The role of acetylcholine on the ß-amyloid-induced cPLA2 activation in the neuroectodermal cell line TB: implications in the pathogenesis of Alzheimer's disease

Polverino A.a,b, Grimaldi M.c, Sorrentino P.d, Jacini F.a, D'Ursi A.M.c, Sorrentino G.a,b

a) Department of Motor Sciences and Health, University of Naples Parthenope, Naples, Italy; b) Institute of Diagnosis and Treatment Hermitage, Naples, Italy; c) Department of Pharmacy, University of Salerno, Salerno, Italy; d) Department of Engineering, University of Naples Parthenope, Naples, Italy.

Introduction

The role of β-amyloid (Aβ) in the pathogenesis of Alzheimer's disease (AD) is still considered crucial. We have previously demonstrated that in the cholinergic LAN-2 cells, Acetylcholine (ACh) is neuroprotective against the toxic effects of Aβ [1]. To better understand the mechanisms of action of Aβ and ACh, we studied the effect of Aβ25-35, the biologically active region of the full length peptide Aβ1-42, on Phospholipase A2 (Ser505, Phospho-cPLA2) in the human non-cholinergic TB cell line [2].

Material and method

TB cells have a neuroectodermal origin and are able to differentiate toward a neuronal phenotype when treated with 10 µM retinoic acid (RA). We evaluated the biological effects of Aβ on cPLA2 in both undifferentiated and RA-differentiated TB cells grown *in vitro* up to 7 days. The effect of ACh on cPLA2 activation by Aβ was also evaluated through Western blot analysis.

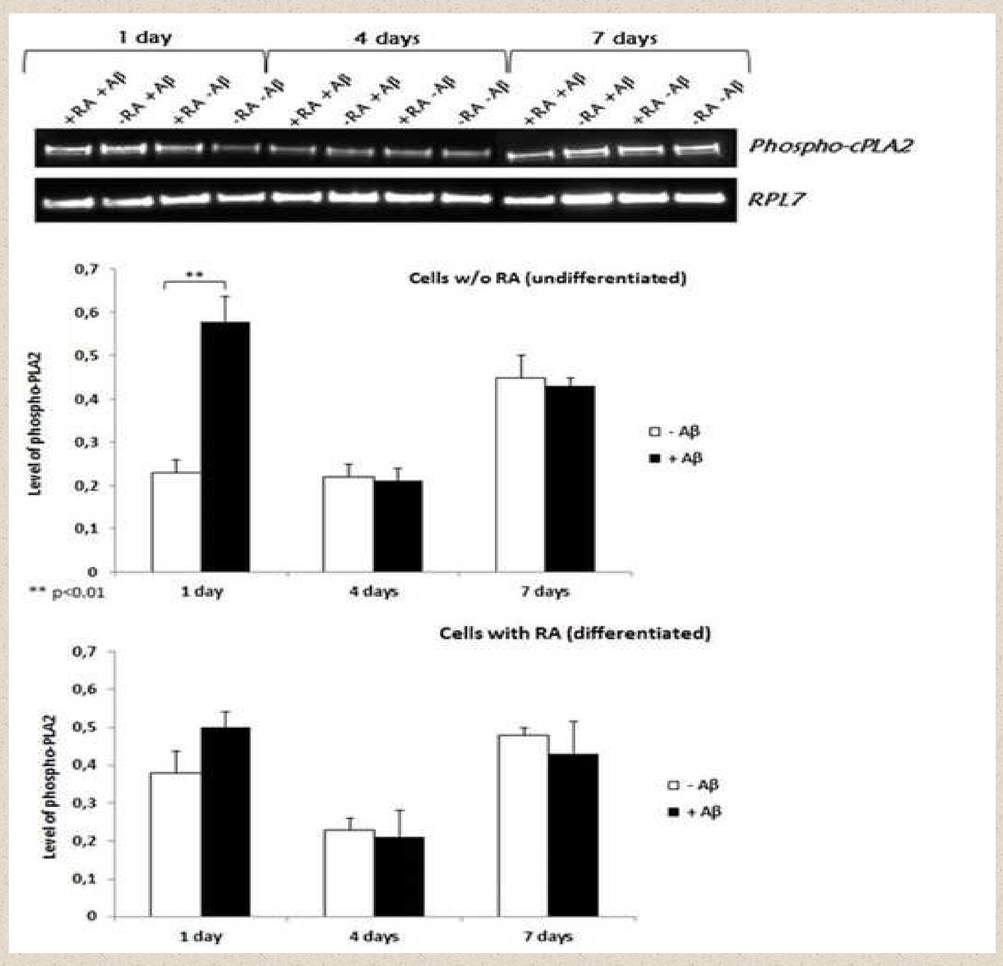

Fig. 1. Western blotting analysis on total proteins (20 µg) extracted from TB differentiated (+RA) and not differentiated (-RA), grown *in vitro* up to 7 days and treated for 1 hour with 20 µM Aβ25-35.

Results

Our results show that in undifferentiated TB cells Aβ induced a 2.5 fold increase of the Phospho-cPLA2 level compared to the control after 24 h *in vitro*, while no significant difference was observed between Aβ-treated and non-treated cells after 4 and 7 days *in vitro* (**Fig. 1**). The RA-differentiated cells were not sensitive to Aβ.

ACh was able to blunt the effect of Aβ in undifferentiated TB cells (**Fig 2A**), while it has no effects in RA-differentiated cells (**Fig 2B**).

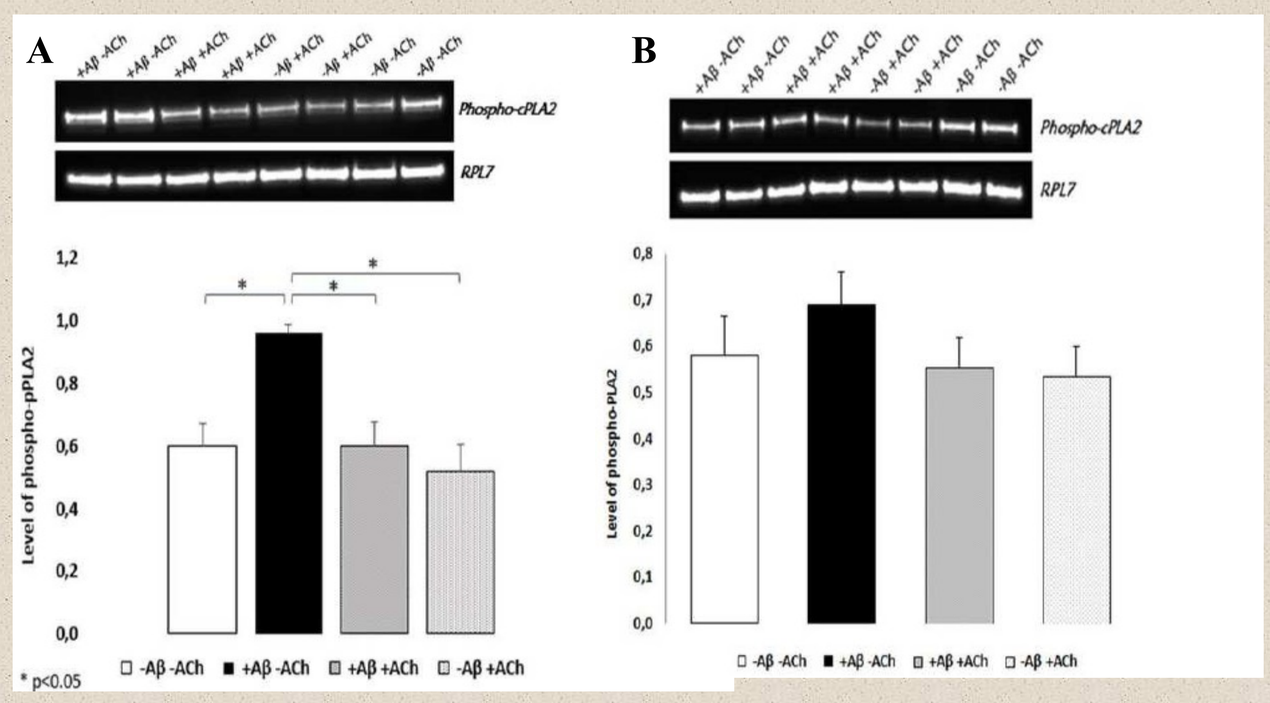

Fig. 2. Western blot analysis on total proteins extracted from TB cells treated for 1 hour with 20 µM Aβ25-35 and 25 µM ACh after 24 h *in vitro* without (**A**) and with (**B**) 10 µM RA.

Discussion and conclusions

The active form of cPLA2 (pSer505) is an index of inflammation and cellular toxicity induced by Aβ25-35, while ACh has a protective role. The mechanisms of action of ACh are still unclear and they could give some helpful information about AD pathogenesis and treatment.

In fact, the ability of ACh to protect non-cholinergic cells against Aβ reinforces the hypothesis that, in addition to its role in cholinergic transmission, ACh could also act as a neuroprotective agent.

[1] Grimaldi M et al. (2016) β-Amyloid-acetylcholine molecular interaction: new role of cholinergic mediators in anti-Alzheimer therapy? *Future Medicinal Chemistry* 8:1179-1189.

[2] Sorrentino G., Monsurrò MR., Pettinato G., Vanni R., Zuddas A., Di Porzio U., Bonavita V. (1999). Establishment and characterization of a human neuroectodermal cell line (TB) from a cerebrospinal fluid specimen. *Brain Res. 1999 May 8;827(1-2):205-9.*