Clinical and pharmacokinetic effects of regional or general anaesthesia on intravenous regional limb perfusion with amikacin in horses

A. T. Mahne[1,*], E. Rioja[1], H. J. Marais[1], N. F. Villarino[2] and L. M. Rubio-Martinez[1]

[1] Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria, South Africa

[2] Department of Biomedical and Diagnostic Sciences, College of Veterinary Medicine, University of Tennessee, USA

[*]Correspondence email: arnold.mahne@up.ac.za

Summary

Reasons for performing study: Antimicrobial intravenous regional limb perfusion (IV-RLP) is clinically performed on anaesthetised or sedated horses with or without regional anaesthesia. To date, no scientific data is available on the clinical and pharmacokinetic effects of these anaesthetic protocols on antimicrobial IV-RLP, which is believed to result in better tourniquet efficiency due to decreased movement.

Objective: To determine the effects of regional or general anaesthesia on the clinical and synovial pharmacokinetic parameters of amikacin administered by IV-RLP to horses.

Methods: Eight healthy horses received 4 treatments of amikacin IV-RLP in a randomised, blinded, cross-over design: standing sedation without regional anaesthesia (CNT), standing sedation with intravenous regional anaesthesia (IVA), standing sedation with perineural regional anaesthesia (PNA) or general anaesthesia (GA). Synovial fluid amikacin concentrations were measured over 24 hours and regional pharmacokinetic parameters calculated. Heart and respiratory rates, visual analogue scale (VAS) of discomfort, number of times the limb was lifted and number of additional sedations administered were recorded. ANOVA cross-over analysis was applied with significance level at $P < 0.05$.

Results: Amikacin concentrations and regional pharmacokinetic parameters did not differ significantly among treatments. Scores of VAS (mean ± SD) were significantly lower with PNA (19 ± 15) versus IVA (69 ± 36) or CNT (81 ± 13) ($P < 0.001$). Significantly less lifting of the limb (mean ± SD) occurred with PNA (20 ± 20) versus CNT (54 ± 22) ($P < 0.04$).

Conclusions: Perineural regional anaesthesia before IV-RLP was most effective in providing comfort to standing, sedated horses without significantly affecting the regional pharmacokinetic parameters of amikacin. High variability of synovial amikacin concentrations was present.

Potential relevance: The comfort of horses undergoing standing IV-RPL can be increased by performing perineural anaesthesia prior to treatment. The use of general anaesthesia for IV-RLP is not justified based on this study.

Keywords: horse; regional limb perfusion; amikacin; general anaesthesia; regional anaesthesia; pharmacokinetics

Introduction

Equine orthopaedic infections are common life-threatening conditions that necessitate early and aggressive treatment including drainage, surgical debridement and antimicrobial therapy. Antimicrobials are frequently administered both systemically and regionally, including intravenous regional limb perfusion (IV-RLP) [1, 2].

Movement of standing, sedated animals undergoing IV-RLP is believed to cause inadvertent leakage of the perfusate into the systemic circulation due to failure of vascular occlusion by the tourniquet [3, 4]. In an attempt to minimise patient movement, perineural or intravenous regional anaesthesia is sometimes combined with antimicrobial IV-RLP to decrease the horse's discomfort [5, 6]. To the authors' knowledge, an objective evaluation of the effects of these regional anaesthetic techniques on the horse's comfort level and synovial antimicrobial concentrations has not been performed.

Performing IV-RLP under general anaesthesia eliminates patient movement, which might increase regional antimicrobial concentrations due to less vascular leakage; however, general anaesthesia carries an increased risk to the patient [7] and additional costs to the client. Therefore, antimicrobial IV-RLP is commonly performed on standing, sedated horses and it provides high regional antimicrobial concentrations [8-10]. However, there is no available comparative data on the synovial antimicrobial concentrations after IV-RLP on the standing versus the anaesthetised horse.

This study examined the effects of intravenous regional anaesthesia, perineural regional anaesthesia or general anaesthesia on the horse's comfort level and synovial antimicrobial pharmacokinetics in horses undergoing IV-RLP with amikacin. It was hypothesised that the addition of regional anaesthesia (intravenous or perineural) during IV-RLP would increase the horses' comfort level and the synovial fluid amikacin concentrations compared to IV-RLP without regional anaesthesia in standing, sedated horses. It was further hypothesised that synovial amikacin concentrations after IV-RLP on standing, sedated horses with the concurrent use of intravenous or perineural regional anaesthesia would be similar to those obtained after IV-RLP under general anaesthesia.

Materials and Methods

Animals

Eight clinically healthy, adult, local breed (detail to be provided on acceptance) mares with mean (range) weight and age of 390 (357 - 430) kg and 7 (5.5 - 10) years, respectively, were included. Inclusion criteria were normal physical examination, absence of lameness at walk and trot, no musculoskeletal abnormalities in the front limbs and no drug administration for at least eight weeks prior to the study. The study was approved by the (detail to be provided on acceptance) Animal Use and Care Committee.

The mares were housed in outdoor sheltered pens with free access to hay and water. Before general anaesthesia food, but not water, was withheld for 6 hours. Daily clinical examinations were performed from the day before treatment until 24 hours after collection of the last sample.

Study design

A prospective, blinded, cross-over study was conducted. Each mare received 4 IV-RLP treatments in a random order, with a wash-out period of one week between treatments. The first treatment was randomly assigned to either the left or right front limb and subsequent treatments alternated between the front limbs.

IV-RLP

All horses received amikacin (Amikacin Fresenius)[a] IV-RLP on 4 occasions: standing sedation without regional anaesthesia (CNT); standing sedation with addition of 20 mL of lidocaine (detail to be provided on acceptance)[b] to the perfusate (IVA); standing sedation 20 minutes after perineural

anaesthesia (ulnar, median and musculocutaneous nerve block with 35 mL of lidocaine [11]) (PNA); and under general anaesthesia (GA). The same author performed the nerve blocks 20 minutes prior to IV-RLP on all horses for treatment PNA and placed a light bandage over the injection sites in all horses undergoing treatments CNT, IVA and PNA to ensure blinding of the primary author.

Horses were sedated with romifidine (Sedivet)[c] (0.03 mg/kg bwt i.v.) and butorphanol (Torbugesic)[d] (0.01 mg/kg bwt i.v.) 5 minutes prior to the placement of the tourniquet in treatments CNT, IVA and PNA. Additional sedation (romifidine [0.015 mg/kg bwt i.v.] and butorphanol [0.01 mg/kg bwt i.v.]) was administered to standing horses during the treatment period if the primary author (blinded to treatment group) considered that the procedure could not continue any longer without additional sedation due the horse's discomfort. The number of additional sedations administered was recorded.

For treatment GA, a 16-gauge, 2-inch over-the-needle catheter (Nipro Safelet Cath)[e] was aseptically placed into the left or right jugular vein. Horses were sedated with romifidine (0.08 mg/kg bwt i.v.) and 5 minutes later anaesthesia was induced with ketamine (Ketamine-Fresenius)[f] (2.2 mg/kg bwt i.v.) and diazepam (0.025 mg/kg bwt i.v.) (A-Lennon Diazepam)[g]. Horses were placed in left or right lateral recumbency with the limb to be treated down. General anaesthesia was maintained with an intravenous continuous rate infusion of a combination of 45 mg romifidine, 50 g guaifenesin (GGE Powder)[h] and 1 g ketamine in 1 L of Lactated Ringer's solution, which was administered to effect for the duration of the treatment. Oxygen (15 L/min) was administered intra-nasally for the duration of general anaesthesia, which ended upon removal of the tourniquet. Recovery from anaesthesia was unassisted.

For all treatments, a 12 cm wide elastic tourniquet was placed by the primary author in the distal aspect of the antebrachium. The primary author injected the perfusate over 60-90 seconds into the cephalic vein at the palmaro-medial aspect of the carpus using a 23 gauge butterfly needle (Scalp vein set)[i]. The tourniquet was kept in place for 30 minutes following the end of the injection in all treatments.

The total volume of the perfusate was 50 mL in all treatments. This volume consisted of 1 g of amikacin in 50 mL of Lactated Ringer's solution[a] in treatments CNT, PNA and GA. In treatment IVA, the volume consisted of 1 g of amikacin in 30 mL of Lactated Ringer's solution and 20 mL of 2% lidocaine.

Sample collection

Samples of synovial fluid (approximately 0.6 mL) were aseptically collected into heparinised tubes[j] from the middle carpal joint via dorsal arthrocentesis using a 22-gauge needle. Samples were collected before IV-RLP and tourniquet placement (T0), 30 minutes after perfusate administration and before tourniquet release (T0.5), and 1.5 (T1.5), 6 (T6), 12 (T12) and 24 (T24) hours post-administration. Blood samples were collected into heparinised tubes by jugular venipuncture at T0 and T0.5. All samples were centrifuged (1.207 g, 8 minutes) and the supernatant was frozen at -80 ^{0}C for amikacin concentration analysis.

Amikacin measurement

Amikacin concentrations were measured using fluorescence polarization immunoassay [12] (Integra 400 Plus)[k], following procedures described by the manufacturer. Synovial fluid was diluted with calibration kit diluent as required for amikacin concentrations to fall within the test range of 3 – 40 µg/mL. The measured values were corrected, accordingly, by the dilution factor to obtain the synovial amikacin concentrations

Pharmacokinetic analysis

Regional synovial pharmacokinetic parameters were estimated by a non-compartmental analysis using a software package (SummitPK Solutions)[l]. The area-under-the-concentration-time curve over 24 h post-administration (AUC_{0-24}), area-under-the-concentration-first-moment-time curve over 24 hours post-administration ($AUMC_{0-24}$), mean residence time (MRT) and terminal elimination half-life ($T_{1/2}$) were estimated. The AUC_{0-24} was calculated by log-linear trapezoidal rule and Lambda-Z (λ_z) was estimated with uniform weighting. The peak concentration (C_{max}) and time to C_{max} (T_{max}) were obtained from inspection of the raw data.

Clinical assessment

Assessment of discomfort of the standing, sedated horses undergoing IV-RLP (CNT, IVA and PNA) was performed by using a visual analogue scale of discomfort (VAS) [13]. The VAS scale consisted of a horizontal line of 100 mm with no markings, with 0 mm representing no discomfort and 100 mm representing severe discomfort (horse becoming aggressive, pawing intensively and/or rearing). The 30-minute period of IV-RLP was subdivided into two 15-minute periods (0 - 15 minutes and 15 - 30 minutes)

and a VAS score was subjectively assigned to each period by the primary author who was blinded to the treatment group. If a horse received additional sedation during the first 15-minute period, a VAS score of 100 was assigned to both the first and second periods. If the first additional sedation was administered during the second period, only the second period was assigned a score of 100. The average of the scores assigned to both periods was calculated and used in the statistical analysis. Lifting of the treated limb during the procedure was also counted and recorded. Heart rate and respiratory rate of each horse were recorded 5 minutes after sedation and before tourniquet placement (baseline) and 15, 30 and 40 minutes after perfusate administration in treatments CNT, IVA and PNA.

Statistical analysis

All the statistical procedures were analysed by Statistical Analysis System (SAS, version 9.2, 2011)[m]. A linear mixed model analysis of variance (ANOVA) cross-over and cross-over repeated measures was used. Analysis of variance was implemented for the statistical comparison of clinical parameters, including VAS, lifting of the limb, heart rate and respiratory rate. The potential effect of lifting of the limb or VAS on systemic or synovial fluid amikacin concentrations at T0.5 was evaluated by correlation analysis. Drug concentrations, AUC_{0-24}, C_{max}, and heart rate at 15 min were logarithmically transformed before analysis to meet normality assumptions. Post-hoc Tukey-Kramer mean separation was used to identify differences among least squared means. Significant difference was set at $P < 0.05$.

Results

All horses received all treatments. All general anaesthesia recoveries were uneventful. One mare became recumbent during CNT 29 minutes after perfusate administration and stood up 10 minutes after removal of the tourniquet. The same mare also became recumbent during IVA at 5 minutes and stood up 26 minutes after perfusate administration. In both instances her clinical parameters were within normal limits at 40 minutes after perfusate administration and T1.5. Due to the fact that the mare was recumbent during two of the procedures to be performed standing, she was removed from statistical analysis.

Amikacin measurements and rPkp parameters

One synovial sample (T0.5, treatment GA) was excluded from the analysis due to measurement error. Synovial fluid amikacin concentrations and estimated regional pharmacokinetic parameters for

different treatments are included in Figure 1 and Table 1 respectively. No significant differences were found among treatments for any of the regional pharmacokinetic parameters or amikacin concentrations at each time point.

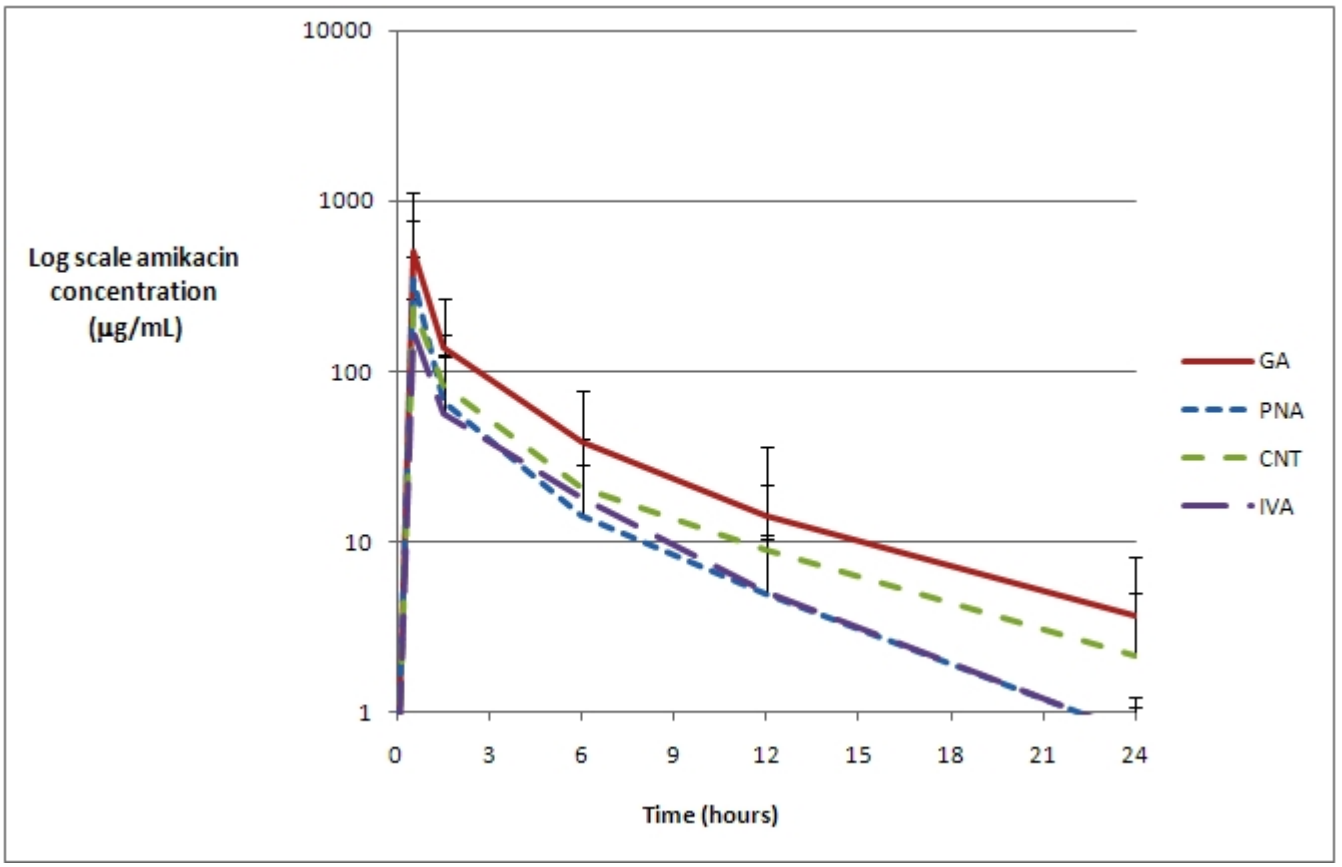

Figure 1. Log scale intercarpal synovial fluid amikacin concentration (µg/ml) over 24 h after amikacin i.v. regional limb perfusion in standing, sedated horses without regional anaesthesia (CNT), with i.v. regional anaesthesia (IVA) or with perineural regional anaesthesia (PNA) and in horses under general anaesthesia (GA). The graph was plotted from the raw data and vertical bars represent the upper range of standard deviation at each time point.

All systemic amikacin concentrations at T0 were below the limit of detection of the analytical method. Mean (range) systemic amikacin concentrations (µg/mL) at T0.5 were 1.45 (<0.3 - 7.59) for GA, 4.62 (1.0 - 10.38) for IVA, 6.23 (<0.3 - 13.8) for PNA, and 9.23 (2.71 - 13.7) for CNT. No significant differences were found among treatments. No significant association between lifting of the limb or VAS and systemic amikacin concentrations at T0.5 or synovial fluid C_{max} was observed.

Table 1. Results of the regional pharmacokinetic parameters (mean ± SD; [range]) of amikacin in intercarpal joint fluid after amikacin IV-RLP in standing, sedated horses without regional anaesthesia (CNT), with intravenous regional anaesthesia (IVA) or with perineural regional anaesthesia (PNA) and in horses under general anaesthesia (GA). No significant differences were found among treatments.

PK parameter	CNT	IVA	PNA	GA
AUC_{0-24} (h*µg/mL)	907 ± 1046 [33.1 - 2942]	518 ± 432 [173 - 1289]	462 ± 454 [132 - 1526]	1075 ± 1213 [165 - 3703]
$AUMC_{0-24}$ (h*h*µg/mL)	3974 ± 4417 [105 - 8070]	1575 ± 1402 [526 - 5179]	2100 ± 1927 [491 - 3894]	4933 ± 5647 [723 - 14877]
C_{max} (µg/mL)	239 ± 231 [6.66 - 615]	172 ± 94 [63.7 - 311]	344 ± 431 [28.3 - 1039]	503 ± 634 [76.6 - 1752]
MRT (h)	4.48 ± 2.48 [2.32 - 4.17]	3.79 ± 0.60 [2.71 - 6.47]	3.60 ± 0.94 [2.39 - 9.25]	4.88 ± 2.06 [2.67 - 5.16]
$T_{½}$ (h)	4.24 ± 1.47 [2.76 - 7.12]	4.42 ± 1.52 [3.01 - 3.89]	3.91 ± 0.65 [2.62 - 4.53]	4.30 ± 0.56 [3.07 - 4.21]
T_{max} (h)	0.5 ± 0 [0.5 - 0.5]	0.5 ± 0 [0.5 - 0.5]	0.5 ± 0 [0.5 - 0.5]	0.5 ± 0 [0.5 - 0.5]

Table 2. Mean ± SD results of lifting of the limb, visual analogue scale of discomfort (VAS), heart rate and respiratory rate in standing, sedated horses undergoing amikacin IV-RLP without regional anaesthesia (CNT), with intravenous regional anaesthesia (IVA) and with perineural regional anaesthesia (PNA). Heart rate and respiratory rate were measured 5 minutes after sedation before tourniquet placement (baseline) and 15, 30 and 40 minutes after perfusate administration. Significant differences (P < 0.05) between groups are depicted with different superscript letters for the specific outcome.

Parameter	CNT	IVA	PNA
Lifting of the limb (number of times)	54 ± 22[a]	53 ± 33[ab]	20 ± 20[b]
VAS	81 ± 13[a]	69 ± 36[a]	19 ± 15[b]
Heart rate (beats/min)			
Baseline	28 ± 5[a]	32 ± 8[a]	29 ± 4[a]
15 min	31 ± 3[a]	35 ± 5[a]	33 ± 3[a]
30 min	35 ± 9[a]	34 ± 4[a]	33 ± 5[a]
40 min	30 ± 4[a]	29 ± 3[a]	33 ± 4[a]
Respiratory rate (breaths/min)			
Baseline	11 ± 3[a]	12 ± 6[a]	11 ± 6[a]
15 min	10 ± 2[a]	13 ± 7[a]	9 ± 1[a]
30 min	12 ± 4[a]	15 ± 7[a]	9 ± 4[a]
40 min	10 ± 3[a]	10 ± 4[a]	9 ± 3[a]

Clinical assessment

Results of VAS scores, lifting of the limb, heart rate and respiratory rate are summarized in Table 2. Significantly lower VAS scores (mean ± SD) were observed with PNA (19 ± 15) compared to CNT (81 ± 13) or IVA (69 ± 36) (P < 0.001). Significantly less lifting of the limb (mean ± SD) was observed with PNA

(20 ± 20) compared to CNT (54 ± 22) ($P < 0.04$). No additional sedations were necessary with PNA in comparison with treatments CNT and IVA, where median (range) of 1.5 (0 - 3) and 1 (0 - 4) additional sedations were administered, respectively. Median (range) time to first additional sedation were 17 (8 - 28.5) and 17.75 (9 - 25.3) minutes in CNT and IVA, respectively. No significant differences in heart rate and respiratory rate were observed among any of the standing treatments at any time.

Discussion

In the present study, the use of perineural regional anaesthesia increased the comfort level of standing, sedated horses undergoing IV-RLP; however, the use of intravenous regional anaesthesia in standing, sedated horses did not have any beneficial clinical effect compared with IV-RLP without regional anaesthesia. In addition, neither the use of regional anaesthetic techniques or general anaesthesia had a significant effect on the regional pharmacokinetic parameters of amikacin in horses undergoing IV-RLP.

The reason for failure of intravenous regional anaesthesia to reduce the discomfort of horses is unclear but might be attributed to pain caused by the tourniquet being located proximal to the desensitized area. In humans, a double cuff tourniquet is used [14, 15], where the proximal cuff is initially inflated to perform the intravenous injection of the local anaesthetic, which will desensitize the appendage distal to the cuff. Thereafter, the distal cuff, positioned immediately distal to the proximal cuff and on the desensitised area, is inflated, which will maintain vascular isolation of the distal appendage for the duration of the procedure. The proximal cuff, which is located outside the desensitized area, is deflated, thus not contributing to pain or discomfort. To the authors' knowledge, no information is available on the use of double cuff tourniquets in horses. When perineural regional anaesthesia was performed in the present study, the tourniquet was placed on the desensitized area. This might explain the increase in comfort levels in standing, sedated horses with the addition of perineural regional anaesthesia in this study.

A wide elastic tourniquet was used in the present study instead of a pneumatic tourniquet because it is more economical and widely used in clinical practice [16]. Recent studies have shown the use of a wide elastic tourniquet to be comparable [12] or superior [16] to a pneumatic tourniquet in achieving high intra-synovial amikacin concentrations with IV-RLP.

A visual analogue scale is commonly used in horses to evaluate pain [13,17] and has been shown to have good intra- and inter-observer agreement [18]. The blinded use of this scale proved to be useful to evaluate discomfort of horses in this study and revealed differences in discomfort among different treatments on standing, sedated horses. The results obtained with VAS were supported by lifting of the limb and the number of extra sedations needed, which showed that treatment PNA provided superior comfort to the horses in this study.

The hypothesis that the use of regional anaesthesia during IV-RLP versus IV-RLP without regional anaesthesia on standing horses would result in a significant increase in synovial fluid amikacin concentrations could not be demonstrated in this study, although mean concentrations and regional pharmacokinetic parameters were numerically higher in PNA treatment. A possible contributing factor is the high variability in the synovial amikacin concentrations as observed in previous studies [12, 19, 20]. This variability might in part be explained by the fact that the dose of amikacin was fixed and not corrected for the animal's weight similar to clinical practice and previous studies [21]. Blood contamination of the synovial fluid samples might have affected the measured intra-synovial amikacin concentrations [21]. However, arthrocentesis of the middle carpal joint is regarded as easy [11] and all arthrocenteses were performed by the primary author in a similar manner.

This study showed that synovial fluid amikacin concentrations after IV-RLP on standing, sedated horses with the concurrent use of regional anaesthesia were similar to those obtained after IV-RLP under general anaesthesia, although mean concentrations and regional pharmacokinetic parameters were numerically higher in GA. Similarly to what has been discussed previously, it is possible that the high variability in the synovial amikacin concentrations precluded any significance. During IV-RLP, venous pressure in the perfused area is influenced by volume of perfusate, rate and site of injection [22]. The maximum venous pressure is the highest achievable intravenous pressure before leakage to the systemic circulation will result and this is a reflection of tourniquet efficacy [22]. Sudden weight shifts on the front limbs of a horse can double intravascular pressure distal to the tourniquet [21], which might exceed the maximum venous pressure and cause leakage of the perfusate from the isolated area. This has been suggested as a possible cause of perfusate leakage [3, 4, 12]. However, in the present study no significant effect of limb movement on synovial or systemic amikacin concentrations could be

demonstrated. It is possible that quantifying the number of times the limb was lifted was not sensitive enough, as either a gentle lift of the limb or an aggressive pawing were quantified similarly.

The reason for recumbency of one mare during CNT and IVA is uncertain, but was interpreted as severe discomfort. This behaviour was not seen in any of the other horses and has not been observed by the authors in the clinical setting. The data obtained from this horse was removed from the analysis since the effect of recumbency on regional pharmacokinetic parameters and clinical parameters is unknown.

This study was performed on healthy horses. In inflamed joints, increased amikacin delivery and clearance from the synovial fluid after IV-RLP have been demonstrated when compared to healthy joints [23]; therefore, the regional pharmacokinetic parameters obtained with the different treatments in the present study cannot necessarily be directly extrapolated to horses with orthopaedic infections.

A shortcoming of this study is that, although fluorescence polarization immunoassay has been used previously to measure amikacin on equine synovial samples [10, 12, 16, 19, 21, 23], we did not perform an in-house validation of the assay.

In conclusion, this study shows that the use of perineural regional anaesthesia proximal to the tourniquet alleviates the discomfort of horses more efficiently than intravenous regional anaesthesia when performing IV-RLP on the standing, sedated horse. Therefore, perineural regional anaesthesia can be recommended as a preferred technique for IV-RLP treatments in standing horses. Additionally, the use of regional or general anaesthesia during IV-RLP with amikacin did not significantly affect the regional pharmacokinetic parameters. Thus the use of general anaesthesia for IV-RLP in horses is not justified based on the results of this study.

Manufacturer's address

a. Bodene (Pty) Ltd., (detail to be provided on acceptance)

b. Bayer, (detail to be provided on acceptance)

c. Boehringer Ingelheim, Ingelheim Pharmaceuticals (Pty) Ltd (detail to be provided on acceptance)

d. Fort Dodge Animal Health, Iowa, USA

e. Shandong Zibo Shanchuan Medical Instrument Co. Ltd., Zibo, China

f. Safeline Pharmaceuticals, (detail to be provided on acceptance)

g. Pharmacare Limited, (detail to be provided on acceptance)

h. Kyron Laboratories (Pty) Ldt., (detail to be provided on acceptance)

i. Niporo Corporation, Osaka, Japan

j. Becton Dickinson (Pty) Ltd., (detail to be provided on acceptance)

k. Roche Products (Pty) Ltd., (detail to be provided on acceptance)

l. Summit PK, Montrose, USA

m. SAS institute, Cary, USA

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