Mechanism of Action of Valproic Acid (VPA) in Spinal Cord Injury (SCI)

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Abstract

Spinal cord injury (SCI) can result in varying degrees of motor dysfunction, partial sensory loss, and sphincter dysfunction. SCI is categorized into two phases: primary injury and secondary injury. Necrosis during the primary injury phase, along with apoptosis, oxidative stress, and autophagy during the secondary injury phase, leads to extensive cellular damage, consequently causing permanent neurological dysfunction. Histone deacetylase (HDAC) plays a pivotal role in regulating cellular activity and gene transcription. The neurological dysfunction resulting from spinal cord injury is associated with an imbalance in protein acetylation levels and related transcriptional dysfunction. Valproic acid (VPA), an HDAC inhibitor, is commonly used clinically as an antiepileptic drug. Research indicates that VPA may possess therapeutic potential for central nervous system diseases. VPA exerts neuroprotective effects by inhibiting HDAC, thereby modulating oxidative stress, cellular autophagy, ionic imbalance, microglial differentiation, and suppressing inflammatory responses. This article reviews the relevant molecular mechanisms underlying VPA treatment for SCI.

Full Text

Study on the Mechanism of Valproic Acid (VPA) in the Treatment of Spinal Cord Injury (SCI)

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Abstract

Spinal cord injury (SCI) causes varying degrees of motor dysfunction, partial sensory loss, and sphincter dysfunction. SCI can be divided into two stages: primary injury and secondary injury. Necrosis during the primary injury phase and apoptosis, oxidative stress, and autophagy during the secondary injury phase lead to extensive cellular damage, resulting in permanent neurological dysfunction. Histone deacetylase (HDAC) plays a key role in regulating cellular activity and gene transcription. The neural dysfunction caused by spinal cord injury is associated with imbalances in protein acetylation levels and related transcriptional dysfunction. Valproic acid (VPA) is an HDAC inhibitor commonly used clinically as an antiepileptic drug. Research suggests that VPA may have therapeutic potential for central nervous system disorders. As an HDAC inhibitor, VPA exerts neuroprotective effects by regulating oxidative stress, autophagy, ion imbalance, microglial differentiation, and inhibiting inflammatory responses. This article reviews the molecular mechanisms of VPA in treating SCI.

Keywords: spinal cord injury; valproic acid; signaling pathways; review

SCI leads to spinal cord dysfunction and is associated with multiple systemic complications, such as cardiovascular, pulmonary, and gastrointestinal diseases [1]. Although the primary injury phase is brief, the direct physical damage to the spinal cord causes irreversible harm. The structural damage resulting from primary injury creates pathological conditions for secondary injury, which in the acute phase involves neuroinflammation, ion imbalance, oxidative stress, glutamate excitotoxicity, neurogenic shock, and a series of cellular and molecular disturbances [2-4].

Studies have shown that VPA can exert therapeutic effects on various conditions including stroke, traumatic brain injury, and Alzheimer' s disease through multiple pathways such as regulating ion imbalance, autophagy, inhibiting inflammatory responses, and reducing oxidative stress [5-7]. In the early stage of SCI, structural damage leads to increased endothelial cell permeability, impaired endothelial vasomotor function, and damage to the blood-spinal cord barrier (BSCB). Following BSCB disruption, blood components enter the spinal cord parenchyma, ultimately causing local edema, ion imbalance, glial and neuronal damage, and the production of inflammatory factors and large amounts of free radicals. Endoplasmic reticulum (ER) stress also plays a significant role in secondary spinal cord injury [8]. In SCI rat models, ER stress can degrade tight junction and adherens junction proteins that constitute the BSCB, and damage to these components not only causes BSCB dysfunction but also allows numerous inflammatory cells to enter the BSCB [9-10].

Current treatment measures for SCI include surgical decompression, medications (such as high-dose steroids, neurotrophic factors, and antibiotics), and rehabilitation therapy [11]. Pharmacological therapy is the most commonly used treatment approach. HDAC inhibitors exert neuroprotective effects by

increasing histone acetylation, regulating gene transcription, upregulating neurotrophic genes, reducing inflammation, and modulating autophagy [12], making them promising therapeutic interventions for spinal cord injury. VPA is a broad-spectrum HDAC inhibitor clinically used to treat various neurological disorders such as epilepsy, psychiatric conditions, and migraine [13]. Existing research indicates that VPA promotes recovery after SCI by attenuating BSCB disruption [14], regulating autophagy and ion imbalance [15], and reducing oxidative stress and inflammatory responses [16].

1. Valproic Acid Reduces Autophagy Flux

Autophagy maintains intracellular homeostasis by recycling and degrading toxic substances, abnormal proteins, and damaged organelles through the lysosomal degradation pathway. Autophagy initiation first involves the formation of an isolation membrane in the cytoplasm that sequesters damaged materials, called a phagophore. After elongation and closure, the resulting double-membrane vesicle (autophagosome) fuses with lysosomes to form an autolysosome, where the contents are degraded and recycled by lysosomes [17]. Microtubule-associated protein light chain 3 (LC3) is an autophagy regulatory protein located on the inner membrane of autophagic vesicles. When autophagy begins, LC3 precursor is processed into LC3-I, which then binds to phosphatidylethanolamine (PE) on the autophagic membrane surface to form LC3-II. LC3-II protein expression directly reflects autophagic capacity, with higher expression indicating stronger autophagic activity. Studies have demonstrated that the autophagy marker LC3-II is upregulated within 24 hours after SCI [18], suggesting that autophagy may be an important component of secondary injury following SCI.

mTOR belongs to the phosphatidylinositol 3-kinase (PI3K) family and participates in regulating DNA repair and growth. The mTOR pathway has two key components: mTORC1 and mTORC2. mTORC1 controls protein synthesis, autophagy, proliferation, cell growth, stress responses, and cellular metabolism, while mTORC2 is involved in regulating cell polarity and survival [19]. mTORC1 regulates autophagy by dephosphorylating and phosphorylating autophagy-related proteins ATG13 and Unc-51-like autophagy activating kinase 1 (Ulk1) [20]. ULK1 is positioned at the most upstream and critical point in triggering autophagy and forms a complex with ATG13, ATG101, and the 200 kDa family interacting protein (FIP200). ULK1 plays a regulatory and mediating role in autophagy initiation. Under nutrient-rich conditions, mTORC1 reduces ULK1 activity by decreasing phosphorylation of ULK1 and ATG13, leading to autophagy inhibition [21]. Conversely, when nutrients are insufficient, mTORC1 enhances ULK1 activity to promote autophagy, a process that also provides nutrients for the organism [22].

In the PI3K pathway, Akt is located upstream of mTORC1 and regulates mTORC1-mediated signaling pathways. Following PI3K activation through different signaling pathways, PIP2 is converted to PIP3, which recruits downstream Akt to the inner membrane and activates it through phosphorylation.

Akt activation reduces the activity of the tuberous sclerosis complex TSC1/2 and regulates mTORC1-mediated autophagy signaling pathways [23]. PTEN is an antagonist of PI3K that reduces production of phosphatidylinositol 3,4-bisphosphate (PIP2) and phosphatidylinositol 3,4,5-trisphosphate (PIP3), and acts as an inhibitor of the Akt/mTOR signaling pathway. PTEN also directly inhibits axon regeneration after spinal cord injury, while PTEN inhibition can promote axon regeneration following SCI [24].

AMPK is a metabolic regulator expressed in eukaryotic cells that senses cellular energy status. It activates catabolic pathways to generate energy and participates in autophagy as a positive regulator [25]. AMPK has an antagonistic relationship with the mTOR signaling pathway. AMPK directly phosphorylates Ser467, Ser555, Thr574, and Ser637 to increase ULK1 activity and inhibits mTORC1 activity by promoting assembly of the TSC1/TSC2 heterodimer [26]. Therefore, AMPK activation can promote autophagy occurrence.

Autophagy plays an important role in maintaining the balance between protein synthesis and degradation. After SCI, autophagy markers such as Beclin-1 and LC3 are upregulated [27], which has positive effects on spinal cord injury recovery because autophagy activation can reduce lesion size, inhibit neuroinflammation, and thereby protect motor neurons [28]. However, autophagy is a double-edged sword, as excessive autophagy activation can exacerbate cell damage. VPA significantly reduces the expression levels of Beclin-1 and LC3 after spinal cord injury [29], maintaining protein degradation in a relatively balanced state and thereby accelerating recovery from SCI.

Autophagy activation can protect microtubules from degradation by superior cervical ganglion protein 10 (SCG10) and promote axon regeneration after SCI [30]. SCG10 is a regulator of microtubule dynamics. JNK1 phosphorylates SCG10 at Ser62/73 and negatively regulates SCG10 activity [31], while mitogen-activated protein kinase phosphatase 1 (MAPK1) activates JNK through the MAPK1-JNK axis by mediating JNK dephosphorylation. VPA can also activate JNK to exert protective effects on microtubule proteins [32]. Netrin-1 is a chemokine that serves as a signal for axon migration during nervous system development and participates in autophagy regulation. In SCI, netrin-1 inhibits mTOR1, activates AMPK to stimulate autophagy, and can also activate MAPK1 to inhibit SCG10 through the MAPK1-JNK axis, thereby improving functional recovery [33]. VPA can also inhibit netrin-1, playing a role in regulating spinal cord autophagy homeostasis and promoting recovery after SCI [34].

2. Valproic Acid Regulates Neural Stem Cells

The adult spinal cord contains a population of multipotent neural stem/progenitor cells (NSPCs) that exhibit the potential to differentiate into neurons. NSPCs isolated from the spinal cord serve as a cellular source for repairing neural damage after SCI [35]. NSPCs possess characteristics of self-renewal, asymmetric division, migration to injury sites, and generation

of new neural cells. They are progenitor cells in the central nervous system capable of differentiating into neurons, astrocytes, and oligodendrocytes. However, endogenous neurogenesis and remyelination are very limited after SCI because activated NSPCs primarily differentiate into astrocytes rather than neurons or oligodendrocytes [36], thereby increasing glial scar formation. Therefore, enhancing NSPCs activity to produce more neurons and fewer astrocytes represents one therapeutic strategy for SCI.

Studies have shown that VPA promotes the neurogenic potential of NSPCs after SCI. In the short-term course of SCI ($\leq$ 7 days), VPA exerts neuroprotective effects by inhibiting inflammatory mediators. In the long-term course ($\geq$ 4 weeks), VPA promotes differentiation of NSPCs into neurons [37]. During the long-term course, VPA significantly increases the expression of the newborn neuron marker doublecortin (DCX) and the mature neuron marker neuron-specific nuclear protein (NeuN) in the injured spinal cord [38], while inhibiting differentiation into astrocytes and oligodendrocytes [39-40]. Octamer-binding transcription factor 4 (Oct4) can convert astrocytes into neurons, and VPA also increases Oct4 promoter activity via the PI3K/Akt/mTOR pathway [41], significantly improving the efficiency of conversion of human astrocytes into neuroblasts while promoting axon regeneration. As an HDAC inhibitor, VPA regulates the expression of many genes while increasing acetylated chromatin levels [42]. Its neuroprotective properties also involve regulation of neurotrophic factors. VPA upregulates nerve growth factors including brain-derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF). BDNF and GDNF can prevent neuronal death and promote regeneration and differentiation of injured neurons, playing important roles in neuronal survival and neurite growth under SCI conditions [43].

3. Valproic Acid Ameliorates Inflammatory Response

Inflammatory response is a protective mechanism of the organism; however, excessive inflammatory response can hinder neural repair and regeneration. Hemorrhage and tissue necrosis after SCI rapidly trigger activation of microglia and astrocytes, activating many inflammation-related pathways [44] while recruiting peripheral immune cells to the lesion area, where inflammatory cell cascade reactions occur, further leading to blood-spinal cord barrier disruption [45]. Microglial activation and inflammatory response ultimately induce neuronal death and cause permanent neurological deficits [46].

Microglial activation is divided into classical activation (M1 type) and alternative activation (M2 type). The M1 type releases pro-inflammatory mediators that cause tissue damage, while the M2 type releases anti-inflammatory and tissue-nourishing factors [47]. The phenotypic shift of microglia from anti-inflammatory (M2-like) to pro-inflammatory (M1) plays an important role in microglial activation and its mediated neuroinflammatory response [48]. SCI-induced M1-type cell activation and subsequent release of inflammatory factors such as interleukins (IL), tumor necrosis factor (TNF), and interferon (INF)

cause direct neuronal death while inducing vascular endothelial cells to express various cell adhesion and chemotactic molecules [49]. These inflammatory factors stimulate NO synthesis, leading to increased capillary permeability and BSCB dysfunction while promoting neuronal apoptosis [50]. SCI also produces large amounts of myelin debris, and the process of phagocytes clearing myelin debris further exacerbates the inflammatory response [51]. Inhibiting SCI-induced microglial activation and subsequent neuroinflammatory response has been shown to improve recovery in SCI patients [52].

VPA treatment shifts microglial polarization toward the M2 phenotype and reduces microglia-mediated inflammatory response. After VPA treatment, expression of M1-type microglial proteins (CD16 and Iba-1) is significantly suppressed, while expression of M2-type microglial protein (CD206) is increased [53]. In other neurological disorders such as traumatic brain injury, VPA also significantly inhibits microglial activation and downregulates IL-1β, IL-6, and TNF-α expression, thereby suppressing inflammatory response [54]. NF- B is a central transcription factor for inflammatory mediators and plays an important role in microglial activation [55-56].

The neuroinflammatory response induced by activated microglia through the NF- B pathway is also a key contributor to secondary injury [57]. The NF- B signaling pathway activates microglia to secrete large amounts of inflammatory cytokines and amplifies inflammatory cascades after necrotic or damaged cell injury [58]. STAT activation has the potential to alleviate various NF- B-driven inflammatory and metabolic disorders [59]. VPA inhibits microglia-mediated central inflammatory response through STAT1-mediated acetylation of the NF- B pathway, which depends on HDAC3 activity after SCI [60]. STAT1 is regulated by HDAC3, and VPA treatment suppresses HDAC3 expression and activity after SCI, thereby enhancing acetylation of STAT1 and NF- B p65. Acetylated STAT1 forms a complex with nuclear NF- B p65, inhibiting NF- B p65 nuclear translocation and expression, and attenuating microglia-mediated central inflammatory response after SCI [61].

4. Valproic Acid Regulates Oxidative Stress and Ion Imbalance

Concurrent with the inflammatory response in SCI, oxidative stress and ion imbalance occur [62]. Overactivation of Na+ channels is one of the common mechanisms exacerbating SCI [63]. Na+ influx through voltage-gated sodium channels (VGSCs) plays an important role in maintaining neuronal excitability and regulating cellular homeostasis [64]. Excessive activation of VGSCs in SCI is also an initiating factor for microglial activation [65]. Excessive Na+ influx can trigger glutamate release in presynaptic neurons, causing excitotoxicity to postsynaptic neurons [66]. High levels of extracellular glutamate leading to overactivation of glutamate receptors can also cause endothelial cell dysfunction. Glutamate excitotoxicity and endothelial dysfunction also result in BSCB dysfunction, ultimately leading to neuronal cell death [67].

Inhibition of Mg2+ homeostasis can also cause glutamate neurotoxicity [68]. Mg2+ is one of the basic cations in organism cells, acting as a cofactor in many enzymatic reactions, synthesizing DNA and proteins, preventing ROS-induced lipid peroxidation, and playing an important role in neurological recovery during cerebral ischemia [69]. SCI can cause a decline in Mg2+ levels and promote the spread of oxidative stress in the spinal cord. In animal experiments, Mg2+ levels in SCI rats treated with VPA increased with VPA administration, and regulating Mg2+ homeostasis and inhibiting specific sodium channels could reduce inflammation and astrocyte proliferation in SCI [70].

Zn is an essential trace nutrient element that functions in various processes including oxidative stress, inflammation, wound healing, and DNA damage repair. Zn2+ can prevent glutamate neurotoxicity by regulating glutamate signaling [71]. Secondary injury in SCI leads to a significant decrease in Zn2+ levels and increased oxidative stress. Increasing Zn2+ levels after SCI is one of the mechanisms by which VPA exerts its neuroprotective effects. Additionally, VPA can prevent excessive increases in levels of pro-oxidant elements such as Cr, Cu, and Fe [72].

[Figure 1: see original paper] Molecular mechanism of valproic acid (VPA) in treating spinal cord injury (SCI)

Concurrent with ion imbalance is oxidative stress. After SCI, the activity of antioxidant enzymes such as TAS, CAT, and GPx decreases. Oxidative stress caused by ROS and reactive nitrogen species (RNS) generated by ischemia/reperfusion stimulation leads to damage to cell membrane lipids, proteins, and DNA. Polyunsaturated fatty acids (PUFA) are major components of cell membranes, and the presence of bis-allylic methylene groups in their structure makes them highly sensitive to ROS and reactive nitrogen species, making them the first target of attack. The spinal cord is highly susceptible to oxidative stress damage after injury due to its high PUFA composition and limited antioxidant capacity [73]. Furthermore, the reaction between ROS and PUFA leads to further spread of oxidative damage and disruption of cell membrane permeability, ultimately resulting in cell death [74].

In vivo studies have found that during secondary injury after SCI, VPA treatment in SCI rats significantly increased the activity of antioxidant enzymes including TAS, CAT, and GPx. Additionally, there is an inverse relationship between Mg2+ levels and ROS-induced lipid peroxidation [75], suggesting that one of the neuroprotective mechanisms of VPA during SCI may be increasing Mg2+ levels to inhibit ROS-mediated oxidative damage.

Summary and Outlook

SCI leads to neuronal damage through necrosis during primary injury and inflammation, autophagy, and oxidative stress during secondary injury, resulting in permanent neurological dysfunction. Although progress has been made in SCI treatment, there are currently no effective therapeutic measures to re-

store patients' motor function. As a broad-spectrum HDAC inhibitor, VPA has potential antioxidant and anti-inflammatory properties and can prevent complications such as oxidative stress after ischemia-reperfusion. VPA protects damaged spinal cord tissue by activating various mechanisms, including regulating autophagy, inhibiting inflammatory responses, modulating ion imbalance and oxidative stress, increasing antioxidant enzyme activity and antioxidant element levels, and protecting the BSCB, thereby attenuating secondary injury. VPA can serve as an adjunctive therapeutic option for existing clinical SCI treatments, but further research is needed on drug combinations and dosages of VPA in clinical SCI treatment.

[1] Eckert MJ, Martin MJ. Trauma: Spinal Cord Injury. Surg Clin North Am. 2017 Oct;97(5):1031-1045.
[2] Fang Y, Huang X, Wan Y, et al. Deficiency of TREK-1 potassium channel exacerbates secondary injury following spinal cord injury in mice. J Neurochem. 2017 Apr;141(2):236-246.
[3] Li MX, Weng JW, Ho ES, et al. Brain delivering RNA-based therapeutic strategies by targeting mTOR pathway for axon regeneration after central nervous system injury. Neural Regen Res. 2022 Oct;17(10):2157-2165.
[4] Zhang D, Wang F, Zhai X, et al. Lithium promotes recovery of neurological function after spinal cord injury by inducing autophagy. Neural Regen Res. 2018 Dec;13(12):2191-2199.
[5] Biesterveld BE, Pumiglia L, Iancu A, et al. Valproic acid treatment rescues injured tissues after traumatic brain injury. J Trauma Acute Care Surg. 2020 Dec;89(6):1156-1165.
[6] Zhang Z, Xu J, Xu P, et al. Quetiapine Combined with Sodium Valproate in Patients with Alzheimer' s Disease with Mental and Behavioral Symptoms Efficacy Observation. J Healthc Eng. 2022 Jan 17;2022:1278092.
[7] Gao X, Zeb S, He YY, et al. Valproic Acid Inhibits Glial Scar Formation after Ischemic Stroke. Pharmacology. 2022;107(5-6):263-280.
[8] Zhang HY, Wang ZG, Lu XH, et al. Endoplasmic reticulum stress: relevance and therapeutics in central nervous system diseases. Mol Neurobiol. 2015;51(3):1343-52.
[9] Zheng B, Zhou Y, Zhang H, et al. Dl-3-n-butylphthalide prevents the disruption of blood-spinal cord barrier via inhibiting endoplasmic reticulum stress following spinal cord injury. Int J Biol Sci. 2017 Nov 27;13(12):1520-1531.
[10] Zhou K, Sansur CA, Xu H, et al. The Temporal Pattern, Flux, and Function of Autophagy in Spinal Cord Injury. Int J Mol Sci. 2017 Feb 21;18(2):466.
[11] Collins-Yoder A, Lowell J. Valproic Acid: Special Considerations and Targeted Monitoring. J Neurosci Nurs. 2017 Feb;49(1):56-61.
[12] Kuboyama T, Wahane S, Huang Y, et al. HDAC3 inhibition ameliorates spinal cord injury by immunomodulation. Sci Rep. 2017 Aug 17;7(1):8641.
[13] Liu S, Li Y, Choi HMC, et al. Lysosomal damage after spinal cord injury causes accumulation of RIPK1 and RIPK3 proteins and potentiation of necroptosis. Cell Death Dis. 2018 May 1;9(5):476.

[14] Lee JY, Kim HS, Choi HY, et al. Valproic acid attenuates blood-spinal cord barrier disruption by inhibiting matrix metalloprotease-9 activity and improves functional recovery after spinal cord injury. J Neurochem. 2012 Jun;121(5):818-29.
[15] Hao HH, Wang L, Guo ZJ, et al. Valproic acid reduces autophagy and promotes functional recovery after spinal cord injury in rats. Neurosci Bull. 2013 Aug;29(4):484-92.
[16] Lee JY, Maeng S, Kang SR, et al. Valproic acid protects motor neuron death by inhibiting oxidative stress and endoplasmic reticulum stress-mediated cytochrome C release after spinal cord injury. J Neurotrauma. 2014 Mar 15;31(6):582-94.
[17] Hu YL, Jahangiri A, Delay M, et al. Tumor cell autophagy as an adaptive response mediating resistance to treatments such as antiangiogenic therapy. Cancer Res. 2012 Sep 1;72(17):4294-9.
[18] Liu S, Li Y, Choi HMC, et al. Lysosomal damage after spinal cord injury causes accumulation of RIPK1 and RIPK3 proteins and potentiation of necroptosis. Cell Death Dis. 2018 May 1;9(5):476.
[19] Kaur A, Sharma S. Mammalian target of rapamycin (mTOR) as a potential therapeutic target in various diseases. Inflammopharmacology. 2017 Jun;25(3):293-312.
[20] Noda T. Regulation of Autophagy through TORC1 and mTORC1. Biomolecules. 2017 Jul 7;7(3):52.
[21] Liu Y, Xu H, An M. mTORC1 regulates apoptosis and cell proliferation in pterygium via targeting autophagy and FGFR3. Sci Rep. 2017 Aug 4;7(1):7339.
[22] Zachari M, Ganley IG. The mammalian ULK1 complex and autophagy initiation. Essays Biochem. 2017 Dec 12;61(6):585-596.
[23] Li X, Wu C, Chen N, et al. PI3K/Akt/mTOR signaling pathway and targeted therapy for glioblastoma. Oncotarget. 2016 May 31;7(22):33440-50.
[24] Tang YJ, Li K, Yang CL, et al. Bisperoxovanadium protects against spinal cord injury by regulating autophagy via activation of ERK1/2 signaling. Drug Des Devel Ther. 2019 Feb 1;13:513-521.
[25] Jo EK, Silwal P, Yuk JM. AMPK-Targeted Effector Networks in Mycobacterial Infection. Front Microbiol. 2019 Mar 15;10:520.
[26] Tamargo-Gómez I, Mariño G. AMPK: Regulation of Metabolic Dynamics in the Context of Autophagy. Int J Mol Sci. 2018 Nov 29;19(12):3812.
[27] Bai L, Mei X, Wang Y, et al. The Role of Netrin-1 in Improving Functional Recovery through Autophagy Stimulation Following Spinal Cord Injury in Rats. Front Cell Neurosci. 2017 Nov 3;11:350.
[28] Meng HY, Shao DC, Li H, et al. Resveratrol improves neurological outcome and neuroinflammation following spinal cord injury through enhancing autophagy involving the AMPK/mTOR pathway. Mol Med Rep. 2018 Aug;18(2):2237-2244.
[29] Hao HH, Wang L, Guo ZJ, et al. Valproic acid reduces autophagy and promotes functional recovery after spinal cord injury in rats. Neurosci Bull. 2013 Aug;29(4):484-92.
[30] He M, Ding Y, Chu C, et al. Autophagy induction stabilizes microtubules

and promotes axon regeneration after spinal cord injury. Proc Natl Acad Sci U S A. 2016 Oct 4;113(40):11324-11329.
[31] Wang YF, Liu F, Lan J, et al. The Effect of Botulinum Neurotoxin Serotype a Heavy Chain on the Growth Related Proteins and Neurite Outgrowth after Spinal Cord Injury in Rats. Toxins (Basel). 2018 Feb 2;10(2):66.
[32] Trolle C, Goldberg E, Linnman C. Spinal cord atrophy after spinal cord injury - A systematic review and meta-analysis. Neuroimage Clin. 2023;38:103372.
[33] Bai L, Mei X, Shen Z, et al. Netrin-1 Improves Functional Recovery through Autophagy Regulation by Activating the AMPK/mTOR Signaling Pathway in Rats with Spinal Cord Injury. Sci Rep. 2017 Feb 10;7:42288.
[34] de Campos Vidal B, Mello MLS. Sodium valproate (VPA) interactions with DNA and histones. Int J Biol Macromol. 2020 Nov 15;163:219-231.
[35] Zhang LQ, Zhang WM, Deng L, et al. Transplantation of a Peripheral Nerve with Neural Stem Cells Plus Lithium Chloride Injection Promote the Recovery of Rat Spinal Cord Injury. Cell Transplant. 2018 Mar;27(3):471-484.
[36] Chaurasia P, Gajzer DC, Schaniel C, et al. Epigenetic reprogramming induces the expansion of cord blood stem cells. J Clin Invest. 2014 Jun;124(6):2378-95.
[37] Chen S, Ye J, Chen X, et al. Valproic acid attenuates traumatic spinal cord injury-induced inflammation via STAT1 and NF- B pathway dependent of HDAC3. J Neuroinflammation. 2018 May 18;15(1):150.
[38] Chu W, Yuan J, Huang L, et al. Valproic Acid Arrests Proliferation but Promotes Neuronal Differentiation of Adult Spinal NSPCs from SCI Rats. Neurochem Res. 2015 Jul;40(7):1472-86.
[39] Farzanehfar P, Horne MK, Aumann TD. Can Valproic Acid Regulate Neurogenesis from Nestin+ Cells in the Adult Midbrain? Neurochem Res. 2017 Aug;42(8):2127-2134.
[40] Teng HF, Li PN, Hou DR, et al. Valproic acid enhances Oct4 promoter activity through PI3K/Akt/mTOR pathway activated nuclear receptors. Mol Cell Endocrinol. 2014 Mar 5;383(1-2):147-58.
[41] Chaurasia P, Gajzer DC, Schaniel C, et al. Epigenetic reprogramming induces the expansion of cord blood stem cells. J Clin Invest. 2014 Jun;124(6):2378-95.
[42] Hasan MR, Kim JH, Kim YJ, et al. Effect of HDAC inhibitors on neuroprotection and neurite outgrowth in primary rat cortical neurons following ischemic insult. Neurochem Res. 2013 Sep;38(9):1921-34.
[43] Guo L, Lv J, Huang YF, et al. Bioinformatics analyses of differentially expressed genes associated with spinal cord injury: A microarray-based analysis in a mouse model. Neural Regen Res. 2019 Jul;14(7):1262-1270.
[44] Kjell J, Olson L. Rat models of spinal cord injury: from pathology to potential therapies. Dis Model Mech. 2016 Oct 1;9(10):1125-1137.
[45] Jiang X, Yu M, Ou Y, et al. Downregulation of USP4 Promotes Activation of Microglia and Subsequent Neuronal Inflammation in Rat Spinal Cord After Injury. Neurochem Res. 2017 Nov;42(11):3245-3253.
[46] Fan H, Tang HB, Shan LQ, et al. Quercetin prevents necroptosis of

oligodendrocytes by inhibiting macrophages/microglia polarization to M1 phenotype after spinal cord injury in rats. J Neuroinflammation. 2019 Nov 7;16(1):206.
[47] Xu H, Wang Z, Li J, et al. The Polarization States of Microglia in TBI: A New Paradigm for Pharmacological Intervention. Neural Plast. 2017;2017:5405104.
[48] Zhang Q, Bian G, Chen P, et al. Aldose Reductase Regulates Microglia/Macrophages Polarization Through the cAMP Response Element-Binding Protein After Spinal Cord Injury in Mice. Mol Neurobiol. 2016 Jan;53(1):662-676.
[49] He Z, Zou S, Yin J, et al. Inhibition of Endoplasmic Reticulum Stress Preserves the Integrity of Blood-Spinal Cord Barrier in Diabetic Rats Subjected to Spinal Cord Injury. Sci Rep. 2017 Aug 9;7(1):7661.
[50] Kopper TJ, Gensel JC. Myelin as an inflammatory mediator: Myelin interactions with complement, macrophages, and microglia in spinal cord injury. J Neurosci Res. 2018 Jun;96(6):969-977.
[51] Zendedel A, Mönnink F, Hassanzadeh G, et al. Estrogen Attenuates Local Inflammasome Expression and Activation after Spinal Cord Injury. Mol Neurobiol. 2018 Feb;55(2):1364-1375.
[52] Chen X, Wang H, Zhou M, et al. Valproic Acid Attenuates Traumatic Brain Injury-Induced Inflammation in Vivo: Involvement of Autophagy and the Nrf2/ARE Signaling Pathway. Front Mol Neurosci. 2018 Apr 17;11:117.
[53] Liu G, Fan G, Guo G, et al. FK506 Attenuates the Inflammation in Rat Spinal Cord Injury by Inhibiting the Activation of NF- B in Microglia Cells. Cell Mol Neurobiol. 2017 Jul;37(5):843-855.
[54] Jiang J, Luo Y, Qin W, et al. Electroacupuncture Suppresses the NF- B Signaling Pathway by Upregulating Cylindromatosis to Alleviate Inflammatory Injury in Cerebral Ischemia/Reperfusion Rats. Front Mol Neurosci. 2017 Nov 6;10:363.
[55] Chen X, Wu S, Chen C, et al. Omega-3 polyunsaturated fatty acid supplementation attenuates microglial-induced inflammation by inhibiting the HMGB1/TLR4/NF- B pathway following experimental traumatic brain injury. J Neuroinflammation. 2017 Jul 24;14(1):143.
[56] Kumar P, Gogulamudi VR, Periasamy R, et al. Inhibition of HDAC enhances STAT acetylation, blocks NF- B, and suppresses the renal inflammation and fibrosis in Npr1 haplotype male mice. Am J Physiol Renal Physiol. 2017 Sep 1;313(3):F781-F795.
[57] Anjum A, Yazid MD, Fauzi Daud M, et al. Spinal Cord Injury: Pathophysiology, Multimolecular Interactions, and Underlying Recovery Mechanisms. Int J Mol Sci. 2020 Oct 13;21(20):7533.
[58] Chen W, Sheng J, Guo J, et al. Cytokine cascades induced by mechanical trauma injury alter voltage-gated sodium channel activity intact cortical neurons. Neuroinflammation. 2017 Mar 31;14(1):73.
[59] Lindsly C, Gonzalez-Islas C, Wenner P. Elevated intracellular Na+ concentrations in developing spinal neurons. J Neurochem. 2017 Mar;140(5):755-765.
[60] Jung GY, Lee JY, Rhim H, et al. An increase in voltage-gated sodium

channel current elicits microglial activation followed inflammatory responses in vitro and in vivo after spinal cord injury. Glia. 2013 Nov;61(11):1807-21.
[61] Nagoshi N, Nakashima H, Fehlings MG. Riluzole as a neuroprotective drug for spinal cord injury: from bench to bedside. Molecules. 2015 Apr 29;20(5):7775-89.
[62] Sámano C, Nistri A. Mechanism of Neuroprotection Against Experimental Spinal Cord Injury by Riluzole or Methylprednisolone. Neurochem Res. 2019 Jan;44(1):200-213.
[63] Huang Z, Filipovic Z, Mp N, et al. AC105 Increases Extracellular Magnesium Delivery and Reduces Excitotoxic Glutamate Exposure within Injured Spinal Cords in Rats. J Neurotrauma. 2017 Feb;34(3):685-694.
[64] Kirkland AE, Sarlo GL, Holton KF. The Role of Magnesium in Neurological Disorders. Nutrients. 2018 Jun 6;10(6):730.
[65] Pappalardo LW, Samad OA, Black JA, et al. Voltage-gated sodium channel Nav 1.5 contributes to astrogliosis in an in vitro model of glial injury via reverse Na+/Ca2+ exchange. Glia. 2014 Jul;62(7):1162-75.
[66] Baltan S, Morrison RS, Murphy SP. Novel protective effects of histone deacetylase inhibition on stroke and white matter ischemic injury. Neurotherapeutics. 2013 Oct;10(4):798-807.
[67] Ulas M, Argadal OG. Trace element, antioxidant and oxidant levels in spinal cord injury: different perspective on the effects of valproic acid. Eur Rev Med Pharmacol Sci. 2023 May;27(9):3892-3905.
[68] Jia Z, Zhu H, Li J, et al. Oxidative stress in spinal cord injury and antioxidant-based intervention. Spinal Cord. 2012 Apr;50(4):264-74.
[69] Shultz RB, Zhong Y. Minocycline targets multiple secondary injury mechanisms in traumatic spinal cord injury. Neural Regen Res. 2017 May;12(5):702-713.
[70] Sperl A, Heller RA, Biglari B, et al. The Role of Magnesium in the Secondary Phase After Traumatic Spinal Cord Injury. A Prospective Clinical Observer Study. Antioxidants (Basel).

Note: Figure translations are in progress. See original paper for figures.