The Effect of Hydrogen-Rich Saline Solution on Erythrocyte Deformability in Lower Limb Ischemia Reperfusion Injury in Rats

Sıçanlarda, Hidrojenle Zenginleştirilmiş Salin Solüsyonunun Alt Ekstremite İskemi Reperfüzyon Hasarında Eritrosit Deformabilitesine olan Etkisi

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ABSTRACT

Aim: We aimed to investigate the effects of HRSS on erythrocyte deformability in lower limb ischemia reperfusion (IR) injury in rats.
Methods: Eighteen male Wistar albino rats used in this study. The animals were randomly divided into three experimental groups as control, IR and IR-HRSS. 20 mg.kg^{-1}HRSS was administered (20 mg.kg^{-1} i.p)30 min before the procedure. An atraumatic microvascular clamp was placed across the infrarenal abdominal aorta in the IR groups. 120 min ischemia and 120 min reperfusion is applied to the groups. Erythrocytes were obtained from heparinized whole blood samples for deformability measurements. Kruskal-Wallis test was used for independent samples and Mann-Whitney U test was used to analyze differences between groups.
Results: Ischemia reperfusion was found to increase relative resistance to the control group. The erythrocyte deformability index was significantly higher in IR and IR-HRSS groups than the control group. HRSS application significantly decreased erythrocyte deformability index compared to IR group.
Conclusion: IR induced rats decreased erythrocyte deformability was partially corrected by HRSS. We believe that the protective effects of HRSS in IR injury and its use indications can be demonstrated in detail as long as the findings we have reached in our study are supported by other studies.

Keywords: Hydrogen-rich saline solution, erythrocyte deformability, ischemia reperfusion.

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ÖZET

Amaç: Hidrojenle zenginleştirilmiş salin solüsyonunun (HRSS) alt ekstremite kaslarındaki iskemi reperfüzyon hasarında eritrosit deformabilitesine olan etkisinin araştırılması hedeflenmiştir.
Yöntem: 18 adet wistar cinsi albino sıçan rastgele olarak kontrol, iskemi reperfüzyon (IR) ve iskemi reperfüzyon + HRSS (IR+HRSS) olmak üzere 3 gruba ayrılmıştır. IR+HRSS grubuna işlemden yarım saat önce intraperitoneal olarak 20 mg.kg^{-1}HRSS uygulanmıştır. IR gruplarında aobdominal aortaya atravmatik mikrovasküler klemp konularak 120 dk süreyle iskemi sağlanmış, sonra klemp kaldırılarak 120 dk reperfüzyon sağlanmıştır. Kan örnekleri heparinize edilerek eritrosit deformabilitesi açısından incelenmiştir. Bağımsız değişkenler Kruskal Wallis testiyle incelenmiş, örnekler arasındaki farklar ise Mann Whitney U testi ile ortaya konulmuştur.
Bulgular: IR gruplarında rölatif direncin kontrol grubuna göre arttığı bulunmuştur. IR ve IR+HRSS gruplarında eritrosit deformabilte indeksinin kontrol grubuna göre anlamlı düzeyde yüksek olduğu görülmüştür. HRSS uygulamasının IR grubuna göre eritrosit deformabilte indeksini ciddi oranda azalttığı tespit edilmiştir.
Sonuç: IR sonucunda eritrosit deformabilitesinde meydana gelen artışın HRSS uygulaması ile kısmen düzeltildiği görülmüştür. IR hasarına karşı HRSS uygulamasının etkili olabileceğini düşünmekte ve bu bulguların diğer çalışmalarla desteklendiği takdirde HRSS kullanım endikasyonlarının şekillenebileceği görüşündeyiz.

Anahtar Sözcükler: Hidrojenle zenginleştirilmiş salin solüsyonu, eritrosit deformabilitesi, iskemi reperfüzyon.

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INTRODUCTION

Ischemia is described as decreased blood supply to a tissue. This reduces the nutrients and and oxygen supply (1,2). Reperfusion may paradoxically cause increased rates of mortality and morbidity as substances produced as a result of oxidation may cause 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 (3–7). Ischemia/reperfusion (IR) is more dangerous than single ischemia (8).

Damage to cells after reperfusion of previously viable ischemic tissues in the lower extremities is a frequent and serious clinical event. Besides oxidative stress, free radical formation and lipid peroxidation are also important in development of IR injury. These factors change membrane of red blood cells (RBC) (9). Optimal erythrocyte deformability is essential for normal circulation as RBCs change shape to get through narrow capillaries or to reduce blood viscosity (10).

Hydrogen-rich Saline Solution (HRSS) has antioxidant, anti-inflammatory effects. Although the effect of HRSS on IR injury is investigated in experimental animal models, there are still few studies about the effect of HRSS on erythrocyte deformability in lower limb IR injury in rats. Therefore, we aimed to investigate the effect of HRSS on erythrocyte deformability in lower limb IR injury in rats.

MATERIALS and METHODS

Animals and Experimental Protocol

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

The subjects in our study were 24 Wistar Albino rats weighing between 180 and 220 g, which were nurtured under the same habitat. The subjects were kept under 20-21 °C within cycles of 12-hour daylight and 12-hour darkness. They were given free access to nutrition until 2 hours before the anesthesia procedure and randomly separated into four equal groups of 6 animals. Ketamine anesthesia was applied prior to midline laparatomy.

Control group (Group C): Midline laparotomy was the sole surgical procedure without any additional intervention. After 4 hours of follow-up, blood sample was collected and subjects were sacrificed.

Ischemia-reperfusion group (Group IR): Midline laparotomy was done in a similar fashion. Infrarenal aorta was left clamped for 2 hours. After removing the clamp, reperfusion was established for another additonal 2 hours. At the end of 4 hours, blood samples were collected from the abdominal aorta and subjects were sacrificed

Ischemia-reperfusion group with Hydrogen-rich Saline Solution (Group IR-HRSS): After following the same steps in IR group, Hydrogen-rich Saline Solution was given (20 $mg.kg^{-1}$) intraperitoneally 30 minutes before the ischemia period. At the end of 4 hours, blood samples were collected from the abdominal aorta and subjects were sacrificed.

Intraabdominal blood samples were collected after ketamine (100 $mg.kg^{-1}$) was given intraperitoneally to all subjects. Erythrocyte packs were prepared with heparinized total blood samples. Erythrocyte suspensions of 5 % hematocrit with phosphate buffered saline (PBS) were used for deformability measurements.

Deformability Measurements:

First the samples were centrifuged for ten minutes at 1000 rpm and then serum and the buffy coat on erythrocytes were removed. Then, isotonic PBS buffer was added to the collapsing erythrocytes. This mixture of PBS and erythrocytes was centrifuged for another ten minutes at the same speed of 1000 rpm. Subsequently, liquid was removed from the upper surface. Finally pure red cell packs were obtained from three consequent washing process. PBS buffer was mixed with erythrocyte packs in order to obtain a value. And those mixed suspensions with 5% hematocrit were used for deformability measurement. These procedures were done at 22 ºC.

Deformability measurement was done with the constant-current filtrometer system. Samples of 10 ml erytrocytes suspension - PBS buffer were prepared for measurement. There was a constant flow rate of 1.5 ml/min through an infusion pump. We used a 28 mm nucleoporin polycabonate filter which has a pore diameter of 5 µm. A transducer detected the pressure changes during the erythroctes passage through the filter and the collected data was transferred to computer with MP 30 data equation system (Biopac Systems Inc, Commat, USA).

The pressure of the system was calibrated before each measurement. Buffer (P_T) and then erythrocytes (P_E) were passed subsequently through from the filtration system and pressure changes were measured.

The relative refractory period value (Rrel) was calculated by relating the pressure value of erythrocyte suspension to pressure value of buffer. Increase in Rrel as the deformability index was interpreted as adverse effect on erythrocyte deformability.

Statistical Analysis

SPSS 17.0 software program was used for statistical analyses and $p<0.05$ was considered statistically significant. The findings were expressed as mean±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 was found to increase relative resistance compared to the control group ($p<0.0001$). The erythrocyte deformability index was significantly higher in IR and IR-HRSS groups than the control group ($p<0.0001$, $p=0.001$, respectively). HRSS application significantly decreased erythrocyte deformability index compared to IR group ($p=0.001$) (Figure 1).

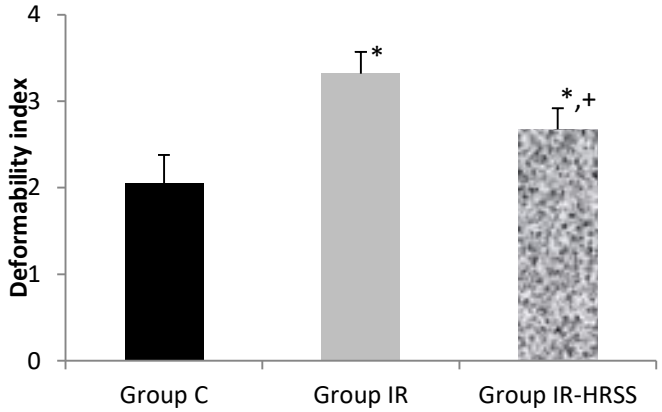

Figure 1: Erythrocyte deformability index values of the groups. Each bar represents the mean ± sd.
* $p<0.05$ compared to the C
+ $p<0.05$ compared to the Group IR

DISCUSSION

In this study, we have reported the protective effects of HRSS on erythrocyte deformability in experimental lower limb IR injury in rats. IR was found to increase relative resistance compared to the control group. Besides, in the IR and IR-HRSS groups, the erythrocyte deformability index was significantly higher than the control group.

IR injury is an inflammatory response accompanied by free radical formation, leucocyte migration and activation, sinusoidal endothelial cellular damage, deteoriated microcirculation and coagulation and complement system activation (11). IR injury causes lipid peroxidation and a complex variety of products ocur and this production causes local and systemic toxic and mutagenic effects (12). These effects lead to damage of the cellular membrane, which contains polyunsaturated fatty acids. Finally, the loss of disintegration of cellular membrane occurs by structural and functional tissue damage.

Erythrocyte deformability is important for organ and tissue perfusion (13). Erythrocytes must have the capability to extend and curve to move in final organ capillaries for delivering oxygen and vital molecules and clearing metabolic wastes. This capacity is called 'deformability' (14). When equilibrium in free radical production and antioxidant defense system is disrupted oxidative damages occur (15). The products of lipid peroxidation caused by oxidative stress damage membrane permeability and micro viscosity. Thus, diminished deformability capacity and survival of the erythrocytes are observed (16).

Erythrocyte deformability and erythrocyte membrane rigidity are affected by several agents. Altered erythrocyte deformability not only changes the oxygen delivery capacity of the erythrocytes but also the survival of the circulating erythrocytes (16, 17).

Hydrogen is the lightest and most abundant chemical element in nature and its reductive potential has been recently recognized (18-22). Besides hydrogen can act as a scavenger and has an increasing focus as it has antioxidant and anti-apoptotic properties (22). As a potent cellular protector, hydrogen especially decreases ROS (reactive oxygen species) (23-29). Therefore, HRSS could be an inexpensive, safe choice for the skeletal muscle IR injury as it is produced, stored, and transported easily (30,31).

In our study, HRSS application significantly decreased erythrocyte deformability index compared to IR group.

As a conclusion the results of this study clearly demonstrate that erythrocyte deformability is significantly altered in experimental lower limb IR injury in rats. We found that in IR induced rats decreased erythrocyte deformability was partially corrected by HRSS. We believe that the protective effects of HRSS in IR injury and its use indications can be demonstrated in detail as long as the findings we have reached in our study are supported by other studies.

Conflict of interest

No conflict of interest was declared by the authors.

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