

Effect on Hep-1 cell Calcium Homeostasis after A β ₂₅₋₃₅ Injury

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Abstract. The purpose of this study was to observe the effect of β -amyloid protein injury on intracellular calcium homeostasis in Hep-1 cells. A β ₂₅₋₃₅ was used to build the Hep-1 cell model of AD. Intracellular Calcium measurements was performed by incubation with the calcium indicator probe and then observed under laser confocal microscopy. Ca²⁺ release from intracellular stores was significantly increased in immortal human endothelial cells (SK-Hep-1) after injury induced by A β ₂₅₋₃₅. No significant difference was found in CCE from injured cells to controls. Increased β -amyloid protein production and intracellular calcium homeostasis disturbance were pathological mechanisms relate to AD.

Introduction

Alzheimer's disease (Alzheimer's disease, AD) is the most common neurodegenerative disease in eldly. And its etiology and pathogenesis is not entirely clear. More and more studies showed that vascular factors play an important role in the pathogenesis of AD. Calcium ions are important intracellular second messenger involved in a large number of signal transduction pathways, including cell proliferation, differentiation, activation of transcription factors and thus affect the synthesis of transcription and expression of genes, proteins and steroids, modification and protein folding, secretion, and apoptosis [2]. Previous studies have shown that intracellular calcium balance disorders can cause a reaction of cell damage, leading to the onset of AD. In the present study, calcium homeostasis changes that were observed in endothelial cells. Intracellular calcium release and capacity-dependent calcium flux (capacitative calcium entry, CCE) changes were detected to investigate its relationship with the pathogenesis of AD. With a view to expand the pathogenesis of AD research and treatment to provide a theoretical basis for the idea.

Materials and methods

Materials and reagents.

A β ₂₅₋₃₅, FBS, DMEM medium, more drugs were purchased from Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd. Calcium fluorescent probe Fluo-3/AM, Pluronic F-127 was purchased from Molecular Probe, USA, Thapsigargin available from AG Scientific, Inc. USA.

Methods.

1) Cell culture and AD cell model construction

SK-Hep-1 cells: SK-Hep-1 cell line was purchased from Shanghai Cell Bank. EMEM medium containing 10% fetal bovine serum, 37 °C, 5% CO₂, saturated humidity cultivation, 0.25% Trypsin / 0.03% EDTA digestion and passage, after disengagement in A β ₂₅₋₃₅ used in the experiment.

2) The A β ₂₅₋₃₅ incubation

A β ₂₅₋₃₅ fragment was selected to incubate the cells, with triple-distilled water formulated to 100 μ mol / L, filtration, packaging, -20 °C cryopreservation before use of the aging process and preparation of the required concentration. (The A β ₂₅₋₃₅ was dissolved in DMSO, diluted with DMEM, incubated at at 37 °C 7-14 d, namely aging)

3) Construction of AD cell model:

AD Group: Using A β ₂₅₋₃₅ induced SK-Hep-1 cells to establish AD cell model, A β ₂₅₋₃₅ final concentration of 20 μ mol/L, the contact 6h, 12h, 24h. Normal control group and the experimental

AD group, AD group into contact with 6h group, the contact group and the contact 24h and 12h.

4) fluo-3/AM stock solution and application of liquid preparation

The fluorescent probe fluo-3 / AM 100ug 88.5ul fully dissolved in anhydrous dimethyl sulfoxide (DMSO) stock solution preparation, storage concentration of about 1000uM, -20 °C dark storage for later use. Application of the liquid prior to use temporary preparation, diluting said fluo-3 / AM diluting said stock solution fluo-3AM was obtained in the culture medium or balanced salt solution with no interference. Which fluo-3AM final concentration 5 ~ 10umol / L in the application solution.

5) Pluronic F-127 stock solution and application of liquid preparation

Paired with DMSO 20% (w/v) stock solution was heated to 40 °C and maintained 20 minutes to facilitate dissolution. With a good F-127 in order to prevent crystallization, storage at room temperature.

6) Intracellular calcium ion concentration calculation

Intracellular calcium ion concentration can be estimated with the following formula: $[Ca^{2+}]_i = K_d (F - F_{min}) / (F_{max} - F)$. Where in the K_d of calcium fluorescent probe solution dissociation constant (in this experiment using a probe to 390nM); F_{min} is the absence of intracellular calcium mean fluorescence intensity; F_{max} fluorescence intensity of intracellular calcium saturation time; F for the intracellular calcium concentration measured under normal intracellular fluorescence intensity and fluorescence probe and calcium. F_{min} of measurement: the extracellular calcium-free conditions, the measured fluorescence intensity F_{min} . F_{max} of measurement: adding calcium-free buffer containing 1% (v / v) Triton CaCL2100 mM X-100, while the addition of calcium ionophore, in intracellular calcium concentration reaches saturation when measured fluorescence intensity F_{max} .

7) Mark intracellular free Ca^{2+} with Fluo-3AM

One day before the assay cells were 50% confluent seeded in Petri dish experiment that experiment, 70% to 80% of the cells accounted bottom of the plate and to ensure the growth of cells dispersed, not accumulation. Measured before, with calcium and magnesium-free PBS cells were rinsed three times, and wash away the residue, and other contaminants on the cell surface. Was added to the Petri dish 5μM Fluo-3 / AM ester, 0.5% (v / v) Pluronic F-127 application solution, so as to completely cover the cell, 37 °C cells were incubated for 30 minutes if there fluo within the first test cell under a fluorescence microscope -3- Ca^{2+} + green fluorescence, marking the completion of confirmation, the fluorescent probe was eluted with PBS extracellular unbound, on the basis of microscopy calcium ion concentration, added Thapsigargin (1.0 uM), continuously record changes in calcium ion concentration is detected, until the calcium plasma levels return to baseline, was added to the cells CaCL2 (1.0 mM), continuous inspection records calcium ions.

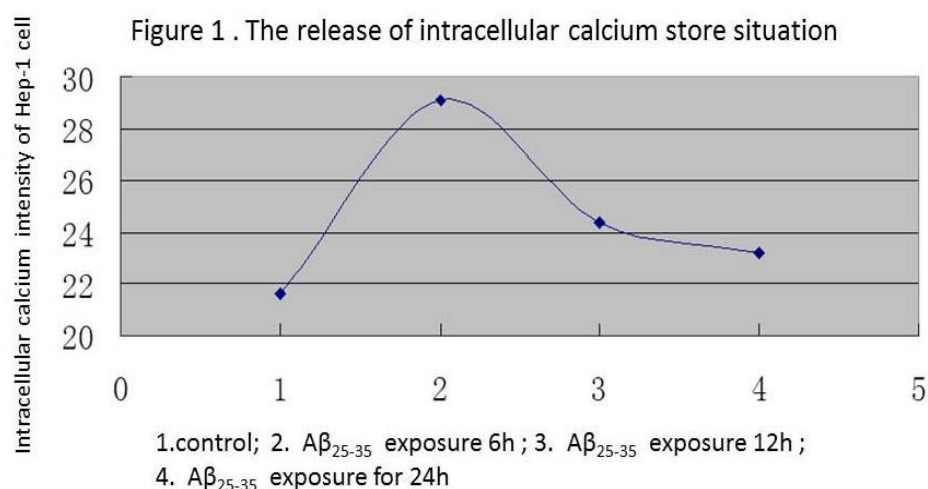
8) Statistical Methods

Results are mean ± standard deviation ($\bar{x} \pm s$) to indicate, using Instat statistical software, groups were compared using t test, $p < 0.05$ was considered statistically significant difference.

Result

2.1 The release of intracellular calcium store situation was detected after adding Tg. Compared with the normal control group, Hep-1 cell lines after exposure $A\beta_{25-35}$ increased release of calcium ions (Mean ± SD = mean peak increase in amplitude ± standard deviation: $A\beta_{25-35}$ contacts 6h group, 29.08 ± 1.01 ; $A\beta_{25-35}$ contact with 12h group, 24.39 ± 1.07 ; $A\beta_{25-35}$ contact with 24h group, 23.18 ± 1.23 ; control group, 21.61 ± 0.82 . $A\beta_{25-35}$ contacts 6h group vs in the control group, $A\beta_{25-35}$ contacts 6h 12h group vs $A\beta_{25-35}$ contacting the contact group and $A\beta_{25-35}$ 24h 12h group vs $A\beta_{25-35}$ exposure group, $p < 0.01$, as shown in Figure 1. The cells within the endoplasmic reticulum calcium store depletion, the addition of calcium load (CaCL2, 1 mM), CCE detected change, compared with the control cells, the outer Hep-1 cell lines after exposure $A\beta_{25-35}$ calcium influx without significant difference.

Discussion



With the entire world's population ages, the incidence of dementia has been increased markedly, AD pathogenesis research has been a hot and difficult field of neurology. Recent years, neurogenic and angiogenic mechanisms researches caused for AD onset made some likely progress, the current findings are more inclined to that the pathogenesis of AD is a result of many factors working together. Different pathogenesis of AD, and also other neurodegenerative diseases, as the process of interaction of multiple factors that may go through a number of common mechanisms, such as inflammatory, metabolic abnormalities and so on. Abnormal amyloid deposition is a major pathological features of AD, suggested that protein metabolism dysfunction plays an important role among the pathogenesis of AD, so as a functional changes of organelle ER in protein metabolism process also become focus in sporadic AD and familial AD pathogenesis researches at present. Wherein intracellular calcium metabolism function changes to reflect the endoplasmic reticulum to a certain extent. In this study, changes in calcium metabolism in endothelial cells after detecting amyloid damage, designed to explore post-amyloid injured endothelial cell calcium metabolism changes in the law, provide the basis for vascular lesions of AD.

Intracellular free Ca^{2+} is the process of cell metabolism important second messenger involved in the regulation of many physiological processes, and its dysfunction involved in many pathological processes. Intracellular Ca^{2+} concentration [1] There are two ways: one is the extracellular Ca^{2+} through the cell membrane into the cytoplasm Ca^{2+} channel, and the other is calcium pool of intracellular calcium released into the cytoplasm, after these additions Ca^{2+} routed through the cell membrane extracellular Ca^{2+} pump or by sarcoplasmic reticulum / endoplasmic reticulum Ca^{2+} -ATP enzyme (sarcoplasmic / endoplasmic reticulum Ca^{2+} + ATPase, SERCA) to re-enter the intracellular calcium pool. American scholar Putney [2] proposed a volume of 1986 Ca^{2+} influx (capacitative calcium entry, CCE) hypothesis, that is, when the endoplasmic reticulum calcium pool Ca^{2+} concentration decreases, can be activated Ca^{2+} channels on the cell membrane caused by Ca^{2+} influx, which species Ca^{2+} influx is also used by some scholars call calcium pool operated Ca^{2+} influx (store-operated calcium entry, SOCE), or calcium storeoperated induced Ca^{2+} influx (store-depletion induced calcium influx) [3].

The intracellular Ca^{2+} concentration is reduced or intracellular Ca^{2+} concentration can lead to cell damage are death. Recent studies suggest that intracellular calcium signal transduction abnormalities are one of a series of features including AD neurodegenerative diseases [4-5]. However, for AD pathology findings of intracellular calcium changes vary greatly: increased [6-7], [8-9], and reduce no significant changes have been reported by [10]. The purpose of this study is to explore the Inner amyloid injured endothelial cells and calcium homeostasis of ways.

In this study, endothelial cell line constructed AD model after model within intracellular calcium homeostasis changes were detected. The results showed that in the Hep-1 cell lines amyloid injury increased release of calcium store and release significant increase in the early injury, described in

the AD pathogenesis reticulum calcium content changes are the main factors involved in amyloid damage a direct result of endothelial cells in the calcium homeostasis can lead to changes in the endoplasmic reticulum dysfunction, but the endothelial cell calcium homeostasis is the cause of AD onset, or the consequences of further damage caused by endothelial cells after onset AD requires further Discussion. And after amyloid damage endothelial cells increase is due to release of calcium needs to be further explored.

The results showed that A β generation increased endothelial cell damage Ca²⁺ balance. A β may cause lipid peroxidation, which in turn acts on the membrane, the membrane ion transport-dependent enzyme damage (including Na⁺ / K⁺ -ATP enzyme and Ca²⁺ -ATP enzyme) and some other activity, so that the latter maintain intracellular Ca²⁺ concentration gradient of disability, leading to intracellular Ca²⁺ balance disorder, causing cell damage. Results amyloid injury on endothelial cell lines of calcium release also further support the hypothesis about pathological changes of endothelial cells in the pathogenesis of AD has an important role for angiogenic doctrine AD provides a theoretical in accordance with.

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