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Anti-arrhythmogenic and anti-inflammatory effects of troxerutin in ischemia/reperfusion injury of diabetic myocardium

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ABSTRACT

Introduction: Medicinal plants are increasingly used in the treatment of cardiovascular diseases due to their multifaceted properties. This study was designed to investigate anti-arrhythmic and anti-inflammatory potentials of the natural bioflavonoid, troxerutin (TXR) in myocardial ischemia/reperfusion (I/R) injury in diabetic rats.
Methods: Male Wistar rats were randomly divided into 4 groups (control, control + TXR [150 mg/kg, daily], diabetic, and diabetic + TXR). Type-1 diabetes was induced by an intraperitoneal injection of streptozotocin (50 mg/kg) and lasted for 10 weeks. After mounting on the Langendorff apparatus, isolated hearts in all groups received a normal Krebs–Henseleit solution for 20 min of stabilization period, followed by 30 min of regional ischemia through ligation of the left anterior descending coronary artery, and 60 min of full reperfusion. During the experiment, the electrocardiograms were recorded and the arrhythmias [number, duration and incidence of premature ventricular complexes (PVC), ventricular tachycardia (VT), ventricular fibrillation (VF), and arrhythmia score] during I/R phases were assessed based on the Lambeth Convention. Ischemic left ventricular samples were used to determine the activities of lactate dehydrogenase (LDH), interleukin-1beta (IL-1β), and tumor necrosis factor (TNF-α).
Results: The arrhythmias induced by I/R were not significantly changed in diabetic group as compared to the control group. However, pretreatment with TXR significantly reduced the number of PVC and duration and incidence of VF in ischemic phase in comparison to the untreated animals ($P < 0.05$). In addition, the duration, and incidence of most arrhythmias during reperfusion phase were significantly declined by TXR administration in both control and diabetic groups ($P < 0.05$). Pretreatment of rats with TXR significantly reduced myocardial inflammatory cytokines TNF-α and IL-1β levels after I/R insult in diabetic as well as control hearts ($P < 0.05$).
Conclusion: Preconditioning with TXR could provide cardioprotection by anti-arrhythmic and anti-inflammatory effects against I/R injury in rat hearts. This effect of TXR can introduce this material as a protective agent in cardiovascular diseases.

1. Introduction

Cardiovascular disease comprises a wide ranges of diseases including ischemia/reperfusion (I/R) injuries, causing human mortality worldwide [1]. It is anticipated that ischemic heart disease will be the most important reason for death by 2020 [2,3]. This high mortality risk is associated with some comorbidities such as aging, high blood pressure, hyperlipidemia, metabolic disorders, and diabetes mellitus [1,4,5]. Undoubtedly, restoration of blood flow (reperfusion) to the ischemic heart lead to decreased mortality rate of ischemic patients [1,6,7], however, reperfusion by itself causes further tissue injury and a sequence of detrimental events including arrhythmias (particularly), microvascular injury, myocardial dysfunction, myocardial stunning, and finally death [8,9].

Myocardial arrhythmias are a common problem of I/R injuries in clinical practice, occurring in up to 80% of patients with acute myocardial infarction [10]. Increased intracellular [Ca^{2+}] has been proposed as a potential culprit for reperfusion-arrhythmogenesis [11,12]. In addition, inflammatory cytokines and free radicals have been implicated in the pathophysiology of reversible post-ischemic contractile dysfunction, cardiac cell death, and electrophysiological derangements [13,14]. The inflammatory response has an important contribution to I/

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R injury [15]. In fact, the occurrence of arrhythmias in myocardial reperfusion might be the direct consequence of increased production of reactive oxygen species (ROS) and inflammatory cytokines during myocardial I/R [16]. Inflammatory signaling cascades triggered during reperfusion injury activate NF-κB, and result in the overexpression of a range of important pro-inflammatory cytokine and chemokine genes, such as TNFα, IL-1, IL-6, and IL-8, initiating myocardial inflammatory responses [17,18]. In the presence of diabetes, the inflammatory response to I/R insult is exaggerated due to the stimulatory role of diabetes in the accumulation of leukocytes and activation of adhesion molecules and cytokines [16].

So far, myocardial injury induced by I/R and other dependent conditions have been studied in healthy subjects, but fewer studies have been done about the interactions of cardiovascular risk factors including diabetes mellitus in this regard [1]. Hyperglycemia is the most important risk factor for diabetic complications, resulting from uncontrolled glucose regulation in diabetic patients [1,4]. Numerous studies demonstrated that the neurohumoral alterations and intracellular kinases dysfunctions in diabetic situations may contribute to the development and progression of diabetic complications and their interference with myocardial I/R injuries and related treatments [1,8,16]. Despite significant advances in therapeutic strategies against all manifestations of myocardial post-ischemic injury, a potent and safe cardioprotective agent have not been defined yet.

Troxerutin (TXR), known as vitamin P_4, is a derivative of the glucosidal natural bioflavonoid, which has various biological effects such as anti-oxidation and anti-inflammation [19–21]. This natural flavonoid is extracted from *Sophora japonica* and *Dimorphandra gardneriana* [21]. In some countries, TXR is used for treatment and prevention of varicose veins, hemorrhoids, and thrombophlebitis [22]. Also, it has antineoplastic, anti-thrombotic, anti-fibrinolytic and rheological activities [19–23]. The effects of TXR on myocardial I/R injury remain still unclear in healthy and diabetic conditions. We previously reported that pretreatment of diabetic rats with TXR can prevent diabetic vascular abnormalities through the reduction of lipid peroxidation and improvement of endogenous antioxidative activity [24]. We have also shown that this agent has anti-apoptotic potential in myocardial reperfusion injury of diabetic rats [25]. In a recent work, we demonstrated that TXR treatment as well as ischemic postconditioning significantly inhibited the activation of leukocyte-endothelial cell interactions and prevented the inflammatory-pathological changes of I/R insults in healthy myocardial cells [14]. Monitoring and lessening of arrhythmias are clinically important in the management of subjects with myocardial infarction and I/R injury, more especially in the presence of diabetes. Thus, due to the cardioprotective potentials of TXR, the aim of the present study was to explore the effects of this agent on the myocardial arrhythmias induced by I/R injuries and the related contribution of myocardial inflammatory response in type-1 diabetic rats.

2. Materials and methods

2.1. Animals

Healthy adult male Wistar rats (230–300 g) were used in this investigation. The rats were taken from the animal center of Tabriz University of Medical Sciences and housed in the animal room under 12-h light/dark cycle at 22 ± 3 °C and 50 ± 10% relative humidity. All animals had free access to sufficient food and water. All the stages of the experiments, animals care and handling were in accordance with guidelines of animal care committee of Tabriz University of Medical Sciences (ethical number: 92-3729).

2.2. Materials

TXR, streptozotocin (STZ), and D-glucose were obtained from Sigma (Germany) and other chemical materials were obtained from Merck (Germany). All of the chemicals and reagents were purchased from the best commercial sources of the highest quality available.

2.3. Induction of diabetes and experimental protocol

Diabetes was induced by a single intraperitoneal injection of STZ (50 mg/kg body weight) in rats. STZ leads to disruption of pancreatic islet β cells and reduces secretion of insulin, resulting in type 1 diabetes mellitus. Development of diabetes was confirmed 72 h later by measuring blood glucose levels using a glucometer device through the sampling of blood from rats' tail vein. The animals with blood glucose levels higher than 300 mg/dl were considered as diabetic and the animals with lower glucose levels were excluded from the experiment. The duration of diabetes was 10 weeks to mimics the chronic nature of the disease [8].

The rats were randomly divided into following 4 groups (n = 6/each group):

- Control group (C) – non-diabetic rats with no TXR treatment.
- Control + TXR group (C + TXR) – non-diabetic rats received orally TXR (150 mg/kg) for a month.
- Diabetic group (D) – diabetic rats with no TXR treatment.
- Diabetic + TXR group (D + TXR) – diabetic rats received orally TXR (150 mg/kg) for a month.

Rats in C + TXR and D + TXR groups received 150 mg/kg daily dose of TXR in distilled water by oral gavage for 4 weeks (at the last month of the diabetic period) (2426). The gavage was performed once a day in the morning for all rats. After ten weeks, the hearts of all rats experienced I/R injury in the Langendorff setting.

2.4. Langendorff setting for isolated hearts perfusion

All animals were heparinized (500 IU) and anesthetized with a mixture of ketamine (60 mg/kg) and xylazine (10 mg/kg), then their hearts were surgically isolated and rapidly mounted on the Langendorff apparatus. The hearts were perfused with Krebs–Henseleit (K–H) solution that contained (in mmol/L): 118 NaCl, 4.8 KCl, 1.2 $MgSO_4$, 1.0 KH_2PO_4, 27.2 $NaHCO_3$, 10 Glucose and 1.25 $CaCl_2$, at a constant perfusion pressure of 75 mmHg throughout the experiment. Also, a mixture of 95% O_2 and 5% CO_2 was bubbled through the perfusion, so that the perfused pH was kept at 7.4. A thermostatically controlled water circulator (Satchwell Sunvic, UK) maintained the perfusate and bath temperatures at 37 °C [25,27].

2.5. Myocardial ischemia/reperfusion

Isolated hearts in the Langendorff perfusion setting in all groups received normal K–H solution within 20 min of stabilization period, 30 min of regional ischemia and 60 min of reperfusion. The regional ischemia was applied through ligation of left anterior descending (LAD) coronary artery and confirmed by reduced coronary flow to about 30–40% of its baseline values. The reperfusion was followed by reopening of LAD and confirmed by restoring of coronary flow [28].

2.6. Electrocardiogram recording and arrhythmias interpretation

Impulsive heart rate and heart electrical activity were monitored through electrocardiograms (ECGs), recorded throughout the experiment. The arrhythmia were continuously recorded and digitized by a data acquisition system (PowerLab, AD Instruments, Australia), displayed on a monitor and analyzed using Chart v7.7 for Windows Software (AD Instruments). Criteria for classification of ventricular arrhythmias was based on the Lambeth Conventions [29]. Accordingly, arrhythmias were categorized as a single ventricular premature beats