LPA1 receptor binding sites are abundant in multiple sclerosis patient rim lesions associated with TSPO activation and disease progression

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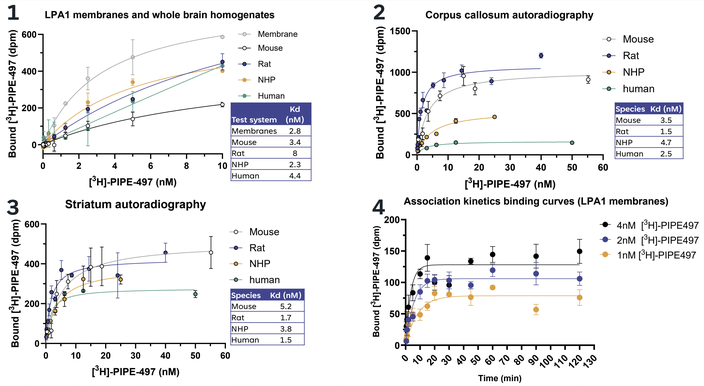
INTRODUCTION

Lysophosphatidic acid (LPA), is a bioactive phospholipid signaling through six G protein-coupled receptors LPA1-6. LPA is elevated in the cerebrospinal fluid (CSF) and serum of patients with multiple sclerosis (MS) compared to non-inflammatory, non-vascular neurological disease patient samples. Through the quantification of mRNA transcripts, Lysophosphatidic acid receptor type 1 (LPA1) was found to be enriched in glial cells and especially in oligodendrocytes. However, the exact cellular localization and distribution of LPA1 have not been well defined due to the lack of reliable tools for its protein detection. To address this, we designed PIPE-497, a highly selective LPA1 antagonist, and its tritiated analogue [^{3}H]-PIPE-497 and characterized their binding properties and compare it with [^{3}H]-DPA-713, a Translocator Protein (TSPO) tracer used in the clinic to label neuroinflammation.

RESULTS

[^{3}H]-PIPE-497 IN RODENT BRAIN HOMOGENATES AND IN HUMAN BRAIN TISSUE SECTIONS



The activity and binding properties of PIPE-497 and [^{3}H]-PIPE-497 were first characterized in a variety of assays. In recombinant human LPA1 calcium flux assay, the IC50 of PIPE-497 for the LPA-mediated response was 37 nM (not shown). **1**, [^{3}H]-PIPE-497 bound with high affinity in B103 cells transfected with human LPA1 membranes and homogenates from whole mouse brain, whole rat brain, non-human primate female cynomolgus monkey brain (NHP) white matter and female human white matter. **2 & 3**, we evaluated the binding of [^{3}H]-PIPE-497 in two brain regions, the corpus callosum and striatum in mouse, rat, NHP and human sections by autoradiography. We confirmed that [^{3}H]-PIPE-497 was concentration dependent, saturable and displaceable with cold PIPE-497 and exhibited similar dissociation constants across all species examined. **4**, [^{3}H]-PIPE-497 bound to human LPA1 in recombinant cells with fast on- and off-rate kinetics with a calculated off-rate of 0.06184 minutes^{-1}, a residence time of 16.2 minutes, a $T_{1/2}$ of 11.2 min and a Kd of 1.09 nM.

[^{3}H]-PIPE-497 EX VIVO AUTORADIOGRAPHY BINDING IN MOUSE (5) AND RAT (6) BRAIN

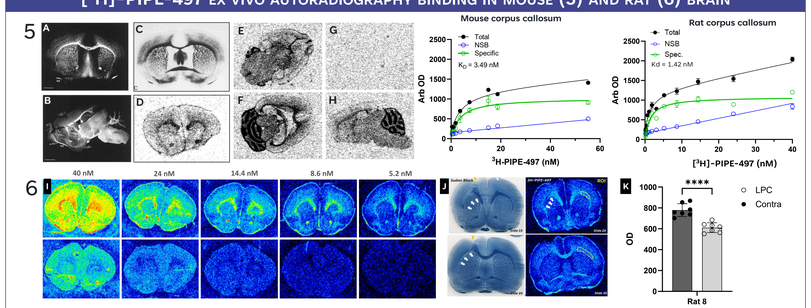


Figure 5: **[^{3}H]-PIPE-497 showed a strong specific binding in mouse coronal (D) horizontal (E, F) and sagittal (H) brain sections.** Non-specific binding (**G**) was low when an excess of PIPE-497 (15 µM) was added to the binding incubation mix. [^{3}H]-PIPE-497 binding in mouse and rat was highest in the white matter tracts. The brain distribution of [^{3}H]-PIPE-497 was comparable to transgenic mice expressing PLP (A & B, Mallon, 2002) and MBP reporter genes to selectively label white matter (C, Gow, 1992). [^{3}H]-PIPE-497 dissociation constant Kd was 4.06 nM and 1.42 nM in the mouse and rat corpus callosum, respectively.

Figure 6: **In rat brain, [^{3}H]-PIPE-497 showed concentration dependent increase in binding with a distribution comparable to mouse (I).** Binding is also observed in gray matter areas especially in the cortex, striatum, thalamus, pons and medulla but with lower intensity. To evaluate the impact of demyelination on [^{3}H]-PIPE-497 binding; Sprague-Dawley rats were injected with 4µL of 1% 14:0 LysoPC in the corpus callosum (**J & K**). Animals were sacrificed seven days later and brain sections were incubated with 8nM [^{3}H]-PIPE-497 or processed for Sudan Black staining to visualize white matter (**J**). The contra lateral side was used as control for myelin levels (yellow box in **J**). Both Sudan black staining and [^{3}H]-PIPE-497 binding were reduced by the LysoPC injection suggesting a decrease in myelin (**K**).

[^{3}H]-PIPE-497 AUTORADIOGRAPHY BINDING IN CYNOMOLGUS MONKEY (7) AND HUMAN (8) BRAIN

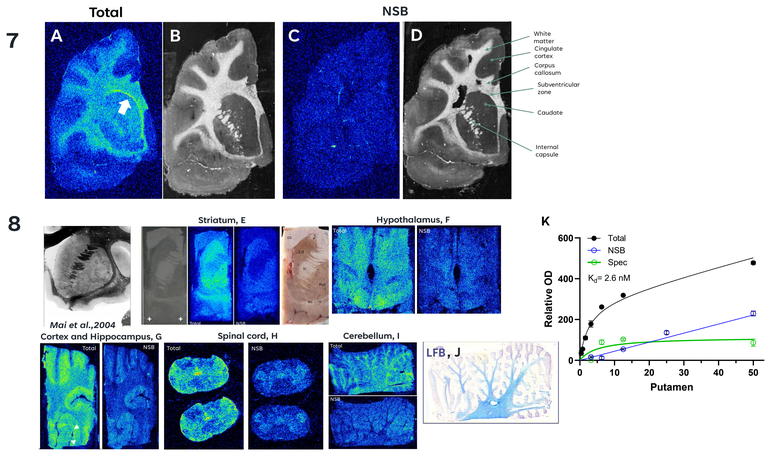


Figure 7: **[^{3}H]-PIPE-497 ex vivo autoradiography binding in female cynomolgus monkey brain.** Like previous findings in mouse and rat, [^{3}H]-PIPE-497 showed a strong specific binding that was concentration dependent and enriched in white matter tract (**A, B** and **C**). The strongest signal was seen in the subventricular zone (SVZ, arrow in **A & D**) with low non-specific binding (**C**).

Figure 8: **[^{3}H]-PIPE-497 ex vivo autoradiography binding in healthy female human brain.** Specific binding was detected in both the white and grey matters with sometime higher intensity in the latter in the striatum (**E**), Hypothalamus (**F**), Cortex **(G),** Hippocampus (**G**, arrowheads), spinal cord (**H**) and cerebellum (**I**). Strong [^{3}H]-PIPE-497 binding was seen in the caudate (Cd), putamen (Pu) and the external capsule (**E**, **ec**). [^{3}H]-PIPE-497 binding was also seen in white matter tracts (corpus callosum, internal and external capsule) and overlapped with Sudan black staining (Mai et al, 2004 Atlas of the human brain). In the cortex, [^{3}H]-PIPE-497 signal was enriched in the deepest layers and stronger than in the white matter **(G).** In the spinal cord **(H)** and hypothalamus **(F),** strong signal could also be seen in grey matter and especially in the medial mammillary nucleus. In the cerebellum, [^{3}H]-PIPE-497 is abundant in the white matter **(I)** as illustrated by myelin staining with Luxol fast blue **(J).** NSB= Nonspecific binding. [^{3}H]-PIPE-497 dissociation constant was determined and found to be 2.8 nM in corpus callosum (not shown) and 2.6 nM in the putamen (**K**).

CHARACTERIZATION OF *IN VIVO* [^{3}H]-PIPE-497 BINDING IN MOUSE BRAIN (9)

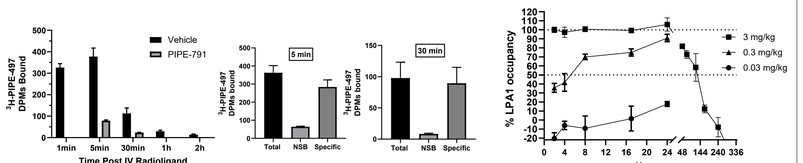


Figure 9 (A-D): [^{3}H]-PIPE-497 binding in mouse brain. A-C, Following IV administration, [^{3}H]-PIPE-497 binding in mouse brain increased rapidly, peaked 5 minutes post dose and declined sharply over the evaluation period (2h). Total binding at 5 minutes and 30 minutes was significantly reduced by PIPE-791, indicating that [^{3}H]-PIPE-497 bound to LPA1 in vivo and with high specific binding and low non-specific binding. **D,** [^{3}H]-PIPE-497 could be used to determine target engagement and occupancy in vivo as illustrated in this graph showing LPA1 antagonist PIPE-791 occupancy across time. Full coverage of LPA1 was maintained for at least 24 hours following a dose of 3 mg/kg and by 5 days declined to 60%. Following a single oral dose of 0.3 mg/kg, the occupancy increased from 35% at 2h post dose to 90% at 24h post dose while $C_{u,brain}$ remained at approximately 2 nM over this time period.

[^{3}H]-PIPE-497 AUTORADIOGRAPHY BINDING IN MULTIPLE SCLEROSIS SPINAL CORD LESIONS (10)

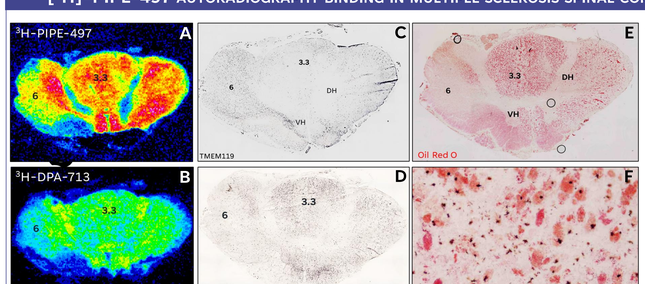


Figure 10 : [^{3}H]-PIPE-497 (A) and [^{3}H]-DPA-713 (B) ex vivo autoradiography binding in spinal cord lesions from Multiple Sclerosis (MS) patients. Tissues were obtained from Netherlands Brain Bank. Mixed active inactive or chronic lesion (score 3.3) and shadow plaque (score 6) displayed intense [^{3}H]-PIPE-497 binding and to a lesser extend [^{3}H]-DPA-713, a TSPO tracer for neuroinflammation. HLA-DR positive immunoreactive cells (**D**) and foamy cell (**E & F**) were abundant in the chronic lesion. TMEM119, a marker for activated microglia immuno-reactivity was more abundant in the shadow plaque (**C**). DH=dorsal horn, VH =ventral horn. TSPO =Translocator protein. ORO= Oil Red O

[^{3}H]-PIPE-497 AUTORADIOGRAPHY BINDING IN MULTIPLE SCLEROSIS BRAIN LESIONS (11)

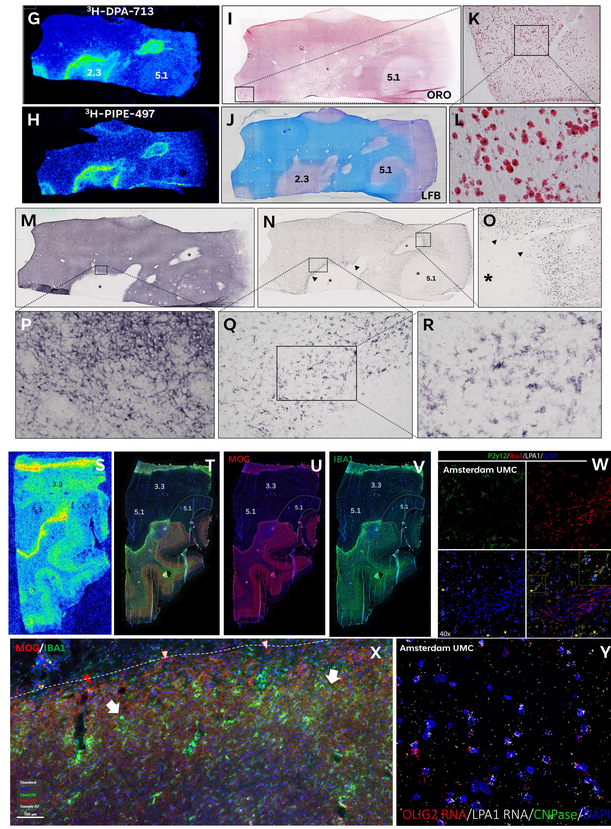


Figure 11 : [^{3}H]-PIPE-497 (H) and [^{3}H]-DPA-713 (G) ex vivo autoradiography binding in brain lesion from Multiple Sclerosis (MS) patients. Tissue blocks with a variety of lesions were obtained from Netherlands Brain Bank from three male patients with age ranging from 45-54 years old and more than 10 years of MS clinical history. For consistency, data from only one patient are illustrated here. [^{3}H]-PIPE-497 **(H)** and [^{3}H]-DPA-713 **(G)** binding signals were increased in white matter (WM) active lesion rim (score 2.3). WM active lesions (**G-L**) contained numerous foamy cells labeled with Oil Red O **(I, K, L)** uniformly distributed in the center of the lesion as well as HLA positive cells (not shown, NBB). An increase in TMEM119 **(N-Q)** and Myelin Oligodendrocyte Glyco-protein (MOG) immunoreactivity was also seen in the active lesion rim **(M, O)**. Interestingly, TMEM119 were not seen in the active lesion center. Together those data suggest an overlap of LPA1 with microglia and oligodendrocytes at the lesion rim. ORO = Oil red O, LFB = Luxol fast Blue.

[^{3}H]-PIPE-497 binding (S), MOG (T, U, X) and Iba1 (T, V, X) immunostaining in MS patient brain lesions. Both [^{3}H]-PIPE-497 (**S**) and [^{3}H]-DPA-713 (not shown) were increased in subcortical chronic active lesion rims. This was consistent with an increase in MOG and Iba1 immunoreactivity **(T-V, X)**. Cellular expression assessed by using RNAscope Multiplex Fluorescent Kit v2 and performed by the Amsterdam University Medical Center, revealed abundant expression of LPA1 receptors mRNA in active lesion rim and core (**Z**). The number of oligodendrocytes expressing LPA1 receptors was increased in active lesion rim/core (**Z, Z' & Z''**). Some cells expressing LPA1 were found to expressed markers of microglia Iba1/P2Y12 (**W**, arrows).

CONCLUSION

- [^{3}H]-PIPE-497, a selective LPA1 receptor antagonist, displays strong specific binding that overlaps with white matter in all species examined.
- In human brain, [^{3}H]-PIPE-497 and [^{3}H]-DPA-713 (a marker of neuroinflammation) are particularly prominent in/near activated multiple sclerosis lesions and follow similar labeling patterns suggesting the participation of activated oligodendrocytes and microglia in brain and foamy cells or macrophages in spinal cord.
- [^{3}H]-PIPE-497 was a suitable tool to measure the LPA1 receptor occupancy of compounds of interest in vivo in a variety of compartments, including the brain.
- Based on these data, PIPE-497 was developed into a PET tracer, [^{18}F]-PIPE-497, and used clinically to assess the level of LPA1 and its distribution in the brain of healthy volunteers and patients with progressive MS.