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Determining the effect of long-term dexamethasone and prednisolone treatment on sugammadex

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Background/aim: We aimed to investigate the effect of long-term use of dexamethasone and prednisolone on the reversal effect of sugammadex.

Materials and methods: TTwenty-four male Wistar albino rats were divided into three groups. Dexamethasone (600 μg/kg) was given to group D, prednisolone (10 mg/kg) was given to group P, and an equivalent volume of saline per day was administered intraperitoneally to group S for 14 days, respectively. The left hemidiaphragm with attached phrenic nerve was maintained in Krebs solution. Sugammadex (30 μmol/L) was applied while rocuronium (10 μmol/L) was present in an organ bath and a single twitch was obtained. The right hemidiaphragm was used for both adult (ε-subunit) and fetal nicotinic acetylcholine receptor (AChR) (ɣ-subunit) determination using polymerase chain reaction.

Results: All animals lost weight, except group S. The mean baseline single-twitch tension was lower in both group D (14.4 ± 1.7 g) and group P (12.68 ± 0.05 g) than group S (16.8 ± 0.5 g) ($P < 0.001$). When sugammadex was added to the organ bath while rocuronium was present, the single twitch was measured to be lower in both group D (11.7 ± 0.7 g) and group P (11.5 ± 0.78 g) than group S (16.5 ± 0.24 g) ($P < 0.001$). ɣ-AChR expression was higher in both dexamethasone and prednisolone than in saline.

Conclusion: Long-term medication with dexamethasone and prednisolone caused muscle weakness, resistance to neuromuscular blockers, and upregulation of immature γ-AChR and reduced the neuromuscular reversal effect of sugammadex.

Key words: Sugammadex, dexamethasone, prednisolone, acetylcholine receptors, neuromuscular blockers

1. Introduction

Glucocorticoid drugs are widely used for the long-term treatment of disease and symptoms such as sepsis, edema, anaphylaxis, and inflammatory disease. Chronic glucocorticoid treatment can cause muscle atrophy and this muscle weakness after glucocorticoid treatment can be associated with increased synthesis and release of acetylcholine; therefore, resistance to the effect of neuromuscular blocking drug occurs (1–6).

The effect of long-term prednisolone medication on atracurium- and rocuronium-induced neuromuscular blockage has already been indicated by Soeltsz et al. The authors showed that patients who received long-term prednisolone medication had shorter duration of atracurium- and rocuronium-induced neuromuscular blockage (7,8).

Nicotinic acetylcholine receptors (nAChRs) have four different subunits in a pentameric structure. Muscle-type nAChRs include two subtypes: the adult or junctional mature form (ε-AChR) comprises an α2βδε subunit, and the fetal immature form (γ-AChR) contains an α2βδγ subunit. The immature fetal form is usually expressed in fetal life. Fetal-type nAChRs have shorter open times than adult-type nAChRs (9). When the muscles are innervated, γ-AChR is replaced by ε-AChR. Some pathologic conditions such as immobilizations, burns, and denervation induce upregulation of expression to immature subtypes (10–13). Furthermore, glucocorticoid treatment upregulates the synthesis and expression of junctional nAChRs (6,14–16).

Sugammadex is a ɣ-cyclodextrin that reverses the neuromuscular block induced by rocuronium and vecuronium. It has a rapid onset and high efficacy in the reversal of deep neuromuscular blockage (17).

In the present study, we aimed to investigate the effect of long-term usage of prednisolone and dexamethasone on

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sugammadex reversal. We hypothesized that sugammadex was less effective with chronic glucocorticoid use and this could be due to both muscle weakness and an increase in immature acetylcholine receptors. Our primary objective was to investigate the effect of long-term use of prednisolone and dexamethasone on sugammadex reversal. The secondary outcome was the detection of immature acetylcholine receptors in glucocorticoid-treated rat diaphragms.

2. Materials and methods

All experiments reported in this paper were performed at the Department of Biophysics and Medical Biology of Çukurova University, Turkey, in collaboration with the Department of Anesthesiology and Intensive Care, Faculty of Medicine, Çukurova University, Turkey. The study was approved by the Çukurova University Ethics Committee of Animal Research (Permission Date: 26 February 2016, Ethics Number: 2/3)

2.1. Animals and muscle preparation

Twenty-four male Wistar albino rats (280–320 g) were used for this study. They were maintained for 1 week in the biophysics laboratory for adaptation. They were fed standard rat chow and tap water ad libitum during the experimental period. The temperature and humidity were monitored continuously throughout the experimental period. The animals were kept in an environmentally controlled room at 21–23 °C and relative humidity of 40%–60%, with a light/dark cycle of 12/12 h. In this study, 14-day glucocorticoid treatment was considered a long-term treatment, as previously reported in the literature (15,18,19). Rats were divided into 3 groups receiving dexamethasone (group D; n = 8), prednisolone (group P; n = 8), or saline (group S; n = 8). In group D, 600 μg/kg dexamethasone (Dekort ampule, 8 mg/2 mL, Deva Holding AŞ, Turkey) was applied intraperitoneally daily for 14 days (dosage was taken from previous studies) (16,20). In group P, 10 mg/kg prednisolone (Prednol-L ampule, 20 mg/mL, Mustafa Nevzat AŞ, Turkey) was administered intraperitoneally for 14 days (dosage as used by Shin et al.) (3). The drug dosages were chosen taking into consideration the equivalents of the lowest doses that could cause muscle atrophy. The saline group (group S) received an equivalent volume of saline per day (0.9% NaCl). All drugs were given at the same time of day. Animal mass was recorded daily and dexamethasone or prednisolone doses were adjusted according to changes in body mass.

Twenty-four hours after the last drug administration (14th day), the animals were anesthetized with sevoflurane. The left hemidiaphragm with attached phrenic nerve was rapidly dissected for muscle preparation, and the right hemidiaphragm was rapidly removed for molecular assessment.

2.2. Measurement of twitch contractions

The dissected rat phrenic nerve-hemidiaphragm muscle strips, weighing 90–120 mg and 7–10 mm in length, were suspended in organ baths in a vertical position, with the ribs supported on silver electrodes for indirect and direct stimulations in the organ baths (4 organ baths) containing 10 mL of Krebs solution (NaCl 117 mmol/L; KCl 4.7 mmol/L; KH_2PO 40.9 mmol/L; $CaCl_2$ 2.5 mmol/L; $NaHCO_3$ 25 mmol/L; glucose 10.1 mmol/L; sodium EDTA 0.03 mmol/L), bubbled with 5% carbon dioxide and 95% oxygen in air, and maintained at 37 °C with a pH of approximately 7.40. After being mounted in the organ baths, each hemidiaphragm with phrenic nerve was allowed to equilibrate at an optimal preload tension (1.0–2.0 g) for 60 min. Then the organ bath solutions were exchanged every 15 min by Krebs solution during the equilibration period. Isometric twitch tension was elicited by direct (muscle) or indirect (phrenic nerve) supramaximal constant-voltage stimulation at 1 Hz (for 0.05 and for 0.2 ms in the cases of direct and indirect stimulation, respectively) using a stimulator. Twitch tensions of phrenic nerve-hemidiaphragm strips were measured using isometric force transducers (MAY FDT-10A Force Displacement Transducer (0–50 g), Commat Ltd., Ankara, Turkey). Normal contraction forces were recorded prior to the phrenic nerve-hemidiaphragm muscle and then the phrenic nerve-hemidiaphragm muscle was pretreated with a sufficient concentration (5 μM) of d-tubocurarine to totally abolish neuromuscular transmission. After the twitch tension was stabilized for at least 15 min, maximal single-twitch tension was determined with an average of five twitches. Contraction durations were measured for each phrenic nerve and diaphragm muscle.

2.3. Rocuronium and sugammadex administration

After the elicited twitch tension for 95%–105% of the initial values (stabilized for 15 min) and baseline twitches were recorded, 10 μmol/L rocuronium was added to the Krebs solution. After stabilization of the drug effect, twitch tensions in the phrenic nerve-hemidiaphragm strips with rocuronium bromide effect were recorded with at least 10-min intervals for 40 min using an MP35 Data Acquisition System (Biopac System, USA). Finally, while rocuronium was present in the organ bath, sugammadex (30 μmol/L) (Bridion, 200 mg/2 mL, Merck Sharp & Dohme Corp Inc., USA) was added to the same organ bath and twitch tensions were determined.

The dry muscle weight was measured and calculated, and the cross-sectional area was estimated based on the following formula: muscle weight (g) / [L (cm) × 1.056 (g/cm^3)] (muscle density = 1.056 g/cm^3) (20).

2.4. Gene expression analyses from diaphragmatic muscle tissue

In this study, RNA was isolated from the diaphragmatic tissues using TRI reagent (Sigma, St. Louis, MO, USA) as

per the manufacturer's instructions. On-column DNAse digestion was performed using a DNAse I kit (QIAGEN, Valencia, CA, USA). The integrity of the isolated RNA was verified by running a total of 5 µg of RNA on a 1% formaldehyde gel. RNA concentration and purity were measured spectrophotometrically at 260 and 280 nm. Complementary DNA synthesis was performed using a High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher, Grand Island, NY, USA) from the obtained RNA samples. Expression profiles of cholinergic receptor nicotinic ε (muscle) (Thermo Fisher, Rn00567899_m1) and cholinergic receptor nicotinic ɣ (muscle) (Thermo Fisher, Rn00569614_m1) genes were analyzed using real-time polymerase chain reaction (RT-PCR) with TaqMan expression assays on an Applied Biosystems 7000 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). Expression levels of all RNA samples were normalized with actin (Actb) (Thermo Fisher, Rn00667869_m1), which was used as an endogenous control.

3. Statistical analysis

At the start of each experimental procedure, twitch tensions of rat phrenic nerve-hemidiaphragm muscle strips in all groups were recorded as a reference response (baseline value). Twitch tensions are expressed in grams and contraction durations are expressed in milliseconds. Contractions are expressed as percentages of the initial reference twitch tension response. Data are presented as mean ± standard deviation (SD) and percentage and were analyzed using two-way analysis of variance (ANOVA). Repeated measures were employed for the comparison of several group means using the Tukey post hoc Bonferroni test (SPSS 22, IBM Corp., Armonk, NY, USA). $P < 0.05$ was considered statistically significant.

4. Results

Twenty-four male rats were used for this experimental study. No deaths occurred in any groups. There was no significant difference in the initial body weight of rats ($P > 0.05$). The animals in group D and group P lost weight but those in group S gained weight. The daily mean weights of all animals until the 14th day are shown in Figure 1.

4.1. Isometric twitch contractions

The mean baseline single-twitch tension was lower in both group D (14.4 ± 1.7 g for muscle, 9.88 ± 0.05 g for nerve) and group P (12.68 ± 0.05 g for muscle, 11.8 ± 0.9 g for nerve) than in group S (16.8 ± 0.5 g for muscle, 14.9 ± 0.4 g for nerve) ($P < 0.001$). When sugammadex was added, twitch tensions were elevated for all groups. The values were higher in group S (16.5 ± 0.24 g for muscle, 14.7 ± 0.3 for nerve) than group D (11.7 ± 0.7 g for muscle, 9.6 ± 0.03 g for nerve) and group P (11.5 ± 0.78 g for muscle, 10.54 ± 0.44 g for nerve) ($P < 0.001$). The percentage of neuromuscular blockade reversal was lowest in group D. Data of the single-twitch tension (g) and percentage of neuromuscular blockade reversal are shown in Table 1.

4.2. The contraction and relaxation properties of the diaphragm and phrenic nerve

The duration of contractions was significantly shorter in group D and group P than group S ($P < 0.001$). The duration of contractions per group are presented in Table 2. The duration of the relaxation time was longer in group D than group P and group S ($P < 0.001$). The percentage of the reversal of relaxation time is shown in Table 3.

4.3. γ-Subunit gene expression analysis

Gene expression analysis of the nAChRs subunit showed that the γ-subunit was significantly increased in both the prednisolone and dexamethasone groups. The increase of the γ-subunit in group P was higher than in group D. M-RNA expressions of the nAChRs γ-subunit for all groups are shown in Figure 2.

5. Discussion

In the present study, the effect of dexamethasone and prednisolone medication for 14 days on sugammadex reversal was investigated. We found that sugammadex was less effective in dexamethasone- and prednisolone-treated rat diaphragms. Long-term medication with dexamethasone and prednisolone caused muscle weakness and upregulation of immature γ-AChR. This upregulation was greater with prednisolone treatment than dexamethasone. The decrease in the effectiveness of sugammadex may be due to both muscle atrophy and the increase in γ-AChR.

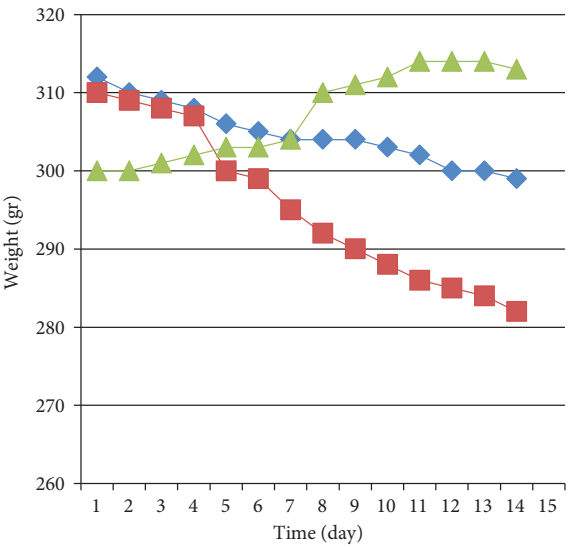

Figure 1. Daily weights of the rats. Green line shows saline group, blue line shows dexamethasone group, red line shows prednisolone group.

Table 1. Single-twitch tension elicited by muscle (direct) or phrenic nerve (indirect) stimulation and percentage of reversal. Data are expressed as mean ± standard deviation. *P < 0.05.

	Dexamethasone		Prednisolone		Saline	
	Twitch tension (g)		Twitch tension (g)		Twitch tension (g)	
	Muscle	Phrenic nerve	Muscle	Phrenic nerve	Muscle	Phrenic nerve
Baseline	14.4 ± 1.7*	9.88 ± 0.05*	12.68 ± 0.3*	11.8 ± 0.9*	16.8 ± 0.5*	14.9 ± 0.4*
Roc	3.8 ± 0.04*	1.6 ± 0.04*	3.4 ± 0.3*	2.4 ± 0.13*	2.8 ± 0.08*	2.9 ± 0.3*
Roc+Sugam	11.7 ± 0.7*	9.6 ± 0.03*	11.5 ± 0.78*	10.54 ± 0.44*	16.5 ± 0.24*	14.7 ± 0.3*
Percentage of reversal (%)	81.9 ± 7.6*	97.2 ± 0.8*	90.62 ± 5.9*	89.6*	98.3 ± 1.9*	99.9*

Table 2. Duration of contraction (ms) of both diaphragm muscle and phrenic nerve. ANOVA was used. Data are presented as mean ± standard deviation. Roc: Rocuronium, Sugam: sugammadex. *P < 0.05.

	Dexamethasone		Prednisolone		Saline	
	Contraction Duration (ms)		Contraction Duration (ms)		Contraction Duration (ms)	
	Muscle	Phrenic nerve	Muscle	Phrenic nerve	Muscle	Phrenic nerve
Baseline	57.8 ± 2.1*	50.1 ± 3.0*	43.7 ± 1.8*	37.7 ± 1.3*	67 ± 1.7*	62.2 ± 0.5*
Roc	50.4 ± 2.5*	40.3 ± 2.6*	36.5 ± 1.3*	36.5 ± 1.2*	40.3 ± 0.5 *	37.2 ± 0.4*
Roc+Sugam	51.03 ± 2*	46.6 ± 1.5*	37.3 ± 0.7*	36.0 ± 1.3*	66.4 ± 0.9*	61.9 ± 0.1*
Percentage of reversal (%)	88.46 ± 5.9*	93.3 ± 4.5*	85.51 ± 4.3*	95.7 ± 4.9*	99.2 ± 2.2*	99.5 ± 0.9*

Table 3. Duration of relaxation (ms) of both diaphragm muscle and phrenic nerve. ANOVA was used. Data are presented as mean ± standard deviation. Roc: Rocuronium, Sugam: sugammadex.

	Dexamethasone		Prednisolone		Saline		P
	Relaxation duration (ms)		Relaxation duration (ms)		Relaxation duration (ms)		
	Muscle	Phrenic nerve	Muscle	Phrenic nerve	Muscle	Phrenic nerve	
Baseline	32.0 ± 1.9	28.7 ± 2.0	27.7 ± 0.9	24.8 ± 1.2	41.1 ± 0.6	40.9 ± 0.2	0.00
Roc	35.9 ± 1.5	32.8 ± 2.2	29.5 ± 1.3	29.0 ± 0.9	48.8 ± 0.6	45.9 ± 0.3	0.00
Roc+Sugam	36.8 ± 2.6	34.5 ± 2.3	27.5 ± 0.9	24.1 ± 1.0	41 ± 0.1	40.7 ± 0.6	0.00
Percentage of reversal (%)	115 ± 11.8	120.76 ± 13.2	99.54 ± 4.9	97.36 ± 3.9	99.75 ± 1.4	99.5 ± 1.8	0.00

Previous studies showed that fetal-type nAChRs were resistant to nondepolarizing neuromuscular blockers and more sensitive to succinylcholine (21,22).

Wang et al. showed that an extended block of synaptic activity triggered prolongation of end-plate current deterioration, and when fetal-type AChRs were inhibited selectively, end-plate current deterioration was reversed at the neuromuscular junction (23). The authors suggested that the prolongation of end-plate current deterioration depended on activation of fetal-type AChRs. Wang et al. emphasized the selective prolongation of end-plate deterioration due to upregulation of fetal-type AChRs. Thus, neuromuscular transmission will be impaired and sufficient muscle tone will not be achieved. Their study supports our hypothesis and results.

The interaction between steroids and neuromuscular transmission is explained through several mechanisms. First, glucocorticoids have a facilitator effect on impulse generation at the neuromuscular junction (24). Second, glucocorticoids can affect the synthesis and release of acetylcholine (25,26). Third, they can depress postsynaptic neuromuscular transmission (27). Rezonja et al. showed

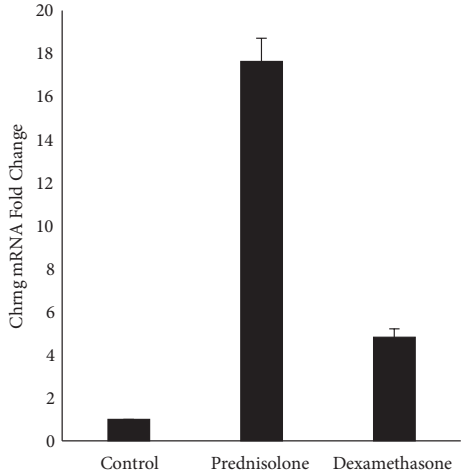

Figure 2. M-RNA expressions of the nAChRs γ for dexamethasone, prednisolone, and saline (control) groups.

that dexamethasone treatment impaired functional innervation by reducing the number of contracting units in in vitro innervated primary human muscle cells (28).

Chen et al. treated rats with dexamethasone for 14 days and investigated the effect of different neuromuscular blockers such as rocuronium, vecuronium, and atracurium on twitch tension and acetylcholine receptor expression. The researchers concluded that chronic dexamethasone administration induced hyposensitivity to nondepolarizing muscle relaxants, which is associated with increased nAChR expression (15).

Kindler et al. showed that both forms of the muscle-type acetylcholine receptor were inhibited by methylprednisolone and hydrocortisone (29). A combination of vecuronium and methylprednisolone showed additive effects on muscle-type acetylcholine receptors. Accordingly, the authors concluded that neuromuscular blockade was augmented with pharmacologic denervation when corticosteroids were combined with vecuronium, resulting in prolonged muscle weakness.

We used a constant concentration of rocuronium (10 μmol) and 3-fold sugammadex (30 μmol) for reverse rocuronium-induced neuromuscular blockade, as used by Rezonja et al., because previous studies indicated that high-dose sugammadex was more effective for neuromuscular blockade reversal (30,31).

It is widely accepted that sugammadex reverses neuromuscular blockage by encapsulating steroidal neuromuscular blocker agents (NMBAs) and removing the NMBA occupation of the pre- and postjunctional nicotinic receptor. The main role in the reversal of the neuromuscular blockade belongs to acetylcholine. If there is no acetylcholine or motor nerve activity, sugammadex cannot be effective in neuromuscular blockade reversal (32). Although sugammadex encapsulates the rocuronium, neuromuscular transmission deteriorates because immature nAChRs have a longer open time than the adult form. This information is the main rationale of our study.

In the present study, despite reversing the neuromuscular blockade with sugammadex, twitch tensions and contraction durations did not achieve baseline values. The percentage of reversal remained at 81.9% and 90.6% for dexamethasone and prednisolone, respectively. It was also observed that immature nAChRs were active. Dexamethasone is a fluorinated steroid and has a greater potency for development of myopathy than prednisolone (nonfluorinated steroid). (33,34) However, M-RNA expression of the γ-AChR is 3-fold higher with prednisolone than dexamethasone, and the percentage of the reversal is higher with prednisolone. This result can be attributed to prednisolone causing less myopathy.

Our study has several limitations. First, we did not measure blood cortisol levels. Second, we used a constant concentration of both rocuronium and sugammadex and they were not used in a dose-dependent manner.

In conclusion, long-term use of dexamethasone and prednisolone causes muscle weakness and induces resistance to rocuronium-induced neuromuscular blockade, which can be related to upregulation of immature nAChR (γ-AChR). Furthermore, these medications attenuate the reversal effect of sugammadex on rocuronium-induced neuromuscular blockade. This was an animal study; therefore, further clinical studies in humans are needed to clarify the effect of long-term glucocorticoid medication on sugammadex reversal.

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