Concentrations of dexmedetomidine and effect on biomarkers of cartilage toxicity following intra-articular administration in horses

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OBJECTIVE
The goal of this study was to determine plasma, urine, and synovial fluid concentrations and describe the effects on biomarkers of cartilage toxicity following intra-articular dexmedetomidine administration to horses.

ANIMALS
12 research horses.

PROCEDURES
Horses received a single intra-articular administration of 1 μg/kg or 5 μg/kg dexmedetomidine or saline. Plasma, urine, and synovial fluid were collected prior to and up to 48 hours postadministration, and concentrations were determined. The effects on CS846 and C2C were determined in synovial fluid at 0, 12, and 24 hours postadministration using immunoassays.

RESULTS
Plasma concentrations of dexmedetomidine fell below the limit of quantification (LOQ) (0.005 ng/mL) by 2.5 and 8 hours postadministration of 1 and 5 μg/kg, respectively. Synovial fluid concentrations were above the LOQ (0.1 ng/mL) of the assay at 24 hours in both dose groups. Drug was not detected in urine samples at any time post-drug administration. CS846 concentrations were significantly decreased relative to baseline at 12 hours postadministration in the saline group and significantly increased in the 5-μg/kg-dose group at 24 hours. Concentrations of C2C were significantly decreased at 12 and 24 hours postadministration in the saline treatment group. There were no significant differences in CS846 or C2C concentrations between dose groups at any time.

CLINICAL RELEVANCE
Systemic concentrations of dexmedetomidine remained low, compared to synovial fluid concentrations. CS846, a marker of articular cartilage synthesis, increased in a dose-dependent fashion. Based on these findings, further dose titration and investigation of analgesic and adverse effects are warranted.

Introduction

The α2-adrenergic agonists are potent analgesic and sedative agents, and while they are commonly used as part of anesthetic protocols, their routine use as analgesic agents is somewhat limited by unfavorable physiologic and behavioral changes.1 Dexmedetomidine is a very potent and highly specific α2-adrenergic agonist used in both human and veterinary medicine. The increased specificity of this drug for the α2-adrenergic receptor and short duration of action suggest that dexmedetomidine may allow for better titration and have a more favorable systemic safety profile as compared to more traditional α2-adrenergic agonists used in equine medicine. The use of intra-articular (IA) medications to treat pain and inflammation associated with chronic use or acute traumatic injuries is commonplace in older and performance horses. Arguably the most used IA drugs in horses are corticosteroids; however, dose-dependent chondrotoxicity has been reported. In human medicine, the use of IA local anesthetics, opioids, and α2-adrenergic agonists has proven an effective means to treat postoperative joint pain.2–4 Reports5 of IA dexmedetomidine in dogs have also been encouraging. In one study, investigators
reported that IA dexmedetomidine alone and in combination with lidocaine provided better intraoperative analgesia than lidocaine alone in dogs undergoing arthroscopy. Although not widely used in the treatment of joint pain in horses as of yet, the success of IA opioids and α₂-adrenergic agonists as postoperative analgesics in humans and dogs is encouraging for their use in horses.⁶⁻⁸ There are limited studies documenting the pharmacokinetics, disposition, and effects of morphine and α₂-adrenergic agonists, namely, xylazine⁷ and detomidine,⁷⁻⁸ following IA administration to horses.

The potential for fewer systemic side effects coupled with its reported efficacy in the treatment/prevention of joint pain in human patients is encouraging for the use of IA dexmedetomidine in the horse. However, to begin to properly evaluate the use of this drug for the treatment of joint pain, a study of the disposition and chondrotoxicity of this compound following IA administration is necessary. To that end, the primary goal of the current study was to assess plasma, urine, and synovial fluid concentrations of dexmedetomidine following intra-articular administration of 2 doses in a small group of healthy sound horses. As the administration of this drug may also be of benefit in performance horses, concentration data would also aid in regulating its use before competition. A secondary goal was to assess the effects of dexmedetomidine on 2 well-established biomarkers of cartilage toxicity following administration into the joint.

Materials and Methods

Animals
Twelve University-owned healthy sound exercised Thoroughbred research horses (7 geldings and 5 mares; 3 to 6 years of age) were studied. All horses were part of the research herd at the University of California-Davis and were exercised 5 days a week, following standard protocols established by our laboratory.³

Horses did not receive any medications for a minimum of 4 weeks before the start of the study. Before beginning the study, a complete blood count (CBC), serum biochemistry panel, and physical exam were performed, and all horses were deemed healthy. The CBC and serum biochemistry panel were performed by the Clinical Pathology Laboratory of the William R. Pritchard Veterinary Medical Teaching Hospital of the University of California-Davis. Each horse also underwent a lameness evaluation by an experienced board-certified equine surgeon before the commencement of the study to ensure there was no overt lameness. Horses were required to walk and trot in a straight line and be scored according to guidelines established by the American Association of Equine Practitioners.¹⁰⁻¹¹ The study was conducted in accordance with the Institutional Animal Care and Use Committee of the University of California-Davis.

Instrumentation and drug administration
A 14-gauge catheter was placed in the left external jugular vein for sampling. Horses were randomly assigned by use of a computerized random number generator to 1 of 3 dose groups. Dose groups included 0.9% NaCl (1 to 5 mL; n = 4), 1 μg/kg dexmedetomidine (Dexdormitor; Zoetis; 4), and 5 μg/kg dexmedetomidine (4). Before drug administration, the skin over the right antebrachiocarpal joint was scrubbed with chlorhexidine solution and 70% isopropyl alcohol, the joint was flexed, and drug or saline was administered aseptically into the joint.

Sample collection
Blood samples for drug concentration determination were collected from all horses at time 0 (immediately before drug administration) and at 5, 10, 15, 30, and 45 minutes and 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 18, and 24 hours after dexmedetomidine administration. Blood samples were collected into EDTA blood tubes and placed on ice (maximum time of 1 hour) until centrifugation for 10 minutes at 3,000 X g. Plasma was immediately transferred into storage cryovials and stored at −20°C until analyzed for concentration determination.

For synovial fluid collection, the skin over the joint was aseptically prepared as described for drug administration. Horses were restrained (without sedation), and synovial fluid samples (approx 0.5 mL/time point) were collected from the right and left antebrachiocarpal joint by aspiration with a sterile needle (20 gauge × 1.5 inch) immediately before injection and at 12 and 24 hours postadministration. All synovial fluid samples were stored at −20°C until analysis for determination of dexmedetomidine and biomarker concentrations.

Urine samples were collected from all horses via free catch, for measurement of dexmedetomidine concentrations. Samples were collected on day 0 (before drug administration) and at approximately 24 and 48 hours after dexmedetomidine administration. Samples were stored at −20°C until analyzed for concentration determination.

Determination of dexmedetomidine concentrations (plasma/synovial fluid/urine drug concentrations)
A previously published liquid chromatography tandem-mass spectrometry (LC-MS/MS) assay for the determination of plasma dexmedetomidine concentrations in horses was utilized in the current study.¹² Per the Food and Drug Administration’s Guidance for Industry on Bioanalytical Method Validation, a partial validation was performed for urine and synovial fluid.

Biomarker concentration determination
Commercially available, competitive ELISAs, previously validated for use in equine synovial fluid, were used to measure the biomarker concentrations of chondroitin sulfate 846 (CS846; a biomarker of aggrecan turnover; IBEX Pharmaceuticals) and the neo-epitope C2C (C2C; a biomarker of type II collagen degradation; IBEX Pharmaceuticals) as described previously.¹² Concentrations were assessed in synovial fluid samples collected before
drug administration and at 12 and 24 hours after drug administration.

Statistical analysis

Differences in biomarker concentrations between time periods, conditional on treatment, were analyzed using a mixed-effects analysis of variance, with the horse as the random effect and time as the fixed effect. Differences in biomarker concentrations between treatments, conditional on time, were analyzed using a one-way analysis of variance. Data were analyzed using Stata/BE 17.0 statistical software. P < .05 was considered statistically significant.

Results

The LC-MS/MS instrument response for dexmedetomidine was linear and gave a correlation coefficient of 0.99 or better. The precision and accuracy of the assay were determined by assaying quality control samples in replicates (n = 6). Accuracy was reported as percent nominal concentration, and precision was reported as percent relative standard deviation. Accuracy and precision were within ±15% of nominal concentrations (Table 1) and were considered acceptable based on the Food and Drug Administration's guidelines for Bioanalytical Method Validation. The technique was optimized to provide a limit of quantitation (LOQ) of 0.005 ng/mL, 0.25 ng/mL, and 0.1 ng/mL in plasma, urine, and synovial fluid, respectively. The limit of detection (LOD) was 0.0025 ng/mL, 0.15 ng/mL, and 0.05 ng/mL in plasma, urine, and synovial fluid, respectively.

At all times postadministration, joints were subjectively assessed for any signs of swelling and heat. In all cases, observable swelling appeared to be subcutaneous in nature. Following saline administration, the treated joint was slightly swollen without noticeable heat at 24 hours in 2/4 horses and slightly swollen with some heat at 48 hours in 3/4 horses. Following administration of 5 μg/kg dexmedetomidine, the treated joint was swollen and slightly warm at 24 hours posttreatment in 3/4 horses and slightly swollen with no noticeable heat in the same 3 horses at 48 hours.

Dexmedetomidine plasma concentrations at several times postadministration are listed in Table 2. Dexmedetomidine was first detected in plasma at 5 minutes postadministration in the 5-μg/kg dose group and at 30 minutes following administration of 1 μg/kg. Plasma concentrations remained low throughout the sampling period with levels in plasma falling below the LOQ in all horses by 2.5 and 8 hours postadministration of 1 and 5 μg/kg, respectively.

Concentrations of dexmedetomidine in the right antebrachiocarpal joint were notably higher in the 5-μg/kg-dose group, compared to horses administered a dose of 1 μg/kg (Table 3). Dexmedetomidine was not detected in the left antebrachiocarpal joint of any horses at any time postadministration. Dexmedetomidine was not detected in urine samples collected at any time postdrug administration.

Concentrations of the markers of cartilage toxicity CS846 and C2C were measured in synovial fluid before and after drug administration. For CS846, concentrations were significantly decreased (P < .05), relative to baseline at 12 hours postadministration in

| Table 1—Accuracy and precision values for LC-MS/MS analysis of dexmedetomidine in equine plasma, synovial fluid, and urine. |
|---|---|---|
| **Matrix** | **Concentration (ng/mL)** | **Intraday accuracy (% nominal concentration)** | **Intraday precision (relative SD)** |
| **Plasma** | 0.015 | 94.0 | 11.0 |
| | 0.15 | 101 | 12.0 |
| | 5.00 | 105 | 6.0 |
| | 45.0 | 98.0 | 11.0 |
| **Synovial fluid** | 0.30 | 103 | 6.0 |
| | 50.0 | 109 | 5.0 |
| | 500.0 | 97.0 | 6.0 |
| **Urine** | 0.30 | 110 | 4.0 |
| | 2.00 | 95.0 | 10.0 |
| | 9.00 | 113 | 3.0 |

Dexmedetomidine concentrations in the saline group were nondetectable at all time points. LOQ = Limit of quantitation. ND = Not detected.
the saline group and significantly increased ($P < .05$) in the 5-μg/kg-dose group at 24 hours (Figure 1). There were no significant differences in CS846 concentrations between dose groups at any time postadministration. The concentrations of C2C were significantly decreased ($P < .05$) at 12 and 24 hours postadministration in the saline treatment group (Figure 2). There were no significant differences in C2C concentrations between dose groups at any time postadministration.

**Discussion**

The current study describes plasma, synovial fluid, and urine concentrations of dexmedetomidine as well as its effects on 2 well-established markers of cartilage toxicity, following intra-articular administration to a small group of horses.

The primary benefit of intra-articular administration of drugs for the treatment of joint-related pain is high local and lower systemic concentrations. Lower systemic concentrations in turn minimize the likelihood of unwanted off-target effects. The disposition of intra-articular dexmedetomidine has not been described in horses. In the current study, the low dose was chosen based on reports in humans, in which an intra-articular dose of 1 μg/kg was found to elicit an analgesic effect. The high dose of 5 μg/kg was chosen based on a previous study describing the pharmacokinetics of dexmedetomidine in horses following IV administration. These doses also encompass those previously studied in rats (1 and 3 μg/kg) and dogs (2.5 μg/kg). In agreement with a previous report describing systemic concentrations of xylazine following intra-articular administration to horses, in the current study, the plasma dexmedetomidine concentrations were low to nondetectable at all time points postadministration. Conversely, the concentrations of dexmedetomidine in the joint were high and remained above the limit of detection for up to 24 hours postadministration, suggesting a prolonged residence time and a slow rate of systemic absorption, thereby decreasing the likelihood of systemic effects. Although no overt signs of sedation or other systemic $\alpha_2$-adrenergic effects were noted, such assessments were beyond the scope of the current study, and a more comprehensive evaluation of systemic effects following IA administration of dexmedetomidine is warranted before recommending this route of administration in clinical cases.

Although there are several reports describing IA use of $\alpha_2$-adrenergic agonists in humans, there are a limited number of studies describing administration via this route in horses. In one such study, investigators reported that IA detomidine was effective in eliciting an analgesic effect, without any adverse systemic effects, when a shoe model of lameness was utilized. In a second study, IA administration of xylazine appeared to improve postoperative outcomes following arthroscopy in horses. Even though the results of these studies are promising, the safety and potential detrimental effects of this class of...
drugs on components of the joint are still not completely understood and results appear to be mixed. In rats, administration of 25 μg of dexmedetomidine into the knee joint did not appear to have detrimental effects on histopathological parameters. 13 Although there are no in vivo studies describing the effects of α2-adrenergic agonists on joint structures following administration to horses, the effects of dexmedetomidine on equine chondrocytes in vitro have been described. 16 Mancini and colleagues 16 reported that exposure to dexmedetomidine impaired mitochondrial and lysosomal functions, altered cell membrane integrity, and induced late apoptosis and necrosis in the joint. These effects did appear to be dose dependent, occurring only at higher concentrations. 16

In the current study, the concentrations of 2 well-established biomarkers of cartilage turnover were studied as indicators of dexmedetomidine-induced cartilage damage. The levels of C2C, a biomarker that reflects articular cartilage degradation by collagenases, and CS846, a marker of articular cartilage synthesis, were studied. These biomarkers have been assessed previously in studies evaluating the effects of the local anesthetic bupivacaine on articular cartilage in horses. In the present study, dexmedetomidine administration did not lead to a significant change in C2C concentrations following treatment with either dose of the drug. Conversely, CS846, was significantly increased at 24 hours post-administration in the 5-μg/kg-dose group. This was not observed in the saline or 1-μg/kg-dose group, suggesting that effects on articular cartilage synthesis may be dose and therefore concentration dependent, similar to what was suggested following exposure of chondrocytes to the drug in vitro. 16 Investigators 13,17 studying the effects of intra-articular bupivacaine speculated that an increase in cartilage synthesis without a concomitant increase in C2C may be the result of a reparative response to a short-term undetected chondrocyte insult attributable to the drug. Although further study is necessary, this may also explain the results of the biomarker analysis in the current study. Several horses in the current study had focal subcutaneous swelling in the region surrounding the joint, with or without heat. This was observed in all treatment groups, suggesting that these clinical signs were a result of the arthrocentesis process. However, it is also important to note that the commercially available dexmedetomidine formulation used in the current study is labeled for systemic administration and contains preservatives that could be irritating to joint structures, and this may also elicit inflammation. In contrast, the most common formulation used in humans for IA administration is preservative free and contains no additives or chemical stabilizers. There are a few notable limitations in the current study. First, a limited number of horses were studied. As this is the first study to describe IA administration of dexmedetomidine in horses, we chose to evaluate 2 doses and include a saline group, limiting the number of horses in each dose group. Although numbers are limited, the results of the current study will help guide future studies. It is also important to note that the doses that were selected for the study may not achieve therapeutic concentrations within the joint. Efficacy studies will be necessary to determine therapeutic joint concentrations. These efficacy studies could include such things as an assessment of lameness following drug administration. Although one of the objectives of the current study was to assess cartilage toxicity induced by IA dexmedetomidine administration, only 2 biomarkers were studied, and histopathological effects were not assessed. Due to a limited synovial fluid sample volume, it was not possible to assess additional biomarkers in the current study. It is also important to note that biomarker concentrations were only assessed up to 24 hours postadministration in the current study. The synovial fluid sampling times were based on predictions related to drug residence time in the joint and limiting the number of arthrocentesis procedures, but it is possible that additional samples collected beyond 24 hours may reveal drug-related effects on biomarkers of chondrotoxicity. Additional studies, with additional endpoints and indicators of cartilage toxicity, are necessary to fully describe the effects on articular cartilage before dexmedetomidine can be recommended for IA administration in the horse. The current study represents another step toward understanding the pharmacokinetics and effects of α2-adrenergic agonists on joint structures and a first assessment of dexmedetomidine following IA administration to the horse.

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References


