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AWARD NUMBER: W81XWH-18-1-0249

TITLE: “Drug-Induced Hypothermia plus Glibenclamide for Rapid, In-Field Treatment of SCI”

PRINCIPAL INVESTIGATOR: J. Marc Simard, M.D., Ph.D.

RECIPIENT: University of Maryland, Baltimore

Baltimore, MD 21201

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PREPARED FOR: U.S. Army Medical Research and Materiel Command

Fort Detrick, Maryland 21702-5012

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Sept 2019	Annual	1 Sept 2018-31 Aug 2019

4. TITLE AND SUBTITLE

"Drug-Induced Hypothermia plus Glibenclamide for Rapid, In-Field Treatment of SCI"

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5b. GRANT NUMBER
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5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

University of Maryland, Baltimore
685 W. Baltimore Street
MSTF, Room 6-30
Baltimore, MD 21201

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

SPECIFIC AIMS: In ***Aim 1***, we will directly compare physical hypothermia and drug-induced hypothermia by DHC, with the goal of demonstrating that in SCI drug-induced hypothermia is therapeutically equivalent to physical hypothermia. In ***Aim 2***, we will directly compare DHC-induced hypothermia vs. glibenclamide, with the goal of demonstrating that in SCI, the combination of the two is superior to either alone.

IMPACT: Establishing the safety and benefit of drug-induced hypothermia could greatly expand the use of hypothermia not only in SCI but in traumatic brain injury and other conditions of importance to the mission of the Department of Defense. This proposed study directly impacts the Area of Encouragement: "Pre- hospital, prolonged field care, en route care, and early hospital management of SCI".

MILITARY RELEVANCE: The proposed research project, by optimizing in-field and initial critical care treatments, holds the promise of reducing the burden of SCI suffered by military Service members, Veterans, and their family members and caregivers.

15. SUBJECT TERMS

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	15	**19b. TELEPHONE NUMBER** *(include area code)*

Table of Contents

The text of the report must include all sections addressed in the table of contents to include the following. **DO** include the bolded section headings, but **DO NOT** include the *italicized* descriptions of section contents in your submitted reports.

1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

Rats are subjected to severe cervical SCI and will be instrumented for telemetric monitoring of temperature, heart rate and activity. The overall approach is to perform a direct head-to-head comparison of treatments, physical hypothermia and chemical hypothermia produced by IV infusion of Dihydrocapsaicin (DHC) outcome measures will include microRNA serum biomarkers, neurological and urological function, body weight, and spinal cord tissue evaluations.
Establishing the safety and benefit of drug-induced hypothermia could greatly expand the use of hypothermia not only in SCI but in traumatic brain injury and other conditions of importance to the mission of the Department of Defense. This proposed study directly impacts the Area of Encouragement: "Pre- hospital, prolonged field care, en route care, and early hospital management of SCI". In addition, because we seek to minimize lesion expansion, we expect that recovery from SCI will be enhanced, and that long-term harmful consequences of SCI, including neurological and urological abnormalities, will be greatly reduced.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

Spinal cord injury, progressive hemorrhagic necrosis, physical hypothermia, chemical hypothermia, dihydrocapsaicin, glibenclamide,

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.*

- **What were the major goals of the project?**
  - *List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project identify these dates and show actual completion dates or the percentage of completion.*

In ***Aim 1***, we will directly compare physical hypothermia and drug-induced hypothermia by DHC, with the goal of demonstrating that in SCI, as has been found in cardiac arrest, that drug-induced

hypothermia is equivalent or superior to physical hypothermia. These experiments will examine different treatment start times, 2–6 hours, for clinical relevance. Outcome measures will include microRNA serum biomarkers, multiple neurofunctional tests, and spinal cord tissue evaluations. In ***Aim 2***, we will directly compare drug-induced hypothermia by DHC vs. glibenclamide, and we will determine whether the combination of the two is superior to either alone. These experiments will be performed at a single treatment start time, 4 hours. Outcome measures will be the same as in Aim 1

- **What was accomplished under these goals?**
  - *For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.*

Statement of Work Subtask 1 completed

Local IACUC Approval

ACURO approved

We are currently in the process of accomplishment of Major Tak1 /Subtasks 2, 3, 4, and 5

4. All equipment used in the study (Telemetry , Ultrasound etc.) was tested prior to the beginning of the experiments.
5. Infusion rates for drug induced hypothermia were tested on naïve animals to optimize the rate to match temperature drop to the animals with physical hypothermia. Average temperature during physical hypothermia 32.8+0.3 ^{O}C, during DHC-induced hypothermia 32.6+0.6 ^{O}C
6. We finished survival surgeries for Experimental group 5 (DHC hypothermia with 4 hours delay), Experimental group 6 (Physical hypothermia with 4 hours delay), and Experimental group 2 . Currently animals are being monitored and undergo blinded testing described under Major Task1/ Subtask 4. Currently tested animals are in the weeks 2-5 after survival surgery.
7. We started survival surgeries for Experimental group 7 (DHC hypothermia with 6 hours delay) and Experimental group 8 (Physical hypothermia with 6 hours delay)

So far major findings are as follows:

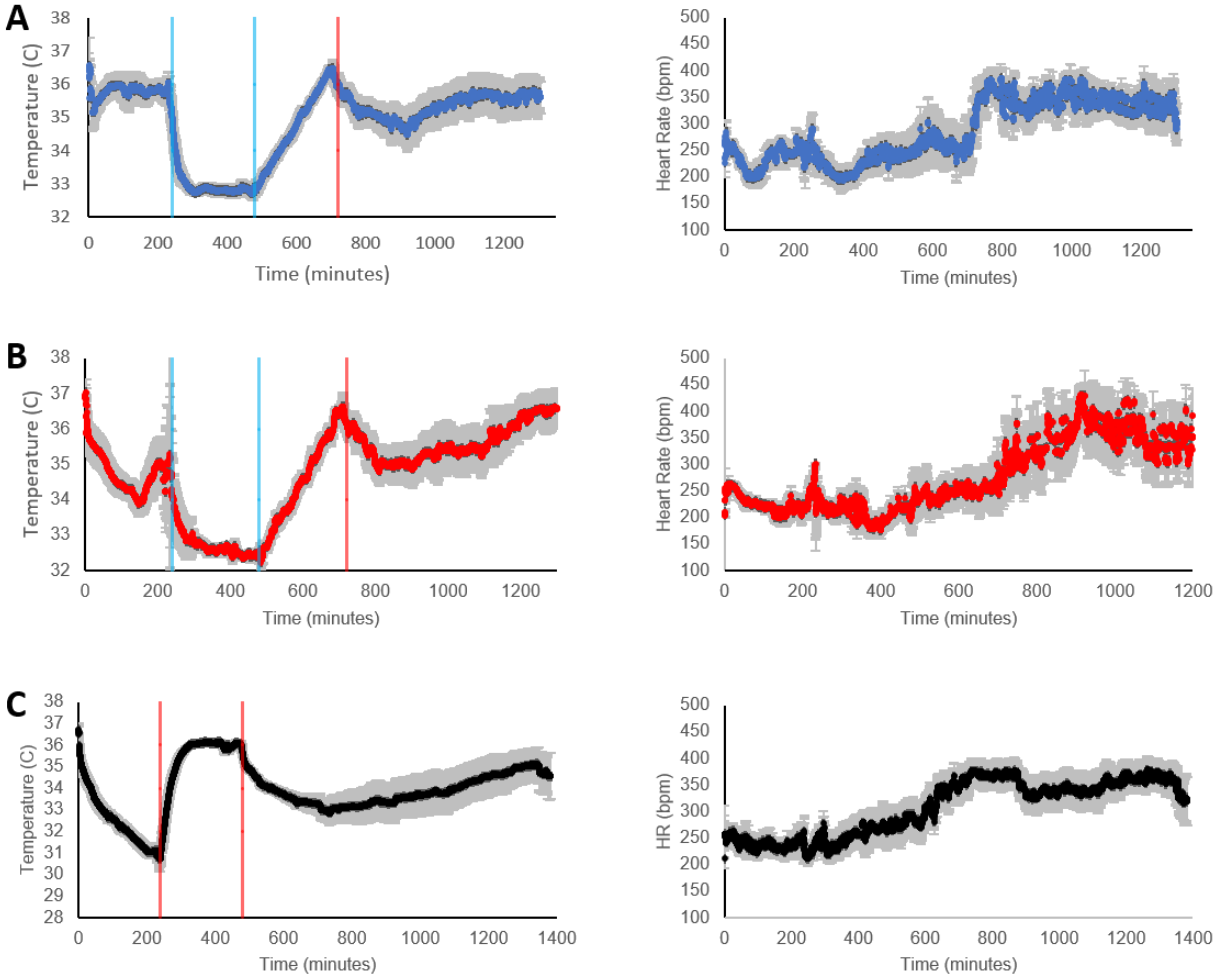


Figure 1: Core temperature and heart rate data. Physical cooling (A) and DHC-induced cooling (B) showed similar cooling trends, shown between blue markers. Rewarming was also consistent between the two groups, up to the red marker. Normothermic controls (C) were maintained at 36-37°C in the warming period shown between red markers but showed a fall in body temperature overnight following treatment. No significant patterns were noted in heart rate data.

Temperature Modulation

Intravenous DHC administration (Figure 1b) reliably produced a sustained reduction in core body temperature to 33.0 ± 1.0°C over the 4h treatment window similar to physical cooling (Figure 1a). In normothermic controls, core temperature data shows that although control animals were warmed to 36-37°C during the treatment period, they were unable to maintain a normothermic core temperature overnight.

No distinct patterns or differences between groups were noted in the heart rate data; specifically, no significant bradycardia was observed in the DHC-cooling group compared to physical cooling.

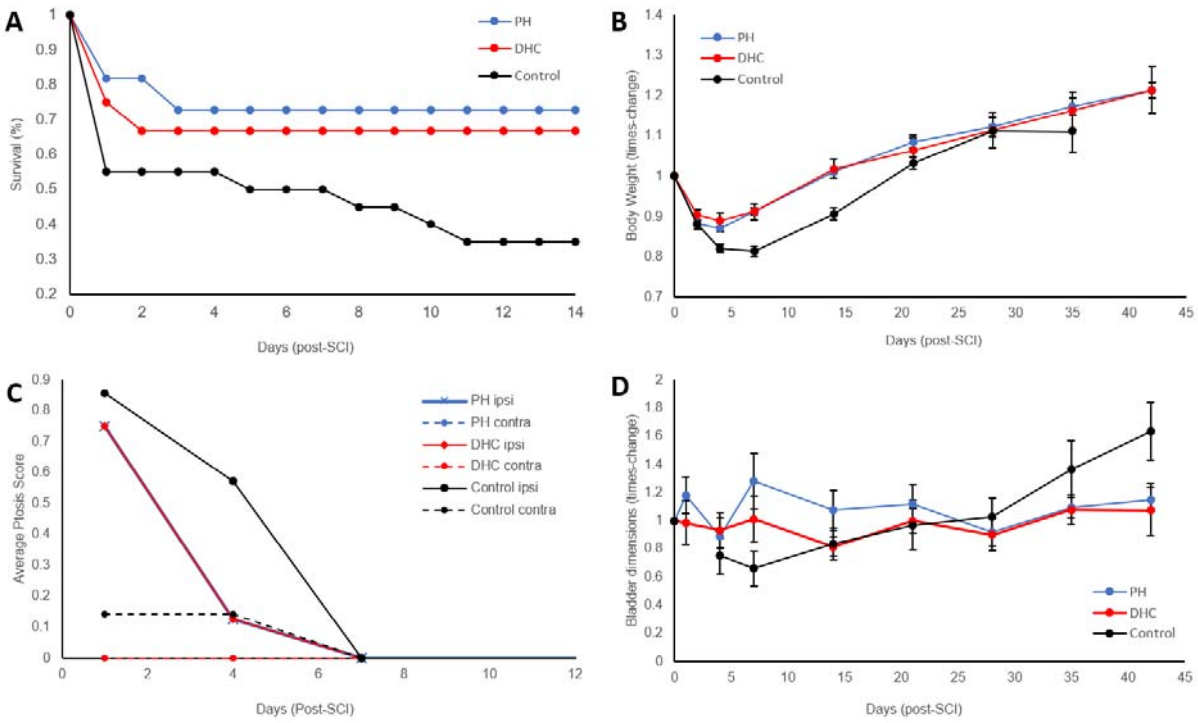


Figure 2: Survival, body weight, ptosis, and bladder dimensions. (A) Both physical cooling (PH) and DHC-hypothermia (DHC) improved survival rates. (B) Body mass (expressed as times-change from pre-SCI weight) demonstrates both hypothermia treatments (PH and DHC) resulted in reduced loss of body mass after SCI. (C) Average ptosis scores over time (1 for ptosis, 0 for none) show that both treatments reduce the incidence of ptosis overall and eliminate its presence entirely in the contralateral eye. (D) Bladder dimensions obtained via ultrasound (expressed as times-change from pre-SCI measurement).

Survival

Survival rate was 72% in the physical hypothermia group (8/11) and 66% (8/12) in the DHC-hypothermia group, while only 35% (7/20) of animals in the normothermic control group survived after 10 days (Figure 2a).

Body Weight

Over the first 3 weeks, both physical cooling and DHC-hypothermia resulted in reduced loss of body mass (times-change) compared to normothermic controls. Over the course of 6 weeks, control animals eventually reached similar body masses to the hypothermia treated groups (Figure 2b).

Ptosis

Ptosis was not seen in any group past 7 days. Average ptosis score (1 for ptosis, 0 for normal eye function) was highest in the ipsilateral eye of normothermic control animals. Furthermore, ptosis was noted in the contralateral eye in 1/7 control animals and was not noted in the contralateral eye of any treated animals (Figure 2c).

Bladder Dimensions

Previous studies have cited urinary retention as a potential consequence of spinal cord injury[51]. However, we did not notice significant patterns of retention in any of the groups (Figure 2d).

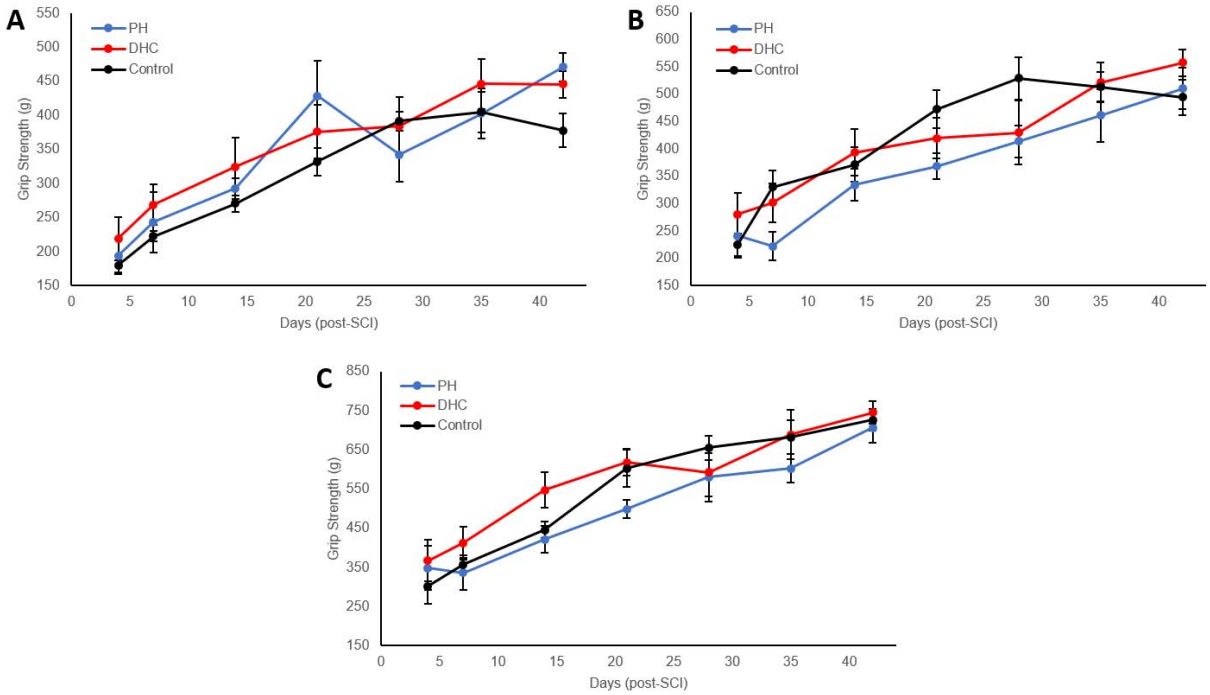


Figure 3: Grip strength. (A) Grip strength (g) in the ipsilateral (left) hindlimb over 6 weeks in physical hypothermia (PH), DHC-hypothermia (DHC) and normothermic control (Control) animals. (B) Grip strength (g) in the contralateral hindlimb over 6 weeks in PH, DHC, and Control animals. (C). Grip strength (g) in both hindlimbs over 6 weeks in PH, DHC, and Control animals.

Grip Strength

Grip strength was tracked over 6 weeks in the ipsilateral (Figure 3a) and contralateral (Figure 3b) hindlimb separately, as well as in both hindlimbs together (Figure 3c). No significant differences were noted between any groups in grip strength.

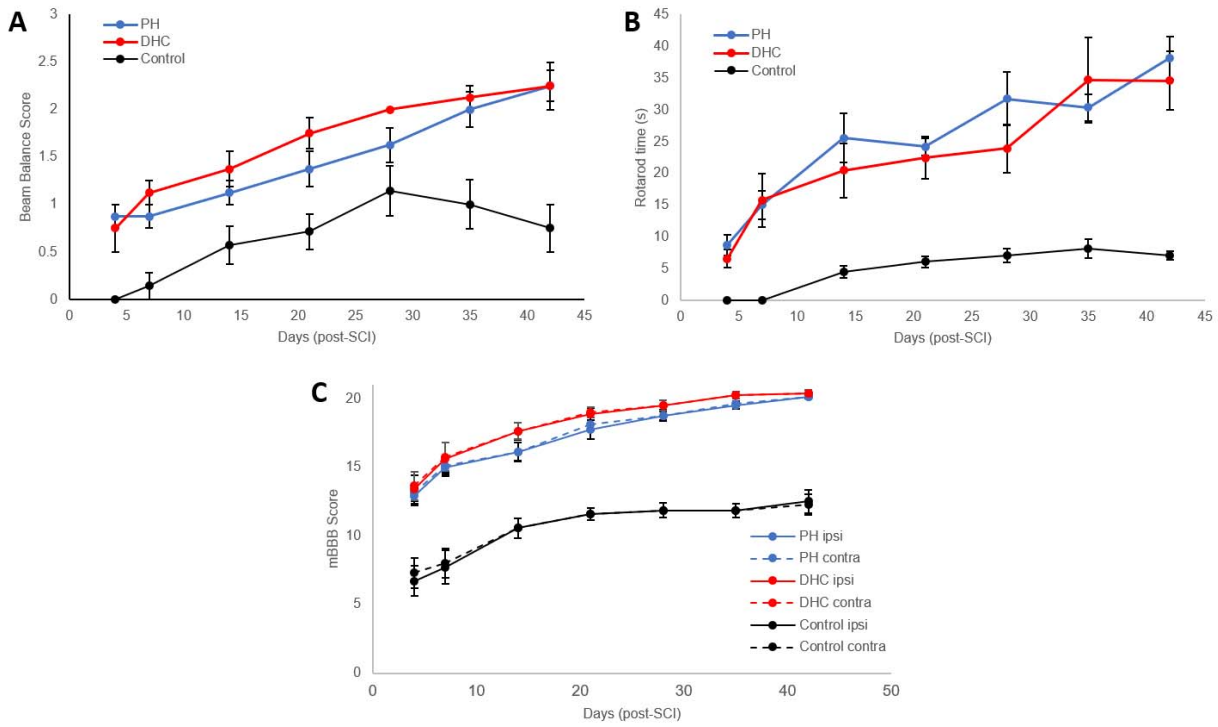


Figure 4: Beam balance, accelerating Rotarod, and mBBB scores. (A) Average beam balance score for physical hypothermia (PH), DHC-induced hypothermia (DHC) and normothermic control (Control) animals over 6 weeks. (B) Average accelerating Rotarod time (time to fall off in seconds) for PH, DHC, and Control animals over 6 weeks. (C) Average ipsilateral and contralateral mBBB score (0-21) for PH, DHC, and Control animals over 6 weeks.

- **What opportunities for training and professional development has the project provided?**
  - *Nothing to report*
- **How were the results disseminated to communities of interest?**
  - *Nothing to report*
- **What do you plan to do during the next reporting period to accomplish the goals?**
  - *If this is the final report, state "Nothing to Report."*
  - *Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.*

8. **IMPACT:** *Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

- **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*

9. **CHANGES/PROBLEMS:** *The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

- **Changes in approach and reasons for change**
  - *No changes*
- **Actual or anticipated problems or delays and actions or plans to resolve them**
  - *There was 1.5 month delay at the beginning of the projects due to the delay in approval of the of the ACURO, however the workflow planned for the first 12 month of the project is completed.*
- **Changes that had a significant impact on expenditures**
  - *No changes*
- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**
  - *No changes*

10. **PRODUCTS:** *Nothing to report*

11. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

- **What individuals have worked on the project?**
  - *Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160*

hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change."

No change from previous submission

Name:	*J.M. Simard*
Project Role:	*Principal Investigator*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*12*
Contribution to Project:	*Dr. Simard worked on SCI+physical and hypothermia experiments Major task1 Subtasks 2,3,4,5 SOW Supervised project participated in survival surgeries.*
Funding Support:	*This award*

Name:	*X.Jia*
Project Role:	*Co-Investigator*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*6*
Contribution to Project:	*Dr. Jia worked on SCI +physical hypothermia experiments Major task1 Subtask 2 SOW. Performed survival surgeries and post op monitoring*
Funding Support:	*This award*

Name:	*O. Tsymbalyuk*
Project Role:	*Co-Investigator*

Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*12*
Contribution to Project:	*Dr. Tsymbalyuk worked on SCI+physical hypothermia experiments Major task1 Subtask 2 SOW. Performed survival surgeries and post op monitoring*
Funding Support:	*This award*

Name:	*K. Keledjian*
Project Role:	*Co-Investigator*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*12*
Contribution to Project:	*Dr. Keledjian worked on SCI+physical hypothermia experiments Major task1 Subtask 2 SOW Performed monitoring , ultrasound.*
Funding Support:	*This award*

Name:	*S. Ivanova*
Project Role:	*Co-Investigator*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*12*

Contribution to Project:	*Dr. Ivanova worked on SCI+physical hypothermia experiments Major task1 Subtask 2 SOW. Performed tissue and blood collection. Behavioral testing., second independent observer*
Funding Support:	*This award*

- **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*
    - **Organization Name:**
    - **Location of Organization:** *(if foreign location list country)*
    - **Partner's contribution to the projec**t *(identify one or more)*
      - **Financial support;**
      - **In-kind support** *(e.g., partner makes software, computers, equipment, etc., available to project staff)*;
      - **Facilities** *(e.g., project staff use the partner's facilities for project activities)*;
      - **Collaboration** *(e.g., partner's staff work with project staff on the project)*;
      - **Personnel exchanges** *(e.g., project staff and/or partner's staff use each other's facilities, work at each other's site)*; and
      - **Other.**

12. **APPENDICES:** *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc. Reminder: Pages shall be consecutively numbered throughout the report.* ***DO NOT RENUMBER PAGES IN THE APPENDICES.***

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

MARKING OF PROPRIETARY INFORMATION: Data that was developed partially or exclusively at private expense shall be marked as "Proprietary Data" and Distribution Statement B included on the cover page of the report. Federal government approval is required before including Distribution Statement B. The recipient/PI shall coordinate with the COR/GOR to obtain approval. **REPORTS NOT PROPERLY MARKED FOR LIMITATION WILL BE DISTRIBUTED AS APPROVED FOR PUBLIC RELEASE.** It is the responsibility of the Principal Investigator to advise the COR/GOR when restricted limitation assigned to a document can be downgraded to "Approved for Public Release." **DO NOT USE THE WORD "CONFIDENTIAL" WHEN MARKING DOCUMENTS. DO NOT USE WATERMARKS WHEN MARKING DOCUMENTS.**

Drug-induced hypothermia plus glibenclamide for rapid, in-field treatment of SCI.
Proposal Log Number SC170199 Task1/Subtask1
Award Number W81XWH-18-1-0249

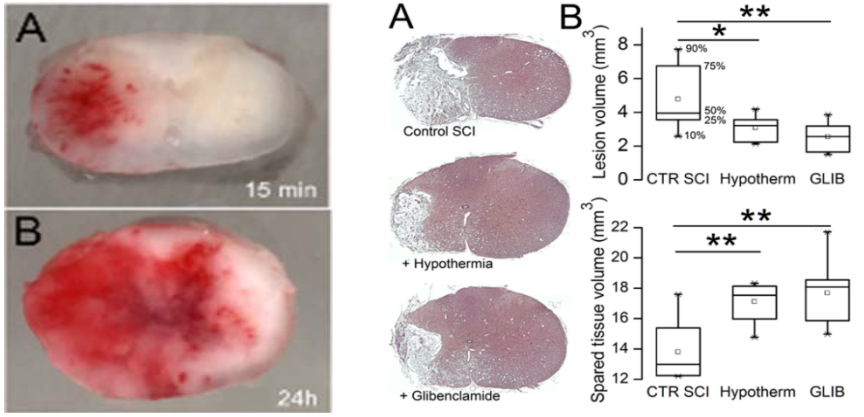
PI: J. Marc Simard **Org:** Maryland, University of, Baltimore **Award Amount:** $500,000

Study Aims

- In ***Aim 1***, we will directly compare physical hypothermia and drug-induced hypothermia by Dihydrocapsaicin (DHC), with the goal of demonstrating that in SCI that drug-induced hypothermia is therapeutically equivalent to physical hypothermia
- In ***Aim 2***, we will directly compare DHC-induced hypothermia vs. glibenclamide, and we will determine whether the combination of the two is superior to either alone.

Approach

Using rodent model of the cervical SCI we will study combination of the glibenclamide and DHC-hypothermia starting 6 hours after trauma. Functional outcomes(body weight, motor and bladder function), serum microRNA biomarkers and spinal cord tissue will be evaluated to asses the extent of protection.



Lesion expansion after traumatic SCI (*left*) is a critical element that significantly determines outcome. Combination of the pharmacological hypothermia and glibenclamide (*right)*could bring acute SCI treatment directly to the patient in the field.

Timeline and Cost

Activities	CY 18	19	20
Specific Aim 1			
Specific Aim1			
Estimated Budget ($K)	**$200**	**$150**	**$150**

Updated: June 30, 2019

Goals/Milestones
CY18 Goal –Origination of the study
X Local IACUC approval
X Finishing Specific Aim1
CY19 Goals – Work on Specific Aims of the study
☐Completion of the Specific Aim1
☐Start of the Specific Aim 2
CY20 Goal – Completion of the study, creating final report
☐Completion of the Specific Aim 2
☐Analysis of the data, creating final report, prepare and submit manuscript for publication
Comments/Challenges/Issues/Concerns
Task1/Subtask2,3,4,5 in process Started Subtask 6
Budget Expenditure to Date
Projected Expenditure: $500,000 (Direct Costs)
Actual Expenditure: $103.619 (Direct Costs)