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Treatment with the neurotoxic Aβ (25–35) peptide modulates the expression of neuroprotective factors Pin1, Sirtuin 1, and brain-derived neurotrophic factor in SH-SY5Y human neuroblastoma cells

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ABSTRACT

The deposition of Amyloid β peptide plaques is a pathological hallmark of Alzheimer's disease (AD). The Aβ (25–35) peptide is regarded as the toxic fragment of full-length Aβ (1–42). The mechanism of its toxicity is not completely understood, along with its contribution to AD pathological processes. The aim of this study was to investigate the effect of the neurotoxic Aβ (25–35) peptide on the expression of the neuroprotective factors Pin1, Sirtuin1, and Bdnf in human neuroblastoma cells.

Levels of Pin1, Sirtuin 1, and Bdnf were compared by real-time PCR and Western blotting in SH-SY5Y cells treated with Aβ (25–35) or administration vehicle. The level of Pin1 gene and protein expression was significantly decreased in cells exposed to 25 μM Aβ (25–35) compared to vehicle-treated controls. Similarly, Sirtuin1 expression was significantly reduced by Aβ (25–35) exposure. In contrast, both Bdnf mRNA and protein levels were significantly increased by Aβ (25–35) treatment, suggesting the activation of a compensatory response to the insult. Both Pin1 and Sirtuin 1 exert a protective role by reducing the probability of plaque deposition, since they promote amyloid precursor protein processing through non-amyloidogenic pathways. The present results show that Aβ (25–35) peptide reduced the production of these neuroprotective proteins, thus further increasing Aβ generation.

1. Introduction

The pathological hallmarks of Alzheimer's disease (AD) are abundant extracellular senile plaques of amyloid β (Aβ) peptides in cerebral blood vessels and brain parenchyma, along with intracellular neurofibrillary tangles of aggregated phosphorylated tau protein (Selkoe, 2011). Aβ peptides derived from the proteolytic cleavage of the large transmembrane polypeptide amyloid precursor protein (APP) are able to form β-sheets structures and fibrillary aggregates (Selkoe, 2011). The accumulation of Aβ plaques in the brain is considered a central event in the etiology of AD, but it is still debated if the plaques themselves represent a primary cause of AD or if they are a by-product of underlying pathological processes (Drachman, 2014). The Aβ (25–35) peptide is regarded as the biologically active fragment of full-length Aβ (1–42), since it is the shortest Aβ peptide retaining full toxicity (Millucci et al., 2010). This peptide displays rapid aggregation properties forming stable fibrils and it is neurotoxic immediately upon dissolution. Moreover, immunohistochemical analyses demonstrated the presence of the Aβ (25–35) peptide in AD brains, suggesting that soluble Aβ (1–40) is released from plaques and converted to the toxic Aβ (25–35) fragment (Millucci et al., 2010).

It is conceivable that the modulation of neuroprotective pathways may contribute to Aβ neurotoxicity by suppressing basal restorative responses. Among neuroprotective proteins, the peptidyl–prolyl *cis*/*trans* isomerase Pin1 plays a role in the alteration of protein phosphorylation state by regulating protein conformation. Pin1 has been implicated in the pathophysiology of AD through a dual mechanism. Since Pin1 binds to phosphorylated tau and promotes its dephosphorylation, reduced protein amount is associated to increased accumulation of phosphorylated tau and formation of neurofibrillary tangles. In addition, Pin1 catalyses the isomerization of APP, favoring its processing in the direction of non-amyloidogenic pathways and reducing Aβ production (Driver et al., 2014).

Abbreviations: AD, Alzheimer's disease; Aβ, amyloid β; APP, amyloid precursor protein; Bdnf, brain-derived neurotrophic factor; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PBS, phosphate-buffered saline; Sirt1, Sirtuin 1.

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Another protein exerting a protective activity against neurodegeneration by reducing APP amyloidogenic processing and Aβ deposition is the histone deacetylase Sirtuin 1 (Sirt1) (Bonda et al., 2011). Sirt1 beneficial effects are mediated through an increased transcription of ADAM10, which activates the non-amyloidogenic α secretase pathway and the notch neurogenetic pathway (Bonda et al., 2011). Moreover, Sirt1 exerts a further neuroprotective activity by promoting the deacetylation and subsequent degradation of tau pathogenic forms (Min et al., 2010).

Growing evidence suggests that reduced brain-derived neurotrophic factor (Bdnf) expression in brain is a common feature of AD and other cognitive dysfunctions (Tapia-Arancibia et al., 2008) and restore of its function has been suggested as a possible therapeutic approach in neurodegenerative diseases (Lu et al., 2013). Neuroprotective effects against Aβ toxicity exerted by Bdnf have been described in cellular and animal models (Tapia-Arancibia et al., 2008; Zhang et al., 2012a). The neuroprotective activity against Aβ toxicity involves several components, including the shifting of APP processing towards the α-secretase pathway (Holback et al., 2005) and the rescue of Aβ-induced inhibition of hippocampal long-term potentiation by enhanced CaMKII autophosphorylation (Zeng et al., 2010). Moreover, available data support the notion that Aβ peptides are able to interfere with Bdnf signal transduction pathways involved in neuronal survival and synaptic plasticity, hampering the transmission of neurotrophic responses (Tong et al., 2004).

The aim of the present study was to investigate the effect of the neurotoxic Aβ (25–35) peptide on the expression of the neuroprotective factors Pin1, Sirt1, and Bdnf in human neuroblastoma cells to help elucidating their role in Aβ toxicity.

2. Materials and methods

2.1. Cell culture and treatments

Human neuroblastoma SH-SY5Y cells, purchased from ICLC-IST (Genoa, Italy), were cultured in Dulbecco's modified Eagle medium, supplemented with 10% foetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin, and 2 mM glutamine (Lonza, Italy) at 37 °C in a humidified atmosphere containing 5% CO_2. Experiments were performed at 80% confluence.

Aβ (25–35) peptide (Sigma–Aldrich, St. Louis, MO, USA) was dissolved at 1 mM in sterile distilled water. The unaggregated peptide was incubated at 37 °C for 72 h, and gently mixed to promote aggregation (Millucci et al., 2009). Treatments were performed with a working solution at 25 μM in cell medium supplemented with 2% serum. The dose was selected based on previous studies reporting toxic effects in neuroblastoma cells and in primary neurons (Croce et al., 2011; Resende et al., 2007; Sultana et al., 2006; Wang et al., 2014; Xi et al., 2012)

2.2. MTT cell viability assay

Cell viability was assessed with a modified MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma–Aldrich) assay. Briefly, cells were plated in 24-well plates at 3×10^4 cells/. After 24 h, the medium was removed; the cells were rinsed with phosphate-buffered saline (PBS) and treated with Aβ (25–35) for 5, 24 or 48 h. At the end-point cells were rinsed with PBS and treated with the MTT solution (0.5 mg/ml in PBS) in medium without phenol red and serum. The cells were incubated

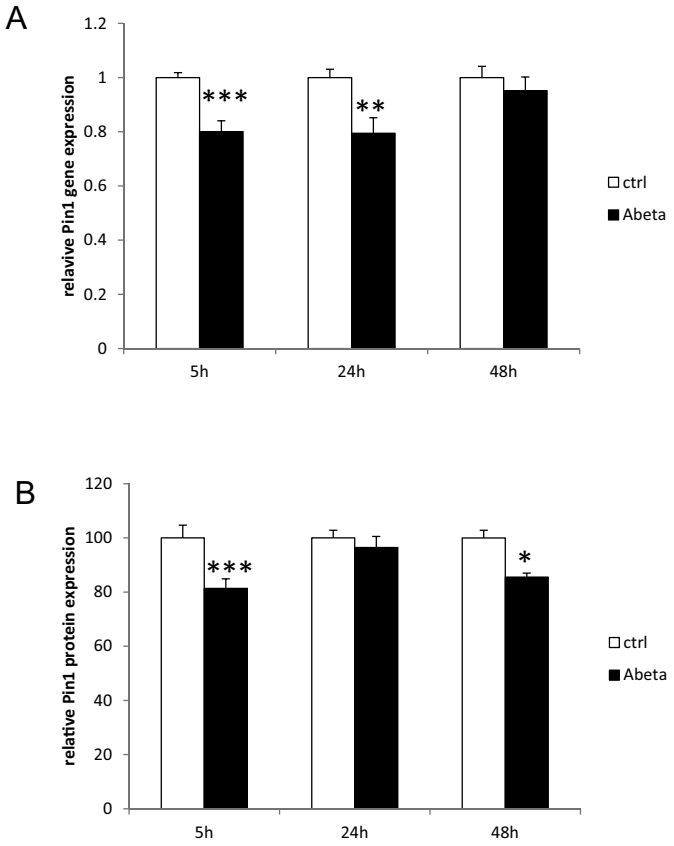

Fig. 1. (A) Pin1 mRNA expression after 25 μM Aβ (25–35) treatment for 5, 24, or 48 h. Gene expression was measured by real-time PCR. Data represent 2^{-DDCt} values normalized to GAPDH levels. Data are expressed as mean ± SEM of controls of three independent experiments. $^{**}p < 0.01$; $^{***}p < 0.001$. (B) Pin1 protein levels. Protein levels were evaluated by Western blot analysis. Data normalized to GAPDH levels are expressed as percentage of controls (mean ± SEM) of three independent experiments. $^{*}p < 0.05$; $^{***}p < 0.001$.