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LPA-Induced Activation of Satellite Glial Cells and Its Modulation by PIPE-791, a novel LPA_1 antagonist, in an *In Vitro* Pain Model

*K.I. LORRAIN, A. R. BROADHEAD, D. BAGNOL, M. M. POON, G. JOSEPH, J.R. ROPPE, A. C. CHEN, D. S. LORRAIN.
Contineum Therapeutics, San Diego CA, USA

NO – PHOTO / RECORD

Introduction

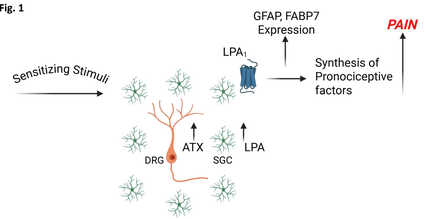
Dorsal root ganglia (DRG), part of the peripheral nervous system (PNS), contain astrocyte-like satellite glial cells (SGCs) that closely envelop neuronal cell bodies. SGCs are implicated in nerve regeneration and pain signaling and express LPA_1 (lysophosphatidic acid receptor 1). Given the established link between LPA signaling in dorsal root ganglia (DRG) and neuropathic pain, we investigated the response of satellite glial cells (SGCs) to LPA stimulation, both in the presence and absence of PIPE-791, a novel selective LPA_1 receptor antagonist. Previous studies have shown that lysophosphatidic acid (LPA) levels increase following injury, leading to the activation of satellite glial cells (SGCs), which in turn amplify pain signaling pathways—an effect primarily mediated by the LPA_1 receptor.

We generated primary rat SGC cultures and confirmed both Glutamine Synthetase (GS) and FABP7 (fatty acid binding protein 7) expression. Previous data has shown that LPA induces rapid calcium release via intracellular stores. We measured calcium mobilization after LPA and observed rapid induction. This was completely inhibited in the presence of PIPE-791, confirming not only that LPA induces calcium release, but that release is mediated by LPA_1. To assess SGC activation, cells were treated with LPA for 3 hours, which led to an upregulation of glial fibrillary acidic protein (GFAP), a well-established marker of SGC activation. Notably, PIPE-791 significantly reduced the expression of GFAP and FABP7, reinforcing the role of LPA signaling in neuropathic pain and demonstrating that LPA_1 antagonism can effectively attenuate glial activation. Collectively, these findings suggest that PIPE-791 may alleviate pain in vivo by modulating LPA-induced activation of satellite glial cells (SGCs).

Pathway

Figure 1: After sensitizing stimuli, Autotaxin is released, which catalyzes the production of LPA. LPA acts on satellite glial cells through the LPA_1 receptor, leading to neuronal hyperexcitability and glial activation. This activation is marked by increased expression of GFAP and FABP7, which contribute to the amplification and maintenance of pain signaling.

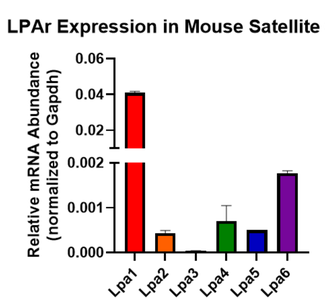
Fig. 1



Mouse Satellite Glia Cells express LPA1

Figure 2: Satellite glial cells (SGCs) express LPA_1. Total RNA was extracted from mouse satellite glial cells using RNeasy Mini Kit columns (Qiagen), following the manufacturer's protocol. Complementary DNA (cDNA) was synthesized from the isolated RNA using the qScript XLT cDNA SuperMix (Quantabio) according to the manufacturer's instructions. Quantitative PCR (qPCR) was performed using PerfeCTa SYBR Green FastMix (Quantabio) on a StepOnePlus Real-Time PCR System (Applied Biosystems). Relative mRNA expression levels were calculated using the ΔCt method, normalized to GAPDH as the endogenous control.

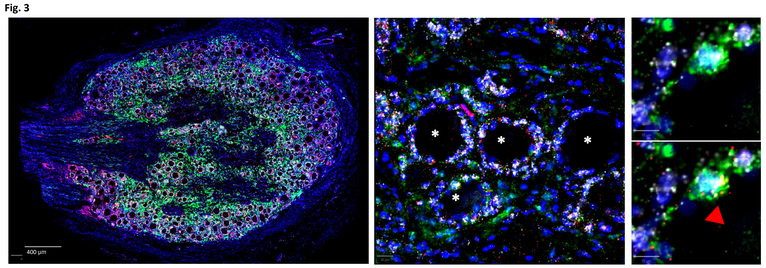
Fig. 2



LPA1, Autotaxin, and Glutamine Synthetase mRNAs are Expressed in Human DRGs

Figure 3: Human fresh frozen sections (14 µm) were hybridized with different probes using the protease-free treatment RNAscope® Multiplex Fluorescent reagent kit v2 according to the manufacturer protocol. Images illustrating the gene expression of LPA_1 (white), Autotaxin (red) and Glutamine synthase (green) in human healthy donor (age 19, male). The expression of LPA_1 mRNA in SGCs surrounding neuron cell bodies are highlighted with white asterisks. SGCs expressed LPA_1 and some express both LPA_1 and ATX (red arrowhead).

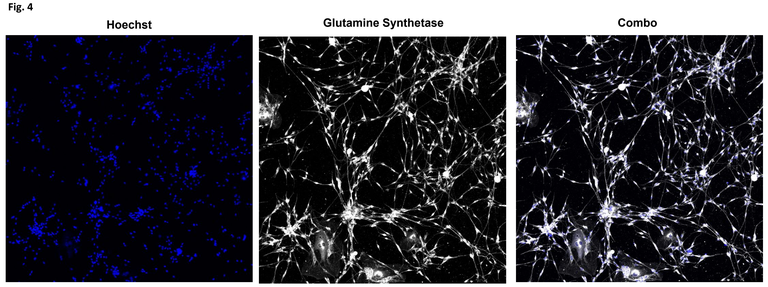
Fig. 3



Cultured Mouse Satellite Glia Cells Express Glutamine Synthetase

Figure 4: Satellite glial cells (SGCs) isolated from dorsal root ganglia (DRG) exhibit an average purity of 93%, as determined by immunostaining for glutamine synthetase. DRGs were harvested from adult CD-1 mice and enzymatically dissociated using collagenase and papain. The resulting cell suspension was plated in 96-well plates and maintained in culture for 14 days, with media refreshed every 2–3 days. Following fixation, cells were stained for Hoechst (blue) and glutamine synthetase (white), a well-established marker of SGCs.

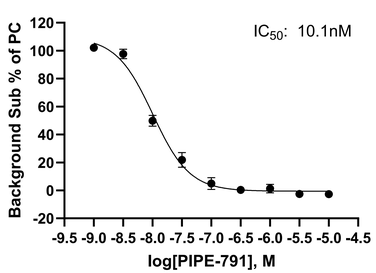
Fig. 4



PIPE-791 Inhibits LPA Induced Calcium Release in Satellite Glia Cells

Figure 5: PIPE-791 inhibits LPA-induced calcium release in satellite glial cells (SGCs). LPA has been shown to mediate calcium signaling in SGCs in response to pain via the LPA_1 receptor (Fischer M et al. (2019) Glia.23585). SGCs isolated from dorsal root ganglia (DRG) were evaluated for inhibition of LPA-induced calcium release at the EC_{80} concentration (1 µM). PIPE-791 produced a dose-dependent reduction in calcium signaling in response to LPA, with a half-maximal inhibitory concentration (IC_{50}) of 10.1 nM (n = 2, error bars are SEM).

Fig. 5



PIPE-791 Inhibits LPA induced Upregulation of GFAP and FABP7

Figure 6: PIPE-791 inhibits LPA induced upregulation of glial fibrillary acidic protein (GFAP) and fatty acid binding protein 7 (FABP7). LPA plays a critical role in pain signaling in the peripheral nervous system by activating the glia cells via the SGC's. This causes increased expression of glial fibrillary acidic protein and fatty acid–binding protein. Upregulation of these markers reflects a reactive glial phenotype, which contributes to neuroinflammatory processes associated with pain sensitization.

Fig 6a. LPA (1 µM) increases GFAP expression in SGCs in an LPA_1-sensitive manner; MCI: mean cell intensity (n=6, error bars are SEM).

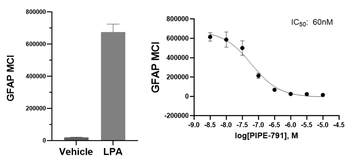


Fig 6b. LPA (1 µM) increases FABP7 expression in SGCs in an LPA_1-sensitive manner; MCI: mean cell intensity (n=6, error bars are SEM).

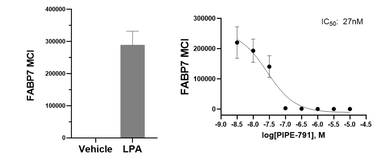
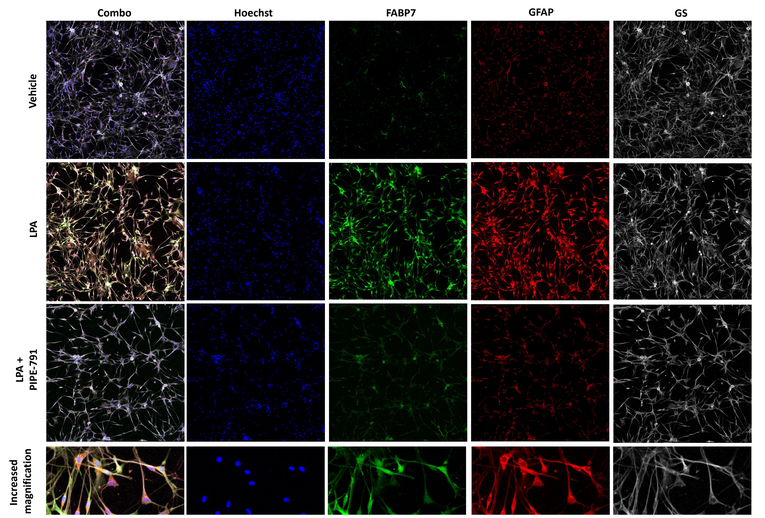


Fig 6c. Representative images show an increase in both FABP7 and GFAP staining with 1µM LPA compared to vehicle control. PIPE-791 inhibits glial activation back to basal levels.

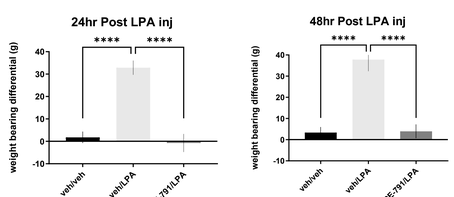


PIPE-791 ameliorates LPA-Induced Rat OA(1)

Figure 7. PIPE-791 ameliorates pain in a LPA-induced osteoarthritis rat model (McDougall et al., 2017). Behavioral assessments indicate that PIPE-791 (3 mg/kg) mitigates LPA-induced joint pain, as evidenced by improved weight-bearing symmetry and reduced mechanical allodynia. Data are presented as mean ± SEM; ****p < 0.001; n = 5 per group.

(1) ***For more information, visit poster PSTR273.01***

Fig.7

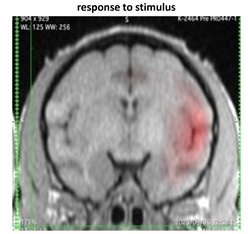


PIPE-791 Suppresses Brain Activation in NHP Nerve Injury Model(2)

Figure 8. PIPE-791 Suppresses CNS Regions Mediating Pain in NHP Model(2). The current study examined the effect of PIPE-791 on regional brain activation in response to non-noxious mechanical stimulation of the foot in male macaques with a unilateral right sciatic nerve injury. Thirteen days after nerve injury, stimuli that did not previously lead to activation in naïve macaques activated the anterior cingulate cortex (ACC) and contralateral insular/secondary somatosensory cortex (Ins/SII). Brain activation was suppressed following 4 weeks of treatment with PIPE-791, dosed once daily at 10 mg/kg. Six weeks after nerve injury (one day after the last dose of PIPE-791) activation of the contralateral Ins/SII was reduced. Results below show non-invasive neuroimaging using fMRI.

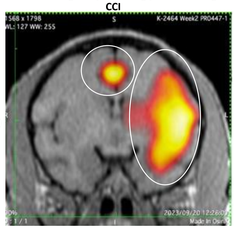
(2) *(Hama et all. Lysophosphatidic Acid Receptor Subtype-1 Antagonist PIPE-791 Reduces Neuropathic Pain in Macaques, IASP 2024)*

Baseline fMRI: minimal activation in response to stimulus

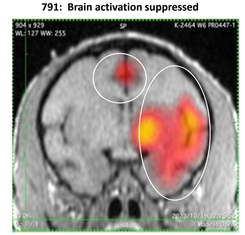


Chronic sciatic nerve constriction injury (CCI)

2 weeks post CC1: Increased brain activation in cortical regions processing pain following CCI



4 weeks post treatment, dosed PO PIPE-791: Brain activation suppressed



Conclusions

- Lysophosphatidic acid signaling in satellite glial cells promotes neuronal hyperexcitability and glial activation through the LPA_1 receptor, contributing to the amplification of pain signaling.
- Contineum Therapeutics has developed PIPE-791, a novel and selective LPA_1 receptor antagonist.
  - Following LPA stimulation, PIPE-791 effectively inhibits calcium signaling in SGCs isolated from mouse DRG cultures.
  - LPA induces upregulation of glial activation markers GFAP and FABP7 in SGCs, reflecting neuroinflammatory processes associated with pain sensitization, while PIPE-791 reverses these effects to basal levels.
  - In an *in vivo* rat model of osteoarthritis, PIPE-791 improved weight-bearing symmetry and reduced mechanical allodynia (Poster PSTR273.01).
  - Furthermore, in a non-human primate model of neuropathic pain, PIPE-791 reduced pain signaling following chronic constriction injury (CCI) of the sciatic nerve (Hama et al., IASP 2024).
  - PIPE-791 is currently being evaluated in a Phase 1b clinical trial for subjects with chronic osteoarthritis pain or chronic low back pain (ClinicalTrials.gov Identifier: NCT06810245).
  - PIPE-791 may alleviate pain *in vivo* by modulating LPA-induced activation of satellite glial cells (SGCs).