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TITLE: Optimizing Hemodynamic Support of Acute Spinal Cord Injury Based on Injury Mechanism

PRINCIPAL INVESTIGATOR: Dr. Brian Kwon

CONTRACTING ORGANIZATION: University of British Columbia, ICORD
Blusson Spinal Cord Centre
818 west 10th ave, Vancouver, BC, Canada, V5Z 1M9

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Dr. Brian Kwon

E-Mail: brian.kwon@ubc.ca

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University of British Columbia, ICORD
Blusson Spinal Cord Centre
818 west 10th ave
Vancouver, BC, Canada

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14. ABSTRACT

This grant is focused on the augmentation of MAP with vasopressors and determining how such MAP augmentation would best be implemented (during compression, after decompression, or both). In **YEAR 2** of this grant we finalized the comparison of two commonly used vasopressors, norepinephrine (NE) and phenylephrine (PE) after SCI. As our data suggested NE would better maintain elevated MAPs, using NE, in **YEAR 3** we studied our first animal surgeries as part of **Aim 1-3** to determine the impact of MAP augmentation during compression and/or decompression, on tissue hemodynamics within the spinal cord after a traumatic SCI. In **YEAR 4**, we finalized a draft manuscript on the role of vasopressor support in SCI. Furthermore, in **YEAR 4** we sent human and pig CSF and serum sample to UofA for metabolomic and lipidomic profiling as part of **TASK 3** of **AIM 2** and **3.** Although, conclusive results on the lipidomics/metabolomics work was not available prior to this report, we are committed to completing the analysis within the next few months (the microdialysis samples have been collected and sent to Dr. Li – we just await the actual mass spectrometry analysis).

15. SUBJECT TERMS
Hemodynamic support, SCI, vasopressors, blood flow, oxygenation, pressure

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TABLE OF CONTENTS

1 INTRODUCTION

There are currently very few treatments to improve the neurologic outcome of individuals who sustain an acute spinal cord injury (SCI). Treatment options include urgent surgical decompression to relieve pressure on the spinal cord and aggressive augmentation of systemic blood pressure to minimize ischemia. However, improved outcomes from these approaches have not been convincingly demonstrated in randomized or controlled clinical trials, and hence they are not considered 'standards of care'. We postulate that the difficulty in unequivocally demonstrating the benefits of aggressive hemodynamic support may be due to this approach eliciting not only beneficial but also adverse effects on the injured spinal cord, depending upon the presence or absence of spinal cord compression. Our overall objective is therefore to determine how hemodynamic support of mean arterial pressure (MAP) in the presence or absence of spinal cord compression affects the vascular, metabolic, biochemical, and behavioral outcomes of traumatic SCI. We hypothesize that well-intended increases in MAP after decompression contribute to detrimental edema/swelling, hemorrhage, and increased intraparenchymal pressure, in addition to exacerbating ischemia-reperfusion injury mechanisms. To test this hypothesis, we will utilize a novel pig model of SCI, for which we have developed innovative techniques for measuring intraparenchymal spinal cord blood flow (SCBF), hydrostatic pressure, and metabolic responses over time. Achieving a better understanding of how the spinal cord responds to alterations in MAP before and after decompression will provide insights that could be deployed rapidly into clinical practice to optimize the hemodynamic management of acute SCI. Such insights have added significance for soldiers injured in combat where sophisticated therapies are likely not available and the early treatment of their SCI may be limited to basic hemodynamic resuscitation and the management of their MAP.

2 KEYWORDS

- Hemodynamic Support
- Spinal Cord Injury Based
- Cord Compression
- Cord Decompression
- Porcine model of SCI
- Spinal Cord Blood Flow
- Microdialysis
- Intraparenchymal Pressure
- Vasopressors

3 ACCOMPLISHMENTS

3.1 Protocol and Activity Status

- **Human Use Regulatory Protocols**
  No human subjects' research will be performed to complete the Statement of Work

- **Use of Human Cadavers for RDT&E, Education or Training**
  No RDT&E, education or training activities involving human cadavers will be performed to complete the Statement of Work

- **Animal Use Regulatory Protocols**

  Total Protocols: 1 animal use research protocol will be required to complete the Statement of Work

- **Protocol:** 1 of 1
- **Protocol [ACURO Assigned Number]:** SC130007
- **Title**: SCI in pigs [IACUC protocol number A13-0013]
- **Target required for statistical significance**: n=6/group
- **Target approved for statistical significance**: n=6/group
- **Submitted to and Approved by**: Bryan K. Ketzenberger, DVM, DACLAM
- **Status:** Approved, 26-March-2015

3.2 Approved Statement of Work

The approved statement of work is described below. A Gantt chart is provided in **Table 1** for reference (see page 5).

Aim 1. Determine the acute vascular, metabolic, and pressure responses to SCI and long-term functional outcome. "SCI with no hemodynamic support"

Task 1: Submit documents for ACURO approval [Month(s) 1-3]
Task 2: Characterize the intraparenchymal metabolic, blood flow, and pressure changes that occur during the first 24 hours post-injury with no hemodynamic resuscitation before or after decompression [Month(s) 4-18]
Task 3: Determine the long-term functional and histologic outcome of SCI with no hemodynamic resuscitation before or decompression [Month(s) 16-36]
Task 4: Evaluate the metabolomic changes that occur within the spinal cord and surrounding CSF and compare these with metabolomic changes within human CSF [Month(s) 7-15 and 22-33]

Milestone(s) Achieved:
(a) Determine baseline intraparenchymal responses to SCI without treatment [Month(s) 18]

(b) Determine how invasive monitoring alone influences functional recovery [Month(s) 36]

Aim 2. Determine the effects of aggressive hemodynamic support during sustained spinal cord compression on the acute physiologic responses to spinal cord injury and long-term functional outcome. "SCI with SCI with hemodynamic support during compression"

Task 1: Characterize the intraparenchymal metabolic, blood flow, and pressure changes that occur during the first 24 hours post-injury with aggressive hemodynamic support provided during sustained spinal cord compression [Month(s) 4-18]

Task 2: Determine the long-term functional and histologic outcome of SCI when aggressive hemodynamic support is provided during sustained compression [Month(s) 16-36]

Task 3: Evaluate the metabolomic changes that occur within the spinal cord and surrounding CSF and compare these with metabolomic changes within human CSF [Month(s) 7-15 and 22-33]

Milestone(s) Achieved:
(a) Determine if hemodynamic support during spinal cord compression can restore perfusion and alleviate intraparenchymal ischemia [Month(s) 18]
(b) Determine if hemodynamic support during spinal cord compression improves functional recovery [Month(s) 36]

Aim 3. Determine the effects of aggressive hemodynamic support after spinal cord decompression on the acute physiologic responses to spinal cord injury and long-term functional outcome.

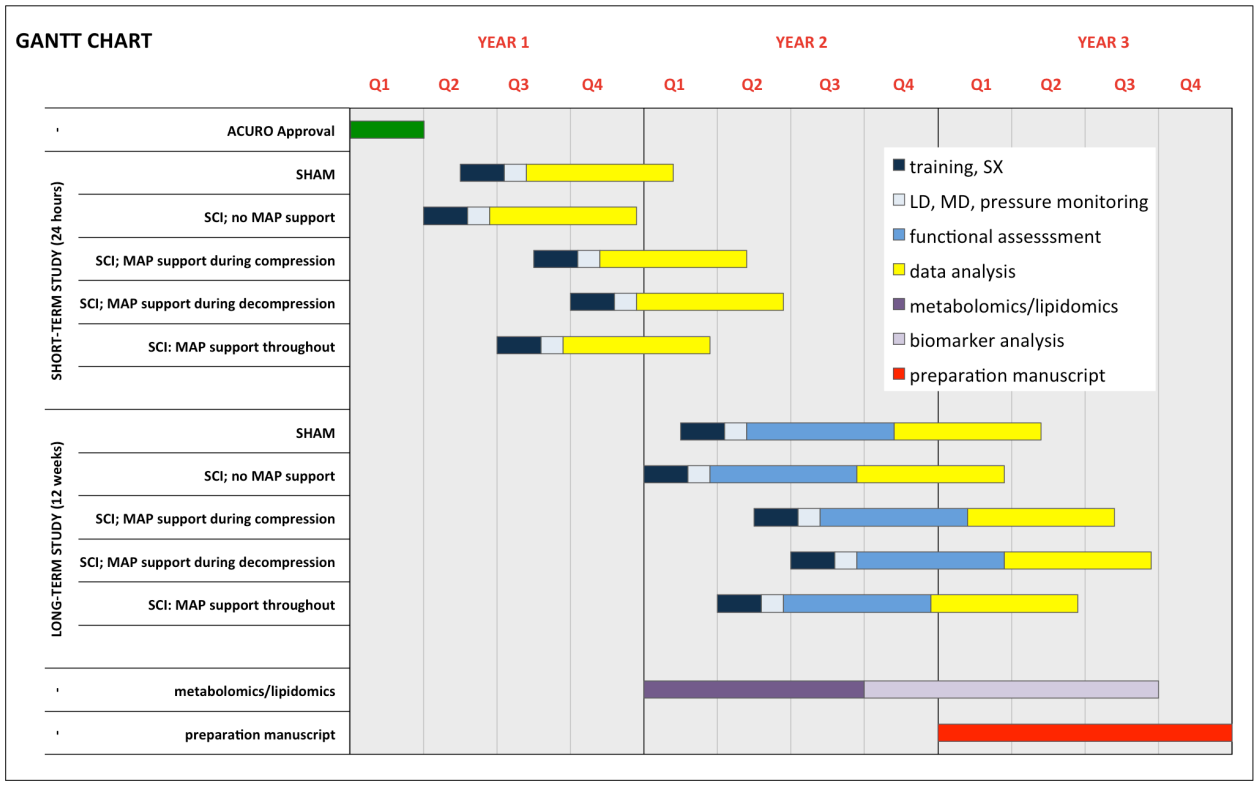
Task 1: Characterize the intraparenchymal metabolic, blood flow, and pressure changes that occur during the first 24 hours post-injury with aggressive hemodynamic support provided after spinal cord decompression [Month(s) 16-36]

Task 2: Determine the long-term functional and histologic outcome of SCI when aggressive hemodynamic support is provided after cord decompression [Month(s) 7-15 and 22-33]

Task 3: Evaluate the metabolomic changes that occur within the spinal cord and surrounding CSF and compare these with metabolomic changes within human CSF [Month(s) 16-36]

Milestone(s) Achieved:
(a) Determine if hemodynamic support after spinal cord decompression induces an ischemia-reperfusion injury [Month(s) 18]
(b) Determine if hemodynamic support after spinal cord compression worsens functional recovery [Month(s) 36]

Table 1. Approved statement of work (Gantt Chart)



3.3 Current Progress on Statement of Work

A Gantt chart of the current work is provided in **Table 2** for reference (page 9). The months in the approved statement of work do not necessarily match with the Gantt chart, since the Gantt chart reflects actual work completed in each year.

Aim 1. Determine the acute vascular, metabolic, and pressure responses to SCI and long-term functional outcome. "SCI with no hemodynamic support"

- **Task 1:** Submit documents for ACURO approval

  Completed. *ACURO approval was granted 26-MARCH-2015.*

- **Task 2:** Characterize the intraparenchymal metabolic, blood flow, and pressure changes that occur during the first 24 hours post-injury with no hemodynamic resuscitation before or after decompression

  Completed.
  A manuscript has been published in Journal of neurotrauma (ID NEU-2017-5034) entitled "Changes in Pressure, Hemodynamics and Metabolism Within the Spinal Cord During the First 7-days After Injury Using a Porcine Model". J Neurotrauma. 2017 Sep 14. doi: 10.1089/neu.2017.5034

- **Task 3:** Determine the long-term functional and histologic outcome of SCI with no hemodynamic resuscitation before or after decompression

  Completed.

- **Task 4:** Evaluate the metabolomic changes that occur within the spinal cord and surrounding CSF and compare these with metabolomic changes within human CSF

  In progress. A manuscript on the changes within serum and CSF metabolome of SCI patients has been currently published in Scientific Reports entitled "Parallel Metabolomic Profiling of Cerebrospinal Fluid and Serum for Identifying Biomarkers of Injury Severity after Acute Human Spinal Cord Injury". Sci Rep. 2016; 6: 38718. This human data set will help identify and interpret patterns of metabolite changes in the pig CSF and spinal cord.

Aim 2. Determine the effects of aggressive hemodynamic support during sustained spinal cord compression on the acute physiologic responses to SCI and long-term functional outcome. "SCI with hemodynamic support during compression"

- **Task 1:** Characterize the intraparenchymal metabolic, blood flow, and pressure changes that occur during the first 24 hours post-injury with aggressive hemodynamic support provided during sustained spinal cord compression

  Completed.

- **Task 2:** Determine the long-term functional and histologic outcome of SCI when aggressive hemodynamic support is provided during sustained compression

  Completed. As mentioned in our previous reports, when looking at our 7-day data, we feel that the natural rise in MAP in the first week actually obscures the data and prevents us from answering this research question. We proposed in the Year 3, Quarter 3 report that the non-survival study during which we examine the responses while the MAP augmentation is actually occurring, will be a better way of addressing the research question. While this was unexpected, it is a change that is related to aspects of the pig model that we were not aware of initially.

  Task 1-2: A manuscript is in preparation for submission to the Journal of Neurotrauma entitled "Early vasopressor administration during spinal cord compression and decompression state is associated with augmented hemorrhaging: observations from a porcine model of traumatic spinal cord injury".

- **Task 3:** Evaluate the metabolomic changes that occur within the spinal cord and surrounding CSF and compare these with metabolomic changes within human CSF

  In progress. A manuscript on the changes within serum and CSF metabolome of SCI patients has been currently published in Scientific Reports entitled "Parallel Metabolomic Profiling of Cerebrospinal Fluid and Serum for Identifying Biomarkers of Injury Severity after Acute Human Spinal Cord Injury". Sci Rep. 2016; 6: 38718. This human data set will help identify and interpret patterns of metabolite changes in the pig CSF and spinal cord.

Aim 3. Determine the effects of aggressive hemodynamic support after spinal cord decompression on the acute physiologic responses to spinal cord injury and long-term functional outcome. "SCI with hemodynamic support after decompression"

- **Task 1:** Characterize the intraparenchymal metabolic, blood flow, and pressure changes that occur during the first 24 hours post-injury with aggressive hemodynamic support provided after spinal cord decompression

  Completed.

- **Task 2:** Determine the long-term functional and histologic outcome of SCI when aggressive hemodynamic support is provided after cord decompression

  Completed. As mentioned in our previous reports, when looking at our 7-day data, we feel that the natural rise in MAP in the first week actually obscures the data and prevents us from answering this research question. We proposed in the Year 3, Quarter 3 report that the non-survival study during which we examine the responses while the MAP augmentation is actually occurring, will be a better way of addressing the research question. While this was unexpected, it is a change that is related to aspects of the pig model that we were not aware of initially.

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Table 2: Gantt chart current work. Tasks performed at the University of British Columbia and University of Alberta (latter - metabolomics assessment only). The Gantt chart reflects actual work completed. Therefore, months in the approved statement of work do not necessarily match with the Gantt chart, since the Gantt chart reflects actual work completed in each year. Note that in Year 3 we proposed that a non-survival study during which we examine the responses while the MAP augmentation is actually occurring, will be a better way of addressing the research question (due to the occurrence of a natural rise in MAP in the first week after SCI, which obscures the data). The gantt chart has been modified to reflect the revised experimental design.

Specific Aim 1: *SCI w/o HDS*	**YEAR 1**				**YEAR 2**				**YEAR 3**				**YEAR 4**			
Activity	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**
ACURO Approval																
Surgery																
Metabolomic /Histologic assessments																
Data analysis /dissemination																

Specific Aim 2: *SCI with HDS - compression*	**YEAR 1**				**YEAR 2**				**YEAR 3**				**YEAR 4**			
Activity	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**
Surgery																
Metabolomic / Histologic assessments																
Data analysis / dissemination																

Specific Aim 3: *SCI with HDS - decompression*	**YEAR 1**				**YEAR 2**				**YEAR 3**				**YEAR 4**			
Activity	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**
Surgery																
Metabolomic / Histologic assessments																
Data analysis / dissemination																

4 OVERALL PROJECT SUMMARY

This grant is focused on the augmentation of MAP with vasopressors and determining how such MAP augmentation would best be implemented (during compression, after decompression, or both). In **YEAR 2** of this grant we finalized the comparison of two commonly used vasopressors, norepinephrine (NE) and phenylephrine (PE) after SCI to determine how these commonly used vasopressors affects spinal cord oxygenation, perfusion, intraparenchymal pressure and downstream metabolic responses. As our data suggested NE would better maintain elevated MAPs, using NE, in **YEAR 3** we studied our first animal survival surgeries as part of **AIM 1-3** to determine the impact of MAP augmentation during compression and/or decompression, on tissue hemodynamics within the spinal cord in the "acute" (0-6 hours post SCI) and "chronic" phases (7-days post SCI) after a traumatic SCI. When looking at our 7-day data, we felt that the natural rise in MAP in the first week after SCI obscures the data and prevents us from answering this research question. Therefore, in Year 3 we proposed that non-survival study during which we examine the responses while the MAP augmentation is actually occurring, will be a better way of addressing the research question. In **YEAR 4**, we completed **TASK 1** and **2** of **AIM 2** and **3** and have finalized a draft manuscript on the role of vasopressor support in spinal cord injury (attached as appendix - notably the interpretation of the results and statistical analysis is still ongoing).

Furthermore, in **YEAR 4** we sent human and pig CSF and serum sample to UofA for metabolomic and lipidomic profiling as part of **TASK 3** of **AIM 2** and **3**. Dr. Li has completed the two labeling experiments to profile the sub-metabolomes of amines/phenols and acids for both human serum and CSF. Currently they are in the process of running samples for the remaining two labeling experiments to profile the sub-metabolomes of hydroxyls and carbonyls. With regards to lipidomics, Dr. Li has completed running of both human serum and CSF samples in positive and negative ion modes and is processing the data now. Lipidomics and metabolomics work on the pig samples is scheduled to commence within a month from now (February 2019).

4.1 Results

Aim 2-3: Determine the effects of aggressive hemodynamic support during sustained spinal cord compression and after spinal cord decompression on the acute physiologic responses to SCI.

For this study, we completed experiments on a total of 24 animals, which were distributed into four groups (n=6/group): (1) no MAP support (i.e. Control group), (2) MAP support during cord compression (i.e. CP group), (3) MAP support after cord decompression (i.e. DCP group), and (4) MAP support during cord compression and after decompression (i.e. CP-DCP group). All four groups received a T10 contusion injury followed by 2 hours of compression and then 2 hours of post-decompression observation. The CP group received a 1.5 hour infusion of NE (4 mg in 250 mL of 0.9% NaCl/1.25% dextrose) 30 minutes after SCI. The DCP group received a 1.5 hour infusion of NE immediately after decompression. The CP-DCP group received a 3.0 hour infusion period of NE 30 minutes after SCI. The control group did not receive MAP support and no NE was administered.

The interpretation of the data from this study was made challenging by the variability in the physiologic responses after SCI that were observed even before initiating any vasopressor support. In theory, the basic physiologic responses to SCI (e.g. SCBF and oxygenation changes), should be the similar in all four groups prior to the initiation of vasopressor support. We did not, however, observe this. Rather, there was considerable variability in these early physiologic responses after the SCI. As reported in our **Year 4, Q3** report, one potential reason for this could be the location of the monitoring probes with respect to gray and white matter. In this study, the probes were intended for placement in the white matter with corresponding lower SCBF and oxygenation (PO_2) values and lesser variability as compared to that in gray matter. It is, however, highly probable that due to inter-animal variability with regards to morphology of the spinal cord and its surrounding CSF space, some probes might have been measuring from the grey matter rather than from white matter. Additionally, in our experiments, baseline monitoring has typically been measured 1.5 hour after probe insertion. Based on pilot data in our lab, this may be sufficient time for equilibration for probes located in the white matter, but not the grey matter. Thus, insufficient stabilization times for "miss-placed" probes located into the grey matter could have potentially contributed to the observed variability in responses after SCI between animals. Although the response to a short hypoxic event seems to be similar between grey and white matter, it is possible that a longer duration of hypoxia, as with sustained compression, might illuminate differences between grey and white matter in SCBF and oxygenation responses.

Using our pig model of SCI, we were able to increase SCBF (**Figure 1**) and PO_2 (**Figure 2**) proximal to the epicenter (2-mm location of the probes) during both the compressed and decompressed state of the spinal cord using NE, as observed for both the CP and DCP group. For the CP-DCP group a similar increase in SCBF and PO_2 was observed during the compressed state, however, after decompression NE infusion did not seem to affect the overall response in this group. While we hypothesized that ongoing NE infusion throughout the compressed and decompressed state (i.e the CP-DCP group) of the spinal cord would induce a state of hyperperfusion following decompression, this was not evident based on the SCBF data.

Additionally, the trajectory of the L/P ratio (**Figure 3**) seem to differ between the groups. All four groups showed an initial rise in L/P ratio values after SCI and during compression, however, in both the CP and CP-DCP, a drop in L/P ratios was observed after the initiation of NE Infusion. After decompression, NE infusion did not seem to affect the overall response in L/P ratio and all groups showed a decline towards baseline values.

SCP values recovered to baseline levels within minutes after decompression and remained unchanged throughout the remainder of the post-injury period in the control, CP, and DCP group. Notably, the CP-DCP group showed a gradual increase in SCP after the initial drop following decompression, with a rise of approximately +5 mmHg compared to baseline at the end of the study. It is interesting to us that a significant rise in parenchymal pressure was

Arguably the most striking observation from this experiment was the increased extent of hemorrhage in the CP-DCP group (**Figure 5**). In other words, the animals that received MAP augmentation with vasopressors both during compression and after decompression had the worst amount of hemorrhage in the spinal cord. While, hemorrhage is a fairly normal and expected

sequelae of traumatic injury and was observed within the spinal cord in all groups near the site of injury, in the CP-DCP group, approximately 25% of the cross-sectional area was occupied by hemorrhage versus ~14% in the CP group, ~16% in the DCP group, and ~17% in controls.

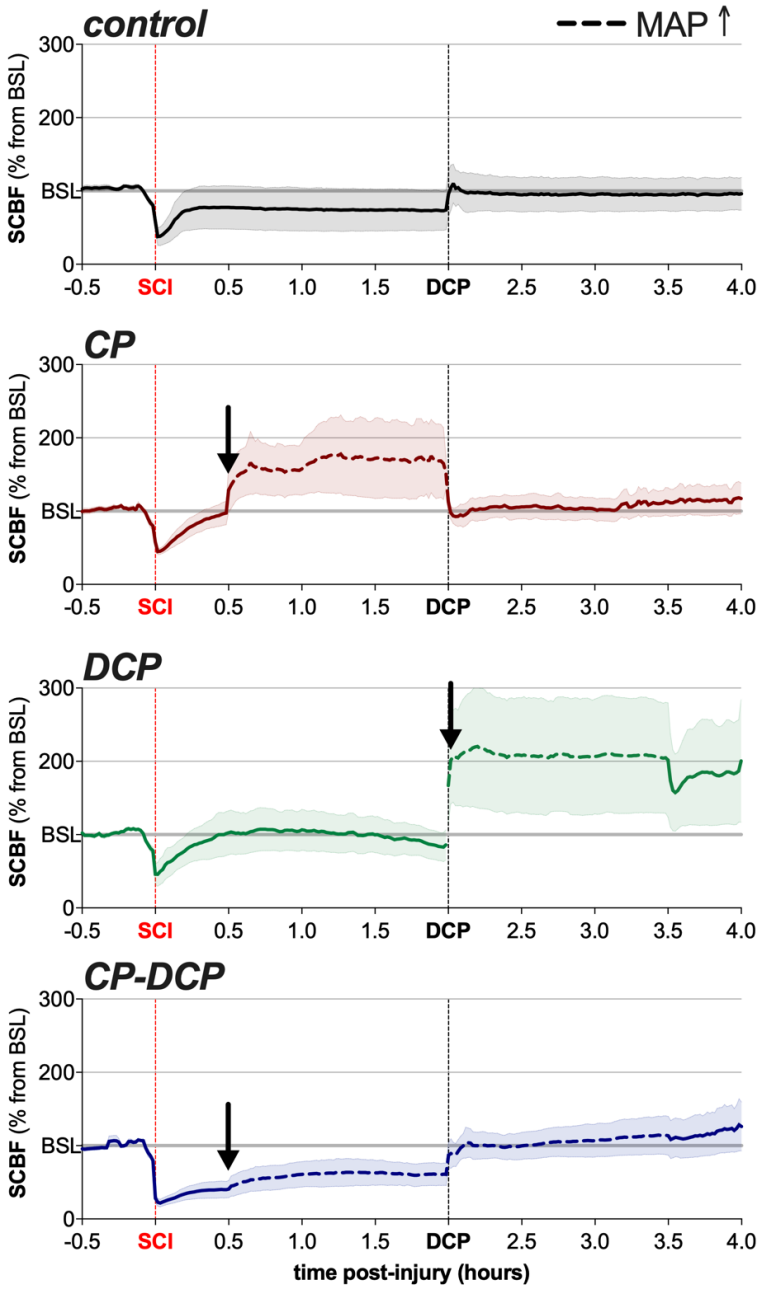

Figure 1. Effect of MAP augmentation by 20 mmHg using NE after SCI on intraparenchymal blood flow (2-mm location). Spinal cord blood flow (SCBF) responses of 1.5 h of MAP augmentation during the compressed (CP group), decompressed (DCP group), and both states (CP-DCP group) of the injured spinal cord. MAP augmentation showed a modest recovery of SCBF in the CP, DCP and CP-DCP groups. Black arrow indicates the start of vasopressor infusion in order to increase MAP by ~20 mmHg; the horizontal dashed line indicates the total duration of vasopressor infusion. Data are expressed as mean ± SEM.

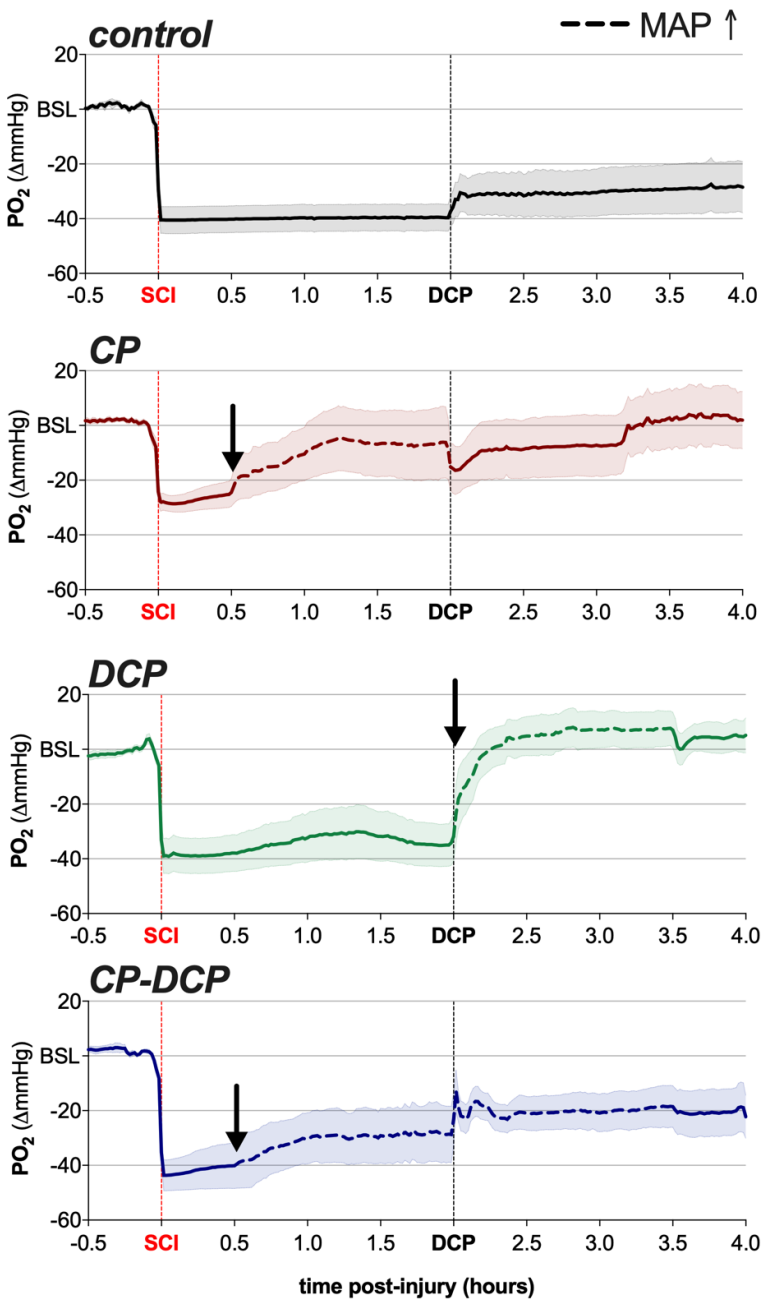

Figure 2. Effect of MAP augmentation by 20 mmHg using NE after SCI on intraparenchymal spinal cord oxygenation (2-mm location). Spinal cord oxygenation (PO_2) responses of 1.5 h of MAP augmentation during the compressed (CP group), decompressed (DCP group), and both states (CP-DCP group) of the injured spinal cord. MAP augmentation resulted in an increase in PO_2 in the CP and DCP group. The CP-DCP group showed a similar increase during compression, however, after decompression NE infusion did not seem to affect the overall response in this group. Black arrow indicates the start of vasopressor infusion in order to increase MAP by ~20 mmHg; the horizontal dashed line indicates the total duration of vasopressor infusion. Data are expressed as mean ± SEM.

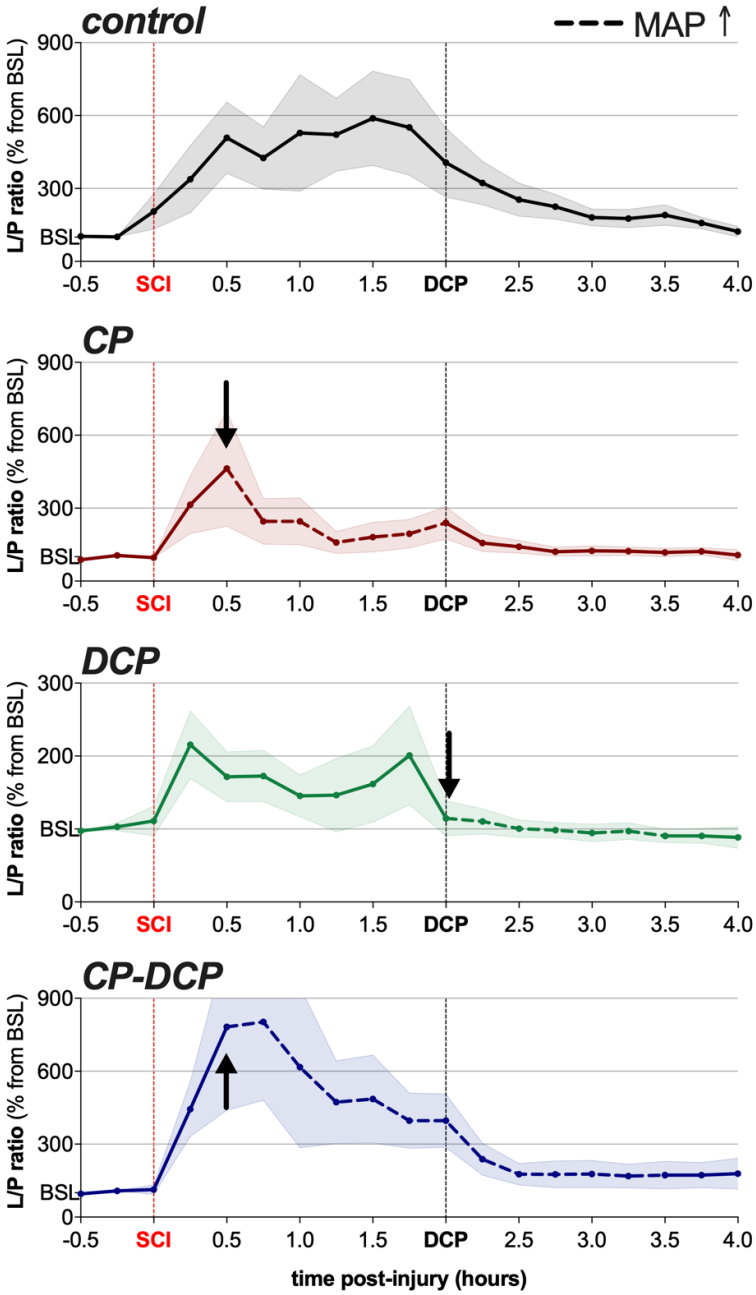

Figure 3. Effect of MAP augmentation by 20 mmHg using NE after SCI on intraparenchymal L/P ratio levels (2-mm location). Lactate/Pyruvate (L/P) ratio response of 1.5 h of MAP augmentation during the compressed (CP group), decompressed (DCP group), and both states (CP-DCP group) of the injured spinal cord. NE infusion decreased the L/P ratio more effectively than MAP augmentation during the compression phase in both the CP and CP-DCP group. Black arrow indicates the start of vasopressor infusion in order to increase MAP by ~20 mmHg; the horizontal dashed line indicates the total duration of vasopressor infusion. Data are expressed as mean ± SEM.

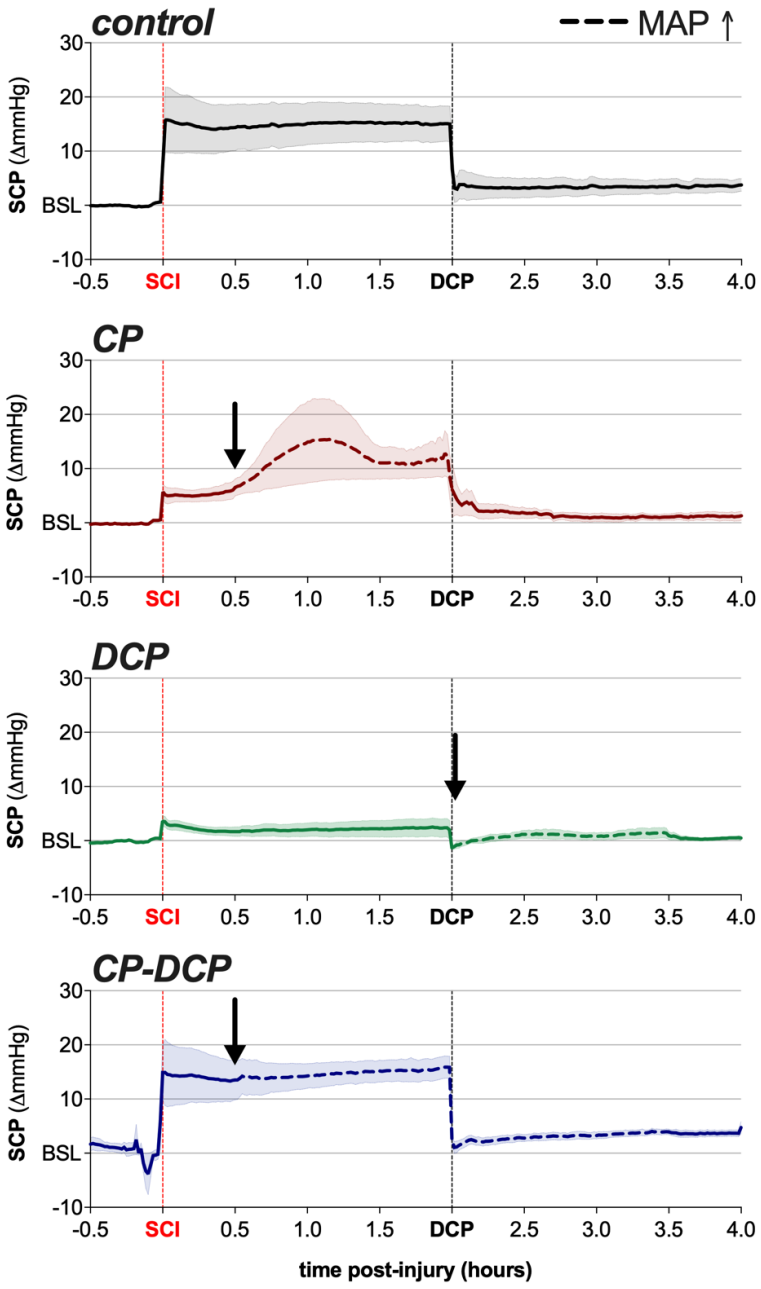

Figure 4. Effect of MAP augmentation by 20 mmHg using NE after SCI on intraparenchymal spinal cord pressure (2-mm location). SCP responses of 1.5 h of NE infusion during the compressed (CP group), decompressed (DCP group), and both states (CP-DCP group) state of the injured spinal cord. NE infusion following decompression resulted in a modest but steady increase in SCP in the CP-DCP group. Black arrow indicates the start of vasopressor infusion in order to increase MAP by ~20 mmHg; the horizontal dashed line indicates the total duration of vasopressor infusion. Data are expressed as mean ± SEM.

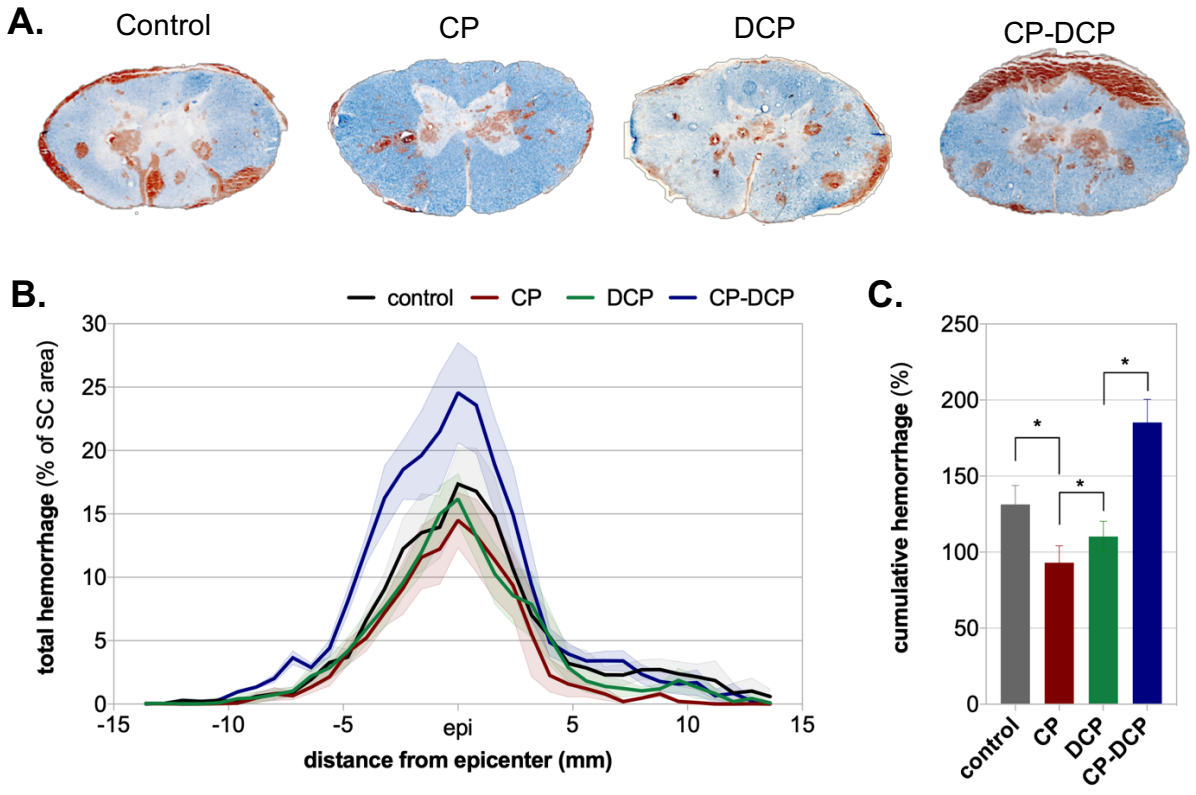

Figure 5. Effect of MAP augmentation by 20 mmHg using norepinephrine after SCI on spinal cord hemorrhage. (**A**) Representative images from Eriochrome Cyanine stained axial sections of the spinal cord from the control, CP, DCP, and CP-DCP group at the epicenter of injury. (**B**) The total hemorrhage calculated as the percentage of the spinal cord area of each axial section. The CP-DCP group demonstrated the greatest average hemorrhage at the epicenter of injury (~25%). (**C**) The total volume of hemorrhage (cumulative hemorrhage) calculated as the area under the curve of total hemorrhage (%) was significantly increased in the P-DCP group compared to the CP group (p=0.0002), DCP group (p=0.002), and controls (p=0.03) (ANOVA). Data are expressed as mean ± SEM.

5 KEY RESEARCH ACCOMPLISHMENTS

Our findings of significantly more extensive intraparenchymal hemorrhage in animals receiving continuous NE infusion during both the compression and post-decompression phase of injury are particularly noteworthy given the clinical practice of augmenting MAP with vasopressors for nearly 7 days post-injury in human patients. Acute increases in blood pressure have been associated with increased hemorrhage by likely increasing systemic vascular resistance to contribute to the rupture of cerebral blood vessels. These findings are relevant to the traumatically injured spinal cord, where the microvasculature of the spinal cord has been shown to be weakened and compromised following injury. A manuscript presenting the results of this study is in preparation for submission to Journal of Neurotrauma entitled (draft manuscript in appendix). These results represent both an important and concerning finding: that aggressive hemodynamic support following acute SCI can lead to detrimental effects. With efforts to improve blood flow and reduce ischemia in the injured cord by augmenting MAP via vasopressors, clinicians may inadvertently promote undesirable bleeding within the spinal cord thereby increasing the size of intraparenchymal hemorrhage at the injury site. This is certainly an observation that warrants followup in a clinical population.

6 CONCLUSION

This study provides a comprehensive description about the acute physiologic and metabolic responses following the hemodynamic management of MAP after traumatic SCI. Our data suggests that MAP augmentation during the presence or absence of spinal cord compression, but not continuously throughout both injury states, modestly restores intraparenchymal blood flow and oxygenation. Elevating MAP by vasopressor administration during cord compression appears to reduce the L/P ratio, reflecting a lesser degree of ischemia in the injured cord. Most importantly, our findings suggest that continuous MAP augmentation during the compressed and post-decompressed state of injury may be detrimental due to increased cumulative hemorrhage near the injury site. Overall, our findings can inform clinicians on the optimal timing and efficacy of MAP augmentation after SCI to optimize the hemodynamic management of acute SCI patients and ultimately improve neurologic recovery for patients with SCI.

Although, conclusive results on the lipidomics/metabolomics work was not available prior to this report, we are committed to completing the analysis within the next few months (the microdialysis samples have been collected and sent to Dr. Li – we just await the actual mass spectrometry analysis). We are excited to provide an update on the discoveries of this work as soon as the data comes available.

7 PUBLICATIONS, ABSTRACTS AND PRESENTATIONS

A manuscript is in preparation for submission to the Journal of Neurotrauma entitled "Early vasopressor administration during spinal cord compression and decompression state is associated with augmented hemorrhaging: observations from a porcine model of traumatic spinal cord injury." (draft version of the manuscript is attached as appendix to this report).

8 INVENTIONS, PATENTS AND LICENSES

Nothing to report

9 REPORTABLE OUTCOMES

Using our pig model of SCI, we showed that:

- NE infusion is better in maintaining elevated MAPs compared to phenylephrine.
- NE infusion results in augmented perfusion, oxygenation and glucose levels during the compressed and decompressed state of the spinal cord.
- NE infusion results in an advantageous drop in L/P ratio values during the compressed state, indicative of reduced ischemia.
- NE infusion throughout the compressed and decompressed state of the spinal cord results in a steady increase in SCP, indicative of progressive edema
- NE infusion throughout the compressed and decompressed state of the spinal cord results in increased hemorrhaging at and near the injury side.

10 OTHER ACHIEVEMENTS

Nothing to report

11 REFERENCES

Nothing to report

12 APPENDICES

Draft manuscript: “Early vasopressor administration during the compressed state of the injured spinal cord is associated with augmented hemorrhaging: observations from a porcine model of traumatic spinal cord injury”.

Early Vasopressor Administration During Spinal Cord Compression and Decompression is Associated With Augmented Hemorrhaging: Observations From a Porcine Model of Traumatic SCI.

A. Cheung[†1], F. Streijger[†1], K. So[1], E.B. Okon[1], N. Manouchehri[1], K. Shortt[1], K.T. Kim[1,2], S.M. Keung[1], A. Fong[1], J. Sun[1], D.E. Griesdale[3], M. Sykhon[4], B.K. Kwon[1,5]

[1]International Collaboration on Repair Discoveries, University of British Columbia (UBC), Vancouver, BC, Canada; [2]Department of Neurosurgery, Kyungpook National University, Kyungpook National University Hospital, Daegu, South Korea; [3]Vancouver General Hospital, Division of Critical Care Medicine, Department of Anesthesiology, UBC, Vancouver, BC, Canada; [4]Vancouver General Hospital, Division of Critical Care Medicine, Department of Medicine, UBC, Vancouver, BC, Canada; [5]Vancouver Spine Surgery Institute, Department of Orthopaedics, UBC, Vancouver, BC, Canada

[†] these authors contributed equally to the work.

Amanda Cheung	amandac.1206@gmail.com	Ph: 604-675-8837, Fax: 604-675-8849
Femke Streijger, Ph.D.	streijger@icord.org	Ph: 604-675-8837, Fax: 604-675-8849
Kitty So, B.Sc.	kitty@icord.org	Ph: 604-675-8837, Fax: 604-675-8849
Elena B. Okon, Ph.D.	okon@icord.org	Ph: 604-675-8837, Fax: 604-675-8849
Neda Manouchehri, B.Sc.	nedamanouchehri@gmail.com	Ph: 604-675-8837, Fax: 604-675-8849
Katelyn Shortt	katelyn.shortt@gmail.com	Ph: 604-675-8837, Fax: 604-675-8849
Kyoung-Tae Kim, M.D., Ph.D.	nskimkt7@gmail.com	Ph: 604-675-8837, Fax: 604-675-8849
Martin Keung	martinksm@hotmail.com	Ph: 604-675-8837, Fax: 604-675-8849
Allan Fong	allanfong96@gmail.com	Ph: 604-675-8837, Fax: 604-675-8849
Jenny Sun	jennysun18@gmail.com	Ph: 604-675-8837, Fax: 604-675-8849
Donald Griesdale, M.D.	Donald.Griesdale@vch.ca	Ph: 604-875-4304, Fax: 604-875-5209
Mypinder Sekhon, M.D.	mypindersekhon@gmail.com	
Brian K. Kwon, M.D. Ph.D.	brian.kwon@ubc.ca	Ph: 604-875-5857, Fax: 604-875-8223

§Corresponding Author (and for Reprints):
Brian K. Kwon, MD, PhD, FRCSC
Canada Research Chair in Spinal Cord Injury
Professor, Department of Orthopaedics, University of British Columbia
6th Floor, Blusson Spinal Cord Centre, Vancouver General Hospital
818 West 10th Avenue, Vancouver, BC, CANADA, V5Z 1M9
PH: 604-875-5857
FX: 604-875-8223
E-mail: brian.kwon@ubc.ca

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Abstract (max. 250 words)

Currently vasopressor support of mean arterial blood pressure (MAP) to improve spinal cord perfusion is one of the few treatment options available for patients who suffer acute spinal cord injury (SCI). Using our porcine model of SCI, we examined the impact of the timing of vasopressor support on intraparenchymal blood flow, oxygenation, hydrostatic pressure, and metabolism.

Yucatan mini-pigs were subjected to a T10 contusion injury followed by 2-hours of sustained compression. After SCI, norepinephrine (NE) was used to elevate MAP by ~20 mmHg while the cord was compressed (CP group), after the cord was decompressed (DCP group), or throughout both injury states (CP-DCP group). Laser Doppler flowmetry/oxygenation, fibre optic pressure, and microdialysis probes were inserted into the spinal cord adjacent to the injury site to measure the intraparenchymal responses.

Our data showed that NE infusion, increased spinal cord blood flow and oxygenation levels in all three groups. Moreover, in the CP and CP-DCP group lactate/pyruvate (L/P) ratio decreased more effectively during MAP support. Contrary to the CP and DCP group, spinal cord pressure seemed to steadily increase in the CP-DCP group. Histological analysis showed augmented hemorrhaging in the spinal cord at and near the injury site in the CP-DCP group.

With efforts to improve blood flow and reduce ischemia in the injured spinal cord by augmenting MAP via vasopressors, inadvertently undesirable bleeding within the spinal cord may be promoted, thereby increasing the extend of intraparenchymal hemorrhaging.

Introduction

There are currently limited early intervention strategies for patients who suffer acute traumatic spinal cord injury (SCI). Early treatment options aim to mitigate the secondary pathological processes, such as hypotension, decreased cord perfusion, and ischemia, that follow primary mechanical impact [1,2]. Hemodynamic management to increase spinal cord perfusion is one of the few treatment options available in clinical care that may benefit patients. Early non-randomized clinical studies lacking appropriate control groups reported that aggressive maintenance of mean arterial blood pressure (MAP) at 85-90 mmHg through intravenous fluid and vasopressor administration for 7 days improved neurological outcome [3,4]. Based on these two studies from the 1990's, many have implemented the clinical practice of elevating MAP to 85-90 mmHg using intravenous fluids and vasopressors for 7 days following acute SCI. In fact, current clinical guidelines of the American Association of Neurological Surgeons/Congress of Neurological Surgeons (AANS/CNS) Joint Section on Spine and Peripheral Nerves recommend this practice [5], despite clear evidence supporting these recommendations. The Consortium for Spinal Cord Medicine suggests that "*Further study is needed to define ideal mean arterial pressure (MAP) and the potential role for elevation of MAP with fluids or pharmacologic treatment*" [6].

Although the approach to minimize ischemia by increasing cord perfusion seems justifiable, aggressive maintenance of MAP at 85-90 mmHg for 7 days continuously in a traumatically injured spinal cord with impaired autoregulation may present consequences. Previous studies using a rodent model of SCI induced by one-minute clip compression showed that MAP augmentation did not improve spinal cord blood flow (SCBF) at the

injury site and can additionally lead to increased hemorrhage and edema [7]. Furthermore, MAP augmentation similarly showed adverse effects in a cat model of contusive SCI, where elevating MAP resulted in worsened spinal cord hemorrhage and greater axonal damage [8]. While several studies have found no benefit between the elevation of MAP and neurological recovery in injured patients (Cohn et al., 2010; Inoue et al., 2014; Kepler et al., 2015; Martin et al., 2015), there is also clinical evidence suggesting the avoidance of hypotension and MAP augmentation can improve neurological outcome (Wolf et al., 1991; Catapano et al., 2016; Dakson et al., 2017). As a result, the hemodynamic management of SCI appears to provide a potential opportunity to alter neurological outcome, however further understanding of post-traumatic intraparenchymal responses in the spinal cord are critical.

Insights into spinal cord blood flow (SCBF) responses after SCI have been widely demonstrated in pre-clinical literature. Interestingly, inconsistent SCBF responses have been observed with different models of SCI where sustained compression varied. The majority of studies utilize rodent models with transient clip compression (about 1 to 40 minutes at most) [17–20], which presents a challenge for the translation to clinical SCI, where the common mechanism of injury is rapid contusion followed by sustained compression. In addition, the Surgical Trial of Acute Spinal Cord Injury Study (STASCIS) [21] revealed that the average time of early surgical decompression was 14.2±5.4 hours and late surgical decompression was 48.3±29.3 hours. This highlights the need to consider the hemodynamic management of SCI in two distinct injury phases: 1. during sustained compression of the contused spinal cord, and 2. the period after decompression of the spinal cord (when compression has been removed).

In the present study, we aimed to determine how the hemodynamic management of MAP in the presence or absence of spinal cord compression affects the acute vascular and metabolic responses after traumatic SCI. Previous work comparing vasopressors used to elevate MAP reported that norepinephrine (NE) was more effective than phenylephrine for restoring blood flow and oxygenation [22]. Therefore, we investigated the effect of MAP augmentation (using NE administration) during the compressed, decompressed, and both injury states on SCBF, oxygenation (in partial pressure of oxygenation; PO2), spinal cord pressure (SCP), and downstream metabolic responses. We used female Yucatan mini-pigs subjected to a T10 contusion injury with 2 hour of compression to evaluate the effect of intervention timing on the acute intraparenchymal and metabolic responses.

Methods

All animal protocols and procedures used in this study were approved by the Animal Care Committee of the University of British Columbia and were compliant with the policies of the Canadian Council of Animal Care and were compliant with the policies of the Canadian Council on Animal Care and the US Army Medical Research and Materiel Command (USAMRMC) Animal Care and Use Review Office (ACURO).

Animals and experimental groups

Female miniature Yucatan pigs (Sinclair Bio-resources, Columbia, MO) weighing 21-30 kg arrived at the animal facility 5 weeks before surgery. Upon arrival, animals were housed in groups of two to four in an indoor pen bedded with sawdust and toys (chains and balls) with access to an adjoining outdoor pen. Animals were given water *ad libitum* and fed 1.5% of their body weight twice a day (Mazuri Mini-Pig Youth; PMI Nutrition International, Brentwood MO).

At the time of surgery, animals were distributed into four groups (n=6/group): (1) no MAP support (control group), (2) MAP support during cord compression only (CP group), (3) MAP support after cord decompression only (DCP group), and (4) MAP support throughout cord compression and decompression (CP-DCP group). All four groups received a T10 contusion injury followed by 2 hours of compression and then 2 hours of post-decompression. To elevate MAP ~20 mmHg from baseline recording, animals in the CP group received a 1.5 h infusion of NE (4 mg in 250 mL of 0.9% NaCl/1.25% dextrose) 30 mins after SCI; the DCP group received a 1.5 h infusion of NE immediately after decompression; The CP-DCP group received a 3.0 hour infusion period

of NE commenced 30 minutes after SCI. The control group did not receive MAP support and no NE was administered. IV maintenance fluids rates (0.9% NaCl/1.25% dextrose) were uniquely adjusted for each animal to ensure a total fluid infusion of 7 mL/kg/h for all experimental groups during NE infusion.

Porcine model of SCI

Tracheal intubation, mechanical ventilation, and carotid artery and jugular vein catheterization to perform NE infusions were performed as previously described [22]. After catherization, the animal was placed into the prone position and a skin incision was made along the dorsal midline of the thoracic region of each animal. Electrocautery (Surgitron® Dual Frequency RF/120 Device; Ellman International, Oceanside, NY), was used to separate the semispinalis, multifidus, and longissimus lumborum muscles from the dorsal spinous processes, laminae, and transverse processes. A laminectomy at T9 to T13 was performed to expose the dura and spinal cord allowing sufficient space for sensor positioning and weight drop injury.

The contusive SCI was induced by a weight drop impactor device, which was securely fixed to the spine using an articulating arm (660; Starrett, Athol, MA) mounted via bilaterally inserted T6 and T8 pedicle screws. This arm allowed the rail (which guided the impactor) to be precisely positioned and aligned, providing a straight vertical impact onto the exposed dura and cord at T10. The tip of the impactor (diameter, 0.953 cm) was equipped with a load cell (LLB215; Futek Advanced Sensor Technology, Irvine, CA) to measure the force at impact. The guide-rail was outfitted with a Balluff Micropulse linear position sensor (BTL6-G500-M0102-PF-S115; Balluff Canada Inc., Mississauga, Ontario)

to record the impactor position from 10 cm above the impact (for calculation of impact velocity and cord displacement). A custom controller was used to operate the impactor device and filter the force, while position data was collected simultaneously with the USB DAQ module (DT9816-S; Data Translation Inc., Marlboro, MA). Remote operation of the device and real-time data collection feedback was employed with the LabVIEW (National Instruments, Austin, TX) program. Immediately after the weight drop contusion injury (weight: 50 g; height: 20 cm), an additional 100 g weight was placed onto the impactor (150 g total) to sustain a 2 hour compression period on the contused spinal cord.

Monitoring of intraparenchymal blood flow, partial pressure of oxygen (PO_2), and pressure

We employed a single multi-parameter probe for simultaneous measurement of SCBF and PO_2, as described in detail previously [22]. Briefly, the probe with a tip diameter of 450 µm (NX-BF/OF/E, Oxford Optronix, Oxford, UK), connects to the OxyLab/OxyFlo combined channel monitor (Oxford Optronix OxyLab, Oxford, UK) which uses the LabChart Pro software for interpretation of recordings (ADInstruments, Colorado Springs, CO). SCBF and PO_2 data were recorded continuously for 5 hour at a sampling rate of 10Hz. A post-processing filter was applied to the SCBF and PO_2 data (smoothing type: median filter, window width: 601 samples/1 min of sampling) to resolve movement artifacts.

SCP was monitored with custom-made fiberoptic combined Fabry-Perot interferometry pressure sensors with a diameter of 333 µm (FOP-LS-NS-1006A, FISO Technologies Inc., Harvard Apparatus, Quebec, Canada). Transducers were connected

to a chassis-mounted signal conditioner module (EVO-SD-5/FPI-LS-10, FISO Technologies Inc., Harvard Apparatus, Quebec, Canada) with internal atmospheric pressure compensation. The data were acquired digitally at a frequency of 1 Hz through the Evolution software (FISO Technologies Inc., Harvard Apparatus, Quebec, Canada).

Monitoring of intraparenchymal spinal cord microdialysis

For sampling of energy-related metabolites in extracellular fluid, we utilized microdialysis probes (CMA11, CMA Microdialysis, Harvard Apparatus, Quebec, Canada) with an outer diameter of 380 µm, 2 mm membrane length, and a 6 kDa cutoff. A subcutaneous implantable micropump (SMP-200, IPrecio, Alzet Osmotic Pumps, Durect Corporation, Cupertino, CA) was employed to continuously perfuse the probes with artificial CSF (Perfusion Fluid CNS, CMA Microdialysis, Harvard Apparatus, Quebec, Canada) at a flow rate of 0.5 µL/minute. Dialysate samples were collected every 15 minutes in microtubes, capped and frozen on dry ice, from the beginning of the baseline period to 4 hour post-injury. This provided a sufficient sample volume of 7.5 µL for the examination of lactate (LAC), pyruvate (PYR), glucose (GLUC), glutamate (GLUT), and glycerol (GLY). The lactate to pyruvate (L/P) ratio was derived from the existing measurements of LAC and PYR concentrations. Samples were analyzed within a week of collection using the ISCUSflex Microdialysis Analyzer (M Dialysis, Stockholm, Sweden). All values are expressed as a percentage (%) from baseline as a function of time (hours) to account for differences in absolute values from the baseline recordings.

Eriochrome cyanine histochemistry

Spinal cord segments were collected and fixed in 4% PFA, then cryoprotected using graded concentrations of sucrose (6, 12, 24% sucrose in 0.1 M Phosphate Buffer each for 48 h). Tissue was then transversely cut in 20 µm sections and eriochrome cyanine R staining was performed on spinal cord sections, as previously described, [22] to differentiate between grey and white matter. Dried sections were cleared in xylene, rehydrated in a reverse ethanol series followed by distilled water (dH2O), then left in a solution containing 0.16% Eriochrome cyanine R, 0.5% sulphuric acid, and 0.4% iron chloride (in dH2O) to stain myelinated fibers. Following staining, sections were differentiated in 0.5% ammonium hydroxide and counterstained in Neutral Red, then rinsed in dH2O. Lastly, sections were dehydrated and cleared, then mounted onto silane-coated SuperFrost™ Plus slides (Fisher Scientific, Pittsburgh, PA). Pictures were taken of sections at 800 µm intervals throughout the lesion site (Zeiss AxioImager M2 microscope) to measure the distribution of hemorrhage (in percentage of the cross-sectional area). Images were analyzed using Zen Imaging Software (Carl Zeiss Canada Ltd., Toronto, Ontario).

Data analysis and statistical analysis

Comparisons between each treatment group for SCBF, PO_2, SCP, and metabolic responses were calculated using repeated measures ANOVA followed by Tukey's multiple comparisons test (GraphPad Prism 7). If more than two consecutive points in the elapsing time were statistically significant at $p < 0.05$, differences between treatment groups were considered significant. Results were plotted as mean values ± SEM for SCBF, PO_2, and SCP recordings, where values were collected at 1 minute intervals, and 15 minute intervals for microdialysis samples.

Data for the hemorrhage analysis are plotted as mean values ± SEM for different positions (spaced 800 μm apart) along the length of the spinal cord (total 5600 μm). The area under the curve (AUC) of total hemorrhage (%) was calculated to measure the cumulative amount of hemorrhage. Comparisons between each treatment group were calculated using a one-way ANOVA followed by Tukey's multiple comparisons test.

Due to the need for appropriate titration of NE dosages throughout the experiment, the surgery team was not blinded to the intervention groups. However, the research team was blinded for the data analysis of the physiological, metabolic, and histological measurements.

Results

Injury biomechanics

Impact force, displacement, and velocity were measured for each animal to evaluate the consistency of the injury (**Supplementary Table 1**). Across all four experimental groups, the average maximum impact force measured from the tip of the impactor was 3296.5 ± 67.9 kdyn (mean ± SEM). The average displacement of the impactor tip upon initial contact with the exposed cord was 5.2 ± 0.1 mm with a velocity of 1836.5 ± 6.4 mm/sec at impact. No differences were observed amongst the treatment groups.

Hemodynamic outcomes

During vasopressor support we aimed to maintain a target MAP of ~20 mmHg above pre-SCI levels. Over time, NE dose was adjusted by increasing the infusion rate to keep target MAP. Average NE dose for the CP, DCP, and CP-DCP group was respectively 26.4 ± 11.2 (range 11.4–81.7), 18.0 ± 5.6 (range 5.7–44.1), and 21.2 ± 6.7 (range 7.2–47.8) µg/kg/min.

No significant differences were observed in target MAP values across the three treatment groups (CP: 82 mmHg; DCP: 83 mmHg; CP-DCP: 88 mmHg)(**Supplementary Figure 1A**). NE infusion additionally resulted in an rise in heart rate in all three group, which declined when NE infusion was ceased. On average the increase in heart rate was lowest in the CP-DCP group (**Supplementary Figure 1B**).

Intraparenchymal responses at proximal measurement site (2mm probe position)

Post-injury changes in intraparenchymal pressure, SCBF, PO_2 and SCP**:** At the proximal measurement site (2-mm position), SCBF immediately dropped below baseline values following SCI in all experimental groups (**Figure 1**). NE infusion resulted in a rise of approximately 1.5- and 2.0-fold in SCBF in respectively the CP and DCP group, reaching average values well above baseline. Notably, however, the within-group variability within these group was relatively high. CP-DCP group also showed a related increase in SCBF upon NE infusion, although the effect was dampened compared to the CP and DCP groups.

PO_2 measurements reflected similar results as the SCBF data (**Figure 2**). PO_2 dropped 30-40 mmHg below baseline values after SCI and during compression in all three groups. While the SCBF seemed to recovery during the subsequent 30 minutes, PO_2 levels remained relatively flat and did not demonstrate any recovery during this period. Upon NE infusion, PO_2 in the COP and DCP group increased to levels close to baseline state values. Similarly an augmented PO_2 response was observed in the CP-DCP group during the compressed and decompressed state of the injured spinal cord, however to a lesser extent and values remained at approximately -20 mmHg below baseline for the duration of the experiment.

As expected, SCP increased after SCI and during compression, however the extent of the increase was relatively variable between groups/animals (**Figure 3**). SCP remained relatively stable throughout the compression period, with no notable differences in SCP responses during NE infusion. While SCP appeared to increase upon NE infusion in the CP group, inter-group variability was relatively large in this group. Upon decompression SCP values recovered to baseline levels within minutes all groups and

remained unchanged throughout the remainder of the post-injury period in the control, CP, and DCP group. Notably, the CP-DCP group showed a gradual increase in SCP after the initial drop following decompression, with a rise of approximately +5 mmHg compared to baseline at the end of the study (4 hpi).

Post-injury changes in microdialysis measurements: Alike SCBF and PO_2, GLUC levels dropped dramatically within minutes after compression (**Figure 4**). Contrary to the gradual drop in GLUC levels for the control group during the 1.5 hour of compression, glucose held steady or increased in respectively the CP-DCP and CP group. During decompression higher GLUC levels were observed for the CP, DCP and CP-DCP group, reaching average values close to or above baseline compared to the control group whose values remained below baseline (50% of baseline).

All four groups showed an initial rise in L/P ratio values within minutes following spinal cord compression (**Figure 5**), however, in both the CP and CP-DCP values steady declined upon NE infusion during this period. Contrary, after an initial rise in L/P ratio, values remained relatively stable over time in the control and DCP group. After decompression, NE infusion did not seem to affect the overall response in L/P ratio and all groups showed a decline towards baseline values.

NE infusion did not appear to influence the SCI-induced rise in glutamate (GLUT) or glycerol (GLY) levels as observed in the CP, DCP and CP-DCP group (**Supplementary Figure 2**).

Intraparenchymal responses at distal measurement site (22mm probe position)

Post-injury changes in intraparenchymal pressure, blood flow, PO_2, and SCP: Compared with the 2mm probe location, slight changes in SCBF, PO_2, and SCP after SCI were observed at the 22mm position (i.e., the more distal of the two probe positions (**Supplementary Figure 3**). NE infusion demonstrated no noticeable effect on SCBF, PO_2 or SCP.

Post-injury changes in microdialysis markers: at the 22mm position GLUC, GLUT, GLY, and L/P ratio responses after SCI were far less pronounced (**Supplementary Figure 4**). Except for GLUC, no significant differences were observed among the experimental groups. Alike the 2-mm location, NE infusion increased GLUC levels in the CP, DCP, and CP-DCP group (**Figure 6**).

Spinal cord tissue hemorrhage

Hemorrhage was observed within the spinal cord in all animals acutely following contusive/compression injury at and near the site of injury (**Figure 7**). The CP-DCP group, which received the longest period of NE infusion, demonstrated significant greater hemorrhage at and up to 5mm rostral to the epicenter when compared to the CP, DCP and control group. On average, more than one fourth of the of the cross-sectional area (27%) was occupied by hemorrhage at the epicentre in the CP-DCP group, versus 14%, 16%, and 17% in respectively the CP, DCP and control group. Similarly, there was more cumulative hemorrhage in the CP-DCP group, calculated as the AUC for the calculated hemorrhage measurements from all sections. No significant differences were observed between the CP, DCP, and control group.

Discussion

Hemodynamic management is one of the few treatment options to improve neurologic recovery of individuals who sustain an acute SCI. Current clinical guidelines recommend elevating MAP to 85-90 mmHg using intravenous fluids and vasopressors for 7 days following acute SCI [6]. Although improved outcomes have been observed from aggressive hemodynamic support (Wolf et al., 1991; Catapano et al., 2016; Dakson et al., 2017), several studies have also found no benefit in neurologic recovery [9,10,12,13]. Examining the difficulty in convincingly demonstrating the benefits of aggressive hemodynamic support is warranted. It is important to consider that this approach may be providing not only beneficial but also adverse effects on the injured spinal cord. The presence or absence of spinal cord compression during periods of aggressive increase in systemic blood pressure to minimize ischemia may play a role. Therefore, understanding how the hemodynamic management of MAP in the presence or absence of spinal cord compression affects the acute vascular and metabolic responses after traumatic SCI is central in providing insight to optimize the hemodynamic management of SCI.

To our knowledge, this represents the first comprehensive study of combined intraparenchymal physiologic and metabolic responses comparing MAP augmentation during the injury phase of sustained compression and the period after decompression of the spinal cord. We investigated the effect of MAP augmentation during either the compressed, decompressed, or both injury states on intraparenchymal SCBF, oxygenation, hydrostatic pressure, and downstream metabolic responses. MAP augmentation was achieved by intravenous NE infusion, which is clinically used to elevate MAP in acute neurotrauma settings. NE has also previously been reported to improve

restoration of blood flow and oxygenation compared to other clinical vasopressors in the injured cord [22]. We measured intraparenchymal SCBF and oxygenation using laser Doppler blood flow/oxygen probes, which collect data using the well-established technique of laser Doppler flowmetry to continuously monitor microvascular changes in tissue perfusion [23] while also using a fluorescence quenching and fibre-optic technology to measure the partial pressure of oxygen [24]. We simultaneously measured hydrostatic pressure with pressure sensors that utilizes a Fabry-Pérot interferometer, a well-known optical pressure transducer [25,26], and measured metabolic responses by collecting analytes from microdialysis probes. Probes were positioned adjacent to one another within the spinal cord proximal (0.2 cm) and distal (2.2 cm) from the site of injury. We have shown that animals received a consistent contusion injury with respect to force, displacement, and velocity.

Elevating MAP by 20 mmHg with NE increased heart rate in the compression and decompression groups. At the proximal measurement site, MAP increase during only the compressed state of injury restores SCBF levels ~174% above pre-injury values in the compression group, while SCBF stays below pre-injury values in the double group. Interestingly, these animals are initially receiving the same treatment, since differences in these group only occur after decompression. Although there were similar target MAP levels in both groups, the significantly higher heart rate in the compression group compared to the double group may explain differences in SCBF recovery with MAP augmentation. Previous studies have also observed increased heart rate [22] and increased cardiac output [27] in animals with improved SCBF. We also observed that MAP augmentation during only the decompressed state of injury restores SCBF levels ~200%

above pre-injury values. Once again, this may likely be due to the pattern of increased heart rate in the decompression group compared to the double group, although much variation is observed in the decompression group. Previous studies reported increased SCBF upon vasopressor administration in both a lamb [28] and porcine [22] model of SCI. SCBF preservation is critical immediately following SCI to limit ischemia and subsequent secondary injury. Experimental studies have shown that reduction in SCBF after spinal cord compression significantly impairs neurological recovery after SCI [29] and is significantly correlated with dysfunction in axonal conduction in motor and somatosensory spinal tracts [30], while increased SCBF is associated with improved axonal function after acute SCI [31]. Our results suggest that MAP augmentation during only the compression or post-decompression phase of injury increases SCBF and consequently microvascular tissue perfusion proximal to the site of injury, while having a minimal effect distal to the site of injury. MAP augmentation during only compression or only post-decompression also appears to significantly increase PO2 levels close to or above pre-injury values proximal to the site of injury and demonstrate an increasing trend distal to the site of injury. PO2 measurements similarly reflect the changes observed in SCBF. Previous work in a canine model of SCI demonstrated that increased perfusion in spinal cord-feeding arteries improved intrathecal PO2 and protected against posttraumatic ischemia [32]. This suggests that MAP augmentation increases cellular oxygen availability proximal to the site of injury to prevent ischemic injury. With respect to tissue hemodynamics directly adjacent to the site of injury, our data suggests that MAP augmentation during the compressed or post-decompressed state, but not continuously throughout both states, modestly improves intraparenchymal blood flow and oxygenation with minimal effect on hydrostatic pressure.

In addition to direct measurements of tissue hemodynamics, we were also interested in examining the effect of MAP augmentation on downstream metabolic responses by measuring metabolic analytes via microdialysis. Previous work suggests monitoring lactate and pyruvate levels as markers of tissue perfusion and tissue oxygen metabolism status, in which an elevated lactate to pyruvate ratio was associated with decreased blood flow to the cord in both a primate model [33,34] and feline model of SCI [35,36]. In such ischemic/hypoxic environments, anaerobic glycolysis occurs to transform glucose to lactate and convert pyruvate to lactate, which ultimately increases the L/P ratio. Our results reveal a decreasing trend in the L/P ratio when MAP was elevated during spinal cord compression, suggesting a reduction of ischemia and hypoxia in the cellular environment adjacent to the site of injury. We also observed an increasing trend in glucose at the distal measurement site upon MAP augmentation during both injury states. This suggests improved energy metabolism can be observed 2 cm away from the injury site when MAP is elevated, likely due to less tissue damage at a distal location. A recent study monitoring spinal cord perfusion pressure (SCPP) and microdialysis directly at the site of injury in human acute SCI patients found that an increased SCPP of 90-100 mmHg resulted in improved metabolism, in which lower L/P ratios and higher glucose levels were observed in patients [37]. Interestingly, hypoperfusion (SCPP below 70 mmHg) and hyperperfusion (SCPP above 100 mmHg) in patients were metabolically detrimental for the injured cord, where higher L/P ratios and lower glucose levels were observed. These findings may explain why MAP augmentation during only cord compression, but not throughout both states of injury where there is an extended period of MAP elevation, resulted in a reduction of the L/P ratio proximal to the injury site.

Our findings of significantly larger intraparenchymal hemorrhage in animals receiving continuous NE infusion during both the compression and post-decompression phase of injury are particularly interesting. Based on studies examining intracerebral hemorrhage, acute increases in blood pressure have been associated with increased hemorrhage [38] by likely increasing systemic vascular resistance [39] to contribute to the rupture of cerebral blood vessels [40]. Others have found that increasing blood pressure elevated levels of superoxide in blood vessels [41], where this increased oxidative stress induced impairment and cell death of endothelium and vascular muscle [42,43] to also impair blood vessels and ultimately increase cerebral hemorrhage [44]. These findings are relevant to the traumatically injured spinal cord, where the microvasculature of the spinal cord has been shown to be weakened and compromised following injury [45]. In a T10 rat model of SCI, increased intraparenchymal hemorrhage was observed after NE infusion in an effort to maintain MAP at hypertensive levels following injury [46]. The authors started NE infusion 15 minutes after injury and already observed increased hemorrhage size 60 minutes post-injury. Another study also found that animals receiving epinephrine to induce hypertension following injury demonstrated hyperemia in the injured cord, which could potentially lead to increased hemorrhage and edema [17]. These results represent both an important and concerning finding: that aggressive hemodynamic support following acute SCI can lead to detrimental effects. With efforts to improve blood flow and reduce ischemia in the injured cord by augmenting MAP via vasopressors, clinicians may inadvertently promote undesirable bleeding within the spinal cord thereby increasing the size of intraparenchymal hemorrhage at the injury site. Studies using magnetic resonance imaging in human patients with SCI found that a greater degree of hemorrhage was

associated with poorer neurological outcomes [47] and similarly in a rat model of SCI, where increased areas of hemorrhage were correlated with greater functional deficits [48,49]. Our demonstration of increased intraparenchymal hemorrhage after continuous NE in both the presence and absence of cord compression may contribute to the explanation of why contrasting findings in neurologic outcome following MAP management have been reported.

This data highlights the elegant proposal by Saadoun and Papadopoulos (2016), that perhaps optimizing not MAP, but MAP minus intraspinal pressure (ISP) to calculate SCPP, may actually be more important. This suggestion is analogous to management in traumatic brain injury (TBI) where instead of ISP, intracranial pressure (ICP) is measured along with MAP, in order to monitor and optimize cerebral perfusion pressure (CPP) [51–53]. Although due to the lack of ISP monitoring that is presently available, utilizing SCPP is still in development. A recent prospective observational study found that SCPP can better predict neurologic recovery following SCI, where cut-off values for SCPP were a more robust marker than cut-off values for MAP in predicting neurologic improvement [54]. Monitoring SCPP, which incorporates MAP measurements, is a novel target to improve neurologic outcome following SCI and in essence, may be a more optimal approach for hemodynamic management.

We recognize there are limitations in this study that are worth discussing. Firstly, pigs and humans are not alike, however we employed a porcine model of SCI due to its translational relevance to the human condition [55]. Contusion injury was induced at T10 as this was the location of injury developed in our pig model in the past [56], but more importantly, the thoracic region from T6-T10 in pigs is most similar to humans [57,58]. In

addition, the vascular supply and cardiovascular physiology in the porcine spinal cord is strikingly similar to humans [59] and porcine models have extensively been used to model several cardiovascular diseases in the literature [55]. Secondly, similar to humans, variabilities are observed in normal MAP measurements of healthy awake pigs, ranging from 85-113 mmHg [60–62]. In this current study, the combination of anesthesia and a traumatic SCI results in a decreased MAP of about 60-80 mmHg and increasing MAP by 20 mmHg actually brings MAP up to normotensive levels. MAP augmentation may perhaps be at hypotensive levels for some animals and not hypertensive. In addition, we recognize this acute investigation is not an accurate representation of the clinical environment where patients are receiving vasopressors and a plethora of other drugs for hours/days post-injury. Future studies can investigate the direct interaction between vasopressors and the combination of drugs patients are receiving after injury. Despite these limitations, given our brief period of MAP augmentation, increased intraparenchymal hemorrhaging following three hours of NE infusion warrants consideration.

This study provides a comprehensive description about the acute physiologic and metabolic responses following the hemodynamic management of MAP after traumatic SCI. Our data suggests that MAP augmentation during the presence or absence of spinal cord compression, but not continuously throughout both injury states, modestly restores intraparenchymal blood flow and oxygenation. Elevating MAP during cord compression appears to reduce the L/P ratio, reflecting a lesser degree of ischemia in the injured cord. Most importantly, our findings suggest that continuous MAP augmentation during the compressed and post-decompressed state of injury may be detrimental due to increased

cumulative hemorrhage near the injury site. Overall, we hope our findings can inform clinicians on the optimal timing and efficacy of MAP augmentation after SCI to optimize the hemodynamic management of acute SCI patients and ultimately improve neurologic recovery for patients with SCI.

References

1. Tator, C.H., and Fehlings, M.G. (1991). Review of the secondary injury theory of acute spinal cord trauma with emphasis on vascular mechanisms. J. Neurosurg. .

2. Senter, H.J., and Venes, J.L. (1978). Altered blood flow and secondary injury in experimental spinal cord trauma. J. Neurosurg. .

3. Levi, L., Wolf, a, and Belzberg, H. (1993). Hemodynamic parameters in patients with acute cervical cord trauma: description, intervention, and prediction of outcome. Neurosurgery .

4. Vale, F.L., Burns, J., Jackson, A.B., and Hadley, M.N. (1997). Combined medical and surgical treatment after acute spinal cord injury: results of a prospective pilot study to assess the merits of aggressive medical resuscitation and blood pressure management. J. Neurosurg. .

5. Walters, B.C., Hadley, M.N., Hurlbert, R.J., Aarabi, B., Dhall, S.S., Gelb, D.E., Harrigan, M.R., Rozelle, C.J., Ryken, T.C., and Theodore, N. (2013). Guidelines for the management of acute cervical spine and spinal cord injuries: 2013 update., in: *Neurosurgery*. .

6. Consortium for Spinal Cord Medicine. (2008). Early acute management in adults with spinal cord injury: a clinical practice guideline for health-care professionals. J. Spinal Cord Med. 31, 403–479.

7. Guha, A., and Tator, C.H. (1988). Acute cardiovascular effects of experimental spinal cord injury. J. Trauma .

8. Rawe, S.E., Lee, W.A., and Perot, P.L.J. (1978). The histopathology of experimental spinal cord trauma. The effect of systemic blood pressure. J. Neurosurg. .

9. Cohn, J., Wright, J., McKenna, S., and Bushnik, T. (2010). Impact of mean arterial blood pressure during the first seven days post spinal cord injury. Top. Spinal Cord Inj. Rehabil. .

10. Martin, N.D., Kepler, C., Zubair, M., Sayadipour, A., Cohen, M., and Weinstein, M. (2015). Increased mean arterial pressure goals after spinal cord injury and functional outcome. J. Emerg. Trauma. Shock .

11. Readdy, W.J., Whetstone, W.D., Ferguson, A.R., Talbott, J.F., Inoue, T., Saigal, R., Bresnahan, J.C., Beattie, M.S., Pan, J.Z., Manley, G.T., and Dhall, S.S. (2015). Complications and outcomes of vasopressor usage in acute traumatic central cord syndrome. J. Neurosurg. Spine .

12. Inoue, T., Manley, G.T., Patel, N., and Whetstone, W.D. (2014). Medical and Surgical Management after Spinal Cord Injury: Vasopressor Usage, Early Surgerys, and Complications. J. Neurotrauma .

13. Kepler, C.K., Schroeder, G.D., Martin, N.D., Vaccaro, A.R., Cohen, M., and Weinstein, M.S. (2015). The effect of preexisting hypertension on early neurologic results of patients with an acute spinal cord injury. Spinal Cord .

14. Wolf, a, Levi, L., Mirvis, S., Ragheb, J., Huhn, S., Rigamonti, D., and Robinson, W.L. (1991). Operative management of bilateral facet dislocation. J. Neurosurg. .

15. Dakson, A., Brandman, D., Thibault-Halman, G., and Christie, S.D. (2017). Optimization of the mean arterial pressure and timing of surgical decompression in traumatic spinal cord injury: A retrospective study. Spinal Cord .

16. Joshua Stephen Catapano, MD, Gregory William John Hawryluk, MD, PhDcorrespondencePress enter key for correspondence information, William Whetstone, MD, Rajiv Saigal, MD, PhD, Adam Ferguson, PhD, Jason Talbott, MD, PhD, Jacqueline Bresnahan, PhD, Sanjay Dh, P. (2016). Higher Mean Arterial Pressure Values Correlate With Neurological Improvement in Patients With Initially Complete Spinal Cord Injuries. World Neurosurg. .

17. Guha, A., Tator, C.H., and Rochon, J. (1989). Spinal cord blood flow and systemic blood pressure after experimental spinal cord injury in rats. Stroke .

18. Kang, C.E., Clarkson, R., Tator, C.H., Yeung, I.W.T., and Shoichet, M.S. (2010). Spinal Cord Blood Flow and Blood Vessel Permeability Measured by Dynamic Computed Tomography Imaging in Rats after Localized Delivery of Fibroblast Growth Factor. J. Neurotrauma .

19. Rivlin, a S., and Tator, C.H. (1978). Regional spinal cord blood flow in rats after severe cord trauma. J. Neurosurg. .

20. Hamamoto, Y., Ogata, T., Morino, T., Hino, M., and Yamamoto, H. (2007). Real-time direct measurement of spinal cord blood flow at the site of compression: Relationship between blood flow recovery and motor deficiency in spinal cord injury. Spine (Phila. Pa. 1976). .

21. Fehlings, M.G., Vaccaro, A., Wilson, J.R., Singh, A., Cadotte, D.W., Harrop, J.S., Aarabi, B., Shaffrey, C., Dvorak, M., Fisher, C., Arnold, P., Massicotte, E.M., Lewis, S., and Rampersaud, R. (2012). Early versus delayed decompression for traumatic cervical spinal cord injury: Results of the surgical timing in acute spinal cord injury study (STASCIS). PLoS One .

22. Streijger, F., So, K., Manouchehri, N., Gheorghe, A., Okon, E.B., Chan, R.M., Ng, B., Shortt, K., Sekhon, M.S., Griesdale, D.E., and Kwon, B.K. (2018). A Direct

Comparison Between Norepinephrine and Phenylephrine for Augmenting Spinal Cord Perfusion in a Porcine Model of Spinal Cord Injury. J. Neurotrauma .

23. Micheels, J., Aisbjorn, B., and Sorensen, B. (1984). Laser doppler flowmetry. A new non-invasive measurement of microcirculation in intensive care? Resuscitation .

24. Griffiths, J.R., and Robinson, S.P. (1999). The OxyLite: A fibre-optic oxygen sensor. Br. J. Radiol. .

25. Roriz, P., Frazão, O., Lobo-Ribeiro, A.B., Santos, J.L., and Simões, J.A. (2013). Review of fiber-optic pressure sensors for biomedical and biomechanical applications. J. Biomed. Opt. .

26. Poeggel, S., Tosi, D., Duraibabu, D., Leen, G., McGrath, D., and Lewis, E. (2015). Optical fibre pressure sensors in medical applications. Sensors (Switzerland) .

27. Wallace, C.M., and Tator, C.H. (1987). Successful improvement of blood pressure, cardiac output, and spinal cord blood flow after experimental spinal cord injury. Neurosurgery .

28. Dyste, G.N., Hitchon, P.W., Girton, R.A., and Chapman, M. (1989). Effect of hetastarch, mannitol, and phenylephrine on spinal cord blood flow following experimental spinal injury. Neurosurgery .

29. Kubota, K., Saiwai, H., Kumamaru, H., Kobayakawa, K., Maeda, T., Matsumoto, Y., Harimaya, K., Iwamoto, Y., and Okada, S. (2012). Neurological recovery is impaired by concurrent but not by asymptomatic pre-existing spinal cord compression after traumatic spinal cord injury. Spine (Phila. Pa. 1976). .

30. Fehlings, M.G., Tator, C.H., and Linden, R.D. (1989). The relationships among the severity of spinal cord injury, motor and somatosensory evoked potentials and spinal cord blood flow. Electroencephalogr. Clin. Neurophysiol. Evoked Potentials .

31. Fehlings, M.G., Tator, C.H., and Linden, R.D. (1989). The effect of nimodipine and dextran on axonal function and blood flow following experimental spinal cord injury. J. Neurosurg. .

32. Ishizaki, M., Sugiyama, S., Uchida, H., Nawa, S., and Shimizu, N. (1999). Identification and selective perfusion of the spinal cord-feeding arteries by intrathecal pO2monitoring for spinal cord protection. Eur. J. Vasc. Endovasc. Surg. .

33. Locke, G.E., Yashon, D., Feldman, R.A., and Hunt, W.E. (1971). Ischemia in primate spinal cord injury. J Neurosurg 34, 614–617.

34. Feldman, R.A., Yashon, D., Locke, G.E., and Hunt, W.E. (1971). Lactate accumulation in primate spinal cord during circulatory arrest. J. Neurosurg. 34, 618–620.

35. Anderson, D.K., Means, E.D., Waters, T.R., and Green, E.S. (1982). Microvascular perfusion and metabolism in injured spinal cord after methylprednisolone treatment. J. Neurosurg. .

36. Braughler, J.M., and Hall, E.D. (1983). Lactate and pyruvate metabolism in injured cat spinal cord before and after a single large intravenous dose of methylprednisolone. J. Neurosurg. 59, 256–261.

37. Phang, I., Zoumprouli, A., Papadopoulos, M.C., and Saadoun, S. (2016). Microdialysis to Optimize Cord Perfusion and Drug Delivery in Spinal Cord Injury. Ann. Neurol. .

38. Metoki, H., Ohkubo, T., Kikuya, M., Asayama, K., Obara, T., Hashimoto, J., Totsune, K., Hoshi, H., Satoh, H., and Imai, Y. (2006). Prognostic significance for stroke of a morning pressor surge and a nocturnal blood pressure decline: The Ohasama study. Hypertension .

39. Talmor, D., Merkind, V., Artru, A.A., Shapiro, O., Geva, D., Roytblat, L., and Shapira, Y. (1999). Treatments to support blood pressure increases bleeding and/or decreases survival in a rat model of closed head trauma combined with uncontrolled hemorrhage. Anesth. Analg. .

40. Caplan, L.R. (1994). Hypertensive intracerebral hemorrhage., in: *IntraCerebral Hemorrhage*. Boston, MA: Butterworth-Heinemann, pps. 99–116.

41. Didion, S.P., Ryan, M.J., Baumbach, G.L., Sigmund, C.D., and Faraci, F.M. (2002). Superoxide contributes to vascular dysfunction in mice that express human renin and angiotensinogen. Am. J. Physiol. Heart Circ. Physiol. .

42. Didion, S.P., and Faraci, F.M. (2003). Angiotensin II produces superoxide-mediated impairment of endothelial function in cerebral arterioles. Stroke .

43. Burlacu, A., Jinga, V., Gafencu, A., and Simionescu, M. (2001). Severity of oxidative stress generates different mechanisms of endothelial cell death. Cell Tissue Res. .

44. Wakisaka, Y., Miller, J.D., Chu, Y., Baumbach, G.L., Wilson, S., Faraci, F.M., Sigmund, C.D., and Heistad, D.D. (2008). Oxidative stress through activation of NAD(P)H oxidase in hypertensive mice with spontaneous intracranial hemorrhage. J. Cereb. Blood Flow Metab. .

45. Cao, Y., Wu, T., Yuan, Z., Li, D., Ni, S., Hu, J., and Lu, H. (2015). Three-dimensional

imaging of microvasculature in the rat spinal cord following injury. Sci. Rep. .

46. Soubeyrand, M., Dubory, A., Laemmel, E., Court, C., Vicaut, E., and Duranteau, J. (2014). Effect of norepinephrine on spinal cord blood flow and parenchymal hemorrhage size in acute-phase experimental spinal cord injury. Eur. Spine J. .

47. Bozzo, A., Marcoux, J., Radhakrishna, M., Pelletier, J., and Goulet, B. (2011). The Role of Magnetic Resonance Imaging in the Management of Acute Spinal Cord Injury. J. Neurotrauma .

48. Noble, L.J., and Wrathall, J.R. (1989). Correlative analyses of lesion development and functional status after graded spinal cord contusive injuries in the rat. Exp. Neurol. .

49. Noble, L.J., and Wrathall, J.R. (1985). Spinal cord contusion in the rat: Morphometric analyses of alterations in the spinal cord. Exp. Neurol. .

50. Saadoun, S., and Papadopoulos, M.C. (2016). Spinal cord injury: Is monitoring from the injury site the future? Crit. Care .

51. Maas, A.I., Stocchetti, N., and Bullock, R. (2008). Moderate and severe traumatic brain injury in adults. Lancet Neurol. .

52. Ghajar, J. (2000). Traumatic brain injury. Lancet 356, 923–929.

53. Carney, N., Totten, A.M., O'Reilly, C., Ullman, J.S., Hawryluk, G.W.J., Bell, M.J., Bratton, S.L., Chesnut, R., Harris, O.A., Kissoon, N., Rubiano, A.M., Shutter, L., Tasker, R.C., Vavilala, M.S., Wilberger, J., Wright, D.W., and Ghajar, J. (2017). Guidelines for the Management of Severe Traumatic Brain Injury, Fourth Edition. Neurosurgery .

54. Squair, J.W., Bélanger, L.M., Tsang, A., Ritchie, L., Mac-Thiong, J.M., Parent, S., Christie, S., Bailey, C., Dhall, S., Street, J., Ailon, T., Paquette, S., Dea, N., Fisher, C.G., Dvorak, M.F., West, C.R., and Kwon, B.K. (2017). Spinal cord perfusion pressure predicts neurologic recovery in acute spinal cord injury. Neurology .

55. Schomberg, D.T., Miranpuri, G.S., Chopra, A., Patel, K., Meudt, J.J., Tellez, A., Resnick, D.K., and Shanmuganayagam, D. (2017). Translational Relevance of Swine Models of Spinal Cord Injury. J. Neurotrauma .

56. Lee, J.H.T., Jones, C.F., Okon, E.B., Anderson, L., Tigchelaar, S., Kooner, P., Godbey, T., Chua, B., Gray, G., Hildebrandt, R., Cripton, P., Tetzlaff, W., and Kwon, B.K. (2013). A Novel Porcine Model of Traumatic Thoracic Spinal Cord Injury. J. Neurotrauma .

57. Bozkus, H., Crawford, N.R., Chamberlain, R.H., Valenzuela, T.D., Espinoza, A.,

Yüksel, Z., and Dickman, C.A. (2005). Comparative anatomy of the porcine and human thoracic spines with reference to thoracoscopic surgical techniques. Surg. Endosc. Other Interv. Tech. .

58. Sheng, S.R., Wang, X.Y., Xu, H.Z., Zhu, G.Q., and Zhou, Y.F. (2010). Anatomy of large animal spines and its comparison to the human spine: A systematic review. Eur. Spine J. .

59. Griepp, E.B., Luozzo, G. Di, Schray, D., Stefanovic, A., Geisbüsch, S., and Griepp, R.B. (2012). The anatomy of the spinal cord collateral circulation. Ann. Cardiothorac. Surg. .

60. Goodrich, J.A., Lackland, D.T., Del Signore, M.J., and Swindle, M.M. (2001). Non-invasive measurement of blood pressures in the Yucatan Micropig (Sus scrofa domestica), with and without midazolam-induced sedation. Comp. Med. .

61. Fernández-Rodríguez, O.M., Palenciano, C.G., Ríos, A., Martínez, L., Arance, M., Segura, B., Martín-Gil, R., Conesa, C., Sansano, T., Acosta, F., Ramírez, P., and Parrilla, P. (2006). Hemodynamic Assessment During Auxiliary Heterotopic Liver Transplantation With Portal Vein Arterialization in a Swine Model: Preliminary Report of 10 Transplants. Transplant. Proc. .

62. Myrie, S.B., McKnight, L.L., King, J.C., McGuire, J.J., Van Vliet, B.N., and Bertolo, R.F. (2012). Effects of a diet high in salt, fat, and sugar on telemetric blood pressure measurements in conscious, unrestrained adult yucatan miniature swine (Sus scrofa). Comp. Med. .

Group	n	Age (days)	Body weight (kg)	Max force (kdyn)	Displacement (mm)	Impact velocity (mm/sec)
Control	6	167 ± 12	24.7 ± 1.3	3372 ± 150	5.1 ± 0.1	1840 ± 12
CP	6	161 ± 8	26.0 ± 1.5	3176 ± 168	5.2 ± 0.1	1841 ± 13
DCP	6	160 ± 7	25.5 ± 1.6	3214 ± 127	5.2 ± 0.2	1825 ± 15
CP-DCP	6	163 ± 6	25.8 ± 1.5	3423 ± 97	5.1 ± 0.1	1841 ± 13

Supplementary Table 1. Injury parameters. The number of animals for each experimental group is shown with their average age and body weight, in addition to their respective maximum force, displacement, and velocity measurements at impact. Injury parameters showed no differences across experimental groups. Data are presented as mean ± SEM.

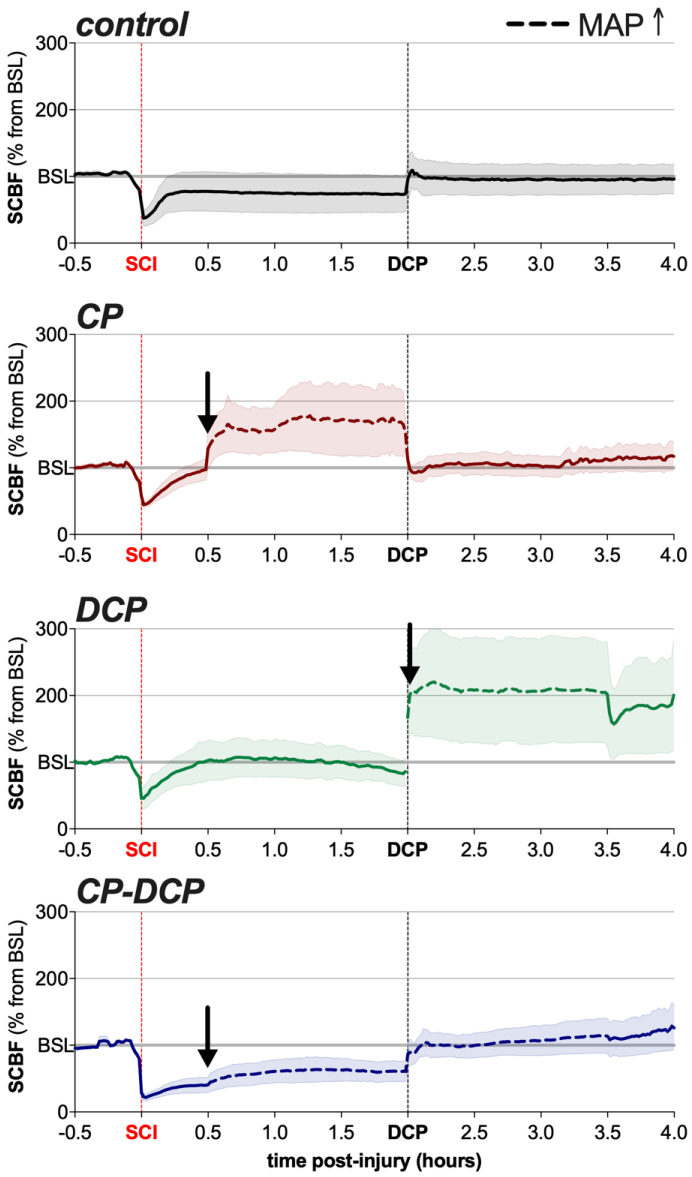


Figure 1. Effect of MAP augmentation by 20 mmHg using NE after SCI on intraparenchymal blood flow (2-mm location). Spinal cord blood flow (SCBF) responses of 1.5 h of MAP augmentation during the compressed (CP group), decompressed (DCP group), and both states (CP-DCP group) of the injured spinal cord. MAP augmentation showed a modest recovery of SCBF in the CP, DCP and CP-DCP groups. Black arrow indicates the start of vasopressor infusion in order to increase MAP by ~20 mmHg; the horizontal dashed line indicates the total duration of vasopressor infusion. Data are expressed as mean ± SEM.

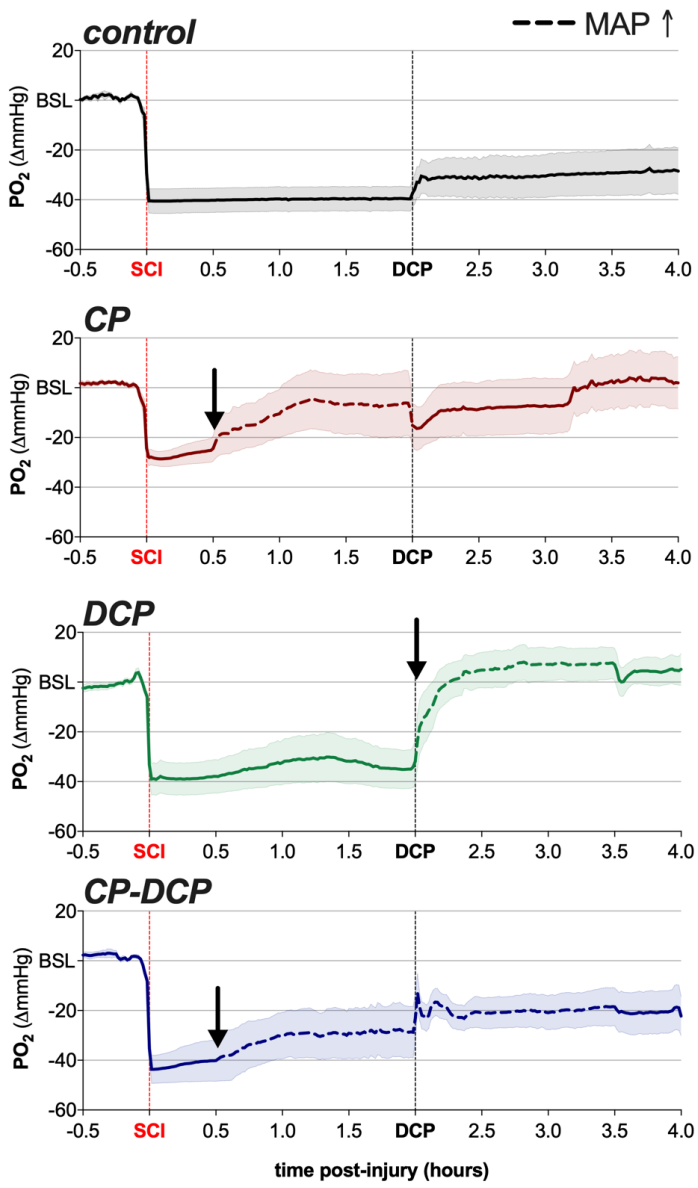


Figure 2. Effect of MAP augmentation by 20 mmHg using NE after SCI on intraparenchymal spinal cord oxygenation (2-mm location). PO_2 responses of 1.5 h of NE infusion during the compressed (CP group), decompressed (DCP group), and both states (CP-DCP group) state of the injured spinal cord. NE infusion resulted in an increase in PO_2 in the CP and DCP group. The CP-DCP group showed a similar increase, however, after decompression NE infusion did not seem to affect the overall response in this group. Black arrow indicates the start of vasopressor infusion in order to increase MAP by ~20 mmHg; the horizontal dashed line indicates the total duration of vasopressor infusion. Data are expressed as mean ± SEM.

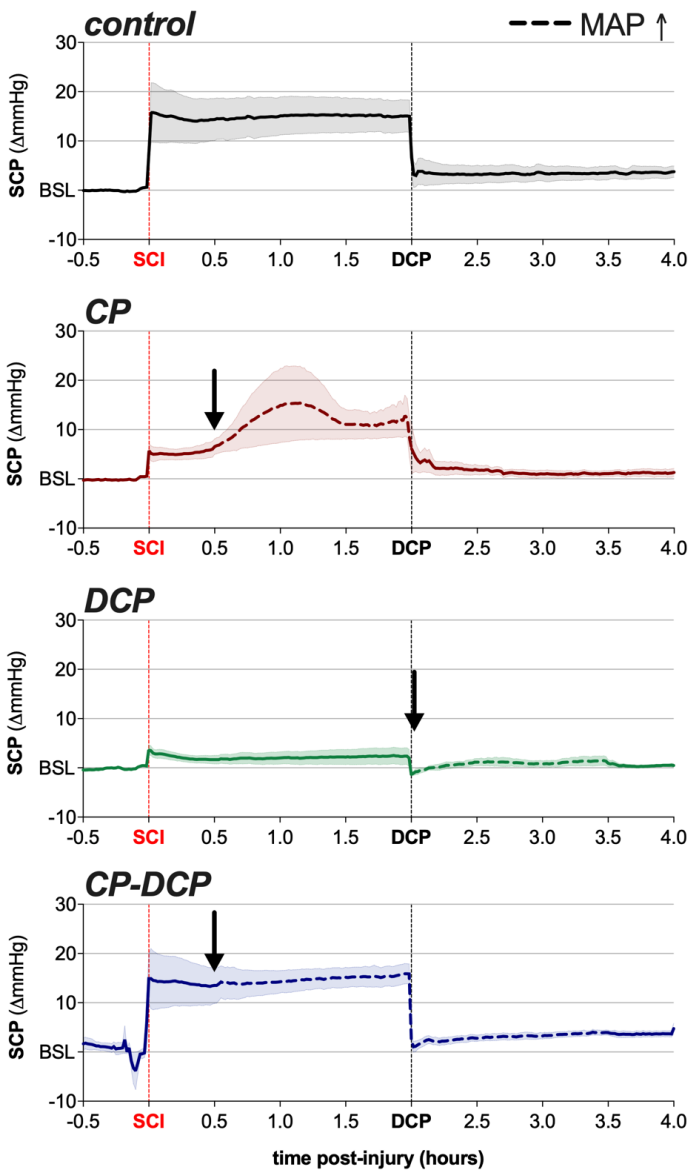


Figure 3. Effect of MAP augmentation by 20 mmHg using NE after SCI on intraparenchymal spinal cord pressure (2-mm location). SCP responses of 1.5 h of NE infusion during the compressed (CP group), decompressed (DCP group), and both states (CP-DCP group) state of the injured spinal cord. NE infusion following decompression resulted in an immodest but steady increase in SCP in the CP-DCP group. Black arrow indicates the start of vasopressor infusion in order to increase MAP by ~20 mmHg; the horizontal dashed line indicates the total duration of vasopressor infusion. Data are expressed as mean ± SEM.

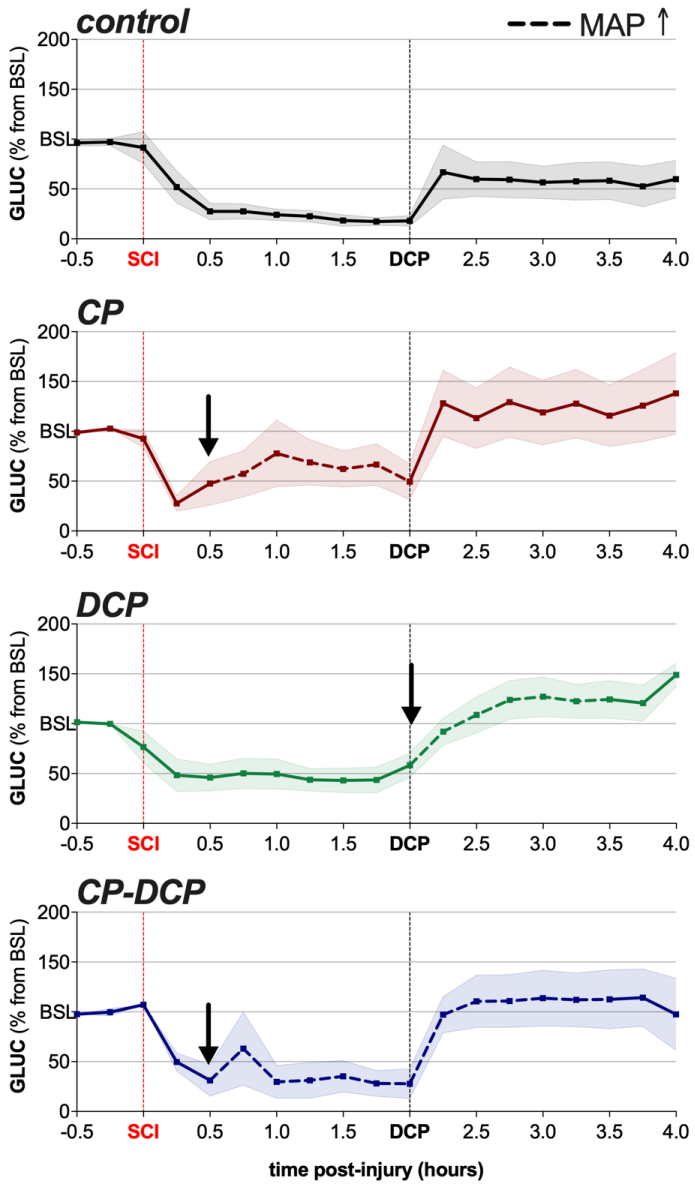


Figure 4. Effect of MAP augmentation by 20 mmHg using NE after SCI on intraparenchymal glucose levels (2-mm location). GLUC response of 1.5 h of MAP augmentation during the compressed (CP group), decompressed (DCP group), and both states (CP-DCP group) of the injured spinal cord. Contrary to the gradual drop in GLUC for the control group during the 1.5 hour of compression, glucose during NE infusion held steady or increased in respectively the CP-DCP and CP group. During decompression higher GLUC levels were observed for the CP, DCP and CP-DCP group,. Black arrow indicates the start of vasopressor infusion in order to increase MAP by ~20 mmHg; the

horizontal dashed line indicates the total duration of vasopressor infusion. Data are expressed as mean ± SEM.

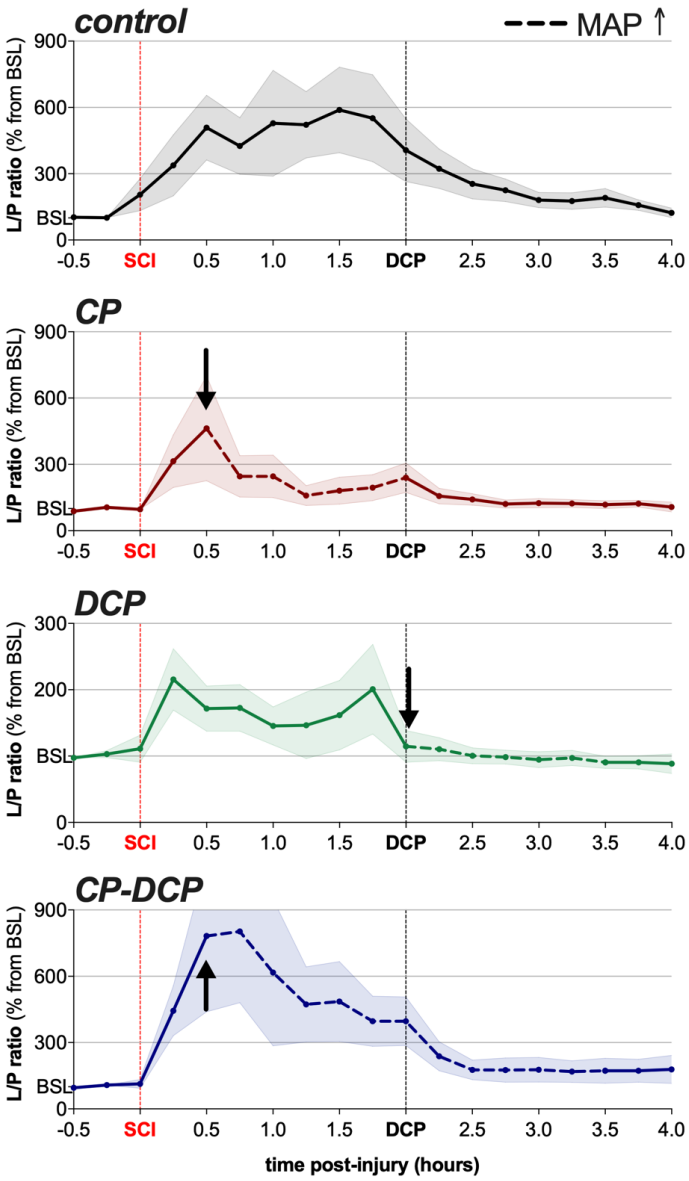


Figure 5. Effect of MAP augmentation by 20 mmHg using NE after SCI on intraparenchymal L/P ratio levels (2-mm location). Lactate/Pyruvate (L/P) ratio response of 1.5 h of MAP augmentation during the compressed (CP group), decompressed (DCP group), and both states (CP-DCP group) of the injured spinal cord. NE infusion decreased the L/P ratio more effectively than NE infusdion during the compression phase in both the CP and CP-DCP group. Black arrow indicates the start of vasopressor infusion in order to increase MAP by ~20 mmHg; the horizontal dashed line indicates the total duration of vasopressor infusion. Data are expressed as mean ± SEM.

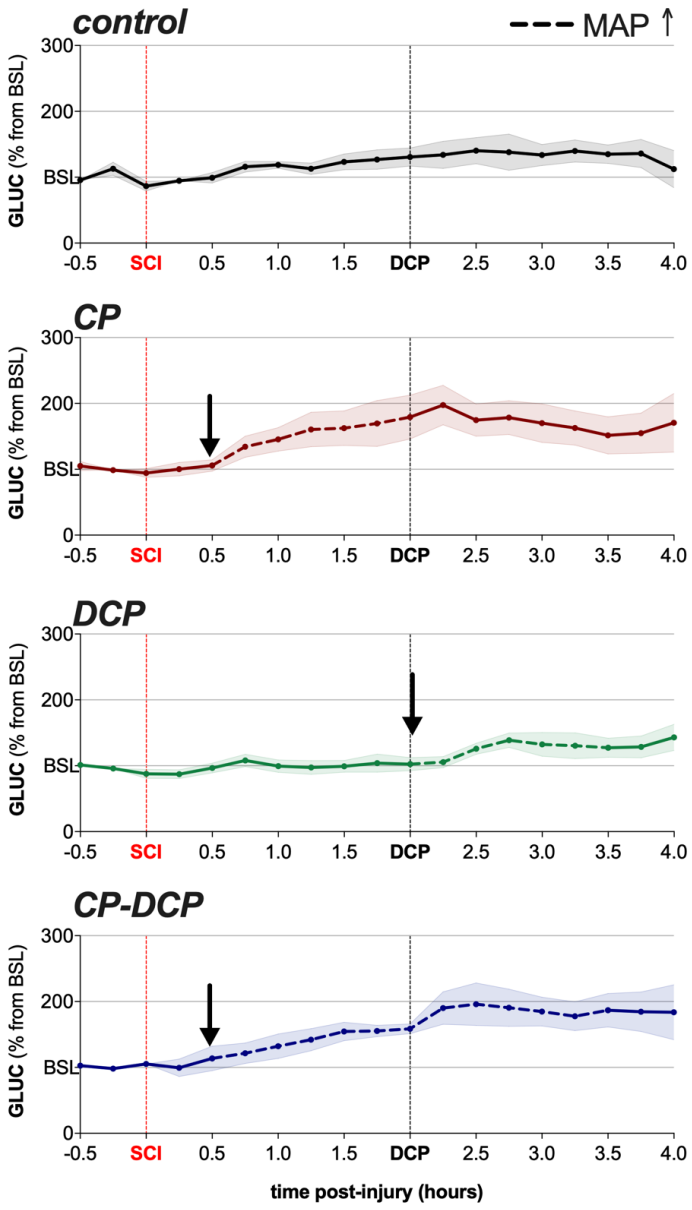


Figure 6. Effect of MAP augmentation by 20 mmHg using NE after SCI on intraparenchymal glucose levels (22-mm location). GLUC response of 1.5 h of MAP augmentation during the compressed (CP group), decompressed (DCP group), and both states (CP-DCP group) of the injured spinal cord. More distal to the epicenter NE infusion increased GLUC levels in the CP, DCP and CP-DCP group. Black arrow indicates the start of vasopressor infusion in order to increase MAP by ~20 mmHg; the horizontal dashed line indicates the total duration of vasopressor infusion. Data are expressed as mean ± SEM.

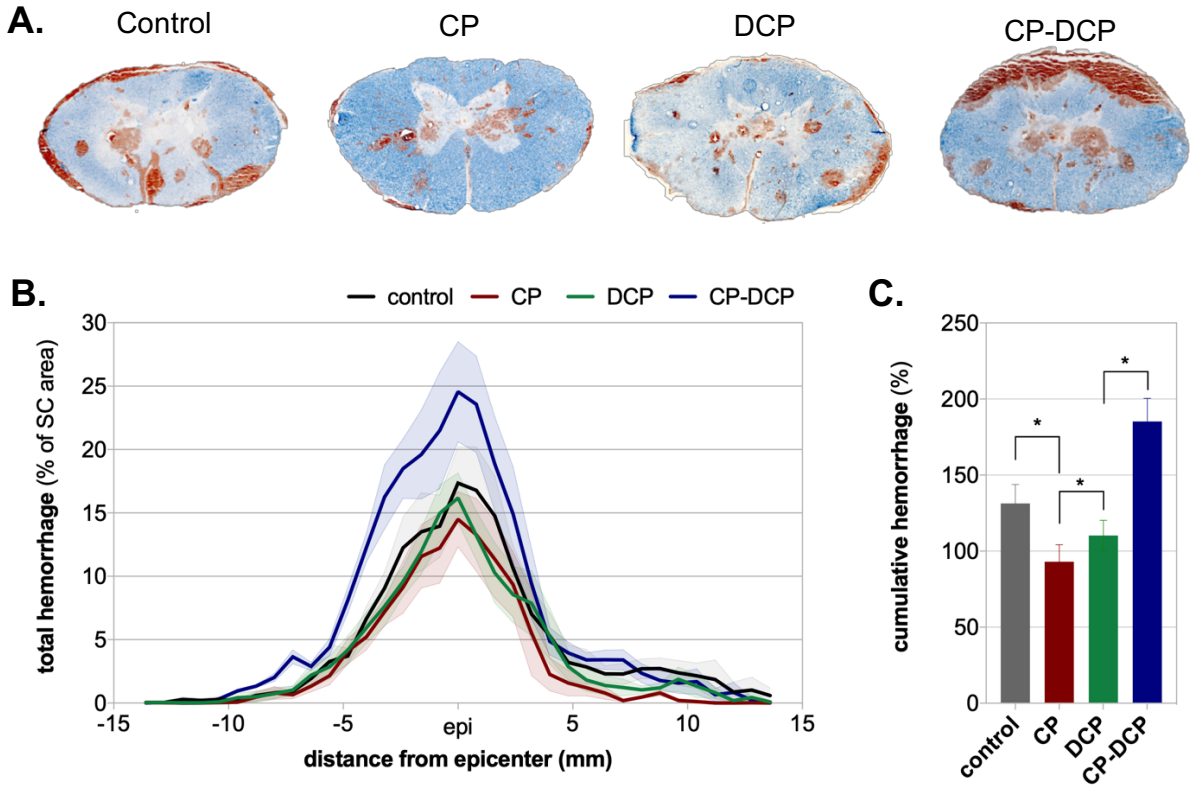
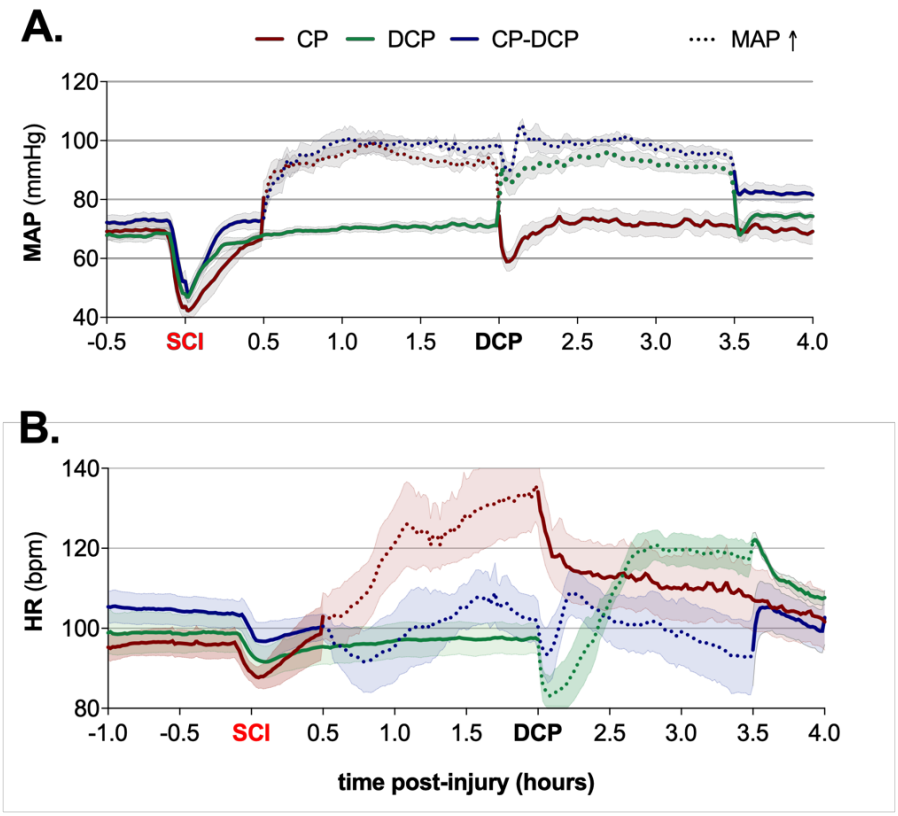
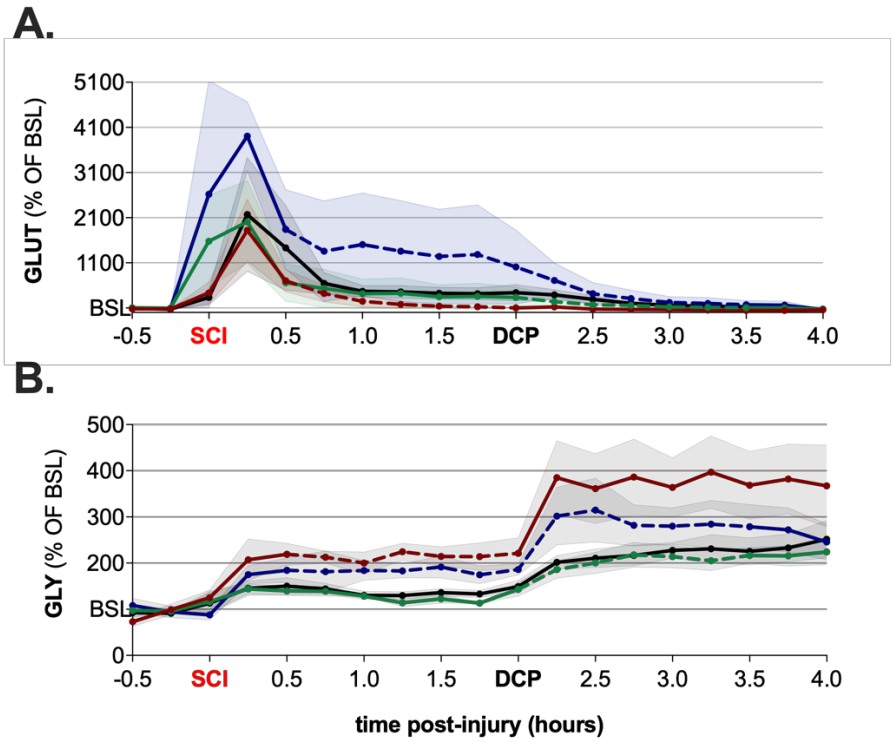


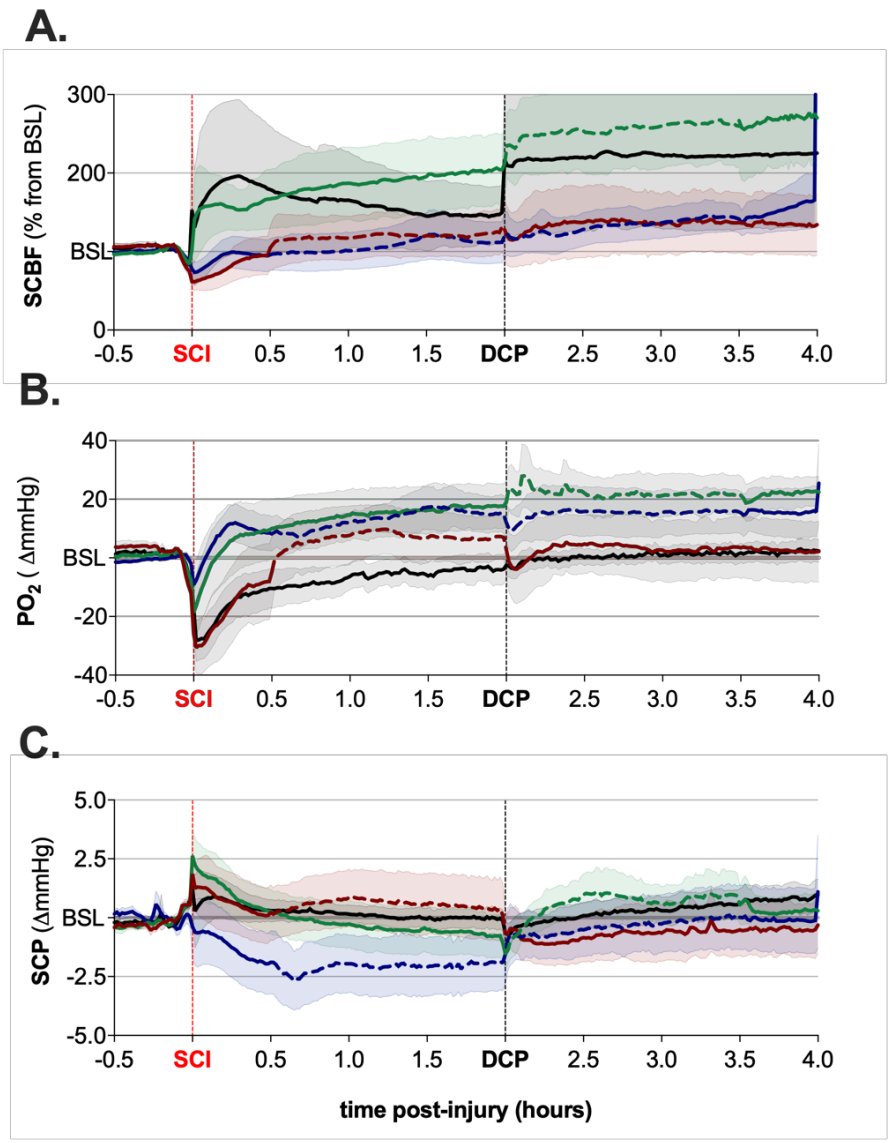
Figure 7. Effect of MAP augmentation by 20 mmHg using norepinephrine after SCI on spinal cord hemorrhage. (**A**) Representative images from Eriochrome Cyanine stained axial sections of the spinal cord from the control, CP, DCP, and CP-DCP group at the epicenter of injury. (**B**) The total hemorrhage calculated as the percentage of the spinal cord area of each axial section. The CP-DCP group demonstrated the greatest average hemorrhage at the epicenter of injury (~25%). (**C**) The total volume of hemorrhage (cumulative hemorrhage) calculated as the area under the curve of total hemorrhage (%) was significantly increased in the P-DCP group compared to the CP group ($p=0.0002$), DCP group ($p=0.002$), and controls ($p=0.03$) (ANOVA). Data are expressed as mean ± SEM.



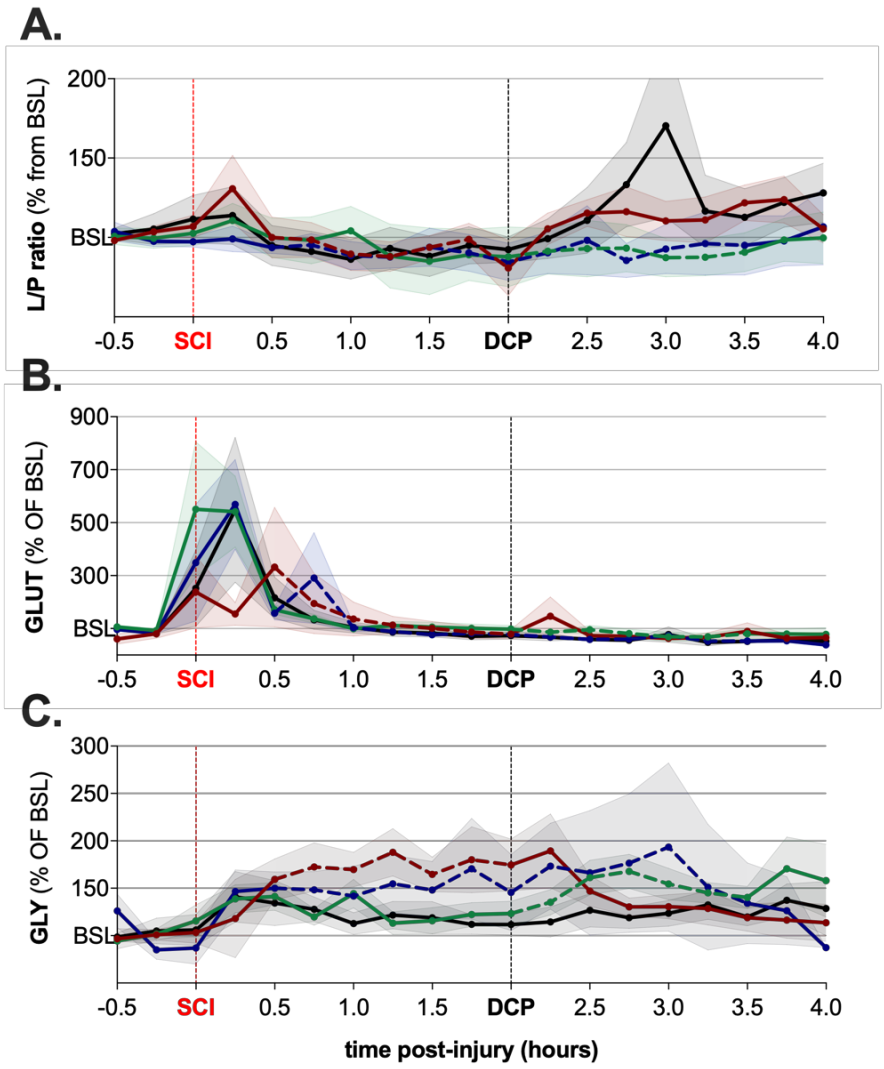
Supplementary Figure 1. Effect of NE administration on MAP and HR measurements. (A) To elevate MAP ~20 mmHg from baseline recording, animals in the CP group received a 1.5 h infusion of NE 30 mins after SCI; the DCP group received a 1.5 h infusion of NE 30 mins after decompression; The CP-DCP group received a 3.0 hour infusion period of NE commenced 30 minutes after SCI. The control group did not receive MAP support and no NE was administered. IV maintenance fluids rates (0.9% NaCl/1.25% dextrose) were uniquely adjusted for each animal to ensure a total fluid infusion of 7 mL/kg/h for all experimental groups during NE infusion. (**B**) Besides an increase in MAP, NE infusion was associated with a rise in heart rate. The horizontal dashed line indicates the total duration of vasopressor infusion. Data are expressed as mean ± SEM.



Supplementary Figure 2. Effect of MAP augmentation by 20 mmHg using NE after SCI on glutamate and glycerol levels (2-mm location). (A) GLUT and (B) GLY responses of 1.5 h of MAP augmentation during the compressed (CP group), decompressed (DCP group), and both states (CP-DCP group) of the injured spinal cord. NE infusion did not have a noticeably effect on GLUT or GLY levels. The horizontal dashed line indicates the total duration of vasopressor infusion. Data are expressed as mean ± SEM.



Supplementary Figure 3. Effect of MAP augmentation by 20 mmHg using NE after SCI on intraparenchymal blood flow, oxygenation and pressure (22-mm location). **(A)** SCBF, **(B)** PO_2, and **(C)** SCP responses of 1.5 h of NE infusion during the compressed (CP group), decompressed (DCP group), and both states (CP-DCP group) state of the injured spinal cord. More distal to the injury, NE infusion did not have a noticeably effect on SCBF, PO2 or SCP. The horizontal dashed line indicates the total duration of vasopressor infusion. Data are expressed as mean ± SEM.



Supplementary Figure 4. Effect of MAP augmentation by 20 mmHg using NE after SCI on the L/P ratio, glutamate and glycerol levels (22-mm location). (A) L/P ratio, **(B)** GLUT**,** and **(C)** GLY responses of 1.5 h of NE infusion during the compressed (CP group), decompressed (DCP group), and both states (CP-DCP group) state of the injured spinal cord. More distal to the injury, NE infusion did not have a noticeably effect on L/P ratio, GLUT or GLY levels. The horizontal dashed line indicates the total duration of vasopressor infusion. Data are expressed as mean ± SEM.