



ORIGINAL ARTICLE

Does Esmolol Havean Effect on Erythrocyte Deformability in Rat Lower Limb Ischemia Reperfusion Injury?

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Abstract

Objective: Ischemia reperfusion (I/R) injury is a common problem in vascular surgery. We aimed to investigate the effects of esmolol on erythrocyte deformability in rat lower limb I/R injury.

Materials and methods: Following Ethics Committee approval 24 rats were randomly divided into 4 groups, Control (Group C), Esmolol (Group E), Ischemia-reperfusion (Group I/R), I/R-Esmolol (Group I/R-E). In I/R group, atraumatic microvascular clamp was placed to infrarenal abdominal aorta. After 120 minutes from ischemia, the clamp was removed and reperfusion was performed for 120 minutes. Esmolol (200 µg/kg/min) was administered 30 minutes before the operation in I/R-Esmolol group. Erythrocytes were obtained from heparinized whole blood samples. A constant flow filterometer was used to measure erythrocyte deformability and relative resistance was calculated.

Results: Ischemia reperfusion increased relative resistance when compared to control group ($p < 0.0001$). The erythrocyte deformability index was significantly higher in the I/R and I/R-E groups than in the control group ($p < 0.000$, $p < 0.000$, respectively). Esmolol application significantly decreased the erythrocyte deformability index according to the I/R group ($p = 0.039$).

Conclusions: It was found that the application of esmolol partially corrects the erythrocyte deformability impairment

in the I/R-generated rats. Findings that we have reached in our study suggest that the protective effects of esmolol in I/R damage will be shown in detail and the indications for use will expand when supported by other studies.

Keywords

Esmolol, Erythrocyte deformability, Ischemia-reperfusion, Lower extremity

Introduction

Lower extremity ischemia reperfusion (I/R) injury is seen especially in patients undergoing major vascular surgery. However, it may be observed as a result of interruption of blood flow after arterial occlusion of the lower extremity and restoration in the follow-up. Under normal conditions, it is known that aerobic metabolism is the main pathway in maintaining the metabolic functions and integrity of the cells and oxygen is the essential component for its maintenance. Anaerobic metabolism is used in the absence of oxygen and as a result of this, lactic acid production increases and in the follow-up, acidosis develops in the cell which disrupts the continuity of the basic functions of the cell. When the interrupted oxygen supply starts again, lots of local and

systemic response occurs. In particular, inflammatory cytokines and reactive oxygen radicals (ROS) released during reperfusion lead to I/R damage. One of the most important damages of I/R is microcirculation. Inflammatory cytokines released from polymorphonuclear leukocytes interact with the endothelium causing tissue damage. Another target of this ROS release is erythrocytes [1,2].

Microcirculation is very important for oxygen delivery to tissue. Proper maintenance of microcirculation depends on erythrocyte deformability. I/R damage due to erythrocyte membrane structure and intracellular ionization disrupts erythrocyte deformability and consequently oxygen supply to tissue [3]. Many agents have been studied to correct this damage partially or completely [4-6]. However, there is no information in the literature about esmolol which is a selective β -1 blocker. Esmolol has previously been found to prevent cardiac I/R damage in cardiac clinical models and animal models, but it has been found to have protective effects in spinal cord I/R injury models [7,8]. The aim of this study was to investigate the effect of esmolol on erythrocyte deformability disorder caused by lower-extremity I/R injury.

Materials and Methods

Animals

Erythrocyte deformability was conducted in the Physiology Laboratory of Kirikkale University upon the consent of Experimental Animals Ethics Committee of Gazi University. All the procedures were performed according to accepted standards of Guide for the Care and Use of Laboratory Animals. 24 male Wistar rats weighing 250-300 g were used in the experiment. Animals were maintained under standard conditions such as stable room temperature (24 ± 3 °C) and a 12-hour light-dark cycle and were allowed access to rat pellets and water.

Experimental model

Before the procedure, the animals were anesthetized with ketamine 100 mg/kg ip and were placed below a heating lamp to maintain a temperature of 37 °C. After obtaining a vein access via tail vein of rats, esmolol infusion was started at a dose of 200 μ g/kg/min through the tail vein to the groups which were receiving esmolol. In this groups laparotomy was performed with an abdominal incision 30 mins after the end of infusion.

Lower-limb ischemia-reperfusion

The lower-extremity ischemia was created by using a microvascular bulldog clamp. Midline laparotomy was done similarly. Infrarenal segment of the aorta was clamped for 2 hours. After removing the clamp, reperfusion was established for another 2 hours. Then rats

were sacrificed after obtaining blood and tissue samples.

Experimental protocol

Group C: Control group

Group E: Esmolol group

Group I/R: Ischemia-reperfusion group

Group I/R-E: Ischemia reperfusion + Esmolol group

Group C: Following anesthesia, and vein cannulation, laparotomy was performed and lower limb I/R was applied then rats were sacrificed after obtaining blood samples.

Group E: Following anesthesia, tail vein was cannulated and 200 μ g/kg/min esmolol was infused. After the infusion ended, the rats were kept without any surgical intervention, then rats were sacrificed after obtaining blood samples.

Group I/R: Thirty mins after the anesthesia, laparotomy then lower limb I/R was applied and following reperfusion blood samples were taken.

Group I/R-E: After the anesthesia and esmolol infusion. Laparotomy was performed and then lower limb I/R-E was created. After the procedures were completed, rats were sacrificed and blood samples were taken.

Deformability measurements

A constant flow filterometer system (MP 30, Biopac Systems Inc., Commat, USA) was used for deformability measurements. Erythrocyte suspension that was delivered at 1 ml/min flow rate was passed through a nucleopore-polycarbonate filter of 5 mm in diameter, and alterations in the filtration pressure corresponding to different flow rates were measured. The alterations in the pressure were transferred to computer medium with an MP 30 data equation system. The ratio of the values of filtration pressure for the cellular suspension and buffer were calculated, and the relative resistance was calculated.

Statistical analysis

SPSS 20.0 software program was used for statistical analyses and $p < 0.05$ was considered statistically significant. The findings were expressed as mean \pm standard deviation. Kruskal-Wallis variance analysis was preferred for data evaluation. The variables with significance were evaluated with Bonferroni corrected Mann-Whitney U test.

Results

Ischemia reperfusion increased relative resistance when compared to control group ($p < 0.0001$). The erythrocyte deformability index was significantly higher in the I/R and I/R-E groups than in the control group ($p < 0.0001$, $p < 0.0001$, respectively). Esmolol application significantly decreased the erythrocyte

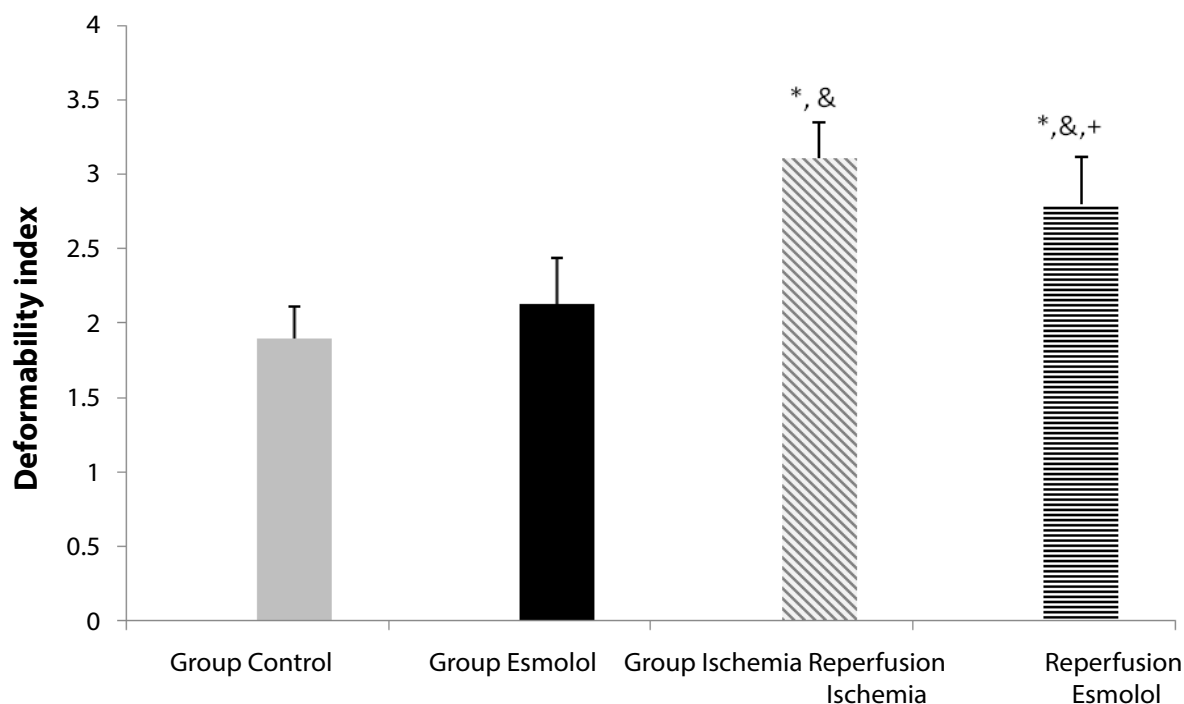


Figure 1: Data's of erythrocyte deformability index (Mean ± Standard Deviation).

*p < 0.05: Compared with control group; & p < 0.05: Compared with esmolol group; +p < 0.05: Compared with ischemia reperfusion group.

deformability index according to the I/R group (p = 0.039), (Figure 1).

Discussion

Erythrocytes have unique characteristics such as deforming through areas where capillary diameters are narrower than erythrocyte diameter and providing gas exchange between blood and tissue. However, this unique feature is affected by many pathophysiological processes such as sickle cell anemia, malaria, and sepsis, leading to disturbances in microcirculation and oxygen supply to the tissue [9]. Ischemia reperfusion disrupts the functions of both Na-K ATPase pumps and deformability by disrupting membrane skeletal structures and membrane fluidity as a result of oxidative stress [3,9]. In order to correct this damage, there is no data about esmolol in the literature.

The deformability of erythrocytes depends on the preservation of membrane properties such as surface area/volume relationship. The most important determinant of this continuity is the continuation of the functions of Na-ATPase pumps located in the erythrocyte membrane. Again, the protection of the ionic gradient on both sides of the membrane depends on these pumps. ATP is also required for proper operation of these pumps. It has been found that the changes occurring during I/R disrupt the lipid peroxidation and impair the Na-K ATPase activity in erythrocytes and consequently increase the deformability [3,10].

Esmolol is a short-acting selective β -1 adreno-receptor blocker and has a rapid time of onset and short duration of action. It has protective effects against I/R damage during cardiac surgery with inotropy, chronotropy and dromotropy. Another reason for this protective effect is due to the antioxidant and cytoprotective properties of free oxygen radicals [11,12]. In previous studies, it has been found that esmolol shows antioxidant properties by reducing lipid peroxidation markers [13]. Esmolol has been found to reduce histological and motor disorders caused by spinal cord I/R damage and has protective effect against spinal cord I/R injury [14]. At the same time, protective efficacy has been determined in transient ischemic attacks [8].

In this study, we want to investigate the effects of esmolol on erythrocyte deformability in rat lower limb ischemia reperfusion injury. We found that lower-extremity I/R injury increased erythrocyte deformability and improved the deformability index and relative resistance of esmolol at a dose of 200 μ g/kg/min before I/R administration. The increase in the deformability index of lower extremity I/R of esmolol was partially corrected by esmolol infusion. Although the underlying mechanism has not been fully elucidated, we believe that this effect is due to the antioxidant activity of esmolol.

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