Interaction of alfaxalone on the neuromuscular blockade of rocuronium in dogs

Rakuno Gakuen University
Graduate School of Veterinary Medicine
Doctoral course

CHEN I-YING

Veterinary Anesthesiology

Supervisor: Prof. Kazuto Yamashita

2022

Contents

page

Abbreviations

AMG	acceleromyography
ANOVA	analysis of variance
BE	base excess
CRI	constant rate infusion
ED	effective dose
$EtCO_2$	end-tidal CO_2
EtSEVO	end-tidal sevoflurane concentration
HR	heart rate
IPPV	intermittent positive-pressure ventilation
IV	intravenous
MAC	minimum alveolar concentration
MAP	mean invasive arterial blood pressure
MIR	minimum infusion rate
NMBA	neuromuscular blocking agent
$PaCO_2$	partial pressure of arterial carbon dioxide
PaO_2	partial pressure of arterial oxygen
pHa	arterial pH
PROP	propofol
RI	recovery index
SD	standard deviation
SEVO	sevoflurane
T1	the first twitch of train of four stimulation
T1C	control T1
T4	the fourth twitch of train of four stimulation
T_{ESO}	esophageal temperature
TIVA	total intravenous anesthesia
TOF	train of four
TOFR	ratio of the T4 amplitude to the T1 amplitude
TOFR0.9	TOFR/control TOFR $\geq$ 0.9
TOFRC	control TOFR
T_S	skin surface temperature

Introduction

Basic elements of general anesthesia include analgesia, amnesia and muscle paralysis [13]. Although some anesthetics also have relaxation effect by depressing the spinal motor neurons [31], the desired degree of muscle relaxation requires deeper plane of anesthesia, which increase the risk of dose-dependent side effects caused by the anesthetics. Therefore, the coadministration of neuromuscular blocking agents (NMBA) with anesthetics can decrease the anesthesia risk by achieving muscle relaxation with reduced anesthetic requirement [26, 61].

In human anesthesia practice and intensive care medicine, NMBAs was used for intubation, mechanically ventilation and therapeutic hypothermia management [26]. Because the intubation of veterinary patient is easier than that of human patient, the use of NMBAs is unessential for most veterinary practitioners [29]. However, the use of NMBAs has been increasing in veterinary clinical practice due to the development of the new drugs with faster onset, shorter duration and recovery time and less adverse effect [49]. NMBAs were used to assist surgical manipulations during fraction reduction, prevent spontaneously breathing during mechanically ventilation, maintain central position of the globe during ophthalmic surgery in veterinary patient [4, 54].

The mechanism of muscle contraction is mediated by the release of acetylcholine from presynaptic motor nerve terminals in neuromuscular junction. The acetylcholine in the synapse cleft binds to the postsynaptic nicotinic receptor, causing the sodium channel located on the muscle plasma membrane to open, and leads to the depolarization of the muscle. The muscle is repolarized after the acetylcholine is degraded by acetylcholinesterase [2, 15]. Depending on the mechanism of action, NMBAs are separated into two types: depolarizing and nondepolarizing. Depolarizing NMBAs can depolarize the muscle fiber, which is similar to the effect of acetylcholine at the neuromuscular junction. However, the repolarization of the muscle will persist because the depolarizing NMBAs are undegradable by acetylcholinesterases. Therefore, the upcoming impulse transmissions are blocked, and flaccid paralysis is achieved. Nondepolarizing NMBAs can bind to postsynaptic nicotinic receptors but do not have the ability to cause muscle depolarization. Therefore, they prevent the acetylcholine from binding to the receptors and block the transmission of action potential [42, 49, 61].

Rocuronium bromide, an aminosteroid, is a nondepolarizing NMBA. It has a faster onset as compared with that of vecuronium, but the duration of action is similar between the two [36, 43, 47]. Currently, rocuronium has widespread clinical application in veterinary practice and has been administered to dogs through intravenous (IV) boluses, incremental IV doses, or constant rate infusion (CRI) to improve skeletal muscle relaxation during surgical procedures under general anesthesia [1, 4, 9, 20, 27]. However, administration of NMBAs may lead to a residual block, which is the presence of a neuromuscular blockade during the postoperative period [32]. Studies in humans have shown that sex, body weight, body temperature, concentration of volatile anesthetic agents and interval between the last NMBA administration and reversal agent administration are associated with a residual block [40, 62, 70, 71].

NMBAs must be co-administered with inhalation or IV anesthetics because they do not have an anesthetic effect [37]. Therefore, to avoid a residual blockade and determine the adequate interval between the last NMBA administration and reversal agent administration, it is important to know if a certain anesthetic can prolong the effect of the NMBAs. In dogs, potent volatile inhalation anesthetics, such as isoflurane and sevoflurane, potentiate the neuromuscular blockade produced by NMBAs such as atracurium, cisatracurium, rocuronium and vecuronium [11, 37, 54, 67]. Conversely, propofol, a short-acting and noncumulative injectable anesthetic that is suitable as a hypnotic for total intravenous anesthesia (TIVA), has little to no effect on the neuromuscular blockade produced by NMBAs in dogs [11, 37, 67].

Alfaxalone is a synthetic neuroactive steroid that has an efficacy and safety similar to that of propofol and is also suitable for TIVA in dogs [6, 7, 19]. However, unlike propofol, alfaxalone causes less apnea [38], does not produce pain after IV injection [51] and is not a legally controlled drug in most countries. Consequently, the use of alfaxalone for TIVA in veterinary practice has increased in recent years [5, 10, 19, 60]. However, the interaction between rocuronium and alfaxalone in dogs remained uninvestigated and information to guide veterinary physicians in clinical practice regarding the effective doses of rocuronium with alfaxalone was insufficient.

Sugammadex, a gamma-cyclodextrin, selectively reverses rocuronium-induced neuromuscular blockade by chemical encapsulation [8, 17, 18]. The structure of sugammadex comprises a lipophilic internal cavity that encapsuled aminosteroid neuromuscular blocking molecules to form an inactive

complex, which is then excreted unchanged via the kidneys and thus reverses the neuromuscular blockade [8, 59]. The reversal effect of sugammadex on rocuronium-induced blockade has been evaluated in dogs under isoflurane anesthesia [52]. They used 8 mg/kg of sugammadex to reverse profound rocuronium block. Recovery of the train-of-four (TOF) ratio (TOFR; ratio of the T4 amplitude to the T1 amplitude) to 0.9 was achieved within 2 min after sugammadex administration. Compared to acetylcholinesterase inhibitor, sugammadex does not have cardiovascular side effects and is able to reverse profound neuromuscular block [63]. However, the encapsulation of other steroidal drugs or endogenous steroids may occur with sugammadex administration [30]. The alfaxalone formulation registered for clinical use in dogs and cats associated with the use of a 2-hydroxypropyl-beta cyclodextrin as a solvent [22]. Therefore, considering that alfaxalone is a steroidal drug and the similar structure between sugammadex and the solvent of alfaxalone, the efficacy of sugammadex in the reversal of rocuronium-induced neuromuscular blockade should be investigated in dogs under alfaxalone anesthesia.

In this study, a series of experiments was performed to clarify the interaction among alfaxalone, rocuronium and sugammadex in dogs. In Chapter I, the interactions of anesthetic on the neuromuscular blockade produced by rocuronium were compared in dogs anesthetized with sevoflurane, propofol and alfaxalone. In Chapter II, the potency of rocuronium was investigated by constructing the dose–response curve in dogs under alfaxalone anesthesia and its ED_{50} and ED_{95} were determined. In Chapter III, the reversal effect of sugammadex on neuromuscular blockade produced by rocuronium was investigated in dogs under alfaxalone anesthesia.

Chapter I

Effects of sevoflurane, propofol or alfaxalone on neuromuscular blockade produced by a single intravenous bolus of rocuronium in dogs

1.1 Preface

Rocuronium bromide is a NMBA that has a faster onset and similar duration of action compared with vecuronium [36, 43, 47]. Currently, clinical applications of rocuronium have widespread in veterinary practice and has been used to improve skeletal muscle relaxation during surgical procedures under general anesthesia in dogs [1, 4, 9, 20, 27]. However, rocuronium must be co-administered with inhalation or IV anesthetics because of its lack of anesthetic effect [37]. In dogs, potent volatile inhalation anesthetics, such as isoflurane and sevoflurane, potentiate the neuromuscular blockade produced by NMBAs such as atracurium, cisatracurium, rocuronium and vecuronium [11, 37, 54, 67]. Conversely, propofol, a short-acting and noncumulative injectable anesthetic that is suitable as a hypnotic for TIVA, has little to no effect on the neuromuscular blockade produced by NMBAs in dogs [11, 37, 67]. Alfaxalone is a synthetic neuroactive steroid that has an efficacy and safety similar to that of propofol and is also suitable for TIVA in dogs [6, 7, 19]. However, unlike propofol, alfaxalone causes less apnea [38], does not produce pain during IV injection [51] and is not a legally controlled drug in most countries. Consequently, the use of alfaxalone for TIVA in veterinary practice has increased in recent years [5, 10, 19, 60]. However, there are no published studies on the interaction between rocuronium and alfaxalone in dogs.

This chapter aimed to compare the effects of sevoflurane, propofol, and alfaxalone on the neuromuscular blockade produced by a single IV bolus of rocuronium in dogs. It was hypothesized that in comparison with sevoflurane, both alfaxalone and propofol would not increase the degree or duration of the neuromuscular blockade induced by rocuronium.

1.2 Materials and methods

1.2.1 Animals

The present study was designed as a randomized, prospective, crossover experiment. A total of eight adult Beagle dogs (four female and four male), weighing 8.9–15.3 kg and aged 5–7 years, were included in this study (Table 1-1). The dogs were deemed healthy based on physical examinations, blood cell counts and serum biochemical profiling. Each dog was anesthetized three times with sevoflurane (SEVO treatment), propofol (PROP treatment) or alfaxalone (ALFX treatment) with a washout period of at least 14 days between each experiment. The order of the dogs and the treatment were randomized using an online randomization system (https://www.randomizer.org/). Food was withheld for 12 hours before each experiment, although the dogs had free access to water. This study was approved by the Animal Care and Use Committee of Rakuno Gakuen University (no. VH20B1) and the dogs were cared for in accordance with the principles of the Guide for the Care and Use of Laboratory Animals prepared by Rakuno Gakuen University.

Table 1-1. Demographic data

Dog number	Sex	Weight (kg)	Age (years)
1	Male	13.7	6
2	Female	12.8	6
3	Female	8.9	7
4	Female	10.5	7
5	Male	15.3	6
6	Male	11.9	5
7	Female	11.2	5
8	Male	14.1	6
Mean ± SD		12.3 ± 2.1	6.0 ± 0.8

1.2.2 Determination of the minimal alveolar concentration (MAC) and the minimum infusion rate (MIR)

Before the induction of anesthesia, the right cephalic vein was catheterized using a 22-gauge, 2.5 cm catheter (Surflo F&F; Terumo Co., Ltd., Tokyo, Japan) for the administration of an IV bolus of rocuronium and for the IV infusion of an isotonic crystalloid fluid. The left cephalic vein was catheterized with a 22-gauge catheter for administration of treatments. Anesthesia was induced with 5% sevoflurane (Sevoflo; DS Pharma Animal Health Co., Ltd., Osaka, Japan) in oxygen through a mask, IV propofol (7 mg/kg; 2% Propofol injection Maruishi; Maruishi Pharmaceutical Co., Ltd., Osaka, Japan) or IV alfaxalone (3 mg/kg; Alfaxan; Meiji Seika Pharma Co., Tokyo, Japan) during the SEVO, PROP and ALFX treatments, respectively. Thereafter, the dogs were orotracheally intubated and the cuffed endotracheal tube was connected to a circle rebreathing system with 2 L/min oxygen inflow from an anesthetic machine (Beaver 20; Kimura Medical Instrument Co., Tokyo, Japan). The dog was placed in right lateral recumbency after intubation. During instrumentation, in the SEVO treatment anesthesia was maintained with 2.2% end-tidal sevoflurane concentration (EtSEVO) using an out-of-circuit vaporizer (Sevorex S-200; Shin-Ei Industries Inc., Tokyo, Japan). Anesthesia was maintained in dogs in treatments PROP and ALFX using a propofol infusion (0.4 mg/kg/min) or alfaxalone infusion (0.12 mg/kg/min), respectively, delivered from a precision syringe infusion pump (TOP-551V; TOP Corporation, Tokyo, Japan). The right or left dorsal pedal artery was catheterized with a 22-gauge, 3.1 cm catheter (Supercath 5; Medikit Co., Ltd., Tokyo, Japan) for invasive arterial blood pressure measurement and arterial blood sampling. After instrumentation, the end-tidal carbon dioxide partial pressure was maintained at 35 ± 1 mmHg by intermittent positive-pressure ventilation (IPPV) using a time-cycled volume-limited ventilator (Nuffield Anesthesia Ventilator Series 200; Penlon, Abingdon, UK). The initial settings comprised a respiratory rate of 12 breaths/min and an inspiratory-to-expiratory time ratio of 1:2. During the entire experiment, lactated Ringer's solution (5 mL/kg/hr; Solulact; Terumo Co., Ltd.) was administered with a precision infusion pump (TOP-221V; TOP Corp.). The esophageal temperature (T_{ESO}) was maintained between 37.5 °C and 38.0 °C with a heating pad and a warm air blanket.

For each dog, the sevoflurane minimal alveolar concentration (MAC), propofol minimum infusion rate (MIR), and alfaxalone MIR were determined by two observers (IC, HT or YW) using electrical stimulation methods [6, 46, 72]. After equilibration for 20, 60 and 90 min during the SEVO [72], PROP

[46] and ALFX [6] treatments, respectively, electrical stimuli (50 V, 50 Hz, 10 msec) were delivered by an electrical stimulator (SEN-3301; Nihon Kohden, Tokyo, Japan). The stimulator was connected to two 25-gauge, 2.5 cm stainless steel needles (Top injection needles; TOP Corp.) that were inserted 5 cm apart subcutaneously on the ventral base of the tail. Electrical stimuli were applied for a maximum of 10 sec or terminated when a purposeful movement was observed. The purposeful movement was observed for 60 sec after the initiation of the electrical stimuli. Tail movements, swallowing, blinking or spontaneous breathing were not considered purposeful movements. In case of disagreements among the observers, the stimulation was repeated. If purposeful movements were elicited by the electrical stimuli, the EtSEVO was increased by 0.2% and maintained for 15 min, while the propofol and alfaxalone infusion rates were increased by 0.025 and 0.01 mg/kg/min, respectively, and maintained for 20 min. If no purposeful movement was observed, the EtSEVO and the propofol and alfaxalone infusion rates were similarly decreased and maintained. The MAC (of sevoflurane) and the MIR (of propofol and alfaxalone) were calculated as the arithmetic mean of three values of the EtSEVO and infusion rates that allowed and abolished purposeful movements, respectively.

1.2.3 Neuromuscular function monitoring

Neuromuscular blockade and neuromuscular function in the dogs were evaluated by responses to train-of-four (TOF) stimulation using an acceleromyography (AMG) monitor (TOF-Watch SX; MSD Co., Inc., Tokyo, Japan) [25, 66]. After predetermination of the MAC or MIR, the EtSEVO was set as 1.25-fold the individual sevoflurane MAC during SEVO treatment, while the propofol and alfaxalone infusion rates were set as 1.25-fold the individual MIRs during the PROP and ALFX treatments, respectively.

When the dog was placed in dorsal recumbency, the uncatheterized pelvic limb was passively extended and immobilized from the femur to the distal tibia using a vacuum pillow (ESF-19BN; Engineering System Co., Ltd., Nagano, Japan) allowing free movement of the hock joint. Two stimulating needles (TOP Injection Needle, 25-gauge, 2.5 cm; TOP Corp.) were placed subcutaneously, approximately 2 cm apart, over the peroneal nerve between the lateral condyle of the femur and the proximal one-third of the fibula, and a negative electrode was placed distally. An acceleration transducer was taped to the dorsal aspect between the third and fourth digits using surgical tape (Yutoku Surgical

Tape, 2.54 cm wide; Yutoku Pharmaceutical Ind. Co., Ltd., Saga, Japan). A thermometer was placed on the skin over the proximal site of the tibialis cranialis muscle and fixed using an adhesive elastic bandage (Elastpore, 2.5 cm wide; Nichiban Co., Ltd., Tokyo, Japan) to measure the skin surface temperature (T_S). The stimulating needles, electrodes, acceleration transducer and thermometer were connected to an AMG monitor. The T_S was maintained at ≥ 33 °C by using a forced-air warmer or by wrapping the dog in a cotton roll. Using the AMG monitor, the peroneal nerve was stimulated with TOF (pulse duration: 0.2 msec, frequency: 2 Hz, duty cycle: 15 sec) for 15 min to allow twitch potentiation [48]. After 15 min of uninterrupted TOF stimulation, the AMG monitor was calibrated using the CAL2 function. The number of twitches (TOF count) and the amplitudes of the first (T1) and fourth (T4) TOF twitches were measured, and the TOF ratio (TOFR; ratio of the T4 amplitude to the T1 amplitude) was calculated. A preload (an object weighing 15 g) was added to the dorsal side between the third and fourth digits if the TOFR varied significantly. If the variation in the TOFR remained < 5% for 2 min, the control TOF (TOFRC) and the supramaximal stimulation current were recorded. The TOF variables were recorded continuously throughout the experiments using the monitor software (TOF-watch SX Monitor Version 2.5.INT; Organon Ltd., Dublin, Ireland).

After 40 min of anesthesia equilibration with 1.25-fold MAC or MIR for each treatment, IV rocuronium bromide (1 mg/kg; Rocuronium Bromide Intravenous Solution; Fuji Pharma Co., Ltd., Tokyo, Japan) was administered. The following times were recorded: time from rocuronium administration to achieving TOF count 0 (onset time), time from achieving TOF count 0 to the reappearance of TOF count 4 (clinical blockade period), time to recovery from 25% to 75% of the TOFRC (recovery index) and time from achieving TOF count 0 to TOFR/TOFRC ≥ 0.9 (total neuromuscular blockade duration).

Before and 2, 5, 10, 15, 20, 30, 45 and 60 min after rocuronium administration, and when a TOFR/TOFRC ≥ 0.9 was achieved (TOFR90), an arterial blood sample was collected into heparinized syringes that were self-prepared by liquid-heparin rinsing (Heparin Sodium Injection 10,000 units/10 mL MOCHIDA; Mochida Pharmaceutical Co., Ltd., Tokyo, Japan). These were immediately analyzed for the arterial pH (pHa), partial pressure of arterial oxygen (PaO_2) and carbon dioxide ($PaCO_2$) (GEM Premier 3500; Instrumentation Laboratory Japan Co., Ltd., Tokyo, Japan). The IPPV was set to maintain the $PaCO_2$ at 40 ± 1 mmHg.

At 60 min after rocuronium administration and when the TOFR90 was achieved, cefazolin sodium hydrate (25 mg/kg; Cefamezin α 1 g for Injection; LTL Pharma Co., Ltd., Tokyo, Japan) was administered IV with buprenorphine hydrochloride (0.01 mg/kg; Lepetan injection 0.3 mg; Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) intramuscularly and meloxicam (0.2 mg/kg; Metacam 0.5%; Boehringer Ingelheim Japan Inc., Tokyo, Japan) subcutaneously. Anesthetic administration was discontinued and the dog was allowed to recover from anesthesia. If delirium during recovery was unacceptable, IV medetomidine (1 μg/kg; Dorbene vet; Kyoritsu Seiyaku Corporation, Tokyo, Japan) was administered. The dog was confirmed its good recovery from anesthesia and returned to the laboratory animal housing facility in our university after each experiment. Furthermore, the dog was confirmed to be in good health until one week after the end of the third experiment.

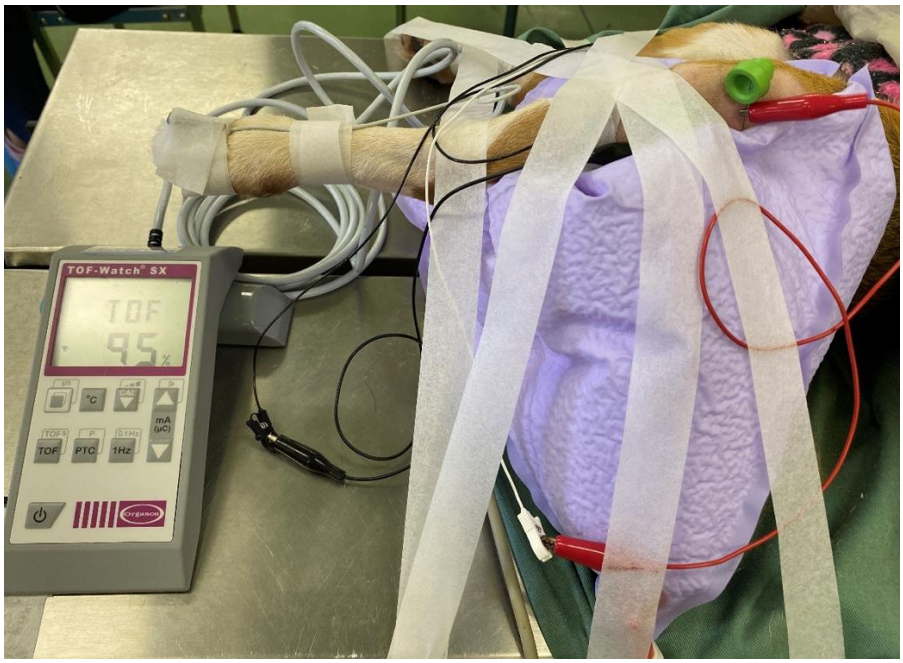

Fig. 1-1. Setting of Neuromuscular function monitor.

The pelvic limb of the dog was fixed with a vacuum pillow. Two stimulating needles were placed subcutaneously, approximately 2 cm apart, over the peroneal nerve between the lateral condyle of the femur and the proximal one-third of the fibula, and a negative electrode was placed distally. An acceleration transducer was taped to the dorsal aspect between the third and fourth digits. A thermometer was placed on the skin over the proximal site of the tibialis cranialis muscle and fixed using an adhesive elastic bandage. A 15 g preload was added to the dorsal side between the third and fourth digits if the TOFR varied significantly.

1.2.4 Cardiopulmonary monitoring

During anesthesia, the cardiopulmonary variables were monitored continuously and recorded every 5 min. These included the heart rate (HR), peripheral hemoglobin oxygen saturation (SpO_2), T_{ESO}, electrocardiography, respiratory rate, systolic, MAP and diastolic invasive arterial pressure and EtSEVO (BP-608V; Omron Colin, Ltd., Tokyo, Japan). The invasive arterial blood pressure was measured by connecting the arterial catheter placed in the dorsal pedal artery to a pressure transducer (BD DTX Plus DT-4812; Japan Becton, Dickinson and Co., Fukushima, Japan) that was zeroed at the level of the mid-sternum or mid-thorax.

1.2.5 Statistical analysis

Data analyses were performed using the SAS statistical software (SAS OnDemand for Academics; SAS Institute Inc., NC, USA). All data were analyzed for normality using the Shapiro–Wilk test. Parametric values are presented as mean ± standard deviation, while nonparametric values are presented as median (interquartile range). Statistical significance was set at $p < 0.05$.

The TOFRC, time for MAC or MIR determination, total anesthesia time, onset time, recovery index and total neuromuscular blockade duration were compared among the SEVO, PROP, and ALFX treatments using a one-way analysis of variance (ANOVA) with the Tukey–Kramer *post hoc* test. The supramaximal current and clinical blockade period were compared among the treatments using the Kruskal–Wallis and Steel–Dwass *post hoc* tests. Changes in the MAP were compared among the treatments using a two-way repeated-measures ANOVA followed by the Tukey–Kramer *post hoc* test. Changes in the HR, pHa, base excess (BE), PaO_2, $PaCO_2$, T_{ESO} and T_S were compared among the treatments using the Friedman test with the Bonferroni *post hoc* test. For each treatment, the MAP was compared to the baseline value by a repeated measures single-factor ANOVA followed by the Dunnett's *post hoc* test. The HR, pHa, BE, PaO_2, $PaCO_2$, $T_{ESO,}$ and T_S were compared to the baseline values by the Friedman and Steel–Dwass *post hoc* tests.

Spearman's rank correlation coefficient was used to identify the variables (*i.e.* sex, age, weight, EtSEVO or infusion rate of PROP or ALFX, the mean of MAP, and the medians of HR, pHa, BE, PaO_2, $PaCO_2$, T_{ESO} and T_S during the experiment) that correlated with the logit transform of the clinical blockade period, RI or duration TOFR90 within each group.

1.3 Results

1.3.1 MAC/MIR determination and total anesthesia time

The MAC/MIR determination time and the total anesthesia time were 209.4 ± 33.6 and 360.8 ± 35.4 min in the SEVO treatment, 378.3 ± 86.2 and 535.0 ± 72.4 min in the PROP treatment, and 340.5 ± 106.4 and 489.8 ± 113.1 min in the ALFX treatment, respectively. The total anesthesia time and the time required for MAC/MIR determination were shorter during the SEVO treatment than during the PROP (p = 0.001 and p = 0.011, respectively) and ALFX (p < 0.001 and p = 0.011, respectively) treatments. The MAC of sevoflurane was 2.18 ± 0.44%, and the MIRs of propofol and alfaxalone were 0.29 ± 0.10 and 0.13 ± 0.04 mg/kg/min, respectively. In all dogs, there was no unacceptable delirium during recovery and therefore medetomidine was not administered.

1.3.2 Changes in the TOFR/TOFRC values

Changes in the TOFR/TOFRC of the SEVO, PROP and ALFX treatments are shown in Figure 1-2. The TOFRC, supramaximal current, onset time and recovery index did not differ significantly among the treatments (Table 1-2). However, the clinical blockade period was longer in the SEVO treatment than in the PROP and ALFX treatments (p = 0.002 and p = 0.017, respectively), and also longer in the ALFX treatment than in the PROP treatment (p = 0.020) (Table 1-2). The total neuromuscular blockade duration was longer in the SEVO treatment than in the PROP and ALFX treatments (p < 0.001 and p = 0.036, respectively).

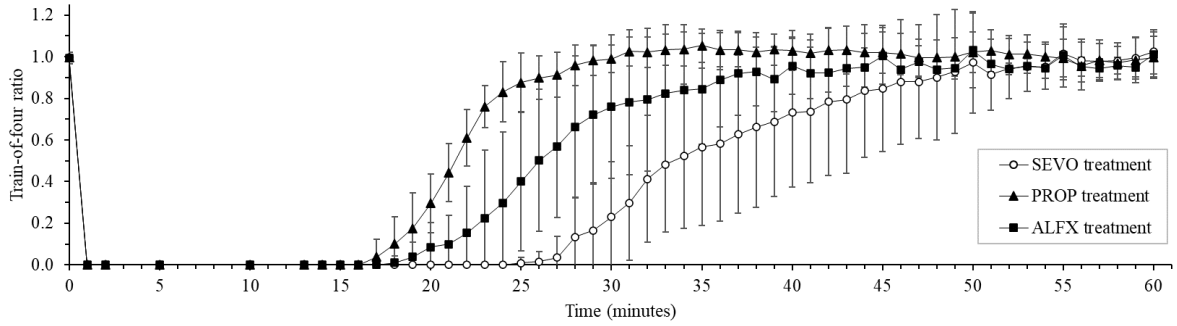

Fig. 1-2. Train-of-four ratio before (baseline) and after rocuronium (1 mg/kg bolus) intravenous administration in eight dogs anesthetized with sevoflurane (SEVO treatment), propofol constant rate infusion (CRI) (PROP treatment) or alfaxalone CRI (ALFX treatment).

Data are presented as mean ± standard deviation.

Table 1-2. The train-of-four (TOF) ratio before rocuronium administration (TOFRC), time from rocuronium (1 mg/kg bolus) intravenous administration to achieving TOF count 0 (onset time), time from achieving TOF count 0 to reappearance of TOF count 4 (clinical blockade period), time to recovery from 25% to 75% of the TOFRC (recovery index) and time from achieving TOF count 0 to achieving TOF ratio/TOFRC ≥ 0.9 (total neuromuscular blockade duration) in eight dogs anesthetized with sevoflurane (SEVO treatment), propofol constant rate infusion (CRI) (PROP treatment) or alfaxalone CRI (ALFX treatment). Data are presented as mean ± standard deviation or median (interquartile range).

Variable	Treatment		
	SEVO	PROP	ALFX
TOFRC	1.08 ± 0.19	1.12 ± 0.12	1.14 ± 0.16
Supramaximal current (mA)	60 (60–60)	60 (60–60)	60 (60–60)
Onset time (sec)	49 ± 14	61 ± 11	48 ± 13
Clinical blockade period (min)	27.3 (26.0–30.3)	16.6 (15.4–18.0) *	22.4 (18.6–23.1) *†
Recovery index (min)	5.8 ± 3.0	4.1 ± 2.0	4.7 ± 1.7
Total neuromuscular blockade duration (min)	43.7 ± 9.9	25.1 ± 2.7 *	32.5 ± 8.4 *

*Significantly different from the period/duration in the SEVO treatment ($p < 0.05$). †Significantly different from the period in the PROP treatment ($p < 0.05$).

1.3.3 Physiological parameters

Compared with the SEVO treatment, HR during the PROP treatment was lower at baseline (p = 0.005) and at 2, 10 and 15 min after rocuronium administration (p = 0.048, p = 0.043 and p = 0.042, respectively) (Table 1-3). Compared with the ALFX treatment, HR during the PROP treatment was lower at baseline (p = 0.004); at 2, 5, 10, 15, 20, 45 and 60 min after rocuronium administration (p-values = 0.042, 0.045, 0.009, 0.005, 0.013, 0.020 and 0.022, respectively); and while achieving TOFR90 (p = 0.031) (Table 1-3). Compared with the PROP treatment, MAP during the SEVO treatment was lower at 2, 5, 15, 20, 30, 45 and 60 min after rocuronium administration (p-values = 0.022, 0.010, 0.010, 0.009, 0.005, < 0.001 and 0.002, respectively) and while achieving TOFR90 (P = 0.002) (Table 1-3). Compared with the ALFX treatment, MAP during the SEVO treatment was lower at the baseline (p = 0.049); at 2, 5, 15, 20, 30, 45 and 60 min after rocuronium administration (p-values = 0.018, 0.013, 0.041, 0.012, < 0.001, < 0.001 and 0.001, respectively); and while achieving TOFR90 (P = 0.002). The BE, PaO_2, $PaCO_2$, T_{ESO} and T_S did not differ among the treatments at baseline or at any other time point (Table 1-3). The HR, MAP, pHa, BE, PaO_2, T_{ESO} and T_S did not differ between baseline and any other time point within each treatment. Statistically significant differences were detected in: 1) the pHa between the SEVO and ALFX treatments at 60 min after rocuronium administration (p = 0.023), 2) the $PaCO_2$ between the baseline and while achieving TOFR90 during the SEVO treatment (p = 0.034), and 3) the $PaCO_2$ between the baseline and 15 (p = 0.039) and 20 min (p = 0.038) after rocuronium administration in the ALFX treatment. However, these differences were considered not clinically significant.

Table 1-3. Changes in the heart rate (HR), mean invasive arterial pressure (MAP), arterial pH (pHa), base excess (BE), partial pressures of oxygen (PaO_2) and carbon dioxide ($PaCO_2$), esophageal temperature (T_{ESO}) and skin surface temperature (T_S) before (baseline) and after rocuronium (1 mg/kg bolus) intravenous administration in eight dogs anesthetized with sevoflurane (SEVO treatment), propofol constant rate infusion (CRI) (PROP treatment) or alfaxalone CRI (ALFX treatment). Data are presented as mean ± standard deviation or median (interquartile range).

Variable	Treatment	Baseline	Time (min)							
			2	5	10	15	20	30	45	60
HR (beats/min)	SEVO	121 (102–133)	123 (108–135)	127 (115–138)	127 (119–137)	127 (117–136)	125 (116–138)	125 (115–136)	128 (122–136)	125 (113–135)
	PROP	82 * (72–94)	93 * (81–106)	109 (95–121)	108 * (91–117)	104 * (90–113)	104 (93–122)	98 (92–128)	94 (84–118)	90 (82–103)
	ALFX	115 † (107–121)	126 † (114–132)	128 † (121–138)	133 † (120–145)	131 † (124–144)	136 † (125–144)	135 (130–151)	137 † (129–143)	130 † (125–144)
MAP (mmHg)	SEVO	88 ± 14	85 ± 17	88 ± 13	92 ± 13	92 ± 11	90 ± 11	89 ± 12	89 ± 12	91 ± 12
	PROP	103 ± 15	107 ± 14 *	109 ± 14 *	109 ± 14	112 ± 14 *	111 ± 12 *	110 ± 11 *	115 ± 9 *	114 ± 13 *
	ALFX	105 ± 12 *	108 ± 15 *	109 ± 13 *	108 ± 14	108 ± 11 *	110 ± 14 *	116 ± 11 *	119 ± 10 *	115 ± 10 *
pHa	SEVO	7.41 (7.40–7.43)	7.38 (7.37–7.39)	7.39 (7.37–7.39)	7.38 (7.35–7.40)	7.38 (7.38–7.38)	7.37 (7.36–7.38)	7.37 (7.36–7.40)	7.39 (7.36–7.41)	7.38 (7.36–7.39)
	PROP	7.42 (7.42–7.43)	7.39 (7.39–7.40)	7.40 (7.39–7.40)	7.38 (7.36–7.39)	7.39 (7.37–7.41)	7.39 (7.37–7.40)	7.40 (7.38–7.41)	7.39 (7.38–7.40)	7.40 (7.38–7.41)
	ALFX	7.43 (7.43–7.45)	7.40 (7.40–7.42)	7.38 (7.37–7.41)	7.39 (7.38–7.41)	7.39 (7.38–7.41)	7.39 (7.38–7.41)	7.40 (7.39–7.40)	7.40 (7.39–7.43)	7.41* (7.40–7.42)
BE (mmol/L)	SEVO	−0.6 (−1.5 to −0.2)	−1.4 (−3.6 to −0.9)	−1.6 (−2.6 to −1.2)	−1.5 (−2.4 to −0.7)	−1.5 (−2.9 to −1.1)	−1.3 (−3.7 to −1.0)	−2.1 (−2.9 to −0.8)	−2.0 (−3.1 to −1.1)	−1.7 (−2.8 to −1.5)
	PROP	−0.2 (−0.8 to 0.2)	−1.3 (−1.8 to −0.1)	−1.5 (−1.9 to −0.7)	−1.8 (−1.9 to −1.2)	−1.6 (−1.7 to −1.1)	−1.5 (−2.2 to −0.9)	−1.7 (−2.1 to −1.1)	−1.2 (−2.2 to −0.4)	−1.3 (−2.7 to 0.2)
	ALFX	−0.2 (−1.1 to 1.6)	−0.7 (−1.1 to 0.2)	−1.2 (−1.4 to 0.2)	−1.2 (−1.8 to 0.4)	−1.2 (−1.8 to −0.3)	−1.1 (−1.7 to −0.1)	−1.2 (−1.5 to −0.3)	−0.8 (−1.6 to −0.2)	−1.1 (−1.4 to 0.1)
PaO_2 (mmHg)	SEVO	550 (503–584)	527 (451–568)	470 (455–580)	522 (431–590)	509 (441–590)	519 (459–584)	549 (513–601)	451 (395–581)	548 (513–592)
	PROP	567 (544–581)	558 (549–566)	551 (515–566)	558 (525–566)	558 (513–565)	542 (503–573)	517 (471–557)	572 (541–576)	563 (554–575)
	ALFX	552 (530–580)	557 (520–567)	572 (521–581)	560 (523–576)	569 (529–592)	573 (527–583)	585 (528–587)	574 (510–585)	555 (528–594)
$PaCO_2$ (mmHg)	SEVO	37 (35–37)	40 (38–41)	40 (38–42)	41 (39–44)	40 (39–40)	41 (39–41)	40 (39–41)	39 (39–40)	39 (38–39)
	PROP	36 (35–36)	39 (38–41)	39 (38–40)	41 (40–41)	40 (40–41)	39 (39–40)	39 (37–40)	40 (39–40)	40 (39–40)
	ALFX	36 (36–37)	38 (38–41)	40 (38–43)	41 (40–41)	39 (39–40) ‡	40 (39–40) ‡	39 (38–40)	38 (36–39)	38 (37–38)

T_{ESO} (°C)	SEVO	37.9 (37.8-37.9)	37.9 (37.8-37.9)	37.9 (37.8-37.9)	37.9 (37.7–37.9)	37.8 (37.8–37.9)	37.8 (37.8–37.9)	37.9 (37.7–37.9)	37.8 (37.8–37.9)	37.8 (37.7–37.9)
	PROP	37.9 (37.8-37.9)	37.9 (37.9-37.9)	37.9 (37.9-37.9)	37.9 (37.9–38.0)	37.9 (37.9–37.9)	37.9 (37.8–37.9)	37.9 (37.8–37.9)	37.7 (37.7–37.8)	37.8 (37.7–37.8)
	ALFX	37.9 (37.7-37.9)	38.0 (37.8-38.0)	37.9 (37.9-38.0)	38.0 (37.9–38.0)	38.0 (37.9–38.0)	38.0 (37.9–38.0)	38.0 (37.8–38.0)	37.9 (37.8–37.9)	37.9 (37.8–37.9)
T_S (°C)	SEVO	35.4 (35.3–35.9)	35.3 (35.1–35.8)	35.1 (35.0–35.5)	34.9 (34.8–35.2)	34.9 (34.9–34.9)	34.8 (34.6–34.8)	34.6 (34.5–34.8)	35.2 (34.8–35.5)	35.3 (34.5–35.8)
	PROP	34.9 (34.6–35.1)	34.9 (34.5–35.1)	34.8 (34.4–35.0)	34.7 (34.3–34.8)	34.5 (34.2–34.7)	34.5 (34.2–34.6)	34.7 (34.5–34.7)	34.8 (34.6–34.9)	34.7 (34.4–34.8)
	ALFX	35.0 (34.3–35.6)	35.1 (34.4–35.6)	34.9 (34.3–35.4)	34.7 (34.1–35.2)	34.5 (34.0–35.1)	34.4 (33.9–35.1)	34.5 (34.1–35.3)	34.6 (34.3–35.6)	34.9 (34.4–35.8)

*Significantly different from SEVO treatment at the same time point ($p < 0.05$). †Significantly different from PROP treatment at the same time point ($p < 0.05$). ‡Significantly different from baseline within the treatment ($p < 0.05$).

1.3.4 Spearman's rank correlation coefficient

By Spearman's rank correlation coefficient, EtSEV correlated with the logit-transformed clinical blockade period in SEV (*rs* = 0.73193, *p* = 0.0390). In PROP and ALFX, the medians of T_{ESO} during the experiment correlated with the logit-transformed clinical blockade period (*rs* = -0.71147 and -0.72591, *p* = 0.0478 and 0.0415, respectively). There was no correlation identified between logit-transformed RI and variables (i.e. sex, age, weight, EtSEV, the mean of MAP, and the medians of HR, pHa, BE, PaO_2, $PaCO_2$, T_{ESO} and T_S during the experiment) in SEV. In PROP, the medians of T_{ESO} during the experiment correlated with the logit-transformed RI (*rs* = 0.87439, *p* = 0.0045). In ALFX, infusion rate of ALFX correlated with the logit-transformed RI (*rs* = 0.84146, *p* = 0.0088). Medians of T_{ESO} and T_S during the experiment correlated with the logit-transformed duration TOFR90 in SEV (*rs* = -0.74882 and -0.73055, *p* = 0.0325 and 0.0396, respectively). In PROP, there was no correlation identified between logit-transformed duration TOFR90 and variables (*i.e*. sex, age, weight, infusion rate of PROP, the mean of MAP, and the medians of HR, pHa, BE, PaO_2, $PaCO_2$, T_{ESO} and T_S during the experiment). In ALFX, the medians of T_{ESO} during the experiment correlated with the logit-transformed duration TOFR90 (*rs* = -0.75094, *p* = 0.0318).

1.4 Discussion

The present study showed that compared with sevoflurane anesthesia at 1.25 × MAC, alfaxalone and propofol infusion at 1.25 × MIR did not prolong the rocuronium-induced neuromuscular blockade to a greater extent in dogs. Furthermore, the duration of clinical blockade differed between alfaxalone and propofol infusions, and results indicated that alfaxalone may induce a greater prolongation of the rocuronium-induced neuromuscular blockade. It is inferred that there is a minor difference in the pharmacodynamic effect on rocuronium-induced neuromuscular blockade between alfaxalone and propofol in dogs.

Sevoflurane inhibits the adult mouse muscle nicotinic acetylcholine receptor (nAChR) by potentiating the functional effect of antagonists, such as vecuronium and d-tubocurarine, at the receptor site in a dose-dependent manner [58]. Another *in vitro* study also reported that sevoflurane enhanced the rocuronium-induced inhibition of adult mouse muscle nAChR in a concentration-dependent manner [44]. Sakata *et al.* [67] reported that sevoflurane prolonged recovery from rocuronium CRI-induced neuromuscular blockade in a dose-dependent manner in dogs; the rocuronium plasma concentrations suggested that sevoflurane enhanced neuromuscular blockade by affecting rocuronium pharmacodynamics at the neuromuscular junction. Conversely, sevoflurane anesthesia may also affect rocuronium metabolism by reducing the cardiac output and hepatic blood flow, because rocuronium is eliminated mainly by the liver [24, 41]. In the present study, the cardiac output and hepatic blood flow may have been decreased by sevoflurane, because the MAP was lower in the SEVO treatment than in the ALFX and PROP treatments. Moreover, according to the result of Spearman's rank correlation coefficient, EtSEVO correlated with the logit-transformed clinical blockade period in SEV and showed that the higher the EtSEVO, the longer the clinical blockade period. Therefore, it was speculated that sevoflurane prolongs rocuronium-induced neuromuscular blockade in dogs by potentiating the inhibition of the nAChR and affecting rocuronium metabolism in a dose-dependent manner.

Compared with sevoflurane, alfaxalone and propofol had lesser effects on rocuronium-induced neuromuscular blockade in the dogs. Nevertheless, the clinical blockade period was slightly, but significantly, longer in the ALFX treatment than in the PROP treatment. This indicated that alfaxalone infusion might increase the degree of rocuronium-induced neuromuscular blockade as compared to

propofol infusion. It has been demonstrated that nondepolarizing NMBAs inhibit the neuronal α3β2 nAChR subtype and muscle nAChRs [34, 35]. An *in vitro* study demonstrated that propofol inhibited the α1β1δε, α3β2, and α7 nAChR subtypes, which can be found at the neuromuscular junction; however, the inhibitory effect occurred at concentrations higher than those required for general anesthesia [33]. Conversely, alfaxalone has been reported to inhibit nAChRs in cultured bovine adrenal chromaffin cells at anesthetic concentrations [68]. Although the inhibited nAChR subtype was not determined in the Shiraishi *et al*. [21] study, α3β2 is one of the most abundant nAChR subtypes in the adrenal medulla. It was postulated that the prolongation of the clinical blockade period in the ALFX treatment was caused by variation in the pharmacodynaics of rocuronium at the neuromuscular junction. In addition, according to the result of Spearman's rank correlation coefficient, infusion rate of ALFX correlated with the logit-transformed RI and also indicated that the higher the infusion rate, the longer the RI. Therefore, compared with propofol, alfaxalone may cause a slightly greater prolongation of rocuronium-induced neuromuscular blockade in dogs.

The medians of T_{ESO} negatively correlated with the logit-transformed clinical blockade period in PROP and ALFX, and negatively correlated with the logit-transformed duration TOFR90 in SEV and ALFX. Hypothermia may influence the neuromuscular blocking effect by reducing muscle strength or drug elimination rate [28]. However, the body temperature range (37.5–38.0°C) in the present study was higher than in daily clinical practice and therefore our results could underestimate the recovery time of rocuronium-induced neuromuscular blockade in clinical situation. The medians of T_{ESO} during the experiment positively correlated with the logit-transformed RI in PROP. Considering the median body temperature was either 37.8 or 37.9°C in PROP and the same trend was not seen in other two treatments, we concluded that further studies are needed to determine if body temperature affects the RI of rocuronium in dogs. The medians of T_S negatively correlated with the logit-transformed duration TOFR90 in SEV. Nevertheless, the skin surface temperature was maintained above 33.0°C in the present study and was considered acceptable because *in vitro* study showed the potency of neuromuscular blocking agent increases at temperatures less than 33.0°C [28].

There are several limitations to the present study. First, the small sample size may increase the probability of a type II error. Although the difference in the clinical blockade period between the PROP and ALFX treatments was significant, no significant differences in the total neuromuscular blockade

duration were detected. However, when analyzed with the Kaplan–Meier survival curve, log-rank tests revealed that the time to TOFR90 was significantly shorter in the PROP treatment than in the ALFX treatment (p = 0.008). Therefore, the total neuromuscular blockade duration may differ between the PROP and ALFX treatments. Moreover, in the present study the individual MAC or MIR were determined before rocuronium administration to minimize any intra-individual variations. However, this resulted in a significant difference in the total anesthesia time among the treatments, and the influence of these variations cannot be ruled out. Conversely, the prolonged anesthesia times in the PROP and ALFX treatments may have resulted in the accumulation of propofol and alfaxalone in the peripheral tissues, respectively. Therefore, propofol and alfaxalone plasma concentration analyses are warranted to determine whether there was an accumulation of IV anesthetic drugs. In addition, a previous study in dogs demonstrated that AMG detected recovery from neuromuscular blockade earlier than electromyography [65]. Thus, the recovery time may have been underestimated in the present study. Nevertheless, this underestimation does not alter the conclusion that compared with propofol and alfaxalone, sevoflurane significantly prolongs rocuronium-induced neuromuscular blockade, and that compared with propofol, alfaxalone prolongs the clinical blockade period to a greater extent.

1.5 Brief summary

This study is randomized, prospective, crossover experimental study which compared the effects of sevoflurane, propofol and alfaxalone on the neuromuscular blockade induced by a single intravenous bolus of rocuronium in dogs.

A total of eight adult Beagle dogs (four female, four male), weighing 8.9–15.3 kg and aged 5–7 years was included. The dogs were anesthetized three times with 1.25 × minimum alveolar concentration of sevoflurane (SEVO treatment) and 1.25 × minimum infusion rate of propofol (PROP treatment) or alfaxalone (ALFX treatment) at intervals of ≥ 14 days. Neuromuscular function was monitored with train-of-four (TOF) stimulation of the peroneal nerve by acceleromyography. After recording the control TOF ratio (TOFRC), a single bolus dose of rocuronium (1 mg/kg) was administered intravenously. The times from rocuronium administration to achieving TOF count 0 (onset time), from achieving TOF count 0 to the reappearance of TOF count 4 (clinical blockade period), from 25% to 75% of TOFRC (recovery index) and from achieving TOF count 0 to TOF ratio/TOFRC ≥ 0.9 (total neuromuscular blockade duration) were recorded.

The onset time and recovery index did not differ among the treatments. The median clinical blockade period was longer in the SEVO treatment [27.3 (26.0–30.3) min] than in PROP [16.6 (15.4–18.0) min; $p = 0.002$] and ALFX [22.4 (18.6–23.1) min; $p = 0.017$] treatments; and longer in the ALFX treatment than in the PROP treatment ($p = 0.020$). The mean total neuromuscular blockade duration was longer in the SEVO treatment (43.7 ± 9.9 min) than in PROP (25.1 ± 2.7 min; $p < 0.001$) and ALFX (32.5 ± 8.4 min; $p = 0.036$) treatments. According to the results, when compared with alfaxalone and propofol, sevoflurane prolonged rocuronium-induced neuromuscular blockade by a significantly greater extent in dogs.

Chapter II

ED_{50} and ED_{95} of rocuronium during alfaxalone anesthesia in dogs

2.1 Preface

The results from Chapter I showed that the single IV dose rocuronium-induced neuromuscular blockade was longer in dogs under alfaxalone anesthesia than those under propofol anesthesia. However, there was still insufficient information regarding to effective doses of rocuronium in dogs under alfaxalone anesthesia. According to the Good clinical research practice in pharmacodynamics studies of neuromuscular blocking agents [25], the effective doses of NMBAs can be evaluated by constructing dose–response curves from a linear least squares regression analysis model. The single bolus method is considered as the 'gold standard' for dose–response curve construction of intermediate and short-acting NMBAs. In this method, the degree of neuromuscular block is measured following administration of different doses of NMBA to determine the median effective dose 50 (ED_{50}) and effective dose required to depress the twitch value by 95% (ED_{95}).

For dose-response curve construction, the ideal stimulation pattern of neuromuscular monitoring is the single twitch stimulation; however, train-of-four stimulation is also an acceptable stimulation pattern [25]. If the first twitch (T1) of train-of-four (TOF) stimulation is used for the dose–response study of an NMBA, the ED_{50} and ED_{95} are defined as the dose required to depress the T1 value by 50% and 95%, respectively [55].

This chapter aimed to investigate the potency of rocuronium by constructing the dose–response curve and determining its ED_{50} and ED_{95} during alfaxalone anesthesia in dogs.

2.2 Materials and methods

2.2.1 Animals

The experimental animals included in this randomized, prospective, crossover experiment were four female and four male adult Beagle dogs, weighing 10.3–14.6 kg, aged 6–8 years, and purpose-bred for research. The dogs were used in this study after confirming their good health condition by physical examination and blood test including complete blood count and serum biochemical analysis. Since there was no current report about confidence interval data for ED_{50} or ED_{95} of rocuronium in dogs, no *a priori* power analysis was performed. Eight dogs were used according to the experiment in Chapter I.

The MIR of alfaxalone for each dog was determined before the experiments, using a method described in Chapter I. The predetermined individual MIR of alfaxalone was 0.13 (0.11–0.14) mg/kg/min [median (25th–75th percentile)]. Each dog was anesthetized three times with alfaxalone infusion at 1.25-fold the individual MIR [1.25 MIR; equal to 0.16 (0.14–0.18) mg/kg/min], during which rocuronium was administered at 100 μg/kg (R100 treatment), 175 μg/kg (R175 treatment), or 250 μg/kg (R250 treatment). Washout period between each experiment was set for at least 14 days. Order of the dogs and the rocuronium doses were randomized by an online randomization system (https://www.randomizer.org/). The randomized result was shown in Table 2-1. Food was withheld from the dogs for 12 hr, but access to water was not restricted, prior to each experiment.

This experiment protocol was approved by the Animal Care and Use Committee of Rakuno Gakuen University (no. VH21B7), and the dogs received care in accordance with the principles of the Guide for the Care and Use of Laboratory Animals in Common Breeding Facilities prepared by Rakuno Gakuen University. The stabling conditions included feeding and environmental cleaning twice a day and walking activity for at least once a week throughout the study period.

Table 2-1. The result of randomization for eight dogs administered rocuronium 100 μg/kg (R100 treatment), 175 μg/kg (R175 treatment) or 250 μg/kg (R250 treatment) during alfaxalone anesthesia.

Dog number	Treatment order		
	1	2	3
1	R250	R100	R175
2	R100	R175	R250
3	R100	R250	R175
4	R100	R175	R250
5	R250	R175	R100
6	R250	R175	R100
7	R250	R100	R175
8	R100	R175	R250

2.2.2 Anesthesia and instrumentation

First, catheterization of the right cephalic vein was performed with a 22-gauge catheter (Surflo F&F, 22-gauge, 2.5 cm; Terumo Co., Ltd.) for rocuronium administration and isotonic fluid infusion, while catheterization of the left cephalic vein was performed with a 22-gauge catheter for alfaxalone administration. Induction of anesthesia was achieved by administering alfaxalone (3 mg/kg IV; Alfaxan; Meiji Seika Pharma Co.) over a period of 1 min and was maintained by alfaxalone infusion of 1.25 MIR using a precision syringe infusion pump (TOP-551V; TOP Corp.). After the induction of anesthesia, the dog was orotracheally intubated with a cuffed endotracheal tube and oxygen inflow of 2 L/min was delivered by a circle rebreathing system of an anesthetic machine (Beaver 20; Kimura Medical Instrument Co.). Then, the dog was placed in dorsal recumbency and lactated Ringer's solution (Solulact; Terumo Co., Ltd.) was administered at 5 mL/kg/hr using a precision infusion pump (TOP-221V; TOP Corp.) during the entire experiment.

Invasive arterial blood pressure was measured by catheterizing the median caudal artery with a 24-gauge catheter (Supercath 5, 24-gauge, 1.9 cm; Medikit Co., Ltd.). Vacuum cushion (ESF-19BN; Engineering System Co. Ltd, Japan) was used to fix the femur and the distal tibia of left pelvic limb to the table, allowing the hock joint to move freely. The peroneal nerve was stimulated by inserting two needles (Top Injection Needle, 25-gauge, 2.5 cm; TOP Corp.) subcutaneously, approximately 2 cm apart, between the lateral condyle of the femur and proximal one-third of the fibula. The stimulation needles were connected to an AMG monitor (TOF-Watch SX; MSD Co., Inc.). An acceleration transducer of the AMG monitor was fixed to the dorsal aspect between the third and fourth digits using surgical tape (Yutoku Surgical Tape, 2.54 cm wide; Yutoku Pharmaceutical Ind. Co., Ltd.). T_S measurement was performed by placing the temperature sensor of the AMG monitor on the skin over the proximal site of the tibialis cranialis muscle.

After instrumentation was completed, the alfaxalone anesthesia was equilibrated for 90 min and the end-tidal partial pressure of carbon dioxide was maintained at the value to control $PaCO_2$ at 40 ± 3 mmHg. The respiratory rate and inspiratory-to-expiratory time ratio of intermittent positive pressure mechanical ventilation (Nuffield Anesthesia Ventilator Series 200; Penlon Ltd.) were initially set at 12 breaths/min and 1:2, respectively. T_{ESO} was controlled between 37.5°C and 38.0°C by a heating pad and

a warm air blanket. If T_S was lower than 33°C, the pelvic limb would be wrapped with a cotton roll and forced-air warmer would be used when necessary.

2.2.3 Rocuronium administration and neuromuscular function monitoring

Neuromuscular function was evaluated using the TOF stimulation mode (pulse duration: 0.2 msec, frequency: 2 Hz, duty cycle: 15 sec) of the AMG monitor. Considering twitch potentiation [48], the peroneal nerve was stimulated for 15 min via TOF stimulation mode before rocuronium administration. CAL2 function of the AMG monitor was then used for calibration. The TOF count, amplitudes of T1 to T4, and TOFR were recorded continuously by the monitor software (TOF-watch SX Monitor Version 2.5.INT; Organon Ltd.). If the TOFR varied significantly, a preload, which was a mass weighing 15 g, would be fixed to the dorsal aspect between the third and fourth digits. If the variation of TOFR value was less than 5% for 2 min after calibration, the TOFRC, control T1 (T1C), and supramaximal stimulation current were recorded.

After 90 min of alfaxalone anesthesia equilibration, rocuronium bromide (100, 175, or 250 μg/kg IV; Rocuronium Bromide Intravenous Solution; Fuji Pharma Co., Ltd.) was administered for each treatment. The following values were recorded: lowest TOFR, the lowest value of TOFR/TOFRC which TOF count = 0–4 was considered TOFR = 0 (*e.g.*, TOF count of 3 would be analyzed as TOFR = 0, not as TOFR = 3 or 0.03); lowest T1, lowest value of T1/T1C; onset time of lowest T1, time from rocuronium administration to achieving lowest T1; duration TOFR0.9, time from a rocuronium administration to TOFR/TOFRC $\geq$ 0.9.

The experiment was terminated when TOFR0.9 was achieved, and 60 min had passed from rocuronium administration. Thereafter, cefazolin sodium hydrate (25 mg/kg IV; Cefamezin α 1 g for Injection; LTL Pharma Co., Ltd.), buprenorphine hydrochloride (0.01 mg/kg for intramuscular injection; Lepetan injection 0.3 mg; Otsuka Pharmaceutical Co., Ltd.), and meloxicam (0.2 mg/kg of subcutaneous injection; Metacam 0.5%; Boehringer Ingelheim Japan Inc.) were administered, the alfaxalone infusion was terminated. If delirium was profound during recovery, medetomidine administration (1 μg/kg IV; Dorbene vet; Kyoritsu Seiyaku Corp.) was considered. Once the dog recovered full consciousness and was able to walk without support, it would go back to the laboratory animal housing facility in our

university. The health condition of the dogs were confirmed until 1 week after the end of the last experiment.

2.2.4 Cardiopulmonary monitoring

Cardiopulmonary variables, including HR, peripheral arterial hemoglobin oxygen saturation, T_{ESO}, electrocardiography, respiratory rate, end-tidal partial pressure of carbon dioxide, systolic, MAP, and diastolic invasive arterial pressure, were monitored continuously. The cardiopulmonary variables except for electrocardiography were recorded every 5 min throughout the experiment, using a patient monitoring device (BP-608V; Omron Colin, Ltd.). The arterial catheter was connected to a pressure transducer (BD DTX Plus DT-4812M; Japan Becton, Dickinson and Co.) that was zeroed and then placed at the level of the shoulder joint during the experiment. Heparinized saline solution (10 unit/mL) was used for the arterial catheter and tubing to the pressure transducer.

Arterial blood gas analysis was performed before and 2, 5, 10, 15, 20, 30, 45 and 60 min after the rocuronium administration, and when a TOFR/TOFRC ≥ 0.9 was achieved. Arterial blood (0.5 mL) was collected from the arterial catheter after discarding 3 mL of blood and immediately analyzed after collection. Liquid-heparin prerinsed syringes was used for arterial blood sample collection. The arterial blood samples were analyzed for the pHa, BE, PaO_2 and $PaCO_2$ using a blood gas analyzer (GEM Premier 3500; Instrumentation Laboratory Japan Co., Ltd.). The results were corrected to concurrent esophageal temperature.

2.2.5 Statistical and dose–response analysis

Statistical analysis was performed using SAS OnDemand for Academics (SAS Institute Inc.). Data are presented as median (25th–75th percentile). Among the treatments, the instrumentation completion time (defined as the time from the induction of anesthesia to instrumentation completion), total anesthesia time (defined as the time from the induction of anesthesia to extubation), TOFRC, T1C, supramaximal current, lowest TOFR, lowest T1, onset time of lowest T1, duration TOFR0.9, HR, MAP, pHa, BE, PaO_2, $PaCO_2$, T_{ESO} and T_S were compared using the Friedman test, with the Dwass, Steel, Critchlow–Fligner multiple comparison analysis. Within each treatment, the HR, MAP, pHa, BE, PaO_2, $PaCO_2$, T_{ESO} and T_S after the rocuronium administration were compared to the corresponding baseline

values by the Friedman and Dwass, Steel, Critchlow–Fligner multiple comparison analysis. A *p*-value < 0.05 was considered statistically significant.

Dose–response curves were constructed by plotting the logit transform of the dose of rocuronium against the logit transform of the depression of T1/T1C values (response) using linear least squares regression models. The 100% depression of T1/T1C was adjusted to 99% and 0% depression of T1/T1C was adjusted to 1% during logit transformation. ED_{50} and ED_{95} were defined as the dose required to achieve T1/T1C = 50% and 5%, respectively. The ED_{50} and ED_{95} values of rocuronium were calculated using the dose–response curve.

2.3 Results

2.3.1 Neuromuscular blocking properties

There was no morbidities, adverse reactions, or mortalities occurred in the dogs during the study period. Only one dog in treatment R100 required preload due to TOFR variation. The differences of instrumentation completion time, total anesthesia time, TOFRC, T1C and supramaximal current among the treatments were small; therefore, it is unlikely that they caused the statistically significant differences of lowest TOFR, lowest T1, onset time of lowest T1 and duration TOFR0.9 found in the study (Table 2-2).

The lowest TOFR decreased dose-dependently with the rocuronium dose and was higher in the R100 treatment than in the R175 and R250 treatments (p = 0.006 and = 0.001, respectively) (Table 2-2). The lowest T1 also decreased dose-dependently with the rocuronium dosage and was higher in the R100 treatment than in the R175 and R250 treatments (both p = 0.002) and in the R175 treatment than in the R250 treatment (p = 0.031) (Table 2-2).

The onset time of lowest T1 was longer in the R250 treatment than in the R100 treatment (p = 0.023) (Table 2-2). The duration TOFR0.9 was prolonged dose-dependently with the rocuronium dosage and its median value ranged from 3.1 min in the R100 treatment to 10.1 min in the R250 treatment (Table 2-2).

Table 2-2. Variables in eight dogs administered rocuronium 100 μg/kg (R100 treatment), 175 μg/kg (R175 treatment) or 250 μg/kg (R250 treatment) during alfaxalone anesthesia.

Variable	Treatment		
	R100	R175	R250
Instrumentation completion time (min)	37 (23–53)	23 (20–69)	22 (18–28)
Total anesthesia time (min)	232 (223–254)	248 (213–263)	230 (217–243)
TOFRC	1.16 (1.08–1.25)	1.11 (1.05–1.21)	1.05 (0.99–1.16)
T1C	0.91 (0.89–0.94)	0.95 (0.90–0.96)	0.94 (0.86–0.94)
Supramaximal current (mA)	55 (41–60)	60 (40–60)	55 (44–60)
Lowest TOFR	0.60 (0.40 –0.67)	0.15 (0.00–0.19) *	0.00 (0.00–0.00) *
Lowest T1	0.91 (0.77–0.93)	0.29 (0.12–0.41) *	0.07 (0.03–0.10) *†
Onset time of lowest T1 (sec)	68 (65–89)	101 (87–107)	112 (107–125) *
Duration TOFR0.9 (min)	3.1 (2.9–4.4)	7.7 (6.9–8.1) *	10.1 (9.2–10.9) *†

Data are presented as median (25th–75th percentile). Abbreviations: TOF, train-of-four; TOFRC, TOF ratio before rocuronium administration; T1C, T1 before rocuronium administration; the lowest value of TOFR/TOFRC (TOFR lowest; TOF count = 0–4 was considered TOFR = 0); lowest T1, the lowest value of T1/T1C; onset time of lowest T1, times from rocuronium intravenous administration to achieving lowest T1; duration TOFR0.9, times from rocuronium intravenous administration to achieving TOF ratio/TOFRC ≥ 0.9. *Significantly different from R100 treatment ($p < 0.05$). †Significantly different from R175 treatment ($p < 0.05$).

2.3.2 Dose–response curve

The constructed dose–response curve of rocuronium is shown in Figure 2-1. The ED_{50} and ED_{95} calculated from the dose–response curve was 175 μg/kg (95% confidence interval = 158–188 μg/kg) and 232 μg/kg (95% confidence interval = 189–236 μg/kg) (R^2 = 0.7313), respectively.

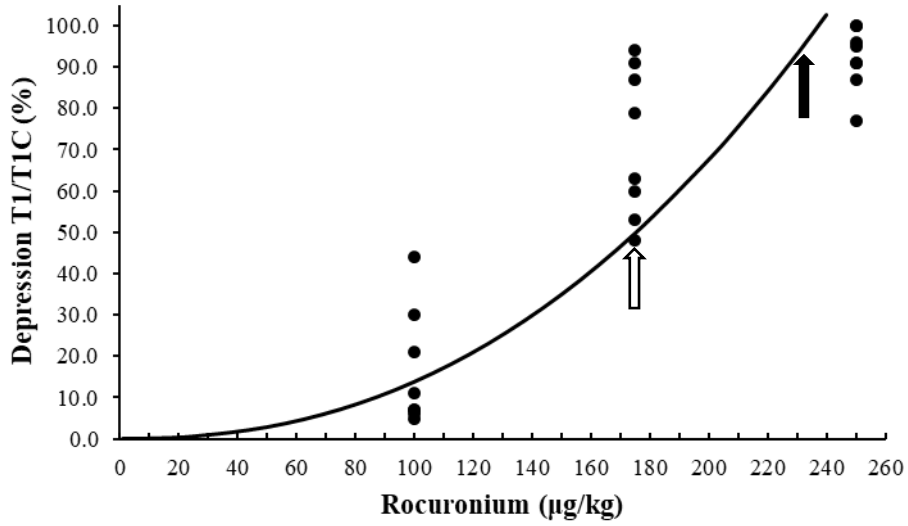

Figure 2-1. Dose–response curve and effective dose 50 (ED_{50}) and effective dose 95 (ED_{95}) of rocuronium in dogs anesthetized with alfaxalone infusion

The dose–response curves were constructed by plotting the logit transforms of the rocuronium dose against that of depression of T1/T1C values (response) using linear least squares regression models. The ED_{50} (open arrow) and ED_{95} (solid arrow) were 175 μg/kg (95% confidence interval = 158–188 μg/kg) and 232 μg/kg (95% confidence interval = 189–236 μg/kg), respectively. Individual data were shown as dots. Abbreviations: TOF, train-of-four; T1, first twitch of TOF; T1C, control first twitch of TOF

2.3.3 Perioperative physiological parameters

The differences of in the HR, MAP, pHa, BE, PaO_2, $PaCO_2$, T_{ESO} and T_S were small among the treatments and within treatment. The overall median (25th–75th percentile) for pHa, BE, PaO_2, $PaCO_2$, T_{ESO} and T_S were 7.40 (7.38–7.42), –0.5 (–1.5 to 1.0) mmol/L, 557 (529–576) mmHg, 39 (37–40) mmHg, 37.9 (37.8–38.0) ℃ and 36.0 (35.5–36.8) ℃, respectively. No significant effect of HR and MAP was seen upon these variables.

2.4 Discussion

The ED_{50} and ED_{95} values were 175 μg/kg (95% confidence interval = 158–188 μg/kg) and 232 μg/kg (95% confidence interval = 189–236 μg/kg), respectively, during alfaxalone anesthesia in dogs. The 95% confidence interval of the ED_{50} and ED_{90} of rocuronium in cats were reported to be 127–179 and 192–300 μg/kg with the tibialis muscle [53]. Although the experimental protocol and specie were different from our study, we considered the accuracy of the estimation in our study was acceptable. In R250 treatment, only two out of eight dogs achieved TOF count = 0. Therefore, for achieving TOF count = 0, the recommended dose ($2 \times ED_{95}$) for rocuronium is 0.5 mg/kg in dogs under alfaxalone anesthesia. The current recommended clinical initial dose of rocuronium is 0.1–0.6 mg/kg in dogs anesthetized with halothane, isoflurane or propofol [1, 3, 4, 14, 20, 39]. The results of this study support the recommended clinical initial dose of rocuronium in dogs anesthetized with alfaxalone.

The ED_{95} of rocuronium in humans under nitrous oxide–opioid anesthesia was reported to be 271 μg/kg [50] and the ED_{90} of rocuronium in cats under α-chloralose–pentobarbital anesthesia was reported to be 246–311 μg/kg [53]. The ED_{95} of rocuronium in dogs under alfaxalone anesthesia appears to be slightly lower than that in previous studies in humans and cats, although a simple comparison of the results was not possible, due to the differences in anesthetic and experimental protocols between the studies. In dogs, compared with potent volatile inhalation anesthetics including isoflurane and sevoflurane, injectable anesthetics including propofol and alfaxalone have little or minimal effect on NMBA-induced neuromuscular blockade [11, 12, 37, 54, 67]. Therefore, further study is required to determine whether alfaxalone would cause a greater augmentation of rocuronium-induced neuromuscular blockade compared with other anesthetics.

In this study, three different IV doses of rocuronium (100, 175 and 250 μg/kg) were employed to construct the dose–response curve for evaluating the ED_{50} and ED_{95}. A low rocuronium dose (100 μg/kg IV) was selected as a dose that would produce a weak neuromuscular blockade in the dogs anesthetized with alfaxalone infusion. This was based on the result of a previous study in which T1 and the TOF ratio decreased to 61% and 45% of each baseline value after rocuronium administration (100 μg/kg IV) in dogs anesthetized with propofol infusion [3]. TOF ratio was not depressed by rocuronium administration of 75 μg/kg IV although the globe was centralized in dogs anesthetized with isoflurane [9]. The high

rocuronium dose (250 μg/kg IV) was selected as a minimum dose that would depress T1 by nearly 100% in dogs anesthetized with alfaxalone infusion. This was based on the results of previous studies in which rocuronium administration (300 and 400 μg/kg IV) produced a TOF count of 0 in dogs anesthetized with isoflurane [3, 20], and alfaxalone anesthesia prolonged rocuronium-induced neuromuscular blockade to a lesser extent than did sevoflurane anesthesia [12]. Thus, in our study, rocuronium doses of 100, 175, and 250 μg/kg IV produced a dose-dependent depression of T1, ranging from 0.09 to 0.93, and allowed construction of the dose–response curve for evaluating the ED_{50} and ED_{95} of rocuronium in the dogs anesthetized with alfaxalone infusion.

According to previous studies, factors that may affect the neuromuscular blocking effect induced by NMBA are sex, age, physical status, body weight, monitoring method [25], anesthetic technique [71], body temperature [28], and acid–base disturbance [56, 57]. In this study, age, physical status, and body weight of the dogs were similar, and therefore, their influence on the effect of the rocuronium-induced neuromuscular blockade was considered minimal. The number of female and male dogs included in the study was identical to offset sex differences. Moreover, the T_{ESO} was controlled between 37.5°C and 38.0°C, and pHa was maintained within a normal range during the experiments. Consequently, the differences in neuromuscular blockade between the treatments were mainly associated with the rocuronium dosage.

Auer [3] reported that the time to T1 depression was detected within 1 min from rocuronium administration and TOF suppression was achieved at 2.0 min and 1.1 min after administration of rocuronium 0.3 mg/kg and 0.6 mg/kg IV in dogs anesthetized with propofol infusion, respectively. The result in Chapter I showed that the time from rocuronium administration (1 mg/kg IV) to TOF count of 0 achievement as 48 sec in dogs under alfaxalone anesthesia [12]. In this study, the onset time of neuromuscular blockade was correlated negatively with the rocuronium dose. This meant that the lower the dose of rocuronium, the higher the value of lowest T1, and which resulted in shorter onset time of the lowest T1.

There are some limitations to the present study. First, this study was conducted with a homogeneous population of dogs and the conditions were also well controlled. Therefore, the results obtained from this study may be less applicable to dogs with different breeds, age, and co-morbidities. Second, no *a priori* power analysis was performed; thus, the small sample size may have led to type II errors. However,

the results of lowest TOFR, lowest T1, onset time of lowest T1 and duration TOFR0.9 from the present study showed significant differences. Moreover, the main objective of this study was to determine the dose–response curve for rocuronium. We consider that the dose–response curve constructed in this study was reliable for the dogs used in the experiment. Third, the MIR of each dog was predetermined rather than being determined on the day of each experiment. The individual MIR may alter due to several factors that may influence the drug clearance (age, diet, level of sex hormones) [19, 23]. Volemia may also alter the clearance of alfaxalone by influencing the hepatic blood flow [19]. However, an adequate level of anesthesia for conducting the experiments could be maintained using the predetermined MIR. Moreover, by separating the MIR determination experiment, a similar anesthesia time could be employed in each treatment, preventing different levels of accumulation of alfaxalone during the rocuronium administration experiment. Fourth, the researcher was not blinded to the dose administered. Nevertheless, the aim of this study was to determine the ED_{50} and ED_{95} of rocuronium by constructing the dose–response curve. The construction of dose–response curve was based on the depression of T1/T1C values, which was an objective value and was unaffected by observers. Fourthly, the potency of the neuromuscular blocking agent tends to be overestimated when using TOF method to determine the dose-response relationship compared with single twitch method [16, 45]. We decided to use TOF method because we desired to investigate the duration TOFR90 in present study. In addition, Sakai *et al.* [64] reported that data obtained with AMG should not be used interchangeably with that of mechanomyography which the gold standard method for assessing neuromuscular function. However, due to the popularity of the use of AMG in current clinical practice, we consider that the results presented here are reliable for daily clinical use.

2.5 Brief summary

The aim of this chapter is to determine the median effective dose (ED_{50}) and effective dose required to depress the twitch value by 95% (ED_{95}) of rocuronium during alfaxalone anesthesia in dogs. This was a randomized, prospective, crossover experimental study which involved a total of eight adult Beagle dogs (four female, four male), weighing 10.3–14.6 kg, and aged 6–8 years.

The dogs were anesthetized three times with 1.25-fold the individual minimum infusion rate of alfaxalone at intervals of ≥ 14 days. Neuromuscular function was monitored with train-of-four (TOF) stimulation of the peroneal nerve by acceleromyography. After recording the control TOF ratio (TOFRC) and first twitch of TOF (T1C), a single bolus dose of rocuronium 100, 175 or 250 μg/kg (R100, R175, or R250 treatment) was administered intravenously. The maximum suppression of the T1 was recorded and calibrated with T1C to construct the dose–response curve, from which ED_{50} and ED_{95} were calculated. Time from rocuronium administration to TOF ratio/TOFRC ≥ 0.9 (duration TOFR0.9) was recorded.

The results showed that ED_{50} and ED_{95} of rocuronium during alfaxalone anesthesia were 175 μg/kg and 232 μg/kg, respectively. The duration TOFR0.9 was longer in the R250 treatment (10.1 [9.2–10.9] min) than in R100 (3.1 [2.9–4.4] min; $p < 0.0001$) and R175 (7.7 [6.9–8.1] min; $p < 0.0001$) treatments; and longer in the R175 than in R100 treatment ($p < 0.0001$).

According to the results in this chapter, the recommended dose ($2 \times ED_{95}$) for rocuronium is 0.5 mg/kg for achieving TOF count = 0 in dogs anesthetized with alfaxalone infusion. The duration TOFR0.9 correlated positively with the dose of rocuronium. These findings support the currently recommended clinical initial dose of rocuronium in dogs. Future research is required to determine the neuromuscular effect and recovery characteristics of rocuronium 0.5 mg/kg during alfaxalone anesthesia in dogs.

Chapter III

Sugammadex for reversal of rocuronium-induced neuromuscular blockade during alfaxalone anesthesia in dogs

3.1 Preface

According to the results in Chapter II, the recommended dose of rocuronium for achieving TOF count = 0 is 0.5 mg/kg in dogs anesthetized with alfaxalone infusion. Investigation of the neuromuscular effect and recovery characteristics of rocuronium 0.5 mg/kg during alfaxalone anesthesia in dogs was required.

The reversal effect of the selective relaxant binding agent, sugammadex, on rocuronium-induced blockade has been evaluated in dogs under isoflurane anesthesia [52]. Compared to acetylcholinesterase inhibitor, sugammadex does not have cardiovascular side effects and is able to reverse profound neuromuscular block [63]. However, the encapsulation of other steroidal drugs or endogenous steroids may occur with sugammadex administration [30].

The alfaxalone formulation registered for clinical use in dogs and cats associated with the use of a 2-hydroxypropyl-beta cyclodextrin as a solvent [22]. Therefore, considering that alfaxalone is a steroidal drug and the similar structure between sugammadex and the solvent of alfaxalone, the efficacy of sugammadex in the reversal of rocuronium-induced neuromuscular blockade should be investigated in dogs under alfaxalone anesthesia.

This chapter aimed to investigate the reversal effect of sugammadex on neuromuscular blockade produced by a single bolus of rocuronium in dogs under alfaxalone anesthesia. Hypothesis was that sugammadex can effectively reverse the rocuronium-induced neuromuscular blockade in dogs under alfaxalone anesthesia.

3.2 Materials and methods

3.2.1 Animals

The experimental protocol was approved by the Animal Care and Use Committee of Rakuno Gakuen University (approval no. VH22B3). Six adult beagle dogs (3 females and 3 males), weighing from 11.3–15.8 kg and aged from 6–8 years old, were included in this study, which was designed as a randomized, prospective, crossover experimental study. Sample size was determined by power analysis with provided parameters: significance level (adjusted for sidedness) = 0.025, standard deviation of recovery time within patients = 5 min, power = 0.8, difference of mean recovery time = 10.2 min.

The dogs were judged to be healthy based on physical examination, blood cell counts and serum biochemical profiling. The dogs were cared for according to the principles of the Guide for the Care and Use of Laboratory Animals in Common Breeding Facilities prepared by Rakuno Gakuen University. Food was withheld for 12 hours before each experiment but with free access to water. Washout period was set for at least 14 days between experiments. The order of the dogs and treatments for each experiment were randomized by using an online randomization system (https://www.randomizer.org/).

3.2.2 Anesthesia and instrumentation

Before the induction of anesthesia, the left and right cephalic veins were catheterized with 22-gauge, 2.5 cm catheters (Surflo F&F; Terumo Co., Ltd.) for IV administration of alfaxalone and IV infusion of an isotonic crystalloid fluid, respectively. Anesthesia was induced with alfaxalone (3 mg/kg IV; Alfaxan; Meiji Seika Pharma Co.) and maintained with alfaxalone infusion at an initial rate of 0.12 mg/kg/min by a precision syringe infusion pump (TOP-551V; TOP Corp.). The dog was orotracheally intubated with a cuffed endotracheal tube and administered an oxygen flow of 2 L/min by a circle rebreathing system of an anesthetic machine (Beaver 20; Kimura Medical Instrument Co.). Lactated Ringer's solution (5 mL/kg/hr; Solulact; Terumo Co., Ltd.) was administered by a precision infusion pump (TOP-221V; TOP Corp.). The right or left dorsal pedal artery was catheterized with a 22-gauge, 3.1 cm catheter (Supercath 5; Medikit Co., Ltd.) for invasive arterial blood pressure measurement and arterial blood sampling. IPPV was started by a time-cycled volume-limited ventilator (Nuffield Anesthesia Ventilator Series 200; Penlon). The respiratory rate and inspiratory-to-expiratory time ratio were set at 12

breaths/min and 1 : 2, respectively. Tidal volume was adjusted to maintain $PaCO_2$ at 40 ± 1 mmHg during the experiment. Also, the dog was warmed by a heating pad and a warm air blanket to maintain T_{ESO} at 37.5–38.0 °C during the experiment.

Electrical stimulation method [6, 12] was conducted to determine MIR of alfaxalone. Two stimulation needles (Top injection needles, 25-gauge, 2.5 cm; TOP Corp.) were inserted on the ventral base of the tail (5 cm apart, subcutaneously) and connected to an electrical stimulator (SEN-3301; Nihon Kohden). After an equilibration of alfaxalone infusion at the initial rate of 0.12 mg/kg/min for 90 min, nociceptive electrical stimuli (50 V, 50 Hz, 10 msec) was applied for 10 sec or stopped once the purposeful movement occurred. Movement of tail, swallowing, blinking, or spontaneous breathing were judged non-purposeful movement. The response of each stimulation was judged by three observers and stimulation would be repeated if there was a disagreement among the observers. If purposeful movements occurred within 60 sec after the initiation of the electrical stimuli, the alfaxalone infusion rate was increased by 0.01 mg/kg/min and maintained for 20 min. If negative, the alfaxalone infusion rate was decreased by 0.01 mg/kg/min and maintained for 20 min. The alfaxalone MIR was calculated as the arithmetic average of three infusion rates that purposeful movements occurred or abolished.

Then, the dog was maintained anesthesia with an infusion of alfaxalone at 1.25-fold of individual MIR in dorsal recumbency and instrumented for neuromuscular function monitoring. The femur to the distal tibia of the uncatheterized pelvic limb was fixed by a vacuum pillow (ESF-19BN; Engineering System Co., Ltd.) and allowing free movement of the hock joint. The positive electrode (proximally) and negative electrode (distally) of AMG monitor (TOF-Watch SX; MSD Co., Inc.) were attached to two stimulating needles (TOP Injection Needle, 25-gauge, 2.5 cm; TOP Corp.) which were inserted subcutaneously, 2 cm apart, over the peroneal nerve between the lateral condyle of the femur and the proximal one-third of the fibula. An acceleration transducer of the AMG monitor was fixed to the dorsal aspect between the third and fourth digits by a surgical tape (Yutoku Surgical Tape, 2.54 cm wide; Yutoku Pharmaceutical Ind. Co., Ltd.). The T_S was measured by the temperature sensor of the AMG monitor which was fixed by an adhesive elastic bandage (Elastpore, 2.5 cm wide; Nichiban Co., Ltd.) on the skin over the tibialis cranialis muscle. When T_S was decreased to less than 33 °C, the limb would be wrapped with a cotton roll and forced-air warmer would be used to maintain T_S.

3.2.3 Neuromuscular function monitoring

The AMG monitor was used to monitor the neuromuscular function by TOF stimulation mode [12, 25, 66]. After the instrumentation was completed, TOF stimulation mode (pulse duration: 0.2 msec, frequency: 2 Hz, duty cycle: 15 sec) was applied to peroneal nerve for 15 min to allow twitch potentiation [12, 48]. The CAL2 calibration mode of the AMG monitor was performed after 15 min of TOF stimulation. Neuromuscular function variables include the TOF count, the amplitudes of T1 to T4, and TOFR. TOFRC was defined as the TOFR when variation remained < 5% for 2 min. The supramaximal stimulation current for TOFRC was recorded. The AMG monitor was connected to the monitor software (TOF-watch SX Monitor Version 2.5.INT; Organon Ltd.) and neuromuscular function variables were continuously recorded.

After stabilizing for 40 min under alfaxalone 1.25 MIR anesthesia, rocuronium bromide (0.5 mg/kg IV; Rocuronium Bromide Intravenous Solution; Fuji Pharma Co., Ltd.) was administered from the 22-gauge catheter placed in the right cephalic vein. When TOF count 1 reappeared after achieving TOF count 0, sugammadex (4 mg/kg IV; Bridion; MSD K.K., Tokyo, Japan) (sugammadex treatment) or equal volume of saline (Isotonic Sodium Chloride Solution; Terumo Co., Ltd., Tokyo, Japan) (control treatment) was administered from the 22-gauge catheter. The following times were recorded: time from rocuronium administration to achieving TOF count 0 (onset time), time from achieving TOF count 0 to the reappearance of TOF count 1 (maximum blockade period), time to recovery from 25% to 75% of the TOFRC (RI), time from achieving TOF count 0 to TOFR/TOFRC ≥ 0.9 (duration TOFR0.9). In sugammadex treatment, time from sugammadex administration to achieving TOFR/TOFRC ≥ 0.9 (sugammadex onset time) was also recorded.

After the TOFR0.9 was achieved, cefazolin sodium hydrate (25 mg/kg IV; Cefamezin α 1 g for Injection; LTL Pharma Co., Ltd.), buprenorphine hydrochloride (0.01 mg/kg intramuscularly; Lepetan injection 0.3 mg; Otsuka Pharmaceutical Co., Ltd.) and meloxicam (0.2 mg/kg subcutaneously; Metacam 0.5%; Boehringer Ingelheim Japan Inc.) were administered to the dog, and alfaxalone infusion was terminated. All dogs were observed at least twice a day for one week after each experiment to confirm their health.

3.2.4 Cardiopulmonary monitoring

Electrocardiography, cardiopulmonary variables including HR, peripheral hemoglobin oxygen saturation, T_{ESO}, respiratory rate and invasive arterial pressure were monitored continuously by a patient monitoring device (BP-608V; Omron Colin, Ltd.). The cardiopulmonary variables were recorded every 5 min during the experiment. The pressure transducer (BD DTX Plus DT-4812; Japan Becton, Dickinson and Co.) used for invasive arterial blood pressure measurement was zeroed and placed at the level of the mid-sternum or mid-thorax when the dog was placed at right lateral recumbency or dorsal recumbency, respectively. Self-prepared liquid-heparin (Novo-heparin for injection; Mochida Pharmaceutical Co., Ltd., Tokyo, Japan) rinsing 2.5 mL-plastic syringes were used to collect arterial blood sample. The pHa, BE, PaO_2 and $PaCO_2$ were measured by a blood gas analyzer (GEM Premier 3500; Instrumentation Laboratory Japan Co., Ltd.) at 10, 30, 60 and 90 min after IPPV initiation, before the rocuronium administration and after TOFR0.9 achievement.

3.2.5 Statistical analysis

Data analyses were performed using the SAS statistical software (SAS OnDemand for Academics; SAS Institute Inc.). All values are presented as median (interquartile range). Statistical significance was set at $p < 0.05$. Wilcoxon signed-rank test was employed to compare the MIR of alfaxalone, TOFRC, supramaximal current, onset time, maximum blockade period, RI and duration TOFR0.9 between treatments. Friedman and Dwass, Steel, Critchlow–Fligner multiple comparison analysis were employed to compare the HR, MAP, T_{ESO} and T_S before (baseline) and after the rocuronium administration in each treatment. Wilcoxon signed-rank test was employed to compare the pHa, BE, PaO_2 and $PaCO_2$ at baseline and after TOFR0.9 achievement in each treatment and between treatments. Wilcoxon signed-rank test was employed to compare the HR, MAP, T_{ESO} and T_S between treatments at each time point.

Spearman's rank correlation coefficient was used to identify the variables (*i.e.* sex, age, weight, infusion rate of alfaxalone, and the pHa, BE, PaO_2, $PaCO_2$ at baseline and after TOFR0.9 achievement and the HR, MAP, T_{ESO} and T_S at 5 min after rocuronium administration) that correlated with neuromuscular blocking properties (*i.e.* onset time, maximum blockade period, RI and duration TOFR0.9) in each treatment.

3.3 Results

3.3.1 MAC/MIR determination and times associated to anesthesia

Alfaxalone MIRs in sugammadex and control treatments were 0.13 (0.13–0.15) mg/kg/min and 0.15 (0.12–0.17) mg/kg/min, respectively. In terms of the MIR of alfaxalone, time from applying the first nociceptive stimulation to judging final response to determine MIR (MIR determination time), time from the first alfaxalone IV to extubation (total anesthesia time) and time from the termination of alfaxalone infusion to extubation with the recovery of laryngeal reflex (extubation time), the differences were small between treatments (Table 3-1).

Table 3-1. The control train-of-four ratio (TOFRC), supramaximal current, onset time, maximum blockade period, recovery index, duration TOFR0.9, MIR determination time, extubation time and total anesthesia time in dogs anesthetized with alfaxalone which sugammadex 4 mg/kg (sugammadex treatment) or equal volume of saline (control treatment) was administered intravenously when the neuromuscular blockade was reappeared from train-of-four (TOF) count 0 to TOF count 1.

Variable	Sugammadex treatment	Control treatment
TOFRC	1.13 (1.12–1.21)	1.02 (1.02–1.04)
Supramaximal current (mA)	60 (56–60)	60 (60–60)
Onset time (sec)	69 (56–72)	60 (49–63)
Maximum blockade period (min)	9.9 (9.6–10.0)	9.9 (8.9–10.9)
RI (min)	N/A	2.8 (2.1–4.2)
Sugammadex onset time (min)	0.8 (0.8–0.9)	N/A
Duration TOFR0.9 (min)	11.4 (11.1–11.5)	21.1 (19.2–22.1) *
MIR determination time (min)	144 (130–167)	120 (126–193)
Total anesthesia time (min)	391 (359–429)	432 (387–448)
Extubation time (min)	37 (21–44)	32 (24–38)

Values are presented as median (interquartile range). Control TOF ratio (TOFRC) defined as the TOF ratio before rocuronium administration; Onset time, time from rocuronium administration to achieving TOF count 0; Maximum blockade period, time from achieving TOF count 0 to TOF count 1 reappearance; Recovery index (RI), time to recovery from 25% to 75% of the TOFRC; TOFR0.9, TOFR/TOFRC of 0.9; Duration TOFR0.9, time from the onset of neuromuscular blockade to attaining TOFR0.9; Total anesthesia time, time from the induction of anesthesia to extubation; Extubation time, time from the termination of alfaxalone infusion to extubation. *Significantly different from sugammadex treatment ($p < 0.05$).

3.3.2 Neuromuscular blocking properties

The differences of TOFRC, supramaximal current, onset time and maximum blockade period were small between treatments (Table 3-1). Changes in TOFR was shown in Figure 3-1. The TOFR increased rapidly after the administration of sugammadex. The RI was not available in sugammadex treatment due to the rapid recovery from the neuromuscular blockade. As shown in Table 3-1, sugammadex onset time was within 1 min and duration TOFR0.9 was shorter in sugammadex treatment than in control treatment (p = 0.031).

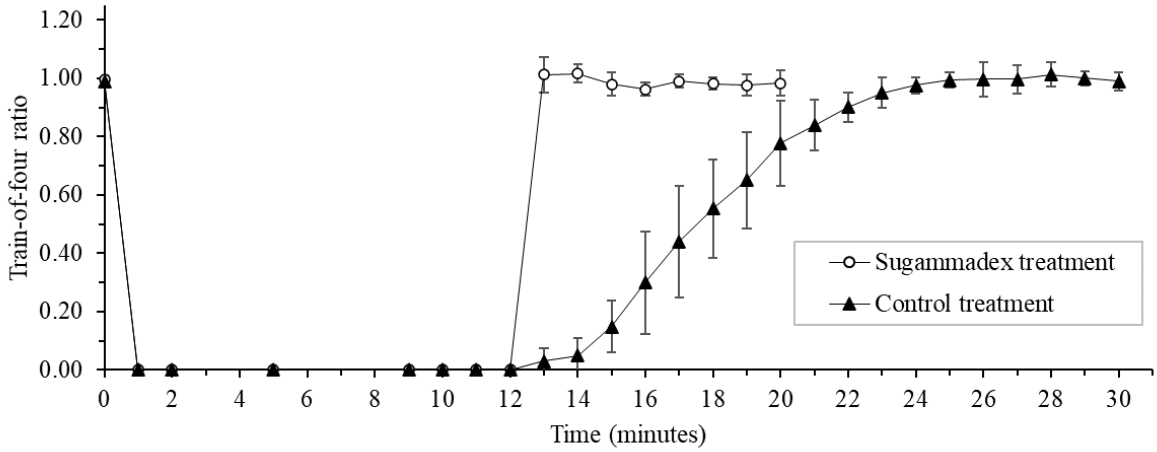

Fig. 3-1. Changes in the train-of-four ratio (TOFR) in each treatment.

Plots and error bars presented median and interquartile range.

3.3.3 Physiological parameters and Spearman's rank correlation coefficient

The differences of the pHa, BE, PaO_2, $PaCO_2$, HR, MAP, T_{ESO} and T_S were small between the treatments and within treatment (Table 3-2 and Table 3-3). By Spearman's rank correlation coefficient, there was no correlation identified between onset time and variables in the control treatment. The onset time of the sugammadex treatment only correlated with the BE at baseline (Spearman ρ = -0.82857, 95% confidence interval = -0.977243 to 0.030871 p = 0.042). The RI of the control treatment only correlated with the age of the dogs (Spearman ρ = 0.83324, 95% confidence interval = -0.016272 to 0.977891; p = 0.039). There was no correlation identified between duration TOFR0.9 and variables in both treatments.

Table 3-2. Arterial pH (pHa), base excess (BE), partial pressures of oxygen (PaO_2) and carbon dioxide ($PaCO_2$) before rocuronium administration (baseline) and after train-of-four (TOF) ratio achieving 0.9 (TOFR0.9) in dogs under alfaxalone anesthesia which sugammadex 4 mg/kg (sugammadex treatment) or equal volume of saline (control treatment) was administered intravenously when the neuromuscular blockade was reappeared from train-of-four (TOF) count 0 to TOF count 1.

Variable	Sugammadex treatment		Control treatment	
	Baseline	TOFR0.9	Baseline	TOFR0.9
pHa	7.39 (7.37–7.41)	7.41 (7.39–7.43)	7.43 (7.42–7.43)	7.41 (7.39–7.42)
BE (mmol/L)	–0.5 (–1.2 to 0.3)	–0.4 (–1.0 to –0.1)	0.4 (–0.9 to 1.6)	0.2 (–0.3 to 0.4)
PaO_2 (mmHg)	558 (537–575)	570 (535–589)	562 (538–583)	554 (528–575)
$PaCO_2$ (mmHg)	40 (40–40)	38 (37–39)	39 (36–40)	40 (38–41)

Values are presented as median (interquartile range).

Table 3-3. Changes in the heart rate (HR), mean direct arterial pressure (MAP), esophageal temperature (T_{ESO}) and skin surface temperature (T_S) before rocuronium administration (baseline) and after rocuronium administration in dogs under alfaxalone anesthesia which sugammadex 4 mg/kg (sugammadex treatment) or equal volume of saline (control treatment) was administered intravenously when the neuromuscular blockade was reappeared from train-of-four (TOF) count 0 to TOF count 1.

Variable	Baseline	Time after rocuronium administration (min)					
		5	10	15	20	25	30
HR (beats/min)							
Sugammadex	129 (120–138)	141 (132–156)	136 (126–155)	134 (126–158)	133 (124–166)	136 (109–157)	124 (101–137)
Control	126 (120–132)	136 (125–139)	135 (129–144)	136 (130–144)	140 (132–145)	136 (133–143)	134 (133–142)
MAP (mmHg)							
Sugammadex	106 (98–115)	111 (102–116)	114 (103–123)	117 (103–123)	116 (104–129)	106 (103–114)	N/A
Control	96 (90–104)	101 (96–105)	110 (98–111)	113 (101–117)	114 (99–119)	114 (103–119)	107 (99–110)
T_{ESO} (℃)							
Sugammadex	37.8 (37.7–37.9)	37.8 (37.8–37.9)	37.8 (37.7–37.9)	37.8 (37.7–37.9)	37.7 (37.7–37.9)	N/A	N/A
Control	37.9 (37.7–37.9)	37.9 (37.8–37.9)	37.9 (37.7–37.9)	37.9 (37.8–37.9)	37.9 (37.8–37.9)	37.9 (37.8–37.9)	37.9 (37.8–37.9)
T_S (℃)							
Sugammadex	36.1 (35.2–36.3)	35.9 (35.2–36.1)	35.7 (35.1–35.8)	35.7 (35.3–35.9)	36.0 (35.9–36.1)	N/A	N/A
Control	35.9 (35.4–36.2)	35.6 (35.2–36.1)	35.4 (35.2–35.9)	35.4 (35.2–35.8)	35.5 (35.3–35.9)	35.8 (35.6–36.1)	35.8 (35.7–36.1)

Values are presented as median (interquartile range).

3.4 Discussion

The results of this chapter showed that TOF ratio/TOFRC ≥ 0.9 was achieved within 1 min after the administration of sugammadex in the dogs paralyzed with rocuronium under alfaxalone anesthesia. As we hypothesized, rocuronium-induced neuromuscular blockade was effectively reversed by sugammadex in dogs under alfaxalone anesthesia. The solvent of alfaxalone seemed to have no effect on the efficacy of sugammadex in the reversal of rocuronium-induced neuromuscular blockade.

The sugammadex dose of 2 mg/kg IV was reported as a dose that would safely reverse a neuromuscular blockade of TOF count 1 [69]. On the other hand, one study in dogs reported that sugammadex of 8 mg/kg can effectively reverse the neuromuscular blockade after 5 min from rocuronium administration [52]. In our study, sugammadex was administered when TOF count 1 reappeared after achieving TOF count 0. That is, sugammadex was administered immediately after the rocuronium-induced neuromuscular blockade had passed its peak. Therefore, sugammadex dose of 2 mg/kg IV should be enough in the dogs under alfaxalone anesthesia. However, considering the lack of knowledge regarding sugammadex reversal in dogs, we chose the sugammadex dose of 4 mg/kg IV to ensure the complete reversal of the neuromuscular blockade.

There were concerns that encapsulation of alfaxalone may occur with sugammadex administration [30]. In our study, there were no signs of light anesthesia such as increases in HR and MAP after administration of sugammadex in the dogs under alfaxalone anesthesia and the difference of extubation time in the sugammadex treatment from those in the control treatment was small. Comparing to the solvent of alfaxalone which is a beta-cyclodextrin containing 7 glucose subunits [22], the gamma-cyclodextrin structure of sugammadex contains 8 glucose subunits [8, 18]. Therefore, the encapsulation of alfaxalone with the sugammadex administration may not occur; or even if it occurs, it may not cause the dog to suddenly recover from the anesthesia.

The differences of HR and MAP were small between the values before rocuronium administration and after sugammadex administration, which indicated that the lack of cardiovascular side effects comparing to acetylcholinesterase inhibitor. On the other hand, the results of Spearman's rank correlation coefficient showed that 1) the onset time of the sugammadex treatment correlated with the BE at baseline and 2) the RI of the control treatment correlated with the age of the dogs. Previous studies

showed that sex, age, physical status, body weight, monitoring method [25], anesthetic technique [71], body temperature [28] and acid-base differences [56, 57] may affect the neuromuscular blocking effect induced by neuromuscular blocking agent. In this chapter, the influence of sex, age, physical status and body weight between treatments were controlled by the crossover method. The T_{ESO} was controlled between 37.5–38.0°C and therefore the influence might be minimal. Although the BE at baseline correlated with the onset time of the rocuronium in the sugammadex treatment, the BE at baseline and after TOFR0.9 achievement were not different in sugammadex treatment or between treatments and pHa was maintained within normal range during the experiments. Therefore, the influence of acid-base differences between treatments was considered minimal.

There are limitations to this chapter that should be noted. First, type II errors are more likely to occur due to the small sample size. Although the sample size of the present study was determined by power analysis, the difference of duration TOFR0.9 between treatments was closed to 10 min. Thus, type II errors still may occur in this study. Secondly, the experiment was not blinded. However, the properties of neuromuscular blocking were determined by the values of AMG monitor, which was an objective value and was unaffected by observers. Moreover, detection of recovery from neuromuscular blockade was earlier by the AMG monitor than by electromyography monitor [65]. Therefore, underestimation of the recovery times in the present study may occur. Nevertheless, the results in the present study were still considered reliable because the AMG monitor was widely used in veterinary practice.

3.5 Brief summary

In this chapter, we investigated the reversal effect of sugammadex on neuromuscular blockade produced by a single bolus of rocuronium in dogs under alfaxalone anesthesia. This randomized, prospective, crossover experimental study involved six adult beagle dogs (three females, three males), weighing 11.3–15.8 kg and aged 6–8 years.

The dogs were anesthetized two times with 1.25 × minimum infusion rate of alfaxalone, with a washout period of at least 14 days between experiments. Neuromuscular function was monitored by an acceleromyography monitor with TOF stimulation of the peroneal nerve. After recording the control TOF ratio (TOFRC), rocuronium (0.5 mg/kg) was administered intravenously. Subsequently, sugammadex (4 mg/kg; sugammadex treatment) or equal volume of saline (control treatment) was administered intravenously when TOF count was returned from 0 to 1 under neuromuscular blockade. Time from rocuronium administration to achieving TOF count 0 (onset time), time from achieving TOF count 0 to the reappearance of TOF count 1 (maximum blockade period), time to recovery from 25% to 75% of the TOFRC (recovery index) and time from achieving TOF count 0 to TOFR/TOFRC ≥ 0.9 (duration TOFR0.9) were recorded.

The onset time and maximum blockade period were not different between treatments. Recovery index of control treatment was 2.8 (2.1–4.2) min [median (interquartile range)], while it was not available in sugammadex treatment due to the rapid recovery. Duration TOFR0.9 was 11.4 (11.1–11.5) min in sugammadex treatment, which was shorter than control treatment [21.1 (19.2–22.1) min] (p = 0.031).

In conclusion, rocuronium 0.5 mg/kg IV provided about 10 min of maximum neuromuscular blockade. Sugammadex 4 mg/kg IV effectively antagonized the rocuronium-induced neuromuscular blockade in dogs under alfaxalone anesthesia within a minute. Rocuronium-induced neuromuscular blockade was effectively reversed by sugammadex in dogs under alfaxalone anesthesia.

Conclusion

Basic elements of general anesthesia include analgesia, amnesia and muscle paralysis. Although some anesthetics also have relaxation effect by depressing the spinal motor neurons, the desired degree of muscle relaxation requires deeper plane of anesthesia, which increase the risk of dose-dependent side effects caused by the anesthetics. Therefore, the coadministration of neuromuscular blocking agents (NMBA) with anesthetics can decrease the anesthesia risk by achieving muscle relaxation with reduced anesthetic requirement.

In this study, a series of experiments was performed to clarify the interaction among alfaxalone, rocuronium and sugammadex in dogs. In Chapter I, the interactions of anesthetic on the neuromuscular blockade produced by rocuronium were compared in dogs anesthetized with sevoflurane, propofol and alfaxalone. In Chapter II, the potency of rocuronium was investigated by constructing the dose–response curve in dogs under alfaxalone anesthesia and its ED_{50} and ED_{95} were determined. In Chapter III, the reversal effect of sugammadex on neuromuscular blockade produced by rocuronium was investigated in dogs under alfaxalone anesthesia.

In Chapter I, a total of eight adult Beagle dogs (four female, four male) were anesthetized three times with 1.25 × minimum alveolar concentration of sevoflurane (SEVO treatment) and 1.25 × minimum infusion rate of propofol (PROP treatment) or alfaxalone (ALFX treatment) at intervals of ≥ 14 days. Neuromuscular function was monitored with train-of-four (TOF) stimulation of the peroneal nerve by acceleromyography, and a single bolus dose of rocuronium (1 mg/kg) was administered intravenously. The median clinical blockade period was longer in the SEVO treatment [27.3 (26.0–30.3) min] than in PROP [16.6 (15.4–18.0) min; $p = 0.002$] and ALFX [22.4 (18.6–23.1) min; $p = 0.017$] treatments; and longer in the ALFX treatment than in the PROP treatment ($p = 0.020$). The mean total neuromuscular blockade duration was longer in the SEVO treatment (43.7 ± 9.9 min) than in PROP (25.1 ± 2.7 min; $p < 0.001$) and ALFX (32.5 ± 8.4 min; $p = 0.036$) treatments. According to the results, when compared with alfaxalone and propofol, sevoflurane prolonged rocuronium-induced neuromuscular blockade by a significantly greater extent in dogs.

In Chapter II, a total of eight adult Beagle dogs (four female, four male) were anesthetized three times with 1.25-fold the individual minimum infusion rate of alfaxalone at intervals of ≥ 14 days. A

single bolus dose of rocuronium 100, 175 or 250 μg/kg (R100, R175, or R250 treatment) was administered intravenously. The maximum suppression of the T1 was recorded and calibrated with T1C to construct the dose–response curve, from which ED_{50} and ED_{95} were calculated. The results showed that ED_{50} and ED_{95} of rocuronium during alfaxalone anesthesia were 175 μg/kg and 232 μg/kg, respectively. According to our results, the recommended dose (2 × ED95) for rocuronium is 0.5 mg/kg for achieving TOF count = 0 in dogs anesthetized with alfaxalone infusion.

In Chapter III, six adult beagle dogs (three females, three males) were anesthetized two times with 1.25 × minimum infusion rate of alfaxalone, with a washout period of at least 14 days between experiments. Rocuronium (0.5 mg/kg) was administered intravenously and sugammadex (4 mg/kg; sugammadex treatment) or equal volume of saline (control treatment) was administered intravenously when TOF count was returned from 0 to 1. Duration TOFR0.9 was 11.4 (11.1–11.5) min in sugammadex treatment, which was shorter than control treatment [21.1 (19.2–22.1) min] (p = 0.031). Rocuronium 0.5 mg/kg IV provided about 10 min of maximum neuromuscular blockade. Sugammadex 4 mg/kg IV effectively antagonized the rocuronium-induced neuromuscular blockade in dogs under alfaxalone anesthesia within a minute.

In conclusion, when compared with alfaxalone and propofol, sevoflurane prolonged rocuronium-induced neuromuscular blockade by a significantly greater extent in dogs. ED_{50} and ED_{95} of rocuronium during alfaxalone anesthesia in dogs were 175 μg/kg and 232 μg/kg, respectively. Rocuronium 0.5 mg/kg IV provided about 10 min of maximum neuromuscular blockade under alfaxalone TIVA in dogs. Rocuronium-induced neuromuscular blockade was effectively reversed by sugammadex in dogs under alfaxalone anesthesia.

Acknowledgement

First and foremost, I am extremely grateful to my supervisors, Prof. Kazuto Yamashita for his invaluable advice, continuous support, and patience during my PhD study. His immense knowledge and plentiful experience have encouraged me in all the time of my academic research and daily life. I would like to express my sincere gratitude to Prof. Shido Torisu, Assoc. Prof. Hiroshi Ohta, Assoc. Prof. Tadashi Sano, Assoc. Prof. Takaharu Itami, Asst. Prof. Keiko Kato for their insightful comments and suggestions.

I am also deeply grateful to associate lecturer Kenjiro Miyoshi for his contribution to the management of the experimental animals. I would like to offer my special thanks to the contract research associates and the graduate students in Veterinary Anesthesia Unit, Dr. Haruka Tamogi, Dr. Taku Hirokawa, Dr. Yixian Wei, Dr. Chihiro Sugita, veterinary nurse Nozomi Daimaruya, and all the students in Companion Animal Medical Field for their assistance at every stage of the research project.

I acknowledge the Japan-Taiwan Exchange Association for providing me with scholarship funding. Finally, I would like to extend my sincere thanks to my family and friends for their unwavering support and belief in me.

References

1. Alderson B, Senior JM, Jones RS, Dugdale AH. 2007. Use of rocuronium administered by continuous infusion in dogs. *Vet Anaesth Analg* **34**: 251-256.
2. Appiah-Ankam J, Hunter JM. 2004. Pharmacology of neuromuscular blocking drugs. *Anaesth Crit Care Pain Med* **4**: 2-7.
3. Auer U. 2007. Clinical observations on the use of the muscle relaxant rocuronium bromide in the dog. *Vet J* **173**: 422-427.
4. Auer U, Mosing M, Moens YP. 2007. The effect of low dose rocuronium on globe position, muscle relaxation and ventilation in dogs: a clinical study. *Vet Ophthalmol* **10**: 295-298.
5. Bennell PM, Whittem T, Tudor E. 2019. A controlled randomized clinical trial to assess postoperative analgesia after thiopental–isoflurane anaesthesia or total intravenous anaesthesia with alfaxalone in dogs. *J Vet Pharmacol Ther* **42**: 268-277.
6. Bennett KJ, Seddighi R, Moorhead KA, Messenger K, Cox SK, Sun X, Pasloske K, Pypendop BH, Doherty TJ. 2019. Effect of fentanyl on the induction dose and minimum infusion rate of alfaxalone preventing movement in dogs. *Vet Anaesth Analg* **46**: 173-181.
7. Bennett RC, Salla KM, Raekallio MR, Scheinin M, Vainio OM. 2017. Effects of the α2-adrenoceptor agonist medetomidine on the distribution and clearance of alfaxalone during coadministration by constant rate infusion in dogs. *Am J Vet Res* **78**: 956-964.
8. Bom A, Bradley M, Cameron K, Clark JK, van Egmond J, Feilden H, MacLean EJ, Muir AW, Palin R, Rees DC, Zhang MQ. 2002. A novel concept of reversing neuromuscular block: chemical encapsulation of rocuronium bromide by a cyclodextrin-based synthetic host. *Angew Chem Int Ed* **41**: 265-270.
9. Briganti A, Barsotti G, Portela DA, Di Nieri C, Breghi G. 2015. Effects of rocuronium bromide on globe position and respiratory function in isoflurane-anesthetized dogs: a comparison between three different dosages. *Vet Ophthalmol* **18**: 89-94.
10. Bustamante R, Gómez de Segura IA, Canfrán S, Aguado D. 2020. Effects of ketamine or midazolam continuous rate infusions on alfaxalone total intravenous anaesthesia requirements and recovery quality in healthy dogs: a randomized clinical trial. *Vet Anaesth Analg* **47**: 437-446.

11. Chen IY, Liang YY, Chen KS, Lee WM, Wang HC. 2020. Comparison of the neuromuscular blocking effects of cisatracurium during isoflurane or propofol anesthesia in dogs. *Vet Anaesth Analg* **47**: 454-462.
12. Chen IY, Tamogi H, Wei Y, Kato K, Itami T, Sano T, Yamashita K. 2022. Effects of sevoflurane, propofol or alfaxalone on neuromuscular blockade produced by a single intravenous bolus of rocuronium in dogs. *Vet Anaesth Analg* **49**: 36-44.
13. Clarke KW, Trim CM, Hall LW. 2014. An introduction to anaesthesia and general considerations. pp. 3-18. In: Veterinary Anaesthesia, 11th ed, (Clarke KW, Trim CM and Hall LW eds.), Elsevier, Amsterdam.
14. Clarke KW, Trim CM, Hall LW. 2014. Relaxation of the skeletal muscles. pp. 169-194. In: Veterinary Anaesthesia, 11th ed, (Clarke KW, Trim CM and Hall LW eds.), Elsevier, Amsterdam.
15. Cookson JC, Paton WDM. 1969. Mechanisms of neuromuscular block. *Anaesthesia* **24**: 395-416.
16. Cooper RA, Mirakhur RK, Elliott P, McCarthy GJ. 1992. Estimation of the potency of ORG 9426 using two different modes of nerve stimulation. *Can J Anaesth* **39**: 139-142.
17. de Boer HD, van Egmond J, van de Pol F, Bom A, Booij LHDJ. 2006. Chemical encapsulation of rocuronium by synthetic cyclodextrin derivatives: reversal of neuromuscular block in anaesthetized Rhesus monkeys†. *Br J Anaesth* **96**: 201-206.
18. de Boer HD, van Egmond J, van de Pol F, Bom A, Booij LHDJ. 2006. Sugammadex, a new reversal agent for neuromuscular block induced by rocuronium in the anaesthetized Rhesus monkey†. *Br J Anaesth* **96**: 473-479.
19. Dehuisser V, Bosmans T, Devreese M, Gehring R, Croubels S, Duchateau L, Polis I. 2019. Alfaxalone total intravenous anaesthesia in dogs: pharmacokinetics, cardiovascular data and recovery characteristics. *Vet Anaesth Analg* **46**: 605–612.
20. Dugdale AH, Adams WA, Jones RS. 2002. The clinical use of the neuromuscular blocking agent rocuronium in dogs. *Vet Anaesth Analg* **29**: 49-53.
21. Fagerlund MJ, Eriksson LI. 2009. Current concepts in neuromuscular transmission. *Br J Anaesth* **103**: 108-114.

22. Ferré PJ, Pasloske K, Whittem T, Ranasinghe MG, Li Q, Lefebvre HP. 2006. Plasma pharmacokinetics of alfaxalone in dogs after an intravenous bolus of Alfaxan-CD RTU. *Vet Anaesth Analg* **33**: 229-236.
23. Fink G, Sarkar DK, Dow RC, Dick H, Borthwick N, Malnick S, Twine M. 1982. Sex difference in response to alphaxalone anaesthesia may be oestrogen dependent. *Nature* **298**: 270-272.
24. Frink EJ Jr., Morgan SE, Coetzee A, Conzen PF, Brown BR Jr. 1992. The effects of sevoflurane, halothane, enflurane, and isoflurane on hepatic blood flow and oxygenation in chronically instrumented Greyhound dogs. *Anesthesiology* **76**: 85-90.
25. Fuchs-Buder T, Claudius C, Skovgaard LT, Eriksson LI, Mirakhur RK, Viby-Mogensen J. 2007. Good clinical research practice in pharmacodynamic studies of neuromuscular blocking agents II: the Stockholm revision. *Acta Anaesthesiol Scand* **51**: 789-808.
26. Greenberg SB, Vender J. 2013. The use of neuromuscular blocking agents in the ICU: where are we now? *Crit Care Med* **41**: 1332-1344.
27. Haga HA, Bettembourg V, Lervik A. 2019. Rocuronium infusion: A higher rate is needed in diabetic than nondiabetic dogs. *Vet Anaesth Analg* **46**: 28-35.
28. Heier T, Caldwell JE. 2006. Impact of hypothermia on the response to neuromuscular blocking drugs. *Anesthesiology* **104**: 1070-1080.
29. Hubbell JAE. 1992. Disadvantages of Neuromuscular Blocking Agents. *Vet Clin North Am Small Anim Pract* **22**: 351-352.
30. Hunter JM, Flockton EA. 2006. The doughnut and the hole: a new pharmacological concept for anaesthetists. *Br J Anaesth* **97**: 123-126.
31. Jinks SL, Bravo M, Hayes SG. 2008. Volatile Anesthetic Effects on Midbrain-elicited Locomotion Suggest that the Locomotor Network in the Ventral Spinal Cord Is the Primary Site for Immobility. *Anesthesiology* **108**: 1016-1024.
32. Jones RS, Auer U, Mosing M. 2015. Reversal of neuromuscular block in companion animals. *Vet Anaesth Analg* **42**: 455-471.
33. Jonsson Fagerlund M, Krupp J, Dabrowski MA. 2016. Propofol and AZD3043 inhibit adult muscle and neuronal nicotinic acetylcholine receptors expressed in Xenopus oocytes. *Pharmaceuticals (Basel)* **9**: 8.

34. Jonsson Fagerlund MJ, Dabrowski M, Eriksson LI. 2009. Pharmacological characteristics of the inhibition of nondepolarizing neuromuscular blocking agents at human adult muscle nicotinic acetylcholine receptor. *Anesthesiology* **110**: 1244-1252.
35. Jonsson M, Gurley D, Dabrowski M, Larsson O, Johnson EC, Eriksson LI. 2006. Distinct pharmacologic properties of neuromuscular blocking agents on human neuronal nicotinic acetylcholine receptors: a possible explanation for the train-of-four fade. *Anesthesiology* **105**: 521-533.
36. Kansanaho M, Olkkola KT, Wierda JM. 1997. Dose-response and concentration-response relation of rocuronium infusion during propofol-nitrous oxide and isoflurane-nitrous oxide anaesthesia. *Eur J Anaesthesiol* **14**: 488-494.
37. Kastrup MR, Marsico FF, Ascoli FO, Becker T, Soares JH, Gomez de Segura IA. 2005. Neuromuscular blocking properties of atracurium during sevoflurane or propofol anaesthesia in dogs. *Vet Anaesth Analg* **32**: 222-227.
38. Keates H, Whittem T. 2012. Effect of intravenous dose escalation with alfaxalone and propofol on occurrence of apnoea in the dog. *Res Vet Sci* **93**: 904-906.
39. Keegan RD. 2007. Muscular relaxants and neuromuscular blockade. pp. 260-276. In: Veterinary Anesthesia and Analgesia (5th ed of Lumb and Jones), (Grimm, KA, Lamont, LA, Tranquilli, WJ, Greene, SA and Robertson, SA eds.), Blackwell, USA.
40. Khamtuikrua C, Suksompong S, Rhoopanwong S, Sangsab P, Chaikittisilpa N, Bormann B. 2017. Risk factors for residual neuromuscular blockade after general anesthesia. *J Med Assoc Thai* **100**: 75.
41. Khuenl-Brady K, Castagnoli KP, Canfell PC, Caldwell JE, Agoston S, Miller RD. 1990. The neuromuscular blocking effects and pharmacokinetics of ORG 9426 and ORG 9616 in the cat. *Anesthesiology* **72**: 669-674.
42. Larijani GE, Gratz, I, Silverberg, M, Jacobi, AG. 1991. Clinical pharmacology of the neuromuscular blocking agents. *DICP : the annals of pharmacotherapy* **25**: 54-64.
43. Larijani GE, Gratz I, Afshar M, McDonald PA, Fisher DM. 1995. The effect of isoflurane versus balanced anesthesia on rocuronium's pharmacokinetics and infusion requirement. *Pharmacotherapy* **15**: 36-41.

44. Liu L, Li W, Wei K, Cao J, Luo J, Wang B, Min S. 2013. Synergistic effect of sevoflurane and isoflurane on inhibition of the adult-type muscle nicotinic acetylcholine receptor by rocuronium. *J Anesth* **27**: 351-358.
45. Maddineni VR, Mirakhur RK, Cooper R, McCoy E. 1993. Potency estimation of mivacurium: comparison of two different modes of nerve stimulation. *Br J Anaesth* **70**: 694-695.
46. Mannarino R, Luna SP, Monteiro ER, Beier SL, Castro VB. 2012. Minimum infusion rate and hemodynamic effects of propofol, propofol-lidocaine and propofol-lidocaine-ketamine in dogs. *Vet Anaesth Analg* **39**: 160-173.
47. Marshall RJ, Muir AW, Sleigh T, Sovage DS. 1994. An overview of the pharmacology of rocuronium bromide in experimental animals. *Eur J Anaesthesiol Suppl* **9**: 9-15.
48. Martin-Flores M, Lau EJ, Campoy L, Erb HN, Gleed RD. 2011. Twitch potentiation: a potential source of error during neuromuscular monitoring with acceleromyography in anesthetized dogs. *Vet Anaesth Analg* **38**: 328-335.
49. Martinez EA. 1999. Newer neuromuscular blockers. Is the practitioner ready for muscle relaxants? *Vet Clin North Am Small Anim Pract* **29**: 811-817.
50. Mellinghoff H, Diefenbach C, Bischoff A, Grond S, Buzello W. 1994. Dose-response relationship of rocuronium bromide during intravenous anaesthesia. *Eur J Anaesthesiol Suppl* **9**: 20-24.
51. Michou JN, Leece EA, Brearley JC. 2012. Comparison of pain on injection during induction of anaesthesia with alfaxalone and two formulations of propofol in dogs. *Vet Anaesth Analg* **39**: 275-281.
52. Mosing M, Auer U, West E, Jones RS, Hunter JM. 2012. Reversal of profound rocuronium or vecuronium-induced neuromuscular block with sugammadex in isoflurane-anaesthetised dogs. *Vet J* **192**: 467-471.
53. Muir AW, Houston J, Green KL, Marshall RJ, Bowman WC, Marshall IG. 1989. Effects of a new neuromuscular blocking agent (Org 9426) in anaesthetized cats and pigs and in isolated nerve-muscle preparations. *Br J Anaesth* **63**: 400-410.
54. Nagahama S, Nishimura R, Mochizuki M, Sasaki N. 2006. The effects of propofol, isoflurane and sevoflurane on vecuronium infusion rates for surgical muscle relaxation in dogs. *Vet Anaesth Analg* **33**: 169-174.

55. Naguib M, Samarkandi AH, Ammar A, Elfaqih SR, Al-Zahrani S, Turkistani A. 1998. Comparative clinical pharmacology of rocuronium, cisatracurium, and their combination. *Anesthesiology* **89**: 1116-1124.
56. Ono K, Ohta Y, Morita K, Kosaka F. 1988. The influence of respiratory-induced acid-base changes on the action of non-depolarizing muscle relaxants in rats. *Anesthesiology* **68**: 357-365.
57. Ono K, Nagano O, Ohta Y, Kosaka F. 1990. Neuromuscular effects of respiratory and metabolic acid-base changes in vitro with and without nondepolarizing muscle relaxants. *Anesthesiology* **73**: 710-716.
58. Paul M, Fokt RM, Kindler CH, Dipp NC, Yost CS. 2002. Characterization of the interactions between volatile anesthetics and neuromuscular blockers at the muscle nicotinic acetylcholine receptor. *Anesth Analg* **95**: 362-367.
59. Peeters P, Passier P, Smeets J, Zwiers A, de Zwart M, van de Wetering-Krebbers S, van Iersel M, van Marle S, van den Dobbelsteen D. 2011. Sugammadex is cleared rapidly and primarily unchanged via renal excretion. *Biopharm Drug Dispos* **32**: 159-167.
60. Quirós-Carmona S, Navarrete R, Domínguez JM, Granados MdM, Gómez-Villamandos RJ, Muñoz-Rascón P, Aguilar D, Funes FJ, Morgaz J. 2017. A comparison of cardiopulmonary effects and anaesthetic requirements of two dexmedetomidine continuous rate infusions in alfaxalone-anaesthetized Greyhounds. *Vet Anaesth Analg* **44**: 228-236.
61. Raghavendra T. 2002. Neuromuscular blocking drugs: discovery and development. *J R Soc Med* **95**: 363-367.
62. Saager L, Maiese EM, Bash LD, Meyer TA, Minkowitz H, Groudine S, Philip BK, Tanaka P, Gan TJ, Rodriguez-Blanco Y, Soto R, Heisel O. 2019. Incidence, risk factors, and consequences of residual neuromuscular block in the United States: The prospective, observational, multicenter RECITE-US study. *J Clin Anesth* **55**: 33-41.
63. Sacan O, White PF, Tufanogullari B, Klein K. 2007. Sugammadex reversal of rocuronium-induced neuromuscular blockade: a comparison with neostigmine–glycopyrrolate and edrophonium–atropine. *Anesth Analg* **104**.

64. Sakai DM, Romano M, Tseng CT, Flanders A, Martin-Flores M. 2018. Bias, limits of agreement, and percent errors between acceleromyography and mechanomyography in anesthetized dogs. *Vet J* **233**: 3-7.

65. Sakai DM, Martin-Flores M, Tomak EA, Martin MJ, Campoy L, Gleed RD. 2015. Differences between acceleromyography and electromyography during neuromuscular function monitoring in anesthetized Beagle dogs. *Vet Anaesth Analg* **42**: 233-241.

66. Sakai DM, Martin-Flores M, Romano M, Tseng CT, Campoy L, Gleed RD, Cheetham J. 2017. Recovery from rocuronium-induced neuromuscular block was longer in the larynx than in the pelvic limb of anesthetized dogs. *Vet Anaesth Analg* **44**: 246–253.

67. Sakata H, Ishikawa Y, Ishihara G, Oyama N, Itami T, Umar MA, Sano T, Yamashita K. 2019. Effect of sevoflurane anesthesia on neuromuscular blockade produced by rocuronium infusion in dogs. *J Vet Med Sci* **81**: 425-433.

68. Shiraishi M, Shibuya I, Minami K, Uezono Y, Okamoto T, Yanagihara N, Ueno S, Ueta Y, Shigematsu A. 2002. A neurosteroid anesthetic, alphaxalone, inhibits nicotinic acetylcholine receptors in cultured bovine adrenal chromaffin cells. *Anesth Analg* **95**: 900-906.

69. Soto Mesa D, Fayad Fayad M, Pérez Arviza L, Del Valle Ruiz V, Cosío Carreño F, Arguelles Tamargo L, Amorín Díaz M, Fernández-Pello Montes S. 2015. Efficacy of different doses of sugammadex after continuous infusion of rocuronium. *World J Clin Cases* **3**: 360-367.

70. Srivastava A, Hunter JM. 2009. Reversal of neuromuscular block. *Br J Anaesth* **103**: 115-129.

71. Vanlinthout LE, Booij LH, van Egmond J, Robertson EN. 1996. Effect of isoflurane and sevoflurane on the magnitude and time course of neuromuscular block produced by vecuronium, pancuronium and atracurium. *Br J Anaesth* **76**: 389-395.

72. Yamashita K, Iwasaki Y, Umar MA, Itami T. 2009. Effect of age on minimum alveolar concentration (MAC) of sevoflurane in dogs. *J Vet Med Sci* **71**: 1509-1512.