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Case Report

Pharmacokinetics of Cephalexin after Intravenous and Single and Multiple Intramuscular Administration to Rabbit

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Abstract

Cephalexin is a first generation cephalosporin widely used in rabbits. Its spectrum includes Pasteurella multocida and Staphylococcus aureus. These bacteria, together with Bordetella bronchiseptica, are the main cause of respiratory infections. Although many textbooks on rabbit therapeutics report the use of cephalexin, including administration schedules, there are not published papers on the pharmacokinetics of cephalexin after IV and IM administration to rabbit. Therefore, the objective of the present study was to describe cephalexin disposition in rabbits after intravenous and single and multiple intramuscular administrations. Three administration schedules were studied: single IV administration (10 mg/kg), single IM administration (10 mg/kg) and multiple IM administration (2.5 mg/kg/6). Serial blood samples were collected over a 24 h period. Cephalexin plasma concentrations were determined by microbiological method using Kocuria rhizophila ATCC 9341 as microorganism test. No statistical differences were observed between routes of administration for any of the estimated PK parameters. The unique difference was observed on bioavailability between intramuscular administration schedules. Elimination half-life was 1.45, 1.09 and 1.91 h for the single IV, single IM and multiple IM administration, respectively. Bioavailability after single and multiple IM administration was 47 and 97.5%, respectively. After multiple IM administration maximum and minimum plasma concentration at steady state were 2.77 and 0.34 μg/ml, while Cmax after single IM administration was 9. 22 μg/ml.

Considering that for betalactams the PK/PD breakpoint recommended for efficacy (T > MIC) should be 50 – 80% and that the reported MIC for most gram-positive organisms and Pasteurella multocida is ⩽1.0 μg/ml, the present study demonstrates that a single IM dose of 10 mg/kg/24 h is enough to maintain therapeutic concentrations for a 24 hours period. When a 2.5mg/kg dose is used administration every 6 hours is recommended.

Keywords: Cephalexin; Rabbit; Pharmacokinetics; Multiple intramuscular administration.

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Introduction

Cephalexin is a first generation cephalosporin with high activity against Gram-positive cocci (staphylococci, streptococci) including many beta-lactamase-producing strains, some Gram-negative Enterobacteriaceae and anaerobes (Prescott, 2006). Cephalexin is, due to its high efficacy against *Pasteurella multocida* and *Staphylococcus aureus,* commonly used in rabbit for the treatment of respiratory infections (Rougier et al., 2006). These bacterial agents, together with *Bordetella bronchiseptica*, are the main cause of respiratory infections, which may give symptoms such as sneezing, coughing, nasal discharge and lethargy. They are also involved in formation of abscesses in subcutaneous tissues, behind the eye bulb or in internal organs as well as inflammation of the mucus membranes of the eyes and in the middle ear.

The reported minimum inhibitory concentration (*MIC)* for most gram-positive organisms and *Pasteurella multocida* is ≤1.0 μg/mL, while for susceptible gram-negative organisms (*E. coli*, and *Klebsiella pneumoniae)* it may be as high as 8 μg/ml (Prescott, 2006).

As for other betalactam antibiotics, cephalexin antibacterial activity is time dependent, i.e. time free drug concentration remains above the MIC (T > MIC) being the PK/PD parameter that relates better with clinical efficacy (Toutain et al., 2002; McKellar et al., 2004).

Cephalexin, in most species, is administered by oral route. However, in rabbits disruption of the normal enteric flora, with the consequent overgrowing of toxin producing Clostridium, is a common side effect for this route of administration for a number of antimicrobials, included cephalexin; therefore its use is not advisable (Carman, 1993).

Although many textbooks on rabbit therapeutics report the use of cephalexin (Morris, 1995; Ivey and Morrisey, 2000; Harcourt-Brown, 2002; Richardson, 2003; Saunders and Davies, 2005; Meredith and Flecknell, 2006), there are not published papers on the pharmacokinetics of cephalexin after IV and IM administration. Therefore, the objective of the present study was to describe the plasma disposition of cephalexin after intravenous and single and multiple intramuscular administration to adult rabbits.

Materials and Methods

The study was carried out in twelve healthy New Zealand White rabbits of weight 3.0- 3.5 kg. They were maintained in cages of 60 cm x 60 cm x 90 cm with free access to water and to a commercial diet (Gepsa Feeds, Pilar Group, Argentina).

All animal procedures were approved by the Institutional Animal Care and Use Committee, School of Veterinary, University of La Plata, Argentina.

Animals were divided into three groups: Group 1 (n=4) received cephalexin lysine salt (cefalexina, Laboratorio Richet, Argentina) intravenously at a dose rate 10 mg/kg, group 2 (n=4) received the same dose but intramuscularly and group 3 (n=4) received also intramuscularly a total of four 2.5 mg/kg doses of cephalexin every 6 hours.

Blood samples (0.4 ml) were collected in K-EDTA, through a 24G x ¾" intravenous catheter (Introcan®, Braun Aß, Germany) placed in the marginal ear vein, at 3, 6, 9, 12, 18 and 24 hours post-administration. For the intravenous administration two additional samples were withdrawn at 0.5 and 1 h post-administration.

Cephalexin plasma concentration was determined by microbiological assay (Bennet et al.,1966) using *Kocuria rhizophila*, formerly classified as *Micrococcus luteus* (American Type Culture Collection 9341) as test microorganism.

Each sample was measured in triplicate and a standard curve was prepared using normal

rabbit plasma. Serial twofold dilutions from 25 to 0.39µg/ml were used. Inhibition zones around the sample wells were measured with a digital calliper and compared with inhibition zones produced by the standards.

The limit of detection and quantification of the method were 0.39 and 0.78 µg/ml, respectively. The method was linear between 0.39 and 25 µg/ml (r = 0.9984). Inter and intra-assay coefficients of variation were 9.39 and 4.87 %, respectively.

Individual cephalexin plasma concentration vs. time curves were analyzed by non-linear least square regression analysis using PCNonlin (SCI Software, Lexington, USA), applying a one compartment open model with first order absorption and elimination. Initial estimates were determined using the residual method (Gibaldi and Perrier, 1982) and refitted by non linear regression. Most pharmacokinetic parameters were calculated using classic equations associated with compartmental analysis (Gibaldi and Perrier, 1982).

Pharmacokinetic parameters are expressed as median and range. Main parameters for each animal were statistically compared for the three administration methods applying Kruskall Wallis test. Differences were considered statistically significant when $p \leq 0.05$.

Results and Discussion

The microbiological assay used in this study was appropriate for the quantification of cephalexin concentrations, as no active metabolite has been identified in rabbits for this drug; therefore, plasma concentration data and pharmacokinetics correlate accurately with total antimicrobial activity. Besides, the good correlation between microbiological assay and other analytical methods, including HPLC has been reported (Steppe et al., 2003).

Adverse effects were not observed during or following cephalexin IV or IM administration in any of the experimental animals.

Cephalexin mean plasma concentration–time curves following IV and IM administrations are shown in Fig. 1. Estimated pharmacokinetic parameters for both routes are summarized in Table 1.

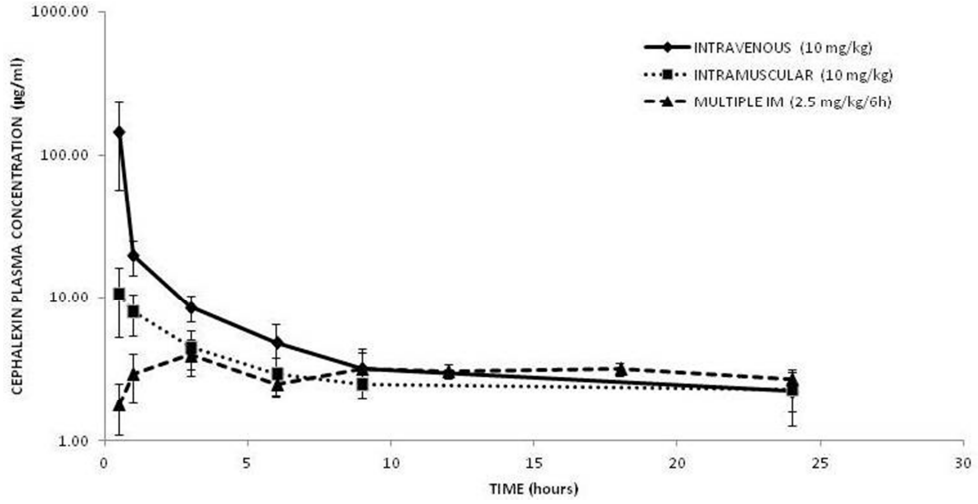

Fig. 1. Mean (±SD) Cephalexin Plasma Concentrations after Intravenous and Single and Multiple IM Administration to Rabbits (N=4)

Table 1: Median (Range) Pharmacokinetic Parameters of Cephalexin after Intravenous and Single and Multiple Intramuscular Administration to Rabbits (N=4)

PK PARAMETER	SINGLE IV DOSE (10 mg/kg)	SINGLE IM DOSE (10 mg/kg)	MULTIPLE IM DOSE (2.5 mg/kg/6h)
Kel (1/h)	0.92(0.36-1.63)	0.63 (0.10-3.55)	0.36 (0.25-0.46)
Ka (1/h)	NA	0.83 (0.27-16.99)	NA*
$AUC_{(0-\infty)}$ (µg/h/ml)	103.02 (94.77-144.99)	64.26 (27.5-90.80)	25.41 (20.38-47.05)
$T_{½}$elim (h)	1.45 (0.42-2.57)	1.09 (0.20-6.74)	1.91 (1.52-2.74)
$T_{½\,(a)}$ (h)	NA	1.12 (0.04-2.60)	NA
$C_{p\,(0)}$ -C_{max} (µg/ml)	85.02 (27.85-236.78)	9.22 (2.74-16.70)	NA
T_{max} (h)	NA	1.09 (0.30-1.40)	NA
Cl_B (ml/kg/h)	0.10 (0.07-0.11)	0.06 (0.05-0.27)	0.08 (0.056-0.124)
Vss- Vss/F (l/kg)	0.21 (0.04-0.36)	0.55 (0.04-3.53)	0.27 (0.12-0.48)
F(%)	NA	47.75 (31.40-76.00)**	97.51 (53.8-135.00)**
Css (max)	NA	NA	2.77 (1.52-3.39)
Css(min)	NA	NA	0.340.16-0.48 (0.16-0.48)
RA	NA	NA	1.21 (1.06-1.31)
T>MIC (h)	24	24	6

Kel elimination rate constant; K_a absorption rate constant; $AUC_{(0-\infty)}$ area under the plasma concentration vs. time curve from 0 to infinite; $t_{½}$ elimination half-life; $t_{½\,(a)}$ absorption half-life; $C_{p\,(0)}$ plasma concentration at 0 time ; C_{max} maximum concentration; T_{max} time of maximum concentration Cl_B body clearance; Vss volume of distribution; F bioavailability; RA accumulation rate ;Css(max) maximum concentration at steady state; Css(min) minimum concentration al steady state. RA accumulation index T>MIC amount of time drug concentrations are maintained above an *MIC of 1µg/ml*

* Since $K_a >> K_{el}$, e^{-Kat} approaches zero, the original model equation $Cp = \frac{KaFD}{V(Ka-Kel)} e^{-kat} - e^{-kelt}$ becomes $Cp = \frac{KaFD}{V(Ka-Kel)} e^{-kelt}$ (Gunaratna, 2001)

**p<0.05

No statistical differences were observed between routes of administration for any of the estimated PK parameters, this could be consequence of the high inter-animal variation. Unique statistical significant difference was observed when comparing bioavailability after IM administrations.

The most common route of administration for cephalexin in most species is the oral. Although, published data suggest actual or potential toxicity related to their use in rabbit, as well as in hamster and guinea pigs (Morris, 1995; Carman, 1993) .The mechanism of toxicity is related to the disruption of normal enteric flora and the consequent overgrowth of toxigenic *Clostridium difficile* and innocuum (Hara-Kudo, et al., 1996).

Another administration route that could be used in rabbits for cephalexin administration is the subcutaneous. However, it is important to highlight that dehydration, commonly observed in sick animals, can reduce the rate and extent of absorption of antimicrobials; this situation is not observed after IM administration (Ballard, 1968).

After IM administration, bioavailability for the single administration was 47.75 %. This values is similar to that reported for dogs (60%) (Carli et al., 1999; Chicoine et al.,

2009). After multiple administration bioavailability was higher ($p<0.05$), this difference could be reflecting the improvement of absorption when small volumes are injected (Zuidema et al., 1994).

Maximum plasma concentration (C_{max}) after single IM administration was almost three times lower compared with values reported for the same dose in dogs (Silley et al., 1988). Comparison of C_{max} after single IM administration with maximum concentration an steady state ($C_{ss(max)}$) after multiple administration, after correcting for dose difference, shows a higher value for the repeated administration schedule. This is reflecting the higher bioavailability of the latter schedule.

No statistically significant differences between administration routes and administration schedules were observed in elimination half-life. This is consequence of the high inter-animal variation observed, especially, after IV and single IM administration. The estimated valueswere similar to that in other species such as, rat (Tsai et al., 2000) dog (Carli et al., 1999) and cat (Albarellos et al., 2011). Accumulation index after multiple administrationswas closed to 1, reflecting cephalexin low accumulation capacity.Similar values are reported for humans after repeated oral administration (Pfeffer et al., 1977).

The antibacterial activity of cephalexin depends on the amount of time drug concentrations are maintained above the *MIC*($T > MIC$) for susceptible bacteria. Current recommendations for cephalosporins suggest that concentrations be maintained above the *MIC* for at least 50% of the dosing interval to achieve maximum activity (McKellar et al., 2004; Toutain et al., 2002). Considering that the reported *MIC* for most gram-positive organisms and *Pasteurella multocida* is ≤1.0 μg/ml (Griffith and Black, 1970; Brown *et al.*, 2004), the present study demonstrates that a single IM dose of 10 mg/kg/24 h is enough to maintain therapeutic concentrations for a 24 hours period. When a 2.5mg/kg dose is used administration every 6 hours is necessary.

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