AWARD NUMBER: W81XWH-16-1-0349

TITLE: Acute and Delayed Systemic Treatment with Cannabinoid Receptor 2 Agonists to Prevent or Treat/Reverse Osteoporosis in a Mouse Model of SCI

PRINCIPAL INVESTIGATOR: Raymond J. Grill, PhD

CONTRACTING ORGANIZATION: University of Mississippi Medical Center
Jackson MS 39216

REPORT DATE: August 2017

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
August 2017	Annual	1 Aug 2016 - 31 Jul 2017

4. TITLE AND SUBTITLE

Acute and Delayed Systemic Treatment with Cannabinoid Receptor 2 Agonists to Prevent or Treat/Reverse Osteoporosis in a Mouse Model of SCI

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-16-1-0349

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Raymond J. Grill, PhD

E-Mail: rgrill@umc.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Mississippi
Medical Center, 2500 North
State Street, Jackson, MS,
39216

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The overall goal of this project is to determine whether a selective agonist for the cannabinoid-2 receptor, when systemically delivered, can prevent the onset of osteoporosis in mice when delivered during the acute phase of apinal cord injury or restore bone density when delivery is delayed until the late, chronic period of injury. During this first year, we have focused on aim 1; testing a range of CB2 agonist concentrations, delivered early but over a maximum of 40 days (longest time group). While we are still assessing bone densities (post-mortem), we noted that both the low and high doses of CB2 agonist appear to elicit neuropathic pain-like symptoms, resulting in the required early euthanasia of those subjects. As a result, we are focusing on the mid-range dose for all subsequent experiments. We have also begun Aim 2 by performing the spinal transection surgeries. These animals will require a 3 month period (to induce chronic stage) before initiation of CB2 agonist treatment.

15. SUBJECT TERMS
spinal cord injury, osteoporosis, neuropathic pain, bone density, acute, chronic

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	8	**19b. TELEPHONE NUMBER** *(include area code)*

Table of Contents

Page

Introduction

The overall goal of this project is to develop a safe and effective therapeutic that can either prevent onset of osteoporosis following spinal trauma or reverse established osteoporosis when treatment is delayed until the chronic period of spinal cord injury (SCI). Osteoporosis afflicts the majority of individuals living with SCI, putting them at great risk for bone fracture. As this often occurs in bones below the level of injury, individuals may be unaware that a fracture has occurred, leading to potentially fatal consequences. My group previously observed a preservation of bone integrity in mice that received a full spinal transection lesion if injured mice received early but sustained treatment with a selective agonist against the cannabinoid receptor-2. Therefore, we wish to determine whether this class of drugs can serve as a safe and novel therapeutic for the treatment of SCI-induced osteoporosis. We are exploring whether the early delivery of a selective cannabinoid receptor-2 agonist, HU-308, will protect bone density if treatment is started within 3 hours of injury. Bone density is monitored by assessing hind limb bone density at 10, 20, 30 and 40 days using post-mortem tissues. As there are hundreds of thousands of individuals living in the United States with a chronic SCI, we are attempting to determine whether a delay in treatment to mice in which osteoporosis has already become established will result in a reversal of bone loss and a restoration of bone density. Such an outcome would result in a significant improvement in an SCI patient's overall quality of life.

Keywords

osteoporosis, spinal cord injury, acute, chronic, cannabinoid, HU-308, micro-CT, longitudinal

Accomplishments:

Major goals of the project:

The major goals of this project include:

1) Determine whether the CB2 agonist, HU-308, can prevent osteoporosis during the acute phase of injury. This aim tests the early intervention and comparison of efficacy of three different concentrations of HU-308 administered within 3 hours of injury and continued daily for up to 40 days. Post-mortem bone density is measured from cohorts collected at 10, 20, 30 and 40 days with subsequent histological measurements of bone integrity performed.

2) In the second aim, we will determine whether a delay in treatment until the chronic phase of SCI (3 months post-SCI) can RESTORE bone density to mice following a full spinal transection injury. Bone density will be measured via micro-CT at the end of a 30 day treatment period followed by histomorphometric assessment of bone integrity.

What was accomplished under these goals:

1) We have finished the surgeries for Aim 1 and continue with the micro-CT analysis of bone density. Progress on analysis has been slowed by reduced access to the computer workstation needed for analysis (see Problems) listed below. This issue has been resolved (also see below).

2) We have initiated the chronic cohorts needed for Aim 2. The first group will be ready for treatment in November, 2017.

What opportunities for training and professional development has the project provided:

Nothing to report.

How were the results disseminated to communities of interest?

Results from Aim 1 are not yet ready for dissemination. We are currently attempting to finish analysis in order to submit an abstract to the Mission Connect Research Consortium Symposium to be held on December 8th, 2017.

What do you plan to do during the next reporting period to accomplish these goals?

1) complete the bone density analysis of the cohorts from Aim 1.

2) complete the surgeries needed for the chronic SCI studies outlined in Aim 2 (should be accomplished either late October, 2017 or early November, 2017.

Impact:

What was the impact on the development of the principal discipline(s) of the project?

Nothing to report.

What was the impact on other disciplines?

Nothing to report

What was the impact on technology transfer?

Nothing to report

What was the impact on society beyond science and technology?

Nothing to report

Changes/Problems:

Changes in approach and reasons for change:

We identified a significant issue in Aim 1 that required a change in approach. We originally proposed a comparison between HU-308 concentrations of 1, 10 or 100 mg/kg vs vehicle in mice after spinal transection. Preliminary data had suggested efficacy at preserving bone density at 10 mg/kg, but we wished to perform a range of drug concentrations to focus on an optimal therapeutic dose. It should be noted that SCI can produce neuropathic pain states in subjects (humans/rodents). In rodents, this can be characterized by autophagia in which the mice will chew at their hind limbs. It is actually a fairly rare event in C57BL6 mice as noted by the virtual absence of this behavior in vehicle-treated subjects. We were very surprised to observe, however, that mice treated with either the low (1 mg/kg) or high (100 mg/kg) doses exhibited nearly 100% autophagia. This was not observed in the 10 mg/kg dose group. This was surprising as it suggest a potentially pathological phasic response to the drug. This has not been observed/reported previously. It should be noted, however, that little work has been done using CB2 agonists as long-term therapeutics. As a result of these observations, we have selected the 10 mg/kg group for ongoing testing in Aims 1 and 2. We are beginning to work with a group here at UMMC to begin to better understand the mechanisms resulting in such pain, but these studies are not included in the current DOD funded project.

Actual or anticipated problems or delays and actions or plans to resolve them:

One issue that has slowed our pace somewhat is reduced access to the computer needed to perform bone density analyses. Currently, one computer was available to both drive the micro-CT in Dr. Chade's laboratory and perform the bone density analysis. High resolution bone scans can take as much as 24 hours to do which significantly tied up this computer. As this instrument is a core piece of equipment, we encountered issues with access for the follow up analyses. To resolve this, Dr. Grill recently purchased a high end Dell computer workstation configured to perform the needed analyses within his own laboratory. This computer was NOT purchased with DOD funding, but rather through Dr. Grill's incentive funds. This computer has been delivered and is now in use with bone density analyses. No additional delays are expected.

Changes that had a significant effect on expenditures:

None

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents:

Significant changes in use or care of human subjects:

None

Not applicable

Significant changes in use or care of vertebrate animals:

None. Subjects that exhibit neuropathic pain/autophagia were humanely euthanized.

Significant changes in us of biohazards and or select agents:

None to report

Products:

None to report as of yet

Participants and Other Collaborating Organizations:

What individuals have worked on the project:

1) Raymond Grill PI Identifier: RGRILL

Nearest person month worked: 1.8 calendar months

contribution to the project: Performed all animal experiments. Trained medical student (not funded off of this project) in bone density measurements)

Funding support: Unchanged

2) Alejandro Chade Co-I Identifier: ACHADE

Nearest person month worked: 1.2 calendar months

contribution to the project: Provides access to the micro-CT and problem-solving guidance in bone density protocols.

Has there been a change in the active other support of the PD/PIs or senior/key personnel since the last reporting period?

No changes that influence the level of support for this active grant.

No other organizations were involved as partners.

Quad Chart: see below

Appendices:

None to include

Acute and delayed systemic treatment with cannabinoid 2 agonists to prevent or treat/reverse osteoporosis in a mouse model of SCI
SC150037
W81XWH-16-1-0349

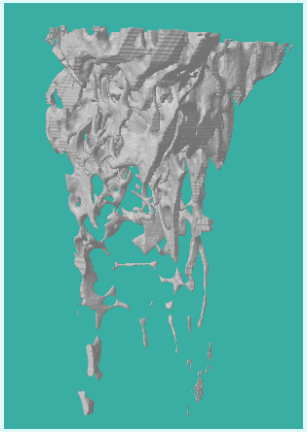
PI: Raymond J. Grill **Org:** University of Mississippi Medical Center **Award Amount:** $337,569

Study/Product Aim(s)

- Determine whether early, acute treatment with a CB2 agonist will preserve hind limb bone density following spinal cord injury in adult, male mice
-
- Determine whether delayed CB2 agonist treatment reverses established osteoporosis in chronically-injured, adult, male mice.

Approach

Mice receive a full spinal transection injury at thoracic level 8. In Aim 1, subjects receive either vehicle or HU-308 at one of three concentrations starting 3 hours post-injury and then daily. Cohorts are sacrificed at days 10, 20, 30 and 40 and hind limb bones imaged via micro-CT and density quantified. In Aim 2, subjects are lesioned, but not treated for three months. Treatment then begins for 1 month followed by bone density analysis as in Aim 1



Left femur trabecular region as 3D reconstruction. Sample taken from a 10 day post-SCI subject.

Timeline and Cost

Activities CY	16	17	18	
Aim 1: Acute studies				
Aim 2: chronic studies				
Text (Major aim/study/milestone)				
Text (Major aim/study/milestone)				
Estimated Budget ($K)		year 1		
		$166,802		

Updated: 10/03/17

Goals/Milestones (Example)

CY16-17 Goals – Aim 1: perform acute therapeutic interventions
perform endstage bone density measurements
Initiate Aim 2
perform spinal surgeries establishing chronic SCI model

CY17-18 Goal –
Continue chronic surgery preparation
initiate therapeutic treatment (delayed) for chronic studies)
complete endstage bone density measurements

Comments/Challenges/Issues/Concerns

- adapt to time delay by purchasing new computer workstation for bone density analysis (completed-note that this did not require DOD funds).

Budget Expenditure to Date
Projected Expenditure:
Actual Expenditure: $166,802