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Expression of nitric oxide-related enzymes in coronary heart disease

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Coronary heart disease is the leading cause of death and disability in both men and women in industrial nations. Nitric oxide (NO), the biologically active component of endothelium-derived relaxing factor, has a critical role in the maintenance of vascular homeostasis. Decreased endothelial NO production, as a result of endothelial dysfunction, occurs in the early stage of atherosclerosis and plays a predominant role in endothelium dysfunction. Based on the theoretical background, enzymes involved in the metabolism of NO and reactive oxygen species (ROS) play an important role in the development of endothelial dysfunction. We, therefore, hypothesized that the pattern of gene expression of these enzymes is altered in atherosclerosis. To prove this hypothesis, myocardial tissue from patients with coronary heart disease (CHD; atherosclerosis group) or without coronary heart disease (control group) was investigated. The level of enzymes related to NO/ROS metabolism was determined both at mRNA level and protein level with help of reverse transcription PCR, real time PCR and western blot. Specifically, the expression of NOS1, NOS2, NOS3 (synthesis of NO), arginase1 (reduction of L-arginine, the substrate of NO-synthesis), p22phox (active subunit of NADPH oxidase, a radical producing enzyme), GTPCH (rate limiting enzyme for tetrahydrobiopterin, an essential cofactor of NOS), SOD1, SOD2, SOD3 (scavengers of superoxide anions), PTMT1, PRMT2, PRMT3, HRMT1L2, and DDAH2 (all involved in the metabolism of ADMA, an antagonist of NOS) was measured at mRNA level and/or protein level.

The results indicate that all the enzymes are expressed in the human heart. There were no differences in the expression of GTPCH, p22phox, SOD1 and SOD2 between the groups. All NOS isoforms were decreased in CHD in protein level, but only the downregulation of NOS 3

expression reached statistical significance. Still, regarding our data, NO synthesis should be decreased in atherosclerosis due to decreased NOS expression.

The protein level of arginase1 was reduced in CHD. The expression of PRMT1 and PRMT3 was increased, which should lead to an elevation of ADMA synthesis. Additionally, hydrolysis of ADMA should be lower in CHD, because lower expression of DDAH2 was found. Thus, local concentration of ADMA, a potent endogenous inhibitor of NOS is likely to be increased in CHD.

Due to unaltered expression of p22phox, superoxide synthesis from NADPH-oxidase should be unchanged. The downregulation of the expression of SOD3 in CHD, however, would indirectly enhance superoxide activity.

There are some limitations of our study. First, the number of samples, especially in the control group, was not very large. Second, there were no true controls since samples from the group without significant atherosclerosis had also heart disease which could influence the expression of the genes investigated. Third, we used tissue from the atrial appendage of the right atrium, and there may be differences in gene expression in different compartments of the heart. However, in this study for the first time, the expression of a number of genes has been investigated in human myocardial tissue which could have impact on a better understanding of the pathogenesis of coronary heart disease and even give clues for future therapeutical approaches such as gene therapy.

Taken together, we found changes in the expression of the gene pattern of enzymes involved in the metabolism of NO and reactive oxygen species in myocardial tissue from patients with atherosclerosis as compared to tissue from patients without significant atherosclerotic disease. Specifically, there was an enhancement of the expression of genes increasing the level of ADMA in combination with a reduction of genes promoting NO synthesis. These results demonstrate an increase of oxidative stress in atherosclerosis on the level of gene expression. Further studies are needed to investigate possible therapeutical strategies to overcome these changes and to inhibit the progression of atherosclerosis.