Hyperglycemia-induced myocardial oxidative stress and inflammation persist despite optimal glycemic control: role of mitochondrial adaptor p66^{shc}

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Introduction: intensive glycemic control does not reduce the risk of heart failure in the diabetic population. Long-lasting effects of hyperglycemia are indeed emerging as a major determinant of cardiovascular morbidity and this phenomenon has been recently described hyperglycemic memory. The mitochondrial adaptor p66^{Shc}, critically involved in reactive oxygen species (ROS) production, mediates hyperglycemia-induced cardiomyopathy. The present study investigates the role of p66^{Shc} as a determinant of persistent oxidative stress in the diabetic heart despite glycemic control. **Methods**: diabetes was induced in wild-type 129sv mice (4-6 months old) by a single i.p. dose of streptozocin. Mice were divided into 5 experimental groups: 1) healthy controls; 2) untreated diabetics; 3) diabetics treated with insulin, 4) diabetics receiving insulin together with p66^{Shc} siRNA or 5) scrambled siRNA (n=6-7/group). Insulin implants were placed subcutaneously 3 weeks after the induction of diabetes for the following 3 weeks. Silencing of p66^{Shc} was obtained by i.v. administration every 5 days. Isolated mitochondria from hearts were used for measurement of superoxide anion (O_2^-) by ESR spectroscopy and mitochondrial swelling. Pull-down assay were performed to show the interaction between p66^{Shc} and cytochrome c. NF-kB activity was assessed by p65 nuclear translocation and binding activity. Chromatin immunoprecipitation (ChIP) was performed to investigate epigenetic modifications of p66^{Shc} promoter. **Results**: O_2^- production and mitochondrial swelling were significantly increased in the heart of diabetic mice and not reverted by glucose normalization with insulin. These findings were associated with persistent mitochondrial translocation of p66^{Shc} and its co-immunoprecipitation with cytochrome c. Moreover, expression of the pro-hypertrophic and pro-inflammatory genes IL-6, MCP-1 and VCAM-1 was elevated in the diabetic hearts and did not change despite intensive glucose control. Interestingly, in vivo siRNA of p66^{Shc} in the context of glucose normalization blunted ROS production, restored mitochondrial integrity and suppressed ongoing myocardial inflammation by inhibiting NF-kB activation. We also show that persistent p66^{Shc} expression was explained by reduced histone 3 deacetylation by SIRT1, leading to an open chromatin and continued gene transcription. **Conclusions**: Our findings suggest that p66^{Shc}perpetuates ROS-mediated myocardial damage even after glucose normalization. Targeting molecular machineries underlying the "hyperglycemic memory" may represent the best option to reduce diabetes cardiovascular health burden.