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

School of Medicine

22 S. Greene Street, Suite S12D

Baltimore, MD 21201

REPORT DATE: SEPTEMBER 2020

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
SEPTEMBER 2020	Annual	1SEPT2019 - 31AUG2020

4. TITLE AND SUBTITLE

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

5a. CONTRACT NUMBER
W81XWH18-1-0249

5b. GRANT NUMBER
W81XWH18-1-0249

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

J. Marc Simard, M.D., Ph.D.

E-Mail: msimard@som.umaryland.edu

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

NONE LISTED

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)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

Table of Contents

1. **INTRODUCTION:**

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:**

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

3. **ACCOMPLISHMENTS:**

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?**

Statement of Work Subtask 1

completed Local IACUC Approval

ACURO approved

1. All equipment used in the study (Telemetry ,Ultrasound etc.) was tested prior to the beginning of the experiments.
2. 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
3. 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.
4. All neurobehavioral testing for Experimental groups 5 and 6 completed.
5. Harvested spinal cords for Experimental groups 5 and 6 are being processed for histochemistry.
6. We finished survival surgeries for Experimental group 7 (DHC hypothermia with 6 hours delay) and Experimental group 8 (Physical hypothermia with 6 hours delay)
7. We finished survival surgeries for Experimental groups 3 (Physical hypothermia with 2 hours delay) and 4 (DHC hypothermia with 4 hours delay),
8. Analysis of the motor function shows that that treatment with physical hypothermia and DHC significantly improves outcomes post SCI with 4 hours delay treatment being more effective compared to 6 hours delay.
9. All spinal cord tissues from Experimental groups 1-8 were fixed, sectioned and processed histochemically (H&E) for lesion size.
10. Analysis of the lesion size shows that treatment with physical hypothermia and DHC significantly reduce lesion size (by 60% with 4 hours delay and by 40 % with 6 hours delay of the treatments.
11. Collected data are organized in the form of the draft of the manuscript addressing Major Task 1

So far major findings are as follows:

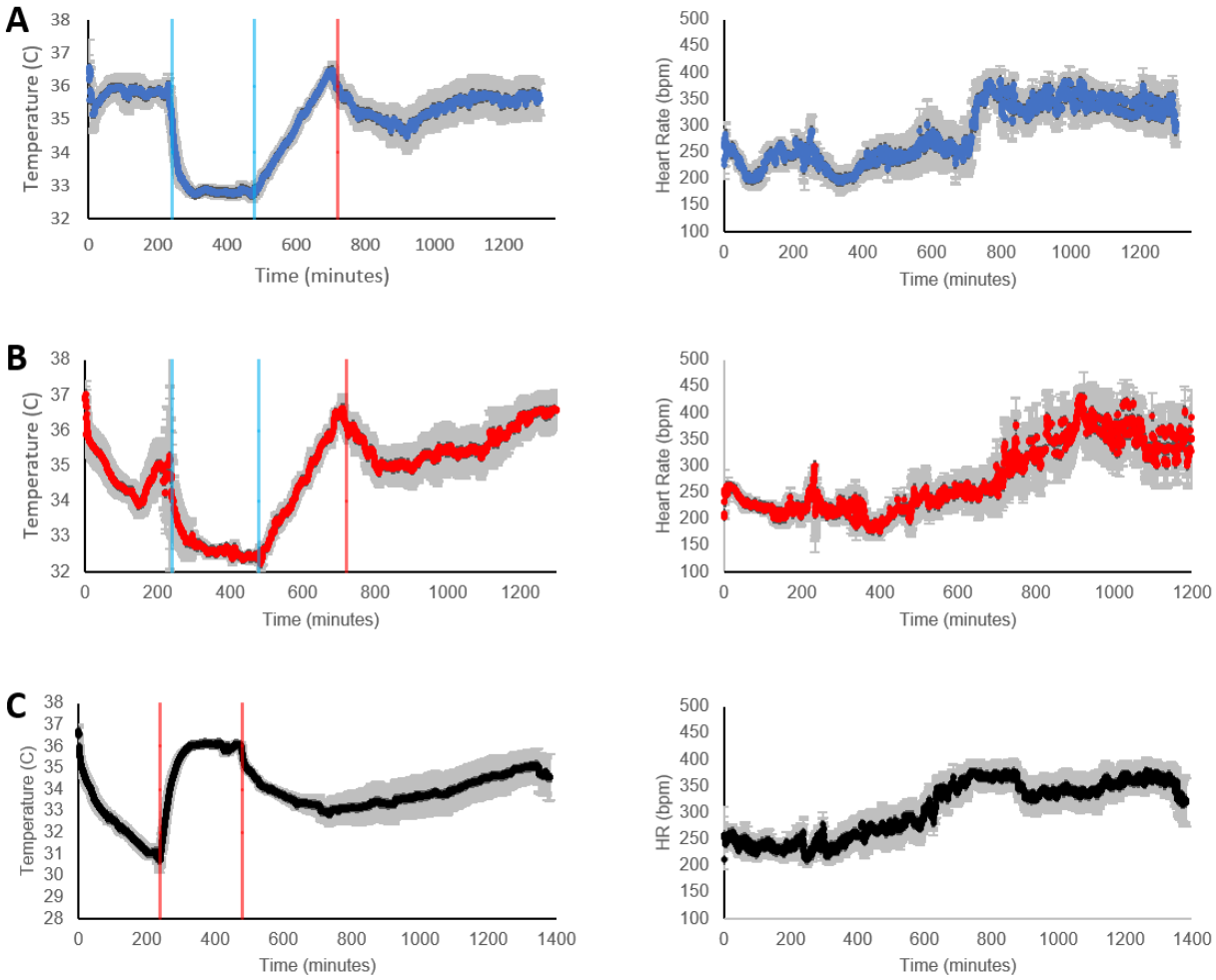


Figure 1: Core temperature and heart rate data. Physical cooling (A) and DHC-induced cooling (B) showed similar cooling trends, shown between blue markers. Rewarming was also consistent between the two groups, up to the red marker. Normothermic controls (C) were maintained at 36-37°C in the warming period 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.

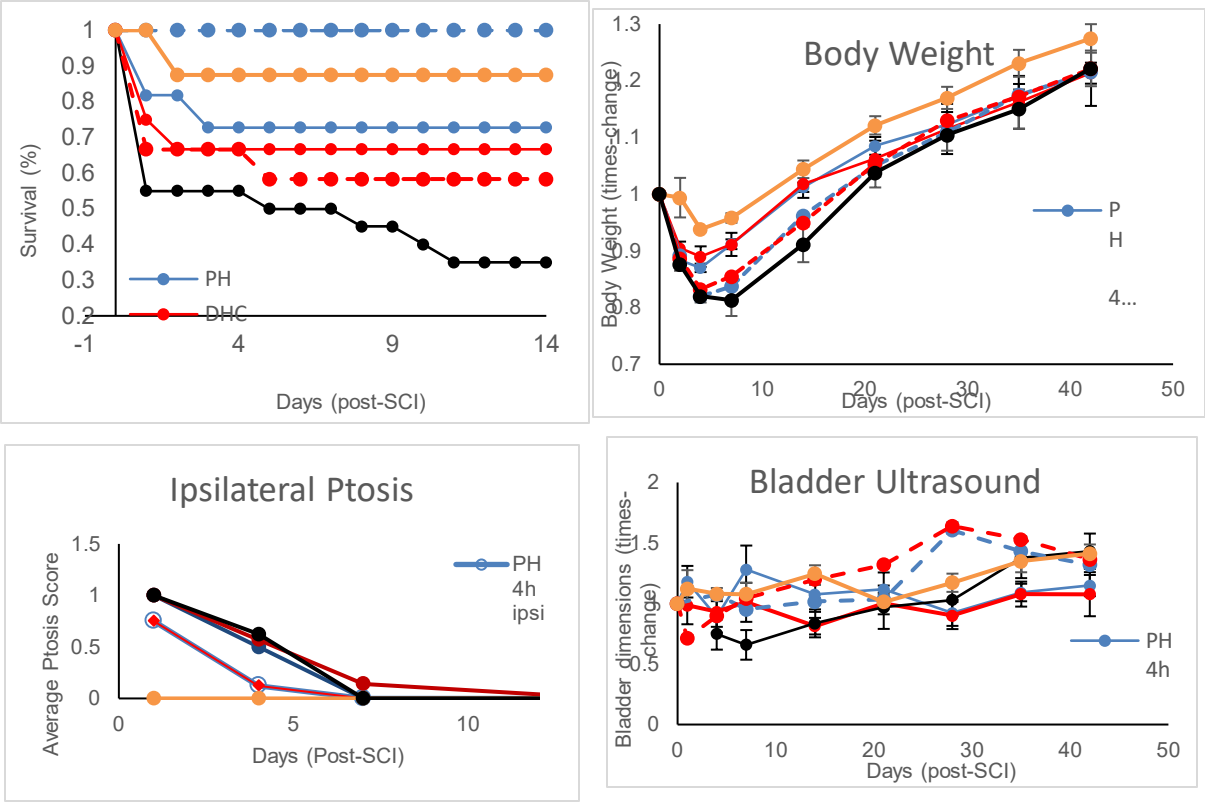


Figure 2: Survival, body weight, ptosis, and bladder dimensions. (A) Both physical cooling (PH) and DHC-hypothermia (DHC) improved survival rates. (B) Body mass (expressed as times-change from pre-SCI weight) demonstrates both hypothermia treatments (PH and DHC) resulted in reduced loss of body mass 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).

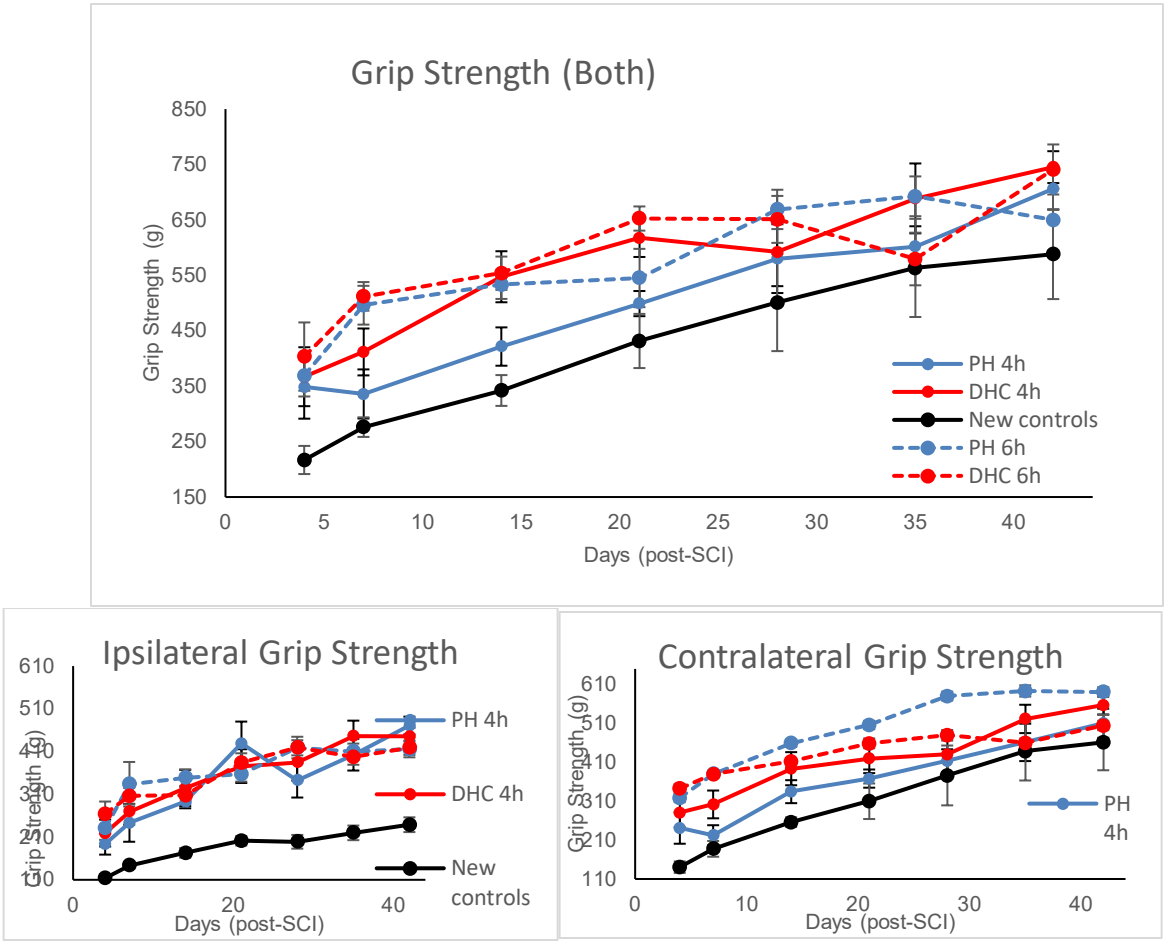


Figure 3: Grip strength. (A) Grip strength (g) in the ipsilateral (left) hindlimb over 6 weeks in physical hypothermia (PH), DHC-hypothermia (DHC) and normothermic control (Control) animals. (B) Grip strength (g) in the contralateral hindlimb over 6 weeks in PH, DHC, and Control animals. (C). Grip strength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

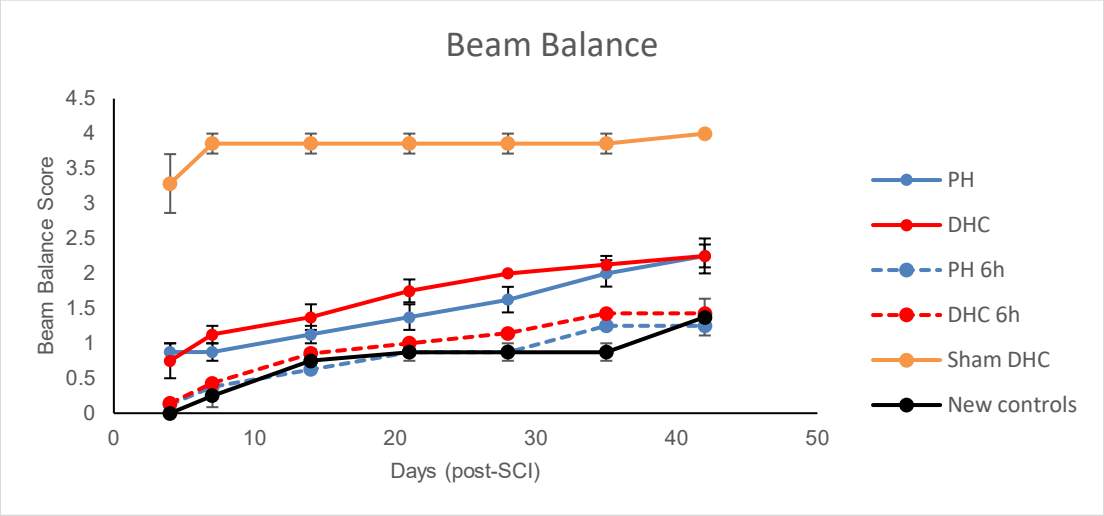
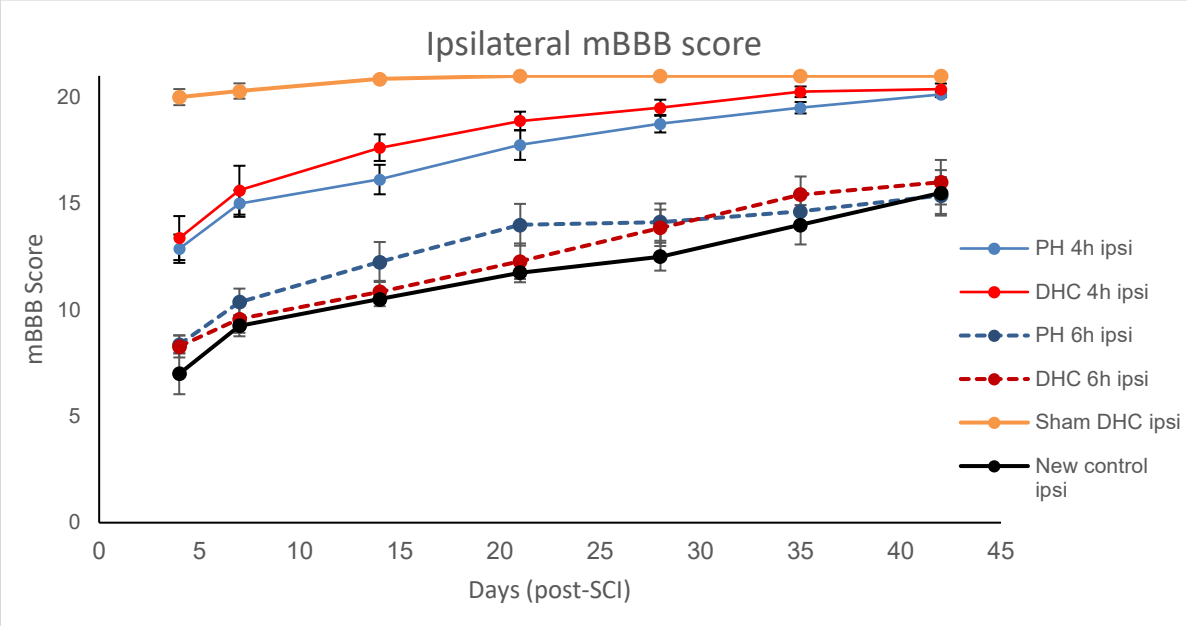


Figure 5: Beam balance,) Average beam balance score for physical hypothermia (PH), DHC-induced hypothermia (DHC) and normothermic control (Control) animals over 6 weeks. (B) Average accelerating Rotarod time (time to fall off in seconds) for PH, DHC, and Control animals over 6 weeks. (C) Average ipsilateral and contralateral mBBB score (0-21) for PH, DHC, and Control animals over 6 weeks.



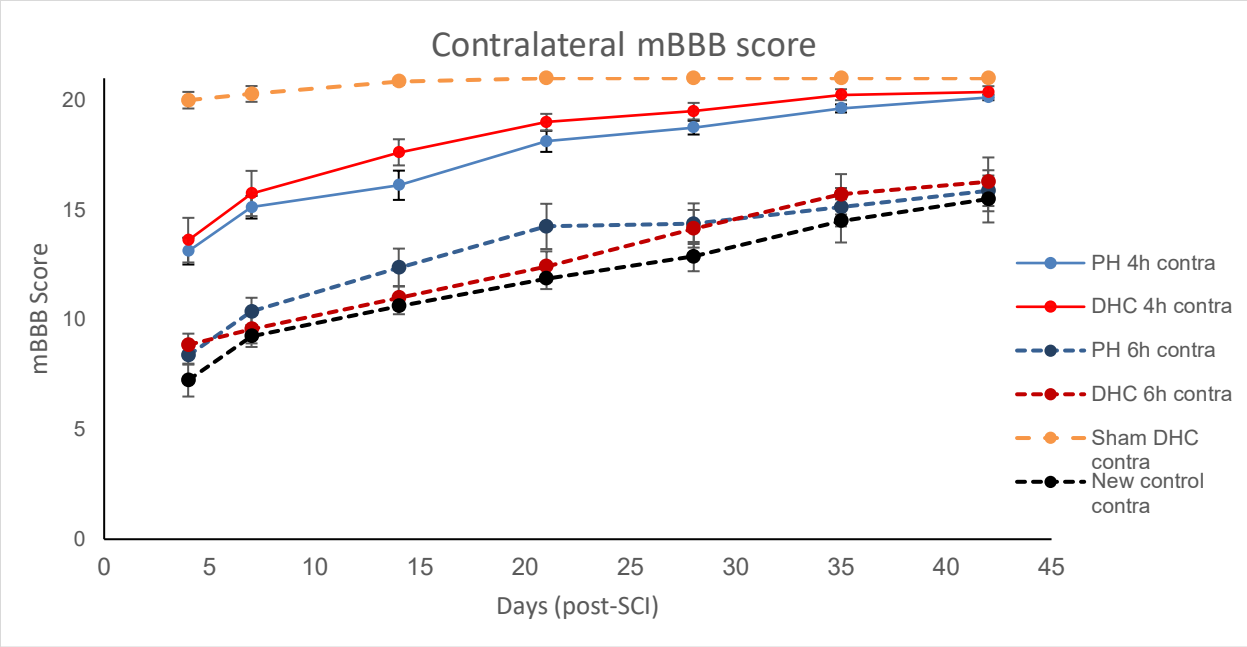


Figure 4: Modified BBB scores for physical hypothermia (PH), DHC-induced hypothermia (DHC) and normothermic control (Control) animals over 6 weeks. Average ipsilateral and contralateral mBBB score (0-21) for PH, DHC, and Control animals over 6 weeks.

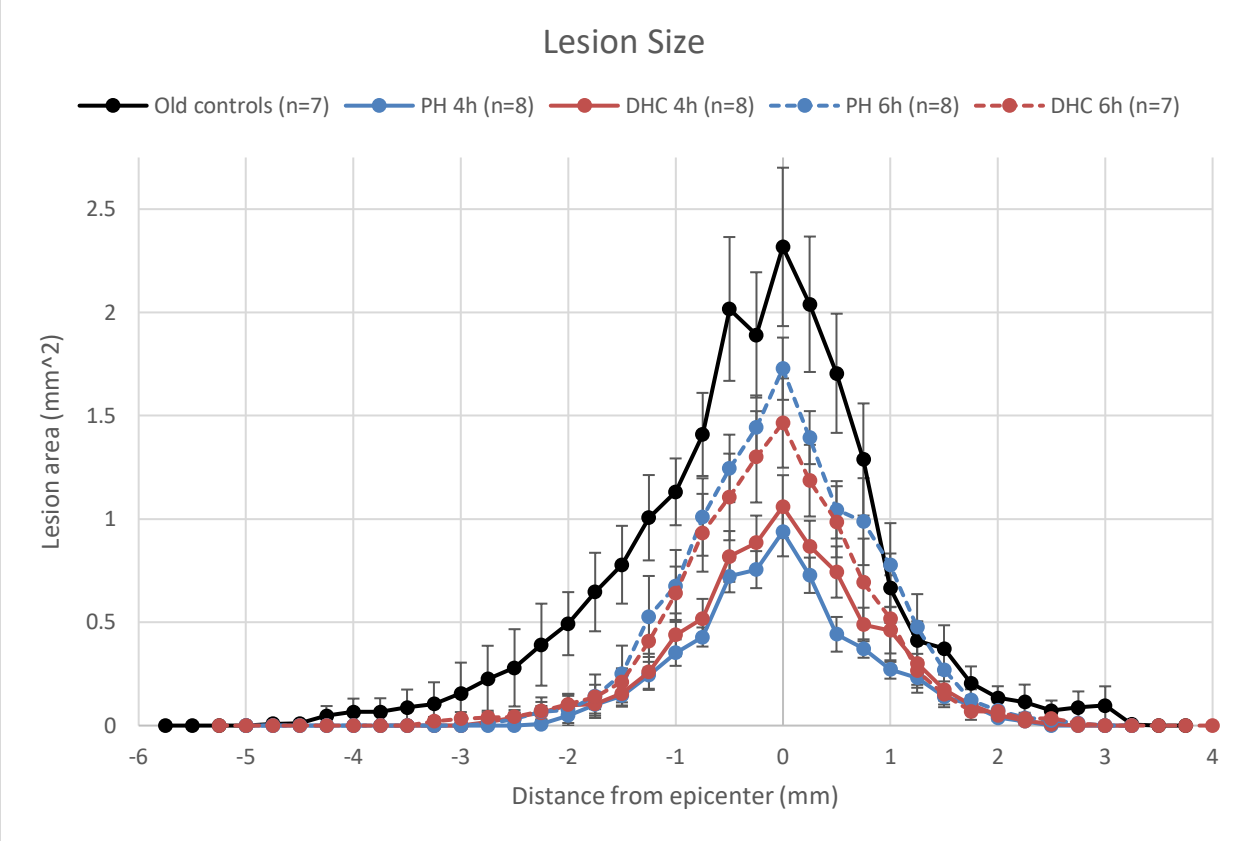


Figure 6:Caudal-Rostral distribution of the spinal cord lesion area e for physical hypothermia (PH), DHC-induced hypothermia (DHC) and normothermic control (Control) animals over 6 weeks. (B) Average accelerating Rotarod time (time to fall off in seconds) for PH, DHC, and Control animals over 6 weeks.

Data collected during previous and current year of the project clearly indicate following:

1. IV infusion of the DHC produces body hypothermia in rats with spinal cord injury.
2. Functional testing indicates that hypothermia induced by IV infusion of DHC after SCI results in neuroprotection comparable to that induced by physical hypothermia.
3. Both pharmacological and physical hypothermia result in neuroprotection when administered to 6 hours after SCI
4. Histological analysis of the spinal cords harvested from animals 6 week after SCI indicates that pharmacological hypothermia induced by DHC results in spinal cord tissue preservation comparable to that induced by the physical hypothermia.

- **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.*

5. **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*

6. **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**

During last cycle the work on this project was conducted remotely. We analyzed acquired data, including videotaped motor and neurobehavioral tests and imaged or scanned histochemical and immunohistochemical spinal cord samples. Additionally, we have worked on the draft of the first manuscript to be reporting results of the experiments were conducted so far. It will report that hypothermia induced by dihydrocapsaicin (DHC) after spinal cord injury provides neuroprotection comparable to the physical hypothermia.

As It was communicated in in previous report, we expected to start new experiments during last quarter. However due to the second wave of the COVID19 cases University kept restrictions on laboratory research. Only COVID19-related projects were allowed to be conducted. Recently University permitted to conduct non-COVID-related research projects with requirement of 25% occupancy and strict social distancing. These conditions did not allow us to start new experiments yet. Surgical procedures, post-op administration of the hypothermia and monitoring animals under inhalation anesthesia have need of at least two researchers to work in proximity.

- **Changes that had a significant impact on expenditures**

The lab is still not up and running fully. However, we are planning to complete Major Tasks of the project, possibly requesting necessitating no-cost extension of the contract. Within next cycles we will have ready manuscript addressing essential component of the project: validation of the DHC as therapeutic agent producing effective neuroprotection due to the induction of pharmacological hypothermia.

- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**
  - *No changes*

7. **PRODUCTS:** *We are currently finalizing the manuscript addressing Major task 1 of the project.*

8. **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. Keledian*
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.**
- **APPENDICES:**