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

Ülkü Sabuncu¹, Ayşegül Küçük², Faruk Metin Çomu³, Nevriye Salman⁴, Gülay Kip⁵, Yusuf Ünal⁵, Mustafa Arslan⁵

1Health Sciences University, Tepecik Training and Research Hospital, Department of Anesthesiology and Reanimation, İzmir, Turkey
2Kütahya Health Sciences University, Medical Faculty, Department of Physiology, Kütahya, Turkey
3Kırıkkale University, Medical Faculty, Department of Physiology, Kırıkkale, Turkey
4Health Sciences University, Yüksek İhtisas Training and Research Hospital, Department of Anesthesiology and Reanimation, Ankara, Turkey
5Gazi University, Medical Faculty, Department of Anesthesiology and Reanimation, Ankara, Turkey

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 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 deformability index when compared to control group (p<0.017).

Conclusion: In this research, esmolol application has improved the erythrocyte deformability 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

Received: 11.26.2018 Accepted: 12.12.2018

ÖZET

Amaç: Esmololün iskemi-reperfüzyon (IR) hasarı üzerine koruyucu etkileri vardır. Bu çalışmanın amacı, esmololün bu etkininın rat karaciğer IR hasarında eritrosit deformabilitesi üzerine etkisini araştırmaktır.

Yöntem: Bu çalışmada 24 adet Wistar cinsi albay rat kullanılmıştır. Ratlar rastgele 4 gruba ayrılmışlardır. Kontrol grubu (grup C; n=6), esmolol grubu intravenöz (iv) 200µg/kg/dk (grup E; n=6), IR grubu (grup IR; n=6) ve IR grubu- esmolol iv 200µg/kg/dk (grup IR-E; n=6). Eritrosit kütleleri heparinize kan örneklerinden hazırlanmıştır ve deformabilite ölçümleri yapılmıştır.

Bulgular: İskemi-reperfüzyonun eritrositlerde kontrol grubuna göre relativ rezistansı arttırdığı bulunmuştur (p<0.0001). Eritrosit deformabilite indeksi kontrol grubu ile karşılaştırıldığında IR ve IR-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 IR hasarında eritrosit deformabilitesini kısmi olarak düzeltebildi. Bununla birlikte, esmololün IR’nün istenmeyen etkileri üzerine de faydalı etkileri vardır. Bu sonuçlar destekleyecek daha geniş ölçekli çalışmalar gerekli olacaktır.

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

Geliş Tarihi: 26.11.2018 Kabul Tarihi: 12.12.2018
INTRODUCTION

The deformability of human mature red cells is defined as their ability to change their shapes while they are passing through narrow cells and this is the major determinant 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 varies 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 cellular responses causing inflammation. 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 previously proven to cause reduction in the IR injury during the cardiac surgery (7). Various agents have been previously studies for their effect on erythrocyte deformability (8-11). The aim of this study is to investigate the effects of esmolol on erythrocyte 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°C) 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°C. After obtaining a vein access 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 laparotomy was performed with an abdominal incision 30 mins after the end of infusion.

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 deformability index when compared to control group (p=0.017), (Figure 1).
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 mitochondrial functions and lipid peroxidation in cell (18).

In hepatic IR injury, reactive oxygen species (ROS) production causes an oxidative damage upon mitochondria and reduces ATP production (12). In liver, the Kupffer cells, sinusoidal endothelial cells and hepatocytes need ATP to maintain their physiological 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 intracellular edema and swelling. Also, in liver tissue the balance of vasoconstrictors- endothelin, thromboxane A2 and vasodilator nitric oxide –(NO)- levels change and this ends with narrowing of the sinusoids. 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 maintained 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 necessary 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 mitochondrial functions and lipid peroxidation in cell (18).

Polyunsaturated fatty acids which are the main membrane phospholipids 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 mitochondria 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 inopportun 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 synthesis, 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 enzymes. calcium channel blockers can inhibit the overload of Ca2+ and reduce cellular damage, thus Ca2+ influx may have an important role in the IR injury process and so end with altered deformability (19).

Esmolol is a highly selective β-1 adrenoreceptor blocker, ultra short acting, a class II anti-arrhythmic agent. In cardiac myocytes it inhibits the β-1 receptors via competitive 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).

**Figure 1:** Erythrocyte deformability index values of the groups. 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
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 against the cytotoxic action of free radicals during reperfusion since they have cytoprotective as well as antioxidant actions (21). Esmolol has 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 deformability can be affected by pathophysiological processes. The normal rheological behavior of RBC is very delicate and strongly based on the proper microenvironment 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 pathophysiological 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 still unclear but various pathophysiological 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.

REFERENCES

1. Radosinska J, Vrbjan N. The role of red blood cell deformability and Na-K-ATPase function in selected risk factors of cardiovascular diseases in humans: focus on hypertension, diabetes mellitus and hypercholesterolemia. Physiol Res 2016; 65:43-54.

2. Simmonds MJ, Meiselman HJ, Baskurt OK. Blood rheology and aging. J Geriatr Cardiol 2013;10: 291-301.

3. Kapan M, Gumus M, Onder A, Firat U, Basarali MK, Boyuk A, et al. The effects of ellagic acid on the liver and remote organs' oxidative stress and structure after hepatic ischemia reperfusion injury caused by pringle maneuver in rats. Bratisl Lek Listy 2012;113:274-281.

4. Pérez JC, Ramírez AC, González LT, Espinosa LE, Quintana MM, Galván GA, et al. Spiironolactone effect in hepatic ischemia/reperfusion injury in wistar rats. Oxid Med Cell Longev 2016:1-9. doi: 10.1155/2016/3196431.

5. Zimmerman MA, Martin A, Yee J, Schiller J, Hong JC. Natural Killer T Cells in Liver Ischemia–Reperfusion Injury. J Clin Med 2017; 6:1-9. doi:10.3390/jcm6040041.

6. Grisham MB, Granger DN. Free radicals: reactive metabolites of oxygen as mediators of postischemic reperfusion injury. In: Martson A, Bulky GB, Fiddian-Green RG, Haglung U, editors. Splanchnic ischemia and multiple organ failure. St. Louis: Mosby; 1989: 135–144.

7. Scorsin M, Mebaza A, Al Attar N, Medini B, Callebert J, Raffoul R, et al. Efficacy of esmolol as a myocardial protective agent during continuous retrograde blood cardioplegia. J Thorac Cardiovasc Surg 2003;125(4): 1022-1029.

8. Arslan M, Çomu FM, Küçük A, Öztürk L, Yavak F. Dexmedetomidine protects against lipid peroxidation and erythrocyte deformability alterations in experimental hepatic ischemia reperfusion injury. Libyan J Med. 2012;7:1-5 doi: 10.3402/ljm.v7i0.18185.

9. Sivgin V, Kucuk A, Comu FM, Kosem B, Kortal S, Turgut HC, et al. Effects of intravenous ibuprofen and lornoxicam on erythrocyte deformability in rats undergoing hind limb ischemia reperfusion injury. Bratisl Med J 2016;117(12):722-725.

10. Kara H, Özer A, Arpaci H, Demirtas H, Comu FM, Oktar GL, et al. Effect of alprostadil on erythrocyte deformability in ischemia reperfusion injury. Bratisl Med J 2015; 116(8):509-511.

11. Tatar T, Polat Y, Comu FM, Kortal S, Arslan M, Kucuk A. Effect of cerium oxide on erythrocyte deformability in rat lower extremity ischemia reperfusion injury. Bratisl Med J 2018;119(7):441-443.

12. Chouchani ET, Pell VR, James AM, Work LM, Saeb-Parsy K, Frezza C, et al. A Unifying Mechanism for Mitochondrial Superoxide Production During Ischemia-Reperfusion Injury. Cell Metab 2016; 23:254-263.

13. Arslan F, de Klein DP, Pasterkamp G. Immune signaling in cardiac ischemia. Nat Rev Cardiol 2011;8(5):292-300.

14. Abu-Amara M, Yang SY, Tapuria N, Fuller B, Davidson B, Seifalian A. Liver ischemia/reperfusion injury: processes in inflammatory networks--a review. Liver Transpl 2010;16(9):1016-1032.

15. Tomaiuolo M, Stalker Tj, Welsh JD, Diamond SL, Sinno T, Brass LF. A systems approach to hemostasis: 2. Computational analysis of molecular transport in the thrombus microenvironment. Blood 2014;124(11):1815-1823.

16. Elimban V, Bartekova M, Xu Yj, Dhallal Ns: Regulation Of Membrane Na+-, K+-, and Ca2+ current. Bratisl Med J 2018;119(7):441-443.

17. Baskurt OK, Meiselman HJ. Blood rheology and hemodynamics. Semin Thromb Hemost 2003;29(5):435-450.

18. Guan LY, Fu PY, Li PD, Li ZN, Liu HY, Xin MG, et al. Effects of ellagic acid on the liver and remote organs' oxidative stress and protective effects of nitric oxide. World J Gastrointest Surg 2014;6(7):122-128.

19. Cannistrà M, Ruggiero M, Zullo A, Galelli G, Serafini S, Maria M, et al. Hepatic ischemia reperfusion injury: A systematic review of literature and the role of current drugs and biomarkers. Int J Surg 2016;33: 57-70.

20. Pevtsov A, Fredlund KL. Esmolol. StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2018 Jul 28.

21. Wallukat G. The beta-adrenergic receptors. Herz 2002;27(7):683-690.

22. Tucker WD, Whitten R. Selective Beta-1-Blockers. StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2018-2018 Aug 30.

23. Daga MK, Chaudhary M, Sharma B, Bhattacharjee J, Ghambhir DS, Arora N, et al. Effect of esmolol on oxidant status and antioxidant activity in acute myocardial infarction. J Assoc Physicians India 2003;51:677-680.

24. Umerhara S, Goyati T, Nishikawa T, Tobe Y, Masaki, Y. Esmolol and lornoxicam: selective β-1 adrenoceptor antagonists, protect neuroprotection against spinal cord ischemia and reperfusion in rats. Anesth Analg 2010;110(4):1133-1137.

25. Goyati T, Horiguchi T, Nishikawa T, Tobe Y, Masaki Y. Neuroprotective effects of selective β-1 adrenoceptor antagonists, lornoxicam and esmolol, on transient forebrain ischemia in rats; a dose-response study. Brain Res 2012;1461:96-101.