Optimization of rat DRG neurite outgrowth assay for peripheral neuropathy prediction

You Feng, Andrew Ramirez, Ben MacDonald, Jenifer Bradley and Christopher J Strock

Cyprotex US, LLC, an Evotec Company, Watertown, MA 02472

Abstract

Many chemotherapeutics induce peripheral neuropathy, with symptoms of numbness, tingling or abnormal sensations. This impacts the long-term quality of patient's life. Animal models used to evaluate this side effect are labor-intensive and may be difficult to interpret. Therefore, we aimed to develop and optimize an in vitro cell-based model for peripheral neuropathy prediction.

Previously, we compared the responses of three cell types in our high content imaging neurite outgrowth assay: rat cortical neurons, IPSC derived human neurons, and rat dorsal root ganglion (DRG) neurons. We compared these cell types using a group of chemotherapeutics from different classes (72 h treatment) and determined that the rat DRG neurons have the greatest neurite outgrowth sensitivity to the chemotherapeutic agents overall.

Here, we continued to optimize the DRG assay conditions by comparing the neurite outgrowth responses to the same group of drugs in 24 h and 72 h, with drug treatment beginning 1 h after cell plating. Our new findings showed that more drugs induced neurite outgrowth inhibition at 24 h before cytotoxicity occurred. Chemotherapeutic compounds such as taxanes (paclitaxel, docetaxel), microtubule interfering agents (vincristine, vinblastine, colchicine, nocodazole) and epothilones (ixabepilone) all showed dramatic inhibition on neurite outgrowth, with minimum effect on cell viability at 24 h. This is a significant improvement of neurite assay sensitivity and specificity compared to 72 h treatment, at which time neuron viability is much lower.

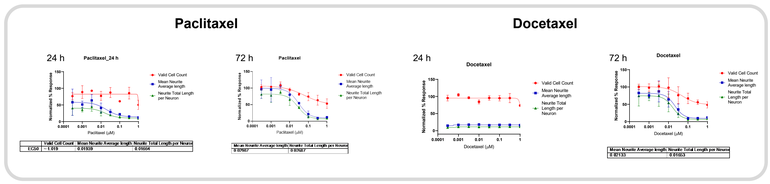
Chemotherapeutics tested

			24 hour Treatment_IC_{50} (μM)			72 hour Treatment_IC_{50} (μM)		
Compound	**Category**	**Top dose tested (μM)**	**Valid Cell Count**	**Mean neurite average length**	**Neurite Total Length per Neuron**	**Valid Cell Count**	**Mean neurite average length**	**Neurite Total Length per Neuron**
Cisplatin	Platinum	200	201.9	195.6	184.2	159.8	222.8	174.8
Oxaliplatin	Platinum	200	>200	94.72	81.65	1.88	45.76	41.07
Bortezomib	Proteasome inhibitor	200	0.06-20	<0.06	<0.06	<0.06	<0.06	<0.06
Paclitaxel	Taxanes (Cytoskeletal drug that targets tubulin)	1	1.02	0.019	0.017	0.063	0.030	0.027
Docetaxel	Taxanes	1	>1	<0.0003	<0.0003	1	0.021	0.017
Ixabepilone	Epothilones	1	>1	<0.0003	<0.0003	1.29	0.90	0.85
Fluoxetine	Antidepressant; Serotonin reuptake inhibitor	50	24.02	22.44	19.74	20.96	42.49	38.04
Vincristine	Microtubule interfering agents	1	>1	<0.0003	<0.0003	<0.0003	<0.0003	<0.0003
Vinblastine	Microtubule interfering agents	1	1.23	<0.0003	<0.0003	0.0064	0.0070	0.0075
Colchicine	Microtubule interfering agents	1	>1	0.026	0.019	0.014	0.537	0.076
Nocodazole	Microtubule interfering agents	1	>1	0.165	0.132	0.425	0.318	0.311
Chlor-promazine	Control (Same response)	100	31.48	14.02	12.75	6.22	12.15	11.63

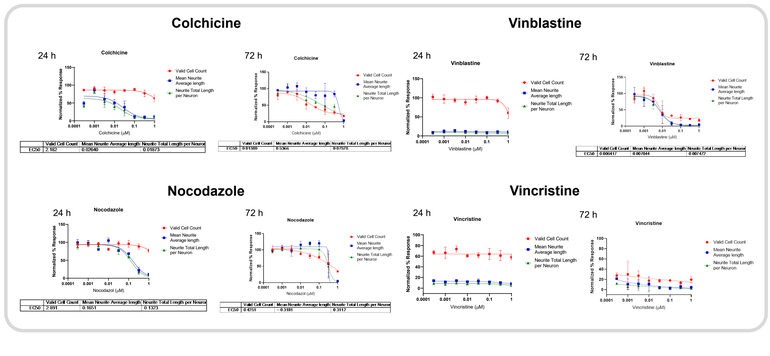
Dose-response curve for Rat DRG at 24 h and 72 h timepoints

Cryopreserved rat dorsal root ganglion cells are plated on laminin-coated 384-well plates 1 hour prior to treatment at a density of 2,000 cells per well. After treatment with test articles and control compounds, the DRG cells are maintained in a humidified environment at 37°C with 5% CO2 for 24-72 hrs. At the end of the treatment period, cells are fixed, permeabilized and stained for evaluation of neurite outgrowth and cell health using the high content imaging platform, CellInsight CX7 High-Content Screening (HCS) Platform (ThermoFisher Scientific), with an optimized neuronal profiling bioapplication.

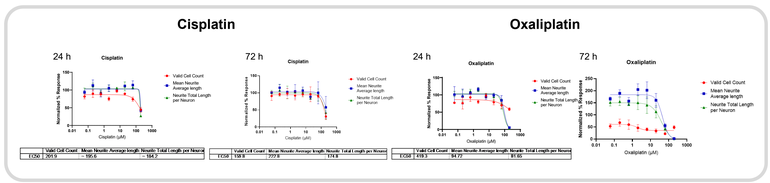
Taxanes



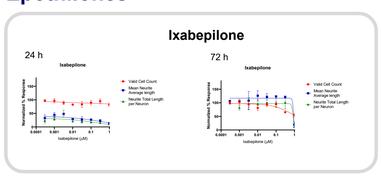
Microtubule interfering agents



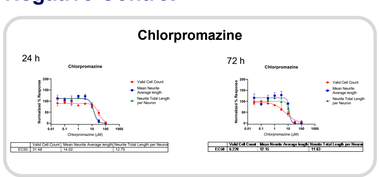
Platinum



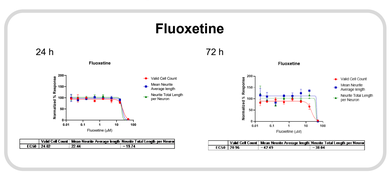
Epothilones



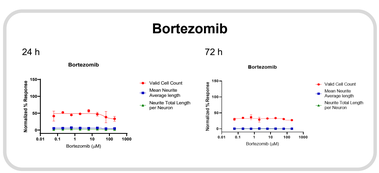
Negative Control



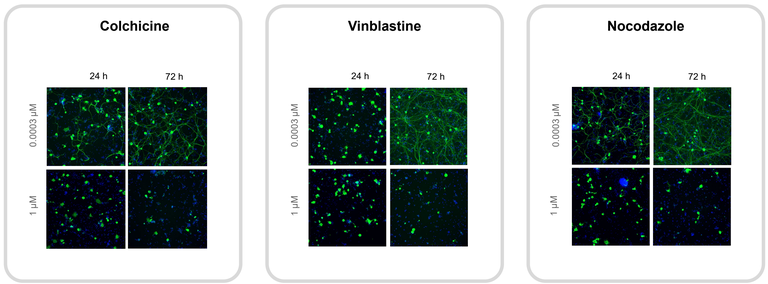
Serotonin reuptake inhibitor

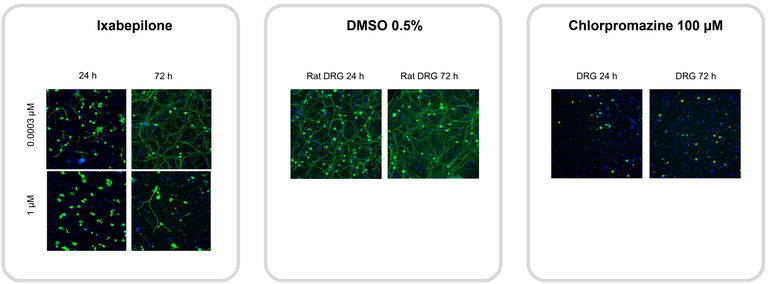


Proteasome inhibitor



Selected neuron images with 24 h or 72 h treatment





Conclusions

- Twelve chemotherapeutic drugs from six different categories were tested against rat DRG sensory neurons with 24 h or 72 h treatment
- Microtubule interfering agents (colchicine, nocodazole, vinblastine, vincristine), taxanes (paclitaxel and docetaxel) and epothilones (ixabepilone) tested all induced more sensitivity in peripheral DRG neurite outgrowth at 24 h
- In summary, the optimized rat DRG peripheral neuropathy assay showed improved sensitivity and specificity compared to the other assays. It picked up a higher percentage of compounds at 24 h than 72 h. Additional assays (MEA, *etc*) will be developed to identify more classes of chemotherapeutics which were not tested/did not respond in this assay