**INTRODUCTION**

- Chemotherapy induced peripheral neuropathies (CIPN) are a serious concern when patients are treated with microtubule dependent chemotherapy drugs such as paclitaxel (Taxol).
- Approximately 250,000 new patients are treated with Paclitaxel each year (American Cancer Society).
- 40-50% of patients undergoing chemotherapy treatment will eventually develop irreversible CIPN which frequently leads to termination of treatment (Ehrlich, 2012).
- Recent studies suggest that chemotherapy induced peripheral neuropathy is linked to the interaction between paclitaxel and the cytoplasmic calcium binding protein, neuronal calcium sensor NCS-1.
- Paclitaxel increases binding of NCS-1 to the inositol 1,4,5 tris-phosphate receptor (InsP3R) causing increased open probability of the InsP3R and activation of the calcium-dependent protease, calpain.
- Calpains cleave NCS-1 and a number of other intracellular proteins, resulting in interrupted calcium signaling and eventually irreversible damage to neurons.
- A mouse knockout model for NCS-1 was generated to help understand the biology of the NCS-1 and its role in chemotherapy induced neuropathies.

**HYPOTHESIS & SPECIFIC AIDS**

- We hypothesized that alterations in NCS-1 (NCS-1 knockout) will change the sensitivity of the system to chemotherapy drugs such as paclitaxel and the subsequent duration and severity of the paclitaxel-induced neuropathy.
- A thorough characterization of the NCS-1 knockout phenotype (and comparison to the wild-type controls) is necessary prior to the paclitaxel-induced neuropathy studies.

**METHODS**

- Subjects: NCS-1 wild-type (WT) (n=29) and knock-out (KO) (n=15) mice. Mice were housed 4 to a housing unit in the University of New England Animal Care facility under standard housing conditions with food and water ad libitum.
- Assay 1 - Cold/Hot Plate (Itpa Basa, Italy): Mice were placed individually on the plate and the latency (s) it took the mouse to respond (i.e., 15s/kg/ shaking of hind paw and/or jumping) was recorded.
- Cold Plate: Temperature set at 10°C (5oC cut-off), 5°C and 2°C (5s cut-off).
- Hot plate: Temperatures tested: 50°C, 52°C and 55°C (15s cut-off).
- Assay 2 - Tail Flick (circular water bath): Mice were restrained, and the distal 1/3 of the tail was immersed into the water and the latency to flick the tail was recorded with testing at: 40°C, 43°C, 46°C and 49°C (15s cut-off and 52°C and 55°C (15s cut-off).
- Assay 3 - Acetic Acid Writhing: Mice were given an intraperitoneal (i.p.) injection of 0.56% glacial acetic acid (AA) at a volume of 10 ml/kg bodyweight and are immediately placed in a clear, plastic observation tank. Mice were then observed individually for a 20 minute session. Total number of writhes within the session was recorded by trained observers blinded to treatment/pheno type. The primary readout was a writh or a lengthwise stretch of the body, characterized by the back arching and the hind paws stretching out behind the mouse so that the abdomen is stretched across the surface of the observation chamber.
- Assay 4 - Inflammatory Pain (carrageenan): Mice were tested in the von Frey assay to obtain a baseline threshold. Following baseline, mice were injected subcutaneously in the left hind paw (paw) with carrageenan at a volume of 50uL. Injections were performed using a glass Hamilton syringe with a 30G needle. Mice were re-tested in the von Frey assay 3.5 hours post-injection.
- Assay 5 - Post-surgical Pain (plantar incision surgery): Mice were tested in the von Frey and Hargreaves assay to obtain a baseline threshold. Mice were anesthetized under isoﬂurane inhalation and the left hind paw was propped for surgery. A 5mm incision was made in the skin (0.5cm distal to the heel) and the plantar muscle was retracted to make a 0.7mm incision lengthwise in the muscle using a #11 surgical needle. The wound was stitched with 2 sutures knots of 4-0 nylon, cleaned with saline and a topical antibiotic was applied to prevent infection. Mice were re-tested in the von Frey and Hargreaves assays 24, 48 and 72 hours post-surgery.

**RESULTS**

All mice were tested in the following assays:

**EXPERIMENTAL DESIGN**

**RESULTS**

- Cold Plate/Hot Plate (Figure 1a/b):
  - In both the cold and hot plate assays, there was a stimulus (temperature) response relationship that was similar in both NCS-1 WT and KO mice.
  - Increasing noxious stimuli led to decreased latency times.
  - At the lower intensity stimuli (cold and hot plate), the NCS-1 KO mice had decreased latencies to respond.
  - At the higher intensity stimuli (cold and hot plate), no notable differences between the NCS-1 KO and WT mice were observed.

- Tail Flick (Figure 2):
  - The results recorded for both WT and KO mice showed a descending trend for both groups as temperatures increased.

- Acetic Acid Writhing (Figure 3):
  - There was no difference observed between NCS-1 KO and WT mice for baseline thresholds.
  - Following carrageenan treatment, both NCS-1 WT and KO mice showed similar level of hypersensitivity to tactile stimuli and no statistical differences were observed.
  - Following plantar incision surgery, both groups remained similar in their level of hypersensitivity to thermal stimuli and no statistical differences were observed.

- Hargreaves (Figure 4):
  - There was no difference in baseline thresholds in the Hargreaves test between NCS-1 WT and KO mice.
  - Following plantar incision surgery, hypersensitivity developed within 24 hours and remained hypersensitive within 3 days.
  - NCS-1 KO mice showed similar hypersensitivity levels on day 1 and reduced effect on days 2 and 3.

- Open-field, Rotarod, Grip-Strength and Elevated Plus Maze
  - Overall, the NCS-1 KO mice had a behavioral phenotype that was close to the WT controls with respect to the motor system (open-field levels of activity, grip strength and coordination) as well as with respect to anxiety-like baseline behaviors.
  - Sensory testing revealed a near normal phenotype with respect to baseline sensitivities to hot and cold stimuli, as well as tactile thresholds.
  - There were some modest differences in reduced latencies (hypersensitivity) at the lower cold and hot stimuli in both the cold and hot plates.
  - Responses to three commonly used noxious stimuli (chemical/inflammatory/mechanical) resulted in expected pain like behaviors including reduced thresholds to response.

**DISCUSSION**

- Overall, the NCS-1 KO mice had a behavioral phenotype that was close to the WT controls with respect to the motor system (open-field levels of activity, grip strength and coordination) as well as with respect to anxiety-like baseline behaviors.
- Sensory testing revealed a near normal phenotype with respect to baseline sensitivities to hot and cold stimuli, as well as tactile thresholds.
- There were some modest differences in reduced latencies (hypersensitivity) at the lower cold and hot stimuli in both the cold and hot plates.
- Responses to three commonly used noxious stimuli (chemical/inflammatory/mechanical) resulted in expected pain like behaviors including reduced thresholds to response.

**FUTURE DIRECTIONS**

- This initial characterization of the NCS-1 mouse knockout model can be expanded in many ways in order to gain a better understanding of the separation of the crucial calcium signaling pathways involving NCS-1 and calcium signaling disruption by chemotherapeutic drugs such as paclitaxel.
- Future studies will focus on assessing nerve injury sequelae following nerve injury in NCS-1 WT and KO controls.
- We will also assess administration of small molecule inhibitor drugs that restore normal calcium signaling through NCS-1 before, during or after paclitaxel in order to identify potential neuroprotective treatments.

**LITERATURE CITED AND ACKNOWLEDGEMENTS**

4. This research was supported in part through a National Institutes of Health - National Institute of General Medical Sciences Grant (R01GM078345).