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Original article

Activation of retinoid receptor-mediated signaling ameliorates diabetes-induced cardiac dysfunction in Zucker diabetic rats

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ABSTRACT

Diabetic cardiomyopathy (DCM) is a significant contributor to the morbidity and mortality associated with diabetes and metabolic syndrome. Retinoids, through activation of retinoic acid receptor (RAR) and retinoid x receptor (RXR), have been linked to control glucose and lipid homeostasis, with effects on obesity and diabetes. However, the functional role of RAR and RXR in the development of DCM remains unclear. Zucker diabetic fatty (ZDF) and lean rats were treated with Am580 (RARα agonist) or LGD1069 (RXR agonist) for 16 weeks, and cardiac function and metabolic alterations were determined. Hyperglycemia, hyperlipidemia and insulin resistance were observed in ZDF rats. Diabetic cardiomyopathy was characterized in ZDF rats by increased oxidative stress, apoptosis, fibrosis, inflammation, activation of MAP kinases and NF-κB signaling and diminished Akt phosphorylation, along with decreased glucose transport and increased cardiac lipid accumulation, and ultimately diastolic dysfunction. Am580 and LGD1069 attenuated diabetes-induced cardiac dysfunction and the pathological alterations, by improving glucose tolerance and insulin resistance; facilitating Akt activation and glucose utilization, and attenuating oxidative stress and interrelated MAP kinase and NF-κB signaling pathways. Am580 inhibited body weight gain, attenuated the increased cardiac fatty acid uptake, β-oxidation and lipid accumulation in the hearts of ZDF rats. However, LGD1069 promoted body weight gain, hyperlipidemia and cardiac lipid accumulation. In conclusion, our data suggest that activation of RAR and RXR may have therapeutic potential in the treatment of diabetic cardiomyopathy. However, further studies are necessary to clarify the role of RAR and RXR in the regulation of lipid metabolism and homeostasis.

1. Introduction

Diabetic cardiomyopathy (DCM), one of the most prevalent cardiovascular complications of diabetes, is characterized by both systolic and diastolic dysfunction, due to reduced contractility, prolonged relaxation and decreased compliance [1]. A growing body of clinical and experimental data suggest that cardiac insulin resistance and metabolic perturbations largely contribute to the development of DCM. The diminished glucose utilization and increased fatty acid oxidation in diabetic heart leads to lipid accumulation in myocardium [2]. Myocardial glucotoxicity and lipotoxicity triggers a series of maladaptive stimuli that result in increased oxidative stress, enhanced expression of renin–angiotensin system components, altered intracellular ion transients and calcium homeostasis, and activated apoptotic and inflammatory signaling pathways, such as mitogen-activated protein kinase (MAPK) and nuclear factor (NF)-κB [3–5]. Elucidation of the molecular and metabolic mechanisms will provide a better understanding of the development of cardiac dysfunction associated with diabetes and the metabolic syndrome.

Retinoic acid-vitamin A metabolites exerts a number of essential biological functions through activation of two classes of nuclear receptors, RAR (α, β and γ), which respond to all-*trans* retinoic acid (ATRA) and 9-*cis*-isomers of RA; and RXR (α, β and γ), which are activated by 9-*cis*-RA exclusively. Studies have shown that ATRA inhibits the development of type 1 diabetes [6]; and reduces body weight and adiposity through regulation of lipid metabolism in the adipose tissue, liver and skeletal muscle of mice [7–9]. RXR agonists also have anti-diabetic effects in type 2 diabetic mouse models [10]. These data indicate the importance of RAR/RXR-mediated signaling in the regulation of glucose and lipid homeostasis. However, the role of RAR and RXR in the regulation of cardiac glucose and lipid metabolism and its relationship with the development of DCM remain unclear. Recently, we reported that the expression and transcriptional activation of RARα and RXRα are significantly suppressed in high glucose (HG)-treated cardiomyocytes and in the hearts of ZDF rats

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[11,12]; and that silencing the expression of RARα and RXRα in cardiomyocytes, further promoted HG-induced cell apoptosis. On the other hand, activation of RAR and RXR, by ATRA and 9-*cis* RA, protected cardiomyocytes from HG-induced apoptosis, through inhibition of oxidative stress-mediated activation of MAP kinases and NF-κB-mediated inflammatory signaling [11,13]. These results led us to hypothesize that RAR and RXR-mediated signaling, through the regulation of systemic and/or cardiac glucose and lipid homeostasis, has an important role in the development of DCM. ZDF rats were utilized to determine whether activation of RAR and RXR has beneficial effects on controlling functional, as well as morphological cardiac damage, through regulation of glucose/lipid metabolism and related signaling pathways in this representative rat model of the human metabolic syndrome.

2. Research design and methods

2.1. Animals

Male ZDF rats and age matched Zucker lean rats (Charles River Laboratories) were housed in a temperature-controlled room under a 12/12 h light/dark cycle, with free access to water and the Purina 5008 diet. Animal use was approved by the Institutional Animal Care and Use Committee of the Texas A&M Health Science Center and conformed to the *Guide for the Care and Use of Laboratory Animals*, published by the National Institutes of Health (NIH Pub. No. 85-23, 1996).

2.2. Experimental protocol

ZDF and lean rats were randomized into 6 groups (8 rats/group) at the age of 9 weeks: (1) control lean rats; (2) lean rats treated with LGD1069 (20 mg/kg body weight/day, from LC Laboratories); (3) lean rats treated with Am580 (1 mg/kg body weight/day, Enzo Life Science); (4) control ZDF rats; (5) ZDF + LGD1069; and (6) ZDF + Am580. Rats were given vehicle (corn oil), LGD1069 or Am580 daily, orally by gastric gavage, for 16 weeks. Body weight and fasting glucose levels were measured weekly. Before sacrificing, an oral glucose tolerance test (GTT) was performed. Additional information regarding short term treatment groups is provided in the Supplemental data.

2.3. Blood chemistry and metabolic analysis

Serum samples were collected from rats that were fasted overnight and insulin levels determined (Insulin ELISA Kit from Millipore). For the GTT, D-glucose (2 g/kg body weight, Sigma Aldrich) was orally administrated after overnight fasting. Blood glucose was measured before, and after 30, 60 and 120 min of glucose uptake, using a commercially available glucometer (Bayer, IN). The area under the glucose curve (AUC) from 0 to 30, 0 to 60 and 0 to 120 min was calculated using the trapezoidal method [14]. Insulin sensitivity of individual animals was evaluated using the previously validated homeostasis model assessment (HOMA) index [15]. The HOMA-IR (HOMA-insulin resistance) is a method used to quantify insulin resistance and HOMA-β% to quantify β-cell function. The formula used was as follows: [HOMA-IR] = fasting serum glucose (mg/dL) × fasting serum insulin (mU/L)/405. [HOMA-β%] = fasting serum insulin × 360/(fasting serum glucose − 63). Plasma total cholesterol was measured using the polychromatic (452, 540, 700 nm) endpoint technique. The triglycerides (TG) and high-density lipoprotein (HDL) were measured using a bichromatic (510/700 nm; 600/700 nm) endpoint technique.

2.4. Histological analysis

Hearts were removed, weighed and separated into 2 halves along the anterior longitudinal mid-line. One half of the heart was fixed in formalin solution, embedded in paraffin and cut into sections in 5 μm thick for H&E, Masson's trichrome (Sigma Aldrich) and TUNEL staining (Promega) [16]. The other half of each heart was frozen in liquid nitrogen and sectioned (20 μm) for Oil Red O staining (Sigma Aldrich), to identify lipid disposition [17] and for dihydroethidium (DHE) staining (Sigma Aldrich) to identify intracardiac superoxide production [16]. Interstitial and perivascular fibrosis were measured, using NIH Image J software. Blue-stained areas and non-stained myocyte areas from each section were determined using color-based thresholding. The percentage area of total fibrosis was calculated as the summed blue-stained areas, divided by total ventricular area. The area of perivascular fibrosis was calculated as the ratio of the area of fibrosis surrounding the vessels, to the total vessel area.

2.5. Echocardiography

Transthoracic echocardiography was performed in anesthetized rats using HP sonos 5500 (Hewlett-Packard) with a 12-MHz imaging transducer. Left ventricle (LV) wall thickness, diameter, systolic and diastolic function were analyzed as described previously [18], by an experienced sonographer who was blinded to treatment.

2.6. Real time RT-PCR

Total RNA was extracted from the left ventricles, using the RNeasy Fibrous Tissue Mini Kit (Qiagen). Real time RT-PCR was performed to analyze mRNA expression for ANP, BNP, interleukin1 (IL-1β), transforming growth factor β (TGFβ), tumor necrosis factor-α (TNF-α), RARα, RXRα, alcohol dehydrogenase (ADH) and retinaldehyde dehydrogenase (RALDH), glucose transporter 1 (GLUT1), GLUT4, aldolase A, hexokinase 2 (HK2), peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), acetyl-CoA C-acyltransferase 1 (Acaa1), Acaa2, Acyl-CoA dehydrogenase (Acad), carnitine palmitoyltransferase 1 (CPT1) and fatty acid binding protein 3 (Fabp3) [11]. The mRNA expression levels were normalized to GAPDH mRNA. Data were shown as mean ± SEM. Primers used were purchased from Applied Biosystems.

2.7. Western blot analysis

Left ventricles were lysed in cell lysis buffer (Cell Signaling Technology) containing protease inhibitor cocktail (Roche Diagnostics). Total protein was extracted and resolved on SDS-PAGE. Expression of Bcl2, Bax (Santa Cruz Biotechnology) and caspase 9, and phosphorylation of IKKα/β (Ser176/180), IκBα (Ser32/36), p65 NF-κB (Ser536), Akt (Ser473), GSK3β (Ser9), ERK1/2 (Thr202/Tyr204), JNK (Thr183/Tyr185) and p38 MAPK (Thr180/Thr182) (Cell Signaling Technology) were determined by Western blot, as described previously [11,13,18].

2.8. Statistical analysis

Data are expressed as the mean ± SEM. Statistical significance between experimental groups was determined using one-way ANOVA, combined with the Tukey–Kramer Multiple Comparisons test. $p<0.05$ was considered statistically significant.

3. Results

3.1. Body weight changes

At 9 weeks of age, ZDF rats had a higher body weight (BW) than lean rats (292 ± 3 g vs 244 ± 5 g. Table 1, supplemental data). Lean control rats gained BW steadily from 9 to 25 weeks. Am580 and LGD1069 did not significantly affect the BW gain in lean rats. ZDF rats gained BW between 9 and 21 weeks, and there was no further gain from 21 to 25 weeks. Their BW was comparable to the lean rats at 21 weeks (380 ± 9 g in ZDF vs 390 ± 6 g in lean group) and