North Clin Istanb 2024;11(1):1–9
doi: 10.14744/nci.2023.07888

Original Article ANESTHESIOLOGY & REANIMATION

Effect of sugammadex, rocuronium and sevoflurane on oxidative stress and apoptosis in cerebral ischemia reperfusion model in rats

Hakan Ciftci,[1] Nilay Tas,[2] Zubeyir Cebeci,[2] Sibel Kokturk,[3] Selma Cirrik,[4] Tevfik Noyan[5]

[1]Department of Anesthesiology and Reanimation, Taskopru State Hospital, Kastamonu, Turkiye
[2]Department of Anesthesiology and Reanimation, Ordu University Faculty of Medicine, Ordu, Turkiye
[3]Department of Histology and Embryology, Istanbul University Faculty of Medicine, Istanbul, Turkiye
[4]Department of Medical Physiology, Ordu University Faculty of Medicine, Ordu, Turkiye
[5]Department of Biochemistry, Ordu University Faculty of Medicine, Ordu, Turkiye

ABSTRACT

OBJECTIVE: Cerebral ischemia-reperfusion (I/R) injury causes neurological dysfunction and cell death. Sugammadex, as a large molecule, is normally difficult to pass through the blood-brain barrier (BBB). In ischemia, molecules can pass into the brain tissue. In this study, we aimed to evaluate the effect of sugammadex in the presence of cerebral I/R damage in rats with a general anesthesia model with sevoflurane and rocuronium.

METHODS: Rats were divided into 7 groups; Group 1 (Control), Group 2 (Sham), Group 3 (Sevoflurane), Group 4 (Sugammadex), Group 5 (Sevoflurane + Rocuronium), Group 6 (Sevoflurane + Sugammadex), Group 7 (Sevoflurane + Rocuronium + Sugammadex). Brain tissues of rats with cerebral I/R damage with bilateral carotid occlusion were removed. Tissue Malondialdehyde (MDA), Myeloperoxidase (MPO), and Superoxide dismutase (SOD) levels were examined with ELISA and apoptosis was examined by Caspase-3.

RESULTS: The number of caspase-3 positive cells decreased the most in Group 4 compared to the other groups. Group 4's mean MDA and MPO levels were lower than Group 2. There was no significant difference in terms of SOD levels.

CONCLUSION: The apoptotic effect of sugammadex was lowest compared to other agent groups, and it did not increase oxidative damage as much as the other groups.

Keywords: Apoptosis; cerebral ischemia reperfusion; oxidative stress; rocuronium; sevoflurane; sugammadex.

Cite this article as: *Ciftci H, Tas N, Cebeci Z, Kokturk S, Cirrik S, Noyan T. Effect of sugammadex, rocuronium and sevoflurane on oxidative stress and apoptosis in cerebral ischemia reperfusion model in rats. North Clin Istanb 2024;11(1):1–9.*

Brain ischemia is a serious health problem that occurs due to arterial obstruction and causes apoptosis and neuronal death [1, 2]. Various surgical procedures such as cardiopulmonary bypass and cerebral aneurysms may cause neurological dysfunctio [3]. Free oxygen radicals (ROS) are mainly responsible for the damage caused by reperfusion [4]. Specifically, inhalation agents and intravenous anesthetics are thought to have neuroprotective roles against cerebral I/R injury. Protecting the brain during surgery has been the most important target for anesthesiologists [3]. Sugammadex is an agent that has a modified gamma cyclodextrin structure and rapidly reverses rocuronium [5]. Sugammadex is normally difficult to pass through the blood-

Received: September 19, 2022 *Accepted:* February 02, 2023 *Online:* January 29, 2024

Correspondence: Hakan CIFTCI, MD. Taskopru Devlet Hastanesi, Anesteziyoloji ve Reanimasyon Klinigi, Kastamonu, Turkiye.
Tel: +90 366 417 10 32 e-mail: hakancft@gmail.com

brain barrier (BBB). In ischemia, the BBB is disrupted and molecules that are not able to pass can pass into the brain tissue. In this study, we aimed to evaluate the effect of sugammadex in the presence of cerebral I/R damage in rats with a general anesthesia model with sevoflurane and rocuronium.

Highlight key points

- General anesthesia model has apoptotic effects on the cerebral I/R injury model in rats.
- Single sugammadex administration has a low effect on apoptosis secondary to I/R damage and has the lowest apoptotic effect compared to other agents.
- Single sugammadex administration does not increase oxidative damage as much as other groups.

MATERIALS AND METHODS

This experimental study was initiated after the approval of the Ordu University Animal Experiments Local Ethics Committee with the number 82678388/07, dated 24.04.2018 and was supported by Ordu University Scientific Research Projects Coordination Department (Project Number: 2018/B-1821) Ordu, Turkiye.

In this study, Sprague-Dawley female rats with an average weight of 200–250 grams were used. The study was conducted in accordance with "Declaration of Helsinki" and "Guide for the Care and Use of Laboratory Animals". Rats were housed in cages at a circadian rhythm of 12 hours at night and 12 hours during the day, at 24–26 °C room temperature and 50–60% humidity before the experiment. Standard conditions were used for feeding rats. Before the surgery, anesthesia was administered by 60 mg/kg intraperitoneal (ip) Ketamine (Ketalar, 500 mg/10 ml, Pfizer Pharmaceuticals / Istanbul) and 10 mg/kg ip Xylazine (XylazinBio 2% 50 ml, Bioveta / Czech Republic). Depth of anesthesia was checked at appropriate intervals by tail compression method, additional doses of 10 mg/kg ip xylazine were administered if needed. During the experiment with pulse oximetry, peripheral oxygen saturation and heart rate monitoring with extremity electrodes were performed. The rats were randomly divided into 7 groups (n=5) in equal numbers. However, due to a problem experienced during tissue removal in one of the animals belonging to Group 1 during the study, this group continued to be 4 animals. In the experimental procedure, the Mapleson B circuit was used in company with Mindray WATO EX-65 Pro anesthesia device.

Experimental procedure: Group 1 (Control); No surgical procedures or drugs were administered. Group 2 (Sham); After anesthesia was administered surgical neck dissection was performed and they were left in cerebral ischemia for 10 minutes by placing a bilateral carotid clamp, and then they were reperfused for 2 hours by opening the clamps. Group 3 (Sevoflurane); After the rats were anesthetized, they were left in cerebral ischemia for 10 minutes by placing bilateral carotid clamps. At the end of ischemia period, the animals were tracheostomized and they were inhaled with 4 L/min 100% oxygen + 2% sevoflurane (Sevorane Liquid, AbbVie, UK) for 2 hours from the beginning of reperfusion. Group 4 (Sugammadex); rats were anesthetized and left in cerebral ischemia for 10 minutes by placing bilateral carotid clamps, and then they were reperfused for 2 hours by opening the clamps. At the beginning of reperfusion, 96 mg/kg sugammadex (Bridion, Organon, Holland) ip was administered to the animals. Group 5 (Sevoflurane + Rocuronium); After the rats were anesthetized, they were left in cerebral ischemia for 10 minutes by placing bilateral carotid clamps. At the end of the ischemia period, the animals were tracheostomized and inhaled with 4 L/min 100% oxygen + 2% sevoflurane for 2 hours from the beginning of reperfusion. At the beginning of the reperfusion, 1 mg/kg rocuronium (Esmeron, MSD, Germany) ip was administered to the animals. Group 6 (Sevoflurane + Sugammadex); After the rats were anesthetized, they were left in cerebral ischemia for 10 minutes by placing bilateral carotid clamps. At the end of the ischemia period, the animals were tracheostomized and inhaled with 4 L/min 100% oxygen + 2% sevoflurane for 2 hours from the beginning of reperfusion. At the beginning of reperfusion, 96 mg/kg sugammadex ip was administered to animals of this group. Group 7 (Sevoflurane + Rocuronium + Sugammadex); After the rats were anesthetized, they were left in cerebral ischemia for 10 minutes by placing bilateral carotid clamps. At the end of the ischemia period, the animals were tracheostomized and inhaled with 4 L/min 100% oxygen + 2% sevoflurane for 2 hours from the beginning of reperfusion. At the beginning of the reperfusion, 1 mg/kg rocuronium ip was injected into the animals. Animals in all groups were sacrificed after 2 hours and cerebral tissue samples were removed.

Histopathological evaluation; Caspase-3 Immunohistochemical Staining Method:

Table 1. Comparison of staining cell count

Groups	n	Mean	SD	Min	Max	p
Group 1	4	0.1125[f]	0.1315	0.0000	0.2500	
Group 2	5	16.280[a]	1.373	14.400	17.500	
Group 3	5	13.760[b]	0.307	13.500	14.250	
Group 4	5	2.650[e]	0.379	2.000	3.000	<0.001 (F=421.31)
Group 5	5	11.730[c]	0.463	11.250	12.400	
Group 6	5	8.400[d]	0.379	8.000	9.000	
Group 7	5	7.8500[d]	0.1369	7.7500	8.0000	

SD: Standard deviation; Min: Minimum; Max: Maximum; F; One-way Analysis of Variance (One-way ANOVA). The difference between groups without a common letter is statistically significant ($p<0.05$).

The left brain lobes of the animals, including the hippocampus, were taken. Immunohistochemistry staining was performed with Caspase-3 antibody (p17 (H-60), sc-98785 polyclonal rabbit antibody, Santa Cruz Biotechnology) using a ready-to-use immune staining kit (Santa Cruz Biotechnology for rabbit primary antibodies). They were photographed with the DP72 Olympus camera under an Olympus BX61 light microscope, covered with a sealing medium. Apoptotic Cell Count: The average of the counts made in at least four different areas of the CA1 region of the sections taken was obtained and thus the mean of the Caspase-3 positive cell counts of the groups was found. Hematoxylin Eosin Staining: Hematoxylin Eosin (H&E) staining was used to examine neuron degeneration in the hippocampus region.

Tissue Biochemical Analysis Procedure: Brain tissues stored in a -80 ^{0}C freezer were homogenized in phosphate buffer (1 gram wet tissue: 9 ml phosphate buffer) and centrifuged at 5000 g for 5 minutes. Malondialdehyde (MDA) (Elabscience, E-EL-0060) and Myeloperoxidase (MPO) (Cloud-Clone Corp, SEA601Ra) levels and Superoxide dismutase (SOD) activity (Elabscience, E-BC-K020) were measured in the supernatants obtained by the ELISA method. The manufacturer's instructions were followed for measurements using commercial kits.

Power analysis: The sample size is calculated as 5 for each group and 35 in total for 90% power and alpha 0.5, taking into account the results of the MDA measurement in the reference publication with the G-Power 3.1 program. determined [3].

Statistical Evaluation: Whether the data used in the statistical analysis conformed to the normal distribution in each group was evaluated by Shapiro-Wilk normality test. It was determined that the groups fit the normal distribution. One-way analysis of variance (One-way-ANOVA) was used in the assessment of the data. Homogeneity control of group variances was analyzed with the Levene test. Tukey's HSD test was used for homogeneous groups and Dunnett's T3 test was used for heterogeneous groups in determining the difference between groups. Tukey test results are expressed in the form of letter representation alongside the averages. All calculations were performed by using SPSS v25 (IBM Inc., Chicago, IL, USA) statistical package program.

RESULTS

Morphological evaluation with Hematoxylin and Eosin: Morphological findings such as pycnotic nucleus (condensed chromatin) and dark staining were observed, which are generally used to detect neuron degeneration in Figure 1.

Caspase -3 apoptotic cell evaluation: Morphological findings obtained from the Caspase-3 immunohistochemical staining method are shown in all groups in Figure 2. Caspase-3 positive apoptotic cells showed a significant increase in Group 2 and all other groups compared to Group 1 ($p<0.05$, Table 1, Fig. 3). Likewise, a significant increase was found in Groups 2, 3, 5, 6, and 7 compared to Group 1 and Group 4 ($p<0.05$, Table 1, Fig. 3). The number of caspase-3 positive cells decreased the most in Group 4 compared to the other groups ($p<0.05$, Table 1, Fig. 3). The arrangement of Caspase-3 positive cells between the groups according to the mean number increase was determined as Group 1, Group 4, Group 7, Group 6, Group 5, Group 3, and Group 2, respectively.

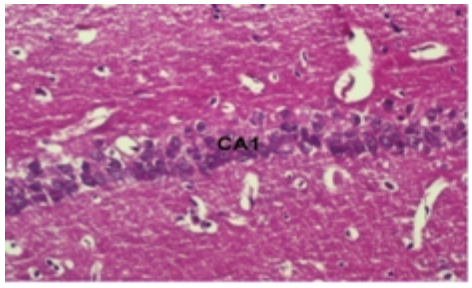

Figure 1a. Group 1, H&E.

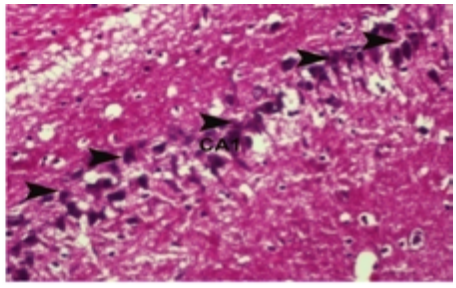

Figure 1b. Group 2, H&E.

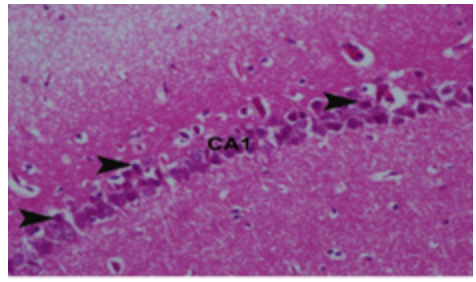

Figure 1c. Group 3, H&E.

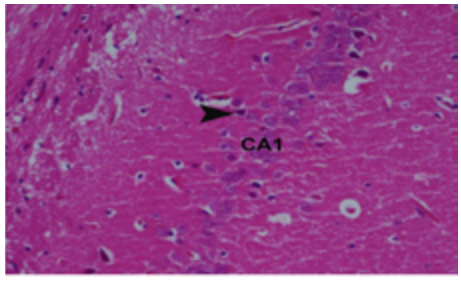

Figure 1d. Group 4, H&E.

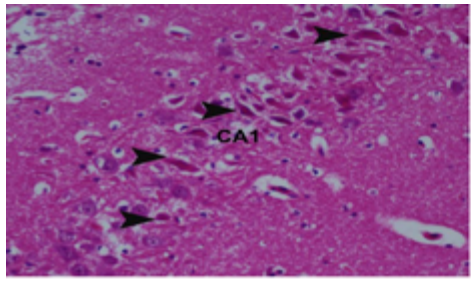

Figure 1e . Group 5, H&E.

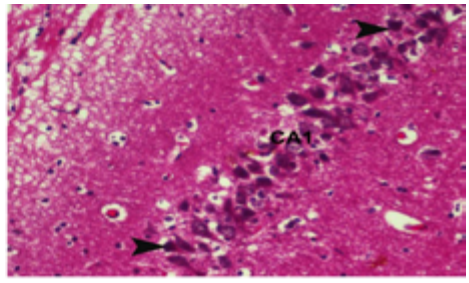

Figure 1f. Group 6, H&E.

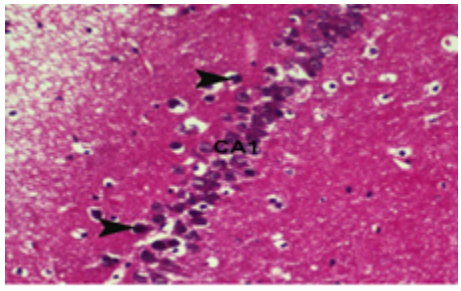

Figure 1g . Group 7, H&E.

Figure 1. Morphological evaluation images with Hematoxylin and Eosin in all groups. **(A)** Neurons showing normal morphology. **(B)** Neurons with degenerative morphology are seen in the CA1 region of the hippocampus with narrowed nuclei, condensed and dark stained. **(C)** Neurons showing degenerative morphology are seen in the CA1 region of the hippocampus. **(D)** Neurons showing degenerative morphology are seen in the CA1 region of the hippocampus. **(E)** Neurons with degenerative morphology are seen in the CA1 region of the hippocampus, whose nucleus is narrowed, condensed, and stained darkly. **(F)** Neurons showing a small amount of degenerative morphology in the CA1 region of the hippocampus. **(G)** Neurons showing a small amount of degenerative morphology in the CA1 region of the hippocampus (X40).

Table 2. Comparison of MDA levels (ng/mg protein)

Groups	n	Mean	SD	Min	Max	p
Group 1	4	9.550^{e}	0.525	8.853	10.096	
Group 2	5	17.380^{bc}	0.359	17.012	17.875	
Group 3	5	18.693^{ab}	0.844	17.602	19.966	
Group 4	5	14.439^{d}	0.806	13.503	15.468	<0.001 (F=92.72)
Group 5	5	19.454^{a}	0.869	18.565	20.679	
Group 6	5	16.430^{c}	0.830	15.474	17.712	
Group 7	5	17.443^{bc}	0.614	16.767	18.070	

MDA: Malondialdehyde; SD: Standard deviation; Min: Minimum; Max: Maximum; F; One-way Analysis of Variance (One-way ANOVA). The difference between groups without a common letter is statistically significant (p<0.05).

Tissue biochemistry evaluation results: In terms of MDA levels; a statistically significant increase was observed in Group 2, Group 3, Group 4, Group 5, Group 6, and Group 7 compared to Group 1 (p<0.05, Table 2). There was no statistically significant difference between Group 2, Group 3, and Group 7. The mean MDA level of Group 4 was lower than Group 2 and the difference between them was statistically significant (p<0.05, Table 2). In terms of MPO levels; A statistically significant increase was observed in Group 2, Group 3, Group 5, Group 6, and Group 7 compared to Group 1 (p<0.05, Table 3). Mean MPO

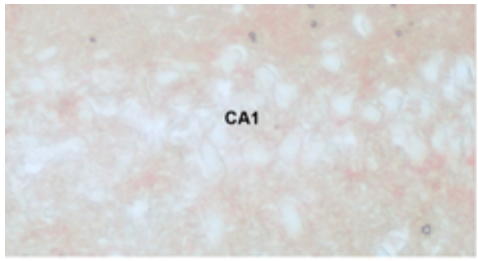

Figure 2a: Group 1, Caspase-3

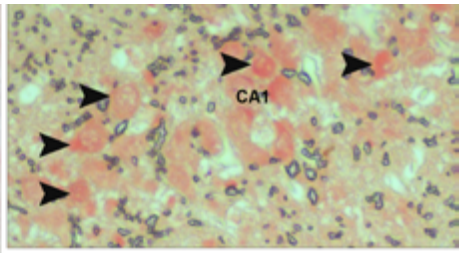

Figure 2b: Group 2, Caspase-3

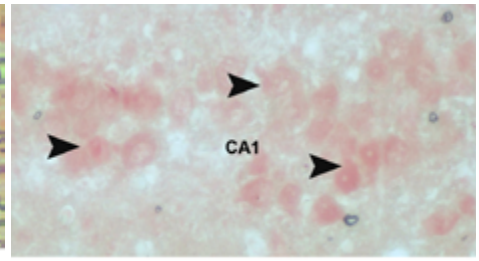

Figure 2c: Group 3, Caspase-3

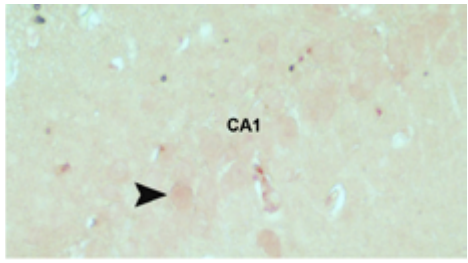

Figure 2d: Group 4, Caspase-3

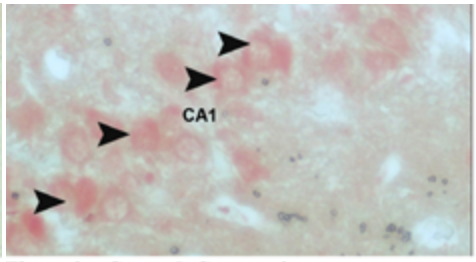

Figure 2e: Group 5, Caspase-3

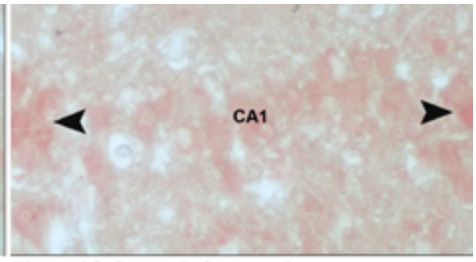

Figure 2f: Group 6, Caspase-3

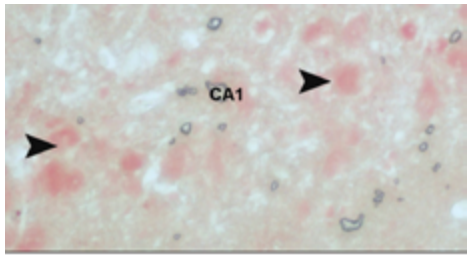

Figure 2g: Group 7, Caspase-3

FIGURE 2. Caspase-3 immunohistochemical staining morphological images in all groups.

TABLE 3. Comparison of MPO (ng/mg protein) levels

Groups	n	Mean	SD	Min	Max	p
Group 1	4	0.2235^{d}	0.0330	0.1937	0.2702	
Group 2	5	0.4595^{b}	0.0511	0.3873	0.5161	
Group 3	5	0.5336^{ab}	0.1130	0.4135	0.6589	
Group 4	5	0.30282^{cd}	0.01964	0.28514	0.33384	<0.001 (F=17.96)
Group 5	5	0.6102^{a}	0.0713	0.5089	0.7023	
Group 6	5	0.4241^{bc}	0.0648	0.3644	0.5304	
Group 7	5	0.4675^{b}	0.0634	0.3919	0.5624	

MPO: Myeloperoxidase; SD: Standard deviation; Min: Minimum; Max: Maximum; F; One-way Analysis of Variance (One-way ANOVA). The difference between groups without a common letter is statistically significant (p<0.05).

levels of Group 4 were found to be lower than Group 2 and the difference between them was statistically significant. Again, there was a statistically significant difference between Group 5 and Group 2 in terms of MPO levels, MPO levels of Group 5 were found to be high (p<0.05, Table 3). In terms of SOD levels; The mean SOD values of Group 2, Group 3, Group 4, Group 5, Group 6, and Group 7 were lower than Group 1 and a statistically significant difference was found between Group 1 (p<0.05, Table 4). There was no statistically significant difference between group 2 and the other groups in terms of SOD levels.

Table 4. Comparison of SOD activity (U/mg protein) levels

Groups	n	Mean	SD	Min	Max	p
Group 1	4	3.200[a]	0.508	2.458	3.603	
Group 2	5	1.211[b]	0.290	0.854	1.566	
Group 3	5	1.1253[b]	0.1629	0.8641	1.2375	
Group 4	5	1.622[b]	0.326	1.353	1.990	<0.001 (F=21.96)
Group 5	5	1.3492[b]	0.1721	1.1632	1.6106	
Group 6	5	1.489[b]	0.396	0.839	1.911	
Group 7	5	1.5711[b]	0.2094	1.3096	1.8026	

SOD: Superoxide dismutase; SD: Standard deviation; Min: Minimum; Max: Maximum; F; One-way Analysis of Variance (One-way ANOVA). The difference between groups without a common letter is statistically significant (p<0.05).

DISCUSSION

Cerebral ischemia causes permanent damage and cell death in the brain [6, 7]. Also in our study, morphological findings such as pycnotic nucleus were observed in animals with I/R damage and an increase was found in apoptotic cells compared to the control group. For anesthesia management, the selection of the most advantageous anesthetic agent in terms of cerebral protection is important [7]. Many studies have shown that volatile anesthetics have neuroprotective effects [8–11]. In the experimental study by Kim et al. [12], it was reported that apoptosis was reduced in the sevoflurane postconditioning group in cerebral ischemia. A theory is that sevoflurane decreases apoptosis-induced cell death [13–16]. In a cerebral ischemia model, it was shown that exposure to sevoflurane for 6 and 10 minutes had a histologically neuroprotective effect in rats [16–18]. In the study of Satomoto et al. [19] on rats, they reported that ultrastructural changes occurred in the BBB in the rat hippocampus with exposure to sevoflurane for 6 hours. It is known that inhalational anesthetics in clinical doses induce neuronal apoptosis, especially in newborn and elderly rodents [17, 18]. It has also been reported that anesthetic agents have a dose-dependent neurodegenerative effect in the growing rat cerebral tissue [18, 20, 21]. In our study, an increase in apoptotic cells was found in all groups treated with sevoflurane compared to the control group, and the arrangement of the sevoflurane groups according to the mean number increase of Caspase-3 positive cells, in order of increase; Group 7, Group 6, Group 5, and Group 3. Among the groups that received sevoflurane, the increase in the count of apoptotic cells in the group given single sevoflurane

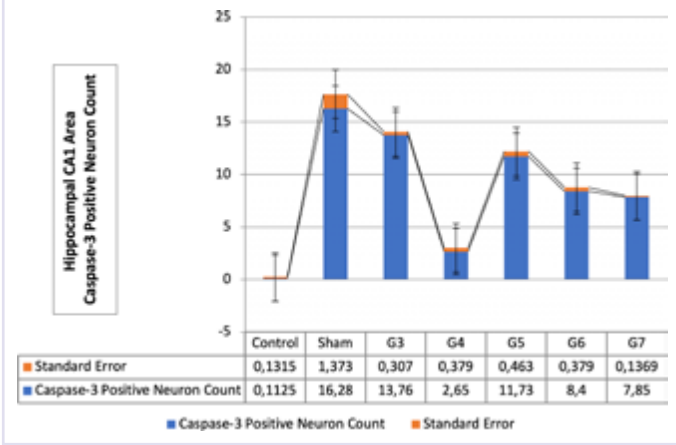

Figure 3. Hippocampal CA1 area Caspase-3 positive neuron count in all groups (p<0.05).

(Group 3) was the highest compared to the other groups that received sevoflurane. It is known that neuromuscular blockers do not exceed BBB due to their large molecular weight (rocuronium; 610 Da) [22]. Aldasaro et al. [23] showed that rocuronium and vecuronium had no direct effects on neurons in primary cultures. Langley et al. [24] showed that rocuronium can pass the BBB. Furthermore, Fuchs-Buder et al. [25] showed that rocuroniumcould not pass the BBB. In our study, the count of apoptotic cells in Group 5, the group in which we gave rocuronium with sevoflurane, and in Group 7, where we gave rocuronium + sugammadex with sevoflurane, were significantly different when compared to Group 2, which was the I/R group. In these groups with rocuronium, the mean increment in the count of Caspase-3 positive cells was found to be less than Group 2 and Group 3. With this result, it was concluded that rocuronium passes through the BBB,

which can undergo ultrastructural changes with the effect of both I/R damage and sevoflurane, and it may have a neuroprotective effect against apoptosis. It has been reported that cyclodextrins have a protective effect on cortical neurons and hippocampal regions [26–28]. Sugammadex is a gamma-cyclodextrin derivative [29–31]. It is thought that sugammadex does not have a toxic effect on the central nervous system, but in cases where the permeability of the BBB changes, the passage of sugammadex to the brain may change [3, 32, 33]. Some studies have also shown that gamma-cyclodextrins have a neuroprotective effect on cerebral hypoxia [3, 12, 16]. However, some experimental studies have concluded that sugammadex induces apoptosis [23, 32]. Ozbilgin et al. [3] concluded in their experimental study that they caused I / R damage by placing a carotid clamp and that the sugammadex they administered to rats improved both functionally and histopathologically on the damage. In this experimental study, the researchers reported that the results of TUNEL and caspase tests in the treatment groups treated with sugammadex were lesser than the results in the I/R group and that 100 mg/kg sugammadex dose was more protective than 16 mg/kg, both in terms of neurological functions and histopathologically. Satomoto et al. [19] reported that ultrastructural changes occurred in the BBB in the rat hippocampus after 6 hours of exposure to sevoflurane. Researchers reported that neuronal apoptosis did not develop with the administration of sugammadex and that administration of sugammadex together with sevoflurane caused a significant increase in apoptosis compared to single sevoflurane group. In this study Satomoto et al., [19] it was concluded that sugammadex, which is thought to not pass the BBB under normal conditions, passes the ENT that is disrupted by the effect of sevoflurane and induces neuronal apoptosis. In our study, it was determined that the count of Caspase-3 positive cells was reduced in Group 4 compared to the other groups. According to histopathological evaluations, it was concluded that sugammadex has a low I/R damage-enhancing effect, may have a neuroprotective effect, and this effect is mostly in Group 4 compared to other groups. It was observed that the apoptotic effect in Group 6 with sugammadex given with sevoflurane was lower than in Group 3, which was given single sevoflurane group. In the group in which sevoflurane, rocuronium, and sugammadex were administered together (Group 7), the count of Caspase-3 positive cells was less than in Group 3, which was given a single sevoflurane group. In our study, tissue MDA and MPO levels as markers of oxidative damage in I/R injury and SOD levels known to be an antioxidant biomarker were examined. Researchs have shown that SORs play a major role in neuronal loss after ischemic damage [33, 34]. SOR-induced lipid peroxidation is indirectly evaluated by the measurement of secondary products such as MDA. MDA is therefore widely used as an indicator of the oxidative state [35]. Acute inflammatory response resulting from neutrophil function during I/R injury can also be evaluated by secreted MPO levels. H_2O_2 (Hydrogen peroxide), $O_2^{\bullet -}$ (Superoxide anion), and $OH^{\bullet}$ (Hydroxyl radical) are produced by the reaction of the oxygen molecules. Antioxidant enzymes such as SOD protect the organism against these radicals [36]. In our study, MDA levels were found to be increased in tissue in all groups compared to Group 1. The mean MDA level of Group 4 was lower than in Group 2 and the difference between them was statistically significant. A significant increase was observed in Group 2, Group 3, Group 5, Group 6, and Group 7 compared to Group 1 in terms of MPO levels studied from tissue samples. Mean MPO levels in Group 4 were lower than Group 2. The most striking point in these results is that single sugammadex administration is the least effective on oxidative damage compared to other anesthetic agents. In our study, tissue SOD levels in all groups were significantly lower than Group 1, but no significant difference was found between the other groups and Group 2. Basal levels and production rates of antioxidant enzymes such as SOD are too slow to balance the production of SOR [37]. Although the increment in the expression of these enzymes may happen in ischemia, the endogenous antioxidant power may be depleted in a short time with the increase in superoxide and H_2O_2 concentrations [38, 39]. We think that the effects of the anesthetic agents we used in our study on the cerebral I/R injury were revealed more clearly than tissue biochemistry markers histopathologically. It is an important aim for researchers to find the most ideal anesthetic agent that can be preferred by considering the risk factors of patients during general anesthesia management.

Conclusion

In this study, we evaluated the oxidative stress and apoptotic effects of the general anesthesia model with amnesia, muscle relaxation, and reversing agent sugammadex on the cerebral I/R injury model. In our experimental research, we determined that the effect of a single sevoflurane on the increment in the count of apoptotic cells was the highest compared to the other groups and that

rocuronium could have a neuroprotective effect against apoptosis in case of I/R damage. The most striking result of our study is that single sugammadex administration has a low effect on apoptosis secondary to I/R damage, has the lowest apoptotic effect compared to other agents, and does not increase oxidative damage as much as other groups. With these results, we think that sevoflurane, rocuronium, and sugammadex can be used safely in the early stages of I/R injury such as hemorrhagic and/or ischemic stroke, with surgical procedures such as carotid endarterectomy, bypass and valvuloplasty.

Acknowledgements: We special thanks to Assist. Prof. Y. K. Arici, Assoc. Prof. E. Canakci, Assist. Prof. I. Coskun, A. Altinbas MD Specialist, A. Kilinc MD and M. S. Alp MD Ordu, Turkiye for helping our experimental research.

Ethics Committee Approval: The Ordu University Animal Experiments Local Ethics Committee granted approval for this study (date: 24.04.2018, number: 82678388/07).

Authorship Contributions: Concept – HC, NT, ZC, SK; Design – HC, NT, SK; Supervision – HC, ZC, SC, TN; Fundings – HC, NT, ZC; Materials – HC, NT, ZC, SC; Data collection and/or processing – HC, NT, SK, TN; Analysis and/or interpretation – SC, SK, TN, NT, HC; Literature review – NT, ZC, HC, TN; Writing – NT, HC, ZC, SC; Critical review – NT, ZC,TN.

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

Use of AI for Writing Assistance: Not declared.

Financial Disclosure: This study was supported by Ordu University Scientific Research Projects Coordination Department (Project Number: 2018/B-1821), Ordu, Turkiye.

Peer-review: Externally peer-reviewed.

REFERENCES

1. Dirnagl U, Iadecola C, Moskowitz MA. Pathobiology of ischaemic stroke: an integrated view. Trends Neurosci 1999;22:391–7. [CrossRef]
2. Bielewicz J, Kurzepa J, Łagowska-Lenard M, Bartosik-Psujek H. The novel views on the patomechanism of ischemic stroke. [Article in Polish]. Wiad Lek 2010;63:213–20.
3. Ozbilgin S, Yılmaz O, Ergur BU, Hancı V, Ozbal S, Yurtlu S, et al. Effectiveness of sugammadex for cerebral ischemia/reperfusion injury. Kaohsiung J Med Sci 2016;32:292–301. [CrossRef]
4. Wilhelm J. Metabolic aspects of membrane lipid peroxidation. Acta Univ Carol Med Monogr 1990;137:1–53.
5. Welliver M, McDonough J, Kalynych N, Redfern R. Discovery, development, and clinical application of sugammadex sodium, a selective relaxant binding agent. Drug Des Devel Ther 2009;2:49–59. [CrossRef]
6. Jeon YT, Hwang JW, Lim YJ, Park SK, Park HP. Postischemic sevoflurane offers no additional neuroprotective benefit to preischemic dexmedetomidine. J Neurosurg Anesthesiol 2013;25:184–90. [CrossRef]
7. Schifilliti D, Grasso G, Conti A, Fodale V. Anaesthetic-related neuroprotection: intravenous or inhalational agents? CNS Drugs 2010;24:893–907. [CrossRef]
8. Codaccioni JL, Velly LJ, Moubarik C, Bruder NJ, Pisano PS, Guillet BA. Sevoflurane preconditioning against focal cerebral ischemia: inhibition of apoptosis in the face of transient improvement of neurological outcome. Anesthesiology 2009;110:1271–8. [CrossRef]
9. Engelhard KC, Reeker WW, Lu H, Möllenberg O, Mielke L, Kochs E. Desflurane and isoflurane improve neurological outcome after incomplete cerebral ischaemia in rats. Br J Anaesth 1999;83:415–21. [CrossRef]
10. Haelewyn B, Yvon A, Hanouz JL, MacKenzie ET, Ducouret P, Gérard JL, et al. Desflurane affords greater protection than halothane against focal cerebral ischaemia in the rat. Br J Anaesth 2003;91:390–6.
11. Werner C, Möllenberg O, Kochs E, Schulte J, Esch AM, Werner C, et al. Sevoflurane improves neurological outcome after incomplete cerebral ischaemia in rats. Br J Anaesth 1995;75:756–60. [CrossRef]
12. Kim HC, Kim E, Bae JI, Lee KH, Jeon YT, Hwang JW, et al. Sevoflurane postconditioning reduces apoptosis by activating the JAK-STAT pathway after transient global cerebral ischemi in rats. J Neurosurg Anesthesiol 2017;29:37–45. [CrossRef]
13. Inoue S, Davis DP, Drummond JC, Cole DJ, Patel PM. The combination of isoflurane and caspase 8 inhibition results in sustained neuroprotection in rats subject to focal cerebral ischemia. Anesth Analg 2006;102:1548–55. [CrossRef]
14. Kawaguchi M, Kimbro JR, Drummond JC, Cole DJ, Kelly PJ, Patel PM. Isoflurane delays but does not prevent cerebral infarction in rats subjected to focal ischemia. Anesthesiology 2000;92:1335–42.
15. Pape M, Engelhard K, Eberspächer E, Hollweck R, Kellermann K, Zintner S, et al. The long-term effect of sevoflurane on neuronal cell damage and expression of apoptotic factors after cerebral ischemia and reperfusion in rats. Anesth Analg 2006;103:173–9. [CrossRef]
16. Park HP, Jeong EJ, Kim MH, Hwang JW, Lim YJ, Min SW, et al. Effects of sevoflurane on neuronal cell damage after severe cerebral ischemia in rats. Korean J Anesthesiol 2011;61:327–31. Erratum in: Korean J Anesthesiol 2020;73:85. [CrossRef]
17. Jevtovic-Todorovic V, Hartman RE, Izumi Y, Benshoff ND, Dikranian K, Zorumski CF, et al. Early exposure to common anesthetic agents causes widespread neurodegeneration in the developing rat brain and persistent learning deficits. J Neurosci 2003;23:876–82. [CrossRef]
18. Sun Z, Satomoto M, Adachi YU, Kinoshita H, Makita K. Inhibiting NADPH oxidase protects against long-term memory impairment induced by neonatal sevoflurane exposure in mice. Br J Anaesth 2016;117:80–6. [CrossRef]
19. Satomoto M, Sun Z, Adachi YU, Makita K. Sugammadex-enhanced neuronal apoptosis following neonatal sevoflurane exposure in mice. Anesthesiol Res Pract 2016;2016:9682703. Erratum in: Anesthesiol Res Pract 2017;2017:1368514. [CrossRef]
20. Loepke AW, Soriano SG. An assessment of the effects of general anesthetics on developing brain structure and neurocognitive function. Anesth Analg 2008;106:1681–707. [CrossRef]
21. Satomoto M, Satoh Y, Terui K, Miyao H, Takishima K, Ito M, et al. Neonatal exposure to sevoflurane induces abnormal social behaviors and deficits in fear conditioning in mice. Anesthesiology 2009;110:628–37. [CrossRef]
22. Pardridge WM. Drug transport across the blood-brain barrier. J Cereb Blood Flow Metab 2012;32:1959–72. [CrossRef]
23. Aldasoro M, Jorda A, Aldasoro C, Marchio P, Guerra-Ojeda S, Gimeno-Raga M, et al. Neuronal effects of sugammadex in combination with rocuronium or vecuronium. Int J Med Sci 2017;14:224–30.
24. Langley RJ, McFadzean J, McCormack J. The presumed central nervous system effects of rocuronium in a neonate and its reversal with sugammadex. Paediatr Anaesth 2016;26:109–11. [CrossRef]

25. Fuchs-Buder T, Strowitzki M, Rentsch K, Schreiber JU, Philipp-Osterman S, Kleinschmidt S. Concentration of rocuronium in cerebrospinal fluid of patients undergoing cerebral aneurysm clipping. Br J Anaesth 2004;92:419–21. [CrossRef]
26. Abulrob A, Tauskela JS, Mealing G, Brunette E, Faid K, Stanimirovic D. Protection by cholesterol-extracting cyclodextrins: a role for N-methyl-D-aspartate receptor redistribution. J Neurochem 2005;92:1477–86.
27. Rufini S, Grossi D, Luly P, Tancredi V, Frank C, D'Arcangelo G. Cholesterol depletion inhibits electrophysiological changes induced by anoxia in CA1 region of rat hippocampal slices. Brain Res 2009;1298:178–85. [CrossRef]
28. Yao J, Ho D, Calingasan NY, Pipalia NH, Lin MT, Beal MF. Neuroprotection by cyclodextrin in cell and mouse models of Alzheimer disease. J Exp Med 2012;209:2501–13. [CrossRef]
29. Fink H, Schaller SJ. Sugammadex as a reversal agent for neuromuscular block: an evidence-based review. Core Evid 2013;8:57–67. [CrossRef]
30. Sokół-Kobielska E. Sugammadex - indications and clinical use. Anaesthesiol Intensive Ther 2013;45:106–10. [CrossRef]
31. Yang LP, Keam SJ. Sugammadex: a review of its use in anaesthetic practice. Drugs 2009;69:919–42. [CrossRef]
32. Palanca JM, Aguirre-Rueda D, Granell MV, Aldasoro M, Garcia A, Iradi A, et al. Sugammadex, a neuromuscular blockade reversal agent, causes neuronal apoptosis in primary cultures. Int J Med Sci 2013;10:1278–85. [CrossRef]
33. Valencia A, Morán J. Reactive oxygen species induce different cell death mechanisms in cultured neurons. Free Radic Biol Med 2004;36:1112–25. [CrossRef]
34. Pekcetin C, Kiray M, Ergur BU, Tugyan K, Bagriyanik HA, Erbil G, et al. Carnosine attenuates oxidative stress and apoptosis in transient cerebral ischemia in rats. Acta Biol Hung 2009;60:137–48. [CrossRef]
35. Draper HH, Hadley M. Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol 1990;186:421–31. [CrossRef]
36. Reiter R, Tang L, Garcia JJ, Muñoz-Hoyos A. Pharmacological actions of melatonin in oxygen radical pathophysiology. Life Sci 1997;60:2255–71. [CrossRef]
37. Xing C, Arai K, Lo EH, Hommel M. Pathophysiologic cascades in ischemic stroke. Int J Stroke 2012;7:378–85. [CrossRef]
38. Fukui S, Ookawara T, Nawashiro H, Suzuki K, Shima K. Post-ischemic transcriptional and translational responses of EC-SOD in mouse brain and serum. Free Radic Biol Med 2002;32:289–98. [CrossRef]
39. Warner DS, Sheng H, Batinić-Haberle I. Oxidants, antioxidants and the ischemic brain. J Exp Biol 2004;207:3221–31. [CrossRef]