Development of an original phospholipase A_2-targeted peptide able to reduce amyloid pathology in a mouse model of Alzheimer's disease

S. André[1], J. Atakana[1], M. Lecomte[1], R.N. Muller[1,2], L. Vander Elst[1], S. Laurent[1,2], C. Burtea[1]

[1] *Nuclear Magnetic Resonance and Molecular Imaging Laboratory, University of Mons-UMONS, 19 Avenue Maistriau B-7000 Mons, Belgium*
[2] *Center for Microscopy and Molecular Imaging, 8 Rue Adrienne Bolland, B-6041 Charleroi, Belgium*

JeanMazarin.ATAKANALUKULU@student.umons.ac.be

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]

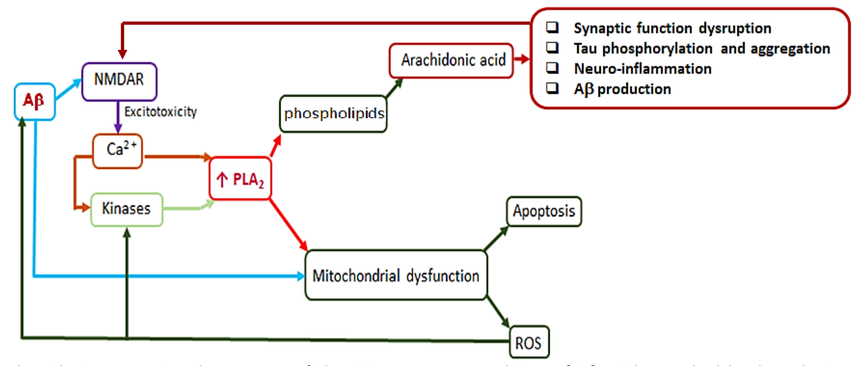


Figure 1. Our hypothesis concerning the progress of the AD process: accumulation of Aβ at the cerebral level results in excessive stimulation of NMDAR, triggering the exacerbated influx of Ca^{2+} which activates kinases responsible of PLA_2 activation. Arachidonic acid (AA) produced promotes synaptic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

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 (PLP_{25}) and rendered <u>able to cross the BBB</u> by coupling to a vector peptide (LRP_2) targeting the LDLR.

RESULTS

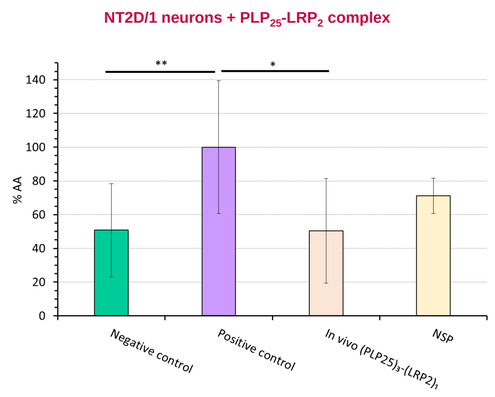


Figure 2. The glutamate shows a stimulatory effect on PLA_2 (positive control) compared to the negative control (non-induced and non-inhibited cells), attested by a significant increase in the released AA. PLP_{25}-LRP_2 complex significantly decreases the released AA levels, whereas the non-specific peptide (NSP) shows no effect (*: p<0,05; **: p<0,01).

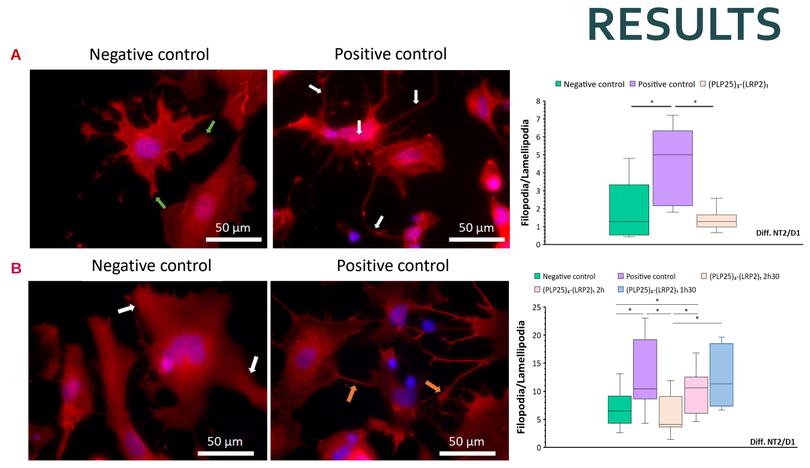


Figure 3. Detection by IF of (**A**) glutamate-induced PLA_2 and (**B**) Aβ-induced PLA_2 on neuron differentiated NT2/D1 cells, highlighted by Dylight 594 (red). Nuclei appear blue using DAPI. PLA_2 migrates to neurites in both stimulations. In both cases, PLP_{25} prevents the migration of PLA_2. * p < 0.05

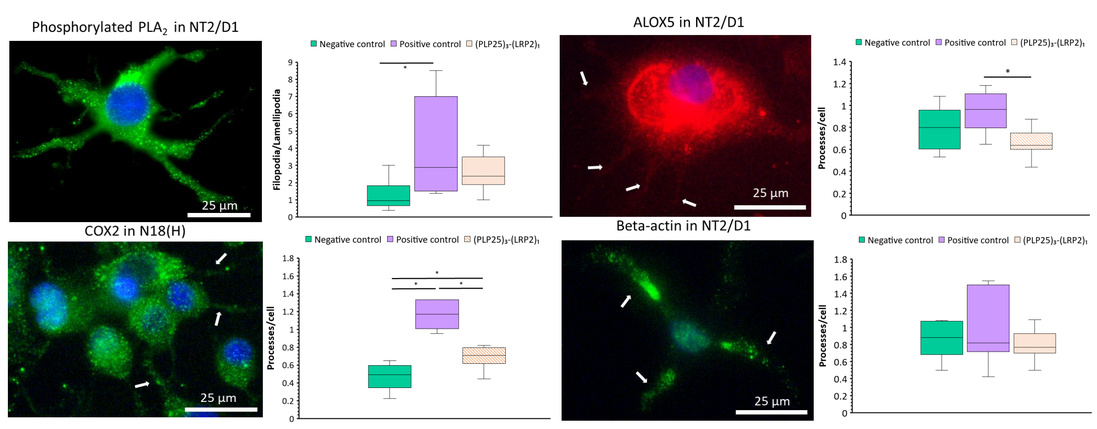


Figure 4. Study of the inhibitory effect of PLP_{25} on cellular biomarkers linked to PLA_2. PLA_2 stimulation by glutamate results in its phosphorylation and migration to membranes and neurites, the activation of COX2 and/or ALOX5 depending on the cell type, the translocation of these enzymes into cell processes and the restructuration of the beta-actin cytoskeleton. PLP_{25} prevents all these phenomena by its interaction with PLA_2. * p < 0.05

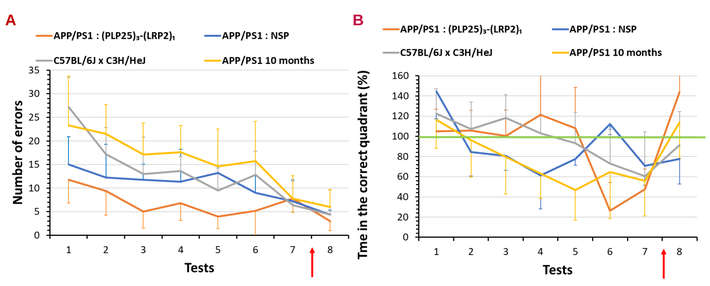


Figure 5. Study of the spatial memory of APP/PS1 mice by the Barnes maze. Mice treated with PLP_{25}-LRP_2 generally made fewer errors (**A**) and spent more time in the correct quadrant (**B**) than the non-treated or NSP-treated mice.

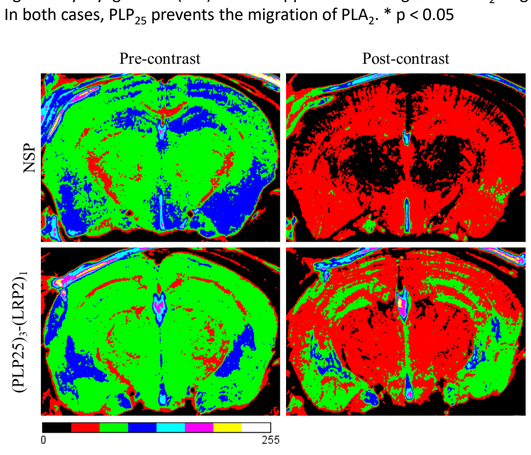


Figure 6. Representative images of mouse brains acquired by MRI on APP/PS1 mice injected with USPIO-PHO targeted to amyloid beta peptide. MRI experiments were performed after the period of treatment. The negative contrast observed for mice injected with NSP reveals a higher amount of Aβ in the brain.

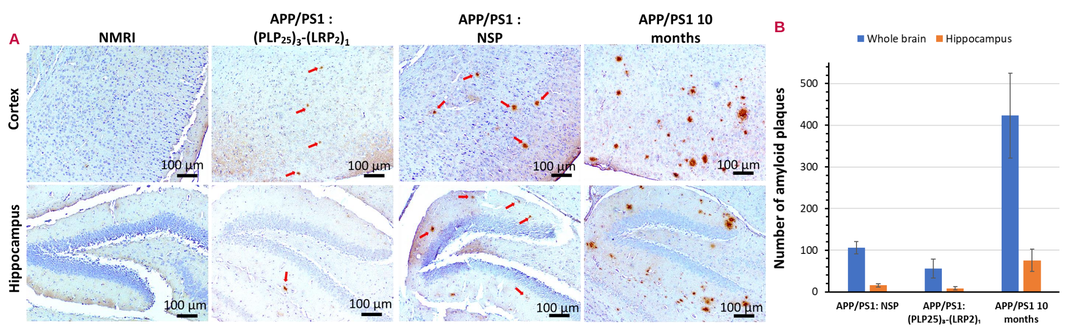


Figure 7. (**A**) Detection of amyloid plaques (AP) by IHC on brain slices of healthy NMRI and APP/PS1 mice, highlighted by brown spots. After 1.5 months of treatment, the injection of PLP_{25}-LRP_2 to APP/PS1 mice reduced the number and size of AP in contrast to NSP injection, whereas older and non-treated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

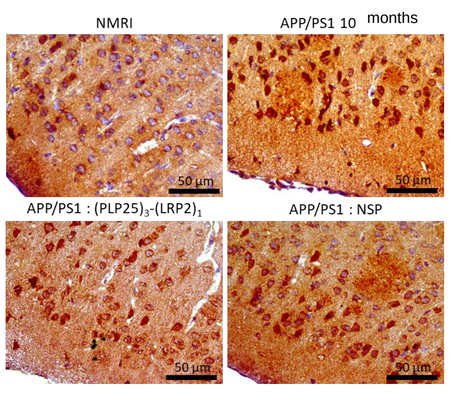


Figure 8. Detection of amyloid precursor protein (APP) by IHC. The labeling obtained for PLP_{25}-LRP_2-treated mice is intermediate between the 10 months-diseased mice (more cytoplasmic) and healthy mice (mainly membranous) with a closer approach to healthy mice.

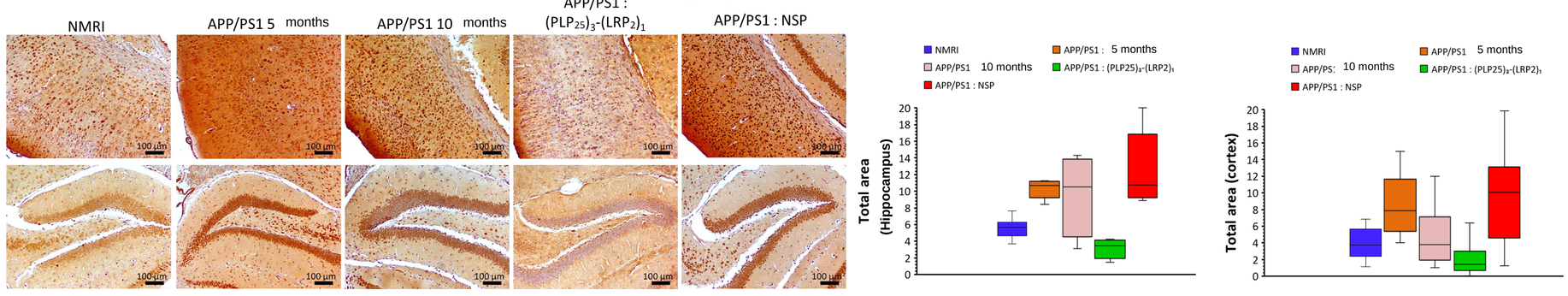
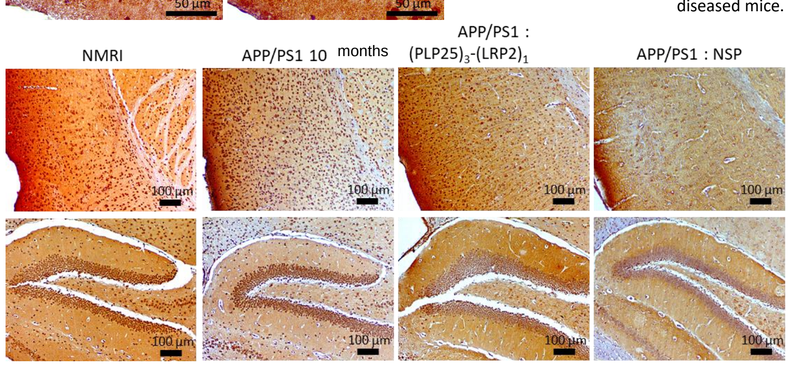


Figure 9. Detection of PLA_2 by IHC. Pathological conditions induce the overexpression of PLA_2 in both the cortex and the hippocampus. Mice treated with PLP_{25}-LRP_2 have a clearly weak overall labeling, unlike mice treated with the NSP, in the range of diseased mice.



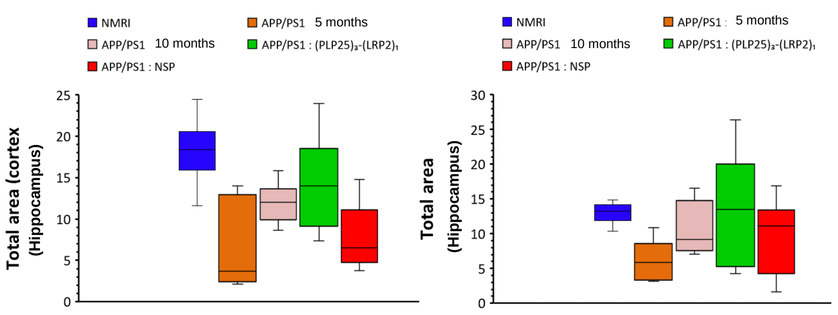


Figure 10. Detection of NMDAR by IHC. The strong cellular NMDAR expression in the cortex and hippocampus of healthy mice is decreased in diseased mice. The treatment with PLP_{25}-LRP_2 allows to increase the NMDAR expression, unlike the treatment with NSP.

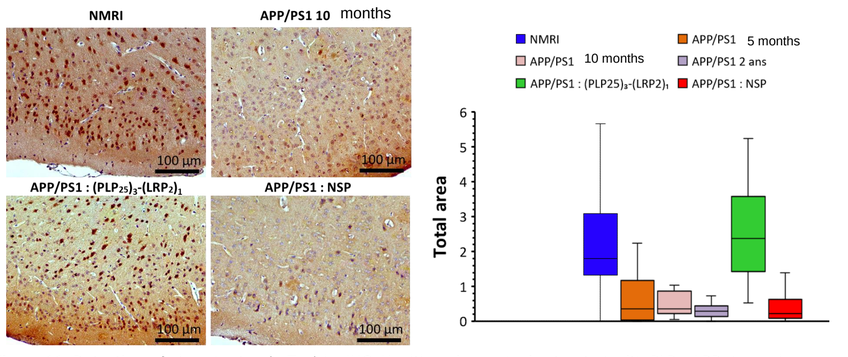


Figure 11. Detection of phospho-tau (p-Tau) by IHC. Healthy mice and mice treated with PLP_{25}-LRP_2 have shown a p-Tau labeling mainly in the cell bodies and in the nucleus, whereas in diseased mice and NSP-treated mice p-Tau is located in axons.

CONCLUSION

Evaluation of the inhibitory potential of the PLA_2-targeted peptide combined with LDLR-targeted peptide

IN VITRO

PLP_{25}-LRP_2 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

PLP_{25}-LRP_2 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.

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 PLP_{25}-LRP_2 or NSP (1.5 month). All performances were recorded and analyzed manually.
- **Immunohistochemistry:** detection of AP, APP, PLA_2, NMDAR and p-tau

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.
ACKNOWLEDGMENTS This work is supported by the Rotary Foundation – Hope in Head campaign 2015, the FRMH (Fond pour la Recherche Médicale en Hainaut) and the grant "Actions de Recherche Concertée" financed by the Fédération Wallonie-Bruxelles, the Waloon Region, Feder, Interreg and COST actions.