Abstract

Introduction: Alzheimer disease (AD) is a specific type of dementia with a complex pathology. A formation of extracellular insoluble amyloid-beta (Aβ**)** fibrils from precursor protein (AβPP) has been identified as one of the main causes of AD. There are several enzymes involved in a production of Aβ**;** β-secretase has been recently considered as a potential target for AD treatment by methods of passive immunization. A monoclonal antibody (2B12) has been developed and proved that it binds in the vicinity of β-secretase cleavage site on AβPP and prevents the cleavage of AβPP by steric hindrance. 2B12 is known to binds to AβPP at the cell surface and the whole complex after internalization inhibits β-secretase activity.

Methodology: The astrocytoma MOG-G-UVW (MOG) and the Human-CNS derived neuroglioma (H4) living cell lines were used as a model of AD. Incubated with 2B12, another AβPP – binding antibody (N-terminal) and several organelle markers (OM) under various conditions, the cells were fixed and stained by the method of sequential immunocytochemistry (ICC) and visualized using fluorescent microscope.

Results: The experiments with MOG/H4 cells demonstrated that the intake of 2B12/N-terminal antibody into the cells is time-dependant; the best labelling was after 4 hours of incubation for 2B12 and MOG /H4 cells and after 2 hours of incubation for N-terminal antibody and H4 cells. Both cell lines have been incubated with 2B12 or N-terminal antibody, with OM and then visualized by sequential ICC staining. The absence or presence of antibody – OM co-localization enabled to determine organelles most likely to be involved in the process of antibody - AβPP complex internalization. However, the co-localization was properly observed only when using H4 cells and N-terminal antibody. Early endosomes appeared to be the compartment most likely to involved in the process of internalization, lysosomes as quite probable compartment, whereas trans-Golgi network, mitochondria, plasma membrane and endoplasmic reticulum as non-involved compartments.

Conclusion: The experiment with living cells confirmed the hypothesis of specific time-dependent internalisation of the 2B12/N-terminal antibody complex and promoted the importance of endocytic (endosomal/lysosomal) pathway in AβPP processing.