Abstracts

EXTRACT OF LIMONIUM GMELINII ATTENUATES Aβ- AND H2O2- INDUCED OXIDATIVE RESPONSE IN CEREBRAL ENDOTHELIAL CELLS

Y.Shayakhmetov1, A.Jaguparov, A.Tsoy*

1) School of Science and Technology, Nazarbayev University; 2) Center for Life Sciences, Nazarbayev University, Astana, Kazakhstan; *Andrey.tsoy@nu.edu.kz

Introduction. Alzheimer’s disease (AD) is a chronic neurodegenerative disorder which affects approximately 10% of the population at age 65 and 40% of people over the age 80. Clinically, AD pathology is characterized by an increased deposition of amyloid-β peptide (Aβ) in the brain, and a progressive impairment of cognition and memory of affected individuals. Blood Brain Barrier (BBB) dysfunction is observed in all of the stages of AD, and may even precede neuron degeneration in AD brains. During the early stages of AD, microvasculature deficiencies and hypertrophy of astrocytes are commonly observed. Numerous in vivo and in vitro studies have demonstrated that the vascular deposition of Aβ induces oxidative stress in cerebral endothelial cells (CECs). Aβ-induced oxidative stress in cells, in turn, initiates a cascade of redox reactions leading to apoptosis and neurovascular inflammation. Consequently, antioxidants are considered as therapeutic agents in Aβ-induced CECs damage.

It has been reported previously, that reach with polyphenols extract of Limonium gmelinii (a plant widespread on the territory of Kazakhstan) exerts a wide range of therapeutic action. Here, we studied the effect of polyphenols extracted from roots, stems and leaves of Limonium gmelinii on the H2O2- and Aβ42-induced oxidative responses in cerebral endothelial cells in vitro.

Materials and methods. Mouse bEnd3 line (from ATCC) of cerebral endothelial cells (CECs) was applied in this research as following: control; cells incubated with 5 μM of Aβ42 for 2 hrs; cells incubated with 0.5 mM of H2O2 for 30 min; cells pretreated with extracts of Limonium gmelinii followed by Aβ42 or H2O2 exposure; cells treated with extracts of Limonium gmelinii only.

The cellular levels of ROS were measured with CM-H2DCF-DA (Invitrogen, Cat. No. C6827). The CM-H2DCF-DA stock solution (5 mM) was dissolved in DMSO and diluted in the culture medium to a final concentration of 1 μM just before addition to the cells. For ROS measurements, cells were starved for 12 hr, rinsed twice with warm phenol free DMEM, and incubated with CM-H2DCF-DA for 1 hr at 37°C in dark. The CM-H2DCF-DA fluorescence was measured on a plate reader Synergy H1 Hybrid Reader with excitation and emission wavelengths of 492 nm and 520 nm.

For cell imaging we applied DHE staining. DHE reacts with O2- to produce oxyethidium (oxy-E), a highly fluorescent product, which binds to DNA and causes an increased fluorescent intensity of the cell nuclei.

Results. We have shown that H2O2, increased CM-H2DCF-DA fluorescence by almost 100%, and Aβ42 - by 75% compared to the control. At the same time, H2O2 stimulated ROS production in CECs was attenuated by the pretreatment with all three extracts of Limonium gmelinii. Similarly, Aβ42-induced oxidative response in cerebral endothelial cells was suppressed by Limonium gmelinii polyphenols as well. As a positive control, the ROS scavenger NAC also reduced Aβ42-stimulated ROS production. Furthermore, we observed significant decrease in CM-H2DCF-DA fluorescence when the cells were treated with extracts of Limonium gmelinii alone.

Conclusions. This study demonstrated that extracts of Limonium gmelinii could attenuate H2O2- and Aβ42-induced reactive oxygen species (ROS) generation in cerebral endothelial cells. Thus, their protective potential requires further investigations.