Amyloid peptide and cytoplasmic TDP-43 accumulation in pathogenesis of ALS: an in vitro study

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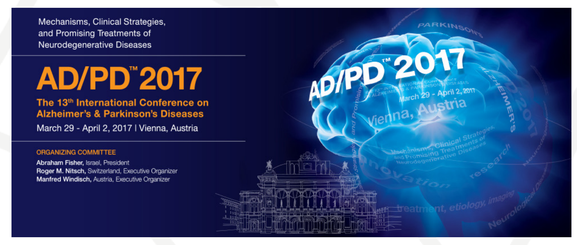


1 Introduction

In amyotrophic lateral sclerosis (ALS), the progressive loss of motor neurons is accompanied by extensive muscle denervation, resulting in paralysis and ultimately death. Disturbances in glutamate homeostasis, which lead to toxic accumulation of this excitatory neurotransmitter in the synaptic cleft, are observed in several neuropathologies notably in ALS. It has been shown in SOD1G93A mouse model as well as in ALS patients, an up-regulation of amyloid beta precursor protein (APP) in muscle fibres coinciding with symptom onset. Additionally, motor neuron (MN) axon defects death have recently been observed in murine models of familial Alzheimer's disease (AD) that produce elevated levels of Aβ, indicating susceptibility of MN to this neurotoxic peptide. It has been shown that neuromuscular junction (NMJ) loss and motor neuron degeneration are substantially reduced in SOD1G93A mice when APP is genetically ablated. In addition, our group showed that NMJs were highly sensitive to oligomeric forms of amyloid peptide (AβO) and that the toxic pathway involves glutamate and NMDAR (Combes *et al.*, 2015).
In ALS, a number of genes cause the disease, when mutated; among these are SOD1, FUS, TARDBP (encoding TDP-43), PRPH (encoding peripherin, a NF protein). TDP 43 (Transactivating response element DNA binding protein 43 kDa), has been shown to accumulate in cytpoplasm of neurons in early and late familial or sporadic Alzheimer's disease (AD) (Hu *et al.*, 2008). Altogether, these data raising the possibility of converging mechanisms leading to disease in AD and ALS.
Here, we provide evidence that AβO (as glutamate) induced the cytoplasmic TDP-43 aggregation in MN. In addition, the role of TDP-43 accumulation into neuronal death was investigated using Guanabenz (an activator of unfolded protein response (UPR)).

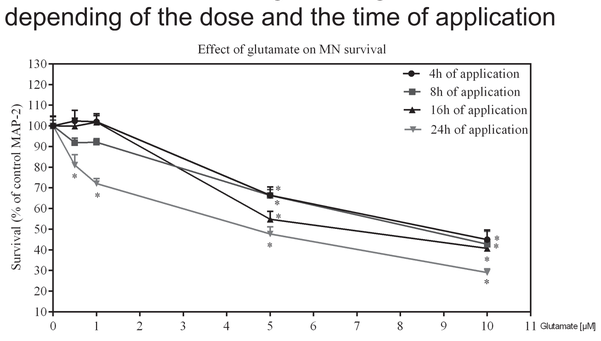
2 Methods

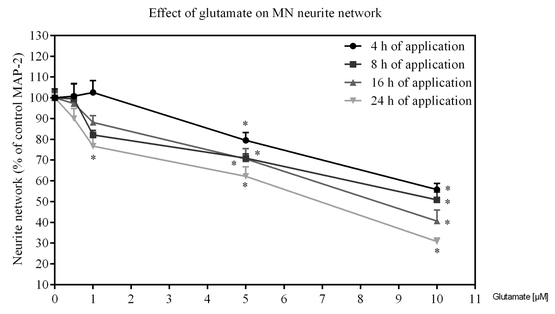
Culture: Rat spinal cord (SC) MN were cultured as described by Wang *et al.*, 2013. Briefly, pregnant female rats of 14 days gestation were killed. The SC primary cells from embryos were seeded at a density of 20,000 per well in 96-well plates (immunostaining) and 112,000 per well in 24-well plates (for Western-blotting, WB) precoated with poly-L-lysine and will be cultured at 37 °C in an air (95 %)-CO2 (5 %) incubator. The medium were changed every 2 days. The spinal cord motor neurons were injured with Glutamate or $A\beta_{1-42}$ peptide after 13 days of culture.
Pharmacological treatments: The $A\beta_{1-42}$ preparation was done following the procedure described by Callizot *et al.*, 2013. The oligomeric fraction of the peptide was dosed in the mother solution, 10 % of the total peptide were oligomers of the peptide. The MN were submitted to different concentration of $A\beta_{1-42}$ for 8, 16 or 24 h. The cultures were injured with glutamate for 20 min. After 20 min, glutamate was washed and fresh culture medium was added for additional 4, 8, 16 and 24 hours.
Guanabenz, an α2 adrenergic receptor agonist centrally acting oral drug approved for the treatment of hypertension, enhances the PERK pathway by selectively inhibiting GADD34-mediated dephosphorylation of EiF2α, was used at 5 µM concentration and was pre-incubated 1 h before $A\beta_{1-42}$ application. 17-β Estradiol (Estradiol) was used here at 100 nM as a neuroprotective reference compound (and was incubated 1 h before $A\beta_{1-42}$ application). Guanabenz and Estradiol were let during the 24 h of $A\beta_{1-42}$ application.
Staining of MNs: After 8, 16 or 24 h, cultures were fixed by a cold solution of ethanol (95 %) and acetic acid (5 %) for 5 min at -20 °C. After permeabilization, cells were incubated for 2 h with:
a) mouse monoclonal Ab anti microtubule-associated-protein 2 (MAP-2) (this antibody stains specifically cell bodies and neurites, allowing study of neuronal cell death and neurite network);
b) rabbit polyclonal antibody anti TDP43, TDP43 (cytoplasmic) were evaluated. These Abs were revealed with Alexa Fluor 488 goat anti-mouse IgG and Alexa Fluor 568 goat anti-rabbit IgG. The immunolabelled cultures were examined with ImageXpress equipped with a LED lamp (excitation 360/480/565 nm; emission 460/535/620 nm) at X 20 magnification. For each condition, 30 fields per well were observed (representing the total surface of the well), and six wells per conditions were analyzed.
EiFα-Phospho evaluation (WB): After 24 h of treatment, cells were lysed with cellytic and immediately frozen at -80 °C. All reagents were prepared and used according to manufacturer's recommendations (Simon™ - ProteinSimple - www.proteinsimple.com). Anti-EiF2α-Phospho, primary antibody was used for WB analysis.

3 Results

Effect of glutamate on MN survival and neurite network:

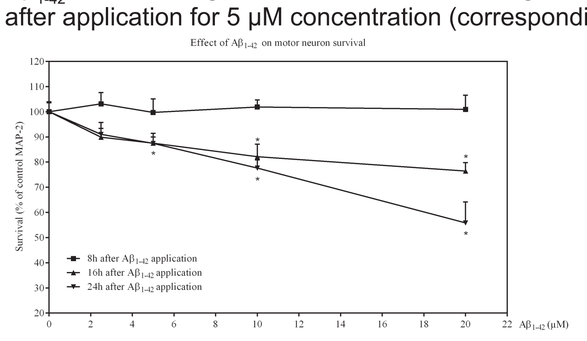
Glutamate induces a large and significant MN death and a large neurite network lose. The toxicity was depending of the dose and the time of application

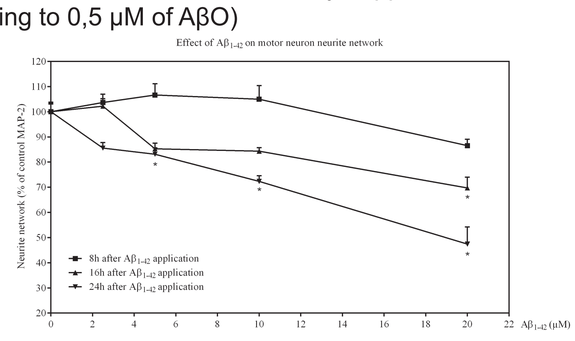




Effect of $A\beta_{1-42}$ on MN survival and neurite network:

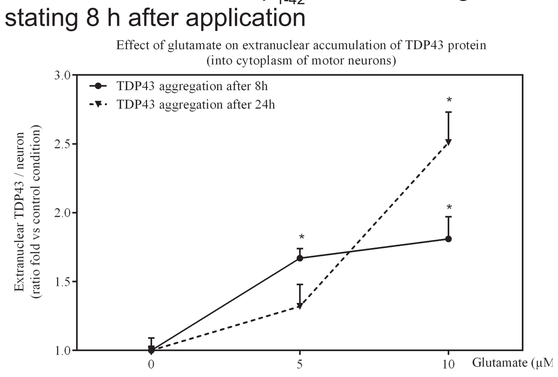
$A\beta_{1-42}$ induces a significant MN death and a large neurite network lose. The toxicity happened 16 h after application for 5 µM concentration (corresponding to 0,5 µM of AβO)

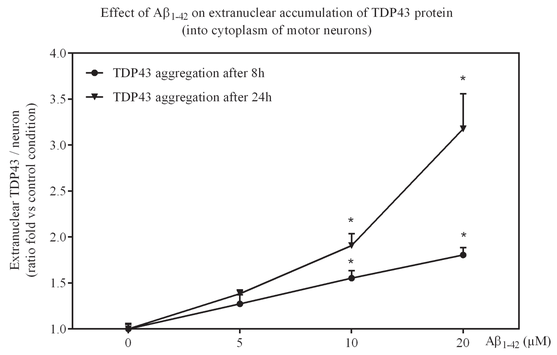




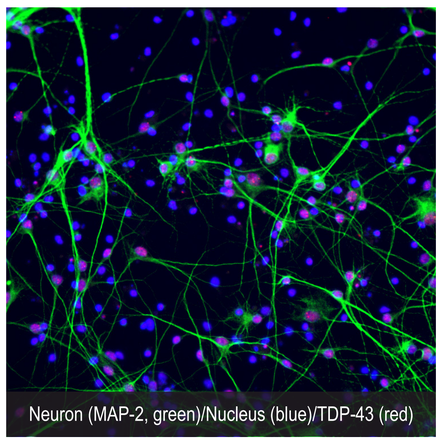
Effect of glutamate and $A\beta_{1-42}$ on extranuclear TDP-43 accumulation in MNs:

Glutamate as well as $A\beta_{1-42}$ induced a large and significant TDP-43 cytoplasmic aggregation into MNs stating 8 h after application

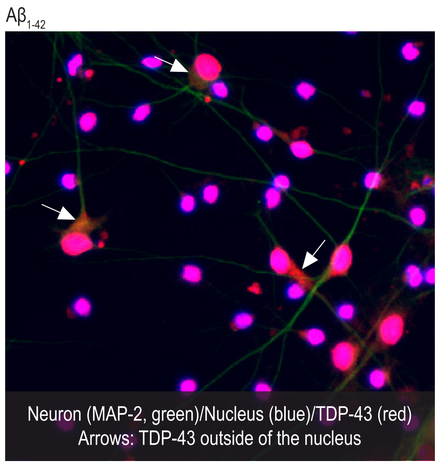




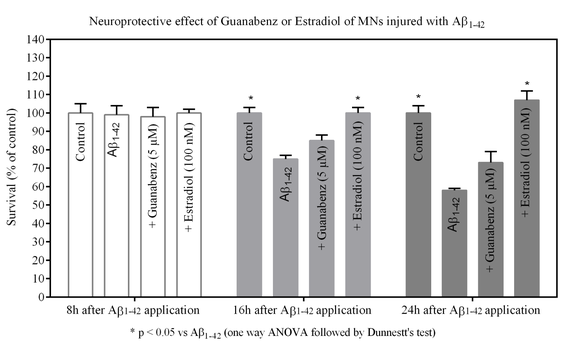
Control

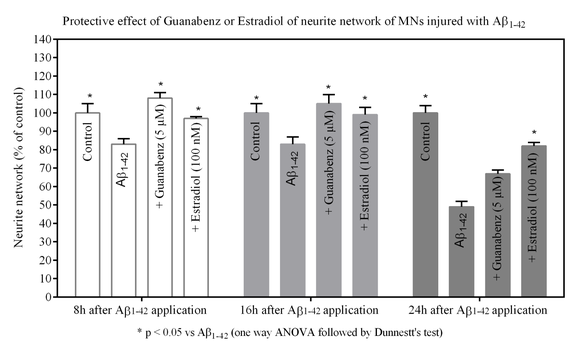


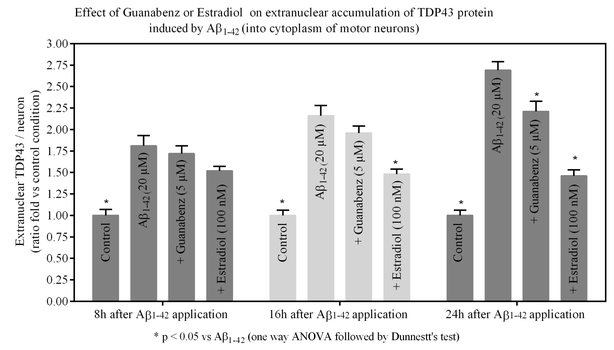
$A\beta_{1-42}$



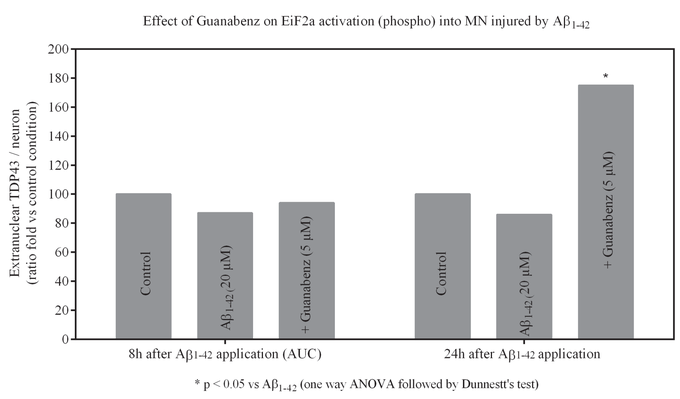
Guanabenz induced a moderate prevention (at 24 h) of TDP-43 inducing a slight neuronal protection. The injuries induced by $A\beta_{1-42}$ were slightly prevented (neurite network) with Guanabenz. A significant reduction of TDP-43 cytoplasmic aggregation was observed (after 24 h). This effect involved the PERK/Eifα pathway via the inhibition of GADD34 induced by Guanabenz

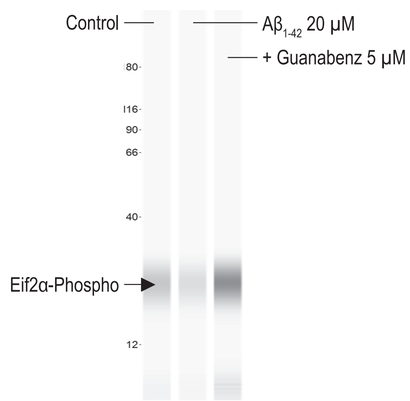


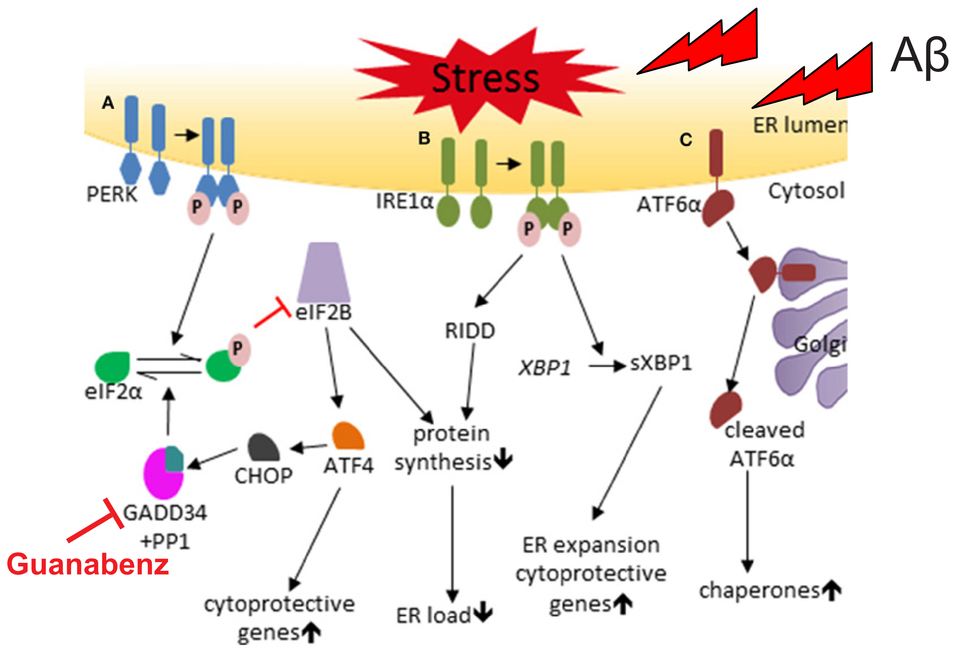




Guanabenz induced (at 24 h) a large activation of Eif2α pathway via the inhibition of GADD34







4 Conclusions

These results showed that Aβ induced a large MN toxicity associated with TDP-43 cytoplasmic aggregtion. Guanabenz (used at 5 µM), activating the UPR pathway via GADD34 inhibition, was able to decrease TDP-43 aggregation into MNs, without protecting neurons from death.

References: Callizot *et al.*,2013 J Neurosci Res. 2013 May;91(5):706-16 - Combes *et al.*, 2015 J Neurosci Res. 2015 Apr;93(4):633-43. - Hu *et al.*, 2008 Acta Neuropathol. 2008 Aug;116(2):215-20. - Wang *et al.*, 2013 Neural Regen Res. 2013 Aug 25;8(24):2213-24.

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