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Evaluatiton of the expresion and co-expresion of endoglin and VCAM-1 in the aorta on apoE-deficient mice
Diploma thesis
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Background: The aim of this work was to monitor the expression and a possible co-expression of endoglin (TGF-β receptor III) and VCAM-1 in mouse aortic endothelium. As an experimental model, we used the mouse strain C57BL/6J, that was genetically modified, and is characteristic by a deficit of apolipoprotein E.

Methodes: In the study, we focused on testing the mouse strain C57BL/6J with gene knockout for apolipoprotein E in different stages of the atherosclerotic process. 10 weeks old female mice were divided into three groups and fed diets with different content of cholesterol. One experimental group was fed a standard diet (called "chow type diet") for a period of two months. The other two groups were fed a diet containing 21% fat (called "Western type diet") for a period of two and four months. For the determination of the levels of total cholesterol, a biochemical analysis of blood was performed. Obtained parts of ascending aorta were analyzed by ImmPRESS^{TM} immunohistochemical method with the detection reagent DAB. Descriptive markers were endoglin (ENG), and VCAM-1.

Results: Biochemical analysis showed levels of cholesterol in a standard laboratory diet group approximately 10mmol/l. There were no atherosclerotic lesions observed in ascending aorta in this group of mice. In contrast, higher levels of cholesterol (and atherosclerotic plaques) were observed in the group of Western type diet fed for a period of two months, where the serum cholesterol levels in the blood were around 30mmol/l. The amount of cholesterol in the group of Western type diet lasting four months reached values in diameter 25mmol/l (with no significant difference in comparison with the previously mentioned group) and there was also an evidence of lipid lesions. Using immunohistochemical methods, we observed the expression of endoglin and VCAM-1 in atherosclerotic lesions dissections of the ascending part of the aorta. Expression of endoglin was detected exclusively in the endothelium of blood vessels.

Generally, there was a presence of VCAM-1 in atherosclerotic lesions and atherosclerotic vessel media. The expression of VCAM-1 was detected in the endothelium of aorta in mice on chow diet (no plaques). Endoglin molecule in this group in the endothelium was not detected, which means no co-expression of these proteins in mice on chow diet. Endoglin positivity was identified in mice on "Western diet" for a period of 2 months (Western 2m), exclusively in the endothelium covering the plaque. In this group, the positivity of VCAM-1 was restricted to the inner parts of atherosclerotic lesions and to areas of endothelium without atherosclerotic plaques. No significant co-localization of endoglin and VCAM-1 in Western 2m group was proved. Similar observations were recorded in the aorta of mice exposed to Western diet for 4 months (Western 4m). Endoglin has been localized to the endothelium of atherosclerotic plaques without significant co-expression with VCAM-1.

Conclusions: The results presented in this thesis demonstrate the effect of the diet with a high content of lipids to increase levels of lipoproteins in the blood, and the progression of atherosclerotic lesions in the dissections of the ascending aorta apoE-deficient mouse model. Expression of endoglin in particular, appeared in the endothelium of atherosclerotic plaques. It is therefore evident that the expression endoglin is stimulated by higher levels of cholesterol. Another result of the work is a probable absence of co-expression of endoglin and VCAM-1 during atherogenesis. It is therefore likely that ENG does not participate in the activation of endothelium, together with adhesion molecules, which are crucial for the vessel inflammation and thus atherogenesis in apoE-deficient mouse. Adhesion molecule VCAM-1 was detected in the non-atherosclerotic endothelium and is supposed to be a determinant of the vessel damage.