# ROLE OF ROS IN A042 MEDIATED CELL SURFACE P-SELECTIN EXPRESSION AND ACTIN POLYMERIZATION

**A. Tsoy*[1,2], S. Askarova[1], T. Shalakhmetova[2], B. Umbayev[1], S. Adambekov[1], Z. Zhumadilov[1]**

1) Center for Life Sciences, Nazarbayev University, Astana, Kazakhstan; *andrey.tsoy@nu.edu.kz; 2) Faculty of Biology and Biotechnology, Al-Farabi Kazakh National University, Almaty, Kazakhstan

**Introduction.** Blood-brain barrier dysfunction plays an important role in the onset and progression of Alzheimer's disease (AD). In the AD brains an increased deposition of A(3 in the cerebral vasculature has been found to correlate with increased transmigration of blood-born inflammatory cells and neurovascular inflammation [1]. Transmigration of leukocytes into brain parenchyma is a sequential process starting with primary capture to the endothelium and rolling adhesion mediated by tethering on selectins and selectin ligands. P-selectin is a type I transmembrane cell adhesion molecule which is stored in cytoplasmic Weibel-Palade bodies (WPb) and can be mobilized on the endothelial cell surface within minutes upon exposure to different pro-inflammatory agents; then it's rapidly cleared through endocytosis in 30 min. It has become evident that A(3 activates cerebral endothelial cells (CECs) and induces mobilization of P-selectin to the cell surface [2]. However, expression mechanisms of this receptor on the surface of brain endothelial cells under administration with A(342 remain unclear. Since P-selectin is stored in WPb, and there is an evidence of active role of ROS (reactive oxygen species) and actin filaments in the different stages of WPb exocytosis, in this study we examined the dynamic of P-selectin expression on the endothelial cell surface activated by A(342 in relation to ROS and actin polymerization.

**Materials and methods.** We used immortalized CECs (bEnd3) as follows: control; cells incubated with A(342 for 10, 30 and 60 min; cells incubated with 30 mM of antioxidant N-acetylcysteine (NAC) for 1 hr; cells pretreated with NAC followed by A(342 exposure. Fluorescent microscopy of anti-P-selectin, dihydroethidium (DHE), and oregon green phalloidin was used to quantify the surface P-selectin expression, ROS production, and actin polymerization.

**Results.** We have observed that A(342 promoted ROS production up to 34.5%, 61%, 83% and increased P-selectin fluorescence up to 28%, 46% and 54% after 10 min, 30 min and 60 min of treatment, respectively. In contrast, pre-incubation of cells with NAC prevented the superoxide production and attenuated A(342 induced P-selectin activation. At the same time, NAC alone significantly reduced DHE fluorescence, but did not affect expression of the adhesion receptor. QIM data also demonstrated that A(342 promoted actin polymerization in cells, while pretreatment with NAC was able to suppress A(3-induced actin polymerization and cytoskeletal reorganization in bEND3 cells.

**Conclusions.** The results of our study have indicated that A(342 induces expression of P-selectin on the surface of bEnd3 cells and promotes actin polymerization in a time dependent manner, and all these events correlate with ROS generation. The rapid, posttranslational cell signaling response mediated by ROS, may well represent an important physiological trigger of the micro vascular inflammatory response in AD and requires further investigations.

**References.**

1. Grammas P., Ovase R. (2001). Neurobiology of Aging, 22: 837-842.
2. Askarova S., Sun Z., Sun G.Y., Meininger G.A., Lee J.C. (2013). PLoS ONE 2013, 8:e60972.