Abstract

Charles University in Prague
Faculty of Pharmacy in Hradec Králové
Department of Biological and Medical Sciences
Candidate: Mgr. Zbyněk Stráský
Supervisor: Doc. PharmDr. Petr Nachtigal, Ph.D.
Title of Doctoral Thesis: Possibilities to influence atherogenesis in experimental animal models and endothelial cells.

This guided final thesis studied the Endoglin and Spirulina platensis in the process of atherosclerosis both *in vivo* and *in vitro* and their effects on specific pro- or anti-atherogenic markers, particularly with regard to the vascular endothelium.

Endoglin (CD 105, TGF-β receptor III ENG) is a homodimeric membrane glycoprotein, which plays a regulatory role in TGFβ signaling. The relation between endoglin and endothelial NO synthase (eNOS) is essential for our study. Endoglin increases eNOS expression and promotes the production of NO, which affects the function of vascular endothelium. Changes in the expression of eNOS and endoglin could thus be associated with the development of endothelial dysfunction, a key step in atherogenesis.

In our studies, we investigated the effects of cholesterol (1% cholesterol diet) and statins (atorvastatin) with respect to endoglin expression and soluble endoglin levels in a mouse model of atherosclerosis. Hypercholesterolemia increased levels of soluble endoglin in blood and reduced its expression in the aorta, together with some protective markers of atherosclerosis, such as p-Smad2 and VEGF. Other results showed that statins increased endoglin levels as well as other protective markers of atherosclerosis and also reduced the size of atherosclerotic lesions beyond the hypolipidemic effect. In addition, atorvastatin treatment simultaneously reduced the levels of soluble endoglin, suggesting that monitoring of endoglin levels might represents a promising marker of the progression or treatment of atherosclerosis.

Spirulina platensis is a blue-green alga belonging to the group of cyanobacteria. It is a human and animal food supplement. It contains protein complexes with linear tetrapyroles called phycobiliprotein composed of protein part (fycocyanins) and linear tetrapyroles (fycocyanobilins). Fycocyanobilin (PCB) is biliverdin derivate, which is converted to fycocyanorubin by the enzyme called biliverdin reductase. This substance is nearly identical in structure as bilirubin, which is known to have protective effects on atherogenesis.

Based on this information we studied the effect of Spirulina and PCB on the expression and activity of heme oxygenase-1 (HMOX1, HO-1), which is the key enzyme in the catabolism of heme with significant atheroprotective properties *in vivo* and *in vitro*. The *in vitro* study on endothelial cells showed a strong activating effect on the expression of HO-1 and the activity of this enzyme not only by Spirulina but also by PCB. We also observed increased levels of endothelial NO synthase and reduction of the expression of cell adhesion molecules VCAM-1 after administration of Spirulina in endothelial cells, which provide evidence of the potential atheroprotective effect.

These *in vitro* data were confirmed in *in vivo* study in ApoE-deficient mice fed by cholesterol. Administration of Spirulina platensis resulted in a significant increase of atheroprotective HO-1 and eNOS in atherosclerotic lesions in the aorta. It seems that the influence of Spirulina to induce HO-1 is universal, because we were able to clearly demonstrate the upregulation of HO-1 transcription and function (enzyme activity) levels in the spleen HMOX-luc Tg mice.

For these reasons, the activation of HO-1 (together with the direct effects of PCB) represents an interesting mechanism to administration of Spirulina platensis, which may contribute to the positive effects on atherosclerosis.

In conclusion, we showed that tissue and soluble endoglin might play role in atherogenic process and soluble endoglin might be promising biomarker of atherogenesis and/or atherosclerosis. In addition, we demonstrated that Spirulina platensis is able to induce expression and activity of atheroprotective enzyme heme oxygenase-1, which makes it together with previously shown antioxidant and hypolipidemic effects an interesting food supplement for cardiovascular prevention.