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

Noelle Callizot, Maud Combes and Philippe Poindron
Neuro-Sys SAS, R&D Department, 410 Chemin Denierbois, 13720 Gardanne, France

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 neuronumcular 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 are 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 200 kDa protein). TDP 43 (Transactivating response element DNA binding protein 43 kDa), FUS (FTT1), SOD1, FUS, TARDBP (encoding TDP-43), PRPH (encoding peripherin, a NF 200 kDa protein). TDP 43 (Transactivating response element DNA binding protein 43 kDa), FUS (FTT1), C9orf72, VCP (pm 150 kDa protein), PRKRA, GOLPH3. 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 200 kDa protein). TDP 43 (Transactivating response element DNA binding protein 43 kDa), FUS (FTT1), C9orf72, VCP (pm 150 kDa protein), PRKRA, GOLPH3. 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 200 kDa protein). TDP 43 (Transactivating response element DNA binding protein 43 kDa), FUS (FTT1), C9orf72, VCP (pm 150 kDa protein), PRKRA, GOLPH3.

Here, we provide evidence that AβO (as glutamate) induced the cytoplasmic TDP-43 accumulation in MN. In addition, the role of TDP-43 accumulation into neuronal structure and its potential effects on neuronal death was investigated using Guanabenz (an activator of unfolded protein response (UPR)).

2 Methods

Culture: Rat spinal cord (SC) MNs 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 (immunospanning) 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 AβO peptide after 13 days of culture.

Pharmacological treatments: The AβO peptide was used during the following procedures 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 AβO 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 AβO application. Guanabenz and Estradiol were let during the 24 h of AβO 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 Aβ 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 secondary Ab.

3 Results

Effect of glutamate on MN survival and neurite network:

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

Effect of AβO on MN survival and neurite network:

AβO induces a significant MN death and a large neurite network loss. The toxicity happened 16 h after application for 5 µM concentration (corresponding to 0.5 µM of AβO).

4 Conclusions

These results showed that AβO induced a large MN toxicity associated with TDP-43 cytoplasmic aggregation. 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


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www.proteinsimple.com). Anti-EIF2a-Phospho, primary antibody was used for WB analysis.