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Diosmin pretreatment improves cardiac function and suppresses oxidative stress in rat heart after ischemia/reperfusion

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ABSTRACT

Reperfusion of ischemic tissue leads to the generation of oxygen derived free radicals which plays an important role in cellular damage. Objective of the current study is to evaluate the cardio-protective and antioxidant effect of diosmin on ischemia–reperfusion related cardiac dysfunction, oxidative stress and apoptosis. Diosmin (50 and 100 mg/kg body weight (bw)) was given every day to the rats orally throughout the experimental period. Ischemia/reperfusion protocol was carried out *ex vivo* using langendorff perfusion method and the cardiac functional recovery was assessed in terms of percentage rate pressure product. Coronary effluents of LDH and CK-MB activities, antioxidant enzyme activities, lipid peroxidation products, activity of TCA cycle enzymes were evaluated. Moreover, *in vitro* superoxide anion and hydroxyl radical scavenging potential of diosmin was also quantified. Finally, quantitative real-time PCR was used for assessing Bcl-2 mRNA expression in heart. Cardiac functional recovery was impaired after reperfusion compared with continuously perfused heart. It was significantly prevented by diosmin treatment. Impaired antioxidant enzyme activities and elevated lipid peroxidation products level were also significantly suppressed. The activity of TCA cycle enzymes was protected against reperfusion stress. Down regulated Bcl-2 was also significantly increased. This study concluded that diosmin pretreatment prevents all the impaired patterns including cardiac function, oxidative stress and apoptosis associated with reperfusion in control heart by its antioxidant role.

1. Introduction

Myocardial ischemic injury results from severe impairment of coronary blood supply and produces a spectrum of clinical syndromes. It is well known that ischemia/reperfusion (I/R) leads to functional, metabolic and structural abnormalities in the myocardium and there is a great deal of evidence showing that both cytosolic Ca^{2+} overload and oxidative stress are pivotal factors underlying ischemia/reperfusion injury in the heart (Buja, 2005; Dhalla et al., 2007; Moens et al., 2005; Powers et al., 2007).

Myocardial ischemia–reperfusion injury contributes to adverse cardiovascular outcomes after myocardial ischemia, cardiac surgery or circulatory arrest. Primarily, no blood flow to the heart causes an imbalance between oxygen demand and supply, resulting in damage or dysfunction of the cardiac tissue. Restoration of blood flow and reoxygenation to the ischemic myocardium, named reperfusion frequently associated with an exacerbation of tissue injury (Frank et al., 2012). As a result of intensive investigation over decades, a detailed understanding is now available about the complexity of response of the myocardium to an ischemic insult. Ischemia/reperfusion (I/R) injury results in cell death of cardiac myocytes and consequently reduced myocardial function.

Endogenous reactive oxygen species appear to play important roles in modulating normal cellular processes and the intracellular antioxidant system can balance the effect of reactive oxygen species under normal conditions, under abnormal condition, the antioxidant mechanism is undermined and reactive oxygen species-induced tissue damage can take place (Law et al., 2013). Mitochondrial dysfunction during cardiac ischemia/reperfusion injury is associated with Ca^{2+} overload, excess emission of reactive oxygen species and reactive nitrogen species that can lead to deleterious post-translational modifications of mitochondrial proteins. Superoxide (O_2^-), the origin of most O_2-derived free radicals, is overproduced and under scavenged during cardiac I/R injury (Yang et al., 2012).

Since oxidative stress is a key factor that contributes to ischemia–reperfusion injury, antioxidant treatment is considered as a potential strategy to prevent myocardial ischemia–reperfusion injury (Aldakkak et al., 2011; Montecucco et al., 2010). Moreover, cluster of studies

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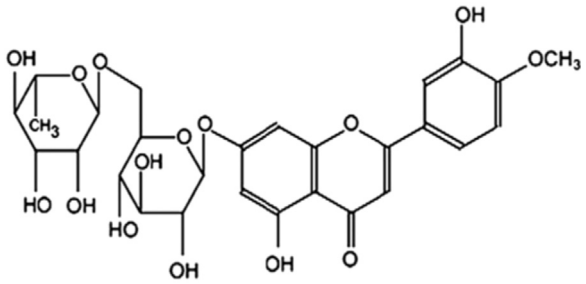

Fig. 1. Structure of diosmin.

found that natural flavonoids may protect heart from ischemia–reperfusion injury by scavenging reactive oxygen species and enhancing antioxidant system gene expression (Akhlaghi and Bandy, 2009). Some flavonoids exhibit direct cardio-protective effects against the injury induced by drastic I/R (Testai et al., 2013).

Diosmin is a flavonoid found in citrus fruits and its structure (3′,5,7-trihydroxy-4′-methoxyflavone-7-ramnoglucoside) was shown in Fig. 1. As a flavonoid, it possesses a multitude of biological activities including antihyperglycemic, anti-lipid peroxidation (Srinivasan and Pari, 2012), anti-inflammatory, antioxidant, antimutagenic properties (Camarda et al., 2007) and antihypertensive properties (Silambarasan and Raja, 2012).

Antioxidant and anti-apoptotic potential of diosmin against I/R injury and its effect on impaired cardiac function were not studied elsewhere. Therefore the aim of the present study was to investigate the effects and mechanism of action of pharmacologically preconditioned heart with diosmin on I/R injury and cardiac functional recovery in the context of oxidative stress, mitochondrial dysfunction and apoptosis related gene expression.

2. Materials and methods

2.1. Animals and chemicals

Male albino Wistar rats, 8–10 weeks old (weighing 180–220 g) were procured for this study. This experimental study was approved by the Ethical Committee of Rajah Muthiah Medical College and Hospital, Annamalai Nagar, Tamil Nadu, India. Diosmin was purchased from Sigma-Aldrich (St. Louis, Missouri, USA). All other chemicals used in this study were of analytical grade obtained from Merck and Himedia, India.

2.2. Diosmin treatment

Each of the following groups consists of ten animals. Diosmin was dissolved in 0.5% dimethylsulfoxide and administered orally to rats daily for 7 days.

Group I – Control animal heart continuously perfused (Control-No-I/R)

Following groups were subjected to ischemia/reperfusion protocol,

Group II – Control-I/R
Group III – Control-I/R+diosmin 50 mg/kg bw
Group IV – Control-I/R+diosmin 100 mg/kg bw

2.3. Langendorff heart ischemia/reperfusion protocol

The animals were anaesthetized with an intramuscular injection of ketamine (75 mg/kg bw). After thoractomy, the hearts were rapidly excised and were mounted on a Langendorff apparatus (AD Instruments, Australia), and retrogradely perfused with modified Krebs–Henseleit (KH) bicarbonate buffer (containing (in mM) 120 NaCl, 25 $NaHCO_3$, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 1.2 $CaCl_2$, and 11 glucose; the perfusion buffer was prepared and filtered) *in vitro* via the aorta at a constant pressure of 80 mmHg. The perfusion medium was continuously bubbled with a mixture of 95% O_2 and 5% CO_2 at 37 °C giving a pH of 7.4. Each heart was housed in a controlled heart chamber maintained at 37 °C (Khan et al., 2006). The ischemia and reperfusion protocol was followed as previously described (Khan et al., 2006; Esterhuyse et al., 2005). After 10 min of stabilization, hearts from I/R protocol groups were then subjected to 30 min of global ischemia and 60 min reperfusion. One group of control rat hearts was subjected to continuous perfusion (Control-No-I/R). Fig. 2 shows the schematic diagram of I/R protocol.

A water-filled balloon, connected to a pressure transducer, was placed into the left ventricle (LV) through a left atrial incision. The balloon volume was adjusted to achieve a stable left ventricular end-diastolic pressure (LVEDP) of 10 mmHg. The rate-pressure product [RPP=(LVSP−LVEDP)×HR] was calculated as percentage by dividing the RPP of reperfusion by the RPP of pre-ischemic and multiplying it by hundred (Esterhuyse et al., 2005; Ferrera et al., 2009).

2.4. LDH and CK-MB activity analysis

Cellular injury was evaluated by measuring the activity of lactate dehydrogenase (LDH) and CK-MB release in the coronary effluent immediately after the reperfusion period. Assay was performed by commercially available kits as per manufacturer's instructions (Agappe diagnostics, India). Results were expressed in unit per liter.

2.5. Enzymatic antioxidants and lipid peroxidation products

Heart tissues were sliced into pieces and homogenized in appropriate buffer in cold condition (pH 7.0) to give 20% homogenate (w/v). The homogenate was centrifuged at 560×*g* for 10 min at 4 °C in refrigerated centrifuge. The supernatant was separated and used for various biochemical estimations.

Heart antioxidant enzyme assay and lipid peroxidation assays had been done by spectroscopic methods. Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) was assayed with the methods of Kakkar et al. (1984), Sinha (1972), and Rotruck et al. (1973) respectively. Reduced glutathione (GSH) was estimated by the method of Ellman (1959). Total protein was assayed by the method of Lowry et al. (1951). The level of thiobarbituric acid reactive substances (TBARS) and lipid hydroperoxides (LOOH) was estimated by the methods of Niehaus and Samuelson (1968) and Jiang et al. (1992).

2.6. Activity of mitochondrial TCA cycle enzymes

Mitochondrial isolation and enzyme assays were performed as explained by Aristatile et al. (2011). The activities of isocitrate

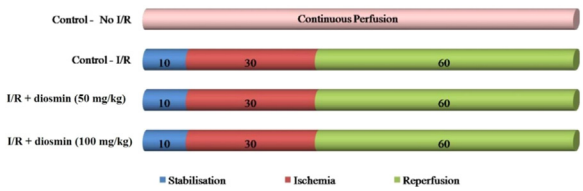

Fig. 2. Schematic diagram of I/R protocol. Diagram explains the time duration of stabilization, ischemia and reperfusion steps in the langendorff experiment.