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 I/R group were significantly higher than those of the control and I/R-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, iloprost, rat.
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:** Similar 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 erythrocytes were done at 22 °C.

The constant-current filtermeter 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 polycarbonate 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 (P₀) and then erythrocytes (Pₑ) passed through from the filtration system and the changes in pressure were measured. The relative refractory period value (R_rel) was calculated by relating the pressure value of erythrocytes suspension to pressure value of buffer. Increasing in R_rel as the deformability index was interpreted as adversely affected ability of erythrocytes deformability (7, 8).

**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. Ilomedin® 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
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.

References


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