

Project Title: Improving neuroregeneration after exposures to multiple mild blasts

Principal Investigator: Denes V. Agoston, M.D., Ph.D.

ABSTRACT

Repeated exposures to low levels of explosive blast are a significant health issue in the US military because they can lead to chronic neurobehavioral abnormalities, including mood and anxiety disorders and memory impairments. We have developed a rodent model to mimic repeated exposures to mild blast and analyzed the behavioral, cellular and molecular outcomes. We are also testing the effect of a “rest” period between sets of blast exposures on these outcomes. Here we report that exposures of rats to multiple mild level of blast overpressure results in: a) changes vital parameters; b) changes in the expression of protein markers in the hippocampus and c) decreased de novo hippocampal neurogenesis. Altered vital parameters in response to blasts include arterial O₂ saturation and heart rate whereas pulse distension and breath rate were minimally or not affected. Proteomics analysis of brain regions that are parts of the anxiety and memory circuitries (prefrontal cortex, amygdala, dorsal and ventral hippocampus) showed elevated expression of the selected markers even after exposure to a single blast. However, there are candidate markers that appear to respond specifically to multiple blasts. While exposure to a single blast increased DCX immunoreactivity in the hippocampus, exposures to multiple blasts resulted in decreased DCX immunoreactivity suggesting that de novo hippocampal neurogenesis is down-regulated by multiple exposures. In summary, the observed changes in vital parameters, protein expressions and de novo hippocampal neurogenesis can contribute to the observed neurobehavioral abnormalities, predominantly increased anxiety and memory impairments observed after exposures to multiple mild blasts.

Project Title: Neural Stem-Progenitor Cell Repair Potential at Distinct Sites of TBI Pathology

Principal Investigator: Regina C. Armstrong, Ph.D.

ABSTRACT

The repair capacity of neural stem/progenitor (NS-P) cells, i.e. endogenous cycling cells, in the adult brain has been revealed by experimental manipulations to increase permissiveness of the tissue environment and by transplantation of neural stem cells that modulate the immune response and stimulate endogenous NS-P cell regenerative responses. Distinct populations of NS-P cell populations persist in the adult brain, including those in germinal zones, such as the subventricular zone (SVZ) of the forebrain and subgranular zone (SGZ) of the hippocampus, but NS-P cells are also found at low frequencies in the white matter and cerebral cortex. Reactive proliferation of neural stem cells (NSC) in the SVZ and SGZ has been reported following TBI but the extent to which these cells can or could, with experimental modifications, contribute to functional repair is not clear. Although cerebral cortex involvement in TBI has long been appreciated, the response of local endogenous cells has not been sufficiently characterized. In addition, in mild TBI white matter tracts are emerging as areas of pathology with high correlation to functional deficits. Interestingly, NS-P cells that populate white matter may share a common precursor with interneurons, which may implicate a shared role in certain neuropsychiatric diseases. Again, the response to TBI and repair potential of NSP cells in white matter is not yet clear. Importantly, mild TBI can result in extensive white matter involvement with axonal injury that does not progress to discontinuity so that denuded axons may be highly vulnerable and potentially protected by remyelination initiated from white matter progenitors. We hypothesize that endogenous NS-P cells at distinct CNS sites may respond differently to mild TBI pathology and possess different repair potential, which can be enhanced by modifications of the local tissue environment. We will use a combination of molecular approaches to monitor in vivo cellular responses in mouse models of TBI.

Project Title: Using the Mouse Olfactory System as a Model for Studying TBI

Principal Investigator: Leonardo Belluscio, Ph.D.

ABSTRACT

Olfactory dysfunction is an early indicator of TBI . The well-defined neural maps within the olfactory system and broad regenerative capacity allow for the investigation of neurodegeneration, recovery, and possible intervention. Here we introduce an olfactory-based model that reproduces many hallmarks associated with human brain trauma. Following a unilateral penetrating impact to the anterior dorsal cortex we examined the piriform cortex and found a selective bilateral disruption. We demonstrate a clear bilateral loss of synaptic contacts in layer 1 but only an ipsilateral glial response. We also assessed a second injury site on the olfactory bulb (OB) and observed a similar ipsilateral glial response. Although, in contrast to the cortical impact, we detect a gradation in OB cell loss with ipsilateral showing a greater reduction than contralateral and both significantly less than control. Moreover, cells in the olfactory epithelium (OE), including olfactory sensory neurons (OSNs) that send axonal projections to the OB and olfactory ensheathing glia, also exhibited a graded expression of injury markers including; activated caspase-3, β -APP and p75NTR with ipsilateral showing higher levels than contralateral and both significantly higher than controls. Importantly, at a behavioral level we also found that both injuries result in a clear loss of olfactory function. Together these data establish the mouse olfactory system as a new model to study TBI serving as a platform to understand neural disruption and the potential for circuit restoration.

Project Title: Characterizing mitochondrial Ca²⁺ homeostasis and the effects of oxidative damage in normal and injured *Drosophila* brains

Principal Investigator: Rachel Cox, Ph.D.

ABSTRACT

TBI is the result of a blow to the head, leading to neuronal death, short- and long-term brain impairment, and even the death of the patient. In Western countries, such as the United States, millions of cases of TBI happen every year. Troops fighting in Operation Iraqi Freedom and Operation Enduring Freedom in Afghanistan have been particularly prone to TBI. These TBIs can occur from either blast waves or direct blows from objects, especially given our advances in protective body armor, which leave the head more exposed. Although TBI is devastating for the individual, there is a lack of effective therapies, either neuroprotective or neurorestorative.

After the initial diffuse or direct blow to the head, there is a cascade of secondary events that result in increased neuronal cell death. These secondary events, which can take place on the order of minutes to days, are potential therapeutic targets. The immediate secondary events are often due to membrane shearing caused by the impact, including disruption of neuronal membranes. Once this happens, neural cells become leaky, altering their cellular homeostasis. One very important downstream event is mitochondrial dysfunction, which primarily results from disrupted calcium regulation and generation of excess reactive oxygen species (ROS). These types of mitochondrial misregulation can lead to cell death.

Although there is ample evidence of mitochondrial dysfunction post-TBI, there are few therapies that ameliorate the effects of TBI on mitochondria. It is imperative we identify additional drugs and treatments. Research using TBI rodent models have examined the global effects of injury on mitochondrial function, using biochemistry to look at changes in mitochondrial function by typically grinding up various regions of the central nervous system (for examples see). While these studies have underscored the immediate role mitochondrial dysfunction plays after TBI, there is a gap in our knowledge at the cellular and sub-cellular level concerning how mitochondria in the effected tissue react to injury. Mitochondria are a heterogeneous population with respect to their biochemistry, and are also very dynamic, changing shape, numbers and location frequently. A better understanding of how mitochondria react to TBI at the organelle and sub-cellular level will allow us not only to better understand the immediate changes in mitochondria, but also allow us to potentially develop better strategies for coping with the enormous loss of mitochondrial function post-TBI.

The purpose of this proposal is to develop a new model for TBI using the *Drosophila melanogaster* larval brain. Using a less complex model system allows us to better image mitochondria, and has a superior genetic advantage to study the genes and molecular mechanisms that control mitochondrial function in the brain. In addition, much is known about the cell types in the larval brain, as well as the neural stem cells present. We are using live-imaging to detect changes in mitochondrial calcium levels and ROS production pre- and post-TBI in order to better understand this process. Our goal is to create a robust *Drosophila* system to study mitochondrial changes in response to TBI. The advantage this model will have over existing rodent TBI models is we can visualize mitochondria at single organelle resolution and use live-imaging to identify the sequence of changes taking place after brain injury. This information will direct future development of therapies targeting mitochondrial function.

Project Title: HDAC inhibitor effects on adult neurogenesis post-TBI

Principal Investigator: Martin L. Doughty, Ph.D.

ABSTRACT

We are interested in the effects of TBI on neural stem cell (NSC) behavior in the adult brain. TBI stimulates increased proliferation of progenitor cells in niches known to harbor NSCs in the adult. Whether these cells replace neurons lost to TBI or are capable of restoring lost function has not been determined. Indeed it remains unclear if TBI-induced stem cell activation has beneficial or adverse effects on recovery. Disconcertingly, epilepsy is a common comorbidity of TBI and is postulated to result from aberrant TBI-induced neurogenesis, highlighting possible adverse side-effects of neurogenesis. Further complicating the issue is our poor understanding of the mechanisms that regulate normal and pathogenic NSC behavior.

The purpose of this project is to expand our basic knowledge of the effects of TBI on NSCs through the careful characterization of the cellular and molecular effects of controlled cortical impact (CCI) on NSCs in the subventricular zone (SVZ) of adult mice. We will focus on the role of epigenetic mechanisms in this process by examining the effects of histone deacetylase (HDAC) inhibition and histone demethylase (HDM) knockdown on adult NSC biology and neuroregenerative responses. Post-translational modifications of histones by HDACs and/or HDMs can lead to transcriptional activation/suppression.

The activities of histone modifying enzymes are functionally linked in the adult brain and TBI induces changes in histone acetylation and histone methylation in adult rats. Pre-clinical treatment models demonstrate HDAC inhibitors (HDACi) exert neuroprotective effects and stimulate neurogenesis in TBI and ischemia, restore learning and memory in TBI and neurodegenerative mice, enhance neuronal differentiation and synaptic plasticity and exert antidepressant-like effects. However these same HDACi have also been reported to both prevent or induce neuronal apoptosis in culture, a contradiction that is likely the result of differences in neuronal cell type, the culture conditions employed and the type of HDACi molecule tested.

The aims of this project are: (1) to characterize the effects of controlled cortical impact on the proliferation, cell fate and gene expression of NSCs in the SVZ of adult mice; and (2) to determine the effects small molecule HDAC inhibitors or shRNA-mediated HDM knockdown on adult NSC biology; and 3) to identify HDAC/HDM-mediated responses that have therapeutic potential in the treatment of TBI. By focusing on the regulatory role of histone acetylation/methylation in NSC biology, we aim to demonstrate that epigenetic therapies can be used to modulate NSC proliferation and neurogenesis to improve outcomes in the treatment of TBI.

Project Title: Genetic identification of neural stem cell self-renewal regulators

Principal Investigator: Yang (Dennis) Du, Ph.D.

ABSTRACT

Cell replacement therapies employing neural stem cells (NSCs) represent promising strategies for the treatment of TBI. The success of such therapies requires stimulating both the self-renewal of NSCs and the subsequent differentiation of their progenies into new neurons for the repair of neuronal damages in TBI. Although it has been established that the self-renewal of NSCs can be stimulated in vitro and in vivo by the treatment of epithelial growth factor (EGF) and/or fibroblast growth factor-2 (FGF-2), NSCs expanded under such conditions preferentially differentiate into glial cells rather than neurons, suggesting that besides stimulating NSC self-renewal these cytokines may also promote commitment of NSCs to the glial lineages. Therefore, improved conditions to stimulate NSC self-renewal without predisposing NSCs to glial differentiation are likely required for successful cell replacement therapies for TBI. However, finding such conditions has been significantly hampered by the poor understanding of the molecular mechanisms that regulate NSC self-renewal. The objective of this study is to achieve a better understanding of the NSC self-renewal process by identifying its critical regulators through retroviral insertional mutagenesis screens. Specifically, we plan to identify genes/pathways capable of supporting the self-renewal of NSCs in the absence of EGF and FGF-2. Characterization of the genes/pathways identified in our screens will likely lead to better strategies for stimulating NSC self-renewal for the treatment of TBI.

Project Title: Generation of iPS cells without vector

Principal Investigator: Ying-Hong Feng, Ph.D.

ABSTRACT

Stem cell transplantation represents a promising potential alternative for the treatment of TBI . This treatment is largely limited by the lack of resources for sufficient amounts of stem cells. Recent breakthrough in generating induced pluripotent stem (iPS) cells through reprogramming of terminally differentiated somatic cells by overexpressing four transcription factors (Oct3/4, Sox2, Klf4, and c-Myc or Oct3/4, Sox2, Nanog and Lin28 has provided an ideal solution. However, generation of iPS cells with the current approaches requires gene transfer, a potential genetic risk, not to mention the extremely low efficiency (0.003-1%), long period of generation time and partial reprogramming. These problems need to be resolved first before the iPS cells can be used in patients. Thus, the proposed project was set to develop a recipe/protocol that generates iPS cells with >10% efficiency, but without use of any genetic material. The 4 specific aims were:

1. Produce 5 reprogramming factor proteins (c-Myc not included) that are biologically active and cell-permeable: Recombinant reprogramming factor proteins are not genetic material and do not have any genetic risk. This aim includes various designs and production of the recombinant proteins.
2. Determine the stability, cell-penetrating efficiency, cell capacity, cytotoxicity, and activity of the 5 factors: This aim include mostly routine experiments that characterize the recombinant proteins. Based on the property of these proteins, refinement will be made to either improve the proteins or modify the recipe/protocol for iPS cell generation.
3. Develop the most efficient (>10%) recipe and protocol for generation of iPS cells: This aim is to use primary keratinocytes, or skin fibroblasts BJ cells and IMR-90 cells or prostate cancer PC3 cells to test the the potency/efficiency of the recombinant proteins for generation of iPS cells. After optimization, a recipe/protocol that produces >10% iPS cells can be developed.
4. Produce iPS cells from skin fibroblasts isolated from GFP-labeled transgenic mice. This aim is to use skin fibroblasts isolated from GFP-labeled transgenic mouse to generate iPS cells using the above developed recipe/protocol.

Project Title: Tracking labeled stem cells in TBI model by cellular MRI

Principal Investigator: Joseph A. Frank, M.S., M.D.

ABSTRACT

At the present time, there is no stem cell treatment paradigm for TBI in patients following bomb blasts or accidents. The pathology of TBI in experimental models includes acute inflammatory reaction, blood brain barrier disruption, hemorrhage, demyelination, axonal transection and chronically with axonal neuronal loss and gliosis. Stem cell (SC) therapy is a potential treatment either as replacement therapy or via paracrine effect with release of growth factors and anti-inflammatory cytokines for TBI injury. Experimental studies in rodent models of TBI have been limited and usually a single dose of cells is administered within 24 to 72 hours after experimental injury. The optimal timing and dose of cell delivery to maximize functional recovery and transplantation survival during the acute inflammatory and edematous phase of damage is unknown. Moreover, there are no reports of multiple dosing schedules of SCs alone or in combination with adjuvant neuroprotective drugs as a possible treatment option for TBI. MRI methods have been developed for evaluating brain damage and also for tracking the migration and homing of magnetically labeled stem cells to target tissues. By using superparamagnetic iron oxide nanoparticles (SPION) magnetic labeling of stem cells can be obtained that can potentially translated to the clinic.

This study has 3 specific aims: Aim 1) Can MRI be used to determine the optimal cell dose and dosing schedule of Bone marrow stromal cells (BMSC) that results in improvement of neurological function and minimizes pathology? Aim 2) Does the combination of neuroprotective agent(s) and stem cell infusions alter the clinical course or pathology in CCI-TBI model and can these results be correlated with MRI metrics and neurological function? Aim 3) To investigate the microenvironment of the CCI-TBI model utilizing micro-dialysis to determine the effect growth factors on BMSC homing and neurogenesis.

Project Title: Does TBI alter extrinsic and intrinsic connectivity in sensory cortex?

Principal Investigator: Sharon L. Juliano, Ph.D.

ABSTRACT

Our overall hypothesis states that a simple CNS circuit can be repaired after TBI using neural progenitor cells. We use the barrel cortex as our primary model of a cortical circuit. The barrel cortex is a region in the rodent CNS that contains a distinct representation of the whiskers. Each whisker is represented by a discrete anatomical structure in the neocortex called a barrel. The cellular structure of a barrel differs slightly from species to species, but basically consists of a ring of cells in layer 4 in the neocortex that connects structurally and functionally to the layers above and below. Each layer within a barrel column has distinct cell types and functions. The task of this proposal will be to reconstruct as closely as possible the distribution of cell types in a brain injured slice in the barrel cortex. Because this region contains a confined iterative architecture it should be possible to reconstruct a circuit responsive to stimulation. Similar possibilities also relate to the olfactory bulb, which contains a simple circuit composed of several well characterized cell types: principal cells receiving afferents from olfactory sensory neurons and interneurons. In addition, cells populating the olfactory bulb are generated throughout life and therefore provide a potential supply of newly differentiated progenitor cells to use for circuit reconstruction after TBI.

Project Title: Modulation of NF- κ B-dependent immune responses for post-TBI neuroregeneration

Principal Investigator: Brian C Schaefer, Ph.D.

ABSTRACT

The injury-stimulated inflammatory response has evolved to promote tissue repair and functional recovery. However, it is clear that not every inflammatory response is beneficial. In the context of the CNS, certain repair responses stimulated by the inflammatory response, such as glial scarring, lead to replacement of CNS tissue with astrocytes and connective tissue, actively inhibiting CNS repair processes. In contrast, increasing evidence indicates that immune cells in the CNS, particularly T cells, may promote the properly targeted migration and engraftment of endogenous neural stem cells, thereby promoting regenerative responses. Our project is designed to better define cellular and molecular mechanisms of the inflammatory response to TBI. We hypothesize that NF- κ B-dependent immune responses are a major determinant of functional recovery, post-TBI. Our goal is to define the contribution of specific NF- κ B signaling pathways and NF- κ B-dependent immune mechanisms to TBI outcome. Specifically, we are performing experiments to characterize the cellular and molecular components of the post-TBI immune response, to determine how TBI outcomes are influenced by MyD88- and NF- κ B-dependent inflammatory responses, and to elucidate the role of NF- κ B dependent T cell responses in determining TBI outcomes. We expect that the data generated in this project will be crucial in assembling a mechanistic understanding of TBI-induced and immune response-mediated loss of CNS function, and for efforts to develop effective treatments and therapies for TBI.

Project Title: Altering the ECM after TBI to enhance regeneration and plasticity

Principal Investigator: Aviva Symes, Ph.D.

ABSTRACT

TBI often leads to permanent disability because injured axons fail to regenerate. This regenerative failure is due, in part, to the inhibitory environment of the glial scar that forms after injury and acts as a molecular and physical barrier to axon regeneration. The sequence of events following TBI that leads to the formation of the glial scar is not understood. Neither are the differences in the glial response to different types of TBI. Scar formation is strongest after penetrating brain injury, but the glial response to other types of TBI is poorly described. As the glial response to injury results in prolonged changes in the environment in which attempts to promote regeneration will occur, it is vital that we understand the potentially harmful alterations in glial function caused by injury and develop ways to counteract them.

Glial cells, specifically astrocytes and oligodendrocyte progenitor cells (OPCs), deposit an injury-induced extracellular matrix (ECM) that contributes to the post-lesion environment preventing neuronal reconnection and plasticity. It is thought that this limited regeneration and plasticity is due to the properties of the central nervous system ECM, especially the role of chondroitin sulfate proteoglycans (CSPGs). Much of this inhibitory biological activity is due to the sulfated glycosaminoglycan (GAG) sugar side chains on CSPGs. Interventions that degrade the GAG chains of the CSPGs with the bacterial enzyme, chondroitinase ABC (cABC), have led to increased functional recovery in rodent models of brain and spinal cord injury. Understanding the mechanisms through which glial cells respond to injury to upregulate synthesis and modification of the CSPGs, may provide a mechanism to intervene to reduce the inhibitory environment post-lesion. Our hypothesis therefore is that the glial response to TBI creates a hostile environment for differentiation and regeneration and thus reduced plasticity. Alteration of the post-lesion ECM, and specifically the level or composition of the CSPGs will promote a more supportive regenerative environment and enhanced plasticity. This project therefore seeks to understand the pathology surrounding the glial response to different types of TBI. We will then, through manipulation of production or levels of CSPGs, seek to determine whether these alterations lead to changes in plasticity or regeneration after TBI. Specifically we will

1) Characterize the glial response and ECM deposition after controlled cortical impact (CCI) in mice. We will compare the activation and proliferation of different glial cells together with changes in the expression pattern of CSPGs produced after CCI.

2) Manipulate the glial response and composition of the ECM after CCI through the use of a mutant mouse with an astrocytic-specific expression of a dominant negative TGF- β receptor. We will determine the effect of these manipulations on the cell fate of endogenous neural stem cells in the subventricular zone of the lateral ventricle through retroviral labeling.

3) Evaluate changes in axonal structure and cortical barrel-field plasticity following siRNA or pharmacological manipulations that affect the composition of the ECM after TBI.

These experiments will elucidate many aspects of the glial response to different types of TBI, determine whether manipulation of the glial-produced ECM may enhance plasticity and regeneration and lay the groundwork for further experiments seeking to manipulate glial responses to achieve greater regeneration after injury.

Project Title: Macrophage subsets in TBI: determination of the roles of M1 and M2 macrophages in inflammation or regeneration in controlled cortical impact model

Principal Investigator: Joseph A. Frank, M.S., M.D.

ABSTRACT

TBI is responsible for 50% of trauma deaths in the U.S. and accounts for a substantial burden of disability in survivors, particularly among the survivors of blast injury. To date, few effective treatments for acute TBI or its long-term sequelae have been identified. The body's inflammatory response to acute injury can be both destructive and/or reparative with macrophages playing a key role. Similar to the two phenotypes (Th1, Th2) of T-cells in autoimmune/inflammatory diseases, two subsets of peripheral macrophages have also been identified in gliomas, breast and lung cancer and mouse contusion spinal cord injury (SCI) model. The M1 phenotype is associated with inflammation generating cytokines (i.e., interleukin (IL), tissue necrosis factor, Interferon γ) and tissue destruction, while the M2 phenotype can facilitate repair through phagocytosing debris, secreting growth factors, and supporting neuronal survival, angiogenesis, axonal regeneration and remyelination. Kigerl has shown that in acute SCI, the ratio of M1:M2 phenotypes are high but changes with over 28 days. Whether a similar schism exists in macrophage phenotypes and function in the response to acute TBI is presently unknown.

This study has two specific aims: Aim 1) What is the ratio of M1:M2 phenotypes and are there temporal changes in the ratio of M1:M2 in the rat CCI model? Aim 2) Will superparamagnetic iron oxide (SPIO labeled) M2 macrophages home and alter M1:M2 ratio in TBI lesions? Will M2 macrophages modify lesion size and improve behavioral scores in the controlled cortical impact (CCI) rat model?

Project Title: Assessment of neurotrophin involvement and anti-inflammatory therapy in TBI

Principal Investigator: Brian C Schaefer, Ph.D.

ABSTRACT

The production of neurotrophins in response to injury is complex, with cells of both the CNS and immune system capable of producing these molecules. An additional level of complexity is that neurotrophins have been shown to have both neuroprotective activities and neurodegenerative effects. Data suggest protection is generally mediated by the mature molecules, and neurodegeneration (via apoptosis) is mediated by the pro-forms; however, the in vivo relevance of pro-forms has also recently been questioned. Importantly, recent studies have reported that administration of neurotrophins has a neuroprotective effect in vivo. Thus, existing data suggest that neurotrophins play a pivotal role in regulation of inflammatory processes and in determining outcomes of TBI. We hypothesize that perturbation of NF- κ B signaling pathways in bone-marrow derived cells and/or non-hematopoietic cells in the brain will alter neurotrophin production, thereby influencing TBI outcomes. The first goal of this project is to define the kinetics of the neurotrophin response following CCI injury, and to assess the contribution of specific NF- κ B signaling pathways to the in vivo neurotrophin response.

NF- κ B is a major mediator of inflammatory reactions. Acute inflammation in the brain, particularly for moderate to severe injuries, is almost certainly a major contributor to morbidity and mortality. Inflammation-induced brain swelling and accompanying changes in blood flow are among the many problems associated with an acute inflammatory response to serious brain injury. Because these acute inflammatory responses are initiated within minutes of TBI, we hypothesize that rapid intervention is desirable to ameliorate consequent inflammation associated damage. A drug that could be administered on the battlefield to troops who are suspected to have sustained a TBI injury is most desirable. The second goal of this project is to explore the potential benefit of two FDA-approved anti-inflammatory drugs for i) limiting the inflammatory response to TBI and ii) improving functional outcomes.

Project Title: Angiotensin receptor blockers as potential therapeutics for TBI

Principal Investigator: Aviva Symes, Ph.D.

ABSTRACT

TBI results in complex pathological reactions – damage caused by the initial lesion followed by a secondary cascade involving inflammation, edema and a strong glial response. The secondary consequences of the initial injury lead to significantly larger lesion volume, worsening of neurological competence and result in an environment around the lesion that is more inhibitory to regeneration. Angiotensin II (AngII) is produced in the brain and is known to be vasoconstrictive and pro-inflammatory. The two major classes of receptors for AngII, AT1 and AT2 receptors are expressed in the CNS and often have opposing actions, AT1 receptors mediating most physiological effects of Ang II. AT1Rs are expressed on neurons and endothelial cells in the CNS, in numerous brain regions including the cortex. In addition to powerful vasoconstriction of the cerebral microvasculature, AT1R signaling also elicits strong pro-inflammatory actions in the brain, through production of reactive oxygen species, inflammatory cytokines and adhesion molecules. Ang II receptor blockers (ARBs) are specific antagonists of the AT1 receptor and are widely used FDA – approved drugs with limited side effects. ARBs, are potent anti-inflammatory agents in the CNS. In rodent models of stroke, treatment with ARBs lessened the lesion penumbra, decreased the number of apoptotic cells and improved neurological outcome. ARBs also have been shown to ameliorate the cognitive impairment seen after whole brain irradiation in rats. Thus, ARBs in addition to their anti-inflammatory actions appear also to be neuroprotective. We therefore hypothesize that ARBs will have efficacy in treating TBI in mice, through decreasing inflammatory sequelae, reducing the lesion penumbra, and limiting secondary neuronal cell death. The objective of this pilot proposal is therefore to examine the effects of the ARB, candesartan, on recovery from controlled cortical impact (CCI) to the cerebral cortex in mice. We will first characterize the expression of components of the Angiotensin II pathway in the CNS at specific time points after CCI injury to mice. Little is known of the response of the Angiotensin II system to TBI. However, AT1R expression is upregulated by inflammation and stress. We will therefore examine expression of the AT1 and AT2 receptors, indicative of activation of the Angiotensin II system in the sham and injured brain at 6 hours, 1, 3, 7 and 28 days after moderate CCI injury by in situ hybridization, autoradiography and immunohistochemistry. Secondly we will determine whether treatment of mice with the ARB, candesartan, after CCI injury, lessens the morphological and functional outcomes of TBI. We will implant a minipump containing candesartan or vehicle 5 hours before CCI of moderate impact. Mice will be sacrificed at 3 and 28 dpi after first assessing motor and cognitive function. Brains will then be examined for lesion volume, cell death, inflammatory markers, glial reactivity and scar formation at these different time points to assess the morphological result of treatment with candesartan. Given the strong evidence in the literature that the ARBs are beneficial to different models of neuronal injury, we anticipate that this project will show that ARBs are efficacious in treating TBI in mice. Immediate treatment with ARBs may reduce inflammation, promote neuronal survival and lessen glial scarring to allow for more successful regenerative approaches.

Project Title: Mesenchymal Stem Cell-Based TBI Treatment and Therapy Effect Monitoring

Principal Investigator: Xiaoyuan Chen, Ph.D.

ABSTRACT

Mesenchymal stem cells (MSCs) can promote the recovery of brain damage caused by TBI. However, when transplanted via intravenous route, only a very small percentage of MSCs reach the cerebral parenchyma and remain there. SDF-1 is a chemical messenger released by infected or damaged cells such as injured brain to form a concentration gradient, which can make MSCs move along the gradient towards the higher concentration of SDF-1 where damage happens. To fully utilize the beneficial effect of MSCs on brain recovery, we plan to improve their homing efficiency by enforcing MSCs to express SDF-1 receptor (CXCR4) on the cell surface and testing different injection routes. To track the distribution of MSCs in vivo, we will introduce a positron emission tomography (PET) reporter gene (Herpes Simplex virus thymidine kinase, TK) into the MSCs. TK can phosphorylate radiolabeled 9-(4-[18F] fluoro-3-hydroxymethylbutyl) guanine (18F-FHBG); and phosphorylated 18F-FHBG will be trapped in the cells. Noninvasive PET imaging of TK expression is advantageous for cell trafficking because the location, magnitude, and temporal dynamics of the cells expressing TK can be easily identified and measured by quantitative 18F-FHBG/PET imaging. PET imaging will also be performed with CXCR4 specific 18F-T140 peptide to determine the expression of CXCR4 on MSCs longitudinally. The most effective injection route which can result in more MSCs homing to the TBI site will be identified. Finally, we will evaluate the treatment efficacy of MSCs by studying the following parameters: brain glucose metabolic rate, angiogenesis, injury site blood flow, behavior changes and other pathophysiological parameters.

Methods:

Rat MSCs will be isolated, characterized and infected with lentiviral particles carrying CXCR4 and TK genes. The multilineage differentiation capability and the tropism to injured brain of MSC-CXCR4-TK will be evaluated in vitro.

The controlled cortical impact (CCI) induced rat TBI model will be established. To track the homing of the MSC-CXCR4-TK to TBI in vivo, each group of rats will receive wild-type MSCs or MSC-CXCR4-TK through different routes, such as intravenous, intracranial, or intra left ventricle. The cell amount homed to TBI site and subsequent cell survival/proliferation will be monitored over time with 18F-FHBG and 18F-T140 PET imaging quantitatively and non-invasively. The in vivo imaging results will be confirmed with ex vivo immunohistological staining results. The purpose of this experiment is to compare the homing properties of wild-type MSCs and MSC-CXCR4 towards TBI and identify the most appropriate cell infusion route.

Once the most effective injection route of MSCs is identified, we will test the therapeutic effect of MSC-CXCR4-TK on the recovery of TBI rats. At different time points after cell administration, MSC-CXCR4-TK cell number will be measured by 18F-FHBG and 18F-T140 PET imaging; brain glucose metabolism rate, angiogenesis, brain injury and brain function will be evaluated by 18F-FDG/PET, 18F-RGD/PET imaging, MR imaging, and neurological severity score (NSS) respectively. In vivo results will be confirmed by ex vivo immunohistochemical staining. These analyses will allow us to measure if MSC treatment can improve the outcome of TBI and if this improvement is dependent on the dose of MSCs.

Significances:

The proposed studies will determine how interaction between chemokines and chemokine receptors can regulate MSC homing property and how enhanced MSC homing to TBI lesion sites can contribute to brain functional recovery. The findings from the current studies may have a significant impact on guiding future studies to optimize these observed regenerative responses.

Project Title: The evolution of Hedgehog signaling and oxidative stress following TBI

Principal Investigator: Amanda Mierzwa, Ph.D.

ABSTRACT

TBI is a major public health concern with an estimated 1.7 million people affected annually with approximately 75% classified as mild. Following primary injury, secondary cellular and molecular processes, such as inflammation or oxidative stress, may contribute to delayed neuronal and glial cell death and dysfunction. Neural stem/progenitor cells in the subventricular zone of the forebrain show increased proliferation in response to injury, indicating the potential to counteract damage. Ultimately, to enhance recovery following brain injury, those processes which damage the brain must be attenuated and those processes that aid recovery must be allowed to function optimally. The studies proposed will examine the sonic hedgehog (Shh) signaling pathway as well as oxidative stress in a model of mild TBI. Through the proposed studies we expect to delineate the interplay of these processes in the regulation of neuroregeneration and neuroplasticity of neural stem/progenitor cells following TBI. Specifically, these studies will define the window for abrogating the effects of oxidative stress and to enhance Shh signaling through therapies that circumvent oxidative stress and/or extend the period of Shh upregulation post-TBI.

Project Title: Modulation of Inflammation after TBI

Principal Investigator: L. Christine Turtzo, M.D., Ph.D.

ABSTRACT

Disproportionately affecting the young, TBI causes a substantial burden of disability among survivors, particularly in accidental trauma and among soldiers subjected to blast injury. Clinical TBI is a heterogeneous disease with a variety of causes and multiple mechanisms resulting in brain injury. No single animal model accurately represents the entire spectrum of human disease after TBI.

The controlled cortical impact (CCI) model of TBI, which produces focal contusions, has been highly utilized by many research laboratories. However, this model does not reproduce the diffuse axonal injury (DAI) and microhemorrhages characteristic of TBI in clinical patients. In contrast, the linear acceleration closed head (LACH) model of diffuse TBI induces DAI but not focal contusions.

After TBI, the temporal relationship driving degenerative versus reparative events may be influenced by the ratio of proinflammatory versus anti-inflammatory cells. The large degree of cortical injury, macrohemorrhage, and necrosis seen in the CCI focal contusion model may result in a different inflammatory profile than seen in the more diffuse LACH injury model. These two different mechanisms of injury may require diverse therapeutic approaches.

The hypothesis driving the research arm of this fellowship project is to examine whether the modulation of inflammation in a clinically relevant model of severe TBI can improve outcomes. In Aim 1, the MRI, histopathological, and neurobehavioral characteristics of the LACH TBI model of diffuse injury will be examined. In Aim 2, cellular therapy to modulate inflammation after TBI will be explored in the LACH TBI model. In Aim 3, the efficacy of pharmacological agents in targeting inflammation and cell death pathways after TBI will be investigated.

For the educational arm of this fellowship, the fellow will acquire expertise in models of TBI and imaging techniques and experience in translational neurology. These will be acquired through both research as well as participation in local and national seminars and meetings.

Project Title: Angiotensin II receptor blockers as potential therapeutics for TBI

Principal Investigator: Sonia Villapol, Ph.D.

ABSTRACT

TBI results in complex pathological reactions. Damage from the initial lesion is worsened by secondary inflammation and edema. Angiotensin II, the major effector molecule of the renin-angiotensinogen system, is produced locally in the brain, and acts via specific AT1 and AT2 receptors that often have opposing actions. In the central nervous system, angiotensin II is a powerful proinflammatory mediator, and regulator of cerebral blood flow, blood pressure and fluid balance. Most actions of angiotensin II are mediated through the AT1 receptor (AT1R), whose expression is increased by stress, inflammation and injury. However, little is known about the response of the renin-angiotensinogen system to TBI. Drugs that antagonize the AT1 receptor, the angiotensin receptor blockers, are potent anti-inflammatory agents in the CNS, that may also have vasodilatory and neuroprotective actions. We hypothesize that inhibition of AT1 receptors after TBI will reduce inflammation, cortical damage and improve neurological outcome. As angiotensin receptor blockers are widely used FDA approved drugs with limited side effects, we are hopeful that success in a pre-clinical model system could quickly be moved to the clinic to help patients with acute TBI. Our preliminary results suggest that treatment of mice with the angiotensin receptor blocker candesartan before controlled cortical impact (CCI) TBI leads to a reduction in lesion volume, reduction in neuronal cell death and improved motor function 3 days post injury. We propose to continue our experiments to determine the mechanisms through which angiotensin receptor blockers act after TBI and the role of angiotensin II in the response to traumatic injury. We will first determine whether treatment of mice with the angiotensin receptor blocker candesartan, pre or post moderate CCI injury, will improve short- and long-term recovery. We will examine morphological and behavioral outcomes at 3 and 28 days after CCI to characterize the effects of angiotensin receptor blocker treatment in mice. We will use two different treatment paradigms - 5 hours before CCI injury, or 2 hours afterwards, to determine whether the beneficial effects of candesartan pre-treatment are maintained if the drug is given in a more clinically relevant manner. Secondly we will characterize the role of the AT1R in the response to TBI, by examining CCI injury in AT1R knockout mice. We will compare the response to CCI in wild type and AT1R knockout mice at 3 and 28 days post injury. We will also determine whether the absence of AT1R leads to changes in cerebral blood flow, or to differences in other aspects of the renin-angiotensinogen system in the brain. As angiotensin receptor blockers are neuroprotective, can prevent vasoconstriction, and also reduce inflammation, they address multiple pathways that contribute to TBI pathology. Data generated from this project, will we hope, provide strong rationale for further development of this drug, to lead to new targeted therapeutics that may help treat damage resulting from TBI.

Project Title: Altering the ECM after TBI to enhance regeneration and plasticity

Principal Investigator: Jae-Hyuk Yi, Ph.D.

ABSTRACT

TBI has long lasting consequences that negatively impact function even when the degree of physical disruption is minimal. Unfortunately, we have yet to understand the events that lead to this prolonged impairment of function, and lack successful ways to treat it. TBI results in the formation of a glial scar much like the one formed after spinal cord injury, where it appears to serve a dual role 1) to contain inflammatory processes and 2) to discourage axonal sprouting. Chondroitin sulfate proteoglycans (CSPGs) are considered to be the major inhibitory component of this glial scar, and thus understanding the role of CSPGs after TBI is essential. CSPGs consist of core proteins decorated with chondroitin sulfate glycosaminoglycan (CS-GAG) sugar chains. Our preliminary findings have shown that there is a localized increase in CSPG core proteins and 4-sulfated CS-GAGs in the injured brain following mild Controlled Cortical Impact (CCI) injury in mice. We will first complete the characterization of CSPG expression and address whether the CSPGs in the glial scar have a positive or negative effect on recovery of function after TBI. We will also address the mechanisms by which neurons are repelled by CSPGs. We posit the presence of a receptor on neurons that reacts to CSPGs. We have identified that, after TBI, there is an upregulation of binding sites for specific GAG chains on neurons, providing preliminary evidence for a specific receptor. Our second aim will be to identify this putative neuronal receptor for CS-GAGs. More specifically, we will:

- 1) Complete the characterization of the spatiotemporal change in CS-GAG chains and their core proteins following mild TBI. Next, we will intracortically deliver chondroitinase ABC (cABC) after CCI to remove CS-GAGs and investigate its effect on axonal sprouting, immune cell invasion and functional recovery. Our hypothesis is that removal of CS-GAG chains by cABC will either enhance axonal regeneration, encourage the inflammatory process, or both. The resulting consequence will be manifested by changes in recovery of function as assessed by behavioral tests.

- 2) Find the cellular localization of CS-A binding site by co-labeling brain sections with markers of neurons, glial cells and immune cells. We will then identify the protein(s) to which CS-A binds by purifying protein(s) from the brain using a CS-A affinity column. Column eluates will be subjected to tryptic digestion, followed by Mass Spectrometry analysis to identify putative proteins that bind CS-A. The binding will be confirmed using a series of approaches, including cDNA cloning and expression of the protein in a heterologous system or knocking down endogenous expression and evaluating changes in CS-A binding.