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Intravenous regional limb perfusion in standing and recumbent horses: a comparative radiographic study

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Abstract:	Although pharmacokinetic studies of drugs administered by intravenous regional limb perfusion (IRLP) to treat equine orthopedic infections suggest efficient drug distribution in the limbs, it remains unclear whether drug perfusion is affected by the position of the horse during the procedure. This study compared the perfusion of a radiopaque contrast into tissues of the extremities of horses maintained in standing and recumbent positions during an IRLP. Radiopaque contrast was administered through IRLP into the cephalic vein of ten healthy adult horses under general anesthesia and right lateral recumbency (RG; n=10) or under sedation and standing (SG; n=10). The same animals were used in both groups, respecting a two-week washout period. Sequential radiographic images were performed immediately after contrast administration (T0) and at 10, 20, 30, 40, and 50 minutes. Tourniquets were removed after 30 minutes. Contrast reached the hooves approximately 2 minutes after the start of administration in SG and 4 minutes in RG. SG showed more uniform perfusion of the limb vessels, whereas RG showed more deposition of the contrast in the lateral digital vein, with smaller amounts reaching the hooves. From T10 onward, soft tissue radiopacity increased, albeit more markedly in standing than in recumbent animals, remaining until T50. Animal movement caused tourniquet failure and contrast leakage into the systemic circulation. Contrast radiography evidenced that IRLP performed in sedated horses in standing position leads to a quicker and more uniform perfusion of the vasculature and a more noticeable diffusion to the surrounding tissues than in anesthetized horses.
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Dear Editor,

We are enclosing herewith the original manuscript entitled **“Intravenous regional limb perfusion in standing and recumbent horses: a comparative radiographic study”** for publication in the “JOURNAL OF EQUINE VETERINARY SCIENCE” for possible evaluation. With the submission of this manuscript we would like to undertake that the above mentioned manuscript is original, has not been published elsewhere, accepted for publication elsewhere or under editorial review for publication elsewhere; and that all authors are fully aware of this submission.

Best regards,

Adriana F. S. Garcia et al.

- **Standing IRLP in horses provides more uniform perfusion of the vasculature.**
- **Standing IRLP in horse provides more uniform diffusion of the perfusate outside the vasculature to the surrounding tissues.**
- **Standing IRLP in horses provides quicker and greater efficiency in reaching the most distal portion of the limb.**
- **Movement of the horse during IRLP lead to tourniquet failure and contrast leakage into systemic circulation.**

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Intravenous regional limb perfusion in standing and recumbent horses: a comparative radiographic study

Abstract

Although pharmacokinetic studies of drugs administered by intravenous regional limb perfusion (IRLP) to treat equine orthopedic infections suggest efficient drug distribution in the limbs, it remains unclear whether drug perfusion is affected by the position of the horse during the procedure. This study compared the perfusion of a radiopaque contrast into tissues of the extremities of horses maintained in standing and recumbent positions during an IRLP. Radiopaque contrast was administered through IRLP into the cephalic vein of ten healthy adult horses under general anesthesia and right lateral recumbency (RG; n=10) or under sedation and standing (SG; n=10). The same animals were used in both groups, respecting a two-week washout period. Sequential radiographic images were performed immediately after contrast administration (T0) and at 10, 20, 30, 40, and 50 minutes. Tourniquets were removed after 30 minutes. Contrast reached the hooves approximately 2 minutes after the start of administration in SG and 4 minutes in RG. SG showed more uniform perfusion of the limb vessels, whereas RG showed more deposition of the contrast in the lateral digital vein, with smaller amounts reaching the hooves. From T10 onward, soft tissue radiopacity increased, albeit more markedly in standing than in recumbent animals, remaining until T50. Animal movement caused tourniquet failure and contrast leakage into the systemic circulation. Contrast radiography evidenced that IRLP performed in sedated horses in standing position leads to a quicker and more uniform perfusion of the vasculature and a more noticeable diffusion to the surrounding tissues than in anesthetized horses.

Keywords: equine; recumbency; regional perfusion; standing position; venography.

1. Introduction

Intravenous regional limb perfusion (IRLP) has been widely used in the treatment of orthopedic infections in horses. Studies on the pharmacokinetics of drugs administered by IRLP indicate that access through the palmar digital vein, cephalic vein, and saphenous vein allows the efficient distribution of antimicrobial drugs in the limbs of these animals [1-3]. IRLP is performed with horses in standing position [4-6], or in lateral recumbency [2,7-8]; however, both positions present indications and contraindications [2,5,9-11].

Typically, horses are not anesthetized to perform IRLP unless they receive anesthesia for another reason or their temperament rejects the execution of standing procedures. One study showed that the concentration of amikacin in samples of synovial fluid collected from the middle carpal joint did not significantly differ between standing and anesthetized horses [12]. However, some important concepts may still affect the effectiveness of drug distribution if IRLP is performed in standing or in recumbent horses. For instance, since cardiovascular variables and blood pressure vary considerably throughout the distal portion of the limb between anesthetized and awake horses, these differences may affect the antimicrobial's distribution during IRLP [2,13]. Also, the laws of physics might influence the diffusion of drugs through the tissues in standing or recumbent limb positionings. Furthermore, one cannot ignore that standing horses' limb movements can cause the loss of tourniquet integrity, perfusate leakage [4,14-18], and, consequently, disturb drug distribution.

Venography enables radiographic visualization of vessels and assessment of vascular perfusion in the limb following the injection of a radiopaque contrast liquid [19]. In equine practice, venography is used mainly to evaluate the microcirculation of the digit in cases of laminitis [20]. After a tourniquet is applied, the circulation in the limb is closed allowing the radiopaque contrast to reveal anatomic structures and soft tissues usually undetectable with plain radiographs [19].

We expected that a comparative venographic study would reveal relevant data to help answer whether the effectiveness of drug infusion through IRLP is affected by the position of horses during the procedure. Thus, herein we qualitatively compared the distribution and perfusion of a radiopaque contrast, administered by IRLP at the cephalic vein, in the extremities of standing and recumbent horses.

2. Material and methods

We used five healthy mares and five healthy geldings of a median age of 8 years (range 4 – 12) and a median weight of 350 (range 300-400 kg). Horses were free of lameness and noticeable vascular abnormalities on the forelimbs. All procedures were approved by the Ethics Committee on Animal Use of XXX, approval number 9519191217.

Horses were moved from pasture to stalls (4 × 4 m) with free access to hay and water two days before the experiments. IRLP with contrast was performed at the cephalic veins of horses in right lateral recumbency under general anesthesia (RG; n=10) and in standing horses under sedation (SG; n=10). The same animals were used in both RG and SG experimental groups, following a two-week washout period between treatments. A 14 G IV catheter was inserted into one of the jugular veins before each experiment.

The ten horses that underwent general anesthesia (RG) received water but food was withheld for eight hours before anesthetic induction. At the moment of assessment, they were sedated with xylazine 2% (1 mg/kg IV), followed by myorelaxation with guaiacol glyceryl ether (100 mg/kg IV). Next, the horses were induced with midazolam (0.1 mg/kg IV) and ketamine (2.2 mg/kg IV), and maintained under general anesthesia with isoflurane. Horses were positioned in lateral recumbency with the right leg down and maintained parallel to the ground. At the moment of the SG assessment, the ten animals were restrained in the stock and sedated with detomidine hydrochloride (20 µg/kg IV).

After aseptic preparation of both experimental groups, a 20-gauge catheter with an injection port was placed into the cephalic vein (RG – right cephalic vein and SG - left cephalic vein), at the level of the chestnut. Before the application of the tourniquet, rolled gauzes were placed over the cephalic vein and the lateropalmar and mediopalmar aspect of the radius, and secured with bandage tape to attain better compression of the vascular structures. A 10-cm-wide pneumatic tourniquet cuff was placed over the bandage tape and inflated to 400 mm Hg. Tourniquet application was always performed by the same individual to limit variation in tourniquet positioning.

One hundred milliliters of low osmolarity contrast (Omnipaque® ioexol 300mg I/ml) was injected into the cephalic vein through the catheter, in a 10 mL/min infusion rate and using of a 60-mL syringe connected to an extension line. There was no exsanguination of the perfused region. After 30 minutes, the pneumatic tourniquet cuff was deflated.

Dispersion of the infused solution was observed in radiographic images collected at six moments: immediately after starting contrast infusion (T0), after 10 minutes (T10), after 20 minutes (T20), after 30 minutes (T30), after 40 minutes (T40), and after 50 minutes (T50). An X-ray unit model POSKON PXP-20HF PLUS from United Radiology Systems and a digital detector from Serv Imagem, model SIMS DR35 were used to acquire the radiographic images. Settings ranged from 76 to 80 Kv and from 3.2 to 4.0 mAs, depending on the region to be radiographed and the size of the animal.

To follow the flow of the contrast through the radiographs, the limb was divided into five regions: radius near the tourniquet, radius distal to the catheter, carpus, metacarpal phalangeal joint, and hoof. These regions were radiographed in mediolateral, dorsolateral-palmaromedial, dorsopalmar, and dorsomedial-palmarolateral projections. Radiographic evaluation of the proximal region near the tourniquet aimed at showing possible tourniquet failure. After 30 minutes of contrast infusion, the tourniquet was deflated and removed, and radiographic assessments were carried out at T40 and T50 to confirm the flow of the contrast after the tourniquet's release and the venous blood return to the limb.

All images were analyzed blindly (individually and without group identification) by two radiologists. Observations were recorded for comparative analysis between the same intervals in the different groups. As this was a qualitative study, no statistical analysis was performed, and results are presented descriptively.

3. Results

Radiographs showed that the contrast injected into the cephalic vein was identified in the circulation of the hoof after 2 minutes of starting contrast infusion in the SG, and after 4 minutes in the RG.

There were observed differences between the experimental groups regarding contrast distribution inside the vessels. In the RG fetlock, contrast was more evident in the lateral digital vein than in the medial digital vein (Fig. 1A), compared to the SG, which showed a more uniform contrast distribution in both these vessels (Fig.

1B). This difference was also evident in the hooves (Fig. 1C and 1D).

We also observed differences between the experimental groups regarding contrast diffusion through extravascular space. At T0, the contrast was evident only inside the vessels of both groups. At T10, soft tissue radiopacity increased in both groups, albeit less markedly in recumbent (Fig. 2A) than in standing animals (Fig. 2B). At T20, increased radiopacity of the tissues and decreased visualization of the vessels, in all regions, were observed in both groups. These results suggest that the contrast spread from the inside of the vessels to the surrounding tissues. Images collected at T30 were similar to those obtained at T20, showing increased radiopacity in the tissues and diminished vessel delimitation (Fig. 2C). Tourniquets were removed at T30, and normal blood flow was re-established. However, radiographic images obtained from both groups at T40 and T50 continued to show soft tissue radiopacity similar to that observed at T30 (Fig. 2D).

Three horses of the SG (30%) displayed sudden movements of the assessed limb at T15. Radiographic images obtained at this time showed the contrast inside the cephalic vein, above the tourniquet (Fig. 3A). The tourniquets were removed immediately and the signs of discomfort ceased. Radiographs taken at T30 revealed a pattern of increased soft tissue radiopacity, without delimitation of the vessels, that was similar to that presented by the images obtained from the horses that remained with the tourniquet, also at T30 (Fig 3B and 3C). These horses' data were excluded from SG at T40 and T50.

5. Discussion

IRLP can be performed in standing sedated horses or under general anesthesia. The positive-contrast dye study performed herein documented the ability of a cephalic intravenous injection to perfuse the soft tissues of the digit of standing and recumbent horses within minutes after injection. Interestingly, the data revealed that the horses position during IRLP led to differences regarding the perfusion of the contrast to the most distal portion of the equine limb.

Considering an infusion rate of 10 mL per minute, the volumes infused to allow the visualization of the contrast in the hooves were approximately 40 mL in RG and 20 mL in SG. These volumes are close enough to those employed in previous studies, which ranged from 20 to 120 mL for IRLP into horses' cephalic vein. These studies investigated drug diffusion into the synovial fluid of metacarpophalangeal/metatarsophalangeal [5,21], distal interphalangeal [6,22-23], and carpal [24-25] equine joints.

Importantly, the present study shows that recumbent and standing horses present different distribution patterns of the contrast. Indeed, contrast flow was quicker in standing horses as the solution reached the hooves in approximately 2 minutes in the SG and in 4 minutes in the RG. Also, contrast was more evident in the lateral digital vein of the limb of recumbent horses. These differences are probably due to the gravity that favors downward flow and consequent deposition of the contrast in more ventral regions. Besides the difference observed between medial and lateral contrast deposition, the vessels inside the hooves were less evidenced in recumbent horses, probably because less contrast reached the region. Aristizabal et al. (2016) [10] showed significantly lower synovial amikacin concentration within the metacarpophalangeal joint in anesthetized compared to standing horses. The authors suggested that anesthetized horses positioned in lateral recumbency have lower venous pressure in the leg compared to standing horses because their systemic blood pressures are likely to be lower due to hydrostatic pressure differences, particularly more distally in the limb. In our study, standing animals showed greater tissue radiopacity than recumbent animals, as observed from T10. It is possible that the infusion of the contrast into a vein with lower pressure (RG animals) may have impaired diffusion of the contrast outside the vasculature at the most distal portion of the limb. Conversely, the effect of gravity on the standing sedated horse probably allowed the perfusate to reach more distal regions of the limb [2,10]. Although a recent pharmacokinetic research concluded that general anesthesia had no effect on the intra-articular concentration of amikacin [12], our study evidenced by contrast images that horses' positioning influences the vascular perfusion of the limb and possible the drug diffusion through the soft tissues.

As iohexol water solubility is low (0.796 mg/mL), diffusion of this contrast into the horses' tissues was probably favored by an increase in vascular permeability promoted by the circulatory stasis caused by tourniquet inflation. The contrast was detected in the soft tissue of the equine limb at T10 and became more evident at T20 and T30 in both groups. Although the tourniquet was removed at T30, the soft tissue remained visible at T40 and T50, even in the absence of the tourniquet. Thus, it is clear that IRLP allows the diffusion of solutions from the intravascular to the extravascular space and that the solution remains in the region for longer than the time under the influence of the tourniquet. Antimicrobials frequently used in horses' IRLP have higher water solubility than the contrast used herein and probably diffuse more promptly into the tissues [22-23].

It is important to highlight that after the drug reaches a specific compartment, such as the synovial fluid, its elimination is conditioned to the tissue metabolism, in this study's case, the joint metabolism. Recent studies show that antibiotics' concentrations greater than minimum inhibitory concentration are observed in joints 15 minutes after their administration, by IRLP, into the cephalic vein of horses in standing position [10,22-23]. These observations and the pattern of the contrast spread reported herein should provide further information to the ongoing discussion about the minimum effective tourniquet time for IRLP in horses, which may well be under 30 minutes, as suggested elsewhere [1,3,5,11,22-24,26].

Although performing IRLP in the standing horse is faster and less costly [5-6,11], the animal's discomfort is a negative aspect that should be considered. It has been suggested that movements of standing sedated animals may affect the performance of the tourniquet. Thus, when the animal moves, peripheral vascular resistance of the limb increases, leading to an intravascular pressure greater than that produced by the tourniquet and allowing an inadvertent leakage of perfusate into the systemic circulation [10]. Moreover, continued arterial blood flow with a greater degree of venous obstruction can cause engorgement of the vessels distal to the tourniquet and contribute to the development of pain, restlessness, and motion of the limb [15]. In the present study, approximately 15 minutes after the tourniquet was placed, 30% of the SG animals showed signs of discomfort, such as agitation, frequent lifting of the limb, and/or pawing. We opted not to perform re-sedation or local anesthetic block in order to illustrate, by radiographic images, the risks of limb movement with an applied tourniquet for the IRLP technique. Accordingly, leakage of the contrast into the systemic circulation was evidenced immediately after the limb's movement. Thus, adequate restraint, sedation, and effective local anesthetic blocks are important steps to minimize the movements of the garroted limb and ensure the effectiveness of the IRLP [1,11,25]. We removed the tourniquet as soon as signs of failure and discomfort to the animals were identified, alleviating the horses' discomfort immediately. This complication was not observed in recumbent horses under anesthesia. Although it is easier and less risky to perform IRLP in horses under general anesthesia, this option is not always suitable. Indeed, treatments based on IRLP with antibiotics may be lengthy, and some may require daily repetitions. Repeated general anesthesia may make to the process expensive and risky, mainly for animals in field conditions.

While we were able to visually show the influence of the horse's position during IRLP on the distribution of the infused solution, we were unable to quantify this difference. Further studies, comparing horses in station and in lateral decubitus regarding the concentration of drugs in samples of interstitial fluid from the lateral and medial aspects of the limb after IRLP, should be performed to reveal the actual quantitative effect.

In conclusion, this study shows that IRLP in standing position leads to a quicker and more uniform diffusion of the contrast and allows the perfusate to reach the most distal regions of the equine limb more efficiently, provided that the limb remains steady. Our results suggest that the horses' positioning during IRLP influences the vascular perfusion of the limb and possibly drug diffusion through the soft tissues.

Author's declaration of interests: The authors declare no competing interests

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Figures legends

FIGURE 1. Radiographic images, in dorsopalmar projection, of the metacarpophalangeal and hoof regions of a horse in recumbency (A and C) and in standing position (B and D). Note a greater presence of the radiopaque contrast in the lateral digital palmar vein (arrow) than in the medial digital palmar vein.

FIGURE 2. Radiographic images of the metacarpophalangeal region, in lateromedial projection, showing the radiopacity of the soft tissues of horses in recumbency (A) and in standing position (B), at T10. Note greater radiopacity in standing than in recumbent horses. The images show increased soft tissue radiopacity in standing horses at T30 (C) and at T50 (D).

FIGURE 3: Radiographic image of the radius region of a horse in standing position showing the presence of contrast above the tourniquet and evidencing perfusate leakage to the circulatory system (A). Radiographic image of the metacarpophalangeal region, in the lateromedial projection, of a horse in standing position at T30 (B); when the tourniquet was removed at 15 minutes. (B) Radiographic image of the metacarpophalangeal region, in the lateromedial projection, of a horse in standing position at T30, in which the tourniquet was kept for 30 minutes (C).

Figure 1 Click here to download Figure FIGURE 1.jpg

Figure 2

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Figure 3 Click here to download Figure FIGURE 3.jpg

Title page_Conflict of Interest

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Ethical animal research: All procedures were approved by the Ethics Committee on Animal Use of the Faculty of Food Engineering and Animal Science, University of São Paulo, approval number 9519191217.

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Autorship: Garcia A.F.S, DVM, MSc contributed to the study execution, radiographic images examination and interpretation and preparation of the manuscript; Dória RGS, DVM, PhD contributed to the study design, study execution, data analysis and interpretation and preparation of the manuscript.; Arantes J.A., DVM, MSc contributed

to the study execution, daily management of the horses and preparation of the manuscript; Reginato G.M., DVM, MSc contributed to the study execution and daily management of the horses; Neubauer F.G., DVM contributed to the radiographic images examination and interpretation; Ferraz, G.R.L., DVM, PhD contributed to the study design, study execution, data analysis and interpretation, and preparation of the manuscript. All authors revised critically and gave their final approval of the manuscript.

Intravenous regional limb perfusion in standing and recumbent horses: a comparative radiographic study

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CERTIFICATE OF ENGLISH LANGUAGE REVISION

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the research manuscript entitled **"Intravenous regional limb perfusion in standing and recumbent horses: a comparative radiographic study"** has been reviewed and edited by a professional scientific editor and an English language copyeditor to improve readability.

Yours faithfully,

Andrea Kauffmann-Zeh, PhD

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