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Neuroprotection in Huntington’s Disease via Transcriptional Modification

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Huntington’s disease (HD) is a progressive autosomal dominant neurodegenerative disorder characterized by motor and cognitive dysfunction.\textsuperscript{1} CAG trinucleotide repeats in the huntingtin (HTT) gene produce glutamine (polyQ) tracts which lead to abnormal protein conformation resistant to degradation.\textsuperscript{2} Accumulation of mutant HTT protein can inhibit normal cell function and results in cell toxicity with atrophy particularly in medium-sized spiny neurons (MSNs) found in the striatum and pyramidal neurons of the cerebral cortex which project to the striatum.\textsuperscript{3,4} Expansion of the trinucleotide repeat produces the phenomenon of anticipation seen with Huntington’s disease.\textsuperscript{5} The age of disease onset is inversely related to the number of trinucleotide repeats and a more severe phenotype is observed in the next generation.\textsuperscript{6}

Mitochondrial dysfunction and metabolic deficits are implicated in the pathophysiology of neurodegenerative diseases such as Parkinson’s disease and HD and have been linked to the transcriptional dysregulation of peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1a).\textsuperscript{7,8,9,10,11} Of the members of the PPAR family of transcription factors which require co-activation by PCG-1a, PPAR delta (PPAR-δ) is most ubiquitous and abundant in the central nervous system. Previous studies have demonstrated that PPAR-δ is dysregulated in HD leading to impairment of mitochondrial function. Furthermore, activation of the PPAR-δ pathway provided neuroprotective effects in HD models, implicating this pathway as an important therapeutic target for HD.\textsuperscript{12}
In order to exert its effects on target genes, PPAR-δ must heterodimerize with retinoid X receptor (RXR) to bind PPAR-responsive elements (PPRE) in the promoter region of gene targets.\textsuperscript{13} In addition to PPAR-δ agonists, RXR agonist can also promote PPAR-δ activation.\textsuperscript{14} A drug currently used for the treatment of cutaneous T cell lymphoma, Bexarotene, has structural similarity to endogenous RXR ligands and acts as a selective agonist of RXR to activate PPAR-δ/RXR heterodimer.\textsuperscript{15} Previous studies demonstrating improved mitochondrial function, protein processing, and other beneficial effects of Bexarotene administration in Alzheimer’s disease mouse model suggest that Bexarotene could confer similar protective effects in HD.

The purpose of this study was to investigate the hypothesis that neuroprotection imparted by Bexarotene in vitro is mediated by the PPAR-δ pathway. Studies in Dr. La Spada’s laboratory have demonstrated Bexarotene modification of PPAR-δ signaling pathway increases target gene expression, improves mitochondrial membrane potential, and reduces neuronal cell death. The specific aim of this project was to demonstrate that neuroprotection conferred by Bexarotene was absent when the PPAR-δ pathway was nonfunctional, supporting the claim that the beneficial effects of Bexarotene are dependent on the activation of the PPAR-δ pathway. Elucidation of the mechanism through which Bexarotene acts has important implication for expanded use as a targeted therapy in HD and possibly other neurodegenerative diseases such as Alzheimer’s and Parkinson’s disease.

METHODS:

To test the hypothesis, in vitro experiments were performed using primary cortical neurons from wildtype and Bacterial Artificial Chromosome Huntington’s Disease (BAC-HD) mice models. Primary cortical neurons were harvested from 0 – 3 day old pups and prepared as
previously described.\textsuperscript{12} Lentiviral transduction with shRNA knockdown of PPAR-δ in the primary neurons was performed 9 – 10 days post-harvest. 24 hour Bexarotene treatment at 1000 nM was initiated 5 days post-transduction. Following completion of Bexarotene treatment, mitochondrial membrane potential was measured in primary cortical neurons using the Tecan M200Pro Reader with administration of a potential sensitive JC-1 dye, which is only taken up in mitochondria if membrane polarization is normal. Carbonyl cyanide m-chlorophenyl hydrazine (CCCP) treatment was used as a positive control for membrane depolarization. Neuronal apoptosis was quantified via immunofluorescence to cell death marker cleaved caspase-3 following treatment with hydrogen peroxide. Statistical analysis was performed with Student t-test, \( P < 0.05 \). All experiments were performed with 3 replicates with a total of 12 replicates per condition for the mitochondrial membrane potential assay and 9 replicates per condition for the apoptosis assay.

RESULTS & DISCUSSION

There was no statistical difference in the measured mitochondrial membrane potential of wildtype and BAC-HD primary cortical neurons with PPAR-δ knockdown following Bexarotene treatment (Figure 1). In neurons transduced with a control shRNA, there was a noticeable, but not statistically significant, increase in mitochondrial membrane potential after Bexarotene treatment in both neuronal types. The larger red/green ratio in the JC1 Assay indicates healthier mitochondria, therefore the lack of ratio increase in BAC-HD neuron with PPAR-δ knockdown treated with Bexarotene demonstrates that mitochondrial health did not improve if the PPAR-δ pathway was not functional.
Figure 1. There was no change in mitochondrial membrane potential suggesting mitochondrial health did not improve if PPAR-δ was not functional.

Cleaved caspase-3 staining, a marker of apoptosis, was significantly increased in both wildtype and BAC-HD primary neurons when the PPAR-δ pathway was inhibited despite administration of Bexarotene (Figure 2a). Increased staining in PPAR-δ shRNA-treated neurons demonstrates loss of Bexarotene-induced rescue. Microtubule-associated protein (MAP) 2, which is an indication of neuronal health, appeared reduced when PPAR-δ was inactivated in both neuron types (Figure 2b). BAC-HD neurons transduced with control shRNA demonstrated a decrease in staining following Bexarotene treatment to levels comparable to that of wildtype neurons. This further supports the hypothesis that the neuroprotective effects of Bexarotene require the presence of PPAR-δ.
Figure 2: (a) Increased cleaved caspase-3 staining in PPAR-δ shRNA-treated neurons demonstrates a significant increase in cell death when the PPAR-δ pathway is inhibited and loss of Bexarotene-induced rescue in the absence of PPAR-δ. This indicates that the neuroprotective effects of Bexarotene require the presence of PPAR-δ.

Figure 2: (b) Neurons stained with apoptosis marker cleaved caspase 3, MAP2 which reflects neuron health, and nuclear stain Hoescht.
CONCLUSION

These results support the hypothesis that the PPAR-δ pathway mediates neuroprotection and mitochondria improvement imparted by Bexarotene in vitro. Treatment with Bexarotene confers neuroprotection when the PPAR-δ pathway is activated, but not when the pathway is inhibited. Thus, the use of Bexarotene has potential beyond the current FDA approval for cutaneous T cell lymphoma as it appears to be a promising treatment for neurodegenerative diseases such as HD. Also, the expansion of Bexarotene’s indication for neurodegenerative disorders rapidly shortens the duration a therapy transitions from the bench to the bedside. The conclusions of this study demonstrate how a better understanding of the mechanism through which medications act can lead to targeted therapies with broader indications.

REFERENCES


