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Irbesartan ameliorates diabetic cardiomyopathy by regulating protein kinase D and ER stress activation in a type 2 diabetes rat model

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ABSTRACT

Recent studies demonstrate an important role of protein kinase D (PKD) in the cardiovascular system. However, the potential role of PKD in the pathogenesis of diabetic cardiomyopathy (DCM) remains unclear. Irbesartan has beneficial effects against diabetes-induced heart damage, while the mechanisms were still poorly understood. Our present study was designed to investigate the effects of irbesartan in DCM and whether the cardioprotective effects of irbesartan were mediated by PKD and endoplasmic reticulum (ER) stress. We induced the type 2 diabetic rat model by high fat diet and low dose streptozotocin injection. The characteristics of type 2 DCM were evaluated by metabolic tests, echocardiography and histopathology. 8-weeks administration of irbesartan (15, 30 and 45 mg/kg/day) was used to evaluate the effect irbesartan in DCM. Diabetic rats revealed severe metabolic abnormalities, left ventricular dysfunction, myocardial fibrosis and apoptosis. PKD and ER stress were excessive activated in the myocardium of diabetic rats. Furthermore, cardiac fibrosis, apoptosis, diastolic dysfunction and ER stress were all significantly related to PKD activation in diabetic rats. Irbesartan treatment attenuated the activation of PKD and ER stress, which paralleled its cardioprotective effects. Our study suggests that irbesartan could ameliorate cardiac remodeling and dysfunction in type 2 diabetes, and these beneficial effects were associated with its ability to suppress the activation of PKD and ER stress.

Introduction

Diabetic cardiomyopathy (DCM), characterized by left ventricular hypertrophy and reduced diastolic function, is a distinct myocardial disease leading to heart failure independently of hypertension and coronary artery disease in patients with diabetes [1]. DCM is the major cause of mortality among diabetic patients, and the number of newly diagnosed cases of DCM is rising at epidemic rates throughout the world [2,3]. Diverse pathogenic mechanisms contribute to diabetic cardiomyopathy, including myocardial cell death and fibrosis [4]. Increased cardiomyocyte apoptosis has been observed in the hearts of diabetic patients and animal models. Accumulating evidence indicates that cardiomyocyte apoptosis, which initiates cardiac remodeling and results in cardiac dysfunction finally, is a key element in the pathogenesis and progression of DCM [5].

The pathogenesis of diabetic cardiomyopathy is a chronic and complex process which is attributed to abnormal cellular metabolism and defects of many organelles, such as mitochondria, endoplasmic reticulum (ER) and sarcolemma. Endoplasmic reticulum (ER) is an organelle involved in the intrinsic pathway of apoptosis. Recently, apoptosis mediated by ER stress was demonstrated to contribute to the progression of diabetic cardiomyopathy [6–8].

Abbreviations: ARB, angiotensin II receptor blocker; Bax, bcl2-associated X protein; Bcl-2, b-cell lymphoma/leukemia-2; CAMK, calcium/calmodulin-dependent protein kinase; DCM, diabetic cardiomyopathy; DM, diabetes mellitus; E/A, peak E to peak A ratio; E′/A′, early (E′) to late (A′) diastolic velocity ratio; ER, endoplasmic reticulum; FBG, fasting blood glucose; FINS, fasting insulin; FS, fractional shortening; GRP78, glucose regulated protein 78 kDa; IPGTT, intraperitoneal glucose tolerance test; IPITT, intraperitoneal insulin tolerance test; Irb, irbesartan; ISI, insulin sensitivity index; LVEDd, left ventricular end-diastolic dimension; LVEF, left ventricular ejection fraction; PKC, protein kinase C; PKD, protein kinase D; RAAS, renin–angiotensin–aldosterone system; TC, total cholesterol; TG, triglycerides; TUNEL, terminal transferase-mediated DNA nick end labeling.

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Protein kinase D (PKD) is a recent addition to the calcium/calmodulin-dependent protein kinase (CAMK) group of serine/threonine kinases, increasingly implicated in the regulation of multiple cell biological processes, including cell survival, migration, differentiation, and proliferation [9,10]. Recent advances demonstrate an important role of PKD-mediated signaling pathways in the cardiovascular system, particularly in the regulation of myocardial contraction and hypertrophy [11–13]. Previous studies also reported a significant increase of PKD expression and activity in the myocardium of spontaneously hypertensive heart failure rats, as well as in heart failure patients [14,15]. Protein kinase C (PKC), as the chief up-streaming kinase of PKD, was demonstrated to play a pivotal role in the pathophysiology of DCM [16]. However, little is known about the potential role of PKD in the development of DCM.

Angiotensin II receptor blocker (ARB) has been demonstrated to offer effective cardioprotection in diabetic patients. In the RENAAL and LIFE studies, losartan significantly lowered the hospitalization rate because of new onset heart failure in diabetic patients [17]. In another clinical trial, irbesartan significantly lowered the incidence of congestive heart failure in patients with type 2 diabetes [18]. Additionally, telmisartan was shown to have an efficacy in improving left ventricular diastolic properties in a diabetic population [19]. Researchers also reported that candesartan induced an improvement in diastolic function parameters in asymptomatic diabetic patients by a decrease in collagen synthesis and an increase in collagen degradation [20]. Previous studies indicate the cardioprotective effects of ARB, such as preventing myocardial fibrosis, reducing cardiomyocyte apoptosis, and improving in Ca^{2+} signaling parameters [21–23]. However, the molecular mechanism of ARB in reversing cardiac remodeling and dysfunction is still incompletely understood.

The present study was designed to investigate the role of PKD in type 2 DCM, and whether the cardioprotective effects of irbesartan (Irb) were mediated by PKD and ER stress.

Materials and methods

Animal model and drug treatment

Sixty male Sprague–Dawley rats weighting 120–140 g were obtained from the experimental animal center of Shandong University of Traditional Chinese Medicine (Ji'nan, China). The animals were maintained under conditions of standard lighting (alternating 12 h light/dark cycle), temperature (20–22 °C) and humidity (50–60%). After 1 week of acclimatization, the rats were then randomly divided into 5 groups ($n = 12$ each): control, diabetes mellitus (DM), DM + Irb-15, DM + Irb-30, DM + Irb-45. The control group received normal chow and the other diabetic groups a high fat diet (34.5% fat, 17.5% protein, 48% carbohydrates; Beijing HFK Bio-Technology, China). After 4 weeks, intraperitoneal glucose tolerance test (IPGTT) and intraperitoneal insulin tolerance test (IPITT) were performed. The rats with insulin resistance in the diabetic groups received an intraperitoneal injection of a single dose of streptozotocin (STZ; 35 mg/kg, in 0.1 mol/L citrate buffer, pH 4.5; Solarbio, China). Rats of the control group received injections of citrate buffer vehicle of equivalent volume. One week later, tail vein fasting blood glucose (FBG) was measured (Roche, Germany) and rats with blood glucose levels ≥11.1 mmol/l were considered diabetic [24,25]. After induction of diabetes for 8 weeks, rats in the DM + Irb-15, DM + Irb-30, and DM + Irb-45 group were treated with Irbesartan at dosages of 15 mg/kg/day, 30 mg/kg/day, and 45 mg/kg/day respectively. The control and DM group received vehicle. Irbesartan (Sanofi-Aventis Pharmaceuticals Company, Hangzhou, China) and vehicle were administered orally by gavage once a day. After 8 weeks of treatment, all rats were killed. The animal experimental protocol was complied with the Animal Management Rules of the Chinese Ministry of Health (document no. 55, 2001) and was approved by the institutional ethics committee of Shandong University.

Serum measurements

After rats fasted overnight, we collected jugular blood samples. Serum triglycerides (TG) and total cholesterol (TC) were determined using an automatic analyzer (Roche, Basel, Switzerland). Serum level of fasting insulin (FINS) was measured by enzyme-linked immunosorbent assay (Jiancheng, Nanjing, China). And the insulin sensitivity index (ISI) was calculated [$ISI = \ln(FBG \times FINS)^{-1}$].

Blood pressure measurements

Heart rate (HR), systolic blood pressure (SBP), and diastolic blood pressure (DBP) were measured with a noninvasive tail-cuff system (Softron BP-98A; Softron, Tokyo, Japan).

Echocardiographic evaluation

Transthoracic echocardiography was performed using the Vevo 770 imaging system (VisualSonics, Toronto, Canada). Images were obtained from two-dimensional, M-mode, pulsed-wave Doppler and tissue Doppler imaging. The derived echocardiography parameters included left ventricular end-diastolic dimension (LVEDd), left ventricular ejection fraction (LVEF), fractional shortening (FS), peak E to peak A ratio (E/A), and early (E′) to late (A′) diastolic velocity ratio (E′/A′). LV internal diameter, thickness of the septum and posterior wall at end-systole and end-diastole were measured from the long-axis view at the level of chordae tendineae. LVEF and FS were then calculated according to these parameters [26]. Transmitral flow velocity variables, including peak E, peak A, and E/A were evaluated by pulsed-wave Doppler imaging from the apical four chamber view. Early (E′) and late (A′) diastolic velocity, and E′/A′ were evaluated by tissue Doppler imaging from the apical four chamber view. Echocardiographic imaging and measurements were performed by the same observer. All measurements were the average of six consecutive cardiac cycles.

Histopathology

Rat hearts were dissected and immediately fixed in 4% formalin. Tissue was paraffin embedded and sectioned (5 μm) for staining with hematoxylin and eosin (H&E) and for immunohistochemistry. For the detection of interstitial collagen deposition, heart sections were stained with Masson's trichrome and Sirius red. The fraction of fibrosis area was quantified by use of Image-Pro Plus 6.0 (Media Cybemetics, US). Immunohistochemistry were performed for detecting the expressions of glucose regulated protein 78 kDa (GRP78). Slides were incubated overnight at room temperature with the primary antibodies rabbit anti-GRP78 (Santa Cruz Biotechnology, USA). Goat anti-rabbit antibody was the secondary antibody.

TUNEL assay

Apoptotic cells in myocardium were detected by the terminal transferase-mediated DNA nick end labeling (TUNEL) assay according to the manufacturer's instructions (ApopTag Plus Peroxidase *In Situ* Apoptosis Detection Kit, Millipore, USA). Briefly, formalin-fixed tissue sections were deparaffinized and treated with 3% hydrogen peroxide in methanol for 10 min. After adding the equilibration