

Protective effect of erdosteine on erythrocyte deformability in a rat model of lower limb ischemia/reperfusion injury

Abdullah ÖZER^{1*}, Hüseyin DEMİRTAŞ², Faruk Metin ÇOMU³, Dilek ERER¹, Yiğit KILIÇ⁴, Barış MARDİN¹, Mustafa ARSLAN⁵, Ayşegül KÜÇÜK⁶, G. Levent OKTAR¹

¹Department of Cardiovascular Surgery, Faculty of Medicine, Gazi University, Ankara, Turkey

²Department of Cardiovascular Surgery, Yüksek İhtisas Hospital, Kırıkkale, Turkey

³Department of Physiology, Faculty of Medicine, Kırıkkale University, Kırıkkale, Turkey

⁴Department of Cardiovascular Surgery, Iğdır State Hospital, Iğdır, Turkey

⁵Department of Anesthesiology and Reanimation, Faculty of Medicine, Gazi University, Ankara, Turkey

⁶Department of Physiology, Faculty of Medicine, Dumlupınar University, Kütahya, Turkey

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Background/aim: The protective effect of erdosteine on local and distant organ injury due to ischemia/reperfusion has been well documented but its effect on erythrocyte deformability needs further investigation. Our aim was to investigate the effect of erdosteine on erythrocyte deformability in the infrarenal aorta of rats undergoing ischemia/reperfusion.

Materials and methods: Our study was conducted with 18 Wistar albino rats. Rats were divided into 3 groups: a randomized control group (group 'control', n = 6), an ischemia/reperfusion group without erdosteine (group 'ischemia/reperfusion', n = 6), and an ischemia/reperfusion group with erdosteine at 150 mg kg⁻¹, intraperitoneally (group 'ischemia/reperfusion - erdosteine', n = 6). Packs of erythrocytes were prepared from heparinized blood samples and deformability measurements were conducted.

Results: Comparisons of the control and ischemia/reperfusion - erdosteine groups revealed similar results (P = 0.051). The values of the ischemia/reperfusion group were significantly higher than those of the control and ischemia/reperfusion - erdosteine groups (P < 0.0001 and P = 0.024, respectively). Relative resistance, a marker of erythrocyte deformability, was increased significantly by ischemia/reperfusion compared to the control and ischemia/reperfusion - erdosteine groups (P < 0.05).

Conclusion: We detected unfavorable effects of ischemia/reperfusion on erythrocyte deformability, which may lead to disturbance in blood flow and hence tissue perfusion in the infrarenal rat aorta. We also found that erdosteine had beneficial effects by reversing undesirable effects of ischemia/reperfusion. However, these promising results should be further supported by more detailed studies with larger volumes.

Key words: Erythrocyte deformability, ischemia reperfusion injury, erdosteine, lower limb

1. Introduction

Damage to cells after reperfusion of previously viable ischemic tissues in the lower extremities is a frequent and serious clinical event. If blood flow is reestablished after reperfusion, substances produced as a result of oxidation may unexpectedly induce increased rates of mortality and morbidity because of systemic complications. Following local edema and muscle tissue necrosis, systemic inflammatory response syndrome and multiple organ failure (e.g., kidney, respiratory, and circulatory systems) generally occur as reperfusion accelerates (1-5).

Erdosteine (N-carboxymethylthioacetyl-homocysteine thiolactone), which is a mucolytic agent, contains two

sulfhydryl groups that are available for free radical scavenging (6). Erdosteine has been used to protect against and cure ischemia/reperfusion (I/R) injury in many organs (1,7-10).

Advantages of erdosteine use in cases of I/R to prevent local and distal tissue injury have been well reported so far. However, not much is known about its protective effect on erythrocyte deformability following I/R injury.

The primary aim of this study was to investigate the effect of erdosteine on lower extremity muscle ischemia and subsequent I/R injury, which may happen frequently after the tourniquet method.

* Correspondence: dr-abdozer@hotmail.com

2. Materials and methods

2.1. Animals and experimental protocol

This study was carried out in the Gazi University Physiology Laboratory with the approval of the Gazi University Ethical Committee of Experimental Animals (20/06/2016-E.7657 7GÜET-16.044). All employed methods were in agreement with approved basics of the Guide for the Care and Use of Laboratory Animals.

The present study was undertaken with 18 albino Wistar rats with body weights ranging between 250 and 300 g and aged between 10 and 12 weeks, raised under identical environmental conditions. The rats were kept at 20–21 °C with a 12:12-h light:dark photoperiod and could access food freely until 2 h before the induction of anesthesia. The 18 Wistar albino rats were randomly divided into three groups, each containing 6 rats. Median laparotomy was done under general anesthesia.

In the control group, median laparotomy was performed alone without any additional surgical intervention. After 2 h, at the end of the procedure, a blood sample was taken from the infrarenal segment of the aorta and the animals were sacrificed.

In the I/R group, median laparotomy was performed as well. We clamped the infrarenal part of the aorta with an atraumatic clamp for 2 h. Then we removed the clamp and allowed reperfusion for 2 more h. In the end, rats were sacrificed after taking blood samples from the infrarenal segment of the aorta.

In the I/R group with erdosteine (I/R-E), similar steps were performed, but additionally, before the ischemia period, erdosteine was given (150 mg kg⁻¹) intraperitoneally for 30 min. Rats were sacrificed at the end of the reperfusion period, which lasted 2 h, after collecting blood samples from the infrarenal segment of the aorta.

All the rats were given ketamine at 100 mg kg⁻¹ intraperitoneally and blood samples were obtained. Heparinized total blood samples were used to prepare erythrocyte packs. Deformability measurements were done using erythrocyte suspensions with 5% hematocrit in phosphate-buffered saline (PBS) buffer.

2.2. Deformability measurements

To prevent hemolysis, samples of blood were collected very carefully and the measurement process was done rapidly. Collected blood was centrifuged at 1000 rpm for 10 min. Blood plasma in the upper phase and the buffy coat were removed. Isotonic PBS buffer was added to collapsing erythrocytes and this mixture was centrifuged at 1000 rpm for 10 min. Liquid from the upper surface was taken. The washing process was repeated three times and finally pure red cell packs were obtained. Erythrocyte suspensions with 5% hematocrit in PBS buffer were used for deformability measurements. Erythrocytes were collected and then deformability measurements were done at 22 °C

Erythrocyte deformability was measured by a constant-current filtrometer system. Samples were prepared as 10 mL of erythrocyte suspension and PBS buffer before measurement. The infusion pump was set at 1.5 mL/min for a constant rate of flow. A 28-mm nucleoprin polycarbonate filter with a 5-µm pore diameter was used. Pressure changes while the erythrocytes passed through the filter were detected by the pressure transducer and the data were transferred to a computer with the help of the MP30 system (BIOPAC Systems Inc., USA). At different times pressure changes were measured by using relevant computer programs for calculations. Pressure calibration of the system was performed each time before measuring the samples. After the buffer (PT) was passed through the filtration system, the erythrocytes (PE) were passed through secondly. Pressure variations were measured. By relating the pressure value of the erythrocyte suspension to the pressure value of the buffer, the relative refractory period value (Rrel) was calculated. The deformability index was interpreted such that as Rrel was increasing the ability of erythrocyte deformability was affected adversely (7,8).

2.3. Statistical analysis

All data were processed by variance analysis using SPSS 17.0 for Windows (SPSS Inc., USA). $P < 0.05$ was considered statistically significant. The data were expressed as mean \pm standard deviation. Variance analysis and the Kruskal–Wallis test were used to evaluate the data. Mann–Whitney U tests with Bonferroni corrections were used to evaluate the variables with significance.

3. Results

This study showed that relative resistance, a marker of erythrocyte deformability, was increased significantly by I/R compared to the control and I/R-E groups ($P < 0.05$; Figure).

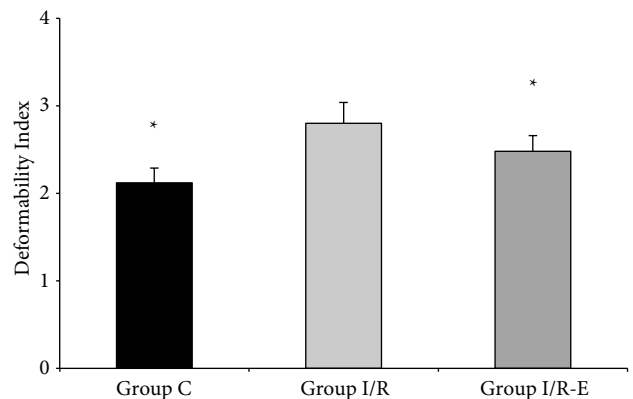


Figure. Erythrocyte deformability index values of the groups. Each bar represents the mean \pm standard deviation.

*: $P < 0.05$ compared to the I/R group.

There were significant differences between the groups according to the comparisons with one-way ANOVA test ($F: 14.937, P < 0.0001$). The results obtained after corrections with the Tukey HSD test were as follows: comparisons of the control and I/R-E groups revealed similar results ($P = 0.051$). The values of the I/R group were significantly higher than those of the control and I/R-E groups ($P < 0.0001$ and $P = 0.024$, respectively).

4. Discussion

Erdosteine inhibits the clustering and activation of platelets. It enhances microvascular perfusion and fibrinolytic activity, inhibits leukocyte/vessel wall interaction, and helps maintain endothelial cell integrity. Ischemic damage results from a decrease in the blood flow of an organ and reperfusion injury results from the enhanced generation of oxygen radicals. These effects together improve the outcome of patients with critical limb ischemia (6,11,12).

Erdosteine is a thiol derivative containing two blocked sulfhydryl groups in the aliphatic side chain, which become free only after hepatic metabolization. Metabolites of erdosteine have two sulfhydryl groups and they take part in free radical scavenging and antioxidant activity (13). Free radical scavenging properties of erdosteine have been shown in experimental and clinical studies (1,6,7,11).

Lipid peroxidation is reduced by erdosteine with direct free radical scavenging properties. This was shown by reducing the level of malondialdehyde (MDA), the end product of lipid peroxidation, by the administration of erdosteine (14).

For migration of oxygen and vital molecules to the final organ capillaries and clearance of metabolic wastes, erythrocytes must be able to extend and curve and have the capability to move in these areas. This capacity, known as “deformability”, becomes more important in

microcirculation. Altered erythrocyte deformability changes not only the oxygen delivery capacity of the erythrocytes but also the survival of the circulating erythrocytes (15–17).

In our study, relative resistance, a marker of erythrocyte deformability, was increased significantly by I/R compared to the control and I/R-E groups ($P < 0.05$; Figure). As comparisons of the values of the control and I/R-E groups were similar ($P = 0.051$), values of the I/R group were significantly higher than those of the control and I/R-E groups ($P < 0.0001$ and $P = 0.024$, respectively). We observed that erdosteine treatment had a protective effect in reducing the oxidative stress after hind limb ischemia in this animal model. The aim of this study was to investigate the antioxidant protective effect of erdosteine on hind limb I/R injury in rats and these results were encouraging.

In a previous study, 150 mg/kg erdosteine was found to be effective in preventing local tissue injury following testicular torsion (18). In another study, Yurdakul et al. reported that levels of MDA and xanthine oxidase were decreased by erdosteine treatment (19). To our knowledge, this is the first study demonstrating the protective effect of erdosteine on I/R injury by erythrocyte deformability in an experimental lower limb I/R injury rat model.

In addition to these data, our findings indicate that in rats undergoing I/R erythrocyte deformability is impaired and this impairment leads to disturbed microvascular perfusion and related problems. We think that measurement of erythrocyte deformability could be useful as a parameter in cases of I/R. We also did not observe a negative effect of erdosteine on maintaining erythrocyte deformability during periods of I/R, but we still think these promising results should be further supported by more detailed studies with larger volumes.

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