Background/Readiness: Chronic, neuropathic pain occurs in 60-80% of persons with spinal cord injury (SCI), yet less than 10% of patients show complete recovery, either spontaneously or following treatment[1]. Alarmingly, clinical approaches are largely ineffective in the treatment of neuropathic pain and adjuvant medications (i.e. antidepressants and anticonvulsants) demonstrate only marginal effectiveness. Although the neurobiological mechanisms which underlie neuropathic pain are poorly understood, a prevalent hypothesis is that SCI induces the formation of reactive oxygen species (ROS) and activation of nuclear factor κB (NF-κB) signaling which leads to inflammation and neuropathic pain (for review see[2]). In support of this hypothesis, a body of research demonstrates that SCI results in formation of ROS[3] and activation of the transcription factor NF-κB[4]. Administration of ROS scavengers reduces neuropathic pain in animal models[5]. Additionally, various methods to inhibit NF-κB activity have been shown to decrease neuropathic pain including the use of NF-κB decoys[6] and inhibition of NF-κB with pyrrolidine dithiocarbamate[7]. We recently found that inhibition of NF-κB with sulfasalazine blocks development of neuropathic pain in a rodent model of cervical SCI, yet the blood-brain barrier (BBB) penetration of sulfasalazine is very limited and thereby necessitated very high and poorly tolerated systemic dosing (unpublished). Collectively, these data demonstrate that dissipating ROS and blocking NF-κB signaling are therapeutic targets to alleviate neuropathic pain after SCI. We hypothesize that a highly efficacious treatment for neuropathic pain after SCI would be a compound that scavenges ROS, inhibits NF-κB activation, is bioavailable to the central nervous system (CNS), and is well-tolerated.

Our group has discovered that a therapeutic approach that ***both*** dissipates ROS ***and*** inhibits NF-κB activation is to employ catalytic oxidoreductants, specifically Mn (III) N-alkylpyridylporphyrins (MnPorphyrins). In contrast to the more passive scavenging activity of "classical" antioxidants, MnPorphyrins are catalytically active oxidoreductants which confer pharmacological properties superior to other compounds (see [8]). They are efficient in dissipating a broad range of ROS and reactive nitrogen species including superoxide, hydrogen peroxide, peroxynitrite, and hypochlorous acid (see [8]). We have extensive experience in the development and characterization of MnPorphyrins and our previous published studies demonstrate marked protection conferred by MnPorphyrins in models of CNS insults and spinal cord injury[9-12]. Intriguingly, in addition to their highly efficacious cytoprotectant effects due to catalytic redox-regulating activity, MnPorphyrins are potent inhibitors of NF-κB signaling[8, 9, 13]. A main limitation in the development of MnPorphyrins as therapeutics for CNS injury has been the CNS bioavailability. To overcome this, we have recently synthesized a highly lipophilic MnPorphyrin, MnTnBuOE (BuOE), and demonstrated robust brain penetration as well as neuroresuscitation in rodent CNS injury models and prevention of morphine tolerance. Moreover, we found that BuOE is also a potent NF-κB inhibitor. Since dissipation of ROS and suppression of NF-κB signaling both inhibit neuropathic pain after SCI, we hypothesize that BuOE can be developed as a novel therapeutic to treat neuropathic pain after SCI by offering the unique and simultaneous chemistry of direct redox-regulation of inflammation and catalytic reduction of superoxide and peroxynitrite. Indeed, our preliminary data in a rat model of cervical SCI strongly support this hypothesis. Thus, the proposed research is a comprehensive animal validation study which will provide the necessary data and infrastructure to plan first-in-human clinical trials as next step in the translational process.

Hypothesis and Approach: The proposed research will test the hypothesis that **post-SCI administration of the catalytic oxidoreductant BuOE will inhibit neuropathic pain after SCI** with the following aims:

Aim 1: Test the hypothesis that post-SCI administration of BuOE decreases ROS levels, inhibits NF-κB signaling, inhibits inflammation, and decreases pain-associated behaviors in a clinically relevant *rat* model of *cervical contusion* SCI.

In an effort to increase the clinical relevance of rodent SCI models, our laboratory has recently characterized a *cervical* contusion SCI model in adult male rats[14]. Of major importance, this model produces a robust neuropathic pain phenotype. Using this model, adult male rats will receive a C5 hemicontusion SCI followed by post-SCI administration of BuOE for 14 days. We will conduct a dose-effect evaluation to assess the effects of BuOE administration on ROS levels, NF-κB signaling, and inflammation in spinal cord tissue as well as on functional recovery and pain-associated behaviors.

Aim 2: Test the hypothesis that post-SCI administration of BuOE reduces ROS levels, inhibits NF-κB signaling, inhibits inflammation, and decreases pain-associated behaviors in *mouse* model of *ischemic* SCI.

A critical step in the translation of a novel therapeutic from the bench to the bedside is the evaluation of the treatment effects in multiple animal models, preferably with divergent injury mechanisms[15]. Thus, we will evaluate the effect of post-SCI administration of BuOE in a mouse model of ischemic SCI. Our group has recently characterized a murine model of ischemic spinal cord injury which produces a highly reproducible pathophysiology and functional deficits[16]. Using this model, adult male mice will receive a minimally invasive thoracic ischemic SCI followed by post-SCI administration of BuOE. We will evaluate the effects of BuOE administration on ROS levels, NF-κB signaling, synthesis of NF-κB-dependent pro-inflammatory cytokines and chemokines, functional recovery, and pain-associated behaviors.

Aim 3: Test the hypothesis that post-SCI administration of BuOE reduces ROS levels, inhibits NF-κB signaling and inflammation after SCI in a *porcine contusion* model.

Recently, models of spinal cord injury in Yucatan minipigs have been developed and characterized as an important intermediate translational step between preclinical rodent studies and first-in-human clinical trials[15]. We will evaluate the dose-response effect of post-SCI BuOE2 administration on ROS levels, NF-κB signaling and inflammation at acute and sub-acute post-injury time points in minipigs. We will also evaluate pharmacodynamic and pharmacokinetic events crucial to future clinical trial design.

Translational Components: The research proposed here is translational in both the central hypothesis and experimental design, as it centers on movement of ideas from the bench to beside in a partnership to develop a novel therapeutic for the treatment for neuropathic pain in persons with SCI. First, we have chosen a well-characterized MnPorphyrin with demonstrated protective efficacy, safety, and bioavailability in CNS injury models. Secondly, our path to translation includes the evaluation of therapeutic efficacy in multiple animal models and animal species, including well-established rodent models and a large animal model which is a key component in successful translation[15]. Third, careful consideration of pharmacodynamics and pharmacokinetic variables is incorporated into all aspects of the design to facilitate translation to first-in-human trials.

Partnership: The interdisciplinary partnership upon which this research is based is a key element to its success. The partnership involves three PIs, each with unique and complimentary expertise. The Initiating PI, Candace L. Floyd, Ph.D., Assistant Professor in the Department of Physical Medicine and Rehabilitation at University of Alabama, Birmingham (UAB), is an expert in traumatic CNS injury animal models. Dr. Floyd's laboratory has recently developed and characterized a clinically-relevant model of cervical SCI in adult male rats that manifests neuropathic pain-associated behaviors. One partnering PI is Hubert M. Tse, Ph.D., Assistant Professor in the Department of Microbiology at UAB who is an expert in immunology, NF-κB signaling, and biological effects of catalytic oxidoreductants. The second partnering PI is David S. Warner, M.D., Professor in the Departments of Anesthesiology and Neurosurgery at Duke University, a well-established physician-scientist dedicated to discovering novel therapeutic approaches for SCI. Dr. Warner is an international expert in the use of catalytic oxidoreductants as protective agents in acute CNS injury.

Impact: Up to 80% of persons with SCI suffer from neuropathic pain yet there are limited treatment strategies that successfully alleviate this pain. If successful, this research would be well suited for rapid translation into clinical trials and could in short-order affect clinical practice in the treatment of post-SCI neuropathic pain.

Military relevance: In the on-going war on terror, vehicular collisions, gunshot wounds, and blast exposure are occurrences that induce SCI in military personnel. Indeed, neuropathic pain is particularly problematic in patients with SCI after gunshot wounds[17]. Thus, development of a novel treatment strategy to reduce neuropathic pain after SCI would yield significant improvement in quality of life for our Nation's injured warfighters and Veterans, as well as other individuals living with SCI.

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