EFFECT OF PERFUSATE VOLUME ON AMIKACIN CONCENTRATIONS AFTER SAPHENOUS INTRAVENOUS REGIONAL LIMB PERFUSION IN STANDING, SEDATED HORSES

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Abstract: The objective of this study was to determine the influence of perfusate volume on synovial fluid amikacin concentrations in the joints of the hind limb after standing saphenous intravenous regional limb perfusion (IV-RLP). A randomized cross-over study design was utilized, and six adult horses served as animal subjects. Saphenous IV-RLP was performed in six standing horses with 1 g of amikacin diluted with 0.9% NaCl to volumes of 10 mL, 60 mL, and 120 mL. Samples of synovial fluid from the tarsocrural, metatarsophalangeal, and hind limb distal interphalangeal joints were collected at 15 and 30 minutes after perfusate administration. Concentrations of 40 μg/mL and 160 μg/mL were considered therapeutic for susceptible and resistant pathogens, respectively. No difference in synovial fluid amikacin concentrations was detected between volumes in any joint ($p=0.4$). All synovial fluid amikacin concentrations were higher at 30 minutes compared to 15 minutes ($p=0.003$). All median synovial fluid amikacin concentrations at 30 minutes were > 40 μg/mL using the 60 mL and 120 mL volumes. Synovial fluid amikacin concentrations > 40 μg/mL were only reached in the distal interphalangeal joint when the 10 mL volume was used. All median synovial fluid amikacin concentrations observed were < 160 μg/mL. Target concentrations for pathogens considered susceptible were consistently reached with perfusate volumes of 60 mL and 120 mL. However, median synovial fluid amikacin concentrations did not reach target levels for resistant pathogens. Perfusate volumes of 60 mL or 120 mL are recommended to treat infections due to susceptible pathogens in the joints of the distal hind limb. These results justify investigation of saphenous IV-RLP with different perfusate volumes using higher doses of amikacin.

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CHAPTER I

INTRODUCTION

Synovial structures include joints, tendon sheaths, and bursae, all of which are closed spaces with a mesenchymal synovial lining that produces synovial fluid as well as maintains a very specific cellular and biochemical environment within the synovial space. Bacterial contamination of these structures can result from penetrating cutaneous wounds, hematogenous spread, local extension of a perisynovial infection, or iatrogenic inoculation during synoviocentesis. Potentiating factors for establishing infection within synovial structures include the presence of foreign material or devitalized tissue; the nature and number of infectious organisms; and immunologic status of the patient (McIlwraith et al. 2015). Regarding synovial sepsis in adult horses, Staphylococcus aureus is the most commonly isolated bacteria (34.3%) (Robinson et al. 2016). In neonates, common bacterial isolates include Escherichia coli, Salmonella, Actinobacillus equuli, Klebsiella spp, Staphylococcus, Streptococcus, and Rhodococcus equi (Glass and Watts 2017). The pathophysiology of synovial sepsis begins with the release of enzymes and free radicals in response to colonization of the synovium. A rapid influx of inflammatory cells follows, predominated by neutrophils (McIlwraith et al. 2015). These neutrophils, along with the activated synoviocytes, produce destructive enzymes such as collagenase, caseinase, lysozyme, elastase, cathepsin G, and gelatinase (Palmer and Bertone 1994; Spiers et al. 1994; McIlwraith et al. 2015). Other inflammatory mediators produced include eicosanoids, interleukins, and tumor necrosis factor (Bertone et al. 1993). These enzymes trigger production of degradative enzymes from the chondrocytes, ultimately leading to deterioration of the articular cartilage. Established synovial

infection frequently produces a fibrinocellular material (pannus) that can house foreign material or devitalized tissue and acts as a nidus for continued bacterial infection (McIlwraith et al. 2015). In treating septic synovial structures, objectives include removal of foreign material and pannus, the debridement of contaminated tissues, elimination of microorganisms, removal of destructive enzymes and free radicals, and restoration of a normal synovial environment (McIlwraith et al. 2015). Prompt, aggressive synovial lavage is the mainstay of treatment. Ideally, this high-volume lavage is performed under general anesthesia with endoscopic guidance. Benefits of endoscopic examination include rapid fluid delivery, ability to remove fibrin/pannus, debridement of devitalized tissue, and thorough evaluation of the joint surface or tendinous structures. In refractory cases, arthrotomy and open drainage may become necessary but carries a higher risk of ascending bacterial infection (Schneider 1998). Judicious use of antimicrobials is also an essential component of successful treatment.

Treatment of horses with synovial sepsis is often a significant challenge for equine practitioners. Approximately 24% of horses reported to survive septic synovitis fail to return to their previous or intended use (Beccar-Varela et al. 2011). This poor response to treatment stems from the poor vascularity of the distal limb joints and the minimal blood supply to the lower limbs of horses in general. Additionally, systemic administration of antimicrobials at recommended doses often does not achieve concentrations in the synovial compartments considered therapeutic for common pathogens. In addition to systemic therapy, local delivery of antimicrobials is associated with improved clinical outcome attributed to extremely high doses of antimicrobials instilled in and maintained close to infected tissues (Ahern and Richardson 2012). Regional limb perfusion (RLP) is one such delivery method. This technique involves isolation of an area of the limb using a single or multiple tourniquets followed by injection of an antimicrobial or other drug either into a peripheral vein (intravenous; IV) or into the medullary cavity of a bone (intraosseous; IO) within the isolated region. IV-RLP has proven to be a very cost-effective, minimally invasive,

and safe technique that achieves high concentrations of an antibiotic in the synovial structures of a limb without subjecting the patient to potential side-effects seen with higher than recommended systemic doses (Rubio-Martinez and Cruz 2006; Rubio-Martinez et al. 2012; Kelmer 2016). In order to address inconsistencies in IV-RLP among practitioners, many experimental studies have been performed focusing on variables such as antimicrobial dose, peripheral vessel used, tourniquet type and number, duration of perfusion, and the effect of recumbency (Levine et al. 2010; Kelmer et al. 2013; Mahne et al. 2014; Harvey et al. 2016; Aristizabal et al. 2016; Kilcoyne et al. 2016; Schoonover et al. 2017). The impact of different perfusate volumes containing the same quantity of antimicrobial has been evaluated for cephalic (Moser et al. 2016; Oreff et al. 2016) and digital (Hyde et al. 2013; Godfrey et al. 2016) IV-RLP but not for IV-RLP administered via the saphenous vein. Saphenous IV-RLP has been shown to result in similar antimicrobial concentrations in the synovial fluid of the fetlock as cephalic IV-RLP using a 100 mL perfusate volume (Kelmer et al. 2013a), but some describe a volume of 60 mLs as typical (Stewart and Richardson 2019) and more commonly used in clinical settings (Rubio-Martinez et al. 2012). Several studies have reported antimicrobial concentrations in the synovial fluid of the metatarsophalangeal joint (MTPJ) (Kelmer et al. 2013a; Kelmer et al. 2013b; Kelmer et al. 2015; Kelmer et al. 2017), and the tarsocrural joint (TCJ) (Scheuch et al. 2002; Snowden et al. 2019; Kilcoyne et al. 2021) after saphenous IV-RLP (Scheuch et al. 2002). However, an extensive review of the current veterinary literature identified no studies evaluating antimicrobial concentrations achieved in the hind limb distal interphalangeal joint (HL-DIPJ) with IV-RLP.

The objective of this study was to establish if volume of perfusate influenced synovial fluid amikacin concentrations of the TCJ, MTPJ, and HL-DIPJ after saphenous IV-RLP perfusate containing 1g amikacin. As previously demonstrated with cephalic IV-RLP (Moser et al. 2016), we hypothesized that volume of perfusate would not significantly affect amikacin concentrations achieved in the synovial fluid of the TCJ, MTPJ and HL-DIPJ after saphenous IVRLP at 15 and

30 minutes. Additionally, similar to cephalic IV-RLP (Moser et al. 2016), we hypothesized the synovial fluid amikacin concentration observed in the joints of the lower limb (HL-DIPJ & MCPJ) would be significantly higher than those achieved in the TCJ at 15 and 30 minutes after saphenous IV-RLP. Lastly, it was hypothesized that the larger perfusate volumes (60 &120 mL) would result in a higher regional intravenous pressure leading to more horses with detectable systemic amikacin concentrations, indicating a higher frequency of leakage of the perfusate beyond the tourniquet into the systemic circulation than the 10 mL volume.

CHAPTER II

REVIEW OF LITERATURE

Early Research

Use of local anesthetic agents to provide regional analgesia after the application of a tourniquet was first reported in humans in 1908 (Bier 1908; Biasutti et al. 2021). Subsequently, researchers began investigating a similar technique to administer chemotherapeutic agents regionally in an attempt to shelter portions of the bone marrow and intestines from the deleterious effects of the drug on the hemopoietic and gastrointestinal systems (Karnofsky et al. 1948a; Karnofsky et al. 1948b). Following these animal studies, the IO regional perfusion technique became a method of delivering systemically toxic chemotherapeutic agents to locally malignant tumors in humans (Creech et al. 1958). In 1961, Ryan et al. reported on the use of regional perfusion of antibiotics in cases of chronic soft tissue infection in human medicine, citing the need for a technique to treat localized infections that were resistant to systemic or topical antimicrobial administration. A number of useful modifications of the regional perfusion method for use in human patients were subsequently discussed and described, including antitoxin and antifungal treatments, and treatment of osteomyelitis (Hurley et al. 1966; Finsterbusch et al. 1970; Finsterbusch and Weinberg 1972; Jones et al. 1973).

In 1992(a), Whitehair et al. described IO-RLP of the equine carpus with gentamicin. Using radiographic contrast medium, they demonstrated that perfusate was uniformly distributed throughout the radiocarpal joint (RCJ) and intercarpal joint (ICJ) synovial membranes and

periarticular tissues, and that high levels of gentamicin were achieved in the RCJ, leading to the conclusion that IO-RLP could be a beneficial adjunctive therapy in the treatment of septic arthritis (Whitehair et al. 1992a). The group went on to perform a study comparing the use of gentamicin IV (systemically) or via IO-RLP in horses with experimentally induced septic synovitis (Whitehair et al. 1992b). The RCJ was subjected to bacterial contamination via intraarticular injection of a *Staphylococcus aureus* suspension. Horses were then treated by either IO-RLP with 1g gentamicin or with 2.2 mg/kg gentamicin IV every six hours. Gentamicin concentrations in the synovial fluid of the horses in the IO-RLP group were significantly higher than those in the group administered gentamicin IV (Whitehair et al. 1992b). The mean leukocyte count in the inoculated joints was also significantly decreased at 24 hours after perfusion compared to immediately before (Whitehair et al. 1992b). Terminal bacterial cultures of the synovial fluid and synovial membranes were negative in two of the horses treated with IO-RLP, while *S. aureus* was isolated from the joints in all three horses treated with gentamicin IV (Whitehair et al. 1992b). The results of the study demonstrated the clinical benefit of RLP with antibiotics when compared to IV administration in an experimentally induced bacterial contamination model. The group also reported the use of RLP with various antibiotics in three horses with osteomyelitis associated with orthopedic implants (Whitehair et al. 1992c). Antimicrobial choices for each case were made based on culture and sensitivity results, and all perfusions were performed under general anesthesia. Additionally, all perfusions were performed via an IO catheter save for one, which was performed via an IV catheter placed in the lateral plantar digital vein. In two of the three horses, infections were reportedly resolved without removal of the orthopedic implants (Whitehair et al. 1992c). Variability existed between drug, dose, frequency, route, and location of the perfusions between horses, but the study demonstrated clinically relevant use of the RLP technique and paved the way for further development of RLP in horses.

From these early studies, the need for further research to establish guidelines for the number and timing of perfusions was recognized. Additionally, assessment of efficacy of various antimicrobials in both humans and horses became a vested topic of interest. Beyond the type of drug used, many variations in the technique have since been studied, including dose, volume and concentration of perfusate, dosing interval, type and duration of tourniquet used, and whether the procedure is performed anesthetized or standing.

Efficacy

The mechanism of action for perfusion of antibiotics to such high concentrations regionally is not entirely understood. Several theories have been proposed. One study suggests an increase in local venous pressure and distention of the vessel with an antibiotic creates a concentration gradient that drives the drug from the isolated peripheral vasculature into the surrounding tissues (Brumbaugh 2005; Rubio-Martinez et al. 2006; Moser et al. 2016). Other studies suggest increased hydrostatic pressure, vessel stretching, and loosening of endothelial cell attachments allows antimicrobials to move into the tissues (Langer et al. 1996; Godfrey et al. 2016; Biasutti et al. 2021). The high regional concentration may also create a depot phenomenon, delaying or preventing return of the drug to systemic circulation (Finsterbusch et al. 1970).

As a therapeutic option, RLP has been evaluated and reported to be beneficial in several retrospective clinical studies. Rubio-Martinez et al. (2012) reported on use of RLP as part of the treatment regimen in 174 horses. 96 horses presented with synovial sepsis, 50 horses presented with extrasynovial lacerations or minimally contaminated intrasynovial lacerations without evidence of infection, and 28 horses had other conditions. The study reported a 53% survival rate for horses with synovial sepsis, with 80% of survivors returning to their previous level of work (Rubio-Martinez et al. 2012). The group of horses with lacerations had a much more favorable survival rate at 92%, and 73% of these horses returned to their previous use (Rubio-Martinez et

al. 2012). However, 99% of cases included in the study also received systemic antimicrobials, and 62.5% had intrasynovial administration of antimicrobials at some point during treatment, making a direct comparison between RLP and outcome very difficult (Rubio-Martinez et al. 2012). In a study by Kelmer et al. (2012), indwelling cephalic or saphenous venous catheters were used for IV-RLP to treat 44 horses with synovial contamination or infection of the distal aspect of the limb. Synovial sepsis of the distal portion of 87% of limbs (39) resolved, and 61% of horses returned to soundness (Kelmer et al. 2012). Catheter-related complications occurred in 27% of the limbs but were not significantly associated with outcome (Kelmer et al. 2012). IV-RLP was performed as an adjunct therapy on 43/62 cases (69.3%) in a retrospective study assessing the effect of arthroscopic lavage and repeated intraarticular administrations of antibiotic in adult horses and foals with septic arthritis (Cousty et al. 2017). The study reported a high success rate (95%), stating that a combination of systemic and regional (intrasynovial +/− IV or IO) administration of antibiotics with synovial lavage appears crucial to eradicate septic synovitis in horses (Cousty et al. 2017). A direct analysis between treatment with RLP and outcome was not made, limiting the validity of this statement within this particular study. In 2018, Rinnovati et al described diagnosis, treatment, surgical management, and outcome in 16 foals affected by septic arthritis of the TCJ. The report suggested that use of RLP after arthroscopic treatment based on sensitivity of bacteria greatly reduced the need to perform a second arthroscopy, but this statement was based on clinical impression and IV-RLP was only performed in 5/16 foals (Rinnovati et al. 2018).

Other studies have suggested that RLP does not improve outcome in adult horses or foals with septic synovitis. Wereszka et al. (2007) found no significant difference between outcome (survival or return to function) and use of secondary treatments, including IV-RLP, in horses treated for septic tenosynovitis. However, only 9/51 horses (18%) received IV-RLP as a secondary treatment, and a direct comparison was not made between horses receiving IV-RLP

and other secondary treatments utilized (implantation of PMMA beads, continuous antimicrobial infusion devices, or ingress-egress drain systems) (Wereszka et al. 2007). In another study, the influence of post-operative management on short- and long-term outcome in horses with synovial structure involvement following solar foot penetrations was assessed (Findley et al. 2014). There was some evidence from the univariable analysis that intraoperative IV-RLP may have a positive effect on survival to discharge, while post-operative IV-RLP was not significantly associated with outcome (Findley et al. 2014). Only 21 cases received intraoperative IV-RLP and 11 cases received post-operative IV-RLP, making it possible that the lack of effect seen was due to insufficient sample size, or because selection of IV-RLP was associated with other treatment factors (Findley et al. 2014).

In several retrospective studies, use of IV-RLP in distal limb injury and septic synovitis is described as an adjunct therapy (Milner et al. 2014; Orsini 2017; Wright et al. 2017). However, direct comparisons between outcome and treatment with IV-RLP are not made, preventing the reader from drawing any conclusions regarding efficacy of IV-RLP from these reports.

Route of Administration

As already implied, early development of the RLP technique involved drilling a tract through the cortex of a long bone and administering the drug directly into the medullary cavity. Although RLP are now typically performed IV, the IO technique was the first to be implemented in early research and clinical application (Whitehair et al. 1992c; Mattson et al. 2004). Suggested advantages of the IO technique were ease of accessibility, should soft tissue swelling or injury preclude the use of the peripheral venous system (Biasutti et al. 2021). However, the technique is much more invasive and requires use of specialized orthopedic equipment. The IV route of administration has since gained favor over the IO route for its ease of application and minimal side effects. Several studies have been performed to compare the two methods. Scheuch et al.

(2002) found higher concentrations of amikacin in the TCJ following IV-RLP when compared to IO-RLP. Another study found higher amikacin concentrations in the forelimb distal interphalangeal joint (FL-DIPJ) with IV-RLP compared to IO-RLP (Butt et al. 2001). However, there were no significant differences in time to peak concentration or elimination half-life between methods for each synovial structure studied, and each technique produced mean peak amikacin concentrations ranging from 5 to 50 times the minimum inhibitory concentration (MIC) (Butt et al. 2001).

The IO-RLP has also been reported to result in more significant adverse effects when compared to IV-RLP. Parker et al. (2010) described a case in which IO-RLP with gentamicin into the proximal phalanx was used to treat FL-DIPJ and navicular bursa synovial sepsis. Reported complications at the perfusion site included persistent osteomyelitis, progressive osteonecrosis, and ultimately pathologic fracture of the proximal phalanx (Parker et al. 2010). While the findings at necropsy were suggestive of a toxic osteonecrosis secondary to IO-RLP (Parker et al. 2010), several other factors such as antimicrobial selection and the high antimicrobial dose used could have played a role in development of these adverse effects. The most common adverse effects associated with the IV-RLP include swelling at the injection site, extravasation of perfusate subcutaneously, phlebitis, or thrombosis of the peripheral vessel (Rubio-Martinez et al. 2012; Biasutti et al. 2021). These complications are minimized with adequate restraint and sedation and are typically easily managed should they occur. Levine et al. (2009) reported a significant decrease in limb swelling after topical application of 1% diclofenac ointment, which can easily be employed in the clinical setting.

Drug Selection

Aminoglycosides are the most commonly utilized antimicrobials for RLP given their concentration-depending pharmacokinetics, free distribution through the interstitial fluid

compartment, and good spectrum of activity against commonly isolated equine orthopedic pathogens (Beccar-Varela et al. 2011; Ahern and Richardson 2012; Biasutti et al. 2021). A peak concentration (Cmax) of 8-10 times the MIC is recommended to obtain suitable bacteriocidal activity (CLSI 2020) and achieving a maximum Cmax:MIC ratio is also suggested to result in enhanced post-antibiotic effect (Murphey et al. 1999; Biasutti et al. 2021).

Of the available aminoglycosides, amikacin is often preferred. Amikacin has a wide spectrum of activity against orthopedic pathogens but is cost-prohibitive and potentially toxic to use systemically in adult horses, limiting its use to regional or local administration. A large range of synovial fluid amikacin concentrations have been reported in the currently available literature (Kelmer et al. 2013a; Mahne et al. 2014; Kilcoyne et al. 2016; Harvey et al. 2016; Moser et al. 2016; Oreff et al. 2016; Kilcoyne et al. 2018; Gustafsson et al. 2020; Kilcoyne et al. 2021). Discrepancies in synovial fluid amikacin concentrations between studies may be explained by the dose of amikacin used, discussed further in an upcoming section.

Gentamicin has also been evaluated for use in RLP treatments in horses (Whitehair et al. 1992b; Mattson et al. 2004; Werner et al. 2005; Hyde et al. 2013). As previously discussed, Whitehair et al. (1992b) compared the use of gentamicin IV with administration via IO-RLP in horses with experimentally induced septic synovitis. Mattson et al. (2004) found that standing IO-RLP of gentamicin resulted in local antibiotic concentrations in the synovial structures and bones of the distal limb that exceed the reported MIC of pathogens commonly implicated in equine orthopedic infections. Werner et al. (2005) noted no difference in bone gentamicin concentration obtained with intraarticular or IV-RLP administration, and synovial fluid concentration remained above MIC for common pathogens for over 24 hours with both methods. Hyde et al. (2013) compared varying perfusate volumes containing gentamicin and found wide variability and low (< 20 µg/mL) gentamicin concentrations in synovial fluid following digital IV-RLP in some of the limbs studied.

Time-dependent antimicrobials have also been used for IV-RLP, with varying results in synovial fluid concentrations. Ceftiofur is reported to be particularly well-suited for RLP in horses due to its broad spectrum of activity, particularly with beta-lactamase producing bacteria, and its proposed efficacy related to peak serum concentrations in veterinary species (Cox et al. 2017; Biasutti et al. 2021). Several studies suggest that ceftiofur administered daily via an IV-RLP may be appropriate for certain cases. Pille et al. (2005) performed an experimental study in normal horses and reported RCJ synovial fluid ceftiofur concentrations above MIC for >24 hours following cephalic IV-RLP with 2g of ceftiofur. Additionally, mean RCJ synovial fluid ceftiofur concentrations were consistently higher after cephalic IV-RLP compared to that after IV administration (Pille et al. 2005). In another study, ceftiofur administration via cephalic IV-RLP maintained plasma concentrations above MIC for 12 hours, while subcutaneous tissue concentrations were maintained above MIC for 24 hours (Cox et al. 2017). Bone concentrations were only above MIC immediately after tourniquet removal (Cox et al. 2017). Using cephalic IV-RLP, Bonilla et al. (2021) reported MCPJ ceftiofur synovial concentrations above MIC in only 72% and 50% of the horses 5 minutes and 8 hours after tourniquet removal, respectively. Synovial fluid ceftiofur concentrations at 24 hours were consistently below the MIC for all horses, suggesting daily cephalic IV-RLP with 2 g of ceftiofur may not achieve therapeutic concentrations in the MCPJ (Bonilla et al. 2021). Additionally, median ceftiofur concentrations did not routinely reach the 8 to 9 times MIC values recommended to increase the efficacy of beta-lactams (Bonilla et al. 2021). A study by Oreff et al. (2017) investigated use of ceftazidime administered via cephalic IV-RLP and found that synovial fluid concentrations in the MCPJ were 15 times higher than the MIC for most bacteria involved in equine orthopedic infections, including resistant pathogens such as *Pseudomonas aeruginosa* (MIC = 16 μg/mL). However, synovial concentrations decreased quickly and remained above the MIC in only 1 horse by 6 hours postperfusion, leading the authors to recommend that once daily IV-RLP with 2 g ceftazidime in standing horses may not be effective in a clinical setting (Oreff et al. 2017). This

study by Oreff et al. clearly demonstrates that the favorable results reported by some studies using IV-RLP of ceftiofur should not be extrapolated to the use of other third-generation cephalosporins.

A number of other antimicrobials have been evaluated for use for RLP in an experimental setting, but use of these antimicrobials in the clinical setting is uncommon. Of the fluoroquinolones, enrofloxacin and marbofloxacin have been studied (Parra-Sanchez et al. 2006; Lallemand et al. 2013). Enrofloxacin concentrations following cephalic IV-RLP have been shown to exceed MIC for approximately 24 hours in subcutaneous tissue and synovial fluid of the RCJ, and for 36 hours in bone marrow of the third metacarpal bone (Parra-Sanchez et al. 2006). However, 3 of 7 study horses developed vasculitis following IV-RLP with enrofloxacin, and the drug is known to cause developmental cartilage abnormalities in young horses; therefore, its use in the clinical setting should be approached with caution (Parra-Sanchez et al. 2006). Marbofloxacin showed high variability of concentrations in the synovial fluid of the RCJ following cephalic IV-RLP (Lallemand et al. 2013). The study noted that single daily IV-RLP of 0.67 mg/kg of marbofloxacin (1/3 the recommended systemic dose) should be effective in treating most important pathogens in horses, but that efficacy variables reported for *Streptococcus* spp. indicate that marbofloxacin administered by IV-RLP might not be effective against this pathogen (Lallemand et al. 2013). Additionally, no clinical or ultrasonographic signs of phlebitis or thrombophlebitis after IV-RLP administration were observed, but a significant increase in thickness of subcutaneous tissue observed on treated limbs compared to the control limbs indicates that potential for local inflammation exists with repeated administration (Lallemand et al. 2013). Chloramphenicol administered via IV-RLP yielded concentrations in the synovial fluid of the MCPJ and MCPJ initially far above the MIC of most susceptible pathogens and significantly higher than the MIC of methicillin-resistant *Staphylococcus aureus* (MRSA), but only remained at these levels for up to 6 hours (Kelmer et al. 2015). The study did not report any

adverse effects with its use (Kelmer et al. 2015). From the macrolide classification of antibiotics, erythromycin was found to consistently reach therapeutic concentrations in the synovial fluid of the fetlock joints when using the saphenous or cephalic veins for IV-RLP, but not when the palmar digital vein is used (Kelmer et al. 2013b). No adverse effects were noted with use of erythromycin (Kelmer et al. 2013b). The use of vancomycin, a glycopeptide antibiotic, was evaluated by Rubio-Martinez et al. (2005). Vancomycin concentrations exceeded MIC for susceptible pathogens in MCPJs for approximately 20 hours following IV-RLP (Rubio-Martinez et al. 2005). Additionally, higher concentrations were reached in FL-DIPJs than in MCPJs (Rubio-Martinez et al. 2005). No complications or significant differences in renal function, lameness, or clinical variables were observed between treatment and control groups (Rubio-Martinez et al. 2005). Carbapenems, a "last-line" group of antibiotics, have been evaluated experimentally but, due to emerging resistance (Biasutti et al. 2021), would be rarely used in the clinical setting. Kelmer et al. (2017) found that the imipenem Cmax in the fetlock joint following cephalic or saphenous IV-RLP of a 500 mg dose was 87 and 60 μg⁄mL, respectively. These concentrations are well above the MIC of most susceptible pathogens, including resistant bacteria such as MRSA and *Pseudomonas aeruginosa* (Kelmer et al. 2017). Meropenem has also been studied, but with less favorable results. Administration of 500 mg meropenem by cephalic IV-RLP resulted in highly variable RCJ synovial fluid concentrations between horses and only achieved levels above clinically relevant MIC for 4 hours (Fontenot et al. 2018). Mosichuk et al. (2021) performed a retrospective analysis of cases of synovial sepsis treated with meropenem or gentamicin via IV-RLP. 23 meropenem- and 37 gentamicin-treated horses were analyzed (Mosichuk et al. 2021). In the meropenem group, 9 horses received meropenem only; the remainder received another antibiotic initially then changed to meropenem. Overall survival to discharge was 86% (52/60), with meropenem at 91% (21/23) and gentamicin at 84% (31/37) (Mosichuk et al. 2021). No significant differences were noted between meropenem or gentamicin groups for overall survival to discharge or outcome after discharge, and susceptibility to other

antibiotics such as ceftiofur (*n* = 22/26), ampicillin (*n* = 18/26), amikacin (*n* = 15/26), or gentamicin (*n* = 12/26) was also frequently present (Mosichuk et al. 2021). Therefore, although meropenem appears to result in clinically positive outcomes when administered via IV-RLP, less crucial antimicrobials may be a viable and more judicious treatment option (Mosichuk et al. 2021). Gustafsson et al. produced two studies in 2021 evaluating various antimicrobials administered via cephalic IV-RLP. The first aimed to describe the pharmacokinetics and safety of the administration of trimethoprim-sulphadiazine through a single treatment of cephalic IV-RLP. Several horses in the study suffered from severe vasculitis, and the resulting MCPJ synovial fluid concentration of trimethoprim-sulphadiazine over time was low (Gustafsson et al. 2021a). In light of these findings, the administration of trimethoprim-sulphadiazine to horses using cephalic IV-RLP appears unjustified (Gustafsson et al. 2021a). The second study was performed to determine the concentration of metronidazole in the FL-DIPJ after IV-RLP. High FL-DIPJ synovial fluid metronidazole concentrations (Cmax of 327 ± 208 µg/mL) were achieved, and concentrations of metronidazole at 15 and 30 minutes after perfusion were significantly higher than concentrations before perfusion (Gustafsson et al. 2021b). However, the concentrations rapidly decreased below the MIC of potential target pathogens, leading the authors to question the effectiveness of metronidazole administered by cephalic IV-RLP as a sole therapy against anaerobic infections of synovial structures of the distal forelimb (Gustafsson et al. 2021b).

The use of antimicrobial combinations administered via IV-RLP in the horse has also been considered. Imipenem and marbofloxacin were evaluated together by Dahan et al. (2017), resulting in synovial fluid concentrations for both drugs far above the MIC, without any negative effects seen. Amikacin and penicillin have been combined in several studies with varying results (Nieto et al. 2016; Dahan et al. 2019). In one study evaluating concentrations within the MCPJ, the Cmax:MIC ratio for amikacin was 8:1 and time above MIC for penicillin was 6 hours (Nieto et al. 2016). At 24 hours, the mean concentration of amikacin in the MCPJ was still above 4

μg/mL (Nieto et al. 2016). The authors noted that while amikacin and penicillin were successfully combined for use of IV-RLP, penicillin may not be practical as rapid clearance from the synovial fluid requires frequent perfusions to maintain acceptable therapeutic concentrations (Nieto et al. 2016). However, in a later study, the Cmax of both amikacin and penicillin was over 10 times the relevant MIC in the MCPJ of all horses and remained above those MICs for at least 24 hours (Dahan et al. 2019). A study by Zantingh et al. (2014) indicated that the combination of amikacin and ticarcillin-clavulinate used for IV-RLP resulted in reduced amikacin concentrations, citing possible drug interactions, or a structural modification of the amikacin.

Since development of the RLP technique, a number of studies have evaluated a variety of antibiotic choices, making drug selection seem like a potentially daunting task. Ideally, selection of an antimicrobial for use in IV-RLP should be made on the basis of a culture and sensitivity, and in light of reported complications with specific antimicrobials. However, in the absence of a positive culture or when IV-RLP is used to treat empirically based on common equine pathogens, the literature suggests that use of an aminoglycoside is a suitable choice (Biasutti et al. 2021).

Standing Sedation vs. General Anesthesia

At the forefront of IV-RLP use in horses, Whitehair et al. (1992a) performed this technique in anesthetized patients. However, use of IV-RLP in standing, sedated horses has gained wide popularity due to its ease of use in the clinical setting, elimination of risks associated with general anesthesia, and cost effectiveness (Biasutti et al. 2021). One study suggests that tissue concentrations achieved in standing, sedated horses may be lower and more variable than those achieved when IV-RLP is performed under general anesthesia, citing decreased movement which can adversely affect the efficacy of the tourniquet (Levine et al. 2010). However, another study found that mean synovial fluid amikacin concentrations in standing sedated horses were significantly higher in the MCPJ at 30 minutes when compared to those in horses under general

anesthesia (Aristizabal et al. 2016). No significant difference was noted in synovial fluid concentrations of the RCJ (Aristizabal et al. 2016). Yet another study showed that synovial fluid amikacin concentrations in the ICJ did not differ significantly between cephalic IV-RLP performed in standing, sedated horses versus IV-RLP performed in anesthetized horses (Mahne et al. 2014). This group also found that performing a perineural regional nerve block resulted in less lifting of the limb and improved visual analog scores when compared with sedation alone or with the addition of local anesthesia to the perfusate (Mahne et al. 2014). Based on these results, the use of general anesthesia does not seem to be warranted for the sole purpose of performing an IV-RLP procedure. Additionally, use of perineural anesthesia prior to placement of the tourniquet may result in more compliant patients.

Dosages

The range of antimicrobial doses reported for use in IV-RLP is undoubtedly variable. For example, doses of amikacin used for IV-RLP vary from 250 mg to 3 g (Biasutti et al. 2021). Some studies report the use of a scaled dose, administering one-third the horse's systemic dose via IV-RLP (Beccar-Valera et al. 2011; Edwards-Milewski et al. 2016; Biasutti et al. 2021). For gentamicin, reported doses range from 500 mg (Hyde et al. 2013) to a full systemic (6.6 mg/kg) dose (Ahern and Richardson 2012). The appropriate dose of antimicrobials other than aminoglycosides has been less-widely evaluated.

When a 250 mg amikacin dose diluted to 60 mL with saline was used for cephalic IV-RLP in one study, amikacin concentrations did not achieve the recommended 8-10 times MIC in any fluid or tissue tested, leading the authors to suggest that this dose may be too low for clinical use (Parra-Sanchez et al. 2006). Harvey et al. (2016) compared a 2 g dose of amikacin to a 3 g dose, finding a higher concentration in the ICJ after IV-RLP with 3 g amikacin when compared with 2 g. Both doses (2 g and 3 g) achieved ICJ synovial fluid concentrations considered therapeutic for

susceptible pathogens (8-10 times MIC) immediately following cephalic IV-RLP, but only the 3 g dose achieved concentrations high enough to be therapeutic for resistant pathogens (Harvey et al. 2016). The same study showed that in the MCPJ, only the 3 g dose of amikacin achieved synovial fluid concentrations considered therapeutic for susceptible pathogens (Harvey et al. 2016). These findings suggest that the appropriate dose of antimicrobials may also depend on the site of infection (Harvey et al. 2016). Moser et al. (2016) evaluated use of 1 g amikacin via cephalic IV-RLP and found higher synovial fluid amikacin concentrations within the FL-DIPJ when compared with the RCJ, supporting the theory that site of infection may influence success of IV-RLP treatment and/or dictate dose required to achieve therapeutic concentrations.

The most commonly reported dose of ceftiofur for IV-RLP is 2 g (Pille et al. 2005; Cox et al. 2017; Bonilla et al. 2021). As previously discussed, use of this dose of ceftiofur for cephalic IV-RLP resulted in concentrations above MIC in the RCJ for >24 hours in one study (Pille et al. 2005) and maintained subcutaneous tissue concentrations above MIC for 24 hours in another study (Cox et al. 2017). Ceftiofur concentrations in the MCPJ were consistently below the MIC for all horses at 24 hours in a more recent study (Bonilla et al. 2021), demonstrating variability in results when the same dose of ceftiofur is used.

Making strict dosing recommendations for clinical application of IV-RLP using antimicrobials is not possible based on the current literature. Dose used appears to be largely arbitrary and based on only a few clinical studies, suggesting that further research is needed.

Frequency of Administration

Frequency of IV-RLP administration is often quite variable, but the literature suggests that daily administration is likely to beneficial (Rubio-Martinez et al. 2012; Kelmer et al. 2012; Harvey et al. 2016; Kelmer 2016; Biasutti et al. 2021). There are no current recommendations on the number of treatments considered ideal, but consensus appears to be that treatments should be

repeated as often as is practical based on patient tolerance and response to treatment. A retrospective study by Rubio-Martinez et al. (2012) reported up to 19 perfusions administered to one patient, while another study reported up to 21 perfusions using an indwelling catheter (Kelmer et al. 2012).

Optimal Vessel

Realistically, any peripheral vessel that can be isolated from systemic circulation using a tourniquet could be utilized for administration of IV-RLP. Most studies have used the cephalic vein, although the saphenous and digital vessels have been evaluated as well (Scheuch et al. 2002; Alkabes et al. 2011; Hyde et al. 2013; Kelmer et al. 2013b; Kelmer et al. 2015; Godfrey et al. 2016; Kelmer et al. 2017; Snowden et al. 2019; Kilcoyne et al. 2021). Use of proximal vessels for treatment of distal limb synovial sepsis or infection appears to be efficacious, as evidenced by high concentrations of antimicrobials found in the distal interphalangeal joint following cephalic IV-RLP (Moser et al. 2016; Schoonover et al. 2017). Advantages of proximal peripheral vessels (namely the cephalic or saphenous veins) include accessibility above bandages or half-limb casts; the unlikelihood that the area of infusion is affected by soft tissue swelling associated with injury to the distal limb; the ability to place indwelling catheters more easily, if desired; and a decreased likelihood that the infusion apparatus is dislodged if movement occurs during perfusion (Biasutti et al. 2021).

Tourniquet Type, Number, and Duration

Several varieties of tourniquets are available for clinical use and have been studied experimentally. Pneumatic tourniquets require specialized equipment but allow for standardization of sub-tourniquet pressures between patients. Alternatively, wide rubber or Esmarch tourniquets are easily placed, require no other equipment, are widely available, and are cost-effective from an investment standpoint. However, application of pressure may vary among

practitioners and is not as easily repeatable. Levine et al. (2010) evaluated the efficacy of 3 tourniquet types (narrow rubber, wide rubber, and pneumatic) and found that the pneumatic tourniquet resulted in the highest metacarpophalangeal synovial fluid amikacin concentrations in all horses. Additionally, both the pneumatic and wide rubber tourniquets achieved adequate amikacin concentrations (Levine et al. 2010). The narrow rubber tourniquet was found to be ineffective in achieving high concentrations of amikacin in the synovial fluid, and the authors suggest this tourniquet type should not be used for IV-RLP above the carpus in the standing horse (Levine et al. 2010). Subtourniquet pressures of at least 100 mmHg above the systolic blood pressure are suggested in order to occlude venous return and prevent leakage beyond the tourniquet (Rubio-Martinez and Cruz 2006; Alkabes et al. 2011). In one study, wide rubber tourniquets applied at the antebrachium, gaskin, and metacarpal regions generated and maintained subtourniquet pressures >100 mmHg above systolic blood pressure for 30 minutes in standing, sedated horses (Plunkett et al. 2019). Application of a wide rubber tourniquet was implicated in another study as being superior to a pneumatic tourniquet, as indicated by lower amikacin concentrations detected in systemic circulation (Alkabes et al. 2011). Clinically, desired pressure ranges of 250-420 mmHg are reported (Levine et al. 2010; Rubio-Martinez et al. 2012; Biasutti et al. 2021) while experimentally, most studies utilize a pneumatic tourniquet pressure of 400-450 mmHg (Scheuch et al 2002; Levine et al 2010; Alkabes et al. 2011; Aristizabal et al. 2016; Godfrey et al. 2016; Harvey et al. 2016; Moser et al. 2016; Schoonover et al. 2017).

The number of tourniquets required to optimize treatment with IV-RLP in horses has also been evaluated, albeit sparingly. Schoonover et al. (2017) found that synovial fluid amikacin concentrations achieved in the RCJ were significantly higher when a proximal pneumatic tourniquet was combined with a distal Esmarch tourniquet 15 and 30 minutes after perfusion, compared to use of a proximal tourniquet alone. Whitehair et al. (1992a) reported the use of two

tourniquets in the forelimb for equine IO-RLP, but evaluation of the effect of this technique was not undertaken as part of that study.

Suggested tourniquet application times range from 10 minutes to 45 minutes (Kilcoyne et al. 2016; Biasutti et al. 2021). Some studies found that maximum amikacin synovial fluid concentrations were reached at 15 minutes after perfusion in the forelimb (Kilcoyne et al. 2018; Gustafsson et al. 2020). Moser et al. (2016) reported no significant difference in synovial fluid amikacin concentrations in either the RCJ or FL-DIPJ at 15 and 30 minutes after perfusion. This result was substantiated by another study performed in 2016 by Kilcoyne et al., which found no significant difference in synovial fluid amikacin concentrations of the RCJ or MCPJ after cephalic IV-RLP comparing tourniquet times of 10 and 30 minutes.

Exsanguination

It is theorized that reducing the volume of blood in the region to be treated via IV-RLP increases the concentration of the perfusate and promotes a more effective concentration gradient for diffusion of the antimicrobial used (Sole et al. 2016; Biasutti et al. 2021). In the horse, limb exsanguination can be performed by the application of an Esmarch bandage around the distal extremity starting at the level of the hoof capsule (Sole et al. 2016). Studies performed to evaluate the need for exsanguination when performing IV-RLP in horses have been contradictory. Sole et al. (2016) reported improved amikacin synovial fluid concentrations in the MCPJ when exsanguination was performed prior to IV-RLP but did not find any significant differences between concentrations in the RCJ. Schoonover et al. (2017) showed no differences in synovial fluid amikacin concentrations in the RCJ and FL-DIPJ when exsanguination was performed prior to low volume (10 mL) IV-RLP. Further evaluation is necessary to determine the potential benefit of exsanguination prior to performing IV-RLP in horses.

Volume of Perfusate

The reported total volume of perfusate varies heavily among studies (Biasutti et al. 2021). The suggested advantages of a smaller perfusate volume include faster infusion of the perfusate, a more concentrated solution that may bolster the subsequent development of a concentration gradient, and reduced vascular pressure that may prevent or reduce leakage of perfusate beyond the tourniquet (Hyde et al. 2013; Moser et al. 2016; Schoonover et al. 2017; Biasutti et al. 2021). From the other perspective, a larger perfusate volume may result in increased vascular pressure that promotes expansion of the spaces between endothelial cells, leading to easier perfusion of the antimicrobial into tissues (Godfrey et al. 2016; Biasutti et al. 2021).

Moser et al. (2016) reported no significant difference in synovial fluid amikacin concentrations in the RCJ and FL-DIPJ after cephalic IV-RLP, comparing 4 perfusate volumes (10 mL, 30 mL, 60 mL, and 120 mL) in standing horses. Similarly, Hyde et al. (2013) reported no significant difference in synovial fluid gentamicin concentrations in the MCPJ after digital IV-RLP comparing 3 volumes (10 mL, 30 mL, and 60 mL) in standing horses. On the contrary, Oreff et al. (2016) reported greater synovial fluid amikacin concentrations in the MCPJ after cephalic IV-RLP, comparing perfusate volumes of 100 mL, 60 mL, and 30 mL. Godfrey et al. (2016) also reported greater synovial fluid amikacin concentrations in the MCPJ after digital IV-RLP using a 60 mL perfusate volume compared to a 10 mL volume in recumbent, anesthetized horses.

Along these lines, and as previously stated, the objective of this study was to establish if volume of perfusate influenced synovial fluid amikacin concentrations of the TCJ, MTPJ, or HL-DIPJ after saphenous IV-RLP perfusate containing 1 g amikacin. We hypothesized that volume of perfusate would not significantly affect amikacin concentrations achieved in the synovial fluid after saphenous IV-RLP at 15 and 30 minutes, and that synovial fluid amikacin concentration observed in the joints of the lower limb (HL-DIPJ & MCPJ) would be significantly higher than

those achieved in the TCJ at 15 and 30 minutes after saphenous IV-RLP. Lastly, it was hypothesized that the larger perfusate volumes (60 &120 mL) would result in a higher regional intravascular pressure leading to more horses with detectable systemic amikacin concentrations, indicating a higher frequency of leakage of the perfusate beyond the tourniquet into the systemic circulation than the 10 mL volume.

CHAPTER III

METHODOLOGY

Animals

Six Quarter Horse type horses (4 geldings, 2 mares) ranging in age from 7 to 20 years (median 15 years) and weighing from 393 to 567 kg (median 535.5 kg) were used. All horses were deemed healthy on physical examination. Close visual inspection and palpation of both hind limbs yielded symmetry, normal vascularity, and no signs of acute or chronic injury. All horses were confined to a large paddock and fed grass hay and a 14% protein pelleted ration before and during the study period. All procedures were approved by and performed in accordance with the Oklahoma State University Institutional Animal Care and Use Committee.

IV-RLP

Three volumes of perfusate (10 mL, 60 mL, 120 mL) made up of 0.9% NaCl containing 1 g of amikacin (250 mg/ml;4 mL) were used for standing saphenous IV-RLP. A randomized, cross-over study design was used so that each horse was administered three IV-RLPs using a different perfusate volume in a predetermined order (2 – 10 mL, 60 mL, 120 mL; 2 – 60 mL, 120 mL, 10 mL; 2- 120 mL, 10 mL, 60 mL). A minimum 2-week washout period was used between perfusions. The hind limb for IV-RLP was chosen at random by a coin toss before the first IV-RLP and subsequent IV-RLPs.

Horses were sedated with detomidine HCL (0.01mg/kg IV) and butorphanol tartrate (0.01 mg/kg

IV) administered in the jugular vein contralateral to the hind limb receiving the IV-RLP. Horses were positioned in stocks and tibial and peroneal perineural anesthesia was performed on the selected hind limb using 20 mL of 2% mepivacaine hydrochloride at each site approximately 10 cm proximal to the point of the calcaneus, as described by Moyer et al. (2011b). The dorsomedial aspect of the tarsus, the dorsal and lateral aspects of the MTPJ, and the dorsal coronet were clipped and aseptically prepared with 4% chlorhexidine gluconate scrub and 70% isopropyl alcohol. A 5.7-inch X 23-inch pneumatic tourniquet cuff (Vari Contour Cuff, Delfi Medical Innovations Inc, Vancouver, BC, Canada) was positioned on the gaskin approximately 15cm proximal to the point of the calcaneus. Two wraps of elastic tape (Elastikon, Johnson & Johnson, New Brunswick, NJ) was placed to secure the cuff to the limb and prevent migration before and during cuff inflation. Immediately before, during and for 30 minutes after perfusate administration, the tourniquet was inflated and maintained at 420 mmHg using a pneumatic inflation system (Delfi P.T.S, Delfi Medical Innovations Inc, Vancouver, BC, Canada). In an effort to decrease variability between horses, the tourniquet was placed by the same investigator.

Samples

Similar to previous cephalic IV-RLP studies (Moser et al. 2016; Schoonover et al. 2017), a 20-gauge 1.5-inch needle was placed into the TCJ (dorsomedial aspect), MTPJ (dorsolateral aspect), and HL-DIPJ (dorsal midline) and capped with a male adaptor injection port, and maintained for synovial fluid sampling. Sterile lactated ringer's solution (LRS) was injected into the TCJ (0.04mL/kg), MTPJ (0.03mL/kg) and HL-DIPJ (0.01mL/kg) at the time of needle placement, but prior to administration of the perfusate, to facilitate fluid recovery from the joint during sampling. The dose of LRS was determined using the high end of the recommended volume of local anesthetic for each individual joint based on a 500 kg horse (Moyer et al. 2011a). Five minutes after the last LRS infusion, baseline samples were collected. Systemic venous blood was collected via direct venipuncture of the ipsilateral jugular vein and synovial fluid (0.5–1.0 mL)

was aspirated from the TCJ, MTPJ and HL-DIPJ via the previously placed needles. Venous blood samples were placed in plain plastic tubes and synovial fluid samples were placed directly into 1mL cryovial tubes (CryoElite, Fisher Scientific, Pittsburgh, PA).

After collection of all baseline samples, the tourniquet was inflated. A 23-gauge winged infusion set was placed in a proximal to distal direction in the ipsilateral saphenous vein distal to the tourniquet. A 3-way stopcock was placed on the winged infusion set tubing and 1 g (4 mL) of amikacin (Amiglyde-V, Zoetis, Florham Park, NJ) combined with either 6 mL, 56 mL, or 116 mL of 0.9% NaCl, the injectate was administered through the right-angled port via a syringe at a steady rate by the same investigator. The amount of time required to administer the entire perfusate volume was recorded. During injection, the injection site was visually monitored for subcutaneous swelling indicating perivascular leakage of the perfusate. Once the entire perfusate volume was administered, a digital pressure gauge (model DPG1000B-760MMHGG; Omega Engineering, Norwalk, Connecticut) was attached to the straight port of the 3-way stopcock. The valve was turned opening the infusion set to the digital pressure gauge and the intravascular pressure was recorded. The needle of the winged infusion set was then removed from its insertion site in the skin and a temporary compressive bandage was placed over the venipuncture site and left in place until the tourniquet was deflated. Ten minutes after the conclusion of the perfusate administration, each horse received an additional sedation dose of detomidine HCL (0.01 mg/kg, IV) by direct jugular venipuncture contralateral to the hind limb receiving the IV-RLP.

Synovial fluid was aspirated from the TCJ, MTPJ and HL-DIPJ, and venous blood was collected from the ipsilateral jugular vein 15 and 30 minutes after perfusate administration. The tourniquet was released after collection of the 30 minute samples and all horses received a single dose of flunixin meglumine (1.1 mg/kg, IV). All venous blood samples were allowed to clot for a minimum of 30 minutes, then centrifuged (Thermo Scientific, Waltham, MA) at 3,500 X *g* for 5 minutes. The serum was drawn off with a plastic pipette and placed into a 1 mL cryovial tube

(CryoElite, Fisher Scientific). All serum and synovial fluid samples were frozen in the cryovial tubes at – 80°C until assayed. Horses were monitored for 2 hours after each IV-RLP procedure to ensure acceptable recovery from sedation and identify any initial complications, after which they were returned to paddock turnout and the IV-RLP site was visually evaluated daily for one week.

Amikacin Concentration Analysis

Amikacin concentration was measured using an enzyme immunoassay (Emit® assay, Syra, Siemens Healthcare Diagnostics Inc, Newark, DE) at the Clinical Pharmacology Laboratory at Auburn University. Briefly, a calibration curve was established using a reagent kit (Ref. No. 6X019UL) consisting of amikacin, human serum, and preservatives. Calibration samples were reconstituted with deionized water to yield final concentrations of 2.5, 5, 10, 20, and 50 mg/mL. Quality control was performed before sample analysis. Samples that yielded very high (>3,000 mg/mL) amikacin concentrations were diluted in 3 stages with dilution factors of 10, 100, and 300. To evaluate precision of the process, the same dilution procedure was applied to a 3,000 mg/mL stock solution. The sensitivity of the assay or lowest measurable concentration of amikacin sulfate was 5.0 µg/mL for synovial fluid and 2.5 µg/mL for serum, thus any measurement equal to or below these concentrations was considered 0.

Target Concentrations

Minimum amikacin concentrations in synovial fluid considered therapeutic were based on the recommendation of the subcommittee on Veterinary Antimicrobial Susceptibility Testing (CLSI 2020). For adult horses, the reported MIC of amikacin for common orthopedic pathogens (*Escherichia. coli, Staphylococcus aureus, Streptococcus equi* subsp. *zooepidemicus* and subsp. *equi* and *Pseudomonas* spp.) is ≤4 µg/mL for susceptible pathogens and ≥16 µg/mL for resistant pathogens (CLSI 2020). Regional administration of aminoglycosides using 8–10 times the MIC as a target concentration is considered effective (Beccar-Varela et al. 2011). Therefore, target

concentrations of 40 μg/mL and 160 μg/mL were established for susceptible and resistant pathogens, respectively.

Statistical Analysis

The mean amikacin concentration from each location (HL-DIPJ, MTPJ and TCJ) for each time point (baseline, 15 minutes, 30 minutes) for each technique (10 mL, 60 mL, 120 mL) was used for analysis. Log(1+y) transformation was used to achieve normality. Tests for main effects (and all possible interactions) of each factor (each combination of factors) were conducted with a linear mixed effects model and if significant, pairwise comparisons using a Tukey-Kramer test were made among the levels of the factor(s). Significance was determined to be any p-value <0.05.

CHAPTER IV

FINDINGS

No long-term complications associated with the IV-RLPs were noted in any horse. Two horses developed mild edema at the site of saphenous venipuncture, one after the second IV-RLP (60 mL) and one after the third IV-RLP (10 mL). In both instances the edema resolved without treatment in 4-5 days. In three horses, soft tissue swelling of the medial gaskin region was noted 2-3 days after completion of the third IV-RLP. The swellings were attributed to sequential tourniquet application and resolved without intervention 3-4 days later. During six IV-RLPs, limb movement resulted in sample collection needle displacement from the skin at various time points (0-29 minutes; 4-HL-DIPJ, 1-MTPJ, 1-TCJ). In each instance, a new sample collection needle was placed, and synovial fluid was retrieved without further complication. During the IV-RLP of the 10 mL perfusate volume of horse 1, an inadequate amount of synovial fluid was obtained from the HL-DIPJ at all three time points. These time points were excluded from analysis.

Mean regional intravascular pressures measured via the injection catheter immediately after perfusion were not different between volumes ($p=0.2$; Table 1). Perivascular leakage during perfusate administration was noted during five IV-RLPs (Table 1). When this occurred, either the perfusate administration was slowed without further enlargement of the swelling (2) or the butterfly catheter was removed and reinserted distal to the swelling (3). Mean (range) time for perfusate administration was 1 minute 38 seconds (1:04-2:03) for the 10 mL volume, 5 minutes 6 seconds (2:25-9:08) for the 60 mL volume, and 9 minutes 27 seconds (6:10-13:06) for the

120 mL volume.

Table 1 Regional intravascular pressure (mmHg) measured immediately after saphenous intravenous regional limb perfusion of 1 g of amikacin using 3 perfusate volumes in 6 standing horses. [a]Denotes perivascular leakage observed at the injection site during perfusion.

Horse #	Perfusate volume		
	10mL	60mL	120mL
1	158	203	136*
2	60	145	144
3	95	105*	110*
4	55	138	147
5	119	94	104*
6	58	114	41*
Mean	91	133	114
Median	78	126	123

All mean baseline synovial fluid amikacin concentrations were below the limit of detection of the assay (5.0 μg/mL). Only one baseline synovial fluid sample yielded an amikacin concentration above this limit; 5.4 μg/mL was measured from the synovial fluid of the TCJ from horse 3 using the 120 mL perfusate volume. The mean synovial fluid amikacin concentrations achieved were not different between perfusate volumes used for any joint studied ($p=0.4$; Table 2.) Additionally, no difference was detected in mean synovial fluid amikacin concentration achieved between joints for any perfusate volume ($p=0.5$; Table 2). For all joints studied, the mean synovial fluid amikacin concentration achieved at 15 minutes was higher than that at baseline using the same perfusate volume ($p=0.0002$). Likewise, the mean synovial fluid amikacin concentrations achieved at 30 minutes were higher than those at baseline ($p<0.0001$) and 15 minutes ($p=0.003$; Table 2). All baseline serum amikacin concentrations were below the detectable limit of 2.5 μg/mL. For the 10 mL volume, all 15 and 30 minute serum concentrations

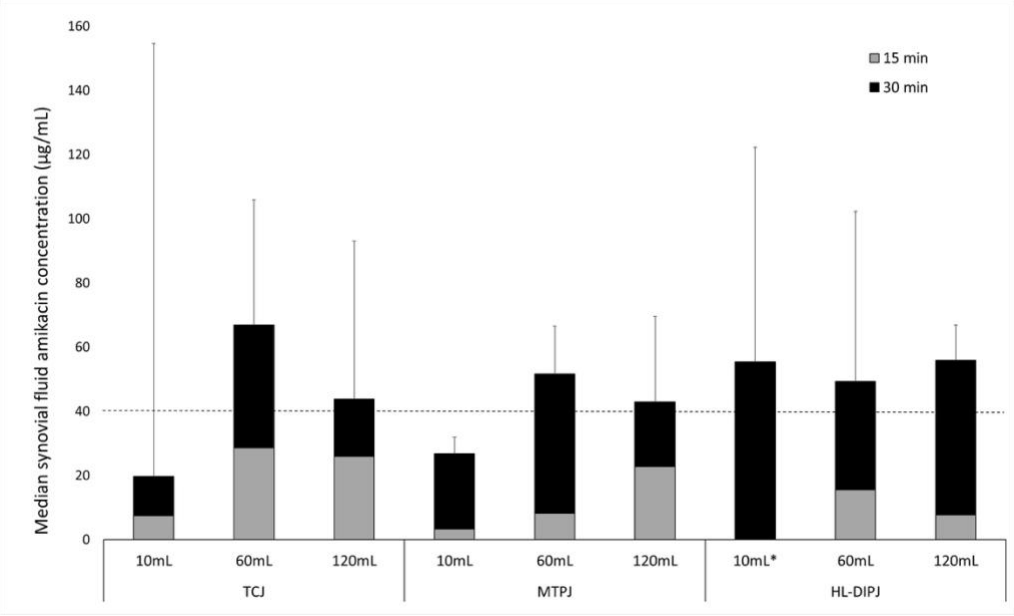
were below 2.5 μg/mL (Table 2). The serum amikacin concentration was above 2.5 μg/mL at 15 minutes for one horse administered the 120 mL volume (4.4 μg/mL). The serum amikacin concentration was above 2.5 μg/mL at 30 minutes for one horse administered the 60 mL volume (3.3 μg/mL) and 2 horses administered the 120 mL volume (3.6 & 2.9 μg/mL).

Table 2 Mean, median, and ranges of amikacin concentrations in the serum (systemic) and the synovial fluid of the tarsocrural joint (TCJ), metatarsophalangeal joint (MTPJ), and hind limb distal interphalangeal joint (HL-DIPJ) of 6 horses at 15 and 30 minutes after saphenous intravenous regional limb perfusion using different perfusate volumes (10 mL, 60 mL, and 120 mL) containing 1g of amikacin. >40 μg/mL is the concentration considered therapeutic for susceptible pathogens. >160 μg/mL is the concentration considered therapeutic for resistant pathogens. *Denotes n=5 due to inability to collect samples in one horse. †Denotes concentration at 30 minutes significantly different than that at 15 minutes (p=0.003.) ‡Denotes median concentrations over the concentration considered therapeutic for susceptible pathogens.

Perfusate volume	Region	Time (min)	Mean (μg/mL)	Median (μg/mL)	Range (μg/mL)	# >40μg/mL	# >160μg/mL
10mL	Systemic	15	0	0	0-0	N/A	N/A
		30	0	0	0-0	N/A	N/A
	TCJ	15	349.0	7.5	0-2056.9	1/6	1/6
		30	177.8†	19.7	0-975.9	2/6	1/6
	MTPJ	15	6.0	3.3	0-20.7	0/6	0/6
		30	27.9†	26.9	11.7-52.1	1/6	0/6
	HL-DIPJ*	15	13.9	0	0-56.1	1/5	0/5
		30	135.3†	55.4‡	0-452.8	3/5	1/5
60mL	Systemic	15	0	0	0-0	N/A	N/A
		30	0.5	0	0-3.3	N/A	N/A
	TCJ	15	28.1	28.6	0-50.2	2/6	0/6
		30	97.9†	67‡	6.8-321.2	4/6	1/6
	MTPJ	15	10.6	8.2	0-24.9	0/6	0/6
		30	60.3†	51.6‡	21.2-124.3	3/6	0/6
	HL-DIPJ	15	73.9	15.6	0-332.3	2/6	1/6
		30	124.5†	49.3‡	0-329.9	3/6	2/6
120mL	Systemic	15	0.7	0	0-4.4	N/A	N/A
		30	1.1	0	0-3.6	N/A	N/A
	TCJ	15	45.8	25.9	0-169.4	1/6	1/6
		30	120.8†	43.8‡	7.3-360.6	4/6	2/6
	MTPJ	15	43.8	22.8	0-122.5	2/6	0/6
		30	77.1†	42.9‡	0-157.5	4/6	1/6
	HL-DIPJ	15	27.9	7.7	0-93.6	2/6	0/6
		30	65.0†	56‡	30.7-122.2	5/6	0/6

The median synovial fluid amikacin concentrations in all joints studied were below the concentration considered therapeutic for susceptible pathogens (40 µg/mL) at 15 minutes for all volumes of perfusate and were below this concentration at 30 minutes for the TCJ and MTPJ using the 10 mL volume (Figure 1). The median synovial fluid amikacin concentrations in all joints were above the concentration considered therapeutic for susceptible pathogens at 30 minutes for the 60 mL and 120 mL perfusate volumes (Figure 1). Only the HL-DIPJ was above this concentration at 30 minutes with the 10 mL volume (Figure 1). No median synovial fluid amikacin concentrations were above the concentration considered therapeutic for resistant pathogens at either 15 or 30 minutes in any joint (Figure 1).

Figure 1 Median synovial fluid amikacin concentrations in the tarsocrural joint (TCJ), metatarsophalangeal joint (MTPJ), and hind limb distal interphalangeal joint (HL-DIPJ) of six horses at 15 and 30 minutes following saphenous intravenous regional limb perfusion using different perfusate volumes (10mL, 60mL, &120mL) containing 1g of amikacin. Error bars denote positive standard error at 30 minutes. Dotted line indicates concentration considered therapeutic for susceptible pathogens (40µg/mL). *Denotes n=5 due to inability to collect samples in one horse.



CHAPTER V

CONCLUSION

The results of the current study support the hypothesis that perfusate volume does not affect synovial fluid amikacin concentrations achieved in the TCJ, MTPJ, and HL-DIPJ with saphenous IV-RLP in standing horses. These results are consistent with Moser et al. (2016) which reported no significant difference in synovial fluid amikacin concentrations in the RCJ and FL-DIPJ after cephalic IV-RLP comparing four perfusate volumes (10 mL, 30 mL, 60 mL, & 120 mL) in standing horses. The results of the current study are also consistent with Hyde et al. (2013) which reported no significant difference in synovial fluid gentamicin concentrations in the MCPJ after digital IV-RLP comparing three volumes (10 mL, 30 mL, & 60 mL) in standing horses. However, the results of the current study are in contrary to Oreff et al. (2016) which reported greater synovial fluid amikacin concentrations in the MCPJ after cephalic IV-RLP using a 100 mL perfusate volume compared to smaller volumes (30 ml & 60 mL) in standing horses. In addition to the limb studied, tourniquet type, and slightly different perfusate volumes, a larger amikacin dose (2 g) was used in this prior study (Oreff et al. 2016) compared to the current study (1 g). These differences in study design could account for the discrepancy in reported results. The results reported here are also in contrast to Godfrey et al. (2016) which reported greater synovial fluid amikacin concentrations in the MCPJ after digital IV-RLP using a 60 mL perfusate volume compared to a 10 mL volume in recumbent, anesthetized horses. Although the same dose of amikacin (1 g) was used in this (Godfrey et al. 2016) and the current study, differences in study design specifics including tourniquet type, limb, position, and vessel used for IV-RLP could

account for the differences in results.

Contrary to our hypothesis, the mean synovial fluid amikacin concentrations were not different among the three joints studied at either 15 or 30 minutes. Kilcoyne et al. (2016) reported similar synovial fluid amikacin concentrations in the RCJ and MCPJ at 10 and 20 minutes after cephalic IV-RLP; however, Moser et al. (2016) described synovial fluid amikacin concentrations in the FL-DIPJ significantly higher than those reported in the RCJ at 15 and 30 minutes after cephalic IV-RLP. Differences in vascular perfusion of the HL-DIPJ and FL-DIPJ could explain this discrepancy but further studies evaluating antimicrobial concentrations in the HL-DIPJ after saphenous IV-RLP are warranted.

In the current study, synovial fluid amikacin concentrations measured at 30 minutes were significantly greater compared to those measured at 15 minutes for all joints studied. These results support the use of a tourniquet for longer than 15 minutes when performing saphenous IV-RLP with 1 g of amikacin and are consistent with Kilcoyne et al. (2021) which reported the time required to achieve amikacin Cmax in the synovial fluid of the TCJ after saphenous IV-RLP with 2 g amikacin was 25 minutes (range 20 to 30 minutes). However, the results of the current study are contrast to several studies in the forelimb evaluating cephalic IV-RLP (Kilcoyne et al. 2016; Moser et al. 2016; Kilcoyne et al. 2018; Gustafsson et al. 2020). Kilcoyne et al. (2016) found no difference in synovial fluid amikacin concentrations of the RCJ or MCPJ after cephalic IV-RLP comparing tourniquet times of 10 and 30 minutes. Additionally, Kilcoyne et al. (2018) found the synovial fluid amikacin Cmax in the FL-DIPJ after cephalic IV-RLP was achieved at 15 minutes, although this study utilized a total tourniquet time of 20 minutes instead of 30 minutes as in the current study. Gustafsson et al. (2020) reported significantly higher synovial fluid amikacin concentrations in the RCJ at 15 minutes compared to 30 minutes with the highest concentration in all 6 horses measured between 10 and 20 minutes. Moser et al. (2016) also reported no significant difference in synovial fluid amikacin concentrations in either the RCJ or FL-DIPJ at 15 and 30

minutes with 30 mL, 60 mL, and 120 mL perfusate volumes. Interestingly, a higher synovial fluid amikacin concentration was seen in the FL-DIPJ at 30 minutes compared to 15 minutes using a 10 mL perfusate volume (Moser et al. 2016). Anatomic differences between the equine forelimb and hind limb may account for the difference in tourniquet time necessary to reach maximum synovial fluid amikacin concentrations comparing saphenous and cephalic IV-RLP studies.

A larger perfusate volume has been advocated to increase the intravascular pressure and result in a higher drug diffusion rate to the surrounding tissues (Whitehair et al. 1992c). In contrast, a higher intravascular pressure may result in more perfusate escaping into the systemic circulation (Grice et al. 1986) or loss of venous integrity at the injection site leading to extravascular perfusate leakage (Moser et al. 2016). In the current study, systemic amikacin concentrations above the lower limit of quantitation (LLQ) (2.5 µg/mL) were only observed for 6 0mL and 120 mL perfusate volumes. Systemic concentrations above the LLQ were not observed during any IV-RLPs using the 10 mL perfusate volume, indicating superior tourniquet effectiveness with this volume, consistent with our hypothesis. However, contrary to our hypothesis, the mean regional intravascular pressures immediately after perfusate administration did not differ between perfusate volume. These results are consistent with those from Moser et al. (2016) investigating standing cephalic IV-RLPs, but contrasts those from Godfrey et al. (2016) which describes higher pressures with a 60 mL compared to a 10 mL perfusate volume during digital IV-RLP in anesthetized horses. Additionally, in the current study, the 120 mL perfusate volume was associated with local swelling indicating perfusate leakage at the site of saphenous venipuncture in 4/6 (66%) horses. Nonetheless, the synovial fluid amikacin concentrations observed for the 6 0mL & 120 mL perfusate volume were not significantly different from the 10 mL volume, questioning the clinical relevance of these observations.

Median target concentrations of 40 µg/mL and 160 µg/mL were considered therapeutic for susceptible and resistant pathogens, respectively based on 10 times the recommended MIC for

common equine orthopedic pathogens (CLSI 2020). Medians rather than means were compared to target concentrations due to the large range of synovial fluid amikacin concentrations seen in the current (Table 2) and other IV-RLP studies (Kelmer et al. 2013a; Mahne et al. 2014; Moser et al. 2016; Oreff et al. 2016; Kilcoyne et al. 2016; Harvey et al. 2016; Kilcoyne et al. 2018; Gustafsson et al. 2020; Kilcoyne et al. 2021). For all joints studied, the median synovial fluid amikacin concentrations observed at 30 minutes for the 60 mL and 120 mL perfusate volumes exceeded the target concentration considered therapeutic for susceptible pathogens; however, no median synovial fluid concentration reached that considered therapeutic for resistant pathogens at either time for any volume. The median synovial fluid amikacin concentrations in the TCJ observed in the current study using 60 mL perfusate volume were well below those reported by Kilcoyne et al. (2021) at 15 minutes (29 µg/mL; range 0-50 vs. 190 µg/mL; range 80–311) and 30 minutes (67 µg/mL; range 7-321 vs. 404 µg/mL; range 285–722). The median synovial fluid amikacin concentrations in the MTPJ at 30 minutes with the 60 mL perfusate volume in the current study (52 µg/mL) were also well below those reported by Kelmer et al. (2012) (363 µg/mL). The current study administered a total dose of 1 g of amikacin whereas Kilcoyne et al. (2021) and Kelmer et al. (2012) both administered twice that (2 g), which likely explains the discrepancy in synovial fluid amikacin concentrations between the studies. A larger amikacin dose (2 g versus 3 g) has been shown to result in higher synovial fluid amikacin concentrations in the ICJ with cephalic IV-RLP (Harvey et al. 2016). In an in vitro study, Pezzanite et al. (2020) found that 24-hour exposure to a concentration of ~800 µg/mL amikacin resulted in the death of 50% of synoviocytes and a concentration of ~300 µg/mL resulted in death of 50% of chondrocytes, indicating amikacin has a dose-dependent cytotoxic effect on articular tissue. Prior studies have shown a rapid rise followed by a rapid decrease in synovial fluid amikacin concentration within the first 24 hours after tourniquet release with cephalic (Kelmer et al. 2013a; Kilcoyne et al. 2016), saphenous (Scheuch et al. 2002; Kelmer et al. 2013a), and digital (Godfrey et al. 2016) IV-RLP. Although the initial synovial fluid amikacin concentrations reported in these

studies may have exceeded those described as cytotoxic by Pezzanite et al. (2020), the rapid decrease in concentration may allow these cells to recover. To the author's knowledge, adverse effects on the articular tissues directly attributed to the clinical use of amikacin via IV-RLP are not reported but they may be difficult to distinguish from those expected from a septic arthritic process and thus overlooked. In vivo studies evaluating the cytotoxic effects of different doses of amikacin administered via IV-RLP are warranted.

Limitations of this study include the use of normal horses free of septic arthritis, and the addition of LRS to the joint compartments to facilitate synovial fluid sampling. The latter created effusion and could have had a dilutional effect, decreasing the synovial fluid amikacin concentrations observed; however, the addition of LRS was done prior to the IV-RLP allowing the total volume (LRS plus the existing synovial fluid) to be perfused. Horses with joint disease, especially those with joint sepsis, generally exhibit considerable joint effusion, thus this model was possibly more clinically accurate. Beccar-Varela et al. (2011) reported inflammation within synovial structures to alter the pharmacokinetics and pharmacodynamics of amikacin, resulting in increased synovial fluid amikacin concentrations and a decreased time to reach maximum synovial fluid amikacin concentration. Clinical studies evaluating synovial fluid amikacin concentrations within septic synovial structures after saphenous IV-RLP are warranted. Additionally, the possibility for Type II error exists within our statistical analysis. A power calculation limiting Type I error to 5% and Type II error to 20% was performed prior to data collection, yielding a minimum sample size of 6; however, increasing the number of horses utilized would have reduced the likelihood of Type II error.

The results of the current study indicate perfusate volumes of 10-120 mL result in similar synovial fluid amikacin concentrations after saphenous IV-RLP with 1 g of amikacin. The 10 mL perfusate volume was associated with less injection complications compared to the larger (6 0mL & 120 mL) volumes. The median synovial fluid amikacin concentration was above that

considered therapeutic for susceptible pathogens in the synovial fluid of the TCJ, MTPJ, and HL-DIPJ at 30 minutes with the 60 mL and 120 mL perfusate volumes. This concentration was not observed with the 10 mL volume in either the TCJ or MTPJ. Concentrations considered therapeutic for resistant pathogens were not achieved with saphenous IVRLP using 1 g of amikacin in any joint studied. Therefore, regardless of perfusate volume used, treatment of septic arthritis with amikacin delivered via saphenous IV-RLP would likely require a dose greater than 1 g to be effective for pathogens considered resistant. Further investigation focused on perfusate volume effect of synovial fluid concentrations using a higher dose of amikacin delivered via saphenous IV-RLP is needed before recommendations related to perfusate volume can be made. This additional research is also essential in determining whether higher doses of amikacin delivered via saphenous IV-RLP can achieve concentrations considered therapeutic for resistant pathogens. Additionally, further research is required to determine the effect of multiple tourniquets on synovial fluid concentrations in the hind limb following saphenous IV-RLP, similar to the work done by Schoonover et al. (2017).

IV-RLP is a very cost-effective, minimally invasive, and safe technique that achieves high concentrations of an antibiotic in synovial structures of the distal limb in horses. Although much has been done in the way of research, much more evaluation is necessary to continue addressing inconsistencies in technique and determine protocols to optimize treatment with IV-RLP in horses.

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APPENDICES

Appendix 1 List of Abbreviations

RLP – Regional limb perfusion

IV – Intravenous

IO – Intraosseus

IV-RLP – Intravenous regional limb perfusion

IO-RLP – Intraosseus regional limb perfusion

MTPJ – Metatarsophalangeal joint

TCJ – Tarsocrural joint

HL-DIPJ - Hind limb distal interphalangeal joint

RCJ – Radiocarpal joint

FL-DIPJ – Forelimb distal interphalangeal joint

MIC – Minimum inhibitory concentration

Cmax – Maximum concentration

MCPJ – Metacarpophalangeal joint

MRSA – Methicillin-resistant *Staphylococcus aureus*

LRS – Lactated ringer's solution

LLQ – Lower limit of quantitation

ICJ – Intercarpal joint

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