ABSTRACT

Charles University, Faculty of Pharmacy in Hradec Králové

Department of Biological and Medical Sciences

Title of Diploma Thesis: Glucose effects on the expression of biomarkers of endothelial dysfunction in endothelial cells

Author: Kristína Schmidtová

Supervisor of Diploma Thesis: prof. PharmDr. Petr Nachtigal, Ph.D.

Background: The aim of this diploma thesis was to determine whether the new monoclonal antibody carotuximab affects the expression of endoglin and its transcription factors (KLF6 and RELA), as well as the expression of enzyme MMP-14 and soluble endoglin, in human aortic endothelial cells that were exposed to high glucose levels over the period of 3 days.

Methods: We used human aortic endothelial cells (HAEC), passage 5, and we exposed them to high glucose levels (45 mmol) for the period of 3 days and to carotuximab (300 μg/ml) for 12 hours. The results were compared with a control group which was exposed to normal glucose levels (5 mmol). Real-time PCR was used to measure the mRNA expression of endoglin, KLF6, RELA and MMP-14. Protein levels of endoglin and the enzyme MMP-14 were measured by flow cytometry. ELISA method was used to measure the level of soluble endoglin.

Results: Gene expression of endoglin, transcription factors and the enzyme MMP-14 was significantly increased after exposure to high glucose levels. Following the addition of carotuximab to the high glucose group, there was a significant decrease in KLF6 mRNA expression. No significant difference was observed for endoglin, RELA and MMP-14 mRNA expression. Significant increase in endoglin protein level was observed in high glucose group but after addition of carotuximab there was a significant decrease in endoglin protein level. The protein level of MMP-14 decreased significantly after exposure to high glucose, however the addition of carotuximab led to increase in MMP-

14 protein level. Soluble endoglin levels in the medium were increased in high glucose group.

Conclusions: The results showed that carotuximab has an effect on the expression of endoglin and its transcription factors, especially KLF6, but also on the enzyme MMP-14 in high glucose-induced endothelial dysfunction. The mechanism by which the TRC causes these changes needs to be the subject of further studies.

Keywords: carotuximab, endoglin, endothelial dysfunction, hyperglycemia, MMP-14, soluble endoglin