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

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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 spinal cord injury or restore bone density when delivery is delayed until the late, chronic period of injury. Dr. Grill, the PI of this project passed away at the very end and did not complete the work. Dr. Grayson and her assembled team has asked to complete the work and publish the findings. Here we lay out the work that was completed by Dr. Grill and the outline of a plan to complete this work by replacement PI, Bernadette Grayson.

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

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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.

Dr. Ray Grill 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, he wished to determine whether this class of drugs can serve as a safe and novel therapeutic for the treatment of SCI-induced osteoporosis.

The goal of this contract is to determine 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 was to be 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

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) **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?

We found all the scans and we were able to finish the analysis of the bones during the intervening time since Dr. Grill's passing and the present time point. This is the data we have identified and analyzed so far:

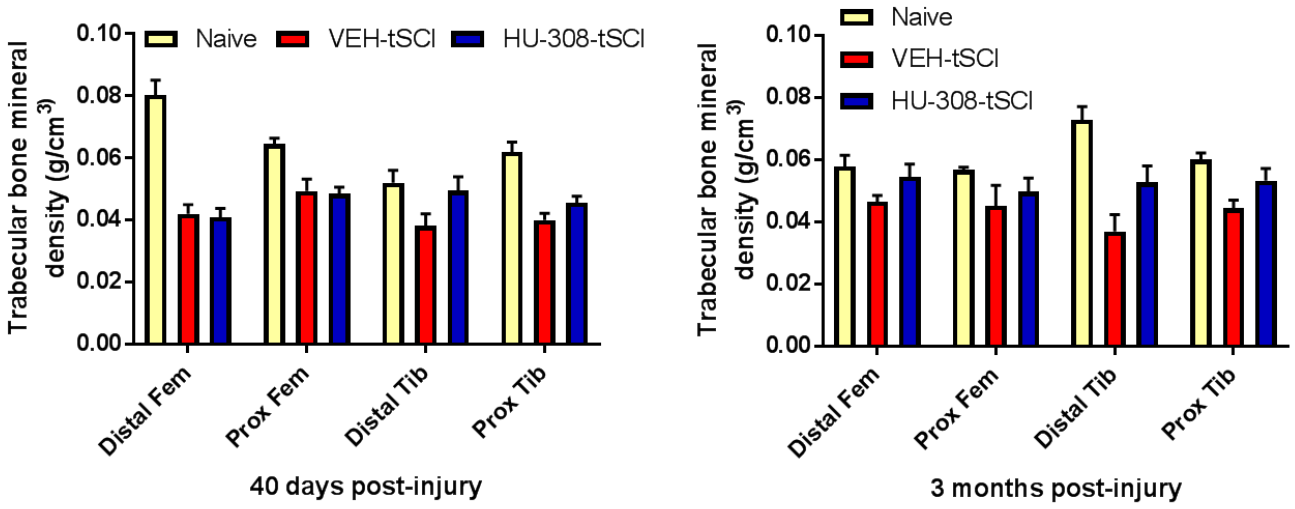


Figure 1: Trabecular Bone mineral density was calculated for C57BL6 mice that were treated either with Vehicle and received tSCI or Cannabinoid agonist (HU-308),. Naïve show greater density at both time points and at all levels. HU308 treatment improves the density when given 3 months after injury.

SPECIFIC AIM 1-PREVENT OSTEOPOROSIS

1) Three doses were to be administered. Two of these doses, namely the 1mg/kg and 100 mg/kg of HU-308 caused illness and autophagy in these animals according to previous reports. Therefore, only the 10mg/kg dose was used.
2) We were able to identify in the freezers the following tissues for SPECIFIC AIM 1
    a. 10 day time point-No samples
    b. 20 day time point-No samples
    c. 30 day time point-No samples
    d. 40 day time point- Naïve, N=11, Vehicle/SCI=12 and 10mg/kg SCI = 12

3) For the 40 day time point, on days 3 and 30 mice were to receive an injection of fluorochrome calcein (Sigma) (15 mg/kg). This was injected but no analysis has been done on this.
4) All the densitometry analysis has been completed on the 40 day animals.
5) No reconstructions of the images have been done for publication prior to the PI transfer
6) No sectioning of the bones was done prior to PI transfer
7) No histology work was done prior to the PI transfer.

Previously Dr. Grill reported that mice treated with either the low (1 mg/kg) or high (100 mg/kg) doses exhibited nearly 100% autophagia and significantly morbidity. He planned on not completing these doses and we will not undertake them.

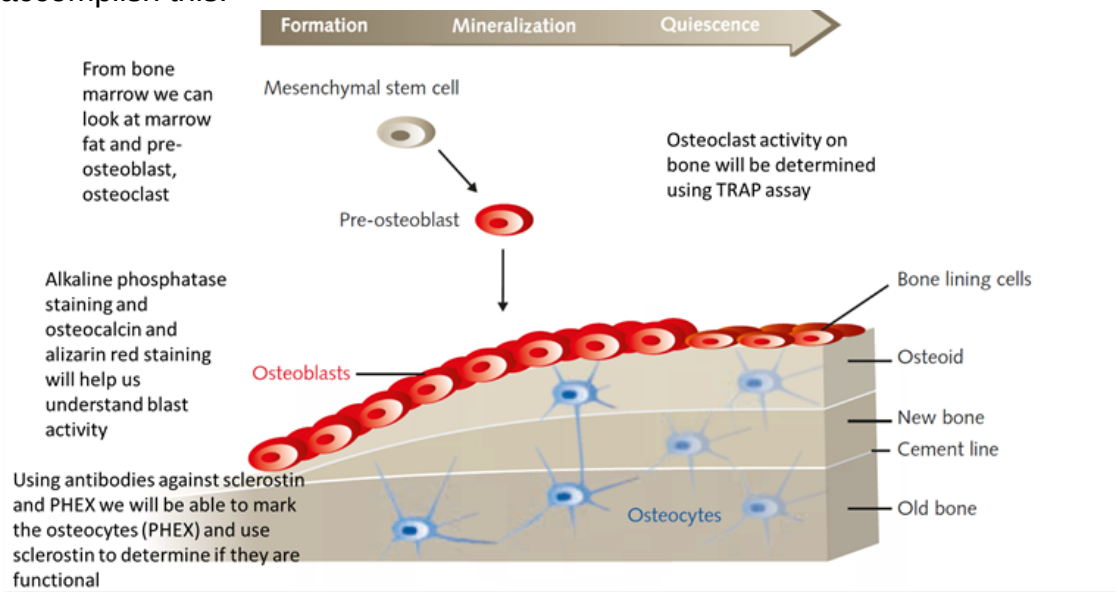
SPECIFIC AIM 2- REVERSING OSTEOPOROSIS

The goal of specific aim 2 was to reverse osteoporosis. The state of this work is as follows:

1) Injured subjects received only supportive, post-operative treatment until the end of month 3 post-SCI. At 3 months post-injury for 30 days SCI treated animals received 10 mg/kg HU-308, Naïve, N=9, Vehicle/SCI=8 and 10mg/kg SCI = 9
2) All densitometry has been completed on the bones.
3) No reconstructions of the images have been done for publication.
4) On days 3 and 29 of month 4, all subjects were to receive an IP injection of calcein. However, this was not completed. The animals were euthanized without the calcein. We will not be able to redo this but we can do histology to determine the amount of improvement in bone density.
5) No sectioning of the bones was done prior to PI transfer
6) No histology work was done prior to the PI transfer.

NEXT STEPS to finish this project

For Specific Aim 1 and 2 we will reconstruct using software those portions of the bone that we are sectioning in order to do side by side comparisons of the bone densitometry with the histologic analysis. This takes an extended period of time. Dr. Tucci has some experience with this and has more time than Dr. Chade at this point. Therefore she will work with the RA3 to accomplish this.



For Specific Aim 1 and 2 we will be sectioning the bones that were identified and then using the following staining methods for both the distal and proximal tibia and femur (See schematic above).

a. APOPTOSIS (cell death)
    i. TUNEL staining will be performed to determine whether the osteocytes are apoptotic.
b. MECHANICAL RESORPTION
    i. Alkaline Phosphatase histology will allow identification of the osteoblasts during matrix maturation during bone development.
    ii. Sclerostin antibody immunohistochemistry will be used to determine the communication from osteocyte to osteoblast.
    iii. PHEX antibody will label osteocytes and allow us to quantify them in the bone.
    iv. TRAP staining- This staining will assay the activity of the Osteoclasts.
c. MINERALIZATION
    i. Alizrin red stains for mineralization of the bone. We will compare the levels of bone mineralization.
d. BONE MARROW ASSESSMENT
    i. Determine the amount of fat present histologically. CB1 agonist should increase bone marrow fat.
e. NEURAL INNVERVATION OF THE BONE- It is possible that the bone is no longer innervated properly after tSCI and that the HU-308 compound protects innervation. Here we will stain for these neural components:
    i. CB1 receptors (cannabinoid receptors)
    ii. NPY Neuropeptide Y
    iii. NPY1 receptors (Neuropeptide Y 1 receptor)
    iv. CGRP (calcitonin gene related peptide)

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

Nothing to report.

How were the results disseminated to communities of interest?

The results have not been disseminated.

What do you plan to do during the next reporting period to accomplish these goals?
1)

Impact:

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

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:

After assessing the work that was completed under Dr. Grill, we are going to work hard to analyze the samples histologically in order to determine how the bone is changing and altering in the treated animals. We are not planning on continuing any more animal work at this time since the work necessary to perform the correct analysis of the bone is extensive.

Changes that had a significant effect on expenditures:

We have revamped the budget for this grant. We are working towards reconstruction of the images, histology and preparing this paper for publication.

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

Dr. Grayson has taken steps to become PI of the IACUC protocol.

Significant changes in use or care of human subjects:

Not applicable

Significant changes in use or care of vertebrate animals:

None.

Significant changes in us of biohazards and or select agents:
None to report

PRODUCTS:

Nothing to report yet.

Other publications, conference papers, and presentations.

No presentations have been made to our knowledge.

Website(s) or other Internet site(s)

Nothing to Report.

Technologies or techniques

Nothing to Report.

Inventions, patent applications, and/or licenses

Nothing to Report.

Participants and Other Collaborating Organizations:

What individuals have worked on the project: from 6/2017-6/2018

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?

Dr. Grayson has asked to be the Replacement PI and to complete the remaining work on this grant. The work to be completed is significant and will take time to complete. Dr. Tucci who has gre

No other organizations were involved as partners.

What NEW individuals will work on the project?

Name:	*Bernadette Grayson*
Project Role:	*PI on this project*
Researcher Identifier (e.g. ORCID ID):	*GRAYSON*
Nearest person month worked:	*20% effort*
Contribution to Project:	*Dr. Grayson is the PI on this project*
Funding Support:	*I also receive NIH funding 1 P20 GM121334 01*

	DOD contract

Name:	*Michelle Tucci*
Project Role:	*Professor*
Researcher Identifier (e.g. ORCID ID):	*None*
Nearest person month worked:	*20% Effort on this project*
Contribution to Project:	*Dr. Tucci is leading the bone histology*
Funding Support:	*Supplemental department funds*

Name:	*Yilliyanis Pride*
Project Role:	*Research Assistant 3*
Researcher Identifier (e.g. ORCID ID):	*None*
Nearest person month worked:	*100% effort*
Contribution to Project:	Cutting tissues, staining and
Funding Support:	

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

Nothing to report

What other organizations were involved as partners?

Nothing to report

SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *Not/applicable*

QUAD CHARTS: *Attached*

APPENDICES: *Attached.*

********** **ADDITIONAL NOTES:**

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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: ***Bernadette E. Grayson*** (Replacement for Raymond Grill **Org:** University of Mississippi Medical Center **Award Amount:** $185.910

Study/Product Aim(s)

PREVENT OSTEOPOROSIS

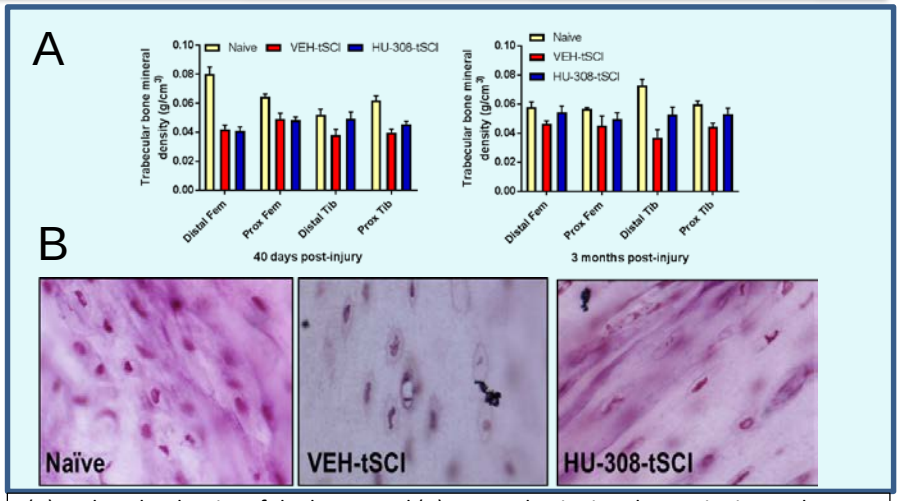
• Determine if CB2 agonist, HU-308, can prevent osteoporosis when administered during the acute phase of SCI.

REVERSE OSTEOPOROSIS

• Determine whether delayed treatment with HU-308 treats or reverses established osteoporosis in a chronic model of mouse spinal transection SCI.

Approach

We use a full spinal transection model in adult male mice to induce rapid and progressive osteoporosis. We assess early vs. delayed treatment with a cannabinoid 2 receptor agonist in either preventing or reversing osteoporosis.



(A) Trabecular density of the bones and (B) We are beginning the sectioning and staining of the bones. Here we see representative images of each of the 3 groups in AIM 1.

Timeline and Cost

Activities CY	F18	W19	SP19	SU19
Aim 1: Sectioning of bones Aim 1: Bone reconstruction				
Aim 2: Sectioning of bones Aim 2: Bone reconstruction				
Aim 1: Histology outlined Aim 2: Histology outlined				
Write and publish paper				
Estimated Budget ($K)	**$000**	**$000**	**$000**	**$185910**

Updated: (10.12.18)

Goals/Milestones (Example)

CY16 Goal – completion of all surgeries in Aim 1a: almost accomplished

CY16 Goals – initiation of surgeries needed to induce chronic spinal cord injury: underway

CY17 Goal – complete bone density assessments in Aim 1, surgery/delayed dosing and bone density assessments in Aim 2.

this is a 2 year grant.

Budget Expenditure to Date

Projected Expenditure: We feel we can complete the work we have set out to accomplish with the plan we have set forth in the current time frame.