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Bupivacaine and liposomal bupivacaine do not produce prolonged perineural anesthesia in a lameness model and are detectable beyond clinical effect in conditioned Thoroughbreds

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OBJECTIVE
To determine (1) the dose of liposomal bupivacaine (LB) to eliminate grade 2 of 5 lameness, the (2) duration of analgesia of LB versus bupivacaine hydrochloride (BH), and (3) LB pharmacokinetics versus BH.

METHODS
A reversible lameness model was validated in conditioned Thoroughbred horses (n = 12), aged 3 to 10 years. A dose-response trial compared subjective and objective lameness following abaxial sesamoid block with 25 mg BH/nerve or 30, 60, or 133 mg LB/nerve (n = 3/group). The LB dose that eliminated lameness and reduced lameness for the longest was used for blinded, randomized, crossover pharmacokinetic/pharmacodynamic trials (n = 12/group). Data were analyzed using a paired *t* test or Wilcoxon signed-rank test, $P < .05$.

RESULTS
The 133-mg/nerve dose of LB eliminated lameness in 3 of 3 horses in the dose-response trial, and lameness returned at 6, 36, and 72 hours. In the pharmacokinetic/pharmacodynamic trials, time to return of lameness greater than or equal to starting lameness was longer for LB compared to BH on subjective (LB, 12 hours, 4 to 24 hours; BH, 4 hours, 4 to 12 hours) and objective (LB, 12 hours, 4 to 24 hours; BH, 4 hours, 2 to 6 hours) evaluations. The terminal half-life was not different between formulations (LB, 17.8 hours ± 10.1; BH, 12.4 hours ± 6.3); however, LB had increased area under the concentration-versus-time curve from time 0 to infinity (LB, 388 ng·h/mL ± 117; BH, 63 ng·h/mL ± 18) and mean residence time (LB, 17.6 hours ± 2.4; BH, 3.9 hours ± 1.6).

CONCLUSIONS
Liposomal bupivacaine analgesia duration was greater than BH, but the median time until lameness returned was only 12 hours. Bupivacaine is quantifiable in serum and urine beyond loss of clinical effect.

CLINICAL RELEVANCE
A single, high-dose injection of LB is not effective for providing perineural analgesia over several days. Bupivacaine is detectable after the effect of the drug has worn off.

Keywords: bupivacaine, perineural anesthesia, lameness, pharmacokinetics, liposomes

Liposomal bupivacaine (LB) was developed to provide prolonged postoperative analgesia to reduce the reliance on opioids for pain control in people.[1] Approval by the FDA was granted to provide local postoperative analgesia for cruciate ligament surgery in dogs and onychectomy in cats for up to 72 hours. The prolonged effect of LB is reported to be due to the nonconcentric distribution of aqueous bupivacaine within lipid bilayer microvesicles. The bupivacaine is gradually released as the bilayer breaks down over 96 hours.[2] Liposomal bupivacaine is not approved for any indication in horses; however, several studies[3–7] have been performed to evaluate its effectiveness for local anesthesia.

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Local anesthetics are commonly used in equine practice for diagnostic anesthesia and intra- and postoperative analgesia. However, the utility of local anesthetics for postoperative analgesia has been limited by a short duration of action. Caudal epidural analgesia and indwelling perineural catheters for continuous injection of local anesthetics have been described to produce a prolonged effect; however, these require advanced techniques and are limited to specific locations.[8,9] Liposomal bupivacaine may provide a simple method for prolonged postoperative analgesia or alleviation of pain in horses with severe lameness due to conditions such as complex subsolar abscesses. However, if prolonged local anesthesia is achieved, the product could have the potential for misuse to mask lameness in equine athletes.

There has only been 1 study[10] on the pharmacokinetics (PK) of LB in horses following intra-articular injection. However, the analgesic effect of the dose was not evaluated. Various studies[3,5,7] using differing models of reversible foot lameness have evaluated local anesthesia of the digit following abaxial sesamoid perineural injection of LB. However, the selection of dose was somewhat arbitrary, and horses demonstrated decreased lameness but were not reported to consistently achieve elimination of lameness. A study in dogs[11] demonstrated differing PK for bupivacaine hydrochloride (BH) and LB, where the plasma concentration of bupivacaine was 4 to 6 times lower in the LB group following local infiltration of equivalent doses of each treatment. Thus, the dose to achieve elimination of lameness and the corresponding PK of that dose compared to BH merit investigation in horses.

The objectives of this study were to determine the dose of LB required to eliminate a grade 2 of 5 lameness based on the American Association of Equine Practitioners (AAEP) scale, the duration of analgesia, and the corresponding PK of LB compared to BH in conditioned Thoroughbred horses. We hypothesized that a higher total dose of LB would be required to eliminate lameness compared to BH and that the local anesthetic effect would be dose dependent for LB. We further hypothesized that LB would reduce lameness for a longer duration and would have a lower maximum concentration and longer elimination than BH.

Methods

Thoroughbred horses (n = 12) of mixed sex and aged 3 to 10 years that were systemically healthy, sound on subjective lameness exam, and able to gallop on a high-speed treadmill were enrolled from a dedicated research herd. Study procedures were approved by the IACUC (protocol 201808925).

Study design

Horses were conditioned on a high-speed treadmill for 2 months, and exercise continued throughout the study except during lameness model evaluations. There were 3 major phases of the study **(Figure 1)**. The first phase validated the lameness model over 96 hours. The second phase was a dose-response study where horses were randomized to 1 of 4 groups, a BH group and 1 of 3 LB dose groups (n = 3 horses/treatment group), to determine the dose of LB that eliminated lameness and provided analgesia the longest and to refine time points for lameness evaluation in phase 3. Finally, the dose selected from the dose-response trial was used in the PK/pharmacodynamic (PD) trials (phase 3). A balanced, randomized, crossover design was used to assign limb for lameness and treatment (BH or LB), with each horse having the opposite limb and treatment in the subsequent trial (n = 12 horses/treatment). All treatment and limb for lameness randomization was performed by 1 author (HAR). All local anesthetic injections were performed by a board-certified surgeon and sports medicine specialist (AJM), and all subjective lameness evaluations were performed by a board-certified surgeon (TMM) blinded to treatment randomization.

Animal care and health assessment

Horses were kept on pasture for the majority of the study but were kept in stalls whenever lameness was being evaluated. No medications were given within 4 weeks of the study, nor during the study, except as described. Horses had general physical examinations, CBCs, and plasma biochemistry to ensure systemic health and subjective lameness examinations to ensure soundness and were weighed prior to starting treadmill training and prior to each local anesthetic phase (Figure 1).

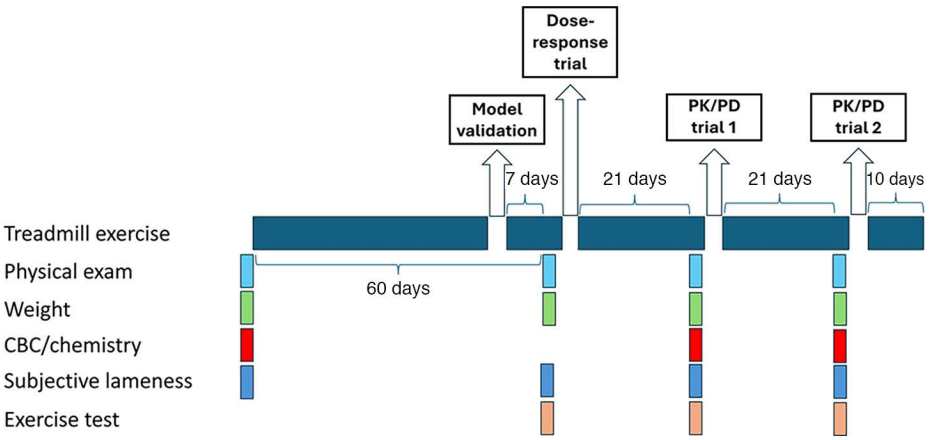

Figure 1—Schematic of overall study design. The color filled boxes represent the approximate timing of each of the interventions listed on the left. The arrows point to boxes indicating the major phases of the study. Phase 1, model validation; phase 2, dose response; phase 3, pharmacokinetic/pharmacodynamic (PK/PD) trials 1 and 2.

Conditioning program

Horses were conditioned on a high-speed treadmill for a minimum of 60 days and maintained using a standard training regimen 3 days per week. Exercise tests were completed at the conclusion of the initial conditioning period (prior to dose-response study) and prior to the PK/pharmacodynamic (PD) trials. The standard training regimen consisted of trotting for 0.6 km at 4 m/s, galloping for 2 km at 8 m/s, and trotting for 0.6 km at 4 m/s. The exercise test consisted of a 4-minute warmup at 4 m/s, followed by galloping at 13 m/s for 1.6 km and a 4-minute cooldown at 4 m/s. Heart rate was monitored every 5 minutes following the test and must have returned to < 50 beats per minute within 40 minutes to pass the test.

Lameness model

Horses were shod on both front hooves with modified keg shoes designed to accept a blunt, headless set screw to apply pressure to the lateral bar as previously described.[12] The hooves were trimmed and shoes reset every 4 to 8 weeks. Shoes were not reset within 2 weeks of lameness trials. To establish lameness, the screw was tightened until it just contacted the sole, and the horse was evaluated for lameness. The screw was progressively turned until the first appearance of a consistent lameness on hard ground at trot, and the number of turns was recorded. To facilitate the repeatability of screw placement, the straight edge of a protractor was aligned with the arm of an Allen key, which allowed precise determination of the number of full turns (1 turn = 360°) and partial turns, which were adjusted in increments as small as 45°. Horses were trotted in a straight line in hand 75 meters on a flat asphalt pathway for lameness evaluation. Lameness assessments were performed in duplicate and required that horses trot consistently in a straight line. Any trials where horses veered off the asphalt path, broke gait, or misbehaved in any way were stopped at the time of inconsistency and excluded. Subjective lameness grade was recorded according to the AAEP lameness grading scheme from 0 to 5. Objective lameness evaluation was performed using a body-mounted inertial sensor system with accelerometers placed midline on the poll and sacrum and a gyroscope secured on the dorsal aspect of the right front pastern (Equinosis Q with Lameness Locator; Equinosis). The screw was removed after each evaluation time point and replaced at the next time point. Once all lameness evaluations had been completed for each phase, the horse's hooves were packed with an iodinated cushion material for 24 hours. The number of screw turns to induce a grade 2 of 5 lameness and objective vector sum (VS) at time 0 (starting lameness = LT0) were established for each horse at the beginning of each phase and the initial and crossover stages of phase 3.

Model validation

Model validation was performed during the seventh week of exercise conditioning. Methodology was modified from Hoerdemann et al.[12] Horses were first determined to be sound prior to placing the screw. The screw was then placed and progressively tightened until the first appearance of a grade 2 of 5 lameness. Once the target subjective lameness was achieved, the VS and percentage of difference between duplicate assessments were recorded (time 0). The number of turns of the set screw to achieve the target lameness was also recorded. The screw was removed, and a single subjective evaluation was performed to ensure that the horse had returned to soundness. The set screw was placed using the same number of turns in duplicate trials at 24, 48, 72, and 96 hours. Replicate evaluations were recorded and the average VS for each time point calculated. The model was considered valid if the horses were graded 2 of 5 on each subjective examination and if the average VS values from duplicate examinations for each horse at each time point were within 20% of each other. The minimum, maximum, and median VS values of all horses were determined.

In order to define the VS values that would be accepted as corresponding to a grade 2 of 5 lameness for the purpose of establishing the lameness model for the dose-response and PK/PD trials, we calculated the smallest VS value that was within 30% of the overall median VS from all horses. Horses were given 1 week of no interventions other than the routine treadmill training before preparatory evaluation for the dose-response phase.

Dose-response

Preliminary work determined that a dose of 25 mg (5 mL) 0.5% BH/nerve injected SC over the medial and lateral palmar digital nerves at the base of the sesamoid bones was required to achieve soundness in this model. Soundness was defined as subjective grade 0 of 5 and VS ≤ 8.5 mm. Horses were randomized to the following treatment groups: 25 mg (5 mL) 0.5% BH/nerve (n = 3), 30 mg (2.25 mL) LB/nerve (LB_{30}; n = 3), 60 mg (4.5 mL) LB/nerve (LB_{60}; n = 3), and 133 mg (10 mL) LB/nerve (LB_{133}; n = 3). Horses were trotted in hand to ensure soundness, and then lameness was induced as described above (LT0). The hair and skin at injection sites were prepared with 4% chlorhexidine and isopropyl alcohol and allowed to dry. A new bottle of LB was used for each horse, and bottles were gently inverted prior to drawing up doses. For LB, doses were drawn into syringes using an 18-g 1.5-inch needle, and injections for BH and LB were performed with 23-g 1-inch needles. All blocks were performed with the limb unweighted, and limbs were rinsed with water before returning horses to their stalls. Subjective and objective lameness was performed at 10, 15, 30, and 60 minutes. If horses had no change in lameness by 60 minutes on both subjective and objective assessment, no further lameness evaluations were performed. Lameness evaluations otherwise continued at 4, 6, 8, 10, 12, 24, 36, 48, 60, 72, 84, and 96 hours. Lameness examinations were discontinued once lameness returned to ≥ LT0 (grade 2 to 5 of 5 and VS greater than minimum target VS; if VS was smaller than the starting

VS, then within 20% of starting VS on duplicate evaluations). Lameness evaluations were only stopped if criteria for both subjective and objective lameness were met for return lameness ≥ LT0. The time to return lameness ≥ LT0 was recorded separately for subjective and objective lameness assessment if these were not in agreement. Horses were evaluated following removal of the set screw at the last evaluated time point to determine if they returned to soundness. The lowest LB dose that achieved complete block (CB) in all horses and longest local anesthesia was selected for the PK/PD trials. Horses had a minimum washout period of 3 weeks where only treadmill training was performed.

Pharmacokinetic and PD trials

Pharmacodynamics

A grade 2 of 5 lameness was induced meeting the established VS criteria, and data were recorded from duplicate lameness examinations performed as described above (LT0). Local anesthesia was performed with either BH or the LB dose established from the dose-response phase according to treatment randomization as described above. Lameness evaluations were planned at 0.5, 1, 2, 4, 6, 12, 24, 36, 48, 60, 72, 84, and 96 hours as modified based on the results of the dose-response study. Lameness evaluations were stopped once lameness evaluation parameters were ≥ LT0 according to criteria described in the dose-response phase. If there was disagreement between subjective and objective assessments for return of lameness, evaluations were continued until there was agreement between both types of evaluation, but the time of return to lameness ≥ LT0 was recorded separately. Horses had a minimum of 3 weeks between PK/PD trials. The crossover trial was completed in the same manner as trial 1 except that the opposite treatment and limb for lameness induction were used. Horses were kept in treadmill training until the last sample for pharmacologic analysis was collected, 10 days after local anesthetic injection in PK/PD trial 2.

Sample collection and pharmacologic analysis

Blood was collected into 13-mL serum separator tubes before injection of BH and LB and at 5, 10, 15, 20, 30, and 45 minutes and 1, 2, 3, 4, 6, 8, 12, 24, 48, 72, 96, 120, 168, and 240 hours. Urine was collected by free-catch method prior to injection of local anesthetic and at 4, 8, 12, 24, 48, 72, 96, 120, 144, 168, and 240 hours. Blood samples were allowed to clot for 30 minutes, following which they were centrifuged and serum collected. Triplicate aliquots of serum and urine samples were stored at −80 °C until analysis.

Serum and urine bupivacaine concentrations were determined by use of a liquid chromatography–tandem mass spectrometry method as previously described.[10] A USP-standard bupivacaine and d9-bupivacaine were used. Calibration curves and negative control samples were prepared fresh for each quantitative assay. In addition, quality control samples (equine plasma fortified with analyte at 3 concentrations within the standard curve) were included with each sample set.

Prior to analysis, 1 mL of matrix was supplemented with 100 µL of methanol containing bupivacaine-D9 internal standard at 0.1 ng/µL, and the samples were vortexed briefly. For serum samples, methyl tert-butyl ether (5 mL) was added. Samples were mixed and then centrifuged at 4,000 X *g* for at least 10 minutes, and the top organic layer was transferred to a glass tube. Urine samples were hydrolyzed for 30 minutes with a β-glucuronidase enzyme at room temperature, then extracted by solid-phase extraction with CSDAU206 columns (UCT Inc). Columns were conditioned with 3 mL methanol, 3 mL deionized water, and 2 mL 1-mM phosphate buffer, pH 6. After sample was added, the columns were cleaned up with 3 mL deionized water and 3 mL methanol, dried for at least 5 minutes at max flow, and eluted with 3 mL 78:20:2 dichloromethane :isopropanol:ammonium hydroxide into glass tubes. Following extraction, all samples were dried under nitrogen at 40 °C, then dissolved in 120 µL of 5% acetonitrile in water with 0.2% formic acid. Twenty µL and 10 µL were injected into the liquid chromatography–tandem mass spectrometry system for serum and urine samples, respectively.

Quantitative analysis of serum and urine was performed on a TSQ Quantum Ultra (ThermoScientific) triple quadrupole mass spectrometer employing positive heated electrospray ionization coupled with an ultra-high performance liquid chromatography system operated in laminar flow mode. Detection and quantification were conducted using selective reaction monitoring of the initial precursor ion for bupivacaine (m/z, 289.2) and the internal standard bupivacaine-D9 (m/z, 298.2). The response for the product ions for bupivacaine (m/z, 84.1, 98.1, 140.1) and the internal standard (m/z, 149.2) were plotted, and peaks at the proper retention time were integrated using Qualbrowser (ThermoScientific) software. Graphing software was used to generate calibration curves and quantitate analytes in all samples by linear regression analysis. A weighting factor of 1/X was used for all calibration curves.

Pharmacokinetic analysis

Serum bupivacaine concentration-versus-time curves were analyzed noncompartmentally using commercial software (Pharmacokinetic Modeling Program; APL Analyst IT Solutions Pvt Ltd). Maximum concentration (C_{max}) was observed directly from the data, as was the time to maximum concentration. Where a second rise in serum bupivacaine concentrations was observed, the second peak C_{max} and second peak time to maximum concentration were noted. The terminal half-life was determined from the terminal slope as ($0.693/\lambda_z$). The area under the concentration-versus-time curve from time 0 to infinity ($AUC_{0-\infty}$) was calculated as the sum of trapezoids extrapolated to infinity. The volume of distribution calculated using the area under the concentration-versus-time curve method and corrected for bioavailability and the mean residence

time were determined using standard noncompartmental equations.

Statistical analysis

For the dose-response and PK/PD phases, subjective and objective lameness data were categorized as follows: CB (grade 0/5 and VS ≤ 8.5 mm), partial block (PB; grade 1/5, VS > 8.5 mm, and less than or equal to target minimum VS), or not blocked (NoB; grade 2 to 5/5 and VS greater than minimum target VS) at each time point. Time, VS, and PK parameters were treated as continuous variables, and AAEP grade and block category were treated as categorical variables. Vector sum, time to achieve CB, and time to return of lameness ≥ LT0 were assessed for normality using a Shapiro-Wilk test and expressed as mean ± SD or median (minimum-maximum) as appropriate. Statistical comparisons were performed using Wilcoxon signed-rank test with significance set at $P < .05$ (Statistix; Analytical Software). Differences between selected PK parameters associated with each bupivacaine formulation were examined using paired *t* tests, with $P < .05$ deemed significant (SigmaPlot, version 14.5; Inpixion).

Results

Seven mares and 5 geldings with a median age of 5.5 years (3 to 10) and median weight of 503 kg (408 to 575) were included in the study.

Model validation

Ten of 12 horses completed the validation of the lameness model. One horse was recovering from a subsolar abscess and was not sound. The second horse became markedly lame after a screw adjustment. Horses were graded 2 of 5 at each assessment once screw position was established. The vector sums were 23.7 ± 3.2 mm, 21.5 ± 7.8 mm, 21.9 ± 10.8 mm, 25.5 ± 6.2 mm, and 22.9 ± 8.5 mm at 0, 24, 48, 72, and 96-hour evaluations. The mean percentage of difference in VS between duplicate examinations was 12.9%. Overall, the median VS corresponding with a grade 2 of 5 lameness was 23.6 mm (16.0 to 29.1). Thus, the minimum target VS for establishing lameness for the dose-response and PK/PD phases was calculated to be 16.5 mm. All 10 horses were sound following screw removal.

Dose response

Complete block was achieved in 3 of 3 horses in the BH group from 15 to 30 minutes after injection. No LB_{30} horses were CB by 1 hour; 2 of 3 were PB starting at 30 minutes, and the final horse was assessed as PB on subjective lameness and NoB on objective evaluation at 60 minutes. In the LB_{60} group, 2 of 3 horses were CB at 30 minutes and 1 of 3 was PB at 1 hour. In the LB_{133} group, horses were CB at 10, 15, and 60 minutes. All horses that were CB or PB on 1 or both assessment methods at 60 minutes had continued evaluations. No PB horses progressed to achieve CB after 60 minutes.

Time to return of lameness ≥ LT0 was 4 hours in 3 of 3 horses in BH. In LB_{30}, 1 horse returned to lameness ≥ LT0 at 4 hours, and 2 horses returned to lameness ≥ LT0 at 6 hours. In the LB_{60} group, 1 horse had return of lameness ≥ LT0 at 6 hours and 1 horse at 10 hours, and 1 horse was PB at 8 hours but could not be evaluated further due to the nut breaking off the shoe during the 10-hour evaluation. In the LB_{133} group, return of lameness ≥ LT0 occurred at 6, 36, and 72 hours. Since the 133-mg/nerve dose (10 mL per nerve) of LB was the only dose to achieve CB in all horses and overall longer local anesthesia times, it was selected as the dose for the PK/PD trials.

Pharmacokinetic and PD trials

Pharmacodynamics

All LB horses and 11 of 12 BH horses achieved CB; 1 BH horse only achieved PB. Two horses in the BH group developed severe lameness (grade 4/5) and were not trotted for objective lameness data collection upon return of lameness. Five horses had lameness severe enough that only 1 subjective and objective lameness data collection was performed, 3 had been treated with BH, and 2 had been treated with LB. One of the 2 horses with significant lameness upon return of lameness in the LB

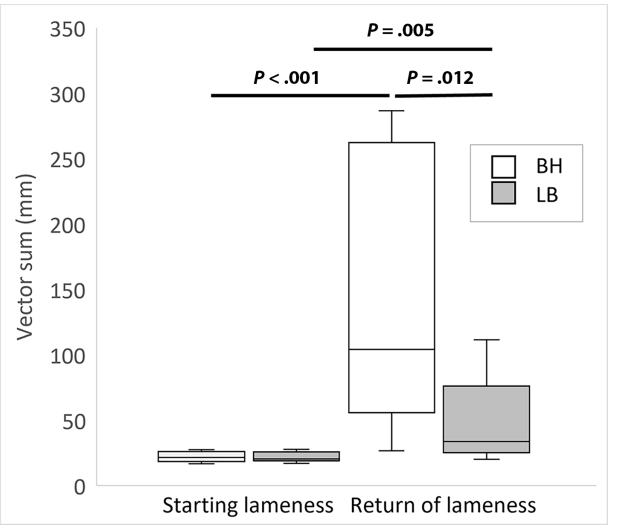

Figure 2—Horses (n = 12) had lameness induced using a reversible lameness model and had abaxial sesamoid nerve blocks performed with 25 mg bupivacaine hydrochloride (BH) per nerve or 133 mg liposomal bupivacaine (LB) per nerve in a randomized crossover design. Objective lameness evaluation using a horse-mounted inertial sensor system produced vector sum (VS) data at various time points. Box and whisker plots show the VS for each treatment group after the lameness was established (starting lameness) and at the time the local anesthetic was no longer effective and lameness returned to greater than or equal starting lameness. For the box and whisker plot, the line within the box represents the median, the upper and lower limits of the box represent the values of the 25th and 75th percentiles, and the whiskers indicate the range. The Wilcoxon signed-rank test was used to assess differences between groups; $P < .05$.

group developed severe swelling of the distal limb at 12 hours that resolved after 3 days of bandaging and anti-inflammatory therapy with 2 g phenylbutazone by mouth per day.

There was no difference in VS between BH and LB at LT0 **(Figure 2)**. At the time of return of lameness ≥ LT0, the VS was increased within both BH (P < .001) and LB (P = .005) compared LT0. The VS for BH at the time of return of lameness ≥ LT0 was higher than for LB (P = .012). The median subjective lameness grade at the time of return of lameness ≥ LT0 for BH was 3 (2 to 4), which was also higher than the median grade of 2 (2 to 3) for LB (P = .039).

The duration of CB was not different between BH and LB on objective evaluation but was longer for LB compared to BH on subjective evaluation (P = .031) **(Figure 3)**. Time to return of lameness ≥ LT0 was significantly longer for LB compared to BH on both subjective (P = .004) and objective (P = .008) evaluations.

Pharmacokinetics

The mean systemic dose of bupivacaine for BH was 0.10 ± 0.01 mg/kg and was 0.52 ± 0.05 mg/kg for LB. Serum bupivacaine concentration-versus-time curves showed a rapid single peak (C_{max}), whereas LB showed a similar C_{max} that was not different from that of BH followed by a second smaller peak in 11 of 12 horses **(Figure 4**; **Table 1)**. The C_{max} of LB was lower than BH when adjusted for the dose that was administered (P < .001). Although there was no difference in terminal half-life or $AUC_{0-\infty}$

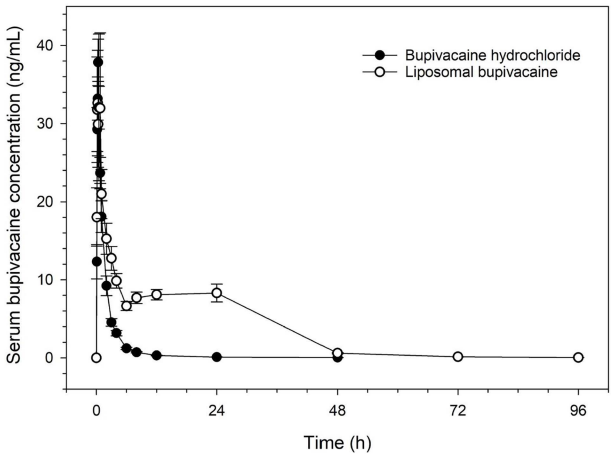

Figure 4—Serum bupivacaine concentration (mean ± SE)-versus-time curve after abaxial sesamoid perineural injection of 25 mg bupivacaine hydrochloride per nerve (total dose 50 mg) or 133 mg liposomal bupivacaine per nerve (total dose 266 mg) to 12 exercise-conditioned Thoroughbred horses in a randomized crossover design.

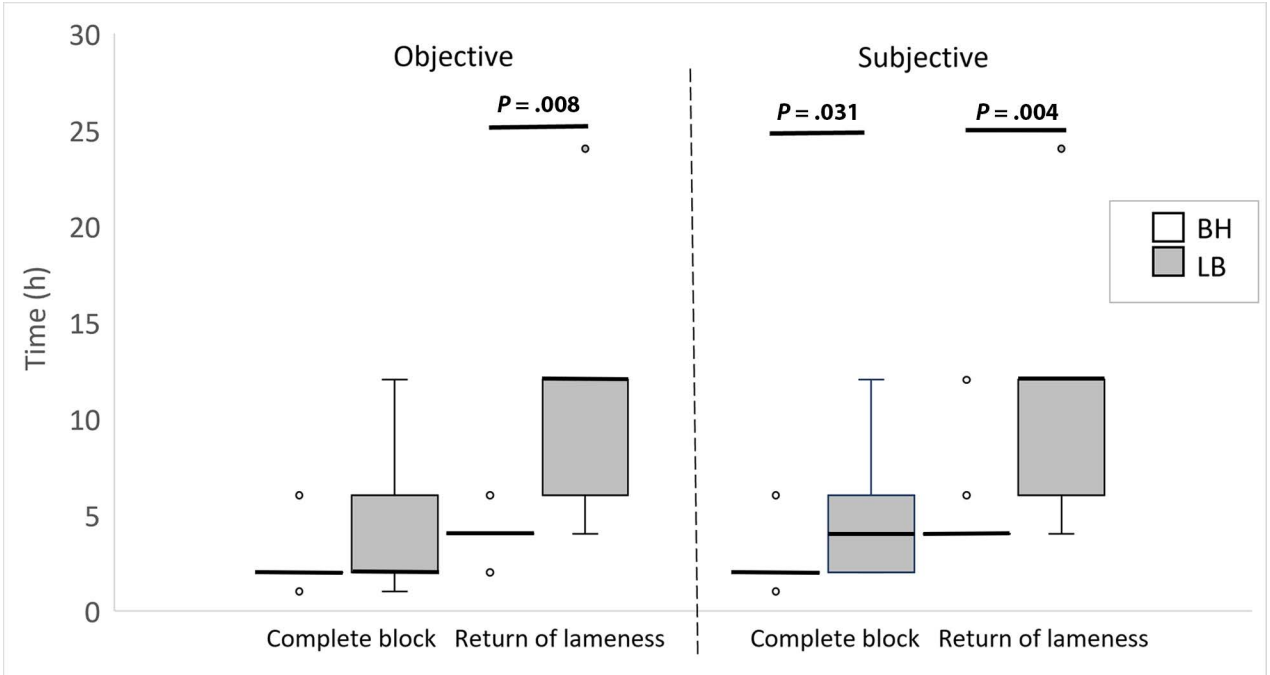

Figure 3—Horses (n = 12) had lameness induced using a reversible lameness model and had abaxial sesamoid nerve blocks performed with 25 mg BH per nerve or 133 mg LB per nerve in a randomized crossover design. Lameness was subjectively graded according to the American Association of Equine Practitioners scheme and objective lameness evaluation was performed using a horse-mounted inertial sensor system at various time points. Horses were categorized as being completely blocked, partially blocked, or not blocked at each time point. Box and whisker plots show the last time at which each treatment group was categorized as being completely blocked and the first time of categorization as no longer being blocked (return of lameness). For the box and whisker plot, the bold line within the box represents the median, the upper and lower limits of the box represent the values of the 25th and 75th percentiles, the whiskers indicate the range, and the dots indicate outlier data points. The Wilcoxon signed-rank test was used to assess differences between groups; P < .05.

Table 1—Pharmacokinetic parameters (mean ± SD) determined after medial and lateral abaxial sesamoid nerve block with a total dose of 50 mg of bupivacaine hydrochloride (BH) or 266 mg liposomal bupivacaine (LB) in conditioned Thoroughbred horses (n = 12).

Parameter	BH	LB
C_{max} (ng/mL)	40.2 ± 9.4	37.4 ± 34.7
C_{max} per mg dose	0.80 ± 0.19[a]	0.14 ± 0.13[b]
t_{max} (h)	0.33 ± 0.08	0.52 ± 0.54
Second peak C_{max} (ng/mL)	—	10.2 ± 2.8[c]
Second peak t_{max} (h)	—	15.1 ± 7.3[c]
$t_{1/2\lambda}$ (h)	12.4 ± 6.2	17.8 ± 10.1
$AUC_{0-\infty}$ (ng·h/mL)	63 ± 18[a]	388 ± 117[b]
$AUC_{0-\infty}$ per mg dose	1.3 ± 0.4	1.3 ± 0.4
MRT (h)	3.9 ± 1.6[a]	17.6 ± 2.4[b]
Vd_{area}/F (L/kg)	28 ± 14	42 ± 22

$AUC_{0-\infty}$ = Area under the concentration-versus-time curve from time 0 to infinity. C_{max} = Maximum serum concentration. MRT = Mean residence time. $t_{1/2\lambda}$ = Terminal half-life. t_{max} = Time to maximum concentration. Vd_{area}/F = Volume of distribution calculated using the area under the concentration-versus-time curve method and divided by bioavailability.

[a–b]Different superscript letters indicate significant difference between groups based on paired *t* tests ($P < .05$). [c]n = 11.

when adjusted for the administered dose, the overall $AUC_{0-\infty}$ ($P < .001$) and mean residence time ($P < .001$) were higher in LB compared to BH.

Urine bupivacaine concentrations were determined for 11 horses and plotted on a concentration-versus-time curve **(Figure 5)**. For 1 horse, both BH and LB urine samples could not be analyzed due to the presence of an interfering analyte.

The limit of quantitation (LOQ) of the assay was 0.010 ng/mL. All horses had no detectable bupivacaine on time-0 serum and urine samples. The median time for horses to have serum levels of bupivacaine below the LOQ was 72 hours (48 to 120) for BH and 168 hours (120 to 240) for LB. The last time

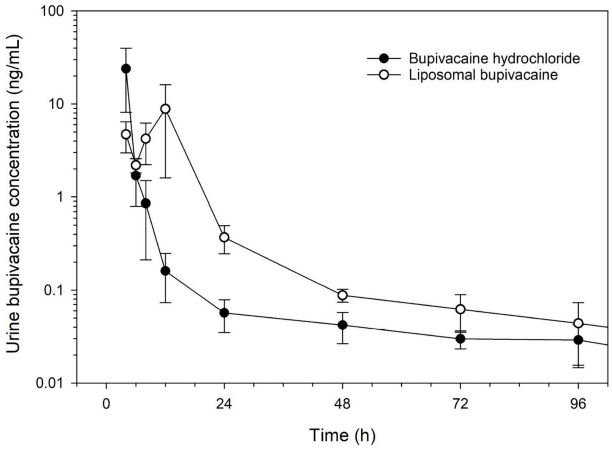

Figure 5—Urine bupivacaine concentration (mean ± SE)-versus-time curve after abaxial sesamoid perineural injection of 25 mg bupivacaine hydrochloride per nerve (total dose 50 mg) or 133 mg liposomal bupivacaine per nerve (total dose 266 mg) to 11 exercise-conditioned Thoroughbred horses in a randomized crossover design.

that urine bupivacaine was above LOQ ranged from 24 to > 240 hours for BH and 96 to > 240 hours for LB. There was no difference between BH or LB for median serum bupivacaine concentration at the last reported time point the horse was documented to be CB (BH, 7.98 ng/mL [1.33 to 20.91]; LB, 7.74 ng/mL [2.80 to 18.32]). However, the median serum concentration at the time of return of lameness ≥ LT0 for BH 2.95 ng/mL (0.32 to 9.61) was lower than for LB 6.36 ng/mL (2.85 to 10.50) ($P = .016$).

Discussion

We found that a higher dose of LB was required to achieve CB in our model compared to the dose of BH; LB at a similar dose to BH did not achieve CB in any horses. Further, we found that the largest dose of LB investigated (133 mg/nerve) was required to consistently eliminate lameness on subjective and objective evaluation and that duration of local anesthesia, although longer than BH, was substantially shorter than 72 hours in all but 1 instance. In a study[3] using a hoof clamp model of lameness, equivalent doses of 10 mg/nerve of BH (2 mL) and LB (0.75 mL) were used. Liposomal bupivacaine initially reduced lameness in 3 of 6 horses. In another study,[5] equivalent volumes (3 mL) of 0.75% BH (22.5 mg) or LB (39.9 mg) per nerve were used for abaxial sesamoid blocks in a reversible sole pressure lameness model using a grade 3 of 5 lameness in 6 horses in a crossover design. Similar to our study, the VS was reduced at 1 hour but not 6 hours postblock for BH and at 1, 6, and 24 hours for LB. Finally, a third study[7] used a reversible frog pressure model to induce a grade 3 of 5 lameness and performed abaxial sesamoid nerve blocks with saline, 0.25 mg/kg LB (62.5 mg or 4.7 mL per nerve for a 500-kg horse), or 0.50 mg/kg LB (125 mg or 9.4 mL per nerve for a 500-kg horse) in 6 horses per group. Kaplan-Meier survival analysis was used to determine a decrease in lameness for 4.5 (3 to 6) hours and 7 (4 to 24) hours and a time of return of lameness of 7 (4 to 24) hours and 9 (3 to 48) hours for low- and high-dose LB, respectively. The different methods of data analysis between studies may explain some of the differences in results. Rather than assessing a decrease in lameness based on numerical comparisons of median values, we required metrics to meet specific targets to be classified as CB, PB, or NoB. Although the time to return of lameness was similar for our 60-mg LB group and the 0.25-mg/kg group in another study,[7] this dose did not eliminate lameness in all horses, which was a criterion for our study. Differences in the lameness model, breed, foot characteristics, and trotting surface may also have played a role. Overall, the studies have found that with sufficient doses, LB produced analgesia longer than BH and that the duration of analgesia was highly variable and fell short of 72 hours.

Dosing for LB has most commonly been expressed as a total body dose. Doses recommended for FDA-approved indications in dogs (5.3 mg/kg) and cats (10.6 mg/kg) are substantially larger than the doses used in any of the lameness studies in horses.[3–5,7]

Equivalent doses in the horse would require injection of approximately 200 to 400 mL of LB for a 500-kg horse, which would be cost prohibitive and impractical in the digit. The probability that action potential blockade will occur is related to dose and concentration of the local anesthetic injected, the length of the nerve exposed to local anesthetic, myelination, and the size of unmyelinated fibers.[13] When LB is injected as a regional block, it is widely dispersed through multiple tissue planes as LB cannot diffuse through tissues; rather, small amounts of bupivacaine are released into the tissue immediately surrounding the liposome, where the bupivacaine interacts with smaller nerve endings.[14] However, in the case of perineural blockade in the horse, relatively large nerves with a high proportion of unmyelinated fibers are the target tissue.[15] Thus, there may be a ceiling where no further addition of volume will increase contact with a single large nerve. Further, as tissues expand during injection, LB may travel along tissue planes away from the nerve. This additional volume may not contribute to blockade, which may explain some of the variation in the duration of effect.

The dose rates of BH (0.1 mg/kg) and LB (0.52 mg/kg) used in this study were substantially lower than in a PK study[11] following brachial plexus perineural injection in rabbits and dogs, which used 9 mg/kg BH and 9, 18, and 30 mg/kg LB. Similar to our study, BH showed a steep peak with rapid elimination. Liposomal bupivacaine had a small initial peak followed by a flat curve that began to decline after 48 hours.[11] In our study, an initial peak similar to BH occurred in the LB group followed by more consistent plasma concentrations that declined more rapidly after 24 hours. The concentration-versus-time curve is consistent with an extended-release product along with the greater area under the concentration-versus-time curve for LB versus BH, whereas terminal half-life did not differ. In addition, the terminal half-life was similar to 0.1 mg/kg LB injected IA in exercised Thoroughbreds, suggesting that terminal half-life is related to the rate of hepatic metabolism and is not affected by total body dose.[10] The more rapid decline in serum bupivacaine concentrations in this study compared to rabbits and dogs may be due to more rapid breakdown of liposomes and/or vascular and lymphatic uptake of bupivacaine in horses, which may also explain the shorter duration of effect. It is also possible that the locomotion required to perform the lameness examinations may have increased the rate of liposomal breakdown and/or bupivacaine clearance due to increased blood flow during exercise. A parallel exercised-versus-sedentary PK study using doses of LB determined to be effective would be required to further evaluate this possibility.

The limitations of this study include the small number of horses used for the dose-response phase and loss of data points due to unexpected lameness or malfunctions of equipment. In addition, although we validated the lameness model in unblocked horses initially, this may not reflect changes in pain stimulus once horses are blocked. It was interesting that many horses had increased lameness metrics at the time of return of lameness ≥ LT0, with horses blocked with BH being significantly lamer than after LB block.

In conclusion, lameness returned at 12 (4 to 24) hours after perineural abaxial sesamoid block of the digit with 10 mL LB per nerve, which is greater than the return of lameness at 4 (4 to 12) hours with 5 mL BH per nerve. The initial C_{max}/mg of bupivacaine for LB is lower than BH, but concentrations of bupivacaine are higher at the end point of clinical effect for LB compared to BH.

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