Effects of Recurrent Sevoflurane Anesthesia on Erythrocyte Deformability in Experimentally Induced Alzheimer Rats

Deneysel Alzheimer Oluşturulmuş Yaşlı Ratlarda Tekrarlayan Sevofluran Anestezisinin Eritrosit Deformabilitesi Üzerine Etkisinin Değerlendirilmesi

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ABSTRACT

Objectives: The aim of this study was to investigate the association between Alzheimer's disease and erythrocyte deformability and to assess whether recurrent sevoflurane anesthesia influenced this association.
Methods: A total of 24 Wistar albino rats were divided into four groups: Control, sevoflurane, Alzheimer, and Alzheimer + sevoflurane. The experimental Alzheimer model was prepared by intracerebroventricular injection of streptozotocin at a dose of 3 mg/kg (10 µl) to the Alzheimer and Alzheimer + sevoflurane groups. Sevoflurane (2.3%) was administered to the sevoflurane and Alzheimer + sevoflurane groups 4 weeks after the surgery for 3 days and lasting for 2 hours per day. Blood samples were then collected for deformability measurements.
Results: The deformability index was significantly increased in Alzheimer-induced rats, but the results for the Alzheimer and Alzheimer + sevoflurane groups were similar. The erythrocyte deformability index was significantly increased in the Alzheimer and Alzheimer + sevoflurane groups (p=0.004 p=0.001 respectively). However, there was no difference in deformability in the sevoflurane group (p= 0.496)
Conclusion: Erythrocyte deformability was increased in the Alzheimer-induced rats; however recurrent sevoflurane anesthesia did not affect erythrocyte deformability.

Keywords: Alzheimer disease, erythrocyte deformability, sevoflurane, rat, animal models experimental.

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ÖZET

Amaç: Alzheimer hastalığı ile eritrosit deformabilitesi arasındaki ilişkiyi araştırıp tekrarlayan sevofluran anestezisinin bu ilişkiyi etkileyip etkilemediğini değerlendirmeyi amaçladık.
Yöntem: Toplamda 24 Wistar Albino rat, her grupta altı adet olmak üzere dört gruba ayrıldı: Kontrol (K), sevofluran (S), Alzheimer (A) ve Alzheimer + sevofluran (AS). Deneysel Alzheimer modeli için 3 mg/kg (10 µl) dozunda streptozotosin (STZ) Alzheimer ve AS gruplarına intraserebroventriküler yoldan enjekte edildi. Sevofluran ve Alzheimer+sevofluran gruplarına cerrahiden dört hafta sonra üç gün boyunca ve günde iki saat sürecek şekilde Sevofluran (%2.3) uygulandı. Daha sonra deformabilite ölçümleri için kan örnekleri alındı.
Bulgular: Yapılan ölçümler sonucunda deformabilite indeksinin Alzheimer oluşturulan ratlarda önemli ölçüde arttığı gözlendi, fakat Alzheimer ve Alzheimer + sevofluran grupları arasındaki sonuç benzerdi. Eritrosit deformabilite indeksi Alzheimer ve Alzheimer + sevofluran gruplarında anlamlı olarak arttı (p=0.004, p=0.001, sırasıyla). Ancak, sevofluran grubunda deformabilite açısından herhangi bir farklılık bulunmadı (p=0.496).
Sonuç: Alzheimer ile indüklenen ratlarda eritrosit deformabilitesi artmaktadır; ancak tekrarlayan sevofluran anestezisi eritrosit deformabilitesini etkilememiştir.

Anahtar Sözcükler: Alzheimer, eritrosit deformabilitesi, sevofluran, rat, deneysel hayvan modelleri.

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INTRODUCTION

Alzheimer's disease is the most common form of dementia, affecting approximately 10% of individuals over 65 years of age and 50% of individuals over 85 years of age (1). Alzheimer's disease was defined by Alois Alzheimer in 1907, and is the most common cause of progressive, irreversible, and advanced age dementia. It is estimated that more than 25 million people are affected, and its prevalence could increase to 100 million cases by the year 2050 (2). The disease begins in the form of forget fulness and leads to progressive cognitive impairment, gradually leaving the patient unable to perform daily tasks (3). The diagnosis is achieved with the patient history, neurological examination, and various tests. However, the definitive diagnosis is achieved with the manifestation of extracellularly accumulated beta-amyloid plaque and extracellular neurofibrillary tangles in the brain (4). With this protein accumulation, the neurons fail to function properly and die in a shorter time period than expected. This accumulation starts much earlier than the symptoms of the disease begin to manifest. The causes of Alzheimer's disease are not yet fully established. It might be caused by genetic factors (5) as well as by sporadic factors. In meta-analyses and systematic reviews, a low cognitive reserve status (education, occupation, mental activity), a lack of physical activity and exercise, obesity, alcohol consumption, and smoking are shown to be preventable causes of Alzheimer's disease (1,4). Several studies have shown that apoptosis plays an important role in cell loss in the course of neurodegenerative diseases such as stroke, Parkinson's disease, and Alzheimer's disease (6–8). Oxidative stress and unusual inflammatory responses have also been shown to be associated with Alzheimer's disease (6).

Low cerebral perfusion as a result of hemodynamic microcirculation insufficiency is one of the factors underlying Alzheimer's disease that leads to progressive cognitive impairment (9). Erythrocytes provide cerebral perfusion and can change their biconcave shape to carry oxygen and other vital substances and remove waste. This shape-changing property is called deformability. Erythrocyte deformability, or the ability of the cell to alter its shape under stress, is important for proper erythrocyte functioning and thus for proper cerebral perfusion. Given the diameter of vessels in the microsirculations is much smaller than the diameter of the erythrocytes, erythrocytes must be able to change their shape topass through these small vessels and provide circulation (10,11).

Sevoflurane, one of the most used volatile anesthetics might affect erythrocyte deformability (12-14).

In this study, we aimed to investigate the effects of recurrent sevoflurane anesthesia on erythrocyte deformability in rats with experimentally induced Alzheimer's disease.

MATERIALS and METHODS

The study was approved by the Ethics Committee of Gazi University Faculty of Medicine (G.Ü.E.T-17.044). A total of 24 Wistar albino rats were divided into four groups of six rats as follows: a control group (group C), sevoflurane group (group S), Alzheimer group (group A), and an Alzheimer + sevoflurane group (group A+S).

After the administration of ketamine (100 mg/kg, intraperitoneally), a stereotaxic apparatus was placed. As follows: after the scalp was cleaned with iodine solution, a midline incision was made, and a burr hole was opened 0.8 mm below the bregma on the scalp 1.4 mm lateral to the mid sagittal line and 3.4 mm below the dura on the scalp according to the stereotaxic atlas of Paxinos and Watson. As in previous studies, the Alzheimer and Alzheimer + sevoflurane groups were injected with a dose of 3 mg/kg of intracerebroventricular streptozotocin to induce an experimental Alzheimer model (15–17). Sevoflurane 2.3% was administered to the Alzheimer and Alzheimer + sevoflurane groups for 3 consecutive days for 2 hours. At 24 hours after the anesthesia, all rats were euthanized under additional anesthesia, and heparinized total blood samples were collected for the erythrocyte suspension. The collected erythrocyte suspensions were used for deformability measurements.

Deformability measurements

The samples were first centrifuged for 10 minutes at 1000 rpm, and then the serum and the buffy coat on the erythrocytes were removed. Next, isotonic phosphate-buffered saline (PBS) buffer was added to the collapsing erythrocytes. This mixture of PBS and erythrocytes was centrifuged for another 10minutes at 1000 rpm. Subsequently, the liquid was removed from the upper surface. Finally, pure red cell packs were obtained from three consequent washing processes. PBS was mixed with the erythrocyte packs to obtain mixed suspensions with 5% hematocrit, which were used for the deformability measurements. All procedures were performed at 22ºC. The deformability parameters were analyzed with the constant-current filtrometer system. 10-ml samples of the erythrocytes suspended in PBS were prepared for measurement. We set a constant flow rate of 1.5 ml/min through an infusion pump, and we used a 28-mm nucleoporin polycarbonate filter with a pore diameter of 5 µm. A transducer detected the pressure changes during the passage of the erythrocytes through the filter, and the collected data were transferred to a computer using MP30 data software (Biopac Systems Inc, Commat, USA). Buffer (P_T) and then eritrocytes (P_E) were passed subsequently through the filtration system and the pressure changes were measured. The relative refractory period value (Rrel) was considered the deformability index and was calculated by relating the pressure value of the erythrocyte suspension to the pressure value of the buffer. Increases in Rrel were considered to indicate decreased erythrocyte deformability.

Statistical Analysis

The SPSS 17.0 software program was used for the statistical analyses and $p<0.05$ was considered statistically significant. The findings were expressed as mean ± standard deviation. A Kruskal–Wallis variance analysis was employed for data evaluation. The variables with significance were evaluated with the Bonferroni-corrected Mann–Whitney U test.

RESULTS

The deformability index was significantly increased in the Alzheimer and Alzheimer + sevoflurane groups ($p=0.004$ and $p=0.001$, respectively), and the results for the Alzheimer and Alzheimer + sevoflurane groups were similar. No differences between the sevoflurane group and the other groups were detected ($p=0.496$) (Figure 1).

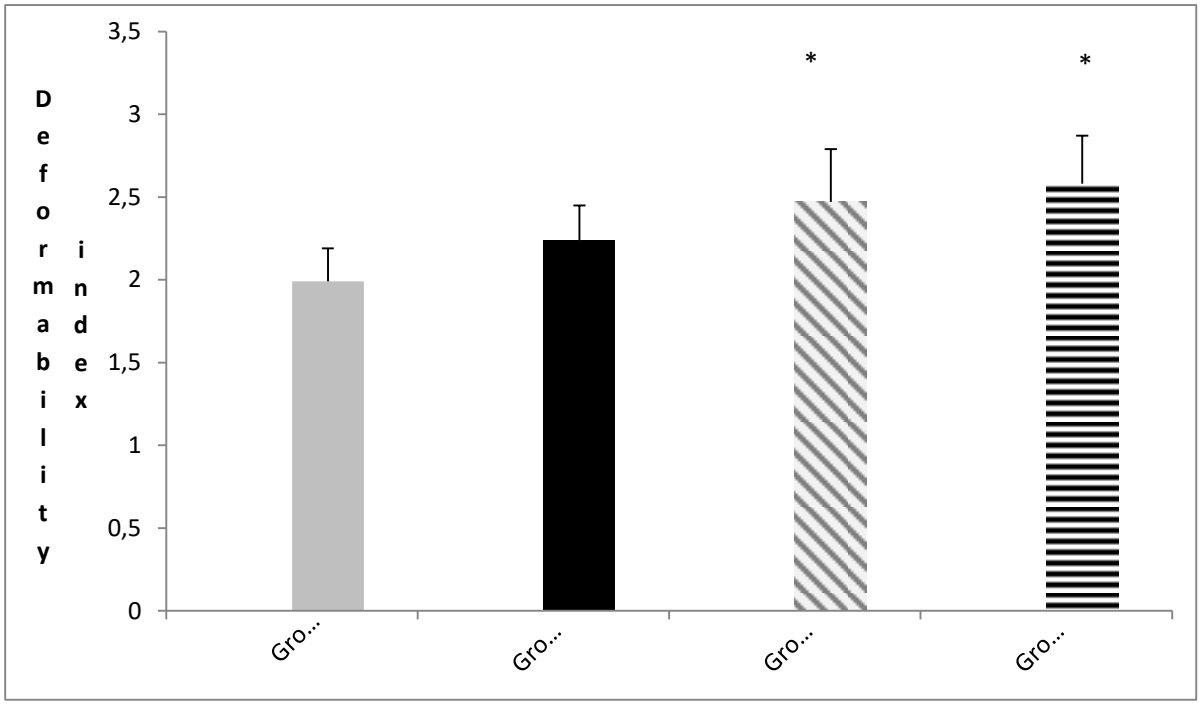

Figure 1: Erythrocyte deformability index values of the groups. Each column represents the mean ± standard deviation; * p <0.05 compared with the Control group.

DISCUSSION

Alzheimer's disease is a slow progressive systemic neurodegenerative disease that many factors play a role in its etiology. It is thought that there is a significant decrease in cerebral perfusion and cerebral energy metabolism due to impaired ability of erythrocytes to carry oxygen to tissues and give oxygen to patients with AD. (9,15) Erythrocytes need to elongate and flex to move through the smallest organ capillaries to transmit oxygen and vital molecules and remove metabolic waste. This capacity is called deformability. Erythrocyte deformability and membrane stiffness are affected by various agents and altered erythrocyte deformability not only modifies their oxygen supply capacity but also affects the survival of circulating erythrocytes (16,17). Arslan et al. showed that the erythrocyte deformability index was increased in diabetic rats undergoing ischemic reperfusion (16). Similarly, in a review, it was shown that erythrocyte membrane deformability was impaired in individuals with hypertension, cardiovascular disease, and diabetes; therefore, their organ blood supply and nutrition was also impaired (18).

In a study conducted on patients with Alzheimer's disease three groups were formed: a healthy control group and two groups with a different severity of the disease (mild and severe). They found that plasma nitric oxide and catalase activities were significantly higher in patients with severe Alzheimer's disease compared with the control group, and glutathione peroxidase activity was significantly lower; however, erythrocyte deformability was not significantly affected by these differences. In that study, it was shown that the oxidant-antioxidant balance changed in Alzheimer's disease but erythrocyte deformability was not affected by this disease. (9). In our study, however, it was found that the deformability index was significantly increased in Alzheimer's-induced rats compared with that of the control group, and this increase was statistically significant.

Inhalation anesthetics are among the frequently used drugs in anesthesia practice, which are known to affect hemodynamic circulation. Various studies have been performed on the effects of inhalation anesthetics on erythrocyte deformability. In a study conducted with desflurane, it was found that the deformity indexes of erythrocytes increased significantly in young rats with desflurane administration and significantly decreased in elderly rats compared with controls. When the young and old rat control groups were compared, the deformability indexes were found to be significantly higher in the elderly rats, and this change was attributed to the deterioration of membrane structures due to old age (13). In a similar study conducted by Aydogan et al., young, and old rats treated with sevoflurane anesthesia were compared with control groups. Although deformability did not change in the young rats compared with the control group, erythrocyte deformability was reduced in the older rats after the sevoflurane anesthesia was administered (12). In our study, however, there was an increase in the erythrocyte deformability index in the sevoflurane group compared with the control group, but the difference was not statistically significant. In addition, although the erythrocyte deformability of the Alzheimer-induced rats was reduced, recurrent sevoflurane anesthesia had no effect on deformability.

CONCLUSION

The deformability of erythrocytes is a factor that provides adequate oxygen supply to the tissue, and its degradation might be a factor underlying many chronic diseases. We found that erythrocyte deformability decreased in Alzheimer-induced rats; yet, recurrent sevoflurane anesthesia did not affect erythrocyte deformability. These results need to be further supported by additional clinical and experimental studies.

Conflict of interest

No conflict of interest was declared by the authors.

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