Original Article

ANATOLIAN CURRENT MEDICAL

DOI: 10.38053/acmj.1088069

Anatolian Curr Med J 2022; 4(3); 244-248

Cardiac effects of Sugammadex and Rocuronium combination in rats: experimental study

Mevlüt Doğukan[1], Nezir Yılmaz[2], Ebru Elibol[3], Zümrüt Doğan[4], Fatih Üçkardeş[5], Öznur Uludağ[1]

[1]Adıyaman University, Faculty of Medicine, Department of Anesthesiology and Reanimation, Adıyaman, Turkey
[2]Adıyaman Training and Research Hospital, Department of Anesthesia and Reanimation, Adıyaman, Turkey
[3]Adıyaman University, Faculty of Medicine, Department of Histology and Embryology, Adıyaman, Turkey
[4]Adıyaman University, Faculty of Medicine, Department of Anatomy, Adıyaman, Turkey
[5]Adıyaman University, Faculty of Medicine, Department of Biostatistics and Medical Informatics, Adıyaman, Turkey

Cite this article as: Doğukan M, Yılmaz N, Elibol E, Doğan Z, Üçkardeş F, Uludağ Ö. Cardiac effects of Sugammadex and Rocuronium combination in rats: experimental study. Anatolian Curr Med J 2022; 4(3); 244-248.

ABSTRACT

Aim: In this experimental study, it was aimed to examine the effects of rocuronium and sugammadex complex on cardiac muscle cells in rats histopathologically and biochemically.

Matherial and Method: 32 adult Sprague-Dawley male rats were divided into four groups with 8 in each group. Group 1 consisted of animals that did not undergo surgical treatment. Group 2 received a volume equivalent to 16 mg/kg sugammadex with 0.9% intravenous saline. Group 3 received 16 mg/kg of intravenous sugammadex. Group 4, 1 mg/kg intravenous rocuronium and 16 mg/kg intravenous sugammadex were administered to rats. After the procedure completed GSH and MDA level evaluated biochemically; and heart tissue evaluated histopathologically.

Results: In group 4, connective tissue edema between muscle fibers was observed to be significantly increased, vessel dilatation and hemorrhagic areas were observed. Groups 3 and 4 were found to cause an increase in GSH level when compared to Groups 1 and 2, and a decrease in MDA level in these two groups compared to the others.

Conclusion: Although sugammadex and sugammadex-rocuronium complex cause biochemical and histopathological effect on the heart tissue, there were no irreversible histopathologic changes and no significant biochemical difference found in this study.

Keywords: Sugammadex, rocuronium, cardiac muscle, histopathology, rat

INTRODUCTION

Neuromuscular blockers are often used to facilitate endotracheal intubation and improve surgical comfort (1). Neuromuscular blockers (NMB) are divided into two groups as depolarizing and non-depolarizing. Rocuronium is a non-depolarizing neuromuscular blocking agent frequently used in clinical practice. To shorten the recovery period, to regain muscle functions and to avoid postoperative pulmonary complications; the neuromuscular blockade created by the NMB agent, needs to be reversed (2). Widely used anticholinesterase agents (including neostigmine) increase the amount of acetylcholine (Ach) at the neuromuscular junction, thereby eliminating the effect of NMB agents by competitive inhibition. Sugammadex, on the other hand, is a modified gamma-cyclodextrin molecule and terminates its effects by encapsulating steroid NMB agents (3).

Many studies conducted since 2008, when it was approved for use in Europe, have proven the efficacy and safety of sugammadex (4-7). According to the results of recent meta-analysis studies, sugammadex used after neuromuscular block provides faster recovery compared to neostigmine (4-6).

Since sugammadex provides rapid and safe recovery, its clinical use is increasing day by day. Although many studies have emphasized that sugammadex is effective and safe, some studies have revealed that it has potential risks (8).

In this experimental study, it was aimed to evaluate the effects of sugammadex on cardiac tissue in rats by histopathological and biochemical methods.

Corresponding Author: Nezir Yılmaz, yilmaznezir@hotmail.com

Received: 15.03.2022 **Accepted:** 14.04.2022

MATERIAL AND METHOD

Ethical Statement

The study protocol and permissions were reviewed and approved by the Animal Experiments Local Ethics Committee of Adıyaman University Faculty of Medicine (Decision No: ADYU-HADYEK: 2018-11/2). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Animals and Experimental Design

A standard diet and water were given to Sprague-Dawley rats. The temperature and humidity of the environment were monitored daily and kept constant with 12-hour light-dark cycles. The animals were divided into four groups:

Group 1: consisted of animals that did not undergo surgical treatment.

Group 2: Rats were administered 0.9% intravenous saline with a volume equivalent to 16 mg/kg sugammadex.

Group 3: Rats received 16 mg/kg intravenous sugammadex (Bridion®; Schering - Plow Corporation, Oss, The Netherlands).

Group 4: 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.

Sample size: Sample size was calculated based on MDA measurement in reference article with G-Power 3.1 program 80% power and 95% confidence (9). Effect size was found 1.45 and 8 rats were needed for each group. 32 Sprague-Dawley rats was planned for 4 groups in this study.

All drugs were administered intravenously to the tail vein. After drug administration, the rats were followed up with ECG (electrocardiography) for 3 days. After the procedure. All animals were euthanized and the heart tissues of the rats were removed under anesthesia.

Histopathological Evaluation

When the experimental application procedures were completed, the heart tissue samples of the groups were taken and fixed in 10% formaldehyde solution for 1 week. After the fixation of the tissues was completed, routine histological tissue follow-up consisting of alcohol, xylene and paraplast chemicals was performed. Tissue samples were then made into paraffin blocks. Thin sections of 5 µm thickness were taken from paraffin blocks for histopathological examination. The prepared sections were deparaffinized using xylene. Stained with Hematoxylin-Eosin (H&E) and Masson triple staining method. The stained sections were examined with a Carl Zeiss brand Axiocam ERc5 model digital camera attached microscope and histopathologically evaluated. Histopathological changes were evaluated in 2 groups as reversible and irreversible (10) (**Table 1**).

Table 1. Reversible-irreversible cell injury

Reversible cell injury	Irreversible cell injury
Cell swelling	Necrosis
Membrane blebs	Fibrosis
Fatty changes	Apoptosis

Biochemical Evaluation

The excised heart tissue samples were washed with saline at +4°C, stored in eppendorf tubes according to cold chain principles and at -70°C until analysis. In tissue samples, homogenates were cold prepared with a 0.15 M KCl (10%, w/v) homogenizer for tissue malondialdehyde (MDA) and glutathione peroxidase (GSH-Px) measurements.

Tissue MDA concentration was prepared according to the Uchiyama method (11). Tissue MDA concentrations were measured below 532 nm in nmol/g tissue.

GSH-Px analysis was performed according to the method described by Ellman (12). It was measured with a spectrophotometer at a wavelength of 410 nm.

Statistical Analysis

One-way analysis of variance (ANOVA) test was used in the analysis of malondialdehyde (MDA) and glutathione peroxidase (GSH-Px) values of the groups. The Mann-Whitney U test was used to compare the groups. Kruskal wallis test was used for data that did not show normal distribution, and Dunnet test was used for multiple comparisons.

RESULTS

In the follow-up period after drug administration, neuromuscular block returned in all rats administered sugammdex after rocuronium in group 4. None of rats was died during procedure. No side effects were observed associated with sugammadex administration (QT prolongation, bradycardia, allergy). In histopathologic examination, no changes was observed in group 1 and group 2. Interstitial edema and vessel dilatation were observed in group 3. Connective tissue edema, vessel dilatation and haemorrhagic areas were observed in group 4. Fibrosis and necrosis was not observed in any group. There was no significant difference found in MDA and GSH levels between groups.

Histopathological evaluation of the groups as a result of histological examination of the sections belonging to Group 1, no pathological findings were found. A structure consisting of dense muscle fibers with normal morphological structure and core placement of the fibers was dominant (**Figure 1. a1** and **a2**). In addition, in the triple staining findings, dilatation, connective tissue fibrosis and inflammation were not found in the vascular structures. In addition, no connective tissue edema was observed between the muscle fibers (**Figure 1 a3**).

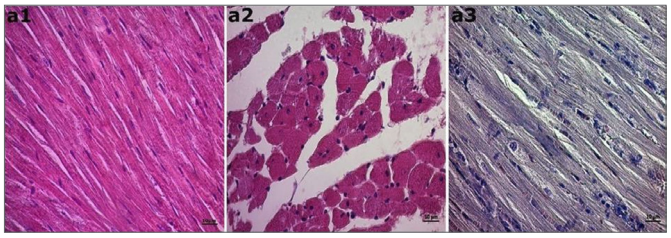

Figure 1. Light microscopy image of group 1; (**a1** and **a2** x40 objective magnification, H&E staining) (**a3** x40 objective magnification, Masson trichrome staining) Healthy tissue image

As a result of histological examination of the sections of group 2, a similar tissue image was found with group 1. There were dense muscle fibers and the connective tissue ratio between muscle fibers was normal (**Figure 2 b1** and **b2**). As a result of the evaluation made with triple staining, no signs of hemorrhage, connective tissue edema, inflammation and fibrosis were found. A healthy tissue image was observed (**Figure 2 b3**).

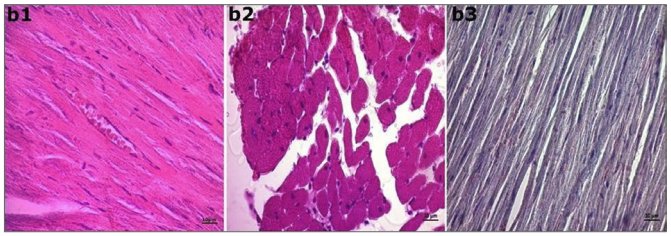

Figure 2. Light microscopy image of group 2; (**b1** and **b2** x40 objective magnification, H&E staining) (**b3** x40 objective magnification, Masson trichrome staining) Group 1 and similar healthy tissue image

As a result of the histological examination of the sections of group 3, there was a slight increase in the number of fibers with interstitial edema and morphological changes in places compared to groups 1 and 2. In addition, signs of vessel dilatation were observed (**Figure 3 c1**, **c2** and **c3**). As a result of the evaluation made with triple staining, no signs of inflammation and fibrosis were found (**Figure 3 c4**).

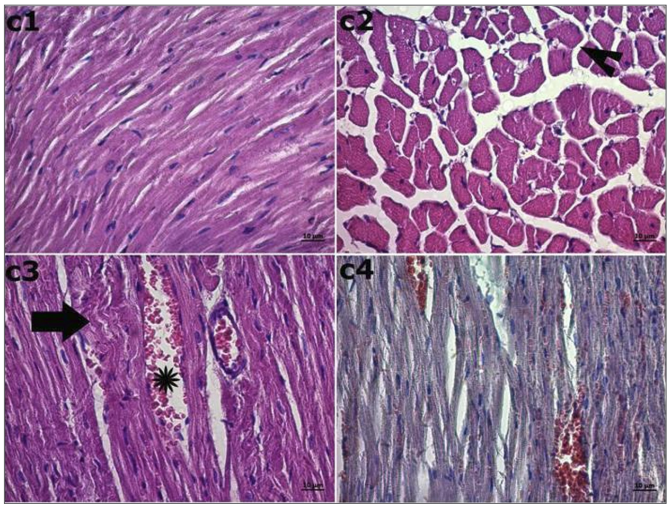

Figure 3. Light microscopy image of group 3; (c1, c2 and c3 x40 objective magnification, H&E staining) (c4 x40 objective magnification, Masson trichrome staining) Black arrow; distorted muscle fiber, star; dilated vessel, black arrowhead; interstitial edema between muscle fibers.

As a result of histological examination of the sections belonging to Group 4, degeneration of muscle fibers was the most intense group compared to other groups. Connective tissue edema between muscle fibers was markedly increased. There was vessel dilatation (**Figure 4 d1, d2** and **d3**). As a result of the evaluation made with triple staining, no signs of inflammation and fibrosis were found. However, haemorrhagic areas were observed (**Figure 4 d4**).

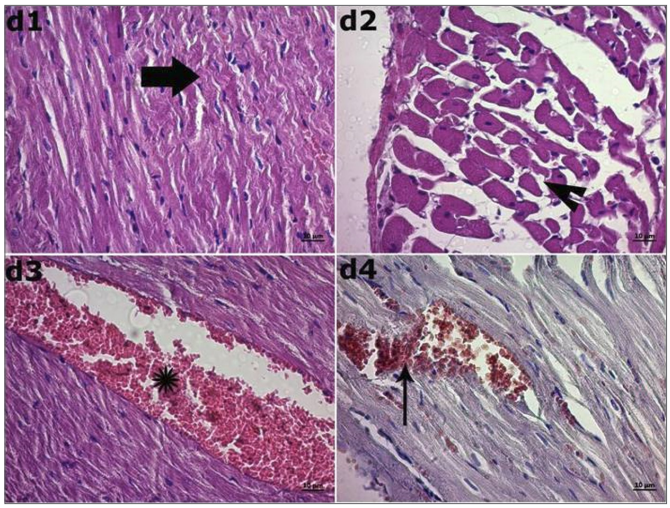

Figure 4. Light microscopy image of group 4; (d1, d2 and d3 x40 objective magnification, H&E staining) (d4 x40 objective magnification, Masson trichrome staining) Black arrow; distorted muscle fiber, star; dilated vessel, black arrowhead; interstitial edema between muscle fibers; thin black arrow; hemorrhagic area.

When the GSH level, a parameter belonging to the antioxidant systems, was compared between the groups, it was determined that group 3 and group 4 increased ($p>0.005$). There was a decrease in MDA level, which is an indicator of lipid peroxidation, in these two groups compared to the others. These increases and decreases were not found to be statistically significant (**Table 2**).

Table 2. Tissue GSH and MDA values

Groups	Statistics	GSH nmol/g	MDA nmol/g
Group 1	Mean	8964.4	288.5
	Median (Min-Max)	9163.1 (4410.1-12403.4)	283.1 (244.6 – 338.4)
Group 2	Mean	7042.2	267.2
	Median (Min-Max)	7262.5 (4628-10172.7)	271.4 (241.2-298.2)
Group 3	Mean	9280.9	259.6
	Median (Min-Max)	9865 (5756.2 -12243.1)	259.6 (227.8-288.1)
Group 4	Mean	9963.5	260.5
	Median (Min-Max)	9874.6 (4487-14871.2)	249.6 (211.1-328.3)
	p	0.272	0.175

Groups 3 and 4 caused an increase in GSH level compared to Groups 1 and 2. We believe that these substances cause an acute increase in GSH level due to the oxidative system. MDA levels did not cause a level of toxicity that

could cause a significant difference between the groups. Sugammadex and rocuronium did not cause oxidative stress-induced lipid peroxidation. The insignificant increase in MDA level suggests that these substances do not show severe toxicity as a result of free radical-induced oxidative stress and lipid peroxidation in heart tissue.

DISCUSSION

Sugammadex has no effect on NM connectivity or any receptor system in the body. This eliminates the need for anticholinergic drugs, which are alternatives and have many side effects. Clinical studies have shown that the side effects of sugammadex are mild and short-lived.

Side effects of sugammadex on the cardiovascular system have been reported as prolongation of the QT interval, bradycardia, hypotension, rhythm disturbances, and in rare cases asystole (13-17).

Sugammadex; although the drug was approved for use in Europe in 2008, the American Food and Drug Administration (FDA) postponed the approval for use until the end of 2015 due to the risk of serious hypersensitivity reactions. In their study, Tsur and Kalansky (18) identified 15 possible hypersensitivity reactions worldwide.

Considering the clinical studies examining the effects of sugammadex on the cardiac system; although high rates of bradycardia are mentioned in some publications, the incidence of bradycardia in patients treated with sugammadex was reported as 2% in a recent meta-analysis study (4).

In studies examining the relationship between sugammadex and QT prolongation, no significant correlation was found even in high-dose sugammadex use (19,20).

Dahl et al. (19) reported that sugammdex-rocuronium complex does not have a muscarinic effect due to its inert structure; therefore, it had no significant effect on hemodynamics and QT interval

Considering the experimental studies, Bostan et al. (21) reported histopathological changes in renal tissue without deterioration of biochemical renal function values in rats administered rocuronium and high-dose sugammadex (96mg/kg). In a similar study, they reported that rocuronium-sugammadex complex caused edema and degeneration in the heart and diaphragm muscle tissues. It has been suggested that the effects may not be directly related to sugammadex, and that the aminosteroid structure of rocuronium may have an effect on myopathy (22). In our study, tissue edema, vessel dilatation and haemorrhagic areas were detected in the histopathological examination of the sugammadex-rocuronium group, but no signs of inflammation or fibrosis were found.

In an I/R study examining the effects of rocuronium-sugammadex complex on brain tissue; neither biochemical values (GSH, MDA) nor histopathologically significant changes were found (9). In our study, no significant difference was found between the groups in terms of GSH and MDA levels.

Considering the recent ischemia-reperfusion (I/R) studies; there are studies in the literature reporting that sugammadex has organ-protective effects. In a recent I/R study, renal ischemia was performed and the nephroprotective effect of high-dose (100mg/kg) sugammadex use was demonstrated (23). In an I/R study performed with bilateral carotid occlusion for 10 minutes, sugammadex was reported to be protective against cerebral ischemia (24).

In this study, the dose of sugammadex that is used safely in humans was used. Although higher doses have been used in some studies, a dose of 16mg/kg has been used in many studies (9,21,22). There was no significant difference found in cardiac and diaphragmatic skeletal muscle with 16 mg/kg and 96 mg/kg sugammadex doses (22). Because of this study aimed to predict the side effects of sugammadex in clinical use, the study was planned to be conducted with this dose.

we observed that sugammadex and rocuronium-sugammadex complex made histopathological changes on the heart muscle in an experimental model in rats. Inthe group given rocuronium and sugammadex, the degeneration of the muscle fibers as a result of the histological examination of the sections was the most intense group compared to the other groups. Connective tissue edema between muscle fibers was markedly increased. Vascular dilatation was present. But no haemorrhagic areas were observed. In the group given sugammadex, less than the group given sugammadex-rocuronium complex, there was an increase in the number of interstitial edema and fibers with morphological changes from place to place. In addition, vascular dilatation findings were observed.

The GSH levels in our experimental groups were found to be higher than those in the control group, we think that this is an acute increase caused by the oxidative system. MDA levels did not cause a level of toxicity that could cause a significant difference between the groups. Sugammadex and rocuronium did not cause oxidative stress-induced lipid peroxidation. The insignificant increase in MDA level suggests that these substances do not show severe toxicity as a result of free radical-induced oxidative stress and lipid peroxidation in heart tissue.

CONCLUSION

As a result, in this experimental study, in parallel with the clinical studies performed, sugammadex and sugammadex-rocuronium complex cause biochemical and histopathological effect on the heart tissue, but no irreversible changes (fibrosis,necrosis) was found in histopathologic evaluation and no significant difference was found in MDA and GSH levels that used as an index for oxidative cellular damage.

ETHICAL DECLARATIONS

Ethics Committee Approval: This study was approved by Animal Experiments Local Ethics Committee of Adıyaman University Faculty of Medicine (Decision No: ADYU-HADYEK: 2018-11/2).

Informed Consent: Because of experimental design of the study informed consent form was not obtained

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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