EXPERIMENTAL STUDY

Investigation of the effects of propofol and vitamin C administration on erythrocyte deformability in rats with streptozotocin-induced diabetes mellitus

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Abstract: Purpose: In the current study we aim to investigate the effects of vitamin C and propofol on red blood cell deformability in diabetic rats

Materials and methods: Twenty-eight Wistar Albino rats were included in the study after streptozocin (60 mg/kg) treatment for 4 weeks of observation for diabetes presence. Twenty-eight rats were allocated to 4 groups. In group DP (n = 7) 150 mg.kg⁻¹ of propofol was injected intraperitoneally. In group DP-vit C (n = 7) rats 100 mg/kg of vitamin C (Ascorbic acid, Redoxon® 1000 mg/5 mL - Roche) were applied one hour before administering 150 mg.kg⁻¹ of propofol, while rats in control group (n = 7), and diabetic control group (n = 7) received intraperitoneally physiological saline. Deformability measurements were achieved by using erythrocyte suspensions with hematocrit level of 5 % in PBS buffer.

Results: Erythrocyte deformability was significantly higher in diabetic control group than in control and vitamin C plus propofol groups (p = 0.00, p = 0.025, respectively). Erythrocyte deformability indexes were found similar in control group and vitamin C plus propofol group (p = 0.949). Relative resistance was increased in diabetic rat model. Conclusions: Erythrocyte deformability was damaged in rats with diabetes. This injury might lead to further problems in microcirculation. Application of propofol did not alter red cell deformability in diabetic rats. Vitamin C supplementation seems to reverse those negative effects and variations in erythrocyte deformability (Fig. 2, Ref. 57).

Key words: erythrocyte deformability, propofol experimental diabet, vitamin C, rat.
jugular venous oxygen saturation (20), induce haemolysis (21), and cause the so-called propofol infusion syndrome, an often fatal cardiac failure (22).

The effect of propofol on insulin secretion is not known. It is well known that diabetic patients have a reduced ability to clear lipids from the circulation (23). Alterations in the pharmacokinetics or pharmacodynamics of propofol in rats with DM were reported in an experimental study (24). There is no data showing whether administration of propofol can alter lipid clearance from the circulation. An experimental study showed that propofol impairs diastolic left ventricular filling in experimental models and produces negative lusitropic effects in diabetic cardiomyocytes (25).

In earlier studies with various methodologies, propofol has been shown to cause OS (oxidative stress), not to affect OS, or to have antioxidant effect (26–29). In in vitro studies, it has been shown to inhibit lipid peroxidation induced by oxidative stress in the liver microsomes, mitochondria, and brain synaptosomes (30). The products that arise due to lipid peroxidation associated with increased oxidative stress significantly affect membrane permeability and microviscosity, thus diminishing the deformability capacity and survival of the erythrocytes (31).

We hypothesized that propofol and vitamin C and/or the emulsifier might do this through a direct action on the biophysical or functional properties of the blood constituents such as the diabetic rat red blood cell (RBC) deformability.

In this study, the effects of propofol anesthesia and administration of vitamin C before application of propofol on the red blood cell deformability of diabetes male rats were evaluated.

Materials and methods

Animals and experimental protocol

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

In the study, 28 male Wistar Albino rats weighing between 250 and 300 g, raised under the same environmental conditions, were used. The rats were kept under 20–21 °C at cycles of 12-hour daylight and 12-hour darkness and had free access to food until 2 hours before the anesthesia procedure. The animals were randomly separated into four groups, each containing 7 rats.

Diabetes was induced by a single intraperitoneal injection of streptozotocin (Sigma Chemical, St. Louis, MO, USA) at a dose of 60 mg.kg⁻¹ body weight. The blood glucose levels were measured at 72 h following this injection. Rats were classified as diabetic if their fasting blood glucose (FBG) levels exceeded 250 mg.dl⁻¹, and only animals with FBGs of > 250 mg.dl⁻¹ were included in the diabetic groups (diabetes only, diabetes plus propofol and diabetes plus vitamin C after propofol). The rats were kept alive 4 weeks after streptozotocin injection to allow development of chronic diabetes before they were exposed to propofol (32).

Rats were anesthetized with intraperitoneal ketamine 100 mg.kg⁻¹. The chest and abdomen were shaved and each animal was fixed in a supine position on the operating table. The abdomen was cleaned with 1 % polyvinyl iodine and when dry, the operating field was covered with a sterile drape and median laparotomy was performed. Twenty-six rats were allocated to 4 groups. In group DP (n = 6) 150 mg.kg⁻¹ of propofol (Propofol 1 % Fresenius 20 mL) was injected intraperitoneally. In group DP plus vitamin C (n = 7) rats were given 100 mg.kg⁻¹ of vitamin C (Ascorbic acid, Redoxon® 1000 mg/5 mL, Roche) 30 minutes before administering 150 mg.kg⁻¹ of propofol, while rats in control (n = 7) and diabetic control groups (n = 6) received intraperitoneally physiologically saline.

Thirty minutes after propofol administration, all rats received ketamin 100 mg.kg⁻¹ intraperitoneally and were euthanized to collect blood samples from vessels in the abdominal cavity. Heparinized total blood samples were used to prepare erythrocyte packs. Deformability measurements were done by erythrocyte suspensions with 5 % htc in phosphate buffered saline buffer.

Deformability measurements

Blood samples were taken very carefully and measurement process was as fast as possible to avoid hemolysis of erythrocytes. The collected blood was centrifuged at 1000 rpm for ten minutes. Serum and buffy coat on erythrocytes were removed. Isotonic PBS buffer was added to collapsing erythrocytes and this was centrifuged at 1000 rpm for ten minutes. Liquid on the upper surface was removed. Finally, pure red cell packs were obtained from the washing process which was repeated three times. Erythrocytes packs were mixed with PBS buffer to generate a suspension with the value of 5 % Htc. Those erythrocyte suspensions were used for the measurement of deformability. Collection and deformability measurements of erythrocytes were done at 22 °C.

The constant-current filtreometer system was used for measurement of erythrocytes deformability. Samples to be measured were prepared as 10 ml of erythrocytes 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. Consisting pressure changes while the erythroctes were passing through from the filter were detected by the pressure transducer and the data was transferred to computer with the help of MP 30 data equation systems (Biopac Systems Inc, Commt, USA). The necessary calculations were performed with related computer programs by measuring the pressure changes at various times. Pressure calibration of the system was performed each time before measuring the samples. First the buffer(P₀) and then the erythroctes (Pₑ) were passed through from the filtration system and the changes in pressure were measured. The relative refractory period value(Rrel) was calculated by relating the pressure value of erythroctes suspension to pressure value of buffer. Increasing in Rrel as the deformability index was interpreted as adversely affecting the ability of erythroctes deformability (33, 34).

Statistical analysis

Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA) 17.0 program was used for statistical analysis. Variations in blood glucose level and erythrocyte deformability were assessed by
using Kruskal-Wallis test. Bonferroni adjusted Mann-Whitney U test was used after significant Kruskal-Wallis to determine which group differs from the other. Results were expressed as mean± standard deviation (Mean ± SD). Statistical significance was set at a p value <0.05.

Results

During the study period 2 of the diabetic rats died (1 in Group DC, 1 in Group DP).

Blood glucose measurements were 83.7 ± 9.1, 374.5 ± 68.6, 375.3 ± 86.7, 368.5 ± 71.3 mg/dL for Group C, DC, DP and DP plus vitamin C, respectively (Fig. 1). Serum glucose was detected to be significantly lower in Group C when compared to Groups DC, DP and DP plus vitamin C (p = 0.001, p = 0.002, p = 0.001, respectively).

Erythrocyte deformability was significantly higher in diabetic control group when compared with the control group and group propofol plus vitamin C (p = 0.002, p = 0.025 respectively). Erythrocyte deformability index was similar in control group and group propofol plus vitamin C (p = 0.949). Also erythrocyte deformability index was found similar in diabetic control group and diabetic propofol group (p = 0.650). However, relative resistance was increased in diabetic group (Fig. 2).

Conclusion

Hemorheological parameters, such as; hematocrit, plasma proteins, erythrocyte aggregation, and erythrocyte deformability are often disturbed in DM (35). Altered erythrocyte deformability not only changes the oxygen delivery capacity of the erythrocytes but also the survival of the circulating erythrocytes (36–38).

Additionally, it has been suggested that the impaired perfusion at the tissue level observed as a complication of diabetes mellitus is primarily due to the reduced erythrocyte deformability (39,40). Besides, metabolic changes and tissue perfusion due to cardiovascular problems may lead to inadequate recovery in plasma viscosity (41).

Barnes et al (42) showed that erythrocyte deformability was lower in the 14 diabetes patients with the most extensive microangiopathy than in 22 diabetes patients with slight or no complications or in controls. They suggested that hyperviscosity and reduced erythrocyte deformability might be important and potentially treatable factors in the etiology or progression of microcirculatory disease in diabetes. Similar to the previous studies, we also found that erythrocyte deformability was decreased in diabetes induced rats.

There are various procedures to measure erythrocyte deformability. The two of the prominent techniques for this measurement are measuring either change in optical diffraction pattern (ektactometry) of erythrocytes or erythrocyte filtration through membrane. In the first technique the diffraction pattern of erythrocytes changes from circular to elliptic form during stationary flow conditions in rhescope or microchannel (43). The erythrocyte filtration through membrane technique is based on the measurement of passage time of erythrocyte suspension through microscope membrane, which is reciprocal to the erythrocyte deformability (43). For better correlation of this measurement the applied pressure should be comparable to that in microcirculation, below 10 Pa. As erythrocytes flowing under low pressure may block the membrane pores, a low hematocrit (less than 10 %) is preferable. The initial flow method, which minimizes the influence of gravitational field by operating within the specified range of applied pressure, has been used to measure erythrocyte deformability under varied conditions (43, 44). The deformability is also measured from the change in erythrocyte count before and after filtration through a membrane under gravitational field (44). Another historical measurement is determining the volume of RBCs (VRBC) filtered per minute through approximately 5 μm pore-size filters. The VRBC was found to be significantly reduced in diabetes patients compared with healthy controls (14).

In our study we used constant flow filtration technique for determining erythrocyte deformability. The filtration technique measurement shows that the erythrocyte deformability is significantly decreased (44–47). Similar decrease in deformability by ektaactometry (48) and transparent microchannels were observed (49).

Erythrocytes are very sensitive to oxidative injury (50). To defend themselves against oxidative stress (OS), erythrocytes are equipped with an effective and complex antioxidant system,
including protective enzymes and biological antioxidants such as superoxide dismutase, catalase, glutathione peroxidase, glutathione, vitamin C, and vitamin E (51).

Among the potential antioxidants in foods, vitamin C and vitamin E are the principal dietary antioxidants that protect erythrocytes from damage caused by reactive oxygen species. Non-enzymatic antioxidants such as vitamin E, vitamin C and L-carnitine act towards reducing the OS (52).

Inhalation and intravenous anesthetic agents are known to affect cardiovascular functions and microcirculation and ongoing studies are investigating the issue. Yesilkaya et al (53) have found that halothane and pentobarbital impair erythrocyte deformability. Yerer et al (54) investigated the effects of desflurane on erythrocyte deformability and found that it impaired the deformability in young and old rats. Aydogan et al (55) showed negative effects of sevoflurane on the deformability of the old rats.

Kim et al (56) showed that verapamil and ascorbic acid have protective effect against tert-butyl hydroperoxide induced oxidative stress. They found that ascorbic acid reverses the effects of tert-butyl hydroperoxide and improves deformability of erythrocytes to the values of non tert-butyl hydroperoxide treated groups'. In addition our previous studies results showed that high dose of Dexametomidine impaired erythrocyte deformability and administration of vitamin C given 1 hour before dexametomidine reversed these negative effects and improved erythrocyte deformability (57).

Our results showed that diabetes mellitus impairs erythrocyte deformability and administration of vitamin C given 30 minutes before propofol reverses this negative effects and improves erythrocyte deformability. Dikmen et al (29) have reported that at clinical doses, propofol andremifentanil have no effect on oxidative stress, and sevoflurane can protect erythrocytes against oxidative stress. In our previous study (33), propofol was found to impair the erythrocyte deformability in both genders, but it was more pronounced in the male rats. This may be accounted for general protective effects of estrogen in female rats.

Erythrocyte deformability was impaired in diabetic rats. These impairments may cause further problems in microcirculations. Additionally, administration of propofol in diabetic rats was observed to have no protective effect on erythrocyte deformability index. It was observed that administration of vitamin C reversed the negative effect on erythrocyte deformability. However, our early results should be confirmed by further detailed clinical and experimental studies on the issue.

References


