

Effect of Levosimendan and Nigella Sativa on Erythrocyte Deformability During Myocardial Ischaemia-Reperfusion Injury in Rats

Ratlarda Miyokardiyal İskemi-Reperfüzyon Hasarı Sırasında Levosimendan ve Nigella Sativa'nın Eritrosit Deformabilitesine Etkisi

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ABSTRACT

Objective: Ischaemia-reperfusion (IR) injury is a chain of events put in place by tissue ischaemia. Reperfusion following the cellular damage causes an active inflammatory response. Erythrocyte deformability and plasma viscosity play a critical role in tissue and organ perfusion. In this study we aimed to evaluate the effect of levosimendan and nigella sativa on erythrocyte deformability during myocardial IR injury in rats.

Methods: Twenty-four Wistar albino rats were included in the study. The animals were randomly divided into four experimental groups. The coronary arteries of rats in Group C (control group) were not occluded or reperfused. Myocardial IR was performed by ligating the left anterior descending coronary artery for 30 min, followed by 2 h of reperfusion in the IR (IR), IR-levosimendan (24µg/kg) (IRL) group and IR-nigella sativa (0.2 mL/kg) (IRNS) group. Erythrocyte suspensions formed with a PBS buffer solution containing 5% htc were used to measure the deformability.

Results: As a result compared to control group, IR increased the relative resistance, a marker of erythrocyte deformability, significantly ($p<0.05$). There were significant differences between the groups according to the comparisons with ANOVA test ($p<0.0001$). The results obtained after corrections with Bonferroni test were as follows: Comparisons of the IRL and IRNS groups revealed similar results ($p=0.764$). The values of the IR group were significantly higher than those of the control, IRNS and IRL groups ($p<0.0001$, $p=0.001$, $p=0.003$, respectively).

Conclusion: Rats with IR injury had decreased erythrocyte deformability. This injury might cause more problems in microcirculation. Levosimendan and nigella sativa may be useful in reducing the adverse effects of this type of injury.

Key Words: Erythrocyte deformability, myocardial ischaemia-reperfusion, nigella sativa, levosimendan, rat

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ÖZET

Amaç: İskemi-reperfüzyon (İR) hasarı, doku iskemisi sonucu gerçekleşen bir olaylar zinciridir. Hücresel hasarı takiben reperfüzyon, aktif bir inflamatuvar yanıtı neden olur. Eritrosit deformabilitesi ve plazma viskozitesi doku ve organ perfüzyonunda kritik bir rol oynar. Bu çalışmada, ratlarda miyokardiyal İR hasarı sırasında levosimendan ve nigella sativa'nın eritrosit deformabilitesi üzerine etkisini araştırmayı amaçladık.

Yöntem: Yirmi dört adet Wistar albino rat çalışmaya dahil edildi. Hayvanlar rastgele dört deney grubuna ayrıldı. Grup K'daki (kontrol grubu) ratların koroner arterlerine oklüzyon veya reperfüzyon yapılmadı. Miyokardiyal İR, sol ön inen koroner arterin 30 dakika süreyle bağlanması, ardından İR (İR), İR-levosimendan (24 µg/kg) (İRL) ve İR-nigella sativa (0.2 mL/kg) (İRNS) gruplarında 2 saat reperfüzyon ile gerçekleştirildi. Deformabiliteyi ölçmek için %5 htc içeren bir PBS tampon çözeltisi ile oluşturulan eritrosit süspansiyonları kullanıldı.

Bulgular: Sonuç olarak, kontrol grubuna kıyasla İR, eritrosit deformabilitesinin bir belirteci olan nispi direnci artırdı ($p<0.05$). Gruplar arasında ANOVA testi ile yapılan karşılaştırmalara göre anlamlı fark vardı ($p<0.0001$). Bonferroni testi ile yapılan düzeltmelerden sonra elde edilen sonuçlar şu şekildedir: İRL ve İRNS gruplarının karşılaştırmaları benzer sonuçlar vermiştir ($p=0.764$). İR grubunun değerleri kontrol, İRNS ve İRL gruplarından anlamlı derecede yüksekti (sırasıyla $p<0.0001$, $p=0.001$, $p=0.003$).

Sonuç: İskemi reperfüzyon hasarı olan ratlarda eritrosit deformabilitesi azaldı. Bu yaralanma mikrodolaşımında daha fazla sorun yaratabilir. Levosimendan ve nigella sativa, bu tip yaralanmaların olumsuz etkilerini azaltmada yararlı olabilir.

Anahtar Sözcükler: Eritrosit deformabilitesi, miyokardiyal iskemide reperfüzyon, nigella sativa, levosimendan, rat

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INTRODUCTION

Cardiac surgery with cardiopulmonary bypass inevitably causes a systemic inflammatory response and ischaemia-reperfusion (IR) injury, which affects multiple organs (1,2). Many tissues and cells can be damaged by free radicals, with red blood cells (RBCs) being one of the most susceptible. During IR, the increased oxidative stress can cause augmented RBC membrane lipid peroxidation, with consequent alteration of cellular deformability. RBC deformability and aggregation have a very important effect on the microcirculation. In the capillaries, where the RBCs must deform to enter and transit vessels smaller than the resting cell diameter, erythrocyte deformability is a crucial factor affecting the flow of blood (3-5).

Levosimendan is a relatively new inotropic and vasodilator agent used in the management of acute and chronic heart failure (6). Its positive inotropic effect is mediated by calcium sensitization of contractile proteins, and its vasodilatory and anti-ischaemic effects are mediated by the opening of adenosine triphosphate-sensitive potassium channels in vascular smooth muscle cells (6-9). The protective effects of levosimendan are not limited to cardiac tissue. Some studies have suggested that levosimendan also attenuates IR injury in the spinal cord, lung and renal tissue (10-12).

Thymoquinone is one of these compounds. It is the main active ingredient of *Nigella sativa*, commonly known as black cumin or black seed, an annual flowering plant native to some areas such as the Mediterranean countries (13). Since its first extraction in 1963 (14), thymoquinone has been shown to act as a potent free radical and superoxide scavenger (15-17). Some studies have suggested that NS also attenuates IR injury in the spinal cord, heart and renal tissue (18-20).

The effects of levosimendan and *nigella sativa* on myocardial injury induced by left anterior descending (LAD) IR have not yet been investigated. The primary aim of this study was to investigate deformability changes and the role of levosimendan and *nigella sativa* in preventing these changes in the erythrocytes of rats in an experimental model of myocardial IR injury.

MATERIALS AND METHODS

Animals and Experimental Protocol

This study was conducted in the GUDAM Laboratory of Gazi University with the consent of the Experimental Animals Ethics Committee of Gazi University. All the procedures were performed according to the accepted standards of the Guide for the Care and Use of Laboratory Animals. In the study, 24 male Wistar albino rats weighing between 250 and 300 g, raised under the same environmental conditions, were used. For at least one week prior to surgery, the animals were housed in standard cages in a pathogen-free environment, with free access to food (until 2 h before the anaesthetic procedure) and water and with a 12 h light/dark cycle. The animals were randomly separated into four groups, each containing six rats.

The rats were anesthetized with an IP injection of 100 mg/kg of ketamine. The trachea was cannulated for artificial respiration. The chest was shaved, and each animal was fixed in a supine position on the operating table. The chest was opened by a left thoracotomy, followed by sectioning the fourth and fifth ribs about 2 mm to the left of the sternum. Positive-pressure artificial respiration was started immediately with room air, using a volume of 1.5 ml/100 g body weight at a rate of 60 strokes/min. Sodium heparin (500 IU/kg) was administered through the peripheral vein in the tail. After the pericardium was incised, the heart was exteriorized with gentle pressure on the right side of the rib cage. An 8/0 silk suture attached to a 10 mm micropoint reverse-cutting needle was quickly placed under the left main coronary artery. The heart was then carefully replaced in the chest, and the animal was allowed to recover for 20 min. Anaesthesia was maintained by repetitive injections of 20 mg/kg ketamine if a positive reaction to surgical stress or intermittent tail pinch could be observed.

There were four experimental groups: Group C (control; $n=6$), Group IR (IR; $n=6$), Group IRNS (IR-*nigella sativa*; $n=6$) and Group IRL (IR-levosimendan; $n=6$). The IRL group underwent left thoracotomy and received IP levosimendan (Simdax 2.5 µg/ml, Abbott*, Orion Pharma, Espoo, Finland) 24 µg/kg diluted in 10 ml of 0.5% dextrose administered intraperitoneally 30 min before ligating the LAD. The IRNS group underwent left thoracotomy and received IP *nigella sativa* (Tymoquinone 1G, Sigma Aldrich*) 0.2 ml/kg administered intraperitoneally 30 min before ligating the LAD. A small plastic snare was threaded through the ligature and placed in contact with the heart. The artery was then occluded by applying tension to the ligature (30 min), and reperfusion was achieved by releasing the tension (120 min). However, after the above procedure, the coronary artery was not occluded or reperfused in the control rats.

Intracardiac blood samples were obtained from all the rats. Heparinized total blood samples were used to prepare erythrocyte packs. Deformability measurements were performed using erythrocyte suspensions with 5% haematocrit (Htc) in a phosphate-buffered saline (PBS) buffer.

Deformability Measurements

Blood samples were carefully taken, and the measurement process was as fast as possible to avoid haemolysis of the erythrocytes. The collected blood was centrifuged at 1000 rpm for 10 min. Serum was removed, in addition to the buffy coat on the erythrocytes. An isotonic PBS buffer was added to the collapsing erythrocytes, and this was centrifuged at 1000 rpm for 10 min. The liquid on the upper surface was removed. Finally, pure red cell packs were obtained from the washing process, which was repeated three times. The erythrocyte packs were mixed with the PBS buffer to generate a suspension with a value of 5% Htc. These erythrocyte suspensions were used for the measurement of deformability. The collection and the deformability measurements of the erythrocytes were performed at 22°C.

A constant-current filterometer system was used in the measurement of the erythrocyte deformability. Samples to be measured were prepared with 10 ml of erythrocyte suspension and PBS buffer. The flow rate was held constant at 1.5 ml/min with an infusion pump. A 28 mm nucleopore polycarbonate filter with a 5 µm pore diameter was preferred. Pressure changes while the erythrocytes passed through the filter were detected by a pressure transducer, and the data were transferred to the computer with the help of an MP30 data equation system (Biopac Systems Inc., Commat, USA). The calculations were performed with related computer programs by measuring the pressure changes at various times. Pressure calibration of the system was performed before each sample measurement. The buffer (P_1) and the erythrocytes (P_2) were passed through the filtration system, and the changes in pressure were measured. The relative refractory period value (Rrel) was calculated by relating the pressure value of the erythrocyte suspension to the pressure value of the buffer. An increasing Rrel in the deformability index was interpreted as adversely affecting the deformability of the erythrocytes.

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA) 12.0 program was used for the statistical analysis. Erythrocyte deformability between the study groups were assessed using the ANOVA test. The Bonferroni-adjusted test was used if the results of the ANOVA test were significant to determine which groups differed from the others. The results were expressed as mean ± standard deviation (mean±SD). Statistical significance was set at a p value of <0.05.

RESULTS

The results of the study indicated that IR significantly increased the relative resistance, a marker of erythrocyte deformability when compared to control group ($p<0.05$), (Figure 1). There were significant differences between the groups according to the comparisons with ANOVA test ($p<0.0001$). The results obtained after corrections with Bonferroni test were as follows: Comparisons of the IRL and IRNS groups revealed similar results ($p=0.764$). The values of the IR group were significantly higher than those of the control, IRNS and IRL groups ($p<0.0001$, $p=0.001$, $p=0.003$, respectively).

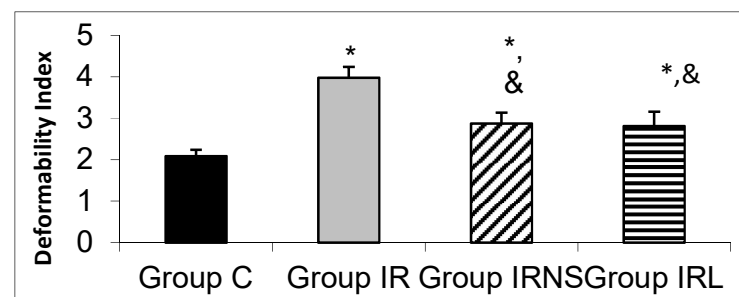


Figure 1: Erythrocyte deformability values of the groups. Each bar represents the mean ± SD.

* $p<0.05$ compared to Group C; & $p<0.05$ compared to Group IR

DISCUSSION

The effects of levosimendan on haemodynamics that include increased contractility, improved ejection fraction, increased cardiac output, reduced cardiac filling pressures and reduced systemic, pulmonary and coronary vascular resistance are based on its positive inotropic, lusitropic and vasodilatory properties (7,21,22). Levosimendan is a calcium sensitizer that increases the sensitivity of the myocardium to calcium, thereby increasing myocardial contractility without a rise in intracellular calcium (23). In addition, levosimendan causes adenosine triphosphate-sensitive potassium channels to open in vascular smooth muscle, resulting in smooth muscle relaxation (23,24). This contributes to vasodilatation and probably anti-ischaemic effects (22,23).

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, called 'deformability', is important in the microcirculation. Altered erythrocyte deformability not only changes the oxygen delivery capacity of the erythrocytes but also the survival of the circulating erythrocytes (25,26).

The administration of levosimendan is also associated with peripheral vasodilation, anti-ischaemic cardioprotection (27), neuroprotection (28) and anti-inflammatory and anti-apoptotic effects (29). We have determined that levosimendan has protective effects on the alteration of erythrocyte deformability during myocardial IR injury in rats (30). The black seed oil and thymoquinone by intraperitoneal injection were found to show protective effects on lipid peroxidation process during IR injury in rat hippocampus (31). Previous studies clearly showed that chronic treatment with NS fixed oil effectively influenced blood homeostasis in rats and seemed to induce transient changes in coagulation activity (32,33).

In this study, for the first time to our knowledge, we have reported that IR of the rat myocardium results in significant negative changes that can be observed in erythrocyte deformability and that levosimendan and nigella sativa, administered at the beginning of myocardial ischaemia, can provide varying degrees of protection against negative effects of variations in erythrocyte deformability.

In conclusion, the results of this study clearly demonstrated that erythrocyte deformability is significantly altered in experimental myocardial IR injury in the rat. This might lead to further problems in microcirculation. Thus, measurement of erythrocyte deformability might have an important impact on the follow-up for IR injury. Additionally, levosimendan and nigella sativa administered before the induction of ischaemia had protective effects on these alterations in myocardial IR injury. Other aspects of these findings, including clinical significance and practical applications, merit further experimental and clinical investigation.

Conflict of interest

No conflict of interest was declared by the authors.

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