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Complete List of Authors:	Briganti, Angela; University of Pisa, Veterinary Science Barsotti, Giovanni; University of Pisa, Veterinary Science Portela, Diego; University of Pisa, Veterinary Science Di Nieri, Camilla; University of Pisa, Veterinary Science Breghi, Gloria; University of Pisa, Veterinary Science
Keywords:	rocuronium bromide, dog, globe position, anaesthesia, ophthalmic procedures, low dose

Effects of rocuronium bromide on globe position and respiratory function in isoflurane-anaesthetized dogs: a comparison between three different dosages

Angela Briganti, Giovanni Barsotti, Diego A. Portela, Camilla Di Nieri, Gloria Breghi.

Department of Veterinary Science, University of Pisa, Pisa, Italy

Correspondence:

Dr Angela Briganti

Department of Veterinary Science, University of Pisa, via Livornese lato monte, 56010, San Piero a Grado, Pisa, Italy

E-mail abriganti@vet.unipi.it

Phone Number: +390502210148; fax number: +39 0502210182

Running title: low dosage of rocuronium in anaesthetized dogs

Abstract

Objective To evaluate the effect on globe position and respiration of three dosages of intravenous rocuronium in isoflurane-anaesthetized dogs.

Animal studied Thirty-two dogs of various ages and breeds, anaesthetized for ophthalmic procedures.

Procedures The dogs were divided into four groups, each of eight animals (G1-G4). G1, G2, G3 received 0.075, 0.05, 0.03 mg/kg of IV rocuronium, respectively; G4 (control group) received 0.9% NaCl IV. Anaesthesia was obtained with dexmedetomidine (2.5 mcg/kg IV), methadone (0.1 mg/kg IV), propofol (2 mg/kg IV) and isoflurane in oxygen. Neuromuscular function was assessed with acceleromyography by the stimulation of the peroneal nerve using the train of four (ToF). Monitoring of cardiovascular and respiratory functions was performed. The change in globe position was recorded.

Results All the three dosages of rocuronium produced the centralization of the globe and the duration of the effect was 24.3 ± 4.2, 23.4 ± 3.6 and 8.7 ± 2.8 minutes, for G1, G2 and G3, respectively. The control group did not show globe centralization. Concerning cardiovascular and respiratory parameters, no significant differences were found among the four groups. Minute volume and ToFR resulted significantly lower in G1 compared to Tbase values.

Conclusions All doses of rocuronium enabled the globe to be centralized. The higher dose provoked a transient respiratory and muscular depression, while the 0.05 mg/kg was not responsible of significant consequences on respiratory activity. The 0.03 mg/kg dosage could be useful for very short ophthalmic procedures or as a bolus prior to IV infusions.

Key words:

rocuronium bromide,

dog,

globe position,

anaesthesia,

ophthalmic procedures

low dosage

Introduction

During ophthalmic diagnostic or surgical procedures, any movement of the eye should be limited or absent and the globe should generally be in a central position. The majority of general anaesthetic drugs cause a ventro-medial deviation of the eye axis resulting in a partial or total covering of the cornea by the third eyelid.(1) The eye can be centralized by very deep anaesthetic planes or by the use of neuromuscular blocking agents (NMBAs). The use of NMBAs produces the complete relaxation of skeletal muscles and also of all the extraocular muscles, thus enabling the eye to return to its frontal position.(1) Most of the NMBAs used during ophthalmic procedures are employed at dosages that also cause diaphragm paralysis. This means that for the duration of the effects of the NMBA, the patient should be mechanically ventilated. Antagonism of the block is possible but it is not free of side effects.(2)

Rocuronium bromide, a NMBA derivative of vecuronium bromide, has been studied in dogs, cats, horses, birds and turtles and its efficacy in producing relaxation of the striated muscular fibres has been demonstrated in several species.(3-11) Particularly in dogs a dosage of 0.4 mg/kg has been efficacious in producing a complete muscular block of approximately 30 minutes.(12) Moreover an infusion

dosage has been studied in dogs and the results have demonstrated that the drug is accumulated to a lesser extent compared to repeated boluses and that an infusion dosage of 0.2 mg/kg/hour was effective in producing a complete muscular block.(13) Some authors compared the effects of high and low doses of pancuronium bromide to obtain the centralization of the globe in spontaneously breathing dogs, and the results of the study showed that a low dose of pancuronium able to produce the centralization of the globe without respiratory impairment was not identified.(14) In a subsequent study a low dosage of rocuronium bromide during ophthalmic procedures in dogs was investigated.(15) The authors demonstrated that a dose of 0.1 mg/kg of rocuronium produced the central position of the globe for at least 20 min, with minimal effects on respiratory and skeletal muscular functions.(15)

The aim of the present study was to evaluate the effects of three dosages of rocuronium bromide of less than 0.1 mg/kg on the ocular globe, ventilation and skeletal muscle relaxation in isoflurane anaesthetized dogs. Our hypothesis was that a dosage of 0.075, 0.05 or 0.03 mg/kg could be as efficacious as 0.1 mg/kg but with less or no muscular and ventilatory effects.

Materials and Methods

Thirty-two client-owned dogs of various breeds, both sexes aged between 2 and 4 years and body weight between 15 and 30 kg, scheduled for diagnostic/surgical ophthalmic procedures (electroretinography, grid keratotomy, superficial keratectomy and conjunctival grafting), were enrolled in the study.

The study was approved by the Ethics Committee on Animal Experimentation of the local University and written consent was obtained from the owners to enrol their dogs in the study.

Before anaesthesia, physical examination, complete blood count, serum biochemistry and electrocardiography were routinely performed.

Dogs were randomly divided into four groups of eight dogs each (G1, G2, G3, G4): G1 received 0.075 mg/kg of rocuronium IV (Esmeron 50mg/5ml, Organon Italia S.p.a.), G2 0.05 mg/kg of rocuronium IV, G3 0.03 mg/kg of rocuronium IV, while G4 received 0.0075 mL/kg of NaCl 0.9% IV. Since this was a double blind study, in order to obtain the same volume of injection for all the groups, the treatments for G2 and G3 were diluted with NaCl 0.9% to administer the same volume as G1 and G4 (0.0075 mL/kg). All the treatment were administered over 10 seconds.

Dogs were premedicated intravenously with dexmedetomidine (Dexdomitor 0.5mg/mL, Pfizer Italia Srl, Div. Vet.) 2.5 mcg/kgand methadone (Eptadone 10 mg/mL, Molteni & C F.lli Alitti S.p.A., Italia) 0.1 mg/kg. They were then induced with propofol 2 mg/kg IV (Propovet 10mg/mL, Esteve S.p.A., Italia), intubated and maintained with isoflurane in oxygen. A circle re-breathing system was used in all the patient with an oxygen flow of 50 mL/kg/min and the adjustable pressure-limiting (APL) valve was set in order not to have a positive pressure in the breathing system and at the same time not to produce a deflation of the reservoir bag.

During the procedure, heart rate (HR) by electrocardiographic evaluation, respiratory rate (RR), mean arterial pressure (MAP) by oscillometric technique, tidal volume (Vt), end-tidal partial pressure for CO_2 ($PE'CO_2$) (both measured at the connection between the tracheal tube and the Y piece of the breathing system), partial saturation of haemoglobin(SpO_2), were monitored using a multiparametrical monitor (Mindray Beneview T5, China) as well as muscular relaxation using an acceleromyograph (TOF-Watch® SX, Organon, Ireland). The evaluation of muscular blockade was done by the train-of-four (ToF) and the ToF ratio (ToFR), throughout the stimulation of the peroneal nerve, placing the stimulating crocodiles in the lateral

aspect of the left knee and the sensor on the dorsal aspect of the metatarsus. The stimulation pattern was obtained with four stimuli delivered over 2 seconds every 15 seconds. The supramaximal stimulation was determined just before the administration of the treatment. Dogs that underwent to surgery were positioned in dorsal recumbency with the head stabilized on a vacuum pillow, whilst dogs that did electroretinography were positioned in sternal recumbency with the caudal portion of the body in right lateral position. In both cases the left hind limb was left free to move in the distal part in response to the neuromuscular stimulation. An eyelid speculum was applied in both eyes of each dog, in order to improve globe exposition. In dogs that showed a partial protrusion of the nictitating membrane, stay sutures were applied to retract the membrane and improve the monitoring of the eye position.

All the parameters were registered in a specific anaesthesia record. Monitoring of the recorded parameters started at Tbase, when an end tidal isoflurane concentration (EtISO) between 1.1 and 1.2 % was reached. After 5 minutes from the registration of Tbase, the treatment was administered (T0) and then all the parameters were recorded every minute for the first 5 minutes, thereafter, parameters were recorded in 5 minutes intervals throughout the procedure. In case of ventilatory depression (respiratory rate under 5 bpm or PE'CO_2 over 55 mm Hg) an assisted or controlled ventilation was considered to be started. Minute volume (MV) was calculated from the tidal volume, the respiratory rate and the body weight. During the procedure the vaporizer setting was variated only in case of hypotension (MAP<60 mm Hg) or in case of anaesthetic plane lightening (increase in RR, presence of palpebral reflex etc.).

Eye position was visually evaluated always by the same clinician. The complete centralization of the globe was considered obtained when the entire cornea and the iris were totally visible in both eyes. The beginning of the ventro-medially movement of the eye was considered as the end of rocuronium effect on the extraocular muscles.

Both the time for the obtainment and the lasting of the centralization were recorded as well as any possible side effects. In the case of the centralization of the globe was not obtained after 5 minutes from the administration of the treatment, stay sutures were applied in order to achieve the correct globe position necessary for the ophthalmic procedure.

Statistical Analysis

Data were analysed for normal distribution using the Kolmogorov-Smirnov test and were represented as mean and standard deviations. A one way analysis of variance for repeated measures with a Dunnet post hoc test was applied to compare the Tbase with the all subsequent times within each group. A one way analysis of variance with a Tukey post hoc test was used to compare the four groups for each time point of monitoring. An unpaired Student T-Test was used to compare values of G1, G2, G3 vs G4 at each time point. Values were considered significant when $P < 0.05$.

Results

On the basis of physical examination and blood exams the dogs included in the study were classified as ASA I-II. The distribution of the ophthalmic procedures in the 4 groups is represented in table 1. No differences were detected among the four groups in terms of age and weight (Table 2) nor were there any differences concerning the BT, PE'CO_2, Vt, EtISO, RR, HR and MAP among the four groups and within each group (figure 1 and 2).

Concerning the minute volume a significant difference was detected between tBase and T1, T2 and T3 in G1, while in the other groups no differences were evidenced (figure 3A).

Before the administration of the treatment all the globes were in ventro-medial position; the dogs of G4 did not show any globe movements while all the dogs of G1, G2 and G3 showed the progressive rotation for the eye to a central position. The time to achieve the centralization was significantly higher in G3 compared to G1 and G2, while the duration of the centralization was significantly lower in G3 than G1 and G2 (Table 3).

The acceleromyography recorded a ToF of 4 and a mean ToFR higher than 80 % in all the groups throughout the monitoring of the neuromuscular block (figure 3B). A difference was highlighted in G1 between Tbase and T1, whereas no differences were detected in the other groups; no differences were evidenced amongst the 4 groups.

All the dogs in G4 needed stay sutures to achieve a central position of the eye to perform the ophthalmic procedure. In all the other groups the duration of the centralization of the globe was sufficient to complete the ophthalmic procedure, with the exception of two cases (conjunctival grafting, superficial keratectomy) in G3, in which the re-acquisition of the ventro-medial position of the eye started before the end of the procedure; in these cases the surgeon applied stay sutures to the globe.

Discussion

The results of this study confirm the efficacy of low dosages of rocuronium in producing extraocular muscle relaxation without clinically affecting the neuromuscular function as measured at the level of the peroneal nerve.

The 0.075 mg/kg and the 0.05 mg/kg dosages gave similar results, but a slight muscular depression could be noticed with the higher dosage. In fact, both doses of rocuronium produced the centralization of the globe and did not affect tidal volume, mean arterial pressure or the heart rate of the patients, with a similar duration of ocular effects (24.3±4.2 minutes and 23.4±3.6 minutes, respectively). With a dose of

0.075 mg/kg, higher PE'CO_2 values were recorded during all the procedure, whereas with the 0.05 mg/kg dosage, PE'CO_2 values always remained within the physiological ranges. Although no significant differences were detected between the two groups regarding PE'CO_2 values ($P>0.1$) and no variation of the Vt was recorded, the 0.075 mg/kg dosage produced a decreasing in the MV for the 3 minutes after the administration and a significant alteration of the ToFR during the first minute after the administration. Because the 0.075 mg/kg and the 0.05 mg/kg dosage gave similar ocular effects, the 0.05 mg/kg dosage could be considered better because it did not produce evident respiratory or muscular depression.

With the 0.03 mg/kg dosage of rocuronium, the duration of centralization was only of 8.7±2.8 minutes. This dosage too was not connected to clinical respiratory or cardiovascular alterations, however the time required to obtain a complete relaxation of the extraocular muscles and the frontal position of the eye was significantly longer compared to the other doses. It is possible that the differences regarding the onset and the duration of the effects between the 0.075-0.05 mg/kg and the 0.03 mg/kg dosages could be due to a dose-dependent effect of the rocuronium with very low dosages.

The dose-dependent effects of rocuronium have been demonstrated in horses (5) with dosages of 0.2, 0.4 and 0.6 mg/kg, and it may also be the same in the canine species.

As the 0.03 mg/kg dosage was as effective as the higher dosages in producing globe centralization, but with shorter duration, this dose could be used for very fast diagnostic procedures (e.g. electroretinography) or as a bolus prior to intravenous infusion.

The low dosages of rocuronium used in this study did not produce any muscular block detectable by the ToF positioned on the peroneal nerve with the exception of the 0.075 mg/kg dosage only during the first minute. However, it is not possible to

exclude a paralysis of facial muscles that could have been shown by placing the ToF on the facial nerve. Because of the clinical nature of this study, the positioning of the ToF in the facial region could represent an impediment for the ophthalmic procedure. Some authors suggested a higher sensitivity of extraocular muscles to neuromuscular blocking agents.(16) Recent studies on rats have shown, in extraocular muscle fibres, a particular subunit of the receptors for acetylcholine that is not present in limb muscles.(17) Differences between the sarcolemmal organization of extraocular muscles compared to other skeletal muscles were also revealed.(18) Those particular features of the extraocular muscles have not yet been demonstrated in dogs. However, it is possible that even in dogs extraocular muscles are more susceptible to the rocuronium blocking effect compared to limb muscles.

A comparison of our results with the study performed by Auer et al. (15), regarding low dosages of rocuronium in dogs highlights that the 0.075 and the 0.05 mg/kg dosages during inhalation anaesthesia produced similar effects to those obtained with the 0.1 mg/kg dosage in propofol-anaesthetized dogs. The 0.05 mg/kg dose did not affect the respiratory function at all, thus could be used instead of other dosages. However, it is important to consider the possibility of a variable response to the drugs: some dogs could not respond to this dosage because of a particular metabolism or due to drug interaction (19) and some other drugs employed during anaesthesia could produce respiratory depression even at low dosages. In fact, strict monitoring of the patient when NMBAs are employed in the protocol is imperative, even with low dosages. Endotracheal intubation, capnography and acceleromiography should be always attained in these patients and extubation should be performed only with ToF ratio higher than 90% or when complete laryngeal reflex is re-achieved.

In conclusion, rocuronium with the dosages used in this study would appear to be effective and safe for the relaxation of extraocular muscles in isoflurane anaesthetized

dogs. The 0.05 mg/kg dosage was as effective as the 0.075 mg/kg dosage, without affecting the PE'CO_2, the ToFR and the minute volume. For this reason it might be considered a better choice for all the ophthalmic procedures that require a central globe for approximately 20 minutes.

The 0.03 mg/kg dosage produced a shorter period of globe centralization and could be useful for very short procedures or as a bolus prior to constant infusion. Further studies are in progress to evaluate the efficacy of a low dose of rocuronium infusion.

Finally, it is important to note that in our study we employed isoflurane anaesthesia thus the efficacy and the duration of action of rocuronium could be different with a different anaesthetic protocol.

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Figure 1: Trend of heart rate (A) (beats per minute) and mean arterial pressure (B) (mm Hg) in the treated with rocuronium: G1 0.075 mg/kg, G2 0.05 mg/kg, G3 0.03 mg/kg and with saline solution G4.

Figure 2: Trend values of PE'ISO (A) (%),PE'CO_2 (B) (mm Hg), respiratory rate (C) (breath per min), tidal volume (D) (mL/kg) and in dogs treated with rocuronium 0.075 mg/kg (G1), 0.05 mg/kg (G2), 0.03 mg/kg (G3) or saline solution (G4).

Figure 3: Trend values of MV (A) (L/min) and ToFR (B) (%) in dogs treated with rocuronium 0.075 mg/kg (G1), 0.05 mg/kg (G2), 0.03 mg/kg (G3) or saline solution (G4). * indicates significantly different from Tbase in G1.

Table 1: Distribution of each ophthalmic procedure in the 4 groups: G1, G2, G3 received rocuronium 0.075, 0.05 and 0.03 mg/kg, respectively. G4 received saline solution.

Table 2: Mean values and standard deviations of weight and age in the four groups. G1, G2, G3 received rocuronium 0.075, 0.05 and 0.03 mg/kg, respectively. G4 received saline solution.

Table 3: Mean values and standard deviations of the time taken to obtain centralization of the globe and the duration of the neuromuscular block; *significantly different compared to G1; † significantly different from G1 and G2.

Table 1: Distribution of each ophthalmic procedure in the 4 groups: G1, G2, G3 received rocuronium 0.075, 0.05 and 0.03 mg/kg, respectively. G4 received saline solution.

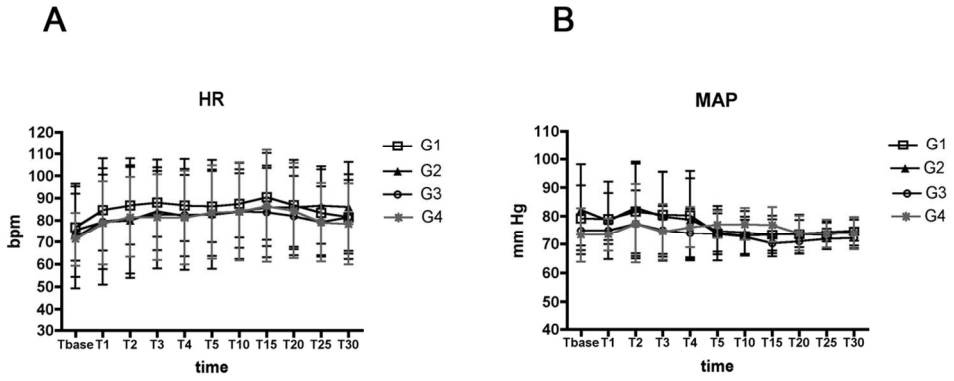
Groups	Electroretinography	Grid keratotomy	Superficial keratectomy	Conjunctival grafting
G1	4	1	1	2
G2	3	2	2	1
G3	3	3	1	1
G4	3	2	1	2

Table 2: Mean values and standard deviations of weight and age in the four groups. G1, G2, G3 received rocuronium 0.075, 0.05 and 0.03 mg/kg, respectively. G4 received saline solution.

	G1	G2	G3	G4
Weight (kg)	27±5	23±4	21±8	23±6
Age (years)	4.5±3	3.1±3	2.4±2	3.8±2

Table 3: Mean values and standard deviations of the time taken to obtain centralization of the globe and the duration of the neuromuscular block; *significantly different compared to G1; † significantly different from G1 and G2.

Groups	Globe centralization time (seconds)	Time of maintained globe central position (minutes)
G1	39.7±5.8	24.3±4.2
G2	48.3±14.5	23.4±3.6
G3	57.8±23.5*	8.7±2.8†
G4	-----	-----



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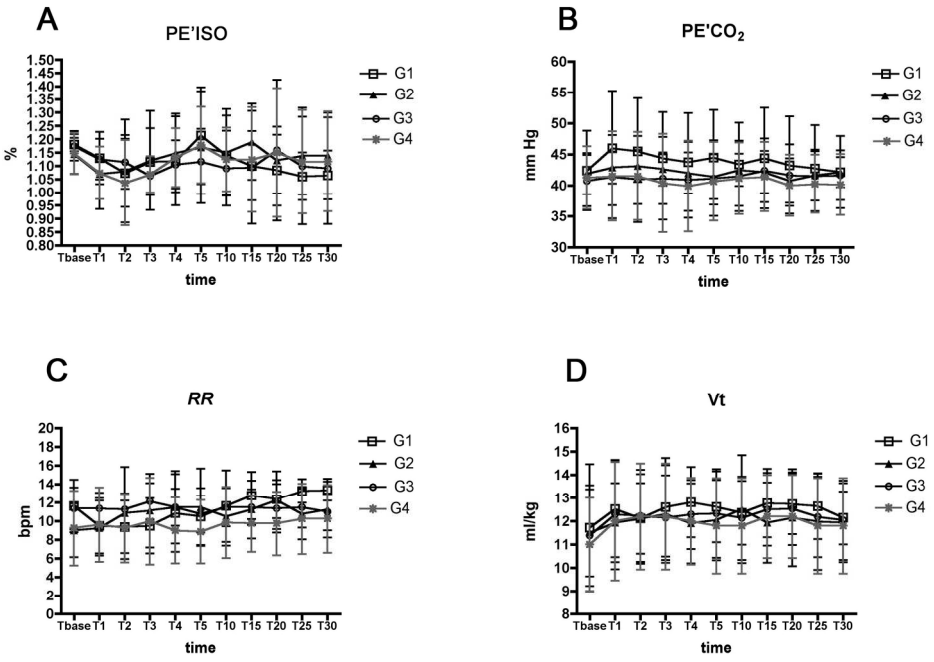

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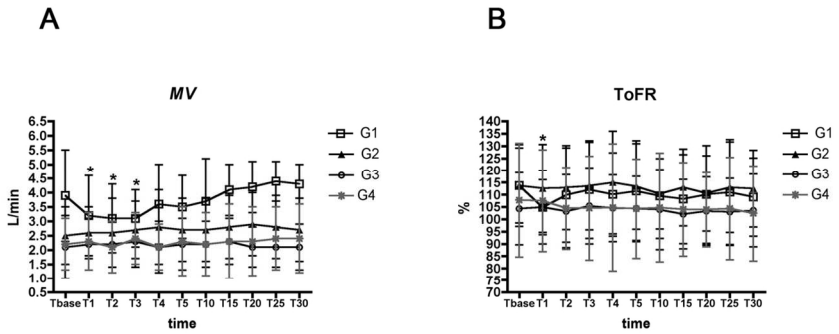

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