SCIRF Award #2016 I-03
PI: Azizul Haque, PhD
Grant Title: Neuron-specific Enolase and SCI
Final Progress Report:

Progress Report (First Ten Months):
Enolase is a multifunctional glycolytic enzyme involved in growth control, hypoxia, immune activation, inflammation, allergic responses, and cancer. 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 can be characterized by an elevation of neuron-specific enolase (NSE), which may play an important role in the secondary injury pathology in SCI. To date, only few studies have focused on the role of NSE in the alterations of inflammatory processes after SCI, and insights into the role of NSE in SCI-mediated dysfunctions remain largely unknown. *We hypothesized that increased NSE expression after SCI promotes inflammatory events in spinal cord, activates plasmin-mediated degradative pathways leading to activation of inflammatory cytokines/chemokines, which aggravate secondary damages of SCI. We also hypothesized that inhibition of NSE by a novel small molecule inhibitor ENOblock (C31H43FN8O3) may attenuate secondary damage following rat SCI, protecting neurons and improving functions.* A long-term goal of this proposal is to develop NSE-targeted therapeutic intervention for treatment of individuals with SCI. To test these hypotheses, we proposed two specific aims:

Specific Aim 1: Examine and compare NSE expression and activity in neurons and glial cells after acute SCI, and investigate whether NSE levels correlate with the inflammatory cell infiltration and production of inflammatory cytokines and chemokines in rats following SCI.

Specific Aim 2: Investigate whether treatment of animals with a novel enolase inhibitor ENOblock attenuates inflammatory events and neuron death after acute and chronic SCI, and improve locomotor function. Studies are planned to determine NSE levels in serum and spinal cord samples, assess cytokine and chemokine levels in sera and tissues, analyze infiltrating cells, inflammatory factors, gliosis, myelin loss, axonal degeneration and neuronal cell death, and evaluate locomotor function (BBB scale) after SCI.

The mechanistic insights gained from this proposal may lead to the novel discovery of enolase as a critical target in SCI, and ENOblock as a potential therapy to promote functional recovery in human SCI.

Spinal cord injury (SCI) is a debilitating condition with life-long consequences that affects an estimated 285,000 people in the United States. SCI has no FDA-approved treatment, even though methylprednisolone is controversially used off-label in many instances of both acute and chronic SCI. As such, research into effective treatment options and alternatives for affected individuals is urgently needed. Although the primary trauma in SCI, the initial mechanical insult to neurons of the CNS, is considered to be irreversible, the secondary injury mechanisms of SCI pathology provide areas of opportunity for researchers to discern effectors of favorable change to injury progression and recovery in sub-acute, acute, and chronic stages. At the sub-acute and acute level, these secondary mechanisms include hypoxia, ischemia, glutamate excitotoxicity, and inflammation that can exacerbate the injury by leading to further neuroinflammation, neurodegeneration, and neuronal cell death. ENOblock is a novel small molecule which is the first, non-substrate analogue that directly binds to enolase and inhibits its expression and activity. It is the first reported enolase inhibitor that is suitable for biological assays, and ENOblock treatment has been shown to target cell-surface-bound enolase to modulate cell growth through downregulation of AKT and BCL-xL.

ENOblock also reduces intracellular enolase expression and activity, and decreases the ability of cells to adapt to the hypoxic inflammatory condition.

A significant progress has been made towards the Specific Aim 1. Our recent studies suggest that NSE is markedly elevated after acute SCI, and ENOblock treatment attenuated NSE levels in serum and spinal cord tissues, decreased inflammatory cytokines/chemokines in serum, and spinal cord tissues (Neurochem Res. 2017 Oct;42(10):2777-2787). Thus, reduction of enolase expression and activity may have therapeutic potential in attenuating inflammatory mediators in acute SCI. Although NSE functions extend beyond glycolytic activity, it lacks traditional sorting signals. Thus, the question remains as to how NSE 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

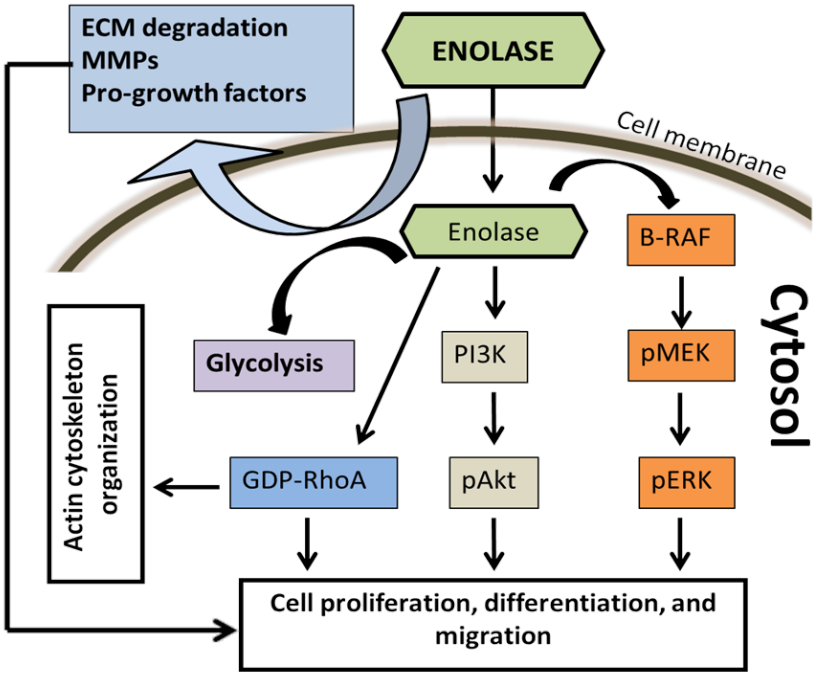


Figure 1. The potential adverse effects of cell surface enolase following SCI. Expression of cell surface enolase promotes cell migration, survival and growth, and initiates inflammatory process after injury. Increased expression of cell surface enolase elicits pro-inflammatory responses to stimulate 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.

an inactive proenzyme, and is converted by tissue-type plasminogen activator (tPA) or urokinase plasminogen activator (uPA) to active plasmin, and to degrade ECM proteins. The diagrammatic representation in **Figure 1** implicates some of the adverse effects of cell surface NSE after SCI (Metab Brain Dis. 2016 Jun; 31(3):487-95).

Our SCIRF Pilot Project as well as Investigator Initiated Research Project examined serum NSE levels and cytokines/chemokines in SCI/vehicle and SCI/ENOblock treated rats, and published the results the journal Neurochemical Research (Neurochem Res. 2017 Oct;42(10):2777-2787). To achieve other goals stated in Aim 1, we evaluated serum metabolic enzymes in both injured and treated animals using Discovery Metabolic Arrays. Briefly, rats were anesthetized with ketamine (80 mg/kg)/xylazene (10 mg/kg), and laminectomy was performed at T10. The spine was immobilized with a spinal stereotactic device, and SCI was induced using a modified method by Perot of dropping a constant weight (5 gm) from a height of 8 cm onto an impounder (0.3 cm in diameter) gently placed on the spinal cord. ENOblock was purchased from Bioscience (Catalog No. A11840), dissolved in vehicle (0.01% DMSO in saline), and was administered (100μg/kg, 100μl in volume) at 15 min and 24 h post-injury via intravenous injection. Vehicle-treated

animals received the same volume of vehicle (100µl). Sham animals received T10 laminectomy. Blood and tissue samples were collected 48h post-injury.

The results indicated alterations of several metabolic factors in ENOblock treated rats. Specifically, we examined the connecting peptide called C-peptide, which can exert insulin-independent biological effects on cells, and is known to act as a bioactive peptide with anti-inflammatory properties. Data from metabolic arrays showed that serum C-peptide levels were significantly elevated in SCI-ENO rats as compared to SCI-vehicle rats **(Fig. 2A)**. We also examined leptin levels in SCI, which are implicated in the pathogenesis of chronic inflammation. The elevated circulating leptin levels increase the risk of developing cardiovascular diseases, type II diabetes, or degenerative disease including inflammatory autoimmune disease (e.g., multiple sclerosis) and cancer. Interestingly, serum leptin levels were significantly inhibited by ENOblock treatment **(Fig. 2B)**. We also examined the peptide hormone, amylin, which is strongly associated with inflammatory markers in a variety of injury conditions. Data suggested that ENOblock treatment significantly reduced amylin levels in SCI-ENO rats as compared to SCI-vehicle rats **(Fig. 2C)**, suggesting that enolase may be an important target to design novel therapeutics for SCI.

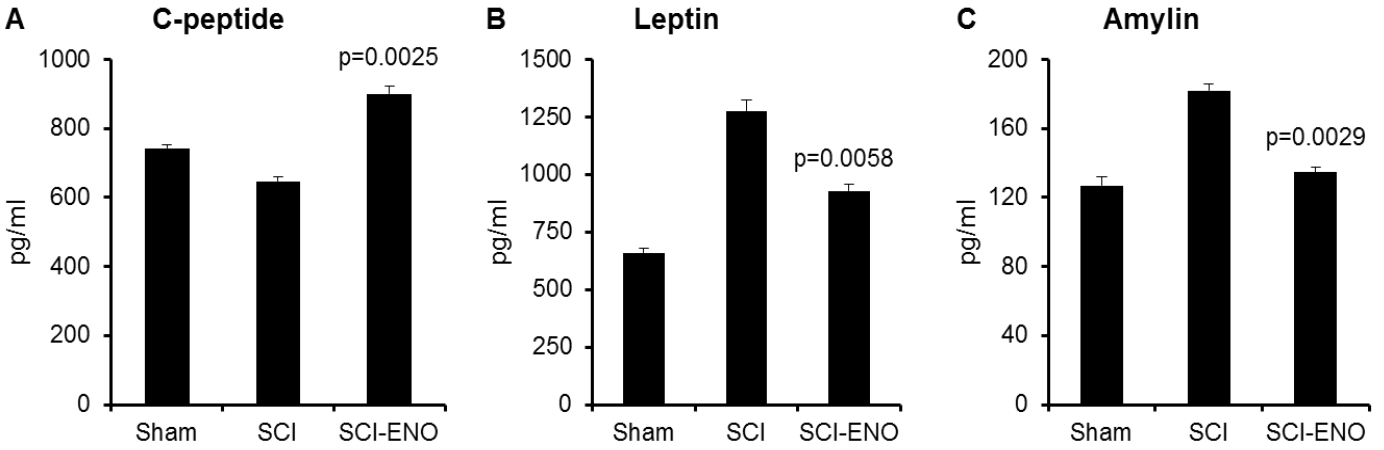
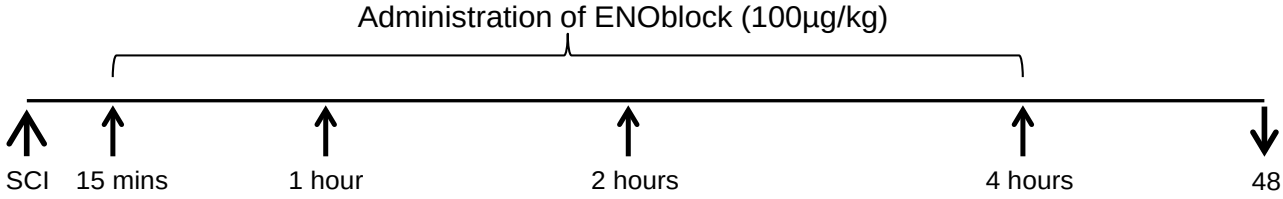


Figure 2. ENOblock treatment differentially influences metabolic enzymes after SCI in rats. SCI rats were treated with vehicle alone or ENOblock (100µg/kg, twice, intravenously), and then blood samples were obtained 48h post-injury. Sham operated rats (T10 laminectomy) were used as controls. Serum c-peptide, leptin, and amylin levels were analyzed by using Discovery rat metabolic arrays. Results obtained from serum samples (duplicate analyses) of three rats suggested that ENOblock treatment significantly downregulated leptin and amylin levels while elevated c-peptide as compared with vehicle treated SCI rats. Statistical analyses were performed using Student's t-test.

Since early administration (15 min) of ENOblock had beneficial effects, we wanted to investigate if injection of ENOblock at later time points (1 h, 2 h and 4 h post-SCI) may yield differential outcomes in SCI rats **(Scheme I)**. Rats were treated with ENOblock (100µg/kg) at 15 min, 1 h, 2 h and 4 h after SCI, followed by analysis of serum samples for c-peptide and leptin levels at 48 h. Data showed that ENOblock



Scheme I: Time-dependent effects of ENOblock on metabolic enzymes in SCI.

treatment of rats at different time points (15 min-4 h) had a similar trend in modulating metabolic enzymes especially c-peptide and leptin after SCI **(Figure 3)**. Thus, administration of ENOblock at a reasonable window of time (15 min-4 h) after SCI contributed to induction of anti-inflammatory responses in the host.

Primary injury to spinal cord would lead to secondary injury causing gliosis, inflammation, and neurodegeneration but treatment of animals with ENOblock may inhibit these inflammatory events in SCI, inducing neuroprotection.

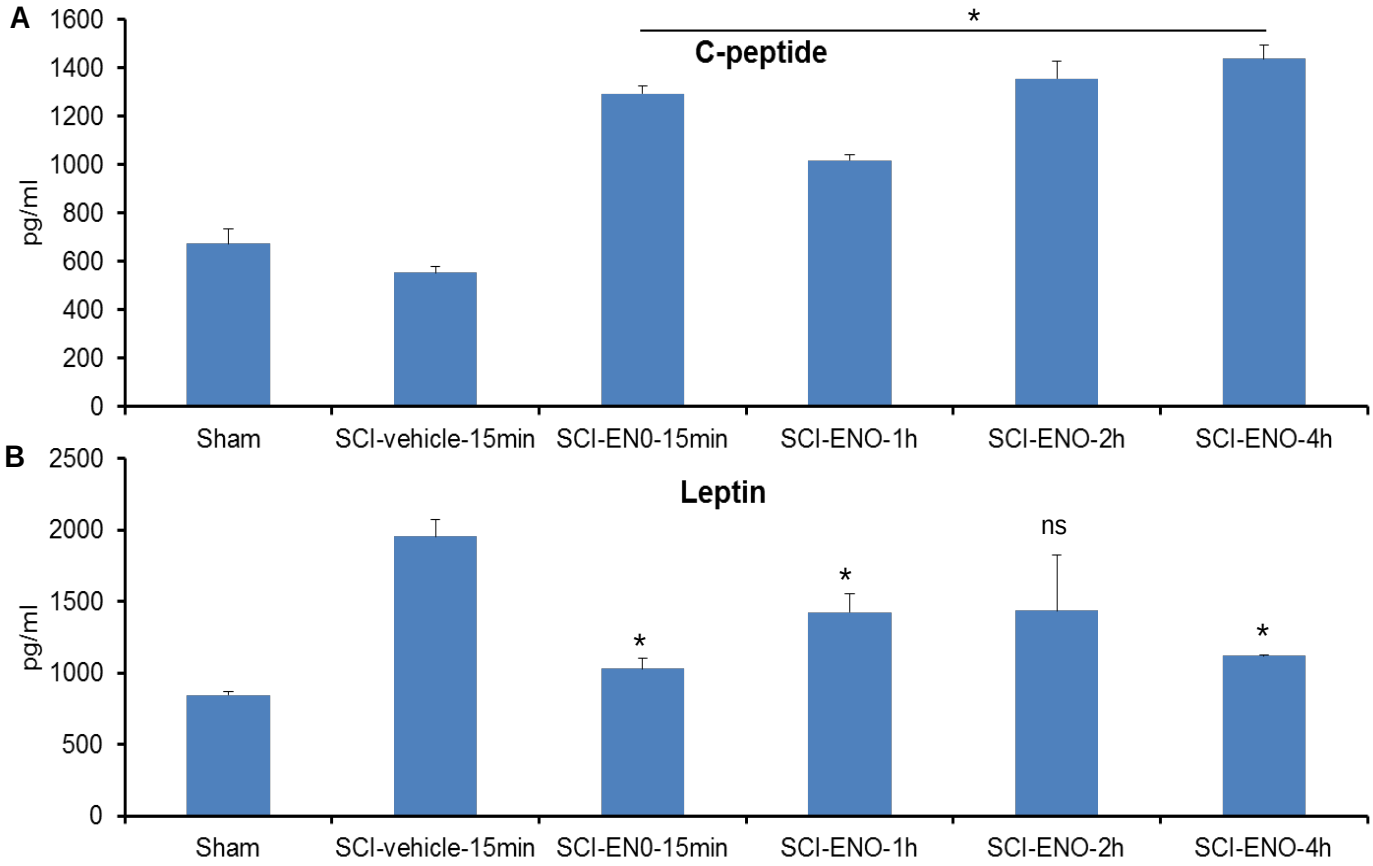


Figure 3. Effects of time-dependent administration of ENOblock on metabolic enzymes after SCI in rats. SCI rats were treated with vehicle alone or ENObloc at different time points as indicated, and blood samples were obtained 48h post-injury. Sham operated rats (T10 laminectomy) were used as controls. Serum c-peptide (A) and leptin (B) levels were analyzed by using Discovery Rat Metabolic Arrays. Data suggest that administration of ENOblock downregulates leptin levels while increases c-peptide levels as compared with vehicle treated SCI rats. Statistical analyses were performed using Student's t-test. *p<0.05, ns=not significant.

The status of inflammatory molecule (MMP-9), microglial activation (Iba1), and astrogliosis (GFAP) was also monitored by immunohistochemistry **(Figure 4)**. Analysis of SCI tissue samples by immunohistochemistry confirmed that ENOblock inhibited MMP-9 protein expression, and decreased gliosis (Iba1 & GFAP), which may have occurred through reduction of elevated NSE in rats. These data are consistent with our earlier findings that serum cytokines/chemokines were also diminished after ENOblock treatment. Overall, elevation of NSE is deleterious as it promotes extracellular degradation and production

of inflammatory cytokines/chemokines and metabolic factors which activates glia and damages neurons. Thus, reduction of NSE by ENOblock may have potential therapeutic implications in acute SCI.

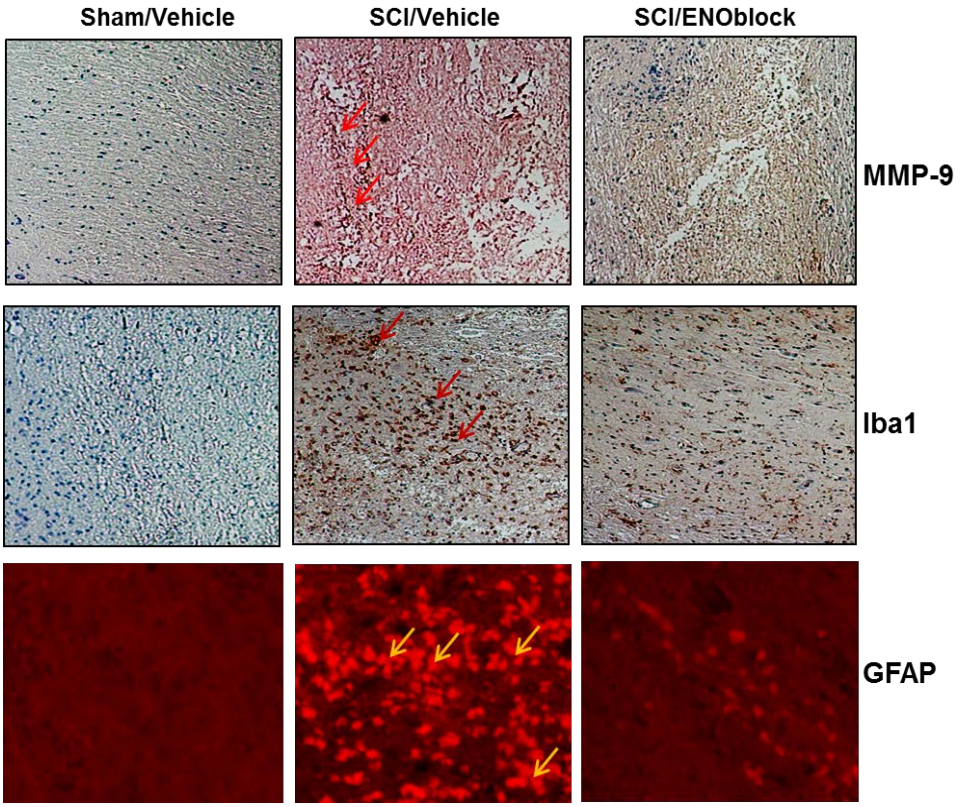


Figure 4. ENOblock treatment attenuates MMP-9 and gliosis (Iba1 and GFAP, glial markers) in acute SCI. Spinal cord injured (T10) rats were treated with vehicle (DMSO, 0.01% in saline) alone or ENOblock (100μg/kg, twice, iv), and spinal cord samples were obtained 48h post-injury. Immunohistochemical analysis was performed for MMP-9 (magnification 10X), Iba1 (microglia, magnification=10X) and GFAP (astrocytes, magnification=20X). Representative figures from three rats show that MMP9 and Iba1/GFAP protein expression (arrows) in SCI rat spinal cords (T10) were markedly upregulated, which were downregulated by ENOblock treatment as compared to vehicle controls. Sham operated (T10) animals (n=2) were used as negative controls.

In addition, during the last 10 months, the PI presented data in the ASN meeting (March 18-22, 2017) in Little Rock, Arkansas. The PI submitted two abstracts for presentation in the ASN-2017 meeting. The PI also submitted a related manuscript for publication in Neurochemical Research and it was published (Neurochem Res. 2017 Oct;42(10):2777-2787). The PI also published a research paper in *Molecular Neurobiology*, and a review article in the *Proceedings of Neurosciences.* We thank SCIRF for supporting this Investigator Initiated Project.

Publications:

Manuscripts accepted or submitted (related):

1. **Haque A**, Capone M, Matzelle D, Cox A, **Banik NL** (2017). Targeting enolase in reducing secondary damage in acute spinal cord injury in rats. Neurochem Res *(submitted).*
2. **Haque A**, Ray SK, Cox A, Banik NL (2016) Challenging pathophysiology in spinal cord injury and promising stem cell therapy. *Proceedings of Neurosciences* (in press).

Manuscripts published (unrelated):

1. Trager NM, Butler JT, Harmon J, Mount J, **Haque A**, Banik NL, Beeson CC (2017). A novel Aza-MBP altered peptide ligand for the treatment of experimental autoimmune encephalomyelitis. *Mol Neurobiol* (*accepted*).

Abstracts Published:

1. **Haque A,** Matzelle D, Capone M, Cox A, Banik NL. 2017. Blockade of enolase activation reduces inflammation and protects spinal cord following injury. American Society for Neurochemistry Annual Meeting (March 18-22) 2017, Little Rock, AR.
2. **Haque A,** Narang A, Das A, Matzelle D, Capone M, Haque A, Banik NL. 2017. Premarin treatment reduces inflammation and degeneration and improves function in SCI. American Society for Neurochemistry Annual Meeting (March 18-22) 2017, Little Rock, AR.

Progress Report (Last Fourteen Months):

Since the last 10-month progress report, we kept working on our other aims and sub-aims as proposed in the application. We made significant progress in investigating the role of enolase/NSE in injury related inflammatory, immunological, biochemical, and neurological aspects in spinal cord. We have recently shown that enolase inhibition with ENOblock treatment of acute SCI in rats significantly decreases levels of pro-inflammatory cytokines/chemokines (TNF-α, IL-1β, IL-6, IP-10, and MIP-1α), alters metabolic hormones (amylin, leptin, and c-peptide, inhibits other inhibitory mediators (evidenced by decreased MMP-9 expression) in serum and tissue samples, and attenuates gliosis (decreased Iba1 and GFAP) (Neurochem Res. 2017 Oct;42(10):2777-2787). An analysis of the metabolic factors c-peptide, amylin, and leptin revealed a connection between reduced enolase/NSE activity and alteration of metabolic hormones with roles in inflammation to favor anti-inflammatory activity. This connection between inflammation, metabolic factors, and gliosis in association with ENOblock treatment of an acute model of SCI in rats led us to question (1) whether this anti-inflammatory activity could also be observed with ENOblock treatment in the sub-acute level of SCI, (2) the extent of enolase/NSE's effects on other metabolic hormones, and (3) how this glycolytic enzyme functions in neuroinflammation and neuronal protection.

In order to evaluate the association between ENOblock treatment and neuroinflammation in a sub-acute and chronic SCI, immunohistochemistry experiments were conducted. Vimentin staining (1:500, Abcam ab92547) of SCI tissue from sham-operated, vehicle-treated, and ENO-treated (100μL at 15min, 24h, and 3d post-injury) rats harvested 7d post-injury indicated an increase in inflammation following injury (**Figure 5A**). Vimentin staining of SCI tissue from sham-operated, vehicle-treated, and ENO-treated (100μL at 15min, 24h, 3d, and 7d post-injury) rats harvested 14d post-injury also indicated an increase in

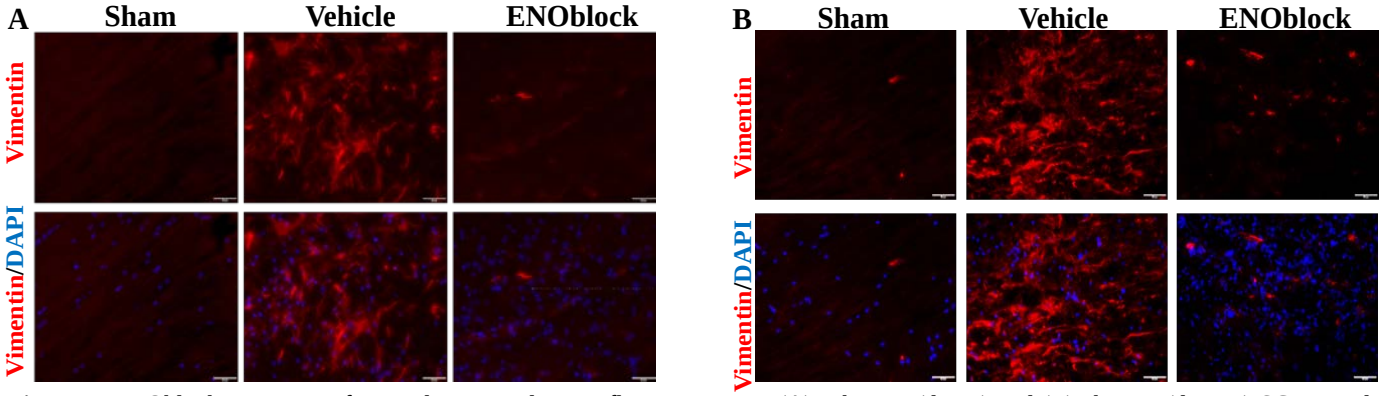


Figure 5. ENOblock treatment of injured tissue reduces inflammation in a (A) subacute (day 7) and (B) chronic (day 14) SCI in male Sprague Dawley rats. Vimentin (red) and DAPI (blue) nuclear staining of SCI tissue at the injury site is shown in sham-operated, vehicle-treated, and ENOblock-treated (ENO) rats. Results show increased vimentin expression in vehicle treated tissue that is attenuated with ENOblock treatment. Representative images were taken at 20X magnification.

inflammation following injury (**Figure 5B**). Results obtained also indicated that vimentin expression was

markedly attenuated with ENOblock treatment in both subacute and chronic SCI (**Figure 5**). This finding aligns with our earlier results from an acute model of SCI and shows for the first time that ENOblock treatment reduces neuroinflammation in a sub-acute to chronic SCI in rats.

GLP-1 receptors (GLP-1R) are found on neurons in the brains of rodents and humans, and they have also been found on microglia and astrocytes in mice. Our metabolic profile of ENOblock treated rats compared to vehicle treated rats showed a significant increase in GLP-1 concentration with ENOblock treatment (**Figure 6A,** p=0.0022). The median concentrations of GLP-1 in SCI-vehicle treated rats compared to SCI-ENO treated rats were 500.1 and 678.3 pg/ml respectively. GIP receptors have also been found on neuronal cells but not on microglia or astrocytes, which may show neuroprotective effects.

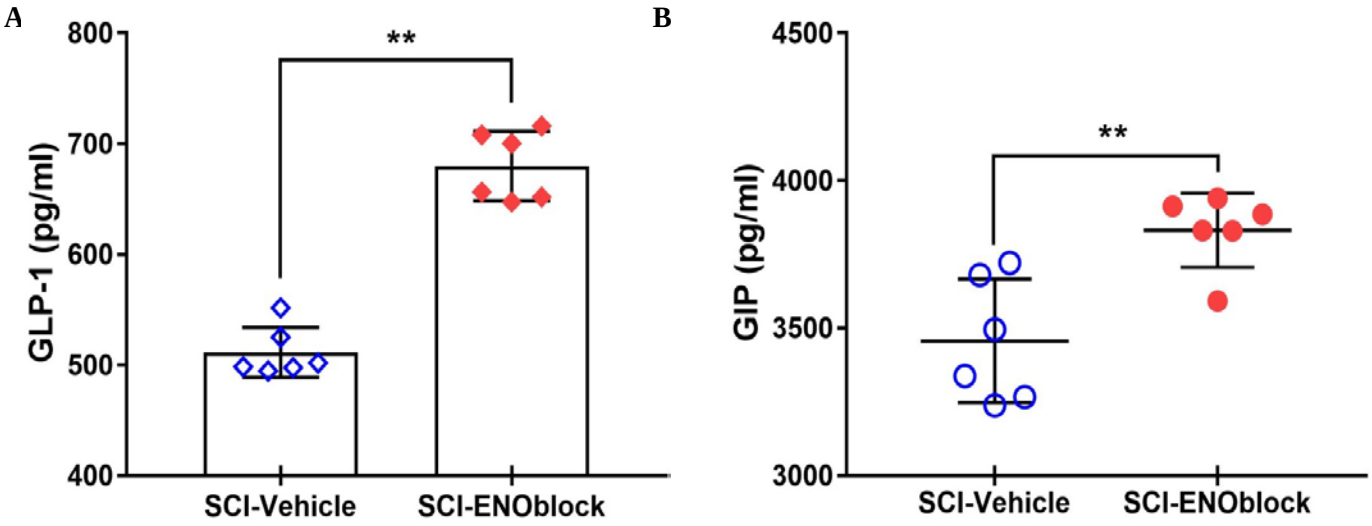


Figure 6. Effect of ENOblock Treatment on Incretin Hormones. SCI rats were treated with vehicle alone or ENOblock (100 µg/kg, four times, intravenously), and then blood samples were obtained 7d post-injury. Sham operated rats (T10 laminectomy) were used as controls. Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotrophic polypeptide (GIP) levels were analyzed by using Eve Tech's Mouse, Rat Metabolic Array (MRDMET) Discovery Assay. (A) Plasma levels of GLP-1 in ENO-treated SCI rat samples were significantly increased as compared to vehicle treated rats. Median concentrations in SCI-vehicle and SCI-ENOblock treated groups were 500.1 and 678.3 pg/ml respectively; the distributions in the two groups differed significantly (Mann–Whitney U = 0, $n_1 = n_2 = 6$, $^{**}p < 0.01$ two-tailed). (B) ENOblock treatment increased GIP levels after SCI in rats. Median concentrations in SCI-vehicle and SCI-ENOblock treated groups were 3416 and 3858 pg/ml respectively; the distributions in the two groups differed significantly (Mann–Whitney $U = 2$, $n_1 = n_2 = 6$, $^{**}p < 0.01$ two-tailed).

Analysis of the plasma sample from ENO-treated and vehicle treated rats indicated a significant increase in GIP levels associated with ENOblock treatment (**Figure 6B**, p=0.0087). The median concentrations of GIP in SCI-vehicle treated rats compared to SCI-ENO treated rats were 3416 and 3858 pg/ml respectively.

Activation of glucagon receptors can lead to an increase in intracellular cAMP and decrease the production of Th2 cytokines, TNF-α, and NK cell activity. Our analysis of the ENO-treated rat plasma indicated a significant increase in glucagon levels as compared to the vehicle-treated levels (**Figure 7A**, p=0.0022). The median concentrations of glucagon in SCI-vehicle treated rats compared to SCI-ENO treated rats were 374.3 and 737.3 pg/ml respectively. Counterintuitively, ENOblock treatment also corresponded to significantly increased levels of insulin (**Figure 7B**, p=0.0022). The median concentrations of insulin in SCI-vehicle treated rats compared to SCI-ENO treated rats were 893.2 and 1264 pg/ml respectively. Insulin typically functions in glucose storage while glucagon functions in glycogen breakdown. However, in the CNS it has been shown that low insulin levels correspond to pro-inflammatory

conditions while high insulin levels promote anti-inflammatory effects. This result aligns with the previously observed reduction of cytokine/chemokine levels and upregulation of amylin levels in

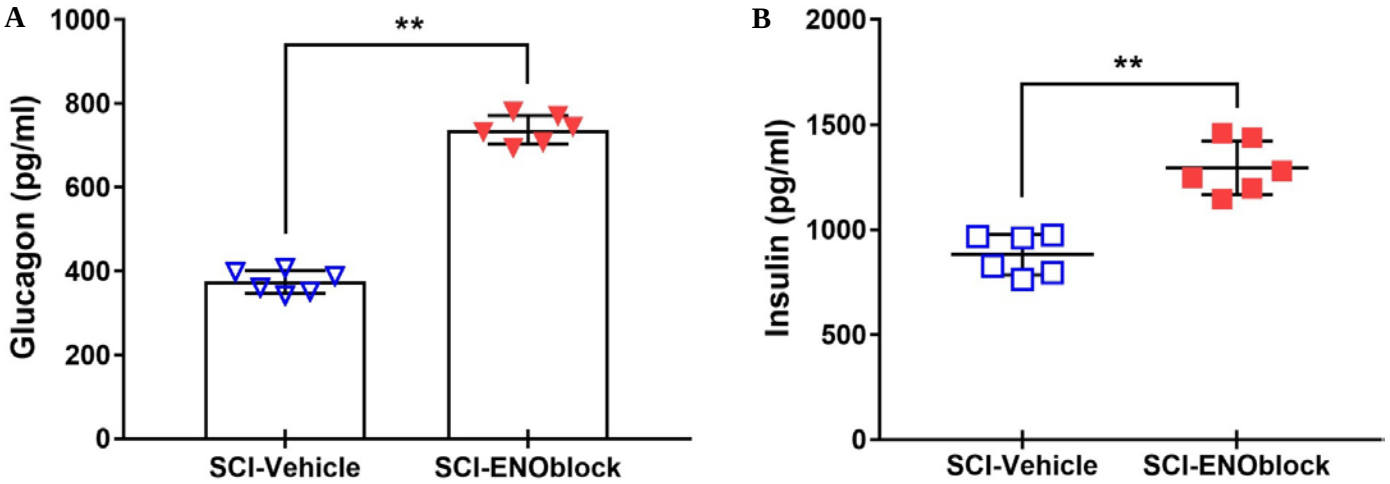


Figure 7. Effect of ENOblock Treatment on Glucagon and Insulin Expression Following SCI in Rats. SCI rats were treated with vehicle alone or ENOblock (100 µg/kg, four times, intravenously), and then blood samples were obtained 7d post-injury. Sham operated rats (T10 laminectomy) were used as controls. Glucagon and insulin levels were analyzed by using Eve Tech's Mouse, Rat Metabolic Array (MRDMET) Discovery Assay. (A) Results obtained from plasma samples indicate that ENOblock treatment increased glucagon levels significantly as compared with vehicle treated SCI rats. Median concentrations in SCI-vehicle and SCI-ENOblock treated groups were 374.3 and 737.3 pg/ml respectively; the distributions in the two groups differed significantly (Mann–Whitney U = 0, $n_1 = n_2 = 6$, $^{**}p < 0.01$ two-tailed). (B) ENOblock treatment increased insulin levels significantly as compared with vehicle treated SCI rats. Median concentrations in SCI-vehicle and SCI-ENOblock treated groups were 893.2 and 1264 pg/ml respectively; the distributions in the two groups differed significantly (Mann–Whitney U = 0, $n_1 = n_2 = 6$, $^{**}p < 0.01$ two-tailed).

association with ENOblock treatment (as amylin and insulin are typically co-secreted) and indicates the anti-inflammatory effects of enolase inhibition in SCI.

We have previously reported that gliosis is attenuated by enolase inhibition in acute SCI (**Figure 4**). We have recently found that microglial and astroglial markers are also reduced in sub-acute SCI (data not shown). Iba1 staining of SCI tissue from sham-operated, vehicle-treated, and ENO-treated rats harvested 7d and 14d post-injury indicated a significant (p=0.02) increase in microglial activation following injury that is attenuated with ENOblock treatment (data not shown). Glial fibrillary acidic protein (GFAP) staining also indicated a significant (p=0.014) increase in astrocyte expression following sub-acute SCI (data not shown). These findings align with our earlier results from an acute model of SCI and show that ENOblock treatment reduces gliosis in a sub-acute and chronic SCI in rats.

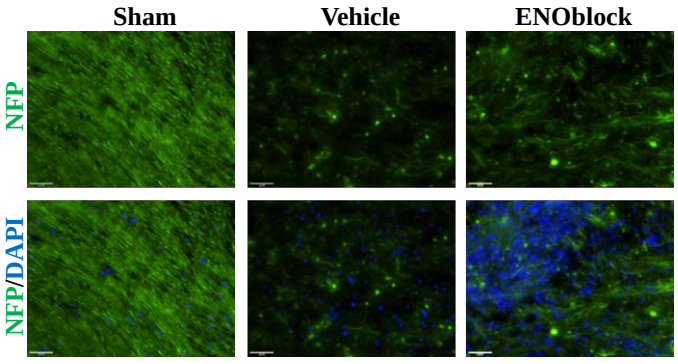


Figure 8. ENOblock treatment induces neuroprotection in sub-acute to chronic (day 14) SCI in rats. Neurofilament protein (NFP, green) and DAPI (blue) nuclear staining of SCI tissue at the injury site. Panel shows representative images taken at 20X magnification.

Neurofilament protein (NFP) staining of SCI tissue from vehicle-treated rats harvested 14d post-injury indicated a significant (p=0.012) decrease in neuronal cytoskeleton following injury (**Figure 8**). This decrease is attenuated with ENOblock treatment. There is also a trend in the recovery of neuronal cytoskeleton with ENOblock tretment rats as

compared to vehicle treated controls, indicating that enolase inhibition may favor neuroprotection in chronic SCI (**Figure 8**). Thus, during the last 24 months, we have examined the role of enolase in acute, sub-acute and chronic SCI to better understand the effects of this enzyme on inflammation, metabolic hormones, glial cell activation, and neuroprotection in SCI rat model, which have important implications for clinical consideration.

The Value to the State of South Carolina

There are many SCI individuals in our state of South Carolina. Current therapeutic strategies often fail to address cellular damages and molecular changes occur in SCI. Thus, development of new therapeutic approaches should help better manage SCI and is highly relevant to the lives of our South Carolina citizens. During the past 24 months, we investigated the potential role of a novel enolase inhibitor ENOblock in a rat model of SCI. Our data suggests that ENOblock treatment offers multisystemic beneficial effects in acute, sub-acute, and chronic SCI. While the effects of ENOblock on locomotor function in chronic SCI are in progress, our current data in preclinical SCI model suggest that ENOblock therapy may significantly impact SCI individuals in our state of South Carolina.

Publications:

Manuscripts published (related):

1. **Haque A**, Capone M, Matzelle D, Cox A, **Banik NL** (2017). Targeting enolase in reducing secondary damage in acute spinal cord injury in rats. Neurochem Res. 42(10): 2777-2787.
2. **Haque A**, Ray SK, Cox A, Banik NL (2016) Challenging pathophysiology in spinal cord injury and promising stem cell therapy. *Proceedings of Neurosciences* (in press).
3. Polcyn R, Capone M, Hossain A, Matzelle D, Banik NL, **Haque** A (2017) Neuron specific enolase is a potential target for regulating neuronal cell survival and death: implications in neurodegeneration and regeneration. Neuroimmunol Neuroinflamm. 4:254-257. PMID: 29423430. PMCID: PMC5800407.
4. **Haque A,** Polcyn R, Matzelle D, Banik NL (2018) New insights into the role of neuron-specific enolase in neuro-inflammation, neurodegeneration, and neuroprotection. *Brain Sci.* 2018 Feb 18;8(2). pii: E33. doi: 10.3390/brainsci8020033. PMID: 29463007
5. Polcyn R, God J, Capone M, Matzelle D, Banik NL and **Haque A** (2018) A missing link between neuron specific enolase release and poor prognosis in aging patients with B-cell lymphoma. J Clin Cell Immunol 2018, 9:3.

Manuscripts published (unrelated):

6. Bryant JM, Bouchard M, **Haque** A (2017) Anticancer Activity of Ganoderic Acid DM: Current Status and Future Perspective. J Clin Cell Immunol. 8(6): 535. PMID: 29399381. PMCID: PMC5795599.
7. Hathaway-Schrader JD, Doonan BP, Hossain A, Radwan FFY, Zhang L, **Haque A** (2018) Autophagy-dependent crosstalk between GILT and PAX-3 influences radiation sensitivity of human melanoma cells. *J Cell Biochem*. 119(2):2212-2221. PMID: 28857256. (PMC in process).
8. Trager NNM, Butler JT, Harmon J, Mount J, Podbielska M, **Haque A,** Banik NL, Beeson CC (2018) A Novel Aza-MBP altered peptide ligand for the treatment of experimental autoimmune encephalomyelitis. Mol Neurobiol. Jan;55(1):267-275. PMID: 28889362.
9. Tanu T, Anjum A, Jahan M, Nikkon F, Hoque M, Roy AK, **Haque** A, Himeno S, Hossain K, Saud

ZA (2018) Antimony-induced neurobehavioral and biochemical perturbations in mice. Biol Trace Elem Res. 2018 Mar 8. doi: 10.1007/s12011-018-1290-5. [Epub ahead of print]. PMID: 29520725.

10. Sambandam Y, Sakamuri S, **Haque A** (2016) RANK Ligand induction of autophagy in oral squamous cell carcinoma tumor cells. *J Cell Biochem.* 117(1):118-25. PMC4675691.

Abstracts Published (related and/or unrelated):

1. Nicole Trager, Jonathan Butler, Jennifer Harmon, Joshua Mount, Maria Podbielska, Denise Matzelle, **Azizul Haque**, Craig Beeson, Naren Banik. A novel altered peptide ligand ameliorates experimental autoimmune encephalomyelitis. American Society for Neurochemistry Annual Meeting (March 24-28) 2018, Riverside, CA

2. **Azizul Haque**, Denise Matzelle, Mollie Capone, Azim Hossain, Rachel Polcyn, Naren Banik. Regulation of neuron specific enolase influences neurotrophic factors and improves neurological function after spinal cord injury. American Society for Neurochemistry Annual Meeting (March 24-28) 2018, Riverside, CA

3. Naren Banik, Mollie Capone, Maria Podbielska, Denise Matzelle, Azim Hossain, Rachel Polcyn, and **Azizul Haque**. Calpain inhibition regulates inflammatory mediators in PBMCs from multiple sclerosis patients. American Society for Neurochemistry Annual Meeting (March 24-28) 2018, Riverside, CA

4. **Azizul Haque**, April Cox, Denise Matzelle, Mollie Capone, Rachel Polcyn, Azim Hossain and Naren Banik. Delivery of estrogen via nanoparticle attenuates gliosis and protects neurons in spinal cord injury in rats. American Society for Neurochemistry Annual Meeting (March 24-28) 2018, Riverside, CA

Manuscripts (submitted):

1. Polcyn R, Capone M, Matzelle D, Kau E, Chandran R, **Haque A**, and Banik NL (2018). Case Report: cytokine/chemokine dysregulation in progressive MS is apparent and susceptible to calpain inhibition.

2. Chandran R, Capone M, Matzelle D, Polcyn R, Kau E, **Haque A**, and Banik NL (2018). Distinct cytokine and chemokine expression in plasma and calpeptin-treated PBMCs of a relapsing-remitting multiple sclerosis patient: a case report.

3. Chandran R, Das A, Samantaray S, Matzelle D, Narang A, Capone M, Wallace G, Taheri S, Reiter R, Varma A, Ray SK, **Haque A,** Banik NL (2018). Premarin reduces neurodegeneration and promotes improvement of function in an animal model of spinal cord injury.

Pending applications:

2 I01 BX001262-05A2 **Banik (PI)** 10/01/2018-09/30/2022
VA BLR&D (Parent I01) Merit Award $250.000.00/yr
Hormonal Intervention Protects Axon-myelin to Promote Functional Recovery in SCI
The goal of this proposal is to determine the role of a novel estrogen-loaded nanoparticle gel patch in protecting neuronal loss in SCI
Role: Co-investigator

1 I01 BX004269-01A1 **Banik/Haque (MPI)** 11/01/2018-10/31/2022
BLR&D (Parent I01) Merit Award $250,000.00/yr

Attenuation of Inflammatory Response in Progressive Neurodegeneration in Parkinson's Disease
The goal of this project is to investigate the role of calpain in the pathogenesis of Parkinson's Disease (PD) in a mouse model of PD
Role: Principal Investigator (MPI)

PVA Research Foundation **Haque (PI)** 01/01/2019-12/31/2020
PVA SCI Award $150,000.00
Inhibition of Enolase Promotes Functional Recovery after Spinal Cord Injury
The goal of this proposal is to investigate the mechanisms of NSE-mediated inflammatory events in SCI.
Role: Principal Investigator

SCIRF **Haque (PI)** 01/01/2019-12/31/2020
Investigator Award $150,000.00
Nanoparticle Delivery of ENOblock and Recovery of Function in SCI
The goal of this project is to determine the efficacy of a novel enolase inhibitor in spinal cord injury.
Role: Principal Investigator