A new treatment for Alzheimer's disease: AZP2006 prevents and reverses amyloid and Tau damages via release of one growth factor and reduction of neuroinflammation.

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Introduction

Alzheimer disease (AD) is pathologically characterized by extracellular senile plaques composed of amyloid-β (Aβ) peptides (Aβ plaques) and intracellular neurofibrillary tangles composed of hyperphosphorylated tau. Synapse loss is widespread and pronounced. In AD, the chronic Aβ accumulation causes cerebral neuroinflammation by activating microglia.

Here we present the effects of a novel small molecule, AZP2006, showing neuroprotective properties, especially on the neuronal deficits after both $A\beta_{1-42}$-induced neuronal damage in cortical neurons in vitro and in $A\beta_{25-35}$-lesioned mice in vivo.

We show that AZP2006 is able to protect and to restore neuronal damages and in vivo cognitive impairment, reducing the microglial inflammation and the hyperphosphorylated Tau protein accumulation. Thus, AZP2006 has the potential not only to delay the progression of Alzheimer's disease but also to reverse existing neurodegenerative damage.

In addition, in a Tauopathy mouse model (THY-Tau22tg mice), associated with a hyperphosphorylation of Tau and Tau tangles, AZP2006 improves memory deficits and reduces the Tau accumulation.

In addition, mode of action studies showed that AZP2006 was able to increase Progranulin (PRGN) a growth factor involved in the neuron survival, neurite outgrowth and in the neuroinflammation modulation. Here, we show that the inhibition of this factor fully abolishes the neuroprotective effect induced by AZP2006.

In addition, previous investigations showed that AZP2006 was able to antagonize Toll-like receptor (TLR, specific subtypes) highly involved in the inflammatory process. This dual effect makes AZP2006 a serious candidate for the treatment of neurodegenerative disorders including AD or Progressive supranuclear palsy.

Methods

In vitro investigations :

Primary culture : Primary rat cortical neurons (E15) were cultured as described by Callizot *et al.*, 2013 with modifications. The cells were seeded at a density of 30,000 per well in 96-well plates (for immunostaining).

Pharmacological treatments: $A\beta_{1-42}$ (20 µM ~2 µM of AβO for 24 h or 5 µM~0,5 of AβO for 72 h) was applied on day 11. Untreated cultures served as controls. AZP2006 (at different concentrations) was applied immediately after the neurotoxic agent. 72 hours after intoxication, the cell culture supernatant was collected for PRGN quantification (Elisa assay Kit). Anti-PRGN Ab was added in the culture medium 1h before AZP2006 and Aβ.

Staining of cortical neurons and microglia and automatic microscopic analysis: After intoxication, neurons were fixed. The cells were incubated with : a) chicken polyclonal antibody anti microtubule-associated-protein 2 (MAP-2), and/or b) mouse monoclonal antibody anti OX-41 (microglia) and/or c) mouse monoclonal antibody anti phospho tau (AT-100) to evaluate hyperpho Tau protein aggregation into neurons. The immuno-labelled cultures were automatically analysed with MetaXpress (Molecular Devices, USA) at X20 magnification.

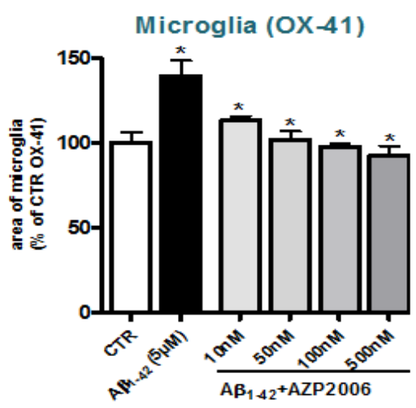
In vivo investigations :

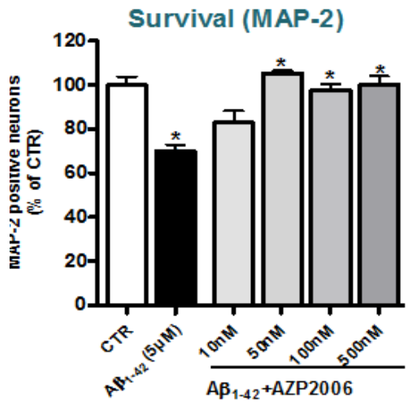
$A\beta_{25-35}$ Mouse model: 2 month-old females C57Bl6 mice (n=12/group) were injected with Aβ 25-35 (i.c.v., 3 µl, 9 nM). AZP2006 (2 mg/kg po) was administrated on day 0 (co-admi. with peptide), on D1 or day 4 (1 or 4 days after the amyloid injection) up to 22 days. Behavioral investigations (Y and water maze) were performed in Amylgen Laboratories (Montpellier) on day 7, 15 and 22 days post amyloid injection.

4R-THY-Tau22 Mice: Three-month-old females C57BL/6 wt and THY-Tau22 (n=10/group) were treated p.o. (drinking water ad libidum) with vehicle, 2.2 or 3.6 mg/kg/day of AZP2006 for 3 months or 5 months. AZP2006 was administered from 3 month-old, age where the pathology in this mice was already well established (Laurent et al. 2017). Abnormal Tau phosphorylation (AT100 immunohistochemistry-IHC) and cognition (learning and memory by Morris Water Maze test –MWM) were performed after 3 and 5-month treatments. This behavioral test relies on distal cues to navigate from start locations around the perimeter of an open swimming arena to locate a submerged escape platform. Spatial learning was assessed across repeated trials and reference memory was determined by preference for the platform area when the platform is absent. Behavioral investigations were performed in Pr Luc Bué Laboratories (INSERM, UMR- S1172 Lille)

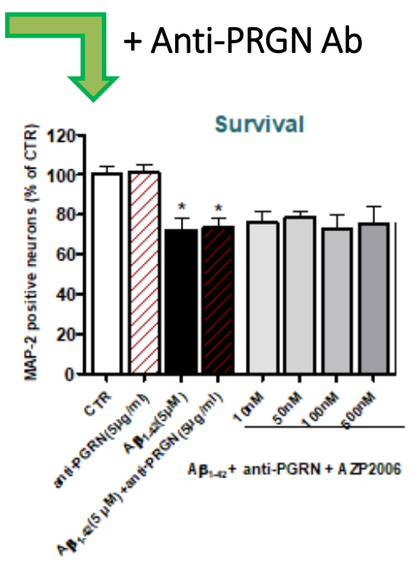
Results

In vitro, AZP2006 fully protects neurons from $A\beta_{1-42}$ injuries and abolishes the neuro-inflammation at nM concentrations : 72h after injuries, AZP2006 (co-administrated with Aβ) , fully protected neurons from the death, and fully abolished the microglia activation (from 10 nM).

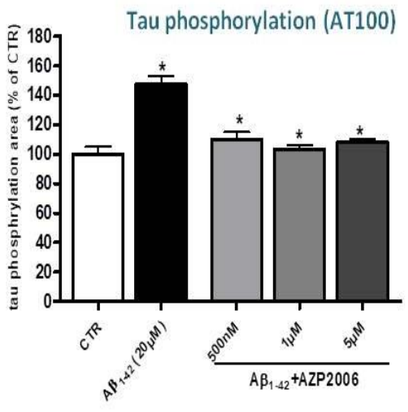


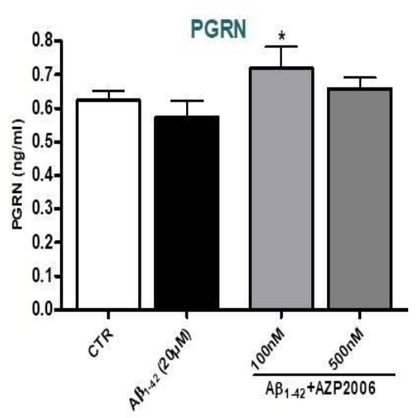


AZP2006 neuroprotective effect occurs via PGRN : In presence of Anti-PRGN, AZP2006 effect was abolished.



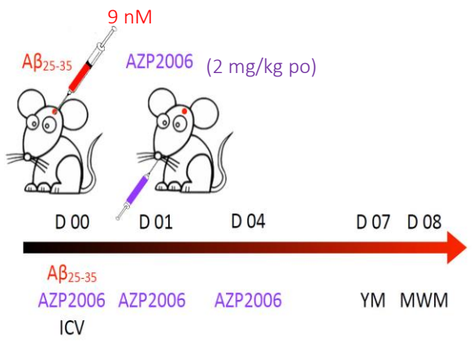
In vitro, AZP2006 fully abolishes the T hyperphosphorylation induced $A\beta_{1-42}$ and increases the PRGN levels in culture medium : 24 h after injuries, AZP2006 (co-administrated with Aβ), fully abolished Tau hyperphospho and significantly increases the PRGN in the extracellular compartment.

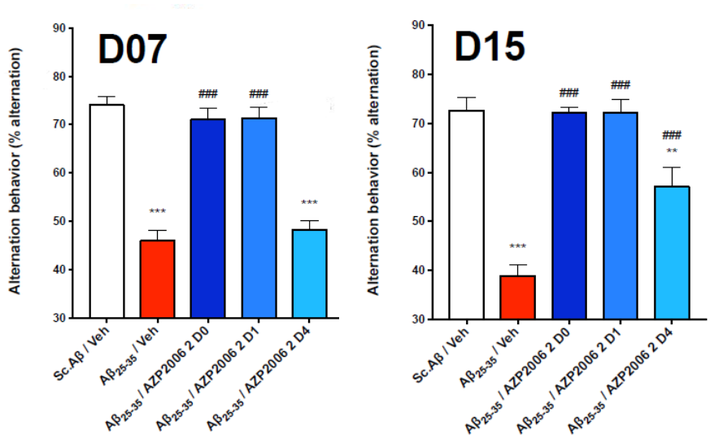




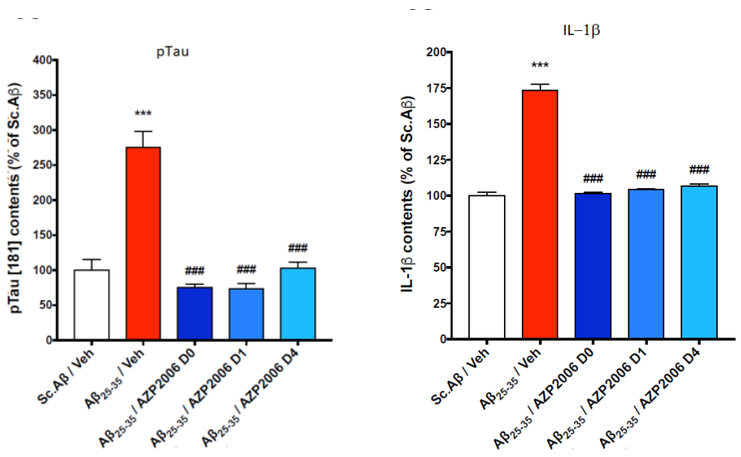
In vivo investigations

* *In vivo* $A\beta_{25-35}$-lesioned mouse model



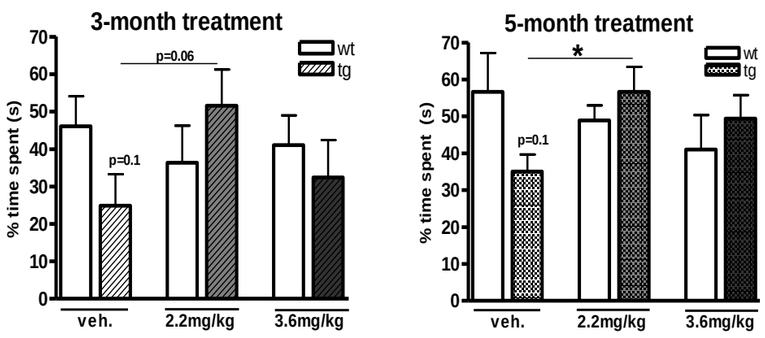


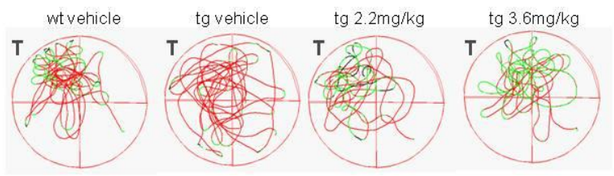
AZP2006 protects and restores the severe memory deficits induced by the amyloid peptide, fully abolished the Tau phosphorylation and the neuroinflammation : AZP2006 fully protected the animals when co-administrated or given 1 day after the amyloid peptide, by contrast partially reverses severe memory deficit (on day 15) when administrated 4 days after the lesion. After 1 additional week of treatment (D21), the deficits were fully reversed, the phosphorylated Tau (pTau) and the inflammation (IL-1β) were abolished.



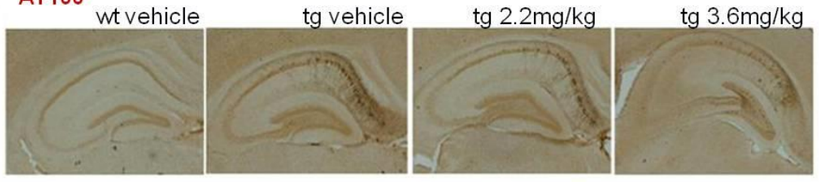
* *In vivo* THY-Tau22tg mouse model

Time on target quadrant





After 3-month treatment, AZP2006 was able to decrease memory deficits , after 5-months a full recovery was observed : *Time spent on target quadrant during MWM test probe trials (retention) on mice of 8 month-old. At 6 and 8 month-old THY-Tau22 mice that received AZP2006 at 2.2 mg/kg dose after 3 and 5 months of treatment prevented the decrease (*p<0.05 Student t test, n=4).*



AZP2006 reduces tau phosphorylation in the in the Hippocampus of THY-Tau 22tg mice : *AT100-immunoreactivity in the hippocampus after 5 months of treatment.*

Conclusions

The results presented here show that AZP2006 is endowed with neuroprotective properties against injuries induced *in vitro* and *in vivo* by $A\beta_{1-42}$ or $A\beta_{25-35}$. In addition, AZP2006 was proved to diminish abnormal τ hyperphosphorylation. In THY-Tau22tg mouse model, associated with a hyperphosphorylation of Tau and Tau tangles, AZP2006 improves memory deficits and significantly reduces the Tau accumulation. AZP2006 has the potential not only to delay the progression but also to reverse existing neurodegenerative damages (as shown in $A\beta_{25-35}$ mouse model). AZP2006 has neuroprotective and neurorestorative potential this effect seemed involving PRGN (a multifunctional growth factor known to increase neuron survival, neurite and synapses growth and to decrease the neuro-inflammation). AZP2006 is currently in clinical development for the treatment of neurodegenerative disorders, including PSP (ODD EU/US granted) and AD. A clinical phase 1 in healthy volunteers (88 patients) has proved the full tolerability and the safety profile of AZP2006. A phase 2a in PSP patients is being prepared. AZP1006 is a very serious candidate for the efficient treatment of tauopathies such as PSP and other neurodegenerative diseases.

Callizot *et al.*, (2013) J Neurosci Res. 2013;91(5):706-16 / Laurent *et al.*, (2017) Brain. 2017 Jan;14(1):184-200 / Meunier et al., (2013) Eur J Pharmacol. 2013 Jan 5;698(1-3):193-9

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