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Deciphering the role of ferulic acid against streptozotocin-induced cellular stress in the cardiac tissue of diabetic rats

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ABSTRACT

The cardiomyocytes are one of the major sources of hyperglycemia induced ROS generation. The present study focuses on the ameliorative role of ferulic acid in combating cardiac complications in diabetic rats. Induction of diabetes by STZ in male Wistar rats (at a dose of 50 mg kg^{-1} body wt, i.p.) reduced body weight and plasma insulin level, enhanced blood glucose, disturbed the intra-cellular antioxidant machineries and disintegrated the normal radiation pattern of cardiac muscle fibers. Induction of ER stress (up-regulation in the levels of CHOP, GRP78, eIF2α signaling, increased calpain-1 expression), caspase-3 activation, PARP cleavage and DNA fragmentation were evidenced from immunoblot analyses and DNA fragmentation assay. However, ferulic acid administration, (at a dose of 50 mg kg^{-1} body wt, orally for eight weeks) in post-hyperglycemia could reverse such adverse effects. Also, the molecule increased GLUT-4 translocation to the cardiac membrane by enhanced phosphorylation of PI3Kinase, AKT and inactivation of GSK-3β thereby altering the hyperglycemic condition in the cardiac tissue of diabetic rats. Therefore, as a potential therapeutic, ferulic acid, exhibiting antioxidant and hypoglycemic effects, may hold promise in circumventing stress mediated diabetic cardiomyopathy in rats.

1. Introduction

Diabetes mellitus (DM) refers to a group of metabolic diseases which are characterized by prolonged maintenance of high blood sugar levels. From 2012 to 2015, diabetes has resulted in 1.5–5.0 million deaths every year. Diabetes occurs either due to insufficient production of insulin from the pancreas or due to lack of response of body cells to insulin. Evidence suggests that, this pathophysiology mainly results due to cellular stress, viz. oxidative and endoplasmic reticulum (ER) stress, followed by hyperglycemia (Kayama et al., 2015; Sinha et al., 2007). Sustained hyperglycemia aggravates reactive oxygen species (ROS) production and further accelerates cellular stress. Diabetic cardiomyopathy refers to the changes in the structure and function of the myocardium, not directly attributable to coronary artery disease or hypertension due to the prolonged maintenance of high blood sugar levels (Abel et al., 1999). In both type 1 and type 2 diabetes, hyperglycemia is associated with increased ROS and reactive nitrogen species (RNS) production in the mitochondria. Within the heart, cardiomyocytes, endothelial cells and neutrophils are the major sources of ROS generation. Increased ROS production causes cardiac dysfunction through direct damage to DNA and proteins, thereby inducing apoptosis (Liu et al., 2014).

Streptozotocin (STZ), a nitrosourea analog, is used for the induction of type 1 diabetes in murine models (Lenzen, 2008). On treatment, it selectively accumulates in the pancreatic β cells via the low-affinity Glucose transporter 2 (GLUT2) in the plasma membrane of such cells and induces hyperglycemic conditions through mitochondrial complication mediated β cell glucotoxicity (Wu and Yan, 2015).

Different natural sources of antioxidants have been reported to exhibit their therapeutic potential in various disease models (Bhattacharya et al., 2013a; Chowdhury et al., in press; Das et al., 2010, 2012a; Ghosh et al., 2009, 2010, 2011, 2015; Manna et al., 2010b; Rashid et al., 2013b; Sinha et al., 2015; Sinha et al., 2007) f including diabetes (Bhattacharya et al., 2011, 2013b; Das and Sil, 2012; Das et al., 2012b; Manna et al., 2010a; Manna et al., 2010b; Manna and Sil, 2012a; Pal et al., 2014; Rashid et al., 2013a; Rashid and Sil, 2015a, b). Ferulic acid (4-hydroxy-3-methoxycinnamic acid) is one such natural potent phytochemical which can be obtained from rice, wheat, barley, apple, orange, coffee, peanuts etc. Ferulic acid exhibits a wide range of biological activities such as

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anti-inflammatory, antimicrobial, hepatoprotective, anticarcinogenic, antiviral, vasodilator actions, etc. (Kumar and Pruthi, 2014). The molecule has already been reported to exhibit antioxidant properties due to lipid peroxidation and effective scavenging of free radicals, by its phenolic hydroxyl group (Srinivasan et al., 2007). In murine models, ferulic acid has been reported to be absorbed intact and possesses the necessary pharmacokinetic properties to be retained in the general circulation for several hours (Priyadarsini, 2014). The anti-hyperlipidemic and anti-hypertensive properties of the molecule holds a promise for its use in cardiovascular diseases. Oral administration of ferulic acid has been reported to reduce blood pressure in a dose-dependent manner in both stroke-prone and spontaneously hypertensive rats (Alam et al., 2013; Ohsaki et al., 2008; Suzuki et al., 2002). The vasodilating effect of the molecule is manifested through the reduced production of NADPH-dependent superoxide anion, increase in nitric oxide synthesis, reduction of angiotensin-II etc. (Suzuki et al., 2002). The cardioprotective effects of the molecule have been reported in the case of both single administrations (Ohsaki et al., 2008) as well as chronic treatment (Alam et al., 2013; Suzuki et al., 2002).

ER stress plays a major role in diabetic conditions. ER stress induces phosphorylation and activation of protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK) which in turn phosphorylates and activates eukaryotic initiation factor 2α (eIF2α). It then activates C/EBP homologous protein (CHOP), a downstream molecule. Phosphorylated PERK releases 78 kDa glucose-regulated protein (GRP78) in the ER lumen. GRP78 is associated with unfolded protein response (UPR) activity. Phospho-PERK (p-PERK) also activates caspase 12 which in turn activates caspase 9 and caspase 3 through subsequent proteolytic events (Morishima et al., 2002). All such activated downstream molecules induce apoptosis. Increase in intracellular Ca^{2+} levels in the myocardial cells of diabetic rats also leads to the activation of calpain-1 which in turn activates caspase 12 by proteolytic cleavage of its procaspase precursor (Xu et al., 2011). On activation, poly-ADP ribose polymerase 1 (PARP1) leads to the nuclear compartmentalization of apoptosis-inducing factor (AIF), thereby inducing cell death (Puthanveetil et al., 2012). However, caspase 3 mediated cleavage induced inactivation of PARP1 facilitates apoptosis by preserving ATP required for the concerned cell death pathway (Rains and Jain, 2011). Oxidative stress leads to the activation of the transcription factors like NF-κB, HIF-1 etc. which on one hand increase the expression of pro-inflammatory genes, while on the other hand, disrupt the phosphorylation of insulin receptor substrate (IRS-1) on binding to the insulin receptor (Rashid and Sil, 2015b). This prevents the activation of the phosphoinositide-3 kinase-protein kinase B (PI3K-Akt) pathway, thereby resulting in reduced translocation of glucose transporter type 4 (GLUT4) which in turn leads to decreased cellular glucose uptake and metabolism. Thus, insulin resistance mediates induction of diabetes (Rains and Jain, 2011). Hyperglycemic conditions prevent insulin signaling by blocking the PI3K-Akt pathway which activates glycogen synthase kinase 3β (GSK-3β) by preventing its phosphorylation which in turn activates caspase 3, thereby inducing apoptosis (Gurusamy et al., 2006).

Literature suggests that ferulic acid exhibits a cardioprotective effect in the diabetic heart (Xu et al., 2012). Since the molecule has already been preliminarily reported to exhibit antioxidant and hypoglycemic effect in STZ induced diabetic rats (Ohnishi et al., 2004), we designed and conducted the present study to evaluate the potential therapeutic role of this molecule in ameliorating STZ-induced and cellular stress mediated cardiac complications using Wistar rats as the working model. Based on the studies on the concerned signaling mechanisms, our results demonstrated that ferulic acid provides protection against cellular stress mediated and ER-dependent apoptotic death of the cardiac cells under diabetic condition.

2. Materials and methods

2.1. Chemicals

STZ was purchased from Sisco Research Laboratory, Mumbai, India. Ferulic acid was purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). BCA (Bicinchoninic assay) kit was obtained from Thermo Fisher Scientific, USA. Antibodies were purchased from Abcam (Cambridge, UK), Cell Signaling (Cell Signaling Technology Inc., Danvers, MA), NeoBioLab, U.S.A., Novus Biologicals, U.S.A. and BioBharti Life Sciences Private Limited, India. All additional chemicals used in the study were of the highest experimental grade available and purchased from Sisco Research Laboratory.

2.2. Animals

Eight weeks old adult male Wistar rats, weighing approximately 180–200 g, were acclimatized under suitable laboratory conditions for two weeks before performing experiments and were maintained under standard conditions of temperature (23 ± 2 °C) and humidity (50± 10%) with alternating 12 h light/dark cycles. The animals were fed with standard pellet diet (Agro Corporation Private Ltd., Bangalore, India) and water ad libitum. All the experiments that had been conducted with animals followed the guidelines approved by the IAEC (InstitutionalAnimal Ethical Committee), Bose Institute, Kolkata [IAEC/BI/3(I) cert./2010] and the study had been approved by both CPCSEA (Committee for the Purpose of Control &Supervision on Experiments on Animals) Ministry of Environment and Forests, New Delhi, India (1796/PO/Ere/S/14/CPCSEA) and IAEC.

2.2.1. Induction of diabetes in experimental animals

Following overnight fasting, diabetes was induced in the experimental rats with a single intraperitoneal injection of STZ (dissolved in 0.1 M sodium citrate buffer, pH 4.5), at a dose of 50 mg/kg body weight (Abdel Aziz et al., 2012). After 7 days of STZ injection, the fasting blood glucose level was determined in the rats using an Advanced Accu-check glucometer (Roche, Germany). The rats with blood glucose above 300 mg/dL were considered to be diabetic and were used for the experiments.

2.3. Solvent and route of administration

STZ was administered intraperitoneally with sodium citrate buffer. On the other hand, ferulic acid was dissolved in water and administered by oral gavage.

2.4. Determination of dose and time dependent effects of ferulic acid

To determine the effective anti-diabetic dose of ferulic acid, dose and time dependent studies were carried out. For this purpose, the rats were randomly divided into 6 groups. Each group had six rats. First two groups served as normal control (received the only vehicle) and diabetic control (received STZ at a dose of 50 mg/kg body weight). Based on the literature, the rest of the four STZ administered diabetic groups were further treated with four different doses of ferulic acid (10, 30, 50 and 70 mg/kg body weight) daily for 8 weeks. The effective dose of ferulic acid and the period of treatment were selected by studying its effect on body weight, fasting blood glucose level and serum insulin level. Eight weeks oral treatment of ferulic acid with a dose of 50 mg/kg body weight daily