Active MS lesions are characterized by high LPA1 expression and PIPE-791 promotes remyelination via LPA1 inhibition in the cuprizone model

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ABSTRACT

Lysophosphatidic acid (LPA), a well-known signaling phospholipid, is elevated in the cerebrospinal fluid (CSF) and serum of patients with MS compared to non-inflammatory, non-vascular neurological disease patient samples. PIPE-791 is an orally bioavailable, brain penetrant LPA1 small molecule antagonist currently in clinical trials. We have shown previously that PIPE-791 improves clinical scores in the mouse MOG-EAE model of inflammatory demyelination by promoting oligodendrocyte differentiation as well as decreasing microglia activation. In the current study, we build upon these findings and show that the combination of PIPE-791 with submaximal efficacious doses of FTY-720 (fingolimod) ameliorates clinical scores beyond FTY-720 alone. Further, using the cuprizone model of acute demyelination, we observe improvements in remyelination and VEP latency, supporting a direct effect on oligodendrocyte differentiation.

A displaceable PET tracer permits real time in vivo characterization of a biological target of interest and provides valuable information on target expression and target engagement by a co-administered competing compound. As such, we identified PIPE-497, an LPA1 antagonist with properties amenable for PET use. For example, in addition to being brain penetrant, PIPE-497 has an LPA1 dissociation $t_{1/2}$ of only 11.2 minutes, versus PIPE-791 which has a significantly longer $t_{1/2}$ of 519 minutes. We characterized ^3H-PIPE-497 binding by autoradiography in rodent, non-human primate, and human brain sections, and observed a similar dissociation constant (Kd) across species. Further, consistent with literature LPA1 expression, ^3H-PIPE-497 displays strong specific binding that overlaps with white matter tracts. Binding was also detected in the gray matter, especially in deep layers of cortex, striatum, thalamus, and medulla.

In human brain sections derived from MS patients, ^3H-PIPE-497 distribution coincided with activated microglia in active and mixed active lesions, as determined using ^3H-DPA-713, a marker of the 18-kDa translocator protein (TSPO). Further, ^3H-PIPE-497 binding overlapped with areas enriched with activated microglia and foamy macrophages known to be associated with chronic inflammation and disease severity. These findings encourage the development of PIPE-497 as PET tracer to evaluate MS lesions in clinical development and support targeting LPA1 for treating MS patients with PIPE-791 with advantages not seen with current immunotherapies.

PIPE-791 IMPROVED DISEASE SCORE IN MOG EAE MODEL
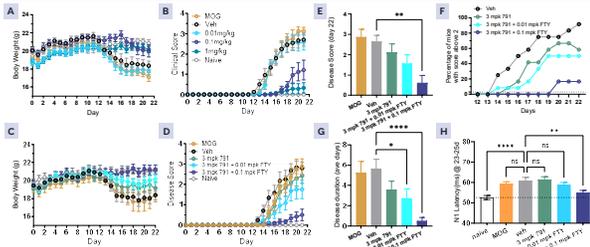
Figure 1: Effect of PIPE-791 in combination with FTY720 on MOG EAE disease score and VEPs. The efficacy of different doses of FTY720 was first evaluated on body weight and disease score (A, B) in MOG EAE mouse model. The efficacy of combining PIPE-791 with subthreshold doses of FTY-720 were subsequently evaluated on body weight and disease score (C, D). The combination of PIPE-791 (3mg/kg, po) with subthreshold doses of FTY720 (0.01 and 0.1 mg/kg, po) improved clinical scores (E, **p< 0.001, mean ± SEM, ANOVA w/ Dunnett's), the number of animal responding to treatment (F), disease duration (G, *p< 0.0285, ***p < 0.0001, mean ± SEM, ANOVA w/ Dunnett's) and visual-evoked potential (VEP, H, **p=0.0022, ****p< 0.0001, mean ± SEM, ANOVA w/ Dunnett's). Interestingly, a dose of FTY720 (0.01 mg/kg) that did not improve disease outcome showed efficacy in combination with PIPE-791 on disease duration (G) and % in disease score above 2 (F).

EFFECT OF PIPE-791 IN THE CUPRIZONE MODEL FOR DEMYELINATION
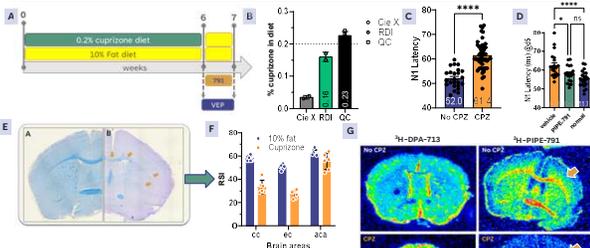
Figure 2: PIPE-791 improved visual evoked potential in the cuprizone-induced demyelination model. C57Bl6/J female mice were fed ad libitum for 6 weeks (A) with a 10% fat diet containing 0.16% of cuprizone (Research Diet Inc (RDI) or a fixed amount of cuprizone spiked in 10% fat diet as measured by LCMS/MS by BioQual Solutions (QC) (B). This treatment induced a statistically significant increase in the VEP N1 wave latency (C) at week 6. Cuprizone was then removed from the diet to initiate remyelination and the cuprizone treated group was dosed with either vehicle or PIPE-791 (3 mg/kg, po) for one week. The N1 latency was decreased for the group treated with PIPE-791 compared to the group of animal fed control diet without cuprizone (D) (mean ± SEM, ANOVA w/ Dunnett's). One week post-recovery, the myelin stain Luxol fast blue was still decreased in the corpus callosum (E & F, cc) and external capsule (E & F, ec) but not in the anterior, anterior commissure (E & F, aca) or the internal capsule (not shown) in cuprizone treated vs naïve mice. Then matched brain coronal sections from mice receiving cuprizone (CPZ) or control diet (no CPZ) were evaluated for translocator protein (TSPO) and LPA1 binding sites one week after cuprizone recovery (G). Both TSPO (^3H-DPA-713) and LPA1 (^3H-PIPE-497) tracers were increased in the corpus callosum and striatum (G). However, the distribution of those tracers appear slightly different suggesting the implication of different cell populations. Further cellular characterization are under way to determine those. Note the decrease of LPA1 binding sites in the cortex in the cuprizone treated mice (arrow in G).

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^3H-PIPE-497 AUTORADIOGRAPHY BINDING IN MOUSE BRAIN
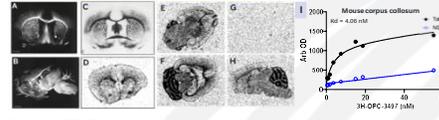
Figure 3: ^3H-PIPE-497 ex vivo autoradiography binding in mouse brain. ^3H-PIPE-497showed strong specific binding in mouse coronal (D) horizontal (E, F) and sagittal (H) brain sections. Non-specific binding (G) was low when an excess of selective cold LPA1 compound (15 μM) was added to the binding incubation mix. The overall brain distribution of ^3H-PIPE-497 was comparable to PLP (A & B, Mallon, 2002) and MBP transgenic mice (C, Gow, 1992). In mouse, ^3H-PIPE-497 Kd is 4.06 nM in the corpus callosum (I).

^3H-PIPE-497 AUTORADIOGRAPHY BINDING IN RAT BRAIN

Figure 4: ^3H-PIPE-497 ex vivo autoradiography binding in rat brain. ^3H-PIPE-497 showed concentration dependent increase in binding with strong signal to noise in rat brain. The distribution of ^3H-PIPE-497 was particularly strong in white matter ie (corpus callosum, anterior commissure, A & B). In the cerebellum, binding sites overlapped with the molecular layer (B). Binding is also observed in gray matter areas (cortex, striatum, thalamus, pons and medulla) but with lower intensity. In the rat corpus callosum, ^3H-PIPE-497 Kd was 1.42 nM (C). To evaluate the impact of demyelination on ^3H-PIPE-497 binding; Sprague-Dawley rats were injected with 4μL of 1% 14:0 LysoPC in the corpus callosum (D & E). Animals were sacrificed seven days later and brain sections were incubated with 8nM ^3H-PIPE-497 or processed for Sudan Black staining to visualize white matter (D). The contra lateral side was used as control for myelin levels (yellow box in D). Both Sudan black staining and ^3H-PIPE-497 binding were reduced by the LysoPC injection suggesting a decrease in myelin (D & E). This result suggests that ^3H-PIPE-497 could be used to trace myelin in rat brain and may offer the opportunity to develop PIPE-497 has a PET tracer.

^3H-PIPE-497 AUTORADIOGRAPHY BINDING IN NHP BRAIN
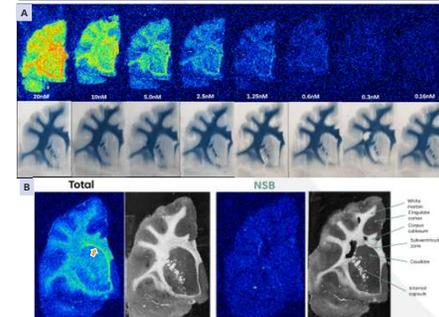
Figure 5: ^3H-PIPE-497 ex vivo autoradiography binding in Cynomolgus female monkey brain. Like previous findings in mouse and rat, ^3H-PIPE-497 showed a strong specific binding that was concentration dependent and enriched in white matter tract (A and B). The strongest signal was seen in the subventricular zone (SVZ, arrow in B). Dissociation constant in the corpus callosum, striatum, SVZ and cortex were 4.5, 2.3, 1.7 and 2.9, respectively.

^3H-PIPE-497 AUTORADIOGRAPHY BINDING IN HUMAN BRAIN
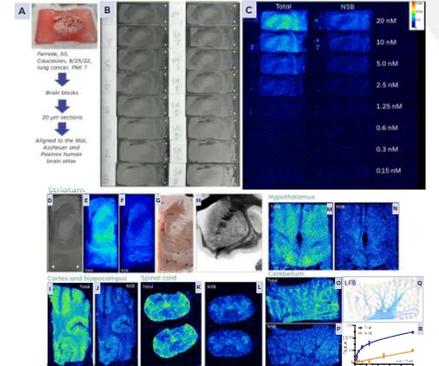
Figure 6: ^3H-PIPE-497 ex vivo autoradiography binding in female healthy brain. Upon receiving, whole human brain was dissected into 1-2 cm blocks and frozen on dry ice (A). Striatum sections were generated (B) and incubated with decreasing concentrations of ^3H-PIPE-497 (C). Dissociation constant was determined and found to be 3.5 nM in corpus callosum (N) and 2.8 nM in the putamen (not shown). Binding sites were present in both the white and grey matters with sometime higher intensity in the later. For example, strong signal was seen in the caudate and putamen as well in the external capsule (D-H). White matter tracts (corpus callosum, internal and external capsule) are clearly seen in G & H (Mai et al, 2004 Atlas of the human brain) and represent a section of the striatum stained with Sudan black. In the cortex, ^3H-PIPE-497 signal was enriched in the deepest layers and stronger than in the white matter (J–P, NSB= Nonspecific binding. In the spinal cord (K, L) and hypothalamus(M, N), strong signal could be seen also in grey matter and especially in the medial mammillary nucleus. In the cerebellum, ^3H-PIPE-497 is abundant in the white matter (O, P) as illustrated by myelin staining with Luxol fast blue (Q).

^3H-PIPE-497 AUTORADIOGRAPHY BINDING IN MS LESIONS
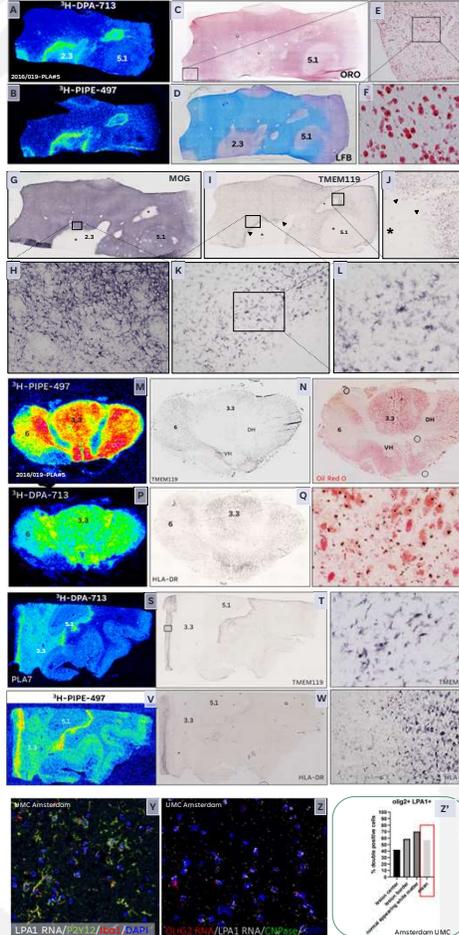
Figure 7: ^3H-PIPE-497 and ^3H-DPA-713 ex vivo autoradiography binding with multiple labeling (A-X) and LPA1 receptor cellular gene co-expression with oligodendrocytes and microglia markers (Y-Z') in multiple sclerosis patient brain lesions. Multiple Sclerosis (MS) tissues blocks from three male patients with age ranging from 45-54 years old and with more than 10 years of MS clinical history were obtained from the Netherlands Brain Bank (NBB, Amsterdam). Those tissues blocks from different brain areas and spinal cord contained lesions dissected on MRI guidance and were selected to represent a variety of lesions ranging from active, chronic/active and mixed in both the white and grey matters following the classification of MS lesion according to Luchetti et al. 2018. For consistency, data from only one patient are illustrated here and showed the binding of TSPO (^3H-DPA-713, A, P, S) and LPA1 receptor (^3H-PIPE-497, B, M, V) tracers. Active lesions (score 2.3) in the white matter (A-F) contained numerous foamy cells labeled with Oil Red O (ORO, C, E, F) uniformly distributed in the center of the lesion as well as HLA positive cells (not shown, NBB). The binding of both tracers at the center of the lesion was relatively low, yet higher then the signal observed in the white matter. Another lesion, this time in the grey matter with ramified HLA positive cells with a of score 5.1 (NBB) seen at the lower edge of the lesion that did not contain any cells labeled with ORO or TMEM119, a marker for activated microglia. Interestingly, the border of the lesion displayed a higher binding signal for both tracers that overlapped with the increase in TMEM119 labeling (J) probably reflecting an accumulation of activated microglia at the lesion border. We cannot rule out that the accumulation of binding signal could also be due to activated oligodendrocytes. Indeed, a slight increase in myelin could be observed in sections labeled with MOG antibody in lesion 2.3 but not 5.1 (G, H). A mixed active/inactive lesion score 3.3 was seen in the thoracic spinal cord (M-R) with abundant HLA positive (Q) and foamy cells (O, R); also displayed very strong binding signal for ^3H-PIPE-497 (M) and a weaker signal for ^3H-DPA-713 (P). A shadow plaque score 6 could also be seen in the lateral column with increased number of activated microglia labeled with TMEM119 (N) but lower level of HLA positive cells and foamy cells displayed a strong binding signal with ^3H-PIPE-497 (M) suggesting that this tracer may label activated microglia as well as foamy macrophages. A subcortical mixed active/inactive lesion score 3.3 as well as grey matter lesion score 5.1 could be seen with intense staining at the lesion border for ^3H-PIPE-497 (V) and ^3H-DPA-713 (S). A similar distribution pattern could be observed by TMEM119 (T, U) and HLA positive cells (W, X). Cellular expression studies performed in collaboration with the Amsterdam University Medical School revealed abundant expression of LPA1 receptors mRNA detected by RNAscope in Iba1/P2Y12 (Y) and Oligo-2 positive cells (Z). The number of LPA1/Oligo-2 cells were found to be increased at the lesions border (Z').

CONCLUSION

- PIPE-791 in combination with FTY-720 improves disease scores and VEP N1 latency in MOG EAE mouse model. PIPE-791 also improved VEP N1 latency in the remyelination phase in mouse cuprizone model.
- ^3H-PIPE-497, a selective LPA1 receptor antagonist, displays strong specific binding that overlaps with white matter tracts in all species examined with a Kd ranging from 1.4–4.5nM. Some signal could also be observed in grey matter areas.
- ^3H-PIPE-497 binding in mouse brain follow a similar dynamic range to myelin in ablation or rescuing models.
- In human brain, ^3H-PIPE-497 and ^3H-DPA-713 are particularly prominent in/near activated multiple sclerosis lesions and follow similar labeling patterns suggesting the participation of activated oligodendrocytes, macrophages, microglia and foamy cells.
- These findings encourage the development of PIPE-497 as PET tracer to evaluate MS lesions in clinical development and support targeting LPA1 for treating MS patients with PIPE-791 with advantages not seen with current immunotherapies.