Effect of anesthetizing individual compartments of the stifle joint in horses with experimentally induced stifle joint lameness

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Objective—To evaluate the effects of sequential anesthesia of the individual compartments of the equine stifle joint on lameness induced by intra-articular deposition of interleukin (IL)-1β.

Animals—6 horses.

Procedures—For each horse, baseline hind limb lameness was first evaluated. A randomly selected compartment of 1 stifle joint was then injected with IL-1β to induce synovitis and lameness; subsequently, the same compartment was anesthetized with 2% mepivacaine hydrochloride, and lameness was reevaluated. Two weeks later, baseline lameness was evaluated, and lameness was similarly induced; thereafter, the 2 synovial compartments of the stifle joint not injected with IL-1β were anesthetized sequentially in random order (ie, first and second blocks); lameness was evaluated after each block. Finally, the IL-1β–treated compartment was anesthetized (third block); lameness was again evaluated. This second experiment was repeated for the contralateral stifle joint 2 weeks later. Throughout the study, lameness was quantified objectively by assessing vertical pelvic movement asymmetry with a wireless, inertial sensor-based system.

Results—Intra-articular deposition of IL-1β induced lameness in all injected limbs. In the first experiment, anesthesia of the compartment injected with IL-1β resulted in a significant decrease in lameness, with vertical pelvic movement asymmetry approaching baseline. In the second experiment, lameness improved significantly after the second and third blocks and was almost completely abolished after all 3 synovial compartments were anesthetized.

Conclusions and Clinical Relevance—In horses, lameness caused by a lesion in 1 compartment of a stifle joint can be improved more by instillation of local anesthetic solution into that compartment than by anesthesia of the other compartments. (*Am J Vet Res* 2014;75:19–25)

The stifle joint is the largest and the most complex of all the articulations in a horse and is composed of 2 joints: the femoropatellar and the femorotibial joints.[1] The femorotibial joint is subdivided into lateral femorotibial and medial femorotibial compartments. In a study by Reeves et al[2] and a study by Vacek et al,[3] injection of a gelatin-based dye or latex into the femoropatellar joint demonstrated a direct anatomic communication between that joint and the medial femorotibial compartment alone in both stifle joints of 15 of 23 (65%) horses and of 18 of 30 (60%) horses, respectively. In the study by Reeves et al,[2] 4 of the 23 (17.4%) horses also had communication between the femoropatellar joint and both compartments of the femorotibial joint. In the study by Vacek et al,[3] latex injected into the medial femorotibial compartment entered directly into the femoropatellar joint of 24 of 30 (80%) stifle joints from 14 horses. Conversely, both studies[2,3] showed poor communication between the lateral femorotibial compartment and the other 2 compartments of the stifle joint (ie, the femoropatellar joint and the medial femorotibial compartment). Authors of one of those studies[2] suggested that chronic effusion of 1 compartment of the stifle joint as a result of inflammation may enable direct communication to develop among all 3 compartments of the stifle joint, whereas others have proposed that inflammation of 1 or more of the compartments could result in obstruction of the anatomic communi-

Abbreviation

IL	Interleukin

Received June 18, 2013.
Accepted July 25, 2013.
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cations.[4–6] More recently, in an in vitro study,[5] diffusion of local anesthetic solution between compartments of the stifle joint was greater than that previously assumed on the basis of results of anatomic, latex injection, and contrast arthrographic studies.[2,3]

Frequently, local anesthetic solution is instilled into 1 of the 3 synovial compartments of the stifle joint of lame horses to isolate the site of lameness-causing pain. The uncertainty regarding the anatomic or functional communication among the synovial compartments of the stifle joint has led to a common recommendation to inject all 3 compartments with a local anesthetic solution to isolate the site of lameness-causing pain.[2,3,5,7]

For research purposes, various techniques have been used to induce lameness localized to a synovial cavity in horses, including intra-articular administration of 1% solution of sterile carrageenan,[8] amphotericin B,[9] *Escherichia coli* lipopolysaccharide,[10] Freund's complete adjuvant,[11] or recombinant equine IL-1β.[12] The cytokine IL-1β is known to play a fundamental role in naturally occurring synovial inflammation.[13]

The objective of the study reported here was to evaluate the effects of sequential anesthesia of the individual compartments of the equine stifle joint on lameness resulting from transient synovitis induced by intra-articular deposition of IL-1β in 1 randomly selected synovial compartment. We hypothesized that deposition of recombinant equine IL-1β into a synovial compartment of the stifle joint would result in clinically apparent lameness and that instillation of local anesthetic solution into any of the 3 compartments would significantly reduce lameness, regardless of which compartment was injected with IL-1β.

Materials and Methods

Horses—Six mares from the University of Tennessee teaching herd, ranging in age from 9 to 16 years (mean, 13 years; median, 12.5 years), were used in the study. Upon enrollment, each horse underwent a preliminary, objective evaluation for lameness while trotting on an asphalt surface in a straight line. Lameness was assessed objectively by measuring asymmetry of vertical pelvic movement with body-mounted, wireless, inertial sensors.[14–18,a] The study was approved by the University of Tennessee Institutional Animal Care and Use Committee.

The study was conducted in 2 parts. First, a randomly selected compartment of 1 stifle joint of each horse was injected with IL-1β to induce synovitis, and the horse was examined for lameness. After lameness was induced, the same compartment of the stifle joint was anesthetized (by means of injection of local anesthetic solution [ie, a block]), and the horse's lameness was reevaluated (experiment 1). Second, a randomly selected compartment of 1 stifle joint of each horse was injected with IL-1β, after which the horse was examined for lameness; subsequently, the 2 synovial compartments of the stifle joint not injected with IL-1β were anesthetized sequentially in random order (ie, first and second blocks); lameness was evaluated after each block. Finally, the IL-1β–treated compartment was anesthetized (third block); lameness was again evaluated (experiment 2). This second experiment was repeated for the contralateral stifle joint 2 weeks later.

Experiment 1—During experiment 1, baseline lameness was objectively assessed, and then 200 ng (2 mL) of recombinant equine IL-1β[b] was deposited into 1 randomly selected compartment of the right stifle joint to induce synovitis-associated lameness. Recombinant equine IL-1β powder was reconstituted with sterile saline (0.9% NaCl) solution to a concentration of 100 ng/mL and stored in 10-mL aliquots at –20°C. Aliquots were thawed 24 hours before intra-articular administration. Radiodense contrast medium (5 mL of radiodense contrast medium[c] diluted to 10 mL with sterile saline solution) was simultaneously injected with the IL-1β; postinjection lateromedial and caudocranial radiographic views of the injected stifle joint were acquired and reviewed to ensure successful deposition of IL-1β into the intended synovial compartment.

Each horse was evaluated objectively for lameness 16 hours after intra-articular deposition of recombinant equine IL-1β. At the 16-hour time point, a sample (1 to 2 mL) of synovial fluid was collected from the compartment injected with IL-1β. The synovial fluid total protein concentration and WBC count were assessed for comparison with reference intervals. Then, the same compartment of the stifle joint that was injected with IL-1β was anesthetized with an injection of a mixture of 20 mL of 2% mepivacaine hydrochloride[d] and 5 mL of radiodense contrast medium. Successful deposition of the local anesthetic solution into the designated compartment of the stifle joint was ascertained by radiographic examination of the stifle joint. The horse was then walked in hand until lameness was evaluated objectively 20 minutes after injection. A final, objective evaluation of lameness was completed 24 hours after intra-articular deposition of the IL-1β. Horses with lameness that persisted to or after the 24-hour time point were treated with phenylbutazone[e] (2.2 mg/kg, IV, q 12 h) until lameness resolved.

Experiment 2—The same horses were used for experiment 2, which was conducted at least 2 weeks after experiment 1. After baseline lameness was evaluated objectively, a randomly selected compartment of 1 stifle joint was injected with 2 mL of recombinant equine IL-1β (100 ng/mL), along with 5 mL of a radiodense contrast medium diluted to 10 mL with sterile saline solution. Successful deposition of the IL-1β solution into the designated compartment of the stifle joint was ascertained by radiographic examination of the stifle joint. Lameness was evaluated objectively 16 hours later. Immediately thereafter, 1 of the 2 synovial compartments of the stifle joint not injected with IL-1β was randomly chosen and injected with 20 mL of 2% mepivacaine hydrochloride and 5 mL of radiodense contrast medium (ie, first block). Successful deposition of the local anesthetic solution into the designated compartment of the stifle joint was ascertained by radiographic examination of the stifle joint. Another objective lameness evaluation was performed 20 minutes after this first block. Immediately after completing the objective lameness evaluation, the other of the 2 compartments of the stifle joint not injected with IL-1β was injected with 20 mL of mepivacaine and 5 mL of radiodense contrast medium (second block). Successful deposition of the local anesthetic solution into the

designated compartment of the stifle joint was ascertained by radiographic examination of the stifle joint. Lameness was evaluated objectively 20 minutes after the second block. Finally, the third compartment of the stifle joint (the one originally injected with recombinant equine IL-1β) was anesthetized with 20 mL of mepivacaine and 5 mL of radiodense contrast medium (ie, third block). Successful deposition of the local anesthetic solution into the designated compartment of the stifle joint was ascertained by radiographic examination of the stifle joint. Lameness was evaluated objectively 20 minutes after the third block. Horses were walked in hand between administration of each intra-articular block and subsequent evaluation of lameness. Each horse was once more evaluated objectively for lameness 24 hours after lameness was induced; if lame at that time point, the horse was administered phenylbutazone (2.2 mg/kg, IV, q 12 h) until lameness had completely resolved.

The same procedures were performed for the contralateral stifle joint of each horse after a washout period of at least 2 weeks. Throughout the study, successful centesis of the synovial compartments of the stifle joint was ensured by examining orthogonal radiographic views of the treated stifle joint obtained immediately after each arthrocentesis. If the radiodense contrast medium was not identified within the compartment intended to be injected, the data were discarded, and the experiment was repeated after a minimum washout period of 2 weeks.

Techniques of arthrocentesis—All arthrocenteses were performed with the horses restrained in a stock, with or without the use of a nose twitch, depending on the horse's demeanor. Arthrocentesis of the femoropatellar joint was performed by inserting a 3.8-cm, 20-gauge hypodermic needle perpendicular to the sagittal plane, approximately 5 cm proximal to the palpable, lateral edge of the lateral tibial condyle and just caudal to the palpable lateral trochlear ridge of the femur.[19] The needle was inserted until its tip contacted bone and was then withdrawn slightly.

Arthrocentesis of the medial compartment of the femorotibial joint was performed by inserting a 3.8-cm, 20-gauge hypodermic needle into an indentation between the medial patellar ligament and the tendon of the sartorius muscle, approximately 2.5 cm proximal to the tibial plateau.[20] The needle was advanced in a cranial-to-caudal direction, parallel to the ground and to the sagittal plane.

Arthrocentesis of the lateral compartment of the femorotibial joint was performed by inserting an 8.9-cm, 20-gauge spinal needle between the middle and lateral patellar ligaments, approximately 2.5 cm proximal to the tibial plateau and advancing the needle in a carnial-to-caudal direction, parallel to the ground and to the sagittal plane. The needle was advanced until it contacted bone at a depth of approximately 2.5 cm.

Evaluation of lameness—In both experiments, lameness of the IL-1β–treated pelvic limb was detected and quantified by measuring asymmetry of vertical pelvic movement with the aid of a commercially available, body-mounted, wireless, inertial sensor system[18] while the horse trotted in a straight line on a hard surface. For this evaluation, a vertical accelerometer was attached between the tubera sacrale on the dorsum of the horse's pelvis, another vertical accelerometer was attached to the center of the poll (ie, the region of the occipital protuberance at the back of the skull [even though data acquired by this latter sensor were not used in the study]), and a gyroscope was placed on the dorsal surface of the pastern of the right thoracic limb. Vertical acceleration of the pelvis and angular velocity of the distal portion of the right thoracic limb were transmitted wirelessly at 200 Hz to a tablet computer and analyzed as described by Keegan et al.[14]

With this system, asymmetry of vertical pelvic movement was determined by measuring differences in maximum and minimum pelvic height (in mm) between right and left halves of the stride for every stride. The difference in maximum pelvic height was obtained by subtracting the maximum height of the pelvis after pushoff of the right pelvic limb from the maximum height of the pelvis after pushoff of the left pelvic limb. Similarly, difference in minimum pelvic height was obtained by subtracting the minimum height of the pelvis during stance of the left pelvic limb from minimum height of the pelvis during stance of the right pelvic limb. By convention, a positive difference in maximum pelvic height indicates deficiency of right pelvic limb pushoff and a negative value indicates deficiency of left pelvic limb pushoff. Similarly, a positive difference in minimum pelvic height indicates deficiency of impact of the right pelvic limb and a negative value indicates deficiency of impact of the left pelvic limb.

In the 2 experiments, a single trial consisted of trotting a horse back and forth in a straight line for 20 m on an asphalt surface. Three trials were collected at each time point (ie, baseline, 16 hours after intra-articular injection of IL-1β, 20 minutes after each anesthesia [single block in experiment 1 and first, second, and third blocks in experiment 2], and 24 hours after injection of IL-1β). The mean differences in maximum and minimum pelvic height for all 3 trials were used as the end measure at each time point for each horse. For this system, the thresholds between nonlame and lame status for maximum and minimum pelvic height differences between the left and right limbs have been experimentally determined to be approximately 3 mm.[18] All differences in maximum and minimum pelvic height after induction of lameness in the left pelvic limb were multiplied by –1 to facilitate statistical analyses.

Statistical analysis—Median differences in maximum and minimum pelvic height at each time point for all horses in each experiment were compared on the basis of the nonparametric equivalent of repeated-measures ANOVA (Friedman test). Post hoc multiple comparisons between time points were determined with the Conover nonparametric test, which is equivalent to the Fisher least significant difference method. Percentage improvement in median differences in maximum and minimum pelvic height after individual blocks was calculated as follows: (difference after IL-1β administration – difference after block)/(difference after IL-1β administration – difference at baseline).

Level of significance was set at a value of $P < 0.05$. All analyses were performed with commercially available statistical software.[f]

Results

Experiment 1—In experiment 1, the right stifle joint was used in 5 horses and the left stifle joint was inadvertently used in 1 horse. During experiment 1, differences in maximum or minimum pelvic height for 3 of the 6 horses before deposition of recombinant equine IL-1β into one of the compartments of the stifle joint were slightly greater than the established threshold of 3 mm, indicating that these 3 horses were subtly lame on 1 pelvic limb. None of these subtly lame horses exceeded the threshold for maximum or minimum pelvic height differences by > 1.3 mm. However, the mean and median differences in maximum and minimum pelvic height for all horses before induction of lameness were less than the established threshold.

Instillation of 200 ng of recombinant equine IL-1β into the medial femorotibial compartment (n = 2) or lateral femorotibial compartment (2) or into the femoropatellar joint (2) was successful for each horse, as indicated by the radiographic views of the treated stifle joints acquired immediately after injection. Results of subjective evaluation indicated that all horses developed easily discernible lameness of the treated limb 16 hours after intra-articular injection of IL-1β. Lameness was confirmed by objective evaluation. Median difference in maximum pelvic height increased from 2.0 to 13.0 mm ($P < 0.001$), and median difference in minimum pelvic height increased from –2.0 to 22.9 mm ($P < 0.001$).

Injection of the local anesthetic solution directly into the compartment of the stifle joint treated with IL-1β caused the median difference in maximum pelvic height to decrease significantly to 2.4 mm, which was not significantly ($P = 0.154$) different from the baseline value. The difference in maximum pelvic height improved at least 50% after each injection of local anesthetic solution except 1, which was administered into the lateral femorotibial compartment of 1 horse. Similarly, median difference in minimum pelvic height (8.0 mm) was also significantly ($P < 0.001$) lower than that measured after lameness was induced but remained significantly ($P = 0.004$) higher than the baseline value. The difference in minimum pelvic height decreased by at least 50% after the injection of the local anesthetic solution for all but 2 evaluations, one after the lateral femorotibial compartment of one horse was injected and the other after the femoropatellar joint of another horse was injected. Twenty-four hours after lameness was induced (also at least 8 hours after intra-articular administration of local anesthetic solution), median differences in maximum (7.9 mm) and minimum (17.3 mm) pelvic height were significantly greater than values at baseline ($P < 0.001$) and values after intra-articular anesthesia ($P < 0.009$; **Figure 1**).

A sample of synovial fluid was collected from the compartment injected with 200 ng of recombinant equine IL-1β from 5 of 6 horses immediately before intra-articular deposition of the local anesthetic solution. The WBC count (mean, 46.76 × 10^3 WBCs/μL [range, 17 × 10^3 WBCs/μL to 87.04 × 10^3 WBCs/μL]; reference interval, < 2 × 10^3 WBCs/μL) and total protein concentration (mean, 5.24 g/dL [range, 4.6 to 5.7 g/dL]; reference interval, < 2.5 g/dL) were consistent with successful induction of synovitis.

Experiment 2—During evaluation for baseline lameness, one of the horses was determined to be subtly lame on 1 pelvic limb, as demonstrated by an increased difference in maximum pelvic height (3.2 mm in trial 1 and 3.8 mm in trial 2). For all horses, however, median baseline differences in maximum (–0.4 mm) and minimum (0.4 mm) pelvic height were less than the established threshold.

Recombinant equine IL-1β was injected successfully into the intended synovial compartment of the stifle joint during 11 of 12 attempts (femoropatellar joint [n

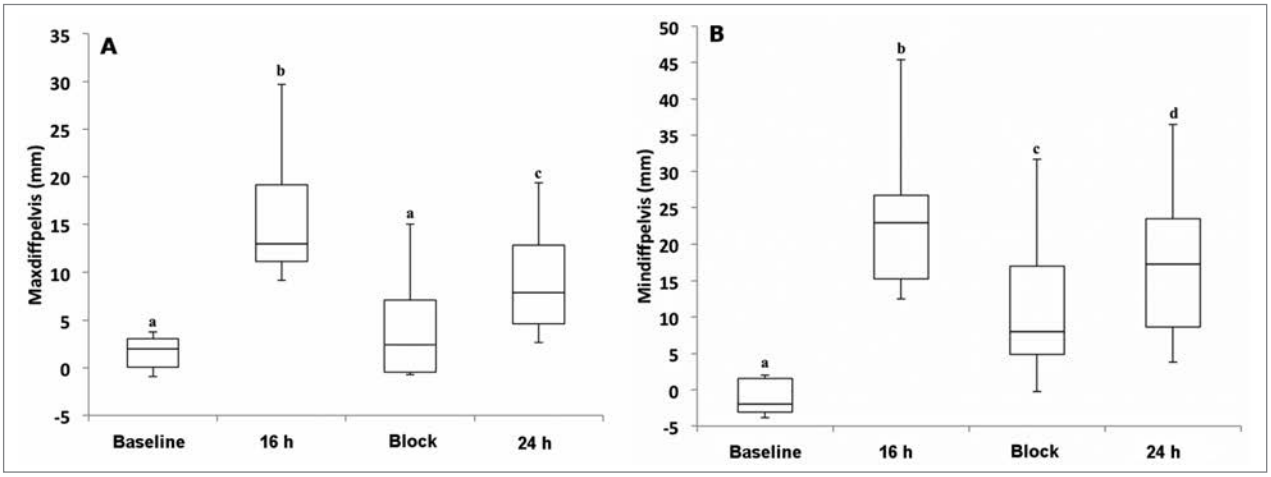

Figure 1—Box-and-whisker plots of differences in maximum (maxdiffpelvis; A) and minimum (mindiffpelvis; B) pelvic height between right and left strides of 6 horses determined while horses trotted in a straight line on a hard surface before (baseline), at 16 hours after deposition of 200 ng of recombinant equine IL-1β in a randomly selected compartment of 1 stifle joint (right [n = 5] or left [1]), at 20 minutes after anesthesia of the compartment of the stifle joint injected with IL-1β (block), and at 24 hours after intra-articular deposition of IL-1β. Compartment anesthesia involved injection of 20 mL of 2% mepivacaine hydrochloride. To enable radiographic evaluation of successful injection of the intended compartment with either IL-β or local anesthetic solution, 5 mL of radiodense contrast medium was also injected. For each box, the horizontal line represents the median, and the upper and lower boundaries represent the 75th and 25th percentile, respectively. The upper and lower whiskers represent the 90th and 10th percentile, respectively. [a–d]For a given variable, median values with different letters differ significantly ($P < 0.05$).

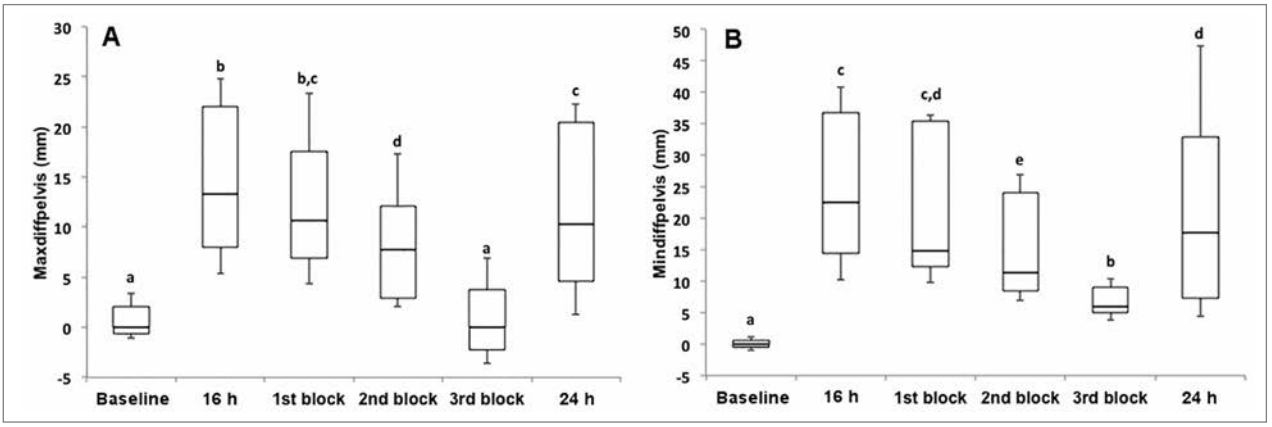

Figure 2—Box-and-whisker plots of differences in maximum (maxdiffpelvis; A) and minimum (mindiffpelvis; B) pelvic height between right and left strides of the 6 horses in Figure 1 determined while horses trotted in a straight line on a hard surface before (baseline), at 16 hours after deposition of 200 ng of recombinant equine IL-1β in a randomly selected compartment of 1 stifle joint, at 20 minutes after anesthesia of one of the compartments of the stifle joint not injected with IL-1β (first block), at 20 minutes after anesthesia of the other compartment of the stifle joint not injected with IL-1β (second block), at 20 minutes after anesthesia of the compartment of the stifle joint injected with IL-1β (third block), and at 24 hours after intra-articular deposition of IL-1β. The intent was to repeat the experiment for the contralateral stifle joint 2 weeks later; however, data were available for only 9 of 12 joints (5 horses). [a–e]For a given variable, median values with different letters differ significantly ($P < 0.05$). *See* Figure 1 for remainder of key.

= 3], lateral femorotibial compartment [4], and medial femorotibial compartment [4]). One horse became agitated during attempted centesis of the femoropatellar joint, preventing completion of centesis. Results of subjective evaluation indicated that all horses successfully injected with IL-1β developed easily discernable lameness in the treated limb 16 hours after injection. Objective measurements of lameness could not be obtained for 1 horse because that horse was unwilling to trot after lameness was induced in either stifle joint; therefore, data obtained from only 9 of 12 attempts at intrasynovial analgesia were available for analyses. The 9 data sets included 3 horses with lameness induced in the femoropatellar joint, 3 horses with lameness induced in the medial femorotibial compartment, and 3 horses with lameness induced in the lateral femorotibial compartment. One of the 3 horses injected with IL-1β in the medial femorotibial compartment developed non–weight-bearing lameness after lameness was induced and could not be trotted. Lameness in this horse did not improve sufficiently to allow trotting until after administration of the third block. Thus, this horse was assigned values for differences in maximum and minimum pelvic height of 60 mm until the lameness reduced to a severity that allowed trotting (ie, after the third block).

After lameness was induced (16 hours after intra-articular injection of IL-1β), median differences in maximum (13.3 mm) and minimum (22.5 mm) pelvic height were significantly ($P < 0.001$) greater than the value at baseline. Subsequent injection of local anesthetic solution into one of the compartments of the stifle joint not injected with the IL-1β did not result in improvement in lameness, as indicated by a lack of significant change in differences in maximum (median, 10.6 mm; $P = 0.196$) and minimum (median, 14.9 mm; $P = 0.161$) pelvic height. However, deposition of local anesthetic solution into the other synovial compartment of the stifle joint not injected with IL-1β improved lameness, as indicated by a significant decrease in differences in maximum (median, 7.7 mm) and minimum (median, 11.3 mm) pelvic height from the values measured at 16 hours after induction of lameness ($P < 0.001$ and $P < 0.001$, respectively) and after anesthesia of the first compartment ($P = 0.002$ and $P < 0.001$, respectively). Nevertheless, the degree of lameness still present after injecting local anesthetic solution into this second compartment of the stifle joint remained significantly more severe than the degree of lameness detected at baseline, as indicated by significantly greater differences in maximum ($P = 0.017$) and minimum ($P < 0.001$) pelvic height.

Injection of local anesthetic solution into the compartment of the stifle joint injected with IL-1β further improved lameness, as indicated by further significant decreases in median differences in maximum (–0.54 mm) and minimum (6 mm) pelvic height from the values obtained at 16 hours after lameness was induced ($P < 0.001$ and $P < 0.001$, respectively) and after administration of the first ($P < 0.001$ and $P < 0.001$, respectively) and second blocks ($P < 0.016$ and $P < 0.001$, respectively). After the third block, the difference in maximum pelvic height was not significantly ($P = 0.99$) different from baseline, but the difference in minimum pelvic height failed to return to baseline ($P = 0.028$).

Twenty-four hours after injection of recombinant equine IL-1β into one of the compartments of the stifle joint (ie, after the effects of intrasynovial anesthesia had dissipated), the lameness returned, as indicated by an increase in differences in maximum (10.3 mm) and minimum (17.7 mm) pelvic height. These differences in maximum and minimum pelvic height were significantly higher than values observed before lameness was induced ($P < 0.001$ and $P < 0.001$, respectively) and values observed after the second ($P = 0.021$ and $P = 0.028$, respectively) or third block ($P < 0.001$ and $P < 0.001$, respectively; **Figure 2**). Administration of phenylbutazone (2.2 mg/kg, IV, q 12 h) 24 hours after intra-articular deposition of IL-1β resulted in complete resolution of lameness in all horses within 36 to 48 hours.

Discussion

The objective of the present study was to evaluate the clinical response to diagnostic analgesia of the various compartments of the stifle joint of horses made lame by induction of transient synovitis in 1 of the 3 compartments of the stifle joint. All horses developed pelvic limb lameness within 16 hours after deposition of 200 ng of recombinant equine IL-1β into 1 of the 3 synovial compartments of the stifle joint. Lameness persisted for at least 24 hours after the injection. Although lameness was not graded subjectively in this study, the perceived lameness of all horses in both experiments of the study ranged in severity from obvious lameness to lameness so severe that the horse was unwilling to trot or would touch only the toe of the injected limb to the ground when trotted. Lameness was assessed objectively in 6 of 6 limbs (6 horses) in experiment 1 and in 9 of 12 limbs (5 horses) in experiment 2.

Development of lameness after intra-articular deposition of 200 ng of recombinant equine IL-1β was most likely attributable to development of acute synovitis, the presence of which was corroborated by a high WBC count and high total protein concentration in synovial fluid samples obtained from the IL-1β–treated stifle joint compartment at 16 hours after injection. In a pilot study,[12] intra-articular deposition of 100 ng of recombinant equine IL-1β induced lameness in 3 of 4 horses. In that study,[12] analyses of synovial fluid samples obtained 1 day after intra-articular deposition of the IL-1β revealed a high WBC count and high total protein concentration indicative of suppurative inflammation. In the present study, lameness was consistently evident 16 hours after intra-articular deposition of 200 ng of recombinant equine IL-1β, but the development of severe (albeit transient) lameness in 2 of the horses calls into question the suitability of use of this higher dose in experimental studies on humane grounds.

Results of experiment 1 indicated that deposition of local anesthetic solution into the synovial compartment injected with 200 ng of recombinant equine IL-1β significantly improved the lameness. This finding has 2 explanations: either recombinant equine IL-1β and mepivacaine were each able to diffuse freely among the compartments of the stifle joint, or the IL-1β remained confined and induced inflammation only in the compartment into which it was deposited. Regardless, results from experiment 2 suggested that diffusion of mepivacaine among the 3 compartments within 20 minutes after its administration was not sufficient to resolve lameness induced by deposition of IL-1β into a different compartment of the stifle joint. This indicates that improvement of lameness in experiment 1 likely occurred because IL-1β remained largely confined to the compartment into which it was deposited.

In experiment 2, persistence of lameness after mepivacaine was injected into either of the compartments of the stifle joint into which recombinant equine IL-1β had not been deposited indicated that mepivacaine diffused insufficiently among the compartments of the stifle joint during the 20 minutes that elapsed between its injection and evaluation of lameness. Close inspection of the data (Figure 2) revealed that sequential deposition of mepivacaine into the 3 synovial compartments of the stifle joint resulted in a gradual decrease in lameness. Although some change was effected by administration of the first block, this decrease in lameness was not significant. However, a significant decrease in lameness was achieved after the second compartment of the stifle joint was anesthetized, and lameness improved again after mepivacaine was injected into the compartment into which IL-1β had been deposited. Perhaps allowing more time to elapse between the first injection of mepivacaine and subsequent evaluation of lameness (eg, a 40-minute interval instead of a 20-minute interval) would have resulted in a more profound improvement of lameness that might have reached significance in the present study. On the basis of the study results, we suggest evaluating the results of diagnostic analgesic blocks of the stifle joint in horses within 20 minutes because this interval appeared sufficient in experiment 1 for lameness to significantly improve following injection of the local anesthetic solution into the compartment of the stifle joint that received IL-1β. In clinical cases, allowing an interval > 20 minutes to elapse between intra-articular deposition of the anesthetic solution and evaluation of the block may result in sufficient diffusion of mepivacaine among the stifle joint compartments to confound interpretation of the results. On the other hand, allowing diffusion of local anesthetic solution for a period > 20 minutes may be necessary to eliminate pain resulting from injury or disease of an extrasynovial structure, such as a cruciate ligament. If > 1 stifle joint compartment is painful, simultaneous instillation of local anesthetic solution into the painful compartments may be necessary to effect gait improvement within a period of 20 minutes.

It is important to realize that in both experiments 1 and 2, anesthesia of the stifle joint compartment into which the recombinant equine IL-1β had been deposited resulted in nearly complete restoration of the baseline gait, indicating that injection of the painful compartment of the stifle joint with local anesthetic solution is both necessary and sufficient to elicit maximal response.

In both experiments, all horses were evaluated for lameness 24 hours after recombinant equine IL-1β was deposited into one of the stifle joint compartments to ensure that improvement in lameness detected during the study was not the effect of the resolution of the induced synovitis. All horses remained consistently lame for 24 hours after deposition of IL-1β, demonstrating the ability of IL-1β to cause lameness lasting for at least 24 hours. The baseline gait of all horses was restored within 36 to 48 hours after IV administration of phenylbutazone.

Because of the low number of horses evaluated in experiment 2, we were unable to determine whether results observed after deposition of recombinant equine IL-1β into a specific synovial compartment of the stifle joint would differ from results observed when the IL-1β was deposited into another specific compartment. For the same reason, we were unable to determine the effects that the order of anesthesia of the 2 compartments of the stifle joint not injected with IL-1β might have in resolving lameness.

In the present study, intra-articular deposition of 200 ng of recombinant equine IL-1β into a stifle joint compartment was an effective method of inducing transient pelvic limb lameness in horses and had no apparent long-term adverse effects. Nevertheless, considering the severity of lameness in 2 of the horses, perhaps a lower dose of IL-1β should be evaluated for use in future studies. Injection of local anesthetic solution into the synovial compartment of the stifle joint previously injected with IL-1β was sufficient to restore the study horses' gait to near baseline level. Conversely, injection of 1 of the other 2 compartments did not result in significant improvement in the lameness when lameness was evaluated 20 minutes after injection.

a. Equinosis LLC, Columbia, Mo.
b. R&D Systems, Minneapolis, Minn.
c. Omnipaque 240 (iohexol), GE Healthcare Inc, Princeton, NJ.
d. Carbocaine-V, Pfizer Inc, New York, NY.
e. Equi-Phar Phenylbutazone Injection 20%, Vedco Inc, St Joseph, Mo.
f. StatsDirect statistical software, version 2.7.8, StatsDirect Ltd, Altrincham, Cheshire, England.

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