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Evaluation of the pharmacokinetics of imipenem following regional limb perfusion using the saphenous and the cephalic veins in standing horses☆

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ABSTRACT

This prospective experimental study goal was to determine the pharmacokinetics of imipenem after intravenous regional limb perfusion (IV-RLP) in standing horses. Nine horses participated in the study; that was approved by the University Animal Care and Use Committee. One thoracic limb or one pelvic limb of each horse was randomly selected. After the veins were catheterized, an Esmarch bandage tourniquet was applied and the catheter was injected with a solution containing 500 mg of imipenem. Synovial fluid samples were collected from the fetlock joint and blood samples were collected from the jugular vein. All samples were analyzed for imipenem concentration using liquid chromatography mass spectrometry. Cmax of imipenem in the fetlock joint using the cephalic and the saphenous vein was 87 and 60 μg / mL, respectively. The results indicate that by performing IV-RLP using the cephalic/saphenous, one can achieve imipenem concentrations in the fetlock joint that are well above the MIC of most susceptible pathogens including resistant bacteria such as Methicillin Resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa*. Thus, with selective; judicious use, RLP with imipenem can markedly increase treatment efficacy of severe distal limb infections in horses.

1. Introduction

Synovial infection of the distal portion of a limb is one of the most common causes of morbidity and mortality in horses (Lugo and Gaughan, 2006). The therapeutic target when treating synovial infection is restoration of the normal synovial environment by eradication of bacteria from the joint, removal of any foreign material and elimination of inflammatory mediators and free radicals. In order to eliminate the pathogenic bacteria, antimicrobial agents need to reach adequate concentration in the injured joints. Systemic administration of antimicrobials is often inadequate to eliminate the infection due to poor blood supply to the distal portion of the limb and insufficient synovial concentrations (Lugo and Gaughan, 2006; Whithair et al., 1992). Blood supply to the distal portion of the limb may even be more compromised after an injury due to tissue trauma, edema and ischemia (Knottenbelt, 1997).

Regional limb perfusion (RLP) is an established method of treating horses with a synovial infection, that significantly decreases morbidity and mortality associated with these injuries (Rubio-Martínez and Cruz, 2006; Rubio-Martínez et al., 2012). In RLP, the antimicrobial drug is most commonly administrated intravenously, into a selected portion of the extremity, which was first isolated from the systemic circulation, by application of a tourniquet. During RLP, high concentrations and pressure gradients between the intravascular and extravascular compartments are obtained. This facilitates diffusion of the antimicrobial drug into the surrounding tissues, including the poorly vascularized tissues where bacteria are protected from systemically circulating antimicrobials (Cruz et al., 2006). Consequently, local antimicrobial concentrations are significantly higher than the concentrations attainable by systemic intravenous administration (Pille et al., 2005). Also, antibiotic

Abbreviations: RLP, regional limb perfusion; MCP, metacarpo-phalangeal; MTP, metatarso-phalangeal; Cmax, maximum concentration; AUC, area under the curve; MRSA, methicillin resistant *Staphylococcus aureus*; PK, pharmacokinetics; MDR, multidrug resistant; PAE, post antibiotic effect.

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induced diarrhoea is a frequent, and potentially fatal complication of systemic use of antibiotics in the horse; thus; avoiding the systemic route is highly advantageous in that respect as well (Cohen and Woods, 1999). In recent years, there is a surge of research on RLP, certain studies investigated some of the procedure's basic concepts such as the required volume, the necessary duration of tourniquet application and regional versus systemic anaesthesia, while other studies explored new horizons such as the efficacy of combining antibiotics in the perfusion (Aristizabal et al., 2016; Hyde et al., 2013; Mahne et al., 2013; Sole et al., 2015; Zantingh et al., 2014).

The goal of antimicrobial therapy is to achieve antimicrobial concentrations above the MIC in the infected tissue without causing any unwanted systemic or local effects. In human patients it has been proven that when the infection is well established (as is often the case in equine medicine), the drug concentration must be 10 to 12 times higher than the MIC to be efficacious (Moore et al., 1984). These high concentrations of antimicrobials are typically impossible to achieve safely by systemic administration but readily attainable by RLP.

Imipenem is a member of the carbapenem antimicrobial family, a sub-group of the beta-lactams. It has a broad spectrum of bactericidal activity, with excellent in vitro efficacy against a wide variety of Gram-positive and negative, aerobic and anaerobic bacteria. Its broad spectrum of activity includes important equine pathogens such as: *Rhodococcus equi*, *Streptococcus equi* subspecies *zooepidemicus*, *S. equi* subsp. *equi*, *S. dysgalactiae* subsp. *equisimilis*, coagulase-positive *Staphylococcus* spp., *Pasteurella* spp., *Salmonella enterica*, *Escherichia coli*, *and Klebsiella* spp. (Orsini et al., 2005).

In this study, we investigated the PK of imipenem after RLP in standing horses. Carbapenems are still one of the last lines of defense against rapidly emerging MDR human pathogens (Prescott et al., 2002). Systemic use of imipenem requires administration of high and frequent doses, which can promote resistance and become cost-prohibitive in equine practice. Thus, the use of carbapenems in equine medicine should be as limited as much as possible. In accordance with that, local vs. systemic use, can aid with regard to the valid concern of increasing resistance to carbapenems among bacterial populations.

Several studies have already evaluated the pharmacokinetics of imipenem following by systemic intravenous administration in horses (Gronwall et al., 1986; Orsini et al., 2005), however, we are not aware of any equine study evaluating imipenem pharmacokinetics following RLP.

2. Materials and methods

2.1. Animals

Nine adult horses weighting, aged 4–14 years (median 7 years), weighing 380–520 kg (mean 420 kg), 7 geldings and 2 mares, 7 local breed, one Warmblood and one Quarter Horse participated in this study, which was approved by the University Animal Care and Use Committee. Horses were housed individually in 4 by 3 meter stalls, had free access to fresh water, and were fed free choice grass hay.

2.2. Study design

One thoracic limb or one pelvic limb of each horse was selected randomly. The cephalic vein was used to perfuse the thoracic limb, and the saphenous vein was used to perfuse the pelvic limb.

2.2.1. Catheter placement

In each horse, a 14G, 12 cm catheter, was introduced into the jugular vein and maintained for 36 h for the delivery of sedation, and blood samples collection.

Before the procedure, each horse was sedated with detomidine hydrochloride (0.005 mg/kg) and butorphanol tartrate (0.01 mg/kg). In the forelimb, the cephalic vein was catheterized at the level of the chestnut, and in the hind limb, the saphenous vein was catheterized at the level of the medial malleolus. The area for perfusion was clipped and scrubbed, and skin over the vein was desensitized with 0.5 mL 2% lidocaine. A Seldinger technique was used as follows. A 16 G needle was introduced into the vein and directed distally, after blood started flowing through the needle, it was inserted deeper into the vein, and a flexible metal guide wire was inserted into it. The needle was removed and a 16 G 10 cm, over-the-wire, polyurethane catheter (Mila International, Erlanger, KY, USA) was threaded onto the wire through its entire length.

A 70 cm-long extension set, filled with heparinised 0.9% saline solution, was connected to the catheter. After blood was observed to flow, the extension set was flushed with 5 mL of heparinised physiological saline solution through an injection port attached to the extension set. The catheter, and the extension set, were then secured to the skin with several simple interrupted sutures of 2-0 polypropylene.

2.2.2. Perfusion

Before the perfusion, a tourniquet (Esmarch Bandage, 8 cm wide) was applied approximately 10 cm proximal to the site of catheterization. The catheter was injected with 10 mL of mepivacaine HCl, and with 500 mg of imipenem (imipenem and cilastatin, Hospira Inc. Lake Forest, IL, USA) all together diluted to 100 mL with 0.9% sodium chloride solution. The solution of the drug was injected over the course of 5 min, and the catheter was then flushed with 5 mL of heparinised 0.9% sodium chloride solution. The tourniquet was kept in place for 30 min after the injection; before removing it gradually over about 1 min.

2.2.3. Sampling for imipenem concentration

A 2 mL sample of synovial fluid was collected from the metacarpophalangeal/metatarsophalangeal (MCP/MTP) joint for joint imipenem concentration. The samples were collected by inserting the shaft of a 21 G, 4 cm needle through the slight depression between the proximal phalanx, the lateral proximal sesamoid and the third metacarpal/metatarsal bones, after horses were sedated with 0.3–0.5 mg/kg IV xylazine.

Samples of synovial fluid and venous blood (2 mL) were collected, before the perfusion, and 30 min and 2, 6, 12, 24 and 36 h after the perfusion. Blood samples were collected from the jugular vein via the jugular catheter.

All samples were centrifuged at 2878 G for 5 min and the supernatant (plasma and synovial fluid) was kept in deep freeze (−80 °C).

2.2.4. Samples analysis

All samples were analyzed for imipenem concentration using liquid chromatography tandem mass spectrometry (LC-MS/MS).

2.2.5. Serum samples preparation

First, 0.05 mL of serum sample were mixed with 0.05 mL of bidistilled water that contained 1 μg/mL ampicillin (used as internal standard), and with 0.1 mL of acetonitrile. Then, the samples were mix-vortexed briefly and centrifuged at 16,000*g* for 5 min at temperature of 4 °C. Finally, 0.04 mL of the supernatant was diluted in 0.16 mL of bidistilled water, and the samples were applied to the LC-MS/MS analysis. Calibration curve was constructed by adding imipenem at known concentrations into the serum of healthy horses that were not treated with this antibiotic before acquisition, to obtain a final concentration range of 0.01 μg/mL to 2.5 μg/mL. Limits of detection (LOD) and quantification (LOQ) were 0.004 and 0.013 μg/mL, respectively. Recovery of imipenem was 92 ± 6% when compared to matrix-matched standard. Intra-day and inter-day precision of low/medium/high level (0.01/0.5/2.5 μg/mL) were 11.8/15.4/13.7% and 13.4/15.6/8.2%, respectively.