Thyroid receptor beta-selective agonist enhances functional brain delivery and accelerates remyelination in *in vitro* and *in vivo* rodent demyelination models

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Introduction

- Thyroid hormone (T_3) is known to induce oligodendrocyte differentiation and remyelination *in vivo*.[1] Brain-directed thyromimetics have potential as therapeutics to enhance remyelination in neurodegenerative diseases such as multiple sclerosis (MS).[2] LL-341070 is a novel thyromimetic prodrug activated by fatty acid amide hydrolase (FAAH)-mediated conversion to LL-341070A, a potent and selective small molecule agonist of thyroid hormone receptor (TR) beta. FAAH-activated delivery allows for enhanced brain penetration of the active molecule.
- 24S-hydroxycholesterol (24OHC) is a primary metabolite of *de novo* cholesterol synthesis in the CNS and can cross the blood brain barrier into the periphery.[3] ~70% of brain cholesterol is myelin and synthesis rate of 24OHC in plasma closely reflects the 24OHC synthesis rate from brain.[3,8] Decreased serum 24OHC is observed in MS patients.[4] Thus, plasma 24OHC is a potential biomarker of myelin synthesis in the CNS. This study measured the effect of thyromimetic treatment on the fractional synthesis of 24OHC in brain and plasma using deuterated water labeling of 24OHC in a cuprizone demyelination model following withdrawal from cuprizone diet, during the period of active remyelination.[5]
- Compound potency, pharmacokinetics and target engagement were confirmed for LL-341070 prior to testing efficacy in remyelination models including oligodendrocyte precursor cell differentiation *in vitro* and experimental autoimmune encephalitis *in vivo*.

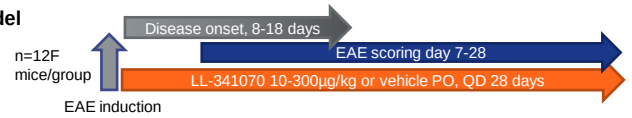
Methods

24OHC Fractional Synthesis Rate (FSR) Measurement: Tissue and plasma underwent alkaline hydrolysis and derivatization for GC/MS or LC/MS analysis of deuterated 24OHC. Plasma analysis was performed by Ardena Biosciences. Fractional synthesis was calculated by Mass Isotopomer Distribution Analysis using precursor ^2H enrichment in body water from liver palmitate [6].

Statistics: Data were analyzed by one-way ANOVA with Tukey's multiple comparisons test and are represented as mean +/- SEM. *p<0.05. Median day of onset in EAE compared using Wilcoxon's survival test.

Cuprizone Model

EAE Model



TRβ Potency and Selectivity

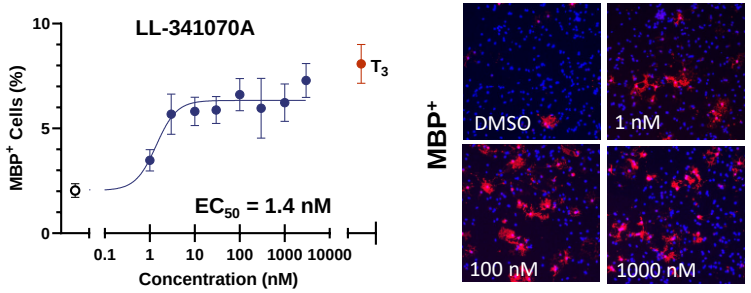
Both the prodrug and active molecule show enhanced selectivity for the thyroid hormone β receptor

Profile	Prodrug LL-341070 (nM)	Active L-341070A (nM)
TRβ EC_{50}	478	7.3
TRα EC_{50}	> 10,000	24
Selectivity*	n/a	9.1

*Selectivity adjusted to TRα-bias of T_3 in the assays.

OPC Differentiation

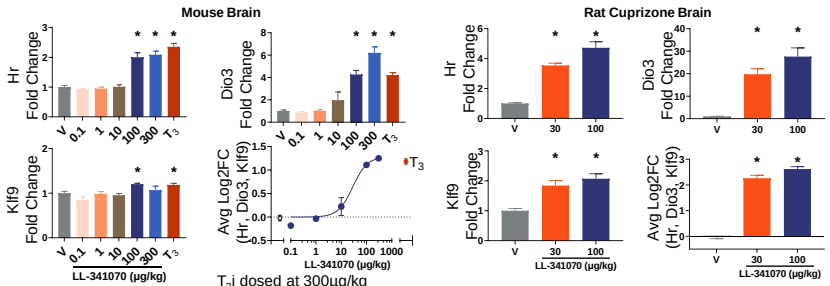
LL-341070A enhances mouse oligodendrocyte progenitor cell (OPC) differentiation *in vitro*



OPC Differentiation Assay: Primary OPC cultures were generated from brains of E14.5 PLP-EGFP C57Bl/6 mouse. OPC differentiation induced with or without compound for 5 days. N=6/concentration; T_3 at 10 ng/mL.

T_3-Target Gene Expression

Target engagement (TE) in rodent brain is demonstrated by increased expression of T_3-responsive genes.[7] LL-341070 or T_3 dose (left) increases expression of *Hr*, *Dio3, Klf9*, and composite average log_2 fold change. TE was confirmed in the rat cuprizone model (right).



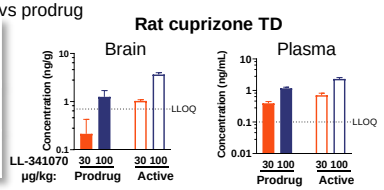
T_3i dosed at 300µg/kg

Tissue Distribution

Tissue distribution (TD) in mouse and rat demonstrates enhances brain exposure of active compound vs prodrug

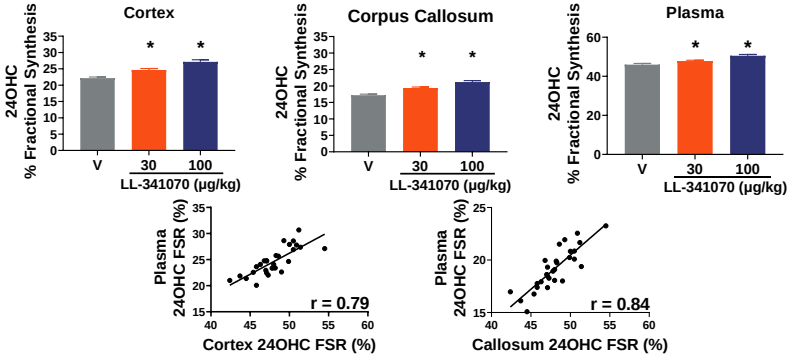
Prodrug @ 100 µg/kg PO	Brain AUC (ng/mL*h)	Plasma AUC (ng/mL*h)	Brain / Plasma AUC Ratio
Prodrug LL-341070	10.9	18.8	0.58
Active LL-341070A	73.9	35.8	2.06

Tissue distribution in male C57BL/6 mouse. AUC is 0-24 hr.



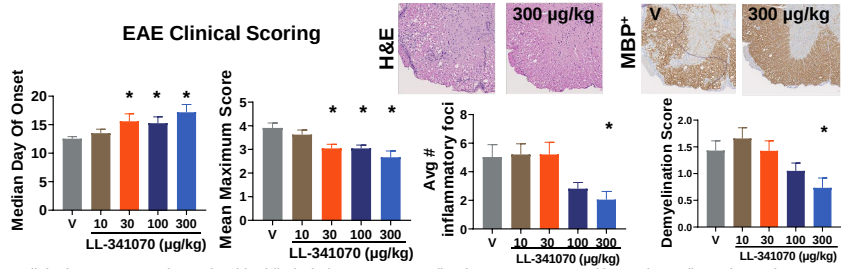
24S-hydroxycholesterol Measurement

Thyromimetic treatment enhances the fractional synthesis rate (FSR) of 24S-hydroxycholesterol in brain and demonstrates a significant correlation with 24OHC FSR in plasma



Efficacy in EAE Model

LL-341070 improves clinical scoring and histological endpoints of inflammation and demyelination in a prophylactic experimental autoimmune encephalitis (EAE) model



Clinical scores were determined by blinded observer. Demyelination score assessed by % demyelinated area in MBP stain, inflammatory foci refers to # of groups of >20 cells / section in H&E stain.

Conclusions

- The thyromimetic FAAH-mediated prodrug LL-341070 demonstrates robust brain penetration and target engagement *in vivo* and has efficacy in demyelination models *in vitro* and *in vivo*
- Thyromimetic treatment enhances the fractional synthesis rate of brain 24OHC during active remyelination in a rat cuprizone model. Brain 24OHC FSR is highly correlated with plasma 24OHC FSR, suggesting the potential of plasma 24OHC FSR via ^2H2O labeling as a biomarker of brain cholesterol synthesis in neurodegenerative disease.

References

1. Slivestroff et al 2012 *Exp Neurol* 2. Hartley et al 2019 *JCI* 3. Leoni & Caccia 2013 *Biochimie* 4. van de Kraats et al 2014 *MSJ* 5. Harsan et al 2008 *JNeurosci* 6. Hellerstein & Neese 1992 *Am J Phys* 7. Dugas et al 2012 *Mol Cell Neurosci. PNAS* 8.Shankaran et al 2017 Neurobiol Dis.