The Effects of Esmolol on Erythrocyte Deformability in Rat Liver Ischemia-Reperfusion Injury

Karaciğer İskemi- Reperfüzyon Hasarında Esmololün Eritrosit Deformabilitesi Üzerine Etkileri

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ABSTRACT

Background: Esmolol has protective effects in ischemia reperfusion (IR) injury. The purpose of our study was to look into the effects of this which esmolol on erythrocyte deformability in rat liver IR injury model.
Materials and Methods: We used 24 Wistar albino rats as subjects in our study. They were divided into 4 groups; randomized control group (group C; n=6), esmolol group 200µg/kg/min intravenously (group E; n=6), IR group (group IR; n=6) and IR group with esmolol 200µg/kg/min intravenously (group IR-E; n=6). Erythrocyte packs were prepared from heparinized blood samples and deformability measurements were performed.
Results: It was discovered that ischemia reperfusion increased the relative resistance when compared to control group (p<0.0001). Erythrocyte deformibility index was found to be higher in IR and IR-E groups compared to control group (p<0.0001, p=0.002, respectively). Esmolol application decreased the erythrocyte deformibiltiy index when compared to control group (p=0.017).
Conclusion: In this research, esmolol application has improved the ertyhrocyte deformibity in liver rat IR injury partially. We also found that esmolol had beneficial effects by reversing undesirable effects of IR. Further studies with larger volume are required to support our promising results.

Keywords: Ischemia-reperfusion, liver, esmolol, erythrocyte deformability, rat

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ÖZET

Amaç: Esmololün iskemi-reperfüzyon (İR) hasarı üzerine koruyucu etkileri vardır. Bu çalışmanın amacı, esmololün bu etkisinin rat karaciğer İR hasarında eritrosit deformabilitesi üzerine etkilerinin araştırılmasıdır.
Yöntem: Bu çalışmada 24 adet Wistar cinsi albino rat kullanılmıştır. Ratlar rastgele 4 gruba ayrılmışlardır. Kontrol grubu (grup C; n=6), esmolol grubu intravenöz (iv) 200µg/kg/dk (grup E; n=6), İR grubu (grup İR; n=6) ve İR grubu-esmolol iv 200µg/kg/dk (grup İR-E; n=6). Eritrosit kütleleri heparinize kan örneklerinden hazırlanmıştır ve deformabilite ölçümleri yapılmıştır.
Bulgular: İskemi- reperfüzyonun eritrositlerde kontrol grubuna göre relatif rezistansı arttırdığı bulunmuştur (p<0.0001). Eritrosit deformabilite indeksi kontrol grubu ile karşılaştırıldığında İR ve İR-E gruplarında daha yüksek bulunmuştur (p<0. 0001, p=0.002, sırasıyla). Esmolol uygulaması kontrol grubuna göre eritrosit deformabilite indeksini düşürmüştür (p=0.017).
Sonuç: Bu çalışmada esmolol uygulaması, karaciğer İR hasarında eritrosit deformabilitesini kısmi olarak düzeltmiştir. Bununla birlikte, esmololün İR'nun istenmeyen etkileri üzerine de faydalı etkileri vardır. Bu sonuçları destekleyecek daha geniş ölçekli çalışmalar gereklidir.

Anahtar Sözcükler: İskemi-reperfüzyon, karaciğer, esmolol, eritrosit deformabilitesi, rat

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INTRODUCTION

The deformability of human matur red cells is defined as their ability to change their shapes while they are passing through narrow cells and this is the major deterinant of red cell survival in the circulation (1). Also, deformability has a supreme role for a proper microcirculatory function and adequate delivery of oxygen to tissues (1). Deformability can be affected by disorders like hemoglobinopathies, genetic disorders and varios during various acquired diseases including infections, circulatory disorders, ischemia and reperfusion, metabolic diseases (e.g., diabetes), and pulmonary disorders (2).

During the hepatic surgery, temporary reduction and restoration in blood supply to liver results in ischemia-reperfusion injury (IR) (3). During the IR injury prolonged oxygen deprivation causes a series of events. Depletion of ATP and cellular conversion to anaerobic metabolism provokes necrosis and cell death. Reestablishment of oxygen creates reactive oxygen radicals and causes direct tissue injury and trigger celluler responses causing inflamattion. Pathologically, tissue injury is characterized by endothelial injury, microvascular disruption, increased apoptosis, and ultimately necrosis (4, 5). Also, oxidative stress, reactive oxygen radicals and lipid proxidation cause serious changes in red blood cell (RBC) membranes (6).

Esmolol is a β-1-adrenoreceptor blocker which has been previosly proven to cause reduction in the IR injury during the cardiac surgery (7). Various agents have been previosly studies for their effect on erytrocyte deformability (8-11). The aim of this study is to investigate the effects of esmolol on erytrocyte deformability following rat liver IR injury.

MATERIALS and METHODS

Animals

This study was conducted in the Physiology Laboratory of Kirikkale University upon the consent of Experimental Animals Ethics Committee of Gazi University. All the procedures were performed according to accepted standards of Guide for the Care and Use of Laboratory Animals. Experiments were performed using 24 male Wistar rats weighing 250-330 g. Animals were maintained under standard conditions such as stable room temperature (24 ± 3^0C) and a 12-hour light-dark cycle and were allowed access to rat pellets and water.

Experimental Model

Before the procedure, the animals were anesthetized with ketamine 100 mg/kg ip and were placed below a heating lamp to maintain a temperature of 37 ^{0}C. After obtaining a vein acces via tail vein of rats, esmolol infusion was started at a dose of 200μg/kg/min through the tail vein to the groups which were receiving esmolol. In this groups laparatomy was performed with an abdominal incision 30 mins after the end of infusion.

Hepatic Ischemia-Reperfusion

The hepatic iscemia was created by using a microvascular bulldog clamp. After the laparatomy and dissection, the clemp was placed after the visualization of left portal triad. Forty-five minutes of partial ischemia was performed and through this partial ischemia method, the congestion of right, caudat lobes and mesenteric area were blocked. Following 45 mins of ischemic period, 2 hours of reperfusion was performed by removing the vascular clemp. Then rats were sacrified after obtaining blood and tissue samples.

Experimental Protocol

Grup C: Control Group
Group E: Esmolol Group
Group IR: Ischemia-reperfusion Group
Group IRE: Ischemia reperfusion+esmolol Group
Group C: Following anesthesia, and vein cannulation, laparatomy was performed and hepatic IR was applied then rats were sacrified after obtaining blood samples.
Group E: Following anesthesia, tail vein was cannulated and 200μg/kg/min esmolol was infused. After the infusion ended, the rats were kept without any surgical intervention, then rats were sacrified after obtaining blood samples.
Group IR: Thirty mins after the anesthesia, laparatomy then hepatic IR was applied and following reperfusion blood samples were taken.
Group IRE: After the anesthesia and esmolol infusion. Laparatomy was performed and then hepatic IRE was created. After the procedures were completed, rats were sacrified and blood samples were taken.

Deformability measucrements

A constant flow filtrometer system (MP 30, Biopac Systems Inc., Commat, USA) was used for deformability measurements. Erythrocyte suspension that was delivered at 1 ml/min flow rate was passed through a nucleopore-polycarbonate filter of 5 mm in diameter, and alterations in the filtration pressure corresponding to different flow rates were measured. The alterations in the pressure were transferred to computer medium with an MP 30 data equation system. The ratio of the values of filtration pressure for the cellular suspension and buffer were calculated, and the relative resistance was calculated.

Statistical analysis

SPSS 20.0 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

It was discovered that IR increased the relative resistance when compared to control group ($p<0.0001$). Erythrocyte deformability index was found to be higher in IR and IR-E groups compared to control group ($p<0.0001$, $p=0.002$, respectively). Esmolol application decreased the erythrocyte deformabiltiy index when compared to control group ($p=0.017$), (Figure 1).

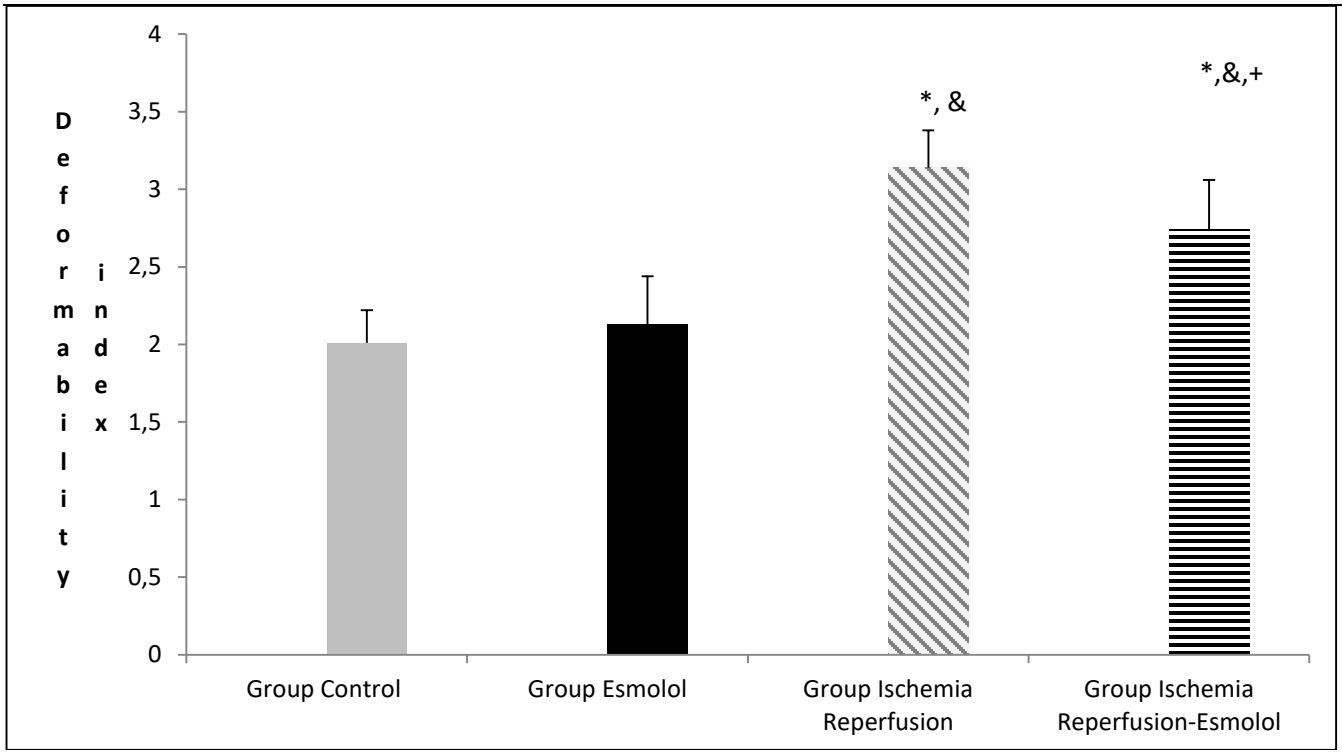

Figure 1: Erythrocyte deformability index values of thegroups. Each bar represents the mean ± sd.
* p<0.05 compared to the Group Control
& p<0.05 compared to the Group Esmolol
+ p<0.05 compared to the Group Ischemia Reperfusion

DISCUSSION

In this study, it has been shown that hepatic IR injury increased the erythrocyte deformability and relative resistance. Also it has been shown for the first time, the esmolol cardioselective β-1 adrenoreceptor blocker application before the IR injury 30 mins ago with a dose of 200μg/kg/min intravenously reduced the deformability index.

In hepatic IR injury, reactive oxygen species (ROS) production causes an oxidative damage upon mitocondria and reduces ATP production (12) . In liver, the Kupffer cells, sinusoidal endothelial cells and hepatocytes need ATP to maintain their phsiological functions. In ischemic period of IR injury, ATP provided for these structures reduces and reduction in ATP levels results in dysfunction of ATP-dependent sodium/potassium plasma membrane pump (Na/K adenosine triphosphatase [ATPase). The dysfunction of the pump leads intracellular Na accumulation which causes intracelluler edema and swelling. Also, in liver tissue the balance of vasoconstrictors- endothelin, thromboxane A_{2-} and vasodilatator nitric oxide –(NO)- levels change and this ends with narrowing of the sinusoidals. Following reperfusion, adhesion and aggregation of neutrophils and platelets in the sinusoids lead to disruption in microcirculation (13,14). RBC deformability is mainly determined by deformability of membrane, surface area/per volume ratio of red cell and intracellular viscosity. (15). Of these two determining the deformability of red cells are depend on the proper activity of the Na, K-ATPase in RBC. The ionic gradient accros the red cell membrane is maintaned by Na, K-ATPase, and the pump plays a major role in signal transduction system in the cell (16). And ATP is the form of energy neccessary for the normal RBC deformability and it is vital for the pumps. The lack of ATP, dysfunction of Na, K-ATPase in RBC membrane and such changes, may also occur in RBCs trapped in ischemic tissue of liver for prolonged periods and may alter the deformability index of red cells (17).

During the IR injury, the increased reactive oxygen radicals are defined as superoxide anions, hydroxyl radicals, and peroxide hydrogen and these radicals on proteins, enzymes, nucleic acids, cytoskeleton. Also lipid peroxides disrupts the mitocondrial functions and lipid peroxidation in cell (18).

Polyunsaturated fatty acids which are the main membrane phospolipids are strongly effected by this oxidative damage and ion hemostasis fails and the cascade ends with cell death. Besides these effects are not limited with cell membrane, even into the organels including mitocondria and nucleus. In the nucleus, DNA can be damaged by ROS and finally protein transcription and translation is disrupted. Antiproteases are inactivated by ROS damage and this inopportune activation worsens the cellular damage (14). The RBC membrane and the cytoskeleton of the RBC are major determinants of cell's mechanical behavior. Nearly the lipid layer of the RBC membrane is poor in viscosity and this lack of viscosity limits the elasticity of the RBC membrane. Furthermore, the cytoskeleton of RBC membrane is mainly responsible for the maintenance of biconcave-discoid shape. The RBC membrane cytoskeleton is a network of proteins lying just beneath the cell membrane. During the IR injury impaired protein cyntesis, lipid peroxidation corruption leads to changes in deformability (17).

Calpains, protein kinase C, and phospholipase C are calcium dependent enzymes. During the IR injury, the accumulation of calcium into the cell leads to overload of calcium and then activation of these calcium dependent enymes. calcium channel blockers can inhibit the overload of Ca^{2+} and reduce cellular damage, thus Ca^{2+} influx may have an important role in the IR injury process and so end with altered deformability (19).

Esmolol is a highly selectibe β-1 adrenoreceptor blocker, ultra short acting, a class II anti-arrhythmic agent. In cardiac myocytes it inhibits the β-1 receptors via competetive antagonism. Esmolol increases atrioventricular refractory time, decreases oxygen demand of the myocardium, and decreases atrioventricular conduction (20). β-1 blockers exert their effect by binding to the beta-1 receptor sites selectively and inhibiting the action of epinephrine and norepinephrine on these sites. These receptors are G-protein-coupled receptors. They show their effects through the cyclic AMP (cAMP)and cAMP-dependent protein kinase action with resultant calcium ion concentration increases. This kind of activation in beta-1 receptors lead to inotropy, chronotropy, and dromotropy (21).

Esmolol is used mainly such indication: supraventricular tachycardia,, urgent care, perioperatively, and postoperatively, sinus tachycardia, tachycardia and hypertension, acute coronary syndrome, non-ST elevation myocardial infarction, hypertensive emergencies, thyrotoxicosis, refractory ventricular tachycardia, ventricular fibrillation, and to decrease catecholamine response during electroconvulsive therapy (20).

They are the best agents againts the cytotoxic action of free radicals during reperfusion since they have cytoprotective as well as antioxidant actions (21). Esmolol have been proven to cause cardioprotection against IR injury (22). During the ischemia β- 1 receptor and protein kinase activity increases and contributes to IR injury and inhibition of these receptors have beneficial role in protecting against IR injury (22) . Also It has been previously proven, esmolol alters the IR injury by reducing malondialdehyde, superoxide dismutase and glutathione peroxidase levels which are markers of oxidant status and lipid peroxidation in human settings (23).

Besides cardiac settings, it can be used for neuroprotection in spinal cord IR injury as well. It enhances the histological and motor impairment following spinal cord IR injury (24). And it enhances the transient forebrain ischemia as well (25).

As a conclusion RBC deformabonlity can be affected by pathophysiological processes. The normal rheological behavior of RBC is very delicate and strongly based on the proper microenviroment and metabolic functions. The chaos either in local or systemic may alter the normal rheological properties of RBC. This study has shown that erythrocyte deformability is reduced in experimental hepatic IR injury in the rat because of the pathophsigological processes during the IR injury. And deformability can be partially corrected which was caused by hepatic IR injury. The exact mechanism underlying this correction is stil unclear but various pathopysiological and molecular mechanisms may have role. In this regard, further investigations are needed to discover the exact mechanisms.

Conflict of interest

No conflict of interest was declared by the authors.

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