Abstract

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Title of Doctoral Thesis: Monitoring the effect of statins and soluble endoglin on markers of endothelial dysfunction in selected cell line and culture.

This doctoral thesis studied the effect of soluble endoglin on endothelial dysfunction markers *in vitro* in primary cell culture HUVEC and cell line HEK293T. Some experiments included in this thesis were made in cooperation in the laboratory of prof. Carmelo Bernabeu in the research center Centro de Investigaciones Biológicas (CIB) in Madrid.

Endothelial dysfunction is damage of endothelium when the balance between vasoconstrictor and vasodilatory mechanisms is disrupted, it´s leading to platelet aggregation, leukocyte adhesion and increased permeability of the endothelium. All these events represent the first step in the development of atherosclerosis and they are part of other cardiovascular diseases such as preeclampsia, type 2 diabetes mellitus, familial hypercholesterolemia. An important role in these diseases play endoglin, eNOS and soluble endoglin (sEng), which is cleaved by MMP- 14 from the membrane endoglin and released into the circulation. It was found that sEng is able to increase expression of cellular adhesion molecules and modify the TGF-β signaling in the endothelium.

Statins, which reduce LDL cholesterol and slightly increase HDL cholesterol and reduce the risk of a coronary event, play a significant role in the treatment of the above-mentioned diseases.

In this thesis, we focused on whether atorvastatin and high levels of sEng cause changes in the expression of markers of endothelial dysfunction, inflammation, oxidative stress, and whether sEng causes changes in TGF-β signaling *in vitro* in HUVEC and HEK293T. In our experiments, we worked with human recombinant endoglin.

Atherosclerosis is considered as an inflammatory disease. To mimic the inflammatory conditions we used TNFα cytokine for the treatment of cells, this resulted in decreased expression of both endoglin and eNOS. Preventive administration of atorvastatin, prior to administration of TNFα to HUVEC, prevented the decrease in endoglin and eNOS expression.

Furthermore, we found out that 40 ng/mL and 500 ng/mL of sEng increased a transcriptional activity of the NFκB and IL-6, that are major proinflammatory markers. We found out increased expression of these markers on the protein level. Therefore, we hypothesized that high levels of sEng can contribute to inflammation/endothelial dysfunction *in vitro* in human endothelial cells. The treatment with sEng induced significant increase in expression of the membrane endoglin. Moreover, we didn´t found any significant effect in expression of markers of oxidative stress HO-1, p22-PHOX, adhesion molecules VCAM-1, ICAM-1, inflammatory markers MCP-1, COX-2, markers of the endothelial function/dysfunction eNOS (Ser^{1177}) phosphorylation, and some of members of the TGF-β signaling cascade ALK-1, ALK-5, TGFβRII, BMPR2, PAI-1, BMP-2, BMP-4, pSmad1/5. Surprisingly, sEng treatment also resulted in an increased expression of the phosphorylated Smad2/3, but without any significant effects on PAI-1, suggesting that this pathway is not fully activated after sEng treatment. Another surprising result was decreased expression ID1.

In conclusion, the positive effect of atorvastatin on endoglin and eNOS signaling is an interesting result of our experiments, which could be the basis for clinical trials. We further showed that sEng treatment results in an activation of the proinflammatory markers NFκB and IL-6. Increased expression of this markers can contribute to the development of endothelial dysfunction, as an initial step in the development of atherosclerosis. The precise mechanism of this activation and its consequences remains to be elucidated.