Delayed Luminescence by an *in vitro* model for the study of mechanism involved in neurodegenerative diseases

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Abstract – The aim of the study was to investigated on Delayed Luminescence emitted by an *in vitro* model concerning the effects of amyloid-β (Aβ). Aβ is a neurotoxic protein overexpressed in Alzheimer's Disease (AD), which is also related to mitochondrial dysfunction. The experiments have been carried out on Olfactory Ensheathing Cells (OECs) cultures. The cells have been exposed to Aβ(1-42) native full-length peptide or to Aβ(25-35), a toxic fragment of Aβ, or Aβ(35-25), a no toxic Aβ fragment both in the absence and in the presence of Astaxanthin, a well-known antioxidant. DL intensity and kinetics changes also as a function of the treatments were measured. In particular, an increase in DL emission, when compared with the untreated cells used as control, was observed when the cells were exposed to Aβ(25-35) fragment. This emission appears quenched in presence of Astaxanthin.

INTRODUCTION

The research and the understanding of the new mechanisms involved in the mitochondrial quality control can allow to identify new therapeutic treatments of neurodegenerative diseases involving mitochondrial dysfunction [1]. In this context the analysis of Delayed Luminescence emitted by *in vitro* models for the study of Alzheimer's disease (AD) can give new insight of the alterations of mitochondria functional state and of the collective properties linked to the electronic transport in the mitochondrial respiratory chain.

It is well known that AD is characterized by intracellular and extracellular protein aggregates in the brain, including microtubule-associated protein tau and cleavage products of the amyloid precursor protein, beta-amyloid (Aβ). The accumulation of Aβ can result in oxidative stress, inflammation, and neurotoxicity, all of which lead to apoptosis and the deterioration of the neurotransmission system seen in AD. Several evidences have shown that elevated Aβ levels contribute to the mitochondrial abnormalities. Amyloid precursor protein (APP) and Aβ are found in mitochondrial membranes and interact with mitochondrial proteins. Overproduction of the APP and Aβ may affect dynamics of mitochondrial fusion/fission, impair mitochondrial transport, disrupt the electron transfer chain, increase reactive oxygen species (ROS) production, and alteration of calcium homeostasis, which are the hallmarks of mitochondrial diseases. Further, a significant reduction of the protein content of Complex I of the respiratory chain, of its activity and of energy production, characteristic signs of the reduction of energy metabolism associated to AD, were observed.

Delayed Luminescence (DL) is the phenomenon of photo-induced and ultra-weak emission of optical photons. Previous researches carried on Jurkat-T leukemic cells, follicular tumors and glioblastoma [2-5], also using substances that target the mitochondria, and in particular the process of electron transfer in Complex I, have shown how the DL is able to detect the activation of apoptotic pathways and oxidative stress.

The investigation was performed on an *in vitro* animal model for the study of AD by using primary cell cultures of Olfactory Ensheathing Cells (OECs) [6], glial cells of the olfactory system and whose loss of functionality is the first marker of the AD. The variation of the DL spectral emissions (both in terms of kinetics and intensity) was evaluated on cell cultures treated with Aβ peptides and fragments also following pretreatment with molecules having antioxidant properties.

METHODOLOGY

OECs were isolated from olfactory bulb of 2-day old mouse pups (P2) provided by Envigo RMS s.r.l. Italy, stock: C57BL6J. The cells were suspended in Dulbecco's Modified Essential Medium (DMEM), containing 10% of heat inactivated Fetal Bovine Serum (FBS), antibiotics, and plated in 75 cm^2 flasks. To reduce fibroblast growth, after 24 h 10 μM cytosine arabinoside was added. Then cell cultures were incubated and maintained at 37° C in ambient of

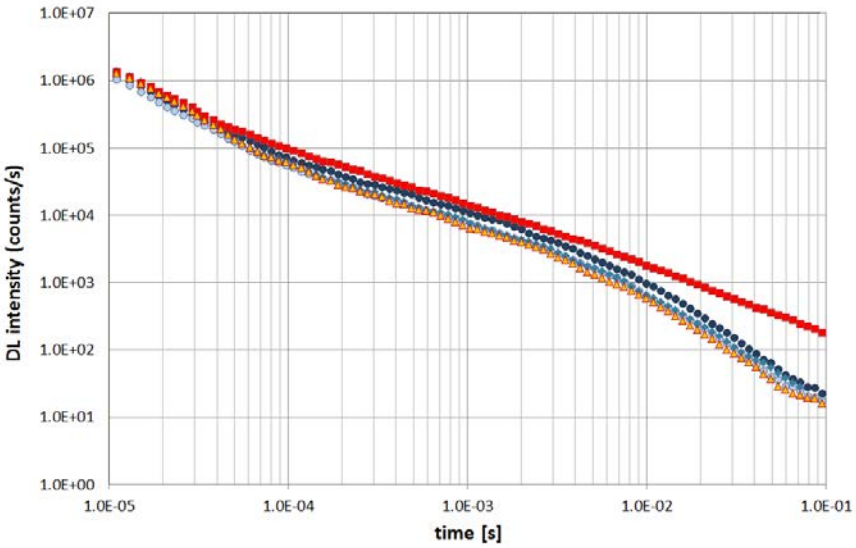

Figure 1: Example of time trend of DL emitted by in visible range (350÷850 nm) by untreated OECs cells cultures (dark grey circle) and OEC cell cultures treated with: (open circle) 10µM DMSO @24h; (diamond) 10µM Aβ(1-42) peptide @24h; (square) 10µM Aβ(25-35) fragment @24h; (triangle) 10µM Aβ(35-25) fragment @24h. Results are expressed as the mean ± SE of the values of at least three technical replicates. Error bars are into the marker sizes.

humidified air and CO_2 (95-5%). Medium was changed 2 times a week.

OECs were exposed for 24 h to 10 µM Aβ(1-42) native full-length peptide or to Aβ(25-35), a toxic fragment of Aβ, or Aβ(35-25), a no toxic Aβ fragment, used as a control, both in the absence and in the presence of 100 µM Astaxanthin, a well-known antioxidant. Since Astaxanthin was solubilized in DMSO, an aliquot of cells was treated with the same concentration of DMSO and used as a control. The biological alterations expressed by Aβ exposure on OECs were evaluated through the study of some cytoskeletal markers, of the proteins involved in excitotoxicity (eg, TG2), of the mitochondrial potential, intracellular ROS levels and activation of the apoptotic pathway. The more adequate concentrations and time of expositions were established by MTT test. The biological results showed a good ability of Astaxanthin to recovery the damage induced by Aβ treatments. To perform DL measurements, after treatments, cells were immediately trypsinized. The pellet was resuspended in 80 µl of fresh PBS and analyzed by DL spectroscopy.

The DL experiments were performed using a dedicated equipment allocated in Catania at Laboratori Nazionali del Sud of National Institute for Nuclear Physicsy 20µl single drop of cell culture suspension having a cell density of not less than 10^6 cells/ml.

RESULTS

DL emitted by at least three biological replicates of treated cell line cultures was studied. During each run DL measurements were performed on at least three technical replicates. The photoemission was recorded between 10 µs and 10 ms after laser (337 nm) excitation. Figure 1 shows typical time trend decays in the visible range (350÷850 nm) by untreated OECs cells cultures and OEC cell cultures treated for 24h with 10µM DMSO, 10µM Aβ(1-42) peptide, 10µM Aβ(25-35) fragment, 10µM Aβ(35-25) fragment, respectively. The effect of amyloid-β fragments on DL kinetic is evident. Astaxantin induced a quenching behaviour on DL emission (data not showed). This investigation correlates the DL emission with the existing damage and the possible recovery induced by treatments aimed at the prevention of the disease.

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