A promising phospholipase A2-targeted peptide slowing amyloid beta pathology in an Alzheimer's disease mouse model

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INTRODUCTION

Alzheimer disease (AD)

- Most common dementia worldwide with 2 main features : extracellular senile plaques of amyloid β (Aβ) & intracellular neurofibrillary tangles of tau protein
- Since 1993, no new drug was approved by FDA (> 95% failed during clinical trials [1])
- Actual therapies are only symptomatic & do not slow the progression of the disease

AD management

- Only symptomatic treatments allowing a better quality of life for patients
- Various phospholipase isoforms involved in memory impairment and neurodegeneration in AD
- PLA_2 signaling pathway involved in AD [2] → PLA_2 inhibition has shown neuron protection against apoptosis induced by Aβ [3]

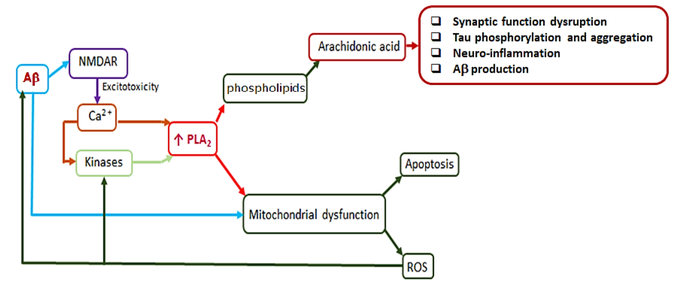

Figure 1. Our hypothesis concerning the progress of the AD process: accumulation of Aβ at the cerebral level results in excessive stimulation of NMDAR, triggering the exacerbated influx of Ca^{2+} which activates kinases responsible of PLA_2 activation. Arachidonic acid (AA) produced promotes synaptic function disruption, tau phosphorylation and aggregation, increased Aβ production and ROS generation, as well as neuro-inflammation and apoptotic death of neurons.

METHODS

- **Inhibitory potential of PL-P25:** Pre-incubation of differentiated NT2/D1 cells during 30 minutes with peptides (20µM) before induction with glutamate (50µM) → AA dosage (AA ELISA kit, Cusabio, USA).
- **Immunofluorescence:** subcellular localization of PLA_2 and p-PLA_2, of AA-depending enzymes such as COX2, ALOX5, and beta-actin.
- ***In vivo* molecular imaging:** APP/PS1 mice (Jackson Laboratory, Maine, USA) were injected with 200µmol Fe/kg b.w of USPIO-PHO [4]. Then, images were acquired at the level of the head with T_2-weighted RARE imaging protocol (TR/TE = 3000/60 ms, RARE factor = 4, NEX = 6, matrix = 512x512, FOV = 2.5cm, slice thickness 1mm, 20 axial slices, spatial resolution = 48µm, TA = 38m24sec).
- **Barnes maze:** study of the spatial memory of non treated healthy mice and APP/PS1 mice during the period of treatment with PLP25-LRP2 or NSP (1.5 month). All performances were recorded and analyzed manually.
- **Immunohistochemistry:** detection of AP, APP, PLA_2, NMDAR and p-tau

Development of a <u>therapeutic strategy</u> by targeting a key actor in the phospholipase (PLA_2) signalling involved in AD using a peptide identified by phage display (PLP25) and rendered <u>able to cross the BBB</u> by coupling to a vector peptide (LRP2) targeting the LDLR.

RESULTS

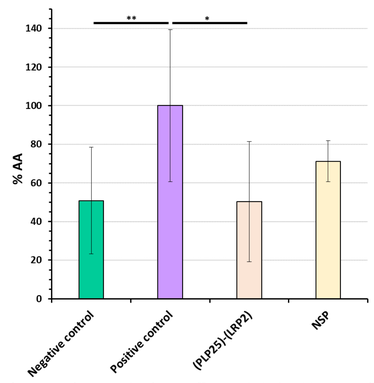

Figure 2. The glutamate shows a stimulatory effect on PLA_2 (positive control) compared to the negative control (non-induced and non-inhibited cells), attested by a significant increase in the released AA. PLP25-LRP2 complex significantly decreases the released AA levels, whereas the non-specific peptide (NSP) shows no effect (*: p<0.05; **: p<0.01).

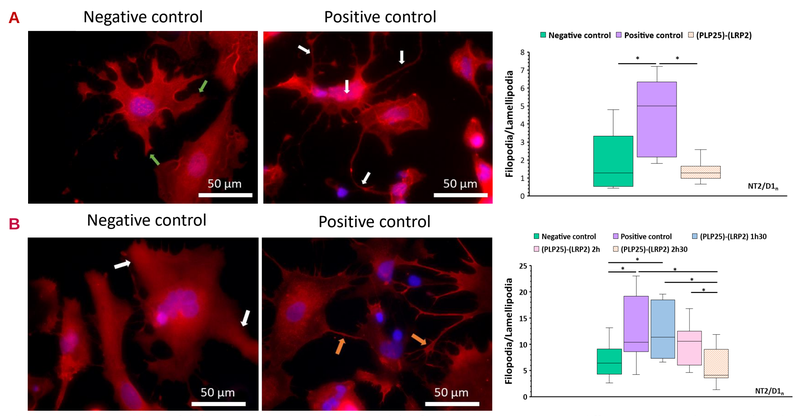

Figure 3. Detection by IF of (**A**) glutamate-induced PLA_2 and (**B**) Aβ-induced PLA_2 on neuron differentiated NT2/D1 cells ($NT2/D1_n$), highlighted by Dylight 594 (red). Nuclei appear blue using DAPI. PLA_2 migrates to neurites in both stimulations. In both cases, PLP25-LRP2 prevents the migration of PLA_2. * $p < 0.05$

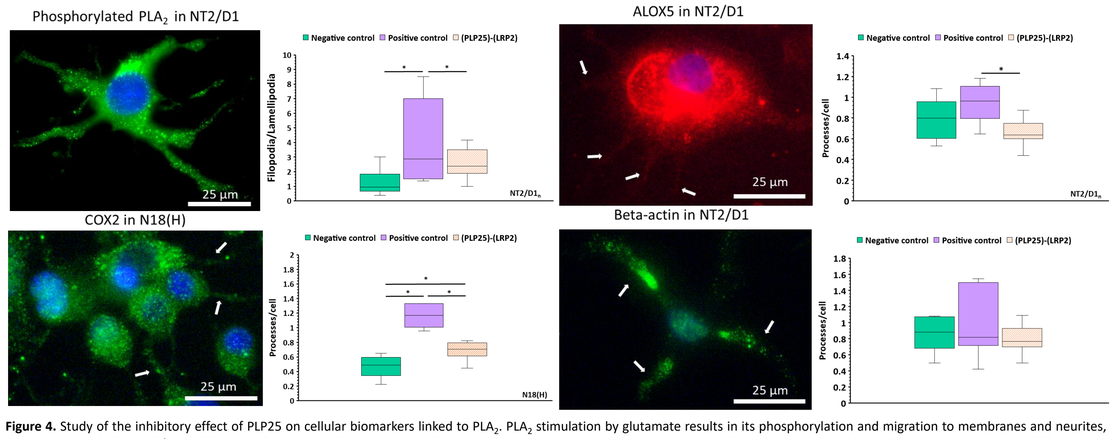

Figure 4. Study of the inhibitory effect of PLP25 on cellular biomarkers linked to PLA_2. PLA_2 stimulation by glutamate results in its phosphorylation and migration to membranes and neurites, the activation of COX2 and/or ALOX5 depending on the cell type, the translocation of these enzymes into cell processes and the restructuration of the beta-actin cytoskeleton. PLP25 prevents all these phenomena by its interaction with PLA_2. * $p < 0.05$

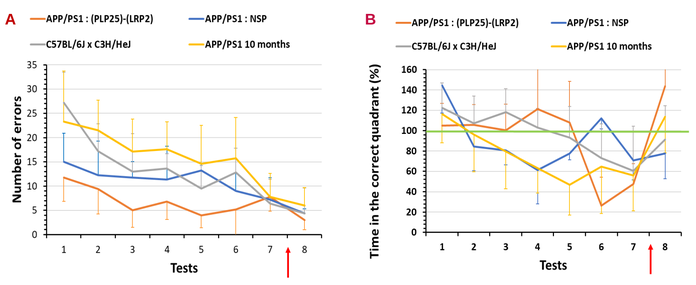

Figure 5. Study of the spatial memory of APP/PS1 mice by the Barnes maze. Mice treated with PLP25-LRP2 generally made fewer errors (**A**) and spent more time in the correct quadrant (**B**) than the non-treated or NSP-treated mice.

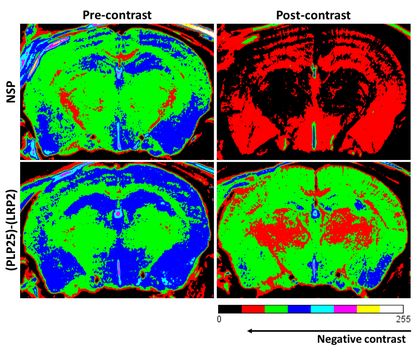

Figure 6. Representative images of mouse brains acquired by MRI on APP/PS1 mice injected with USPIO-PHO targeted to amyloid beta peptide. MRI experiments were performed after the period of treatment. The negative contrast observed for mice injected with NSP reveals a higher amount of Aβ in the brain.

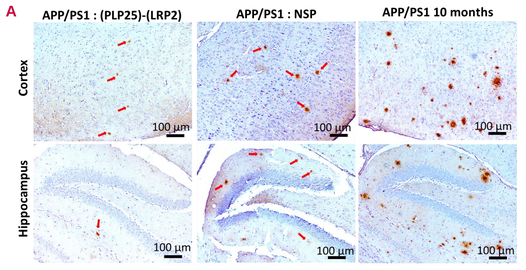

Figure 7. (**A**) Detection of amyloid plaques (AP) by IHC on brain slices of healthy NMRI and APP/PS1 mice, highlighted by brown spots. After 1.5 months of treatment, the injection of PLP_{25}-LRP_2 to APP/PS1 mice reduced the number and size of AP in contrast to NSP injection, whereas older and non-treated mice show AP progression. (**B**) AP were counted manually in the whole brain and the hippocampus of mice treated with PLP_{25}-LRP_2 or NSP, and in older APP/PS1.

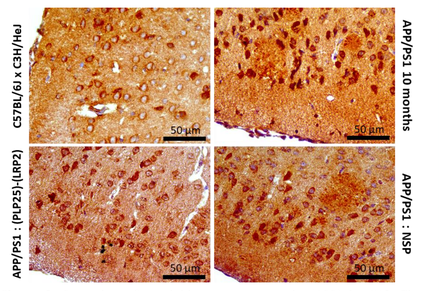

Figure 8. Detection of amyloid precursor protein (APP) by IHC. The labeling obtained for PLP25-LRP2-treated mice is intermediate between the 10 months-diseased mice (more cytoplasmic) and healthy mice (mainly membranous) with a closer approach to healthy mice.

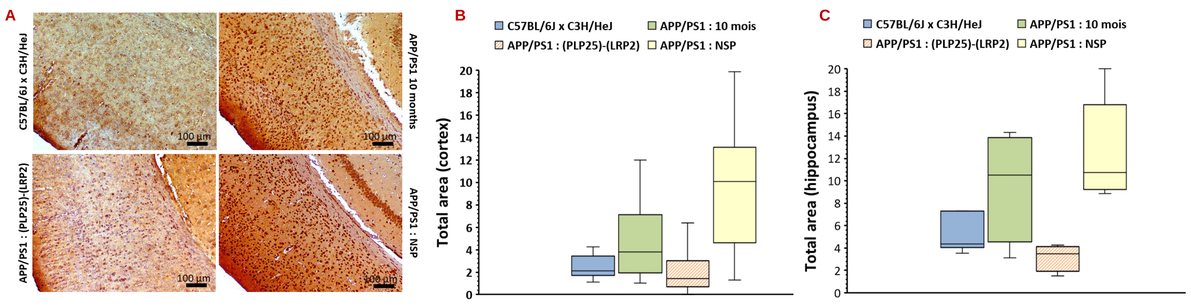

Figure 9. Detection of PLA_2 by IHC. Pathological conditions induce the overexpression of PLA_2 in both the cortex and the hippocampus. Mice treated with PLP25-LRP2 have a clearly weak overall labeling, unlike mice treated with the NSP, in the range of diseased mice.

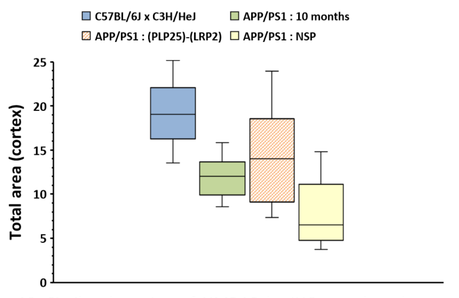

Figure 10. Similar detection of NMDAR by IHC reveals a decreased expression of NMDAR in diseased mice. The treatment with PLP25-LRP2 allows to increase its expression, unlike the treatment with NSP.

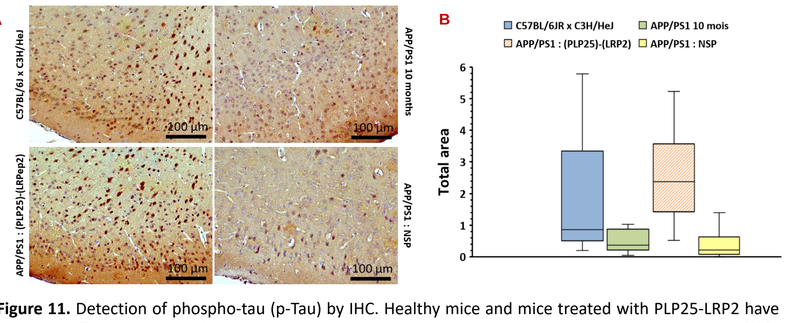

Figure 11. Detection of phospho-tau (p-Tau) by IHC. Healthy mice and mice treated with PLP25-LRP2 have shown a p-Tau labeling mainly in the cell bodies and in the nucleus, whereas in diseased mice and NSP-treated mice, p-Tau is located in axons.

CONCLUSION

IN VITRO

PLP25-LRP2 incubation with cells shows to:

- Prevent the production of AA by Glu-stimulated cells;
- Prevent the Glu- and Aβ-induced PLA_2 translocation to cell membranes
- Prevent COX2 and ALOX5 translocation after Glu stimulation

IN VIVO

PLP25-LRP2 injection to APP/PS1 mice allows to:

- Improve their cognitive abilities (Barnes maze results)
- Reduce the amount of amyloid plaques unlike NSP injection

EX VIVO

IHC on mice brain slices showed that PLP_{25}-LRP_2 injection allows to restore in the range of healthy mice the expression, cellular localization and activity of selected biomarkers of interest for AD.

REFERENCES [1] Cummings JL et al. Alzheimers Res Ther. 2014;6: 37. [2] Schaeffer EL et al. Prog Neuropsychopharmacol Biol Psychiatry. 2010;34: 1381–1389. [3] Farooqui AA et al. Pharmacol Rev. 2006;58: 591–620 ; [4] André et al. J Alzheimers Dis. 2017;60: 1547–1565.

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