Evaluation of the Effects of Recurrent Dexmedetomidine on Cognitive Functions and Brain Tissue in Streptozotocin-Induced Rats with Alzheimer's Disease

Streptozosin ile Alzheimer Oluşturulan Yaşlı Ratlarda Tekrarlayan Deksmedetomidinin Kognitif Fonksiyonlar ve Beyin Dokusu Üzerine Etkilerinin Değerlendirilmesi

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ABSTRACT

Objective: The aim of this study was to evaluate the effects of recurrent dexmedetomidine on cognitive functions and brain histopathology in the elderly rat model which has Alzheimer disease created with streptozocin (STZ).
Materials and Methods: Totally 24 aged Wistar Albino rats were divided into 4 equal groups; control (Group C), sham (Group S), Alzheimer (Group A) and Alzheimer + dexmedetomidine (Group AD). All rats in Group A and Group AD received stereotaxic injection under ketamine (100 mg / kg, i.p.) anesthesia. Midline burr hole was entered under the dura. Group A and Group AD 3 mg/kg (10 ml) were induced by administering STZ experimental Alzheimer intracerebroventricularly. Four weeks after the surgery, Group S and Group AD received 100 µg/kg (i.p) dexmedetomidine for 3 consecutive days. Each group were tested with RAM test. After 24 hours, all rats were euthanized under anesthesia and brain tissue was taken. Hippocampus tissues were evaluated Biochemical and histopathologically.
Results: At the beginning, the number of The radial arm maze test (RAM) input-output is similar in all groups, but 3 weeks after the Alzheimer's formation RAM input-output decreased significantly. In Group AD, the number of RAM input-outputs increased significantly compared to Group A after 2nd and 3rd anesthesia applications. Glial fibrillary acidic protein levels were significantly higher in Group A compared to C and S groups in hippocampus tissue. In Group AD, it was found to be significantly lower than Group A. In group A Catalase (CAT) and Paraoxonase 1 (PON-1) activities and Thiobarbituric acid reactive substances (TBARS) level of brain tissue were found higher than Group C and S. TBARS levels of Group AD brain tissue was significantly lower than Group A.
Conclusion: We concluded that recurrent dexmedetomidine administration in rats treated with STZ positively affects cognitive functions evaluated with RAM in the presence of recurrent dexmedetomidine. Also histopathological and biochemical markers supported these findings. We concluded that observational studies should be performed in large series to correlate these results with clinical applications.

Keywords: Alzheimer, dexmedetomidine, Radial Arm Maze Test, rat, GFAP, TBARS, Catalase

ÖZET

Giriş ve Amaç: Deneysel araştırmada streptozosin (STZ) ile Alzheimer oluşturulan yaşlı rat modelinde, tekrarlayan deksmedetomidinin, kognitif fonksiyonlar ve beyin histopatolojisi üzerine etkilerinin değerlendirilmesi amaçlanmıştır.
Gereç ve Yöntem: Etik Kurulu izni alınarak gerçekleştirilen deneyde; 24 yaşlı Wistar Albino rat, 4 eşit gruba [kontrol (Grup K), sham (Grup S), Alzheimer (Grup A) ve Alzheimer+deksmedetomidin (Grup AD)] ayrıldı. Tüm gruplara Radial Arm Maze Test (RAM) uygulandı. Grup A ve Grup AD'deki tüm ratlara ketamin (100 mg/kg, i.p.) anestezisi altında stereotaksik başlık takıldı. Orta hattan burr hole ile dura altına girildi. Grup A ve Grup AD'ye 3mg/kg (10 µl) STZ intraserebroventriküler verilerek deneysel Alzheimer oluşturuldu. Cerrahiden 4 hafta sonra Grup S ve Grup AD'ye birbirini izleyen 3 gün 100 µg/kg deksmedetomidin i.p. uygulandı. Grup S ve Grup AD'de her anestezi uygulamasını takiben (Grup K ve Grup A'da da eş zamanlı) RAM yapılarak veriler kaydedildi. Bu uygulamadan 24 saat sonra tüm ratlara anestezi altında ötenazi yapılıp beyin dokusu alındı. Biyokimyasal ve hipokampüs dokuları histopatolojik olarak değerlendirildi.
Bulgular: Tüm gruplarda RAM giriş-çıkış sayıları başlangıçta benzer iken, Alzheimer oluşturulduktan sonra; 3. haftadan itibaren Grup A ve Grup AD'de anlamlı olarak azaldı. Grup AD'ise ise 2. ve 3. anestezi uygulamalarından sonra RAM giriş-çıkış sayısı Grup A'ya göre belirgin olarak arttı. Ratların hipokampus dokusunda Glial fibriler asidik protein düzeyi Grup A'da K ve S gruplarına göre anlamlı yüksek bulundu. Grup AD' de ise Grup A'ya göre anlamlı düşük bulundu. Grup A'da beyin dokusu katalaz ve PON-1 aktiviteleri ve TBARS düzeyi Grup K ve S'ye göre yüksek bulundu. Grup AD beyin dokusu TBARS seviyesi Grup A'ya göre anlamlı düşük bulundu.
Tartışma/Sonuç: Streptozosin ile deneysel Alzheimer oluşturulan yaşlı ratlarda, tekrarlayan deksmedetomidin uygulamasının RAM ile değerlendirilen kognitif fonksiyonları tekrarlama sayısı ile korele şekilde olumlu yönde etkilediği, histopatolojik ve biyokimyasal belirteçlerin de bu bulguları desteklediği saptanmıştır. Elde edilen bu sonuçların klinik uygulamalarla korelasyonu için geniş serilerde gözlemsel çalışmalar yapılması gerektiği kanısındayız.

Anahtar Sözcükler: Alzheimer, deksmedetomidin, Radial Arm Maze Test, rat, GFAP, TBARS, Katalaz

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INTRODUCTION

Alzheimer's disease (AD), which was described by Alois Alzheimer in 1907, is the most common form of progressive, irreversible dementia in the elderly. It affects more than 25 million people worldwide; without a major therapeutic breakthrough, its prevalence is expected to increase more than 100 million by 2050 (1).

Worldwide, 200 million patients receive anesthesia every year. As overall life expectancy has increased, an increasing number of elderly patients are receiving anesthesia (1). Concerns that anesthesia may increase AD are evaluated in a theoretical framework. Anesthetic management at elderly or patient with AD is very important.

Patient history, neurological examination, neuropsychiatric tests are helpful in diagnosing Alzheimer's Definitive diagnosis of AD disease is made post mortem histopathologically. The two histopathological hallmarks of AD are senile plaques composed of extracellular aggregates of the beta-amyloid peptide and intraneuronal neurofibrillary tangles, composed of abnormally hyperphosphorylated tau protein assembled into paired helical filaments (2). Predisposing factors for AD are not yet fully understood. It is thought to be affected by many environmental factors as well as genetic transition (3,4).

The role of anesthesia in the development of postoperative cognitive dysfunction (POCD) is still unclear. In recent studies, there is an increasing concern that volatile anesthetics may impair cognitive functions of older patients (5-9). In recent studies show that there may be a relationship between drugs used in general anesthesia and AD progress. We do not use ether anesthesia for our current anesthesia practice but Ikeada et al. have demonstrate that ether anesthesia increases tau phosphorylation in rat hippocampus (10). It has been shown that volatile anesthetics can induce synaptic dysfunction and neuronal apoptosis and thus lead to cognitive disorders in elderly brains. It is thought that intracellular β-amyloid levels increase the synthesis of β-amyloid and tau protein and disrupts the intracellular calcium metabolism (1). Isoflurane has been shown to increase caspase activity and increase amyloid β in vivo in many researches (11,12). There are also some research suggesting that sevoflurane and desflurane can cause to POCD and may progress to AD (13,14). The situation for dexmedetomidine differs from inhaler anesthetics. Studies have shown that dexmedetomidine is neuroprotective and does not affect short and long term memory (15,16).

In this experimental research; The radial arm maze test (RAMT) was used for evaluating of the cognitive function in rats (17). Oxidative stress was evaluated with as antioxidant enzymes CAT, PON 1 activities, as oxidants TBARS levels in brain tissue caspase activity and GFAP.

In this study, we aimed to determine whether there is any change in cognitive function after repeated dexmedetomidine anesthesia in elderly rats with streptozotocin-induced Alzheimer's diseases and also demonstrate biochemical, immunohistochemical and pathological evidence of this change.

MATERIALS and METHODS

Animal models and experimental protocol

This study was carried out in the GUDAM Laboratory of Gazi University with the approval of the Experimental Animals Ethics Committee of Gazi University (GÜET-17.090). All procedures were carried out in accordance with the standards in the Guide for the Care and Use of Laboratory Animals. In this study, 24 elderly Wistar Albino rats weighing between 250 and 300 g, raised under the same environmental conditions, were used. The 24 rats were randomly separated into four groups. Control group (n=6, Group C), sham (n=6, Group S), Alzheimer's (n=6, Group A) and Alzheimer's + dexmedetomidine (n=6, Group AD). 100 mg/kg ketamine (Ketalar; Parke-Davis; Pfizer, Inc, New York, NY, USA) was applied intraperitoneally to all rats in group A and group AD and stereotactic head was placed. It was entered under the dura with burr hole from the mid-line. As in previous research, experimental Alzheimer's disease was created by giving 3 mg/kg (10 µl) streptozotocin (Sigma Chemical, St. Louis, MO, USA), to Group A and Group AD intracerebroventricularly (18-20). Radial Arm Maze Test was applied to all groups. The original design of Radial Arm Maze consisted of a 34 cm wide central platform with eight equal-length arms radiating out. Each arm has food sites at the end that are not visible from the central area; four arms have food at the distal end, and the other four arms

do not have any. During a trial of the RAMT, an animal must acquire food from all four areas of the maze (17,21). Each animals were placed individually in the center of the maze. In the RAM test, the number of input-output of the rats in the arms was studied. Four weeks after surgery, Group S and Group AD were administered dexmedetomidine 100 µg/kg (i.p) for 3 consecutive days. Following each anesthesia in Group S and Group AD, RAMT was administered (simultaneously in Group C and Group A), and data were recorded. After 24 hours from this administration, all rats were euthanized under ketamine anesthesia and their blood was taken. Brain tissue samples were evaluated biochemically and hippocampus tissues were evaluated with histopathologic.

Histochemical and immunohistochemical evaluation

Coronal cut sections were performed on formaline fixed brain tissues of the rats. Thus, slices containing hippocampus were obtained. After tissue processing, embedding in paraffin and sectioning immunohistochemistry for GFAP was performed on 3 micrometer cut sections of the 16 samples using automated Leica Bond-max system. After de-paraffinization and dehydration Bond Epitope Retrieval solution (Leica Microsystems) was used for antigen unmasking for each antibody. After peroxide block placement the slides were incubated with antibodies against GFAP (GA-5, NeoMarkers, 1:300) for 15 minutes. The incubation with Post Primary reagent (Leica microsystems) was followed by washing with Bond Wash solution. Bond Polymer placement is followed by DAB (3,30 diaminobenzidine tetrahydrochloride) as a chromogen for 6 minutes, hematoxylin counterstaining and mounting of the slides.

Biochemistry

Fasting blood samples were obtained from volunteers to plain tubes. Sera were separated after centrifugation at 1600 g for 10 minutes and stored at -80 C^0 until the analyzing time. The brain tissues were first washed with cold deionized water to discard blood contamination and then homogenized in a homogenizator (Heidolph DIA 900 Model) at 3.000 rpm for 3 min. After centrifugation at 10.000 x g for 60 min. the upper clear layer was taken. CAT, PON 1 enzyme activities and TBARS, protein amount were measured as described (22-25) respectively in this fraction.

The CAT activity is based on the measurement of absorbance decrease due to H_2O_2 consumption at 240 nm. PON-1 activity was measured the rate of hydrolysis of paraoxon by monitoring the increase of absorbance at 405 nm and at 25 °C. The basal assay mixture included 1.0 mM paraoxon and 1.0 mM CaCl2 in Tris/HCl buffer (pH: 8.0, 100 mM). The definition of 1 unit of paraoxonase activity was taken as 1 millimole of p-nitrophenol formed per minute. The TBARS assay was carried out to determine lipid peroxidation using the thiobarbituric acid method. TBARS measurements were conducted based on the reaction of MDA with thiobarbituric acid (TBA), which form a pink pigment with an absorption maximum at 532 nm in acid pH, and 1,1,3,3-tetraethoxypropane was used as a standard MDA solution.

The CAT and PON 1 activities were given in IU/mg protein, TBARS levels were given nmol/mg protein.

Statistical Analysis

All the data were processed by variance analysis in The Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA) 20.0 program for Windows statistical software. ANOVA test was used to assess the results. Bonferroni adjusted Bonferroni test was used after significant ANOVA to determine which group differs from the other. The data were expressed as mean ± standard deviation (Mean±SD). A P-value less than 0.05 was considered statistically important.

RESULT

At the beginning, the number of RAM input-output is similar in all groups, but 3 weeks after the Alzheimer's formation RAM input-output decreased significantly. In Group AD, the number of RAM input-outputs increased significantly compared to Group A after 2nd and 3rd anesthesia applications (Table 1). Glial fibrillary acidic protein levels were significantly higher in Group A compared to C and S groups in hippocampus tissue (Table 2, Figure 1-4). In Group AD, it was found to be significantly lower than Group A. In group A catalase and PON-1 activities and TBARS levels of brain tissue were found higher than Group K and S. TBARS level of Group AD brain tissue was significantly lower than Group A (Table 3).

Table 1. Input-output RAM data of rats [Mean ± SD]

Rat	Group C (n=6)	Group S (n=6)	Group A (n=6)	Group AD (n=6)	P**
First	6.83±1.60	8.00±2.06	7.17±1.83	6.17±1.32	0.352
1th. week	8.33±2.66	6.67±2.16	6.33±1.21	5.33±1.50	0.097
2th. week	7.33±2.58	5.50±3.18	4.83±1.72	4.33±1.50	0.053
3th. week	6.80±3.65	5.33±2.06	2.83±1.17*	3.33±1.75*	0.028
4th. week	8.17±3.54	6.33±1.75	2.80±0.75*,+	2.83±1.47*,+	<0.0001
After the first dexmedetomidine	6.17±2.48	5.60±1.86	2.67±1.21*,+	4.00±1.67	0.021
After the second dexmedetomidine	6.33±2.25	5.35±1.76	2.65±0.82*,+	4.83±0.98&	0.001
After the third dexmedetomidine	6.60±2.64	6.17±1.47	2.56±1.37*,+	5.50±0.54&	<0.0001

P**: Level of significance with the ANOVA test p< 0.05
*p<0.05: Compared to Group C, +p<0.05: Compared to Group S, &p<0.05: Compared to Group A

Table 2. Hippocampus GFAP data of rats [Mean ± SD]

|	Group C (n=6)	Group S (n=6)	Group A (n=6)	Group AS (n=6)	P**
GFAP	1.17±0.41	1.50±0.55	2.83±0.41*,+	1.83±0.67*,&	<0.0001

P**: Level of significance with the ANOVA test p< 0.05
*p<0.05: Compared to Group C, +p<0.05: Compared to Group S, &p<0.05: Compared to Group A

Table 3. Brain tissue TBARS, PON-1, catalase data of rats [Mean ± SD]

	Group C (n=6)	Group S (n=6)	Group A (n=6)	Group AS (n=6)	P**
TBARS (nmol/mg protein)	2.08±0.59	2.67±0.83	4.99±0.84 *,+	2.97±1.18 &	<0.0001
PON-1 (IU/mg protein)	0.73±0.24	0.88±0.20	1.38±0.58*,+	1.03±0.37	0.041
Catalase (IU/ mg protein)	60.92±30.82	66.53±30.72	120.78±36.38*,+	90.95±15.47	0.008

P**: Level of significance with the ANOVA test p< 0.05
*p<0.05: Compared to Group C, +p<0.05: Compared to Group S, &p<0.05: Compared to Group A
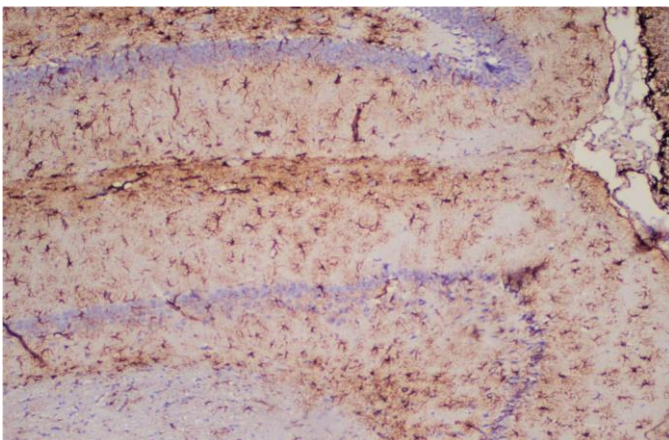
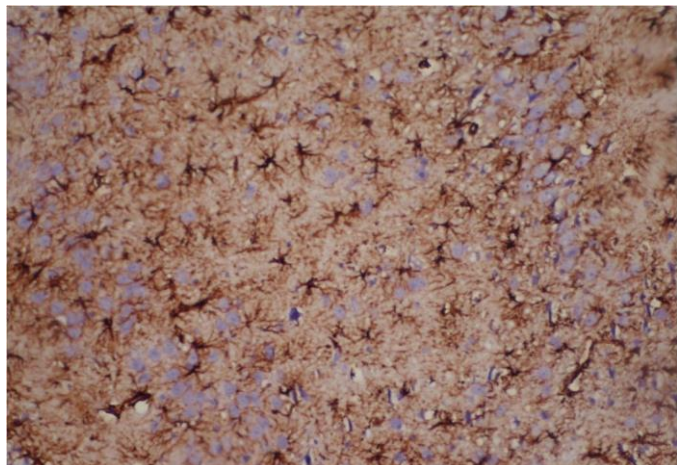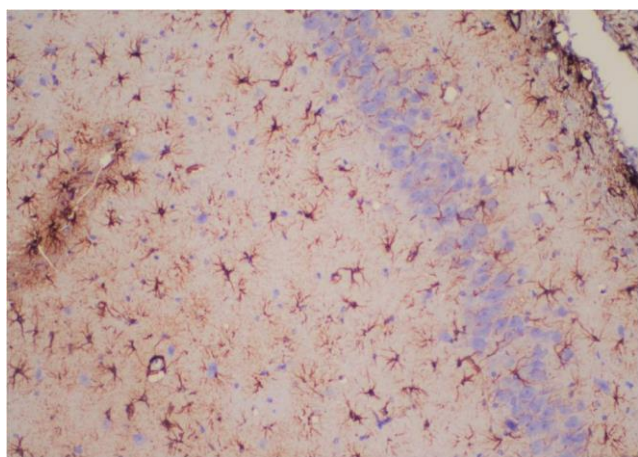**Figure 1:** GFAP (X100) in hippocampus tissues of rats in control group
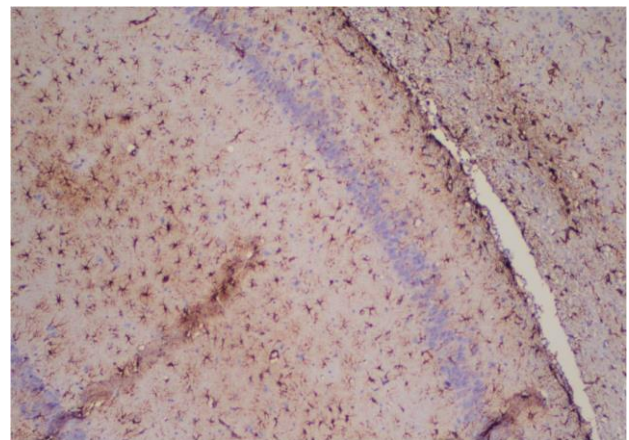
Figure 2: GFAP (x 100) in hippocampus tissues of rats in dexmedetomidine group

Figure 3: GFAP (x 200) in hippocampus tissues of rats in Alzheimer's group

Figure 4: GFAP (X200) in hippocampus tissues of rats in Alzheimer's-dexmedetomidine group

DISCUSSION

The mean life expectancy increases and the incidence of AD increases. There are findings similar to anesthesia toxicity in AD where cholinergic system function is adversely affected. Anesthesia is claimed to increase the severity of Alzheimer's (2) In our study, we determined that there was a impairment in cognitive functions after repeated dexmedetomidine anesthesia in rat with STZ induced AD disease and we have demonstrated this worsening immunohistochemically and pathologically. In a study by Eser et al., dexmedetomidine was observed to be neuroprotective (15). tIn a study by Whittington et al., dexmedetomidine did not affect long-term and short-term memory, but low tau phosphorylation was observed (16). The role of anesthesia on cognitive function is still controversial. It is thought that not only anesthetic agents but also neurotoxicity, inflammation, stress, intraoperative factors (e.g. hypotension, hypoglycemia) or type of surgery have a role in the development of POCD (1,26).

In a meta-analysis of 19 randomized controlled trials of 879 patients, dexmedetomidine reduced inflammatory cytokines (Tumor necrosis factor (TNF), Interleukin 6 (IL6), cortisol level, decreased C-reactive protein (CRP) increase) compared to placebo (27).

In the study of 50 patients undergoing carotid endarterectomy, 25 patients receiving dexmedetomidine had better cognitive performance (28). In a study of 120 patients; The effects of dexmedetomidine on POCD and perioperative inflammation in patients over 60 years of age with cholecystectomy were evaluated. On postoperative day 1, the dexmedetomidine group showed better results than the control group in the mini mental state test; The increase in IL 1B, IL 6 and CRP levels was found to be less in dexmedetomidine group compared to control group (29).

Therefore, anesthesia and surgery are assumed as risk factors that accelerate the onset of AD. Although many reports indicate that there is relationship between cognitive dysfunction and anesthesia in patients with AD, there are many research showing the opposite (30-32). Bohnen et al. evaluated the patients who were followed-up between 1974-1984 years and who had been anesthetized at least once before becoming AD. Overall, they point out that multiple exposure to general anesthesia had no effect on AD development (30). There was no correlation between the risk of general anesthesia exposure and the development of AD in systemic review and meta-analysis by Seitz et al (32).

In our study, we performed a RAMT to evaluate the cognitive functions after creating Alzheimer model with STZ induced and repeated dexmedetomidine anesthesia. The RAMT was designed by Olton & Samuelson. RAMT has been commonly used by researchers for measuring spatial learning and memory in rodents; it allows for the evaluation of spatial working and reference memory (17). In the RAM test we observed the number of input-output on the arms. There was no difference between the rats before the dexmedetomidine exposure. There is a significant difference between the rats when the RAM test is applied after repeated dexmedetomidine anesthesia.

Potential mechanisms for either postoperative cognitive disorder or neurodegeneration are numerous, ranging from N-methyl-D-aspartate-mediated excitotoxicity to oxidant stress or suppression of cholinergic signaling.

Finally, the relationship between AD and anesthesia is not well understood. In elderly rats with STZ-induced experimental AD, it was found that the dexmedetomidine anesthesia has negatively affected cognitive functions evaluated with RAMT at minimum level, and histopathological and biochemical markers supported these findings. In order to correlate these results with clinical applications, we think that observational studies should be conducted in large series.

Conflict of interest
No conflict of interest was declared by the authors.

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