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The effects of iloprost on ischemia-reperfusion injury in skeletal muscles in a rodent model

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ABSTRACT

Purpose: The aim of this study was to investigate the effects of iloprost (IL) on ischemia-reperfusion injury in a rodent model.

Materials and methods: Twenty-four Wistar Albino rats were randomized into four groups ($n = 6$). Laparotomy was performed in all groups under general anesthesia. Only laparotomy was applied in group S (Sham). Ischemia-reperfusion group (group I/R) underwent ischemia and reperfusion performed by clamping and declamping of the infrarenal abdominal aorta for 120 min. The iloprost group (group IL) received intravenous infusion of IL 0.5 ng/kg/min, without I/R. Group I/R + IL received intravenous infusion of IL 0.5 ng/kg/min immediately after 2 h period of ischemia. At the end of the reperfusion period, all rats were killed under anesthesia and skeletal muscle samples of lower extremity were harvested for biochemical and histopathologic analyses.

Results: Tissue levels of endothelial nitric oxide were significantly higher in I/R groups than those in groups S and IL. The heat shock protein 60 levels were higher in group I/R than the other groups. But the heat shock protein 60 levels in group I/R + IL were found to be similar with the groups S and IL. Malondialdehyde levels were significantly higher in group I/R. On the other hand, in group I/R + IL, malondialdehyde levels were higher than those in groups S and IL but lower than those in group I/R. Superoxide dismutase (SOD) enzyme activities were found to be significantly lower in group I/R than the other groups. Also in group I/R/I, the SOD enzyme activities were higher than those in group I/R. But, in group I/R + IL, SOD levels were found to be higher than those in group I/R but lower than those in groups S and IL.

Conclusions: These results indicate that IL has protective effects on I/R injury in skeletal muscle in a rodent model.

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1. Introduction

Despite improved invasive and noninvasive treatment modalities in vascular surgery, ischemia/reperfusion (I/R) injury in skeletal muscle is still a challenge increasing mortality and morbidity. It is known that the major part of the damage occurs during the reperfusion period and that reactive oxygen substances (ROS) play an important role in this process [1].

The generation of ROS is a complex process involving endothelial cells and neutrophils. The main factor triggering the injury is suggested to be the endothelial cell damage [2,3].

Swelling of the cells, degeneration of the cell skeleton structure, and loss of selective membrane permeability are characteristic features of reperfusion injury. These changes all end up with tissue edema and decreased capillary blood flow [4].

Iloprost (IL) is a synthetic analog of epoprostenol (prostaglandin I_2 – PGI_2, prostacyclin) which is synthesized from arachidonic acid primarily by endothelial cells. IL has vasodilatory, antiplatelet, and cytoprotective effects and it induces fibrinolytic activity, smooth muscle cell proliferation, and expression of leukocyte-endothelial adhesion molecules. It is also useful in maintaining tissue microcirculation by reducing levels of certain cytokines (tumor necrosis factor-α, interleukin-1, and interleukin-6) [5,6].

Benefits of IL use to prevent local and distal tissue injury due to I/R have been well documented so far. However, little is known about its protective effect on skeletal muscle after I/R injury. Our study aims to investigate the effect of IL on lower extremity muscle ischemia and subsequent I/R injury, which may happen frequently after aortic occlusion and I/R method.

The present study was designed to evaluate the effectiveness of IL in an established *in vivo* rodent model of skeletal muscle I/R injury. The purpose was also to explain the potential mechanisms involved in any beneficial effect with different parameters, such as endothelial nitric oxide (eNOS) apoptosis and heat shock protein 60 (HSP60) expression, and oxidative stress parameters, such as malondialdehyde (MDA) levels and superoxide dismutase (SOD) enzyme activities.

2. Materials and methods

2.1. Experimental groups

Twenty-four adult Wistar-Albino rats, weighing 250–300 g were used in this study. Rats were housed in cages at an average temperature of 21–22°C in a light-dark cycle–controlled environment with free access to food and tap water. The protocols of this experimental study were approved by the Animal Ethics Committee of Gazi University. All animals received human care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and the Use of Laboratory Animals" prepared by the National Academy of Science and published by the National Institutes of Health (NIH publication Nr. 85–23, revised in 1985).

2.2. Study design

Rats were randomized into four groups ($n = 6$/group). The Sham group (group S) underwent midline laparotomy and dissection of the infrarenal abdominal aorta without cross-clamping; ischemia-reperfusion group (group I/R) underwent laparotomy and cross-clamping of the infrarenal abdominal aorta for 120 min and then 120 min of reperfusion; group IL underwent laparotomy and received intravenous infusion of IL (0.5 ng/kg/min), without I/R; group I/R + IL received intravenous infusion of IL (0.5 ng/kg/min) immediately after 120 min of ischemia.

2.3. Aortic occlusion and I/R

Rats were anesthetized with ketamine hydrochloride (Ketalar, 50 mg/kg, intramuscularly; Parke-Davis, Eczacibasi, Istanbul, Turkey) and xylazine hydrochloride (Alfazyne, 2%; Ege Vet, Izmir, Turkey). Anesthesia was maintained by an additional muscular injection of ketamine hydrochloride and xylazine hydrochloride. The surgical procedures were performed while the rats were placed in a supine position under a heating lamp. The abdomen was shaved, the skin was prepared aseptically, and a midline laparotomy was performed. The abdominal aorta was exposed and clamped using an atraumatic microvascular clamp. The aortic occlusion was confirmed by the loss of the distal arterial pulsation. The skin incision was closed and covered with a plastic wrap to maintain the body temperature and fluid balance. After 120 min of ischemia, the microvascular clamp was removed and lower extremities were reperfused for 120 min. At the end of the reperfusion period, all rats were killed under anesthesia and skeletal muscle samples of lower extremity were harvested for biochemical and histopathologic analyses.

2.4. Biochemical analysis

The skeletal muscle tissues were first washed with cold deionized water to discard blood contamination and then homogenized in a homogenizator. Measurements on cell contest require an initial preparation of the tissues. The preparation procedure may involve grinding of the tissue in a ground glass tissue blender using a rotor driven by a simple electric motor. The homogenizator as a tissue blender similar to the typical kitchen blender is used to emulsify and pulverize the tissue (Diax 900; Heidolph Instruments GmbH&Co KG, Schwabach, Germany) at 1000 U for about 3 min. After centrifugation at 10,000*g* for about 60 min, the upper clear layer was taken.

MDA levels were determined using the method of Van Ye *et al.* [7] based on the reaction of MDA with thiobarbituric acid (TBA). In the TBA test reaction, MDA and TBA react in acid pH to form a pink pigment with an absorption maximum at 532 nm. Arbitrary values obtained were compared with a series of standard solutions (1,1,3,3-tetraethoxypropane). Results were expressed as nmol/L.

In the upper clear layer, total superoxide dismutase, an enzyme, activity was measured as described by Durak *et al.* [8].