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Comparison of two tourniquet application times for regional intravenous limb perfusions with amikacin in sedated or anesthetized horses

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ABSTRACT

Regional limb perfusion (RLP) in horses has proven to be a simple and effective technique for the treatment of synovial and musculoskeletal infections in the distal portion of the limbs. The ideal tourniquet time needed to achieve therapeutic synovial concentrations remains unknown. The pharmacokinetic effects of general anesthesia (GA) versus standing sedation (SS) RLP on synovial amikacin concentrations are not completely understood. This study investigated the pharmacokinetic effects of RLP under general anesthesia (GA) versus standing sedation (SS) on synovial amikacin concentration following 20 or 30 min tourniquet time. Using 1 g of amikacin RLP was performed in two groups of six horses (GA and SS). A pneumatic tourniquet was applied proximal to the carpus and maintained for 20 or 30 min. Two weeks later, the opposite treatment (20 or 30 min) was randomly performed in the opposite limb of horses in each group (GA and SS).

Synovial fluid samples were collected from the metacarpophalangeal (MCP) and radiocarpal (RC) joints. Amikacin was quantified by a fluorescence polarization immunoassay. Regardless of the group, no significant difference in the synovial amikacin concentrations was noted between 20 and 30 min RLP. Mean synovial concentrations of amikacin in the standing sedated horses were significantly higher in the MCP joint at 30 min ($P = 0.003$) compared to horses under general anesthesia. No significant difference was noted for the RC joint.

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Introduction

Synovial sepsis is a common cause of morbidity and mortality in horses that can lead to irreversible cartilage damage and chronic lameness, often jeopardizing the horse's athletic career or life (McIlwraith, 1983; Schneider et al., 1992). Regional limb perfusion (RLP) has been described as a simple and effective technique for antimicrobial delivery to the distal limb tissues. The technique has been used successfully in the management of clinical and experimental musculoskeletal infections in the distal part of the limbs in horses (Whitehair et al., 1992a; Whithair et al., 1992). In a recent clinical retrospective study (Kelmer et al., 2012), 14/44 horses with synovial sepsis of the distal portion of the limb were treated with systemic antibiotics only for the initial 24 h after admission and then received RLP as the sole mode of antimicrobial therapy. The authors stated that in select cases, using RLP as a sole mode of antimicrobial delivery could potentially avoid complications associated with systemic therapy such as antimicrobial-related diarrhea.

The antimicrobial concentrations achieved in synovial fluid and bone tissue after RLP are much higher than those after systemic administration (Whitehair et al., 1992a; Rubio-Martinez et al., 2006). For some antimicrobial agents, synovial levels remain above the minimum inhibitory concentration (MIC) for the most frequently isolated microorganisms, for periods >24 h post administration (Pille et al., 2005). Furthermore, RLP is more effective in attaining higher antimicrobial levels in bone and periarticular tissue compared to intra-articular injection (Whitehair et al., 1992b; Murphey et al., 1999; Pille et al., 2005).

Concentration dependent antimicrobial agents, such as aminoglycosides, are ideal for RLP because the rate and extent of bacterial killing is related to the high maximum concentration (C_{max}) in relation to the MIC. A greater bactericidal effect and longer post antibiotic effect is associated when amikacin dosing achieves a high peak C_{max}:MIC ratio (Murphey et al., 1999). The optimal dose and frequency of administration of antibiotics for distal limb perfusion as well as the optimal C_{max}:MIC ratio for the treatment of orthopedic sepsis requires further investigation. However, in experimental septic arthritis, high concentrations of aminoglycosides in synovial fluid resulted in rapid elimination of bacteria (Lloyd et al., 1990), suggesting that high antibiotic concentration would be beneficial in the treatment of septic arthritis.

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The range of amikacin sulfate routinely administered to horses via RLP is 500 mg–2.5 g diluted with saline (0.9% NaCl) to a volume of 40–100 mL (Pille et al., 2005; Kelmer et al., 2009; Levine et al., 2010; Alkabes et al., 2011). Amikacin is advantageous in RLPs because it has been shown to be effective against the most commonly isolated bacteria from equine orthopedic infections (Snyder et al., 1987; Moore et al., 1992; Ahern et al., 2010). Compared to other aminoglycosides, bacterial resistance to amikacin sulfate is less likely to occur due to its high degree of resistance to aminoglycoside inactivating enzymes (Price et al., 1974; Orsini et al., 1989).

RLP can be performed in standing sedated horses or under general anesthesia. Movement of standing sedated animals could potentially affect the performance of the tourniquet, allowing inadvertent leakage of perfusate into the systemic circulation (Gilliam et al., 2008; Levine et al., 2010). A recent study (Mahne et al., 2014) evaluated the effects of RLP using a wide elastic tourniquet under general anesthesia, standing sedation without regional anesthesia, standing sedation with intravenous (IV) regional anesthesia and standing sedation plus perineural anesthesia, concluding that general anesthesia had no effect on the intra-articular concentration of amikacin.

Most of the studies evaluating RLP in horses have been performed using 30 min of tourniquet time (Murphey et al., 1999; Pille et al., 2005; Alkabes et al., 2011; Beccar-Varela et al., 2011; Kelmer et al., 2012; Mahne et al., 2014). However, in practice standing sedated horses commonly move by the end of the RLP requiring additional sedation or earlier release of the tourniquet. Therefore some clinicians perform RLP for only 20 min. The purposes of the present study were to determine if RLP with 1 g of amikacin for 20 min will attain synovial amikacin concentrations comparable to 30 min and to compare amikacin concentrations in synovial fluid of the metacarpophalangeal (MCP) and radiocarpal (RC) joints of standing versus anesthetized horses following RLP using a pneumatic tourniquet set at 420 mmHg at the level of the antebrachium.

We hypothesized that synovial concentration of amikacin in the RC and MCP joints of horses following RLP is comparable after 20 or 30 min of tourniquet time, as well as between standing sedation and general anesthesia groups.

Materials and methods

Animals

Twelve clinically healthy horses were used in the study. Horses were free of lameness and obvious vascular abnormalities of the forelimbs. There were nine geldings and three mares of median age of 16 years (range 6–24) and median weight of 540 kg (range 425–660 kg). All procedures were approved by the Animal Use and Care Committee of the University of California Davis, approval number 17435 dated 23 February 2013 and approval number 17650 dated 12 June 2013.

Horses were weighed and moved from pasture to stalls (3.5 × 4 m) with free access to hay and water 2 days before the experiments. For the six horses that underwent general anesthesia, food but not water was withheld for 8 h before anesthetic induction. Clinical examinations were performed twice daily from the day before treatment until 24 h after collection of the last sample. A 14 G 13.3 cm IV catheter was inserted into one of the jugular veins before each experiment. Forelimb and tourniquet duration treatment assignments were determined using an online randomization tool.[1]

Study design

Standing sedation (SS) group

Fifteen minutes before the RLP, six horses received a median, ulnar and musculocutaneous nerve block using 35 mL of 2% lidocaine as previously described (Moyer et al., 2007). Horses were then sedated with detomidine hydrochloride (0.01 mg/kg IV) and butorphanol tartrate (0.01 mg/kg IV). Based on the degree of limb movement determined by one of the authors (BM), an additional quarter of the original dose was administered if necessary to maintain adequate sedation. A pneumatic tourniquet with a 12 cm wide cuff (Delfi PTS Portable Tourniquet System, Delfi Medical Innovations) was applied around the antebrachium 10 cm proximal to the proximal border of the accessory carpal bone. A gauze roll was placed underneath the tourniquet cuff and over the cephalic vein. Tourniquet application was always performed by the same individual to limit variation in the tourniquet position. After aseptic preparation of the cephalic injection site, the tourniquet was insufflated to a pressure of 420 mmHg and a 24 G IV catheter (2.5 cm long) inserted into the ipsilateral cephalic vein at the level of the chestnut.

One gram of amikacin sulfate (TEVA) diluted in 0.9% saline to a total volume of 60 mL was administered over 1 min (approximate rate: 1 mL/s) using an extension set. The pneumatic tourniquet pressure was maintained for 20 or 30 min from the beginning of the perfusion, depending on the randomization. After a 2-week washout period, horses received the opposite treatment (20 or 30 min) in the contralateral limb. After completion of each procedure, a bandage was applied over the injection site and maintained for 24 h.

The total amount of sedation administered and the number of times horses required re-sedation were recorded.

General anesthesia (GA) group

Six horses were sedated with dexmedetomidine (3.5 mg/kg IV). Horses were induced with midazolam (0.06 mg/kg IV) and ketamine (2.2 mg/kg IV), and maintained under general anesthesia with sevoflurane at 1.25 × MAC[2] end-tidal concentrations delivered in 100% oxygen. IV crystalloid fluids were administered at 10 mL/kg/h and dobutamine (0.5–2 mg/kg/min) was administered as needed to maintain direct mean arterial blood pressure >70 mmHg. Anesthesia time was 1 h for all of the horses. Horses were positioned in lateral recumbency with the designated leg down and maintained parallel to the ground. Horses under general anesthesia underwent the same protocol for randomization, treatment, duration and sampling as for the standing sedated horses.

After completion of the procedure, a bandage was applied over the injection site, then horses were fitted with a padded helmet and moved to a padded recovery stall and allowed to recover from anesthesia under direct observation but without assistance. The endotracheal tube was secured to the mandible and kept in place during recovery. Oxygen was insufflated via the endotracheal tube at 15 L/min.

Collection of samples

Synovial fluid samples (1 mL) were collected in lithium heparin blood collection tubes by direct arthrocentesis using aseptic technique from the ipsilateral MCP and RC joints. Synovial samples were collected 1 min and 24 h after releasing the tourniquet. Blood samples from the jugular vein were also collected in heparinized tubes 1 min before and 1 min after releasing the tourniquet. However, in the SS group the samples collected 1 min before releasing the tourniquet went missing. All samples were immediately centrifuged at 4440 *g* for 5 min and the supernatant was collected and stored at –80 °C until analysis.

Measurement of amikacin sulfate concentrations

The samples were assayed by a Roche/Hitachi 917 Cobas c311 analyzer using an in vitro test for quantitative determination of amikacin by fluorescence polarization immunoassay. This technique has been previously validated in plasma (Bucki et al., 2004) and synovial fluid of horses (Parra-Sanchez et al., 2006), and several studies have used the same methodology for the measurement of amikacin in blood and synovial fluid (Levine et al., 2010; Mahne et al., 2014). Furthermore, in preliminary experiments we collected blood and tarsocrural joint fluid in heparinized collection tubes from a clinically healthy horse. Samples were centrifuged, aliquoted and primed with known amounts of purified amikacin standards at concentrations ranging from 1 to 50 μg/mL (analyzer range for amikacin 0.8–60 μg/mL). The analyzer was calibrated using a 6-point calibration before measurements using USP reference standards. Plots of measured concentrations versus expected amikacin concentrations were linear with a coefficient of determination (r^2) of 0.996 for plasma and 0.995 for synovial fluid. Samples above the limit of the analyzer (0.8–40 μg/mL) were diluted with Preciset TDM II (Roche Diagnostics) and the measured values were corrected accordingly by the dilution factor to obtain the synovial amikacin concentration.

Data analysis

Data were evaluated for normality using the Shapiro–Wilk test. Non-normal data were log-transformed ($\log_{10}$) to achieve normality. Amikacin concentrations were compared within and between groups by using paired *t* test and unpaired *t* test respectively. In the SS group, the number of times horses were sedated during the procedure was compared by the Wilcoxon signed-rank test. The data including power calculation were analyzed using commercial statistical software (SPSS Statistics for Windows, Version 19.0). A *P* value of >0.05 was considered to be significant. Data are presented as means ± SD.

[1] See: www.random.org (accessed 26 September 2015).

[2] MAC, minimal alveolar concentration required for anesthesia.