

The effect of rocuronium, sugammadex, and their combination on cardiac muscle and diaphragmatic skeletal muscle cells

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Abstract

Purpose Rocuronium has been associated with muscle weakness when administered in prolonged infusions. The effect of sugammadex and rocuronium together on muscle is unknown. In this study, we examined the effects of rocuronium and sugammadex, and the complex formed by these agents, on cardiac and diaphragmatic muscle cells.

Methods Forty-two Sprague–Dawley male rats were divided into six groups. Group I received only rocuronium at a dose of 1 mg/kg and groups II and III received sugammadex alone at doses of 16 and 96 mg/kg, respectively. Groups IV and V received 1 mg/kg rocuronium plus 16 mg/kg

sugammadex and 1 mg/kg rocuronium plus 96 mg/kg sugammadex, respectively. Group six was the control group and received only 0.9 % NaCl without any drug.

Results Histopathological examination demonstrated that rocuronium and high doses of sugammadex accumulated in both cardiac and diaphragm muscle tissues. We also observed intense edema and degeneration in diaphragmatic and myocardial cells when the rocuronium-sugammadex complex was used. Rocuronium and sugammadex remain in the circulation for a long time and they may cause skeletal muscle myopathy, vacuolization, pyknotic nuclear clumps, and hypertrophy, and weaken the muscle fibers.

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Conclusion Rocuronium, sugammadex, and rocuronium–sugammadex complexes cause histopathological changes and immunoreactivity to calcineurin in muscle cells.

Keywords Sugammadex · Muscle · Diaphragmatic muscle cell · Calcineurin · Rat

Introduction

Rocuronium is an aminosteroidal neuromuscular blocker frequently used in general anesthesia and in intensive care units [1–3]. Rocuronium is a non-depolarizing neuromuscular agent with relatively safe properties in applications such as electroconvulsive therapy and thymectomy [4, 5]. However, the use of non-depolarizing neuromuscular blocking agents, specifically rocuronium, has been questioned because of the development of myopathy and muscle weakness after prolonged infusions [6, 7]. Animal studies have demonstrated significant muscle protein degradation [7] and the accumulation of rocuronium in interstitial muscle tissue after the use of this agent [8]. The mechanism underlying these results is unclear; however, aminosteroidal structures have been held to be responsible for such results in previous studies.

The fact that rocuronium dissolves well in cyclodextrins provided an opportunity to eliminate the rocuronium molecules in the blood. Based on this finding, sugammadex has been developed [9]. Sugammadex, a modified gamma cyclodextrin, is a recently developed muscle relaxant binding agent that is used to reverse rocuronium-induced muscle relaxation during anesthesia [10]. Rocuronium is bound within the lipophilic core of the sugammadex molecule, forming a rocuronium–sugammadex complex. Sugammadex–rocuronium complexes are highly hydrophilic, and are eliminated rapidly by the kidneys [11]. These complexes need to be studied to determine whether they have a detrimental role in muscle changes and to determine whether, by increasing the elimination of rocuronium, the complexes can reduce the possible accumulation of rocuronium. Our literature review has not revealed any study investigating these two drugs and their immunohistochemical effects on muscle cells.

Calcineurin (CN) has an important regulatory role in muscle differentiation, muscle hypertrophy, and atrophy [12]. CN and rocuronium both affect calcium release and influence muscle structure. However, there are no studies evaluating possible relationships between rocuronium, sugammadex, sugammadex–rocuronium complex, and CN.

In this study, we aimed to investigate the histopathological and immunohistochemical changes of rocuronium, sugammadex, and the sugammadex–rocuronium complex in muscles. We planned to look at the diaphragmatic and

cardiac muscles for this interaction. Additionally, we investigated the association between changes in blood glucose, calcium, chloride, potassium, magnesium, and sodium levels and changes in muscle tissues. We chose to investigate the diaphragmatic muscle as representative of skeletal muscle as it is the only active skeletal muscle during anesthesia.

Materials and methods

The study protocol and permissions were reviewed and approved by the Local Ethics Committee for Animal Experiments, School of Medicine, Rize University of Turkey (date: 31.03.2011, meeting number: 03).

Animals

Forty-two Sprague–Dawley male rats (Laboratory Animals of Experimental Surgery, Trabzon, Turkey), weighing 300–350 g, were used. Room temperature and humidity were maintained at 21 ± 3 °C and 50–55 %, respectively. White fluorescent lighting was used between 0600 and 1800 hours. All animals were provided with standard 7- to 8-mm pellets (Bayramoglu Yem Sanayii, Erzurum, Turkey) and water ad libitum. The experiments were performed according to the National Institute of Health Guide for the Care and Use of Laboratory Animals.

Study groups

The animals were divided into six groups (one control and five treatment groups), each of which had seven animals ($n = 7$). The following combinations of drugs, which represent the minimum and maximum tolerable amounts for the desired clinical outcome in rats and humans [13–17] were given intravenously (caudal vein).

Group I received 1 mg/kg rocuronium (Esmeron ampule; Organon, PA, USA);

Group II received sugammadex 16 mg/kg (Bridion; Schering-Plough, Oss, Netherlands);

Group III received sugammadex 96 mg/kg;

Group IV received 1 mg/kg rocuronium + 16 mg/kg sugammadex;

Group V received 1 mg/kg rocuronium + 96 mg/kg sugammadex;

Group VI (control) received 0.9 % NaCl.

Rats receiving rocuronium were ventilated with an Ambu ventilator (PlusMED, İstanbul, Turkey) [18] until resolution of spontaneous ventilation started (approximately 3–5 min) and followed in the cage for approximately 72 h.

Tissue preparation processes

After 3 days, the rats were anesthetized with thiopental sodium at a dose of 50 mg/kg i.p. Following the anesthesia, the animals were intracardially perfused with 4 % formaldehyde at room temperature. Both skeletal muscle and cardiac muscle were excised to prepare histopathological specimens. Skeletal muscle was chosen from diaphragm with abdominal muscle.

The muscle tissues were removed and stored in fixative (10 % formaldehyde) overnight at 4 °C. The next day, the samples went through a graded alcohol series, and were then embedded in liquid paraffin at 60 °C. Suitable sections were cut, at a thickness of 4–6 µm, with a microtome (RM2255; Leica, Nussloch, Germany). Tissue sections of each block were collected on glass slides for histopathological examination. Sections stained with hematoxylin–eosin (H&E) were made ready for review and were examined under a light microscope (BX51; Olympus, Tokyo, Japan) with a digital camera (DP72; Olympus). The photographs were taken at 40× and 10× magnifications.

Calcineurin levels were detected by the streptavidin–biotin peroxidase method. Muscle tissue samples were deparaffinized in xylene and rehydrated through an ethanol series. The sections were incubated with 3 % H₂O₂ in order to block endogenous peroxidase activity. Normal bovine serum was used to bind and remove nonspecific antibodies in the samples. The following primary antibody [purified mouse anti-calcineurin (clone 29/calcineurin), (dilution; 1:250), reactivity (chicken, frog, human, mouse, rat) BD Biosciences, Franklin Lakes, NJ, USA] was used for 60–75 min and a biotinylated secondary antibody (Universal LSAB Code: K0690; DAKO, Glostrup, Denmark) was used for 30 min. The sections were incubated with streptavidin–horseradish peroxidase (DAKO-Universal LSAB Kit-K0690) and the antibody binding sites were visualized with 3,3'-diaminobenzidine (DAB), and washed in phosphate-buffered saline (PBS). Nuclei were stained with EnVision™ FLEX Hematoxylin, Code K8018 (DAKO), and the sections were dehydrated through an increasing ethanol series, and cleared in xylene. The treated sections were then mounted with Entellan (Code 107960; Merck, Darmstadt, Germany). Antibody binding was examined by light microscope and the sections were photographed.

Biochemical processes

Blood samples were withdrawn from the tail vein to measure the electrolytes calcium (Ca), magnesium (Mg), chloride (Cl), potassium (K), and sodium (Na); this measurement was done by a standard autoanalyzer technique (Architect c16000 Autoanalyzer; Abbott Diagnostics, Worcester, MA, USA).

Statistical analysis

Statistical analyses of differences in Ca, K, Na, Cl, and Mg values in the six groups were done by one-way analysis of variance (ANOVA) followed by multiple comparison post-hoc Tukey HSD test and the Kruskal–Wallis test (all statistical analyses were done with SPSS 18 for Windows; IBM, Chicago, IL, USA).

None of the immunoreactivity score data showed normal distributions according to the Kolmogorov–Smirnov and Shapiro–Wilk tests. Score data were analyzed using the Kruskal–Wallis test. All the groups were separately compared using the Mann–Whitney *U*-test. The *p* value used for multiple comparisons was calculated by dividing 0.05 by fifteen. The *p* values were considered statistically significant at ≤0.003.

Results

Histopathological results

Group I Intense edema and degeneration were observed in diaphragmatic and myocardial cells (Fig. 1a, d). Mild vacuolization was observed in the interstitial vessels of the diaphragm muscle, but vacuolization was more intense in the myocardium.

Group II and Group III In addition to the findings seen in *Group I*, we also observed pyknotic nuclear clumps, hypertrophy in myocardial and skeletal muscle cells, mild interstitial edema, and degeneration in the cells of the diaphragm and myocardium in both *group II* (Fig. 1b, e) and *group III* (Fig. 1c, f). In the histopathological examination of the tissues, muscle fibers were weakened in *group II*. However, we also found swelling and sloughing of endothelial cells and edema in some places. Moreover, vacuolization was observed more frequently in *group I* than in *groups II and III*. The density of hypertrophic cells was lower in *group I* than in *group II* and *group III*, and there were slight histopathological differences between these two groups. *Group IV* The diaphragm and heart muscle tissues had mild cell degeneration in the muscle fibers (Fig. 2a, d). Moreover, these cells appeared along with pyknotic nuclear clumps. Edema was detected in both the diaphragm and the heart muscle tissues. In particular, there was edema in the arterial endothelial cells of the intersitium of diaphragmatic muscle.

Group V We observed dense edema, pyknotic nuclear clumps, hypertrophy, and degeneration in muscle cells (Fig. 2b, e). Also, muscle fibers were weakened and acidophilic, and increased vacuolization was observed (Fig. 2b, e). In addition, arterial endothelial cells showed

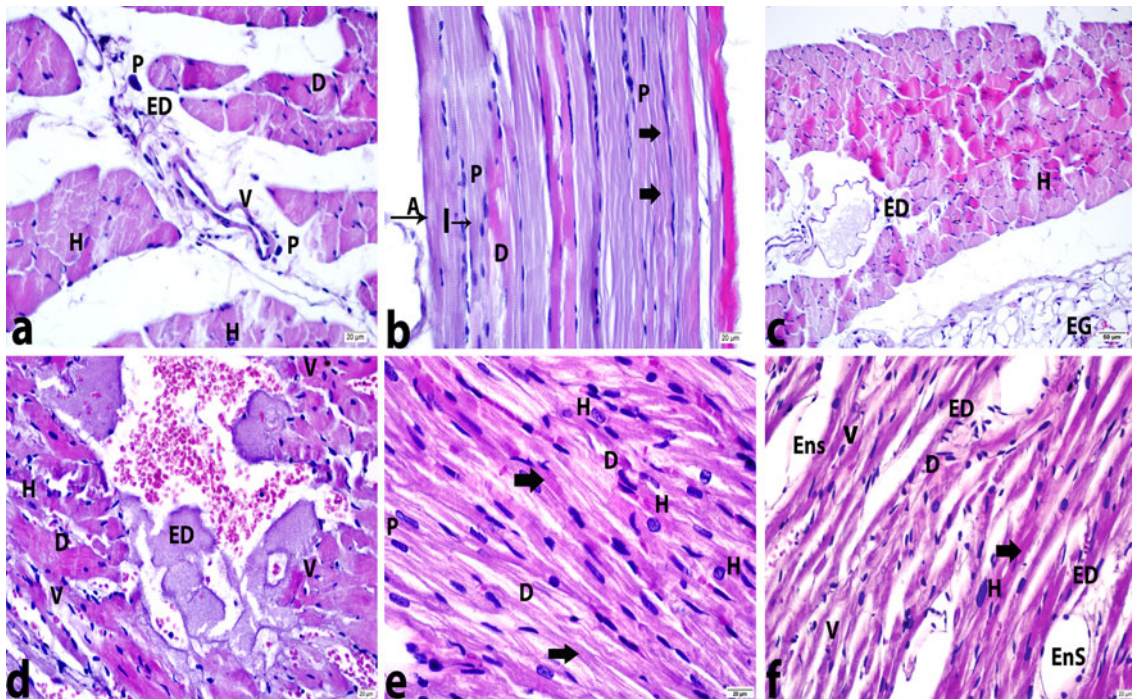


Fig. 1 Histopathological changes on light microscopy of the diaphragm (a–c) and cardiac muscle tissues (d–f); rats received rocuronium 1 mg/kg (a, d), sugammadex 16 mg/kg (b, e), and sugammadex 96 mg/kg (c, f). **a** Myofibers of the diaphragm muscle in cross section; *D* muscle degeneration, *P* pyknotic nuclear clumps, *H* myocardial cell hypertrophy, *ED* edema, *V* vacuolation, H&E stain $\times 40$, bar 20 μm . **b** Myofibers of the diaphragm muscle in longitudinal section; *P* pyknotic nuclear clumps, *A*, *I* bands in myofiber, *thick arrow* myofibers, *D* muscle degeneration, *P* pyknotic nucleus, H&E stain $\times 40$, bar 20 μm . **c** Myofibers of the diaphragm in cross-section; *ED* edema, *H* myocardial cell hypertrophy, *EG* eosinophil

granulocytes, H&E stain $\times 20$, bar 50 μm . **d** Myofibers of cardiac muscle in cross-section; *ED* edema, *H* myocardial cell hypertrophy, *D* muscle degeneration, *V* vacuolation, H&E stain $\times 40$, bar 20 μm . **e** Myofibers of cardiac muscle in longitudinal section; *thick arrow* bands in myofibers, *P* pyknotic nuclear clumps, *H* myocardial cell hypertrophy, *D* muscle degeneration, H&E stain $\times 40$, bar 20 μm . **f** Myofibers of cardiac muscle in longitudinal section; *H* myocardial cell hypertrophy, *thick arrow* bands in myofibers, *V* vacuolation, *D* muscle degeneration, *EnS* endothelial cell swelling, *Ens* endothelial cell sloughing, H&E stain $\times 40$, bar 20 μm

mild degrees of edema, swelling and sloughing. In this group, vacuolization and arterial dilatation was observed more frequently than in the other five groups.

Group VI/control group The histological structure in the control group appeared normal (Fig. 2c, f).

Immunoreactivity results

In the evaluation of skeletal muscle calcineurin immunoreactivity, *group I* was found less immune-positive than other groups with immune-histochemical stain of the tissues. The immunoreactivity of skeletal muscle was similar in *groups II* and *III*, and was milder compared to *groups IV* and *V*. The immunoreactivity of the heart muscle was more prominent in *groups IV* and *V* than in all the other groups. Staining in *Group I* was light-colored compared with that in *groups II* and *III*. Densities of calcineurin immunoreactivity in the skeletal muscle were significantly lowered by rocuronium. In contrast, densities of calcineurin immunoreactivity in the heart muscles were unchanged by rocuronium.

More immunopositivity was observed in and around hypertrophied cells and fibrotic muscles (Figs. 3, 4).

There was a significant difference in the immunoreactivity values of the heart muscles between *groups I, II*, and *III* and *group V* ($p < 0.003$).

Biochemical results

Some biochemical values in the experimental groups were higher than those in the control group (Fig. 5). Electrolyte levels appeared to be within the normal range in all groups (Ca 5.3–13 mg/dL, Cl 100–110 mmol/L, K 5.4–7 mmol/L, Mg 3.3–5.6 mg/dL and Na 143–156 mmol/L) [19].

There were statistically significant differences between the groups in the values of Ca, K, and Na ($p < 0.05$). Ca and Na values in *group VI* were significantly different from the values in the other groups ($p < 0.05$). There was also a significant difference in K values between *groups I* and *II* and *group VI* ($p < 0.05$). Cl and Mg were similar in all groups ($p > 0.05$) (Fig. 5).

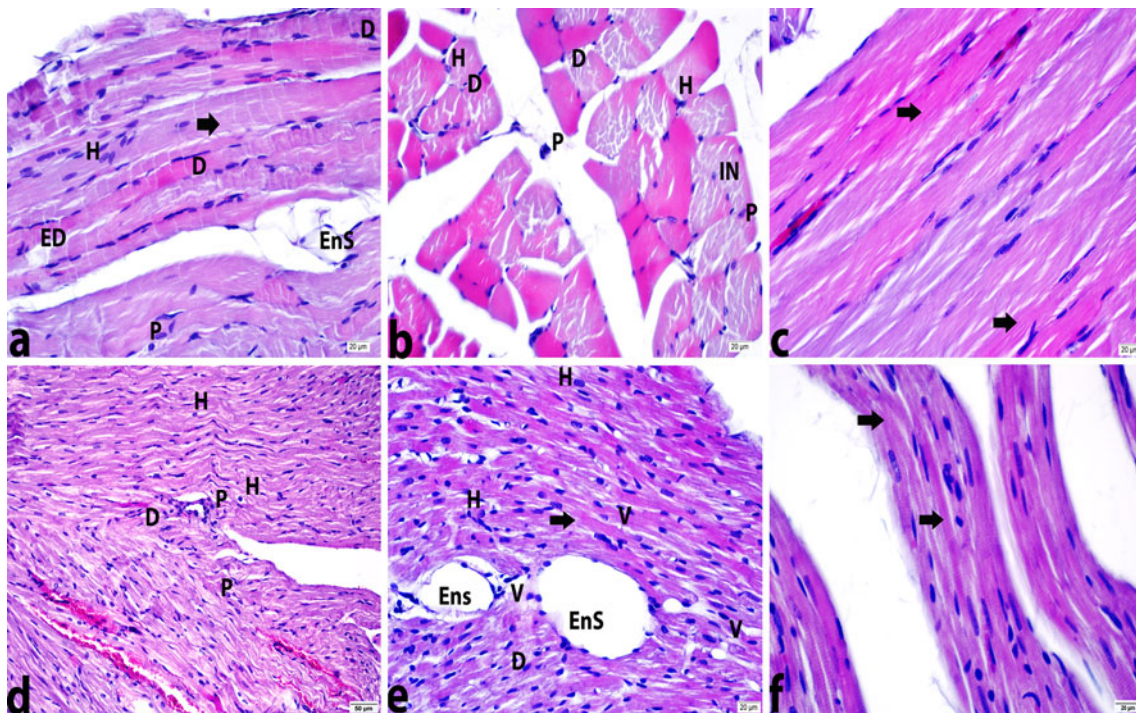


Fig. 2 Histopathological changes on light microscopy of the diaphragm (a–c) and cardiac muscle tissues (d–f); rats received rocuronium 1 mg/kg + sugammadex 16 mg/kg (a, d), rocuronium 1 mg/kg + sugammadex 96 mg/kg (b, e), and saline (control) (c, f). **a** Myofibers of the diaphragm muscle in longitudinal section; *D* slight muscle degeneration, *EnS* endothelial cell swelling, *P* pyknotic nuclear clumps, *H* myocardial cell hypertrophy, H&E stain $\times 40$, bar 20 μm . **b** Myofibers of the diaphragm muscle in cross-section; *H* myocardial cell hypertrophy, *D* slight muscle degeneration, *P* pyknotic nuclear clumps, *IN* internal nuclei in some fibers, H&E stain $\times 40$, bar 20 μm . **c** Myofibers of the diaphragm muscle in

longitudinal section, *thick arrow* bands in myofiber, H&E stain $\times 40$, bar 20 μm . **d** Myofibers of cardiac muscle in longitudinal section; *D* slight muscle degeneration, *H* myocardial cell hypertrophy, *P* pyknotic nuclear clumps, H&E stain $\times 40$, bar 20 μm . **e** Myofibers of cardiac muscle in longitudinal section, *thick arrow* bands in myofiber, *H* myocardial cell hypertrophy, *D* muscle degeneration, *V* vacuolation, *EnS* endothelial cell swelling, *Ens* endothelial cell sloughing, H&E stain $\times 40$, bar 20 μm . **f** Myofibers of cardiac muscle in longitudinal section; *H* myocardial cell hypertrophy, *thick arrows* bands in myofiber, *V* vacuolation, H&E stain $\times 40$, bar 20 μm

Discussion

In this study, we observed significant histopathological and immunoreactivity changes in diaphragmatic and myocardial cells in rats treated with rocuronium. Interestingly, the effect on myocardial muscle cells was more prominent than the changes seen in diaphragmatic cells. Our result is consistent with previous clinical reports demonstrating potential drug accumulation and a detrimental effect on muscle function with prolonged infusion of rocuronium in the intensive care setting [20].

We found that sugammadex, at both low and high doses, induced more significant histopathological changes and a further detrimental effect than rocuronium alone. The mechanism behind this is not clear and needs further investigation. After the administration of sugammadex, sugammadex-rocuronium complexes are rapidly formed and are eliminated via the kidneys instead of rocuronium being eliminated by biliary excretion. Previous studies have shown that steroidal drugs staying in the circulation for a long time or prolonged steroid treatments are known

to cause skeletal muscle changes and myopathy. Myopathy associated with steroids is more frequent with the use of fluorinated steroids, such as dexamethasone [21]. The use of sugammadex-rocuronium complexes has also been questioned, because of the potential for drug accumulation due to the steroid content in the structure. Steroids are myotoxic, and complex compounds used with steroids can also lead to severe myopathy. In the present study, pathological changes similar to myopathy were observed in the groups given rocuronium; furthermore, the sugammadex-rocuronium groups had significant changes as well.

Calcineurin (CN) has been shown to induce cardiac and diaphragm muscle cell hypertrophy [22]. In the present study, CN immunoreactivity in the rocuronium-alone group was relatively less than that in the other groups [8]. CN immunoreactivity in the cardiac muscles in our groups IV and V was greater than that in all the other groups. Because CN plays an important role in the pathological processes of muscle cells, a greater level of staining basically indicates hypertrophy and atrophy [23–25]. In our groups I, II, and III, CN immunopositivity was decreased;

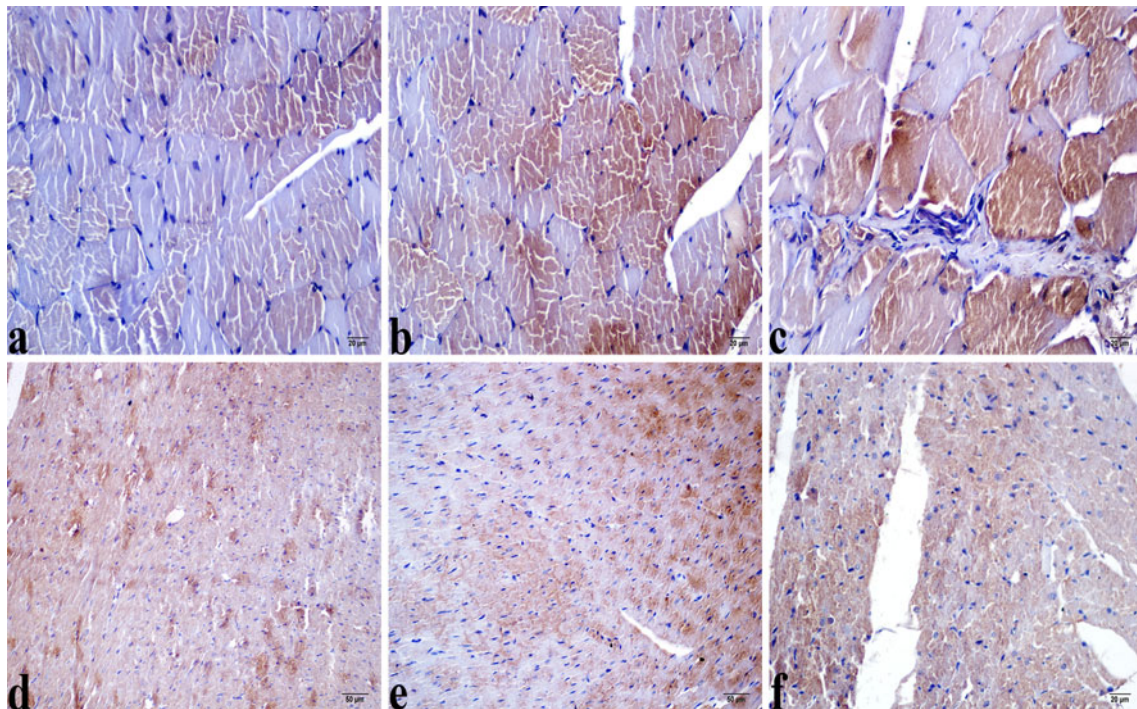


Fig. 3 Immunohistochemical changes on light microscopy of the diaphragm (a–c) and cardiac muscle tissues (d–f); rats received rocuronium 1 mg/kg (a, d), sugammadex 16 mg/kg (b, e), and sugammadex 96 mg/kg (c, f)

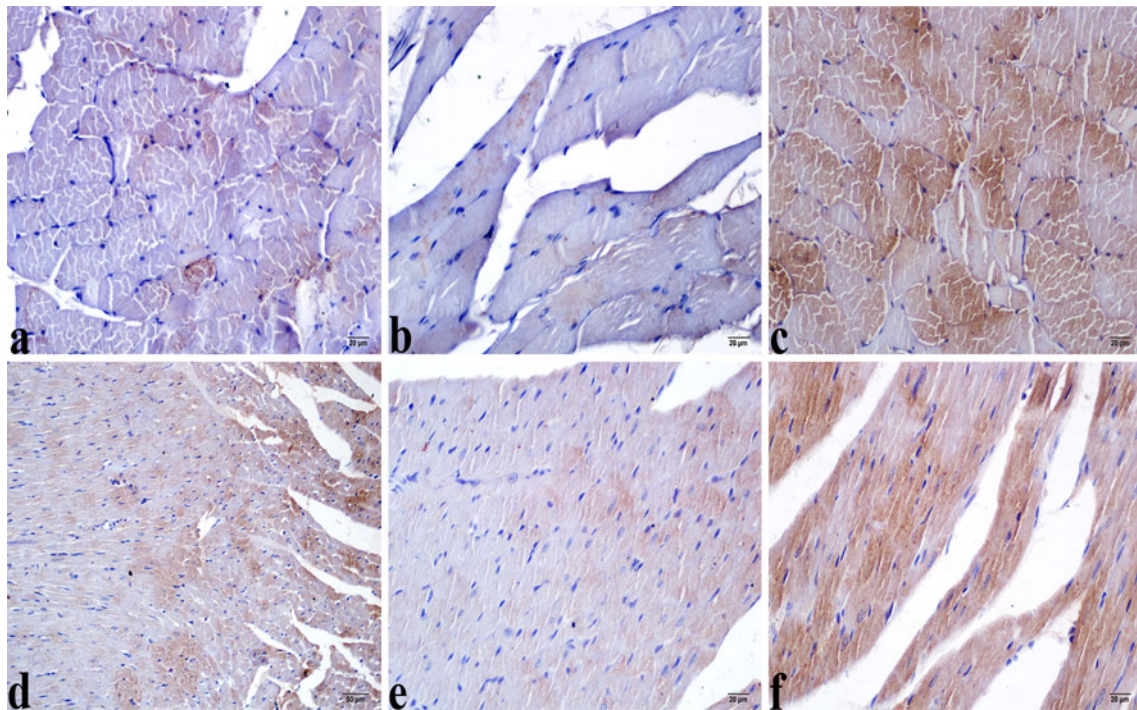


Fig. 4 Immunohistochemical changes on light microscopy of the diaphragm (a–c) and cardiac muscle tissues (d–f); rats received rocuronium 1 mg/kg + sugammadex 16 mg/kg (a, d), rocuronium 1 mg/kg + sugammadex 96 mg/kg (b, e), and saline (control) (c, f)

however, sugammadex seemed to reduce the effect of rocuronium. Also, we observed slight myopathy, marked by muscular degeneration and fiber weakness in *groups I, II*

and III. We also saw more intense edema and degeneration in the diaphragmatic and myocardial cells in *group I* than in the other groups.

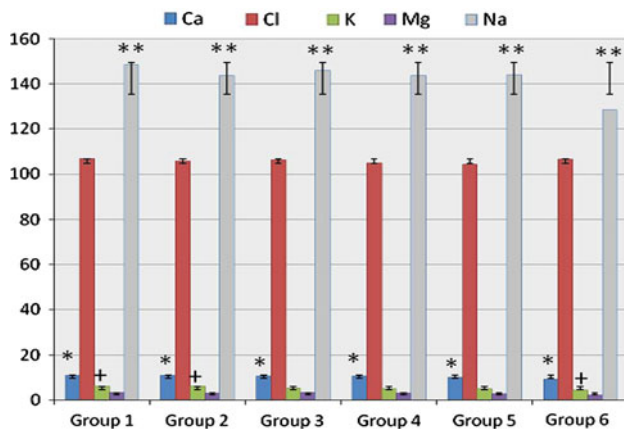


Fig. 5 Results of statistical analysis of differences in Ca, Cl, K, Mg, and Na among all groups. * $p < 0.05$ Ca, ** $p < 0.05$ Na, + $p < 0.05$ K

Interestingly, we found that muscle degeneration and vacuolization were more significant in cardiac cells than in skeletal muscle. According to Testelmans et al. [20], H&E staining showed no histopathological changes in the diaphragm or the gastrocnemius muscle with 24-h infusion of rocuronium and cisatracurium [20]. We observed histopathological changes in the diaphragmatic and the cardiac muscle cells 72 h after the administration of rocuronium and rocuronium–sugammadex. This suggests that rocuronium requires to be administered for a longer time to show its detrimental effect on muscle cells. That is, our finding indicates that the possible harmful effect of rocuronium takes longer than 72 h to be recognized on histopathological examinations.

Clinical consequences of the reversal of neuromuscular blockade by sugammadex have been demonstrated in several animal and human studies [2, 3, 26]. Our review of the literature did not reveal any study directly related to histopathological changes in muscle with rocuronium and sugammadex. There are important future clinical implications of the present study; rocuronium, sugammadex, and the resulting complex cause significant histopathological changes in diaphragm and cardiac muscles. The clinical consequences of the prolonged infusion of rocuronium are associated with muscle weakness; however, the cardiac effects are unknown and need further investigation. Furthermore, it is still not known whether rocuronium and rocuronium–sugammadex complexes have an effect on the cells of other muscles, such as uterine muscles.

Although some biochemical values in our experimental groups were higher than those in the control group, all the electrolyte levels appeared to be within the normal ranges [19, 27]. There was a significant difference in the immunoreactivity values of the heart muscles between groups I, II, and III and group V ($p < 0.003$). There were no clinically important changes after the high-dose administration of either sugammadex or rocuronium.

Conclusion

In summary, our results have demonstrated that rocuronium and sugammadex have degenerative effects on cardiac and diaphragmatic muscle cells. The effects of this degeneration on the function and structure of cardiac and skeletal muscle in a clinical setting in humans are unknown and need to be further studied.

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