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CNS/PNS IN VITRO DRUG DEVELOPMENT

The Chemotherapy-induced peripheral neuropathy involves specific neural or Schwann cell targets. The case of Cisplatin, Taxol and Vincristine

PNS PERIPHERAL NERVE SOCIETY

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INTRODUCTION

Chemotherapy-induced peripheral neuropathy (CIPN) is the most common side effect caused by the antineoplastic treatment (Taxane, vinca alkaloids platinum (Pt) compounds).
Paclitaxel (Taxol®) is commonly used for the treatment of several solid tumors such as breast, ovarian and lung cancers, it is a microtubule-binding compound that is able to cross plasmatic membrane through passive diffusion and bind the N-terminal region of β-tubulin monomer of microtubules. Paclitaxel may induce the onset of neuropathy characterized by paraesthesia and sensory loss, frequently associated with the development of neuropathic pain.
Cisplatin was the first Pt compound to be used, primarily in the treatment of metastatic ovarian and testicular cancers, its mechanism of action is based on the inhibition of DNA synthesis by formation of Pt adducts inter and intra-strands. Peripheral neurotoxicity is prevalently characterized by distal painful paresthesia and, at high doses, by severe sensory ataxia.
Vincristine is an important anticancer drug mainly employed in the treatment of hematologic cancers and pediatric sarcomas. Vincristine is known to impair microtubule dynamics, assembling and disassembling, resulting in cell cycle arrest at metaphase. They develop disturbances in both motor and sensory functions with early numbness, tingling in hands and feet and ankle jerks. Moreover, neuropathic and muscle pain is frequently present, as well as the loss of temperature sensation.
In this work, the effect of these 3 agents were evaluated on rat primary DRG cocultured with SCs. Different culture protocols using a) myelinated sensory neurons (to evaluate the effects on the myelin sheaths) or b) sensory neurons cocultured with non-myelinating SCs (to evaluate the effects on each cell types) were used. Toxicity of the drugs (applied at different times and doses) was deeply investigated on the neuronal and SC survival, neurite network and myelin sheaths.

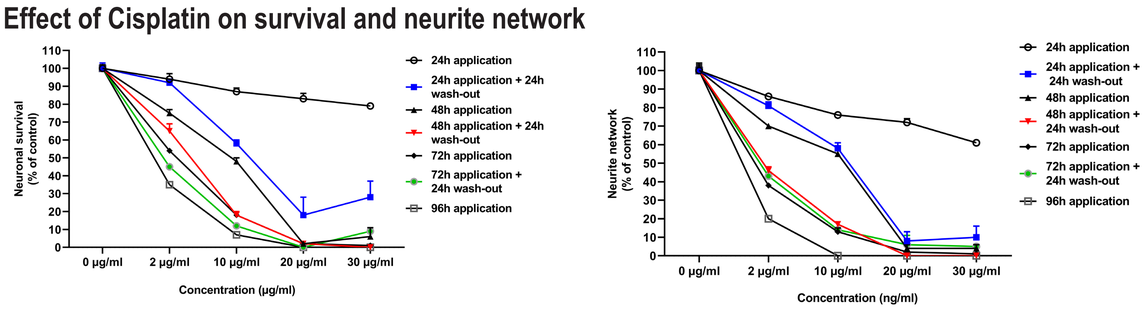
METHODS

Primary culture: Primary coculture of sensory neurons and Schwann cells were cultured as described by Callizot *et al.*, 2011 Exp Cell Res, (for myelination protocol and adapted for unmyelinated cultures). Briefly, DRG (15-day-old SD Rat embryos) cells were seeded at a density of 12,500 cells/well in 96 well-plates coated with poly-L-lysine and laminin. The cultures were added with Ascorbic acid (AA) to induce myelination, no AA was added in unmyelinated cultures.
Pharmacological treatments: *For myelinated cultures*: On D7 of culture, the co-cultures were treated in with addition of 50 µg/mL of L-ascorbic acid (AA), and half of the medium was then changed every day. On D17 (10 days post AA), toxic agents were added at different doses and application time. *For non myelinated cultures*: On D13 (post seeding) toxic agents were added at different doses and application times.
Immunostaining: At different days of culture, cultures were fixed with a solution of acetic acid (5 %) and ethanol (95%). The cells were incubated with a polyclonal antibody anti-NF (neuronal marker) and monoclonal Ab anti-MBP, or anti-MAG. Secondary, Alexa488 and Alexa568 antibodies were used. Pictures (20x or 60x magnification) were acquired an on automated microscope with MetaExpress software and automatically analyzed with Custom Module Editor (Molecular Devices).

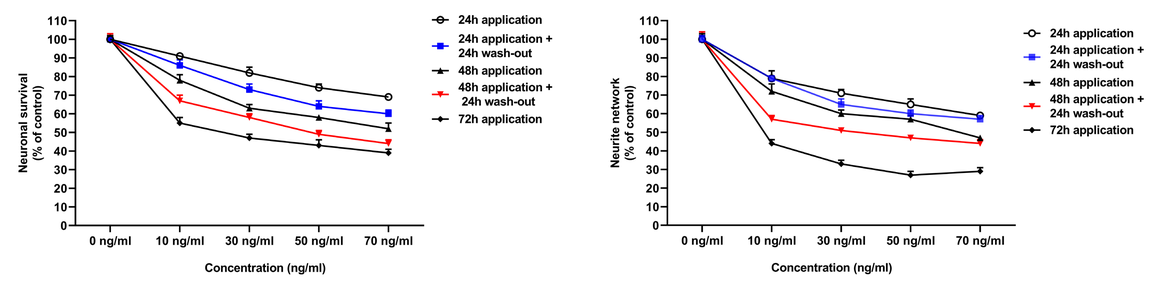
RESULTS

EFFECT OF CISPLATIN, VINCRISTINE AND TAXOL ON NON MYELINATED SENSORY NEURONS

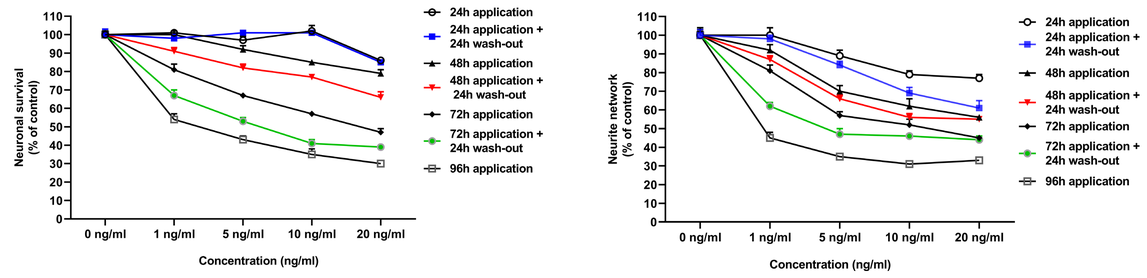
Effect of Cisplatin on survival and neurite network



Effect of Taxol on survival and neurite network



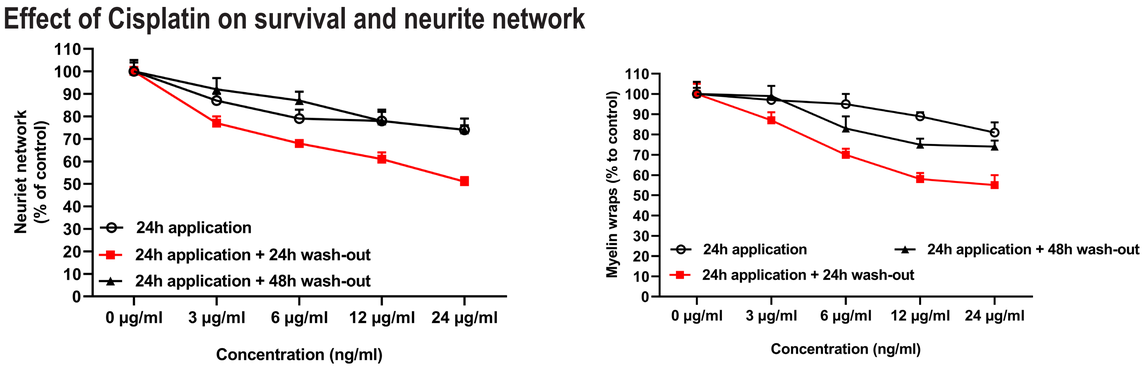
Effect of Vincristine on survival and neurite network



- Cisplatin induced a large and massive neuronal death increasing with dose and time of application. Washout (whatever the time of application) did not abolish or reduce the toxicity.
- Vincristine induced a dose dependent neurotoxicity associated with a large neurite network destabilization, no recovery was observed after washout.
- Taxol induced a large reduction of neurite length followed by neuron death, dependent on time and dose of application.

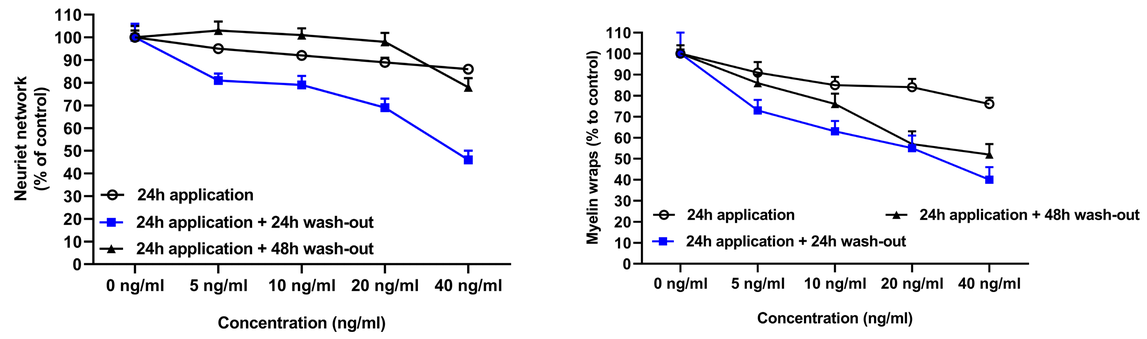
EFFECT OF CISPLATIN, VINCRISTINE AND TAXOL ON MYELINATED SENSORY NEURONS

Effect of Cisplatin on survival and neurite network

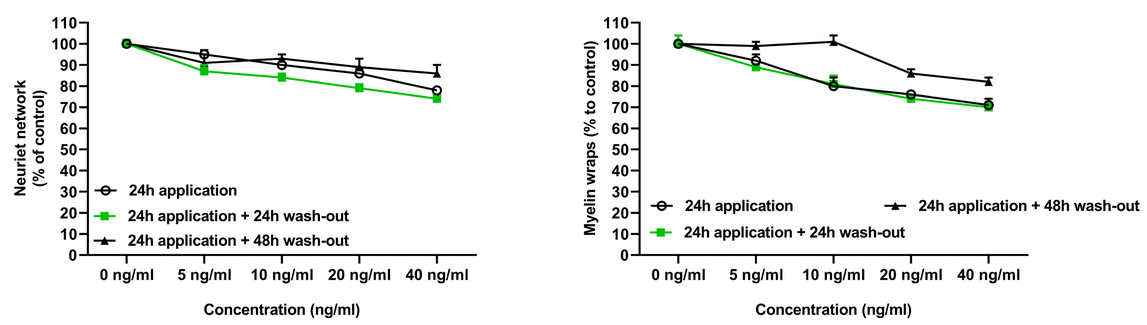


- Cisplatin induced a moderate axonal damages on myelinated fiber, the damages were reversible after 48 h washout time.
- Vincristine showed demyelination followed by axonal damages at the highest doses and time of exposition.
- Taxol induced a demyelination of axons associated with neurite damages, interestingly these damages were reversible after 48 h washout.

Effect of Vincristine on survival and neurite network



Effect of Taxol on survival and neurite network



CONCLUSIONS

Here, we showed that each toxin induced a toxicity preferentially taking place in DRG, sensory neurons, and/or Schwann cells.

- Taxol induced a reduction in neurite length that are dependent on time and dose. When Taxol was added directly on unmyelinated axons, axon length is reduced suggesting direct action of Taxol on the axon. No major damages were observed on myelinated fibers.
- Vincristine is well-known to disrupt the β-tubulin assembly and disassembly leads to severe alterations in axonal microtubules in myelinated and unmyelinated fibers. Here we observed a large toxicity of unmyelinated fibers (when applied directly on axons). No major damages on myelinated fibers was observed by contrast the myelination was largely impacted and led to axonal degeneration.
- Cisplatin applied directly on neurons, induced a large and massive neuronotoxicity (neuronal death), the damages were preferentially focused on unmyelinated fibers.