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TITLE: Spinal Cord Swelling and Alterations in Hydrostatic Pressure After Acute Injury

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14. ABSTRACT

In YEAR 4 we completed the statistical analysis of the existing duraplasty data as part of **AIM 3** and are currently finalizing a manuscript on the role of duraplasty in a porcine model of SCI and its effects on intraparenchymal hemodynamics, metabolism, histological and behavioral recovery. Furthermore, as part of **AIM 4**, we evaluated the pressure inside the injured spinal cord (the "spinal cord pressure", or "SCP") and outside the injured spinal cord within the intrathecal space (the "intraspinal pressure" or "ISP") using our pig model of SCI. We furthermore evaluate how their relationship to one another evolve with **time** over the first week post-injury, and how they may be influenced by **location** within the spinal cord relative to the site of injury. Lastly, we evaluated if CSF pressure, cord pressure, and spinal cord blood flow was associated with impairment or preservation of PRx.

15. SUBJECT TERMS
duraplasty, SCI, spinal cord swelling, pressure reactivity index, porcine model of SCI

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TABLE OF CONTENTS

1 INTRODUCTION

After acute spinal cord injury (SCI) the spinal cord is frequently found to have swollen dramatically, particularly after it has been surgically decompressed. In traumatic brain injury (TBI), brain swelling and increases in intraparenchymal pressure are routinely considered in both the surgical and hemodynamic management of such patients. However, this swelling has largely been neglected in SCI, despite being consistently observed. Even after surgical decompression, such swelling may result in the cord being subjected to significant pressure, either due to constriction by the pia mater, the dura mater, or both. The physiologic consequences of this are poorly understood, and many fundamental questions remain about its impact on intraparenchymal pressure, spinal cord perfusion, and downstream metabolic responses. Determining the physiologic/biologic consequences of this swelling and how they can be mitigated to reduce secondary injury will guide the optimal clinical management of acute SCI. As an example of how swelling, increased intraparenchymal pressure, and its effects on perfusion are factored into clinical decision-making, TBI investigators have established the Pressure Reactivity Index (PRx) to identify where autoregulation remains intact and to guide optimal perfusion support based on that. The PRx has not been investigated in SCI, but given that the cord also swells and has impaired autoregulation, it is likely applicable here as well. This promising approach opens the possibility that we could individualize and optimize the hemodynamic support of acute SCI patients in order to support perfusion without exacerbating deleterious increases in intraparenchymal pressure.

2 KEYWORDS

- Spinal Cord Swelling
- Hydrostatic Pressure
- Spinal Cord Injury
- Pressure Reactivity Index
- Porcine model of SCI

3 ACCOMPLISHEMENTS

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]:** SC130008
- 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).

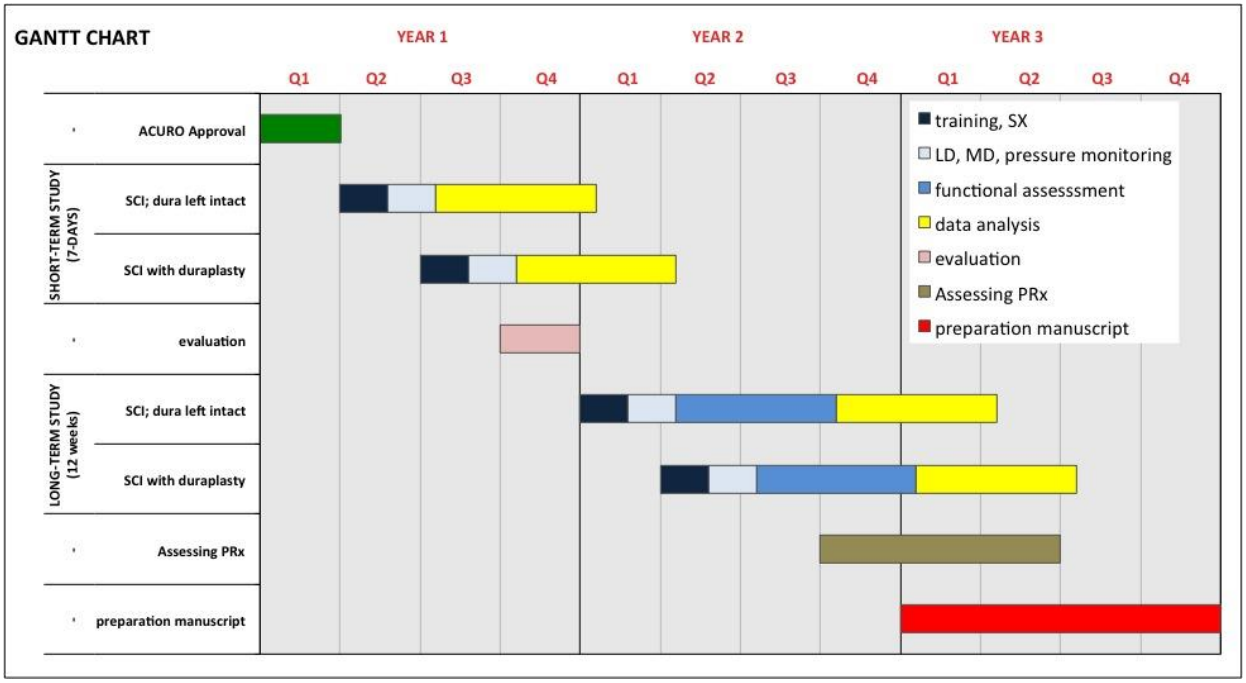
Specific Aim 1: Evaluate if compression by the surrounding dura produces increased intraparenchymal pressure within the injured, swollen cord.	**Months**	**Site**
Subtask 1: Submit documents for ACURO approval	1-3	UBC
Milestone(s) Achieved: Obtain ACURO approval	3	
Subtask 2: Characterize the CSF pressure changes for 7-days after SCI and decompression	3-13	UBC
Subtask 3: Characterize the cord intraparenchymal pressure changes for 7-days with probes positioned in close proximity of the epicenter and a more distal segment (control)	3-13	UBC
Milestone(s) Achieved: Characterization of spatial and temporal hydrostatic pressure changes in the epidural space and spinal cord after SCI and decompression (without dural decompression)	10-15	

Specific Aim 2: To evaluate if dural compression contribute to progressive deficit in blood perfusion and contribute to the pathophysiology of secondary damage after traumatic SCI	**Months**	**Site**
Subtask 1: Characterize the metabolic and spinal cord blood flow (SCBF) changes for 7-days with probes positioned in close proximity of the epicenter and a more distal segment (control)	6-18	UBC
Subtask 2: Determine the effects of surgically opening the dura and expanding the subarachnoid space with a duraplasty will alter intraparenchymal spinal cord pressure, SCBF, and metabolic responses	6-18	UBC
Subtask 3: Examine the histopathological changes in the harvested spinal cord at the 7-day time point	12-18	UBC
Milestone(s) Achieved: Definition of any relation between changes in systemic pressure and SCBF when the spinal cord is decompressed with or without opening of the overlying dura.	18-24	UBC

Specific Aim 3: Evaluate behavioral recovery for a total of 12 weeks following SCI with or without duraplasty	**Months**	**Site**
Subtask 1: Assess hindlimb motor function during overground ambulation (PTIBS)	12-32	UBC
Subtask 2: Neurophysiologic monitoring with transcranial motor-evoked potentials	12-32	UBC
Milestone(s) Achieved: Definition of any relationship between functional recovery after spinal cord decompression with or without dural decompression; preparation of manuscript	32-36	UBC

Specific Aim 4: Evaluate if a moving correlation index exists between mean arterial blood pressure and CSF/cord pressure (pressure reactivity index; PRx)	**Months**	**Site**
Subtask 1: Determine the temporal profile of spinal cord autoregulation following SCI during the first 7-days after SCI	21-30	UBC
Subtask 2: identify any variables - blood pressure, spinal cord perfusion, intraparenchymal pressure, or CSF pressure - associated with impairment or preservation of PRx	21-30	UBC
Milestone(s) Achieved: Quantification of any relation between arterial blood pressure or spinal cord perfusion and CSF pressure; preparation of 1-2 peer reviewed papers	30-36	UBC

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 7). 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: Evaluate if compression by the surrounding dura produces increased intraparenchymal pressure within the injured, swollen cord.

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

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

- **Task 2:** Characterize the CSF pressure changes for 7-days after SCI and decompression

  Completed.

- **Task 3:** Characterize the cord intraparenchymal pressure changes for 7-days with probes positioned in close proximity of the epicenter and a more distal segment (control)

  Completed.

  Task 2-3. 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

Aim 2: To evaluate if dural compression contribute to progressive deficit in blood perfusion and contribute to the pathophysiology of secondary damage after traumatic SCI (7-day evaluation)

- **Task 1:** Characterize the metabolic and spinal cord blood flow (SCBF) changes for 7-days with probes positioned in close proximity of the epicenter and a more distal segment (control)

  Completed.

- **Task 2**: Determine the effects of surgically opening the dura and expanding the subarachnoid space with a duraplasty will alter intraparenchymal spinal cord pressure, SCBF and metabolic responses

  Completed.

- **Task 3**: Examine the histopathological changes in the harvested spinal cord at the 7-day time point

  Completed.

  Task 1-3. A manuscript is in preparation for submission to Journal of Neurotrauma entitled "Duraplasty in Traumatic Thoracic Spinal Cord Injury: The Impact on Spinal Cord Hemodynamics, Tissue Metabolism, Histology and Behavioral Recovery Using a Porcine Model"**.**

Aim 3: Evaluate behavioral recovery for a total of 12 weeks following SCI with or without duraplasty

- **Task 1:** Assess hindlimb motor function during overground ambulation (PTIBS)

  Completed.
  1. A manuscript is in preparation for submission to Journal of Neurotrauma entitled "Duraplasty in Traumatic Thoracic Spinal Cord Injury: The Impact on Spinal Cord Hemodynamics, Tissue Metabolism, Histology and Behavioral Recovery Using a Porcine Model"**.**
  2. A second manuscript is under review in Journal of Neurotrauma entitled "Inherent Variability in Morphometric Measures of the Uninjured Porcine Spinal Cord Affects Histological and Functional Outcomes after SCI".

- **Task 2:** Neurophysiologic monitoring with transcranial motor-evoked potentials

  Completed.
  A manuscript has been published in Annals of Clinical and Translational Neurology entitled "Sensorimotor Plasticity after Spinal Cord Injury: A Longitudinal Translational Study". ACTN, Dec 2018 10.1002/acn3.679

Aim 4. Evaluate if a moving correlation index exists between mean arterial blood pressure and CSF/cord pressure (pressure reactivity index; PRx)

- **Task 1:** Determine the temporal profile of spinal cord autoregulation following SCI during the first 7-days after SCI

  Completed.

- **Task 2:** Identify any variable – blood pressure, spinal cord perfusion, intraparenchymal pressure, or CSF pressure – associated with impairment or preservation of PRx

  Completed.

Table 2: Gantt chart of current work. 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.

Specific Aim 1+2: *7-d Evaluation of Duraplasty*	**YEAR 1**				**YEAR 2**				**YEAR 3**				**YEAR 4**			
Task:	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**
1. ACURO Approval																
2. Animal training / Surgery																
3. monitoring of SCP, PO2, SCBF, and MD																
4. Histologic assessments																
5. Data Analysis / Dissemination																
Specific Aim 3: *12-wk Evaluation of Duraplasty*	**YEAR 1**				**YEAR 2**				**YEAR 3**				**YEAR 4**			
Task	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**
1. Animal training / Surgery																
2. Behavioural / functional assessments																
3. Histologic assessments																
4. Data Analysis / manuscript generation																
Specific Aim 4: *Evaluation of PRx*	**YEAR 1**				**YEAR 2**				**YEAR 3**				**YEAR 4**			
Task	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**
1. Evaluate spinal cord autoregulation 7days after SCI																
2. Identify variable that associated with PRx																

4 OVERALL PROJECT SUMMARY

In YEAR 1-4 we completed the statistical analysis of the existing duraplasty data and are currently finalizing a manuscript on the role of duraplasty in a porcine model of SCI and its effects on intraparenchymal hemodynamics, metabolism, histological and behavioral recovery. Furthermore, we evaluated the pressure inside the injured spinal cord (the "spinal cord pressure", or "SCP") and outside the injured spinal cord within the intrathecal space (the "intraspinal pressure" or "ISP") using our pig model of SCI. We furthermore evaluate how their relationship to one another evolve with **time** over the first week post-injury, and how they may be influenced by **location** within the spinal cord relative to the site of injury. Lastly, we evaluated if CSF pressure, cord pressure, and spinal cord blood flow was associated with impairment or preservation of PRx.

Below a summary of the various aims (1-4) as described in the SOW as initially proposed. Post-award approval, the approved SOW included only two aims corresponding to aim 3-4 of the pre-award SOW.

4.1 Results

Aim 1: Evaluate if compression by the surrounding dura produces increased intraparenchymal pressure within the injured, swollen cord.

In Year 1-4, we characterized spatial and temporal hydrostatic pressure changes in the spinal cord after SCI and decompression (without dural decompression). In our large animal model, we observed a ~10-20 mmHg increase in intraparenchymal pressure near the site of injury in the first 48 hours following SCI. While, a slight decrease in pressure was observed thereafter, values remained ~5 mmHg above baseline levels for the remainder of the 7 days. More distal to the impact border (22 mm location), SCP in the controls SCP remained increased during the entire post-injury period, suggesting a rostro-caudal expansion of dural compression.

Aim 2: To evaluate if dural compression contribute to progressive deficit in blood perfusion and contribute to the pathophysiology of secondary damage after traumatic SCI (7-day evaluation)

Following duraplasty, enlargement of the subarachnoid space was observed in the hours, days and weeks following SCI. Lower spinal cord pressures were observed when duraplasty surgery was performed, however, besides a treatment effect this could potentially be explained by the variability in baseline dural sac size between groups. The most prominent difference observed was an increased drop in tissue oxygenation. Moreover, in the acute post-injury period, a brief period of improved blood flow and L/P ratio was observed in the duraplasty group, however, there was no clear evidence of longer-term hemodynamic of metabolic benefits from this procedure to expand the subarachnoid space around the injured spinal cord.

Aim 3: Evaluate behavioral recovery for a total of 12 weeks following SCI with or without duraplasty

In the first study (**Study 1**, n=9/group) we tested the effects of duraplasty surgery on spinal cord hemodynamics and pressure. For technical reasons, in this study duraplasty surgery and insertion of monitoring probes were performed before SCI. SCI-induced changes in spinal cord pressure (SCP), local blood flow (SCBF), oxygenation (PO_2) and glycolytic metabolites (glucose, lactate, and pyruvate, glutamate, glycerol) were simultaneously monitored for 7 days in all animals. Subsequently, in half of the animals monitoring probes were removed and their behavioral recovery assessed for up to 12 weeks.

Table 1. Lists of animals used for study 1:

SX date	Group	ID#	Name	Species	Injury details	Force (Kdyne)	BW (kg)
2016-02-09	CNTR, Study 1	8124	Quidditch	Yucatan	**Contusion:** 50gr/20cm **Compression:** 150gr/5min	2941	24.5
2016-02-17	CNTR, Study 1	8141	Tennis	Yucatan		3354	22.0
2016-03-08	CNTR, Study 1	8156	UFC	Yucatan		3292	28.0
2016-03-10	CNTR, Study 1	8157	Volleyball	Yucatan		3160	29.0
2016-03-29	CNTR, Study 1	8065	Wrestling	Yucatan		2849	33.0
2016-08-23	CNTR, Study 1	8371	Badminton	Yucatan		3227	33.0
2016-09-06	CNTR, Study 1	8372	Diving	Yucatan		2154	34.0
2016-09-21	CNTR, Study 1	8437	Gymnastics	Yucatan		2538	32.0
2016-09-27	CNTR, Study 1	8400	Heptathlon	Yucatan		2539	34.0

SX date	Group	ID#	Name	Species	Injury details	Force (Kdyne)	BW (kg)
2015-12-07	DPLS, Study 1	7985	Orienteering	Yucatan	**Contusion:** 50gr/20cm **Compression:** 150gr/5min	1531	28.0
2015-12-09	DPLS, Study 1	7983	Polo	Yucatan		2129	25.5
2016-02-11	DPLS, Study 1	8064	Rugby	Yucatan		2242	21.5
2016-02-15	DPLS, Study 1	8112	Soccer	Yucatan		2250	22.5
2016-03-31	DPLS, Study 1	8140	Xare	Yucatan		1780	24.0
2016-08-25	DPLS, Study 1	8436	Croquet	Yucatan		2058	29.0
2016-09-11	DPLS, Study 1	8376	Enduro	Yucatan		2116	34.5
2016-09-19	DPLS, Study 1	8443	Frisbee**	Yucatan		2043	34.0
2016-09-29	DPLS, Study 1	8464	Ironman	Yucatan		2304	34.0

** animal suffered a vertebral fracture and was excluded from further analysis

In a second study (**Study 2**, n=9/group), the effect of duraplasty on behavioral recovery during a 12 week time period was studied. In these animals no probes were inserted into the spinal cord and the duraplasty procedure was performed after SCI with the purpose to get the more equivalent impact between these two groups.

Table 2. Lists of animals used for study 2:

SX date	Group	ID#	Name	Species	Injury details	Force (Kdyne)	BW (kg)
2015-07-20	CNTR, Study 2	7762	Bocce	Yucatan		2421	20.5
2015-07-22	CNTR, Study 2	7743	Football	Yucatan		3488	18.5
2015-07-29	CNTR, Study 2	7751	Hockey	Yucatan	**Contusion:**	2941	20.0
2015-08-04	CNTR, Study 2	7744	Judo	Yucatan	50gr/20cm	2692	19.5
2015-08-19	CNTR, Study 2	7791	Noodling	Yucatan		2157	24.0
2015-08-19	CNTR, Study 2	7800	Maraton	Yucatan	**Compression:**	X	X
2017-04-25	CNTR, Study 2	9036	Outrigger	Yucatan	150gr/5min	3589	25.0
2017-05-02	CNTR, Study 2	9034	Qianball	Yucatan		4208	23.5
2017-04-18	CNTR, Study 2	8998	Minigolf	Yucatan		3689	23.5

SX date	Group	ID#	Name	Species	Injury details	Force (Kdyne)	BW (kg)
2015-07-20	DPLS, Study 2	7753	Archery	Yucatan		2959	21.5
2015-07-21	DPLS, Study 2	7731	Curling	Yucatan		2981	19.0
2015-07-22	DPLS, Study 2	7732	Equestrian	Yucatan	**Contusion:**	2028	18.5
2015-07-29	DPLS, Study 2	7730	Golf	Yucatan	50gr/20cm	2611	19.5
2015-08-04	DPLS, Study 2	7736	Ice Skating	Yucatan		2910	19.0
2015-08-05	DPLS, Study 2	7758	Karate	Yucatan	**Compression:**	3052	21.5
2017-04-03	DPLS, Study 2	8996	Javelin	Yucatan	150gr/5min	3581	21.5
2017-04-12	DPLS, Study 2	9043	Luge	Yucatan		3638	19.5
2017-04-20	DPLS, Study 2	8981	Netball	Yucatan		3160	24.5

As mentioned above, in **study 1** the duraplasty procedure was performed before the SCI, which made the data analyses extremely challenging. In our previous reports, we described a notable improvement in functional recovery after duraplasty surgery with a significant difference in PTIBS (Porcine Thoracic Behavior Scale) score between the groups of study 1 (**Figure 2**). Moreover, the white matter tissue was better preserved in the duraplasty group than in the control group at both the 7-day and 12 week time point (**Figure 3**).

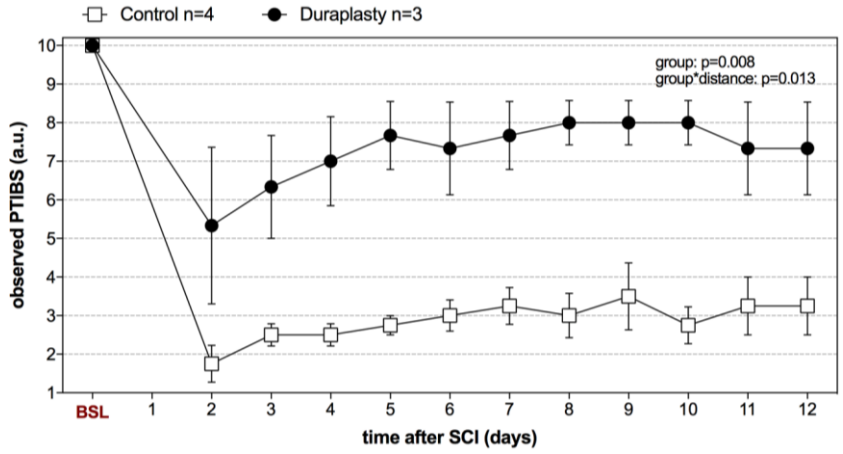


Figure 2. Assessment of functional recovery over the 12 week study period using the Porcine Thoracic Injury Behavior Scale (Study 1). Porcine Thoracic Injury Behaviour Scale (PTIBS) scores were measured before injury (BSL, baseline) and weekly for 12 weeks post injury.

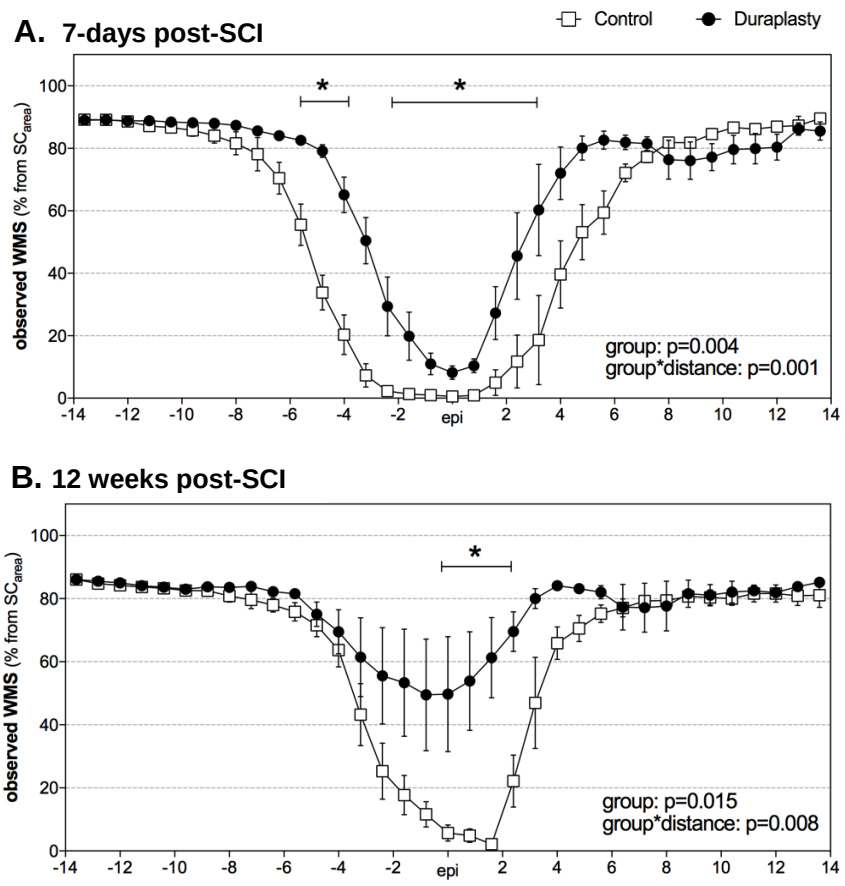


Figure 3. Spinal cord lesion at 7-days and 12-weeks post-injury, quantified as the percentage spared white matter tissue around the injury site (Study 1). Percentage of white matter sparing (WMS) at (**A**) 7 days and (**B**) 12 weeks post-SCI by area measurements taken from axial spinal cord sections at 800 μm increments, extending 14 mm on either side of the lesion epicenter (epi). The lesion epicenter was defined in cross sections as the region with the least total tissue sparing (i.e. gray plus white matter sparing).

However, when examining the dimensions of the uninjured spinal cord right before the drop, we observed relatively large differences between both groups in CSF space around the spinal cord at the T10 region where the injury occurs. Such heterogeneity in CSF space may contribute to the variability in final histological and functional outcome induced by SCI in our pig model, with more CSF around the spinal cord serving to "cushion" the spinal cord from the impactor as it lands. As we reported in **YEAR 4, Q2**, dural sac size (i.e dorso-ventral height of the dural sac, DS_{dv}) can indeed be a strong modulator of the initial severity and outcome in large animal models like the pig. Moreover, although we tried to create the same injury severity between animals that have an artificial dura (duraplasty) overlaying the cord versus the native dura by withdrawing 1-2ml of CSF ~15 minutes before the drop, the duraplasty group demonstrated lower SCI force values.

Our results demonstrated that for both these variables (force and dural sac size) the duraplasty group displayed more biomechanically favorable parameters (lower force values and higher DS_{dv} values) than the control group. Therefore, we conducted a multiple regression analysis to examine the significance and the percentage of variance accounted for by these variables and different SCI outcome parameters. For white matter sparing (WMS), PTIBS, and spinal cord pressure (SCP, 2 mm location only), the final model included only the variable DS_{dv} and explained respectively 92.4%, 85.5%, and 47.6% of the variability. Based on these results, DS_{dv} was included as a potential covariate to control for baseline differences in this study. The results demonstrated that the differences at 12 weeks and the PTIBS scores did not persist once the covariate DS_{dv} was included in the model. Only the significant difference between groups for WMS at the 7 day timepoint remained, with the duraplasty group displaying more spared white matter caudal to the epicenter (**Figure 4**).

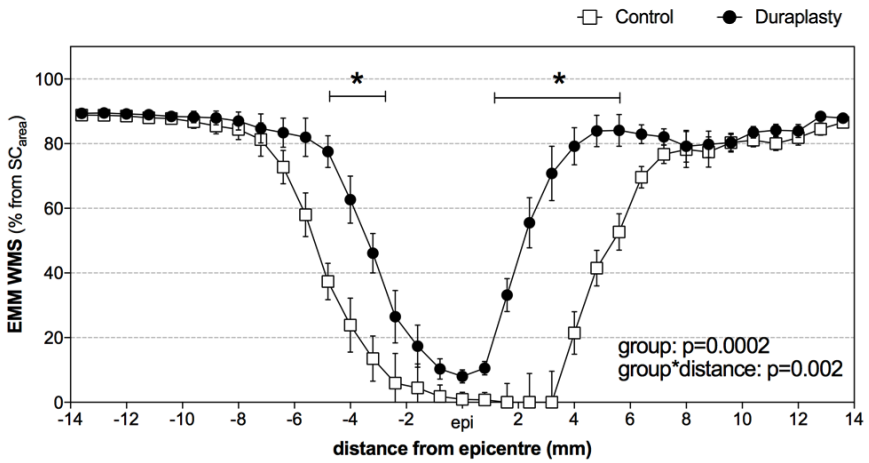


Figure 4. The estimated marginal mean (EMM) of WMS derived from the repeated-measures generalized linear mixed model (GLMM) after adjusting for pre-SCI dorso-lateral thickness of the dural sac (DS_{dv}). Group means, SEM, and p-values of the overall repeated measures ANOVA calculations are displayed. * significanlty different from control group ($p<0.05$).

Similarly, no differences in PTIBS scores or white matter sparing were evident at the 12 week post-SCI time point between groups in **study 2**, where no monitoring probes were inserted into the spinal cord and a more equivalent impact force between these two groups were observed.

Our results also demonstrated a clear association between baseline DS_{dv} and post-SCI spinal cord pressure distal to the impact site (i.e the 2 mm location of the probes), with smaller dural sac size resulting in lower pressures post-injury. Such correlation, however, was not observed for the more proximal location (22-mm location) and in the animals that received expansive duraplasty, spinal cord pressure appeared to be lower in the days following SCI (**Figure 5**).

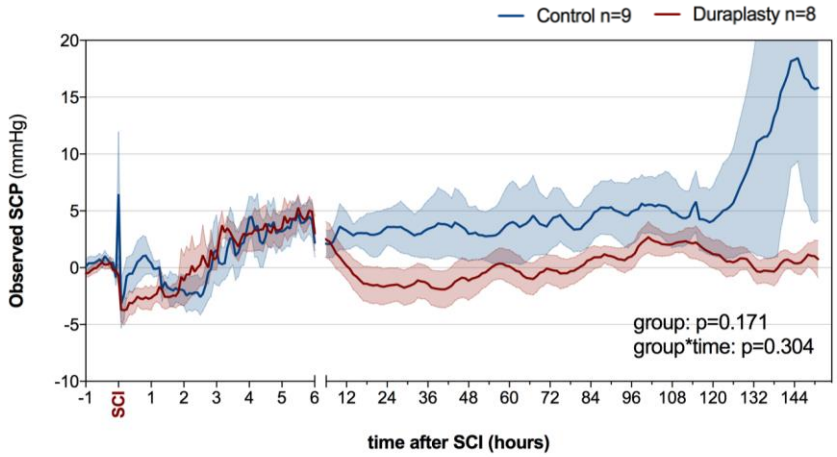


Figure 5. Trajectory of pressure distal to the impact zone (22-mm caudal). Absolute changes (Δ) of spinal cord pressure (SCP) during the first 7 days after SCI. Group means, SEM, and p-values of the overall repeated measures ANOVA calculations are displayed.

Interestingly, neither DS_{dv} nor the impact force were relevant in explaining the response variability in gray matter sparing (GMS), spinal cord blood flow (SCBF), oxygenation (PO_2), or any of the microdialysis parameters *(*L/P ratio, glucose, glutamate, glycerol). Of these parameters, the most prominent difference observed was an increased drop in tissue oxygenation in the duraplasty group (**Figure 6**) and a brief period of improved L/P ratio in the acute post-injury period (**Figure 7**), both at the 2 mm location.

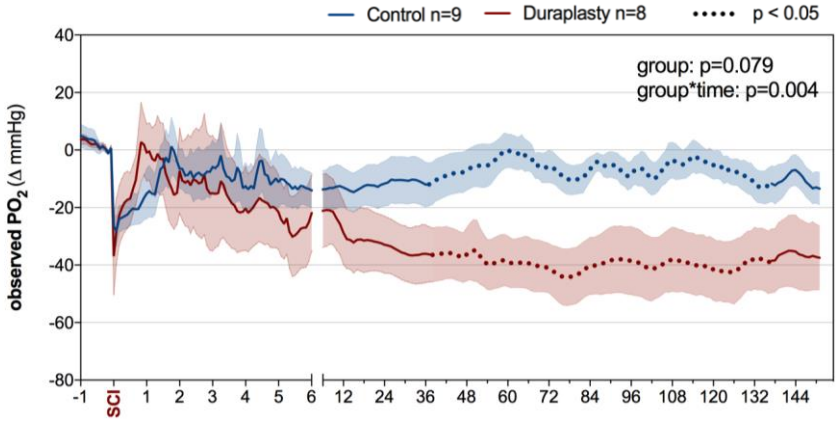


Figure 6. Trajectory of spinal cord pressure in the penumbra of the impact zone (2-mm caudal). Absolute changes (Δ) of spinal cord pressure (SCP) during the first 7 days after SCI. Group means, SEM, and p-values of the overall repeated measures ANOVA calculations are displayed. The dashed red and blue line illustrates significant differences between groups using a one-way ANOVA at each time point

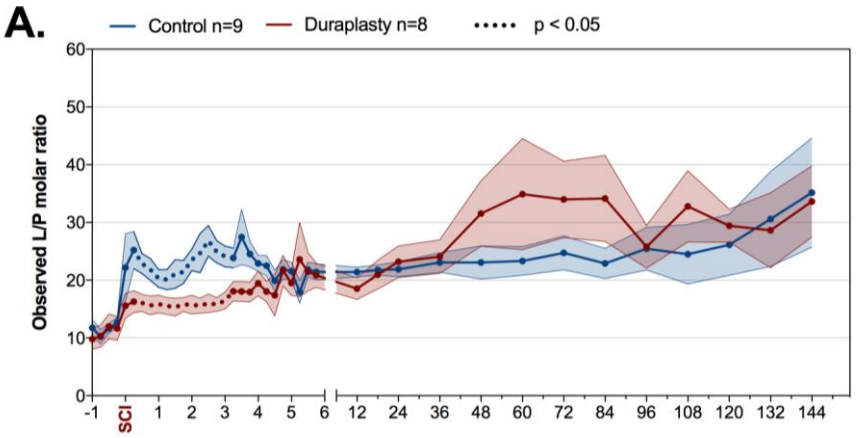


Figure 7. Trajectory of L/P molar ratio in the penumbra of the impact zone (2-mm caudal). Absolute changes (Δ) of Lactate/Pyruvate (L/P) ratio during the first 7 days after SCI. Group means, SEM, and p-values of the overall repeated measures ANOVA calculations are displayed. The dashed red and blue line illustrates significant differences between groups using a one-way ANOVA at each time point.

Aim 4. Evaluate if a moving correlation index exists between mean arterial blood pressure and CSF/cord pressure (pressure reactivity index; PRx)

Ground-breaking studies conducted by Marios Papadopoulos and his colleagues have introduced the concept of monitoring intraspinal pressure (ISP) with a pressure probe inserted into the intrathecal space directly at the site of SCI [1-4]. When the injured spinal cord swells and fills the intrathecal space, the intrathecal pressure probe is pushed up against the dura, and the resultant pressure reflects the pressure inside the spinal cord – a measure that Papadopoulos has called the "intraspinal pressure", or "ISP". By using the ISP to calculate SCPP (SCPP=MAP-ISP), Papadopoulos has shown that the autoregulatory function of the injured cord can be assessed with the spinal pressure reactivity index (sPRx), and that this may help to define the optimal SCPP (SCPPopt) for a given patient. Indeed, Papadopoulos has shown that different patients can have different optimal SCPPs based on the sPRx, and thus, an individualized approach to their hemodynamic management that takes these measures into account may improve neurologic outcome.

The work of Papadopoulos and colleagues is truly paradigm-shifting, and has the exciting potential to significantly improve the way we treat acute SCI. The basic notion is that when the injured cord swells and completely fills the intrathecal space, the pressure probe is pushed up against the dura and the ISP as measured by this probe outside the cord will reflect the pressure inside the spinal cord (which we will refer to as the "spinal cord pressure" or SCP). When the cord is swollen to the extent that it is pushed up against the dura, the ISP may indeed represent the pressure inside the spinal cord (the SCP). When the cord is swollen but not compressed by the dura, the ISP likely does not represent the pressure inside the cord but rather the intrathecal CSF pressure around the cord (which could actually then be measured more easily and safely with a lumbar intrathecal catheter). Because the assumption that ISP reflects the pressure inside the

spinal cord is reliant upon the cord swelling to the point where the dura becomes a source of compression, understanding how the measurement of ISP over time and at different locations of the spinal cord is important, particularly given that the ISP is fundamental to the calculation of spinal cord perfusion pressure.

With a pressure probe inserted into the spinal cord parenchyma and the intrathecal space at the SCI site, we demonstrated that the ISP/SCP relationship becomes stronger after the first 6 hours post-SCI, both at the rostral and caudal location (**Figure 8**). This would suggest that at the 6 hour timepoint the probe is pushed up against the dura by the swollen cord and the CSF pressure post-SCI reflect the pressure inside the spinal cord itself.

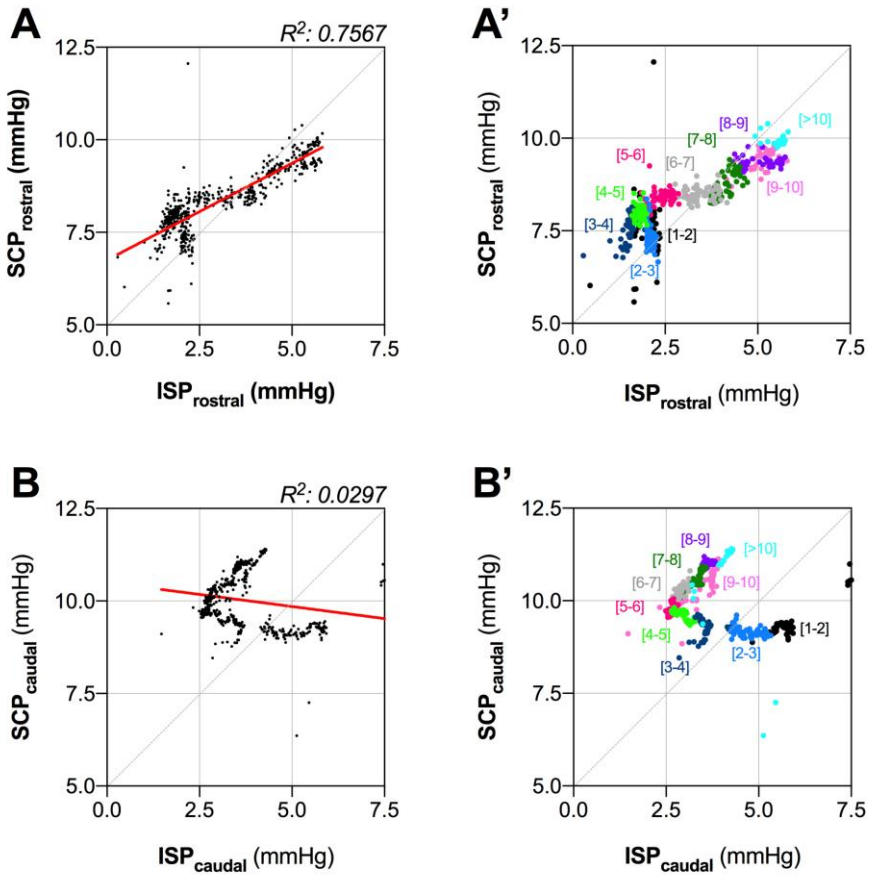


Figure 8. SCP vs. ISP measured 2mm rostrally and caudally from the edge of the impact over a 10+hour period. (A) Rostral to the epicentre (i.e 2-mm location), the relationship between SCP and ISP over a 10+ hours is relatively close (red line: linear regression line). (B) Caudally the ISP/SCP relationship is almost non-existent. (A'-B') When graphed in 1 hour bins, it becomes apparent that the ISP/SCP relationship becomes stronger after the first 6 hours post-SCI, both at the rostral and caudal location.

Our data also demonstrated that in the ensuing days extent of post-decompression swelling can be extremely different between animals even with biomechanically identical injuries (50g weight drop contusion at T10, 1 hr compression), which most certainly affect how the spinal cord "pushes" the pressure probe against the dura and will therefore influence the resultant measure of ISP over time.

Figure 9 shows data from n=4 pigs, where the SCP and ISP were continuously measured for 7 days after SCI. At the epicenter of injury, the relationship between SCP and ISP over 7d is very close in Pig B and D (see red line). This is actually very consistent to the result that was published in a case report by Papadopoulos where he put a pressure probe into both the intrathecal space and into the spinal cord parenchyma in a single patient with a severe T3 SCI, and reported very close correlation between the two pressures. So, the ISP/SCP correlation in this one human patient is indeed replicated in pig B and D. However, in for example pig A, the ISP/SCP relationship is almost non-existent. Also, 20 mm distal to the epicenter (still within the "penumbra" of injury), the relationship between SCP and ISP is poor in this pig, showing the influence of spatial distribution. These results not only illustrate the heterogeneity even in animals with biomechanically identical injuries (50g weight drop contusion at T10, 1 hr compression), but also demonstrate how the measurement of "intraspinal pressure", or ISP, with an intrathecal catheter outside the spinal cord can provide a misleading measurement of the true pressure inside the spinal cord.

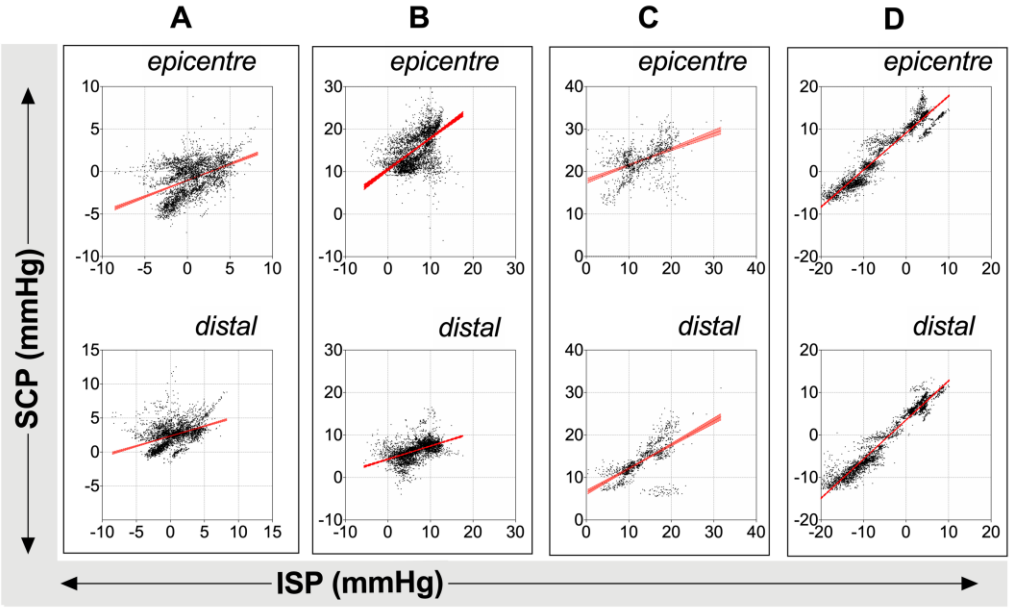


Figure 9. SCP vs ISP and trendline measured over a 7-day period after SCI in four pigs.

Although over a 7-day period a clear correlation was observed between ISP and SCP for pig B and D, when graphing the calculated sPrx as the moving correlation coefficient between MAP and cord pressure (either ISP or SCP), the resultant sPRx values (and their interpretation) were quite different (**Figure 10**). In pig B, the upper limit (UL) and lower limit (LL) of autoregulation was clearly identified by slope analysis of sPRx versus MAP. For this pig, UL was identified at a MAP of 130mmHg and LL at 80 mmHg with an optimal SCBF of approximately 500 PU. Notably, in pig D, the calculation of the sPRx using ISP was in poor agreement with the sPRx derived using the directly measured intraparenchymal pressure (SCP). While ISP-derived sPRx suggest a narrow range of autoregulation, SCP-derived sPRx indicated impaired autoregulation throughout the MAP range, which was in excellent agreement with the obtained blood flow data.

Thus, these two measurements would lead to differing interpretations of the hemodynamic status of the cord and how to best manage it.

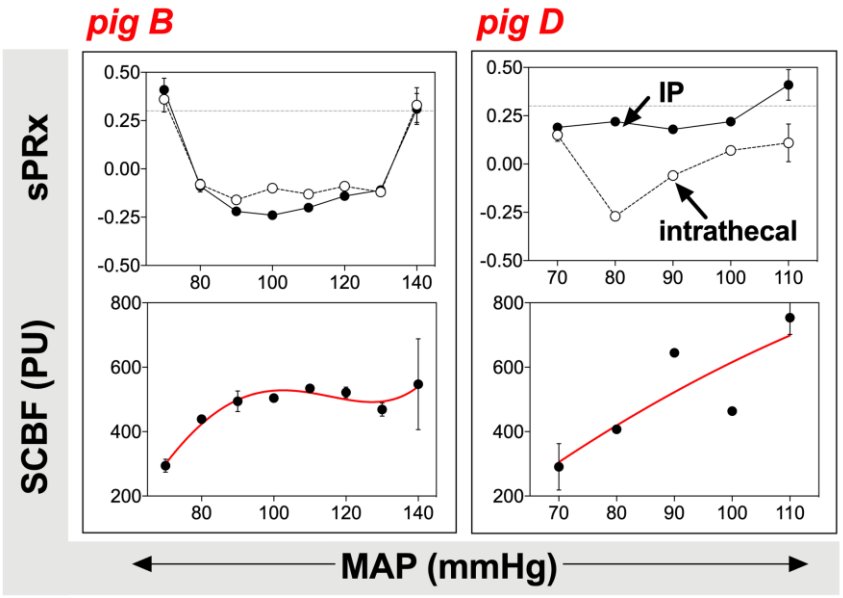


Figure 10. Changes in spinal cord blood flow and sPRx calculations using SCP and ISP measurements plotted against MAP in two pigs. in Pig D, the ISP-measured sPRx with a pressure probe in the CSF space would not detect autoregulatory dysfunction.

5 KEY RESEARCH ACCOMPLISHMENTS

In Year 1-4 we have shown that expansion duraplasty of three levels following thoracic SCI may confer some benefits acutely; however, in our porcine model of SCI there is no clear evidence of longer-term benefit from duraplasty surgery. A manuscript presenting the results of the duraplasty study is in preparation for submission to *the Journal of Neurotrauma* (draft manuscript in appendix). This is a key research accomplishment because expansion duraplasty is being considered as a surgical intervention for acute SCI patients.

Moreover, we have demonstrated that inter-animal variability in dural sac morphometry could provide a potential biological explanation for the observed heterogeneity in histological and behavioral outcomes in our pig model of SCI. Such knowledge is helpful for appropriately balancing experimental groups, and/or varying impact parameters to match cord and CSF layer dimensions, for future studies. A manuscript has been submitted to *the Journal of Neurotrauma.* This is a key research accomplishment because it will help us balance experimental groups in future studies using the pig model of SCI.

In Year 1-4 we demonstrated that subject- and time-dependent differences in ISP and SCP are considerable, even in a controlled experimental setting. Furthermore, we showed that both SCBF and SCP associated with impairment and preservation of sPRx, while for ISP such an association was not necessarily always seen. This is a key research accomplishment because it highlights how heterogeneous the physiologic responses to acute SCI are within the penumbra of the SCI – this is important given that the measurement of ISP and SCP is being proposed for human SCI.

What is the significance of these findings? (The "so what?")

1. We undertook this experimental animal study because of the clinical observation that considerable swelling of the spinal cord often occurs in the hours to days post-injury, and that this swelling might contribute to increased pressure inside the spinal cord if the cord expanded to fill the entire intrathecal space and became "constricted" by the dura. We found that there is indeed an increase in pressure inside the spinal cord after injury, but this pressure increase occurs to only a certain point (around 200% of normal) and then diminishes over time. This suggests to us that while the cord might expand, once it hits the dura, the pressure does increase to some extent but rather than just continue to increase, the pressure spreads through the rostro-caudal axis of the spinal cord (much like when you blow up a long party balloon, the balloon does not expand at one point only – it expands along the length of the balloon.

What does this mean? This finding is significant because it suggests to us that there is indeed an increase in pressure inside the spinal cord as it swells, and that there may be some added pressure as it expands against the dura. But this added pressure is limited as the pressure distributes rostro-caudally through the cord. This tells us that opening the dura with a 'duraplasty' may only relieve the pressure to some extent, and that it needs to be done over multiple spinal levels to mitigate against the swelling that has occurred rostro-caudally.

2. The technique for measuring PRx that has been pioneered by Dr. Papadopoulos depends upon a pressure monitor that is inserted into the intrathecal space right at the spinal cord injury site, and when the cord swells it pushes the pressure monitor up against the dura. It is this pushing up against the dura that allows this pressure probe (sitting OUTSIDE) the spinal cord, to reflect the pressure INSIDE the spinal cord. But our findings of the varying morphometry of the spinal cord in the pigs (which incidentally resembles the anatomic morphometric variations in humans) suggested to us that the injury to the cord and its swelling response would be quite influenced by the cord morphometry. For example, in a situation where the spinal cord occupies the vast majority of the intrathecal space and there is little CSF to "soften" the weight drop blow, the injury itself will be more severe, and even a small amount of swelling might push the pressure probe up against the dura. But if the spinal cord occupies a relatively small portion of the intrathecal space and there is much more CSF around the cord, a large amount of swelling may not allow it to expand to even touch the dura. We began to realize as we were doing our experiments that this variability in cord morphometry would influence the sPRx values (see Figure 9).

What does this mean? This finding is significant because it suggests to us that while sPRx values can be gleaned as the moving correlation coefficient between MAP and "intraspinal pressure", that this sPRx value will be dependent upon how the "intraspinal pressure" is actually measured. Measurement within the intrathecal space (i.e. the Papadopoulos method) is fine so long as the swelling of the cord expands against the dural sac. But if the spinal cord morphometry is such that this cord swelling does not expand against the dural sac, then this measurement of intraspinal pressure is different than the measurement of pressure INSIDE the spinal cord. What this indicates is that while sPRx may be a helpful way of monitoring hemodynamics in the injured spinal cord, the technique of measuring intraspinal pressure is important, and this is dependent upon 1. The degree of swelling (which is affected by injury severity and is differs along the rostro-caudal length of the cord) and 2. The native spinal cord morphometry.

3. We expended considerable effort to address the question of whether expansion duraplasty was a beneficial neuroprotective strategy for the spinal cord, and what it actually does to the physiologic parameters within the cord (e.g. spinal cord blood flow, oxygenation, pressure, metabolism). In the end, we did not find a beneficial effect, although the data – like many in vivo experiments – was abit mixed and did not all fit into one tidy, clear narrative. When we randomized animals to duraplasty vs no duraplasty, there was indeed no marked improvement in hindlimb function in the duraplasty animals. The physiologic effects were somewhat surprising though. There was indeed a lowering of the pressure within the cord in the duraplasty animals, but this was not always associated with the expected improvements in oxygenation and blood flow. So, the exact underlying mechanisms promoted by expanding the intrathecal space were somewhat inconsistent. Part of this challenge we recognize was experimental – it was, simply put, very difficult to create biomechanically severe injuries in the duraplasty and non-duraplasty animals once we had done the duraplasty, and in order to measure the physiologic parameters we needed to do the probe insertion and duraplasty BEFORE the injury.

What does this mean? This finding is significant because it suggests that while a duraplasty might expand the thecal sac and lower the pressure within the spinal cord, that the downstream effects in terms of improving acute physiologic responses and long-term hindlimb function are not that beneficial. This in part might be explained by points 1 and 2 described above. Firstly, the pressure we observed around the injury site in the pig spinal cord appears to increase but only to a point (and then we assume that the pressure is transmitted rostro-caudally); and secondly, the differences in spinal cord morphometry might mean that in some cases the swollen cord does not actually expand against the spinal cord. These are important considerations to consider then in the design of a clinical trial (which I understand is actually being designed by Dr. Papadopoulos in the United Kingdom).

What might we do next from a scientific standpoint?

Like many researchers, we were really hopeful that our duraplasty vs no duraplasty randomized trial would show a benefit to duraplasty. It could be argued that our paradigm of doing the duraplasty right after the injury would be made more clinically relevant by waiting a period of time to simulate the kind of delays that human patients would incur. But given that there was no convincing improvement with the intervention early on, we feel it unlikely to show a benefit when delayed.

The next direction that seems logical would be to further explore the issues of swelling and cord pressure in the context of different injury severities, different locations along the rostro-caudal aspect of the cord, and different spinal cord morphometries. This is important because there is clearly heterogeneity in the human condition with respect to injury severity and spinal cord morphometry, and these can be modeled well in the pig (where we can experimentally alter injury severity, and spinal cord morphometry also differs from animal to animal). Similarly, an evaluation of how the measurement of intraspinal pressure differs between a probe INSIDE the spinal cord and a probe OUTSIDE the spinal cord would be warranted, given what we observed.

These ideas were actually, coincidentally, formalized in a submission to the DoD SCIRP program in October 2018; we applied for an Investigator Initiated Research Award to continue on this line of investigation in our pig model of SCI to evaluate spinal cord pressure (measured inside and outside the spinal cord and at different places along the rostro-caudal axis of the cord) in the context of different injury severities and different cord morphometries. We were unsuccessful in this highly competitive competition (only 6 of 67 grants were funded, 9% success rate). But we did receive a respectable score of 2.0, “Excellent” from the grant reviewers and are considering a resubmission of this grant, either in the fall or as part of the newly announced Expansion awards.

6 CONCLUSION

Duraplasty vs no duraplasty: our study showed that early duraplasty did not improve behavioral outcome**,** but did increase white matter sparing and reduce the L/P ratio acutely after SCI. We acknowledge that the current spinal cord injury protocol requires decompressive duraplasty prior to producing the contusive spinal cord injury, which does not mimic a true clinical SCI scenario where a displaced spinal column may cause the primary injury and result in residual cord compression. We previously reported that after a moderate severity SCI using our pig model of SCI, only a subset of animals displayed a rapid onset of subarachnoid space occlusion at the site of injury (<6 hours post-SCI)[5]. These observations point to pronounced heterogeneity in timing and profile of spinal cord swelling after SCI, even with a pre-defined drop height and compression time. Such inconsistency in the changes in cord swelling may partly explain the observed variable responses in our study and might have negated some more acute benefits of decompressive duraplasty.

ISP vs SCP: caution is warranted in assuming that the ISP is always equivalent to the SCP – it certainly may be equivalent in some cases (for example, in the most severe injuries, as shown by Papadopoulos in the n=1 case report of thoracic SCI), or in some cases *for part of the time* (for example, in the first few days post-injury), or in some cases *in part of the spinal cord* (for example, at the epicenter). These caveats are important to consider because the ISP is used to calculate spinal cord perfusion pressure (SCPP), which is then used to determine the sPRx, which is then used to identify the optimal SCPP, which may then be used to guide the hemodynamic management.

7 PUBLICATIONS, ABSTRACTS AND PRESENTATIONS

1. "Duraplasty in Traumatic Thoracic Spinal Cord Injury: The Impact on Spinal Cord Hemodynamics, Tissue Metabolism, Histology and Behavioral Recovery Using a Porcine Model". Publication to be submitted to Journal of Neurotrauma **(draft manuscript in appendix).**

2. "Inherent Variability in Morphometric Measures of the Uninjured Porcine Spinal Cord Affects Histological and Functional Outcomes after SCI". Manuscript is under review in Journal of Neurotrauma.

3. "Sensorimotor Plasticity after Spinal Cord Injury: A Longitudinal Translational Study". Manuscript has been published in Annals of Clinical and Translational Neurology ACTN, Dec 2018 10.1002/acn3.679

4. Poster presentation, Society for Neuroscience 2018, San Diego, CA, Nov 3-7:

   Title: Differences in morphometric measures of the uninjured porcine spinal cord and dural sac predict histological and behavioral outcomes after traumatic sci
   Session Type: Poster
   Session Number: 568
   Session Title: Spinal Cord Injury and Plasticity: Animal Models and Human Studies
   Date and Time: Tuesday Nov 6, 2018 1:00 PM - 5:00 PM
   Abstract Control Number: 8202

8 INVENTIONS, PATENTS AND LICENSES

Nothing to report

9 REPORTABLE OUTCOMES

- Decompressive duraplasty of three levels following thoracic SCI confers acute benefits
- There is no clear evidence of longer-term benefit from duraplasty surgery
- Subject- and time-dependent differences in ISP and SCP are considerable following SCI
- sPRx calculations are feasible in our pig model of SCI
- Both SCBF and SCP associate with impairment and preservation of sPRx
- ISP/sPRX association is not necessarily always seen

10 OTHER ACHIEVEMENTS

Nothing to report

11 REFERENCES

1. Phang, I. and Papadopoulos, M.C. *Intraspinal Pressure Monitoring in a Patient with Spinal Cord Injury Reveals Different Intradural Compartments: Injured Spinal Cord Pressure Evaluation (ISCoPE) Study.* Neurocrit Care, 2015.
2. Varsos, G.V., et al. *Intraspinal pressure and spinal cord perfusion pressure after spinal cord injury: an observational study.* J Neurosurg Spine, 2015. **23**(6): p. 763-71.
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5. Jones, C.F., et al. Gross Morphological Changes of the Spinal Cord Immediately After Surgical Decompression in a Large Animal Model of Traumatic Spinal Cord Injury SPINE, 2012. **37**(15):, E890–E899

12 APPENDICES

- “Duraplasty in Traumatic Thoracic Spinal Cord Injury: The Impact on Spinal Cord Hemodynamics, Tissue Metabolism, Histology and Behavioral Recovery Using a Porcine Model”. Publication to be submitted to Journal of Neurotrauma.