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Treatment with angiotensin-(1-9) alleviates the cardiomyopathy in streptozotocin-induced diabetic rats

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ABSTRACT

Diabetic cardiomyopathy, a disorder of the heart muscle in diabetic patients, is one of the major causes of heart failure. We hypothesized that angiotensin-(1-9) [Ang-(1-9)] attenuates cardiomyopathy in streptozotocin (STZ)-induced diabetic rats. Rats were injected with a single intraperitoneal injection of STZ (55 mg/kg body weight) to induced diabetic cardiomyopathy. 4 weeks later, diabetic rats were treated with Ang-(1-9) (200 ng/kg/min), angiotensin type 2 receptor (AT2R) blocker PD123319 (100 ng/kg/min), or Mas antagonist A779 (100 ng/kg/min) for 4 weeks. Although Ang-(1-9) treatment did not affect blood glucose and insulin levels, it significantly attenuated cardiac hypertrophy, reduced cardiac fibrosis and improved ventricular function in STZ-induced diabetic rats. Ang-(1-9) treatment suppressed cardiac NADPH oxidase activity and reduced formation of reactive oxygen species. Ang-(1-9) suppressed NFκB activation and reduced myeloperoxidase (MPO) activity and mRNA levels of TNFα and IL-1β in hearts of diabetic rats. In addition, Ang-(1-9) treatment suppressed activity of ACE and reduced angiotensin II (Ang II) formation in hearts of diabetic rats. The beneficial effect of Ang-(1-9) was blunted by coadministration of PD123319 but not by coadministration of A779. Finally, it was found that Ang-(1-9) treatment could alleviate STZ-induced cardiomyopathy in a dose-dependent manner. In conclusions, Ang-(1-9) attenuates cardiac dysfunction in STZ-induced diabetic rats. The Ang-(1-9)/AT2R axis should be investigated as a novel target for treatment of type 1 diabetic cardiomyopathy.

1. Introduction

The incidence and prevalence of diabetes mellitus are both increasing rapidly in societies around the globe. Diabetic cardiomyopathy, defined as ventricular dysfunction that occurs independent of vascular or valvular pathology, is a severely disabling complication of diabetes mellitus and the leading cause of increased morbidity and mortality in the diabetic population [1,2]. The diabetic cardiomyopathy is considered to involve myocardial hypertrophy, myocardial fibrosis, metabolic disturbances, small vessel disease, and cardiac autonomic neuropathy [3,4]. Presently, therapy for patients with diabetes focuses largely on glucose control. However, recent studies highlight that specific elements of the pathogenesis of diabetic cardiomyopathy are unique [5], raising the prospect of diabetes-specific therapeutic intervention.

Involvement of the renin-angiotensin system (RAS) in diabetic cardiomyopathy has been demonstrated by experimental and clinical studies [6,7]. High glucose induced aberrant activation of intracellular RAS in cardiomyocytes [8], cardiac fibroblasts [9], VSMCs [10], and renal mesangial cells [11]. Clinical and experimental studies have shown beneficial effects of RAS inhibitors, mainly including angiotensin type 1 receptor (AT1) blockers (ARBs) and ACE inhibitors, in diabetes-induced organ damages [12,13]. However, current therapeutic modalities utilizing ACE inhibitors and ARBs may only be partially effective in diabetic cardiomyopathy

Abbreviations: ACE, angiotensin converting enzyme; Ang II, angiotensin II; Ang-(1-9), Angiotensin-(1-9); AT2R, angiotensin type 2 receptor; BNP, brain natriuretic peptide; BW, body weight; EF, ejection fraction; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HW, heart weight; LVEDP, left ventricle end-diastolic pressure; MAP, mean arterial pressure; MDA, malondialdehyde; MPO, myeloperoxidase; O_2^-, superoxide; qRT-PCR, quantitative real time polymerase chain reaction; ROS, reactive oxygen species; STZ, streptozotocin; TGF-β, transforming growth factor beta; β-MHC, β-myosin heavy chain.

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[14,15]. Angiotensin-(1-7), as a heptapeptidic hormone formed by angiotensin-converting enzyme type 2 (ACE2), constitutes an important functional end product of the RAS that acts to balance the physiological actions of ANG II. Treatment with angiotensin-(1-7) ameliorated cardiomyopathy in db/db mice [16], adriamycin-treated rats [17], and myocardial ischemia/reperfusion [18]. In addition, ACE2 overexpression improved left ventricular remodeling and function in a rat model of diabetic cardiomyopathy [19]. More recently, another ACE2 metabolite, angiotensin-(1-9) [Ang-(1-9)], has been reported to antagonise pro-hypertrophic signalling in cardiomyocytes, attenuate cardiac fibrosis in angiotensin II infusion model, renal artery clipping model [20], myocardial infarcted rats [21], and stroke-prone spontaneously hypertensive rats [22]. In vitro, Ang-(1-9) treatment attenuated cardiomyocyte hypertrophy induced by AngII, vasopressin [23], and norepinephrine [21].

The aim of this study was to investigate the therapeutic effect of Ang-(1-9) on streptozotocin (STZ)-induced type I diabetic cardiomyopathy in rats. Ang-(1-9) can also be converted to Ang-(1-7) by ACE, so Mas antagonist A779 was applied in this work to elucidate whether the effect of Ang-(1-9) were derived from activity of the Mas receptor, which was activated by Ang(1-7).

2. Methods

2.1. Materials and animals

Ang-(1-9) (Phoenix Pharmaceuticals, Burlingame, CA, USA), Mas antagonist A779 (Bachem, Rhein, Germany), AT2R antagonist PD123319 (Sigma, St Louis, MO, USA) were used in this work. All other reagents were purchased from Sigma–Aldrich (St. Louis, MO, USA) unless otherwise noted. Sprague–Dawley (SD) rats (male, 2 months old) were purchased from Sino-British SIPPR/BK Lab Animal Ltd. (Shanghai, China) and housed at controlled temperature (23–25 °C), humidity (50%) and lighting (12-h light/dark cycle) with food and water provided ad libitum. All the rats used in this work received humane care in compliance with institutional animal care guidelines, and were approved by the Local Institutional Committee. All the surgical and experimental procedures were in accordance with institutional animal care guidelines. The animal procedures were performed conform the NIH guidelines (Guide for the care and use of laboratory animals).

2.2. Animal model and study design

Rats were randomized to receive either 55 mg/kg of streptozotocin (STZ; Sigma, St Louis, MO, USA) diluted in 0.1 mol/l citrate buffer pH 4.5 (diabetic) to induce experimental type 1 diabetes or citrate buffer alone (non-diabetic control) by tail vein injection following overnight fasting. The diabetic state was confirmed 72 h later by the determination of blood glucose concentrations ≥16.7 mmol/l. Diabetic animals received 2–4 units of isophane insulin (Humulin NPH; Eli Lilly and Co., NSW, Australia) intraperitoneally three times per week to maintain blood glucose levels, promote weight gain and reduce mortality. Last inject of isophane insulin was at 3 days before sacrifice. 4.3% of rats that died and 88.1% of rats that became diabetic in our study.

2.2.1. Experiment 1

Four weeks later osmotic minipumps (Alzet, CA, USA) were subcutaneously implanted to secrete Ang-(1-9) (200 ng/kg/min), AT2R blocker PD123319 (100 ng/kg/min), or Mas antagonist A779 (100 ng/kg/min) for 4 weeks. For osmotic minipump implantation rats were anesthetized with 2.5% isofluorane in 1.5 l/min O_2 for the duration of the surgical implantation procedure. Control rats were implanted with a minipump secreting dH_2O (n = 42–46 total in each group).

2.2.2. Experiment 2

Four weeks later osmotic minipumps (Alzet, CA, USA) were subcutaneously implanted to secrete Ang-(1-9) (50, 200, or 500 ng/kg/min) for 4 weeks. Control rats were implanted with a minipump secreting dH_2O (n = 30–35 total in each group).

2.3. Measurement of cardiac function

Following anesthesia (pentobarbitone sodium, 30 mg/kg body weight, i.p.) in rats, the neck skin was cut open and the right common carotid artery was fully exposed. A micromanometer tipped catheter was inserted into the left ventricle through the right common carotid artery for measurement of left ventricular pressure. Left ventricular end-diastolic pressure (LVEDP), the maximal rate of rise and decline of ventricular pressure (±dp/dt [max]), and ejection fraction (EF) were obtained by BL-410 Bio-signal analysis system (Chengdu TME Technology Co. Ltd., Sichuan, China).

2.4. Blood sample

At the end of the treatment period, the animals were kept on an overnight fast, and were euthanized via an anaesthetic overdose (200 mg/kg of ketamine mixed with 40 mg/kg of xylazine delivered by intraperitoneal injection). Then, the blood was collected and the hearts were isolated for further analysis. Serum glucose and insulin were analysed using enzymatic methods with an automatic analyzer (JCA-BM8060, JEOL Ltd., Tokyo, Japan).

2.5. Morphological examination

Hearts were excised, washed with phosphate-buffered saline (PBS), and fixed in 10% formalin. Hearts were then transversely cut close to the apex to visualize the left ventricles. Four micron sections of the heart were prepared, stained with hematoxylin and eosin (H&E), visualized by light microscopy and photographed. The myocyte outlines were traced and the cell areas measured using "lasso" tool in Adobe Photoshop.

The percentage area of myocardial fibrosis was calculated in 3Mallory-Azan-stained sections of each animal [24].

2.6. RT-PCR

Total RNA was extracted from 50 mg snap-frozen tissue of left ventricles by homogenization in peqGold Trifast reagent (Peqlab, Erlangen, Germany). Residual genomic DNA was removed with Turbo DNAse (Applied Biosystems, Foster City, CA, USA). The RNA concentration and purity were assayed by spectrophotometry. First-strand cDNA synthesis was performed with random hexamer primer and 1 μg RNA using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instruction. A total of 10 ng cDNA in 5 μl was normally used as template for amplification. An additional 0.5 μl of each primer (5 pmol, listed in Table 1) and 12.5 μl of Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) were added and diluted with water to a volume of 25 μl. The PCR was performed on an Mx3000P thermal cycler (Stratagene, La Jolla, CA, USA). Reaction conditions were 95 °C for 10 min, 40 cycles at 95 °C for 15 s, and 60 °C for 60 s, followed by an examination of the melting curve. All samples were analysed in triplicate. Expression of the gene of interest was divided by the housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and expressed as fold-change compared with the corresponding control rats group.