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Effect of oleuropein on myocardial dysfunction and oxidative stress induced by ischemic-reperfusion injury in isolated rat heart

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ABSTRACT

Background: Studies have reported antioxidant effect of oleuropein in isolated rat heart.
Objective: This study was conducted to investigate whether perfusion of isolated rat heart with oleuropein, before induction of ischemia or at the onset of reperfusion, had any effect on the hemodynamic parameters, infarct size and biochemical factors following ischemic – reperfusion injury.
Materials and methods: Forty-eight male Wistar rats were divided into 6 groups: the control groups (Con-P and Con-T groups), O10-P and O50-P groups perfused with 10 and 50 μg/g heart oleuropein 5 min before the induction of ischemia and O10-T and O50-T groups perfused with 10 and 50 μg/g heart oleuropein at the beginning of the reperfusion, respectively. All hearts were subjected to 30 min global ischemia and 90 min reperfusion. Hemodynamic parameters were monitored throughout the experiment. The creatine kinase (CK) and malondialdehyde (MDA) level of coronary outflow were assayed and the infarct size measured at the end of reperfusion.
Results: We found hemodynamic parameters namely heart rate, left ventricular end diastolic pressure (LVEDP), left ventricular developed pressure (LVDP), ±dp/dt and coronary outflow significantly improved in all groups that received oleuropein compared to the control groups. Also, the infarct size was smaller and the coronary outflow levels of CK and MDA were lower in the oleuropein groups compared to the control groups.
Conclusions: The findings suggest that perfusion of isolated rat heart with oleuropein would lead to improved myocardial dysfunction following ischemic-reperfusion injury. Our findings confirm the antioxidant potential of oleuropein.

1. Introduction

Biologically active substances from plants attract the interest of many scientists in the era of modern pharmacotherapy worldwide [1–3]. It has been documented that the side effects of natural substances are much less than that of synthetic drugs [4]. Oleuropein is a natural substance present in olive leaves in a high concentration (6–9% of dry weight) [5,6]. It is a polyphenolic constituent with high antioxidant capacity [7–9] comparable to a hydrosoluble analog of α-tocopherol or vitamin E [10]. Various studies shown that oleuropein has many biological benefits in animals and human beings including antioxidant [9,11,12], anti-inflammatory [13], antidiabetic [7,14], anticancer [15,16], hypoglycemic [17], hypolipidemic [17]. Almost all previous studies attribute the beneficial biological effects of olive leaf extracts to oleuropein [9,14,19–22].

So far, few studies have been conducted on the effect of oleuropein on cardiovascular system in animal models to study cardioprotective effects [4,23–28]. However there is no study about the direct effect of oleuropein on myocardial dysfunction following ischemic – reperfusion injury. Because previous studies reported that oleuropein immediately after absorption in the gastrointestinal tract was converted to its conjugated and non-conjugated metabolites [24,29–31], its plasma concentration will be very low. Petkov and Malonov (1978) for the first time, using the *in vitro* and *in vivo* animal models reported that oleuropein has anti-arrhythmic

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and vasodilatory effects in dogs, cats, rabbits and rats [4]. Manna and co-workers in 2004 noticed that perfusion of isolated rat hearts with oleuropein (20 μg/g heart) before the induction of ischemia and reperfusion injury had cardioprotective effects that were evident by reduced creatine kinase (CK) and malondialdehyde (MDA) and glutathione peroxidase levels in coronary outflow. They had no data on the effect of oleuropein on myocardial dysfunction, infarct size and the magnitude of arrhythmia [25]. Recently, Nekooian and colleagues (2014) indicated that pre-treatment of hypertensive rats with type 2 diabetes with oleuropein (20, 40 and 60 mg/kg) for 16 weeks attenuated cardiovascular complications [27].

This study, investigated whether perfusion of isolated rat hearts with oleuropein (10 and 50 μg/g heart) before the induction of ischemia or at the onset of reperfusion had any effects on cardiac dysfunction, infarct size and the magnitude of arrhythmia. There are main differences between this study and Manna's study [25]: first, they only used a single dose of oleuropein (20 μg/g heart); second, they only infused oleuropein before the induction of ischemia; and third, they did not study the effect of oleuropein on cardiac dysfunction, infarct size and the magnitude of arrhythmia.

2. Materials and methods

2.1. Animals

In this study, forty-eight male Wistar rats, weighing 300–350 g, were used. All animals were kept under the standard conditions (12 h light/dark cycle, 22 ± 2 °C, and humidity 55%) with free access to food and water in the animal house of Medical College of Shahid Sadoughi University of Medical Sciences, Yazd, Iran. All procedures were according to the international guide for the and approved by the ethics committee of Shahid Sadoughi University of Medical Sciences, Yazd, Iran (No. 1493).

2.2. Experimental groups

The study had five experimental groups.

Group 1 as the Con-P group: isolated rat hearts were perfused with Krebs – Henseleit solution before induction of ischemic – reperfusion injury (n = 8).

Group 2 as the O10-P group: isolated rat hearts were perfused with 10 μg/g heart oleuropein before induction of ischemic – reperfusion injury for 5 min (n = 8).

Group 3 as the O50-P group: isolated rat hearts were perfused with 50 μg/g heart oleuropein before induction of ischemic – reperfusion injury for 5 min (n = 8).

Group 4 as the Con-T group: isolated rat hearts were perfused with Krebs – Henseleit solution at the reperfusion period (n = 8).

Group 5 as the O10-T group: isolated rat hearts were perfused with 10 μg/g heart oleuropein for five min at the beginning of the reperfusion (n = 8).

Oleuropein was purchased from Extrasynthèse (Geney, France).

2.3. Isolation of the heart

Animals were anesthetized with Sodium Thiopental (75 mg/kg, i.p) and heparinized (1000 IU heparin, i.p) to prevent the occlusion of coronary vessels during the removal of the heart. Then, the chest was opened and the heart removed. To perfuse the coronary vessels, aorta was cannulated and perfused retrogradely with Krebs -Henseleit solution under Langendorf apparatus with constant pressure of 70–80 mmHg. Krebs -Henseleit solution contained (mM), NaCl (118), KCl (4.7), $NaHCO_3$ (25), KH_2PO_4 (1.2), $MgSO_4$ (1.2), $CaCl_2$ (1.25) and glucose (11). Next, a full-fill water balloon was inserted into the left ventricle through the left atrium and connected to a transducer pressure (NARCO Bio – System, USA) to record intraventricular pressures including left ventricular end diastolic pressure (LVEDP), left ventricular developed pressure (LVDP), ventricular pressure time changes (max and min dp/dt) and cardiac contractility. The pressures were monitored using power lab data acquisition system (ADInstrument, Australia) with Lab Chart pro 7 software. Subsequently, the volume of the balloon was gradually increased to adjust the LVEDP to 4–7 mmHg. To monitor the electrical activity of the heart, two electrodes were placed on the base and top of the ventricles. Finally, a warm water jacket was placed around the heart to maintain the temperature at approximately 37 °C [49].

2.4. Induction of ischemic reperfusion injury

To induce global ischemic – reperfusion injury, twenty min after stabilization of the heart under the Langendorff apparatus, the coronary flow was completely occluded for 30 min and then reperfused for 90 min.

2.5. The effect of oleuropein on coronary outflow

To determine the effect of oleuropein on the rate of coronary outflow, it was measured manually 1 min before administration of oleuropein, before the induction of ischemia and 5, 10, 30, 60 and 90 min during reperfusion.

2.6. Measurement of CK activity and MDA in coronary outflow

Following 10 min of reperfusion, a sample of coronary outflow was collected to measure CK activity as a marker of ischemic injury and MDA level as a marker of lipid peroxidation [25,32]. CK activity was measured using a standard kit (Man CO, Iran) with catalog number of 101031 and MDA level measured manually. In brief, 100 μl of coronary outflow was mixed with 100 μl of SDS 8.1% and 750 μl acetic acid 20% and the volume was made up to 2 ml with distilled water. Then, 10 μl of Butylated hydroxytoluene 1%, 70 μl thiobarbituric acid 0.8% were added and vortexed. Next, this solution was heated to 95 °C for 1 h and at 4 °C (refrigerator) for 10 min, respectively. The solution was then centrifuged at 3000 g for 15 min. Finally 2.5 ml N-butanol – pyridine (15:1) was added and the absorbance of supernatant was read at 532 nm. The MDA concentration was calculated using tetraethoxypropane standard curve as a positive control substance.

2.7. Measurement of the infarct size

At the end of 90 min reperfusion, left ventricle was separated and frozen at −24 °C for 24 h. Then, it was cut into sections of 2 mm and stained with triphenyltetrazolium chloride (TTC) 1% at 37 °C for 30 min. TTC causes the viable tissues to obtain red color and the dead tissues to appear white in color. To increase the contrast between the colors, the sections were incubated in formalin 10% for 1 h. Finally, photos were taken from both sides of sections and the infarct size was determined as a percent of total area of the left ventricle using Photoshop 8 CS image-analysis software.

2.8. Statistical analysis

Data were analyzed using GraphPad Prism 5 (San Diego, CA) and expressed as mean ± SEM and the percentage of incidence. One – way and two – way analysis of variance (ANOVA) was used to analyze the difference between means and Fisher exact test to

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