Development of rat DRG model for predicting peripheral neuroinflammation and neurotoxicity of therapeutic agents

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Abstract

Dorsal root ganglion (DRG) toxicity is one of the major concerns for several therapies but no optimized *in vitro is* available for predicting DRG toxicity or spinal nerve/root injury for various therapeutic agents. Here we have developed a rat DRG-microglia co-culture model. We have optimized the culture conditions and validated it using inflammatory agents and neurotoxic tool compounds. Microglial activation and disorganization of neurites was observed with LPS + with IFNγ treatments. Paclitaxel, known to cause peripheral neuropathy in patients showed neurites disorganization without any microglial activation. Colchicine, known to cause gliosis, showed microglial activation (iNOS induction) and neurotoxicity in a dose dependent manner. Evaluation of several exploratory compounds from different modalities reveled that this model has potential for predicting neurotoxicity of small molecules, oligonucleotides and AAVs. In conclusion, here we have developed a rat DRG-microglia co-culture model that can predict peripheral neurotoxicity potential of seral therapeutic modalities.

Development of rat DRG-microglia co-culture model for predicting neurotoxicity

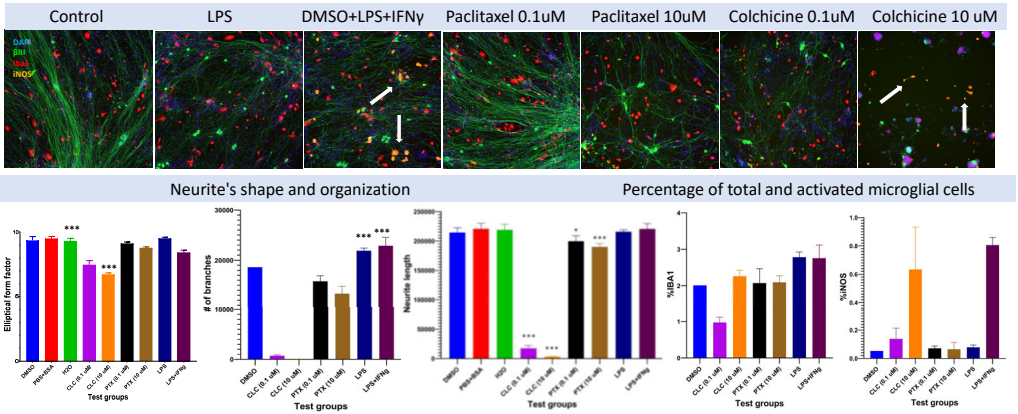

Figure: Rat DRG model treatment with (A) LPS (100ng/ml) caused neurite disorganization without inducing iNOS expression. (B) LPS (100ng/ml)+IFNγ(50U/ml) caused microglial activation (iNOS induction) and disorganization of neurites. (C) Paclitaxel caused disorganization of neurites in dose dependent manner. (D) Colchicine caused neurotoxicity and clumping of nucleus in a dose dependent manner. At 10uM dose Colchicine treatment caused microglial activation.

Optimization of oligonucleotide delivery and AAV transduction in DRG culture

Tominersen delivery

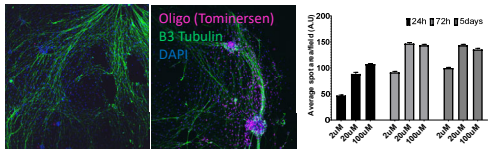

Figure: Gymnotic delivery of Tominersen in the rat DRG culture. Dose dependent increase in delivery was observed at 24, 72h and 5days with highest delivery at 72h and 5 days. PS: gymnotic delivery is dependent on oligonucleotide chemistry.

rAAV9-GFP transduction

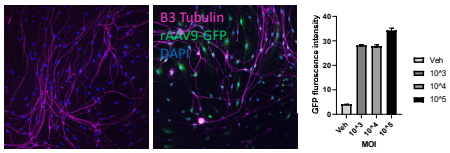

Figure: Transduction of AAV in the rat DRG culture. Dose dependent increase in transduction was observed at 72h post treatment. PS: Transduction were observed in neurons and resident fibroblasts but not in satellite cells.

Exploratory study of rat DRG-microglia co-culture model with various therapeutic modalities

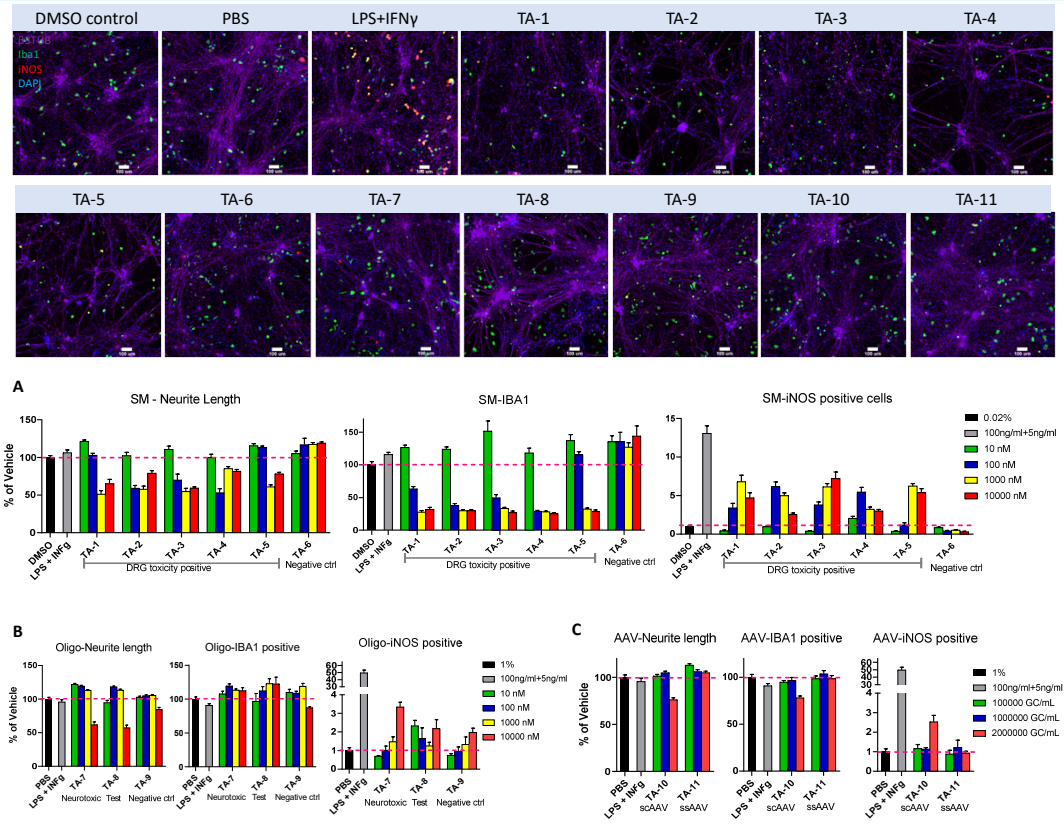

Figure: Testing exploratory compounds in rat DRG-microglia co-culture model: A. Small molecules selected for their DRG toxicity (TA-1, TA-2, TA-3, TA-4 and TA-5) have shown decrease in the neurite length and microglial density with increase in microglial activation as compared to vehicle and negative control (TA-6) compounds. B. Oligonucleotide and C. rAAV the tool compounds that were predictive to be neurotoxic/neuroinflammatory have shown decrease in the neurite length and increase in microglial activation.

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