Chemotherapeutic induced neuropathy (CIPN)

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Introduction

- Chemotherapeutic induced neuropathy (CIPN) is a side effect of many anti-cancer drugs that limits the drug dose during treatment (1). Symptoms of CIPN include tingling and numbness at the extremities (hands and feet) and loss of coordination (1).

- In this experiment, we quantified the degree of axonal neurodegeneration caused by the following chemotherapeutic drugs: Oxaliplatin, Vincristine, and Paclitaxel. Oxaliplatin kills cancer cells by forming DNA adducts to block DNA replication and transcription (2). Accumulation of DNA adducts in DRG neurons is the cause of the neuropathy (2). Vincristine and Paclitaxel kill tumour cells by binding to β-tubulin and destroying the mitotic spindle (1). Vincristine and Paclitaxel cause neuronal die-back by binding to β -tubulin and damaging microtubules to interfere with axonal transport (1).

Methods

- **Cell culture:** DRG neurons were cultured according to protocol for 15 days, after which various concentrations of Oxaliplatin (1, 2, 5, 10, 25, 50, 75, 100 *u*M) were administered and left for 48 hours. Mouse ES cells were also differentiated into a neuronal population using the Bains protocol, and subjected to Vincristine and Paclitaxel doses (Vincristine 5 and 20 *n*M, and Paclitaxel 10 and 50 ng)(3). After 48 hours, cells were fixed in 4% PFA (paraformaldehyde).

- **Immunohistochemistry:** Firstly the cells were incubated with primary anti-rabbit β -tubulin II, diluted in 5 %NGS 1:200.They were then incubated with FITC conjugated secondary antibody diluted in 5% NGS 1:200. Hoesct, diluted in dH_2O 1:200, was used to stain the nuclei.

- **Neurite tracer program:** 25 images of neurons were taken from each concentration using the axio-imaging microscope. The level of axon degeneration was measured through automated analysis on image J with the neurite tracer module. This software measures both neurite length and nuclei number.

Results

- **DRG neurons:** Apart from the data for 10 *u*M Oxaliplatin, the graph in figure 1 shows a decrease in neurite length as the concentration of Oxaliplatin is increased. Average neurite length of neurons treated with 1 *u*M of Oxaliplatin is 98.4% of the control neurite length, whereas at 100 uM Oxaliplatin, neurite length is only 3.4%. Figure 1a shows the qualitative decrease in neurite density as neurons are treated with increasing Oxaliplatin doses.

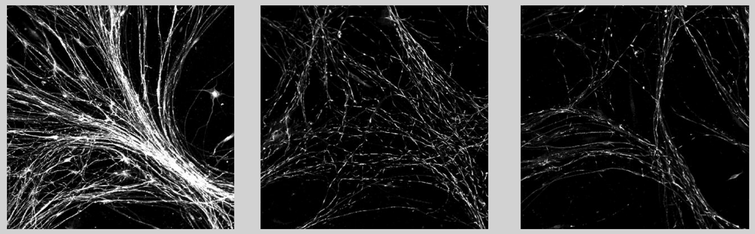

Figure 1a – Media only neurons compared to neurons treated with 50 *u*M and 75 *u*M Oxaliplatin (from left to right)

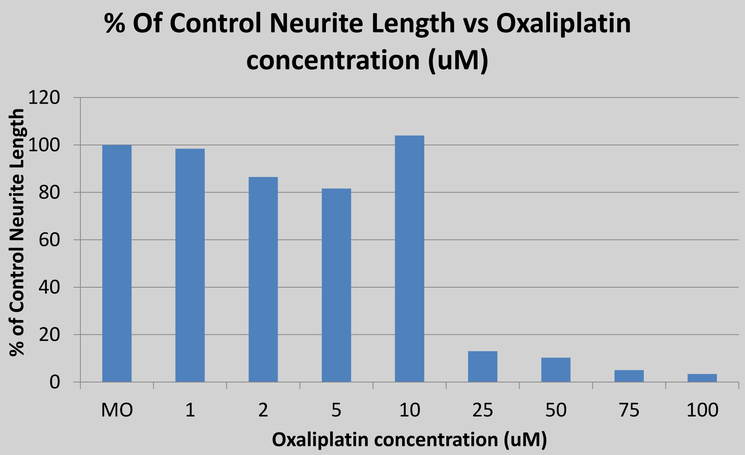

Figure 1 – Quantitative measure of the degree of axonal neurodegeneration at Oxaliplatin concentrations.

- **ES derived neurons:** The graph in figure 2 displays a gradual decrease in neurite length as the concentrations of Vincristine and Paclitaxel are increased. Increasing the Paclitaxel concentration from 10 ng to 50 ng results in a 27% higher decrease in neurite length. The images in figure 2a shows qualitative decreases in neurite density as neurons are treated with increasing concentrations of Paclitaxel and Vincristine.

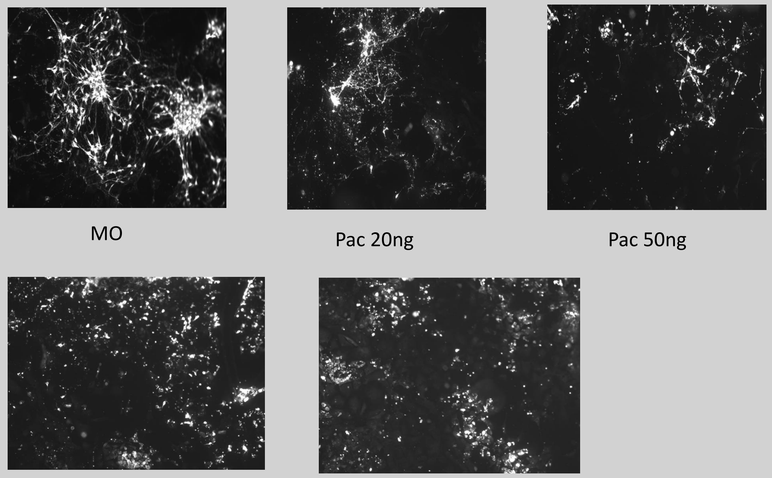

Figure 2a – Images of neurons taken by the axio-imaging microscope.

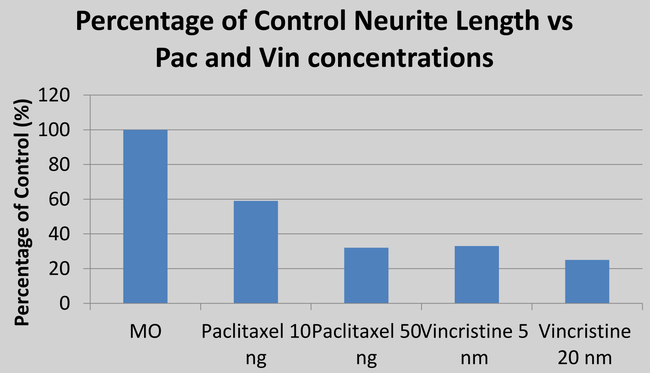

Figure 2 – Quantitative measure of the degree of axonal neurodegeneration at Paclitaxel and Vincristine concentrations.

Conclusion

- CIPN is a painful side effect of these anti-cancer drugs that limits the dose of the drug that can be administered. As a result of this painful side effect, patients are unable to receive stronger doses required to more effectively kill tumor cells.

- Quantifying the level of degeneration that occurs at each specific dose of these drugs means we now have a phenotypic screen on which to test novel therapeutic compounds. If any of these compounds are effective in reducing axonal degeneration they could be taken further with the ultimate goal of using in clinic.

References:
(1) Amteshwar Singh Jaggi, Nirmal Singh. "Mechanisms in cancer chemotherapeutic drugs-induced peripheral neuropathy". Toxicology vol 291 (2012): 1-9.
(2) Lauren E. Ta, Laura Espeset, Jewel Podratz, Anthony J. Windebank. "Neurotoxicity of oxaliplatin and cisplatin for dorsal root ganglion neurons correlates with platinum-DNA binding". Neurotoxicology vol 27 (2006): 992-1002
(3) Gerard Bain, Daniel Kitchens, Min Yao, James E. Huetner, David I. Gottlieb. "Embryonic Stem Cells Express Neuronal Properties *in Vitro*". Developmental Biology vol 168 (1995): 342 – 357