# RAGE TARGETED STRATEGIES FOR ALZHEIMER'S AMYLOID B PEPTIDE INDUCED BLOOD BRAIN BARRIER DYSFUNCTIONS

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**INTRODUCTION.**

Alzheimer's disease (AD) is a chronic neurodegenerative disorder which affects approximately 10% of the population at the age of 65 and 40% of people over the age 80. In Kazakhstan, by the end of 2011, 7.4% of the population was aged over 65. Currently, AD is on the list of the diseases with no effective treatment, thus, study of molecular and cellular mechanisms of the AD progression is of a high scientific and practical importance. ROS (reactive oxygen species) overproduction plays an important role in the pathogenesis of AD. Although there is evidence that amyloid-β-peptide (Aβ) mediates oxidative damage to astrocytes and cerebral endothelial cells (CECs) mainly through the activation of NADPH oxidase, the mechanism of NADPH oxidase activation by Aβ has yet to be elucidated. Since the receptor for advanced glycation endproducts (RAGE) has been postulated to function as a signal transducing cell surface acceptor for Aβ, we hypothesize that $A\beta_{1\text{-}42}$ oligomers induce ROS generation from NADPH oxidase and trigger downstream signaling pathways for phosphorylation of cytosolic phosphorylase A2α (cPLA2α) through the binding of Aβ to RAGE.

**MATERIALS AND METHODS.**

In this study, immortalized CECs (bEnd3) and primary rat astrocytes were applied. To confirm Aβ-RAGE binding, we examined the quantitative immunofluorescence microscopy (QIM) of cellular surface RAGE for primary astrocytes and bEnd3 cells pretreated with oligomeric Aβ1-42 at +4°C (conditions in which the internalization of surface receptors is suppressed) and stained with antiRAGE primary antibody ($Ab_{RAGE}$). To quantify the ROS production, we applied fluorescence microscopy of dihydroethidium (DHE) in the cells. Confocal immunofluorescence microscopy of double immunofluorescent labeled gp91-phox and p47-phox subunits was performed to characterize NADPH oxidase complex assembly. Western blot analysis was used to characterize phosphorylation of cPLA2α

**RESULTS.**

QIM analysis has demonstrated that $A\beta_{1\text{-}42}$ competes with $Ab_{RAGE}$ to bind to RAGE at the surface of the cells. Besides, $Ab_{RAGE}$ totally inhibited $A\beta_{1\text{-}42}$ induced ROS production in the CECs and astrocytes. Confocal microscopy study has demonstrated that $A\beta_{1\text{-}42}$ increased the colocalization of cytosolic subunit p47-phox of NADPH oxidase with its membrane subunits gp91-phox, suggesting that Aβ enhances NADPH oxidase complex assembling, which was also suppressed by $Ab_{RAGE}$. Western blot analysis has shown that incubation of both CECs as well as astrocytes with $A\beta_{1\text{-}42}$ significantly increased phosphorylation of cPLA2α, while pre-treatment of the cells with $Ab_{RAGE}$ prior to stimulation with $A\beta_{1\text{-}42}$ totally inhibited cPLA2α phosphorylation.

**CONCLUSIONS.**

This study demonstrated that Aβ binding to RAGE at the CECs and astrocytes surface is required for NADPH oxidase complex assembling, resulting in subsequent ROS generation and phosphorylation of cPLA2α. Therefore, understanding precise molecular mechanisms underlying Aβ mediated oxidative damage may provide new insights into the development of preventive and treatment strategies for AD.