Neuromuscular blocking effects of cisatracurium and its antagonism with neostigmine in a canine model of autosomal-recessive centronuclear myopathy

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Abstract

Background: Centronuclear myopathy (CNM) is a rare congenital condition associated with skeletal muscle weakness. Patients with CNM may have decreased acetylcholine receptor expression and a reduced number of releasable quanta. Such perturbations could affect the time-course of neuromuscular blocking agents (NMBAs) and their antagonism with cholinesterase inhibitors. As a result of the rarity of CNM, prospective data regarding NMBA use in this subpopulation is scarce. We evaluated the neuromuscular blocking effects of cisatracurium and its antagonism with neostigmine in a canine model of CNM.

Methods: Six dogs with congenital autosomal-recessive CNM and six controls received cisatracurium 0.15 mg kg$^{-1}$ i.v. under general anaesthesia and intermittent positive pressure ventilation. Neuromuscular function was monitored with acceleromyography. When the second response (T2) to train-of-four (TOF) stimulation returned, neostigmine 0.04 mg kg$^{-1}$ (with glycopyrrolate) were administered i.v. The onset time, time to spontaneous return of T2, and the time to reach a TOF ratio $\geq 0.9$ after neostigmine administration were recorded.

Results: Onset time was no different between groups. Median (interquartile range) time to return of T2 was 27 (24–31) min for control dogs and 26 (22–31) min for CNM dogs ($P=0.93$). After neostigmine administration, a TOF ratio $\geq 0.9$ was reached in 12 (10–15) min and 17 (16–19) min in control and CNM, respectively ($P=0.005$).

Conclusions: The spontaneous return of T2 was not different between groups. However, neostigmine-facilitated recovery was significantly slower in dogs with CNM. Canine autosomal-recessive CNM does not preclude the use of cisatracurium or its antagonism with neostigmine.

Key words: myotubular myopathy; neuromuscular nondepolarizing agents
Editors key points

- Centronuclear myopathy (CNM) is a rare condition causing muscle weakness, occurring in dogs and humans.
- There is uncertainty about the use of neuromuscular blocking agents and cholinesterase inhibitors.
- This study used dogs with congenital CNM to examine the effects of cisatracurium and its antagonism with neostigmine.
- CNM does not preclude the use of cisatracurium or its antagonism with neostigmine in dogs and there is no reason to expect any difference in the human condition.

Neuromuscular blocking agents (NMBAs) and the efficacy of antagonism with cholinesterase inhibitors, given the latter’s dependency on acetylcholine (ACh) release.

As a result of its rarity, documented cases of CNM requiring general anaesthesia are scarce and information regarding the use of NMBAs in these individuals is lacking. From the available reports, it is apparent that NMBAs are often avoided in patients with CNM. A canine form of the disease was first described in 1976 and a number of cases in dogs have been reported since. Canine CNM can be transmitted as an autosomal recessive disease with signs of generalized muscle weakness, abnormal posture and exercise intolerance; the histological and clinical characteristics of canine CNM closely resemble those of autosomal recessive CNM in humans. To our knowledge, this is the closest, and possibly the only, animal model that might serve to investigate the effects of anaesthetic drugs on patients with CNM.

In this investigation we evaluated the neuromuscular blocking effects of cisatracurium and its antagonism with neostigmine monitored with acceleromyography in dogs with CNM. We hypothesized that the duration of action of cisatracurium would be longer in dogs affected with CNM than in normal animals, and that antagonism with neostigmine would occur at a slower rate in affected animals, because of reduced ability to release quanta. In addition, we evaluated the performance of acceleromyography in different muscle groups, as the severity of weakness and atrophy secondary to CNM might vary among different muscles.

Methods

Animals

A group of six adult Labrador retriever dogs with autosomal-recessive CNM, weighing 20.7–28.0 kg, and a control group of six healthy adult purpose-bred beagles, weighing 6.2–10.8 kg, were used. Sample size was limited to six animals per group because of the availability of dogs with CNM. Autosomal-recessive canine CNM was diagnosed through DNA testing by an independent laboratory (DDC Veterinary, Fairfield, OH). All procedures were approved by the Cornell University Institutional Animal Care and Use Committee and followed relevant aspects of the ARRIVE guidelines.

General anaesthesia and neuromuscular monitoring

Food was withheld overnight before anaesthesia. All dogs received dexmedetomidine (Dexdomitor, Orion Corporation, Espoo, Finland) 2 μg kg⁻¹ i.v. Anaesthesia was induced with propofol (Propoflo, Abbot Laboratories, North Chicago, IL) 2 mg kg⁻¹ i.v. and the trachea was intubated without the use of NMBAs. General anaesthesia was maintained with isoflurane 1.3–1.5% end-tidal (Isothesia, Butle Schein Animal Health, Dublin, OH) in oxygen. The lungs were ventilated to maintain normocapnia. Dexmedetomidine 2 μg kg⁻¹ h⁻¹ and lactated Ringer’s solution (5 ml kg⁻¹ h⁻¹) were infused throughout the procedure. The electrocardiogram, pulse oximetry, capnography, end-tidal isoflurane concentration, oscillometric arterial bp (every 2 min), and oesophageal temperature were monitored continuously. Oesophageal temperature was maintained between 36 and 38°C by the use of a forced warm air device.

Neuromuscular function was assessed on a thoracic limb with acceleromyography (AMG; TOF Watch, Organon, Ireland) as previously described. Briefly, with the dog in supine position, the limb was held extended with the anti-brachium parallel to the table. A 150 g elastic preload was applied to the paw to facilitate return of the carpus to an extended position (Supplementary data, Fig.S1). Stimulating needles were placed subcutaneously over the ulnar nerve as it runs medially to the medial epicondyle of the humerus, immediately cranial to the insertion of the medial and long heads of the triceps muscle (Supplementary data, Fig.S1). The ulnar nerve was located using anatomical landmarks and direct percutaneous palpation. The acceleration-sensitive crystal was taped to the palmar aspect of the paw, and flexion of the carpus was evoked. The AMG monitor was calibrated, and train-of-four (TOF) stimulation was applied every 15 s (2 Hz, 50 mA). As muscular weakness in our animals was more severe in the pelvic limbs, a second AMG monitor was placed on the pelvic limb to evaluate its performance in muscles producing smaller contractions; the peroneal (common fibular) nerve was stimulated, as it crosses the lateral head of the gastrocnemius muscle, lateral to the femorotibial joint; anatomical landmarks were used in order to locate the nerve (Supplementary data, Fig.S1). Flexion of the tarsus was evaluated. An elastic preload was also applied to the tarsus (and attached to a fixed stand), in order to keep the tarsal-metatarsal joint in an extended position. TOF stimulation on the pelvic limb began with a 5-s delay respective of the thoracic limb in order to avoid interference between monitors because of excessive movement. After at least 30 min of general anaesthesia (and 15 min of neuromuscular monitoring), cisatracurium (Cisatracurium besylate, Sandoz Inc, Princeton, NJ) 0.15 mg kg⁻¹ was administered i.v. over 5 s. This dose was selected based on our clinical experience, and from reports documenting that 0.1 mg kg⁻¹ had been insufficient to produce complete block in anaesthetized dogs. A stable TOF ratio (<5% variation in T1 and TOF ratio) for at least five min was established in every dog before cisatracurium administration, as suggested by the good clinical research practice guidelines. Neuromuscular function was monitored until the second response to TOF (T2) returned spontaneously and could be visually detected in the thoracic limb. At that time, glycopyrrolate (Glycopyrrolate, American Regent Inc, Shirley, NY) 0.01 mg kg⁻¹ was administered i.v. followed immediately by neostigmine (Neostigmine, West-Ward, Eatontown, NJ) 0.04 mg kg⁻¹ i.v. The TOF ratio was observed until it reached ≥0.9, normalized to baseline.

After experimentation the animals remained within the College for use in other studies as appropriate.

Data collection and statistical analysis

For each dog, the baseline value consisted of the average of the five consecutive TOF ratio values measured immediately before cisatracurium administration. The TOF ratio was recorded every
15 s for five min after cisatracurium administration, to measure onset time. Thereafter, TOF stimulation continued every 15 s but the TOF ratio was recorded every min. Onset time was defined as the time when the lowest TOF count was observed after cisatracurium administration. Duration of neuromuscular block was defined as the interval between cisatracurium administration and the spontaneous return of T2. The recovery period was defined as the time between neostigmine administration and a TOF ratio ≥0.9. All TOF ratio values obtained after cisatracurium administration were normalized to the baseline TOF ratio of each dog.

Data describing the time-course of cisatracurium are presented from the AMG monitor placed in the thoracic limb. Data from the TOF-Watch on the pelvic limb are shown only for comparison with those from the thoracic limb.

Data are presented as median and interquartile range (IQR) and the significance of differences between groups was evaluated with the Mann-Whitney U-test (Minitab 16, Minitab Inc). Significance of differences within a group of animals was evaluated with the Wilcoxon Signed-Rank test. Differences were considered significant when P<0.05.

**Results**

All dogs recovered uneventfully from general anaesthesia. The baseline TOF ratio at the thoracic limb was 0.90 (0.86–0.99) and 0.96 (0.91–0.99) for control and CNM, respectively (P=0.57). Complete neuromuscular block was achieved in both groups, with the exception of one dog per group in which one response to AMG TOF could always be observed. Onset time was 3.8 (2.6–5) min for control and 4.8 (4.0–5) min for CNM (P=0.28).

Duration of neuromuscular block to return of T2 was 27 (24–31) min for control and 26.5 (22–31) min for CNM dogs (P=0.93; Fig. 1A). The recovery time after neostigmine administration, to TOF ratio ≥0.9, was significantly longer in the CNM group: control 12 (10–15) min vs CNM 17 (16–19) min; P=0.005 (Fig. 1B).

Baseline TOF ratio measured at the pelvic limbs were 0.97 (0.89–1.0) and 0.96 (0.92–0.97) for the control and CNM groups, respectively (P=0.5). Within groups, these values were no different than those obtained at the thoracic limb for each group (control P=0.18; CNM P=0.6). Discrepancies between simultaneous thoracic and pelvic limb AMG TOF ratio after cisatracurium administration were found in three of the six CNM dogs, but in none of the control animals. In two of those dogs, the pelvic limb AMG monitor continued to display a TOF ratio of 1.0 during otherwise complete neuromuscular block (no twitches could be observed or palpated during TOF stimulation, and no responses were elicited in the thoracic limb either). In the remaining dog, the lowest TOF ratio recorded at the pelvic limb was 0.25 during complete block. In those three animals, the AMG monitor in the pelvic limb erroneously reported the presence of four twitches with a fade (incomplete neuromuscular block) even when no muscular twitches could be observed or palpated and when the AMG monitor in the thoracic limb displayed a TOF count of zero (Fig. 2).

**Discussion**

The results of our investigation show the time for a spontaneous return of T2 after cisatracurium block did not differ between normal dogs and those affected with CNM. This is in accord with an earlier study where the spontaneous recovery to a TOF ratio ≥0.9 did not differ between dogs with CNM and a matched control group of normal Labrador retriever dogs, after administration of vecuronium 0.1 mg kg⁻¹. While spontaneous recovery from non-depolarizing block may depend on redistribution and/or bio- transformation, pharmacological antagonism with neostigmine relies on the release and accumulation of ACh at the endplate. Recovery to a TOF ratio ≥0.9 after administration of neostigmine was approximately 40% (5 min) slower in dogs with CNM. Others have reported that the availability of releasable ACh quanta was reduced in a patient with CNM. In agreement with that report, it is possible that the longer recovery times after neostigmine in the dogs with CNM can be attributed to a slower rate of accumulation of ACh.

Neuromuscular blocking agents have traditionally been avoided in patients with CNM. It may be argued that many of these individuals might not require neuromuscular block to improve surgical conditions. Evoked force of contraction was measured in a patient with CNM and it was found that the force generated was much less than in normal individuals; under those circumstances the anaesthetists deemed the use of NMBAs unnecessary. It is also possible that lack of information might deter anaesthetists from utilizing NMBAs in these patients, presumably from concerns about prolonged duration.
were injected until the desired depth of relaxation was administered to an infant with X-linked CNM, small aliquots and corresponding response to TOF stimulation could be seen 30 min after the last dose of atracurium was administered. The potency and duration of action of vecuronium, and the duration of action of succinylcholine were evaluated in a canine model of CNM and compared with control dogs; in neither experiment were differences detected between the control and CNM individuals. When considered together, these data suggest the possibility that CNM might not affect the potency or duration of NMBAs significantly.

The severity of weakness in animals with CNM can vary between muscle groups. In several of our dogs, clinical examination revealed more severe weakness in the pelvic limbs than in the thoracic limbs. During pilot studies we observed that calibration of the AMG monitor was often more difficult to perform at the pelvic limb, where the evoked response was visibly smaller than it was at the thoracic limb. Therefore, we decided to monitor with AMG on the thoracic and pelvic limbs simultaneously. In healthy anaesthetized ponies no difference between the thoracic or pelvic limbs in their response to AMG monitoring was found and our observations in all the unaffected dogs concur with this observation. However, in three dogs with CNM, the AMG monitor on the pelvic limb failed to provide reliable results after cisatracurium administration, and it reported positive TOF values during the period that complete neuromuscular block was measured at the thoracic limb. In those dogs, the TOF ratio values displayed at the thoracic and pelvic limbs converged as recovery of neuromuscular function progressed (i.e., as evoked twitches became larger in magnitude; Fig. 2). TOF Watch monitors adjust their gain automatically during the calibration routine; presumably this facilitates analysis when the signal generated by slow peak acceleration is small. It is likely that small evoked signal in the pelvic limbs of the most affected animals initiated substantial signal amplification by the monitor during calibration. We speculate that under these circumstances, background ‘noise’ from small movements (e.g., arterial pulse, heartbeat, or surgical table movements) might have been misinterpreted by the monitor as evoked neuromuscular transmission, and lead to the error described above. A similar artifact was observed in dogs with myopathy when succinylcholine was evaluated with single twitch 0.1 Hz stimulation. In that experiment, a TOF Watch SX monitor was used to measure neuromuscular transmission. It was found that during calibration of the AMG, the sensitivity used by the monitor was higher in myopathic animals, indicating that evoked twitches in those dogs produced a smaller response than in controls even before neuromuscular blocking agents were administered. Because no muscular twitches could be observed or palpated, it is unlikely that this artifact is the result of direct muscle stimulation. The use of AMG in muscles that produce a small displacement of the acceleromyographic transducer has also been problematic in humans. Hammerling and colleagues reported problems presumably related to small evoked signals, when the AMG was used to monitor the corrugator supercilii muscle. Diessen and colleagues reported problems calibrating an AMG monitor in infants; this was more common in babies below 4 kg in weight. When using electromyography (EMG) in humans, a similar problem to the one described in our dogs was reported by Dubois and colleagues. Those authors reported that despite having obtained complete neuromuscular block, an EMG monitor erroneously displayed TOF ratios close to one. While EMG and AMG technologies are different from each other, it appears that this artifact can be found with either device.

Limitations of our investigation need to be considered: first, we investigated a canine model of a naturally occurring autosomal-recessive form of CNM. While the histological and clinical

![Fig 2 Time course after cisatracurium (0.15 mg kg⁻¹) of TOF ratio measured at the thoracic and pelvic limbs in three dogs with CNM. Discrepancies between simultaneous thoracic and pelvic limbs AMG can be seen in the middle and bottom traces. In the top trace TOF ratio in both limbs behaved similarly. In the middle trace the lowest TOF ratio (~0.25) in the pelvic limb coincided with a TOF ratio of zero in the thoracic limb. In the bottom trace the TOF ratio in the pelvic limb was 1.0 when the TOF ratio in the thoracic limb was zero and no movement could be seen.](https://academic.oup.com/bja/article-abstract/115/6/927/241848)
signs of CNM between the dog and man are practically identical, we cannot exclude that there may be species differences in the response to NMBAs. Second, we used a control group of beagles, which not only are of a different breed than the animals with CNM but also of smaller size/weight. Beagles have historically been used for research and as far as we are aware, no breed differences have been reported regarding the potency or duration of NMBAs in dogs. Moreover, the fact that we found no differences in the spontaneous recovery from cisatracurium in this experiment, or from succinylcholine in our earlier experiments, supports this. Third, we did not measure force of contraction, nor did the AMG monitor allowed us to measure the absolute acceleration of evoked twitches; AMG monitors display values that are relative to those obtained after calibration. Nevertheless, although absolute measurements are lacking, the AMG is probably the most commonly used objective neuromuscular monitor in a clinical setting. Lastly, our sample size was small because of the rarity of CNM, which could result in insufficient power, at least for some outcomes. Such could be the case of the spontaneous return of T2, where no differences were detected between groups.

In conclusion, the duration of neuromuscular block after cisatracurium (0.15 mg kg\(^{-1}\)) in dogs with autosomal-recessive CNM, was indistinguishable from that in control animals. However, after antagonism with neostigmine, return to a TOF ratio ≥0.9 was delayed by approximately 40% in CNM dogs. Acceleromyographic monitoring was apparently reliable in the least affected limbs whereas in some dogs with CNM, acceleromyography was unreliable when applied to the more severely affected limbs. Although a rare condition, dogs with CNM may require general anaesthesia and we have shown that canine autosomal-recessive CNM does not preclude the use of cisatracurium or its antagonism with neostigmine during general anaesthesia.

Authors’ contributions
Study design/planning: M.M.F., R.D.G.
Study conduct: M.M.F., M.D.P.
Data analysis: M.M.F., L.C., R.D.G.
Writing paper: M.M.F., M.D.P., L.C., R.D.G.
Revising paper: all authors

Supplementary material
Supplementary material is available at British Journal of Anaesthesia online.

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References