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How does the Exponential Increase in Rocuronium Dose Effect the Train of Four Parameters in Rats Reversed with Sugammadex? An Animal Model

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Abstract

BACKGROUND/AIMS: Sugammadex is a gamma cyclodextrin structured agent used for reversing the effect of steroidal neuromuscular blocking (NMB) agents. The first aim of this study was to evaluate the dose of rocuronium required to re-establish NMB when administered 2 min after its reversal with sugammadex in rats. Also, to monitor the onset times and durations of NMB achieved by variable doses of rocuronium after reversal with sugammadex.

MATERIALS AND METHODS: Thirty-five Sprague-Dawley rats were randomly divided into groups including control and four experimental groups. The control group was designed to determine the onset time and duration of NMB induced by 1.2 mg/kg rocuronium. In the control group, no sugammadex was applied. In the experimental groups, rocuronium (1.2 mg/kg) was reversed with sugammadex (4 mg/kg). Subsequently, experimental groups were administered various doses of rocuronium. Groups were named according to the rocuronium dose administered (group 2.4, group 3.6, group 4.8 and group 6.0). Rats in all groups were monitored with train of four.

RESULTS: In group 2.4, rocuronium did not ensure NMB. In group 3.6, NMB occurredin only 3 rats. All rats in groups 4.8 and 6.0 achieved complete NMB. There was no statistically significant difference in the onset time of NMB in 4.8 and 6.0 groups ($p<0.05$). The mean duration of NMB in the experimental groups was significantly shorter than that in the control group ($p<0.01$).

CONCLUSION: Sufficient muscle relaxation and intubation conditions could be achieved with 3.6 mg/kg, 4.8 mg/kg, and 6.0 mg/kg doses of rocuronium as short as 2 min after sugammadex.

Keywords: Sugammadex, rocuronium, neuromuscular block

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INTRODUCTION

Sugammadex is a gamma cyclodextrin structured agent used for reversing the effect of steroidal neuromuscular blocking (NMB) agents such as rocuronium and vecuronium via encapsulation.[1] In clinical practice, sugammadex is widely used to reverse rocuronium-induced NMB in postoperative care and emergency medicine.

Reoperation within the early postoperative period and re-intubation due to respiratory complications or allergic reactions are undesired situations that require NMB.[2] Theoretically, agents other than steroid-structured NMB should be used to re-establish NMB after reversal with sugammadex.[3] However, rocuronium is an alternative choice because of its rapid onset time and minimal hemodynamic changes even at high doses.[4] There are several case reports and studies on rocuronium being reused after reversal with sugammadex.[5-8] In these studies, there is no data on the onset time or duration of NMB with rocuronium when it is usedto re-establish NMB after administration of sugammadex. The aim of this experimental animal study was to evaluate the dose of rocuronium required to re-establish NMB when administered 2 min after its reversal with sugammadex. Secondary end points of this study were to monitor the onset times and durations of NMB achieved by variable doses of rocuronium after reversal with sugammadex.

MATERIALS AND METHODS

Study Subjects and Study Design

This experimental, randomized, controlled, and blinded animal study was approved by the Yeditepe University Local Animal Studies Ethical Board (approval number: 478, date: 19.08.2015). All invasive procedures, anesthesia, animal care, etc. were conducted in accordance with international guidelines on experimental animal studies.[9]

Thirty-five female Sprague-Dawley rats weighing 180-300 gr were equally randomized into four experimental and one control groups. Rats were maintained at a temperature (22-24 ºC) and humidity-controlled environment with free access to food and water. Sugammadex (Bridion; 200 mg/2 mL, Schering-Plough, Türkiye) and rocuronium (esmeron; 50 mg/5 mL Merck Sharp Dohme Ilac, Türkiye) were the agents used in this study.

Anesthesia and procedural preparation: Following intraperitoneal ketamine administration (60 mg/kg), intravenous access was achieved using a 20-22 G cannula in the tail lateral vein. Rats were secured on the dissection tray supinely, and 1 L/m O_2 was administered. Following a 0.5 cm incision on the midline of the neck, surgical dissectors were used to locate the trachea and place a 18 G cannula as a tracheostomy (Figure 1a, b). Thereafter, 1 mL of serum physiology was administered to maintain hemodynamic stability due to possible blood loss.

The effect of NMB was monitored using train of four (TOF) (TOF-WATCH S, Oragon; Dublin, Ireland). The TOF stimulating part was placed using platinum needles neighboring the sciatic nerve and its receiving part was placed in a pocket formed with surgical scissors between the gastrocnemius muscle and skin (Figure 1c). Stimulation with 2 Hz 0.2 ms was administered for 1.5 seconds every 15 seconds. Supramaximal current was determined to be T1/T4: 1.0 for the mentioned muscle groups.

Intravenous rocuronium (1.2 mg/kg) was administered to all rats with TOF measurements taken every 15 seconds. Rats were placed on 850 NEMI Scientific mechanical ventilators (respiratuar rate: 80-100, tidal volume 10 mL/kg) when their respiratory effort was lost. The control group was designed to determine the onset time and duration of NMB induced by 1.2 mg/kg rocuronium. In the control group, no sugammadex was applied.

In all experimental groups, rocuronium (1,2 mg/kg) was applied, then time until TOF <0.2 was recorded as *t1*. Sugammadex at a dose of

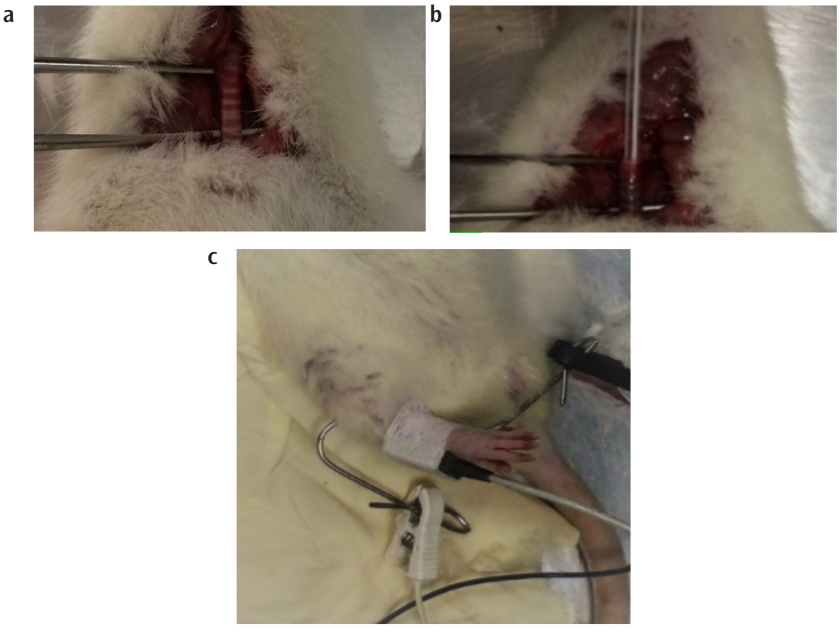

Figure 1. Steps of our study

4 mg/kg was applied when TOF <0.2. After that, the time between the administration of sugammadex and TOF >0.9 was observed and recorded as *t2*. Two minutes after TOF >0.9, groups were administered various doses of rocuronium. Groups were named according to the rocuronium dose performed (group 2.4, group 3.6, group 4.8 and group 6.0). After second doses of rocuronium were applied in experimental groups, the time until TOF <0.2 was defined as *t3*. The duration of action of rocuronium applied to the experimental groups for the second time and the duration of action of rocuronium applied to the control group was defined as *t4*. All steps of our study have been demonstrated in Figure 1.

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics 16 (IBM SPSS, Türkiye). Kolmogorov-Smirnov test was used to determine the normal distribution. One-Way ANOVA with post-hoc Tukey HSD was used to compare the groups. Statistical significance was set as $p<0.05$.

RESULTS

The average age of rats was 137.70±4.06 (133-145 days) and average weight was 187.24±15.43 Gr (171-208). There was no difference between the groups with regard to age, weight, basal body temperature, or basal respiratory weight ($p>0.05$).

There was no statistically significant difference between the groups with regards to *t1* and *t2*. NMB was achieved in no rats in group 2.4 and only 3 rats in group 3.6, whereas all rats in groups 4.8 and 6.0 achieved complete NMB. When groups 3.6, 4.8, and 6.0 were compared according to *dt3*, no statistically significant difference was found. While a statistically significant difference was found between the experimental and control groups for *t4*, there was no difference between the experimental groups (Table 1).

The relationship between time and mean TOF values after the second dose of rocuronium the following sugammadex administration is shown in Figure 2. Comparison of *t4* between groups is shown with box plot in Figure 3 and Table 2.

DISCUSSION

This experimental study demonstrated that increasing dosages of rocuronium can be used for the re-establishment of NMB after the reversal of rocuronium-induced NMB with sugammadex. However, re-used rocuronium has a shorter duration of action when it is performed after sugammadex administration.

Rocuronium is the most commonly used steroid-structured NMB agent. The use of steroidal neuromuscular agents for general anesthesia after the reversal of NMB with sugammadex is controversial. If NMB is required after routine dosage of rocuronium (0.6 mg/kg) has been reversed by 4 mg/kg of sugammadex, 1.2 mg/kg of rocuronium or 0.6 mg/kg of rocuronium can be applied at the 5th minute or 4th hour respectively.[3]

The timing and time to the effect of recurrent doses of rocuronium are open to debate. In a case report, 0.6 mg/kg of rocuronium was

Table 1. Comparison of *t1, t2, t3, t4* times according to groups and number of curarized rats per group

	Group 2.4	Group 3.6	Group 4.8	Group 6	Control group	p
t1 (sec.)	77.14±21.38	74.3±22.3	71.4±25.4	77.1±21.4	68.6±18.6	0.935
t2 (sec.)	68.57±19.51	71.4±25.4	88.6±19.5	80.0±28.3	-	0.395
t3 (sec.)	-	145.0±26.9	120.0±23.1	100.0±24.6	-	0.080
Curarized rats (n)	0/7	3/7	7/7	7/7	-	-
t4 (sec.)	-	310.0±57.7	311.4±33.8	360.0±86.4	514.3±53.8	0.001

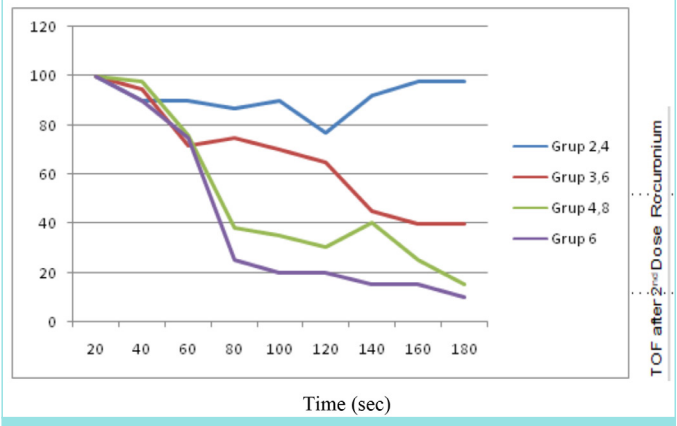

Figure 2. Relationship between time and TOF values after the second dose of rocuronium following sugammadex administration.

TOF: Train of four.

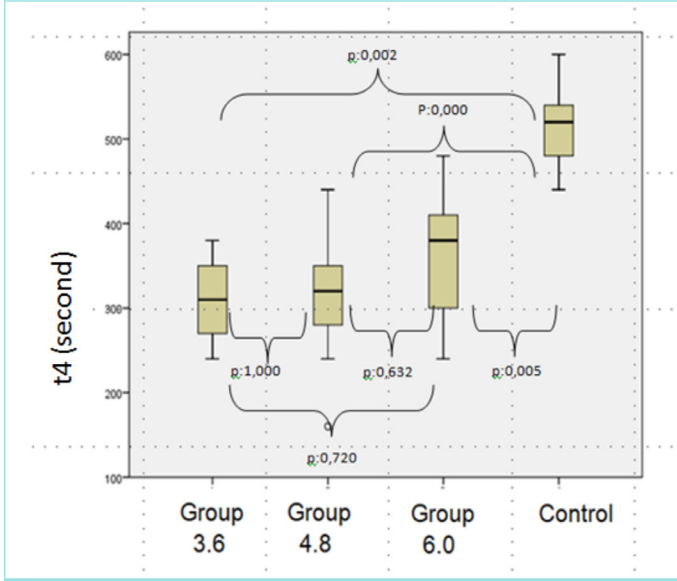

Figure 3. *t4* demonstrated as box plot.

Table 2. Comparison of t4 between groups					
	Control, (n=7)	Group 3.6, (n=4)	Group 4.8, (n=7)	Group 6.0, (n=7)	p
Mean ± SD	514.29±53.80	310.00±57.73	311.43±33.76	360.00±86.41	0.001*
SD: Standard deviation.					

reversed by 4 mg/kg of sugammadex. Emergency resurgery was required 30 min later, and adequate NMB was achieved at the 6th minute following 2 mg/kg of rocuronium.[10] In another case report, 0.6 mg/kg of rocuronium led to adequate NMB for intubation in 102 s following the reversal of rocuronium with 2 mg/kg sugammadex 6 h previously.[8] The authors reported that as the half-life of sugammadex is 2 h, a normal dose of rocuronium (0.6 mg/kg) would be adequate after 3 half lives or more had passed.[8] Case reports have reported varying times for administration of rocuronium following NMB reversal with sugammadex, with even more variation for dosages of 1.2 mg/kg and 3.4 mg/kg.[2,11,12] Although our study supports the hypotheses suggested by these case report examples, our goal is not only to determine the rocuronium dose after sugammadex in rats but also to project the rocuronium doses in humans.

In a study by de Boer et al.[13], body distribution and drug pharmacodynamics and pharmacokinetics were used to determine the dosage of sugammadex required to reverse an initial dose of 0.6 mg/kg rocuronium and the required repeat dose of rocuronium to achieve NMB after sugammadex. The investigators determined that adequate conditions for intubation would be achieved with 1 mg/kg rocuronium for 2 mg/kg sugammadex, 1.5 mg/kg rocuronium for 4 mg/kg sugammadex, and 2.25 mg/kg rocuronium for 8 mg/kg sugammadex. However, these findings do not correlate with previous case reports, and the onset time of rocuronium was not specified in this dose determination study.

Cammu et al.[5] reported a pilot study where the effect of 1.2 mg/kg of rocuronium following 4 mg/kg sugammadex was evaluated in 16 healthy volunteers. 1.2 mg/kg rocuronium was applied at different times after reversal of NMB and their time to reach T1: 0% and TOF rate 0.9 were determined. They were grouped according to the time when rocuronium was performed after sugammadex [5 min (n=6), 5-25 mins (n=6) and after 25 min (n=5)]. Average time to T1: 0% and time to TOF: 0.9 was 3.06 m and 25.3 m for the 5 m group, 3.09 m and 24.8 m for the 5-25 m group, and 1.73 m and 38.2 m for the over 25 m group, respectively. In a later group, NMB commencement in volunteers was found to be significantly shorter. The study reported that the differing time of effect for rocuronium after sugammadex was not predictable in all patients. Therefore, the authors concluded that rocuronium usage after reversal with sugammadex was not a safe and feasible option.[5]

To our knowledge, there are no animal studies regarding the re-use of rocuronium after sugammadex, therefore, our study is the first in the literature on this matter. When published case reports and volunteer-based studies are taken into consideration, the literature generally reports re-use of rocuronium 5 minutes after sugammadex, more often than not 30 minutes after.[8,11,12,14] However, a recently published systematic review reported that allergic reactions and respiratory complications occur within the first 3 min after sugammadex administration.[15] Instead of evaluating redose of rocuronium after 5 min, we therefore decided to evaluate the effect of various doses of rocuronium 2 min after reversal with sugammadex.

Some unforeseen complications have also been described after the widespread use of sugammadex in anesthesia practice. Cases of acute coronary syndrome resulting from a strong allergic/immune reaction to any drug or product, also called Kounis syndrome, have been associated with the use of sugammadex.[16] This newly described-allergic condition has been described in many clinical presentations, from atropine-resistant bradycardia to sudden cardiac arrest.[16-19] Both the aforementioned post-extubation respiratory complications and allergy-related coronary symptoms are conditions that develop in a short time, and the findings of our study may guide clinicians in dealing with these clinical scenarios in case of re-intubation. We demonstrated that the time for reversal of NMB of rocuronium after sugammadex reversal of initial NMB was statistically shorter for the control group than for the other groups. Clinicians should closely follow up for motor blockage in the event of such re-intubation.

In our study, the first dose of rocuronium was administered at 1.2 mg/kg. The suggested induction dose of rocuronium in humans is 0.6 mg/kg. However, due to faster metabolism, this dose does not provide adequate NMB. Differing doses of rocuronium are used in rats, while in most studies a high dose of 3.5 mg/kg is used.[20,21] It has been reported that this dose corresponds to 0.6 mg/kg in humans. However, most studies have used lower doses of 1.2-1.5 mg/kg.[22,23] Our primary aim was not to determine the optimal human dosage but to evaluate differing doses according to total effect time and time to NMB. Therefore, we used the minimum accepted dosage of 1.2 mg/kg for rocuronium. We prevented mortality and complications by keeping experiment time and time on mechanical ventilation to minimum. Intravenous sugammadex demonstrated linear pharmacokinetic properties over the dose range of 1-16 mg/kg. On the other hand, when sugammadex is administered at high doses, the unbound sugammadex molecules will remain free, increasing the possibility of inducing toxic effects. Studies have shown that 1 mg of sugammadex is equivalent to 4 mg in rats.[23,24] Therefore, we used 4 mg/kg of sugammadex to correspond to the minimum dosage in humans. To our knowledge, there are no animal study, rat model, or a pilot study similar to ours in the literature. There is also no study similar to ours in which TOF usage in rats is demonstrated in detail.

In summary, the above-mentioned studies and case reports report that after reversal of NMB with sugammadex, high dosage (four times of normal) of rocuronium leads to NMB and an increase in rocuronium dosage after sugammadex lengthens the time for beginning of its effect. In our study we used 2-5 times more dosage to determine the effect of rocuronium after reversal with sugammadex. While 4-5 times higher dose led to NMB, there was no difference between the time of effect start between 4 and 5 times dosages.

Study Limitations

Our study has some limitations. Due to technical reasons, we were unable to monitor blood gases and other physiological responses in rats. We had to ignore the factors such as the metabolic rate that would affect the effect time and metabolism of drugs. To prevent any negative effects of mechanical ventilation, we kept the rocuronium doses low.

We were therefore unable to administer higher doses of rocuronium. Also, we did not perform any pathophysiological evaluation of the end organ effects of our experimental drugs.

CONCLUSION

Adequate NMB for intubation is possible when rocuronium is applied 2 min after sugammadex. However, the total dose of rocuronium, sugammadex and the time required for intubation after sugammadex should all be kept in mind. A non-steroidal non-depolarising NMB should be used in this case. Rocuronium can be used in 3-4 times of normal dose when other medications are not available. However, studies on the end-organ effect of these dosages of rocuronium must be evaluated.

ETHICS

Ethics Committee Approval: This experimental, randomized, controlled, and blinded animal study was approved by the Yeditepe University Local Animal Studies Ethical Board (approval number: 478, date: 19.08.2015).

Informed Consent: Patient approval has not been obtained as it is performed on animals.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: H.C.K., S.O.A., T.K., Ö.T., S.T., Design: H.C.K., S.G.K., S.O.A., T.K., Ö.T., S.T., Supervision: H.C.K., Ö.T., S.T., Fundings: H.C.K., S.G.K., S.O.A., Ö.T., Materials: H.C.K., S.G.K., S.T., Data Collection and/or Processing: H.C.K., S.G.K., S.T., Analysis and/or Interpretation: T.K., Ö.T., S.T., Literature Search: H.C.K., S.G.K., S.T., Writing: H.C.K., S.G.K., S.T., Critical Review: H.C.K., T.K., Ö.T., S.T.

DISCLOSURES

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

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REFERENCES

1. de Boer HD. Sugammadex, a new reversal agent for rocuronium-induced neuromuscular block: a step forward in improving patient's safety. [Sl: sn]; 2008.
2. Iwasaki H, Sasakawa T, Takahoko K, Takagi S, Nakatsuka H, Suzuki T, et al. A case series of re-establishment of neuromuscular block with rocuronium after sugammadex reversal. J Anesth. 2016; 30(3): 534-7.
3. Bridion 100 mg/ml solution for injection (emc) [Internet]. [cited 2017 Apr 4]. Available from: https://www.medicines.org.uk/emc/product/6409
4. Williamson RM, Mallaiah S, Barclay P. Rocuronium and sugammadex for rapid sequence induction of obstetric general anaesthesia. Acta Anaesthesiol Scand. 2011; 55(6): 694-9.
5. Cammu G, de Kam PJ, De Graeve K, van den Heuvel M, Suy K, Morias K, et al. Repeat dosing of rocuronium 1.2 mg kg-1 after reversal of neuromuscular block by sugammadex 4.0 mg kg-1 in anaesthetized healthy volunteers: a modelling-based pilot study. Br J Anaesth. 2010; 105(4): 487-92.
6. Matsuki G, Takahata O, Iwasaki H. Repeat dosing of rocuronium after reversal of neuromuscular block by sugammadex. Can J Anaesth. 2011; 58(8): 769-70.
7. Kim YH. Repeat dosing of rocuronium-sugammadex: unpredictable. Korean J Anesthesiol. 2014; 67(1): 1-3.
8. Sasakawa T, Iwasaki H, Kurosawa A, Kikuchi C, Takahata O, Iwasaki H. Case report: a normal dose of rocuronium achieved the desired effect in a short time after the administration of sugammadex during reoperation. Masui. 2011; 60(5): 621-4.
9. National Research Council, Division on Earth and Life Studies, Institute for Laboratory Animal Research, Committee on Guidelines for the Use of Animals in Neuroscience and Behavioral Research. Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research. National Academies Press; 2003.p.224.
10. Nishi M, Fujii S, Nitta S. A two-year-old patient who received readministration of rocuronium for re-operation 30 minutes after sugammadex reversal. Masui. 2011; 60(10): 1189-91.
11. Sakai Y, Tsutsumi YM, Wakamatsu N, Soga T, Tanaka K, Oshita S. A case where rocuronium was unable to achieve neuromuscular block immediately after sugammadex administration. J Med Invest. 2011; 58(1-2): 163-5.
12. Real C, Silva J, Esteves S, Machado HS. The use of rocuronium 20 minutes after sugammadex administration - a case report. Glob Anesth Perioper Med. 2015; 1(4): 118-20.
13. de Boer HD, Driessen JJ, van Egmond J, Booij LH. Non-steroidal neuromuscular blocking agents to re-establish paralysis after reversal of rocuronium-induced neuromuscular block with sugammadex. Can J Anaesth. 2008; 55(2): 124-5; author reply 125-6.
14. Cammu G, de Kam PJ, De Graeve K, van den Heuvel M, Suy K, Morias K, et al. Repeat dosing of rocuronium 1.2 mg kg-1 after reversal of neuromuscular block by sugammadex 4.0 mg kg-1 in anaesthetized healthy volunteers: a modelling-based pilot study. Br J Anaesth. 2010; 105(4): 487-92.
15. Tsur A, Kalansky A. Hypersensitivity associated with sugammadex administration: a systematic review. Anaesthesia. 2014; 69(11): 1251-7.
16. Kounis NG, Koniari I, Soufras GD, Tsigkas G, Plotas P, Davlouros P, et al. Sugammadex-induced atropine-resistant bradycardia: clinical, pathophysiologic, and electrocardiographic considerations. JA Clin Rep. 2020; 6(1): 31.
17. Yanai M, Ariyoshi K. Two Cardiac Arrests that Occurred after the Administration of Sugammadex: A Case of Kounis Syndrome. Case Rep Emerg Med. 2020; 2020: 6590101.
18. Yoshida T, Sumi C, Uba T, Miyata H, Umegaki T, Kamibayashi T. A rare case of atropine-resistant bradycardia following sugammadex administration. JA Clin Rep. 2020; 6(1): 18.
19. Yang HS, Kim HJ, Koh W. Effects of sugammadex on the coronary circulation: direct effects on coronary vessels or hypersensitivity (Kounis syndrome)? Korean J Anesthesiol. 2017; 70(3): 363-4.
20. Et T, Topal A, Erol A, Tavlan A, Kılıçaslan A, Uzun ST. The Effects of Sugammadex on Progesterone Levels in Pregnant Rats. Balkan Med J. 2015; 32: 203-7.
21. Eikermann M, Zaremba S, Malhotra A, Jordan AS, Rosow C, Chamberlin NL. Neostigmine but not sugammadex impairs upper airway dilator muscle activity and breathing. Br J Anaesth. 2008; 101(3): 344-9.
22. Tomak Y, Yılmaz A, Bostan H, Tümkaya L, Altuner D, Kalkan Y, et al. Effects of sugammadex and rocuronium mast cell number and degranulation in rat liver. Anaesthesia. 2012; 67(10): 1101-4.
23. Platt PR, Sadleir PH, Clarke RC. Sugammadex, rocuronium and mast cell numbers in the rat liver. Anaesthesia. 2013; 68(2): 208-9.
24. Hoffmann U, Grosse-Sundrup M, Eikermann-Haerter K, Zaremba S, Ayata C, Zhang B, et al. Calabadion: A new agent to reverse the effects of benzylisoquinoline and steroidal neuromuscular-blocking agents. Anesthesiology. 2013; 119(2): 317-25.