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EXPERIMENTAL STUDY

Effects of dexmedetomidine and thymoquinone on erythrocyte deformability in lower limb ischemia reperfusion injury in streptozotocin-induced diabetic rats

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ABSTRACT

OBJECTIVE: In this study we aimed to evaluate the effect of dexmedetomidine and thymoquinone on erythrocyte deformability in lower limb ischaemia-reperfusion (IR) injury in streptozotocin-induced diabetic rats.

MATERIAL AND METHODS: Thirty Wistar albino rats were equally divided into 5 groups (n = 6); randomized control group (Group C), diabetes control group (Group DC), DIR group (Group DIR), DIR group with thymoquinone 25 mg.kg^{-1} intraperitoneally (Group DIRT) and Group DIR with dexmedetomidine 100 μg.kg^{-1} intraperitoneally (Group DIRD). Erythrocyte packs were prepared from heparinized blood samples and deformability measurements were performed.

RESULTS: IR significantly increased the relative resistance, a marker of erythrocyte deformability when compared to control group ($p < 0.05$). There were significant differences among the groups in comparisons with ANOVA test ($p < 0.0001$). Comparisons of the groups DIRD and DIRT revealed similar results ($p = 0.824$). The values of Group DIR were significantly higher than those of the control, DC, DIRD and DIRT groups ($p < 0.0001$, $p = 0.001$, $p = 0.004$, $p = 0.002$, respectively). The values of the DC, DIR, DIRD and DIRT groups were significantly higher than those of the control group ($p < 0.0001$, all).

CONCLUSION: Erythrocyte deformability may cause more problems in microcirculation. Dexmedetomidine and thymoquinone may be useful in reducing the adverse effects of this type of injury *(Fig. 1, Ref. 41).*

KEY WORDS: erythrocyte deformability, lower limb ischemia reperfusion injury, dexmedetomidine, thymoquinone, diabetes, rat.

Introduction

Ischemia is defined as blood supply reduction to a tissue. Thus, oxygen and supply of nutrients decrease (1, 2). Reperfusion induces inflammation and causes remote organ injury (3). Ischemia/reperfusion (IR) is more harmful than single ischemia (4).

Oxidative stress plays a main role in the etiology of both diabetic complications and IR injury (5, 6). In diabetes, oxidation of glucose and glycosylation of proteins cause the produce of free oxygen radicals and these radicals also play the main role in IR injury (5, 6). 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) (7). Optimal erythrocyte deformability is essential for normal circulation as RBCs change shape to get through narrow capillaries or to reduce blood viscosity (8).

Dexmedetomidine is a selective α-2 adrenoceptor agonist agent. US Food and Drug Administration approved dexmedetomidine in 1999 as a sedative drug to use for patients in intensive care units. Also, it has indications for regional and general anesthesia, too (9, 10). It was previously shown that dexmedetomidine has protective effect on renal, focal cerebral, cardiac, testicular, and tourniquet-induced IR injury (11–16). Arslan et al reported that dexmedetomidine has protective effect on hepatic IR injury (15). Also Si et al reported that dexmedetomidine treatment weakens the renal IR injury by inactivating JAK/STAT signaling pathway (16).

Thymoquinone (TQ) is Nigella sativa (NS)'s main active ingredient. It is generally called as black cumin or black seed. Black seed is an annual flowering plant native to some areas like Mediterranean countries (17). 1963 was the year thymoquinone was first extracted as the main active ingredient of NS (18) and it was described as a potent superoxide scavenger and free radical (19–21). However, NS also has an antioxidative effect on the spinal cord, heart and renal tissue IR injury (22–24).

We evaluated the protective effects of dexmedetomidine and thymoquinone on erythrocyte deformability in lower limb IR injury in streptozotocin-induced diabetic rats.

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Materials and methods

Animals and experimental protocol

After the approval of the Experimental Animals Ethics Committee of Gazi University the study was carried out in the GUDAM Laboratory of Gazi University. All employed methods were in agreement with approved basics of the Guide for the Care and Use of Laboratory Animals. In the study, 30 male Wistar albino rats weighing between 250 and 300 g, raised under the same environmental conditions, were used. At least one week before the surgery in a pathogen free environment we housed the animals in standard cages. During this time they were free to access food (until 2 h earlier than the procedure of anesthesia) and water. Under 12 h dark-light cycle and the animals were seperated into four groups of six rats randomly. The animals were randomly separated into five groups, each containing six rats. IP 100 $mg.kg^{-1}$ ketamine was used for the anesthesia of rats. 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.

Diabetes was induced by a single injection of streptozotocin (Sigma Chemical, St. Louis, MO, USA), at a dose of 55 mg/kg (i.p) body weight. 72 hours afeter the injection the blood glucose levels were measured. Rats were classified as diabetic if their fasting blood glucose (FBG) levels exceeded 250 mg/dl, and only animals with FBGs of > 250 mg/dl were included in the diabetic groups (diabetes, diabetes+ischemia-reperfusion diabetes+thymoquinone-ischemia-reperfusion and diabetes+dexdetomidine-ischemia-reperfusion). The rats were kept alive for four weeks after streptozotocin injection to allow the development of chronic diabetes before they were exposed to IR.

The animals were randomly separated into five groups, each containing 6 rats. Midline laparatomy was done under ketamine anesthesia.

Control group (Group C): Midline laparotomy was done alone without any additional surgical intervention. Blood sample was collected after 4 hours of follow-up and animals were sacrificed eventually.

Diabetes-Control group (Group DC): Midline laparotomy was done alone without any additional surgical intervention. Blood sample was collected after 4 hours of follow-up and animals were sacrificed eventually.

Diabetes-Ischemia-reperfusion group (Group DI/R): Midline laparotomy was done similarly. Infrarenal segment of the aorta was clamped for 2 hours. After removing the clamp, reperfusion was established for another 2 hours. Finally, rats were sacrificed after collecting blood samples from their abdominal aorta.

Diabetes-Ischemia-reperfusion group with dexmedetomidine (Group DI/R-D): Similar steps were followed but in addition to the procedure mentioned above, cerium oxide was given (Precedex 100 µg/2 ml, Abbott, Abbott Laboratory, North Chicago, Illinois, USA 100 $\mu g.kg^{-1}$) intraperitoneally 30 minutes before the ischemia period. Rats were sacrificed at the end of reperfusion period which lasted 2 hours after collecting blood samples.

Diabetes-Ischemia-reperfusion group with thymoquinone (Group DI/R-T): Similar steps were followed but in addition to the procedure mentioned above, cerium oxide was given (Tymoquinone 1G, Sigma Aldrich 25 $mg.kg^{-1}$) intraperitoneally 30 minutes before the ischemia period. Rats were sacrificed at the end of reperfusion period which lasted 2 hours after collecting blood samples.

All the rats were given ketamine 100 $mg.kg^{-1}$ intraperitoneally and intraabdominal blood samples were obtained. Heparinized total blood samples were used to prepare erythrocyte packs. Deformability measurements were performed using erythrocyte suspensions with 5 % hematocrit in 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 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 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_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.

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 $p < 0.05$.

Results

The results of the study indicated that IR significantly increased the relative resistance, a marker of erythrocyte deformability when

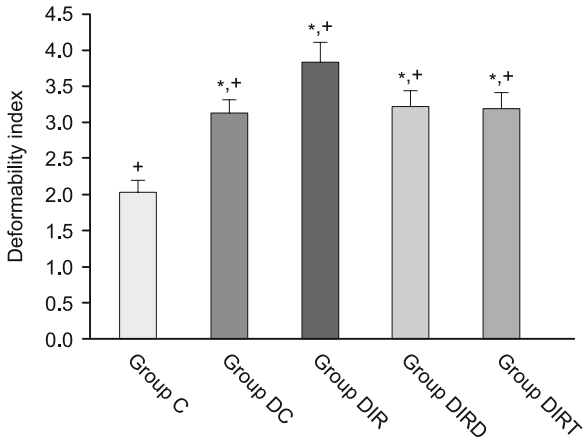

Fig 1. Erythrocyte deformability index values of thegroups. Each bar represents the mean ± SD. * p < 0.05 compared to the Group C, + p < 0.05 compared to the Group DIR.

compared to control group ($p < 0.05$) (Fig. 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 DIRD and DIRT groups revealed similar results ($p = 0.824$). The values of the DIR group were significantly higher than those of the control, DC, DIRD and DIRT groups ($p < 0.0001$, $p = 0.001$, $p = 0.004$, $p = 0.002$, respectively). The values of the DC, DIR, DIRD and DIRT groups were significantly higher than those of the control group ($p < 0.0001$, all).

Discussion

In this study, we have reported the protective effect of dexmedetomidine and thymoquinone on erythrocyte deformability in experimental lower limb IR injury in streptozotocin-induced diabetic rats. Besides, relative resistance was significantly higher in all the groups compared to the control group ($p < 0.05$) and differences between the groups were also significant according to the comparisons with ANOVA test ($p < 0.0001$).

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 (5). IR injury causes lipid peroxidation and a complex variety of products ocur and this production causes local and systemic toxic and mutagenic effects (25). 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 (26). 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' (27). When equilibrium in free radical production and antioxidant defense system is disrupted oxidative damages occur (28). 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 (29).

Hemorheological parameters like hematocrit, plasma proteins, erythrocyte aggregation, and erythrocyte deformability are often disturbed in Diabetes mellitus (30). Barnes et al (31) reported that erythrocyte deformability was lower in the 14 diabetes patients with the most extensive micro-angiopathy than in the controls or the 22 diabetes patients with slight or no complications. They suggested that hyperviscosity and reduced erythrocyte deformability may be important and potentially treatable factors in the aetiology or progression of microcirculatory disease in diabetes. Similar to these previous studies, we also found that erythrocyte deformability was decreased in diabetes induced rats.

Dexmedetomidine which is a potent α-2 agonist has sedative, hypnotic properties. It is also important in prevention of renal, focal, cerebral, cardiac, testicular and tourniquet-induced IR injury (11–16). In this study; we have shown that dexmedetomidine has a protective effect on erythrocyte deformability in lower limb IR injury in diabetic rats similar to TQ induced group ($p = 0.824$).

Hosseinzadeh et al showed that TQ has protective effects on lipid peroxidation after IR injury in rat hippocampus (32). In various experimental studies, TQ's protective effects including antioxidant and free radical scavenging activity were shown (19-21). Additionally, TQ inhibits the some inflammatory mediators' production (33–39). NS seed oil treatment was shown to reduce renal histopathological score, improve renal function and serum and tissue anti-oxidative parameters following IR injury by Bayrak et al (40) In this study; we have shown that TQ has a protective effect on erythrocyte deformability in lower limb IR injury in diabetic rats similar to dexmedetomidine induced group.

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 (29, 41).

As a conclusion the results of this study clearly demonstrate that erythrocyte deformability is significantly altered in experimental lower limb IR injury in streptozotocin-induced diabetic rats. In addition, dexmedetomidine and TQ was observed to protect against these alterations in lower limb IR injury in streptozotocin-induced diabetic rats, when given before induction of ischemia. Other aspects of these findings including clinical significance and practical applications, merit further experimental and clinical investigation.

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