ABSTRACT

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Title of Doctoral Thesis **The effects of statins on TGF-β1 signaling in vivo and in vitro.**

This doctoral dissertation summarizes the effects of statins and inflammation on TGF-β1 signaling both in vivo and in vitro, especially the role of endoglin in the endothelial cells.
Transforming growth factor-β (TGF-β) is a multifunctional factor that regulates various cell specific functions in atherogenesis. Endoglin (TGF-βRIII, CD105) is known as accessory TGF β receptor, and it is able to modulate activity of TGF-β1 and TGF-β receptors. Previous studies demonstrated relation between endoglin and eNOS expression in blood vessels suggesting crucial role of endoglin in endothelial cell functions.
Our in vivo experimental studies, were focused on the expressions of TGF-β family members, on endoglin expression in aorta and its blood serum levels. We wanted to evaluate, whether these proteins are affected by cholesterol levels or atorvastatin treatment in different types of diet in mice. Immunohistochemical analysis showed the expression of endoglin in intact endothelium and in endothelium covering atherosclerotic lesions. Moreover, co localization of endoglin, Smad2, pSmad2/3 and eNOS in aortic endothelium was described. Cholesterol feeding resulted in an increased cholesterol and soluble endoglin levels in blood serum, with concurrent decrease of its expression in aorta. Atorvastatin treatment in mice on chow diet resulted in a significant increase of cholesterol, whereas endoglin levels in blood serum remained unchanged. Moreover, Western blot analysis revealed that atorvastatin treatment significantly increased the expression of endoglin and other proteins of TGF-β signaling in aorta beyond its lipid lowering effects. The administration of atorvastatin in cholesterol fed mice resulted in a significant decrease of cholesterol and endoglin levels in blood serum, with concurrent increase of endoglin expression in aorta. These data show that endoglin blood serum levels and its expression in aorta are affected by both atorvastatin treatment and cholesterol levels in ApoE/LDLR double knockout mice. Moreover, changes in blood serum levels are conversely related to endoglin expression in aorta. Thus, we propose that endoglin might be interesting blood marker, which could reflect atherosclerotic process in the vessel wall and efficiency of statin treatment. Moreover, activation of endoglin/ALK 5/Smad2/3 signaling might represent protective mechanism in aortic endothelium.
In our in vitro study, we focused on endoglin and eNOS expression during inflammation and after atorvastatin treatment in HUVEC cells. We also hypothesized whether statin induced eNOS expression depends on endoglin. Atorvastatin treatment significantly increased endoglin and eNOS expression in HUVECs. TNFα treatment for 16h significantly reduced endoglin expression, together with significant increase of soluble endoglin in medium. Atorvastatin pretreatment, before TNFα exposure, significantly prevented decrease of endoglin and eNOS expression mediated by TNFα. These results showed that inflammation reduces expression of endoglin and eNOS in HUVECs, which could be prevented by atorvastatin treatment. Moreover, endoglin siRNA experiment showed, that atorvastatin induced eNOS expression seems to be dependent on proper endoglin expression.
Endoglin and eNOS play important role in various cardiovascular pathologies including atherosclerosis, hypertension, diabetes, preeclampsia and hereditary hemorrhagic telangiectasia. For these reasons, we propose that statin effects on endoglin signaling and its regulation of eNOS expression may contribute to the positive effects on atherosclerosis and other diseases where lower levels of endoglin and eNOS were described including hereditary hemorrhagic telangiectasia. However, this hypotheses must be evaluated in clinical studies.