Different resuscitation strategies and novel pharmacologic treatment with valproic acid in traumatic brain injury

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ABSTRACT

Traumatic brain injury (TBI) is a leading cause of death in young adults, and effective treatment strategies have the potential to save many lives. TBI results in coagulopathy, endothelial dysfunction, inflammation, cell death, and impaired epigenetic homeostasis, ultimately leading to morbidity and / or mortality. Commonly used resuscitation fluids such as crystalloids or colloids have several disadvantages, and might even be harmful when administered in large quantities. There is a need for next-generation treatment strategies (especially in the pre-hospital setting) that minimize cellular damage, improve survival, and enhance neurological recovery. Pharmacologic treatment with histone deacetylase inhibitors, such as valproic acid, have shown promising results in animal studies of TBI, and may therefore be excellent example of next-generation therapies. This review briefly describes traditional resuscitation strategies for TBI combined with hemorrhagic shock, and describes preclinical studies on valproic acid as a new pharmacologic agent in the treatment of TBI. It finally discusses limitations and future directions on the use of histone deacetylase inhibitors for the treatment of TBI.

INTRODUCTION

Despite optimization of prehospital and intrahospital treatment strategies, traumatic brain injury (TBI) remains a leading cause of morbidity and mortality in young adults (Faul, 2010; Centers for Disease Control and Prevention, 2011). TBI is often paralleled by hemorrhagic shock (HS), and this combination is especially lethal, with studies showing that hemorrhagic shock can significantly worsen the TBI-associated morbidity and mortality (Wald, 1993; McMahon, 1999). TBI and hemorrhagic shock result in secondary conditions such as inflammation, endothelial dysfunction, coagulopathy, hypoxia, and cerebral edema. Such secondary insults are highly linked, and include cross-talk between endothelial and coagulation pathways as key mediators. The primary insult to the brain occurs in the prehospital setting, involves irreversible destruction of neuronal tissue and cannot be treated. Hence, treatment strategies necessarily focus on prevention of secondary brain injury by maintaining adequate cerebral oxygenation and perfusion (Badjatia, 2009). Fluid resuscitation has historically been the mainstay in treating traumatic injuries. It plays a critical role in restoring and maintaining systemic and cerebral circulations in TBI patients (Myburg, 2007). An ideal resuscitation fluid would therefore minimize cerebral edema, as well as attenuate neuronal damage, inflammation, and coagulopathy. However, current resuscitation strategies have failed to prevent this secondary brain injury. For this reason, recent advances in trauma research have focused on next-generation fluids and pharmacologic agents that may attenuate secondary brain injury. The aim of this review is to describe traditional resuscitation strategies for TBI combined with hemorrhagic shock, and discuss the novel use of valproic acid (VPA) as a new pharmacologic agent in the treatment of TBI.

CONVENTIONAL RESUSCITATION TREATMENTS

TBI is often associated with hypovolemia, hemorrhagic shock, and coagulopathy. In addition, microvascular injury may alter the permeability of the blood brain barrier, resulting in intravascular fluid leakage and cerebral edema. The ideal resuscitation strategy should aim to not only replace lost blood, but also minimize secondary brain injury. Accordingly, current strategies focus on hemodynamic stabilization, maintaining systemic circulation, and optimizing cerebral perfusion (Myburg, 2007; Hammell, 2009). However, there is considerable debate about the most effective methods for treating blood loss associated with TBI.

Fluid resuscitation

Prompt fluid resuscitation is the first-line therapy to restore the lost intravascular volume. As blood products are often unavailable at the scene of the injury, prehospital management is usually limited to infusion using crystalloids or colloids. Isotonic crystalloids are typically preferred over colloids (Myburgh, 2007; Perel, 2012), and have served as the standard resuscitation fluid for some time. Yet, traditional resuscitation fluids such as crystalloids have no inherent pro-survival properties (Santry, 2010), and in severely bleeding patients, aggressive crystalloid resuscitation does not result in any survival benefit (Bickell, 1994; Kwan, 2003). Moreover, several clinical studies have identified shortcomings of fluid resuscitation, such as hemodilution, hemostatic derangements, brain edema, and inflammation (Grande, 1997; Peiniger, 2011). Such aggressive administration may even worsen outcomes by further exaggerating the cellular damage suffered during shock (Santry,

2010). Preclinical studies showed that ongoing hemorrhage and coagulopathy are frequently not corrected by current resuscitation protocols using crystalloids and packed red blood cells (PRBC) (Selby, 1996). In addition, massive fluid resuscitation itself may even result in coagulopathy and hemodilution (Selby, 1996; Sondeen, 2003). Compared to other resuscitation fluids such as fresh frozen plasma (FFP) or colloids, normal saline resuscitation is furthermore associated with increased brain swelling (Jin et al. 2012b; Imam, 2014), metabolic derangements (Hwabejire, 2013a), elevated circulating markers of injury (Sillesen, 2013b), and increased activation of the endothelial, coagulation, and anticoagulation systems (Dekker, 2014c; Sillesen, 2014).

These findings suggest that traditional fluid resuscitation with crystalloids is mostly supportive, and does not address the specific cellular dysfunction caused by shock and injury. Due to the increased awareness of the negative effects of massive transfusions, trauma care now favors “damage control resuscitation”, consisting of minimal use of crystalloids, early hemorrhage control, and early administration of blood products (PRBC, FFP, and platelets in an appropriate ratio) (Kwan, 2003).

Blood product resuscitation

Commonly used blood products are PRBC, FFP, platelets, and fibrinogen. There is limited evidence concerning the use of PRBC in the setting of isolated TBI. The combination of TBI and hemorrhagic shock likely mandates PRBC transfusion, with clinical data suggesting a survival benefit when FFP:PRBC ratios are maintained above 1:2 (Peiniger, 2011). The benefits of FFP on the control of acute traumatic bleeding and coagulopathy is furthermore demonstrated in multiple clinical studies

(Borgman, 2007; Duchesne, 2008; Maegele, 2008; Scalea, 2008; Zehtabchi, 2009; Wafaisade, 2010). In the setting of TBI, however, clinical data is conflicting. Some studies have indicated adverse outcomes associatied with FFP administration (Etemadrezaie, 2007; Anglin, 2013) while others have identified outcome benefits (Peiniger, 2011). Pre-clinical studies demonstrated that early administration of FFP also appears to alter the clinical course following TBI by attenuating the degree of neurologic impairment, improving the rate of recovery, and preserving cognitive functions (Halaweish, 2015a). These effects of blood products can even be detected at the level of gene transcription (Sillesen, 2016a), effecting the expression of genes involved in metabolism, platelet signaling, and inflammation. These data suggest that the therapeutic benefits of plasma resuscitation might be more extensive than solely through hemodynamic stabilization.

However, FFP transfusion is not without risk (Nascimento, 2010; Rossaint, 2016). Such risks include transfusion-associated acute lung injury and circulatory overload, allergic reactions, and transmission of infectious diseases (Nascimento, 2010). Moreover, the use of FFP and other blood products is challenging in pre-hospital settings or in underdeveloped areas due to logistical issues such as limited availability, requirement for refrigeration, short life after thawing, and the need for immediately available universal donor plasma. These challenges of FFP resuscitation have fueled the initiative to develop next-generation blood products that are low-volume and shelf-stable.

Future directions of blood products and antifibrinolytics in traumatic bleeding

Lyophilized plasma, a freeze-dried plasma product developed in the 1930's, may solve the shortcomings of FFP resuscitation. It is logistically superior to FFP, as it can be stored as a powder for as long as 30 years, and subsequently reconstituted and administered within minutes following rehydration with water (Fu, 1977). Additionally, lyophilized plasma retains its factor function much better than FFP, and can be stored in ambient temperatures for extended periods of time. Freeze-dried plasma has been shown to be as effective as FFP in large animal models of TBI and hemorrhage (Imam, 2013a; Halaweish, 2016). Freeze dried plasma is approved for clinical use in Europe, and has been used by NATO forces for many years with good results (Alam, 2011a; Pusateri, 2016). However, it is currently not approved by the Food and Drug Administration (FDA) for use in the USA. In the coming years, there is an urgent need for prospective investigations of blood products such as lyophilized plasma and fibrinogen, and anti-fibrinolytic drugs such as tranexamic acid (TXA). There is currently a paucity of data concerning the use of TXA in TBI. A recent Cochrane review concluded that TXA may reduce mortality although the quality of the evidence is low and uncertainties remain (Ker, 2015). Furthermore, studies have suggested a reduction in the incidence of intrancranial hemorrhage following TBI associated with administration of TXA (Zehtabchi, 2014). Future randomized controlled trials should report on the clinical outcomes of treatment with TXA and fibrinogen to address some of the key unanswered questions in trauma-induced coagulopathy (Wong, 2016a).

NOVEL RESUSCITATION TECHNIQUES – EPIGENETIC MODULATION USING VALPROIC ACID

At present, there are no proven pharmacologic treatment options for TBI. However, the search for such therapeutic treatment of TBI has received considerable attention in recent years (Ichiyama, 2000; Fukudome, 2012). A therapeutic strategy for modulating the cellular response to injury may be at the level of the epigenome. One class of promising drugs that affect the epigenome is histone deacetylase inhibitors (HDACI). In pathologic conditions, such as TBI and hemorrhagic shock, there is a decrease in histone acetylation, which limits gene transcription and impairs cellular homeostasis. However, agents such as valproic acid (VPA) have been shown to act as histone deacetylase inhibitors, thus altering gene transcription and thereby inducing a “pro-survival phenotype”. The precise mechanisms underlying these protective effects are an active area of current research.

Traumatic brain injury creates a genomic storm

The genomic response to injury is an important area of current research. Recent studies have revealed that traumatic injuries result in epigenetic changes via DNA methylation, phosphorylation, and acetylation (Wong, 2016b). The so-called “genomic storm” following injury occurs on an epigenetic level, and modulates numerous cellular functions, protein expression, and pathways. These processes are considered “epigenetics”, meaning that they affect the gene expression and resultant phenotype of the cell, but do not alter the genome itself (Bernstein, 2007; Goldberg, 2007). Alam et al. (2002) showed that during hemorrhage and resuscitation, at least 7% of the genes and downstream pathways are differentially expressed. Xiao et al. (2011) previously showed that following traumatic injury, expression of over 80% of the leukocyte transcriptome is altered. These changes occur rapidly following trauma, and exhibit a long-lasting effect for months after the injury (Xiao, 2011; Lipponen,

2016). Modulating these epigenetic changes may thus be an important goal of therapy in patients with TBI.

Acetylation status regulates gene expression and protein functions

Briefly, the human genome is organized into chromatin, a highly conserved complex of DNA and histone proteins. DNA transcription, and hence gene expression, is regulated by the acetylation and deacetylation of these histones. Histone deacetylases are enzymes that remove the acetyl group from the histone, making the DNA-histone structure more compact and therefore limiting transcription. In contrast, histone acetyltransferases weaken DNA-histone attraction, thereby unfolding the complex and making DNA more available for transcription (Delcuve, 2012). A homeostatic balance between histone deacetylases and histone acetyltransferases typically exists in cells. However, dysregulation of acetylation homeostasis has been suggested to play a key role in the pathogenesis of cancers and neurodegenerative diseases. Moreover, as noted above, dysregulation of acetylation homeostasis has also been implicated as an important pathologic mechanism in the body's response to traumatic injuries. Thus, pharmacologic agents that can alter histone acetylation may be promising new therapeutic strategies for trauma, as they can rapidly and reversely modify the transcription of desirable genes.

Although histones were considered the main target of HDACI, we now know that non-histone proteins involved in key cellular functions such as cell cycle, stress response, cytoskeleton dynamics, signaling, repair/healing/remodeling, communication, and proliferation are equally involved (Glozak, 2005). At least 50 such non-histone proteins have been well-characterized (Kim, 2011), but this list is

rapidly growing. In one study, 3,600 acetylation sites (in 1,750 human proteins) were identified, which regulated nearly all nuclear and many key cytoplasmic processes (Choudhary, 2009). This makes acetylation a regulatory mechanism that is as prevalent and important as phosphorylation (Kouzarides, 2000; Norris, 2009). Acetylation of non-histone proteins is actually even more important than histones in the setting of severe shock and/or TBI. While it takes some time to produce phenotype changes through modulation of gene transcription, direct (non-transcriptional) acetylation of regulatory proteins is extremely fast and thus more relevant for rapidly lethal conditions such as shock or TBI. In short, there are many features that make acetylation an attractive therapeutic target: it is *rapid*, completely *reversible*, and can be altered by drugs that are already in clinical use.

Valproic acid restores normal cellular acetylation

A growing body of literature suggests that valproic acid may alter gene expression and protein functions following TBI (Dekker, 2014b; Halaweish, 2015b; Bambakidis 2016). Valproic acid has been in clinical use as a mood stabilizing and anti-epileptic and drug since the 1970s. In high doses, however, valproic acid acts as a histone deacetylase inhibitor. High-dose valproic acid has been shown to improve survival in otherwise lethal models of hemorrhagic shock, polytrauma (Alam, 2009; Alam, 2011b), sepsis, and combined TBI with hemorrhagic shock (Jin, 2012a; Jepsen, 2014; Halaweish, 2015b,c). Yet, the precise protective mechanisms of valproic acid have not been well defined. A conceptual model for the main protective effects of valproic acid is as follows: TBI and hemorrhagic shock result in decreased acetylation of histones and non-histone proteins, which impairs normal gene expression and alters many homeostatic pathways, leading to cell death. In contrast,

valproic acid acts as a HDACI, which restores normal acetylation homeostasis and restores normal cellular functions, ultimately leading to a pro-survival phenotype. HDACIs, such as valproic acid, are a promising therapeutic approach as they are already FDA approved for clinical use for a variety of other illnesses. Importantly, valproic acid is effective as treatment for hemorrhagic shock even when administered in a single bolus in the absence of fluid resuscitation or blood transfusion (Alam, 2009). Thus, valproic acid treatment is very appealing as it can be rapidly administered in the prehospital setting or battlefield environment. Table 1 summarizes the key benefits of pharmacologic treatment with VPA in the setting of central nervous system injury.

Valproic acid alters cell survival and cell death pathways

One of the first studies that investigated the effects of hemorrhage and resuscitation on histone acetylation demonstrated that crystalloid resuscitation results in a predominantly de-acetylation profile in animal models, and that treatment with HDACI was able to reverse it (Lin, 2006). Our research group sought to better understand this effect of HDACI treatment at the level of gene expression. Using a porcine model of TBI+HS, we performed a high throughput analysis of cerebral gene profiling following resuscitation with either hextend or hextend+VPA. We hypothesized that treatment with valproic acid would significantly alter the early transcription of genes in pathways related to cell survival, which may explain its previously observed neuroprotective effects such as reduced brain lesion size and swelling. We found that 1,668 probe sets mapping to 370 known genes were differentially expressed between hextend versus hextened+VPA groups. These genes were mapped to, among others, pathways related to cell death, apoptosis, and necrosis (Dekker,

2014b). These findings support our hypothesis that valproic acid alters important genes and pathways that could improve survival.

Valproic acid reduces inflammation and blood-brain-barrier dysfunction

As described previously, secondary brain injury includes an acute inflammatory response with blood–brain-barrier (BBB) disruption, activation of immune cells, and cerebral edema. The anti-inflammatory properties of valproic acid treatment have been described previously in models of sepsis, hemorrhage, and TBI (Shang, 2010; Jin, 2012a; Liu, 2014). In addition, Bambakidis et al. (2016) showed that valproic acid modulates genes related to inflammation, cell signaling, cell adhesion, and endothelial growth. Dash et al. (2010) furthermore showed that valproic acid treatment significantly decreased Evans Blue dye extravasation in a TBI rat model, indicating that valproic acid might restore BBB function following trauma. Protective effects of valproic acid on BBB function were also demonstrated by Wang et al. (2011) and Lee et al. (2012) who found a valproic acid-induced reduction of matrix metalloproteinase-9 (mmp-9), a protease that disrupts blood-brain-barrier function. This was furthermore associated with decreased degradation of tight junction and basement membrane associated proteins, such as zona occludin-1 (ZO-1), and claudin-5 (Lee, 2012; Yang, 2007). This is in line with data from Nikolian et al., (2016b) who found that valproic acid significantly increases expression of ZO-1, laminin, and claudin-5. Expression of glucose transporter 1 **(**GLUT-1), a marker of barrier-type endothelial cells, was also increased in the valproic acid treatment group. Moreover, *in vitro* monolayers treated with valproic acid significantly decreased permeability relative to anoxic controls (Nikolian 20016b). Taken together, these results suggest that protective mechanisms of valproic acid may involve decreasing

inflammation and correcting blood-brain-barrier dysfunction.

Valproic acid attenuates platelet dysfunction

Coagulopathy plays a major role in the mortality of patients with TBI and hemorrhagic shock. A particularly important mechanism of TBI related-coagulopathy is platelet dysfunction. For example, TBI and hemorrhagic shock induce a combination of platelet activation but decreased function compared with general trauma patients (no TBI) (Kutcher, 2012). The precise mechanisms of this platelet dysfunction remain unclear, but it may be mediated by the so-called “exhausted platelet syndrome”. This syndrome involves initial platelet hyperactivation with subsequent depletion of intracellular mediators, ultimately resulting in platelet hypofunction (Pareti, 1980). Sillesen et al. (2013a) showed that valproic acid may improve platelet functions after TBI and hemorrhagic shock, but the precise mechanisms remain unknown. One of valproic acid’s protective mechanisms might be its effect on coagulopathy by preventing platelet hyperactivation, which would thereby preserve long-term platelet function. Dekker et al. (2014a) demonstrated that the addition of valproic acid to FFP resuscitation results in preservation of platelet activation 8 hours after the TBI, compared to FFP alone. This was reflected in both circulatory as well as cerebral level platelet activation. However, it remains unclear whether this was a direct effect of valproic acid on platelets, or the establishment of an overall pro-survival phenotype in animals treated with valproic acid. Bambakidis et al. (2017) recently conducted *ex vivo* experiments to test the direct effect of valproic acid on platelet function and coagulation. Results showed that valproic acid attenuates platelet activation and improves clot dynamics (strength and rate of formation) in blood from animals with TBI and hemorrhage shock. Importantly, valproic acid did not appear to alter platelet

or coagulation functions in blood from healthy controls.

Valproic acid improves neurological recovery

While recent studies demonstrated that VPA treatment reduces brain lesion size and attenuates damage to tissues, cells, and proteins, understanding longer-term functional outcomes remains an important hurdle to clinical translatability. Halaweish et al. (2015b) recently conducted a 30-day survival model of TBI+HS. Compared to normal saline resuscitation, VPA resuscitation (150 mg/kg) resulted in significantly decreased neurological impairment, significantly faster rate of neurologic recovery, and smaller brain lesion size. Moreover, although NS- and VPA-treated animals reached similar final cognitive function scores, the VPA group reached cognitive normalization significantly faster than the NS controls. In addition, small animal studies showed improved spatial memory (Dash, 2010) and functional recovery (Dash, 2010; Yu, 2013; Tai, 2014) when VPA was added to the treatment protocol. In animal models of spinal cord trauma, VPA treatment was associated reduced secondary damage, improved locomotor scores (Abdanipour, 2012; Darvishi, 2014), and more rapid recovery (Abdanipour, 2012) (Table 1).

FUTURE DIRECTIONS

Valproic acid treatment shows a promising translation to human patients

One of the challenges with new treatment strategies is the translation of outcomes from animal models to patients in the clinical setting. Animal models are imperfect, and there are several differences between porcine and human species in both physiology and genome. For example, the porcine physiology is hypercoagulable

relative to the human coagulation system. One of the main limitations of research in traditional and pharmacological resuscitation is the lack of human studies. Importantly, Sillesen et al. (2016b) demonstrated that histone deacetylase gene expression patterns are also associated with outcomes in actual trauma patients. Furthermore, our laboratory is currently conducting a United States FDA approved phase 1, double-blind, placebo-controlled trial to evaluate the safety and tolerability of valproic acid in healthy volunteers and trauma patients. The first results of this study showed that valproic acid caused differential expression of a total of 173 proteins. Gene enrichment analysis from these human subjects at 4-hour post infusion showed an up-regulation of pathways related to cell death, apoptosis, necrosis, and abnormal morphology of cells and neurons. Eight hours post-infusion, steroid metabolism, lipid synthesis, and vitamin metabolism were also up-regulated (Georgoff, 2016). In part 2 of the ongoing phase I trial, the effects of valproic acid in trauma patients with hemorrhagic shock will be studied (ClinicalTrials.gov Identifier: NCT01951560). Phases 2 and 3 trials have already been approved for funding, and will commence in the next few years.

Valproic acid dosing needs further refinement

Most experiments described in this review used a dose of 300 mg/kg, which is six-fold higher than the dose used in humans for the treatment of seizures. While these high doses improve outcomes in animal models, they may be associated with side effects in human patients. For example, several investigators have shown that 300 mg/kg and 400 mg/kg doses of valproic acid may be toxic, with respiratory and cardiac arrest occurring shortly after infusion (Burns, 2012). Acute valproic acid overdose results in hypotension, respiratory depression, thrombocytopenia, and

metabolic disorders (Manoguerra, 2008). In addition, chronic valproic acid use (>4 weeks) has been associated with thrombocytopenia, platelet dysfunction (Gidal, 1994; Gesundheit, 2002; Kis, 2002; Manoguerra, 2008; Davidson, 2011), and acquired von Willebrand's disease (Verrotti, 1999; Seraroglu, 2002). A dose as low as 60 mg/kg a day has been found to be biologically active in cancer studies, which led to our hypothesis that lower doses might also be beneficial for trauma patients. As detailed above, our recent test of lower-dose VPA (150 mg/kg) in a TBI+HS survival model showed promising results in terms of neurologic recovery, cognitive outcomes, and lesion size (Halaweish 2015b). A low-dose valproic acid strategy was further supported by the first results of our phase 1 trial demonstrating that valproic acid is biologically effective in healthy humans when given at a dose of 120 mg/kg.

There are potential problems with pan-histone deacetylase inhibitor treatment

Valproic acid is cheap, well tested in multiple animal models, and approved worldwide by regulatory agencies. However, it has been shown to affect several different types of histones. Briefly, there are 5 different histone deacetylase classes: Class I, IIa, IIb, III, and IV. Within these classes, there are 18 different histone deacetylase isoforms (HDAC 1-11, and SIRT 1-7). All isoforms have very different physiological functions, cellular locations, and organ distributions (Halaweish, 2015c). As valproic acid is a non-specific pan-inhibitor, it may create a significant potential for toxicities and side effects. An important area of current research is the development of isoform-specific histone deacetylase inhibitors that are both more potent, and also target specific organs. Our research group is currently comparing isoform specific-histone deacetylase inhibitors and pan-histone deacetylase inhibitors, and investigating the synergistic effects between various histone deacetylase inhibitors

and other cytoprotective strategies.

In particular, the use of HDAC6 inhibition has been shown to be effective in models of sepsis (Li, 2015). Previous agents used in such studies have demonstrated poor brain bioavailability, and as such, have had limited application in the setting of TBI. Recent studies evaluating the use of agents that may provide a higher brain bioavailability (ACY-183, Acetylon Pharmaceuticals) have shown promise, with preclinical and in vitro data demonstrating neuroprotective properties that are superior to non-selective inhibitors such as valproic acid (Nikolian, 2016a).

CONCLUSION

TBI results in coagulopathy, endothelial dysfunction, inflammation, cell death, and impaired epigenetic homeostasis, ultimately leading to severe injury or death. Traditional resuscitation fluids such as crystalloids and colloids are unable to reverse these imbalances, and might even be harmful when administered in large quantities. There is a need to develop next-generation resuscitation strategies that can minimize cellular damage, improve survival, and be administered in the pre-hospital setting. A next-generation resuscitation protocol should involve a combination of fluid resuscitation, blood products, and powerful pharmacologic agents. Histone deacetylase inhibitors, such as valproic acid, have shown promising results after injury, and are therefore excellent examples of these next-generation therapies. However, there is need for further refinement, such as isoform-specific treatment, and further safety and efficacy testing in human patients.

CONFLICT OF INTEREST STATEMENT

No author has a conflict of interest. Dr. Alam has received numerous federal research grants from the National Institutes of Health (NIH), Office of Naval Research (ONR), Defence Advanced Research Projects Agency (DARPA) and the US Army Medical Research and Materiel Command (USAMRMC).

SIGNIFICANCE STATEMENT

Traumatic brain injury (TBI) is a leading cause of death in young adults, and effective treatment strategies have the potential to save many lives. However, there is considerable debate about the most effective methods for treating blood loss associated with TBI. This article describes different resuscitation strategies and novel drug treatment for TBI using valproic acid. Limitations of current strategies as well as future research opportunities using valproic acid and related drugs are discussed.

AUTHORS' CONTRIBUTIONS

All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: SED, HBA. Drafting of the manuscript: SED, VCN, MS, TB. Critical revision of the manuscript for important intellectual content: VCN, MS, TB, PS, HBA. Obtained funding: HBA. Study supervision: HBA.

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Table 1. Key effects of valproic acid treatment following central nervous system injury, with selected studies.

Effect	Model	Reference
Increased cell survival, and decreased ~~,~~ apoptosis~~,~~ and necrosis	TBI animal model	Dekker, 2014b
	Spinal cord injury animal model	Abdanipour, 2012
	Hypoxia-induced neuronal apoptosis in vitro model	Li, 2008; Jin 2014
Decreased brain lesion size	TBI animal model	Jin 2012a; Imam, 2013b; Yu, 2013; Jepsen, 2014; Tai, 2014; Halaweish, 2015b
Improved survival, faster recovery, improved cognitive function	TBI animal model	Dash, 2010; Yu, 2013; Tai, 2014; Halaweish, 2015b
	Spinal cord injury animal model	Abdanipour, 2012; Darvishi, 2014
Reduced inflammation	TBI animal model	Jin, 2012a; Tai, 2014; Bambakidis, 2016; Nikolian, 2016b
	Glioma cell in vitro model	Ichiyama, 2000
	Spinal cord injury animal model	Abdanipour, 2012; Darvishi, 2014
Restoration of BBB function	TBI animal model	Yu, 2013; Nikolian, 2016
	Focal cerebral ischemia animal model	Wang, 2010
	Spinal cord injury animal model	Lee, 2012
Attenuated platelet dysfunction	TBI animal model	Sillesen, 2013a; Dekker 2014a; Bambakidis, 2016
Decreased m~~M~~etabolism and attenuated mitochondrial dysfunction	TBI animal model	Hwabejire, 2013b; Dekker, 2014b
Modulation of cell signaling	TBI animal model	Dekker, 2014b; Nikolian, 2016b