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The Effects of Sugammadex on Vitamin D Levels in Rabbits Under General Anesthesia

Genel Anestezi Altındaki Tavşanlarda Sugammadeksin D Vitamini Düzeylerine Etkileri

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Abstract

Objective: Sugammadex is a widely used anesthetic agent for reversing of the non-depolarizing block. It reduces blood levels of steroid compounds by encapsulation. The aim of this study was to investigate the effect of sugammadex on the blood levels of vitamin D levels, which has a steroid structure.

Materials and Methods: A total of 15 adult male New Zealand white rabbits weighing 2-2.5 kg were randomized into three groups according to decurarization: Group S [sugammadex (16 mg/kg), n=5], group N [neostigmine (0.05 mg/kg), n=5], group C (control group, n=5). Vitamin D levels from venous blood were measured at baseline, 20 minutes and 24 hours after general anesthesia.

Results: Mean ± SD vitamin D levels before anesthesia, 20 minutes and 24 hours after anesthesia were 4.42±0.60 ng/mL, 4.93±0.72 ng/mL, and 4.66±0.94 ng/mL for group S, 4.92±0.45 ng/mL, 5.02±0.41 ng/mL, and 5.41±0.56 ng/mL for group N, and 5.15±0.82 ng/mL, 4.57±1.10 ng/mL, and 5.21±1.05 ng/mL for group C, respectively. There were no significant differences in the mean vitamin D levels between the groups at baseline, 20 minutes, and 24 hours.

Conclusion: Contrary to expectations, it was found that sugammadex did not have a statistically significant effect on blood levels of vitamin D.

Keywords: Vitamin D, general anesthesia, steroids, sugammadex

Öz

Amaç: Sugammadeks non-depolarizan bloğun geri döndürülmesi için yaygın kullanılan bir anestezik ajandır. Enkapsülasyon yoluyla steroid bileşiklerin kan düzeylerini azaltır. Bu çalışma, sugammadeksin steroid yapılı olan D vitamininin kan düzeyleri üzerine etkisini araştırmak amacıyla yapıldı.

Gereç ve Yöntem: 2-2,5 kg ağırlığında toplam 15 yetişkin erkek Yeni Zelanda ırkı beyaz tavşan, dekürarizasyona göre randomize olarak üç gruba ayrıldı: Grup S [sugammadeks (16 mg/kg), n=5], grup N [neostigmin (0,05 mg/kg), n=5], grup K (kontrol grubu, n=5). D vitamini düzeyleri; bazal, genel anestezi sonrası 20. dakika ve genel anestezi sonrası 24. saatte venöz kandan ölçüldü.

Bulgular: Anestezi öncesi, anestezi sonrası 20. dakika ve 24. saatte ortalama ± SS D vitamini seviyeleri sırasıyla; grup S için 4,42±0,60 ng/mL, 4,93±0,72 ng/mL ve 4,66±0,94 ng/mL, grup N için 4,92±0,45 ng/mL, 5,02±0,41 ng/mL ve 5,41±0,56 ng/mL ve grup C için 5,15±0,82 ng/mL, 4,57±1,10 ng/mL, 5,21±1,05 ng/mL idi. Gruplar arasında ortalama D vitamini düzeylerinde bazal, 20. dakika ve 24. saatte anlamlı fark yoktu.

Sonuç: Beklenenin aksine, sugammadeksin D vitamini kan düzeyleri üzerinde istatistiksel olarak anlamlı bir etkisi olmadı.

Anahtar kelimeler: D Vitamini, genel anestezi, steroidler, sugammadeks

Introduction

Vitamin D are related to calcium-phosphorus metabolism and bone mineralization (1). In recent years, vitamin D deficiency has been linked to many chronic diseases including various cancers, cardiovascular diseases, metabolic syndrome, infections, and autoimmune diseases (2-4). Therefore, it is very important to reveal the factors affecting vitamin D. Can an anesthetic drug be medication that reduces vitamin D levels?

Today's medical advances have made significant progress in increasing the reliability of anesthetics and the overall quality of anesthesia. Therefore, there have been significant increases in the number of surgical procedures. Turkey's health ministry datas showed that the number of surgical procedures is seen as closer to 5 million per year (5).

Muscle relaxant drugs significantly contribute to the reduction of the medications used in anesthesia. With the widespread

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use of muscle relaxants, the need for agents to antagonize the effects of medium and long-acting muscle relaxant agents in clinical use has increased (6). Representing an alternative to traditional decurarization provided by cholinesterase inhibitors, sugammadex is a widely used cyclodextrin analog. It has been shown to achieve a fast and safe elimination of the non-depolarizing block (7). Sugammadex is a modified γ-cyclodextrin drug used to antagonize the effect of steroid-based non-depolarizing muscle relaxants such as rocuronium and vecuronium. It binds to the circulating steroid muscle relaxants (encapsulation), forms a complex, and is excreted in the urine without metabolizing (8). Despite this advantages, sugammadex can causes side effects on some steroid drugs. Studies showed that sugammadex may interact with toremifene (selective estrogen receptor modulator) and some antibiotics (flucloxacillin, fusidic acid). It can also bind and interact with oral contraceptive drugs taken on the same day (9,10). It reduces both the activity of sugammadex and the efficacy of steroid drugs, so it can lead to undesirable effects.

In recent studies, the effects of sugammadex on endogenous steroid hormones and hormone-like substances are also investigated (11,12). Vitamin D is one of the fat-soluble vitamins from a group of sterols that are hormone and hormone precursors, which can be synthesized endogenously in the appropriate biological environment. But there is no study on the effects of sugammadex on steroid-based vitamin D.

This study aimed to investigate the possible effects of sugammadex on blood vitamin D levels.

Materials and Methods

Trial Design

A randomized-controlled, animal study was planned. Ethical approval of the study was obtained from the local ethics board of the Çanakkale Onsekiz Mart University Ethical Board of Animal Studies (IRB number 2017/42962, date 27/11/2017). The study reporting was done following the CONSORT guidelines (13,14).

Participants

In this study, 15 adult male New Zealand White rabbits weighing 2-2.5 kg were used. All rabbits were examined before the study clinically for the behavioral, respiratory, and cardiovascular systems and no issues were detected.

Study Settings

The experiments were carried out at the Çanakkale Onsekiz Mart University Experimental Research Center during December 2017. All experiments were performed between 09:00-17:00 hours. All rabbits were housed in appropriate plastic cages in an animal room maintained at a standard humidity (45%-50%) and temperature 21±2 °C with 12 hours light and 12 hours darkness, and were fed with standard food (Bil-Yem Ltd. Co., Ankara, Turkey) and water ad libitum. The experiment was started after one week of acclimatization.

Interventions

The rabbits have fasted for 8 hours before the intervention. After randomization, the animals received the following interventions: Group S [sugammadex (16 mg/kg), n=5], group N [neostigmine (0.05 mg/kg), n=5], and group C [0.9% saline (0.05 mL/kg), n=5]

The rabbits receiving general anesthesia were given 10 mg/kg intramuscular ketamine for premedication. electrocardiogram and arterial blood pressure monitoring were performed to the experimental animals during general anesthesia. The mean arterial pressure (59-91 mmHg) and heart rates (137-246/min) of all experimental animals were within physiological limits.

After 20 minutes, vascular access was made with a 22-24 G branule from the ear vein. Two cc blood samples were taken from each rabbit to measure vitamin D levels. Group S and group N were given intravenous (IV) propofol 2 mg/kg, fentanyl 1 mcg/kg, and rocuronium 0.6 mg/kg for general anesthesia. Then, V-gel® Rabbit (V-gel® Rabbit R-3 Docsinnovent ® Ltd. London, UK) was placed in all experimental animals to ensure airway safety, and the animals were placed on the anesthesia machine (Anesthesia Machine w/O2 Flush Model M3000PK Parkland Scientific Lab and Research Equipment. Florida, USA) and inhaled manually. The anesthesia was maintained with 50% oxygen, 50% air mixture and 1 minimum alveolar concentration isoflurane.

The experimental animals were manually ventilated by the same anesthesiologist at a pressure of about 15 cm H_2O (about 10 mL/kg) and a rate of 40/minute ensuring appropriate respiration for rabbit physiology. To secure sufficient oxygenation, blood gases were checked the 10^{th} minute of the procedure and 20 minutes after the removal of the V-gel® Rabbit removal) using the Radiometer ABL 800 device. The analyzed blood gases were within the physiological limits.

Twenty minutes after induction of anesthesia, group S received 16 mg/kg IV sugammadex, group N received 0.05 mg/kg IV Neostigmine plus 0.01 mg/kg atropine, and Group C received IV 0.05 mL/kg 0.9% saline. After sufficient spontaneous breathing was observed, the V-gel® Rabbit was removed, and the animals were allowed to rest for 20 minutes. Then, 2 cc blood samples were taken from all rabbits for a repeat vitamin D analysis. After 24 hours, the last sample of 2 cc blood was taken from all animals.

Outcomes

The main outcome of the study was blood vitamin D levels. Blood vitamin D levels were measured from all rabbits before anesthesia, 20 minutes after anesthesia, and 24 hours after anesthesia. Blood samples were collected into vacuumed gel tubes. The samples were incubated at room temperature for 30 minutes and then centrifuged at 4000 rpm for 10 minutes. The 25(OH) D3 levels were quantified using commercial kits (cat. no: 201-09-3871, Sunred biological technology, Shangai, China) based on the quantitative sandwich Enzyme-Linked Immuno Sorbent Assay (ELISA) method. The results were analyzed with the ELX 808 IU model ELISA reader.

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Sample Size

Sample size calculation was based on the mean differences in vitamin D levels between the three groups. A post hoc sample size calculation demonstrated that 15 cases produce a power of 83% in comparing the three means with an alpha error of 5% and an effect size of 0.95 (15).

Randomization

The 15 rabbits were randomly divided into 3 groups. Randomization was done by giving the rabbits sequential numbers and randomly assigning to groups using a random numbers table. The groups were; group S - Sugammadex group (n=5), group N - neostigmine group (n=5), and group C - control group (n=5) (Figure 1).

Blinding

Data were collected by an independent researcher who was not part of the study. Postoperative scoring of the masks was done by a nursing staff member who was unaware of the grouping.

Statistical Analysis

Data were presented as the mean ± standard deviation for numerical variables and n (%) for categorical variables. Normal distribution of the numerical variables was checked with the Shapiro-Wilk test (statistic; p for Vitamin D levels at baseline, 20 minutes, and 24 hours, 0.959; 0.709, 0.962; 0.753; and 0.923; 0.241, respectively). The one-way ANOVA and repeated measures ANOVA tests were used to compare changes in Vitamin D levels over time. The value of $p<0.05$ was considered significant.

Results

Participant Flow

Results for a total of 15 rabbits were analyzed (Figure 1).

Losses and Exclusions

All randomized rabbits could proceed to the end of the experiment; no rabbits were excluded (Figure 1).

Recruitment

The rabbits were supplied by the Experimental Research Center of the Çanakkale Onsekiz Mart University (Turkey).

Baseline Data

Mean weight of the rabbits was 2.39±0.23 kg. There were no differences in the weights of the rabbits between groups ($p>0.05$).

Outcomes and Estimation

The mean blood Vitamin D levels of the 15 rabbits at baseline was 4.80±0.65 ng/mL. There were no significant differences in the mean Vitamin D levels between the three groups at baseline, 20 minutes, or 24 hours (Table 1).

Also, there was no significant change in the mean Vitamin D levels of the groups over time, neither was an interaction between measurement time and groups (tests of within-subject effects $F=0.859$, $p=0.437$, tests of between-subjects effects $F=1.887$, $p=0.197$, Figure 2).

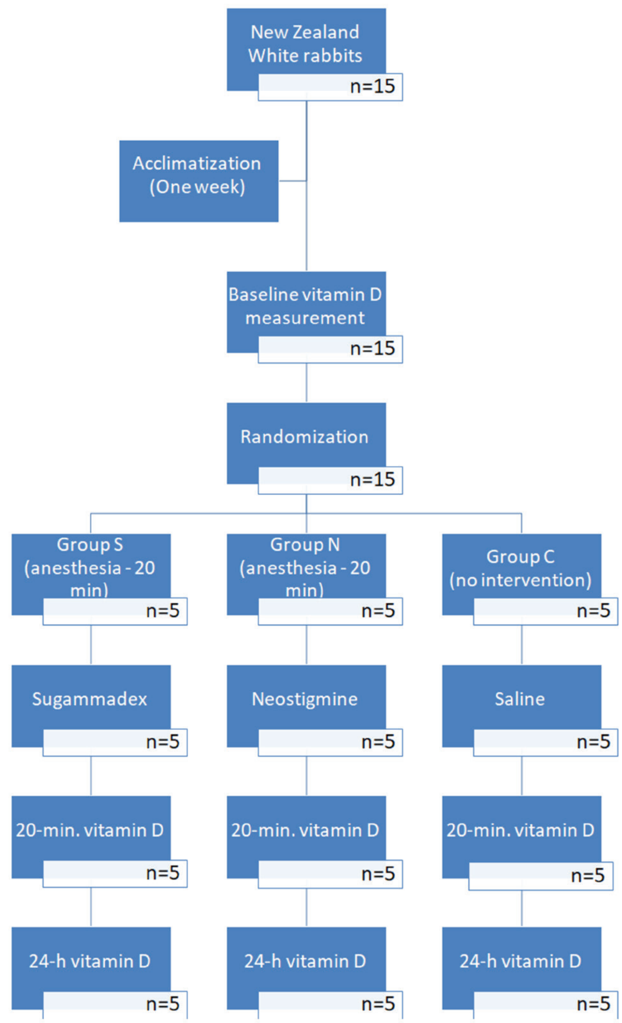

Figure 1. Experiment flow diagram

Table 1. Comparison of the mean Vitamin D levels between the groups

	Sugammadex		Neostigmine		Control	
	Mean ± SD	**F, p**	**Mean ± SD**	**F, p**	**Mean ± SD**	**F, p**
Baseline	4.42±0.60	1.692, 0.229	4.92±0.45	0.449, 0.649	5.15±0.82	0.996, 0.398
20-min.	4.93±0.72	-	5.02±0.41	-	4.57±1.10	-
24-h	4.66±0.94	-	5.41±0.56	-	5.21±1.05	-

SD: Standard deviation

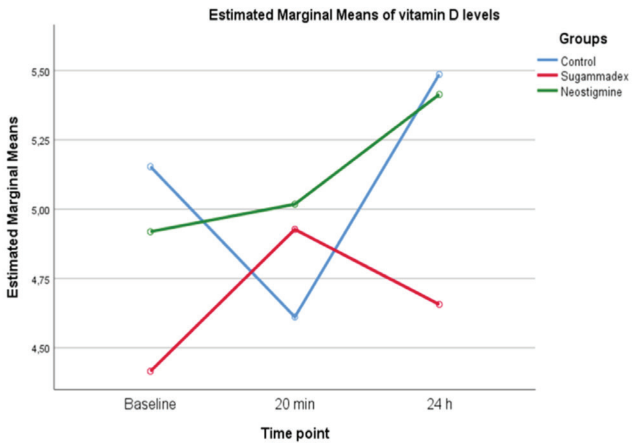

Figure 2. Changes of the mean Vitamin D levels between the groups overtime

Discussion

Study Limitations

Although in this trial we had randomly selected animals with possibly similar confounding factors, we did not check for other variables which could influence the results. Also, we had a low sample size, and we did not check for different doses of sugammadex.

Generalizability

There was no statistically significant difference in the vitamin D levels of rabbits who received sugammadex compared to those who received neostigmine or saline.

Interpretation

According to the prospectus information, it is stated that sugammadex may interact with toremifene (selective estrogen receptor modulator) and some antibiotics (flucloxacillin, fusidic acid). It can also bind and interact with oral contraceptive drugs taken on the same day (9). Since sugammadex acts by encapsulating steroid neuromuscular blockers, the effect of other molecules, hormones, and drugs on the plasma levels of the steroidal structure has been investigated before (16,17).

Ozdemirkan (11) investigated the effects of sugammadex on the levels of stress hormones in the postoperative period. Serum cortisol, insulin, aldosterone, and glucose levels were examined. It has been found that stress response to surgery has occurred in patients using both neostigmine and sugammadex, and the use of sugammadex does not affect this stress response and the levels of stress hormones. However, Ozer et al. (16) demonstrated an earlier reversal of neuromuscular block by sugammadex in patients receiving steroids, and particularly dexamethasone, claiming a potential interaction between sugammadex and steroids. On the other hand, Gulec et al. (18) evaluated dexamethasone's effects on the reversal time of sugammadex in children undergoing tonsillectomy and demonstrated that dexamethasone did not interfere with the reversal time of sugammadex. Similarly, in another study, the administration of dexamethasone to anesthetized patients did not delay neuromuscular block reversal by sugammadex (19). In another paper, it was suggested that sugammadex is not associated with adverse effects on steroid hormones progesterone and cortisol, while it may lead to a temporary increase in aldosterone and testosterone (12).

As seen in the literature, there are different results on the effects of sugammadex on endogenous steroids. Different anesthetic methods may have different effects on the hormonal autonomic responses, and various plasma concentrations of anesthetic medications may cause differences in endocrine responses. Therefore, it may be difficult to evaluate the hormonal response caused by anesthesia and surgery and to compare study results. Vitamin D is a steroid-structured molecule, so sugammadex may interact it. We want to investigate the possible effects of sugammadex on blood vitamin D levels.

In contrast with what expected, it was found that sugammadex has no effects on blood levels of vitamin D. There were no significant differences in the mean Vitamin D levels between the three groups at baseline, 20 minutes, or 24 hours. There may be two possible causes. The first one is that endogenous steroids and steroid drugs have a low affinity when binding to sugammadex because they do not contain quaternary ammonium ions such as steroid drugs (rocuronium, vecuronium). The steroid structure of the hormones in the plasma to bind to specific protein carriers have also been shown to be another reason for low affinity (20). In their review of the interaction of sugammadex with other molecules with the isothermal titration microcalorimetry method, Zhang (21) investigated the tendency of sugammadex to complex with steroid and non-steroidal compounds such as cortisone, atropine, and verapamil. They reported that the inclination of sugammadex to complex with these compounds was clinically insignificant and that this tendency was about 120-700 times less than the tendency to complex with rocuronium. They stated that sugammadex may form a complex with molecules of steroid structure, but with a very low affinity. As an explanation for the lack of statistically significant effect of sugammadex on the vitamin D levels, it was claimed that due to the high affinity of sugammadex for rocuronium, even if it binds to steroid molecules, the ratio may not be not enough to reach a significance level.

The other possible cause may be related to the structure of vitamin D. Although vitamin D is known as a steroid, vitamin D is produced from the cyclopentanoferon ring, and some reports are suggesting that it is not considered steroid hormone because of its four-ring structure and it has a secosteroid structure (22). Therefore, it may be considered that sugammadex actually does not have vitamin D binding properties.

Conclusion

In conclusion, clinicians prefer drugs that can turn down the unwanted side effects of neuromuscular blocking medications without serious side effects. Although sugammadex has been recently introduced, it has been suggested that it is superior to other neuromuscular blocking antagonists concerning its effect velocity and low side effect potential. Although there are many publications in the literature about sugammadex drug interactions and clinical effects, there is no study in the literature on the impacts of sugammadex on vitamin D levels. As to our knowledge, this is the first research in this field. Further clinical studies are needed to prove our conclusions.

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Ethics

Ethics Committee Approval: The study were approved by the Çanakkale Onsekiz Mart University Experimental Research Application and Research Center with approval of Local Animal Experimentation Ethics Committee. (protocol number: 1421)

Informed Consent: It was taken.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: T.H., H.B.A., Concept: H.B.A., Design: T.H., H.B.A., Data Collection or Processing: T.H., H.B.A., Analysis or Interpretation T.H., H.B.A., Literature Search: T.H., Writing T.H., H.B.A.

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