

EXPERIMENTAL STUDY

The effects of propofol and memantine on erythrocyte deformability

Unal Y¹, Comu FM², Emik U¹, Alkan M³, Pampal HK¹, Arslan M¹

Department of Anaesthesiology and Reanimation, Gazi University Medical Faculty, Ankara, Turkey.
marslan36@yahoo.com

Abstract: *Objective:* Propofol is an intravenous general anesthetic with a primary hypnotic effect. Memantine is an NMDA receptor antagonist that has been shown to reverse changes in memory and synaptic plasticity in animal models. This study aims to investigate whether propofol and/or memantine has any effects on erythrocyte deformability. *Methods:* 24 Wistar albino rats were divided randomly into four groups. Group P received 150 mg.kg⁻¹ propofol intraperitoneally (ip); Group M received 1 mg.kg⁻¹ memantine (ip); Group PM received 1 mg.kg⁻¹ memantine mg.kg⁻¹ ip 30 minutes before the administration of 150 mg.kg⁻¹ propofol; and the control group (Group C) received saline ip. Euthanasia was performed in all rats by using intraabdominal blood uptake. The heparinized whole blood samples were used to prepare erythrocyte suspensions, from which erythrocyte suspensions were formed with a PBS buffer solution containing 5 % htc, and the deformability parameters were measured. *Results:* Erythrocyte deformability was significantly higher in Groups P, M and PM when compared to the Group C ($p=0.007$ and $p=0.001$, $p<0.0001$, respectively); while the erythrocyte deformability indices were similar in groups P, M and PM. *Conclusion:* The administration of propofol and memantine altered the erythrocyte deformability in the rats, which may lead to further problems in microcirculation. The administration of memantine to the propofol-treated rats did not alter the erythrocyte deformability; however the early results should be verified through further experimental and clinical studies (Fig. 1, Ref. 23). Text in PDF www.elis.sk.
Key words: propofol, memantine, erythrocyte deformability, rat.

Memantine, a competitive non-NMDA receptor antagonist is used in the treatment of many neurological disorders, including Alzheimer's disease. It is believed that memantine blocks the current through the NMDA receptors (a kind of glutamate receptor family that is widely influential in brain functions) with high concentrations of glutamate (1).

Propofol is an intravenous general anesthetic with a primary hypnotic effect. Although the exact mechanism is unknown, such as barbiturates, it is thought to act by reducing the separation of GABA from the receptors. It has been known to increase the function of the beta-1 subunit of the GABA and the inhibitory synaptic activation through the activation of the chlorine channels; and it suppresses the central nervous system by acting on both postsynaptic and presynaptic levels (2, 3). In in vitro studies, it has been shown to inhibit lipid peroxidation induced by oxidative stress in the liver microsomes, mitochondria, and brain synaptosomes (4). 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 (5).

The aim of the current study is to investigate the possible effects of memantine and/or propofol red blood cell (RBC) deformability.

Materials and methods

Animals and experimental protocol

This study was conducted in the Animal Laboratory of Gazi University upon the approval of the Experimental Animals Ethics Committee of Gazi 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, 24 female Wistar Albino rats weighing between 250 and 300 g 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 and water until 2 hours before the anesthesia procedure.

Twenty-four rats were allocated into 4 groups. In group P (n=6) 150 mg.kg⁻¹ of propofol (Propofol 1 % Fresenius 20 mL) was injected ip. In group M (n=6) 1 mg.kg⁻¹ of memantine (Memantine hydrochloride, Sigma mg.ml⁻¹) was injected ip. In group PM (n=6) rats were given 1 mg.kg⁻¹ of memantine before administering 150 mg.kg⁻¹ of propofol, while rats in Group C (control, n=6) received intraperitoneal physiological saline.

Thirty minutes after the administration of the study drugs all rats were euthanized with ip 100 mg.kg⁻¹ ketamine. Laparotomy was performed to collect blood samples from vessels in the abdominal cavity. Heparinized total blood samples were used to prepare erythrocyte packs. Deformability measurements were

¹Department of Anaesthesiology and Reanimation, Gazi University Medical Faculty, Ankara, Turkey, ²Department of Physiology, Kirikkale University Medical Faculty, Kirikkale, Turkey, and ³Gazi University, Faculty of Dentistry, Department of Oral and Maxillo Facial Surgery, Besevler, Ankara, Turkey

Address for correspondence: M. Arslan, MD, Gazi University Medical Faculty, Department of Anesthesiology and Reanimation, 06510 Ankara, Turkey.

Phone: +90.312.2026739, Fax: +90.312.2024166

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 filtrometre 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 nucleoprin polycarbonate filter with a 5 µm pore diameter was preferred. Consisting pressure changes while the erythrocytes 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, Commat, 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. Firstly buffer (P_T) and then erythrocytes (P_E) 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 erythrocytes suspension to pressure value of buffer. Increasing Rrel as the deformability index was interpreted as adversely affected ability of erythrocytes deformability (6, 7).

Statistical analysis

Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA) 17.0 program was used for statistical analysis. A variation in erythrocyte deformability was assessed by using Kruskal–Wallis test. Bonferroni adjusted Mann–Whitney U test was used to identify the significant differences between groups. Results were expressed as mean± standard deviation (mean±SD). Statistical significance was set at a p value <0.05.

Results

The results of the study indicated that propofol, memantine and propofol memantine increased the relative resistance, a marker of erythrocyte deformability of control rats ($p=0.003$) (Fig.1).

Erythrocyte deformability was significantly higher in Group P, M and PM when compared to Group C ($p=0.007$ and $p=0.001$, $p < 0.0001$, respectively), while the erythrocyte deformability indices were similar in groups administered with different drugs (Fig. 1).

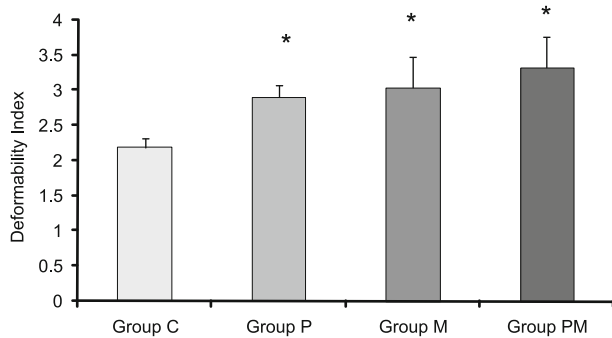


Fig. 1. Erythrocyte deformability values of the groups. Each bar represents the mean ± SD. * $p < 0.05$ compared to the Group C.

Discussion

Recent studies have indicated that the NMDA receptors have an active role in intracellular calcium haemostasis. Memantine, a synthetic NMDA receptor antagonist, prevents excessive adherence to the cells by reducing the level of intracellular calcium (8–10).

In a study of healthy volunteers, Reinhart et al (11) demonstrated that the activation of NMDA receptors by homocysteic acid or the inhibition of the receptors by memantine did not affect the rheological properties of the red blood cells, such as deformability and agreeability. Their results showed that there were no significant changes in the bloating viscosity, measured by the red blood cell deformability. It was also mentioned that minor changes in the red blood cell deformability could not be excluded, measured by methods of higher sensitivity such as Sita cytometry, RBC filtration and micropipette aspiration methods. In contrast to Reinhart et al (11), in the present study it was found that the administration of memantine changed the erythrocyte deformability; however, when applied prior to the administration of the anesthetic agent propofol, no additional negative effects were detected. This was probably due to the higher sensitivity of the method used in the measurement of erythrocyte deformability.

Reinhart et al (11) reported that memantine did not affect blood flow, although there red blood cells contain functional NMDA receptors.

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 (ektacytometry) 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 rheoscope or microchannel (12). The erythrocyte filtration through membrane technique is based on the measurement of passage time of erythrocyte suspension through microscope membrane, which is reciprocal of the erythrocyte deformability (12). For better correlation of this measurement the applied pressure should be comparable to that as 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 (12, 13). The deformability is also measured from the change in erythrocyte count before and after filtration through a membrane under gravitational field (13). 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 (13–17). Similar decrease in deformability by ektacytometry (18) and transparent microchannels has been observed (19).

Inhalation and intravenous anesthetic agents are known to affect cardiovascular functions and microcirculation and ongoing studies are investigating the issue. Yesilkaya et al (20) have found that halothane and pentobarbital impair erythrocyte deformability.

Yerer et al (21) investigated the effects of desflurane on erythrocyte deformability and found that it impaired the deformability in young and old rats. Aydogan et al (22) showed the negative effects of sevoflurane on the deformability of the old rats.

The results of the present study show that propofol and memantine impair erythrocyte deformability, and that the administration of memantine prior to administering propofol does not have any additional negative effects.

Dikmen et al (23) have reported that propofol/remifentanyl has no effect on oxidative stress at therapeutic doses. In contrast they showed that sevoflurane has protective effects on erythrocytes against oxidative stress.

In our previous study (6), propofol was found to impair the erythrocyte deformability of both genders, but it was more pronounced in the male rats. This may be accounted for by general protective effects of estrogen in female rats.

The administration of propofol and memantine alters erythrocyte deformability in rats, which is a disturbance that can lead to further problems in microcirculation. The administration of memantine to propofol-treated rats did not alter the erythrocyte deformability, however these early results should be verified through further experimental and clinical studies.

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