ABSTRACT

Charles University, Faculty of Pharmacy in Hradec Králové

Department of Biological and Medical Sciences

Western blot analysis of selected oxidative stress markers in aorta of apoE/LDLR

deficient mice

Author of diploma thesis: Bc. Martina Čermáková

Supervisor: PharmDr. Jana Rathouská, Ph.D.

Background: The aim of this diploma thesis was to evaluate expression of selected oxidative stress molecules (SOD-3, iNOS, catalase, HO-1) in cardiac aorta and nitrate and nitrite levels in plasma in two groups of mice. These two groups of mice varied in genetic features - the monitored group was, compared to control group of mice, deficient for apolipoprotein E and LDL receptor.

Methods: For the study, 2 months old male mice were fed a standard rodent diet. The males were divided into two groups – control (C57BL/6J) and monitored (apoE^{-/-}/LDLR⁻ /-). Western blot method was used to evaluate and quantify iNOS, SOD-3, catalase and HO-1 molecules in the aorta. HPLC method was used to quantify nitrate and nitrite levels in plasma.

Result: HPLC method did not show statistically significant differences in the nitrate and nitrite levels in plasma between the two groups of examined mice. Western blot analysis of aortic samples showed statistically significantly higher SOD-3 expression (208.39 %) and a significantly higher iNOS expression (207.62 %) in the apoE^{-/-}/LDLR^{-/-} mice group compared to the control group (100.00 %). In contrast, HO-1 molecule showed significantly lower expression (42.22 %) in the apoE^{-/-}/LDLR^{-/-} mice group compared to the control group (100.00 %). Analysis of the catalase molecule expression did not show a significant difference in expression between the groups of examined mice.

Conclusions: The results of our analyses did not show a clear increase in oxidative stress in apoE^{-/-}/LDLR^{-/-} mice compared to the C57BL/6J group of mice. However, in order to make the final conclusions, the results will need to be broadened by other methods.

Key words: endothelial dysfunction, atherosclerosis, oxLDL, oxidative stress, HO-1, catalase, SOD-3, iNOS