Original Article

Repeatability and comparison of train-of-four responses at thoracic and pelvic limbs using electromyography in anesthetized dogs

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Abstract

The aims of this study were to evaluate the repeatability of neuromuscular transmission (NMT) monitoring by electromyography and to compare train-of-four (TOF) responses of thoracic limbs to those of pelvic limbs in dogs after atracurium administration. Seven healthy dogs were enrolled in the study. The dogs were anesthetized with midazolam, morphine, propofol and isoflurane three times with a washout period of seven days. Two treatments of atracurium and electromyography-based neuromuscular monitoring were performed under each anesthesia. The dogs were randomly assigned to undergo either left or right side and either thoracic or pelvic limb for the first treatment and then allocated to the other for the second treatment. Ulnar and peroneal nerves were stimulated with a TOF pattern every 15 seconds. Onset time, time to recovery of the first ($TOFcount_1$), second ($TOFcount_2$), third ($TOFcount_3$) and fourth ($TOFcount_4$) twitches, and time to a TOF ratio of 0.9 ($TOFR_{0.9}$) were recorded. Atracurium (0.2 mg/kg) was administered intravenously at time zero. Means and standard deviations of time to $TOFR_{0.9}$ were used for coefficient of variation (CV) calculation. Statistical analyses revealed that the onset time was not different between the limbs. The times to $TOFcount_{1-4}$ and $TOFR_{0.9}$ were significantly shorter at the pelvic limbs (all $P < 0.05$). The CV of NMT monitoring at the thoracic and pelvic limbs were 14.87 and 11.03%, respectively. In conclusion, the NMT monitoring by electromyography at both limbs in dogs was repeatable with low CV. Nevertheless, the pelvic limb monitoring was prone to underestimate the residual neuromuscular blockade.

Keywords: atracurium, dogs, electromyography, pelvic limb, train-of-four, thoracic limb

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Introduction

Muscle relaxants or neuromuscular blocking agents (NMBAs) antagonize the function of acetylcholine by binding to acetylcholine receptors postsynaptically, resulting in paralysis of the skeletal muscles. The use of NMBAs has been recommended for facilitating mechanical ventilation, achieving rapid airway control, reducing or preventing responses to electrocauterization, improving surgical access to thoracic and abdominal cavities, and facilitating orthopedic and ophthalmologic surgeries (Martinez, 1999). The problem related to the use of NMBAs is postoperative residual curarization. Approximately 40% of humans recover from anesthesia with incomplete neuromuscular function (Murphy and Brull, 2010). A small amount of residual NMBAs is able to induce postoperative respiratory complications, such as hypoxia, upper airway obstruction, aspiration from relaxation of pharyngeal and atelectasis (Eikermann et al., 2007; Murphy and Brull, 2010; Murphy et al., 2011).

To prevent residual paralysis and muscle weakness in the recovery period, a neuromuscular monitoring device should be utilized for evaluating the degree of neuromuscular blockade and determining the appropriate time for safe extubation (Brull and Murphy, 2011; Fabregat et al., 2012). The device provides an electrical stimulus to a peripheral nerve and monitors responses of the innervated muscle (Martin-Flores et al., 2008; Murphy et al., 2011). Electromyography (EMG)-based devices detect the electrical activity within the stimulated skeletal muscles. This technology is potentially the most precise method for the measurement of synaptic transmission (Brull and Kopman, 2017). Train-of-four (TOF) is the most popular pattern of neuromuscular stimulation. A TOF pattern consists of four supramaximal stimuli which are separated by 0.5 sec (2 Hz). The stimulation is repeated every 15 or 20 seconds. The ratio of the amplitude of the fourth twitch (T4) to that of the first twitch (T1), known as the T4/T1 or TOF ratio (TOFR), estimates the neuromuscular blocking effect. In the absence of NMBA, the ratio should approximate 1.0. After NMBA administration, T4 decreases preferentially, leading to TOFR less than 1.0. In human, it is accepted that a patient recovers from laryngeal or pharyngeal paralysis when TOFR is greater than 0.9 (McGrath and Hunter, 2006).

The selection of a proper site of the neuromuscular stimulation depends on several factors. A superficial nerve, such as ulnar, peroneal and tibial nerves, is commonly chosen because of the accessibility. However, the neuromuscular monitoring at great toe (tibial nerve stimulation) produced a more rapid recovery time than that at thumb (ulnar nerve stimulation) in human adults (Kitajima et al., 1995; Saitoh et al., 1998; Heier and Hetland, 1999). In contrast, in infants, there were no significant differences between those nerve stimulations (Kitajima et al., 1996). The neuromuscular monitoring of great toe, therefore, may result in an underestimation of the degree of neuromuscular block in adults. In animals, a previous study reported that there was no difference in recovery of $TOFR_{0.9}$ between the extensor muscles of pelvic limb (peroneal nerve stimulation) and thoracic limb (radial nerve stimulation) after sugammadex administration to reverse profound rocuronium-induced block in ponies (Mosing et al., 2010).

In dogs, ulnar and peroneal nerves can be easily accessed for NMT monitoring. Nonetheless, to our knowledge the repeatability of both stimulation techniques and the differential of NMT monitoring between thoracic and pelvic limbs have not been investigated.

This study aimed to evaluate the repeatability of NMT monitoring in dogs using electromyography and to compare the NMT monitoring between two different muscles: flexor digitorum superficialis muscle of the thoracic limb (ulnar nerve stimulation) and cranial tibialis muscle of the pelvic limb (peroneal nerve stimulation) after the administration of atracurium.

Materials and Methods

This experiment was approved by the Kasetsart University Institutional Animal Care and Use Committee (#ACKU03760). A prospective, randomized complete block design was used. Seven healthy cross-bred dogs (2 males, 5 females) aged between 2-8 years and weighing between 15-30 kg were included in this study. The animals were evaluated to be healthy based on physical, biochemical and hematological examinations. All dogs were classified as American Society of Anesthesiologists (ASA) status I. Informed owner consents were obtained before the study began.

Food was withheld for twelve hours prior to anesthesia. Midazolam (0.1 mg/kg; Midazolam Sandoz®; EVER Pharma Jena GmbH, Germany) and morphine (0.3 mg/kg; Morphine Sulfate Injection; M & H Manufacturing, Co., Ltd. For Food and Drug administration, Thailand) were administered intramuscularly (IM) as premedications. Fifteen minutes after the premedications, anesthesia was induced with intravenous (IV) administration of propofol (2-4 mg/kg; ANEPOL®Inj.; Hana Pharm.Co., Ltd., Korea), and then maintained with isoflurane (Attane™; Piramal Critical Care, Inc., USA) in oxygen 100%. The isoflurane vaporizer was initially set at 3.5% until adequate anesthetic depth was achieved, then decreased to a minimum concentration required to prevent movement during nerve stimulation with the exception of evoked muscle contractions. A 22 GA catheter (BD Insyte™, Becton Dichinson Infusion Therapy System Inc., USA) was placed in a saphenous vein. Acetated Ringer's Injection (R-Cetate; General Hospital Products Public Co., Ltd., Thailand) was infused through the IV catheter (10 mL/kg/hour). A cuffed endotracheal tube was placed for airway management. Monitoring during general anesthesia consisted of an electrocardiogram (ECG), hemoglobin oxygen saturation (SpO_2), body temperature (esophageal probe), oscillometric non-invasive arterial blood pressure measurement and end-tidal carbon dioxide ($ETCO_2$) (Datex-Ohmeda B650 monitor; GE Healthcare, Finland). The lungs were mechanically ventilated to maintain $ETCO_2$ between 35 and 45 mmHg. The dogs were positioned on lateral

recumbency and a blanket with circulating warm water and forced-air warmer were used to maintain esophageal temperature between 36 and 38 degrees Celsius.

Each dog was anesthetized three times with a washout period of seven days. Two treatments of atracurium and electromyography-based neuromuscular monitoring (E-NMT-00 module of a Datex-Ohmeda B650 monitor, GE Healthcare, Finland) were performed under each anesthesia. The second treatment began at thirty minutes after TOFR return to 1. The dogs were randomly assigned to undergo either thoracic or pelvic limb for NMT monitoring of the first atracurium treatment and then allocated to the other in the second treatment. The left or right side was also chosen randomly for the first and second treatments. The procedure was repeated on the same limbs in similar order in the next two anesthetic sessions. The studied limb was clipped and cleaned with alcohol. Stimulating electrodes (NMT electrodes; GE Healthcare, Finland) were attached over the ulnar nerve on the medial aspect of the elbow, and the peroneal nerve on the lateral head of the gastrocnemius muscle at the level of the femorotibial joint for the thoracic and pelvic limb stimulations, respectively. Recording electrodes (NMT electrodes, GE Healthcare, Finland) were placed over the belly of flexor digitorum superficialis muscle, and cranial tibialis muscle for the monitoring at thoracic and pelvic limbs, respectively. Ground electrode was placed between stimulating and recording electrodes. Each electrode was secured in place with adhesive tape. TOF stimulation setting (pulse width, 0.2 ms; frequency, 2 Hz; duty cycle, 15 seconds) was applied independently to each limb. Calibration of the EMG monitor was performed with the start-up function of the E-NMT monitor. The maximal response to nerve stimulation during calibration was set to 100% and supramaximal current (up to 70 mA) was detected automatically. Atracurium (0.2 mg/kg; Atra Injection; Hana Pharm. Co., Ltd., Korea) was administered intravenously over 14 seconds. TOFR more than 0.9 was documented in all dogs prior to extubation.

Data collection: The average of three consecutive values for the amplitude of T1 and TOFR obtained (every 15 seconds) prior to atracurium administration was used as baseline. Onset time was defined as the time when TOFR of 0 was first achieved after atracurium administration. Time to $TOFcount_{1-4}$ and time when TOFR first returned to 0.9 were determined relative to the time that atracurium was administered (time zero). Values for all variables were recorded every 15 seconds.

Data analysis: Data were summarized as mean ± standard deviation (SD) using NCSS software, version 2007 (NCSS 2007, Kaysville, Utah, USA). Normal distribution was confirmed by Shapiro-Wilk test. Generalized linear model (GLM) was used. The onset times and times to reach target values ($TOFcount_{1-4}$ and $TOFR_{0.9}$) were compared between thoracic and pelvic limbs. Statistical significance was considered at a P value < 0.05. Means and standard deviations of time to reach $TOFR_{0.9}$ were used to calculate coefficient of variation of the stimulations at thoracic and pelvic limbs.

Results

All dogs recovered from general anesthesia without complications. Mean arterial blood pressure was never lower than 65 mmHg while SpO_2 was never less than 96% for any dog at any timepoint during anesthesia. No abnormality in electrocardiogram was detected in this study. Prior to atracurium administration, the averages of the amplitude of T1 and TOFR in all dogs were 100% and 1, respectively. The endotracheal tube was removed after TOFR > 0.9 and swallowing reflex returned.

The average CV of thoracic and pelvic stimulations were 14.87 and 11.03%, respectively (Table 1). The onset time at the pelvic limbs was not significantly different from that at the thoracic limbs. The time to $TOFcount_{1-4}$ was consistently faster at the pelvic limbs (all $P < 0.05$) (Table 2). The recovery time of $TOFR_{0.9}$ measured at pelvic limbs was significantly faster than that at thoracic limbs (51.1 ± 11.9 vs 70.6 ± 19.6 minutes, $p = 0.000006$). When the TOFR measured at pelvic limb reached 0.9, the TOFR measured at thoracic limb was lower than 0.6 (0-0.6) in all dogs (Figure 1).

Discussion

The results of this study revealed that the NMT monitoring at pelvic and thoracic limbs using electromyography was precise and repeatable. The dispersion of time to recovery of $TOFR_{0.9}$ from triplicate tests at both limbs was low with CV < 15%; the accepted value for the precision of bioanalytical method (Kadian et al., 2016). The lower average CV of the NMT monitoring at pelvic limbs compared to that at thoracic limbs (11.03 vs 14.87%) may yield a better repeatability. Additionally, the CV values calculated from thoracic limb EMG in three dogs were higher than 15% (Table 1). The reason may be that the stimulating site of ulnar nerve was located at the far caudal aspect of the elbow in the medial side, resulting in difficult access to the nerve.

Another main finding of this experiment was that the recovery of neuromuscular function from atracurium occurred earlier at the pelvic limb than at the thoracic limb. All times to return of twitch (T1-T4) and time to $TOFR_{0.9}$ obtained from the pelvic limb were consistently shorter. These indicate that the pelvic limb EMG may overestimate the thoracic limb EMG during recovery from atracurium. In other words, the pelvic limb EMG may underestimate the residual neuromuscular blockade. The results agree with previous studies in humans (Kitajima et al., 1995; Saitoh et al., 1998; Heier and Hetland, 1999). In those experiments, the electric stimulation of tibial nerve (pelvic limb) produced a more rapid recovery time than that of ulnar nerve (thoracic limb) in adults (Kitajima et al., 1995; Saitoh et al., 1998; Heier and Hetland, 1999). The explanation is the morphological differences between the evoked muscles: the flexor hallucis brevis muscle innervated by tibial nerve contains more type 2 fibers than does the adductor

pollicis muscle innervated by ulnar nerve (Kitajima et al., 1995; Saitoh et al., 1998; Heier and Hetland, 1999). In addition, type 2 muscle fibers are more resistant to non-depolarizing neuromuscular blocking drugs than type 1 muscle fibers (Johnson et al., 1973; Secher et al., 1982; Saitoh et al., 1998). In agreement with previous canine studies, flexor digitorum superficialis muscle in the thoracic limb contains more type 1 and less type 2 fibers than does cranial tibialis muscle in the pelvic limb (Newsholme et al., 1988; Kuzon et al., 1989; Evans and De Lahunta, 2013). Furthermore, the different responses between the evoked muscles may be influenced by variation of regional blood flow, muscle temperature, size and composition of the fibers, and density and location of acetylcholine receptors (Ibebunjo and Hall, 1993; Kelly and Brull, 1993).

Table 1 Coefficients of variation (%) during recovery from non-depolarizing neuromuscular block with atracurium (0.2 mg/kg, IV) in seven anesthetized dogs measured at thoracic and pelvic limbs

DOG	Time to TOFR 0.9 (minutes)							
	Thoracic limb				Pelvic limb			
	Repeat 1	Repeat 2	Repeat 3	% CV	Repeat 1	Repeat 2	Repeat 3	% CV
1 (♀, 4 y/o)	62	54	51.75	9.63	55	57.75	45.75	11.90
2 (♀,6 y/o)	64	46	57.25	16.31	40.75	49	40	11.55
3 (♂, 4 y/o)	59.25	78.5	73.50	14.14	63	74	70.5	8.13
4 (♀, 8 y/o)	52	68.25	59.75	13.55	39	30.5	41.25	15.36
5 (♀, 2 y/o)	68	62	61.25	5.80	42.25	42	37.25	6.96
6 (♂, 5 y/o)	113.75	92.75	82.50	16.54	64.75	54.75	52.75	11.20
7 (♀, 2 y/o)	92	118.75	66.75	28.11	63.75	59.25	50	12.16
average	73	74.32	64.68	14.87	52.64	52.46	48.21	11.03

Table 2 Mean ± SD (range) of onset time, time to $TOFcount_{1-4}$ and time when TOFR first returned to 0.9 ($TOFR_{0.9}$) measured at thoracic and pelvic limbs in seven dogs after administration of atracurium (0.2 mg/kg, IV)

	Thoracic limbs	Pelvic limbs	P value
Onset (seconds)	48.6 ± 17.7	51.4 ± 16.1	0.61
Time to $TOFcount_1$ (minutes)	51.2 ± 14.3 (31-85)	37.5 ± 11.6 (21-67)	0.000097
Time to $TOFcount_2$ (minutes)	61.4 ± 17.2 (40-104)	43.5 ± 11.2 (26-68)	0.000015
Time to $TOFcount_3$ (minutes)	66.6 ± 19.8 (45-118)	46.9 ± 11.6 (30-69)	0.000019
Time to $TOFcount_4$ (minutes)	68.4 ± 20.2 (45-118)	48.2 ± 11.9 (30-70)	0.000015
Time to $TOFR_{0.9}$ (minutes)	70.7 ± 19.6 (46-118)	51.1 ± 11.9 (30-74)	0.000006

In this study, the onset time of neuromuscular blockade (complete abolition of all twitches; TOFR of 0) after 0.2 mg/kg atracurium administration was within one minute at both limbs. All twitches returned ($TOFcount_4$) at 68.4 ± 20.2 and 48.2 ± 11.9 minutes after the drug administration at the thoracic and pelvic limbs, respectively. It may be concluded that the duration of neuromuscular blockade (absence of all twitches to return of all four) measured at the thoracic and pelvic limbs were 67.5 ± 18.2 and 47.4 ± 11.3 minutes, respectively. These outcomes disagree with a previous study in 1983 (Jones et al., 1983). In that experiment, atracurium had an onset of approximately 5 minutes and duration of action of approximately 30 (17-28.9) minutes in dogs (Jones et al., 1983; Keegan, 2015). This dissimilarity may be because of the different NMT monitoring techniques used for the investigations. Gender may also play a role as human females are more sensitive to atracurium than men (Xue et al., 1999). If this is true for female dogs, the greater number of females in this study (5 females and 2 males) might have caused the rapid onset and longer recovery. In addition, the anesthetic protocol of the current study consisted of midazolam, morphine and volatile anesthetic agents which may alter the neuromuscular blocking action of the muscle relaxant (Hughes and Chapple, 1981; Chapple et al., 1983; Vanlinthout et al., 1996; Kastrup et al., 2005).

The neuromuscular response to TOF stimulation can be used to classify the depth of nondepolarizing block. According to the current definitions of depth of neuromuscular block in 2017, profound and deep block are defined when there are no returns of any twitch ($TOFcount_0$); moderate block is determined when TOFcount is equal to 1-3; and light to minimal block are stated when all twitches return ($TOFcount_4$) (Brull and Kopman, 2017). According to the results of our investigation at thoracic limbs (Table 2), therefore, profound and deep block might last approximately 51.2 ± 14.3 minutes and then decreased to moderate block for approximately 17 minutes until the beginning of minimal block at 68.4 ± 20.2 minutes in the dogs after the administration of atracurium.

Some limitations of this study should be addressed. First, the sample size was small for a research study. However, statistical differences were found for all recovery variables and all dogs reacted consistently in the same direction. The statistical power calculated by NCSS & PASS software was 99%. Second, surface electrodes instead of subcutaneous (needle) electrodes were applied for the electric nerve stimulation in this experiment. Apparently, the

advantage of using surface electrode is that it has no potential to penetrate and harm the nerve and surrounding tissue, in particular when a patient moves unintentionally. Nevertheless, the surface stimulating is likely to be affected by physical factors such as obesity, displacement of electrodes and cooling of skin (Kelly and Brull, 1993). To ensure that the supramaximal stimulation was applied on every occasion, thus, a current of 70 mA was used, all electrodes were secured in place with adhesive tape, and the body temperature was maintained between 36 and 38 degrees Celsius in all dogs.

In summary, our results show that NMT monitoring at pelvic and thoracic limbs using electromyography was precise and repeatable in dogs. The EMG monitoring at thoracic limbs detected the presence of residual neuromuscular blockade when neuromuscular function recovered completely at the pelvic limbs. Therefore, EMG monitoring at a thoracic limb may be more reliable for excluding the residual neuromuscular block than that at a pelvic limb in dogs.

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บทคัดย่อ

ความเที่ยงตรงและการติดตามการทำงานของระบบประสาทและกล้ามเนื้อจากการกระตุ้นไฟฟ้าแบบ train-of-four ด้วยเทคนิคอิเล็กทรอไมโอกราฟบริเวณขาหน้าเปรียบเทียบกับขาหลังในสุนัข

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การศึกษาครั้งนี้ มีวัตถุประสงค์ในการประเมินประสิทธิภาพการวัดซ้ำ และเปรียบเทียบการติดตามการทำงานของระบบประสาทและกล้ามเนื้อ (NMT) ของเครื่องมืออิเล็กทรอไมโอกราฟที่ตำแหน่งขาหน้าและขาหลังของสุนัข โดยศึกษาในสุนัขจำนวน 7 ตัว แต่ละตัวได้รับการวางยาสลบทั้งหมด 3 ครั้ง ห่างกันครั้งละ 7 วัน ด้วยยา midazolam, morphine, propofol และ isoflurane ในการวางยาสลบแต่ละครั้งสุนัขได้รับยาหย่อนกล้ามเนื้อ atracurium (0.2 มิลลิกรัมต่อกิโลกรัมเข้าเส้นเลือดดำ) และติดตาม NMT 2 รอบ โดยสุ่มเลือกขาหน้าหรือขาหลังในการทดสอบรอบแรกและสลับเป็นอีกขาในรอบถัดไป พร้อมกับการสุ่มข้างซ้ายหรือขวา ทำการทดสอบซ้ำในการวางยาครั้งที่ 2 และ 3 โดยใช้ขาข้างเดิมกับครั้งที่ 1 เส้นประสาท ulnar และ peroneal ถูกกระตุ้นด้วยกระแสไฟฟ้ารูปแบบ train-of-four ทุก 15 วินาทีสำหรับขาหน้าและขาหลัง ตามลำดับ ทำการบันทึกระยะเวลายาเริ่มออกฤทธิ์ ระยะเวลาที่พบการกลับมาตอบสนองของกล้ามเนื้อจากการกระตุ้นครั้งที่ 1 ($TOFcount_1$), 2 ($TOFcount_2$), 3 ($TOFcount_3$) และ 4 ($TOFcount_4$) และเวลาที่อัตราส่วนของการตอบสนองของการกระตุ้นครั้งที่ 4 ต่อการตอบสนองของการกระตุ้นครั้งที่ 1 เท่ากับ 0.9 ($TOFR_{0.9}$) และคำนวณค่าสัมประสิทธิ์ความผันแปร (CV) จากค่าเฉลี่ยเวลาที่ $TOFR_{0.9}$ การวิเคราะห์ทางสถิติพบว่า ระยะเวลาการกลับคืนมาของ $TOFcount_{1-4}$ และ $TOFR_{0.9}$ ของขาหลังน้อยกว่าขาหน้าอย่างมีนัยสำคัญ ($P < 0.05$) ค่า CV ที่ขาหน้าและขาหลังมีค่าเท่ากับ 14.87 และ 11.03% ตามลำดับ สรุปได้ว่าการติดตาม NMT ด้วยเทคนิคอิเล็กทรอไมโอกราฟที่ตำแหน่งขาหน้าและขาหลังมีประสิทธิภาพในการวัดซ้ำเนื่องจากมี CV ที่ต่ำ แต่การติดตามที่บริเวณขาหลังอาจประเมินการคงค้างของยาหย่อนกล้ามเนื้อต่ำกว่าความเป็นจริง

คำสำคัญ: ยาหย่อนกล้ามเนื้อ สุนัข อิเล็กทรอไมโอกราฟ ขาหลัง การกระตุ้นแบบ train-of-four ขาหน้า

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