Sex Differences in the Development of Chemotherapy Induced Peripheral Neuropathy

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ABSTRACT

Various clinical and preclinical studies have reported that females exhibit greater sensitivity than males in the development of pain. However, the sex differences in the development of chemotherapy induced peripheral neuropathy (CIPN) is poorly elucidated. CIPN is damage to peripheral nerves caused by common chemotherapy treatments that affects 70% of chemotherapy treated patients. In many cases, it is a dose-limiting side-effect and has been linked to poor survival rates. After cessation of treatment, CIPN becomes chronic in 30-40% of patients and dramatically hinders their quality of life by generating pain, burning, and mechanical sensitivity in the extremities. Unfortunately there is no well-established mechanism or treatment for CIPN. Here we investigated the differences between male and female rodents in the development of chemotherapy induced neuropathy. To assess mechanical sensitivity, we used the von Frey behavioral model as well as immunohistochemistry to explore the differences in the development of CIPN and the anatomical manifestations of peripheral neurons after the onset of neuropathy in both males and females. Our research will lead to a better understanding of CIPN progression and considerations in therapeutics in treating CIPN.

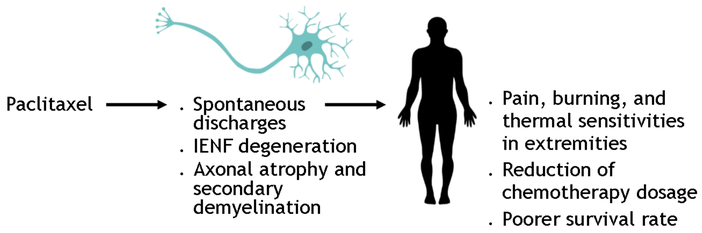

FIGURE 1. Chemotherapy Induced Neuropathy symptoms. The affect CIPN has on the micro and macro scales of the human body.

MATERIALS AND METHODS

ANIMALS

Adult (8 weeks of age) male and female C57BL/6J mice were utilized.

PACLITAXEL INJECTIONS

Paclitaxel diluted in vehicle was administered at a dose of 4 mg/kg intraperitoneally on days 0 and 2. Control group was administered an equal volume of vehicle alone.

BEHAVORIAL TESTING

We used the von Frey assay to test the mechanical sensitivity. Mice were placed in an acrylic chamber with a wire mesh floor for 1 to 2 hours before testing. With calibrated von Frey filaments, we tested for a response using the up-down method. Animals were tested on days 0, 1, 3, 5, 6, and 7 post injection. Data was then expressed as mean ± SEM. Statistical analysis was performed using repeated measures two way ANOVA with statistical significance set at $p < 0.05$.

IMMUNOCHEMISTRY

On day 8 post paclitaxel treatment, we perfused, post-fixed, and cryo-protected hind paw and sciatic nerve. The hind paws were cut in 25 micron sections. Sections were then blocked, incubated in primary solution overnight, incubated in secondary solution for 1 hour, stained with DAPI, and then mounted. Sciatic nerves were stained free floating, incubated in primary for 3 days, washed with PBS, incubated in secondary for 1 day, incubated with DAPI and then whole mounted. Antibodies used in the staining of the paw include anti-IB4+(1:500) and anti-PGP9.5 (1:500). Antibodies used to stain the sciatic nerves included CD-3γ(1:1000) and DAPI. Images of the tissue samples were taken using an Olympus FluoView 1200 single-photon confocal microscope with a 40x and 20x objective. All images are presented as z-projections of z-stacks.

RESULTS

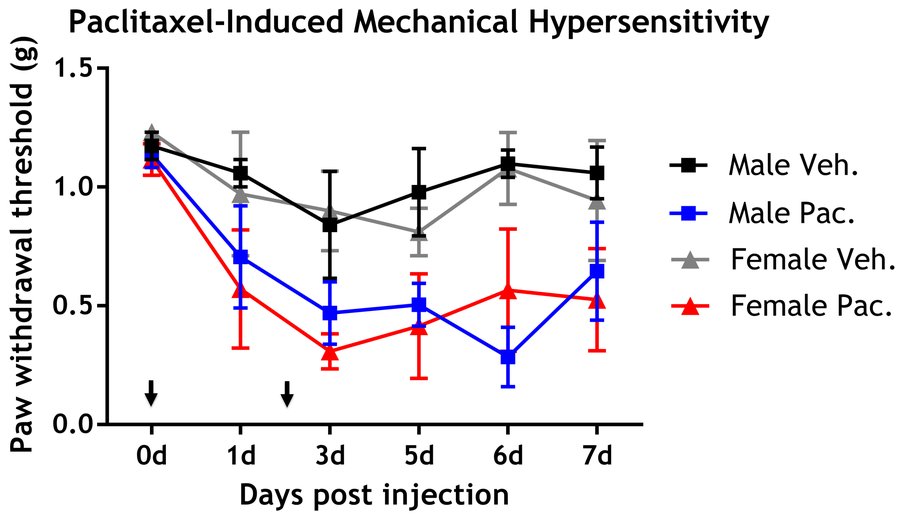

FIGURE 2. The contribution of sex in paclitaxel-induced mechanical allodynia. Paclitaxel (4 mg/kg) or vehicle was administered to WT (C57BL/6) mice on day 0 and day 2. Mechanical allodynia was measured using von Frey hairs and the paw withdraw threshold was calculated using the up-and-down method. n=4-5 .

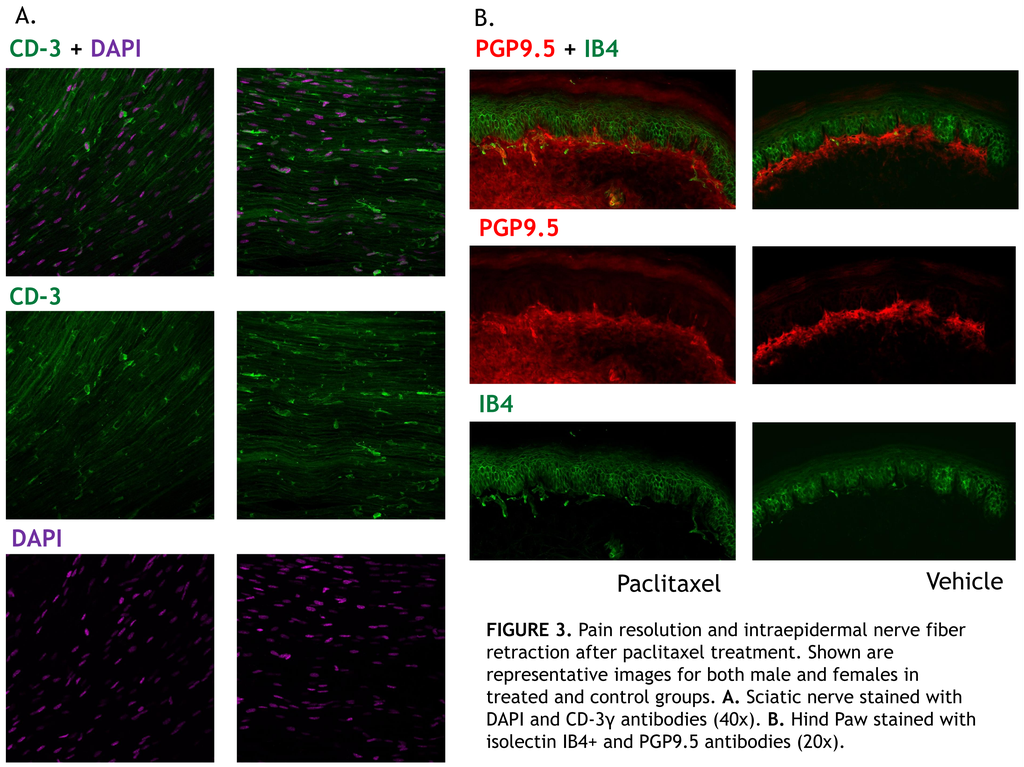

FIGURE 3. Pain resolution and intraepidermal nerve fiber retraction after paclitaxel treatment. Shown are representative images for both male and females in treated and control groups. **A.** Sciatic nerve stained with DAPI and CD-3γ antibodies (40x). **B.** Hind Paw stained with isolectin IB4+ and PGP9.5 antibodies (20x).

CONCLUSION

Our initial studies presented here found no significant difference in the development of CIPN-induced mechanical hypersensitivity between males and females. Moreover our assessment of the recruitment of CD3+ T cells during the onset of pain resolution in the sciatic nerves demonstrated no significant difference between males and females treated with paclitaxel. Our analysis of the hind paws also showed no significant difference in IENF retraction between sexes. It could be interesting to investigate if there are any sex differences in the resolution of as opposed to the onset of CIPN. Our study does provoke the question of why certain types of pain exhibits sexual dimorphism while others do not. Further elucidation of the sex differences could lead to an improved understanding of the underlying mechanism of CIPN and could be considered in therapeutics in treating CIPN.

FUTURE INVESTIGATIONS

- Utilize transgenic mice to assess cell-specific roles in the development of CIPN to explore the mechanism important for therapeutics.
- Further explore the possible differences between males and females during the resolution of CIPN development.
- Investigate possible sex differences in the development of CIPN that is induced by the treatment of other common cancer treatments such as cisplatin.

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