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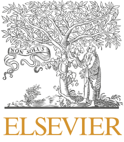

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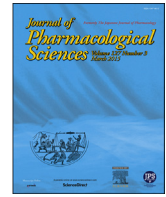

Full paper

Sitagliptin alleviated myocardial remodeling of the left ventricle and improved cardiac diastolic dysfunction in diabetic rats

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A B S T R A C T

Objective: Sitagliptin, a dipeptidyl peptidase IV (DPP-IV) inhibitor, has a biological role in improving the serum levels of glucagon-like peptide 1 (GLP-1). Hence, we sought to determine the effect of sitagliptin on myocardial inflammation, collagen metabolism, lipid content and myocardial apoptosis in diabetic rats.
Materials and methods: The type 2 diabetic rat model was induced by low-dose streptozotocin and a high-fat diet. Characteristics of diabetic rats were evaluated by electrocardiography, echocardiography and blood analysis. Cardiac inflammation, fibrosis, cardiomyocyte density, lipid accumulation, and receptor-interacting protein kinase 3 (RIP_3) level, related to apoptosis, were detected by histopathologic analysis, RT-PCR and western blot analysis to evaluate the effects of sitagliptin on myocardial remodeling of the left ventricle.
Results: Diabetic rats showed myocardial hypertrophy or apoptosis, inflammation, lipid accumulation, myocardial fibrosis, elevated collagen content, RIP_3 overexpression, and left-ventricular dysfunction. Sitagliptin could reverse the overexpression of RIP_3 and alleviate cellular apoptosis in myocardial tissues. It could significantly improve left-ventricular systolic pressure and +dp/dt max, reduce the E/E′ ratio, left ventricular end diastolic pressure, −dp/dt max and Tau in diabetic rats.
Conclusions: Sitagliptin might have a myocardial protective effect by inhibiting apoptosis, inflammation, lipid accumulation and myocardial fibrosis in diabetic rats, for a potential role in improving left-ventricular function in diabetes.
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1. Introduction

Accumulating evidence indicates that diabetic cardiomyopathy (1,2) is a disorder of myocardial lesions induced by high glucose. It features myocardial hypertrophy or apoptosis (3), inflammation, lipid accumulation, and myocardial fibrosis (4). As well, it might feature systolic and/or diastolic dysfunction of the heart and increased mortality (5). Sari et al. (6) declared that cardiac endoplasmic reticulum stress (ERS) and ERS-initiated apoptosis were involved in diabetes. Receptor-interacting protein 3 (RIP_3), an important signal molecule, is involved in tumor necrosis factor α (TNF-α)-mediated apoptosis and related to mitochondrial energy metabolism (7,8), but its expression in the diabetic heart is not known.

Sitagliptin is a dipeptidyl peptidase IV (DPP-IV) inhibitor and has a role in improving the activity of glucagon-like peptide-1 (GLP-1) (9), an incretin hormone secreted from the distal intestine L cells with glucose-dependent release that promotes the secretion of postprandial insulin (10). GLP-1 receptors (GLP-1Rs) exist widely in islets, myocardium and brain tissue. Relatively low GLP-1 concentrations as compared with *in vivo* blood concentrations promoted insulin secretion independent of the cAMP-protein kinase A pathway (11). With decreased activity of

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GLP-1 in diabetic patients, endogenous GLP-1 could be dissolved rapidly by DPP-IV (12).

Sitagliptin could prevent GLP-1 degradation in the short term, elevate serum levels of GLP-1 and enhance or delay the release of insulin, to lower blood glucose (13,14). Furthermore, recent reports demonstrated that GLP-1 has a key role in the inhibiting apoptosis during ischemia-reperfusion (I/R) injury and improved cardiac function (15).

Hence, we evaluated the effect of sitagliptin on inflammation, collagen metabolism, lipid content, myocardial apoptosis (RIP_3 expression) and cardiac function in diabetic rats to detect additional benefits for sitagliptin beyond its blood glucose-lowering effect.

2. Materials and methods

2.1. Animals and supplementation

We purchased 70 male, 8-week-old Wistar rats (120–140 g) from the experimental animal center of Shandong University (Jinan, China). All experimental procedures were performed in accordance with animal protocols approved by the Shandong University Animal Care Committee. Rats were housed at 22 °C with 12-h light/dark cycles. All mice were fed a high-fat diet (34.5% fat, 17.5% protein, 48% carbohydrate; Beijing HFK Bio-Technology). Four weeks later, the intraperitoneal glucose tolerance test (IPGTT) and intraperitoneal insulin tolerance test (IPITT) were performed and blood was sampled by the jugular vein. Fasting insulin and fasting blood glucose (FBG) were measured, and the insulin sensitivity index [$ISI = \ln(FBG \times \text{fasting insulin})^{-1}$] was calculated. Control rats received citrate buffer (intraperitoneally) alone and rats with insulin resistance received a single intraperitoneal injection of STZ (Sigma, St. Louis, MO; 30 mg/kg intraperitoneally in 0.1 mol/L citrate buffer, pH 4.5) as described (16). At one week after STZ administration, rats with FBG > 11.1 mmol/L in 2 consecutive analyses were considered the diabetic model. Animals were then randomized to one of 2 groups for treatment: control rats ($n = 10$), normal chow; or diabetes rats, induced with DPP-IV inhibitor (low-dosage sitagliptin, 30 mg/kg/d) ($n = 20$) [DPP-IVi (Low)] and (high-dosage sitagliptin, 50 mg/kg/d) ($n = 20$) [DPP-IVi (High)]. Treatment with sitagliptin started 4 weeks after STZ injection. The rats were killed after 17 weeks of diabetes.

2.2. Blood analyses

Blood was collected from the jugular vein after rats fasted for 8 h. FBG and circulating levels of cholesterol, triglycerides, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) were analyzed with use of the Bayer blood chemistry analyzer (Bayer, Tarrytown, NY). Free fatty acid (FFA) concentrations were measured by use of an enzymatic test kit (CSB-E08770r; HuaMei BIO-TECH, Wuhan, China). ISI was calculated. Plasma insulin levels were measured by use of the rat insulin ELISA kit based on the direct Sandwich ELISA technique (Mercodia AB, Sweden). Hemoglobin A1C (HbA_{1C}) level was measured by the Nycocard Reader (Ranbaxy, India).

2.3. Electrocardiography and echocardiography

ECG was recorded with the limb leads (I, II, III, aVR, aVL, aVF) to evaluate cardiac electrical activity. Echocardiography involved use of the Philips IE33 imaging system (S12-4). Images were obtained from 2D, M-mode, pulsed-wave Doppler and tissue Doppler imaging (TDI). All measurements were performed by the same researcher and were averaged from 6 consecutive cardiac cycles. Wall thickness and LV dimensions, including LV end systolic diameter (LVEDs) and LV end diastolic diameter (LVEDd), were obtained from the long-axis view. LV ejection fraction (LVEF) and fractional shortening (FS) were measured according to American Society of Echocardiography guidelines (16,17). The mitral-valve pulsed Doppler recordings were obtained from the apical four-chamber view. After pulsed Doppler, transmitral flow velocity variables, including peak E, peak A, and E/A ratio were evaluated (the early (E′) and late (A′) diastolic velocity were analyzed and E′/A′ and E/E′ values were calculated) and tissue Doppler imaging of the mitral annulus was obtained from the apical four-chamber view.

2.4. Hemodynamics by Millar catheter

Rats were anesthetized with urethane (1 g/kg), then the right carotid artery was cannulated and a transducer was advanced into the left ventricle to measure the rate of pressure changes (±dp/dt max), blood pressure, and heart rate with a microtip pressure transducer (Millar Instruments) and LVED pressure (LVEDP) was measured. Tau was calculated with the LV time constant $Tau = P/(-dp/dt)$.

2.5. Morphometric analysis

Heart tissues were fixed in 10% formalin, embedded in paraffin, and sectioned at 5-mm thick. A single myocyte was measured with images captured from H&E-stained sections. The myocyte cross-sectional area was assessed by ×400 magnification within the LV, and a mean was obtained by quantitative morphometry with automated image analysis (Image-Pro Plus 5.0; Media Cybernatics, Houston, TX).

Dark green–stained collagen fibers were quantified to measure fibrosis in Masson trichrome-stained sections. The collagen volume fraction (CVF) and perivascular collagen area to luminal area (PVCA/LA) were analyzed by quantitative morphometry with automated image analysis (Image-Pro Plus 5.0). CVF was calculated as reported previously (18). Perivascular collagen was excluded from the CVF measurement. To normalize the PVCA around vessels with different sizes, perivascular collagen content was represented as the PVCA/LA ratio. Interstitial and perivascular fibrosis was evaluated by Picrosirius red staining. Sections were stained with 0.5% sirius red (Sigma) in saturated picric acid for 25 min. Collagen was stained red. Myocardial frozen sections (5 mm) were stained with Oil-red O (Sigma) for 10 min, washed, then counterstained with hematoxylin for 30 s. A Nikon microscope (Nikon, Melville, NY) was used to capture images.

An *in situ* cell death detection kit (Roche GmbH, Germany) was used for TUNEL assay. Briefly, slides were deparaffinized, rehydrated with xylene; underwent a graded ethanol series; and were permeablized with hot 0.1 M citrate buffer, pH 6.0; incubated with reaction mixture containing TdT; and labeled with dUTP for 1 h at 37 °C. Images were captured by confocal laser scanning microscopy (Zeiss LSM510). For a negative control, TdT was omitted from the reaction mixture.

2.6. Immunohistochemical staining

Paraffin sections underwent immunohistochemistry by a microwave-based antigen retrieval method. Sections were incubated with primary antibodies for rabbit polyclonal collagen I and III, tumor necrosis factor TNF-α, and interleukin 6 (IL-6) (Abcam, Cambridge, MA) overnight, then with biotinylated secondary antibody for 30 min at 37 °C. Negative controls were omission of the primary antibody. Stained sections were developed with diaminobenzidine and counterstained with hematoxylin. Sections

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