The role of acetylcholine on the cPLA2 in cultured cells treated with β-amyloid: implications in the pathogenesis of Alzheimer's disease

Polverino A.a,b, Valente M.T.b, Rodriquez M.c, D'Ursi A.M.c, Sorrentino G.a,b

a) University of Naples Parthenope, Naples, Italy; b) Institute of Diagnosis and Treatment Hermitage, Naples, Italy; c) Department of Pharmacy, University of Salerno, Fisciano (SA), Italy.

Introduction

Neurofibrillary tangles (NT) and senile plaques (SP) are the hallmarks of AD [1]. We evaluated the biological effects of Aβ25-35 peptide, which is the biologically active region of the full length peptide Aβ, in two human cell lines suitable as a model of AD: LAN-2 and TB cells. Aβ25-35 peptide is able to induce arachidonic acid production in LAN-2 cells as a result of the cytosolic phospholipase A2 (cPLA2) phosphorylation [2], while it has never been tested in TB cells.

We tried to understand the role of acetylcholine (ACh) on the cytotoxic effects of Aβ25-35.

Material and method

LAN-2 is a human cholinergic neuroblastoma cell line, while TB cells have a neuroectodermal origin and are able to differentiate toward a neuronal phenotype when treated with retinoic acid (RA) [3]. We evaluated the biological effects of Aβ25-35 on these cell lines and the role of ACh in the process by Western blot analysis.

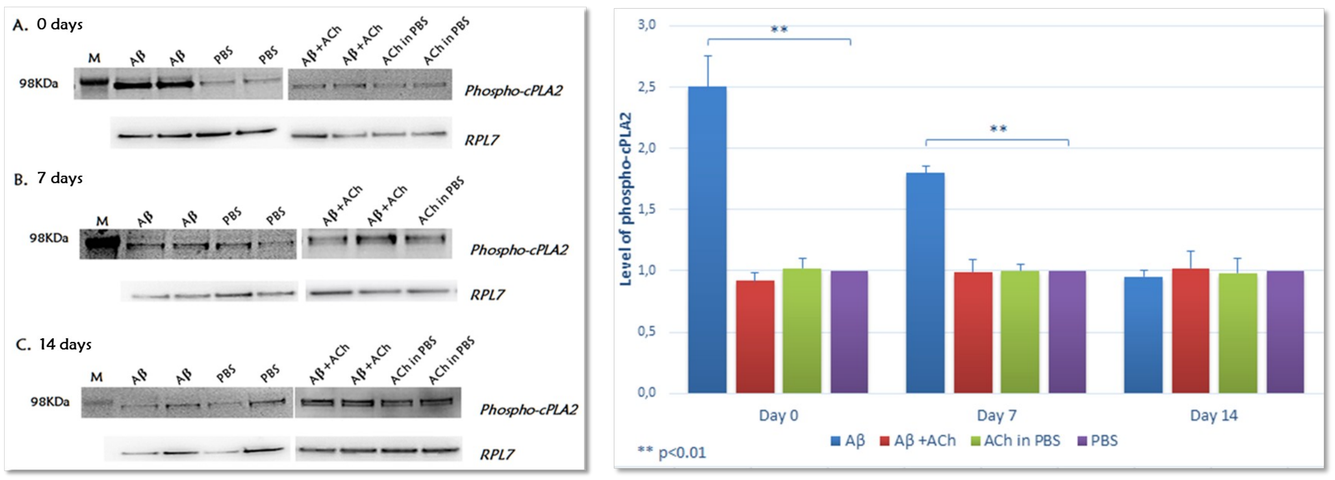

Fig. 1. Western blotting analysis on total proteins (20 µg) extracted from the LAN-2 cells treated with Aβ25-35 and ACh, using an antibody specific for the phosphorylated form of cPLA2. The treatments with Aβ peptide were performed after 0 days (**A**), 7 days (**B**) and 14 days (**C**) of Aβ aggregation. M: molecular weight marker.

Results

Aβ25-35 peptide is able to increase cPLA2 phosphorylation (p-cPLA2) in LAN-2 cell line, while ACh blunts Aβ25-35 cytotoxicity (**Fig. 1**). In TB cells, Aβ25-35 increases p-cPLA2 levels in both undifferentiated and differentiated cells (**Fig. 2**). ACh is still able to blunt Aβ effects (**Fig. 3**).

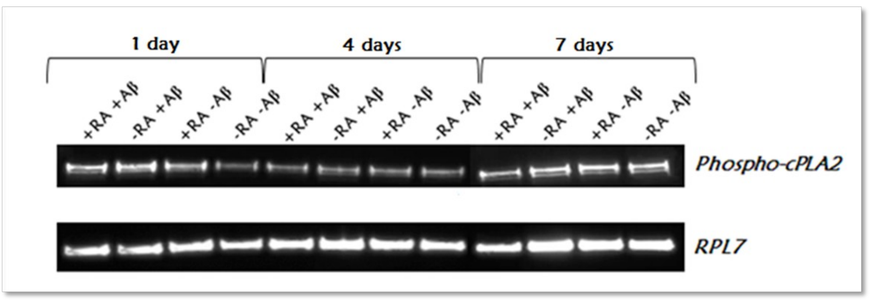

Fig. 2. Western blotting analysis on total proteins (20 µg) extracted from TB cells differentiated with retinoic acid (+RA) and not differentiated (-RA), treated with Aβ25-35 5 µM after 1 day, 4 days and 7 days of incubation with RA.

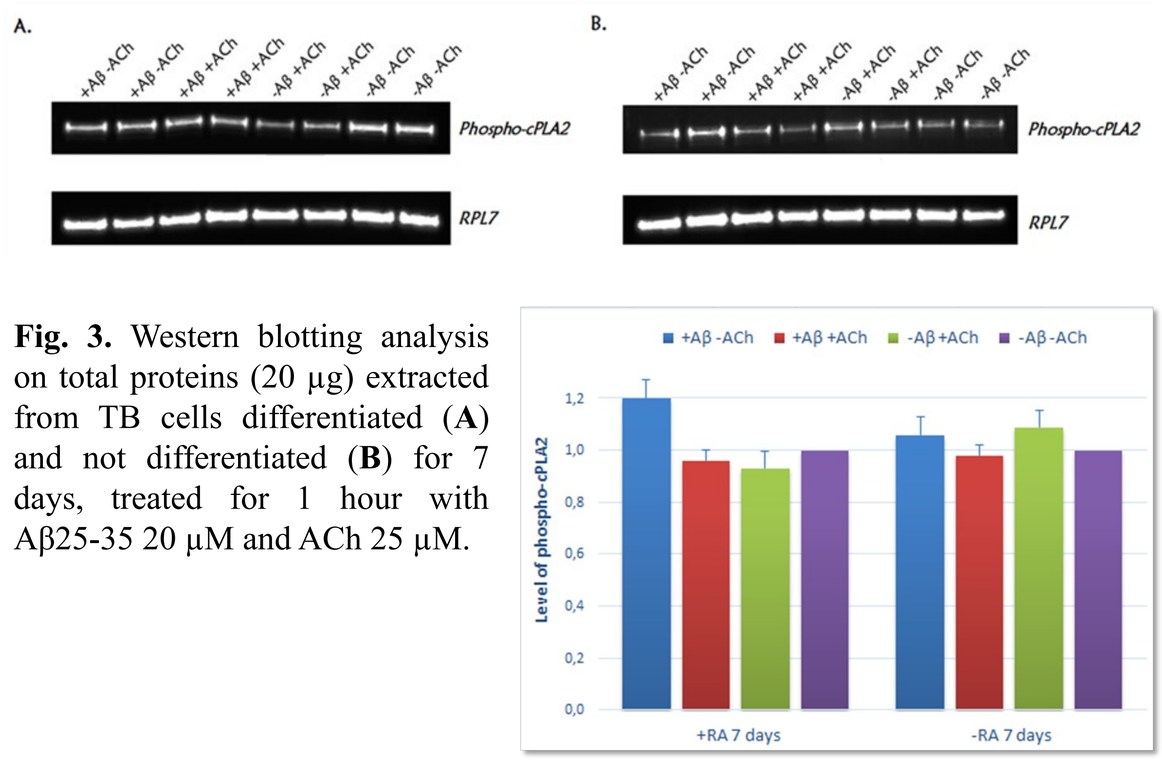

Fig. 3. Western blotting analysis on total proteins (20 µg) extracted from TB cells differentiated (**A**) and not differentiated (**B**) for 7 days, treated for 1 hour with Aβ25-35 20 µM and ACh 25 µM.

Discussion and conclusions

The active form of cPLA2 (pSer505) is both 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.

[1] Fiandaca MS., Mapstone ME., Cheema AK., Federoff HJ. (2014). The critical need for defining preclinical biomarkers in Alzheimer's disease. *Alzheimer's & Dementia 10 (2014) S196-S212.*

[2] Kanfer JN., et al. (1996). Phospholipid metabolism in Alzheimer's disease and in a human cholinergic cell. *J Lipid Mediat Cell Signal. 14(1-3):361-3.*

[3] 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.*