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Original Article / Orijinal Araştırma

Comparative Evaluation of the Effects of Sevoflurane and/or Dexmedetomidine on Behavior, Neuro-inflammation and Apoptosis in Pups Rat

Sevofluran ve/veya Deksmedetomidin'in Sıçan Yavrularında Davranış, Nöroinflamasyon ve Apoptoz Üzerindeki Etkilerinin Karşılaştırmalı Değerlendirilmesi

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Abstract

Aim: Developing brain is vulnerable to side effects of anesthetics. Neurotoxic and cognitive alterations have been documented in several species, and there is concern that small children could be affected adversely if they are exposed for long periods or recurrently to inhalation anesthesia In this experiment we aim to evaluate behavioral and neurotoxic effects of sevoflurane (SEVO) and/or dexmedetomidine (DEX) exposure in pup rats.

Material and Method: Postnatal 21 days old 36 rat were randomly divided into 6 groups (Group I (control); Group II:2.5% SEVO for 4 hours; Group III:2.5% SEVO for 4 hours+intraperitoneal (i.p.) 0.5 µg.kg-1 DEX; Group IV:2.5% SEVO for 4 hours+i.p. 5 µg.kg-1 DEX; Group V: i.p. 0.5 µg.kg-1 DEX; Group VI: i.p. 5 µg.kg-1 DEX was given). Behavior of the rat were examined with the modified Radial Arm Maze test. Histopathological evaluation of the pups' rat brain for neuroinflammation and apoptosis was performed. Statistical evaluation was carried out using the SPSS 20.0, P value <0.05 was considered statistically significant.

Results: Single 2.5% SEVO exposure for 4 hours during early life period in rats is although not show neuroinflammation signs the brain tissue histologically but impaired learning and memory in behavior test (P<0.05). In CA3 stage of the brain tissue apoptosis percentage was diminished in SEVO+DEX groups for comparison with control and single SEVO groups (P<0.05).

Conclusions: Adding DEX to SEVO caused less impairment in memory and learning function. But single 5 µg.kg-1 DEX negatively affected learning and memory function but not locomotor activity and anxiety.

Keywords: Sevoflurane, dexmedetomidine, pup rats, nteurotoxic, cognitive alterations

Öz

Giriş: Gelişen beyin anesteziklerin yan etkilerine karşı savunmasızdır. Birçok türde nörotoksik ve bilişsel değişiklikler belgelenmiştir ve küçük çocukların uzun süreler veya tekrar tekrar inhalasyon anestezisine maruz kalmaları durumunda olumsuz etkilenebileceği endişesi vardır. Bu deneyde sevofluranın (SEVO) davranışsal ve nörotoksik etkilerini değerlendirmeyi amaçlıyoruz. ve/veya yavru sıçanlarda deksmedetomidin (DEX) maruziyeti.

Gereç ve Yöntem: Postnatal 21 günlük 36 rat rastgele 6 gruba ayrıldı (Grup I (kontrol); Grup II: 4 saatlik %2,5 SEVO; Grup III: 4 saatlik %2,5 SEVO+intraperitoneal (i.p.) 0,5 µg). kg-1 DEX; Grup IV: 4 saat için %2,5 SEVO+i.p. 5 µg.kg-1 DEX; Grup V: i.p. 0.5 µg.kg-1 DEX; Grup VI: i.p. 5 µg.kg-1 DEX verildi) . Sıçanların davranışları modifiye Radial Arm Maze testi ile incelendi. Yavruların sıçan beyninin nöroinflamasyon ve apoptoz için histopatolojik değerlendirmesi yapıldı. İstatistiksel değerlendirme SPSS 20.0 kullanılarak yapıldı, P değeri <0,05 istatistiksel olarak anlamlı kabul edildi.

Bulgular: Sıçanlarda erken yaşam döneminde 4 saat boyunca tek seferlik %2,5 SEVO maruziyeti histolojik olarak beyin dokusunda nöroinflamasyon belirtileri göstermemekle birlikte davranış testinde öğrenme ve hafızada bozulmaya yol açmıştır (P<0,05). Beyin dokusunun CA3 aşamasında apoptoz yüzdesi SEVO+DEX gruplarında kontrol ve tek SEVO grupları ile karşılaştırıldığında azaldı (P<0.05).

Sonuç: SEVO'ya DEX eklenmesi, hafıza ve öğrenme işlevinde daha az bozulmaya neden oldu. Ancak tek 5 µg.kg-1 DEX, öğrenme ve hafıza işlevini olumsuz etkiledi, ancak lokomotor aktiviteyi ve kaygıyı etkilemedi.

Anahtar Kelimeler: Sevofluran, deksmedetomidin, yavru fareler, nörotoksik, kognitif değişiklikler

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INTRODUCTION

Diagnostic and therapeutic procedures are becoming more common worldwide as technology advances. This development has increased anesthesia exposure for people of all ages, including fetuses. More fetuses and children under three are expected to be subjected to general anesthesia each year. During their early years, children are more vulnerable than adults to the side effects of volatile anesthetics. Neurotoxic and cognitive changes have been observed in several species, raising concerns that small children may be harmed if exposed to inhalation anesthesia for prolonged periods or regularly.[1,2]

Sevoflurane (SEVO) is a GABAergic inhalational anesthetic agent commonly used for pediatric anesthesia due to its rapid onset of action, short recovery time, and non-irritation of the upper airway.[3] Today, GABA and NMDA-mediated neuronal activity play a crucial and well-documented role in cognitive processes.[4] On the other hand, dexmedetomidine (DEX) is a relatively new agent that is a selective agonist of α2-receptors with sedative, anxiolytic, analgesic, and anesthetic properties. [5] In addition, the neuroprotective effects of DEX have been reported in different animal models.[6]

People are becoming increasingly interested in the relationship between early anesthesia exposure and cognitive function. The primary emphasis of experimental pediatric anesthesia research is on the effects of anesthetics on the brain during the fetus's rapid neuromotor development and shortly after birth. Experimental studies in recent years suggest that inhalation anesthetics may have long-lasting and permanent effects in neurodevelopmental stages by increasing neuronal cell death (apoptosis) and decreasing neurogenesis.[7]

During synaptogenesis, human brain tissue is vulnerable to neurotoxic agents, particularly during the third trimester of pregnancy and the first 2–3 years of life.[8] Therefore, for the sake of public health, it is crucial to determine whether anesthetic toxicity manifests itself during the rapid neurodevelopmental period. As a result, more research is required to determine the potential neurotoxic effects of various anesthetic agents at different developmental stages, the factors that increase and reduce anesthesia-induced neurotoxicity, and possible mechanisms.

This experimental research aims to evaluate pup rats exposed to SEVO and DEX anesthesia during their early life stage by analyzing the immediate histological damage and behavioral changes in the pups.

MATERIAL AND METHOD

In this experiment, 36 Wistar albino rat pups (postnatal 21 days old [PD21]) were used (sample size calculated by power analyses). The rats were housed under standard laboratory conditions (12-hour daytime lighting [lights on from 07:00 to 19:00] and 12-hour nighttime lighting, 20–22°C room temperature, 50–60% humidity, ad libitum feeding) from birth. The day of birth was designated as post-natal day zero (P0). All pups were carefully monitored throughout the experiment for weight and general appearance. The rats were randomly assigned to the anesthesia or control groups (n=6 per group).

Olton and colleagues introduced the radial maze task in 1976 as a measure of working memory for spatial information.[9] This experiment was performed on an eight-arm maze apparatus. To determine behavioral manifestations of any possible acute developmental deficiency in the brain, behavioral parameters were collected for each animal before sacrifice, before, and on day three after anesthetic exposure.

PD21 infant rats were exposed to study drugs with compatible groups. A 2.5% SEVO concentration and 4 h of SEVO exposure were chosen based on previous studies for an induced apoptotic response without mortality.10 Effective anesthesia level was measured by the tail pinch test, with 3–4 lb pressure on the tail root for 15 seconds.

Group I: Control Group, 100% O_2 3L/min, 0.1 mL serum saline three times at the zeroth, second and 4th hour(h) time points intraperitoneally (IP) for determination of placebo effects (n= 6); Group II: 4 h 2.5% SEVO, (AbbVie Tıbbi Ilaclar San. ve Tic. Ltd. Sti. Istanbul-Türkiye) in 100% O_2 3L/min-1 (n=6); Group III: 4 h 2.5% SEVO in 100% O_2 3L/min-1 + 0.5 µg/kg-1 DEX, (Precedex-Abbott-USA) three times at 0., 2.,4. h time points IP (n=6); Group IV: 4 h 2.5% SEVO in 100% O_2 3L/min-1 + 5 µg/kg-1 DEX three times at 0., 2.,4. h time points IP (n=6); Group V: 100% O_2 3L/min-1 + 0.5 µg/kg-1 DEX three times at 0., 2.,4. h time points IP (n=6); Group VI: 100% O_2 3L/min-1 five µg/kg-1 DEX three times at the zeroth, second and 4th hour h time points IP (n= 6).

Each rat in six groups in every test received a 10-minute session on the RAM platform. A modified RAM test was performed twice during the pre-anesthesia and post-anesthesia periods. During this process, video recordings were taken by the researcher. All behavioral testing was conducted during the light cycle by an experimenter. Video records were analyzed blinded to the group allocation of the assessed animal. Pellets were placed on RAM platform arms 1, 2, 3, and 6, and the rats were then placed on the RAM setup to evaluate the hippocampal destruction.

The rats' learning (Reference Memory Error [RME]), memory (Working Memory Error [WME]), locomotor activity (Total Distance [TD]), and anxiety behavior (Rearing [R]) were evaluated respectively by utilizing the total number of the entries to the arm, number of the entries to the baited arm.

In the first and second experiment, the number of the entries to the arm and the number of the entries to the baited arm, respectively, were noted down. At the second experiment, if the rats entered the non-baited arm more than once, their rate was recorded as WME. Finally, the number of entries for the arm and arm length (number of arms entered × 2 × arm length) were calculated and recorded as TD. Rats' behavior, like standing on their rear limbs, was recorded as R. Supported rearing behavior, in which the animal rears against the arena walls, could also be observed in this test but was ignored in this experiment.

The survival rate and time of death at the time of exposure to anesthesia were recorded before the trial ended.

Tissue Sampling and Histopathological Evaluation

After the second RAM test, the rat pups were sacrificed while being put to sleep with ketamine (Alfamine 10%; Ata Fen Veteriner Malzemeleri, Izmir/Türkiye) and 5 mg/kg xylazine (Xylazinbio 2%; Intermed Ecza Deposu, Ankara/Türkiye) by intracardiac blood aspiration. Neonatal rat bilateral hippocampal brain tissues were harvested for neuroinflammation and apoptosis tests. Their brains were extracted from their bodies and coded at random. A 10% formol solution was used to protect the coded brain tissues. The brain samples were paraffin-embedded and stained with hematoxylin and eosin. (H&E). Taking Bregma Point into account, the rat brain tissue was coronally cut 4 µm thick. CA1, CA2, and CA3 sectors are given granular lamel neurons and were evaluated for inflammation, degenerative changes (necrosis, hyperemia, gliosis, and spongy changes), and apoptosis in the hippocampus and cornu ammonia. (×400 Olymphos). Apoptotic cells were counted and examined blindly at ×400 magnification.

Ethics

On February 21, 2020, Gazi University Local Ethics Committee for Animal Experiments in Ankara, Turkey, granted ethics clearance for this research (G.Ü. ET. 20.013 code number). The trial was carried out at Gazi University Experimental Research Center between the dates of 07.27.2020 and 08.21.2020. The experiments were carried out per the "Guide for the Care and Use of Laboratory Animals"

Statistical Analyses

Statistical analysis was performed in the SPSS 20.0 program. Statistical analysis data are expressed as mean±standard deviation (SD), median, minimum, maximum, and n (%). The p-value used to define statistical significance was $p<0.05$. The Shapiro–Wilk test was applied to the measurable parameters (number of entries to the arm and number of entries to the baited arm at the RAM setup) to determine whether the distribution was normal or not. The one-way ANOVA test in independent groups was used to determine whether there was a significant difference between groups. In case of a significant difference, a comparison was made between groups with Bonferroni correction for the post hoc analysis test. A Greenhouse–Geisser correction was used for sphericity analysis in repeated measurements. Statistical evaluations of histopathological data were made with the Kruskal–Wallis test. A post hoc analysis with Mann–Whitney U was performed, which obtained statistically significant differences between groups. Apoptotic cell density data are presented as mean±standard error of the mean and comparison between means was determined using one-way ANOVA followed by the Bonferroni post hoc test for all statistical tests.

RESULTS

All the rats in the experiment were PD21. Their mean weights±SD were as follows: Group I: 31.4±2.04 g, Group II: 31.4±1.7 g, Group III: 30.6±1.7 g, Group IV: 30.05±1.34 g, Group V: 31.3±2.1 g, Group VI: 29.7±1.5 g, and no statistically significant difference was found (p=0.370). No rats perished during anesthesia exposure on the third day. One rat in Group IV died before the second RAM test on the day of the trial. As a result, the data from only 35 rodents was included in this study.

The RAM test experiment for the rats was assessed before and after anesthesia. The number of entries to the arm, the number of non-baited arms, the number of re-entries to baited arms, and the TD and R numbers were all recorded during the first and second experiments. The number of entries to the non-baited arm and the number of re-entries to the baited arm were quantified as RME and WME during the second experiment.

The RME/TEN ratio was then determined. The values in each category were similar, but they had significant differences. For example, when the RME ratio was compared between the groups, there were substantial differences between Group II and Groups I, III, and IV (P=0.015, P=0.004, and P= 0.001). There was also a variation between Groups IV and VI (P=0.028). **Table 1** displays the RME value to TEN percentage (**Figure 1**).

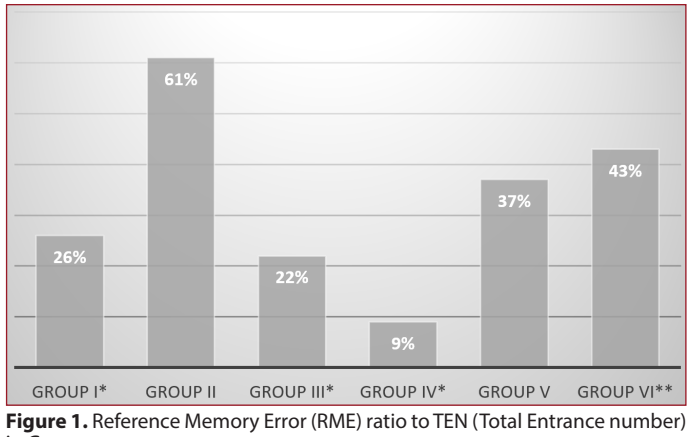

Figure 1. Reference Memory Error (RME) ratio to TEN (Total Entrance number) in Groups
*p<0,05 Comparison with Group II, **p<0,05 Comparison with Group IV
Comparison with Group I to Group II (p=0,015), Comparison with Group II to Group III (p=0,004), Comparison with Group II to Group IV (p<0,001), Comparison with Group IV to Group VI (p=0,028).

Table 1. Reference Memory Error (RME) in Groups (Mean±SD)

	Group I n=6	Group II n=6	Group III n=5	Group IV n=6	Group V n=6	Group VI n=6
TEN	14,67±7,91	19,33±7,528	18,50±6,71	12,37±12,3	10,17±3,601	15,83±2,229
RME	2,67±1,211	10,67±2,503	3,50±1,975	1,60±1,67	3,83±2,041	7±2
%RME	%25,80	%60,40	%21,30	%9,50	%37,26	%43,60

n: Rat number, RME: Referance Memory Error, TEN: Total Entrance Number (Mean) Values, %RME: Ratio of RME to TEN

Working memory errors were determined using the number of re-entries to the baited arm and TEN variables (**Figure 2**). The percentage of WME to TEN was calculated, and there was a significant variation between groups (p=0.001). Furthermore, a significant difference in WME was observed when comparing Group II to Groups I, III, IV, V, and VI (respectively, p=0.001, p=0.001, p=0.001, p=0.001, p=0.001, p=0.001, p=0.017, p=0.030). In addition, the comparison of Group IV to Group VI's variables revealed significant variations (p=0.039).

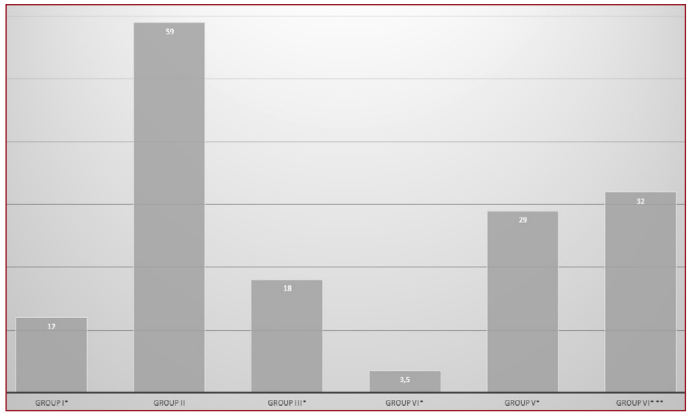

Figure 2. Working memory error (WME) ratio to total entrance number (TEN), (%)
*p<0,05 Comparison with Group II, **p<0,05 Comparison with Group IV
*(Comparison for Group II with Group I (p=0<0.001), Group III (p<0,001), Group IV (p<0,001), Group V (p=0.017), and Group VI (p=0.030)
**(Comparison for Group IV with Group VI (p=0,039)

TEN variables were used to evaluate learning behavior before and after anesthesia. Group II had a considerably higher total entrance number at pre-anesthesia versus post-anesthesia (p=0.045). Although TEN was was significantly higher (p=0.05) in Group VI, it was not in Groups I, III, IV, or V (p=0.671, p=0.590, p=0.278, and p=0.395) (**Table 2**).

Group VI had substantially longer pre-anesthesia and post-anesthesia TDs (p=0.009) than the other groups (**Table 3**) (**Figure 3**). TD was greater in some groups but not statistically significant in others, with the exception of Group VI (p=0.009). To evaluate anxiety, the rats' rearing number was measured during the post-anesthesia interval. On the second try, the rats were expected to exhibit less anxiety and increased R behavior (increase in R number). Heightening behavior, as if on two limbs, was commonly observed in Group IV but was not identified as R behavior. Groups V and VI saw an increase in the number of rearings. Group VI saw a significant increase (p=0.032) (**Table 4**).

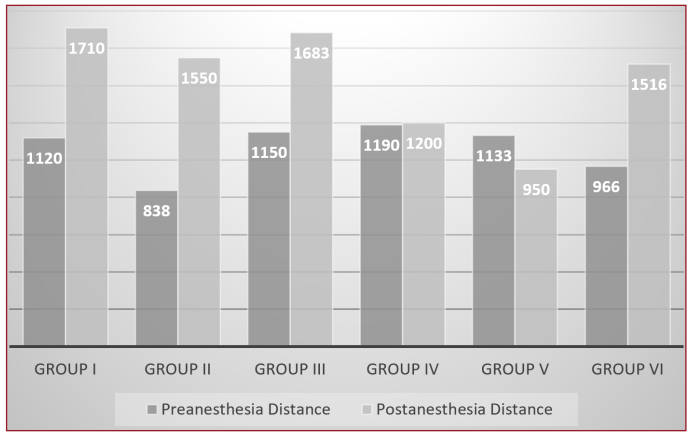

Figure 3. Preanesthesia and postanesthesia total distance variables in groups (cm)

Table 2. Comparison of preanesthesia and postanesthesia TEN values in Groups (Mean±SD).

	Group I n=6	Group II n=6	Group III n=5	Group IV n=6	Group V n=6	Group VI n=6
TEN Preanesthesia	16,17±7,25	10,17±3,971	20±3,162	17,8±5,167	14±8,602	11,5±2,950
TEN Postanesthesia	14,67±7,91	19,33±7,528	18,50±6,71	12,37±12,3	10,17±3,601	15,83±2,229
P	0,671	0,045*	0,59	0,278	0,395	0,05*

n: Rat number, TEN: Total Entrace Number (Mean) *p≤0.05 *(Preanesthesia versus Postanesthesia is significantly different in Group II, and Group VI)

Table 3. Preanesthesia and postanesthesia total distance (Mean±SD).

	Group I n=6	Group II n=6	Group III n=5	Group IV n=6	Group V n=6	Group VI n=6
Preanesthesia distance	1120±461,7	838±373,9	1150±384,7	1190±469,5	1133±753,4	966±273,2
Postanesthesia distance	1710±980	1550±788	1683±756	1200±1141,8	950±387	1516±248,3
p	0,171	0,137	0,146	0,982	0,673	0,009*

*p<0.05

Table 4. Rearing Number of the Groups (Mean)

	Group I n=6	Group II n=6	Group III n=5	Group IV n=6	Group V n=6	Group VI n=6
Rearing number Preanesthesia	1,83±1,6	0,83±0,75	3±3,6	1,2±1,7	4,33±2,7	0,83±0,73
Rearing number Postanesthesia	0,5±0,8	0,50±0,8	2,67±2,42	0	6±5,02	4±2,2
P	0,121	0,576	0,75	0,208	0,352	0,032*

Preanesthesia versus postanesthesia (Mean±SD), n= rat number, * p=0,05

No signs of neuro-inflammation were found in the histopathological evaluation (H&E staining) of the brain tissues in the groups. There was no statistically significant difference in apoptosis percentages between the hippocampus's CA1 (p=0.122) and CA2 (p=0.121) stages. However, in the CA3 stages, apoptosis percentages showed a significant difference among the groups (p=0.015) (**Figure 4**). CA3 region apoptosis ratio was higher in Group III than in Groups I, II, and IV and higher in Group IV than in Groups I, II, and VI. The significant difference was determined for comparison between Group III and Groups I (p=0.017), II (p=0.041), and VI (p=0.049), and for comparison between Group IV and Groups I (p=0.004), II (p=0.01), and VI (p=0.013) as a result of the post hoc analysis. Histological images of brain slices for apoptosis in groups are shown in **Figure 4**.

DISCUSSION

Pre-anesthetic and post-anesthetic cognitive functions (learning, memory, locomotor activity, and anxiety) of rats were measured in this experiment (PD21). An eight-arm RAM platform was used to assess RME, WME, TD, and R behavior in Wistar albino rats. Histopathologic techniques were also used to examine pup rat brain slices for neuro-inflammation and apoptosis after H&E staining.

There were more impairments in memory and learning in the experimental group than in the control group. Additionally, memory and learning ability impairments were reduced when DEX was added to SEVO. However, a single large dose of DEX (5 g/kg-1) had a more negative impact on learning and memory function than in the control group but not on locomotion or anxiety. The apoptosis ratio in the CA1 and CA2 regions of the hippocampus did not vary significantly between the groups when histopathological characteristics of the brain tissue were compared. However, it varied significantly between the groups in the CA3 region during the same period. Compared to the control, one 2.5% SEVO, and five g/kg-1 DEX applied groups, two groups exposed to SEVO with DEX showed reduced levels of apoptosis.

It is well known that Wistar albino rats can be used in studies performed to reveal neuro-apoptosis and cognitive tests. Both their genetic similarities to humans and their ability to perform in behavioral tests create a suitable basis for them to be used in studies of this nature. The brain in rats is structurally similar to that of humans (prosencephalon, mesencephalon, rhombencephalon-hindbrain). There are also similarities in terms of the development of neurological tissues. Synaptogenesis in rats develops between 7 and 30 days after birth.[11,12] One human year equals approximately two rat weeks (365÷26.7=13.8 rat days).[13] In this experiment, 21-day-old rats with the highest synaptogenesis were preferred to evaluate the rapid brain development phase.

Hemodynamic stability during anesthesia exposure is an important issue. Studies have shown that 3% SEVO exposure induces hypoxia or respiratory depression in rats.[14,15] Additionally, it is reported that the motor functions were not impaired in a study conducted with 30-day-old rats given SEVO for 4 h. However, in terms of learning and memory, SEVO caused cognitive deficits. Therefore, in this experiment, rats were exposed to 2.5% SEVO for 4 h to avoid hypoxia and respiratory depression.

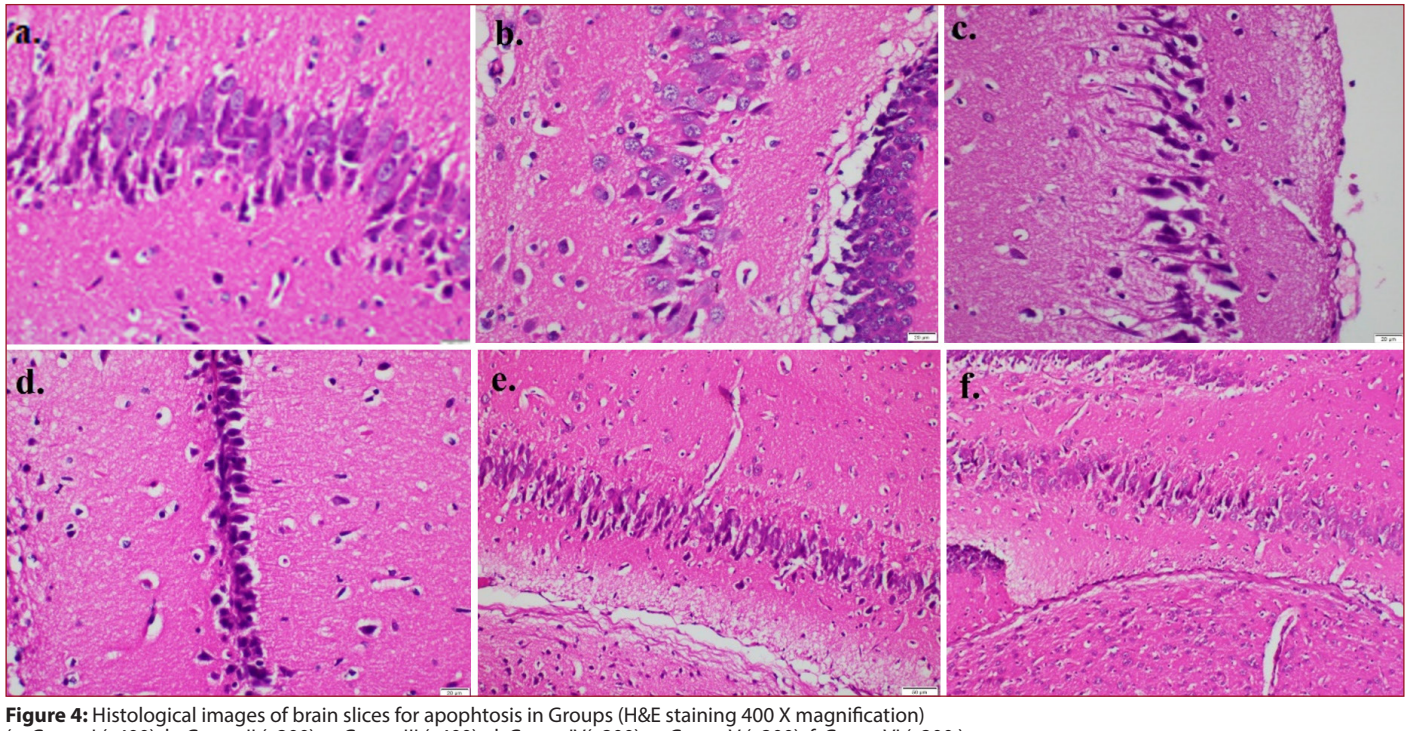

Figure 4: Histological images of brain slices for apophtosis in Groups (H&E staining 400 X magnification)
(a. Group I (x400), b. Group II (x200), c. Group III (x400), d. Group IV(x200), e. Group V (x200), f. Group VI (x200)

Reference memory error and WME are evidence of learning and memory problems. Learning behavior can be assessed with ten arms.[16,17] In addition, TD was measured to reveal locomotor activity before and after anesthesia. Pirke et al. also evaluated locomotor activity using a similar method.[18] Sagvolden et al. have also associated these behaviors with hyperactivity.[19]

Apoptosis is triggered by extrinsic and/or intrinsic cellular pathways. The central role of mitochondria in the intrinsic apoptotic death pathway has been established. Hippocampal neural apoptosis is related to the mitochondrial pathways, and this effect could be dose-dependent; however, studies on the pathways involved in the SEVO-induced development of brain apoptosis are limited. Shen et al. demonstrated that a higher dose of SEVO exposure at PD7 in Sprague Dawley rats leads to histopathological changes and apoptosis in the neonatal rat hippocampus and temporal neuro-cognition deficits.[20]

The hippocampus is a brain area that has an important role in spatial learning and memory functions.[21] Studies show that interrupting the hippocampal pathways causes significant memory deficits in the radial arm labyrinth test.[22] Recently, the CA3 region has attracted major attention for its specific role in memory processes and neuro-degeneration. CA1 region neurons receive and process information from the entorhinal cortex or CA3 region. A solid CA3 and CA1-CA3 connection is necessary for the reference memory. CA3 subfields have richer internal connectivity in hippocampal regions. CA3 pyramidal cells make excitatory contacts with neighboring inhibitory and excitatory neurons. This circuit is implicated in episodic memories and encoding spatial representations. The CA3 region receives inputs from the entorhinal cortex via mossy fiber connections. These connections are essential for memory formation.[23] In this study, baby rats' CA1, CA2, and CA3 brain regions were evaluated histologically.

Neurons are particularly sensitive during the synaptic plasticity phase. Inhalation anesthetics can induce neuronal apoptosis or programmed cell death in the developing brain, leading to long-term cognitive impairments. Exposure to SEVO during the early life period induces neuronal apoptosis, and cognitive dysfunction in a dose-recurrence and time-dependent manner in baby rats has been shown experimentally.[24] Furthermore, it has been demonstrated that higher doses of SEVO can cause histopathological changes and induce apoptosis in the neonatal rat hippocampus.[25] In our study, the number of RME and WME increased significantly in a single SEVO-exposed rat group compared to the control and other groups. But the evaluation time of the rats' RAM experiment was relatively earlier than in Li et al.'s experiment. It was revealed that the administration of a single dose of 2.5% SEVO for 4 h negatively affected cognitive functions in terms of behavior. This result is consistent with the research conducted by Perez-Zoghbi et al.[26]

A decrease in the number of neuronal cells can lead to decreased brain functions. Our experiment shows that a single 2.5% SEVO exposure for 4 h during the early stages of life in rats does not show histologically detectable neuroinflammation signs in the brain tissue. But, in the CA3 stage of the brain tissue, the apoptosis percentage was diminished in the SEVO+DEX groups compared to the control and single SEVO groups.

Not all experiments on rats report behavioral deficits after exposure to SEVO. For example, Chen et al. found that a lower dose of SEVO promotes hippocampal neurogenesis in neonatal rats and facilitates their experiment in dentate gyrus-dependent learning tasks.[27] In our experiment, post-anesthetic learning, evaluated on the RAM platform, was decreased in rats exposed solely to SEVO or SEVO + high-dose DEX.

Dexmedetomidine is a potent α2-adrenergic receptor agonist that is an adjunct to general anesthesia, reduces anesthetic doses, and provides analgesia and sedation in the perioperative period.

The findings from the published animal research on the comparative effects of SEVO and/or DEX exposure in the early life period in rats are contradictory. Goyagi reported that SEVO-dependent neurodegeneration decreased with DEX.[28] However, even DEX has been reported to decrease neuron apoptosis, and cognitive decline is caused by ketamine, isoflurane, and propofol.[29] In addition, DEX has been reported to have a protective effect in hypoxic-ischemic neonatal brains.[30] Perez-Zoghbi et al. reported that DEX reduces SEVO-induced apoptosis in several brain regions when used at 1 μg/kg^{-1} doses. But co-administration of DEX at 5 μg/kg^{-1} during SEVO anesthesia increased mortality.[31]

In this study, 0.5 μg/kg^{-1} and 5 μg/kg^{-1} DEX doses were used, and DEX added to SEVO caused less memory and learning function impairment. However, high doses (5 μg/kg-1) of DEX exposure negatively affected the learning and memory functions of the rats but not their locomotor activity or anxiety. In addition, one rat died just before the second RAM test experiment in the 5 μg/kg^{-1} DEX with SEVO group (Group IV).

The hippocampus is crucial for cognitive functions such as learning and memory in humans and animals. Several experimental studies have indicated that exposure to DEX ameliorates oxidative stress-induced cognitive deficits and restorative abnormal hippocampal synaptic plasticity.

The solely 2.5% SEVO-exposed group in this research had a higher RME and WME than the other groups and a higher WME than the high-dose DEX+SEVO group. Although there was no statistically significant difference between the DEX-only groups (Groups V and VI), they performed better regarding RME and WME than the control group. However, only the 5 g/kg^{-1} DEX group outperformed the 2.5% SEVO+5 g/kg^{-1} DEX groups regarding RME and WME.

The results of our experiment agreed with those of another study, which found that DEX reversed the negative effects of isoflurane and ketamine on learning. This implies that the negative impacts of SEVO can be mitigated with DEX.

Several pieces of literature have only used DEX to judge how well cognitive functions work.[16] According to, DEX enhances learning and spatial memory at a dose of 20 g/kg^{-1}. Another study in rats with increasing DEX doses found cerebral blood flow decreased, arterial blood pressure rose (with 10 g/kg^{-1} DEX), and cerebral vascular resistance rose.[32] An increase in RME and WME percentages in rats given five g/kg^{-1} DEX alone may be due to its impact on cerebral blood flow in our experiment. The healing impact of DEX, when coupled with SEVO, led us to believe that this effect was caused by compensation of cerebral perfusion pressure. According to Goyagi et al., 6.6, 12.5, and 25 g/kg-^{1} DEX heal long-term memory deficits and neuro-degeneration induced by SEVO in rats.[28] According to Perez-Zoghbi et al.[31] SEVO enhances brain cell apoptosis. Nevertheless, co-administration of SEVO with a low dose of DEX (1 g/kg^{-1}) did not affect the animal's reaction to external stimuli or apoptosis. However, larger doses of DEX (5–25 g/kg^{-1}) combined with SEVO increased brain cell apoptosis.

Rearing behavior in rats is related to anxiety control; therefore, anxiety was expected to diminish in rats that came to the RAM platform for the second time.[33] This research found diminished anxiety in solely DEX-administered rat groups (Groups V and VI). Morena et al.[34] reported that rat anxiety was increased with IP 300 mg/kg-1 propofol and 100–125 mg/kg^{-1} ketamine but decreased with 0.4 mg/kg^{-1} DEX. In our experiment, rats' anxiety was diminished in solely DEX- administered groups.

A low dose of SEVO (1.1%) did not cause apoptosis, but a high dose (2.5%) and long exposure time (6 h) were related to an intense apoptosis rate.[26] In our experiment, neuro-inflammation was not observed with H&E staining in groups. In the CA3 region, the apoptosis rate was lower (p=0.015) in DEX plus SEVO performed groups than in solely SEVO, control, and solely high DEX performed groups. This result was compatible with the conclusions of previous studies.[35,36] During the experiment, in the groups with SEVO plus DEX, exposure resulted in a lower apoptosis rate than in control and solely SEVO groups. Only a little research has examined a single DEX's apoptotic effect. Hoffmann et al.[37] reported that DEX improved neurological return in rats following transient brain ischemia. In addition, DEX can reduce neuroinflammation by diminishing the release of the pro-inflammatory cytokines IL1β and IL-6. Except for this, studies show that DEX decreases perinatal hypoxic brain damage by increasing neurotrophic factor expression. However, normally developing brain tissue was not examined in these studies. Based on this research, it might not be a safe anesthetic for babies, but rather only a safe anesthetic dose concentration and exposure time.

Limitations of the study

There were a number of limitations in this study. Initially, only the tail-pinch test was done under deep anesthesia, but studies indicate that the anesthesia level is adequate if it achieves immobility and unresponsiveness to the tail pinch test during anesthesia.[38] Furthermore, in rat experiments, the tail-clamp technique can be used to measure the minimum alveolar concentration. In a recent experiment, no delayed recovery and an absence of rat mortality during exposure to anesthesia was interpreted as indicating the proper depth of anesthesia. Second, to better comprehend drug effects, the blood level of DEX or inspiratory or expiratory SEVO concentration could have been measured. Unfortunately, a more sophisticated technique of histopathological evaluation could have been used. We were unable to assess this due to technical constraints. Finally, while all groups were exposed to the same oxygen concentration, a high (100% O_2) concentration may have impacted the findings.

CONCLUSION

The results depended on the properties of the chosen anesthetic agent, the doses of the agent, the time course of the application, the length of exposure, the cognitive experiment tests chosen, and the length of time these anesthetics are used. So, it can be said that SEVO and high-dose DEX, whether temporary or not, are not healthy for a rat's brain as it grows.

Cognitive functions are considerably impaired after SEVO anesthesia. DEX changes the effect of SEVO on cognitive functions in a dose-dependent manner, and DEX may cause increased locomotor activity at a high dose (5 µg/kg^{-1}). Since the negative effects are reduced using SEVO and DEX together, mechanisms other than apoptosis and inflammation should be kept in mind.

More experimental and clinical research can be done to fully understand how this effect happens, find ways to reduce it, and find drugs that are less likely to cause clinical neurotoxicity. First, we must look at other causes and mechanisms of the learning problems, such as how the blood flows through the hippocampus, parthenogenesis, and synaptogenesis.

ETHICAL DECLARATIONS

Ethics Committee Approval: On February 21, 2020, Gazi University Local Ethics Committee for Animal Experiments in Ankara, Turkey, granted ethics clearance for this research (G.Ü. ET. 20.013 code number). The trial was carried out at Gazi University Experimental Research Center between the dates of 07.27.2020 and 08.21.2020. The experiments were carried out per the "Guide for the Care and Use of Laboratory Animals"

Informed Consent: Written informed consent was obtained from all participants who participated in this study.

Referee Evaluation Process: Externally peer-reviewed.

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