Block duration is substantially longer with a liposomal suspension of bupivacaine than with 0.5% bupivacaine HCl potentiated with dexmedetomidine following an ultrasound-guided sciatic nerve block in Beagles

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OBJECTIVE
To compare the duration of bupivacaine liposome suspension in the dog with that of bupivacaine and dexmedetomidine following a perineural injection.

ANIMALS
8 healthy Beagles.

PROCEDURES
The left sciatic nerve of each dog was randomly assigned to an ultrasound-guided perineural injection with either bupivacaine liposome suspension (BLS) or with 0.5% bupivacaine with dexmedetomidine (1 µg/mL) (BUP-DEX). The contralateral nerve was assigned to the alternate agent. The sensory, motor, and proprioceptive functions were evaluated before the injection (baseline) and at 4, 10, 24, 48, 72, and 96 hours.

RESULTS
The block in 1 limb in the BLS treatment appeared to have failed (data set excluded). The motor scores of 2 individuals could not be evaluated leaving 5 limbs to evaluate in the BLS treatment and 6 in the BUP-DEX.

A total of 6 out of 7 limbs in the BLS achieved a complete sensory block. In 3 out of 5 treatments with BLS, motor block was only partial and in 2 not apparent at all. Proprioceptive block was partial in 5 out of 7 dogs in the BLS treatment. All functions were still completely obliterated at 10 hours in 6 cases in treatment BUP-DEX. All functions were restored in all cases by 96 and 24 hours after administration of BLS and BUP-DEX, respectively.

CLINICAL RELEVANCE
The blockade characteristics of bupivacaine liposome suspension were effective and long lasting. Motor and proprioceptive deficits may be inconsistent over time.

The introduction of ultrasound guided locoregional techniques has increased the accuracy, efficacy, reliability, predictability, and the safety of locoregional anesthesia.1,2 As a result, it has revolutionized the way clinicians can manage perioperative pain.

Bupivacaine is a popular local anesthetic widely used in surgical procedures such as stifle arthroplasty in dogs due to its long duration of action. A single perineural injection of 0.5% bupivacaine has been reported to provide analgesia lasting for 14 (6 to 24) hours.3 Additives, such as dexamethasone or dexmedetomidine, have failed to reliably extend the duration of the analgesic effect of bupivacaine beyond 24 hours.4–7

Bupivacaine liposome injectable suspension (Nocita; Aratana Therapeutics, Inc) is a newer formulation currently available in the United States in which bupivacaine (13.3 mg/mL) is contained within a multivesicular lipid-based suspension approved for veterinary use. Once injected into tissue, the liposomes break down over time gradually releasing the bupivacaine contained within. According to the manufacturer, it can provide analgesia for up to 72 hours when used as labeled. This might be an attractive alternative to the use of 0.5% bupivacaine HCl for perioperative use, even when dexmedetomidine is used as a coadjuvant, as the duration of the analgesic effect of this formulation would potentially extend into the early recovery and rehabilitation period.
In this study, our goal was to compare the duration of the sensory, motor, and proprioceptive function blockade following a perineural injection of bupivacaine liposome suspension (BLS) around the sciatic nerve in the dog with 0.5% bupivacaine with dexmedetomidine (1 µg/mL) as a coadjuvant (BUP-DEX). We hypothesized that an ultrasound-guided perineural injection of the bupivacaine liposome suspension around the sciatic nerve in the dog would produce deficits that would outlast those of the perineural injection of 0.5% bupivacaine augmented with dexmedetomidine (1 µg/mL).

Materials and Methods

This study was approved by the Institutional Animal Care and Use Committee (protocol number: 2016-0046). A total of 8 Beagles (4 males, 4 females) aged 7 (1 to 8) years, and weighing 12 ± 0.4 kg and belonging to a research colony, were enrolled in this study. Based on physical examination and medical records, the dogs were classified as American Society of Anesthesiologists physical status 1 (healthy) or 2 (patient with mild systemic disease). Prior to the experiment, neither lameness nor neurological deficits were detected on physical examination in any of the dogs. Additionally, baseline values regarding sensory (skin pinch at the relevant dermatomes), motor (video recording of the dog being walked on a leash with subsequent visual evaluation of gait), and proprioception (knuckling test) were obtained (Tables 1-3). Under block randomization (closed envelope), the left sciatic nerve of each dog was assigned to be treated with either 0.5% bupivacaine (Bupivacaine HCl injection USP; Hospira) enhanced with dexmedetomidine (Dexdomitor, Zoetis) at 1 µg/mL at a total volume of 0.1 mL/kg (BUP-DEX) or a bupivacaine liposome suspension (Nocita) containing 13.3 mg/mL bupivacaine at 0.1 mL/kg (BLS). The right sciatic nerve was, therefore, assigned to the alternate treatment.

An intravenous catheter was placed in a cephalic vein. Nerve blocks were carried out with the dogs under procedural sedation. The sedation was achieved and maintained by a target-controlled infusion of 10 mg/mL propofol (Sagent Pharmaceuticals) at a target plasma level set at 5 µg/mL during the procedure and 2.5 µg/mL during the postprocedural waiting period using an infusion pump (Graseby 3400; Graseby Medical) controlled by Computer Control Infusion Pump (CCIP) software (Version 2.4; Department of Anaesthesia and Intensive Care; The Chinese University of Hong Kong) and using kinetic data previously published by Beths et al. The software was set to reach the target plasma concentration using the “V1 (plasma)” function without concurrent administration of a bolus. Monitoring included pulse oximetry, electrocardiography, and noninvasive blood pressure (Cardell Touch; Veterinary Monitor; Midmark Corporation).

After skin infiltration at the puncture site with 1 mL of buffered 1% lidocaine (AuroMedics Pharma), the sciatic nerve was identified under ultrasound guidance (Sonosite Edge; Fujifilm Sonosite) with a high-frequency (15- to 6-MHz) linear array transducer (HFL50; Fujifilm Sonosite) and using a 21-gauge, 100-mm insulated needle (NB2110; Mila International). All blocks were carried out by the same clinician and using similar technique as previously described elsewhere. Either side was immediately followed by the contralateral side. The dogs were maintained under sedation and in lateral recumbency for 45 minutes following the second injection. Subsequently, sensory, motor, and proprioceptive functions were scored bilaterally at 4, 10, 24,

| Score | Description |
|-------|-------------|
| 0     | Normal: no evidence of block detected. Immediate postural changes, voluntary limb withdrawal, head turning, or behavior associated with nociception in response to pinching when tissue forceps were lightly applied |
| 1     | Partial block: minimal changes. The legs swing a little outwards or there is some evidence of pseudohypermetria, bringing the knee up in an exaggerated manner |
| 2     | Complete: Unable to flex the foot, obvious outward swing and pseudohypermetria. |

Evaluators tested at the tibial (plantar aspect of paw) and peroneal (dorsal aspect of paw) dermatomes by pinching the skin using mosquito forceps. Evaluators observed the response evoked (or lack thereof). Dogs were all blind folded to minimize possible anticipation.

| Score | Description |
|-------|-------------|
| 0     | Normal: no evidence of block detected. No abnormalities observed in the gait of the evaluated limb |
| 1     | Partial block: minimal changes. The legs swing a little outwards or there is some evidence of pseudohypermetria, bringing the knee up in an exaggerated manner |
| 2     | Complete: Unable to flex the foot, obvious outward swing and pseudohypermetria. |

Each limb of each dog was evaluated independently following review of video footage of the subjects being walked on a leash.

Table 3—Descriptors of proprioceptive function scores.

| Score | Description |
|-------|-------------|
| 0     | Normal: immediate reposition of paw. Normal positioning of the paws at walk |
| 1     | Partial block: reduced or delayed reposition of the paw. Occasional knuckling when walked |
| 2     | Complete: foot is permanently flexed (knuckled) following flexion. When walked, dog constantly knuckled (walked on the dorsal aspect of the foot) |

A knuckling test was bilaterally carried out: With the dog blind folded, the foot was flexed so that the dorsal aspect of the foot was in contact with the ground. Evaluators scored how quickly (if at all) the foot was repositioned. Dogs were also walked on a leash. Occasional or permanent knuckling was evaluated.

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48, 72, and 96 hours after the second injection. The sensory function was evaluated at the tibial (plantar aspect of paw) and peroneal (dorsal aspect of paw) dermatomes by briefly (less than 1 second in duration) pinching the skin using mosquito forceps and observing the response evoked (or lack thereof). The dogs were all blindfolded to minimize possible anticipation. The evaluated evoked responses included postural changes, conscious limb retraction (voluntary withdrawal) with possible head turning, and/or vocalization. The concurrent head turn along with limb withdrawal helped evaluators distinguish this response from a withdrawal reflex (Table 1). The motor function consisted of video gait evaluation of the subjects being walked on a leash looking for evidence of outward leg swing on the stride or pseudohypermetria (exaggerated elevation of the stifle to compensate for the inability to flex the foot during the stride). The video footage was taken with a mobile (cell) phone camera (Google Pixel 2XL), and the dogs were walked up and down initially going away from the camera and then coming back toward it and going past and away in the opposite direction. The video files contained information of dog identification and time point but no treatment allocation. Motor function evaluations were conducted by 3 individuals blinded to treatment allocation and their scores averaged. All evaluators received previous training by the principal investigator in order to standardize scoring among them (Table 2). Finally, proprioception was assessed by conducting a knuckling test consisting on flexing each foot until the dorsal aspect (top of the foot) was in contact with the ground. The evaluator would then observe how quickly the dog would reposition the paw (plantar side touching the ground). Additionally, dogs were observed for the presence of foot dragging leading to nail scrapping or walking on top of their feet when walked on a leash (Table 3).

Statistical analysis
Sample size was calculated “a priori.” With the assumption that bupivacaine 0.5% potentiated with dexmedetomidine and the liposomal encapsulated formulation would produce a sensory disruption for 15 ± 7 (mean ± SD) hours and 48 ± 12 hours, respectively (based on unpublished personal data), a sample size of 6 dogs per treatment was calculated assuming one tail, matched pairs, and the duration of sensory blockade as the main outcome variable. For the purpose of this calculation, alpha and the desired statistical power were set at 0.05% and 95%, respectively (G*Power Version 3.1.5). Data were tested for normality by the Shapiro-Wilk normality test and by observing the distribution of the residuals. Linear mixed effect models were used for each response (sensory at each dermatome, motor, and proprioception) with the fixed effects of treatment, time, and the interaction of treatment and time. To account for the repeated measurements of each dog and of each pelvic limb of each dog, random effects of each individual dog and the interaction of dog and treatment were included in the models. Survival analysis was used for all the “time to” variables such as the duration of the motor deficits (i.e. time to return to normal function). Additionally, the Mantel-Cox test was used to compare the duration of effect between treatments. Duration of effect for each treatment function was defined as the time at which a subject first achieved a score of 0 following verification of scores of 0 for 2 or more consecutive check points.

Gwet’s agreement coefficient (AC2) test was used to rate interobserver agreement for the motor scores. An interobserver agreement > 0.80 was considered acceptable.10 Variables with poor interobserver agreement (Gwet’s AC1 < 0.80) were excluded from further analysis.10

Results of parametric data are presented as means ± SD and as median (minimum – maximum) for nonparametric (skewed) data. All statistical analyses were carried out using JMP Pro Statistical package version 13.2.1 (SAS Institute) for the mixed effect model and GraphPad Prism 6 (GraphPad Software) for the survival analysis and R Version 4.1.1 (R Foundation for Statistical Computing) for the Gwet’s agreement coefficient. Significance was set at P ≤ 0.05.

Results
All dogs recovered uneventfully. The block in 1 limb in the BLS treatment appeared to have failed. Subsequently, this data set was excluded from further analysis leaving 7 limbs in the BLS treatment. Sensory function at both tibial and peroneal dermatomes was significantly affected by time, treatment, and their interaction (all P < 0.0001). A total of 6 (out of 7) limbs in the BLS achieved a complete sensory block. The sensory block in the remaining limb was only partial (grade 1) (Supplementary Figures S1–S2).

Due to a technical problem (corrupted video files), the motor scores of 2 individuals could not be evaluated, and therefore, these 4 scores (2 for BLS and 2 for BUP-DEX) were excluded from the motor function analysis data set leaving 5 limbs to evaluate in the BLS treatment and 6 in the BUP-DEX. Motor function was significantly affected by time (P < 0.0001) and by the interaction of treatment and time (P = 0.0002). The motor block was only partial (grade 1) in 3 treatments with BLS and, in 2, it was not apparent at all (despite exhibiting sensory and proprioceptive blocks) (Supplementary Figure S3). Gwet’s coefficient values were 0.936 and 0.892 for the right and left sides, respectively.

Proprioception was significantly affected by time (P < 0.001) and the interaction of treatment and time (P < 0.0001). Proprioceptive block was also partial in 5 dogs in the BLS treatment (Supplementary Figure S4). There was only 1 dog with complete block of all functions in the BLS treatment and that was at 48 hours postblockade. All 3 functions were still completely obliterated (grade 2) 4 hours postblockade in all limbs except for 1 and, in 6 cases, at the 10-hour time point in treatment BUP-DEX.
Duration of sensory (tibial $P = 0.0002$; peroneal $P = 0.0003$), motor ($P = 0.0343$), and proprioceptive block ($P = 0.0243$) was consistently longer for the limbs corresponding to the BLS treatment. Sensory function was fully restored in all individuals by 96 hours (both dermatomes) in the BLS treatment and by 24 and 48 hours at the tibial and peroneal dermatomes, respectively, in the limbs corresponding to the BUP-DEX treatment. Motor and proprioceptive function deficits were no longer noticeable in any animals by 96 and by 48 hours in the BLS and BUP-DEX treatments, respectively (Figures 1–3; Supplementary Table S1).

**Discussion**

Liposomal encapsulation of a local anesthetic is one of the newer proposed pharmacological formulations in response to the search for longer duration in nerve conduction blockade. The results of this study showed that the duration of the sensory, motor, and proprioceptive blocks lasted substantially longer with the liposomal formulation of bupivacaine than with 0.5% bupivacaine HCl potentiated with dexmedetomidine (1 µg/mL). They also showed that the motor and proprioceptive deficits were inconsistent among patients and over time.

A commercially available bupivacaine liposome suspension (in the United States) (Nocita at 5.3 mg/kg [0.4 mL/kg]) was found to provide pain control for up to 72 hours in a multicenter, placebo-controlled, randomized study with dogs undergoing cruciate ligament stabilization surgery and following infiltration injections into the tissue layers at the time of incisional closure (package insert). Our study confirmed a similar duration of the sensory blockade. However, in this instance, it was used as part of a peripheral nerve block (sciatic nerve) at previously published recommended volumes for this nerve block (0.1 mL/kg) and not as tissue infiltration. When a liposome encapsulated suspension of local anesthetic is used as part of a peripheral nerve block, it has the potential to offer 2 additional advantages over tissue infiltration: first, it can be used preoperatively and thus enhance intraoperative analgesia; and second, it is deposited in a remote location, away from the surgical field, therefore, minimizing possible surgical tissue–drug interactions and potential contamination of the surgical site.

An interesting finding of this study was the quality of blockade for all 3 evaluated functions. Inconsistent quality of sensory, motor, and proprioceptive block...
was observed in the limbs treated with BLS. These fluctuations were not observed in the limbs treated with BUP-DEX. In some cases, scores decreased over time only to be followed by an increase in the subsequent observation. Furthermore, in 2 separate dogs, a sensory score of 0 was recorded. In both instances, it occurred at the 10 hour postblock time point. These data may suggest a similar nonlinear release rate of free bupivacaine from the vesicles, perhaps as a result of the rate of degradation and thus release of free drug into the perineural tissue. A similar observation was reported in the pharmacokinetic data from the safety studies conducted by Aratana Therapeutics in which a dual peak in the free bupivacaine fraction was observed (package insert). This information may question relying completely on the encapsulated formulation as a sole agent included in a peripheral nerve block to provide immediate postoperative analgesia or when a complete and dense block may be desirable.

Survival analysis showed that duration of perceived conduction blockade was substantially longer for BLS than for BUP-DEX. By 24 hours after injection, all functions evaluated were completely restored in all cases treated with BUP-DEX, whereas the sensory and motor functions as well as proprioception were still affected in 37%, 83%, and 71% of individuals, respectively, at the 72-hour check point in the limbs treated with BLS and all functions recovered by the 96-hour check point.

These observations may be important to convey when this formulation is intended to be used as part of a conduction block in a nerve that contains motor fibers (e.g. femoral or sciatic nerves) since it is possible to see the occasional subject with prolonged motor and/or proprioceptive deficits past 72 hours postblockade. While extended motor block may be considered a disadvantage, partial motor deficit may not preclude assisted ambulation and might not necessarily have to delay discharge from the hospital. Certainly, further studies may be needed before we can categorically recommend its use in this application.

Limitations to our study included simultaneous bilateral evaluations of motor function, since a bilateral block may have increased the difficulty to precisely evaluate motor deficits. This study design was based on the 3Rs (Reduction, Refinement, Replacement). However, the main outcome of this study was to observe the difference in duration of the 2 candidate treatments. This study design allowed us to accomplish just that. Another limitation included the inability to capture data regarding the motor function in 2 individuals. This study was initially powered using the sensory function as main outcome variable. However, the remaining data regarding the motor function still showed statistical significance regarding time and the interaction of time and treatment. Lastly, a risk of biasing data may have been introduced since we had more than one person performing motor function evaluations. Gwet’s AC2 scores were > 0.8 and therefore considered acceptable. Additionally, and even though a defined scoring system was used, perhaps, a more objective, standardized, and quantitative nociceptive threshold may have been a more sensitive method of evaluation.

In conclusion, the blockade characteristics of the BLS were effective and long lasting. Motor and proprioceptive deficits may be inconsistent amongst patients and over time and may be affected for prolonged and similar duration as the sensory block.

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Supplementary Materials

Supplementary materials are posted online at the journal’s website: avmajournals.avma.org