PIPE-791, a potent brain-penetrant selective LPA1 antagonist, reduces neuroinflammation *in vivo*

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Introduction

Lysophosphatidic acid (LPA) is a naturally occurring inflammatory lipid that is dysregulated in multiple sclerosis – an immune mediated demyelinating disease in the CNS. LPA activates the LPA1 receptor leading to aberrant cytokine and chemokine levels in the CNS, infiltration of peripheral immune cells (particularly autoreactive T cells), as well as microglial and astrocyte activation[1]. Profiling of the inflammatory signaling in the mouse MOG-EAE model of demyelination demonstrated an upregulation of cytokines and chemokines in the CNS that was responsive to LPA1 antagonism. The inflammatory signaling factors induced in the EAE model are also increased after challenge with LPS (lipopolysaccharide), an endotoxin frequently used to induce widespread inflammation. LPA1 antagonists, such as AM152, are effective at reducing LPS-induced inflammation[4]. However, since AM152 is peripherally-restricted, its ability to impact inflammation in the CNS is limited. To address this deficiency, Pipeline Therapeutics has identified PIPE-791, a potent, selective, and brain-penetrant LPA1 antagonist.

To demonstrate the need for a brain-penetrant LPA1 antagonist, PIPE-791 was characterized in an LPS model of CNS inflammation. LPS rapidly increases cytokine and chemokine levels in the CNS and this upregulation is inhibited by PIPE-791, but not by the peripherally-restricted antagonist, AM152. In this model, PIPE-791 was effective at not only reducing cytokine and chemokine levels in the CNS, but also successful at reducing microglial activation in the retina. While these data highlight the role of LPA1 in neuroinflammation, they also underscore the need for a brain-penetrant antagonist, such as PIPE-791.

PIPE-791 is a promising therapeutic for multiple sclerosis as it not only promotes remyelination[5], but also reduces neuroinflammation in vivo. As such, PIPE-791 shows promise for many CNS dysfunctions caused by neuroinflammation.

MOG-EAE spinal cord inflammation gene expression

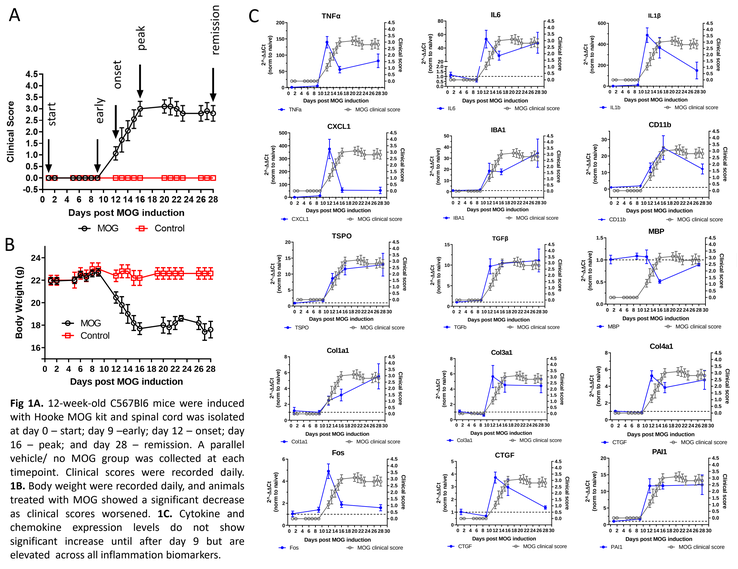
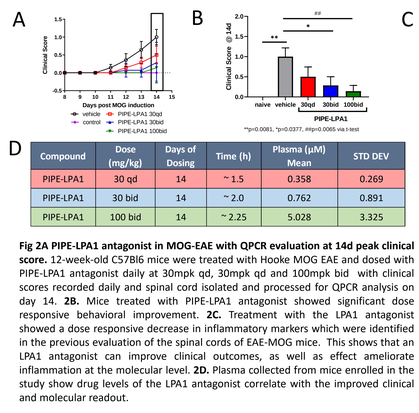


Fig 1A. 12-week-old C567Bl6 mice were induced with Hooke MOG kit and spinal cord was isolated at day 0 – start; day 9 –early; day 12 – onset; day 16 – peak; and day 28 – remission. A parallel vehicle/ no MOG group was collected at each timepoint. Clinical scores were recorded daily. **1B.** Body weight were recorded daily, and animals treated with MOG showed a significant decrease as clinical scores worsened. **1C.** Cytokine and chemokine expression levels do not show significant increase until after day 9 but are elevated across all inflammation biomarkers.

MOG EAE systemic inflammation is reduced by LPA1 antagonism



D

Compound	Dose (mg/kg)	Days of Dosing	Time (h)	Plasma (μM) Mean	STD DEV
PIPE-LPA1	30 qd	14	~ 1.5	0.358	0.269
PIPE-LPA1	30 bid	14	~ 2.0	0.762	0.891
PIPE-LPA1	100 bid	14	~ 2.25	5.028	3.325

Fig 2A PIPE-LPA1 antagonist in MOG-EAE with QPCR evaluation at 14d peak clinical score. 12-week-old C57Bl6 mice were treated with Hooke MOG EAE and dosed with PIPE-LPA1 antagonist daily at 30mpk qd, 30mpk qd and 100mpk bid with clinical scores recorded daily and spinal cord isolated and processed for QPCR analysis on day 14. **2B.** Mice treated with PIPE-LPA1 antagonist showed significant dose responsive behavioral improvement. **2C.** Treatment with the LPA1 antagonist showed a dose responsive decrease in inflammatory markers which were identified in the previous evaluation of the spinal cords of EAE-MOG mice. This shows that an LPA1 antagonist can improve clinical outcomes, as well as effect ameliorate inflammation at the molecular level. **2D.** Plasma collected from mice enrolled in the study show drug levels of the LPA1 antagonist correlate with the improved clinical and molecular readout.

PIPE-791 reduces neuroinflammation *in vivo*

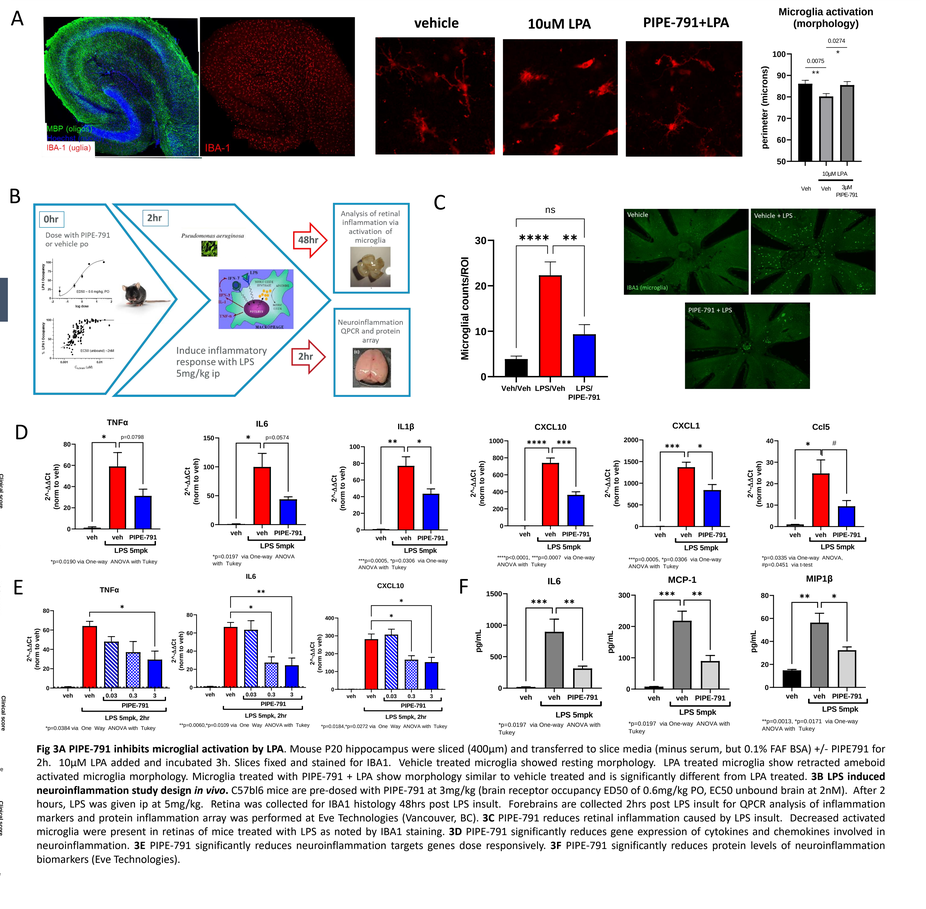
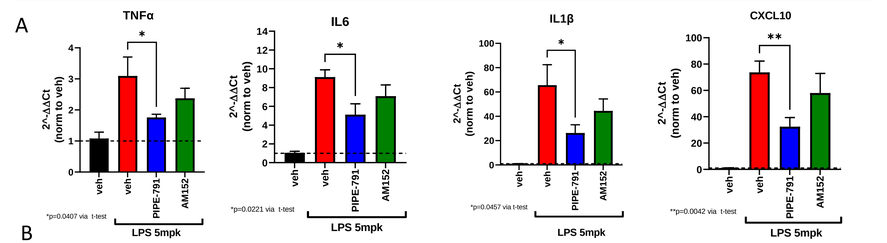


Fig 3A PIPE-791 inhibits microglial activation by LPA. Mouse P20 hippocampus were sliced (400μm) and transferred to slice media (minus serum, but 0.1% FAF BSA) +/- PIPE791 for 2h. 10μM LPA added and incubated 3h. Slices fixed and stained for IBA1. Vehicle treated microglia showed resting morphology. LPA treated microglia show retracted ameboid activated microglia morphology. Microglia treated with PIPE-791 + LPA show morphology similar to vehicle treated and is significantly different from LPA treated. **3B LPS induced neuroinflammation study design *in vivo*.** C57bl6 mice are pre-dosed with PIPE-791 at 3mg/kg (brain receptor occupancy ED50 of 0.6mg/kg PO, EC50 unbound brain at 2nM). After 2 hours, LPS was given ip at 5mg/kg. Retina was collected for IBA1 histology 48hrs post LPS insult. Forebrains are collected 2hrs post LPS insult for QPCR analysis of inflammation markers and protein inflammation array was performed at Eve Technologies (Vancouver, BC). **3C** PIPE-791 reduces retinal inflammation caused by LPS insult. Decreased activated microglia were present in retinas of mice treated with LPS as noted by IBA1 staining. **3D** PIPE-791 significantly reduces gene expression of cytokines and chemokines involved in neuroinflammation. **3E** PIPE-791 significantly reduces neuroinflammation targets genes dose responsively. **3F** PIPE-791 significantly reduces protein levels of neuroinflammation biomarkers (Eve Technologies).

PIPE-791 reduces neuroinflammation, AM152 does not



B

Compound	Dose	Time	Brain (μM) Mean	$C_{u,brain}$ (μM) Mean
PIPE-791	3 mg/kg PO	4 hr	0.107	0.004
AM152	30 mg/kg PO	3 hr	< 0.041	< 0.0002

Fig 4 Comparison of LPA1 antagonists - peripherally restricted AM152 (30mg/kg) and CNS penetrant PIPE-791 (3mg/kg) in the LPS induced neuroinflammation model. PIPE-791 was pretreated 2hrs prior and AM152 was pretreated 1hr prior to LPS insult to ensure both compounds were at peak concentration before LPS insult. Brain was collected 2hrs post LPS insult and analyzed for QPCR. **4A.** PIPE791 shows a reduction of neuroinflammation gene expression whereas peripherally restricted AM152 does not. **4B.** Corresponding neuro PK analysis show PIPE-791 present in the brain and AM152 is not detected in the brain.

PIPE-791 does not impact phagocytosis *in vivo*

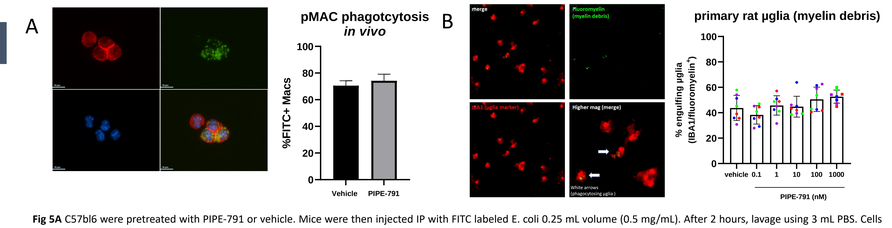


Fig 5A C57bl6 were pretreated with PIPE-791 or vehicle. Mice were then injected IP with FITC labeled E. coli 0.25 mL volume (0.5 mg/mL). After 2 hours, lavage using 3 mL PBS. Cells are stained with F4/80, imaged and quantified. PIPE-791 treated mice showed equal activity to vehicle mice in FITC labeled macrophages showing that PIPE-791 did not impact phagocytosis. **5B.** Cortical cultures generated from P2 rats and plated in DMEM 10% FBS for 10 days. Microglia were mechanically isolated and plated in 96 well plates for 3days in growth media. Media replaced with growth media containing PIPE-791 (0.1nM to 1μM) and incubated for 3h. Fluoromyelin labeled myelin debris was added to each well and incubated for 90 minutes. Cells fixed and stained for IBA-1, imaged and quantified for IBA co-localization with fluoromyelin. Rat microglia treated with vehicle and PIPE-791 (0.1-1000nM) showed no difference in clearing fluoromyelin debris showing that M2 microglia involved in phagocytosis are not affected by PIPE-791.

Conclusions

- PIPE-791, a CNS penetrant, orally bioavailable LPA1 antagonist, reduces neuroinflammation and promotes functional remyelination *in vivo*[5] and is thus a promising treatment for multiple sclerosis.
- PIPE-791 reduces LPS-induced microglia activation and inflammatory cytokines/chemokines *in vivo*.
- PIPE-791 reduces neuroinflammation, whereas the peripherally restricted AM152 does not, emphasizing the benefit of a brain penetrant LPA1 antagonist.
- PIPE-791 does not impact macrophage nor microglia phagocytosis.
- PIPE-791 shows promise for additional neuroinflammatory indications such as spinal cord injury or hydrocephalus.

References

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