Effect of Chronic Exposure to Dexamethasone on Rocuronium-induced Neuromuscular Blockade and Sugammadex Reversal: an in Vivo Study on Rats

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Research Article

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Abstract

Background: Chronic exposure to glucocorticoids is associated with resistance to nondepolarising neuromuscular blocking agents. Therefore, we hypothesised that sugammadex-induced recovery in subjects with chronic exposure to dexamethasone was faster than that in subjects without dexamethasone exposure.

Objective: To evaluate the recovery profile of rocuronium-induced neuromuscular blockade after sugammadex administration in rats.

Design: An *in vivo* study on rats.

Setting: Asan Institute for Life Sciences, Asan Medical Center, Korea, from April 2017 to October 2017.

Animals: Thirty-six male Sprague-Dawley rats.

Intervention: Sprague–Dawley rats were allocated to three groups (dexamethasone group, control group, and pair-fed group) for the *in vivo* study. Dexamethasone group received daily intraperitoneal injections of dexamethasone 500 μg kg^{-1} or 0.9% saline for 15 days. On the sixteenth day, 3.5 mg kg^{-1} of rocuronium was administered to achieve complete neuromuscular blockade.

Main outcome measures: The recovery time to a train-of-four ratio

Results: There were no significant differences in the recovery time to train-of-four ratio to 0.9 among the groups ($P = 0.531$). The time to second twitch of train-of-four recovery that indicated the duration of rocuronium-induced neuromuscular blockade was significantly shorter in Group D than in Groups C and P ($P = 0.001$).

Conclusion: As previously reported, resistance to rocuronium was observed in rats with chronic exposure to dexamethasone. However, the neuromuscular recovery time after sugammadex administration was not significantly different between groups.

Key Points

- Chronic dexamethasone exposure shortened the duration of rocuronium-induced neuromuscular block.
- Sugammadex-induced neuromuscular recovery was not affected by chronic dexamethasone exposure.
- Diet and muscle mass decreased by chronic dexamethasone exposure did not affect neuromuscular blockade and recovery.

I. Introduction

Glucocorticoids have been prescribed for diseases caused by inflammation, such as chronic obstructive pulmonary disease, allergies, rheumatoid arthritis, osteoarthritis, inflammatory bowel diseases, eczema, and other allergic skin conditions. It is also used to achieve immunosuppression after organ transplantation. Glucocorticoids are used in all medical specialties. However, they are a double-edged sword, as anticipated clinical effects could be accompanied with adverse outcomes despite proper dosage and duration of use. Neuromuscular blocking agents (NMBAs) are important for maintaining good surgical conditions and mechanical ventilation under general anaesthesia. Long-term glucocorticoid-induced resistance to numerous nondepolarising NMBAs, such as atracurium, vecuronium, and rocuronium, has been observed in studies[1, 2].

Chen et al. reported that chronic dexamethasone treatment increased the percentage of immature subunits (γ-subunit) of the nicotinic acetylcholine receptor (nAChR) and increased the expression of nAChRs in Sprague–Dawley rats treated with daily dexamethasone for 14 days[1]. These phenomena would increase the amount of NMBAs required to competitively block nAChRs. In addition, chronic exposure to glucocorticoids is known to lead to a reduction in type II fibres in the muscle, which are more sensitive to NMBAs than type I fibres[2]. These factors could lead to resistance to NMBAs.

Sugammadex is a cyclodextrin molecule with an ability to encapsulate lipophilic compounds[3]. It selectively binds to the rocuronium molecule, which has a steroidal nucleus. This binding prevents rocuronium from binding to nAChRs and allows rapid reversal of neuromuscular blockade (NMB). Considering the resistance to NMBA caused by chronic exposure to dexamethasone, it is reasonable to assume that sugammadex-induced recovery of NMB may be affected.

However, clinical studies investigating dexamethasone long term treatment with dose known to cause muscle atrophy are still unavailable and large-scale investigations are not feasible due to ethical constraints. Therefore, we designed specific environmental conditions of dexamethasone long term treatment under in vivo conditions to demonstrate the effects of rocuronium and sugammadex using the animals

We hypothesised that sugammadex-induced NMB recovery in rats with chronic dexamethasone exposure is faster than that in rats without dexamethasone exposure. The objective of this study was to evaluate the recovery profile of rocuronium-induced NMB after chronic exposure to dexamethasone using sugammadex as a reversal agent, since the *in vivo* effect of sugammadex reversal on rocuronium-induced NMB in subjects with chronic dexamethasone exposure has not been investigated yet.

The primary outcome was the recovery time to a train-of-four (TOF) ratio ≥ 0.9 (TTOFr), which is the time it takes for the TOF ratio (TOFr) to recover to 0.9 or higher after the injection of sugammadex. Secondary outcomes were time of T1 (the first twitch of TOF) recovery to 95% (TT1) and the recovery index (RI). TT1 is the time taken for the first twitch of TOF to recover to 95% of the baseline T1 after the injection of sugammadex. RI is the time taken for T1 of TOF to recover from 25–75% of the baseline T1.

Ii. Methods

1. Animals and group assignments

Ethical approval for this study was provided by the Institutional Animal Care and Use Committee of the Asan Institute for Life Sciences, Asan Medical Center (Seoul, Republic of Korea) on 3 March 2017 (Protocol No. 2016-13-067; Chairperson Professor Jong Yeun Park), and this experiment was reviewed and performed in accordance with the guidelines and regulations established by the Institutional Animal Care and Use Committee of Asan Institute for Life Sciences, Asan Medical Center. The committee abides by the Institute of Laboratory Animal Resources guide. The rats were obtained commercially from Orientbio Company (Orientbio, Sungnam, Republic of Korea). This animal study complied with the ARRIVE 2.0 guidelines[4].

There are numerous controversies regarding the calculation of the number of samples in animal experiments. We did not estimate the sample size statistically. Previous studies have conducted experiments with about 10 animals per group to achieve statistically significant results [5–7]. In a previous study that investigated the effects of dexamethasone on sugammadex reversal in rocuronium-induced NMB in animals, it was suggested that 10 animals in one group are sufficient to produce significant results[6, 8, 9]. To allow for attrition, thirty-six adult male Sprague–Dawley rats (7-week-old; weighing 213–253 g) were randomly divided into three groups (n = 12 per group). Sorting was accomplished using a random number generator in Microsoft Excel 2013 (Microsoft, Redmond, WA, USA). To apply the chronic dexamethasone exposure with a dose previously shown to cause muscle atrophy, the dexamethasone group (Group D) received daily intraperitoneal injections of 500 μg kg^{-1} dexamethasone disodium phosphate (Yuhan, Seoul, Republic of Korea) for 15 days [1, 2, 10]. One millilitre of 0.9% saline was used to suspend 500 μg of dexamethasone. Thus, rats weighing 213–253 g were injected with 106.5–126.5 μg of dexamethasone in a 0.2–0.25 mL volume. The control group (Group C) only received an equivalent volume of 0.9% saline daily for 15 days. The rats in the pair-fed group (Group P) were fed daily the same amount of food as was consumed by rats in Group D for 15 days. All treatments were done in the laboratory.

The authors weighed the amount of food consumed by Group D daily and provided the same amount of food to Group P daily. Group P was pair-fed with the Group D for 15 days to evaluate whether muscle dysfunction following dexamethasone treatment was caused by anorexia typically associated with glucocorticoid therapy. Food was available *ad libitum* to rats in Groups C and D. Weights of the rats were recorded daily, and dexamethasone doses were adjusted in accordance with change in body weight. Water was available *ad libitum* for all groups. All mice were bred in the laboratory animal breeding room at the Laboratory Animal Research Center, Asan Institute for Lifesciences. The animals were housed in an individually ventilated cage system (Tecniplast, USA) under specific pathogen-free conditions. The rats were raised at a constant temperature of 22°C, humidity 50 ± 10%, laboratory rodent chow and were

maintained under a regular diurnal (12-h light and 12-h dark) cycle. All injections were administered at the same time of the day. Figure 1 summarises the treatments.

2. General surgical procedures

Twenty-four hours after the final drug administration, the rats were anaesthetised with an intraperitoneal injection of Alfaxan^{TM} (Jurox Pty. Limited, New South Wales, Australia) at 40 mg kg^{-1} of body mass, and an adequate depth of anaesthesia was confirmed by the absence of a withdrawal response to toe clamping[11]. When there was a withdrawal response, an additional 10–20 mg kg^{-1} was injected if necessary. The animals were tracheotomised, mechanical ventilation was applied to maintain normal breathing throughout the surgery, and the jugular vein was catheterised to inject drugs. Body temperature was monitored using an oesophageal temperature probe (Regulation to 37 ± 1°C), and a warming pad and light source were used to maintain proper body temperature. The tibialis anterior muscle was exposed, and the distal part of the tendon was tied with 3-0 black silk. It was then connected to a force displacement transducer (Grass FT03, Grass Instrument Co., Quincy, Massachusetts, USA) to measure the isometric contraction of the tibialis anterior muscle at a resting tension of 2 g. The sciatic nerve was exposed and connected to the bipolar platinum electrodes to evaluate neuromuscular transmission.

3. Assessment of neuromuscular transmission

Using a nerve stimulator (S88, Grass) and stimulation isolation unit (SIU5, Grass), TOF stimulation (frequency 2 Hz, duration 0.2 ms) consisting of four supramaximal square-wave pulses were applied to the sciatic nerve via the bipolar platinum electrodes every 12 s throughout the study. Muscle contraction responses were recorded and digitalised with a PowerLab acquisition system (ADInstruments, Austin, Texas, USA) and stored in LabChart7 (ADInstruments, Colorado Springs, CO, USA), which is a computer with data charting software. In all groups, contraction responses were stabilised for at least 10 min after the initiation of TOF stimulation. The height of T1 was measured as the baseline T1 after 10 min of stabilisation. After administration of 3.5 mg kg^{-1} of rocuronium (Esmeron^{TM}, MSD, Oss, The Netherlands), a dose ensuring that complete NMB is achieved in rats[1], via the jugular vein catheter, complete NMB was induced[12]. The time from rocuronium injection to the appearance of the second twitch of TOF (TT2) was recorded. When T2 appeared, sugammadex (Bridion^{TM}, MSD, Oss, The Netherlands) 0.5 mg kg^{-1} was administered, and the TTOFr, which was our primary outcome, was recorded. RI and TT1 were recorded as secondary outcomes. Figure 2 summarises the overall experiment.

4. Specimen measurement

On completion of the *in vivo* study, the rats were sacrificed for specimen examination.

5. Data and statistical analysis

The primary outcome of this study was the TTOFr. The secondary outcomes were the TT1 and RI. Data are expressed as the mean ± standard deviation or median [Interquartile range] unless otherwise specified. Quantile–quantile plots were used to assess the normality assumption. One-way analysis of

variance followed by the Tukey *post hoc* test was applied to analyse the weight of rats, temperature, weight of the tibialis anterior muscle, TT2, TTOFr, TT1, and RI. The Kruskal–Wallis test was used to analyse the length and width of the tibialis anterior muscle. Statistical significance was set at P values < 0.05, and all statistical tests were two-sided. SAS statistical software (version 9.3; SAS Institute Inc., Cary, North Carolina, USA) was used for statistical analysis.

Iii. Results

1. Animal and specimen data

Thirty-six rats were allocated to three groups, with 12 rats assigned to each group. One rat in Group C was administered with rocuronium overdose and another rat died during the surgical procedure; thus, data from the two rats could not be used. We could not use data of two rats in Group D because of a ventilator breakdown resulting in respiratory failure in the rat, and a computer system shutdown during the recovery process resulting in recording error. In Group P, data of two rats could not be used. Incomplete NMB even after administration of the proper dosage of rocuronium with sufficient time for onset of the drug, and another rat showed incomplete recovery of TOFr. Therefore, a total of 30 rats (10 each in Groups C, D, and P) were included in the analysis (Figure 3).

There were no significant differences in the initial body weight before the experimental treatment, but the final body weight after the experimental treatment significantly differed between the groups (Table 1). Food intake decreased markedly among the rats in Group D, as previously reported[13, 14]. The size and weight of the tibialis anterior muscles were smaller in Group D than in Groups C and P. Though rats in Groups D and P were fed the same amount of food, the degree of weight loss, muscle size reduction, and muscle weight loss were greater in Group P.

Table 1
Baseline comparison of animals in the study groups

		Condition		
		Group C (n = 10)	Group D (n = 10)	Group P (n = 10)
Weight (g)	Initial	241 ± 7	232 ± 19	236 ± 17
	Final	392 ± 15	219 ± 17*†	322 ± 18*
Temperature (°C)		37.6 ± 0.1	37.6 ± 0.2	37.5 ± 0.2
Tibialis anterior muscle	Weight (mg)	687 ± 65	365 ± 48*†	565 ± 21*
	Length (mm)	22 [21 to 22]	19 [18 to 21]	21 [21 to 21]
	Width (mm)	11 [11 to 12]	8 [8 to 9]	10 [9 to 10]

Data are presented as mean ± standard deviation or median [Interquartile range]; Group C, control group; Group D, dexamethasone group; Group P, pair-fed group. Group C received only the amount of 0.9% saline received by Group D each day. Group D received a daily intraperitoneal injection of 500 μg kg^{-1} dexamethasone, suspended in 1 mL of 0.9% saline. Group P was fed daily with the same amount of food consumed by group D. Body temperature was monitored with an oesophageal temperature probe and maintained with a warming pad and a light source for warming within the range of normal body temperature. *P < 0.001 vs. Group C. †P < 0.001 vs. Group P. One-way analysis of variance was performed followed by the Tukey *post hoc* test.

2. NMB induction

After administration of 3.5 mg kg^{-1} (estimated 2-fold ED_{90})[15] of rocuronium via the jugular vein, complete NMB was induced in each group.

3. Recovery profiles

TTOFr, the primary outcome, was not significantly different between the groups (4.3 ± 2.3, 4.0 ± 3.0, and 3.1 ± 1.8 min, P = 0.531 in Groups C, P, and D, respectively) (Table 2). TT1 was significantly shortened in Group D than in Groups C and P (3.6 ± 2.1 min vs. 4.2 ± 2.2 and 4.4 ± 2.5 min, P = 0.001, respectively) (Table 2). However, RI was not significantly different between the groups (1.5 ± 0.6, 2.2 ± 1.8, and 1.3 ± 0.6 min, in Groups C, P, and D, P = 0.531, respectively) (Table 2). TT2, which shows the duration of rocuronium-induced NMB, was significantly shortened in group D when compared with that in Groups C and P (2.9 ± 1.0 min vs. 5.0 ± 1.1 and 5.1 ± 1.4 min, P = 0.001, respectively), while no significant difference was observed between Groups C and P (P = 0.996).

Table 2
Effects of chronic exposure to dexamethasone on duration and sugammadex reversal of neuromuscular blockade

		Condition		
		Group C (*n* = 10)	Group D (*n* = 10)	Group P (*n* = 10)
Duration of neuromuscular blockade	TT2 (min)	5.0 ± 1.1	2.9 ± 1.0*†	5.1 ± 1.4
Recovery profiles after sugammadex administration	TTOFr (min)	4.3 ± 2.3	3.1 ± 1.8	4.0 ± 3.0
	TT1 (min)	4.2 ± 2.2, (*n* = 10)	3.6 ± 2.1, (*n* = 9)	4.4 ± 2.5, (*n* = 7)
	RI (min)	1.5 ± 0.6	1.3 ± 0.6	2.2 ± 1.8

Data are presented as mean ± standard deviation; Group C, control group; Group D, dexamethasone group; Group P, pair-fed group. Group C received only the amount of 0.9% saline received by Group D each day. Group D received a daily intraperitoneal injection of 500 μg kg^{-1} dexamethasone, suspended in 1 mL of 0.9% saline. Group P was fed daily with the same amount of food that was consumed by Group D. Times and recovery indices are presented as minutes; TOF, train-of-four; T1, the first twitch of train-of-four; T2, the second twitch of train-of-four; TT2, the time from rocuronium injection to appearance of T2; TTOFr, time taken for the TOF ratio to recover to 0.9 or higher after injection of sugammadex; TT1, the time taken for the T1 to recover to 95% of the baseline T1 after injection of sugammadex; RI, recovery index (the time taken from T1 of 25% of the baseline T1 to T1 of 75% of the baseline T1). *P = 0.001 vs. Group C. †P = 0.001 vs. Group P. One-way analysis of variance was performed followed by the Tukey *post hoc* test.

Vi. Discussion

The primary outcome of this study was that chronic exposure to dexamethasone, while inducing resistance to rocuronium, did not have any significant effect on sugammadex reversal in this *in vivo* study on rats.

This result was contrary to our hypothesis. We considered the following reasons for this unexpected result. First, in rats, the circulation time is faster than that in humans. As the speed of the reversal was extremely fast, it was hard to determine differences in the recovery profiles that may have existed. The use of a single twitch stimulation would have been better. As TOF stimulation usually occurs every 10–

15s, 1 Hz single twitch stimulation could have provided the more appropriate resolution to identify the fast recovery from deep neuromuscular block[15].

Second, we used only healthy young rats in this study. Patients in the intensive care unit may develop resistance to NMBAs during chronic treatment with glucocorticoid; additionally, co-administration of corticosteroid and NMBA can lead to prolonged weakness and even acute myelopathy[16]. Hence, the use of sugammadex as an NMB reversal agent might have resulted in different recovery profiles if rats sick enough to receive ICU care were used in the study.

Third, chronic exposure to dexamethasone is known to induce nAChR upregulation and expression of the immature form of the receptor subunit, causing resistance to NMBA. However, sugammadex encapsulates NMBA molecules, regardless of the receptor's sensitivity. This may be one of the reasons for no significant difference in the recovery profile with administration of sugammadex, despite the shorter duration of NMB in the group with long-term dexamethasone exposure. Use of anticholinesterases such as pyridostigmine and neostigmine as reversal agents would have resulted in different recovery profiles.

There are few *in vivo* studies on rats that demonstrate the effect of chronic dexamethasone exposure on sugammadex reversal. Previous studies have reported that resistance to rocuronium-induced NMB was observed in rats with chronic exposure to dexamethasone. The effects of glucocorticoids can be categorised as short-term and long-term treatment effects. The presynaptic effects have been observed during short-term treatment. Synthesis and increased release of acetylcholine have been observed[7].

The nAChR in the muscle forms a heteropentamer consisting of two alpha, one beta, and one delta subunit with one gamma subunit in the fetal AChR isoform, which is replaced by an epsilon subunit in the adult AChR isoform. During long-term treatment, changes occur in the nAChRs subunit[1]. The epsilon subunit turns into the gamma subunit, which is an immature form that is resistant to NMBAs. Functional upregulation of the nAChRs is observed during both short-term and long-term glucocorticoid treatment, and this has been documented in burns and immobilisation injury[1, 17, 18].

According to a study by Lee et al., resistance to nondepolarising NMBAs, which occurs after immobilisation, might be related to the upregulation of α7-nAChRs[17]. It has also been shown that α7-nAChRs expression occurs after protracted dexamethasone treatment[19].

One of the limitations is the dosage of sugammadex used in the study. There is no consensus on the recommended dose of sugammadex for an *in vivo* study using rats, as there are few such studies[15, 20]. Therefore, the dose of sugammadex was determined in a pilot study. The dose of sugammadex that resulted in faster recovery without resulting in an extremely rapid recovery from NMB was chosen. However, when a reduced dose of sugammadex was used, there was no significant difference in TTOFr. However, the concentration (dexamethasone 500 μg kg^{-1}) used in this study far exceeds the typical clinical doses. In clinical concentrations of steroids, there would be no effect on sugammadex reversal.

In conclusion, chronic exposure to dexamethasone while inducing resistance to rocuronium did not have any significant effect on sugammadex reversal in this *in vivo* study on rats.

Declarations

Availability of data and materials

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Acknowledgments relating to this article

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Conflicts of interest

The authors certify that there is no competing interest with any financial organization regarding the material discussed in the manuscript.

Consent for publication

Not Applicable

Presentation

Preliminary data for this study was presented as a poster presentation at the Euroanaesthesia Congress on, 2 to 4 June 2018, Copenhagen, Denmark.

Author contribution

JI, HSY and HYP have made substantial contributions to the conception and design of the work. HYP and JI carried out the experiment. JI and HYP have made substantial contributions to the acquisition, analysis. HC, YBK, TK and SKO have made substantial contributions to interpretation of data. HYP and JI have drafted the work or substantively revised it. All authors have approved the submitted version (and any substantially modified version that involves the author's contribution to the study). All authors have agreed both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature. All authors have read and approved the manuscript.

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Figures

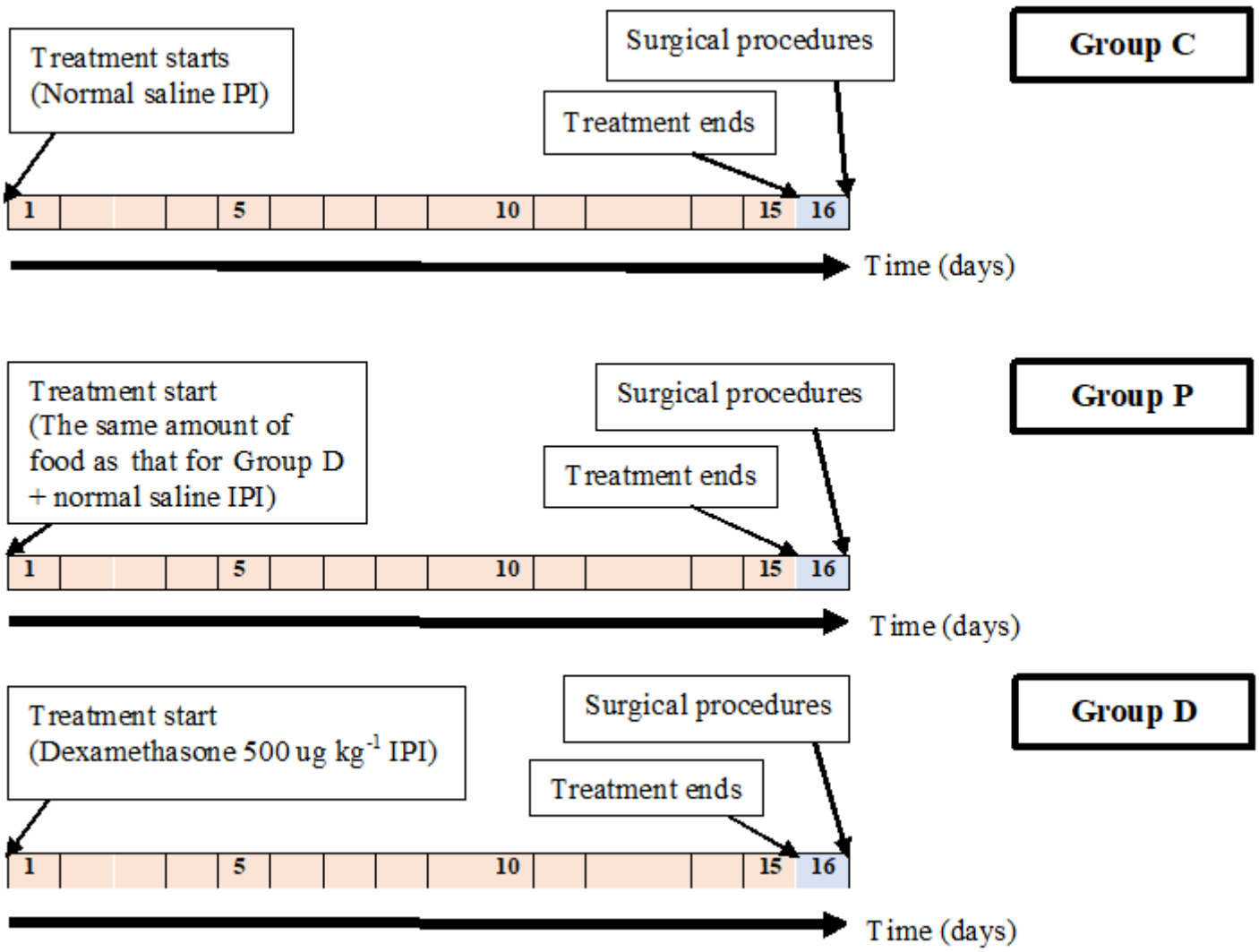

Figure 1

Flow diagram of the treatment IPI, intraperitoneal injection; Group C, control group; Group D, dexamethasone group; Group P, pair-fed group. Group C received the same volume of 0.9% saline received by Group D each day. Group D received a daily IPI of 500 μg kg-1 dexamethasone suspended in 1 mL of 0.9% saline. Food and water were provided ad libitum. Group P received the same volume of 0.9% saline received by Group D each day and fed daily with the same amount of food and water as consumed by Group D.

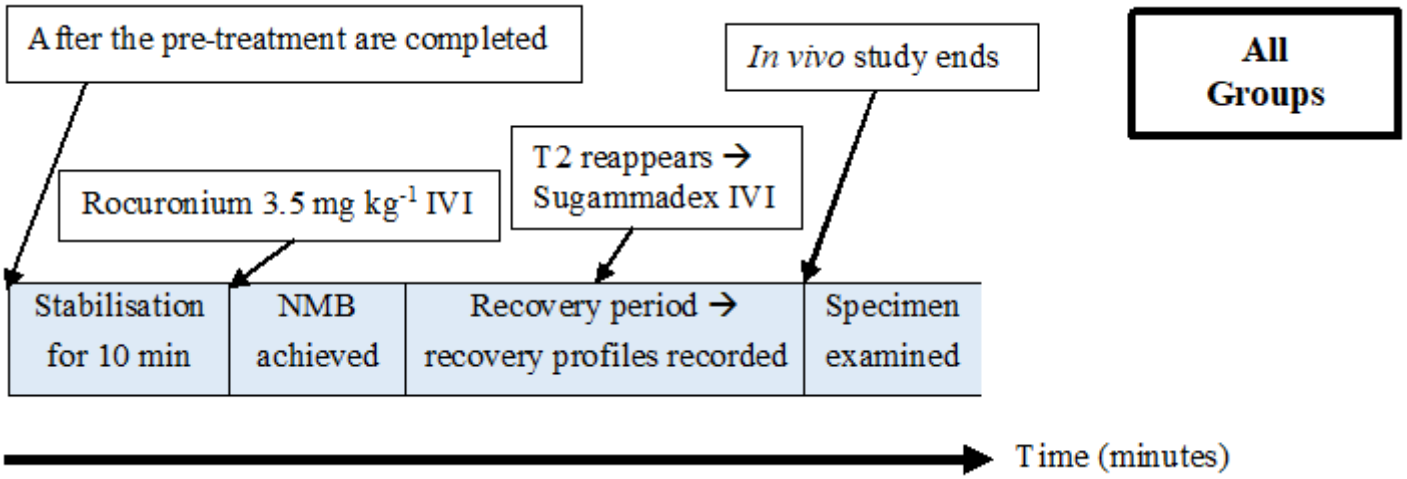

Figure 2

Flow diagram of the experiment Groups included: control group (Group C), dexamethasone group (Group D), and pair-fed group (Group P); NMB, neuromuscular blockade; T2, the second twitch of train-of-four stimulation; IVI, intravenous injection; IPI, intraperitoneal injection. Group D received a daily IPI of 500 μg kg-1 dexamethasone suspended in 1 mL of 0.9% saline. Food and water were provided ad libitum. Group C received the same volume of 0.9% saline received by Group D each day. Food and water were provided ad libitum. Group P received the same volume of 0.9% saline received by Group D each day and were fed daily with the same amount of food and water as consumed by Group D.

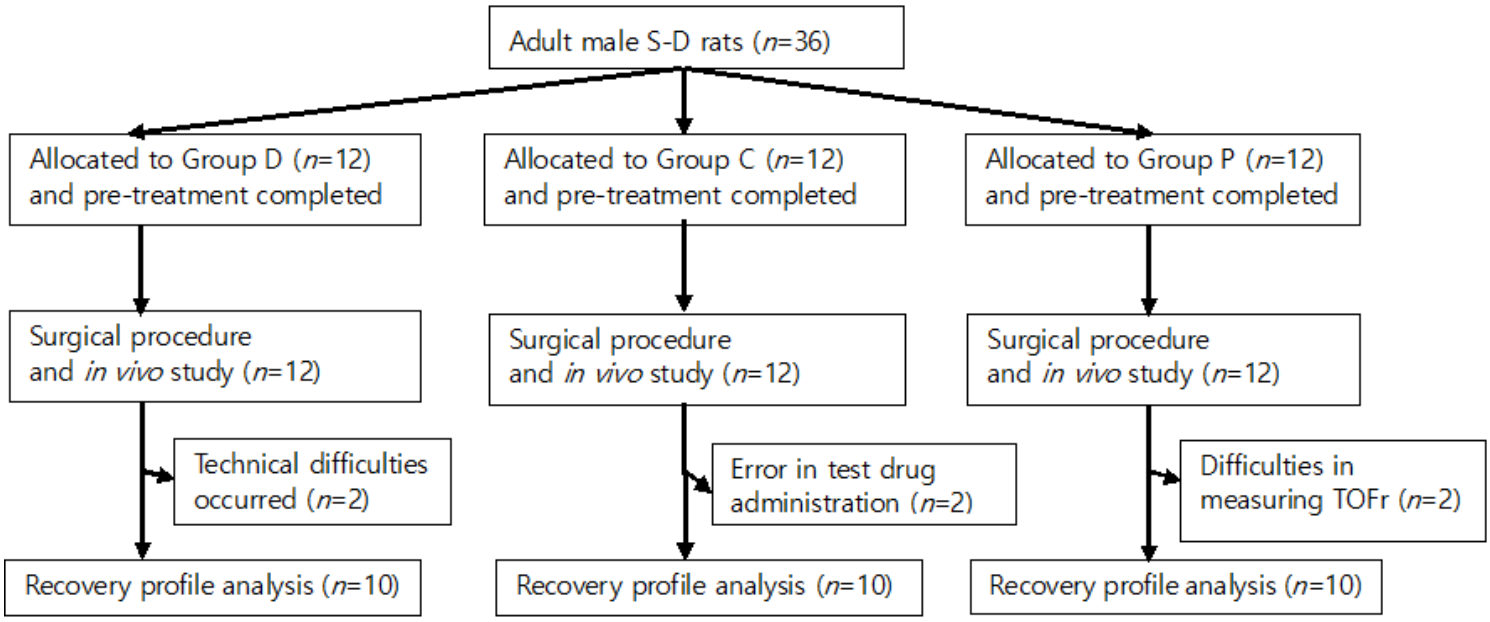

Figure 3

Flow chart of experimental procedure Group C, control group; Group D, dexamethasone group; Group P, pair-fed group; S-D, Sprague–Dawley; NMB, neuromuscular blockade, TOFr; train-of-four ratio.