HHS Public Access

Author manuscript

Metab Brain Dis. Author manuscript; available in PMC 2017 June 01.

Published in final edited form as:

Metab Brain Dis. 2016 June ; 31(3): 487–495. doi:10.1007/s11011-016-9801-6.

Neuron specific enolase: a promising therapeutic target in acute spinal cord injury

Azizul Haque[1,*], **Swapan K. Ray**[2], **April Cox**[3], and **Naren L. Banik**[3,4]

[1]Department of Microbiology and Immunology, Hollings Cancer Center, Medical University of South Carolina, Charleston, SC 29425, USA

[2]University of South Carolina School of Medicine, Department of Pathology, Microbiology, and Immunology, Columbia, SC 29209, USA

[3]Department of Neurosurgery, Medical University of South Carolina, Charleston, SC 29425, USA

[4]Ralph H. Johnson Veterans Administration Medical Center, 109 Bee Street, Charleston, SC, 29401

Abstract

Enolase is a multifunctional protein, which is expressed abundantly in the cytosol. Upon stimulatory signals, enolase can traffic to cell surface and contribute to different pathologies including injury, autoimmunity, infection, inflammation, and cancer. Cell-surface expression of enolase is often detected on activated monocytes/macrophages, microglia and astrocytes, promoting extracellular matrix degradation, production of pro-inflammatory cytokines/chemokines, and invasion of inflammatory cells in the sites of injury and inflammation. Inflammatory stimulation also induces translocation of enolase from the cytosolic pool to the cell surface where it can act as a plasminogen receptor and promotes extracellular matrix degradation and tissue damage. Spinal cord injury (SCI) is a devastating debilitating condition whose progressive pathological changes include complex and evolving molecular cascades, and insights into the role of enolase in multiple inflammatory events have not yet been fully elucidated. Neuronal damage following SCI could be characterized by an elevation of neuron specific enolase (NSE), which is also known to play a role in the pathogenesis of hypoxic-ischemic brain injury. Thus, NSE is now considered as a biomarker in ischemic brain damage, and it has recently been suggested to be a biomarker in traumatic brain injury (TBI), stroke and anoxic encephalopathy after cardiac arrest and acute SCI as well. This review gives an overview of current basic research and clinical studies on the role of multifunctional enolase in neurotrauma, with a special emphasis on NSE in acute SCI.

[*]Correspondence: Department of Microbiology, and Immunology, Medical University of South Carolina, 173 Ashley Avenue, BSB-201, Charleston, SC 20425. BSB-201, Charleston, SC 29425; Tel: 843-792-9466, Fax: 843-792-2464, haque@musc.edu.

Disclosures

The authors have no financial conflict of interest.

Keywords

Neuron specific enolase (NSE); plasmin; matrix metalloproteinase (MMP); major histocompatibility complex (MHC); inflammation; neuronal death; spinal cord injury (SCI)

Introduction

Enolase is a multifunctional glycolytic enzyme that acts as a plasminogen binding protein and it is found to be involved in hypoxia and ischemia [1-3]. Enolase plays multiple roles in growth control, hypoxia, immune tolerance, and allergic responses [4-6]. Enolase can stimulate immunoglobulin production, which in turn may activate humoral and cellular immune responses in the host [7]. Enolase is increased after injury leading to activation of endothelial cells and neutrophils through plasmin activity and PAR-2 (protease activated receptor-2) activation [8]. Enolase has also been detected on the surface of pathogens and activated immune cells, where it can serve as a plasminogen receptor and participates in systemic infection or tissue invasion [9, 10]. However, depending on inflammatory signals, it can be differentially localized to the cell surface, promoting a number of pathologies in injury, infection, transplantation, and autoimmunity [1, 4, 11]. Enolase can also act as a heat-shock protein as it binds to cytoskeletal and chromatin structures, and it can play crucial roles in a variety of pathophysiological processes. Enolase expression and trafficking could also be considered as a marker of pathological stress in a wide variety of diseases [12].

Another important issue is that posttranslational modification of enolase is shown to affect the biological functions of enolase in the host [2]. Although enolase does not have classical cellular machinery for surface transport, it often appears on the cell surface through unknown mechanism(s) under inflammatory conditions. Cell surface enolase, which may also serve as a separate function in some organisms, assists in the invasion of their host cells by binding to plasminogen [13]. The bound plasminogen is then cleaved by specific proteases to generate active plasmin, which degrades ECM, facilitating pathogen invasion. Enolase interacts with urokinase-type plasminogen activator (uPA), uPA receptor (uPAR) and plasminogen, and blockade of enolase reduces extracellular matrix (ECM) degradation and cell invasion [1, 14, 15]. It has been shown that cell surface plasminogen activation in activated monocytes, B-cells, T-cells, and granulocytes, is promoted by enolase [10, 15, 16]. Thus, it is possible that a close association between enolase and the uPA/uPAR complex may be responsible for the presence of enolase on the cell surface.

Enolase expression is found throughout the body, however, different isoforms are found to be tissue specific. A switch from enolase-1 (α) to enolase-2 (γ) occurs in neuronal cells. Similarly, a switch from enolase-1 (α) to enolase-3 (β) occurs in muscle tissue during development in rodents. Recent studies suggest that γ-enolase or neuron specific enolase (NSE) levels in cerebrospinal fluid (CSF) can be a useful diagnostic biomarker capable of predicting complete vs. incomplete spinal cord injuries in humans [17]. Studies in brain injury have also found increases in NSE in CSF and peripheral blood, with increases correlating to injury severity [18]. Enolase is expressed in astrocytes and oligodendrocytes (OLGs) and exerts autocrine and paracrine effects on glia, neurons, and microglia. Elevated

levels of NSE are also toxic and may stimulate the expression of pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α), inducing apoptosis in neuronal cells (Figure 1). Surface enolase on monocytes/macrophages, microglia, and astrocytes may induce reactive oxygen species (ROS), nitric oxide (NO), as well as inflammatory cytokines (IL-1β, TGF-β, and TNF-α) and chemokines (MCP-1, MIP-1α), and may modulate diverse cellular events in secondary damage after spinal cord injury (SCI).

Individuals with SCI usually have devastating neurologic deficits and disability, and depending on severity of injury, can often lead to paralysis. [19, 20]. Acute SCI can be considered as a two-step process involving primary and secondary injury mechanisms. Primary injury results from external mechanical forces leading to irreversible tissue detrition and necrosis at the lesion site. Secondary injury, on the other hand, involves multiple cellular and molecular events such as: ischemia, edema, excitotoxicity, inflammation, electrolyte imbalance, free radical damage, and delayed cell death or apoptosis [21-24]. Secondary injury develops during the days to weeks following primary injury where enolase may play a role in promoting neuronal cell death. A primary component of secondary injury, inflammation and release of toxic molecules from the injured cells, further contributes to additional neuronal and oligodendroglial cell death [25-27]. These processes driving secondary injury may be reversible, and inhibition of enolase at multiple time points may attenuate this damage after SCI.

One of the major pathologies contributing to secondary injury of SCI is the loss of OLGs, with subsequent Wallerian degeneration. OLGs are thought to be susceptible to the toxicity of the acute lesion environment after SCI as they undergo both necrosis and apoptosis in the acute phase while apoptosis may be prevalent in chronic phases of the injury [28, 29]. Loss of OLGs causes demyelination and impairs axon function and survival. Oligodendrocyte precursor cells (OPCs) are potential sources for replacement of OLGs after SCI [29]. It is believed that OPCs act rapidly following injuries, proliferate at a high rate, and can differentiate into myelinating OLGs. Thus, a better understanding of OPC differentiation and maturation could contribute to the development of novel therapies for SCI. It still remains unknown whether cell surface enolase on microglia disrupts the differentiation and maturation of OPCs and promotes demyelination.

Given the potential pathological role that enolase may play in neurodegeneration, studies are underway investigating how enolase inhibition affects neurodegenerative processes. Blocking enolase expression by antibody or shRNA plasmid has been shown to reduce plasmin and MMP-2/9 activation, and ECM degradation, suggesting that enolase inhibitor can be used as a modulator of inflammation. Thus, targeting cell surface enolase may offer a novel therapeutic strategy in individuals suffering from a variety of inflammatory and malignant diseases. This review discusses the current status of a potential neurochemical biomarker NSE and its multifunctional role in astroglial and microglial activation and induction of neuronal damage in SCI.

Enolase structure, isoforms and functions

Enolase was discovered by Lohmann and Meyerhof in 1934. It is a glycolytic enzyme that catalyzes the conversion of 2-phosphoglycerate to phosphoenolpyruvate. The reaction is reversible, depending on environmental concentrations of substrates. In addition to its glycolytic function, enolase plays a variety of roles in pathophysiological settings including ischemia, oncogenesis, tumor progression, and bacterial infection [6]. Enolase exists as cell-type specific isoforms such as αα in most adult tissues, αγ and γγ in neurons, and αβ and ββ in muscle cells. The crystal structure of enolase at high resolution (2.25 Å) has been determined [30]. Enolase can be localized in cytoplasm, nucleus, and cell surface of various mammalian cells to mediate distinct functions [3]. However, upon stimulatory signals, enolase can translocate to cell surface and contribute to the induction of inflammatory immune responses in the host.

Cell-surface expression of enolase is often detected on activated macrophages, microglia, neurons and astrocytes (Figure 1), which may lead to the activation of different cellular events and production of pro-inflammatory cytokines (TNF-α) and chemokines (MCP-1), and invasion of inflammatory cells [10, 31, 32] . Enolase on the cell surface induces surface-associated proteolysis, which favors migration of phagocytic cells to the site of inflammation. Enolase also binds plasminogen at the cell surface, and increases local plasmin production, further contributing to enhanced monocyte migration through epithelial monolayers, and matrix degradation [31]. Surface enolase is also likely to concentrate plasminogen on the cell surface, which promotes pericellular plasminogen activation and ECM degradation, and increases invasion and metastasis of tumor cells [33]. NSE, which is the neuronal form of the glycolytic enzyme enolase, is found almost exclusively in neurons and cells of neuroendocrine origin [34]. NSE influences neurotrophic activity and is believed to regulate growth, differentiation, survival and even regeneration of neurons via the activation of PI3K/Akt pathway (Figure 2). It has also been shown that NSE-mediated PI3K activation regulates RhoA kinase [35], and the inhibition of Rho-associated downstream kinase ROCK results in enhanced NSE-induced cellular proliferation, actin polymerization and overall cell growth.

NSE is a 78 kDa γ-homodimer and represents the dominant enolase-isoenzyme found in neuronal and neuroendocrine tissues. It is also known as enolase 2, and is encoded by the ENO2 gene in humans. A switch from α-enolase to γ-enolase also occurs in neural tissue during development in rats and primates. NSE consists of two γ subunits and converts 2-phosphoglycerate into phosphoenolpyruvate [2]. NSE is thought to be a marker of ischemic brain damage, and it has been shown to be elevated in stroke [36], traumatic brain injury (TBI) [37, 38], ischemia-reperfusion injury (IRI) [39], and SCI [40, 41].

NSE expression in neuronal and glial cells

Enolase molecules are commonly known as dimers, which are composed of two of the three distinct subunits coded by separate genes originally designated as α (liver), β (muscle) and γ (brain) forms. While αα isoenzyme is present in all fetal tissues and most adult mammalian tissues, ββ and γβ forms are found predominantly in skeletal and heart muscle. β-enolase is

found in skeletal muscle cells in the adult where it may play a role in muscle development and regeneration. As mentioned above, a switch from α-enolase to β-enolase occurs in muscle tissue during development in rodents. On the other hand, the γγ-enolase and αγ-enolase are frequently referred to as NSE or γ-enolase, and they are found in neuronal and neuroendocrine tissues in mammals. A switch from α-enolase to γ-enolase occurs in neural tissue during development in rats and primates, which play multiple roles in glial and neuronal activation leading to neuroinflammation. The biological half-life of NSE in body fluids is approximately 24 hours.

NSE expression is also detected in cultured OLGs and elevated during the differentiation of OLG precursors into mature OLGs [42], although it is believed to be repressed in fully mature OLGs. NSE is detectable in glial neoplasms and reactive glial cells while undergoing morphological changes [43]. It is also expressed in astrocytes but at much lower levels than in neurons and OLGs [43]. Surface enolase can drive spinal microglial activation with upregulation of major histocompatibility complex-1 (MHC-1) and MHC-II [44, 45], which in turn may induce inflammatory responses after acute SCI. Our recent study suggests that an increased enolase expression enhances MHC-II-mediated antigen presentation by B-cells, macrophages, and dendritic cells, and also activates CD4+ T cells [46]. Studies have also found measurable NSE in cerebrospinal fluid (CSF) and serum, which is elevated in different inflammatory diseases involving neuronal destruction [47]. Thus, elevated NSE is sometimes considered as a valuable marker in neuronal injury.

NSE in neuroinflammation

NSE is the neuronal form of the glycolytic enzyme enolase. It is a dimeric form compounded of two γ subunits that converts 2-phosphoglycerate into phosphoenolpyruvate. NSE is found almost exclusively in neurons and cells of neuroendocrine origin, and is measurable in blood and CSF [48]. NSE is likely to concentrate plasminogen on the cell surface and promotes pericellular plasminogen activation, ECM degradation, proliferation of inflammatory glial cells, and invasion and metastasis of tumor cells [33]. The ECM exists in different biochemical and structural forms, and alters basic functions that are important for the inflammatory process, such as immune cell migration into inflamed tissues and production of inflammatory cytokines and chemokines.

Studies have tested many different molecules (e.g., tau protein, amyloid precursor protein, α-synuclein etc.) in search for specific biochemical markers of brain injury with special attention on NSE and Ca^{2+}-binding protein S100B [49, 50]. NSE is predominantly present in neurons, and it can be released into blood after brain injury [51]. Interactions of NSE with many different nuclear, cytoplasmic, or membrane molecules in the CNS raises the possibility that it can also interact with S100B, and proceed with the neuronal damage. It has been reported that S100B protein is mainly expressed and secreted by astrocytes in the central nervous system (CNS) [52, 53], and frequently detected in a variety of pathologic insults to the brain, where NSE is also elevated. Additionally, increased plasma concentrations of NSE and S100B have been detected in patients with SCI [54]. Since S100B negatively regulates astrocytic and microglial responses to neurotoxic agents and

causes neuronal death [53, 55], it may be interesting to look at whether regulation of NSE influences S100B and protects neurons against apoptosis.

Although enolase functions extend beyond glycolytic activity, it lacks traditional sorting signals. Thus, the question remains as to how this protein becomes surface exposed and play multiple roles in hypoxia, ischemia, and possibly neuronal death after SCI. Cell surface enolase activates plasminogen, which is a serine protease present in serum as an inactive proenzyme, and is converted by tissue-type plasminogen activator (tPA) or urokinase plasminogen activator (uPA) to active plasmin [32], and to degrade ECM proteins (Figure 2). Then plasmin can degrade fibronectin, penetrate the endothelium, and activate matrix metalloproteinases (MMPs). A number of studies suggest that MMPs are instrumental in the production and maintenance of a pro-inflammatory microenvironment [56-58], which upregulates NSE and promotes ECM degradation, aggravating ischemic neuropathology. Thus, therapies targeting ECM degradation and progressive neuroinflammation could also be designed by inhibition of NSE. Cell surface enolase also influences MMPs that can help process the inflammatory cytokines TNF-α and IL-1β to their biologically active forms [59, 60], which in turn may further enhance inflammatory responses. Both MMP-2 and MMP-9 play important roles in the inflammatory process [61]; thus, targeting enolase may be considered for limiting ECM- and MMP-mediated damages after injury.

NSE in other acute injuries to CNS

NSE is believed to be an important marker that directly assesses functional damage to neurons. NSE levels are often elevated in patients with CNS disorders associated with serious secondary brain injury. Serum NSE has also been tested as a marker of brain injury after global cerebral ischemia, and found to be markedly elevated, suggesting that detection of NSE is a valuable diagnostic tool in clinical management in cerebral ischemia and can be a prognostic parameter during the post-ischemic course [62]. NSE concentrations have also been found to be elevated in rat CSF after occlusion of the middle cerebral artery where sham operated animals showed only minimum damage and base level NSE at the site of surgery [63]. Thus, NSE is now a known biomarker of ischemic brain damage and has already been evaluated in TBI [37], stroke [36], and anoxic encephalopathy after cardiac arrest [64, 65].

NSE levels are persistently elevated in TBI patients [18, 38], although it is not always used as a biomarker in clinical practice. Similar observation has been reported by another group where NSE was measured in CSF samples from 23 infants and children with severe TBI [66]. This study detected 10-fold increase in NSE (100.62 ± 17.34 ng/ml vs. 8.63 ± 2.76 ng/ml, $p = 0.0002$) after TBI in infants and children. It has also been shown that NSE levels are relatively low at the beginning of ischemic brain injury, with low predictive power in the first 6 hours. However, detection of NSE could be useful if measured after 24-48 h of TBI [67, 68]. Other study has found that elevated levels of NSE can be detected at an earlier time, and could be used as a prognostic marker [69]. In that study, NSE levels were measured at any time between 12 and 36 h, and the authors were able to detect NSE levels as a marker of prognosis after cardiac arrest. This was consistent with previous findings [50, 70], where NSE levels were shown to be higher and correlated with a worse outcome.

Significantly increased NSE levels were also found in a study using ischemic stroke patients (n=90) as compared to controls [71]. Similarly, serum NSE levels are also thought to be important in the patients with acute intracerebral hemorrhage (ICH), as it is closely related to the volume of hemorrhage and the condition of the disease. A recent study analyzed the expression profile of NSE in patients with intracerebral hemorrhage (ICH) at 24 h [66], and found that NSE concentrations were markedly elevated in these patients, suggesting that higher levels of serum NSE could be associated with poor outcome in ICH. As mentioned above, NSE is localized in neurons, and secreted into the blood and CSF at the time of neural injury. Like TBI, ischemia, and ICH, NSE levels are increased in subarachnoid hemorrhage (SAH) patients. Using 71 patients, a significant association between higher levels of NSE and worse outcomes has recently been reported [72], suggesting that NSE may hold promise for screening SAH patients.

NSE expression is upregulated in SCI

SCI is a devastating neurotrauma whose progressive pathological changes include complex and evolving molecular cascades [24, 73], and insights into the alterations of these dysfunctions have not been fully elucidated. Neuroprotective agents (e.g., methylprednisolone, gacyclidine, thyrotropin releasing hormone, nimodipine etc.) against secondary injury in SCI, together with neuroregenerative agents (e.g., gangliosides, Rho antagonist, anti-Nogo antibodies, acidic fibrobast growth factor) have been used in different clinical settings of SCI with mixed outcomes [24, 73, 74]. In spite of these studies, there is currently no FDA approved pharmacotherapeutic available for the treatment of SCI. Nonetheless, we recently discussed the potential beneficial effects of estrogen, which is an important neuroprotective agent in the treatment of SCI and TBI [75, 76]. Thus, the underlying pathophysiology of SCI with special emphasis on multimodal therapy targeting novel neuroprotective as well as neurogenerative agents should be explored.

The expression levels of enolase have been tested in adult rat spinal cord following contusion injury [40]. This study showed that enolase expression significantly increased after SCI with a peak increase at day 5, and then returned to a level seen in sham-operated animals. These data suggest that enolase protein expression is upregulated after SCI, which could activate many different inflammatory pathways leading to long-term secondary injury in the host. An increased level of enolase was also detected in both grey matter and white matter subjects in that study. Further study with specific markers for neurons, astrocytes and microglia in both sham and injured sections of SC has confirmed the changes of enolase expression after SCI. This group also performed co-immunofluorescence studies for detecting localization of enolase and determining expression of neuronal (NeuN), astroglial (GFAP), and microglial (CD11b) markers after SCI [40]. Interestingly, the investigators have found that enolase levels were markedly increased in astrocytes and microglia after injury but not in neurons when compared with sham-operated spinal cord, suggesting that differential activation of enolase in the brain may induce different pathologies after injury. Since enolases are metalloenzymes and can function as plasminogen receptors [77], surface enolase may have the ability to initiate MMP activation cascade by cleaving pro-MMP [78]. Downregulation of enolase on immune cells as well as neuronal and glial cells may be a new avenue for suppressing neuroinflammation in secondary injury of SCI.

Regulation of NSE after SCI

Accumulating evidence suggests contribution of immune cells to the SCI pathology and repair. The infiltration of polymorphonuclear leucocytes (PMNs), macrophages/microglia, B-cells and T-cells exacerbates poor recovery from SCI [79-82], where elevated NSE may play critical roles in the inflammatory process. A recent study has shown that NSE expression is increased in astroglial and microglial cells after SCI [40], which may activate inflammatory cytokines and chemokines leading to axonal damage and neuronal death (Figure 3). Our group has shown that calpain content progressively increased in the SCI lesion by 22% at 30 min to 91% at 4 h after trauma compared to control and then decreased but remained elevated for up to 72 h following injury [83]. Like calpain, NSE is also elevated early in injury and is involved in the breakdown of cytoskeletal proteins after injury. An effective enolase inhibitor ENOblock has recently been discovered as the first non-substrate analog inhibitor that directly binds to the enzyme [84]. While enolase inhibitors are thought to be anti-diabetic and anti-metastatic, ENOblock has only been tested in a preclinical xenograft model. So far, clinical trials of this drug have not been performed.

Our unpublished data indicate that inhibition of enolase by ENOblock attenuates calpain levels in glial and immune cells, suggesting that ENOblock treatment may help decrease inflammatory events in SCI and brain injury. Thus, it would be interesting to look at whether use of ENOblock alone or in combination with some of these available therapeutic approaches additively or synergistically prevent secondary damages for improving locomotor function in chronic SCI. It is possible that increased NSE expression in neuronal and glial cells after SCI [40], will activate inflammatory cascades, and enolase inhibition by a novel small molecule ENOblock may attenuate inflammation in secondary damages of SCI (Figure 3). Studies in our laboratory are underway to determine the effect of ENOblock after SCI in rodents.

Conclusions

This review mainly summarizes the detrimental role of NSE mainly in acute SCI. NSE and S100B have the potential to be important biomarkers for neuronal death in different CNS injuries. Enolase has also neurotrophic properties on a broad spectrum of CNS neurons. However, current studies strongly implicate that targeting NSE with enolase inhibitor ENOblock can be an important therapeutic strategy for prevention of inflammation and neurodegeneration in acute SCI. Further, combination of ENOblock and calpain inhibitor may work additively or synergistically for very effective neuroprotection with subsequent improvements in locomotor function in both acute and chronic SCI in experimental animals. Success of this strategy in preclinical models of SCI may encourage its clinical trials for alleviation or even elimination of most of the detrimental consequences of SCI in humans in the near future.

Acknowledgements

This work was supported by grants from the South Carolina Spinal Cord Injury Research Fund (SCIRF 2015 P-01), National Institutes of Health (R01 CA129560 and R01 CA129560-S1), and MUSC Center for Global Health Faculty Pilot Program to A. Haque.

References

1. Pancholi V. Multifunctional alpha-enolase: its role in diseases. Cellular and molecular life sciences : CMLS. 2001; 58:902–920. [PubMed: 11497239]
2. Diaz-Ramos A, Roig-Borrellas A, Garcia-Melero A, Lopez-Alemany R. alpha-Enolase, a multifunctional protein: its role on pathophysiological situations. Journal of biomedicine & biotechnology. 2012; 2012:156795. [PubMed: 23118496]
3. Chen SH, Giblett ER. Enolase: human tissue distribution and evidence for three different loci. Annals of human genetics. 1976; 39:277–280. [PubMed: 1275442]
4. Fan SS, Zong M, Zhang H, Lu Y, Lu TB, Fan LY. Decreased expression of alpha-enolase inhibits the proliferation of hypoxia-induced rheumatoid arthritis fibroblasts-like synoviocytes. Modern rheumatology / the Japan Rheumatism Association. 2015; 25:701–707. [PubMed: 25736363]
5. Kuehn A, Fischer J, Hilger C, Sparla C, Biedermann T, Hentges F. Correlation of clinical monosensitivity to cod with specific IgE to enolase and aldolase. Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology. 2014; 113:670–671. e672.
6. Fukano K, Kimura K. Measurement of enolase activity in cell lysates. Methods in enzymology. 2014; 542:115–124. [PubMed: 24862263]
7. Vermeulen N, Arijs I, Joossens S, Vermeire S, Clerens S, Van den Bergh K, Michiels G, Arckens L, Schuit F, Van Lommel L, Rutgeerts P, Bossuyt X. Anti-alpha-enolase antibodies in patients with inflammatory Bowel disease. Clinical chemistry. 2008; 54:534–541. [PubMed: 18218721]
8. Bock A, Tucker N, Kelher MR, Khan SY, Gonzalez E, Wohlauer M, Hansen K, Dzieciatkowska M, Sauaia A, Banerjee A, Moore EE, Silliman CC. alpha-Enolase Causes Proinflammatory Activation of Pulmonary Microvascular Endothelial Cells and Primes Neutrophils Through Plasmin Activation of Protease-Activated Receptor 2. Shock. 2015; 44:137–142. [PubMed: 25944790]
9. Kolberg J, Aase A, Bergmann S, Herstad TK, Rodal G, Frank R, Rohde M, Hammerschmidt S. Streptococcus pneumoniae enolase is important for plasminogen binding despite low abundance of enolase protein on the bacterial cell surface. Microbiology. 2006; 152:1307–1317. [PubMed: 16622048]
10. Bae S, Kim H, Lee N, Won C, Kim HR, Hwang YI, Song YW, Kang JS, Lee WJ. alpha-Enolase expressed on the surfaces of monocytes and macrophages induces robust synovial inflammation in rheumatoid arthritis. Journal of immunology. 2012; 189:365–372.
11. Shi J, Li Y, Yang X, Yang D, Zhang Y, Liu Y. Upregulation of alpha-enolase in acute rejection of cardiac transplant in rat model: implications for the secretion of interleukin-17. Pediatric transplantation. 2014; 18:575–585. [PubMed: 25041443]
12. Pouw MH, Hosman AJ, van Middendorp JJ, Verbeek MM, Vos PE, van de Meent H. Biomarkers in spinal cord injury. Spinal cord. 2009; 47:519–525. [PubMed: 19153591]
13. Ghosh AK, Jacobs-Lorena M. Surface-expressed enolases of Plasmodium and other pathogens. Memorias do Instituto Oswaldo Cruz. 2011; 106(Suppl 1):85–90. [PubMed: 21881761]
14. Redlitz A, Fowler BJ, Plow EF, Miles LA. The role of an enolase-related molecule in plasminogen binding to cells. European journal of biochemistry / FEBS. 1995; 227:407–415. [PubMed: 7851415]
15. Sawhney S, Hood K, Shaw A, Braithwaite AW, Stubbs R, Hung NA, Royds JA, Slatter TL. Alpha-enolase is upregulated on the cell surface and responds to plasminogen activation in mice expressing a 133p53alpha mimic. PloS one. 2015; 10:e0116270. [PubMed: 25643152]
16. Lopez-Alemany R, Longstaff C, Hawley S, Mirshahi M, Fabregas P, Jardi M, Merton E, Miles LA, Felez J. Inhibition of cell surface mediated plasminogen activation by a monoclonal antibody against alpha-Enolase. American journal of hematology. 2003; 72:234–242. [PubMed: 12666133]
17. Pouw MH, Kwon BK, Verbeek MM, Vos PE, van Kampen A, Fisher CG, Street J, Paquette SJ, Dvorak MF, Boyd MC, Hosman AJ, van de Meent H. Structural biomarkers in the cerebrospinal fluid within 24 h after a traumatic spinal cord injury: a descriptive analysis of 16 subjects. Spinal cord. 2014; 52:428–433. [PubMed: 24710150]

18. Berger RP, Pierce MC, Wisniewski SR, Adelson PD, Clark RS, Ruppel RA, Kochanek PM. Neuron-specific enolase and S100B in cerebrospinal fluid after severe traumatic brain injury in infants and children. Pediatrics. 2002; 109:E31. [PubMed: 11826241]
19. Sribnick EA, Samantaray S, Das A, Smith J, Matzelle DD, Ray SK, Banik NL. Postinjury estrogen treatment of chronic spinal cord injury improves locomotor function in rats. Journal of neuroscience research. 2010; 88:1738–1750. [PubMed: 20091771]
20. McDonald JW, Sadowsky C. Spinal-cord injury. Lancet. 2002; 359:417–425. [PubMed: 11844532]
21. Dumont RJ, Okonkwo DO, Verma S, Hurlbert RJ, Boulos PT, Ellegala DB, Dumont AS. Acute spinal cord injury, part I: pathophysiologic mechanisms. Clinical neuropharmacology. 2001; 24:254–264. [PubMed: 11586110]
22. Oyinbo CA. Secondary injury mechanisms in traumatic spinal cord injury: a nugget of this multiply cascade. Acta neurobiologiae experimentalis. 2011; 71:281–299. [PubMed: 21731081]
23. Zhang N, Yin Y, Xu SJ, Wu YP, Chen WS. Inflammation & apoptosis in spinal cord injury. The Indian journal of medical research. 2012; 135:287–296. [PubMed: 22561613]
24. Chamberlain JD, Meier S, Mader L, von Groote PM, Brinkhof MW. Mortality and longevity after a spinal cord injury: systematic review and meta-analysis. Neuroepidemiology. 2015; 44:182–198. [PubMed: 25997873]
25. Tator CH, Fehlings MG. Review of the secondary injury theory of acute spinal cord trauma with emphasis on vascular mechanisms. Journal of neurosurgery. 1991; 75:15–26. [PubMed: 2045903]
26. Cristante AF, Barros Filho TE, Marcon RM, Letaif OB, Rocha ID. Therapeutic approaches for spinal cord injury. Clinics. 2012; 67:1219–1224. [PubMed: 23070351]
27. Mortazavi MM, Verma K, Harmon OA, Griessenauer CJ, Adeeb N, Theodore N, Tubbs RS. The microanatomy of spinal cord injury: a review. Clinical anatomy. 2015; 28:27–36. [PubMed: 25044123]
28. Almad A, Sahinkaya FR, McTigue DM. Oligodendrocyte fate after spinal cord injury. Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics. 2011; 8:262–273. [PubMed: 21404073]
29. Li N, Leung GK. Oligodendrocyte Precursor Cells in Spinal Cord Injury: A Review and Update. BioMed research international. 2015; 2015:235195. [PubMed: 26491661]
30. Lebioda L, Stec B. Crystal structure of enolase indicates that enolase and pyruvate kinase evolved from a common ancestor. Nature. 1988; 333:683–686. [PubMed: 3374614]
31. Wygrecka M, Marsh LM, Morty RE, Henneke I, Guenther A, Lohmeyer J, Markart P, Preissner KT. Enolase-1 promotes plasminogen-mediated recruitment of monocytes to the acutely inflamed lung. Blood. 2009; 113:5588–5598. [PubMed: 19182206]
32. Hsiao KC, Shih NY, Fang HL, Huang TS, Kuo CC, Chu PY, Hung YM, Chou SW, Yang YY, Chang GC, Liu KJ. Surface alpha-enolase promotes extracellular matrix degradation and tumor metastasis and represents a new therapeutic target. PloS one. 2013; 8:e69354. [PubMed: 23894455]
33. Shih NY, Lai HL, Chang GC, Lin HC, Wu YC, Liu JM, Liu KJ, Tseng SW. Anti-alpha-enolase autoantibodies are down-regulated in advanced cancer patients. Japanese journal of clinical oncology. 2010; 40:663–669. [PubMed: 20395242]
34. Oliva D, Cali L, Feo S, Giallongo A. Complete structure of the human gene encoding neuron-specific enolase. Genomics. 1991; 10:157–165. [PubMed: 2045099]
35. Hafner A, Obermajer N, Kos J. gamma-Enolase C-terminal peptide promotes cell survival and neurite outgrowth by activation of the PI3K/Akt and MAPK/ERK signalling pathways. The Biochemical journal. 2012; 443:439–450. [PubMed: 22257123]
36. Anand N, Stead LG. Neuron-specific enolase as a marker for acute ischemic stroke: a systematic review. Cerebrovascular diseases. 2005; 20:213–219. [PubMed: 16123539]
37. Pleines UE, Morganti-Kossmann MC, Rancan M, Joller H, Trentz O, Kossmann T. S-100 beta reflects the extent of injury and outcome, whereas neuronal specific enolase is a better indicator of neuroinflammation in patients with severe traumatic brain injury. Journal of neurotrauma. 2001; 18:491–498. [PubMed: 11393252]

38. Cheng F, Yuan Q, Yang J, Wang W, Liu H. The prognostic value of serum neuron-specific enolase in traumatic brain injury: systematic review and meta-analysis. PloS one. 2014; 9:e106680. [PubMed: 25188406]
39. Zhang M, Ma YF, Gan JX, Jiang GY, Xu SX, Tao XL, Hong A, Li JK. Basic fibroblast growth factor alleviates brain injury following global ischemia reperfusion in rabbits. Journal of Zhejiang University Science B. 2005; 6:637–643. [PubMed: 15973765]
40. Li M, Wen H, Yan Z, Ding T, Long L, Qin H, Wang H, Zhang F. Temporal-spatial expression of ENOLASE after acute spinal cord injury in adult rats. Neuroscience research. 2014; 79:76–82. [PubMed: 24321872]
41. Cao F, Yang XF, Liu WG, Hu WW, Li G, Zheng XJ, Shen F, Zhao XQ, Lv ST. Elevation of neuron-specific enolase and S-100beta protein level in experimental acute spinal cord injury. Journal of clinical neuroscience : official journal of the Neurosurgical Society of Australasia. 2008; 15:541–544. [PubMed: 18343116]
42. Krohn M, Dressler J, Bauer M, Schober K, Franke H, Ondruschka B. Immunohistochemical investigation of S100 and NSE in cases of traumatic brain injury and its application for survival time determination. Journal of neurotrauma. 2015; 32:430–440. [PubMed: 25211554]
43. Isgro MA, Bottoni P, Scatena R. Neuron-Specific Enolase as a Biomarker: Biochemical and Clinical Aspects. Advances in experimental medicine and biology. 2015; 867:125–143. [PubMed: 26530364]
44. Li K, Tan YH, Light AR, Fu KY. Different peripheral tissue injury induces differential phenotypic changes of spinal activated microglia. Clinical & developmental immunology. 2013; 2013:901420. [PubMed: 23818916]
45. Mueller CA, Schluesener HJ, Conrad S, Pietsch T, Schwab JM. Spinal cord injury-induced expression of the immune-regulatory chemokine interleukin-16 caused by activated microglia/macrophages and CD8+ cells. Journal of neurosurgery Spine. 2006; 4:233–240. [PubMed: 16572623]
46. God JM, Cameron C, Figueroa J, Amria S, Hossain A, Kempkes B, Bornkamm GW, Stuart RK, Blum JS, Haque A. Elevation of c-MYC disrupts HLA class II-mediated immune recognition of human B cell tumors. Journal of immunology. 2015; 194:1434–1445.
47. Royds JA, Parsons MA, Taylor CB, Timperley WR. Enolase isoenzyme distribution in the human brain and its tumours. The Journal of pathology. 1982; 137:37–49. [PubMed: 7045308]
48. Johnsson P, Blomquist S, Luhrs C, Malmkvist G, Alling C, Solem JO, Stahl E. Neuron-specific enolase increases in plasma during and immediately after extracorporeal circulation. The Annals of thoracic surgery. 2000; 69:750–754. [PubMed: 10750755]
49. Goncalves CA, Leite MC, Nardin P. Biological and methodological features of the measurement of S100B, a putative marker of brain injury. Clinical biochemistry. 2008; 41:755–763.
50. Fogel W, Krieger D, Veith M, Adams HP, Hund E, Storch-Hagenlocher B, Buggle F, Mathias D, Hacke W. Serum neuron-specific enolase as early predictor of outcome after cardiac arrest. Critical care medicine. 1997; 25:1133–1138. [PubMed: 9233737]
51. Cooper EH. Neuron-specific enolase. The International journal of biological markers. 1994; 9:205–210. [PubMed: 7836797]
52. Tramontina AC, Tramontina F, Bobermin LD, Zanotto C, Souza DF, Leite MC, Nardin P, Gottfried C, Goncalves CA. Secretion of S100B, an astrocyte-derived neurotrophic protein, is stimulated by fluoxetine via a mechanism independent of serotonin. Progress in neuro-psychopharmacology & biological psychiatry. 2008; 32:1580–1583. [PubMed: 18582527]
53. Dang X, Guan L, Hu W, Du G, Li J. S100B ranks as a new marker of multiple traumas in patients and may accelerate its development by regulating endothelial cell dysfunction. International journal of clinical and experimental pathology. 2014; 7:3818–3826. [PubMed: 25120758]
54. Woodcock T, Morganti-Kossmann MC. The role of markers of inflammation in traumatic brain injury. Frontiers in neurology. 2013; 4:18. [PubMed: 23459929]
55. Sorci G, Riuzzi F, Arcuri C, Tubaro C, Bianchi R, Giambanco I, Donato R. S100B protein in tissue development, repair and regeneration. World journal of biological chemistry. 2013; 4:1–12. [PubMed: 23580916]

56. Manicone AM, McGuire JK. Matrix metalloproteinases as modulators of inflammation. Seminars in cell & developmental biology. 2008; 19:34–41. [PubMed: 17707664]
57. Svedin P, Hagberg H, Savman K, Zhu C, Mallard C. Matrix metalloproteinase-9 gene knock-out protects the immature brain after cerebral hypoxia-ischemia. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2007; 27:1511–1518. [PubMed: 17301159]
58. Leonardo CC, Pennypacker KR. Neuroinflammation and MMPs: potential therapeutic targets in neonatal hypoxic-ischemic injury. Journal of neuroinflammation. 2009; 6:13. [PubMed: 19368723]
59. Gearing AJ, Beckett P, Christodoulou M, Churchill M, Clements J, Davidson AH, Drummond AH, Galloway WA, Gilbert R, Gordon JL, et al. Processing of tumour necrosis factor-alpha precursor by metalloproteinases. Nature. 1994; 370:555–557. [PubMed: 8052310]
60. Schonbeck U, Mach F, Libby P. Generation of biologically active IL-1 beta by matrix metalloproteinases: a novel caspase-1-independent pathway of IL-1 beta processing. Journal of immunology. 1998; 161:3340–3346.
61. Bruschi F, Bianchi C, Fornaro M, Naccarato G, Menicagli M, Gomez-Morales MA, Pozio E, Pinto B. Matrix metalloproteinase (MMP)-2 and MMP-9 as inflammation markers of Trichinella spiralis and Trichinella pseudospiralis infections in mice. Parasite immunology. 2014; 36:540–549. [PubMed: 25124689]
62. Horn M, Seger F, Schlote W. Neuron-specific enolase in gerbil brain and serum after transient cerebral ischemia. Stroke; a journal of cerebral circulation. 1995; 26:290–296. discussion 296-297.
63. Hardemark HG, Persson L, Bolander HG, Hillered L, Olsson Y, Pahlman S. Neuron-specific enolase is a marker of cerebral ischemia and infarct size in rat cerebrospinal fluid. Stroke; a journal of cerebral circulation. 1988; 19:1140–1144.
64. Martens P, Raabe A, Johnsson P. Serum S-100 and neuron-specific enolase for prediction of regaining consciousness after global cerebral ischemia. Stroke; a journal of cerebral circulation. 1998; 29:2363–2366.
65. Pfeifer R, Borner A, Krack A, Sigusch HH, Surber R, Figulla HR. Outcome after cardiac arrest: predictive values and limitations of the neuroproteins neuron-specific enolase and protein S-100 and the Glasgow Coma Scale. Resuscitation. 2005; 65:49–55. [PubMed: 15797275]
66. Varma S, Janesko KL, Wisniewski SR, Bayir H, Adelson PD, Thomas NJ, Kochanek PM. F2-isoprostane and neuron-specific enolase in cerebrospinal fluid after severe traumatic brain injury in infants and children. Journal of neurotrauma. 2003; 20:781–786. [PubMed: 12965056]
67. Bottiger BW, Mobes S, Glatzer R, Bauer H, Gries A, Bartsch P, Motsch J, Martin E. Astroglial protein S-100 is an early and sensitive marker of hypoxic brain damage and outcome after cardiac arrest in humans. Circulation. 2001; 103:2694–2698. [PubMed: 11390339]
68. Rosen H, Sunnerhagen KS, Herlitz J, Blomstrand C, Rosengren L. Serum levels of the brain-derived proteins S-100 and NSE predict long-term outcome after cardiac arrest. Resuscitation. 2001; 49:183–191. [PubMed: 11382525]
69. Rech TH, Vieira SR, Nagel F, Brauner JS, Scalco R. Serum neuron-specific enolase as early predictor of outcome after in-hospital cardiac arrest: a cohort study. Critical care. 2006; 10:R133. [PubMed: 16978415]
70. Schoerkhuber W, Kittler H, Sterz F, Behringer W, Holzer M, Frossard M, Spitzauer S, Laggner AN. Time course of serum neuron-specific enolase. A predictor of neurological outcome in patients resuscitated from cardiac arrest. Stroke; a journal of cerebral circulation. 1999; 30:1598–1603.
71. Pandey A, Saxena K, Verma M, Bharosay A. Correlative study between neuron-specific enolase and blood sugar level in ischemic stroke patients. Journal of neurosciences in rural practice. 2011; 2:50–54. [PubMed: 21716874]
72. Tawk RG, Grewal SS, Heckman MG, Rawal B, Miller DA, Edmonston D, Ferguson JL, Navarro R, Ng L, Brown BL, Meschia JF, Freeman WD. The Relationship Between Serum Neuron-Specific Enolase Levels and Severity of Bleeding and Functional Outcomes in Patients With Nontraumatic Subarachnoid Hemorrhage. Neurosurgery. 2015
73. Bragge P, Piccenna L, Middleton J, Williams S, Creasey G, Dunlop S, Brown D, Gruen R. Developing a spinal cord injury research strategy using a structured process of evidence review

and stakeholder dialogue. Part II: Background to a research strategy. Spinal cord. 2015; 53:721–728. [PubMed: 26099209]

74. Hawryluk GW, Rowland J, Kwon BK, Fehlings MG. Protection and repair of the injured spinal cord: a review of completed, ongoing, and planned clinical trials for acute spinal cord injury. Neurosurgical focus. 2008; 25:E14. [PubMed: 18980474]
75. Chakrabarti M, Das A, Samantaray S, Smith JA, Banik NL, Haque A, Ray SK. Molecular mechanisms of estrogen for neuroprotection in spinal cord injury and traumatic brain injury. Reviews in the neurosciences. 2015
76. Chakrabarti M, Haque A, Banik NL, Nagarkatti P, Nagarkatti M, Ray SK. Estrogen receptor agonists for attenuation of neuroinflammation and neurodegeneration. Brain research bulletin. 2014; 109:22–31. [PubMed: 25245209]
77. Floden AM, Watt JA, Brissette CA. Borrelia burgdorferi enolase is a surface-exposed plasminogen binding protein. PloS one. 2011; 6:e27502. [PubMed: 22087329]
78. Lo EH, Dalkara T, Moskowitz MA. Mechanisms, challenges and opportunities in stroke. Nature reviews Neuroscience. 2003; 4:399–415. [PubMed: 12728267]
79. Popovich PG, Stokes BT, Whitacre CC. Concept of autoimmunity following spinal cord injury: possible roles for T lymphocytes in the traumatized central nervous system. Journal of neuroscience research. 1996; 45:349–363. [PubMed: 8872895]
80. Jones TB, Hart RP, Popovich PG. Molecular control of physiological and pathological T-cell recruitment after mouse spinal cord injury. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2005; 25:6576–6583. [PubMed: 16014718]
81. Saville LR, Pospisil CH, Mawhinney LA, Bao F, Simedrea FC, Peters AA, O'Connell PJ, Weaver LC, Dekaban GA. A monoclonal antibody to CD11d reduces the inflammatory infiltrate into the injured spinal cord: a potential neuroprotective treatment. Journal of neuroimmunology. 2004; 156:42–57. [PubMed: 15465595]
82. Nguyen HX, O'Barr TJ, Anderson AJ. Polymorphonuclear leukocytes promote neurotoxicity through release of matrix metalloproteinases, reactive oxygen species, and TNF-alpha. Journal of neurochemistry. 2007; 102:900–912. [PubMed: 17561941]
83. Banik NL, Matzelle DC, Gantt-Wilford G, Osborne A, Hogan EL. Increased calpain content and progressive degradation of neurofilament protein in spinal cord injury. Brain research. 1997; 752:301–306. [PubMed: 9106471]
84. Jung DW, Kim WH, Park SH, Lee J, Kim J, Su D, Ha HH, Chang YT, Williams DR. A unique small molecule inhibitor of enolase clarifies its role in fundamental biological processes. ACS chemical biology. 2013; 8:1271–1282. [PubMed: 23547795]

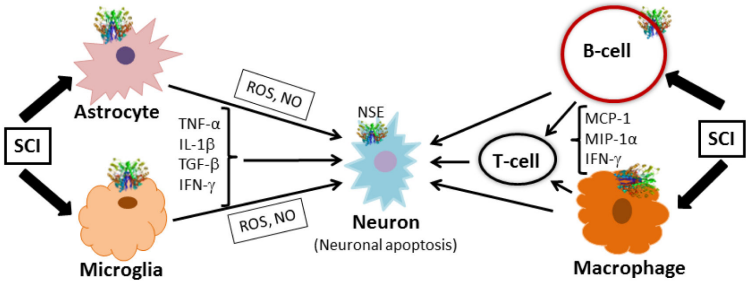

Figure 1.

The potential adverse effects of cell surface enolase in the alterations of neuronal, glial, and immune cell functions, and induction of inflammatory responses following injury. NSE or γ-enolase expression is elevated in microglia and astrocytes following SCI. Similarly, α-enolase expression is increased in macrophages and B-cells following activation or injury. Rapid translocation of enolase from cytosol to the cell surface drives a number of events such as production of reactive oxygen species (ROS), nitric oxide (NO), inflammatory cytokines (e.g., TNF-α, IL-1β, IFN-γ, TGF-β) and chemokines (e.g., MCP-1, MIP-1α), which contribute to neuronal cell death. Macrophages, microglia, and B-cells can enhance antigen presentation, T cell activation, and production of inflammatory cytokines, which may aggravate inflammatory process and neuronal death after injury.

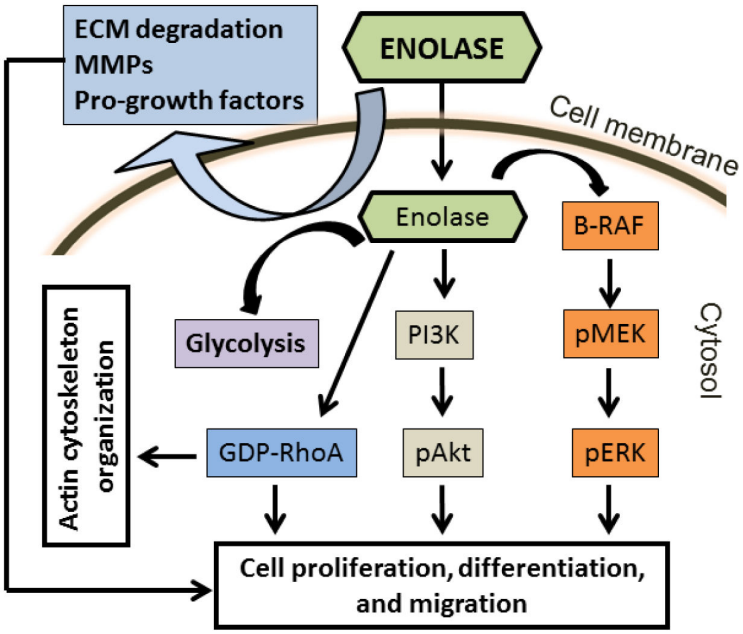

Figure 2.

Expression of cell surface enolase promotes cell migration, survival and growth, and initiates inflammatory process after injury. Increased expression of cell surface enolase elicits pro-inflammatory responses to stimulate plasminogen/plasmin system, promoting activation of MMPs, pro-growth factors, and ECM degradation. Elevation of enolase also promotes glycolysis, cellular proliferation and migration via the PI3K/Akt pathway. Enolase-mediated activation of PI3K also regulates RhoA kinase, which can influence actin cytoskeleton reorganization, induction of neurite outgrowth, and growth arrest in neuronal cells. Upon activation of plasmin/MMPs by increased enolase expression may also lead to MEK/ERK and Rho pathways, inducing cellular proliferation, differentiation and migration.

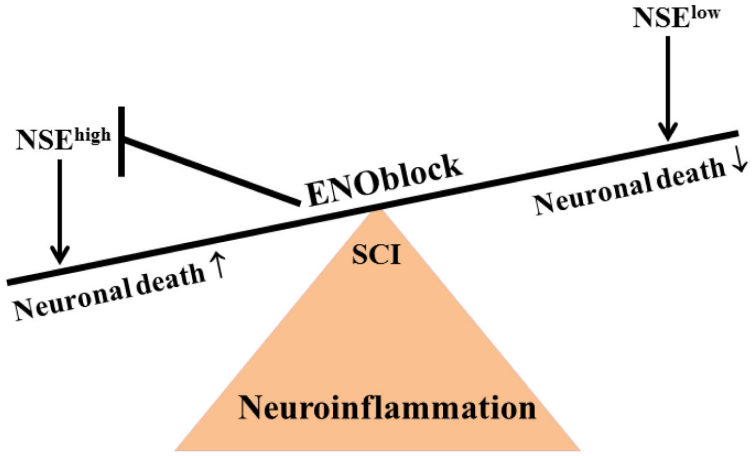

Figure 3.

Elevation of neuron-specific enolase (NSE) following SCI. Cell surface expression of NSE in neuronal and glial cells is detrimental to neuronal survival as it promotes inflammation after SCI. Treatment with enolase inhibitor ENOblock may attenuate inflammatory events in acute SCI and reduce secondary damages of the injury, supporting neuronal survival.