Webinar Q&A Report:
Cardiovascular Pathophysiology In the Setting of Spinal Cord Injury

What happened to cardiovascular function when/if you introduced ganglionic drugs?

Following the administration of HEX in animals that had received an acute SCI we found no further reduction in either LV Pmax or LV dP/dTmax. As an aside, we have done this experiment the other way around and performed an SCI after delivering i.v. HEX and again found no further decrease in either LV Pmax or LV dP/dTmax when SCI is performed after HEX. Both data sets help us determine that the reduction in pressure generating capacity in the heart after SCI is of neural origin.

Intermittent hypoxia induces hypertension via increased respiratory modulation of vasomotor sympathetic activity. Have you looked at respiratory modulation of vasomotor sympathetic activity following chronic intermittent hypoxia in spinal cord injured rats?

This is correct and absolutely something that is of interest to us. We are currently performing pilot experiments to optimize the preparation necessary to answer this question in our SCI animals. We plan to answer this by performing concurrent phrenic/renal/splanchnic recordings in addition to art pressure and LV pressure before, during and after an AIH protocol. This is a complex prep made hard by the fact that our SCI animal start at a MAP of about 65mmHg. My hunch is that respiratory modulation of SNS will be diminished in our SCI animals, but this remains to be seen.

For your PV data collection, have you done any work on the right side of the heart? What effects are seen there in terms of contractility?

We have started to perform biventricular synchronous PV loops in the lab but have not yet performed this preparation in our SCI animals. Based on histological findings from the RV and LV post-SCI we expect that the magnitude of reduction in RV and LV function will be similar in our SCI rats.

What is the role of the inflammatory response in your system?

We do not yet know the answer to this question. Most of our experiments are performed in the chronic setting, after which any acute inflammatory response induced by the SCI is complete. That said, evidence suggests that individuals (and rats) have chronic increases in inflammation. We plan to investigate the role of inflammation on CV dysfunction post-SCI in the coming years.

Did you assess the effect of cold stimuli on the spinal cord nerve activity and potential association with intrathecal administration of pharmacological agents?

Interesting question. No, we have not yet done this in our SCI animals. I have performed a significant number of cold pressor tests in humans with SCI but unfortunately not with concurrent MSNA recordings. We have also not yet performed intrathecal administration of any drugs. We are moving forward with intraspinal injections due to the improved ability to target individual levels more specifically within the spinal cord.

What was the dosage of hypoxia? How did you decide on that dosage? There is a growing amount of literature showing MIH can reduce blood pressure.

In our SCI experiments under anesthesia, we use the following: 10 x 1min FiO2 0.1, interspersed with 2 mins of FiO2 of 1.0. In chronic (i.e., awake) SCI experiments we use the same protocol except with an FiO2 of 0.28 in between our hypoxic bouts (this maintains a PaO2 of approx. 105mmHg). We settled on these doses after pilot testing (we also tried 10 x 45s, 3 x 5 min, and a range of FiO2 between hypoxic bouts). I agree on the MIH comment – we are targeting more towards the severe end of the spectrum as we think targeting the spinal (and not suprapsinal) pathways may be more efficacious post-SCI.

What does intermittent hypoxia look like in a rehab setting?

Few thoughts here: 1) I think performing a short AIH protocol could 'enable' an improved CV response during exercise and therefore augment the efficacy of exercise to offset CV disease risk in those with high-SCI; 2) I think we need to do a lot of work still on cross-organ optimization of protocols. For instance, is the optimum dose/timing the same for CV/Motor/Respiratory. This is akin to the epidural stimulation challenge too. Hopefully we can find a protocol that can optimize the function of multiple bodily systems post-SCI; 3) We are going to need to figure out what the optimum 'device' is to deliver the AIH and what role CO2 plays in this as well before we can truly progress with home-based systems and/or remotely delivered interventions.

Do you see a recovery in sympathetic nerve activity after SCI, or does activity stay reduced long-term?

Great question – my hunch is it is reduced in the first day or so after injury due to neurogenic shock and then recovers and remains at a new 'lower' level. Unfortunately, we do not yet have longitudinal data in our hands to show this. Only 1 study has used telemetry to look at long-term SNA changes after SCI and found it stabilizes pretty well after injury and then doesn't really change. However, that study investigated only changes in the 'global signal'. We do not yet know about burst frequency / incidence / respiratory modulation / or even whether they are still baro-sensitive.

Do you see a difference between Renal and Splanchnic nerve activity changes post-SCI?

I can only imagine yes, as even in our non-SCI rats we see these differences, but we have not yet performed these comparisons.

Do you think there is a confounding contribution to decreased heart size/mass due to atrophy associated from reduced activity?

Tough question to answer but my hunch is that the reduced heart mass is more a direct role of the loss off SNS input, and maybe a SCI-induced reduction in blood volume. There are some terrific papers coming out of Italy showing the role of the SNS in tuning cardiomyocyte size – so I suspect this is the bigger contributing factor. See here for an example paper: https://pubmed.ncbi.nlm.nih.gov/23090606/

Have you investigated the molecular changes in heart tissue following SCI? transcriptomics? proteomics? metabolomics?

Yes, we have but we are not yet at liberty to share these results yet as they are incomplete. It seems that the molecular pathways controlling cell size are changing post-SCI (confirmed via qPCR and Western blot) and there is increased expression of key genes involved in cell signaling, metabolism and ischemia post-SCI.

Have you tested Dobutamine effects in your chronic rat model of SCI? Do the hearts still have the capacity to increase contractility?

We are performing this experiment as I write this response. In our pig model, we found that at 12 weeks following T2-SCI the maximum Ees (i.e., the Ees achieved under DOB infusion) was hugely reduced, suggesting an inability increase contractility post-SCI. This is actually easy to answer in the pig model as performing IVC occlusions under DOB stimulation in rats is very challenging to maintain an optimum PV loop.

The hexamethonium experiments are an excellent test. Have you considered targeting the direct role of stellate ganglia in the control of cardiovascular phenotypes in SCI?

We are currently repeating the blockade experiments under separate and combined beta-blockade and vagal-blockade to answer this question more specifically. Isolating the stellate is hard if not impossible whilst retaining pulmonary pressures around the heart. I would like to deliver CTB-conjugated to a fluorophore into the pericardial sac to back-trace and visualize the ventricular projecting SNS fibers and see how these change with SCI. Functionally, the only way I can think to do this is to develop a cre-based transgenic rat that expresses ChR2 in nor-adrenergic neurons. From here we could then optogentically stimulate the stellate in an open chest ventilated rat whilst measuring PV indices in vivo in rats with and without SCI to see how the stellate regulation of contractility changes with injury.

Could intermittent hypoxia resemble ischemic conditioning particularly in those with SCI and therefore be considered of potential benefit to cardiac function?

This is absolutely our working hypothesis, yes.

For RV PV loops, have you investigated direct stab approaches?

We have tried this, and it definitely works, but we lose the pulmonary pressure around the heart, so there are pros and cons to this approach.

Is there any evidence of remote ischemic reperfusion having a positive effect in SCI?

Yes, see here for an example paper:
https://www.sciencedirect.com/science/article/pii/S0022522320307935

I am aware only of studies that have looked at response in the spinal cord post-SCI, not sure in the CV system.

Have you measured cardiomyocytes following intermittent hypoxia? Since all tissues are exposed to fluctuating patterns of oxygen, could IH elicit benefits in the periphery (in addition to activating neural circuitry)?

We are actively answering this question in the lab, not sure on the answer yet. But my hunch is yes!

Are you thinking too much about drugs to get cardiac function up in the acute setting? Simple cardiac pacing at a higher than normal rate might do the same thing without the hemorrhage risk. It's probably impossible in rats, but it should work in pigs.

Fair point, but I am not sure this will help. My hunch is that we need to pair the hemodynamic management with a strategy to dilate the local cord vasculature and help 'steal' blood away from the hemorrhaging vessels that were damaged as a result of the injury itself. This is at least the line of investigation we are currently following.

Is there any consideration for using rodent models for SCI-CV assessments, given that rodents are more sympathetically driven than large animals/humans, which are more PS-driven?

This is definitely a concern, and partly why we have also been developing the pig model.

In regards to the reduction in sympathetic activity after spinal cord injury, does it result in a reduction in the population of axons in the parasympathetic system with time?

This is an interesting question and one we have not yet investigated in our model. The only paper on this post-SCI is this one by the DiCarlo group, but note they use a mid-thoracic injury model which preserves descending SNS input to the heart.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4097824/

Did you measure lung function in this model?

We have not performed lung function tests in the T2 model. Most research in SCI uses the cervical hemisection or compression model for investigating changes in respiratory function after SCI.

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