Mouse astrocytes exhibit agonist-induced functional S1P₁ receptor antagonism

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Poster Number: P0357
Disclosures

Nada Ben Yakoub, Tatjana Uffelmann, Sarah Tisserand and Marc Bigaud are employees of Novartis.

The study was funded by Novartis Pharma AG, Basel, Switzerland.

Medical writing support was provided by Richa Chhabra and Anuja Shah (employees of Novartis Healthcare Pvt. Ltd., Hyderabad, India). The final responsibility for the content lies with the authors.
Background and objective

- Sphingosine-1-phosphate (S1P) receptor subtype 1 (S1P₁) plays a key role in regulation of lymphocyte trafficking¹
- In multiple sclerosis, S1P₁ agonists such as fingolimod and siponimod induce S1P₁ down modulation (i.e. receptor internalization and degradation) inhibiting the egress of pathogenic lymphocytes to the CNS - a phenomenon also known as functional S1P₁ antagonism²,³,⁴
- However, no evidence of this phenomenon exists in the cells of the CNS

Objective

To investigate the presence of S1P₁-functional antagonism by assessing agonist-induced S1P₁ down modulation in the astrocytes using a Ca²⁺ signaling assay

Ca²⁺, calcium; CNS, central nervous system; S1P₁, sphingosine-1-phosphate receptor subtype 1

Methodology: Agonist-induced Ca\(^{2+}\) signaling

**Fluorescent Ca\(^{2+}\) probe and Fluorescent Imaging Plate Reader (FLIPR)**

**Pre-incubation of murine astrocytes (C8-D1A) with vehicle or an S1P\(_1\) agonist\(^\#\) (S1P, AUY954, fingolimod or siponimod) at different concentrations (0.0001 µM up to 30 µM) (overnight incubation)**

**Washing and Ca\(^{2+}\) probe loading (incubation for an hour) followed by ATP priming (10 µM) to activate the Ca\(^{2+}\) pumps (incubation for 30 minutes)**

**Compound stimulation and FLIPR readout (3 minutes - Quadruplicate)**

**Outcomes**

Dose response curves using agonist-induced Ca\(^{2+}\) signaling, measured as an increase in the intracellular fluorescence via FLIPR

\(^\#\)S1P (natural ligand for S1PRs), AUY954 (selective S1P, agonist), fingolimod (S1P\(_{1,3,4,5}\) agonist), or siponimod (S1P\(_{1,5}\) agonist)

ATP, adenosine triphosphate; Ca\(^{2+}\), calcium; FLIPRs, fluorescent imaging plate reader; S1P, sphingosine-1-phosphate; S1PR, sphingosine-1-phosphate receptor subtype 1; S1PR, sphingosine-1-phosphate receptor
Results

Astrocytes pre-incubated with vehicle (n=3 each)

Dose-dependent increase in the intracellular Ca^{2+} signals in response to all tested S1PR agonists, with EC_{50} values being within the range of 8–70 nM

Ca^{2+}: calcium; EC_{50}: concentration of a drug that gives half-maximal response; EC_{90}: concentration of a drug that gives 90% response; fingolimod-P, fingolimod-phosphate; S1P, sphingosine-1-phosphate receptor; S1PR, sphingosine-1-phosphate receptor
**Results**

**Astrocytes pre-incubated with S1P (1µM) (n=3 each)**

Pre-incubation with natural ligand S1P (S1P<sub>1,2,3,4,5</sub> agonist) did not alter the effects of the S1PR agonists confirming that S1P does not induce down modulation of its own receptors (S1PRs).

- **Target: S1P<sub>1,2,3,4,5</sub>**
  - EC<sub>90</sub> (µM): 1.8±0.4
  - EC<sub>50</sub> (µM): 0.03±0.02

- **Target: S1P<sub>1,3,4,5</sub>**
  - EC<sub>90</sub> (µM): 0.7±0.3
  - EC<sub>50</sub> (µM): 0.05±0.02

- **Target: S1P<sub>1,5</sub>**
  - EC<sub>90</sub> (µM): 0.5±0.2
  - EC<sub>50</sub> (µM): 0.04±0.02

- **Target: S1P<sub>1</sub>**
  - EC<sub>90</sub> (µM): 8.0±1.4
  - EC<sub>50</sub> (µM): 0.09±0.04

Ca<sup>2+</sup>: calcium; EC<sub>50</sub>: concentration of a drug that gives half-maximal response; EC<sub>90</sub>: concentration of a drug that gives 90% response; fingolimod-P, fingolimod-phosphate; S1P, sphingosine-1-phosphate; S1P<sub>1</sub>, sphingosine-1-phosphate receptor subtype; S1PR, sphingosine-1-phosphate receptor receptor subtype
Results

Astrocytes pre-incubated with AUY954 (1µM) (n=3 each)

- Pre-incubation with AUY954 abolished effects of fingolimod, siponimod, AUY954, and induced down modulation of S1P₁ receptors
- In astrocytes, effects of fingolimod and siponimod were principally S1P₁-dependent
- Similar observations were observed after pre-incubation with fingolimod or siponimod

Ca²⁺, calcium; EC₉₀, concentration of a drug that gives half-maximal response; EC₉₀, concentration of a drug that gives 90% response; fingolimod-P, fingolimod-phosphate; n.a., not applicable; S1P, sphingosine-1-phosphate; S1P₁, sphingosine-1-phosphate receptor subtype 1

Target: S1P₁,₂,₃,₄,₅
EC₉₀ (µM): 7.4±1.8
EC₅₀ (µM): 0.12±0.09

Target: S1P₁,₃,₄,₅
EC₉₀ (µM): n.a.
EC₅₀ (µM): n.a.

Target: S1P₁,₅
EC₉₀ (µM): n.a.
EC₅₀ (µM): n.a.

Target: S1P₁
EC₉₀ (µM): n.a.
EC₅₀ (µM): n.a.
Conclusions

• First evidence of agonist-induced S1P₁ down modulation in murine astrocytes
• Similar investigations on other neural and glial cell types are warranted to establish agonist-induced S1P₁ down modulation as a general phenomenon in the CNS
• Translational and clinical studies are warranted to further validate this hypothesis
Thank you