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TITLE: Development of Novel Combinatorial Treatment to Prevent Chemotherapeutic Resistance and Enhance Efficacy of Riluzole in a Rodent Model of SCI

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14. ABSTRACT

Overall goal of this proposal is to use a pharmacological approach to prevent or significantly reduce the onset of chemotherapeutic resistance that we have recently observed/described following spinal cord injury (SCI). In our initial, published report, we found that SCI produced a sustained upregulation of P-glycoprotein (Pgp) within the spinal cord that prevented access of systemically (intraperitoneally) administered riluzole. This chemotherapeutic resistance was found to be permanent within the spinal cord as we continued to detect elevated Pgp at the lesion site as well as in the cervical and lumbar cords out to at least 10 months post-injury. While our rodent study was ongoing, a multi-center clinical trial was performed assessing riluzole as an acute treatment for SCI. While showing a trend toward improved function, the results were not statistically significant. Pharmacokinetic assessment of orally-riluzole bioavailability, however, showed a dramatic reduction of plasma concentrations of riluzole between 3 and 14 days of treatment. Based on our rodent spinal cord data, we asked whether this could suggest a Pgp-dependent reduction of orally-administered riluzole. We subsequently showed (in preliminary data for this project) that SCI produced a rapid induction of Pgp protein expression in the gastrointestinal tract of rats. This lead us to hypothesize that: 1) SCI produces systemic chemotherapeutic resistance, 2) that targeting the inflammatory pathways that promote Pgp induction will prevent onset of chemotherapeutic resistance, 3) that co-administration of riluzole with the anti-inflammatory treatment will both suppress GI Pgp and enhance plasma concentrations of riluzole, and 4) that this combinatorial treatment will lead to a preservation of motor function and an attenuation in long-term pathologies like neuropathic pain in rats following an acute therapeutic intervention. Within this second year, our efforts have focused on completing Aim 1 (generating a surgical time course in which we are collecting GI and spinal tissues for the assessment of Pgp expression). Despite problems with this assay, we have finally resolved the issue of Pgp detection and are moving on through our banked tissues for analsis We have also been working with our Co-Investigator, Stanley Smith, Ph.D., to to measure plasma and spinal bioavailability of orally-administered riluzole.

15. SUBJECT TERMS

spinal cord injury (SCI), gastrointestinal (GI), P-glycoprotein (Pgp), chemotherapeutic resistance, mass spectrometry, riluzole, licofelone, neuropathic pain, locomotor, bioavailability

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Introduction:

The overall goal of this DOD-funded proposal is to target the pathological process known as chemotherapeutic resistance in spinal cord injury (SCI), a process we recently identified, in order to enhance the bioavailability and efficacy of riluzole, an FDA-approved neuroprotective drug. In a previously published study, we demonstrated that SCI resulted in the upregulation of P-glycoprotein (Pgp), an energy driven pump that sequesters and limits the amount of a wide range of substances, both endogenous as well as exogenous (such as drugs) from tissues. Pgp is a significant contributor to the process of chemotherapeutic resistance in many forms of cancer that blocks access of systemically-administered drugs into tumors, reducing their therapeutic efficacy. As there have been no pharmacological treatments shown to be effective in the clinic for the treatment of SCI, we hypothesized that spinal trauma may establish chemotherapeutic resistance in a manner that is analogous to what is observed in cancer. We demonstrated/published these results showing that riluzole, an FDA-approved drug that has been under evaluation in the clinic for acute treatment of SCI, was excluded from the spinal cords of injured rats. We also demonstrated that this effect was due to an SCI-dependent induction of Pgp within the traumatized spinal cord. Finally, we further demonstrated that if we targeted inflammatory conditions using a novel, dual inhibitor of both cyclooxygenase AND 5-lipoxygenase (licofelone) we could prevent the SCI-dependent induction of Pgp. If we then co-administered licofelone with riluzole, this dramatically enhanced the intraspinal bioavailability of riluzole. As mentioned above, riluzole was undergoing a clinical trial as an early intervention for SCI. While showing a strong trend towards efficacy, the results were not significant. Of significant interest to us though, a pharmacokinetic analysis from the clinical trial showed that plasma concentrations of orally-administered riluzole plummet between 3-14 days of treatment. When we examined Pgp expression within the gastrointestinal tract, the sole route through which orally-administered riluzole would gain access to the plasma, we found a more than two-fold induction of Pgp. Thus, the primary goal of this study is to determine whether orally-administered licofelone can suppress SCI-dependent increases in GI and spinal Pgp expression and enhance the systemic and intraspinal bioavailability and efficacy of riluzole. We are measuring the time-dependent induction of Pgp after SCI under vehicle vs. licofelone-treatment (Aim 1a) and assessing the effects of co-administered riluzole bioavailability in plasma and spinal cord (Aim 1b). The second aim will explore the effects of this novel combinatorial treatment on both locomotor and neurosensory function. During this second year we have focused on Aim 1a and Aim 1b and have begun the experiments in Aim 2.

Keywords:

Spinal cord injury (SCI), chemotherapeutic resistance, P-glycoprotein (Pgp), gastrointestinal (GI), riluzole, licofelone, inflammation, anti-inflammatory, pharmacokinetics, plasma

Accomplishments:

During this second year, we have been focused on generating and collecting the tissues to address Aim 1a and Aim 1b. We have also initiated the behavioral studies described in Aim 2. Early, we encountered a problem that we will describe in greater detail below. This issue was an inability to successfully employ the Western Blot method that we previously used to detect Pgp in spinal cord and gastrointestinal tissues. Over the past year we have focused on identifying an alternative approach and have, within the last month, identified a commercially-available Enzyme-linked-adsorbent-assay (ELISA) that works. The benefit to this method is that it is quantitative rather than qualitative and is more sensitive to the previously used Western blot method. In pilot studies, we have found that this ELISA can detect Pgp in rat brain and rat gastrointestinal tissues. We are now moving ahead with this assay through the banked tissues that we have been storing from Aim 1a (spinal cord and GI tissues). In optimizing the conditions of this Pgp ELISA assay, we chose to use spare tissue from other ongoing projects in the Grill laboratory so as not to risk the SC140017 experimental tissues. We first tested brain tissues from a developmental study and detected a significant difference in Pgp expression between control and experimental conditions (not shown). This gave us confidence that this was the ELISA that we could use for this project. We next wanted to test this in GI tissues from naïve vs. spinal cord injury subjects. Again, we did not want to test this new assay on tissues collected for this study so instead, we chose to run the ELISA on ileum from rats with a chronic spinal contusion injury (1.5 years post-SCI) with uninjured, age-matched control rat ileum. The results are shown in Fig. 1. Our pilot data suggests a rapid induction of Pgp within the GI tract of rats within 48 hours of SCI (Fig. 2). This initial data served as the core driving factor for our hypothesis that SCI elicits a systemic response characterized by a state of chemotherapeutic resistance. An induction of Pgp within the gut would lead to reduced transit of Pgp-substrates such as riluzole from the lumen of the gut into the blood stream. As riluzole is delivered via the oral delivery route in the ongoing Phase II riluzole/SCI clinical trial, this would represent a significant impediment to drug bioavailability, and thus,

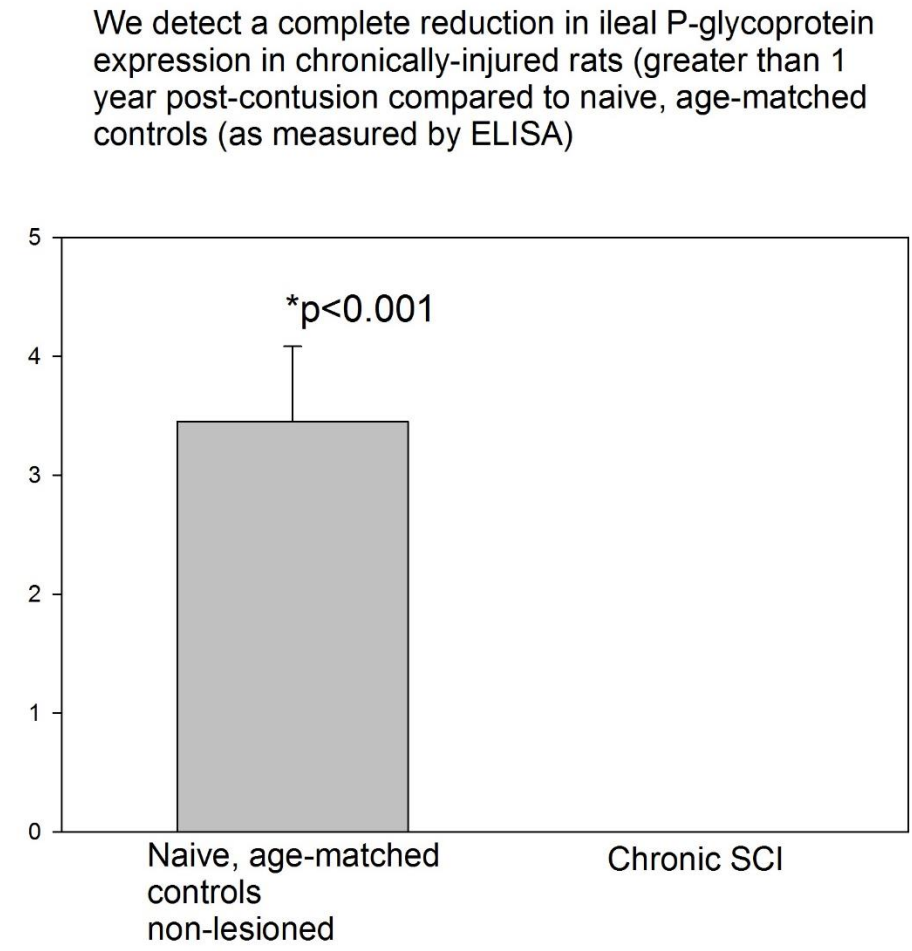


Fig. 1: Testing a new, quantifiable ELISA method to detect Pgp in GI tissues. We detect a significant loss of Pgp expression in the ileum of chronically-injured adult, male Sprague-Dawley rats compared to uninjured, age-matched controls (t-test, *p<0.001)

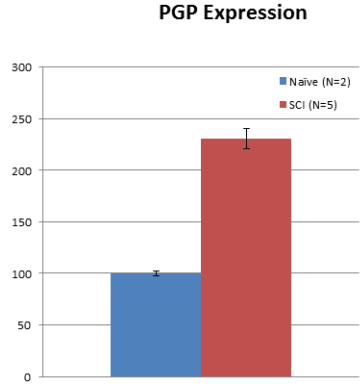
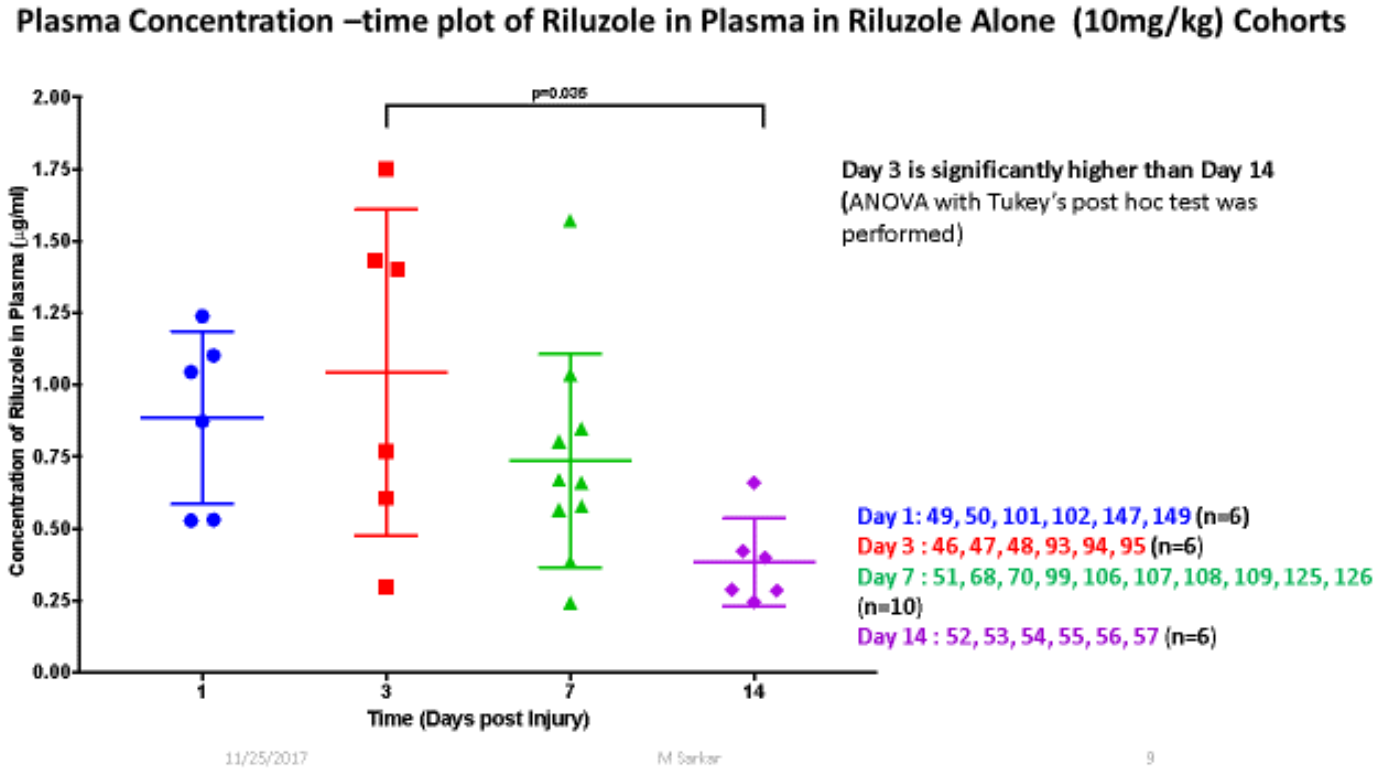


Fig. 2: In a pilot study, we observed a more than two-fold increase in GI expression of Pgp in rats 48 hours post-SCI compared to naïve, uninjured control rats.

potential efficacy. The results shown in Fig. 1 are significant, however, for two reasons: 1) they demonstrate that we have found an effective alternative method to detect Pgp in rat tissues. This has been a driving concern for the entire year as we encountered an inability to get the Pgp antibody previously used to work in our Western blot studies. With the success of this assay, we are now able to perform the studies outlined in Aim 1. 2) While not directly tied to the goals of this current project, our findings using this new Pgp ELISA with tissue from chronically-injured subjects indicates that Pgp expression, crucial to the regulation of transport of materials from the GI lumen to the blood stream, is significantly degraded and is the opposite of what we observe in regards to spinal expression of Pgp in the chronically-injured rat spinal cord. We've previously reported that Pgp is rapidly induced in the injured spinal cord, but remains elevated throughout the life of the rat; contributing to a permanent state of chemotherapeutic resistance. Our observation of reduced GI Pgp expression in far-chronically-injured rats suggest that the expression of Pgp throughout the body post-SCI can differ dramatically over time with potentially serious ramifications for chronic treatment strategies. For instance, opiates, a line of treatment for some patients living with chronic neuropathic pain following SCI, are substrates for Pgp. Lack of opiate regulation/absorption may dramatically enhance risk of overdose as well as addiction in these individuals. I am starting to work with Dr. Kevin Freeman here at UMMC to explore opiate metabolism in chronic SCI as well as alternatives that reduce risk of addiction. This does not involve funding from this current study but represents future collaborative efforts that evolved from this current work.

Aim 1b: In last year's report, we demonstrated the ability to detect riluzole in the plasma of naïve rats. We can now report that orally administered riluzole undergoes an initial rise in plasma concentration between days 1 and



3 post-SCI, but plasma riluzole concentration drops between days 3 and 14 post-treatment in injured subjects. This finding is highly significant for two reasons: 1) this finding is opposite of what we found in our previously published plasma Pk studies in rats following SCI. In those studies, we observed a time-dependent increase in plasma riluzole concentrations following daily intraperitoneal injections of riluzole. In our current study, however, we are using the more clinically-relevant oral delivery route for riluzole treatment. This suggests that oral delivery of riluzole encounters an issue(s) that impede translocation of riluzole from the GI lumen to the plasma compartment that were not an issue with intraperitoneal injection. 2) Our findings coincide with the published Pk studies performed on the Phase I riluzole clinical trial performed for the acute treatment of spinal cord injury through the North American Clinical Trials Network. They reported a similar Pk response observed over a 14 day treatment paradigm initiated within 12 hours of patient receipt. Thus, our rodent Pk data matches

that observed in the human clinical trial supporting our hypothesis of chemotherapeutic resistance induction attenuating access of riluzole when administered along the clinically-relevant oral route.

Summary: With the recent success of the Pgp ELISA, we have broken a significant bottleneck that has slowed progress on this project. We are now focusing significant efforts in the analysis of both spinal and GI tissues for Pgp expression post injury both with and without licofelone treatment. We also continue our Pk analysis to determine whether licofelone co-treatment will affect riluzole's observed Pk parameters after injury.

Training/Personal Development:
We recently hired a new Research Assistant III who is currently being paid off of "start up" funds by Dr. Grill. William Lawson has been key in getting the new Pgp ELISA up and running. He is in the process of training Ms. Sereduck (the RAIII currently assigned to this project) in the methods. Together they will be processing our extensive backlog of spinal and GI tissues in order to close out Aim1a.

Results Disseminated:
No major results to report at this stage.

Plans for next reporting period:
Our goal is to catch up and complete the analyses of Aims 1a and 1b in this next quarter (year 3, quarter 1). We are also currently performing the behavioral analyses described in Aim 2 that will likely take us into year 3, quarter 3 to complete.

Impact:

Our initial pilot study assessing the GI expression of Pgp in the gut has great promise to shape impact in subsequent chronic therapeutic approaches. The PI, Dr. Grill, has already reached out to investigators at UMMC and from his former institution at UT-Health in Houston to develop novel studies that will assess the ramifications of a loss of Pgp in the GI tissues of chronic SCI subjects.

Changes/Problems:
We encountered one significant problem during the second year of the study. As described above, we were unable to get the Western blot technique previously used to measure spinal and GI Pgp concentrations to work. This led to a nearly one year analysis of alternative methods of tissue preparation and assay methods that resulted in our current approach, the use of a commercially-available Pgp ELISA that reliably and with high-sensitivity provides a quantitative assessment methodology that works in both CNS and GI tissues. Thus, this problem has been resolved, opening the door to our analysis of banked tissues so that we may complete this project in a timely manner.

Products:

Nothing to report

Participants:

1) Raymond J. Grill, Ph.D.
PI
ORCID ID: unsure of this
Nearest person month: 1.8 mo
Contribution to Project: 1) General oversight, 2) surgical procedures and post-operative care, 3) providing training on surgical procedures and post-operative care to Research Assistant and Neurosurgical Resident.
Funding support: This grant

2) Stanley Smith, Ph.D.
Co-Investigator
Nearest Person Month: 1.2
Contributions to Project: Establishing detection protocols for measuring riluzole concentrations in plasma and spinal cord using mass spectrometry.
Funding Support: This grant

3) Ms. Suzanne Sereduck (MA)
Research Assistant III
Nearest Person Month: 12
Contributions to project: 1) post-operative care, 2) assisting in tissue collection, 3) sample preparation for mass spectrometry analysis.
Funding Support: This grant

4) William Lawson, MA
Research Assistant III
Nearest Person Month: N/A
Contributions to project: Optimizing Pgp ELISA methods and training Ms. Sereduck in this procedure
Funding Support: University of Mississippi Medical Center, Department of Neurobiology and Anatomical Sciences, Start up funds from Dr. Grill's laboratory

Development of novel combinatorial treatment to prevent chemotherapeutic resistance and enhance efficacy of riluzole in a rodent model of SCI

Insert ERMS/Log Number and Task Title Here: SC140017

Insert Award Number Here: W81XWH-15-1-0668

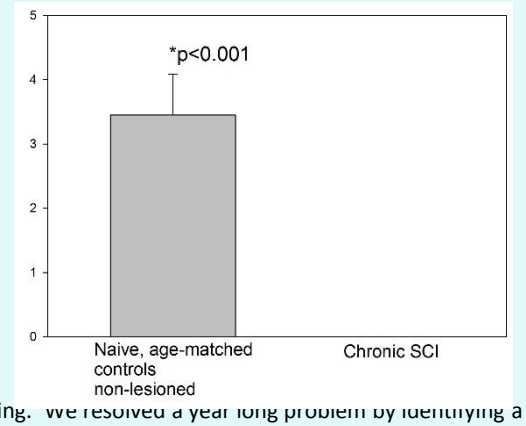
PI: Raymond J. Grill **Org:** University of Mississippi Medical Center **Award Amount: $479,697**

Study/Product Aim(s)

- **Specific Aim 1:** Determine whether orally-delivered licofelone will prevent SCI-dependent increases in both GI and intraspinal Pgp expression and enhance plasma and intraspinal bioavailability of orally-delivered riluzole following SCI.
-
- **Specific Aim 2:** Determine whether combined oral administration of licofelone and riluzole enhance locomotor outcome and prevent onset of neuropathic pain after SCI.

Approach

All studies will use the Infinite Horizons spinal impactor to deliver a moderate-to-severe contusion injury to adult, male Sprague-Dawley rats (225-250 grams at the time of SCI). Aim 1 will assess the ability of licofelone to suppress GI and spinal Pgp and enhance bioavailability of riluzole. Aim 2 will determine whether a combination of riluzole and licofelone will improve locomotor function and prevent onset of neuropathic pain



Significant finding. We resolved a year long problem by identifying a commercially-available ELISA for the detection of Pgp. This will allow us to now process our banked tissue to complete Aim 1a. The results from our pilot study are also highly clinically-relevant in its own right suggesting that GI Pgp expression is dramatically reduced in chronic SCI (1.5 years post injury compared to age-matched controls. This observation opens great new opportunities to assess drug treatment and metabolism in chronic SCI (serving as a launching point for new collaborative efforts evolving from this study.

Timeline and Cost

Activities CY	15	16	17	18
Specific Aim 1				
Specific Aim 2				
Estimated Budget ($K)	**$000**	**$000**	**$000**	**$000**

Updated: 12/6/17

Goals/Milestones (Example)

CY16-17 Goals – System validation

☐ Determine whether licofelone can suppress GI and spinal Pgp expression following SCI-in progress (Aim 1)

☐we can now complete this aim due to the adoption of this Pgp ELISA methodology

☐Determine whether licofelone can enhance orally-administered riluzole bioavailability-in progress (Aim 1)

☐Ongoing work

☐ Assess whether licofelone/riluzole combination can attenuate neuropathic pain and enhance locomotor function-initiated and ongoing (Aim 2)

☐We believe the successful resolution of the Pgp assay problem to represent an important milestone that will aid in study completion.