Singlet Oxygen Prevents the Mitochondrial NADH Depletion in β-amyloid Induced Neurotoxicity

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Abstract - This paper demonstrates the results of the application of singlet oxygen in β-amyloid induced neurotoxicity. The experimental results of the use of 1267 nm laser for generating singlet oxygen in primary co-culture of cortical cells addition β- amyloid peptide fragment 25-35 (5 μM) are described.

Keywords: amyloid, mitochondria, neurons, singlet oxygen

I. INTRODUCTION

Alzheimer's disease is associated with the accumulation of aggregated β-amyloid (Aβ) in the senile plaque in the brain. Aggregated Aβ is neurotoxic and is very often used as a model of neurodegeneration of Alzheimer's disease. Aβ can directly affect the mitochondrial function of astrocytes and neurons by directly affecting mitochondrial complexes or through the production of superoxide anion which oxidises DNA and activates DNA-repairing enzyme poly(ADP-ribose)-polymerase (PARP) which consumes nicotinamide adenine dinucleotide, reducing the availability of the substrate, that lead to metabolic failure and cell death [1].Since Aß is a peptide containing ROS-sensitive residues, the conversion of these residues by singlet oxygen generated by a laser with a wavelength of 1267 nm alters the properties of Aß and may interfere with its self-assembly.[2]. Thus, the aim of this work was to apply singlet oxygen to reduce the toxicity of β-amyloid.

II. MATEIALS AND METHODS

For this study, we used a laser diode LD-1267-PM-500 from Innolume GmbH, which emits laser radiation at a wavelength of 1267 nm. The laser radiation was delivered to the study object via a specially manufactured fiber-optic cable made of quartz. This cable ensured minimal signal attenuation during transmission in the spectral range 400-2000 nm, with a numerical aperture of 0.22. To ensure the stability of our experimental studies, we installed the F280FC-C collimator (Thorlabs Inc.), which forms a parallel beam of laser radiation from the fiber-optic cable after it. This eliminated any dependence of the laser dose on the distance between the cable and the study object. The beam diameter at the collimator output was 3.4 mm. We prepared cortical cell co-cultures as described previously [3] from 2- to 4-day-old Wistar rat pups. Cortical cells were kept at 37 °C in an atmosphere containing 5% CO2 and 95% air, and fed twice a week. The cells were used after 12 to 15 days in vitro (DIV), unless otherwise specified. The cells treated with Aβ25-35 at a concentration of 5 μM (Bachem) were subjected to laser irradiation at a dose of 100 J/cm².For toxicity analysis, 20 μM of propidium iodide (PI), which has red fluorescence in damaged cells, and 4.5 μM of Hoechst 33342, which stains the nuclei blue, were simultaneously injected into the cells in order to count the total number of cells.

The autofluorescence of NADH co-cultures of neurons and astrocytes was imaged using an Olympus IX73P1F fluorescence microscope. To measure the dynamic range of the signal in relation to the total mitochondrial NADH pool and to normalize the data, the cells were exposed to two chemicals. Carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP) was used at a concentration of 1 μM to stimulate respiration and achieve maximum NADH oxidation. Sodium cyanide (NaCN) was also used at a concentration of 1 mM to inhibit respiration and achieve maximum NADH reduction.

III. RESULTS AND DISCUSSION

The results of the work showed that the use of Aβ caused a progressive decrease in the fluorescence of NAD(F)H of co-cultures of cortical cells, so that the autofluorescence signal dropped by 43±5.6% in 30 minutes. Exposure to singlet oxygen removed this effect. Singlet oxygen generated by laser exposure increased the NADH pool from 101,9±6.2 to 143,7±4,3 under conditions of Aβ neurotoxicity, and it also reduced the percentage of dead cells from 42,3±6,1%, to 15,0 ±1,9%. We can suggest that singlet oxygen can neutralize the toxicity of Aβ by changing the oligomerization of this peptide or protects DNA against oxidation that inhibits PARP. Noninvasive optical generation of singlet oxygen leads to increased cell survival and can evens of mitochondrial NADH depletion Aβ induced neurotoxicity.

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