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Sitagliptin attenuates myocardial apoptosis via activating LKB-1/AMPK/Akt pathway and suppressing the activity of GSK-3β and p38α/MAPK in a rat model of diabetic cardiomyopathy

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ABSTRACT

The present study aimed to investigate the protective effect of sitagliptin, a dipeptidyl peptidase-4 inhibitor, on diabetic cardiomyopathy (DCM)-associated apoptosis and if this effect is mediated via modulating the activity of the survival kinases; AMP-activated protein kinase (AMPK) and Akt & the apoptotic kinases; glycogen synthase kinase-3 β (GSK-3β) and p38 mitogen-activated protein kinase (p38MAPK). Diabetes was induced by a single intraperitoneal injection of streptozotocin (55 mg/kg). Diabetic rats were treated with sitagliptin (10 mg/kg/day, p.o.) and metformin (200 mg/kg/day, p.o. as positive control) for six weeks. Chronic hyperglycemia resulted in elevation of serum cardiac biomarkers reflecting cardiac damage which was supported by H&E stain. The mRNA levels of collagen types I and III were augmented reflecting cardiac fibrosis and hypertrophy which was supported by Masson trichome stain and enhanced phosphorylation of p38MAPK. Cardiac protein levels of cleaved casapse-3, BAX were elevated, whereas, the levels of Bcl-2 and p-BAD were reduced indicating cardiac apoptosis which could be attributed to the diabetes-induced reduced phosphorylation of Akt and AMPK with concomitant augmented activation of GSK-3β and p38MAPK. Protein levels of liver kinase B-1, the upstream kinase of AMPK were also supressed. Sitagliptin administration alleviated the decreased phosphorylation of AMPK and Akt, inactivated the GSK-3β and p38 AMPK, therefore, attenuating the apoptosis and hypertrophy induced by hyperglycemia in the diabetic heart. In conclusion, sitagliptin exhibits valuable therapeutic potential in the management of DCM by attenuating apoptosis. The underlying mechanism may involve the modulating activity of AMPK, Akt, GSK-3β and p38MAPK.

1. Introduction

Diabetic cardiomyopathy (DCM) is a hyperglycemia-induced cardiac dysfunction that is characterized by myocardial hypertrophy, fibrosis and apoptosis [1]. Diabetes results in increased circulating FFAs and elevated cardiac uptake and oxidation leading to the accumulation of toxic lipids which trigger the cardiomyocyte apoptosis [2,3] and DCM [4]. Apoptosis is mediated by the activation of caspase-3 and is regulated by the Bcl-2 protein family. The members of this family may be pro-apoptotic such as Bcl-2–associated X protein (BAX) and Bcl-2-associated death promoter (BAD) -which transfer to the mitochondria and alter mitochondrial membrane integrity- or anti-apoptotic proteins such as B-cell lymphoma 2 (Bcl-2) which inhibit the recruitment of pro-apoptotic members to the mitochondria [5]. Phosphorylation of BAD is important in maintaining cell survival, since phosphorylated BAD is unable to transfer to mitochondria [6].

Akt is one of the most commonly described kinases that protect cardiomyocytes from apoptosis through inactivation of caspase-3 [7–9] and phosphorylating BAD, thus inhibiting its pro-apoptotic effect [6–8]. The most-characterized Akt substrate is glycogen synthase kinase-3β (GSK-3β) which is constitutively active in its dephosphorylated form and its activation plays an important role in apoptosis and DCM [10–12]. Under normal conditions, Akt inhibits GSK-3β activity by phosphorylation, however, in diabetes, the ability of Akt to

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phosphorylate GSK-3 is reduced, subsequently; GSK-3β remains active leading to cardiac apoptosis [10–12]. Increased activity of GSK-3β in diabetic cardiomyocytes has been demonstrated and leads to apoptosis via caspase-3 activation [12,13] and stimulating the opening of mitochondrial permeability transition pore (mPTP) [11,13]. Collectively, activation of Akt and inhibition of GSK-3β activity may be a promising strategy to reduce cardiac complications in diabetic patients.

In the heart, the 5′-AMP-activated protein kinase (AMPK) is an upstream kinase that activates Akt and the subsequent phosphorylation of GSK-3β [14,15]. In addition, the activation of AMPK has many cardioprotective effects against ischemic injury, cardiac hypertrophy and cardiac apoptosis in diabetic conditions [14–19]. The activation of AMPK is triggered by its phosphorylation on Thr172 by AMPK kinases; one of them is liver kinase B-1 (LKB-1) which is the major upstream kinase of AMPK in the heart [20]. However, in diabetes, the hyperglycemia results in significant reduced activity of myocardial LKB-1 and AMPK [18,21] which constitutes an important event in the development of DCM [21]. The restored activation of AMPK reversed the adverse effects on diabetic heart; therefore, AMPK signaling has been emerged as an important strategic therapeutic target to ameliorate cardiomyopathy associated with diabetes.

The mitogen-activated protein kinase (MAPK) signaling pathways are effectively involved in the pathogenesis of DCM [22–27]. As a member of MAPKs, p38 has been known to be activated and involved in cardiac hypertrophy [22–24], myocardial fibrosis [19,25] and apoptosis [26,27]. The p38 MAPK consists of four isoforms [α, β, γ and δ], among them p38α is the major form expressed in a healthy heart [28]. The activation of p38αMAPK by hyperglycemia leads to DCM [27,28], meanwhile, its inhibition significantly suppresses the cardiomyocyte apoptosis and the development of DCM [27,29]. Therefore targeting p38αMAPK is another therapeutic strategy in the management of cardiac apoptosis in DCM.

Although remarkable developments have been made in diabetes management, deaths associated with cardiac complications are increasing alarmingly. Metformin is the most commonly used oral antidiabetic drugs and is well known for its cadioprotective effects in diabetic heart [30,31]. However, metformin is used cautiously in patients with heart failure due to the potential risk of lactic acidosis development that may be accentuated in heart failure [32]. More efforts are necessary to develop safe anti-diabetic medications that provide both glycemic control and cardioprotection. Glucagon like peptide-1(GLP-1) provides both goals [8,9]; however, it has a short half-life being metabolized by dipeptidyl peptidase-4 (DPP-4) to inactive form. DPP-4 inhibitors (DPP-4i) are a new class of oral anti-diabetic agents that act by increasing the levels of circulating GLP-1 [33]. Sitagliptin is a selective DPP-4i with cardioprotective effects. Sitagliptin protects against acute myocardial infarction in both normoglycemic and diabetic mice [34]. In addition, sitagliptin revealed cardioprotective effects in diabetic patients presenting with acute coronary syndrome [35] and coronary artery disease [36]. However, the sitagliptin-mediated cardioprotection in DCM and the underlying mechanisms, particularly the anti-apoptotic mechanism are not fully investigated. The purpose of this study was to investigate the protective effects of sitagliptin on DCM-associated apoptosis and whether these effects are mediated via modulating AMPK/Akt/GSK-3β, and p38αMAPK signaling pathways.

2. Materials and methods

2.1. Experimental animals

Adult male albino Wistar rats (200-250 g) were obtained from The Animal Care Centre at the College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. The rats were housed in standard cages, under controlled temperature (25 ± 1 °C) with a 12 h light/dark cycle. Rats were provided with standard rodent chow and tap water *ad libitum*. Animals were allowed to acclimatize to the laboratory conditions for one week before the commencement of the experiments. All experiments were performed in accordance with the guidelines of National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by The Experimental Animals Ethics Committee of the King Saud University.

2.2. Drugs, kits and antibodies

Streptozotocin (STZ) and metformin hydrochloride were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sitagliptin phosphate monohydrate was purchased from BioVision, Inc. (Milpitas, California, USA). Glucose kit was obtained from BioSystem S.A. (Barcelona, Spain). Serum levels of GLP-1, FFA and the biomarkers of cardiac damage [creatine kinase-MB (CK-MB) and cardiac troponin I (cTn-I)] were measured using rat ELISA kits from MyBiosource, Inc. (San Diego, CA, USA). Primary antibodies including anti-BAD, anti-phospho-BAD (p-BAD-Ser136), anti-cleaved caspase-3, anti-Akt, anti-p-Akt (Ser473), anti-GSK-3β, anti-p-GSK-3β (Ser9), anti-AMPK, anti-p-AMPK(Thr172), anti-p38MAPK and anti-p-p38αMAPK (Thr180/Tyr182) were purchased from Cell Signaling Technology Inc. (Beverly, MA, USA). Polyclonal antibodies including anti-BAX, anti-Bcl-2, anti-LKB-1, and anti-GAPDH were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). The secondary antibody; anti-rabbit horseradish peroxidase-conjugated antibody, was purchased from Sigma-Aldrich (St. Louis, MO, USA). Rabbit polyclonal antibodies for immunostaining of caspase-3, BAX and Bcl-2 were purchased from Abcam (Cambridge, UK). All other standard chemical reagents and buffers were provided by Sigma-Aldrich (St. Louis, MO, USA) and Bio-Rad laboratories (Hercules, California, USA).

2.3. Induction of diabetes

After overnight fasting, rats were rendered diabetic using a single intraperitoneal injection of STZ (55 mg/kg) dissolved in a freshly prepared 0.1 M citrate buffer (pH 4.5) [37]. Based on our previous work [38] and recent studies [39,40], i.p. injection of low dose of STZ induces only partial destruction of pancreatic beta cells establishing a model of type 2 diabetes. Seventy-two hours after STZ injection, the rats were tested for hyperglycemia by measuring fasting blood glucose level in tail vein blood samples using Accu-check Advantage II Blood Glucose Monitor (Roche Diagnostic, Indianapolis, USA). Rats with blood glucose levels ≥ 200 mg/dl were considered diabetic and were used in the study [37].

2.4. Experimental design

Rats were weighed, randomly divided into four groups (20 rats each) and treated daily by oral gavage for six weeks as follows: Group I: Normal non-diabetic control rats were administered normal saline solution (the drug vehicle); Group II: Untreated diabetic rats were given normal saline solution; Group III: Diabetic rats treated with 10 mg/kg/day sitagliptin dissolved in 0.9% NaCl and Group IV: Diabetic rats treated with 200 mg/kg/day metformin (the positive control) dissolved in 0.9% NaCl [41]. The dose of sitagliptin was selected based on previous studies that reported this dose effective for cardioprotection [41,42]. All diabetic rats were treated with a sub-therapeutic dose of Lantus insulin (2–3 units) every other day to decrease the mortality rate [43].

At the end of the treatment, all rats were re-weighed after overnight fast, anesthetized and sacrificed by decapitation. Trunk-blood samples were collected to obtain serum. The hearts were rapidly removed, rinsed with ice-cold phosphate buffered saline and weighed. The ratio of heart weight to body weight (HW/BW) was calculated as an index of cardiac hypertrophy. Half of at least three heart tissues from each group were fixed in 4% formaldehyde for histopathological and immunohistochemical studies. The remaining excised heart tissues were