

Pharmacokinetics of regional limb perfusion using a combination of amikacin and penicillin in standing horses

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Abstract – The objectives of this study were to evaluate the compatibility and the pharmacokinetic properties of combined amikacin and penicillin administration by intravenous regional limb perfusion (IVRLP) in horses. A tourniquet was applied proximal to the carpus of 7 clinically healthy adult horses and 2 g of amikacin and 10×10^6 IU of penicillin (100 mL total volume) were sequentially injected into the cephalic vein just distal to the tourniquet. Synovial samples were collected from the joint at several times after injection. All samples were analyzed for amikacin and penicillin concentration. The mean maximum concentration (C_{max}) of both amikacin and penicillin was over 10-fold the relevant minimal inhibitory concentration (MIC) for all horses and remained above those MICs for at least 24 hours. The results of this study indicate that combining amikacin with penicillin during IVRLP in normal horses delivers high therapeutic synovial concentrations of both drugs.

Résumé – **Pharmacocinétique de la perfusion régionale des membres en utilisant une combinaison d'amikacine et de pénicilline chez des chevaux debout.** Les objectifs de cette étude consistaient à évaluer la compatibilité et les propriétés pharmacocinétiques de l'administration combinée d'amikacine et de pénicilline par perfusion intraveineuse régionale des membres (PIRM) chez les chevaux. Un tourniquet a été appliqué proximement au carpe de sept chevaux adultes en bonne santé clinique et 2 g d'amikacine et 10×10^6 UI de pénicilline (volume total de 100 ml) ont été injectés en séquence dans la veine céphalique légèrement distale au tourniquet. Des échantillons synoviaux ont été prélevés de l'articulation plusieurs fois après l'injection. Tous les échantillons ont été analysés pour la concentration d'amikacine et de pénicilline. La concentration maximale moyenne (C_{max}) de l'amikacine et de la pénicilline était plus de 10 fois supérieure à la concentration inhibitrice minimale (CIM) pertinente pour tous les chevaux et est demeurée au-dessus de ces CIM pendant au moins 24 heures. Les résultats de cette étude indiquent que la combinaison de l'amikacine avec la pénicilline durant la PIRM chez des chevaux en santé offre des concentrations synoviales thérapeutiques élevées des deux médicaments.

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Introduction

Intravenous regional limb perfusion (IVRLP) is a well-established method for delivering high antimicrobial concentrations to the equine distal limb for preventing and treating equine orthopedic and soft tissue infections (1,2). The IVRLP antimicrobial drug selection should ideally be based on culture and sensitivity results. It is, however, common practice to use

an empirical broad-spectrum antibiotic for infection prevention early in the course of therapy, when microbial culture and susceptibility data are not available or when there is no positive bacterial culture (3). Broadening the antimicrobial spectrum by systemic administration of combined antimicrobial drugs was shown to improve treatment efficacy. Antibiotic combinations may be used to broaden the antimicrobial spectrum when mixed

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Table 1. Pharmacokinetic values, mean, median (50th), 25th and 75th percentiles for amikacin and penicillin in serum and in synovial fluid in the MCP joint after regional limb perfusion. C_{max} = maximal concentration ($\mu\text{g}/\text{mL}$), AUC_{0-36} = area under the concentration time curve from 0 to 36 h ($\mu\text{g}\cdot\text{h}/\text{mL}$).

Antibiotic	Dose	Sample source	C_{max} Percentiles				AUC_{0-36} Percentiles			
			Mean (95% CI)	25th	Median 50th	75th	Mean (95% CI)	25th	Median 50th	75th
Amikacin	2 g	Plasma	15 (7 to 23)	7	15	20	74 (43 to 104)	49	72	92
		Joint	1391 (192 to 2590)	209	1306	2623	2127 (381 to 3874)	817	3350	4586
Penicillin	10 MU	Plasma	8 (2 to 14)	3	7	12	23 (2 to 44)	13	16	30
		Joint	507 (224 to 791)	252	464	777	1374 (418 to 2330)	572	1155	2202

bacterial infections are known or suspected to be present, to help prevent or overcome resistance, or to improve efficacy through synergistic effects (4,5). Since similar antimicrobial drugs are used in IVRLP, and similar bacterial populations are targeted, it is reasonable to extrapolate from these data and predict that use of antimicrobial drug combinations in IVRLP will lead to similar advantages.

Penicillin belongs to the beta-lactam class of antimicrobials; it is effective mainly against Gram-positive bacteria and anaerobic bacteria by exerting a bactericidal effect. Penicillin has been the backbone of equine antibacterial drug therapy for many years and is still one of the most commonly used prophylactic antibiotic drugs (6). Although it is used clinically in IVRLP (1), a single IVRLP pharmacokinetic study using penicillin was only recently reported (7).

Amikacin belongs to the aminoglycoside class of antimicrobials; it has a broad spectrum of activity and is the most effective drug against commonly isolated bacteria causing orthopedic infections in horses (8). Moreover, amikacin is the most commonly used and studied antibiotic in IVRLP (1–3,7,9–14).

The beta-lactam-aminoglycoside combination is one of the most frequently used antimicrobial drug combination therapy, commonly used systemically (1,15). Clinically, this combination was used effectively as a local therapy both by IVRLP and by intra-synovial lavage (1,16). The beta-lactam-aminoglycoside combination has a synergistic effect that is exerted *via* bacterial cell wall destruction by beta-lactam antibiotics, facilitating the aminoglycoside transport into the bacterial cell, exerting its bactericidal effect by interfering with ribosomal protein synthesis (17). Although there is supportive evidence of synergism, the beta-lactam-aminoglycoside combination also proved to have a negative drug interaction in confined compartments in an *in vitro* study (18). Moreover, a recent equine study showed that the combination of amikacin and ticarcillin/clavulanate, used for IVRLP, resulted in reduced amikacin concentrations and antimicrobial activity in the synovial fluid (11).

Our goal was to evaluate the PK properties of the combination of penicillin G and amikacin sulfate delivered by IVRLP through the cephalic vein to standing horses and sampling the metacarpophalangeal (MCP) joint.

We hypothesized that the amikacin-penicillin combination delivered by IVRLP would yield maximal MCP synovial

penicillin and amikacin concentrations (C_{max}) well above the relevant minimal inhibitory concentration (MIC_{90}) and that these concentrations would remain high for at least 24 h following the perfusion.

Materials and methods

Animals

Seven adult mixed breed horses participated in the study, including 5 geldings and 2 mares. The average age was 8 y (range: 4 to 15 y) and the average weight was 408 kg (range: 320 to 500 kg). Horses were clinically healthy according to physical evaluation, and all were sound when trotted. Horses were housed in separate stalls, had continuous access to fresh water, and were fed grass hay. The study design was approved by the University Animal Care and Use Committee.

Antimicrobial perfusion

Catheter application, horse sedation, and limb IVRLP treatments followed by tourniquet removal were performed as previously described (19). The antibiotic solutions were prepared just prior to performing the perfusion. Two grams of amikacin sulfate were diluted with isotonic saline in three 20-mL syringes (Amikacin-fresenius; Boden, Port Elizabeth, South Africa) and 10×10^6 IU benzylpenicillin sodium (Penicillin G Sodium; Sandoz GmbH, Kundl, Austria) were diluted with isotonic saline solution in two 20-mL syringes, to a total volume of 100 mL. The prepared solutions were administered sequentially, through the cephalic catheter, in five 20-mL syringes, over approximately 2 min.

Sample collection

Blood samples from the jugular catheter and synovial fluid samples from the metacarpophalangeal (MCP) joint of the treated limb were taken before IVRLP as described (19). Sedation with xylazine 0.5 mg/kg body weight (BW), IV was used as needed to prevent discomfort and movement during the sampling procedure.

Following aseptic preparation of the centesis site, aspiration of synovial fluid from the MCP joint was carried out using an approach described by Bassage et al (20). Blood sampling and treatment, acquisition of synovial fluid followed by phenylbutazone administration, amikacin sulfate instillation into each MCP

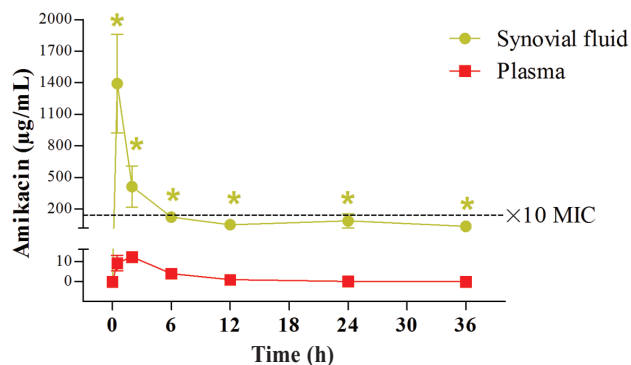


Figure 1. Amikacin concentrations over time in the synovial fluid and in the serum after IV-RLP with the combination of penicillin and amikacin. Over time, amikacin concentrations (Mean \pm SEM) in the synovial fluid were significantly higher compared with the pre-treatment levels, up to at least 36 h after injection (marked by green star; $P < 0.05$, Repeated measures ANOVA). Furthermore, Amikacin concentrations after the IV-RLP were significantly higher in the synovial fluid than in the serum, in all time points up to 36 h ($P < 0.05$). The black dotted line represents the $\times 10$ MIC concentration (160 $\mu\text{g/mL}$).

joint, and bandaging were all as described (19). Horses were confined to a stall for physical examination, with an emphasis on the sites of perfusion and sampling along with a brief daily lameness evaluation during the study and for an additional 7 d after the last sampling.

Antibiotic analysis

Antibiotic analysis was based on established methodology in a previously reported PK study (14). Concentrations of amikacin and penicillin G in serum and in synovial fluid at each time point were determined by mass spectrometry [liquid chromatography/tandem mass spectrometry (LCMSMS)] and preparation of serum samples was as described (14). Concentrations above the upper limit of quantification were determined by diluting the samples with extracted drug-free serum/synovial fluid.

Liquid chromatography parameters for analysis of amikacin: A mixed-mode SiELC, Obelisc R column (5 μm , 100 \times 2.1 mm, SiELC Technologies, Wheeling, Illinois, USA) was used for separation as previously described (19) except that the mobile phase consisted of 0.5% formic acid and acetonitrile at a flow rate of 0.6 mL/min. A gradient was applied by decreasing the acetonitrile concentration from 65% at 1 min to 5% at 3 min. This ratio was maintained for 0.5 min and then brought back to the initial conditions. The column compartment was kept at 30°C, and the injection volume was 10 μL .

Liquid chromatography parameters for analysis of penicillin-G: A C18 Symmetry column (3.5 μm , 100 \times 2.1 mm, Waters, Ireland) was used for separation. The mobile phase consisted of 0.2% formic acid (Sigma-Aldrich, Israel) and acetonitrile at a flow rate of 0.35 mL/min. A gradient was applied by increasing the acetonitrile concentration from 5% at 0 min to 50% at 5 min. This ratio was maintained for 11 min and then brought back to the initial conditions. The column compartment was kept at 25°C, and the injection volume was 20 μL .

Turbo ion spray ESIMSMS in positive ion mode was operated at a temperature of 550°C and 450°C for amikacin and

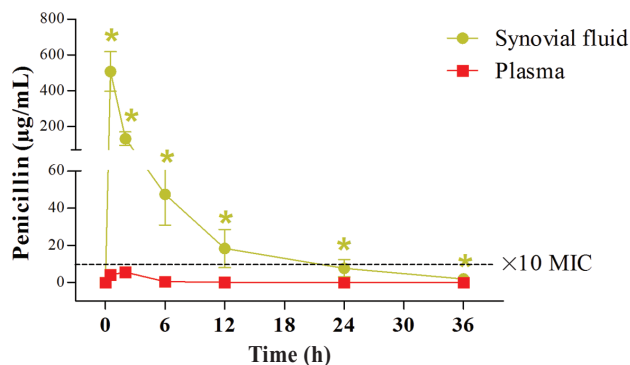


Figure 2. Penicillin concentrations over time in the synovial fluid and in the serum after IV-RLP with the combination of penicillin and amikacin. Over time, penicillin concentrations (Mean \pm SEM) in the synovial fluid were significantly higher as compared to the pre-treatment levels, up to at least 36 h post injection (marked by green star; $P < 0.05$, Repeated measures ANOVA). Furthermore, penicillin concentrations after the IV-RLP were significantly higher in the synovial fluid than in the serum, in all time points up to 36 h ($P < 0.05$). The black dotted line represents the $\times 10$ MIC concentration (10 $\mu\text{g/mL}$).

penicillin-G, respectively. Multiple reactions monitoring (MRM) was applied. The precursor ion was 586.3 m/z for amikacin and 357 for penicillin G. The product quantifier ions were 163.3 and 197.9 m/z for amikacin and penicillin G, respectively. The qualifier ions were 425.3 and 181.9 m/z for amikacin and penicillin G, respectively. Drug-free serum/synovial fluids were fortified with amikacin and penicillin G to obtain calibration curves in the range of 0.5 to 250 $\mu\text{g/mL}$ in synovial fluid and 0.25 to 25 $\mu\text{g/mL}$ in serum. Three replicates of calibration samples were prepared on 3 different days. The accuracy and intra-day and inter-day precision were calculated for 0.25 $\mu\text{g/mL}$, 2.5 $\mu\text{g/mL}$, 25 $\mu\text{g/mL}$ in serum and 0.5 $\mu\text{g/mL}$, 25 $\mu\text{g/mL}$, 250 $\mu\text{g/mL}$ in synovial fluid. These concentrations represented the lower level of quantification, the mid-level of quantification, and the high level of quantification.

The calibration curves for each of the antibiotics in both serum and in synovial fluid were linear over the tested range with a correlation coefficient of 0.991 (serum) and 0.963 (synovial) for penicillin-G and 0.981 (serum) and 0.978 (synovial) for amikacin. Accuracy was 95% to 120% (serum) and 90% to 113% (synovial) for penicillin-G and 98% to 112% (serum), 96% to 109% (synovial) for amikacin. Precision was 8% to 27% (serum) and 9% to 14% (synovial) for penicillin-G and 18% to 20% (serum), 14% to 18% (synovial) for amikacin.

Pharmacokinetic analysis

Amikacin and penicillin in the serum and the synovial fluid were assessed using a pharmacokinetic program (PK solutions 2.0; Summit Research Services Montrose, Colorado, USA), which computed (area under the curve) AUC_{0-36} using the trapezoidal rule. Serum and synovial fluid maximum synovial fluid concentration (C_{max}) of both drugs and the time of maximum synovial fluid concentration of both drugs (T_{max}) were determined by viewing graphs of the time course of each drug in synovial fluid. To evaluate the predicted efficacy of penicillin, we recorded the duration of time that the drug remained above the MIC_{90}

($T > MIC_{90}$) and calculated the AUC_{0-36}/MIC_{90} ratio. To evaluate the predicted efficacy of amikacin C_{max}/MIC_{90} ratio and AUC_{0-36}/MIC_{90} ratio were calculated. Amikacin and penicillin MIC_{90} for common susceptible equine bacterial pathogens were defined as 16 $\mu\text{g}/\text{mL}$ and 1 $\mu\text{g}/\text{mL}$ respectively, (21,22).

Statistical analysis

Statistical analyses were performed with computerized software (Statistix 8 Student edition, Analytical Software, Tallahassee, Florida, USA). A repeated measures analysis of variance (ANOVA) test followed by Tukey's HSD all-pairwise comparisons test were used to compare the differences in penicillin or amikacin concentrations in the different time points after regional limb perfusion. These tests were also used to compare concentrations in the plasma and synovial fluid. Comparisons of C_{max} , T_{max} , and AUC between the plasma and the synovial fluid were made with Wilcoxon signed-rank test. Differences were considered significant at $P \leq 0.05$. Unless otherwise noted, results are presented as median (1st quartile, 25%; 3rd quartile, 75%), or as mean \pm standard error of the mean (SEM).

Results

Other than 1 horse with local edema at the cephalic catheter site and temporary lameness, none of the horses showed clinically noticeable adverse effects during the study. That 1 horse moved following injection of the entire perfusate, dislodging the cephalic catheter which resulted in immediate swelling at the site of venipuncture. In spite of this incident, the tourniquet remained in place and all synovial and blood samples were taken from this horse according to the protocol. The horse was mildly lame at a walk and had significant edema of the affected leg. After the last sample, the horse was treated with phenylbutazone, 2.2 mg/kg BW, q12h, PO for 5 d and application of 1% diclofenac sodium liposomal cream to the venipuncture site (Voltaren Emulgel 1%; Novartis Consumer Health SA, Nyon, Switzerland), q12h for 5 d and the limb was bandaged. Both lameness and edema resolved after 1 wk. One horse was excluded from the study because of aggressive behavior during the injection, which led to tourniquet release resulting in low synovial antibiotic concentrations.

Synovial fluid was successfully obtained from the MCP joint of all horses at all time points.

Pharmacokinetic analyses

Amikacin and penicillin were not detected in the serum or in the synovial fluid before perfusion. Median and percentiles of AUC and mean, median, and percentiles of C_{max} of amikacin and penicillin measured in the serum and synovial fluid of the MCP joint are summarized in Table 1. The concentrations of amikacin and penicillin over time in the synovial fluid and in the serum are presented in Figures 1 and 2, respectively. The amikacin concentration in the synovial fluid of the MCP was well above the MIC of most susceptible pathogens and remained above the MIC in all horses for at least 24 h. The C_{max}/MIC_{90} ratio for amikacin in the synovial fluid was > 10 for all horses.

The T_{max} for both amikacin and penicillin in the synovial fluid was at the time of the tourniquet release (0.5 h). T_{max} for

amikacin and penicillin in the serum was 1.92 h and 1.5 h, respectively. The concentration of penicillin in the synovial fluid of the MCP joint was initially greater than the minimal inhibitory concentration (MIC) of most susceptible pathogens and the time greater than the MIC ($T > MIC$) was at least 24 h. The AUC_{0-36}/MIC_{90} ratios for penicillin in the synovial fluid were > 125 for all horses.

Discussion

We demonstrated that amikacin and penicillin administered sequentially by IVRLP resulted in a high synovial fluid concentration of both drugs. Although we did not administer amikacin alone in this study, our amikacin C_{max} concentrations in the synovial fluid, using a combination of amikacin-penicillin, were higher than in a recent similar study (7). In that study identical doses of the amikacin-penicillin combination were used but the synovial amikacin concentration turned out to be an order of magnitude lower than in the current study. In addition, 12 h after perfusion, penicillin was detectable in only 1 horse, compared to all horses in the current study. These differences may be partially related to the fact that in our study a higher volume of perfusate was used (100 mL compared to 60 mL). Two recent studies, using significantly different experimental setups, have both shown that using a higher volume in IVRLP is associated with significantly higher antibiotic concentrations than with lower volumes (13,14). However, 2 other studies found no significant effect of perfusate volume on synovial antibiotic concentrations and the lower volumes had a trend towards higher concentrations (12,23). Another potential explanation for the discrepancy between the 2 studies, is that in Nieto et al (7) the tourniquet may have failed to completely prevent blood flow and thus some of the perfusate may have leaked into the systemic circulation while the tourniquet was still on. This is consistent with the fact that not only the penicillin but also the amikacin concentration appeared markedly lower in that study compared to the current one. Also, the absence of systemic antibiotic concentration measurements that prevented assessment of tourniquet failure was stated by these authors as a limitation (7). Finally, Nieto et al (7) mixed both antibiotics together in the same syringe while *in vitro* studies have demonstrated that aminoglycosides and penicillin can have mutual antagonist effects (18,24). All the above may explain our significantly higher antibiotic concentrations.

The amikacin-penicillin combination in the present study also yielded seemingly markedly higher amikacin synovial PK variables compared with previously reported synovial fluid amikacin IVRLP PK studies in which amikacin was used as the sole drug (10,12–14). For example, current results include a mean C_{max} of 1391 (95% CI: 192 to 2590) versus 277 and 579 $\mu\text{g}/\text{mL}$ and a mean AUC of 2127 (95% CI: 381 to 3874) versus 499 and 1042 $\mu\text{g}\cdot\text{h}/\text{mL}$ in 2 of these previous PK studies (10,14). Moreover, the synovial amikacin PK/PD most critical parameter, C_{max}/MIC_{90} ratio, was > 10 not just on an average but in each individual horse, and that is associated with an optimal bactericidal effect and with a reduced selection for resistance (25).

A comparison to other independent studies may have limitations. Variables such as the strength of the applied tourniquet

by different persons, the different breeds, and thus different volumes of the distal limbs, different temperaments of the horses, and different environmental conditions, could all potentially reduce the reliability of the comparison. Nevertheless, due to the markedly positive PK results demonstrated in this study, it may be concluded that the sequential delivery of the combination therapy: amikacin and penicillin, during IVRLP, has a potential therapeutic advantage. Adding penicillin to the amikacin IVRLP does not seem to have a negative effect on amikacin concentrations in the MCP joint and may even be associated with an increase in synovial fluid amikacin concentrations in the MCP joint after cephalic IVRLP.

One horse in this study developed regional swelling around the perfusion site accompanied by lameness. According to our clinical and research experience, this reaction is very unusual in IVRLP with amikacin alone and is likely associated with the use of penicillin. Thus, when using IVRLP with penicillin, this potential of local adverse reaction should be taken into consideration. In our clinical use, edema continued to be observed when penicillin was diluted with 40 mL; however, after increasing the dilution volume to 60 mL for penicillin in over 20 additional cases, no edema was observed.

Although systemic antimicrobial combinations have been used for many years in equine medicine (15), very few studies evaluated antimicrobial drugs combinations in IVRLP (7,11,19). While one IVRLP PK study found reduced antimicrobial concentration of amikacin when combined with ticarcillin/clavulanate (11), another IVRLP PK study reported a higher imipenem concentration when it was combined with marbofloxacin (19). In both of these studies, the two antibiotic drugs were injected sequentially, each antibiotic drug in separate syringes. Thus, each antimicrobial combination should be tested in its relevant setting (e.g., IVRLP) and the PK achieved by systemic combined drug administration cannot be automatically assumed to be similar when IVRLP is used.

Traditionally, concentration-dependent antimicrobials are considered more suitable for use in IVRLP. The reason is that the PK of this group, with the emphasis being on a high peak concentration as opposed to a sustained concentration of drugs, best matches the technique. For the sake of the animal's welfare the technique involves sedation of the horse to ameliorate the pain elicited from the tourniquet. Thus, when considering the potential risk of frequent sedation (26), IVRLP is typically used once a day in a clinical setting (1,9). However, rather than systemic administration, the results presented here suggest that the use of time-dependent penicillin antimicrobials for IVRLP may be justified because this technique delivers much higher therapeutic concentrations of the antimicrobial in infected ischemic tissues for a longer period (27). In addition, after the tourniquet is released the high antimicrobial concentrations diffuse from the surrounding tissues which serve as a depot (3). The reason is that the pharmacokinetic/pharmacodynamic (PK/PD) relations of this group correlate well with the therapeutic efficacy of the various beta-lactam antibiotics including penicillin, especially the duration of time that the drug concentration is greater than the relevant MIC ($T > MIC$) (28). Our high synovial penicillin concentrations were greater than the 1 $\mu\text{g/mL}$ MIC break

point for its susceptible pathogens (22) and remained greater for 24 h. The other critical PK/PD parameter for beta-lactam antibiotics is AUC_{0-36}/MIC_{90} and in certain situations, such as osteomyelitis or other isolated infections, the C_{max}/MIC_{90} is also a valuable predictor of efficacy (29). The penicillin AUC_{0-36}/MIC_{90} ratio was > 125 , and the C_{max}/MIC_{90} ratio was > 10 ; these values are indicative of highly effective bacterial killing that leads to minimal development of bacterial resistance (29). These results justify the once-a-day use of penicillin by IVRLP in the clinical setting. Although previous studies also enabled the goal of $C_{\text{max}}/MIC_{90} > 10$ to be reached, we believe the higher amikacin concentration in the synovial fluid achieved in the current study, along with the combination of amikacin-penicillin, may possess a clinical advantage. It was recently shown *in vitro*, that some isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) have greater MIC values for amikacin, some of them exceeding the upper limit of the E-test strips (256 $\mu\text{g/mL}$) (30). As MRSA is a common and problematic pathogen in equine practice (31), reaching greater concentrations ($> 256 \mu\text{g/mL}$) in the joint during IVRLP may be helpful, and even crucial in some clinical cases. Also, according to a thorough recent *in vitro* study on septicemic human neonatal blood (32), the combination of aminoglycosides-beta-lactams resulted in marked synergism against resistant coagulase negative *Staphylococci* and can be used effectively to treat neonatal septicemia. Thus, using the combination reported herein has the potential to effectively combat MRSA and other resistant bacteria in horses as well.

Our results contradict the Zantingh et al (11) recommendation that the combination of aminoglycoside-beta-lactams should not be used in IVRLP. This contradiction indicates that each different drug combination should be evaluated for its own PK properties. The PK data acquired in the present study support our working hypothesis that the amikacin-penicillin combination delivered through IVRLP is potentially efficacious for horses with bacterial infection in the distal portion of the limb. One major drawback in this study design is the lack of IVRLP with amikacin and IVRLP with penicillin separate groups. This would have enabled a direct comparison between the IVRLP with each antibiotic compared to the other and to both combined. The small study group is another limitation of this study, one that is common to most equine research studies. However, in this case the most important PK/PD values (C_{max}/MIC and AUC/MIC) of amikacin and penicillin were uniformly above the ideal threshold in all tested horses. Thus, due to these unequivocal consistent results, the small study group was not an obstacle to the power of statistics.

Another limitation is the use of healthy horses and the lack of a biological assay to assess the activity of the antimicrobial drugs and not only their synovial concentrations. In addition, the lack of published PK data regarding penicillin use in IVRLP did not enable us to compare our penicillin PK values with another study in which penicillin was used as a sole IVRLP antibiotic. Nevertheless, in a similar study setting we did perform a preliminary study (unpublished), conducted on 2 healthy adult horses, using solely 10×10^6 MU benzylpenicillin sodium. In these 2 horses we found lower penicillin concentrations in the MCP joint (mean: C_{max} 67 $\mu\text{g/mL}$) than in the current combination

study (mean: C_{\max} 556 $\mu\text{g/mL}$). Thus, the amikacin in the combined perfusion did not seem to inhibit the accumulation of penicillin in the joint, it may have facilitated penicillin accumulation, but that is yet to be elucidated in future studies.

In summary, our results indicate that properly combining penicillin and amikacin during IV-IVRLP in healthy horses delivered high therapeutic concentrations of both drugs. Therefore, this drug combination should be considered for use in IVRLP in order to broaden the antimicrobial coverage when dealing with treatment or prevention of distal limb infections in horses.

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