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Pharmacokinetics of a cephalone (CQ-M-EPCA) in rats after oral, intraduodenal and intravenous administration

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Abstract

As part of the development of a new series of antibacterial agents derived from coupling a β-lactamic precursor with a fluoroquinolone and named cephalones, the pharmacokinetics of one derivate: CQ-M-EPCA in rats after intravenous, intragastric and intraduodenal routes, was carried out. After the IV injection of 20 mg/kg or 40 mg/kg of this cephalone, plasma concentrations at the time zero (C_{p0}) were 3.1 and 11.26 μg/ml, respectively. Plasma concentrations decreased rapidly to almost disappear in both instances. Forty-five minutes later, a surge in concentrations, in the 40 mg/kg group, with a maximal plasma concentration (C_{pmax}) of 2.97 μg/ml was observed. An elimination half-life ($T_{(1/2)el}$) of 2.36 ± 0.33 h. was calculated. The drug was undetected by the ninth hour. Intragastric administration of the drug resulted in C_{pmax} of 3.78 ± 0.26 μg/ml with a time to reach C_{pmax} (T_{max}) of 25 min and $T_{(1/2)el}$ = 3.22 h. Same variables after intraduodenal administration were C_{pmax} 4.71 μg/ml; T_{max} 1 h, and $T_{(1/2)el}$ 3.41 h. Outstandingly high bioavailabilities after intragastric and intraduodenal administration (169 and 246%, respectively), together with the shape of the concentration versus time profiles after IV administration suggest that the drug undergoes a complex redistribution phenomenon, while showing high tissue diffusion with an apparent volume of distribution of 3.33 l/kg.

Keywords: Pharmacokinetics; Cephalone; CQ-M-EPCA; Rat

1. Introduction

Mexican researchers have patented a series of broad-spectrum antibacterial agents named cephalones which are hybrids of cephalosporins (7-ACA) and fluoroquinolone precursors,[1] obtained by formation of a carboxamide bond between the 7-amino group of the β-lactam nucleus and the carboxyl moiety in position 3 of the fluoroquinolone structure (Johnson et al., 1996).

The so-called cephalone derivative here analyzed has been code-named CQ-M-EPCA (see Fig. 1). It is a broad-spectrum antibacterial agent which presents in vitro activity against both Gram-positive and Gram-negative bacteria, many of which are regarded as clinically important, such as *Staphylococcus* sp., *Escherichia coli*, *Salmonella* sp., among others (Méndez, 1996; Sumano et al., 1998a,b). Previous studies car-

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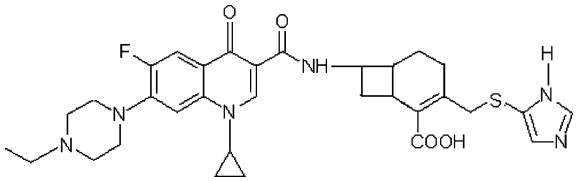


Fig. 1. Chemical structure of cephalone (CQ-M-EPCA).

ried out in dogs showed its high clinical efficacy and its unusually high apparent volume of distribution values in this species (Vd_c 2.5 l/kg; Vd_{ss} of 4.03 l/kg). This cephalone has an elimination half-life of 1 h or less; considering this and its in vitro biocide activity, a dose interval of 6–8 h has been proposed for bacterial infections within the spectrum of this antibacterial agent (Ovalle, 1996; Sumano et al., 1998a,b). Throughout its pharmaceutical development CQ-M-EPCA has shown, after many standard toxicological tests, lack or low toxicity (Data on Files; Laboratorio Aranda[2]). As a standard step in drug development, the pharmacokinetics of cephalone has been defined in dogs and cattle (Sumano et al., 1998a,b; Macouzet et al., 2000). This study defines the pharmacokinetics of CQ-M-EPCA in rats by the oral-intragastric, the intraduodenal, and the intravenous routes.

2. Material and methods

Forty healthy male Wistar rats (300–350 g, 3 months old) were obtained from the animal house at the "Dr. Joaquín Cravioto" Research-Tower of the National Institute of Pediatrics (INP) of the Mexican Ministry of Health (SSA). Four experimental groups of 10 rats each were randomly formed in accordance with the drug administration route: group 1a and 1b intravenous (IV) route with two doses, 20 and 40 mg/kg, respectively; group 2 intragastric (IG) route, and group 3 intraduodenal (ID) route.

This study was approved by the Institutional Committee of Research, Care and Use of Experimental Animals (CICUAL), according to de Mexican Official Regulation NOM-062-ZOO-1999.

In all animals the external jugular vein (right or left) was dissected by longitudinal incision in the middle third of the sternocleiomastoid muscle, a Silastic®[3] No. 20 catheter, previously purged with a solution containing 50 IU heparin/ml of physiological saline solution was placed, and connected to a 20 ml syringe. The catheter was then fixed with 6/0 silk suture-thread and the distal end exteriorized through a skin incision in the nape. Then the syringe was removed and the catheter connected to a heparinized Punzocat^{MR}[4] No. 20 that was left in place. The surgical wound was sutured with 3/0 silk. This route remained permeable along the trial. Then, the same procedure was repeated in the contralateral jugular vein of rats from groups 1a and 1b for the administration of CQ-M-EPCA.

Preliminary trials in rats had shown that the usual dose of 10 mg/kg were only capable of producing very low plasma concentrations in rats, insufficient for the analytical sensitivity of the HPLC method here implemented. Thus, for the purposes of this study, doses of 20 and 40 mg/kg of CQ-M-EPCA were chosen. The cephalone was administered diluted in physiological saline solution with a pH adjusted to 8.2 with KOH (final volume of approximately 0.4 ml). In the intravenous groups, the drug was injected through the left external jugular vein. A 0.5 ml loss in the catheter system was considered and total dose adjusted consequently. Blood sample collection for antibiotic determination was done using the contralateral catheter, previously purging and discarding the heparin–blood mixture left in the catheter. Then, a sample of 0.4 ml was collected. Blood samples were placed in heparinized vials and plasma separated from globular fraction by centrifugation at $3000 \times g$ at $-4\,^{\circ}C$ for 10 min.

The antibiotic was also administered to rats in group 2 through a gastric probe,[5] and to animals in group 3 by syringe with a 22-gauge needle, with previous longitudinal midline laparotomy to expose the duodenum. For this latter procedure and to catheterize jugular veins rats were previously injected with a 0.04 mg/kg dose of atropine, then anesthetized with mixture of 3 mg/kg of xylazine IM[6] and 35 mg/kg

[2] Drug files, Laboratorio Aranda, SA de CV. Querétaro, Qro. Mexico, 1999.

[3] Silastic®, Laboratory Tubing, Dow Corning Corporation Midland, Michigan, USA.

[4] Punzocat^{MR} Equipo Médico Vizarra, Mexico City.

[5] Gastric probe for premature children, sterile, disposable, D-732-E, diameter 1.9 mm, DESVAR de México.

[6] Rompun, Bayer of Mexico.

of ketamine[7] administered intraperitoneally. In these rats 0.4 ml blood samples were also collected, one before the administration of CQ-M-EPCA and at 2, 5, 10 and 45 min and 1, 3, 6 and 9 h after drug administration. Difference between determined time for sampling and actual time achieved were never longer than 1 min. In all rats 0.5 ml of saline solution were injected IV after obtaining each blood sample.

Due to some structural similarities, the technique described by Elkhaili et al. (1997) for the determination of ceftriaxone in biological samples was adapted to determine the plasma concentrations of this cephalone. The equipment used was a Hewlett-Packard 1050 high performance liquid chromatograph (HPLC), detector with deuterium lamp of 991-diode arrangement and 512 wavelength diodes, quaternary pump and automatic injector, HP3396 series II integrator, and a Waters 10 μm μBondapakC18 reverse-phase column (125 Å, 3.9 mm × 150 mm).[8] The analytical method used in this study was validated and showed the following results: inter- and intra-essay errors of 3%, a recovery ratio of 96–102%, accuracy of 99.7%, and quantification and detection limits of 0.01 and 0.008 μg/ml, respectively. Standards of 7-aminocephalosporanic acid and enrofloxacin were run to identify possible rupture of the carboxamide linkage of these two precursors of the cephalone in study. However, no such fractions were identified in actual experimental samples.

Compartmental and non-compartmental analysis using the PKAnalyst software[9] and WinNonlin[10], respectively, were applied for the pharmacokinetic analysis. Although no ideal model was found for the patterns of this cephalone after IV administration of 40 mg/kg, PKAnalyst results were adjusted to Model 7 according to the best correlation value possible, higher than 98% in all cases. Model 3 was used for groups 2 and 3 and correlations were also higher than 98%. General formulae are:

- Model 7:

$$\text{Concentration (time)} = A\,e^{-\alpha\times\text{Time}} + B\,e^{-\beta\times\text{Time}}$$

- Model 3:

$$\text{Concentration (time)} = \frac{\text{Dose}\times K_{AB}}{\text{Volume}\times K_{AB} - K_{elim}} \times e^{-K_{elim}\times\text{Time}} - e^{-K_{AB}\times\text{Time}}$$

Pharmacokinetic variables determined for groups 1a and 1b were: area under the time versus drug concentration curve (AUC), elimination constant (K_{elim}), the hybrid rate constant β, area under the moment curve (AUMC), residence time (t_{res}), plasma concentration at the time zero (C_{p0}), apparent volume of distribution of AUC (Vd_{AUC}), apparent volume distribution at steady state (Vd_{ss}) and clearance from blood (Cl_B).

Pharmacokinetic variables calculated for groups 2 and 3 were:, elimination constant (K_{elim}), absorption half-life ($t_{(1/2)ab}$), maximum plasma concentration (C_{pmax}), time to reach C_{pmax} (t_{max}), area under the curve (AUC), area under the moment curve (AUMC), residence time (t_{res}), clearance from blood (Cl_B) and bioavailability (F).

Data are expressed as the mean ± 1 standard deviation. By means of SPS software[11] Mann–Whitney U-test was used to compare T_{max} and C_{pmax}, and due to lack of variance homogeneity and normality Kruskall–Wallis test was used to compare $T_{(1/2)el}$ and Cl_B. For AUC, AUCM, t_{res} and Vd_{AUC} analysis of variance was utilized.

3. Results

Pharmacokinetic values derived from inserting raw data into PKAnalyst or WinNonlin were very similar in spite of the fact that the former software used a compartmental model and the latter a non-compartmental one (see Table 1). Cephalone administered by IV route presented a C_{p0} value of 8.07 and 3.1 μg/ml after administering 40 and 20 mg/kg, respectively. Plasma concentrations decreased rapidly in both cases and in few minutes the drug reached a trough with almost undetectable levels. Forty-five minutes later, a surge in concentrations of this cephalone peaked to reach a C_{pmax} value of 2.97 or 0.7 μg/ml, followed by an

[7] Ketavet, REVETMEX of Mexico.
[8] The column contained dimethyl-octadecylsily bonded amorphous silica, water.
[9] PKAnalyst. Micromath, Salt Lake City, Utah, 1995.
[10] WinNonlin Profesional 3.2 SCI Scientific Software.
[11] SPS software.

Table 1
Mean ± 1 S.D. of pharmacokinetics variables of CQ-M-EPCA in rats after intravenous, oral and intraduodenal administration

Variables	Group							
	PKAnalyst				WinNonlin			
	IV (20 mg/kg)	IV (40 mg/kg)	IG (40 mg/kg)	ID (40 mg/kg)	IV (20 mg/kg)	IV (40 mg/kg)	IG (40 mg/kg)	ID (40 mg/kg)
$T_{(1/2)el}$ (h)[a]	3.99±0.4	2.36 ± a 0.33	3.22 ± b 0.62	3.41 ± b 0.36	2.72 ± 0.40	2.96±0.43 a	6.48 ± 0.77 b	3.84±0.90 c
β (h)	0.173 ± 0.01	0.30 ± 0.04	–	–	0.25 ± 0.002	0.24±0.003a	0.11 ± 0.001 b	0.19±0.000 c1
AUC[b] (μg/h ml)	5.77±1.25	11.64 ± 2.02 a	19.10 ± 3.19 b	28.26 ± 4.24 b	6.86 ± 1.27	12.28 ± 1.72 a	25.96 ± 2.29 b	26.71±4.12 b
AUCM[b] (μg/h ml)	31.76±8.04	38.81 ± 11.81 a	82.32 ± 38.66 b	150.70 ± 34.95 c	19.78 ± 6.5	47.08 ± 7.74 a	228.79 ± 39.59 b	155.82±49.89 c
Residence time (h)[b]	5.50±0.95	3.27 ± 0.48 a	4.76 ± 0.89 b	5.27 ± 0.57 b	2.88 ± 0.26	3.84 ± 0.37 a	8.78 ± 1 b	5.75±1.07 b
C_{p0} (μg/ml)	8.01 ± 0.2	11.26 ± 0.19	–	–	8.31 ± 0.2	11.25 ± 0.63	–	–
Vd_{AUC}[b] (l/kg)	3.1±0.21	3.33 ± 0.35 b	3.08±0.44 b	3.03±0.44 a	7.78 ± 0.96	4.64 ± 0.93 b	4.77 ± 0.78 b	3.76±0.78 a
Vd_{ss} (l/kg)	5.44±0.95	3.23 ± 0.33	–	–	5.71 ± 0.95	3.98 ± 0.02	–	–
Cl_B (ml/min/kg)[a]	0.99±0.16	0.99 ± 0.18 a	0.24 ± 0.02 b	0.20 ± 0.02 b	1.98 ± 0.22	1.08 ± 0.15 a	0.50 ± 0.004 b	0.50±0.007 b
T_{max} (h)[c]	–	–	0.41 ± 0.03 a	0.98 ± 0.32 b	–	–	0.16 ± 00.2a	0.75±0.003b
C_{pmax} (μg/ml)[c]	–	–	3.78 ± 0.26 a	4.71 ± 0.53 b	–	–	5.04 ± 0.028a	6.46±0.44b
F[d] (%)		–	–	–			96.6	167

Different letters indicate statistically significant mark pharmacokinetic differences, among groups, $P < 0.05$.

[a] Due to lack of variance homogeneity and normality, Kruskall–Wallis test was used as indicated.

[b] Due to lack of variance homogeneity and normality, Tukey's test was used as indicated.

[c] Due to lack of variance homogeneity and normality Mann–Whitney *U*-test was used as indicated.

[d] Calculated considering $T_{(1/2)el}$, as follows: $F = (\text{AUC} \times \text{IG} \times T_{(1/2)el} \times \text{IV/AUC} \times \text{IV} \times T_{(1/2)el} \times \text{IG}) \times 100$.

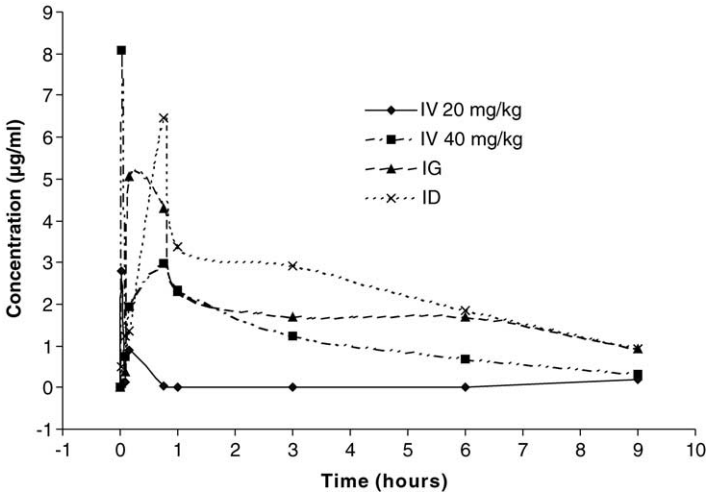


Fig. 2. Mean of plasma concentration values in time of CQ-M-EPCA after intravenous, intragastric and intraduodenal administration of a single dose of 40 mg/kg of the drug.

elimination phase with a $t_{(1/2)el}$ of 2.36 ± 0.33 or 3.99 ± 0.2 h for the 40 and 20 mg/kg dose, respectively. The drug was undetected by the ninth hour in the group that received the 40 mg/kg dose. However in the group dosed with 20 mg/kg the drug was undetected and reappeared in various occasions as shown in Fig. 2.

When CQ-M-EPCA was administered by intragastric route (group 2), C_{pmax} was 3.78 ± 0.26 μg/ml with a T_{max} of approximately 25 min after administration. Elimination was slower than the one shown in the IV groups and the drug was still detected by the ninth hour ($t_{(1/2)el}$ = 3.22 h). In the intraduodenal group, C_{pmax} was 4.71 μg/ml with a T_{max} of almost 1 h, and presented an elimination phase similar to that of the intragastric group ($t_{(1/2)el}$ 3.41 h). Comparison of AUC values after intragastric and intraduodenal administration revealed no statistically significant difference when non-compartmental analysis was considered. However a statistically significant difference was seen after processing data with PKAnalyst ($P < 0.05$) (AUC intragastric = 19.10 ± 3.19 μg/h ml versus AUC intraduodenal = 28.26 ± 4.24 μg/h ml). Also with this latter software program comparisons between the intragastric and intraduodenal groups revealed that $t_{(1/2)ab}$, t_{max}, C_{pmax}, Cl_B were higher for the intraduodenal route ($P < 0.05$). The obtained F was 169% for the intragastric group and 246% for the intraduodenal route.

Fig. 2 shows the mean plasma concentrations of CQ-M-EPCA in rats after two IV doses, intragastric and intraduodenal administration. Table 1 shows pharmacokinetic variables obtained and significance derived from statistical analysis.

4. Discussion

The unique shape of the plasma concentrations of CQ-M-EPCA versus time curve obtained after the IV administration of the drug rendered inapplicable most readily available compartmental pharmacokinetic models. Yet, for most pharmacokinetic variables the non-compartmental values obtained through WinNonlin software resulted in data statistically undistinguishable from corresponding one derived from the compartmental PKAnalyst software program, using model 7. This is not surprising because in either instance, data is evaluated by exponential curve fitting whether model-derived or independent. However, various differences were detected between these two programs when data from the intragastric and intraduodenal routes were compared.

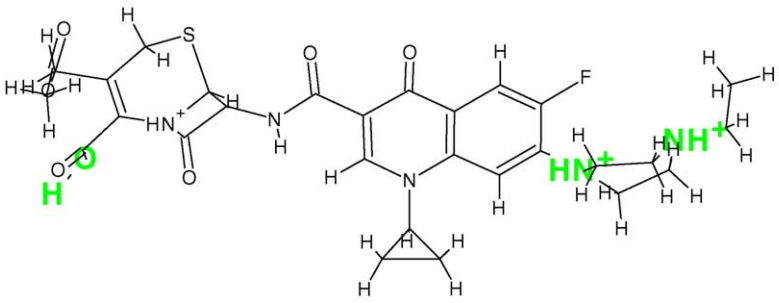
The intravenous administration of CQ-M-EPCA (20 or 40 mg/kg) shows an initial phase of a rapid plasma-concentration decline and is almost undetected in only few minutes. Then, a second phase develops in the group dosed with 40 mg/kg of CQ-M-EPCA

showing a steady increase in plasma concentrations to reach a C_{pmax} of 2.97 μg/ml with a T_{max} of 45 min. A relatively slow elimination phase is observed subsequently ($\beta = 0.29$, $k_{(1/2)el} = 0.715$ h and $t_{(1/2)el} = 2.36$ h) (see Fig. 2). In the group injected IV with a dose of 20 mg/kg, plasma concentrations approach zero μg/ml 1 h post-administration. The reappearance of the drug noted in various occasions must be due to select rats having measurable concentrations well beyond the 3 h sampling time. An attempt to provide mechanistic interpretations of this unique profile is impeded by the limited data provided in this study. Unusual drug distribution patterns can sometimes reflect extravascular administration of a fraction of the dose or in vivo generation of a metabolite. The use of a surgically placed catheter in the jugular vein disregards the former consideration. However, metabolic modification of CQ-M-EPCA is an option that deserves further studies, in spite of the fact that no traces of either a free-fluoroquinolone fraction or 7-ACA were identified in any experimental sample. Thus, it is tempting to speculate that the plasma profile here described does not appear to be an artifact derived from biotransformation of this cephalone. The fluoroquinolonic precursor is known to be only partly biotransformed into ciprofloxacin in mammals. This will give place to an ethyl-free cephalone metabolite that could be feasibly detected during the HPLC analysis. No such compound was recognized during the analytical phase of this study. The other precursor: 7-ACA, does not undergoes any metabolism in mammals (Mandell and Petri, 1996). Nevertheless, mass-spectrometric analysis will be needed to confirm the proposed absence of biotransformation of this cephalone. Although these results lack experimental support to advance a mechanistic statement for the peculiar plasma profile of CQ-M-EPCA, available data allows the speculation that pharmacokinetic behavior of this drug could be an indication of a redistribution phenomenon, and it appears to be consistent with previous descriptions of the pharmacokinetics of this drug in dogs (Ovalle, 1996; Sumano et al., 1998a,b), cows (Macouzet et al., 2000) and pigs (Vargas, 2001).

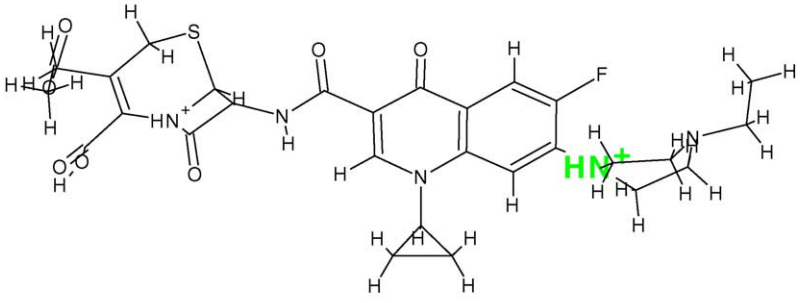
Using WinNonlin, C_{pmax} was clearly higher after the intraduodenal route ($P < 0.05$). Conceding that intraduodenal route offers higher plasma concentrations of this cephalone than the intragastric administration, it may be possible to correlate this with higher ionization of the drug at gastric pH. Through a computer assisted modeling program (ACD/ChemSketch[12]), it has been calculated that CQ-M-EPCA would be highly protonized at pH values above 9.96 and below 3.86, while behaving as zwitterion at pH between 7 and 8.49. The molecule is almost always charged, but more lipid-soluble at standard duodenal pHs as shown in Fig. 3. Differences in both pharmacokinetics variables and consequently in plasma profiles should be taken into account when designing a pharmaceutical preparation for oral use in monogastric species. For most drugs, whether weak acids or weak bases, absorption occurs primarily in the intestine, mainly in the duodenum, even in the case of drugs less dissociated at gastric pH (Insel, 1996). Considering that higher C_{pmax} values have been regarded as key for β-lactam and fluoroquinolone antibacterials (Mandell and Petri, 1996), a similar profile would also appear desirable for this cephalone.

To avoid indiscriminate interpretation based on output from two software programs F values were obtained from WinNonlin software output, based on its model-independent approach. Also, $T_{(1/2)el}$ for the IV and the IG and ID routes were considered, as suggested by Riviere (1999). Then the ID route had the greatest bioavailability (169% for the ID route versus 96.6% for the IG route). This distinctive feature of CQ-M-EPCA may be ultimately explained in terms of either an artifact or a subtle mechanistic effect. Hence, this latter data should be taken cautiously until a better understanding of the peculiar plasma concentration profile of CQ-M-EPCA after IV administration, is acquired, and because the lack of statistically significant differences after processing data with WinNonlin. However, if indeed a high F is obtained after intraduodenal and intragastric administration of CQ-M-EPCA and considering the rapid disappearance from plasma, the apparent redistribution phenomenon, and the high apparent volume of distribution values, overall data comply well with outstanding clinical efficacy so far reported for this cephalone for the treatment of mastitis in cows (Sumano et al., 1996); respiratory bacterial infections in dogs (Sumano et al., 1998a,b), and outbreaks of respiratory bacterial diseases in broilers (Sumano et al., 1988).

[12] Advanced Chemistry Development Inc., 3.00 133 Richmond St. West Suite 605, Toronto, Ont., Canada M5H 2L3, 1997.



Negatively charged CQ-M-EPCA below 3.61



CQ-M-EPCA as zwiterion in pH below 8.49

Fig. 3. Chemical structure of CQ-M-EPCA at pH values that would occur in the stomach and in the duodenum.

Chemically, CQ-M-EPCA carboxamide linkage resembles third generation cephalosporins, and it has been suggested that cephalones can be regarded as β-lactam derivates. If this molecule is regarded as such, large values of the apparent volume of distribution variables ($Vd_{AUC} = 3.33$ l/kg; $Vd_{ss} = 3.23$ l/kg) will not comply with the expected behavior for a β-lactam derivate (Mandell and Petri, 1996), giving this cephalone a unique feature among β-lactams. Additionally, it is important to notice that the outstanding oral bioavailability shown by this cephalone may prove to be advantageous for easy administration, while maintaining both high tissue penetration and bioavailability, ideal features for the treatment of bacterial infections in outpatients.

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