Neuroprotective and Oligodendrocyte Precursor Cell Differentiation Promoting Activities of the RXR Nuclear Receptor Agonist IRX4204 are Potentiated by Thyroid Hormone

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ABSTRACT

OBJECTIVES: (1) determine if the RXR nuclear receptor agonist IRX4204 is effective in an in vivo neuroprotection (NP) model; (2) evaluate the effects of combination treatment with thyroid hormone and IRX4204 in this model; (3) determine if adding thyroid hormone increases the previously reported stimulatory effect of IRX4204 on in vitro differentiation of oligodendrocyte precursor cells (OPC) into oligodendrocytes (OL).

BACKGROUND: IRX4204 is a potent, highly selective RXR agonist compound being developed for treatment of multiple sclerosis (MS) and other neurologic diseases. Central hypothyroidism is a documented side effect of treatment with RXR agonists, including IRX4204. Since thyroid hormone is critical for CNS functions, we studied the effects of combination treatment with IRX4204 and thyroid hormone in an in vivo model of NP, and on OPC differentiation in vitro.

METHODS: Mice were administered cuprizone to induce demyelination; and rapamycin to prevent remyelination. NP was assessed by quantitation of SMI-32 immunostained axonal ovoids (transected axons) in the corpus callosum. Quantitation of OPC differentiation was performed by high-content screening of OPCs, which express PLP-EGFP when differentiated into OL; and by MBP immunostaining.

RESULTS: Combination treatment with thyroid hormone and IRX4204 in the NP model resulted in reduction in axonal transection compared to vehicle, and statistically significant approximately three-fold greater reduction compared to IRX4204 alone (-38.3% vs -13.6%). Addition of thyroid hormone with IRX4204 to OPC cultures resulted in statistically significant additive increase in myelin producing OL versus IRX4204 alone.

CONCLUSIONS: IRX4204 demonstrated neuroprotective effects in vivo; and OPC differentiation promoting effects in vitro; both of which were potentiated by thyroid hormone. RXR agonists should be co-administered with thyroid hormone, not only to maintain euthyroid status, but also to obtain improved efficacy in MS and other neurologic diseases patients treated with these agents.

OBJECTIVES

1. Determine if the RXR nuclear receptor agonist IRX4204 is effective in an in vivo neuroprotection (NP) model.
2. Evaluate the effects of combination treatment with thyroid hormone and IRX4204 in this model.
3. Determine if adding thyroid hormone increases the previously reported stimulatory effect of IRX4204 on in vitro differentiation of oligodendrocyte precursor cells into myelin producing oligodendrocytes.

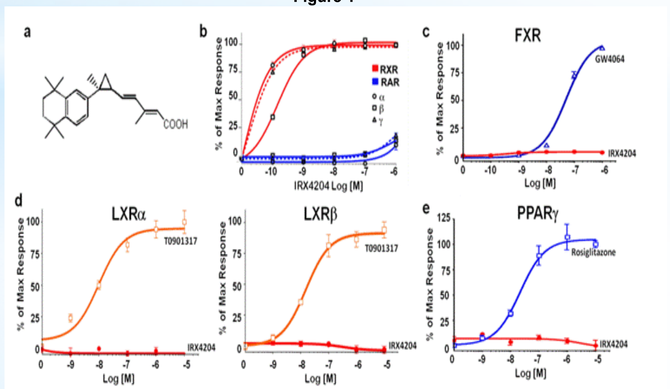
BACKGROUND

IRX4204 is a second-generation Retinoid X Receptor (RXR)-specific agonist (rexinoid). By binding and activating RXR nuclear receptors, rexinoids regulate differentiation, proliferation and survival of neurons and oligodendrocytes, in normal tissue development, and in studies in vitro and in animal models of various neurodegenerative diseases. We have previously reported preclinical studies with IRX4204 demonstrating neuroprotective activity of IRX4204 on in vitro survival of dopaminergic neurons and activity in the 6-hydroxy dopamine induced model of PD in rats (Wang J, et al, Oncotarget, 2016) with improved motor functions and in vivo neuroprotection demonstrated by preserved tyrosine hydroxylase midbrain neurons on histopathology. We reported previously (Chandraratna R et al, Am J Tranl Res, 2016), and in other abstracts in this conference, that IRX4204 also is active in preclinical models of MS, and in models of AD. We also previously reported that IRX4204 promotes differentiation of OPCs into oligodendrocytes in vitro.

RXR agonists, including IRX4204 are known to cause centrally-mediated hypothyroidism by suppression of TSH synthesis. In a phase 1 clinical trial of IRX4204 in patients with Parkinson's disease, reported elsewhere in this conference, we observed an inverse dose response relationship for improvements in motor scores and UPDRS, with reduction of TSH and T4. The patients that experienced the greatest clinical improvement were the patients who had the least reduction in thyroid function. This observation prompted us to investigate possible interactions of RXR agonism mediated by IRX4204, with thyroid hormone actions, in assays and models relevant to neurodegenerative diseases.

Figure 1 shows the molecular structure of IRX4204. Although the compound has two chiral centers, it is synthesized as an optically pure isomer. Figure 1 also shows that IRX4204 is a potent agonist of RXR nuclear receptors in transactivation assays, with EC_{50} less than 1 nM, and EC_{90} of approximately 1 nM for each of the three RXR isoforms. IRX4204 has high selectivity for RXR, with no agonist activity for RAR, FXR, LXR, or PPARγ heterodimers with RXR.

Figure 1



METHODS

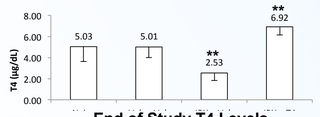
Mice were administered cuprizone to induce demyelination; and rapamycin to prevent remyelination. NP was assessed by quantitation of SMI-32 immunostained axonal ovoids (transected axons) in the corpus callosum.

In vivo Neuroprotection Study Design

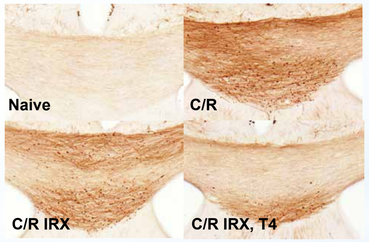
Group	Time point	Treatment	N
1	6+0	No treatment	N=6
2	6+0	Vehicle + Vehicle	N=12
3	6+0	IRX4204 + Vehicle	N=12
4	6+0	IRX4204 + Thyroxine	N=12

In vitro quantitation of OPC differentiation was performed by high-content screening of OPCs, which express PLP-EGFP when differentiated into OL; and by MBP immunostaining.

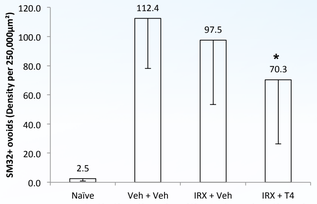
RESULTS



End of Study T4 Levels



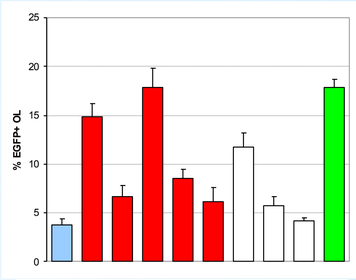
SMI-32 Representative Images



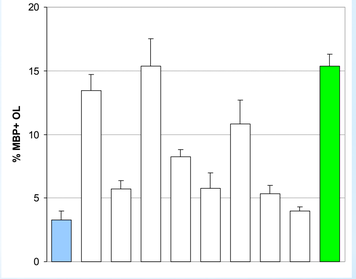
There was a statistically significant reduction in the axonal transection as shown by the number of SMI-32 positive ovoids in the IRX4204+T4 treatment group when compared to vehicle.

In Vitro OPC Differentiation

Percent of EGFP+ Oligodendrocytes



Col	2	3	4	5	6	7	8	9	10	11
IRX	0	10uM			1nM		0	0	0	
T3	0	0	0	0.1	0.01	0.001	0.1	0.01	0.001	10 ng/ml



Percent of MBP+ Oligodendrocytes

CONCLUSIONS

IRX4204 demonstrated neuroprotective effects which were potentiated by thyroid hormone, in the in vivo cuprizone neurorprotection model in mice.

IRX4204 promoted differentiation of OPCs into myelin producing OL in vitro, which also was potentiated by thyroid hormone.

RXR agonists should be co-administered with thyroid hormone, not only to maintain patients' euthyroid status, but also potentially to obtain improved efficacy in MS and other neurologic diseases treated with these agents.

Author Disclosure Information: **M.E. Sanders:** Chairman and Chief Executive Officer, Io Therapeutics, Inc.; stock in Io Therapeutics, Inc.; **R. Chandraratna:** Director, President and Chief Scientific Officer of Io Therapeutics, Inc., Stock in Io Therapeutics, Inc.

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