *BDNF*, brain-derived neurotrophic factor.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript