Lysophosphatidic Acid Receptor Subtype-1 Antagonist PIPE-791 Reduces Neuropathic Pain in Macaques

TH721

Aldric Hama[2], Karin Stebbins[1], Chris Baccei[1], Daniel Lorrain[1], Austin Chen[1], Takahiro Natsume[2], Hiroryuki Takamatsu[2]

[1]Contineum Therapeutics, San Diego, CA USA. [2]Hamamatsu Pharma Research, Inc., Hamamatsu, Shizuoka, Japan.

Introduction

- CNS lysophosphatidic acid (LPA) could underlie initiation neuroinflammation which in tern could initiate neuropathic pain
- Blocking LPA could be a novel mechanism of action for new analgesics
- Mechanism-based outcome measure for pain and analgesic efficacy are lacking
- Weak association between therapeutic efficacy observed in rodent pain models and clinical efficacy.

Aim

❑ **Explore CNS mechanism of action of PIPE-791 in a nonhuman primate model of neuropathic pain.**

Methods and Materials

This study was reviewed and approved by the HPR Animal Care and Use Committee.

Animals

Five male *Macaca fasicularis* (3-4 kg; 4-5 y.o. EBS, Hashimoto, Japan) were individually housed with free access to food and water and had visual, aural and olfactory access to conspecifics. Housing followed the *Guide for the Care and Use of Laboratory Animals (8th ed.)*. Hamamatsu Pharma Research, Inc. is an AAALAC International accredited facility.

Chronic constriction Injury (CCI) surgery

Five macaques underwent CCI surgery, however, only four (4) were selected for dosing. (One macaque was excluded from treatments due to GI distress.) Under ketamine anesthesia and using aseptic technique, the right common sciatic nerve was identified. A 1 cm length of PVC tubing was applied around the sciatic nerve and then the overlying muscles were closed in layers and the skin sutured shut. Antibiotics and analgesics were administered for three days following surgery.

Functional magnetic resonance imaging (fMRI)

Macaques were sedated by continuous intravenous infusion of propofol (20 mg/kg/hr) and a Signa Explorer 1.5T MRI system (GE Healthcare, Milwaukee, WI) was used to observed stimulus evoked brain activation. The anatomical MRI protocol consisted of a T1-weighted fast spoiled gradient-recalled (FSPGR) sequence (repetition time (TR)/echo time (TE), 15.8/7.0 ms; number of averages, 1; flip angle, 12º; field of view, 150mm_150 mm; matrix, 256 x 224; slice thickness/interval, 1.0/0.5 mm; number of slices, 168). Functional scan sequences consist of field-echo, echo-planar imaging (TR/TE, 3000/35 ms; flip angle, 90º; field of view, 140 mm x 140 mm; matrix, 64x 64; slice thickness, 2.4 mm; number of slices, 30).

A block design stimulation protocol was used: 10 sets of mechanical stimulations using von Frey filaments (modified Hama et al., 2021, Mol. Pain). One stimulation set consisted of 9 sec. of an “OFF” stimulus, a 1 g von Frey filament applied by hand to rest perpendicularly on the center of the plantar foot, followed by 9 sec. of an “ON” stimulus, an 8, 15 or 26 g von Frey filament. For each set, 10 frames were acquired, for a total of 100 frames per functional scan. A 10 sec. interval without stimulation separated each set. The filaments were tested in ascending order. Only the ipsilateral foot was tested.

PIPE-791

PIPE-791 is a brain-penetrant, orally bioavailable, small molecule LPA1 receptor antagonist (Poon et al., 2024). xxx

PIPE-791 receptor pharmacology	
Properties	**In vitro profile**
Radioligand binding Ki (nM)	0.752
Functional LPA1 Ca^{+2} mobilization (nM, 24h)	9.9
Fold selectivity over LPA2	35.4x
Fold selectivity over LPA3	35x
Summary of PIPE-791 in vitro radioligand binding selectivity profile in Ca^{+2} mobilization.	

Beginning two weeks after CCI, four (4) macaques were p.o. dosed with PIPE-791, 10 mg/kg, once daily for 28 days.

Determination of drug levels and LPA bioanalysis

Calibration standards and QC solutions of PIPE -791 and LPA species were prepared in solvent and spiked into matching naive matrixes to generate calibration curves. For PIPE-791 analysis, protein precipitation was performed on all samples/stds/QCs using 1:4 v/v ratio of acetonitrile with internal standard (IS). Following vortexing and centrifugation, supernatants were analyzed by LC/MS/MS (Sciex 4000 QTrap). For LPA analysis, methods described by Li et al. (2021) were used in which samples/stds/QCs were quenched with a sodium citrate buffer with IS and 1-butanol, vortexed, centrifuged, and supernatants were dried down under nitrogen gas and reconstituted in methanol for LC-MS/MS analysis (Sciex 6500+). All quantitation was performed by comparison of peak area ratios of analyte/IS to the prepared calibration standards using Sciex Analyst software.

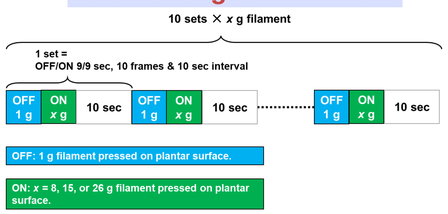
Inflammatory biomarkers in CSF

CSF was analyzed for a panel of non-human primate cytokines and chemokines using a MILLIPLEX® magnetic bead-based multiplex assay system based on the Luminex® xMAP® technology.

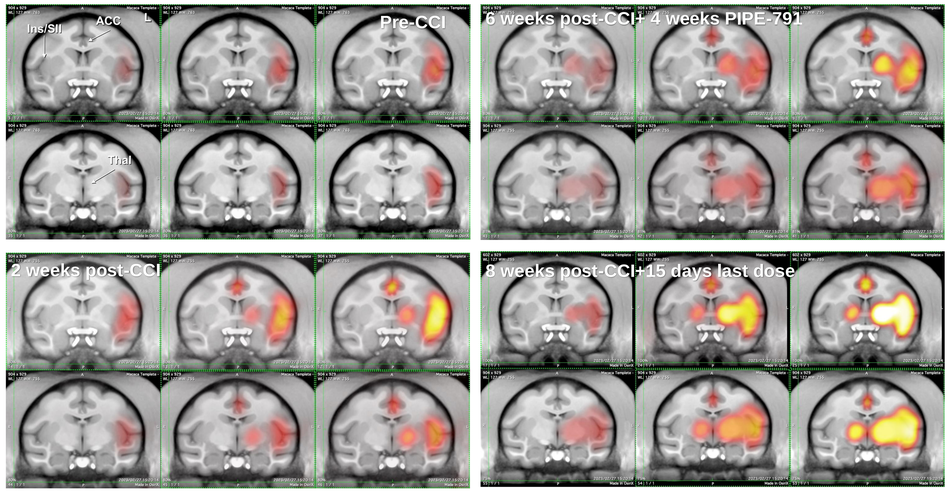
Overall schedule

	Pre-CCI	Post-CCI									
Week	**-1**	**0**	**1**	**2**		**3**	**4**	**5**	**6**	**7**	**8**
Day				**13**	**14**				**42**	**43**	**56**
Habit.	○										
fMRI	○			○					○		○
CCI		○									
Select					○						
Plasma	○				○					○	○
CSF	○				○					○	
Dose					○	○	○	○			

Block design stimulation



Results



Averaged from 4 macaques

Mean z-scores

CCI 2 weeks				
Stimulation	Region			
	Insular cortex (Left side)	Insular cortex (Right side)	Anterior cingulate cortex	Thalamus (Left side)
8 g	1.28	0.73	0.73	0.91
15 g	2.41	0.81	1.92	1.32
26 g	3.18	0.91	2.77	1.93

CCI 6 weeks (+PIPE-791)				
Stimulation	Region			
	Insular cortex (Left side)	Insular cortex (Right side)	Anterior cingulate cortex	Thalamus (Left side)
8 g	1.06	0.77	0.81	1.07
15 g	1.78	0.79	1.59	1.81
26 g	2.47	0.82	1.91	2.59

Contrast maps

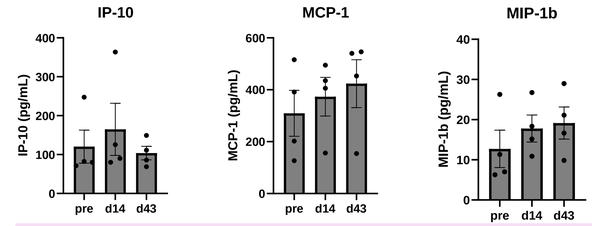
6 weeks > 2 weeks *Decrease*				
Stimulation	Region			
	Insular cortex (Left side)	Insular cortex (Right side)	Anterior cingulate cortex	Thalamus (Left side)
8g	0.91	0.67	0.51	0.39
15 g	1.48	0.59	1.19	0.53
26 g	2.07	0.72	1.61	0.51

8 weeks > 6 weeks				
Stimulation	Region			
	Insular cortex (Left side)	Insular cortex (Right side)	Anterior cingulate cortex	Thalamus (Left side)
8 g	1.09	0.58	0.83	1.1
15 g	1.43	0.69	1.38	1.48
26 g	2.13	0.78	2.08	2.48

PIPE-791 concentrations in CSF and plasma, Day 43

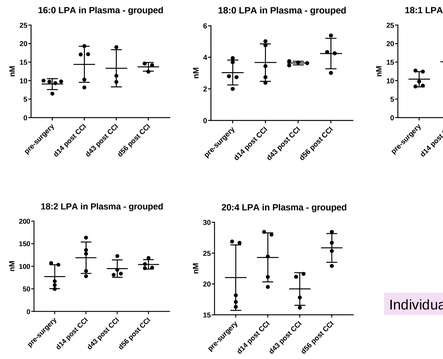
Subject	Treatment period	PIPE-791 Plasma Conc. (nM)	UNBOUND PIPE-791 Plasma Conc. (nM)	PIPE-791 CSF Conc. (nM)	CSF/Unbound plasma ratio
K-2130	Day 14-42	26.5	0.9	< 2.1	-
K-2372		69.6	2.4	5.0	2.08
K-2411		385.8	13.5	14.6	1.08
K-2464		113.7	4.0	3.9	0.98
Mean		**148.9**	**5.2**	**7.8**	**1.38**

Dosing began two weeks after CCI surgery for 28 days.
Collection: 43 days post-CCI, approximately 48 hours after the final oral gavage dose.

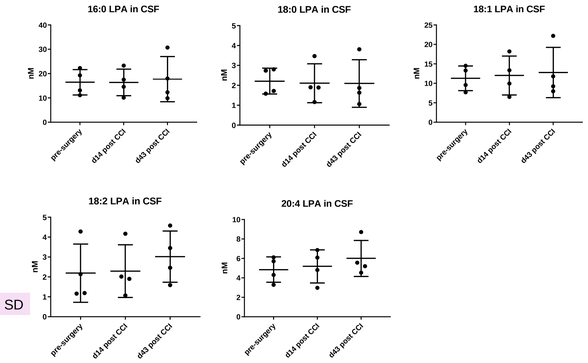


Individual CSF and mean (±SD) cytokine concentrations before and then following CCI surgery.
IFNg, IL-6, IL1b, and TNFa were *BLOQ*:
INFγ < 1.27 pg/mL; IL-6 < 0.30 pg/mL; IL-1β < 0.42 pg/mL; TNFα < 2.45 pg/mL
K-2477 values excluded from mean graphs. N = 4.

Plasma LPA



CSF LPA



Individual data. Mean ± SD

- ❑ Significant evoked brain activation in the macaque model of neuropathic pain
- ❑ Reduced activation of contralateral Ins following PIPE-791 treatment. Possibly reduced activation of the ACC and ipsilateral thalamus as well.
- ❑ Block of LPA1 receptor leads to reduced neural activation
- ❑ Possible role of either PNS or CNS LPA1 receptor.

Acknowledgement
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