EXPERIMENTAL STUDY

Effect of iloprost on erythrocyte deformability in rat's lower extremity undergoing an ischemia reperfusion injury

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Abstract: *Aim:* Ischemia reperfusion injury (I/R) in lower extremity is a frequent and important clinical phenomenon. The protective effect of iloprost on local and distant organ injury due to I/R has been well documented but its effect on erythrocyte deformability needs further investigation. Our aim was to investigate the effect of iloprost on erythrocyte deformability in the infrarenal aorta of rats undergoing I/R.
Materials and methods: Our study was conducted with 18 Wistar albino rats. Rats were divided into the 3 groups; the randomized control group (group C; n=6), I/R group without iloprost (group I/R; n=6) and I/R group with iloprost – 10 mcg.kg^{-1}, 30 min infusion (group I/R-I; n=6). Packs of erythrocytes were prepared from heparinized blood samples and deformability measurements were done.
Results: The comparisons of the control and I/R-I groups revealed similar results (p=0.951). The values of the IR group were significantly higher than those of the control and IR-I groups (p=0.006, p=0.011, respectively).
Conclusion: In our study, we detected the unfavourable effects of I/R on erythrocyte deformability, which may lead to disturbance in blood flow and hence tissue perfusion in the infrarenal rat aorta. We also found that Iloprost had beneficial effects by reversing the undesirable effects of I/R *(Fig. 1, Ref. 15)*. Full Text in PDF *www.elis.sk.*
Key words: erythrocyte deformability, ischemia reperfusion, ilioprost, rat.

The ischemia reperfusion injury (I/R) in lower extremity is a frequent and important clinical phenomenon. Reperfusion period following an ischemic insult may paradoxically cause increased rates of mortality and morbidity due to systemic complications. Local edema and muscle tissue necrosis are likely to be followed by systemic inflammatory response syndrome and multiple organ failure (kidney, respiratory and circulatory system etc.) as reperfusion advances (1–3).

Swelling of cells, degeneration of cell skeleton structure and loss of selective membrane permeability are the characteristic features of the reperfusion injury. All these changes end up with tissue oedema and decreased capillary blood flow (4).

Iloprost is a synthetic analog of epoprostenol (prostoglandin I_2 – PGI_2, prostacyclin), which is synthesized from arachidonic acid primarily by endothelial cells. Iloprost has vasodilatory, antiplatelet and cytoprotective effects as well as 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 (TNF-α, IL-1, IL-6) (5, 6).

The benefits of iloprost to prevent local and distal tissue injury due to I/R has been well documented so far. However, little is known about its protective effect on erythrocyte deformability after I/R injury. Our study aimed to investigate the effect of iloprost on lower extremity muscle ischemia and subsequent I/R injury, which may happen frequently after the tourniquet method.

Materials and methods

Animals and Experimental Protocol

This study was conducted in the Physiology Laboratory of Gazi University upon the consent of Experimental Animals Ethics Committee of our university. All procedures were performed according to accepted standards of the Guide for the Care and Use of Laboratory Animals.

In our study, 18 Wistar Albino rats weighing between 250 and 300 g, raised under the same environmental conditions, were used. The rats were kept under 20-21 °C at cycles of 12-hour daylight and 12-hour darkness and had free access to food until 2 hours before the anaesthesia procedure. The animals were randomly separated into the four groups, each containing 6 rats. A midline laparatomy was done under a general anesthesia.

Control group

The midline laparotomy was done alone without any additional surgical intervention. Blood sample was collected after 2 hours of follow-up and animals were sacrificed eventually.

I/R group: The midline laparotomy was done similarly. The

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infrarenal segment of the aorta was clamped for 2 hours. After removing the clamp, reperfusion was established for another 2 hours. Finally, rats were sacrificed after collecting blood samples from their abdominal aorta.

I/R group with iloprost: Similiar steps were followed but in addition to the procedure mentioned above, iloprost was given (10 mcg.kg^{-1}) through the tail vein for 30 minutes starting simultaneously with the reperfusion period. Rats were sacrificed at the end of the reperfusion period, which lasted 2 hours after collecting blood samples.

All the rats were given ketamine 100 mg.kg^{-1} intraperitoneally and intracardiac 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.

Deformability measurements

Blood samples were taken very carefully and measurement process was done as fast as possible to avoid hemolysis. Collected blood was centrifuged at 1000 rpm for ten minutes. Serum and buffy coat on erythrocytes were removed. Isotonic PBS buffer was added to collapsing erythrocytes and this mixture was centrifuged at 1000 rpm for ten minutes. Liquid on the upper surface was removed. Finally, pure red cell packs were obtained from the washing process which was repeated three times. Erythrocyte packs were mixed with PBS buffer to generate a suspension with the value of 5 % Htc. Those erythrocyte suspensions were used for the measurement of deformability. Collection and deformability measurements of erytrocytes were done at 22 °C.

The constant-current filtrometer system was used for measurement of erythrocytes deformability. Samples to be measured were prepared as 10 ml of erythrocytes suspension and PBS buffer. The flow rate was held constant at 1.5 ml/min with an infusion pump. The 28 mm nucleoporin polycabonate filter with a 5 μm pore diameter was preferred. The pressure changes, while the erythrocytes passed through the filter, were detected by the pressure transducer and the data was transferred to computer with the help of MP 30 data equation systems (Biopac Systems Inc, Commat, USA). The necessary calculations were performed with related computer programs by measuring the pressure changes at various times. A pressure calibration of the system was performed each time before measuring the samples. At first, buffer (P_T) and then erythrocytes (P_E) passed through from the filtration system and the changes in pressure were measured. The relative refractory period value (Rrel) was calculated by relating the pressure value of erythrocytes suspension to pressure value of buffer. Increasing in Rrel as the deformability index was interpreted as adversely affected ability of erythrocytes deformability (7, 8).

Statistical analysis

The statistical analyses were performed with the SPSS 17.0 software program and $p < 0.05$ was considered statistically significant. The findings were expressed as the mean ± standard deviation. The data was evaluated with the Kruskal–Wallis variance analysis. The variables with significance were evaluated with the Bonferroni corrected Mann–Whitney U test.

Results

The results of the study indicated that IR significantly increased the relative resistance, a marker of erythrocyte deformability, when compared to the control and IR-I group ($p < 0.05$) (Fig. 1).

There were significant differences between the groups according to the comparisons with the one way ANOVA test (F: 9.034, p=0.004). The results obtained after corrections with Tukey HSD test were as follows: Comparisons of the control and IR-I groups revealed similar results (p=0.951). The values of the IR group were significantly higher than those of the control and IR-I groups (p=0.006, p=0.011, respectively).

Discussion

Iloprost suppresses platelet clustering and activation. It enhances microvascular perfusion and fibrinolytic activity, inhibits leukocyte – vessel wall interaction and helps maintaining endothelial cell integrity. These effects altogether improve the outcome of patients with critical limb ischemia (9).

It has been reported that decrease in blood viscosity and deformation of erythrocytes do not occur with doses of 1–2 ng/kg/min (10) but increased extravasation of red blood cells are observed within doses of 2–4 ng/kg/min (11). Furthermore, in a previous study, it was shown that microvascular perfusion was improved with doses of 0.5–2 ng/kg/min (12).

Normal blood pressure values were obtained after oral admission of the drug in hypertensive rats. Vascular resistance in lower extremity (13) and mesenteric vessels was lowered and blood flow was increased (14) without causing hypotension and tachycardia. İlomedin® shows therapeutic effect in occlusive peripheral artery disease in patients because it causes antiaggregation, vasodilatation, and inhibition of thrombocyte functions in addition to leukocyte adhesion (15).

All these data and our findings indicate that erythrocyte deformability is impaired in rats subjected to I/R. and this impairment leads to disturbance of microvascular perfusion and related
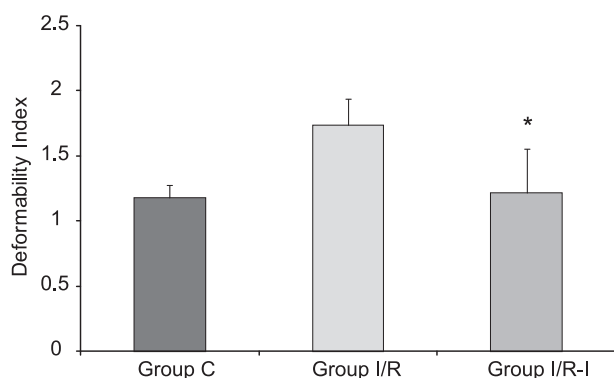

Fig. 1. Erythrocyte deformability index values of the groups. Each bar represents the mean ± SD. * p<0.05 compared to the Group I/R.

problems. So, we think that measurement of erythrocyte deformability can be useful as a parameter in cases of I/R. We also observed the beneficial effect of iloprost infusion on maintaining erythrocyte deformability during periods of I/R but we still think these promising results should further be supported by more detailed and extensive studies.

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