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ORIGINAL RESEARCH **ANESTHESIOLOGY**

The effects of fullerenol nanoparticles on erythrocyte deformability in sevoflurane applied rats

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Abstract

Background & objective: Oxidative damage causing alterations in erythrocyte deformability due to anesthesia might be one of the factors responsible for the deterioration of the tissue and organ perfusion. The antioxidant properties of fullerenol nanoparticles, used in medical field together with the developing technology, have been shown in the literature. We investigated the effects of fullerenol nanoparticles, used before sevoflurane anesthesia, on the erythrocyte deformability in the rats.

Methodology: Twenty-four male Wistar Albino rats were used in this study and randomly divided into four groups, six in each group; Group C (Control Group), Group S (Group Sevoflurane), Group F (Group Fullerenol), Group FS (Group Fullerenol-Sevoflurane). Fullerenol was given to the Group F and Group FS at a dose of 100 mg/kg. Sevoflurane was administered to rats in the Groups S and FS at 3% concentration. Erytrocytes deformability was measured by the constant-current filtrometre system and the deformability index was interpreted. All the data were processed by variance analysis in SPSS 22.0 for Windows statistical software. Variance analysis and Kruskal-Wallis test were used to evaluate the data. Mann–Whitney U test with Bonferroni correction were used to evaluate the variables with significance.

Results: Relative resistance increased in all groups due to sevoflurane administration ($p < 0.0001$). The erythrocyte deformability index was significantly higher in the sevoflurane group than in the control and fullerenol groups ($p < 0.0001$, $p = 0.002$, respectively). Fullerenol administration, before 30 min of sevoflurane treatment, was found to decrease erythrocyte deformability index significantly compared to sevoflurane group ($p = 0.017$).

Conclusion: Administring 100 mg/kg fullerenol nanoparticles intraperitoneally 30 min before sevoflurane reduces erythrocyte deformability.

Key words: Animals; Rats; Sevoflurane; Fullerenol; Fullerenes* / pharmacology; Nanoparticles; Oxidative Stress; Erythrocyte deformability

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1. Introduction

Hemorheology examines the behavior of intracellular cells and plasma elements such as erythrocytes, leukocytes and platelets, and their effects on blood flow. Hematocrit (Hct), blood and plasma viscosity, erythrocyte deformability and erythrocyte aggregation are used during this examination.[1] The biochemical and biophysical properties of erythrocytes, due to their

characteristic structures, are of great importance in terms of their functions. Spectrin protein units and other cytoskeletal proteins form the basis of their unique biconcave cell skeleton. This skeleton is responsible for the hemorheological properties of the normal erythrocytes, such as deformability and mechanical resistance to shear stress by providing stability, flexibility and elasticity.[2, 3] Erythrocytes are very sensitive to lipid peroxidation due to free radicals because erythrocyte membranes are rich in polyunsaturated fatty acids, are constantly exposed to oxygen molecules at high concentrations and contain hemoglobin capable of catalyzing lipid peroxidation.[4]

Damage to the erythrocyte membrane components leads to increased hardness/loss of flexibility and fragility, intravascular hemolysis and release of hemoglobin in the plasma,[5, 6] Hypertension, diabetes mellitus, anemia, chronic diseases that cause endothelial dysfunction, cause a decrease in red cell deformability.[7-9] In many studies, antioxidants such as quersetin, anserine, carnosine, vitamins C and E, alpha–1 acid glycoprotein and alpha tocotrienol were used to improve the reduced erythrocyte deformability caused by reactive oxidants.[4,10-14]

With the rapid development of nanotechnology in the world, nanomaterials are being used in many technological fields including mechanics, materials science, mechanical engineering, construction, electronics, optics, medicine, pharmacology, food and cosmetics.[15] Fullerenes are a group of compounds of interest due to their technical and potential medical applicability. They are promising agents for the treatment of diseases caused by free oxygen radicals as they have specific antioxidant and radical scavenging effects.[16]

General anesthesia, whether volatile or nonvolatile, may affect cardiac and vascular functions as well as microcirculation hemodynamics.[17] In many previous studies, changes in blood rheology due to anesthetic agents were considered as the cause of deterioration of tissue and organ perfusion.[18, 19]

Pharmacodynamic and pharmacokinetic properties and the lack of major side effects on different organ systems have led to the acceptance of sevoflurane as a reliable volatile anesthetic agent for clinical applications in various settings worldwide. ([20]). However, the detoriating effects of sevoflurane on erythrocyte deformability presented in recent papers, and efforts for attenuating or eliminating these effects, are still the subject of many studies. The aim of this study was to evaluate the effects of fullerenol nanoparticles on erythrocyte deformability alterations in sevoflurane treated rats.

2. Methodology

The study was conducted in the Laboratory of Experimental Animals Center (GUDAM) with the approval of Gazi University Experimental Animals Ethics Committee. All procedures were performed in accordance with the accepted standards of the Guide for the Care and Use of Laboratory Animals.

Twenty-four male Wistar Albino rats weighing between 250 and 300 g were used in this study. The rats were cared for at a room temperature of 20–21°C, with 12 h of daylight and 12 h of dark cycles, and allowed free access to food for up to 2 h prior to the experimental procedure. Before the experiment, the rats were randomly divided into four groups, six in each group. Group C (Control Group), Group S (Group Sevoflurane), Group F (Group Fullerenol), Group FS (Group Fullerenol-Sevoflurane).

Sevoflurane was administered to rats in Groups S and FS at 3% concentration with a minimum alveolar concentration (MAC) of 1.3. Sevoflurane was applied in a covered transparent glass container having an input and output hole for the anesthetic gas, with 100% oxygen in a fresh gas flow of 4 L/min. After 180 min of administration of sevoflurane, the rats were sacrificed in both groups. Nanoparticles were administered to the Group F at a dose of 100 mg/kg intraperitoneally.[21,22] Rats in this group were sacrificed after 210 min in order to equalize the exposure times to nanoparticles. In Group FS 100 mg/kg fullerenol nanoparticles were given intraperitoneally 30 min before the inhalation anesthesia. After administration of the nanoparticles, sevoflurane was applied for 180 min and after a total of 210 min rats were sacrificed.

In all groups, sacrification was performed by intracardiac blood collection under anesthesia with 50 mg/kg ketamine (Ketalar®, Parke-Davis, USA), 10 mg/kg xylazine (Alfazyne, 2%, Ege Vet, Izmir, Turkey). Blood samples were stored in heparin contained tubes and deformability measurements were performed as soon as possible. Collected blood was centrifuged at 1000 rpm for ten min. Blood plasma in the upper phase and the buffy coat, which is a thin layer of leukocytes mixed with platelets in the middle over the erythrocytes, were removed. Isotonic phosphate buffered saline (PBS) was added to collapsing erythrocytes and this mixture was centrifuged at 1000 rpm for ten min. Liquid on the upper surface was taken. Washing process was repeated three times and finally pure red cell packs were obtained. Erythrocyte suspensions with 5% hematocrit in PBS buffer were used to do deformability measurements. Erytrocytes were collected and then deformability measurements were done at 22 ^{o}C.

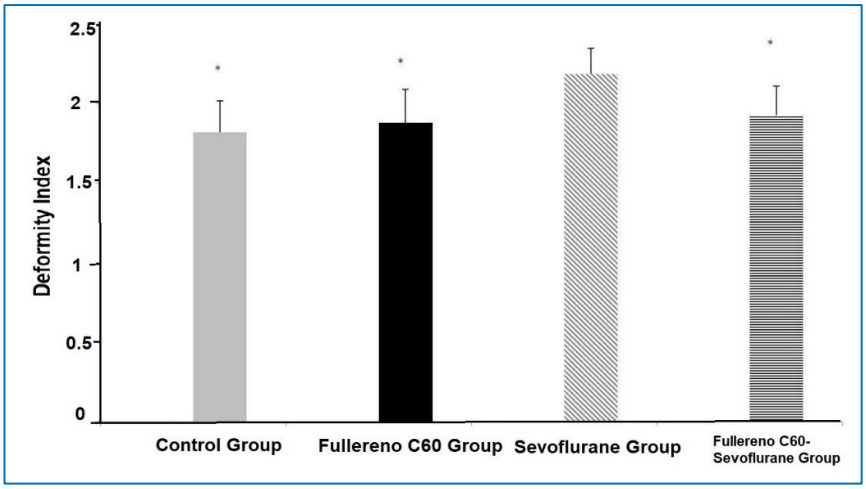

Figure 1: Deformability Index in the four groups.

Constant-current filtrometer system is used to measure erytrocyte deformability. Samples were prepared as 10 ml of erytrocytes suspension and PBS buffer before measurement. The infusion pump was set at 1.5 ml/min for a constant rate of flow. A 28 mm nucleoporin polycabonate filter with a 5 µm pore diameter was used. Constant pressure changes, while the erythrocytes passed through the filter, were detected by the pressure transducer and the data was transferred to computer with the help of MP 30 data acquisition systems (Biopac Systems Inc, Commat, USA). At different times pressure changes were measured by using relevant computer programs for calculations. Pressure calibration of the system was performed each time before measuring the samples. After buffer (P_T) was passed through the filtration system the erythrocytes (P_E) were passed next. Pressure variations were measured. By relating the pressure value of erythrocytes suspension to pressure value of buffer, the relative refractory period value (Rrel) was calculated. The deformability index was interpreted; as Rrel increased the ability of erythrocyte deformability was affected adversely. An increase in the erythrocyte deformability index is a sign of reduced erythrocyte deformability. Erythrocytes with a 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.

2.1. Statistical Analysis: All the data were processed by variance analysis in SPSS 22.0 for Windows statistical software. A p-value less than 0.05 was considered statistically significant. The data were expressed as

mean ± standard deviation. Variance analysis and Kruskal-Wallis test were used to evaluate the data.

Mann–Whitney U test with Bonferroni correction were used to evaluate the variables with significance.

3. Results

Relative resistance was significantly higher in Group S when compared to all other groups ($p < 0.0001$). The erythrocyte deformability index was significantly higher in Group S than in Groups C and F ($p < 0.0001$, $p = 0.002$, respectively). Erythrocyte deformability index was significantly lower in Group SF than in Group S ($p = 0.017$) (Figure 1).

4. Discussion

Erythrocyte deformability is an important physiological factor/phenomenon which plays an essential role for delivering oxygen to the tissues. In addition to other well-known mechanisms, alterations in deformability may cause tissue perfusion problems that may contribute to vascular complications encountered during post-anesthesia period. It is important for its contribution to a poor prognosis.[23] Previous studies have shown that volatile anesthetics induce inflammatory response and induce oxidative stress. The pathophysiological mechanism attributed to this effect is either reducing the antioxidant defense mechanism or inducing oxidative stress by producing toxic free radicals such as superoxide anion.[24, 25]

Yeşilkaya et al. showed in their study investigating oxidative stress in RBCs that free radical formation was observed during halothane metabolism and a decreased red blood cells (RBC) superoxide dismutase activity, consequently resulting in deterioration of erythrocyte stability under halothane anesthesia.[26]

In another study Aydoğan et al. compared the effects of sevoflurane on deforrmability of erythrocytes in old and young rats and concluded that sevoflurane only reduced the deformability of erythrocytes in older rats. They attributed these effects of sevoflurane to the flexibility of young erythrocytes, thus tolerating environmental changes and that demaging effects of inhalation anesthetics are more prominent in the elderly.[27] However, Dikmen et al. observed an increase in antioxidant potential of erythrocytes in sevoflurane treated rats. Therefore, the authors argued that sevoflurane anesthesia may protect erythrocytes against increased oxidative stress, especially in the postoperative period.[28] In the light of this information, in order to avoid contradictory results, we used rats older than 12 weeks in our study and found that sevoflurane caused an increase in erythrocyte deformability index.

In many previous experimental and clinical studies, various antioxidants have been evaluated for treating erythrocyte deformability due to oxidative stress. In their study, Begum et al. found that cigarette tar increased membrane lipid peroxidation and decreased erythrocyte deformability and they showed that this deterioration could be reversed with quersetin – an agent with antioxidant properties.[10] The histidine dipeptides, anserine and carnosine, are antioxidants obtained from animal sources, and play role in defense mechanisms against reactive oxidant damage. An antioxidant preparation of anserine-carnosine mixture, prevented oxidative stress by reactive oxygen species. Loss of deformability in human erythrocytes and protein degradation caused by reactive oxygen species were completely inhibited.[11] One of the main functions of carnosine, which is abundant in skeletal muscle and human brain, is antioxidant and radical scavenger. In this study, it was shown that carnosine molecule had a dose-dependent positive effect on impaired erythrocyte deformability due to oxidative stress induced by hydrogen peroxide (H_2O_2), especially in young rats.[12]

In vitro and in vivo studies have shown that the water soluble fullerenol nanoparticles have strong antioxidative potential. It may function as a free radical scavenger in biological systems. Injac et al. designed a study to investigate the nephroprotective effects of fullerenol nanoparticles. They investigated the antioxidant effects of intraperitoneal 100 mg/kg fullerenol nanoparticles in which nephropathy was induced with 8 mg/kg doxorubicin in rats. Increased oxidative stress in kidney tissue was detected after doxorubicin administration. At the end of the study, pre-treatment of fullerenol was found to prevent oxidative stress, lipid peroxidation and oxidant-antioxidant imbalance caused by doxorubicin in renal tissue.[29] In previous studies, the antioxidant effect of fullerenol administered intraperitoneally at a dose of 100 mg/kg was also detected in different tissues such as testis, heart and liver.[30-32]

Membrane proteins play crucial roles in maintaining plasma membrane function. They are responsible for selective transport, the shape and architecture of the cell and signal transduction. Contrary to the current knowledge, Grebowski et al. argued that fullerenol can diffuse in to membrane phospholipids and interact with membrane proteins, thus influencing their functions. Thus, fullerenol may cause hemolysis due to erythrocyte membrane structural alterations. The authors concluded that changes in the activities of membrane ATPases caused by fullerenol could be the result of its direct and/or indirect (via membrane fluidity changes) interaction with the enzymes, so it could modulate ion homeostasis, which regulates cell death.[33] However, we did not observe hemolysis or organ toxicity with fullerenol in doses used in our study.

In this study, we showed that 100 mg/kg of fullerenol nanoparticles, which gains more popularity for treatment of various pathologies for their antioxidant propoerties, administered 30 min before sevoflurane anesthesia, attenuated the reduction of erythrocyte deformability caused by sevoflurane.

5. Conclusion

On the basis of the results of our study, we believe that fullerenol nanoparticles can be used for diseases in which the underlying pathophysiological mechanism is the reduction in erytrocyte deformability, including exposure to sevoflurane. However, there is need for further experimental investigations and clinical trials.

6. Conflict of interest

None declared by the authors

7. Authors' contribution

All authors contributed in the connduct of this investigation as well as in literature search and manuscript preparation.

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