Abstract

Introduction: Hypertrophic cardiomyopathy is a congenital cardiac disease with autosomal dominant pattern of inheritance and incomplete penetrance. With the knowledge of the responsible genes, the ability to detect the underlying genetic change and with the study of functional analysis of defected protein, we might be able to determine whether specific genotypes lead to different phenotypes.

Aims of Study: To comprehensively analyze the mechanism of genesis of hypertrophic cardiomyopathy in Czech patients afflicted with this disorder from molecular genetic point of view (*MYH7, TNNT2* gene) to functional analysis of the 3D molecular model of defected β-myosin heavy chain protein *in silico*. Beside these aims of the study, the reduction of production of inflammatory aggregates in the cardiovascular system was studied in patients with type 2 diabetes mellitus. The reason of this study was to look into possibilities of therapeutical effect on selected cardiovascular risks in patients with hypertrophic cardiomyopathy simultaneously suffering from type 2 diabetes mellitus. Both of these groups of patients have substantially increased risk of cardiovascular diseases due to development of premature atherosclerosis.

Material and Methods: A total of 170 probands were enrolled in this study of *MYH7* gene. DNA samples were analyzed (PCR, sequence analysis) for mutations in the specific functional regions of *MYH7*. The 3D model of human β-MHC was built using the X-ray structure of nucleotide-free scallop myosin S1 as the structural template. *De novo* structure prediction of two peptides (mutant and wild type variant) spanning the 769–788 region of the β-MHC were performed. A total of 181 probands were enrolled in the study of *TNNT2* gene. DNA samples were genotyped (PCR, sequence analysis) for mutations in the specific binding regions of *TNNT2* gene. The study with rosiglitazone included 33 patients with type 2 diabetes mellitus and 32 normal controls. The expression of leukocyte markers was measured by an immunofluorescence method using single-step staining with monoclonal antibodies. The fluorescence was quantified by the flow cytometry.

Results: The $Asp^{778}Val$ amino acid alteration was found in patient with severe form of hypertrophic cardiomyopathy. This variation was chosen for subsequent 3D molecular modeling *in silico*. The mutation of the Asp by Val not only changes the character of the interaction pattern with other amino acids or ions but Val being a small hydrophobic amino acid can completely change the stability of the region. We hypothesize that it can change the dynamics and flexibility of the long helical part or it can modify its interaction property. In the study with diabetic patients leukocyte expression of uPAR and PSGL-1 was significantly higher in patients than in controls. Leukocyte-platelet aggregates and uPAR and PSGL-1 expression significantly decreased after rosiglitazone treatment.

Conclusion: The mutation location in the *MYH7/TNNT2* genes and therefore changes in amino acid composition may have crucial negative impact on the disease outcome in patients with hypertrophic cardiomyopathy. In addition, a mutation that changes the charge of the amino acid is more likely to affect protein function than a conservative mutation. In the rosiglitazone study we observed substantial lowering of the expression of thrombogenic markers on leukocytes after the treatment, suggesting that rosiglitazone leads to the reduction of atherothrombotic complications.