Abstract

Cardiovascular complications characterized by cardiac dysfunction are a leading cause of morbidity and mortality associated with diabetes. There are ample evidences that excess generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), largely due to hyperglycemia, causes oxidative and nitrosative stress, which further exacerbates the development and progression of diabetic cardiomyopathy. Overproduction and/or insufficient removal of these free radicals result in cardiac dysfunction, damage to cellular proteins, membrane lipids and nucleic acids. Despite overwhelming evidence on the damaging consequences of oxidative and nitrosative stress and its role in experimental diabetes, large scale clinical trials with classic antioxidants failed to demonstrate any benefit for diabetic patients with cardiovascular complications. As our understanding of the mechanisms of free radical generation evolves, it is becoming clear that rather than merely scavenging reactive radicals, a more comprehensive approach aimed at preventing the generation of these reactive species as well as scavenging may prove more beneficial. High glucose (HG) generates reactive oxygen species (ROS) as a result of glucose auto-oxidation. Since glucose oxidase catalyses the oxidation of D-glucose in vitro, we exposed H9c2 cardiac myoblast cells to high glucose (33 mM) and glucose oxidase (1.6 mU/ml) to generate ROS and/or RNS in vitro, and termed it G/GO. Using this model, we tested the hypothesis that NAC, catalase and GSH may exert a beneficial effect in preventing high-glucose mediated cardiac cell apoptosis. Our invitro studies indicate that NAC, catalase and GSH exerts a protective effect on G/GO-induced apoptosis in H9C2 cardiac muscle cells via inhibition of ROS and RNS generation and mitochondrial death pathways. The mitochondrial apoptosis pathway involves the Bcl2/Bax molecules and activation of caspase. However, the mechanism underlying this protection against apoptosis in cardiac myocytes is not well understood. Further, we investigated the protective role of multiple antioxidants (MA) on rat model of diabetic cardiomyopathy. Hyperglycemia induced oxidative stress was confirmed by increase in the lipid peroxidation, activity of SOD, catalase, expression level of hemoxygenase-1 in cardiac tissue and nitrite level in serum. Hyperglycemia increased glycosylation of hemoglobin
HbA1), increased the levels of triglyceride, cholesterol, LDL and decreased HDL in serum. There were upregulation of cytokines TNF-α, IFN-γ, TGF-β, IL-10 and downregulation of IL-4 in response to hyperglycemia. Hyperglycemia also induced left ventricular dysfunction like decrease in +Δp/Δt, -Δp/Δt, heart rate and increase in blood pressure. In this study, we further examined the molecular pathways of diabetes induced cardiomyopathy. There were increased expression of the ROS-generating enzymes XO, MAO-A and inflammation inducing proteins like 5-LO, COX-2. There was also a significant increase in expression of pro-apoptotic molecule Bax and reduction in expression of anti-apoptotic molecule Bcl-2. These changes were significantly attenuated in the diabetic group supplemented with multiple antioxidants (MA). We conclude that cardiac dysfunction is associated with oxidative stress and suggest that antioxidant therapy might be beneficial in diabetic cardiomyopathy. Since multiple factor play a role in diabetic cardiomyopathy mainly due to oxidative and nitrosative stress, supplementation with MA can provide effective protection against oxidative damage. These insights might have therapeutic implications for human diabetic cardiomyopathy also.