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Research paper

Impaired redox signaling and mitochondrial uncoupling contributes vascular inflammation and cardiac dysfunction in type 1 diabetes: Protective role of arjunolic acid

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ABSTRACT

Vascular inflammation and cardiac dysfunction are the leading causes of mortality and morbidity among the diabetic patients. Type 1 diabetic mellitus (T1DM) is associated with increased cardiovascular complications at an early stage of the disease. The purpose of the present study was to explore whether arjunolic acid (AA) plays any protective role against cardiovascular complications in T1DM and if so, what molecular pathways it utilizes for the mechanism of its protective action. Streptozotocin (STZ) was used to induce T1DM in experimental rats. Alteration in plasma lipid profile and release of membrane bound enzymes like LDH (lactate dehydrogenase) and CK (creatine kinase) established the association of hyperlipidemia and cell membrane disintegration with hyperglycemia. Hyperglycemia altered the levels of oxidative stress related biomarkers, decreased the intracellular NAD and ATP concentrations. Hyperglycemia-induced enhanced levels of VEGF, ICAM-1, MCP-1 and IL-6 in the plasma of STZ treated animals indicate vascular inflammation in T1DM. Histological studies and FACS analysis revealed that hyperglycemia caused cell death mostly via the apoptotic pathway. Investigating molecular mechanism, we observed NF-κB and MAPKs (p38 and ERK1/2) activations, mitochondrial membrane depolarization, cytochrome C release, caspase 3 activation and PARP cleavage in apoptotic cell death in the diabetic cardiac tissue. Treatment with AA (20 mg/kg body weight) reduced hyperglycemia, membrane disintegration, oxidative stress, vascular inflammation and prevented the activation of oxidative stress induced signaling cascades leading to cell death. Results suggest that AA possesses the potential to be a beneficial therapeutic agent in diabetes and its associated cardiac complications.

1. Introduction

Diabetes mellitus (DM) is one of the major health problems worldwide. The persistence hyperglycemia is responsible for the various organ and tissue damage in diabetic patients [1]. Vascular inflammation and the cardiac complications are well known among type 1 diabetic subject [2,3]. It has been observed that type 1 diabetes is associated with increased oxidative stress and elevated levels of various inflammatory biomarkers such as CRP (C-reactive protein), monocyte activity, sICAM (intercellular adhesion molecule), sE-selectin, sP-selectin, and sCD40L [4,5]. The mechanism of hyperglycemia induced cardiovascular lesions appears to be multifactorial. Oxidative stress plays a major role in the development of cardiovascular disease (CVD) in T1DM [6,7]. Although the precise source of reactive oxygen species in diabetic pathophysiology has not been clarified yet, auto-oxidation of glucose per se, AGEs (advanced glycation end products) producing processes, mitochondrial dysfunction, and others have been reported as the possible sources [1]. The mechanism of oxidative stress induced CVD in T1DM has been extensively studied and several reports suggest that oxidative stress may disrupt endothelial cell function which may be related to the vascular inflammation and cardiac dysfunction [8–10]. Besides the investigation on the molecular mechanism of CVD in TIDM, the prevention or the reduction of these complications in diabetic population is the most imminent issue in the field of clinical diabetology in many countries. Increased glucose utilization or glycemic control is one of the most effective means to prevent the appearance of diabetic pathophysiology. Therefore, a rationale therapeutic modality would be a pharmacological compound that can stimulate insulin mediated glucose metabolism in the target tissues without any significant

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side effects. Arjunolic acid (AA: 2,3,23-trihydroxyolean-12-en-28-oic acid), a natural pentacyclic triterpenoid saponin, is well known for various biological functions [11–14]. In our earlier investigation, we have found that AA acts as a prophylactic agent against different drugs and toxin (environmental pollutant) induced oxidative cardiac dysfunction [15,16]. In addition we also reported its beneficial role in reducing streptozotocin (STZ, a commonly used agent in experimental diabetes) induced type 1 diabetes and its associated organ pathophysiology like liver, kidney and spleen via the mitochondria dependent as well as independent pathways [17–20]. However, there is no report in the literature describing the effect of this compound against cardiovascular dysfunction in T1DM. The present study was, therefore, designed to investigate the mode of action of AA in the vascular inflammation as well as cardiac pathophysiology in T1DM.

2. Materials and methods

2.1. Chemicals

Streptozotocin (STZ) anti cleaved caspase-3, anti ERK1/2 and anti phosphorylated ERK1/2 antibodies were purchased from Sigma–Aldrich Chemical Company (St. Louis, MO, USA). Anti-NF-κB (p65 subunit), anti phosphorylated NF-κB (p65 subunit), anti p38 and anti phosphorylated p38 antibodies were purchased from Cell signaling technology (Danvers, MA, USA). Other antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). All other chemicals were purchased from Sisco research laboratory (Mumbai, India) unless otherwise mentioned.

2.2. Isolation and characterization of arjunolic acid (AA)

In our laboratory arjunolic acid (AA) has been extracted previously from the bark of *Terminalia arjuna* and the compound has been characterized using NMR (1H, 13C), DEPT, IR, Mass spectroscopy and optical rotation studies [21]. For the present study, the same procedure has been used for the extraction and the purity has been checked with the standard.

2.3. Animals

Swiss albino male rats, weighing approximately 120–130 g were acclimatized under laboratory condition for two weeks prior to the experiments. All the experiments with animals were carried out according to the guidelines of the institutional animal ethical committee and full details of the study was approved by the CPCSEA, Ministry of Environment & Forests, New Delhi, India (the permit number is: 95/99/CPCSEA).

2.4. Induction and selection of diabetic animals

Diabetes was induced in the experimental animals with an intraperitoneal injection of STZ at a dose of 65 mg/kg body weight dissolved in citrate buffer (0.1 M, pH 4.5). STZ-injected animals exhibited massive glycosuria and hyperglycemia within a few days and the diabetic nature of the animals was confirmed by measuring blood glucose concentration 72 h after STZ injection in the overnight-fasted rats. The rats with blood glucose above 350 mg/dL were considered to be diabetic and then they were used for the experiments as necessary.

2.5. Studies on the effect of AA

The animals were divided into four groups (consisted of six rats in each) and they were treated as follows (Fig. 1):

Group 1 (Control) – Animals received only water as vehicle.

Group 2 (STZ treated group) – T1DM group.

Group 3 (T1DM+AA) – AA was administered orally at a dose of 20 mg/kg body weight for 4 weeks after diabetic induction.

Group 4 (T1DM+Insulin) – A volume of 0.5 ml of a 2 mg/mL aqueous insulin solution was administered orally for 2 weeks after diabetic induction. This group served as a positive control through the entire study.

At the end of the experiments, all the animals were euthanized after 12 h fasting. Blood samples were drawn from the caudal vena cava, collected in heparinized tubes, and centrifuged at 1500 *g* for 10 min to obtain serum. For the biochemical analysis and immunoblotting, cardiac tissues were lysed in Radioimmunoprecipitation Assay (RIPA) buffer (50 mM Tris pH 8, 150 mM NaCl, 1% NP-40, 0.5% deoxycholic acid, 0.1% SDS) supplemented with protease and phosphatase inhibitors (1 mM PMSF, 5 μg/mL leupeptin, 2 μg/mL aprotinin, 1 mM EDTA, 10 mM NaF, and 1 mM $NaVO_4$). Lysates were cleared by centrifugation and total protein concentrations were determined by BCA assay (Pierce/Thermo Scientific, Rockford, IL, USA). For histopathological examinations the cardiac tissues were either fixed in 10% formalin.

[The dose schedules of STZ and insulin have been chosen based on the earlier reports of Dias et al. [22] and Von et al. [23] respectively. In our earlier investigations [17–20] we have found that at

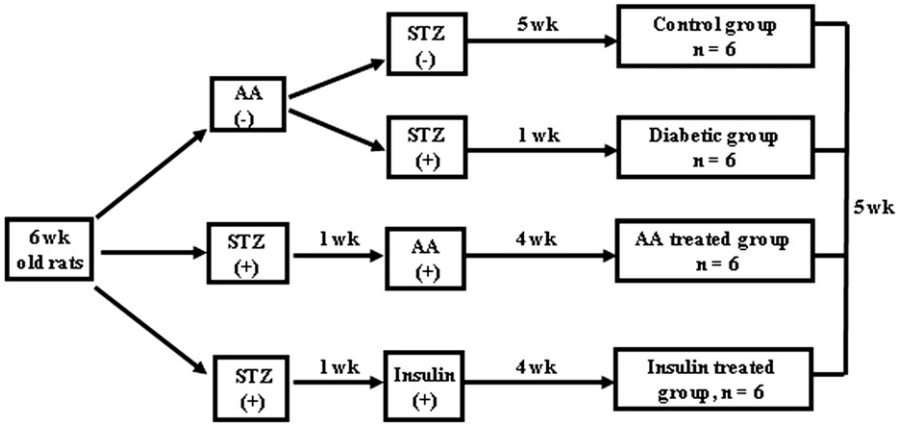

Fig. 1. Schematic diagram of in vivo experimental protocol.