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TITLE: Novel High-Throughput Drug Screening Platform for Chemotherapy-Induced Axonal Neuropathy

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14. ABSTRACT

Peripheral nerve system is vulnerable to the chemotherapy drugs in breast cancer patients. In particular, Taxol can cause severe pain and discomfort in breast cancer patient during the treatment. In order to reduce the pain induced by Taxol, we have identified 4 drugs potentially neuroprotective to chemotherapy induced axonal neuroapthies from drugs candidates. Currently, the in vivo animal studies are ongoing. We believe that these drugs can be potential drugs for chemotherapy induced axonal neurpathies.

15. SUBJECT TERMS
None provided.

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Introduction:

Taxol is an antineoplastic agent, which is used for the treatment of various cancer types such as breast, ovarian, lung, bladder, etc. It disrupts the microtubule assembly and accumulates in the dorsal root ganglion (DRG). The side effect of Taxol is peripheral neuropathy. DRG neurons are sensory nerve cells, and their cell bodies are bundled together lying outside of the spinal cord - ganglia. DRG neurons are accessible and convenient to harvest in embryonic animals and they survive well in culture.

Results:

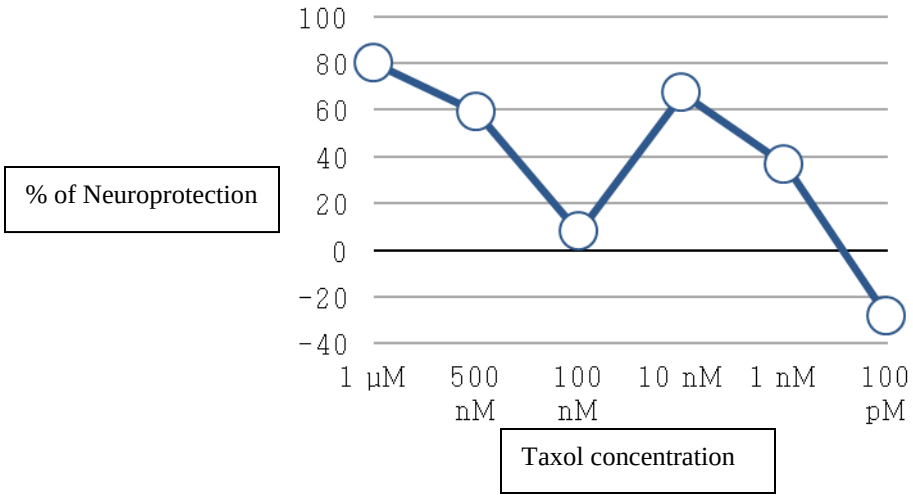
DRG from pregnant rats at 15th day of gestation were aseptically dissected and treated with collegenase and trypsin, mechanically dispersed by trituration and filtered to make a cell suspension. The cell suspension is diluted with Neurobasal Medium containing GDNF and B-27 supplement. The dissociated neurons are loaded at an appropriate density, to 96 well tissue culture plates that are coated with first with PDL and then with Laminin. The amount of neurons are 5000 to 8000 cells per well. The DRG cells cells are incubated in humidified 37 ºC, 5% CO2 incubator and their neurite growth and differentation is visible under inverted light microscope after 24 hours of culture.

100 nMTaxol is added, in order to analyze the cytotoxic action of Taxol. Taxol with a final concentration of 100 nM, and the screened drugs at a final concentration of 1 µM are added to the wells.

The cells are incubated for 24 hours and cytotoxicity and therapeutic effect of drugs together with taxol are evaluated by the ATP assay, which is based upon the bioluminescent measurement of ATP that is present in all metabolically active cells. Since cells need ATP to remain alive the assay can be used for the direct assessment of cell numbers and viability. The amount of light measured by the luminometer is directly proportional to the amount of living cells present each well.

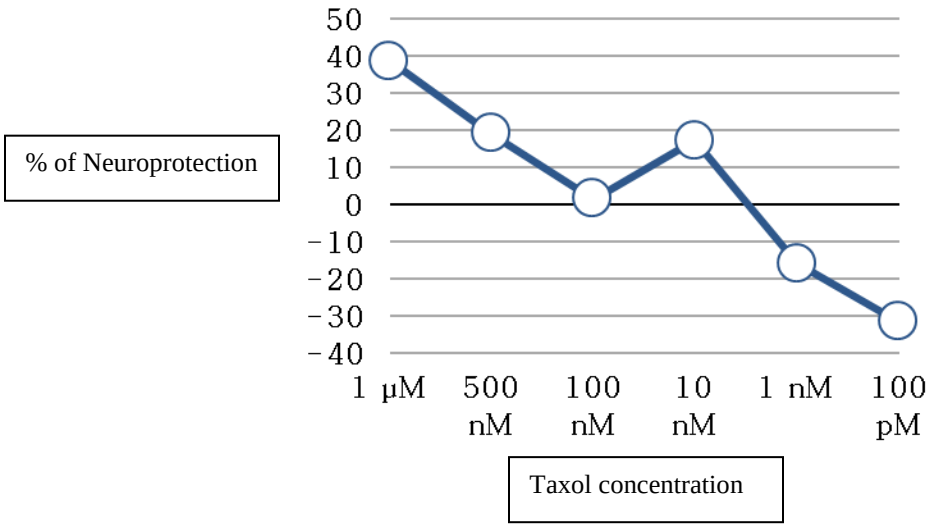
Each drug is screened twice and their average cytotoxicity/protectivity is calculated.
The percentage neuroprotection of the drugs are calculated according to formula:

[(Viability of cells with drug+taxol)- (Viability of cells with taxol)] / [(Viability of cells in control)-
(Viability of cells with taxol)]

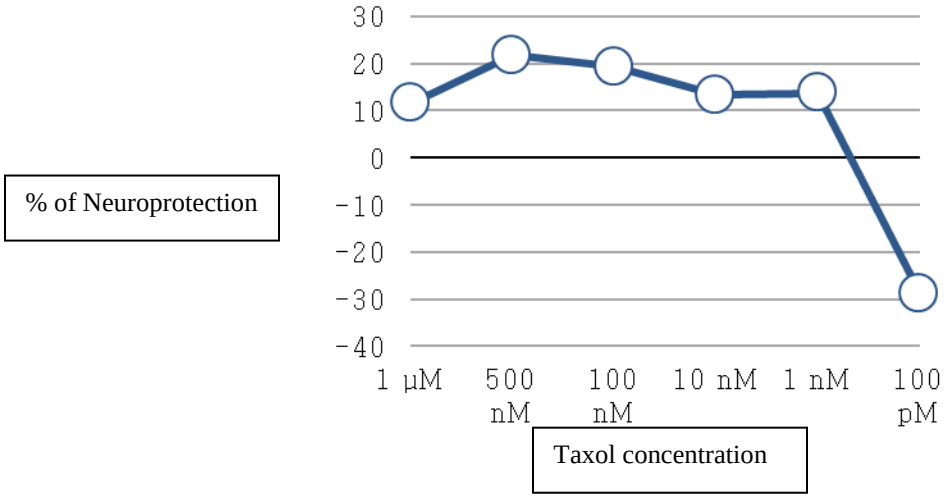


Drug Name: 1FM, Potential role: Antiinflamatory

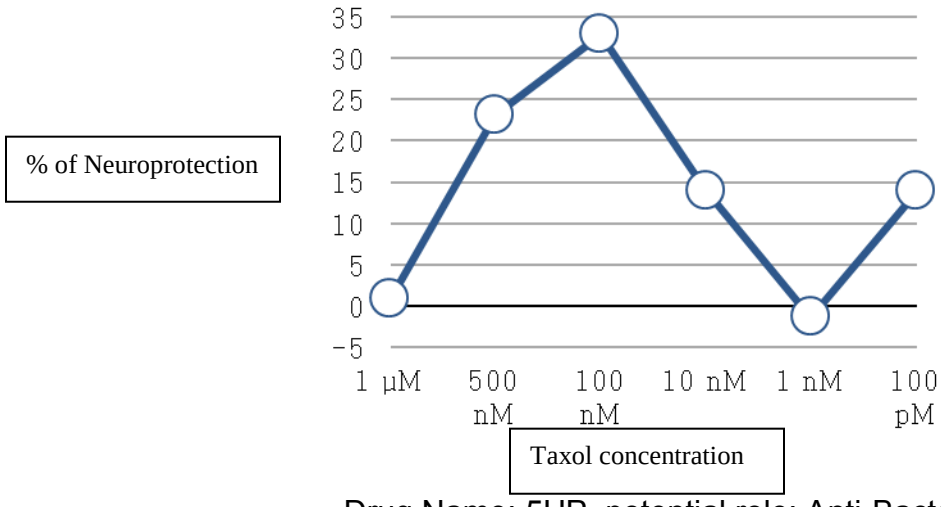
Chart 4



Drug name: 2FA, potential role: Anti-inflammatory.



Drug name: 4FA, potential role: Anti-Bacterial



Drug Name: 5HP, potential role: Anti-Bacterial

Key outcome from this research: From this research, we have identified 3 potential drugs for Taxol induced axonal neuropathies from 1470 drugs. Currently, we are testing the neuroprotective effect in Taxol neuropathy mouse model.

Conclusion: Taxol (paclitaxel and docetaxel) stabilize microtubules effectively treating various types of solid tumors. Through the disruption of microtubels of the mitotic spindle and the subsequent interference in axonal transport, Taxol induces toxic effect in peripheral neuron. From our drug screening, we have identified 4 potential drug candidates for Taxol neuropathies. Identified drugs are FDA approved drugs which readily is applied to clinical field. Identified drugs are 2 anti-inflammatory drugs and 2 anti-bacterial drugs. We don't know the neuroprotective mechanisms of identified drugs. The neuroprotective mechanisms of drugs, sites of actions, and in vivo testing of identified drugs are still on going. Also, the effect of identified drugs on tumors should be examined. We believe that the identified drugs will be beneficial to reduce the pain and discomfort in peripheral system of patients experiencing chemotherapy induced axonal neuropathies.