Neuromuscular blocking effects of vecuronium in dogs with autosomal-recessive centronuclear myopathy

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OBJECTIVE  
To evaluate the potency of vecuronium and duration of vecuronium-induced neuromuscular blockade in dogs with centronuclear myopathy (CNM).

ANIMALS  
6 Labrador Retrievers with autosomal-recessive CNM and 5 age- and weight-matched control dogs.

PROCEDURES  
Dogs were anesthetized on 2 occasions (1-week interval) with propofol, dexmedetomidine, and isoflurane. Neuromuscular function was monitored with acceleromyography and train-of-four (TOF) stimulation. In an initial experiment, potency of vecuronium was evaluated by a cumulative-dose method, where 2 submaximal doses of vecuronium (10 µg/kg each) were administered IV sequentially. For the TOF’s first twitch (T1), baseline twitch amplitude and maximal posttreatment depression of twitch amplitude were measured. In the second experiment, dogs received vecuronium (50 µg/kg, IV) and the time of spontaneous recovery to a TOF ratio (ie, amplitude of TOF’s fourth twitch divided by amplitude of T1) ≥ 0.9 and recovery index (interval between return of T1 amplitude to 25% and 75% of baseline) were measured.

RESULTS  
Depression of T1 after each submaximal dose of vecuronium was not different between groups. Median time to a TOF ratio ≥ 0.9 was 76.7 minutes (interquartile range [IQR; 25th to 75th percentile], 66.7 to 99.4 minutes) for dogs with CNM and 75.0 minutes (IQR, 47.8 to 96.5 minutes) for controls. Median recovery index was 18.0 minutes (IQR, 9.7 to 23.5 minutes) for dogs with CNM and 20.2 minutes (IQR, 8 to 25.1 minutes) for controls.

CONCLUSIONS AND CLINICAL RELEVANCE  
For the study dogs, neither potency nor duration of vecuronium-induced neuromuscular blockade in dogs with autosomal-recessive CNM.

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ther the potency of vecuronium nor the duration of vecuronium-induced neuromuscular blockade would differ between affected and unaffected dogs. A secondary aim was to evaluate the performance of electromyography as an alternative monitoring technique for assessment of recovery from neuromuscular blockade in dogs with CNM.

Materials and Methods

Animals

Six adult (1- to 5-year-old) Labrador Retrievers (4 males and 2 females) with CNM and 5 age-matched (1 to 5 years) and weight-matched dogs (4 Labrador Retrievers and 1 hound; 5 males) that were free of the disease (control group) were used in the study. For dogs with CNM, median body weight was 20.2 kg (IQR, 18.8 to 26.1 kg). For control dogs, median body weight was 27.4 kg (IQR, 25.3 to 32.5 kg). In the affected dogs, CNM was diagnosed through DNA testing by an independent laboratory. Sample size was limited by availability of dogs with CNM and age- and weight-matched controls. All procedures were approved by an institutional animal care and use committee.

Experiments

Each dog was anesthetized on 2 occasions (1-week interval). One experiment was performed to assess the potency of vecuronium in dogs with and without CNM. The other experiment was performed to determine the duration and recovery profile of vecuronium-induced neuromuscular blockade in dogs with and without CNM.

Anesthesia

For each dog, food was withheld overnight (approx. 12 hours) prior to anesthesia. Each dog was administered dexmedetomidine (1 µg/kg, IV). After approximately 3 minutes of O2 supplementation via face mask, anesthesia was induced with propofol administered IV to effect. Propofol was administered manually at a rate of approximately 2 mg/kg/min until the eyelash reflex could no longer be elicited and the dog’s mouth could be opened without resistance. The trachea was intubated with a cuffed tube when coughing or swallowing reflexes were not present, and anesthesia was maintained with isoflurane in O2 (end-tidal concentration, 1.2% to 1.5%). The lungs were ventilated to maintain normocapnia. Dexmedetomidine (1 µg/kg/h) and lactated Ringer’s solution (5 mL/kg/h) were infused throughout the experimental procedure. Electrocardiography and capnography were performed continuously, oxygen saturation as measured by pulse oximetry, end-tidal isoflurane concentration, oscillometric arterial blood pressure recordings, and esophageal temperature were monitored continuously. Esophageal temperature was maintained between 36° and 38°C by use of a forced warm air device.

Neuromuscular monitoring

Each dog was placed in left lateral recumbency. An acceleromyography monitor was used on the left thoracic limb. Subcutaneous needles were placed over the ulnar nerve and connected to the stimulating electrodes of the acceleromyograph. The acceleration transducer was taped to the ventral aspect of the paw to measure the evoked responses to nerve stimulation (flexion of the manus). To return the manus to a fixed position between muscular twitches as effectively as possible, a 150-g elastic preload was applied, similar to a procedure described previously. The acceleromyography monitor was calibrated with a calibration function that automatically detects supramaximal current and sets the amplitude of twitch acceleration to 100%. The acceleromyography monitor reports stimulating current and sensitivity (gain amplification), which are detected during calibration; these data were obtained in each experiment for analysis. Train-of-four stimulation (2 Hz) was begun at 15-second intervals. The amplitudes of T1 and fourth twitch of the TOF were measured, and the TOF ratio (ie, the amplitude of fourth twitch of the TOF divided by the amplitude of T1) was calculated. To allow for twitch potentiation and stabilization of twitch amplitude to occur, at least 15 minutes of TOF stimulation was delivered before data collection began. In each dog, stable twitch amplitudes were established for at least 3 to 5 minutes before vecuronium administration. All data regarding the potency and duration of action of vecuronium were evaluated with acceleromyography.

Evaluation of potency of vecuronium

To evaluate each dog’s susceptibility to vecuronium, a submaximal dose of 10 µg/kg was administered IV as a 5-second bolus. Depression of T1 amplitude was measured by acceleromyography until it reached its nadir. Once the T1 amplitude had no further reduction over 3 consecutive measurements, a second dose of 10 µg of vecuronium/kg was administered IV. If no depression of T1 amplitude was detected after the first dose, the second dose was administered 5 minutes later. The maximum decrease in T1 amplitude relative to baseline after each dose was recorded.

Duration of action and recovery profile of vecuronium

In a separate anesthetic period, vecuronium (50 µg/kg, IV) was administered as a single bolus to each dog. The time to onset of blockade (interval between vecuronium injection and establishment of complete neuromuscular blockade) was recorded, as were the times for T1 amplitude to spontaneously return to 25% and 75% of baseline following vecuronium injection, recovery index (interval between return of T1 amplitude to 25% and 75% of baseline), and the time to attain a TOF ratio ≥ 0.9 following vecuronium injection as measured by the acceleromyograph.

Electromyography

In each experiment to determine the duration of action and recovery profile of vecuronium, an electromyograph was also used to monitor neuromuscular...
function on the right pelvic limb of each dog. Subcutaneous stimulating needles were placed over the peroneal nerve of the nondependent right pelvic limb. The skin was clipped of hair and cleansed. An adhesive silver-silver chloride recording electrode was placed over the belly of the right flexor cranial tibial muscle, and secured with flexible cohesive bandage. The electromyograph was calibrated by means of the start-up function; similar to the acceleromyographic calibration process, the maximal response to nerve stimulation during calibration was set to 100%, and supramaximal current was detected automatically. Train-of-four stimulation every 15 seconds began after calibration.

Statistical analysis

The order of the 2 experimental procedures was randomized for all dogs. The dose of propofol administered to dogs with and without CNM in each experiment was compared with the Mann-Whitney U test. Stimulating current and sensitivity (gain amplification) were obtained by means of the acceleromyography monitor for each dog in each experiment; pooled values of each were compared between groups by means of the Mann-Whitney U test. Baseline T1 amplitudes and TOF ratio were determined by averaging the 3 values recorded immediately prior to vecuronium administration. Depression of T1 amplitude (expressed as a percentage) during the potency experiment was calculated as follows:

\[
(1 - \frac{\text{minimum T1 amplitude}}{\text{baseline T1 amplitude}}) \times 100\%
\]

where minimum T1 amplitude was the lowest T1 amplitude recorded after vecuronium administration. All acceleromyographic data were expressed relative to their baselines (ie, data were normalized to baseline). The significance of differences between groups for each neuromuscular variable was tested by means of the Mann-Whitney test. Values of \( P \leq 0.05 \) were considered significant. Results are summarized as median and IQR.

To assess the usefulness of electromyography as a neuromuscular monitor in clinical situations, we measured and compared the time to attain a TOF ratio \( \geq 0.9 \) after administration of vecuronium (50 µg/kg) to dogs in both groups. The TOF ratios determined by means of electromyography were also normalized to their respective baselines.

Results

All dogs recovered uneventfully from both anesthetic episodes and experimental procedures. The dose of propofol administered during either phase of the study did not differ between groups. During the experiments to assess the potency of vecuronium, the median propofol dose was 3.8 mg/kg (IQR, 3.2 to 4.3 mg/kg) for the dogs with CNM and 3.6 mg/kg (IQR, 2.8 to 4.7 mg/kg) for the control dogs (\( P = 0.9 \)).

Stimulating current and sensitivity obtained during calibration of the acceleromyography monitor in each experiment to determine potency and duration of action of vecuronium were pooled for each group of dogs. Stimulating current for the CNM (median, 39 mA; IQR, 33 to 54 mA) and control (median, 60 mA; IQR, 28 to 60 mA) groups did not differ significantly (\( P = 0.5 \)). The pooled sensitivities (between 1 and 512, where 512 represents the highest sensitivity) were significantly (\( P = 0.001 \)) higher for the CNM group (median, 351; IQR, 315 to 366) than for control dogs (median, 220; IQR, 174 to 255).

Evaluation of potency of vecuronium

Depression of T1 amplitude after two 10 µg/kg doses of vecuronium in dogs with CNM and control dogs was evident (Figure 1). Depression of T1 amplitude did not differ between groups after each dose of vecuronium (all \( P > 0.7 \)).

Duration of action and recovery profile of vecuronium

Administration of vecuronium (50 µg/kg, IV) resulted in complete ablation of responses to TOFs in all
dogs. Median time to onset of neuromuscular blockade was 157 seconds (IQR, 159 to 184 seconds) for dogs with CNM and 150 seconds (IQR, 120 to 165 seconds) for control dogs (P = 0.64). There were no differences between groups for any of the recovery variables. The median time for T1 amplitude to spontaneously return to 25% of baseline was 34.6 minutes (IQR, 22.1 to 41.2 minutes) for dogs with CNM and 31.5 minutes (IQR, 21.5 to 36.2 minutes) for control dogs (P = 0.64). The median time for T1 amplitude to spontaneously return to 75% of baseline was 60.2 minutes (IQR, 37.7 to 62.2 minutes) for dogs with CNM and 55.2 minutes (IQR, 29.5 to 59.6 minutes) for control dogs (P = 0.53). The median recovery index (interval between return of T1 amplitude to 25% and 75% of baseline) was 18.0 minutes (IQR, 9.7 to 23.5 minutes) for dogs with CNM and 20.2 minutes (IQR, 8.0 to 25.1 minutes) for control dogs (P = 0.83). The median time to attain a TOF ratio ≥ 0.9 was 76.7 minutes (IQR, 66.7 to 99.4 minutes) for dogs with CNM and 75.0 minutes (IQR, 47.8 to 96.5 minutes) for control dogs (P = 0.64).

**Electromyography**

For all dogs, electromyographic signals were obtained and the monitor was successfully calibrated at first attempt. By visual inspection, biphasic electromyographic curves obtained from dogs with CNM and control dogs were indistinguishable. The median time to attain a TOF ratio ≥ 0.9 during electromyography was 58 minutes (IQR, 43 to 68.5 minutes) for dogs with CNM and 61 minutes (IQR, 44 to 88.5 minutes) for control dogs (P = 0.53).

**Discussion**

In the present study, there were no differences in the effects of vecuronium in dogs with autosomal recessive CNM and unaffected control dogs. The study was performed in 2 phases with the intent of evaluating both the susceptibility of these dogs to vecuronium and the duration of action and recovery profile of a clinically useful dose of the drug.

Potency of an NMBA in a given species is typically established by measuring T1 amplitude depression following administration of submaximal doses of the drug. The results obtained by that approach can be managed in different ways. When only 1 submaximal dose is administered to each study animal, a minimal number of individuals must be evaluated before potency estimation can be achieved. Notwithstanding the limitations of the cumulative-dose method, T1 amplitude depression after the second dose was approximately 95% in each group. This finding is not substantially different from previous findings for healthy Beagles, in which a vecuronium dose of 25 µg/kg (as a single bolus) produced T1 amplitude depression > 80%. Bom et al previously reported that, for vecuronium, the dose required to produce a 90% effect is 90 µg/kg. However, Kariman and Clutton showed that complete neuromuscular blockade in anesthetized dogs is achieved with 50 µg of vecuronium/kg. Moreover, when we studied recovery from vecuronium-induced neuromuscular blockade in the present study, a dose of 50 µg of vecuronium/kg produced complete neuromuscular blockade in both groups of dogs. Our results and those of Kariman and Clutton suggest that a 50 µg/kg dose is sufficient for producing complete neuromuscular blockade in this species.

As part of the present investigation, the study dogs were administered a clinically useful dose of vecuronium that produced complete paralysis and descriptors of spontaneous recovery were measured. No differences were found in any indicator of recovery: following vecuronium injection, times for T1 amplitude to spontaneously return to 25% and 75% of baseline, recovery index, and the time to attain a TOF ratio ≥ 0.9 were each similar in the 2 groups. Taken together, these data suggested that dogs with autosomal-recessive CNM are not more sensitive to vecuronium than healthy dogs, and the duration of neuromuscular blockade or the recovery characteristics do not differ between dogs with and without CNM.

In the present study, the acceleromyography monitor was used with an elastic preload, as described previously. During calibration, the monitor automatically detects supramaximal current and adjusts the gain of the acceleration signal so that the response is set to 100%; a value of sensitivity (gain amplification) is reported by the device. Hence, lower sensitivity indicates higher acceleration, and vice-versa. Sensitivity was consistently higher in dogs with CNM in the present study, indicating that the magnitude of the signal they produced was lower and that gain amplification was greater than findings in dogs without CNM. Although we did not measure force of contraction in the dogs with CNM, the responses to neuromuscular stimulation obtained appeared to be of a smaller magnitude than usual, especially in the most affected dogs.
This was expected because CNM results in muscle atrophy. In accordance with our observations, reduced force of contraction during TOF stimulation has been reported in a human subject with CNM requiring general anesthesia. 19

To our knowledge, this is the first prospective investigation of an NMBA in dogs with CNM. There are only isolated reports of the use of NMNBAs in humans with CNM; in most of those patients, administration of an NMBA was in fact avoided during anesthesia. The use of atracurium in a patient with X-linked CNM has been reported. 20 A cumulative dose of 0.5 mg of atracurium/kg was administered before TOF response was abolished; however, a clinically relevant increased sensitivity was not reported. 20

In the present study, we evaluated the performance of an electromyography monitor because during our pilot investigations, we experienced substantial difficulties with the acceleromyography monitor; we were unable to calibrate the acceleromyography monitor when placed on the pelvic limb in several of the affected dogs that were subsequently used in the study. Such difficulties had not been encountered during our previous experiences with acceleromyography in healthy dogs. Judging by the degree of muscle atrophy and the poor ability to support body weight, the pelvic limbs appeared to be substantially more affected than the thoracic limbs in the dogs with CNM. Difficulties calibrating the electromyography monitor in human neonates and infants have been reported; for those individuals, the magnitude of evoked twitches are presumably small. 21 It is likely that low signal intensity was the reason that calibration was difficult to obtain in the dogs with CNM in the present study. Unlike acceleromyography monitoring, the electromyography signal does not depend on movement but on muscle cell activation (compound motor action potential); therefore, we speculated that some of the difficulties encountered during acceleromyography would not occur during electromyography. The electromyography monitor was successfully used and calibrated in all dogs. This monitor displays the biphasic curves that are detected during neuromuscular stimulation and reports the amplitude of T1 and the TOF ratio. By inspection, the electromyographic curves of all dogs with CNM were identical to those obtained from dogs without CNM in the present study.

Furthermore, no differences were found when the duration of neuromuscular blockade as measured by electromyography was compared between dogs with CNM and control dogs. It appears that electromyography may offer some advantages when measurement of neuromuscular function of muscles that produce low signals (small contraction) is required. In the present study, we elected to assess the return of the TOF ratio to ≥ 0.9 with electromyography to simplify the amount of data presented. The objective for evaluating electromyography was to assess its usefulness as a means to clinically monitor recovery from neuromuscular blockade when muscle contraction is decreased due to myopathy, but not to evaluate the bias or agreement with acceleromyography.

Results of the present study indicated that there was no difference in the potency or duration of action of vecuronium in dogs with autosomal-recessive CNM and unaffected control dogs. Acceleromyography was useful for quantifying neuromuscular transmission in dogs with myopathy, although calibration of the acceleromyography monitor in the pelvic limbs of some dogs was difficult. Electromyography was successfully calibrated in the pelvic limbs of all study dogs, even in limbs for which acceleromyography calibration had failed.

Footnotes

a. DDC Veterinary, Fairfield, Ohio.
b. TOF-Watch SX, Organon, Swords. County Dublin, Ireland.
c. Vecuronium bromide, SUN Pharmaceutical Industries, Gujarat, India.
d. Dacet Ohmeda AS/J, GE Healthcare, Helsinki, Finland.
e. Cleartrace, Conmed Corp, New York, NY.
g. Minitab, version 16.2.4, Minitab Inc, State College, Pa.

References


