

EXPERIMENTAL STUDY

Does vitamin C prevent the effects of high dose dexmedetomidine on rat erythrocyte deformability?

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Abstract: *Purpose:* Dexmedetomidine is an anesthetic agent frequently used for sedation at the intensive care units and during general anesthesia. The purpose of our study was to investigate whether vitamin C prevents the effect of high dose dexmedetomidine on erythrocyte deformability in rats.

Methods: The study was performed on 21 male rats, with 7 rats in each study groups and the control group. The rats in the study groups were treated with intraperitoneal dexmedetomidine (10 µg/kg) and intraperitoneal dexmedetomidine plus Vitamin C (ascorbic acid) (100 mg/kg ascorbic acid administered 1 hour before administration of 10 µg/kg dexmedetomidine), respectively. Intraperitoneal physiological saline was administered in the control group. Erythrocyte packs were prepared using heparinized total blood samples. Deformability measurements were done by erythrocyte suspensions in phosphate buffered saline (PBS) buffer. A constant flow filterometer system was used to measure erythrocyte deformability and the relative resistance was calculated.

Results: Erythrocyte deformability was significantly higher in dexmedetomidine group than in control and vitamin C plus dexmedetomidine groups ($p=0.003$, $p=0.013$, respectively). Erythrocyte deformability indexes were found similar in the control group and in the vitamin C plus dexmedetomidine group ($p=0.383$)

Conclusions: High dose dexmedetomidine may cause functional deterioration in blood flow and tissue perfusion with negative effects in erythrocyte deformability. Vitamin C supplementation seems to reverse those negative effects and variations in erythrocyte deformability. However, our preliminary results should be confirmed in wider serious of experimental and clinical trials (Fig. 1, Ref. 27). Full Text in PDF www.elis.sk.

Key words: erythrocyte deformability, α_2 agonist, dexmedetomidine, vitamin C, rat.

General anesthetics are known to affect cardiovascular functions and microcirculation dynamics (1). However, whether these agents change plasma rheology and/or anesthesia may result in deterioration of tissue perfusion remains controversial. Changes in plasma viscosity have been listed among the factors associated with anesthesia procedures responsible for deterioration of tissue and organ perfusion (2, 3). Erythrocyte deformability and increased aggregation may be observed after surgical procedures performed under general anesthesia (3).

Hemorheological factors are sensitive to metabolic changes and may be affected by tissue perfusion due to cardiovascular problems. Disorders in the hemorheologic state may lead to an inadequate recovery in plasma viscosity (2). Erythrocyte deformability and plasma viscosity are important factors that affect organ and tissue perfusion (4). For migration of oxygen and vital molecules to the final organ capillaries and clearance of metabolic

wastes, erythrocytes must be able to extend and curve and have the capability to move in these areas. This capacity is termed as “deformability” (5).

Erythrocytes are very sensitive to oxidative injury (6). 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 (7).

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-enzymic antioxidants such as vitamin E, vitamin C and L-carnitine act towards reducing the OS (8).

Dexmedetomidine (Dex) is highly selective α_2 -adrenoreceptor agonist recently introduced to the anaesthesia practice (9). Dexmedetomidine highly selective α_2 -adrenogenic receptor agonist, used as sedative, is an anxiolytic, analgesic, and sympatholytic drug (10).

This drug reduces blood pressure (BP) and heart rate (HR) in a dose dependent manner and has a sedative effect (11).

Its cardiovascular side effects have been described in various studies. It can decrease BP and HR (12), cause transient sinus arrest (13), severe bradycardia (13, 14), postoperative bradycardia (HR less than 40 beats per minute) (15) and a significant hypotension (16).

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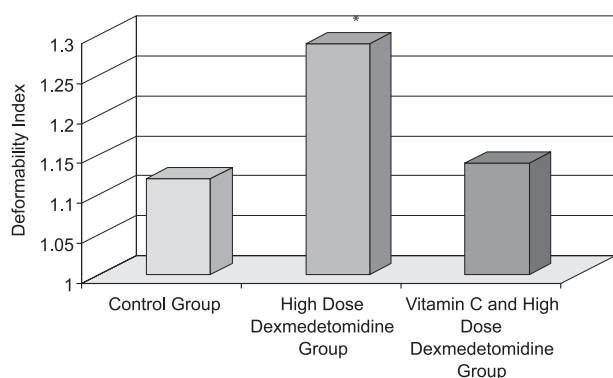


Fig. 1. Erythrocyte deformability values of the groups. * $p < 0.05$ compared to the Control and the vitamin C and high dose dexmedetomidine Groups.

We hypothesized that Dex might do this through a direct action on the biophysical or functional properties of the blood constituents such as the red blood cell (RBC) deformability.

General anesthesia, either with an inhalation or nonvolatile anesthetics, is known to affect the overall cardiovascular function as well as the microcirculatory hemodynamics. Alterations in blood rheology under the influence of anesthesia have been observed and discussed among the responsible factors for the deterioration of tissue and organ perfusion related to anesthetic procedures. Dexmedetomidine is one of the sedative, anxiolytic, analgesic and sympatholytic drug, which is widely used during general anesthesia and at the intensive care units.

In this study, the effects of high dose Dex and vitamin C on the red blood cell deformability of young male rats were evaluated.

Materials and methods

This study was conducted in the Physiology laboratory of the Kirikkale University upon the consent of the Experimental Animals Ethics Committee of Kirikkale University.

In the study, 21 male Wistar Albino rats (total number 21) of 250–300 g in weight, 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.

Twenty-one rats were allocated to 3 groups. In the group D ($n=7$), 10 $\mu\text{g}/\text{kg}$ of Dex (Precedex; Hospira, Inc., Lake Forest, IL) was injected intraperitoneally. In the group DC ($n=7$), rats were given 100 mg/kg of vitamin C (Ascorbic acid, Redoxon® 1000 mg/5 mL- Roche) one hour before administrating 10 $\mu\text{g}/\text{kg}$ of Dex, while rats in the control group ($n=7$) received intraperitoneal physiological saline.

Thirty minutes after Dex administration, all the rats were given ketamin 100 mg/kg intraperitoneally and 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.

Erythrocyte deformability was measured using a constant flow filterometer system. Erythrocyte suspension that was delivered at 1ml/min flow rate was passed through a nucleopor-polycarbonate filter of 5 μm in diameter, and alterations in the filtration pressure corresponding to different flow rates were measured. The alterations in the pressure were transferred to a 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 Analyses

The statistical analyses were performed with the SPSS 12.0 software program and $p < 0.05$ was considered statistically significant. The findings were expressed as the mean \pm standard deviation. The data were evaluated with the Kruskal-Wallis variance analysis. The variables with significance were evaluated with the Bonferroni corrected Mann-Whitney U test.

Results

The results of the study indicated that a high dose Dex significantly increased the relative resistance, a marker of erythrocyte deformability when compared to the control and vitamin C and high dose Dex groups ($p < 0.05$) (Fig. 1).

There were significant differences between the groups according to the comparisons with the Kruskal-Wallis test ($p=0.004$). The results obtained after the corrections with Mann Whitney U test were as follows: Comparisons of the control and vitamin C and high dose Dex groups revealed similar results ($p=0.383$). The values of the high dose Dex group were significantly higher than the control group and the vitamin C and high dose Dex group ($p=0.003$, $p=0.013$, respectively).

Discussion

The effects of anesthetic agents and their metabolites on hemorheologic factors may be in a direct or indirect manner. The effects of anesthetic agents on microcirculation are specific and dose dependent. The mechanisms that cause this interaction may be associated with oxidative disorders that occur during or after various anesthetic procedures (18–20).

Alterations in the erythrocyte deformability may result in poor perfusion that can contribute to vascular complications during post anesthetic period that may arise in addition to other well-known mechanisms. This may lead to inadequate recovery (19).

Erythrocyte deformability method used in this study is composed of hole of similar size to those in the capillary system, and thus, it is a reliable experimental model for microcirculation. An increase in the erythrocyte deformability index is a sign of reduced erythrocyte deformability. Erythrocytes with low deformability index have a high capacity for deformability. They easily change forms while passing through the holes, and thereby are filtered in a short time.

Dexmedetomidine is a selective α_2 -adrenergic agonist, which produces anxiolysis, amnesia, sedation, potentiation of analgesia,

and sympatholysis. α_2 receptors are found in platelets and in many other organs, including the liver, pancreas, kidney, and eye. The responses from these organs include decreased secretion, salivation, and bowel motility; increased glomerular filtration, secretion of sodium and water, and inhibition of renin release in the kidney; decreased intraocular pressure; and decreased insulin release from the pancreas. The most frequently observed adverse events include hypotension (30%), hypertension, nausea/vomiting (11%), sinus bradycardia (8%), atrial fibrillation (7%), fever, hypoxia (6%), sinus tachycardia, and anemia (3%) (21, 22).

Ayoglu et al (23) investigated the effects of 2 different doses of Dex (5 and 10 $\mu\text{g}/\text{kg}$). They administered Dex 1 and 24 hours after an acute cerebral vasospasm and evaluated oxidative stress in a rat subarachnoid hemorrhages model.

Inhalation and intravenous anesthetic agents are known to affect cardiovascular functions and microcirculation and ongoing studies investigate the issue. Yesilkaya et al (24) have found that halothane and pentobarbital impair erythrocyte deformability.

Erdogan et al (1) have shown that midazolam does not impair the erythrocyte deformability index and does not affect plasma viscosity.

Yerer et al (25) investigated the effects of desflurane on erythrocyte deformability and found that it impaired the deformability in young and old rats. Aydogan et al (26) showed the negative effects of sevoflurane on the deformability of the old rats.

In an earlier study, we found that propofol impairs the erythrocyte deformability in both genders, but more pronounced in the male rats. This may be attributed to the general protective effects of estrogen in female rats (27).

We also showed the negative effects of high dose Dex on the erythrocyte deformability of the rats (28).

Kim et al (29) showed that verapamil and ascorbic acid have protective role against tert-butyl hydroperoxide induced oxidative stress. They found that ascorbic acid reverse the effects of tert-butyl hydroperoxide and improve deformability of erythrocytes to the values of non tert-butyl hydroperoxide treated groups. Our results showed that high dose Dex impairs erythrocyte deformability and administration of vitamin C given 1 hour before Dex reverses this negative effects and improves erythrocyte deformability.

Conclusions

In conclusion, high dose Dex, a sedative, anxiolytic, analgesic and sympatholytic drug, may lead to negative alterations in the erythrocyte deformability, which may result in functional disorders in blood flow and tissue perfusion. On the other hand, vitamin C can reverse these effects.

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