Effect of Low and High Dose Sugammadex on Erythrocyte Deformability in Streptozotocin-Induced Diabetic Rats

Streptozosinle Diyabet Oluşturulmuş Sıçanlarda Düşük ve Yüksek Doz Sugammadeksin Eritrosit Deformabilitesi Üzerine Etkisi

Hasan Ali Kiraz[1], Hüseyin Cihad Turgut[2], Seyfi Kartal[3], Faruk Metin Comu[4], Gülay Kip[5], Metin Alkan[6], Muhammed Enes Aydın[6], Meral Erdal Erbatur[6] , Mustafa Arslan[6], Yusuf Ünal[6]

[1]Canakkale Onsekiz Mart University Faculty of Medicine, Department of Anesthesiology and Reanimation, Canakkale, Turkey
[2]Gazi University Faculty of Dentistry, Department of Oral and Maxillofacial Surgery (Anesthesiology and Reanimation Specialist), Ankara, Turkey
[3]Turgut Ozal University Faculty of Medicine, Department of Anesthesiology and Reanimation, , Ankara, Turkey
[4]Kirikkale University Faculty of Medicine, Department of Physiology, Kırıkkale, Turkey
[5]Gazi University Faculty of Dentistry, Department of Pediatric Dentistry (Anesthesiology and Reanimation Specialist), Ankara, Turkey
[6]Gazi University Faculty of Medicine, Department of Anesthesiology and Reanimation, Ankara, Turkey

ABSTRACT

Objective: Erythrocyte deformability is a function of specially designed erythrocyte membrane properties and allows for the oxygen delivery without cell fragmentation. Impaired erythrocyte deformability in diabetes is one of the suspected factors that result in erythrocyte aggregation and the microvascular circulatory arrest. In this study, we aimed to investigate low versus high doses of sugammadex on erythrocyte deformability in streptozototocin-induced diabetic rats.

Methods: Twenty-four male Wistar albino rats weighing between 225 and 300 gr were randomly divided into 4 groups. Group C (control; *n*=6), Group DC (diabetes control; *n*=6), Group DR-16S (diabetes-rocuronium-16mg sugammadex; *n*=6) and Group DR-96S (diabetes- rocuronium-96mg sugammadex; *n*=6). Rats in control and diabetes groups received a 0.9% NaCl solution at the same volume. Diabetes was induced by a single IP injection of streptozotocin (Sigma Chemical, St. Louis, MO, USA) at a dose of 55 $mg.kg^{-1}$ body weight, and animals were kept alive for 30 days. At the end of the follow-up period animals' erythrocyte deformability was measured from blood samples.

Results: Serum glucose was significantly lower in Group C as compared to Groups DC, DR-16S and DR-96S (p<0.0001). The deformability index was significantly increased in the diabetic rats (p<0.0001). It was significantly increased in Group DR-96S as compared to Group C and DC (p<0.0001, p=0.028, respectively).

Conclusion: In this study, we showed the safety profile of low dose sugammadex in diabetic rats in terms of the erythrocyte deformability. Our findings may lead to future animal and human studies investigating sugammadex effects on erythrocyte deformability and micro/macrovascular circulation.

Key Words: Erythrocyte deformability, diabetes, sugammadex, microcirculation

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ÖZET

Amaç: Eritrosit deformabilitesi eritrosit membranının özel yapısı ile ilgili bir fonksiyon olup, hücrenin dağılmadan oksijen taşımasına olanak sağlar. Diyabette görülen bozulmuş eritrosit deformabilitesi eritrosit agregasyonu ve mikrovasküler düzeydeki dolaşım bozukluğunun etkenlerinden biridir. Bu çalışmada sıçanlarda streptozosinle indüklenen diyabette yüksek ve düşük doz sugammadeksin eritrosit deformabilitesi üzerindeki etkisini araştırmayı amaçladık.

Yöntemler: Ağırlıkları 225-300 gram arasında değişen 24 erkek Wistar albino sıçan rasgele 4 gruba ayrıldı. Grup K (kontrol; *n*=6), Grup DK (diyabet kontrol; *n*=6), Grup DR-16S (diyabet-rokuronyum-16mg sugamadeks; *n*=6) ve Grup DR-96S (diyabet- rokuronyum-96mg sugammadeks; *n*=6). Kontrol ve diyabet gruplarındaki sıçanlara aynı hacimde %0.9 NaCl verildi. Diyabet oluşturmak için tek intraperitoneal enjeksiyonla 55 $mg.kg^{-1}$ streptozosin (Sigma Chemical, St. Louis, MO, USA) uygulandı. Hayvanlar 30 gün süre ile izlendi ve takip süresinin sonunda kan örneklerinden eritrosit deformabilitesi ölçümü yapıldı.

Bulgular: Kontrol grubundaki serum glukoz düzeyi DK, DR-16S ve DR-96S gruplarındakilerden anlamlı olarak düşük bulundu (p<0.0001). Diyabet oluşturulan sıçanlarda deformabilite indeksi anlamlı düzeyde yüksek bulundu (p<0.0001). Eritrosit deformabilitesi DR-96S grubunda Kontrol ve DK gruplarındakinden anlamlı olarak yüksek bulundu (p<0.0001 ve p=0.028).

Sonuç: Bu çalışmada diyabetik sıçanlarda düşük doz sugammadeksin güvenli olduğunu gösterdik. Çalışmamazın sonuçları sugammadeksin eritrosit deformabilitesi ve mikro/makrosirkülasyon üzerindeki etkilerini araştıracak insan ve hayvan çalışmaları için yol gösterici olabilir.

Anahtar Sözcükler: Eritrosit deformabilitesi, diabet, sugammadeks, mikrosirkülasyon

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Address for Correspondence / Yazışma Adresi : Mustafa Arslan, MD, Gazi University Medical Faculty, Department of Anaesthesiology and Reanimation, 06510 Ankara, Turkey, Tel: 90 312 202 67 39 E-mail: marslan36@yahoo.com mustarslan@gmail.com

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INTRODUCTION

Erythrocyte deformability is an essential function that facilitates erythrocyte movements in micro and macro vessels and results in rapid and sufficient gas and metabolic product exchanges in vital microcirculation (1,2). Several internal factors include cell shape and geometry (diameter -8 micrometer-, average membrane surface area -135 micrometer square- and mean cell volume 90 micrometer cube), skeleton proteins, membrane elements and hemoglobine concentration as important deformative feature of erythrocyte (1,3). Diabetes mellitus is a metabolic disorder characterized with high plasma glucose levels due to either the impaired insulin production or the elevated insulin resistance at target tissues. Functional and structural impairements occur in the erythrocyte membrane and intracellular elements secondary to the diabetes-specific events (4,5). Diabetes-related oxidative stress leads alterations in endoplasmic reticulum and protein synthesis (6-8). This process results in the pathological protein production (9). Other important pathological changes are alterations in membran lipid ratio, glicolisation in skeletal proteins, alterations in several enzyme functions (10,11). Additionally plasma levels of fibrinogen, albumin change and a tendency towards platelet and erythrocyte aggregation emerges (12-14). This multifactorial process results in the decreased erythrocyte deformability, which leads to circulation and nutritional impairements. Tissue and organ ischemia is the end point of the disease process.

Sugammadex is a new agent used for the rapid reversal of steroid-structured non-depolarizan muscle relaxants' (NDMR) (primarily rocuronium and in a less extent vecuronium) effect in tissue (15). Safety profile and use of sugammadex at 2 $mg.kg^{-1}$ and 4 $mg.kg^{-1}$ has been shown (15). Reported side effects of sugammadex include non-spesific hypotension, cough and with ongoing debate- anaphylaxis (16). Additionaly, several studies suggested polonged clotting time with sugammadex although a recent study showed no significant difference between measured clotting time values of patients treated with sugammadex and controls (17). In this study we investigated the effect of the low and high dose sugammadex on the erythrocyte deformability of streptozotocin-induced diabetic rats.

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 225 and 300 g, raised under the same environmental conditions, were used. For at least one week prior to the 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.

Diabetes was induced by a single IP injection of streptozotocin (Sigma Chemical, St. Louis, MO, USA) at a dose of 55 $mg.kg^{-1}$ body weight. The blood glucose levels were measured 72 h after this injection. The rats were classified as diabetic if their fasting blood glucose (FBG) levels exceeded 250 $mg.dl^{-1}$, and only animals with FBG levels > 250 $mg.dl^{-1}$ were included in the diabetic groups. The rats were kept alive for four weeks after the streptozotocin injection to allow the development of chronic diabetes (18).

At the end of the 30-day period, 100 $mg.kg^{-1}$ ketamine was administered intraperitoneally and tracheostomy was performed in all animals at a supine position via 12G cannula. Intubation was performed on all animal. Eusophagus of animals were strictly protected when tracheostomy was performed. Intubation cannula was fixed carefully. Ventilation was maintained with ventilatory machine at the room air. The rate was 70-100/min. The tidal volume was 2.5-3 ml, and PEEP was 2 mmHg.

There were four experimental groups: Group C (control; *n*=6), Group DC (diabetes control; *n*=6), Group DR-16S (diabetes-rocuronium-16mg sugammadex; *n*=6) and Group DR-96S (diabetes- rocuronium-96mg sugammadex; *n*=6). Rats in DR-16S ve DR-96S groups were received a single dose of sugammadex either at 16 or 96 $mg.kg^{-1}$ intravenously. Rats in control and diabetes groups received 0.9% NaCl at the same volume. Following the reversal of the muscle relexant effect of rocuronium, rats were allowed to breathe spontaneously.

Anaesthesia was maintained by repetitive injections of 20 $mg.kg^{-1}$ ketamine if a positive reaction to surgical stress or intermittent tail pinch could be observed.

After the two hour of the follow-up period, intracardiac blood samples were obtained from all rats. Heparinized total blood samples were used to prepare erythrocyte packs. Deformability measurements were performed using erythrocyte suspensions with 5% haematocrit in a phosphate-buffered saline (PBS) buffer.

Deformability Measurements

Blood samples were carefully taken, and the measurement process was carried out as fast as possible to avoid the 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 22º C.

A constant-current filtrometer 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 nucleoporin polycarbonate filter with a 5 µm pore diameter was preferred. Pressure changes were detected by a pressure transducer while the erythrocytes passed through the filter, and the data were transferred to a 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. The pressure calibration of the system was performed before each sample measurement. The buffer (P_T) and the erythrocytes (P_E) 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 (18).

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA) 12.0 program was used for the statistical analysis. Variations in blood glucose levels, erythrocyte deformability and rat weights between the study groups were assessed using the Kruskal–Wallis test. The Bonferroni-adjusted Mann–Whitney *U* test was used if the results of the Kruskal–Wallis 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 for all the analyses and $p<0.033$ (0.1/3) for the Bonferroni-adjusted Mann–Whitney *U* test.

RESULTS

Blood glucose measurements were 85.3±11.4, 335.1±33.2, 328.0±43.5 and 318.5±38.8 mg/dL for Group C, DC, DR-16S and DR-96S, respectively. Serum glucose was significantly lower in Group C as compared to Groups DC, DR-16S and DR-96S ($p<0.0001$).

The deformability index was significantly increased in the diabetic rats ($p<0.0001$). However, it was similar in Group DC and DR-16S ($p=0.152$), Group DR-16S and DR-96S ($p=0.393$). It was significantly increased in Group DR-96S as compared to Group C and DC ($p<0.0001$, $p=0.028$, respectively) (Figure 1). The relative resistance was increased in the high dose sugammadex aplication.

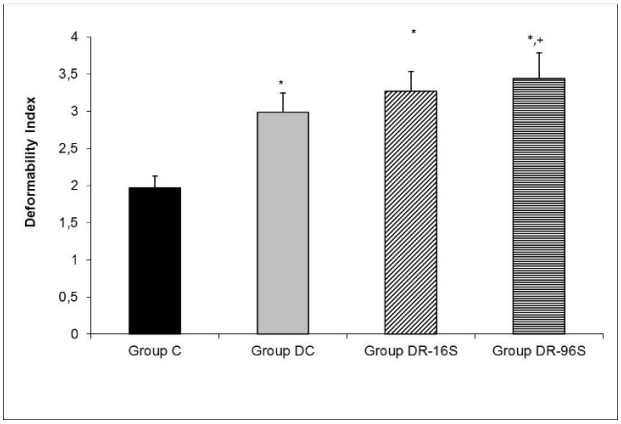

Figure 1: Erythrocyte deformability values of the groups. Each bar represents the mean ± sd. * p<0.05 compared to Group C; +p< 0.05 compared to Group DC

DISCUSSION

Erythrocytes are fundamental elements of blood. They are responsible for vital functions such as oxygen/carbon dioxide and metabolic product exchange. Changes in erythrocyte shape and geometry generate the most important proportion of flow resistance. Erythrocyte deformability, aggregation, plasma fibrinogen and globulins have a key role in maintaining a sufficient flow pattern (19). Biochemical alterations in plasma and erythrocytes that are seen in diabetes directly affect cell structures and functions (19). The elevated glucose level increases HbA1c level. The elevated HbA1c, in turn, result in decreased erythrocyte deformability. It has been shown that at a 50 mmol/l level of plasma glucose, the erythrocyte deformability decreases significantly (10, 20). In our study we used deformability index as the indicator of erythrocyte deformability. Deformability index is in inverse relationship with the erythrocyte deformability. We detected an increase in deformability index in diabetic (mean plasma glucose level was 300 mmol/l) rats in line with previous studies (18,21).

In diabetics high glucagon levels have been identified. With elevated malondialdehyde and decreased gluthation peroxidase levels, high glucagon levels trigger an oxidative stress cascade (7). Even for a short interval, the oxidative stress permanently damages erythrocyte membran proteins (4). The cholesterol phospholipid ratio of cell membrane is an important factor in maintaining deformability. Phospholipid levels increase cholesterol levels four fold in diabetes (4). This allows for a more rigid membrane structure and a decrease in deformability. Erythrocytes with a rigid membran structure can be easily distorted in microcirculation. Consequently, reticulocyte counts increase two fold and old erythrocyte counts decrease in 1/4 (22). Another negative effect of high glucose levels is glycosylation of skeletal proteins such as beta spectrin, ankyrin and protein 4.1. Glycosylation results in decreased viscoelastic properties of erythrocytes (10).

Cellular Na/K ATPase levels decrease and intracellular Na and extracellular Na/K increases in diabetes (11). These metabolic alterations result in the accelerated cellular aging and the increased cellular fragility (23). This process may be responsible for microcirculatory impairements seen in diabetic patients.

Erythrocyte aggregation with erythrocyte deformability leads micro vascular and cardiovascular complications of diabetes (13). Anionic charge in cell membranes decreases in diabetes, resulting in erythrocyte aggregation. Erythrocyte aggregation facilitates intra vascular acidosis and plathelet aggregation (13,14). In combination with the decreased erythrocyte deformability, erythrocyte aggregation complicates disease outcomes.

In our study, we found that a high dose of sugammadex is related to the impaired erythrocyte deformability while there was no difference between erythrocyte deformability index of rats treated with a low dose of sugammadex and the non-treated (control) group.

CONCLUSION

According to our study results it can be stated that the low-dose sugammadex produces no harmful effect on erythrocyte deformability in diabetic rats. This is the first study in the literature investigating the effects of sugammadex on erythrocyte deformability of diabetic rats. Future human studies may provide a better insight into the sugammadex effect on human erythrocyte deformability and microcirculation.

Conflict of Interest

No conflict of interest was declared by the authors.

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