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Original article

Greater propensity of diabetic myocardium for oxidative stress after myocardial infarction is associated with the development of heart failure

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Abstract

Diabetic patients manifest an increased incidence of heart failure (HF) after myocardial infarction (MI), which presages an increase in morbidity and mortality. Although oxidative stress has been implicated in diabetic complications, oxidative stress status associated with comorbid conditions that frequently accompany diabetes remains unknown. Therefore, we examined antioxidants and oxidative stress in the surviving myocardium in relation to ventricular function during diabetic HF following MI. MI was produced in diabetic and nondiabetic rats by ligation of the left coronary artery. At 4 weeks post-MI, LV systolic pressure (LVSP), rate of pressure rise (+dP/dt), and rate of pressure decay (–dP/dt) were depressed to a significantly greater extent in diabetic compared to nondiabetic MI animals. Higher levels of myocardial 8-isoprostane (8-*iso* $PGF_{2\alpha}$), oxidized glutathione (GSSG), as well as greater upregulation of superoxide dismutase (SOD) and catalase (CAT) protein expression paralleled by increases in enzymatic activity was observed in the diabetic MI animals, indicating higher oxidative stress. These data demonstrate a greater derangement of oxidative stress in the surviving tissues of diabetic post-MI rat hearts concomitant with an increased functional severity of HF, and suggest that chronic antioxidant therapy may be useful for the prophylaxis of subsequent HF after MI associated with diabetes.

Keywords: Diabetes mellitus; Myocardial infarction; Heart failure; Antioxidants; Oxidative stress

1. Introduction

Myocardial Infarction (MI) is a dominant reason for the cardiovascular morbidity and mortality seen in patients with diabetes mellitus. Diabetes is associated with a nearly two-fold higher mortality after MI [1,2]. Subgroup analyses from randomized clinical trials have suggested that modern treatment strategies (thrombolytic agents, beta blockers, antiplatelets, angiotensin converting enzyme [ACE] inhibitors) have been unable to reduce the differences in survival after MI between diabetic and nondiabetic patients [3–5]. The excess mortality rate among diabetic MI patients is related to an increased incidence of heart failure (HF). HF has been reported to occur at twice the rate in patients with diabetes after MI compared with nondiabetic patients despite similar-sized infarcts [6,7] and less ventricular enlargement [7]. These findings indicate that other factors must account for the higher frequency of this adverse event in diabetic patients after MI.

Free-radical mediated oxidative stress has been shown to be intimately involved in the pathogenesis of HF [8–10]. The myocardium is equipped with a variety of enzymatic antioxidants (superoxide dismutase [SOD], catalase [CAT], and glutathione peroxidase [$GSHP_x$]) and nonenzymatic antioxidants (glutathione, vitamin E) that act to neutralize the damaging effects of free radicals produced during normal cellular metabolism. However, during pathological conditions, the delicate balance between free-radical production and the protective antioxidant defense system may shift in favor of a relative increase in free-radical mediated oxidative stress. It has been reported that HF under both acute and chronic conditions is associated with increased oxidative stress [10–13]. Expired breath pentane levels, as an index of lipid peroxidation, were found to be elevated in patients with HF as well [14]. Although it has been shown that an increased level of oxidative stress also accompanies diabetes [15–20], little is

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known regarding oxidative stress associated with diabetic comorbid conditions (e.g. hypercholesteremia, hypertension, left ventricular hypertrophy, MI) as these comorbidities have seldom been incorporated into experimental studies. Recently, it has been shown that oxidative stress is greater in patients with diabetes than in those without diabetes who undergo coronary bypass grafting surgery for coronary artery disease [21]. However, direct information on myocardial antioxidant changes and oxidative stress in diabetic hearts during HF after MI is lacking.

In view of the increased risk for HF after MI associated with diabetes, we measured myocardial antioxidant enzyme protein expression and activity along with concomitant oxidative stress in the surviving left ventricle (LV) of diabetic and nondiabetic rats in relation to hemodynamic function at 4 weeks after left ventricular MI. The results demonstrate, for the first time, that after MI, oxidative stress burden is significantly greater in the diabetic compared to the nondiabetic myocardium. The greater propensity for myocardial oxidative stress injury among the diabetic MI animals was accompanied by poorer residual LV function, suggesting that further severe derangement in oxidative stress may play a role in the pathogenesis and increased frequency of HF following MI associated with diabetes.

2. Materials and methods

2.1. Experimental animals

All experiments were performed in accordance with the protocols approved by the Institutional Animal Care and Use Committee and conforms with the *Guide for the Care and Use of Laboratory Animals* of the US National Institutes of Health.

2.2. Animal models and study groups

2.2.1. Induction of diabetes

Hyperglycemia was induced in male Sprague-Dawley rats (150 ± 10 g) by administering a single intraperitoneal (i.p.) injection of streptozotocin (STZ) (65 mg/kg body wt) prepared daily in citrate buffer pH 4.5 for maximal stability. The control group was injected with the vehicle only. To ensure that the animals were diabetic (D), urine analysis was done after 24 h by Chemstrip uGK (Roche Diagnostics, Indianapolis, IN). Rats with urine glucose values of > 2000 mg/dl with polyuria 24 h after STZ injection were considered to be diabetic. Rats with urine glucose values of < 2000 mg/dl after 24 h were not considered to be diabetic and were excluded from further study. Two weeks after induction of diabetes, diabetic animals underwent coronary artery ligation to induce MI.

2.2.2. MI

MI was produced in diabetic and nondiabetic male Sprague-Dawley rats via occlusion of the left coronary artery as described in detail previously [10,12]. Briefly, after isoflurane anesthesia, a left intercostal thoracotomy was performed, by cutting of the third and fourth ribs proximal to the sternum, and the heart was exteriorized through the intercostal space. The left coronary artery was ligated 1–3 mm from its origin with a suture (6-0 silk), and the heart was repositioned in the chest. Excess air was removed with a syringe and the thoracic cavity was closed with a purse string suture. Throughout this surgical procedure, a maintenance dose of anesthesia (1.5–3% isoflurane) was delivered with a positive-pressure ventilation system consisting of 95% O_2. A sham operation in which the left coronary artery was not ligated was also performed in additional groups of vehicle-injected (sham MI group) and STZ-diabetic rats (sham diabetes group). The mortality among the coronary artery-ligated animals was ~30% within the first 24 h, with no differences observed between diabetic and nondiabetic animals, which is consistent with previous findings [22]. Animals were used at 4 weeks of post-surgical duration for different studies.

2.2.3. Hemodynamic studies

Rats were anesthetized with ketamine- xylazine (90:10 mg/kg i.p.). The right carotid artery was cannulated with a Millar miniature catheter (SPC-320, 2F, Millar Instruments, Houston, Texas) and advanced into the aorta to record arterial pressure. The aortic catheter was then advanced into the LV for recording of the following pressures: Left ventricular end-diastolic pressure (LVEDP); Left ventricular systolic pressure (LVSP); rate of pressure rise (+dP/dt); and rate of pressure decay (–dP/dt). After these assessments, the rats were killed, and the heart was removed for further studies.

2.2.4. Biochemical assays

For the studies of antioxidants and oxidative stress, only the viable portion of the LV, remote from the site of infarction, was utilized. The LV was isolated, weighed, and then immediately frozen in liquid nitrogen and stored at –80 °C until use.

2.3. Myocardial oxidative stress

2.3.1. 8-Isoprostane (8-iso $PGF_{2\alpha}$) content

Tissue was homogenized in 0.1 M phosphate, pH 7.4, containing 1 mM EDTA. An equal volume of 15% wt/vol KOH was added to the tissue homogenate and incubated for 60 min at 4 °C. 2–4 volumes of ethanol containing 0.01% BHT was added to the sample and incubated for 5 min at 4 °C and then centrifuged at 1500 × *g* for 10 min to remove precipitated proteins. The supernatant was then decanted into a clean test tube and the ethanol was evaporated to < 10% v/v under a gentle stream of nitrogen. The samples were then reconstituted with EIA buffer, vortexed, and then analyzed for 8-isoprostane using a commercially available kit (8-isoprostane EIA Kit, Cayman Chemical Company, Ann Arbor, MI) and expressed as picogram per miligram protein.

2.3.2. Reduced and oxidized glutathione

Tissue was homogenized in 5–10 ml of cold buffer (i.e. 50 mM MES, pH 6–7, containing 1 mM EDTA) per gram