Absorption kinetics and bioavailability of cephalixin in the dog after oral and intramuscular administration

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INTRODUCTION

Cephalixin is a first generation cephalosporin with bactericidal action and a wide spectrum of activity including Gram-positive and Gram-negative bacteria. It combines resistance to the action of penicillinases produced by Staphylococcus aureus isolates (Muggleton et al., 1968) with activity against the majority of ampicillin-resistant Escherichia coli strains (Foord, 1969). In vitro, more than 95% of β-haemolytic Streptococci, Pasteurella multocida and Staphylococcus intermedius strains isolated from dogs are inhibited by cephalixin concentrations of ≤ 2 μg/mL and more than 50–60% of Bordetella bronchiseptica, Proteus mirabilis, Escherichia coli and Klebsiella pneumoniae strains by 2–4 μg/mL (Silley & Brewster, 1988; Noble & Kent, 1992; Aucoin, 1993; Lloyd et al., 1996; Campbell & Rosin, 1998).

Cephalixin has proved to be effective in the treatment of a wide range of current bacterial infections, and in the dog it is used particularly for pyoderma, folliculitis and furunculosis (Griffith & Black, 1970; Pfeffer et al. 1977; Kietzmann et al., 1990; Frank & Kunkle, 1993; Carlotti & Leroy, 1995).


The pharmacokinetics of cephalixin, a first generation cephalosporin, were investigated in dogs using two formulations marketed for humans, but also often employed by practitioners for pet therapy. Cephalixin was administered to five dogs intravenously and intramuscularly as a sodium salt and by the oral route as a monohydrate. The dosage was always 20 mg/kg of active ingredient. A microbiological assay with Sarcina lutea as the test organism was adopted to measure cephalixin concentrations in serum.

The mean residence time (MRT) median values after intravenous (i.v.), intramuscular (i.m.) and oral administration (p.o.) were 86 min, 200 min, and 279 min, respectively.

After i.m. and oral dosing the peak serum concentrations (24.2 ± 1.8 μg/mL and 20.3 ± 1.7 μg/mL, respectively) were attained at 90 min in all dogs and bioavailabilities were 63 ± 10% and 57 ± 5%, respectively.

The time course of the cephalixin serum concentrations after oral administration was best described by a model incorporating saturable absorption kinetics of the Michaelis-Menten type; thus in the gastrointestinal tract of dogs a carrier mediated transport for cephalixin similar to that reported in humans, may exist.

The predicted average serum concentrations of cephalixin after repeated i.m and oral administration indicated that, in order to maintain the therapeutic concentrations, the 20 mg/kg b.w. dosage should be administered every 6–8 h.

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Although some cephalixin preparations are available for veterinary use in Italy, none is confined to the treatment of dogs and cats. Thus the formulations intended for human therapy are usually employed by practitioners for the treatment of pets at dosage schedules extrapolated from those for humans. The purposes of this study were to determine the intramuscular and oral bioavailability and to define the optimal therapeutic dosage regimen of cephalixin in dogs treated with formulations marketed in Italy for human beings.

Data from the literature showed that the intestinal absorption of cephalosporins in humans and rats was a saturable process (Reigner et al., 1990; Liu et al., 1997; Ruiz-Balanguer et al. 1997) and that a carrier-mediated uptake of cephalixin occurred in human intestinal cells (Dantzig & Bergin, 1988, 1990). Therefore a model incorporating the Michaelis-Menten type absorption characteristics was used to fit concentration vs. time data obtained after cephalixin oral administration and compared to the classical model with first order drug input.
MATERIALS AND METHODS

Animals

Five healthy Beagle dogs (two males and three females), 10–14 kg b.w. and aged 2–5 years, were used. The animals were purchased from an authorised breeding establishment, Greenhill (Montichiari, Brescia, Italy) and individually caged in temperature-controlled rooms (20–22°C and 50–60% humidity) with 12 h light cycles. The dogs were fed standard laboratory dogfood and were given tap water ad libitum. The care and handling of the animals was in accordance with the provisions of the European Economic Community (EEC) Council Directive 86–609, recognised and adopted by the Italian Government (D.L. 27/01/1992 n°116).

Drug formulations and treatments

Intravenous (i.v.) and intramuscular (i.m.) administrations were carried out using CEPOVEN® (Glaxo, Verona, Italy) which is marketed as vials containing 1 g of dried cephalixin sodium salt together with vials containing 4 mL water for injection. A solution of the active substance (100 mg/mL) in injection water was freshly prepared before each administration. Each dog received cephalixin (20 mg active ingredient per kg b.w.) intravenously into the right cephalic vein and intramuscularly in the deep muscles of the thigh.

Oral administration was performed using KEFOREL® (Eli Lilly, Sesto Fiorentino, Firenze, Italy) which is marketed as capsules containing 500 mg of cephalixin monohydrate. The active substance was administered at the dose of 20 mg/kg b.w. by adjusting the amount in each capsule to the body weight of the dogs.

Dogs were fasted for 24 h prior to treatment by i.v., i.m. and oral route according to a crossover design with a washout period of 20 days between each phase.

Sample collection

A venous catheter (Surflo 18C, Terumo Europe N.V., Leuven, Belgium) was placed in the cephalic vein of each dog before starting the trial. Blood samples were collected before each treatment and at 5, 15, 30, 45, 60, 90 min and 2, 3, 4, 6 and 8 h after i.v. injection; the same sampling scheme with an additional sample at 12 h was adopted after i.m. administration. Following the oral administration blood samples were collected at 0.5, 1, 1.5, 2, 3, 4, 6, 8 and 12 h. After each sampling the catheter was flushed with saline containing 1% heparin.

Serum was obtained by centrifugation at 1500 × g and stored at−20°C pending assay.

Microbiological assay

Serum concentrations of cephalixin were determined by the agar plate diffusion method, using 7 mL Antibiotic Medium 1 (Difco Laboratories, Detroit, MI, USA) poured into Petri dishes (90 mm) and Sarcina lutea ATCC 9341 as test organism (Grove & Randall, 1955).

To prepare cephalixin stock solution (1000 µg/mL) 21.2 mg of cephalixin sodium salt (941 µg/mg active ingredient) were dissolved in 20 mL phosphate buffer pH 6.6. The stock solution was diluted with antibiotic-free serum to obtain five calibration concentrations of 2, 1, 0.5, 0.25 and 0.12 µg/mL.

The paper disks (Schleicher & Schull Dassel, 9 mm) were charged with 50 µL of each standard solution or serum sample. Serum samples with cephalixin concentrations greater than the upper limit of quantification (2 µg/mL) were diluted in control serum to obtain a final concentration within the calibration range. Plates were preincubated for 2 h at room temperature (22°C) and then incubated overnight at 37°C.

The method was linear within the range of the calibration curve ($r^2 = 0.988$). Intra-day precision and accuracy were determined for concentrations over the range of the standard curve. The intra-day precision was calculated as the standard deviation (RSD) from six determinations and ranged from 3.8 to 5.4%. The accuracy of the method, calculated as mean error (ME = (observed concentration-added concentration)/added concentration × 100), ranged from −4.1 to 5.3%. The limit of quantification (LOQ) was 0.12 µg/mL with an RSD of 4.6% and an ME of −3.2%.

Pharmacokinetic analysis

Serum vs. time data for each dog after i.v., i.m. and oral administration were fitted to compartmental models by a nonlinear fitting programme (Easy Fit, Istituto Mario Negri, Milano) using a weighted least squares regression analysis. After i.v. injection the time course of serum concentrations was given as a sum of exponential terms as:

$$C_t = \sum_{i=1}^{n} C_i \exp\left(-\lambda_it\right)$$

where $C_t$ is the drug concentration in serum at time $t$, $C_i$ is the intercept and $\lambda_i$ is the exponential term. Each half-life ($t_{1/2}$) was calculated as $\ln2/\lambda_i$ and $C_0$ (the serum concentration at time 0) as the sum of intercepts. Following i.m. and oral administration the serum vs. time data for each dog were fitted to the general equation:

$$C_t = \left[\sum_{i=1}^{n} C_i \exp\left(-k_at\right)\right] - \sum_{i=1}^{n} C_i \exp\left(-k_{at}\right)$$

where $k_a$ is the rate constant for the absorption phase and $k_{el}$ is the rate constant for the elimination phase.

$C_{max}$ was the highest recorded concentration and $t_{max}$ was the time when $C_{max}$ was achieved.

Non compartmental analysis based on statistical moments was also performed (Riegelman & Collier, 1980). The area under serum concentration–time curve (AUC) and the area under the moment curve (AUMC) were calculated by the method of trapezoids and extrapolation to infinity was made as follows:

$$AUC_{last→\infty} = C_{last}/\lambda_i$$

$$AUMC_{last→\infty} = tC_{last}/\lambda_i + C_{last}/\lambda_i^2$$
where \( t_{\text{last}} \) is the last time with measurable concentration (\( C_{\text{last}} \)) and \( \lambda_e \) is the rate constant of the elimination phase. The system moment mean residence time (MRT), the body clearance (Cl), the volume of distribution by area (\( V_{\text{area}} \)) and the volume of distribution at steady state (\( V_{\text{dis}} \)) were determined from the equations:

\[
\text{MRT} = \frac{\text{AUMC}}{\text{AUC}}
\]

\[
\text{Cl} = \frac{\text{Dose}}{\text{AUC}}
\]

\[
V_{\text{area}} = \frac{\text{Dose}}{\text{AUC}\lambda_e}
\]

\[
V_{\text{dis}} = \text{Cl} \times \text{MRT}
\]

After the i.m. and oral administration the mean absorption time (MAT) value was calculated as the difference between the MRT obtained after each administration and the MRT after the i.v. administration.

Following the oral administration, the cephalixin concentration time course was fitted to a model incorporating Michaelis-Menten type absorption (Reigner et al., 1990). In this model the rate of absorption is measured as the rate of change of the drug quantity in the gut (\( \text{Ag} \)):

\[
\frac{d\text{Ag}}{dt} = -\frac{V_{\text{max}} \cdot C_a}{K_m + C_a}
\]

and the rate of change of drug quantity in the body (\( \text{A} \)) is:

\[
\frac{d\text{A}}{dt} = \frac{V_{\text{max}} \cdot C_a}{K_m + C_a} - \text{Cl} \cdot C
\]

where \( C_a \) and \( C \) are the drug concentrations in gut and in serum, respectively. \( V_{\text{max}} \) is the maximum rate of absorption, \( K_m \) is the value of \( C_a \) at which the absorption rate is one-half the maximum, and \( \text{Cl} \) is the body clearance.

To express the model in terms of concentrations the two equations were divided by the apparent volumes of distribution of the drug in the body (\( V \)) and letting \( C_u = V_a \cdot C_a/V, K' m = V_a \cdot K_m/V \) and \( V'_{\text{max}} = V_{\text{max}}/V \), where \( V_a \) is the gut volume. As no analytical solutions are available, the set of differential equations was solved using the Runge-Kutta fourth order numerical integration method, with adaptive stepsize to estimate the values of \( C \) as function of time (Wolfram, 1991).

The average serum concentrations of cephalixin at steady state after repeated i.m. and oral administrations were estimated according to the following equation:

\[
\frac{\tau}{\tau} \cdot \frac{\text{AUC}}{\tau}
\]

where \( \tau \) is the dosing interval (Gibaldi & Perrier, 1982).

**Statistical analysis**

To choose the best representation of the time course plots the weighted (1/observed y) residual sums of squares (WS), \( r^2 \) and AIC (Akaike information criterion-Yamaoka et al., 1978) were calculated.

Serum concentrations of cephalixin, intercepts and exponential terms were given as mean values (± SD). The other pharmacokinetic parameters, which were not normally distributed, were reported as medians and range. Half-lives were expressed as harmonic mean ± pseudo standard deviations (PSD) determined by the jack-knife technique (Lam et al., 1985).

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**Fig. 1.** Serum concentrations of cephalixin in five dogs treated intravenously (i.v.), intramuscularly (i.m.) and orally (p.o.) with 20 mg/kg.
RESULTS

The serum concentration–time course of cephalixin in the five dogs following i.v., i.m. and oral administration are presented in Fig. 1. Table 1 shows the pharmacokinetic parameters calculated after i.v., i.m. and oral dosing by compartmental and non-compartmental analysis. After i.v. injection the serum concentration–time data were best described by the two-compartment open model and those after i.m. administration by the one-compartment model with first order absorption. After the oral treatment the curve of cephalixin serum concentrations was not adequately described by the compartmental model assuming first order absorption (M1) as the $r^2$ value was > 0.93 in only one out of the five dogs. The fitting of the oral data to the Michaelis-Menten absorption model (MM) improved $r^2$ (> 0.93 for four animals). Figure 2 compares the curves generated for a representative dog (animal 2) using the M1 and the MM models.

A number of animals had concentrations substantially greater than the LOQ at the last sampling point: range values were 0.27–1.14 μg/mL, 0.30–2.4 mg/mL and 1.06–2.02 mg/mL after i.v., i.m. and oral administration, respectively. However, after i.v. and i.m. injection all the AUC_{last–infty} calculated were lower than 5% of the total AUC, and after oral administration only two out of the five AUC_{last–infty} were greater (6 and 7% of the total AUC), showing the adequacy of blood sample timing. The predicted steady state serum concentrations of cephalixin during a multiple dosage regimen were estimated as follows: 14.3 ± 0.5 μg/mL and 10.7 ± 0.4 μg/mL after i.m. administration at time intervals of 6 and 8 h, respectively; 13.0 ± 1.3 μg/mL and 9.8 ± 1.0 μg/mL after oral administration every 6 and 8 h, respectively.

DISCUSSION

The time course of cephalixin in serum of dogs treated by the i.v. route was best described by a two-compartment model; both distribution and elimination were fast, as already reported in the literature for other animal species (Carli et al., 1983; Soback et al., 1987; Garg et al., 1990, 1996).

In humans, the urinary recovery of cephalixin accounts for about 80% of the dose and the drug is cleared from the kidneys mainly by glomerular filtration combined with active tubular secretion (Barbhaiya, 1996). In the present study the mean value of Cl (2.5 mL/min/kg) is lower than the average glomerular filtration rate (GFR) reported for dogs (4.5 ± 2.0 mL/min/kg–Bagger, 1977: 2.8 ± 0.96 mL/min/kg–Finco, 1997), suggesting the involvement of tubular reabsorption. Granero et al. (1994) reported that in the rat cephalixin acted as a competitive inhibitor of cefadroxil tubular reabsorption.

The concentration–time data obtained after the oral administration of cephalixin to dogs fitted the MM absorption model. The Michaelis-Menten equation commonly describes capacity limited processes such as the carrier-mediated transport, and underlies the absorption mechanism of many β-lactam antibiotics including cephalixin. However, cephalixin concentrations were only measured in serum of dogs and absorption rate reflects more than just the movements of the drug across gastrointestinal membranes. Carrier-mediated transport in the gastrointestinal tract of the dog is the hypothesis suggested by the results of the present study and has been reported for humans and rats (Dantzig & Bergin, 1988, 1990; Reigner et al., 1990; Liu et al., 1997; Ruiz-Balanguer et al., 1997).

After i.m. administration the bioavailability of cephalixin was 63 ± 10%; similar values have been reported for the cephalixin sodium salt (73.9 ± 6.3%–Carli et al., 1983), monohydrate.
Fig. 2. Cephalixin serum concentrations in one dog treated orally (animal 2). The curves result from the fit of the models with first order (--) and Michaelis-Menten absorption (--). (67.5 ± 2.9%--Garg et al., 1990; 81.9 ± 4.2%--Garg et al., 1992) and lysine salt (89.6 ± 1.0%--Carli et al., 1983) in other species.

The oral bioavailability of cephalixin in dogs (57 ± 5%) calculated in the present experiment was greater than that calculated in calves (about 35%) by Soback et al. (1987), but lower compared to the urinary recovery (74.1–88.7%) of oral doses in humans reported by Barbhaiya (1996).

The serum Cmax found by Campbell & Rosin (1998) in fasting and non-fasting dogs, treated orally with 30 mg/kg of cephalixin every 12 h, were higher than in the present study and ranged from 23.8 to 46.2 μg/mL and from 21.9 to 84.6 μg/mL. Moreover, after the i.m. (oily suspension) and oral (tablets) administration of cephalixin sodium salt (10 mg/kg) to dogs the mean serum concentrations were 31.9 ± 1.08 μg/mL and 18.6 ± 1.70 μg/mL, respectively, with tmax at 1.8 ± 0.11 and 0.9 ± 0.11 h (Silsley et al., 1988).

In veterinary practice, several regimens are reported for the treatment of recurrent pyoderma with cephalixin: 15–20 mg/kg administered every 12 h, as well as 20–22 mg/kg every 8 h for at least 3 weeks are recommended (Guaguère & Picard, 1990; Frank & Kunkle, 1993; Carlotti & Leroy, 1995; Rosser, 1997).

The persistence of antibiotic concentrations in serum and tissues above the minimum inhibitory concentrations is a pharmacodynamic variable related to the clinical efficacy.

The predicted average serum concentrations of cephalixin in dogs after repeated i.m. and oral administration, suggested that the 20 mg/kg b.w. dosage should be administered every 6–8 h in order to attain serum concentrations greater than 5 μg/mL. Longer time intervals between dosing cause a drop in serum concentrations below the MIC values (≤ 2 μg/mL) reported for cephalixin-sensitive bacteria (Silsley & Brewster, 1988; Noble & Kent, 1992; Aucoin, 1993; Lloyd et al., 1996; Campbell & Rosin, 1998).

REFERENCES


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