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Original article

Effects of gallic acid on hemodynamic parameters and infarct size after ischemia-reperfusion in isolated rat hearts with alloxan-induced diabetes

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ABSTRACT

Diabetic rats are more susceptible to myocardial ischemia-reperfusion injury than control rats. The aim of the present study was to evaluate the cardioprotective effect of gallic acid (GA) on isolated rat hearts with alloxan-induced diabetes mellitus. Adult male Sprague-Dawley rats were divided randomly into three groups: control, untreated diabetic and diabetic animals treated with (GA, 25 mg/kg). Diabetes was induced by 120 mg/kg alloxan injection. Eight weeks after GA administration, the hearts were isolated and exposed to myocardial ischemia-reperfusion. The body weight, blood glucose, hypertrophy index, left ventricular function, infarct size, cardiac markers and oxidative stress were measured. In the diabetic group, body weight, cardiac contractility (± dp/dt), glutathione peroxidase (GPx) level ($p < 0.001$), left ventricular developed pressure (LVDP), rate pressure product (RPP), superoxide dismutase (SOD) and catalase (CAT) levels ($p < 0.01$) as well as the heart weight ($p < 0.05$) significantly reduced. However, blood glucose, infarct size, hypertrophy index, lactate dehydrogenase (LDH), creatine kinase-MB (CK-MB, $p < 0.001$) and troponin-I (cTnI) levels ($p < 0.05$) significantly increased in the diabetic rats compared with the control group. Nevertheless, administration of GA improved significantly LVDP, ± dp/dt, infarct size, LDH, CK-MB ($p < 0.001$), blood glucose, the heart weight ($p < 0.01$), body weight, RPP, hypertrophy index, antioxidant enzyme and cTnI levels ($p < 0.05$) in the diabetic rats. The results of this study indicated that in the diabetic rats, left ventricular dysfunction and hypertrophy significantly induced possibly by oxidative stress. Moreover, GA as a potent antioxidant improved both left ventricular dysfunction and hypertrophy.

1. Introduction

Diabetes mellitus (DM) is a metabolic disorder that described by chronic high blood glucose due to the defect in insulin release, insulin effect, or both [1]. It has become a crucial health problem due to the rising prevalence and its association with cardiovascular diseases. Furthermore, DM is a significant risk factor for the induction of ischemic heart disorders [2]. In fact, 80% of cardiac patients are diabetic and ischemic heart disease is an important cause of death in diabetic individuals [3].

Clinical evidences demonstrated that the diabetic heart was higher sensitive to ischemia-reperfusion injury (IRI) [4,5]. Additionally, oxidative stress caused by the overproduction of reactive oxygen species (ROS) in the absence of sufficient antioxidant defense has been regarded as a possible mechanism in the increase of susceptibility to cardiac IRI [6]. Although, ROS at low physiological levels play a beneficial role in cardiac molecular signaling, but at the high pathological concentrations status (such as diabetes) they exert harmful effects on the heart. On the other hand, during ischemia and at the beginning of reperfusion, ROS production is intensified which can aggravate cardiac IRI [7]. Reperfusion, such as angioplasty and thrombolysis may cause coronary blood flow restoration and infarct size reduction, but can trigger cardiac tissue damage, namely, IRI [8]. The high ROS production and oxidative stress during reperfusion play a pivotal role in the progression of IRI [9]. Thus, the foundation of a novel approach to reduce myocardial infarction during ischemia-reperfusion is clinically important. The high blood glucose and ROS production in DM have been documented, and are involved in the pathogenesis of different cardiovascular disorders [10]. Therefore, among the different therapeutic approaches, anti-hyperglycemic and antioxidant compounds

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can be beneficial in the prevention of cardiovascular disorders associated with DM [10].

Gallic acid (GA) is one of the most prominent polyphenol compounds in plants. It is found in various berries, oak bark, sumac, green tea, pineapple, grapes and other fruits and it has been exhibited to possess free radical scavenging, antioxidant and anti-inflammatory effects [11,12]. It has been demonstrated that GA is involved in the amelioration of cardiac disorders associated with alloxan-induced DM [10]. GA also has a preventive activity in cardiotoxicity induced by isoproterenol via antioxidant and anti-lipoperoxidative properties in Wistar rats [13]. In addition, previous study presented that treatment of rats with grape seed extract and exercise training significantly decreased LDL-c level and cardiovascular diseases [14]. However, the beneficial effect of GA on cardiac IRI in diabetic animals did not well investigated. Therefore, the current study aims to evaluate the effects of GA on hemodynamic parameters and infarct size after ischemia-reperfusion in isolated rat hearts with alloxan-induced DM.

2. Materials and methods

2.1. Drugs

Heparin, GA, Triphenyltetrazolium chloride (TTC) and Alloxan monohydrate were bought from Sigma-Aldrich (St. Louis, MO, USA). Sodium hydrogen carbonate, Sodium chloride, Potassium chloride, Potassium hydrogen phosphate, Magnesium sulphate, D-glucose and Calcium chloride were obtained from Merck Company (Merck & Co., Inc, USA). Ketamine hydrochloride and Xylazine hydrochloride were purchased from Alfasan Co. (Woderen- Holland).

2.2. Experimental animals

Male Sprague-Dawley rats weighing 250 ± 20 g were purchased from Ahvaz Jundishapur University of Medical Sciences, Animal House Center, Ahwaz, Iran. They were kept in an animal house under standard environmental conditions (light: dark cycle of 12 h at 20–25 °C) and fed chow pellets and water ad libitum. The study was certified by the Animal Ethics Committee of Ahvaz Jundishapur University of Medical Sciences (grant No. APRC-94-25). After seven days acclimatization to the laboratory, the animals were randomly allocated into three groups (8–9 rats in each group): control (C, received saline and distilled water by gavage and intraperitoneal administration, respectively), diabetes model group (D), diabetes + GA (D + G, 25 mg/kg). GA was dissolved in saline and given once daily via gavage for 8 weeks after verification of diabetes in the diabetic rats [15]. Diabetes was induced using a single intraperitoneal (IP) administration of alloxan (120 mg/kg) dissolved in distilled water. Fasting blood glucose was estimated by glucometer. Rats showing fasting blood glucose ≥250 mg/dL four days after alloxan administration were considered in the following investigation as diabetic rats

At the beginning of the study and eight weeks after induction of diabetes, the blood glucose level and body weight were measured [16].

2.3. Preparation and perfusion of isolated heart

The animals were anesthetized by IP injection of ketamine and xylazine (50 and 5 mg/kg, respectively) contained 1000 U/kg heparin. After cannulation of trachea, the animals were ventilated using a small animal ventilator (UGO BASILE, model: 7025). Procedure of aortic cannulation was performed through a central incision in the aorta and hold in place with suture after thoracotomy. Subsequently, the hearts were hanged on the langendorff system and were perfused continuously. Retrograde heart perfusion was carried out using Krebs-Henseleit bicarbonate buffer solution at 37 °C, pH 7.4, constant flow of 8 ml/min and the solution was oxygenated by 95% oxygen and 5% carbon dioxide. A water latex balloon was placed in the left ventricle for the measurement of left ventricular pressure (LVP) by a pressure transducer and Power Lab system (AD Instruments, Australia). Left ventricular end diastolic pressure (LVEDP) was regulated approximately 5–10 mmHg via balloon volume. The maximum and minimum rates of LVP (± dp/dt), Left ventricular end diastolic and systolic pressure (LVEDP, LVSP) and Left ventricular developed pressure (LVDP) were assessed. Moreover, the rate pressure product (RPP) was computed using following formula

$$RPP = LVDP \times \text{heart rate}$$

The isolated hearts were subjected to ischemia and reperfusion (30 and 60 min, respectively) after an equilibration period of 30 min.

2.4. Infarct size measurement

Infarct size measurement was determined after the ischemia and reperfusion periods. The isolated hearts of rats were frozen and transversely sliced into several sections of 2 mm thickness. The hearts were incubated in 1% TCC and then fixed in 10% formalin for 20 and 10 min, respectively. Images were analyzed using ImageJ software and expressed as the ratio of infarct size of the total area [17].

2.5. Hypertrophy index measurement

After the final administration of GA and body weight measurement, the rats were anesthetized. The heart of each rat was excised and placed in a dish with physiological saline and then was placed on filter paper for measurement of the heart weight. Subsequently, hypertrophy index was calculated using the heart weight to body weight ratio [18].

2.6. Endogenous antioxidant enzymes assay

After ending of reperfusion, 100 mg of heart tissue was removed and frozen in liquid nitrogen and stored at −80 °C. Frozen tissue samples in phosphate buffered saline (PBS; 50 mM at pH of 7.4) were homogenized via a Homogenizer (Heidolph Silenterosher M, Germany), and centrifuged at 14000*g* for 15 min. The measurement of enzyme activities such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) was carried out on supernatant. To prevent inter assay variation; all measurements were carried out on the same day. SOD and GPX were evaluated by Randox kits (Randox Lab, UK) and CAT activity was measured by Zellbio kit (Zellbio Lab, Ulm, Germany).

2.7. Cardiac marker enzymes assay

The measurement of cardiac markers such as lactate dehydrogenase (LDH), myocardial creatine kinase (CK-MB) and cardiac troponin I (cTnI) levels were carried out on supernatant from heart tissue to evaluate of cardiac injury. LDH and CK-MB levels were measured spectrophotometrically by standard kits (Pars Azmun, Tehran, Iran) and cTnI was measured by Zellbio kit (Zellbio, Lab, Germany). The results expressed as units per g tissue.

2.8. Statistical analysis

Data were analyzed with SPSS version 16 and expressed as mean ± SEM. Differences among groups were carried out by paired *t*-test, two-way repeated measures and one-way ANOVA followed by LSD post hoc test. P-values of less than 0.05 and the 95% confidence interval (CI) were regarded as significant.