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Effects of rocuronium-induced neuromuscular blockade with sugammadex on diaphragm in rats

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Abstract

Aim: Sugammadex is a recently developed γ-cyclodextrin agent that can reverse the effects of blockers such as rocuronium used to maintain non-depolarizing neuromuscular blockade. In this study, it was aimed to determine the effects of rocuronium and sugammadex applied to rats on the diaphragm.
Materials and Methods: A total of 32, 8-10 week old Sprague-Dawley male rats were used in the study. Experimental animals were divided into 4 groups with 8 animals in each group. The control group did not receive any application with an experimental period. The sham group was given a volume of physiological saline equivalent to 16 mg/kg Sugammadex. Sugammadex group received 16 mg/kg sugammadex and sugammadex+rocuronium group received 16 mg/kg sugammadex followed by 1 mg/kg rocuronium. At the end of the experiment, diaphragms of all rats were removed. Tissues were evaluated histopathologically for the location of nuclei in the fibers, their serosal structures, cell infiltrations, fiber diameter and changes in morphology.
Results: The cores of the fibers were located in the peripheral areas in sugammedex examinations. In this group, interstitial edema and an increased number of fibers were detected with some morphological changes compared to the Sham and Control Group. The diameters of the fibers were decreased compared to the Sham and Control Group. In the cross-sectional images of the rocuronium-sugammadex Group, it was found that the fiber diameter decreased compared to other groups, and edema increased in the interstitial area. This difference was found to be significant according to the statistical analysis using histopathological scoring ($p < 0.05$).The increase in the fibers undergoing morphological changes was at a significant level.
Conclusion: Sugammadex and Rocuronium-Sugammadex Complexes cause histopathological changes in diaphragmatic cells.

Introduction

A neuromuscular blocking agent is used frequently in patients who undergo surgery that requires general anesthesia to improve surgical conditions. Rocuronium is a rapid-medium onset and non-depolarizing neuromuscular blocking agent [1]. The neuromuscular block must be reversed at the end of the surgery. For rocuronium, reversal agents include Anticholinesterases and Sugammadex. anticholinesterases act by competing with neuromuscular blocking agents for acetylcholine receptors and restoring neurotransmission into its initial state. However, sugammadex is a neuromuscular reverse agent that acts with a different mechanism than acetylcholinesterase inhibitors [2]. It is in the aminosteroid neuromuscular blocking drugs group. Sugammadex reverses the blockage by encapsulating the neuromuscular blocking agents [3]. The diaphragm is the most important inspiratory muscle. The use of neuromuscular blockers is related with postoperative pulmonary complications. Decreased diaphragm activity may causethat postoperative atelectasis develops [4]. The exacteffect of neuromuscular blockers and reversal agents on the diaphragm muscle has not yet been identified well. For this reason, our purpose in this study was to evaluate the effects of the reversal of the neuromuscular blockage induced by Rocuronium with sugammadex on the diaphragm in rats with histopathological methods.

Materials and Methods

Local Ethics Committee Approval was obtained from Adiyaman University Animal Experiments Department for the study (Protocol No: ADYU-HADYEK 2018/027). Animal experimental protocols and institutional guidelines were obeyed. A total of 32 adult 8-10 week old Sprague-Dawley male rats were given a standard diet and water in the study. The temperature and humidity of the setting

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of the animals were monitored daily and werekept stable with a 12-hour light-dark cycle. The animals were divided into four groupswith 8 in each group.

Control Group: This group consisted of animals that were not given drug treatment.

Sham Control Group: The rats were administered 0.9% intravenous saline, and a volume that was equivalent to 16 mg / kg Sugammadex.

Sugammadex Group (SG): Sugammadex was administered to the rats intravenously at16 mg / kg (Bridion®; Schering - Plough Corporation, Oss, the Netherlands).

Rocuronium and Sugammadex Group (R-SG): The rats were administered 1 mg/kg intravenous Rocuronium (Esmeron®; Organon, Istanbul, Turkey), and 16 mg / kg intravenous Sugammadex (Bridion®; Schering - Plow Corporation, Oss, the Netherlands) three minutes later.

All the drugs were administered intravenously into the tail vein. The diaphragmatic tissues of the rats were removed under anesthesia after this procedure. All animals were euthanized at the end of the procedure.

Histopathological Evaluations

When the experimental application procedures were completed on the animals, the diaphragm tissue samples of the groups were taken and embedded in 10% formaldehyde solution for fixation for approximately 1 week. Following this, a routine histological tissue follow-up procedure was performed with alcohol, xylene and parablast chemicals. Then, the tissue samples were embedded in paraffin blocks; and 5-µm thin sections were taken from the paraffin blocks for histopathological examination. These sections were deparaffinized with xylene; and stained with Hematoxylin-Eosin (H&E) and Masson Triple Staining Method before they were examined with Carl Zeiss brand Axiocam ERc5 model digital camera attachedwith a microscope, and were evaluated histopathologically for the location of the nuclei in fibers, serosal structures, cell infiltrations, changes in fiber diameters, and in terms of morphology.

Histopathological Scoring

In this study, semi-quantitative assessment was made using Arnardottir 's histological scoring system [5]. Classification as 0 (normal), 1 (mild), 2 (moderate), and 3 (severe) was made in each animal for findings such as morphological change in muscle fiber, interstitial edema, inflammation, fibrosis and dilatation of a blood vessel.

Statistical Analysis

Whether the data showed normal distribution was determined by Shapiro-Wilk normality tests. The data that did show normal distribution were performed with One-Way ANOVA, a parametric test, and comparisons between groups were shown using Tamhane's test which is done the variances are not homogeneously distributed. The upper limit of significance level was taken as $p = 0.05$. The data were analyzed with IBM SPSS Statistics 25.

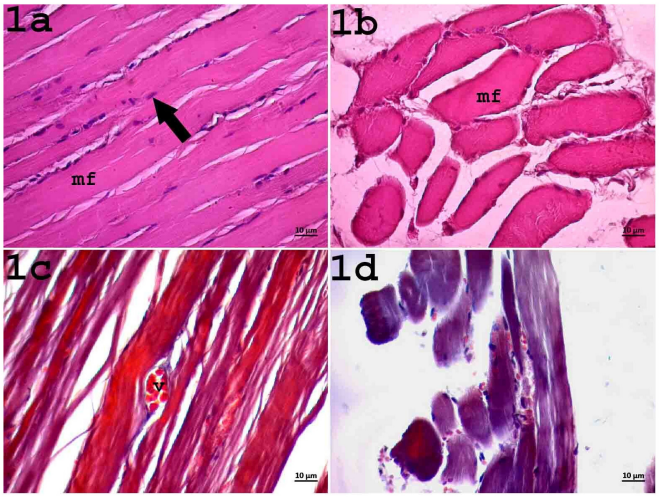

Figure 1. Histological sections of the control group Longitudinal section light microscopic view of diaphragm tissues of group 1a (x40, Hemotoxylin-Eosin staining)Cross-section light microscopic view of diaphragmatic tissues of group 1b (x40, Hemotoxylin-Eosin staining)Light microscope longitudinal section (x40, triple staining) of diaphragmatic tissues belonging to group 1c, cross-section of diaphragmatic tissues belonging to group 1d (x40, triple staining), black arrow;nuclei of the fiber, mf; muscle fiber, *; interstitial tissue, v;vascular tissue

Results

As a result of the histological examinations of the sections that were stained with Hematoxylin-Eosin, no pathological findings were detected in the images of the Sham and Control Group. The morphological structure of the fibers and muscular structureshad normal nuclei in these groups (Figures 1a and 1b, 2a and 2b). Also, no dilatations, connective tissue fibrosis, and inflammation were detected in the vascular structures in triple staining (Figures 1c and 1d, 2c and 2d). The nuclei of the fibers were located in the periphery in Group 3, whichalso had a small amount of intestinal edema and an increased number of fibers with some morphological changes compared to the Sham and Control Group. Also, fiber diameter was reduced when compared to Group 1 and Group 2 (Figures 3a and 3b). In cross-sectional images in Group 4, the decrease in the fiber diameter when compared to other groups, and depending on this, the increase in edema in the interstitial area were noted. Also, the increase in fibers with morphological changes was at significant levels (Figures 4a and 4b). As it was the case in other groups, there were no findings of dilatation, connective tissue fibrosis, and inflammation in vascular structures in triple staining in Group 3 and Group 4, (Figures 3c and 3d and Figures 4c and 4d).

Histopathological Scoring Results

In the study, no significant pathological finding was identified in tissue samples from group 1 and group 2 ($p > 0.05$). An increasing degree of pathological findings was revealed by the assessment of interstitial edema in the tissue samples from the group 4 compared to group 1 and group 2

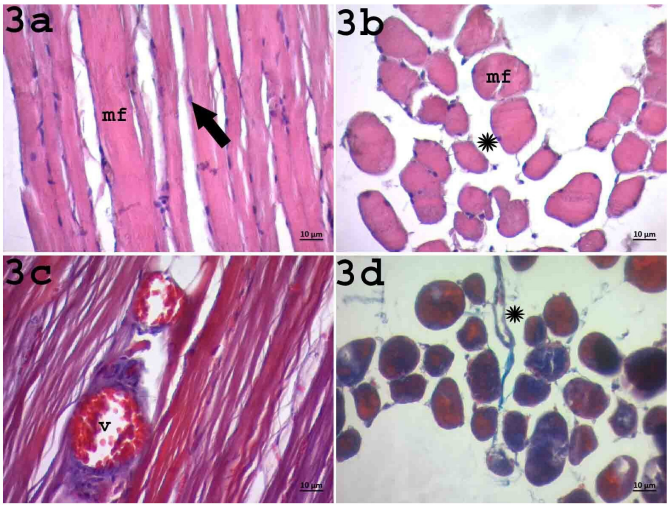

Figure 2. Histological sections of the sham control group Longitudinal section light microscopic view of diaphragm tissues of group 2a (x40, Hemotoxylin-Eosin staining)Cross-section light microscopic view of diaphragmatic tissues of group 2b (x40, Hemotoxylin-Eosin staining)Light microscope longitudinal section (x40, triple staining) of diaphragmatic tissues belonging to group 2c, cross-section of diaphragmatic tissues belonging to group 2d (x40, triple staining), black arrow nuclei of the fiber, mf; muscle fiber, *; interstitial tissue, v;vascular tissue

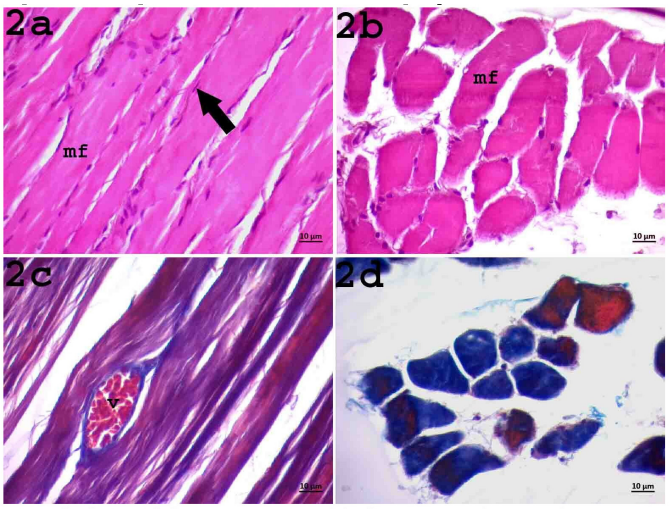

Figure 3. Histological sections of the sugammadex group Longitudinal section light microscopic view of diaphragm tissues of group 3a (x40, Hemotoxylin-Eosin staining)Cross-section light microscopic view of diaphragmatic tissues of group 3b (x40, Hemotoxylin-Eosin staining) Light microscope longitudinal section (x40, triple staining) of diaphragmatic tissues belonging to group 3c, cross-section of diaphragmatic tissues belonging to group 3d (x40, triple staining), black arrow;nuclei of the fiber, mf; muscle fiber, *; interstitial tissue, v;vascular tissue

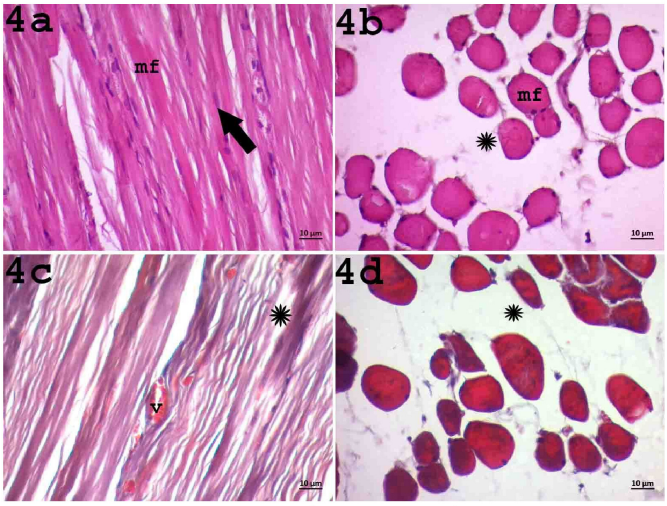

Figure 4. Histological sections of the rocuronium-sugammadex group
Longitudinal section light microscopic view of diaphragm tissues of group 4a (x40, Hemotoxylin-Eosin staining)Cross-section light microscopic view of diaphragmatic tissues of group 4b (x40, Hemotoxylin-Eosin staining)Light microscope longitudinal section (x40, triple staining) of diaphragmatic tissues belonging to group 4c, cross-section of diaphragmatic tissues belonging to group 4d (x40, triple staining), black arrow; nuclei of the fiber, mf; muscle fiber, *; interstitial tissue, v;vascular tissue

($p < 0.001$). There was no significant difference between the groups in terms of inflammation, fibrosis and dilatation of a blood vessel ($p > 0.05$) (Table 1).

Discussion

The diaphragm has a muscular-membranous structure with a membrane in the middle separating the abdominal and thoracic cavities from each other, and its edges consist of muscles. The muscle tissue forming the edges of the diaphragm consists of striated muscles. The diaphragm is the most important inspiratory muscle. Decreased activity of the diaphragm is associated with the development of atelectasis in the postoperative period [6, 7]. Several histological and biochemical changes were reported in the diaphragms of animals in the study conducted by Jaber et al. Among these, the most prominent was muscle fiber atrophy as animportant change as a result of decreased protein synthesis and increased proteolysis [8]. In the study conducted by Maes et al., the diaphragm was examined histopathologically 24 hours after Rocuronium administration, and no significant differenceswere detected in the cross-sectional area of the fibers in the control groups. However, the diaphragm cross-sectional area of type IIx / b fibers tended to decrease at a rate of 13% after Rocuronium was administrated [9].

Supporting the effects of drugs that are used in clinical practice with animal experiments conducted under proper conditions is important in terms of the contribution to human healthcare [10]. The clinical outcomes of the re-

Table 1. Descriptive analysis of histopathological parameters scores

	Group 1	Group 2	Group 3	Group 4
Morphological changes	0.63 ± 0.744	0.88 ± 0.835	1.38 ± 0.744	1.88 ± 1.126
Interstitial edema	0.75 ± 0.463	1.00 ± 0.535	1.50 ± 0.756	2.38 ± 0.744*
Inflammation	0.38 ± 0.518	0.75 ± 0.463	0.50 ± 0.535	0.38 ± 0.518
Fibrosis	0.25 ± 0.463	0.88 ± 0.835	0.63 ± 0.744	0.50 ± 0.756
Dilatation	0.50 ± 0.535	0.63 ± 0.744	0.38 ± 0.518	0.63 ± 0.518

* It expresses the highly significant difference between the group 4 with group 1 and group 2 ($p < 0.005$)

versal of the neuromuscular blockage induced by Rocuronium with Sugammadex werereported in many animal and human studies [1, 4, 11]. Our literature review did not reveal any direct studies that were conductedon Rocuronium and Sugammadex with histopathological changes in the diaphragmatic muscle. Previous studies also showed that circulating steroidal drugs or steroid treatments that lasted for longer durations caused skeletal muscle changes and myopathy. The myopathy related to steroid use is more common with the use of steroids with Fluorsuch as Dexamethasone [9]. In the literature review, it was found that the use of Sugammadex-Rocuronium complexes was questioned because of the drug accumulation potential with the steroid content in the structure, and it was shown that the drugs with steroidal structure remaining in the circulation for longerdurations caused skeletal muscle changes [12, 13].

In our study, significant histopathological changes were detected in the diaphragmatic cells of the rats that were treated with Rocuronium and Sugammadex. We also observed pathological changes similar to Myopathy in the Sugammadex Group; and significant changes were detected in the Sugammadex-Rocuronium Group. Our results are consistent with previous clinical studies which showeddetrimental effects on muscle functions [13]. Testelmans et al. [13] reported that they did not observe histopathological changes with H&E Staining in the diaphragmatic cells or gastrocnemius muscle after 24-hour Rocuronium and Cisatracurium infusion.

In their study, Yoshioka et al. investigated the effects of Sugammadex on bronchial smooth muscle function in rats, and they showed that it had no effects on smooth muscle functions [14]. Eikermann et al. reported by evaluating the EMG of the genioglossus muscle that reversesthe Sugammadex dose administered after Rocuronium-induced neuromuscular block did not affect upper airway dilator muscle activity, but said that Neostigmine impaired muscle activity at significant levels [15]. Although it had no effects on smooth muscle functions, its effects on striated muscle functions werenoteworthy.

In the study of Ünal et al., muscle tissue was examined after 24 hours in rats that given sugammadex, and muscle cell loss and intense glycogen increase were observed [16].

Kalkan et al. In his study, they investigated the effect of sugammadex and rocuronium on rat heart and diaphragm muscle [17]. When sugammadex is administered; reported that pycnotic nuclear clusters and hypertrophy in the heart and skeletal muscle, degeneration and edema in the heart muscle and diaphragm muscle were observed. In addition, they reported that muscle fibers were weakened in tissues where 96 mg of suggammadex alone was applied, swelling and edema in some areas. Diaphragm and heart muscle treated with rocuronium+sugammadex; reported that mild cell degeneration, intense edema, hypertrophy, pycnotic nuclear clusters, degeneration, weakening of muscle fibers and vacuolization were observed in muscle fibers.

In our study, 72 hours after sugammadex and rocuronium-sugammadex administration, diaphragmatic examinations of rats given diaphragmatic only sugammadex showed an increase in the number of fibers with peripherally located muscle fiber nuclei, a small amount of interstitial edema and morphological changes from place to place. In diaphragmatic examinations of rats given sugammadex -recordunium, in the cross-sectional images of the diaphragm, it was evident that the fiber diameter decreased, and accordingly, edema in the interstitial area and an increase in the fibers undergoing morphological changes were evident. Our finding shows that it takes more than 24 hours for the possible harmful effects of sugammadex and rocuronium to be recognized in histopathological examinations.

Limitations and Recommendations

The lack of EMG tests regarding the muscle contraction was a limitation of the present study.Further studies can be conducted at other values within this range because the dose range used for Sugammadex was 2-16 mg in the present study.

Conclusion

Sugammadex and Rocuronium-Sugammadex complexes cause degenerative and histopathological changes in the diaphragm cells. It is important to pay attention to this condition in clinical practice.

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References

1. Brett K, Farrah K. Sugammadex for the Reversal of Rocuronium-Induced Neuromuscular Blockade in Surgical Patients: A Review of Clinical Effectiveness [Internet]. Ottawa (ON): Canadian Agency for Drugs and Technologies in Health. 2019.

2. Kawano T, Yokoyama M. Can sugammadex encapsulation eliminate the antigenic activity of aminosteroidal neuromuscular blocking agent? J Anesth [Internet]. 2011;25(6):953–4.
3. Uludağ O. Effect of Sugammadex and Rocuronium Combination on Cranial Neurotoxicity in Rats: Experimental Study. Kafkas Üniversitesi Veteriner Fakültesi Dergisi [Internet]. 2019;25.
4. Grosse-Sundrup M, Henneman JP, Sandberg WS, et al. Intermediate acting non-depolarizing neuromuscular blocking agents and risk of postoperative respiratory complications: prospective propensity score matched cohort study. BMJ [Internet]. 2012;345(oct15 5):e6329.
5. Arnardottir S, Alexanderson H, Lundberg IE, Borg K. Sporadic inclusion body myositis: pilot study on the effects of a home exercise program on muscle function, histopathology and inflammatory reaction. J Rehabil Med [Internet]. 2003;35(1):31–5.
6. Karayiannakis AJ, Makri GG, Mantzioka A, et al. Postoperative pulmonary function after laparoscopic and open cholecystectomy. Br J Anaesth [Internet]. 1996;77(4):448–52.
7. Yuzkat N, Kati I, Isik Y, et al. Effects of theophylline with methylprednisolone combination therapy on biomechanics and histopathology in diaphragm muscles of rats. Inflammation [Internet]. 2016;39(5):1635–41.
8. Jaber S, Jung B, Matecki S, Petrof BJ. Clinical review: Ventilator-induced diaphragmatic dysfunction - human studies confirm animal model findings! Crit Care [Internet]. 2011;15(2):206.
9. Maes K, Testelmans D, Thomas D, et al. High dose methylprednisolone counteracts the negative effects of rocuronium on diaphragm function. Intensive Care Med [Internet]. 2011;37(11):1865–72.
10. Uludag O. History and importance of ethical rules in animal experiment studies. Adıyaman Üni Sağlık Bilimleri Derg [Internet]. 2019;5(1):1401–13.
11. Cammu G, De Kam PJ, Demeyer I, et al. Safety and tolerability of single intravenous doses of sugammadex administered simultaneously with rocuronium or vecuronium in healthy volunteers. Br J Anaesth [Internet]. 2008;100(3):373–9.
12. Inder WJ, Jang C, Obeyesekere VR, Alford FP. Dexamethasone administration inhibits skeletal muscle expression of the androgen receptor and IGF-1-implications for steroid-induced myopathy. Clinical endocrinology. 2010;73(1):126–32.
13. Testelmans D, Maes K, Wouters P, et al. Infusions of rocuronium and cisatracurium exert different effects on rat diaphragm function. Intensive Care Med [Internet]. 2007;33(5):872–9.
14. Yoshioka N, Hanazaki M, Fujita Y, et al. Effect of sugammadex on bronchial smooth muscle function in rats. J Smooth Muscle Res [Internet]. 2012;48(2_3):59–64.
15. Eikermann M, Zaremba S, Malhotra A, et al. Neostigmine but not sugammadex impairs upper airway dilator muscle activity and breathing. Br J Anaesth [Internet]. 2008;101(3):344–9.
16. Ünal N. Suna Danışman Sugammadeks' in sıçanların iskelet kas yapısına etkisi (Ankara Üniversitesi Fen Bilimleri Enstitüsü Doctoral dissertation). 2018.
17. Kalkan Y, Bostan H, Tumkaya L, et al. The effect of rocuronium, sugammadex, and their combination on cardiac muscle and diaphragmatic skeletal muscle cells. Journal of anesthesia. 2012;26(6):870–7.