mdbiosciences.
Neuroscience Discovery Services

Poster number: NBI-0409-034

From in Vitro to in Vivo Chemotherapy-Induced Peripheral Neuropathy

Yoav Shulman, Keren Kigen Zur, Elhanan Achituv, Matan Nachliely, Elena Finkelstein, Ayelet Weksler, Jonathan Aran, Garry Slosman, Yossie Ofir, Castel David and Sigal Meilin

MD Biosciences, R&D Neurology unit, Rehovot, Israel

Introduction

Chemotherapy-induced peripheral neuropathy (CIPN) is a debilitating side effect of cancer treatment. CIPN manifests as pain, tingling, and numbness, significantly impacting patients' quality of life. This poster aims to establish and correlate in vitro and in vivo models of chemotherapy-induced neurodegeneration to better understand CIPN pathophysiology.

Acetylated alpha tubulin, a post-translational modification of the microtubule protein α-tubulin, plays a vital role in maintaining the stability and functionality of neuronal microtubules, which are essential for axonal transport and overall neural health. Disruptions in tubulin acetylation have been implicated as a potential mechanism driving neuronal damage. Chemotherapeutic agents that target microtubules can alter the balance of alpha tubulin acetylation, leading to destabilized microtubules and impaired intracellular trafficking such as mitochondrial trafficking, which are key pathological features in neurodegenerative diseases. Reduced levels of acetylated alpha tubulin have been linked to impaired axonal transport, disrupted neuronal architecture, and increased susceptibility to neuronal death.

This study examines:

A. Cisplatin-induced neurodegeneration in vitro and associated changes in pain behavior in vivo.
B. Paclitaxel (Taxol) effects on mitochondrial trafficking in DRG cells in vitro along with acetylated alpha tubulin levels in the DRG of Taxol-exposed rats.

Methods

An in vitro model using primary dorsal root ganglia (DRG) neurons from adult rats was developed. Neurons were cultured for 10 days in vitro (DIV), with 0.5 mM cisplatin or Taxol introduced from DIV 5 to DIV 10. At DIV 10, neurons were fixed and stained with anti–β-tubulin III antibody or Mitotracker Green, then analyzed for neurite area, bleb formation, and mitochondrial trafficking and mass. Acetylation levels in the DRG were assessed using western blot (WB).

For the in vivo model, C57BL/6 mice received intraperitoneal injections of cisplatin (2.3 mg/kg) or saline on a 5-day on, 5-day off, 5-day on regimen. Mechanical allodynia, somatosensory-evoked potentials (SEP), and beam walking were evaluated.

Conclusions

In vitro assay using rat DRG are helpful in screening for new technologies designed to treat cryotherapy induced neuropathy. The main read outs are neurite area and neurodegeneration index. Complementary in vivo assay are required to assess the behavior of the new neuroprotectant technologies as pain behavior can only be assessed in the whole organism.

In addition, these assays can serve as a powerful tool to assess MoA of any neuroprotectant technology. They enable the assessment of microtubule integrity and mitochondrial function and main neurodegeneration outcome in case of chemotherapy induced peripheral neuropathy.

Cisplatin in Vitro Assay

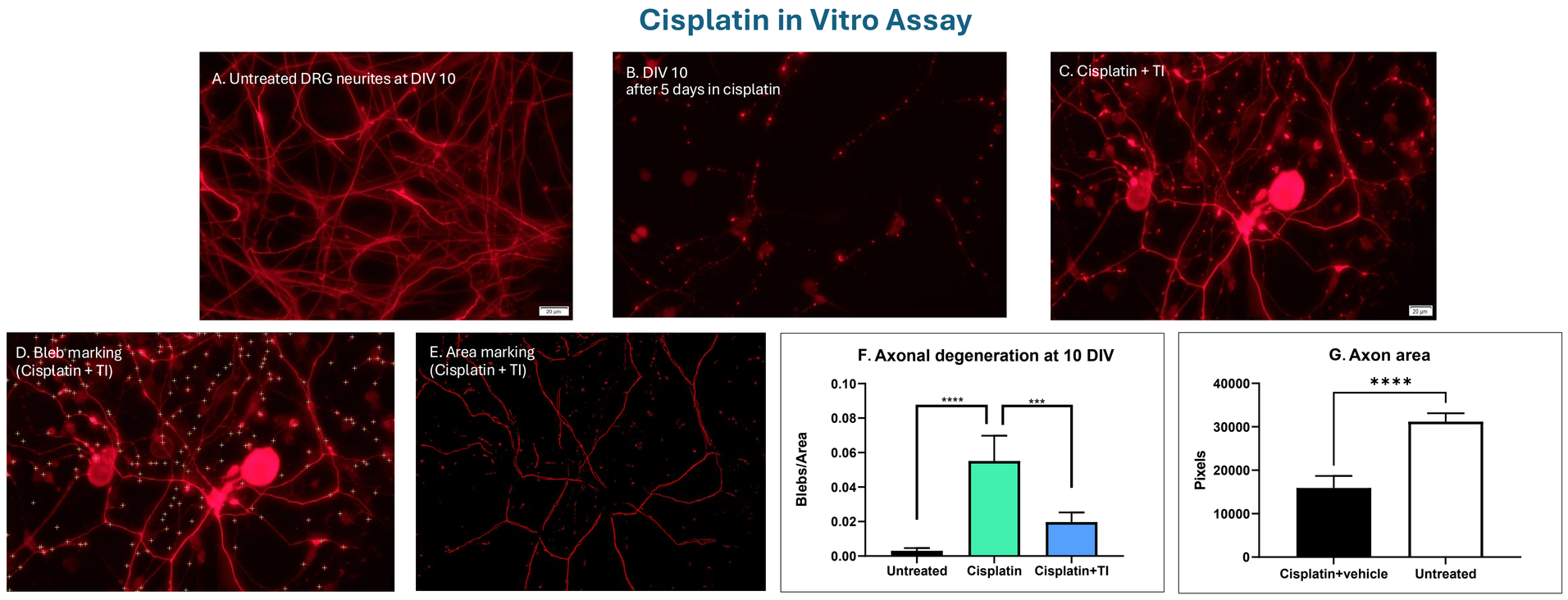


Figure 1: In vitro assay for cisplatin-induced degeneration. (A) Normal DRGs stained with β-tubulin III. (B) Rat DRG neurites 5 days after cisplatin incubation show clear degeneration. (C) DRG cells incubated with cisplatin and treated with a test item display improved neurite growth and fewer blebs. (D) Blebs, a hallmark of degeneration, are marked using computerized imaging. (E) Neurite area is marked in red. (F) Cisplatin exposure increases the axonal degeneration index, while treatment significantly reduces it. (G) Total axonal area is significantly decreased after cisplatin exposure. These findings suggest that the DRG tissue culture assay can be used to screen novel therapies for chemotherapy-induced neurodegeneration.

Cisplatin in Vivo Models

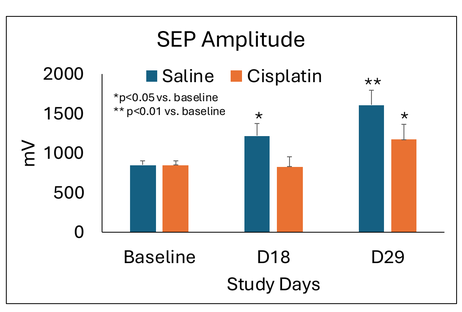


Figure 2: SEP recordings in cisplatin-treated mice. A significant decrease in SEP amplitude was observed at days 15 and 29 post-cisplatin exposure, indicating sensory system dysfunction.

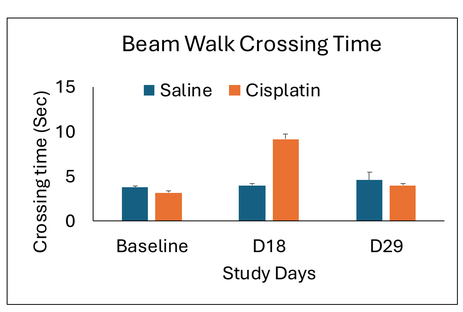


Figure 3: Beam walk crossing time. Cisplatin treatment reversibly affected beam walking. At 18 days after the first dose, crossing time was significantly increased in cisplatin-treated animals. By day 29, no difference was observed between saline- and cisplatin-treated groups.

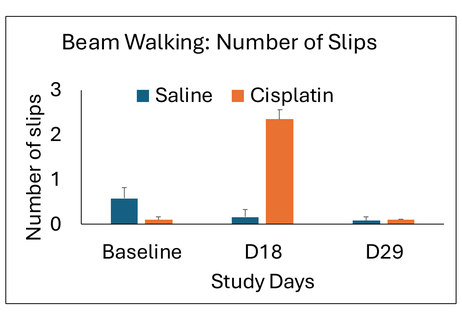


Figure 4: Number of slips on the beam walk. Cisplatin treatment reversibly impaired beam walking. At 18 days after the first dose, cisplatin-treated animals showed an increased number of slips, suggesting disrupted proprioception and impaired beam walking.

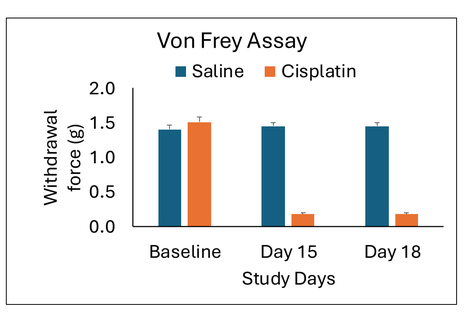


Figure 5. Von Frey assay. Cisplatin administration induced mechanical allodynia, expressed as a reduced withdrawal threshold in response to von Frey filaments.

The Effect of Paclitaxel (Taxol) on Mitochondrial Mass and Transport in In Vitro and Ex Vivo Assays

Taxol stops mitochondrial transport in vitro (Mitotracker staining)

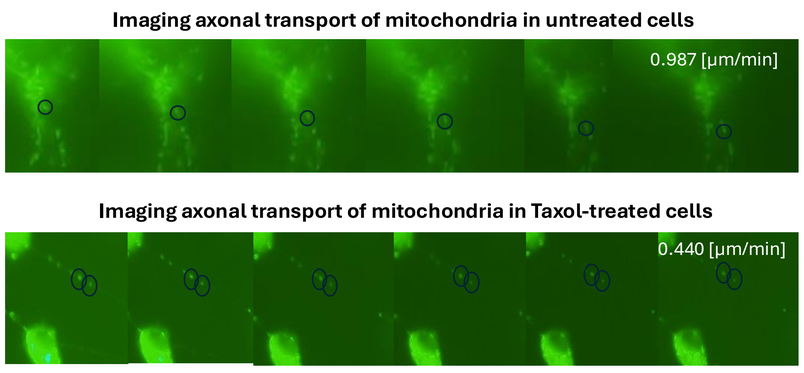


Figure 6: Slides from live imaging of mitochondrial transport. Upper panel: axonal transport of mitochondria in healthy, untreated axons, with a measured speed of 0.987 mm/min. Lower panel: axonal transport of mitochondria in DRG cells treated with Taxol, showing slower transport at 0.440 mm/min.

DRG mitochondrial mass ex vivo (Mitotracker staining)

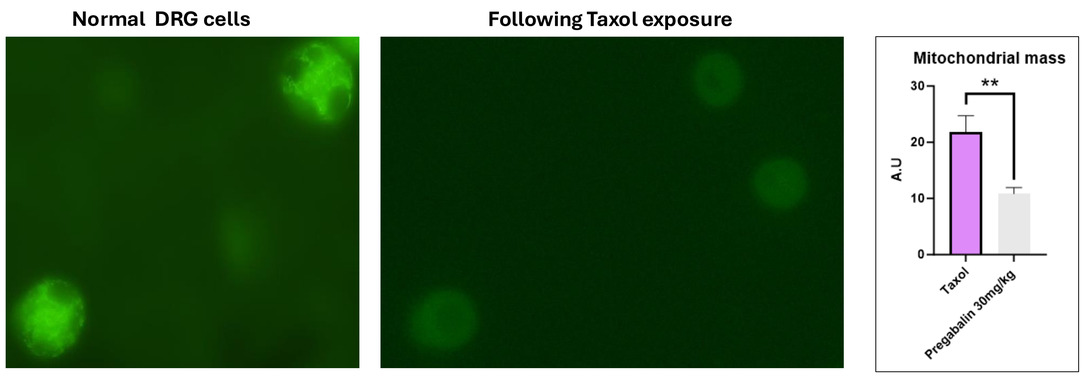
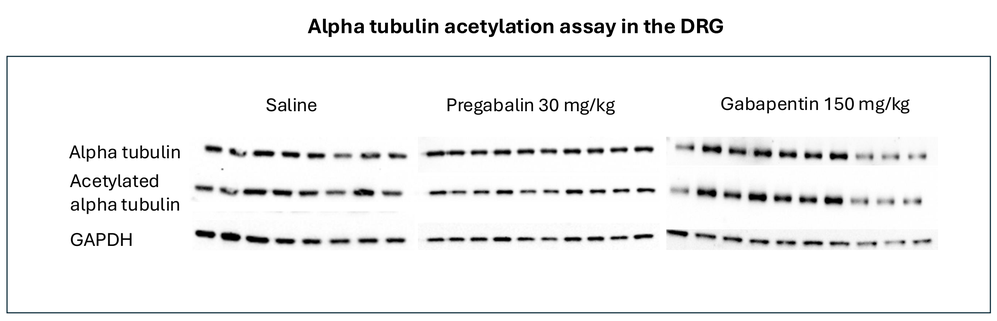


Figure 7. Mitotracker staining of mitochondria. Left: DRG cells from untreated animals showing clear mitochondrial staining. Middle: DRG cells from Taxol-treated animals, showing a significant reduction in mitochondrial mass. Right: DRG cells from Taxol-treated animals given pregabalin, showing a rescue of mitochondrial mass.

Acetylation level of α tubulin in the DRG of Taxol-treated animals



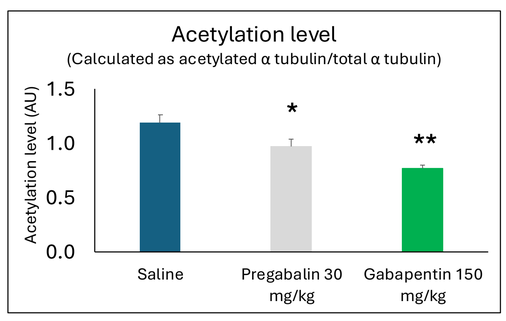


Figure 8: Acetylation level of α-tubulin in the DRG of rats dosed with Taxol. Acetylation was calculated as acetylated protein/total protein. Left: western blots, with each column representing one animal. Right: mean group levels of acetylation. Following treatment with gabapenoids, acetylation is reduced, consistent with the decreased mitochondrial mass observed in Figure 7.