Abstract

Charles University, Faculty of Pharmacy in Hradec Kralove
Department of Biological and Medical Sciences
Candidate: M. Sc. Matej Vicen
Supervisor: Prof. PharmDr. Petr Nachtigal, Ph.D.
Title of Doctoral Thesis: Membrane endoglin and its role in pathogenesis of endothelial dysfunction *in vitro*

Membrane endoglin (Eng) is a transmembrane glycoprotein that acts as a co-receptor in the transforming growth factor β (TGFβ) signalling cascade. Its expression and function have been extensively studied predominantly in relation to endothelial function under various pathological conditions, including hypercholesterolemia and atherogenesis. However, its role in the development of endothelial dysfunction remains controversial. Eng contributes to the formation of nitric oxide (NO) and is crucial in *in vivo* conditions for the development of the cardiovascular system and especially the heart. On the other hand, it may play an important role in inflammatory adhesion and transmigration of leukocytes to the endothelium, thus contributing to the development of endothelial dysfunction as the first stage of atherogenesis.

Experiments on endothelial cells isolated from various blood vessels are used to study short-term effects of substances and drugs on the endothelium. One of the effects studied in endothelial cell cultures is the ability of the substance to induce endothelial dysfunction. Endothelial dysfunction is defined in *in vitro* conditions as a state with increased expression of adhesion molecules and increased adhesion and transmigration of immune cells through the endothelial monolayer.

The aim of this dissertation thesis is to investigate the effects of soluble endoglin (sEng), oxidized cholesterol, and selected macrophage secretes on the vascular endothelium. The main focus is on monitoring the membrane endoglin (Eng) expression and the expression of individual members of its signalling cascade and the expression of pro-inflammatory biomarkers of endothelial dysfunction in relation to the development of endothelial dysfunction *in vitro*.

Firstly, we investigated the role of sEng in the process of endothelial dysfunction development. Human umbilical vein endothelial cells (HUVEC) were treated with two different

doses of sEng (40 or 500 ng / mL) for 16 hours. We demonstrated the induction of inflammatory response by increased activity of nuclear factor kappa B (NF-κB) and interleukin-6 (IL-6) and an increase in membrane endoglin expression. This suggests to pro-inflammatory effects of soluble endoglin and a significant role of membrane Eng in the early stages of endothelial dysfunction development.

Subsequently, we focused on the role of Eng in the development of oxidized cholesterol-induced endothelial dysfunction. In this case, we used human aortic endothelial cells (HAEC) pretreated with various oxidized cholesterols (oxysterols). Preliminary experiments showed that only 7-ketocholesterol (7K) was able to induce endothelial dysfunction. 7K was able to increase the expression of adhesion molecules and Eng as well as increase adhesion and transmigration of monocytes through the endothelial monolayer. The decrease in Eng expression through gene silencing resulted in decreased adhesion and transmigration of monocytes across the monolayer. The results of this study emphasize the crucial role of Eng in the development of endothelial dysfunction under *in vitro* conditions.

Finally, we focused on the effect of reduced Eng on the surface of endothelial cells. HUVECs were incubated in medium containing macrophage-derived matrix metalloproteinase 12 (MMP12). MMP12 was able to cleave Eng from the cell surface, resulting in the development of endothelial dysfunction characterized by the reduced ability of cells to form vessels and heal wounds under *in vitro* conditions.

The results of this dissertation thesis confirm the importance of physiological expression of membrane endoglin by endothelial cells and suggest that altered Eng expression may lead to the development of pathological changes in the vascular endothelium.