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 were kept stable with a 12-hour light-dark cycle. The animals were divided into four groups: with 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 at 16 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 - Plough 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 attached with 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.

**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 structures had 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, which also 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).

**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 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 an important 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 differences were 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-
versal of the neuromuscular blockage induced by Rocuro-
nium with Sugammadex were reported in many animal and
human studies [1, 4, 11]. Our literature review did not
reveal any direct studies that were conducted on Rocuro-
nium 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 poten-
tial with the steroid content in the structure, and it was
shown that the drugs with steroidal structure remaining in
the circulation for longer durations 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 ob-
served pathological changes similar to Myopathy in the
Sugammadex Group; and significant changes were de-
tected in the Sugammadex-Rocuronium Group. Our re-
sults are consistent with previous clinical studies which
showed detrimental effects on muscle functions [13]. Testel-
mans et al. [13] reported that they did not observe
histopathological changes with H&E Staining in the di-
aphragmatic 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 evalu-
ating the EMG of the genioglossus muscle that revers-
esthe 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 stri-
ated muscle functions were noteworthy.

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 pyknotic 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 sugammadex alone was applied, swelling
and edema in some areas. Diaphragm and heart muscle
were treated with rocuronium+sugammadex; reported that
mild cell degeneration, intense edema, hypertrophy, py-
notic 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 lo-
cated 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 di-
aphragm, it was evident that the fiber diameter decreased,
and accordingly, edema in the interstitial area and an in-
crease 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 exami-
nations.

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
decay degenerative and histopathological changes in the
diaphragm cells. It is important to pay attention to this
condition in clinical practice.

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