Abstract

Charles University, Faculty of Pharmacy in Hradec Kralove

Department of Biological and Medical Sciences

Candidate: Msc. Barbora Vitverova

Supervisor: prof. PharmDr. Petr Nachtigal, Ph.D.

Title of Doctoral Thesis: Soluble endoglin role in the pathogenesis of endothelial dysfunction

The endothelium is a major regulator of vascular homeostasis and exerts a number of vasoprotective effects, including production of vasodilating agents, suppression of smooth muscle cell growth, or inhibition of inflammatory responses. Most of these effects are mediated by nitric oxide (NO), the most effective endogenous vasodilator. Decreased NO production or its activity is a hallmark of endothelial dysfunction leading to an impaired endothelial-dependent vasodilation. Endothelial dysfunction is the first and key step in the development of atherosclerosis and can be detected before structural changes in the vessel wall. Atherosclerosis is a complex inflammatory disease underlying many cardiovascular disorders including ischemic heart disease and represents the major cause of morbidity and mortality in developed countries. Statins are drugs of choice in the treatment of atherosclerosis and ischemic heart disease, which have been shown to reduce LDL cholesterol levels and modulate endothelial function with their pleiotropic effects.

Mouse models are currently the most commonly used animal model in the field of atherosclerosis research. Atherosclerotic changes can be achieved in a relatively short time frame, especially due to easy genetic manipulations and dietary interventions. These changes are detectable at both morphological and functional levels. Signaling pathway of transforming growth factor β (TGFβ) has one of the most significant roles during these processes.

The role of membrane endoglin (TGFβ receptor III, Eng), its soluble form and other members of TGFβ signaling pathway in the development of endothelial dysfunction in selected mouse models of atherosclerosis, was the key topic of manuscripts in this summary dissertation thesis. The main objective of this thesis was focused on the role of soluble endoglin (sEng) in the pathogenesis of endothelial dysfunction, especially because of increased sEng levels were detected in patients with hypercholesterolemia, atherosclerosis, arterial hypertension, and type II diabetes mellitus.

To determine the role of sEng in the development of endothelial dysfunction, we used transgenic mouse model with high levels of human sEng in plasma (Sol-Eng^{+} high) and their age matched littermates with low plasma levels of human sEng (Sol-Eng^{+} low) as a control group. Both groups were fed high fat diet to induce hypercholesterolemia and thus simulate the pathophysiological basis in patients with atherosclerosis in clinical practice. Study results showed, that increased sEng levels combined with mild hypercholesterolemia develop endothelial dysfunction like phenotype characterized by significantly impaired vasodilation and vasoconstriction in aortas of Sol-Eng^{+} high mice. Combination of increased sEng levels and hypercholesterolemia led to decreased expression of all members Eng/Smad 2/3/eNOS signaling in aorta resulting in impaired eNOS-dependent vasodilation and the development of endothelial dysfunction.

The next study was focused on the changes in membrane Eng expression and sEng levels during early development of endothelial dysfunction. We used two-month-old female apolipoprotein E and LDL receptor double deficient mice (ApoE$^{-/-}$/LDLR$^{-/-}$) and female C57BL/6J mice at the same age as a control group. Mice were fed chow diet and spontaneous development of hypercholesterolemia was expected in ApoE$^{-/-}$/LDLR$^{-/-}$ group. Study outcomes demonstrated, that hypercholesterolemia is accompanied by increased sEng levels, proinflammatory state and a disruption of NO metabolism. Furthermore, the results suggest that hypercholesterolemia leads to a decrease in membrane Eng expression and all members of its signaling resulting in the development of endothelial dysfunction in ApoE$^{-/-}$/LDLR$^{-/-}$ group.

The role of sEng as a possible inducer of endothelial dysfunction, that contributes to the development of hypertension was evaluated at the myocardial level. Transgenic mouse model expressing human sEng was used (Sol-Eng^{+} high and Sol-Eng^{+} low group). Mice were fed either chow or high fat diet. We assessed the hypothesis that increased sEng levels may result in expression changes in TGFβ signaling (Eng, Smad proteins), in expression of markers reflecting heart remodeling (PDGF, Co11A1), and inflammatory markers (VCAM-1, ICAM-1). Study results did not demonstrate the significant impact of increased sEng levels on morphological structure of myocardium (profibrotic and degenerative cardiomyocyte changes), development of inflammation or influence of TGFβ signaling.

The results of the studies helped to clarify the role of both forms of endoglin in the development of endothelial dysfunction. Decreased membrane Eng expression accompanies the development of endothelial dysfunction in mouse aorta. It can be also concluded, that increased sEng levels can be considered as a risk factor of cardiovascular disease development.