Role of β-Amyloid (Aβ) Oligomeric fractions in ALS disease.

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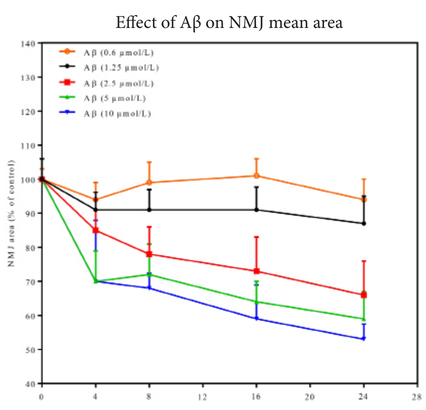
1 Introduction

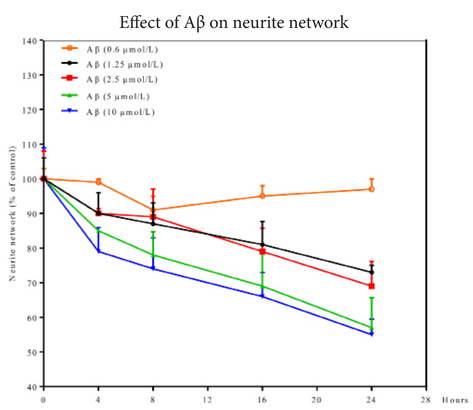
In amyotrophic lateral sclerosis (ALS), the progressive loss of motor neurons is accompanied by extensive muscle denervation, resulting in paralysis and ultimately death. Disturbances in glutamate homeostasis, which lead to toxic accumulation of this excitatory neurotransmitter in the synaptic cleft, 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 axon defects and motor neuron death have recently been observed in murine models of familial Alzheimer's disease (AD) that produce elevated levels of Aβ, indicating susceptibility of motor neurons to this neurotoxic peptide. It has been recently shown that neuromuscular junction (NMJ) loss and motor neuron degeneration are substantially reduced in SOD1G93A mice when APP is genetically ablated, All together, these results indicate that endogenous APP and Aβ may contribute to ALS pathology in humans (Bryson *et al.*, 2012).
The aim of this study was to investigated the role of Aβ on an in vitro model of functional NMJ, in order to better understand its involvement in the NMJ death and the neurodegenerative process on motor neurons.

2 Methods

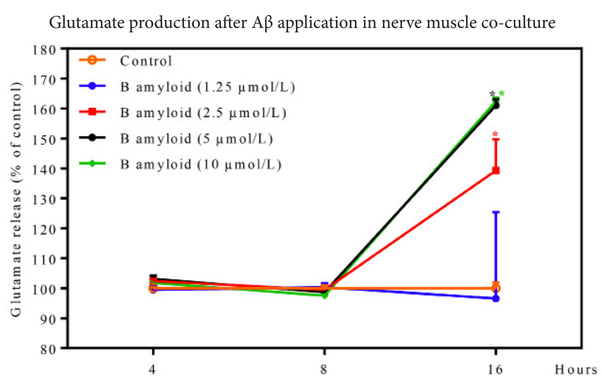
Culture: The nerve/muscle cocultures were cultured according to Askanas *et al.*, 1987; Braun *et al.*, 1996. Briefly, human muscle cells (SkMC) were plated onto gelatin-coated wells in 48-wells plates and grown in a proliferation medium. The medium was changed every 2 days. Five days after the beginning of the culture, immediately after satellite cell fusion, whole transverse slices of 13-day-old rat Wistar embryos spinal cord with dorsal root ganglia (DRG) were placed onto the muscle cell monolayer. After 24 h of co-culture, neurites were observed growing out of spinal cord explants. These neurites made contacts with myotubes and induce the first contractions after ~ 8 days.
Pharmacological treatments: On day 33 following innervation, the co-cultures were injured with glutamate at different concentrations (0.1, 0.5, 1, 10, 30 µmol/L) and/or with Aβ solution (Callizot *et al.*, 2013) at 0.6, 1.25, 2.5, 5 and 10 µmol/L for 4, 8, 16 and 24 hours. Untreated cultures served as controls. MK801, a non-competitive N-methyl-D-aspartate receptor (NMDAR) antagonist was diluted in culture medium and used at 20 µmol/L.
Staining of NMJs and immunostaining of axons: After 4, 8, 12 or 24 h, co-cultures were incubated with 500 nmol/L α-bungarotoxin (αBgt) coupled with Alexa 488 to detect NMJs. Co-cultures were incubated with a mouse monoclonal anti-200 kDa neurofilament (NF) Antibody (Ab) to stain the axons of motor neuron. These Ab were revealed with Alexa Fluor 568 goat anti-mouse IgG.
The mean size of NMJs (evaluated as apparent area of αBgt-labelled zones) was measured to assess the quality of innervation, (the larger the NMJs, the stronger the innervation). The mean size of NMJs was assessed. The total length of neurite network was also measured to verify the quality of muscle innervation.
The immunolabelled cultures were examined with InCell Analyzer TM1000 (GE Healthcare, Cardiff, United Kingdom) equipped with a xenon lamp (excitation 360/480/565 nm; emission 460/535/620 nm) at X 20 magnification. For each condition, 63 fields per well were observed (representing the total surface of the well), and six wells per conditions were analyzed.
Glutamate dosing: After 4, 8 and 16 h, glutamate was dosed in supernatant with Amplex red glutamic acid kit (Molecular Probes).

3 Results

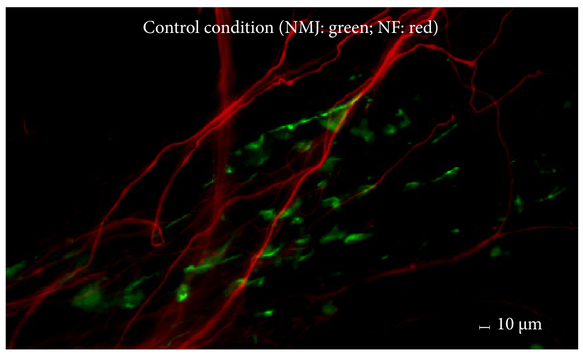


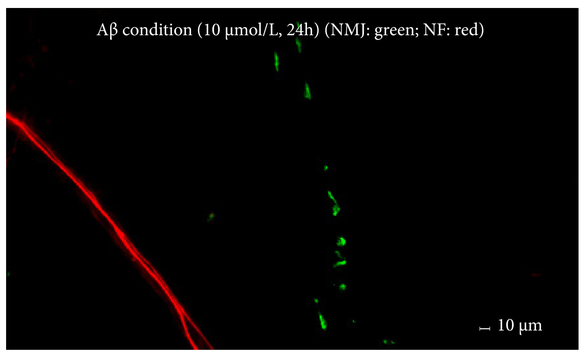


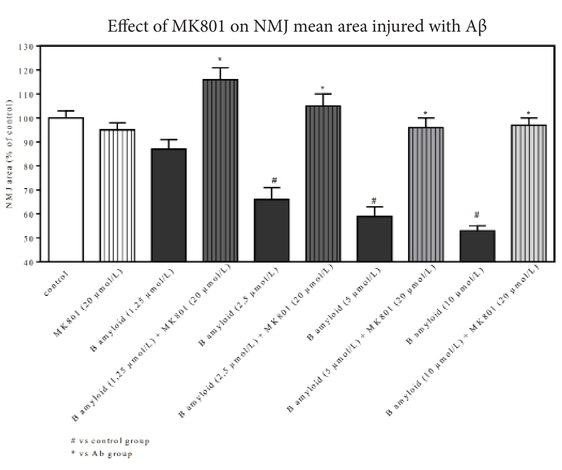
Effect of Aβ on NMJ mean area and neurite network: Only a minor effect was observed on the NMJ mean size when Aβ was applied at 0.6 and 1.25 µmol/L. At highest concentrations, the toxic effect on the mean size and number of NMJs was significant. The extent of injury was dependent on the time of exposure and concentrations of Aβ.
Aβ exerted its toxic effect on neurite network at all concentrations tested (except for the lowest one).

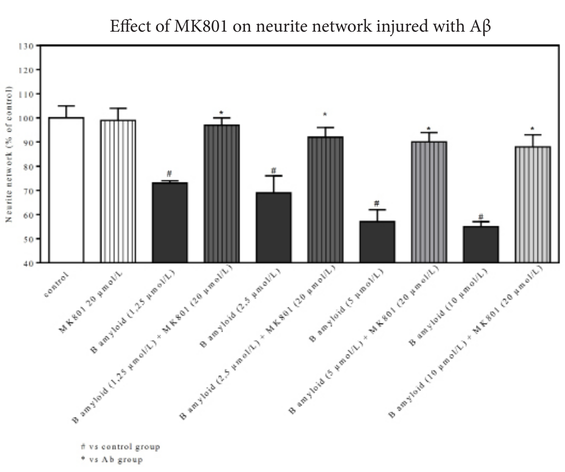


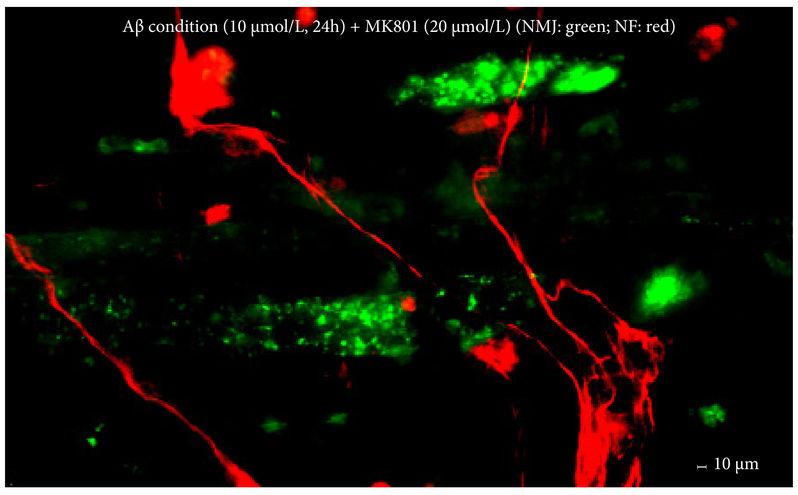
Glutamate production after Aβ application: After 16 h of Aβ treatment, a large glutamate release was observed in the supernatant of the nerve muscle co-cultures. This production was significant for all concentrations of peptide tested, except for the lowest one (1.25 µmol/L).











Effect of MK801 on NMJ mean area and neurite network injured with Aβ : MK801 (used at 20 µmol/L), exerted a significant protective effect against Aβ-induced injuries (all concentrations tested); same conclusions could be drawn concerning the protective effect of this drug on the neurite network.
MK801 fully abolished the direct toxic effect of Aβ1-42 on NMJs and on the neurite network sprouting from spinal cord explant and innervating the muscle cells.

4 Conclusions

We showed that Aβ application on functional co-culture induced a progressive loss of NMJ and motor neurons degeneration. Additionally we proved that low concentrations of Aβ (at a nontoxic level) induced a large glutamate production. The glutamate release was dependent of time of application as well as concentration dependent. Interestingly, application of MK801 fully protected coculture from injuries. All together these results suggest that glutamate is involved in the process of degeneration induced by Aβ suggesting that Aβ could be an important element triggering excitotoxicity event. Together with previous findings, our results suggest that targeting Aβ may be a promising road in ALS therapy.

Callizot *et al.*, (2013) J Neurosci Res. 2013;91(5):706-16 / Braun, S. *et al.*, (1996) J Neurol Sci 136: 17-23 /Askanas V., *et al.*, (1987). J Neurocytol., 16(4):523-37 / Bryson *et al.*, (2012) Hum Mol Genet. 1;21(17):3871-82