Plasma and tissue clindamycin antimicrobial activity after parenteral administration to cats under surgical conditions

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Abstract

Clindamycin plasma and tissue disposition in cats under surgical conditions after a single intravenous (IV), intramuscular (IM) and subcutaneous (SC) administration at a dose rate of 10 mg/kg were studied. After intravenous, intramuscular and subcutaneous administration, peak plasma concentrations were 10.93±3.78 µg/mL ($C_{p(0)}$), 5.93±1.18 µg/mL (C_{max}) and 6.30±0.88 µg/mL (C_{max}), respectively. Eight hours after clindamycin IV, IM and SC administration plasma concentrations declined to 2.01±0.61 µg/mL, 2.96±0.43 µg/mL and 3.36±0.97 µg/mL, respectively. Sixty to 90 minutes after clindamycin administration, tissue concentrations ranged from a minimum in subcutaneous tissue of 4.90 µg/g (IV), 3.06 µg/g (IM) and, 3.13 µg/g (SC) to a maximum in uterus of 13.41 µg/g (IV), 14.07 µg/g (IM) and, 14.44 µg/g (SC). The lowest tissue/plasma concentration ratio for the three administration routes was observed in subcutaneous tissue, while the highest was observed at genital level (ovary for IV and IM and uterus for SC). Estimated efficacy predictor (AUC/MIC), considering MIC breakpoint for bacteria isolated from animals, indicates that clindamycin administered IV, IM or SC at the studied dose is appropriated for perioperative prophylactic protocols and that given with a dose interval of 12 hours would be effective for susceptible infection treatment in cats.

Introduction

Clindamycin is a lincosamide antibiotic more active than lincomycin. It is a basic compound with a pKa of 7.6 and high lipid solubility. It has wide distribution and large tissue penetration. It can be administered by both oral and parenteral routes, being metabolized in liver to active (N-dimethyl clindamycin and clindamycin sulfoxide) and inactive (glucuronide) metabolites which are eliminated through bile and urine.[1-3] Clindamycin antibacterial spectrum includes gram-positive cocci (*Staphylococcus* spp., streptococci beta-hemolytic group), anaerobic bacteria, mycoplasma and many protozoa (*Toxoplasma gondii*).[4,5] Minimum inhibitory concentration (MIC) breakpoint for bacteria isolated from animals has been set in ≤0.5 µg/mL.[6] According to their bacterial killing kinetics, lincosamides are classified as time-dependent with moderate to prolonged persistent effects. The ideal dosing regimen for these antibiotics maximizes the amount of drug received. Therefore, the $AUC_{(0-24)}$/MIC ratio is the parameter that correlates with efficacy. Maximum killing is seen when $AUC_{(0-24)}$/MIC is 25-35.[7-10]

Clindamycin has many therapeutic indications in small animal veterinary medicine. The main indications are infections caused by Gram-positive aerobic bacteria, including staphylococcal infections, streptococcal toxic shock syndrome and diseases caused by anaerobic microorganisms, in penicillin-hypersensitive patients. It is also highly indicated for many soft tissue infections as well as periodontal disease and osteoarticular infections. It could be used as monodrug therapy or, for the treatment of serious life-threatening infections (sepsis, peritonitis, osteomyelitis), in combined protocols. It is also indicated for antibiotic prophylaxis in some clean-contaminated and contaminated surgical wounds.[4,5,11,12] In spite of its good oral absorption and bioavailability, parenteral dosing is preferred for the treatment of serious infections and prophylaxis of surgical wound infections. In such situations, recommended dosage scheme is 5-30 mg/kg every 12-24 hours.[4,13-15]

There are few reports of clindamycin plasma pharmacokinetics and tissue disposition in dogs and cats. Lavy *et al*.[16] described clindamycin pharmacokinetics in dogs after intravenous (IV), intramuscular (IM) and, subcutaneous (SC) administration and Batzias *et al*.[17] oral bioavailability in the same species. Clindamycin pharmacokinetics in cats has been described only after oral administration.[18-20] However, to the authors' knowledge there are no reports characterizing neither plasma nor tissue disposition after parenteral administration. So, the aim of the present study is to describe clindamycin disposition in plasma and in some selected tissues after single IV, IM and SC administration to cats.

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Materials and Methods

Experimental animals

Experimental animals were twenty-three young (1-2 years old) mixed-breed cats with an average weight of 3.18±0.58 kg. All cats were healthy as determined by clinical examination, complete blood and serum biochemical analysis and urinalysis. Animals were client-owned cats presented for standard surgical procedures of ovariectomy or orchiectomy. All animal handlings were approved by the Institutional Animal Care and Use Committee, School of Veterinary Science, University of Buenos Aires, Argentina.

Dosage form

An aqueous 15% clindamycin phosphate salt solution (Clindamicina Richet®, Richet, Buenos Aires, Argentina) was used. Clindamycin was administered at a dose rate of 10 mg/kg intravenously (cephalic vein), over a 2 minute period, to 11 cats (8 females and 4 males), intramuscularly (dorsal lumbar muscles) to six cats (6 females) and subcutaneously (lateral ribcage skin) to six cats (5 females and one male).

Experimental design

A prospective randomized study was designed. Prior starting the study, two

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catheters (Jelco®, Smith Medical International Ltd., Italy) were placed into the cephalic veins. One of them (24G), was for antibiotic and fluid (NaCl 0.9%, 10 mL/kg/h) administration; and, the other (22G), for blood sample withdrawal.

Surgical procedures (ovariectomy or orchidectomy) were performed according to conventional standard techniques under general parenteral anaesthesia (midazolam 0.2 mg/kg, Midazolam®, Richmond Vet Pharma, Argentina; ketamine 10 mg/kg, Ketonal 100®, Richmond Vet Pharma, Argentina; xylazine 0.5 mg/kg, Rompun®, Bayer S.A., Argentina). Clindamycin was administered IV, IM or SC 1-1.5 hours prior first surgical incision.

Blood sampling

Blood samples (0.7 mL) were collected with a heparinised syringe at 0.08; 0.16; 0.33; 0.50; 0.75; 1; 1.50; 2; 3; 4; 6 and 8 hours after clindamycin administration.

Samples were centrifuged at 1500 ×g, for 15 min, and then plasma was harvested and stored at -20°C until analysis.

Tissue sampling

Tissue samples (0.1-0.5 g) were taken at 1-1.5 hours after clindamycin administration. Selected tissues/organs were skin, subcutaneous tissue, muscle, ovary, uterus, testicle and epididymis. Samples were rinsed briefly with saline solution and dried through gentle pressure with sterile gauze for removing blood contamination. Then, they were weighed and stored at -20ºC.

Clindamycin was eluted from tissue samples following a technique described by Bamberg *et al*.[21] Briefly, samples were cut into small pieces and diluted with phosphate buffer pH 7 (in a ratio 1:2 w/v) and incubated for 24 h at 4ºC, applying agitation during the first 40 min of incubation. Finally, they were centrifuged (1500 g for 15 min) and the supernatant harvested till assayed. All collected samples (plasma and tissue) were assayed within two weeks after collection.

Plasma and tissue drug analysis

Clindamycin plasma and tissue concentrations were determined by microbiological assay,[22] using *Kocuria rhizophila* ATCC 9341 as test microorganism. Standard curves were prepared on normal cat plasma and phosphate buffer pH 7, depending on the sample matrix to be quantified Each sample was seeded in triplicate and each standard dilution in quintuplicate. Limit of detection and quantification of the method was 0.78 µg/mL. The method was linear between 0.39 and 50 µg/mL (r=0.9985). Inter and intra-assay coefficients of variation were less than 10%.

Pharmacokinetic parameters

Reported pharmacokinetic parameters were determined using standard noncompartmental analysis (Phoenix® WinNonlin® 6.3, 2005-2012, Certara, L.P.) The areas under the concentration versus-time curves from time zero to time of last sampling time (AUC_{0-t}) were calculated using a combination of the linear and logarithmic trapezoidal rules. Values for maximum concentration (C_{max}) and time to C_{max} (T_{max}) were determined visually from the graphs.

Statistical analysis

Pharmacokinetic parameters, tissue concentrations and, tissue/plasma ratios are reported as mean±standard deviation (SD).

Area under plasma concentration vs time ($AUC_{(0-t)}$), last measured plasma concentration, tissue concentrations and, tissue/plasma ratios after for the three administration routes were statistically compared applying ANOVA followed by Tukey. Peak concentration (C_{max}) and T_{max} were compared using Student's t test. Differences were considered significant when $P<0.05$.

Results

Adverse effects were not observed during or after IV, IM or SC clindamycin administration in any of the cats. Mean plasma concentration vs time curves for the three studied routes are given in Figure 1 and the correspondent pharmacokinetic parameters in Table 1. Clindamycin plasma disposition after IM and SC administration was rather similar; however, administered IM clindamycin seems to be absorbed faster as reflected by the shorter T_{max} ($P<0.05$).

Plasma concentrations of clindamycin were above the limit of detection for up to 8 hours for the three administration routes. However, at this time, extravascular administration routes lead to significant higher concentrations (2.01±0.61 µg/mL, 2.96±0.43 µg/mL and 3.36±0.97 µg/mL, for IV, IM and SC, respectively).

Clindamycin tissue concentration and tissue/plasma concentration ratios are shown in Table 2. For most of the studied tissues, tissue/plasma ratios were above 1, reflecting clindamycin wide tissue penetration. Exception for the three studied administration routes was the subcutaneous tissue.

Extravascular routes showed, compared to the IV route, lower ratios; for both routes, ratio lower than 1 was observed for skin, subcutaneous tissue and muscle. A lower than 1 ratio was observed for testicles and epididymis after SC administration.

Discussion

Parenteral administration of clindamycin is frequently used in cats for the prophylaxis of surgical wound infections and, also for the treatment of serious infections. However, in this species there are not reports on clindamycin plasma or tissue disposition after parenteral administration. The present study is a contribution to the knowledge of clindamycin pharmacology because it describes plasma and some tissue clindamycin concentration after IV, IM and SC administration to cats. It is important to highlight that the present study has some weaknesses. First, related to analytical method used for clindamycin quantification. Since microbiological assay cannot distinguish between clindamycin and its active metabolites (clindamycin sulfoxide and N-dimethyl clindamycin) reported concentrations actually represent total clindamycin antimicrobial activity. On the other hand, restricted blood sampling period do not allowed an integral pharmacokinetic description. It is important to highlight that the applied experimental design was done under genuine standard surgical procedures (ovariectomies and orchidectomy). Therefore, tissue samples were limited to some soft tissues, representatives of different degree of tissue perfusion at a fixed time point (1-1.5 h post-administration). This was a disadvantage because it did not allow knowing or predicting later tissue concentrations. However, previous reports indicate that for macrolides and lincosamides tissue concentrations decline in parallel with plasma concentrations.[23,24] After extravascular administration, clindamycin was rapidly absorbed. Intramuscular administration showed the shortest T_{max}, implying the fastest absorption. This finding is not unexpected, based on the higher irrigation of muscles compare to subcutaneous space. No differences were observed on C_{max}, reflecting that, with temporal differences, both administration routes reach the same drug level. Slightly lower C_{max} (4.4 *vs* 5.93 µg/mL) and longer T_{max} (1.22 *vs* 1.00 h) have been reported in dogs after IM administration.[16] On the contrary, after SC administration to dogs, reported C_{max} are much higher (20.8 *vs*. 6.30 µg/ml) and T_{max} shorter (0.78 *vs* 1.13 h) than in cats.[16] Clindamycin plasma disposition curves after the three administration routes were very similar as reflected by their similar $AUC_{(0-t)}$ values. Also, plasma concentrations were well above MIC breakpoint (0.5 µg/mL) even at the last sampling time. At this time higher concentrations were observed for the IM and SC routes, clearly

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reflecting the absorption process and suggesting that, compared to IV, extravascular clindamycin administrations could allow longer dose intervals.

The AUC/MIC ratio estimated for the three studied administration routes was well above the value associated with maximum killing (25-35),[7-10] allowing to predict that clindamycin administered IV, IM or SC at a dose rate of 10 mg/kg will be highly effective. In the present study, clindamycin concentrations in all the sampled tissues were always higher than bacterial MIC breakpoint. The higher clindamycin tissue concentrations were observed after IV administration; statistical differences between the three administration routes were detected only for skin. For most of the studied tissues, plasma/tissue ratios were above 1, reflecting clindamycin wide tissue penetration. Exception for the three studied administration routes was the subcutaneous tissue. Similar ratios have been reported by Brown *et al.*[20] after multiple oral clindamycin dosage to cats. This scenario is not unexpected considering the high lipophilicity of clindamycin.

Extravascular routes showed, compared to the IV route, lower ratios; not unexpected due to the higher concentration gradient achieved after intravenous administration. For both routes plasma/tissue ratios lower than 1 were observed for skin, subcutaneous tissue and muscle. The observed differences between tissues can be attributed to anatomical and physiologic tissue particularities (such as composition, tissue blood perfusion rate, etc.).

Conclusions

The present results suggest that clindamycin administered by IV, IM or SC route in cats at a dose rate of 10 mg/kg

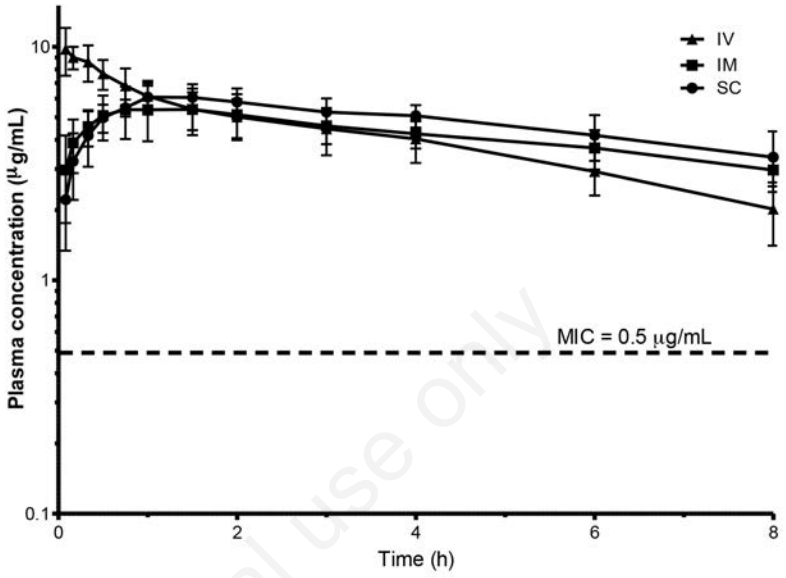

Figure 1. Mean (±SD) clindamycin plasma concentration-time profile after IV (▲), IM (■) and SC (●) administration to cats at a dose of 10 mg/kg. MIC value corresponds to *Staphylococcus* spp. MIC breakpoint for veterinary pathogens (MIC≤0.5 µg/mL).

Table 1. Pharmacokinetic parameters (mean±SD) of clindamycin after intravenous, intramuscular and subcutaneous administration to cats at a dose rate of 10 mg/kg.

Pharmacokinetic parameter	Intravenous administration	Intramuscular administration	Subcutaneous administration
AUC(0-t) (µg.h/mL)	32.06±5.55	33.72±5.24	36.67±6.49
Cp(0) (µg/mL)	10.93±3.78	-	-
Cmax (µg/mL)	-	5.93±1.18	6.30±0.88
Tmax (h)	-	1.00±0.38	1.13±0.31*
AUC(0-t) /MIC	64.12±11.10	67.45±10.49	73.33±12.98

$AUC_{(0-t)}$ area under the plasma concentration vs time curve from 0 to the last sampling time; $C_{p(0)}$ plasma concentration at 0 time; C_{max} maximum plasma concentration; T_{max} time of maximum plasma concentration; MIC minimum inhibitory concentration. *Significant differences (P≤0.05).

Table 2. Clindamycin tissue concentrations (mean±SD) taken between 1-1.5 h after clindamycin administration and, tissue/plasma concentration ratio after IV, IM and SC administration (10 mg/kg) to cats.

Tissue	IV tissue concentration (µg/g) (n)	IV tissue/plasma concentration ratio	IM tissue concentration (µg/g) (n)	IM tissue/plasma concentration ratio	SC tissue concentration (µg/g) (n)	SC tissue/plasma concentration ratio
Skin[a]	9.45±3.15 (11)	1.64±0.39	4.76±2.73 (6)	0.91±0.52	4.22±2.50 (6)	0.68±0.37
Subcutaneous	4.90±2.96 (8)	0.81±0.39	3.06±2.60 (4)	0.47±0.48	3.13±3.30 (5)	0.55±0.56
Muscle	8.15±3.75 (7)	1.36±0.47	3.78±3.02 (6)	0.67±0.48	4.95±2.64 (3)	0.80±0.47
Ovary	12.10±5.37 (14)	2.38±0.77	13.28±8.47 (12)	2.46±1.53	11.25±5.76 (10)	1.96±1.01
Uterus	13.41±2.02 (7)	1.96±0.80	14.07±2.43 (6)	2.23±1.41	14.44±3.11 (3)	2.15±0.85
Testicle	9.61±4.26 (6)	1.68±0.67	-	-	3.96±0.11 (2)	0.57±0.02
Epididymis	9.59±3.26 (6)	1.67±0.47	-	-	5.00±0.49 (2)	0.71±0.07

[a]Significant differences between clindamycin skin concentrations and between skin/plasma concentrations rate after intravenous vs. extravascular administrations.

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reach concentrations in plasma and tissues well above of bacterial MIC breakpoint (0.5 µg/mL), with and AUC/MIC twice that reported as optimal for maximal bacterial killing. Therefore, it will be highly effective for the prophylaxis of surgical wound infection and for preventing bacteria colonization in abdominal surgery. Also, for treatment of serious infections 10 mg/kg/12 h would be highly effective.

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