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Original Research Article

Disposition kinetics of ceftriaxone and determination of its therapeutic dose in dogs

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Abstract

Purpose: *To evaluate the disposition kinetics of ceftriaxone (CFZ) in dogs with a view to determining its therapeutic dose and dosing frequency.*
Methods: *Twelve (12) Basenji dogs (n = 4), divided into 3 groups (A, B and C), were used for the study. Ceftriaxone was administered intramuscularly at doses of 12.5, 25, and 50 mg/kg once to groups A, B and C respectively. Plasma CFZ concentration was determined by agar well diffusion assay at 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, and 24 h post-treatment, and the pharmacokinetic parameters were determined.*
Results: *Intramuscular injection of CFZ to dogs resulted in rapid absorption, distribution and elimination ($p < 0.05$). The elimination half-life was short and did not change significantly with increase in dose. Serum concentration of CFZ changed significantly ($p < 0.05$) with increase in dose of CFZ. The maximum serum concentration (C_{max}, 15.00 ± 1.18, 141.37 ± 15.87 and 259 ± 5.21 µg/mL) for groups A, B and C respectively were significantly ($p < 0.05$) different. The steady state CFZ concentrations; 0.94, 8.81 and 16.19 µg/mL for groups A, B and C, respectively, were significantly ($p < 0.05$) different. However, there was no significant difference in the time to reach steady state concentrations (T_{max}, 00±0.021, 4.00±0.10 and 4.30±0.12 for groups A, B and C respectively). The therapeutic dose of CFZ was therefore determined to be 25 – 50 mg/kg every 4 h.*
Conclusion: *Ceftriaxone undergoes rapid elimination in dogs with a short elimination half-life, thus making it an inconvenient prescription for out-patients in veterinary clinics.*

Keywords: *Ceftriaxone, Pharmacokinetic profile, Dogs, Therapeutic dose, Veterinary clinic*

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INTRODUCTION

Ceftriaxone (CFZ) is a third-generation semi-synthetic cephalosporin introduced in the 1980's [1]. It comes as a white to pale yellow powder. [2]. It is packaged as CFZ sodium at different concentrations and labeled a prescription medication with broad spectrum activity [3]. Its established long half-life in humans means that it can be effective even after a single administration [4], making its utilization in human clinical practice very advantageous [5]. Ceftriaxone has high tissue penetrability and crosses the blood brain barrier as well as blood

placenta barrier and found in low concentrations in breast milk [6].

The pharmacokinetics of CFZ is non-linear due to plasma protein binding. However, extensive pharmacokinetic studies have not been carried out with CFZ using animal models. Ismail, [7] reported a peak plasma concentration (C_{max}) of 23.6µg/mL following intramuscular injection of 20 mg/kg CFZ in goats, whereas, in sheep, Goudah *et al*, [8] reported a C_{max} of 23.16±2.94 µg/mL after intramuscular administration of 10 mg/kg CFZ. Furthermore, Maradiya *et al*, [9] reported a C_{max} of 15.34±2.39 µg/mL at 0.25 h after intramuscular injection of 10 mg/kg using neonatal calves. Ceftriaxone must be administered by parenteral route so as to achieve a higher serum level [10]. Extensive use of CFZ in veterinary clinical practice is limited by its status as a drug of last resort. However, in recent times this drug has been extensively used by pet clinicians in Nigeria and other parts of the world as an off-label drug. The doses used are those extrapolated from humans and ranges from 15 – 50 mg/kg.

Nevertheless, drug doses cannot easily be extrapolated between different animal species because of differences in drug absorption, distribution, metabolism and excretion between species. This underscores the need for pharmacokinetic studies of each drug in each species to determine the effective dose and dosing frequency. This will help reduce the incidences of overdosing, under-dosing, toxicity and bacterial resistance due to under-dosing. The pharmacokinetics of ceftriaxone has not been extensively studied in dogs. More so, therapeutic dose of ceftriaxone has not been established in dogs.

The objective of this study was to use the agar well diffusion assay to establish the therapeutic dose of CFZ in dogs by measuring the serum concentrations of CFZ over time and estimation of the elimination half-life ($t_{1/2}$).

EXPERIMENTAL

Animals

A total of 12 female Basenji dogs were used for the study. On arrival, they were all vaccinated against rabies and dewormed. They were housed in standard dog kennel and fed on dry dog feed. Clean drinking water was provided ad *libitum.* The dogs were acclimatized for 2weeks before the experiment commenced in other to allow them adapt to the environment [11].

Ethics statement

The animal experimental protocol was approved (approval no. UNFVM/11/17/009) by Faculty of Veterinary Medicine University of Nigeria Nsukka experimental animal ethics committee and in compliance with Principles of Laboratory Animal Care" (NIH publication no. 85-23, revised 1985) [12].

Experimental drugs

Rocephin® (Roche Ltd Switzerland) was obtained from a registered pharmaceutical premise in Nigeria. The active ingredient is ceftriaxone sodium (1g per vial). Each vial comes with lignocaine hydrochloride (3.5 ml) as solvent for reconstituting the drug for intramuscular administration.

Ceftriaxone sodium USP (350mg pure ceftriaxone powder) Rockville MD USA, for preparation of the ceftriaxone standard.

Experimental design

The Twelve Basenji dogs were randomly assigned to 3 groups (A, B and C, n = 4). Dogs in each group were treated with 12.5, 25 and 50 mg/kg CFZ, respectively, once at the thigh muscle. These doses were based on the extrapolated human dose used in pet clinics in Enugu state Nigeria.

Blood sample collection

Prior to drug administration, 3ml control blood sample was collected from the cephalic vein with the aid of a syringe and needle. Following drug administration, blood samples (3 mL) was collected from the cephalic vein at 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, and 24 h. The blood samples were centrifuged at 2000 rpm for 15 min after clotting to obtain the serum. The serum was preserved at -20ºC pending analysis.

Media and media preparation

Muller Hinton agar was used for this study. The agar was prepared according to manufacturer's direction in 8.5cm diameter petri dish to a thickness of about 4mm of agar using 20mL of the agar and sterilized in an autoclave at 121ºC for 15min. The media was incubated at 37ºC for 24 h to test for sterility before the organism was inoculated.

The organism (pure culture of *Bacillus sp*.) used in this study was collected from the stock culture

from the Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka.

Preparation of ceftriaxone standard

Serial dilution; 100, 50, 25, 12.5, 6.25, 3.13, 1.56, 0.78 and 0.39µg/mL of standard sample of CFZ were made in sterile test tubes using sterile injection water. An agar well diffusion assay [13] was used to determine the minimum inhibitory concentration (MIC) of CFZ for the *Bacillus sp.*

Agar well diffusion assay

This was done according to the method of Sen and Batra [13]. The *Bacillus* organism was diluted in normal saline and evenly inoculated on the agar plate using a sterile swab stick and allowed on the bench for 10 min. Four (4) wells were bored into the inoculated plate using a sterile cork borer. Fifty (50) microliter of different serum containing CFZ were dispensed into the wells, labeled properly and allowed on the bench for 30 min to diffuse into the agar. The plates were then incubated at 37ºC for 24 h. The inhibitory zone diameter of each serum containing CFZ was determined by measuring with a meter rule and expressed in millimeter (mm). All assays were carried out in triplicate. Then by comparing the obtained result with that of the standard, the concentrations of CFZ in the serum collected at the stated time intervals were determined.

Determination of pharmacokinetic parameters

The following parameters were calculated using standard methods [14].

1. Maximum drug concentration in serum (C_{max})
2. Time of maximum drug concentration in serum (T_{max})
3. Area under curve (AUC) = Dose/clearance
4. Elimination rate constant (Ke)

   Ke = -(Ln C1- Ln C2)/t_1-t_2 (Ln: natural logarithm; C1 t_1: first concentration/time pair; C_2 t_2: second concentration/time pair)

5. Elimination half- life ($T_{1/2}$Ke)

   $T_{1/2}$Ke = Ln2/Ke = 0.693/Ke (Ln2: natural log of 2; Ke: elimination rate constant)

6. Steady state serum concentration (SS_c) = 5 half-lives of the peak serum concentration
7. Time to attain steady state (T_{ss})

   T_{ss} =$T_{1/2}$Ke x 5($T_{1/2}$Ke; elimination half-life)

8. Total clearance (CL)

   CL = Dose/AUC

9. Volume of distribution (Vd)

   Vd = CL/ke (CL: total clearance; Ke: elimination rate constant)

10. Ka: Absorption rate constant (Ka): obtained by the method of residuals [14].
11. Absorption half-life ($T_{1/2}$ka)

    Ln2/Ka = 0.693/Ka.

Statistical analysis

Data obtained were analyzed using one-way analysis of variance using SPSS version 15 for Windows. Variant means were separated using least significant difference and significance was accepted *at* $p < 0.05$. Serum concentrations and pharmacokinetics parameters are presented as mean ± standard error of mean (SEM).

RESULTS

Serum drug concentrations

Significantly higher serum concentration of CFZ *(*$p < 0.05$) was obtained from dogs in group C compared to those in groups A and B. However, the concentration of CFZ obtained from the serum of dogs in group B was significantly *(*$p < 0.05$) higher than those in group A. The semi log plot of the concentration-time curve of the different doses of CFZ showed a double compartment model that yielded a biphasic line (Figure 1).

Pharmacokinetic parameters of CFZ in dogs

The pharmacokinetic parameters following intramuscular administration of the different doses of CFZ in dogs are given in Table 1.

Maximum drug concentration (C_{max}) and Time to attain maximum drug concentration (T_{max})

The C_{max} of group C was significantly ($p < 0.05$) higher than those of groups A and B, but the

C_{max} of group B was significantly ($p < 0.05$) higher than that obtained in group A. The T_{max} did not vary significantly in the 3 doses used.

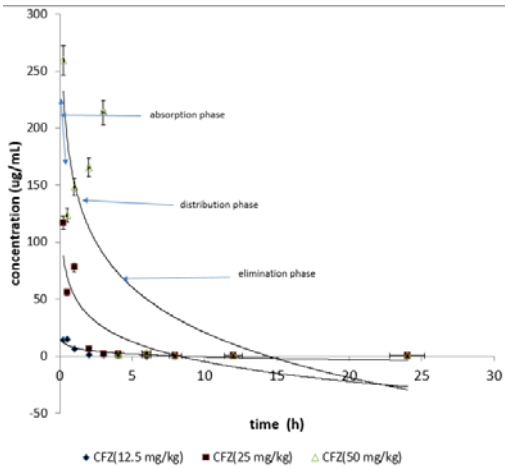

Figure 1: Semi-Logarithmic plot of serum ceftriaxone concentration (µg/ml) ± SEM versus time in dogs after intramuscular administration of graded doses

Absorption half-life and rate constant ($T_{1/2}$Ka and Ka)

The mean absorption half-life of CFZ in the dogs in group A was significantly higher than those observed in the dogs in groups B and C. But the absorption rate constant obtained from groups B and C were significantly higher than that of group A.

Elimination half-life and rate constant ($T_{1/2}$Ke and Ke)

There was no significant variation in the $T_{1/2}$ke between the groups. However, the ke of group A was significantly ($p < 0.05$) greater than that of group C.

Steady state CFZ concentration (SS_c) and time to steady state (T_{ss})

The steady state CFZ concentration in group A was significantly ($p < 0.05$) lower than those of groups B and C, while that of group B was significantly ($p < 0.05$) lower than that of group C. There was no significant difference in the time to attain steady state in the CFZ doses used.

Total clearance (CL)

The mean CL of group A (12.5mg/kg) was significantly ($p < 0.05$) greater than those of groups B and C. There was no significant difference in the mean CL of group B (25mg/kg) and group C (50mg/kg).

Area under the concentration-time curve (AUC)

The AUC of group A was significantly ($p < 0.05$) lower than those of groups B and C, while the AUC of group C was significantly ($p < 0.05$) higher than both groups A and B.

Volume of distribution (Vd)

The volume of distribution (Vd) for group A (12.5 mg/kg) was significantly ($p < 0.05$) higher than those of the other two groups, but there was no significant difference between the volume of distribution in groups B and C.

DISCUSSION

Following intramuscular administration of CFZ in dogs, there was rapid absorption, dose-dependent serum levels and rapid elimination. The peak serum concentration of CFZ was attained in approximately 0.25 h at all the administered doses,

Table 1: Pharmacokinetic parameters of ceftriaxone in dogs after intramuscular administration

Parameter	CFZ (12.5 mg/kg)	CFZ (25 mg/kg)	CFZ (50 mg/kg)
Cmax (µg/mL)	15.00 ± 1.18^a	141.37 ± 15.87^b	259.33 ± 5.21^c
Tmax (h)	0.42 ± 0.08	0.42 ± 0.08	0.33 ± 0.08
Ka (h^{-1})	1.26 ± 0.18^a	4.24 ± 0.55^b	6.75 ± 0.56^c
Ke (h^{-1})	1.05 ± 0.09^a	0.99 ± 0.04	0.56 ± 0.20^b
$T_{1/2}$ka (h)	0.57 ± 0.08^a	0.17 ± 0.03^b	0.11 ± 0.01^b
$T_{1/2}$ke (h)	0.59 ± 0.09	0.80 ± 0.10	0.85 ± 0.08
SS_c (µg/mL)	0.94 ± 0.01^a	8.81± 0.13^b	16.19± 0.11^c
T_{ss}	3.00 ± 0.21	4.00 ± 0.10	4.30 ± 0.12
Cl (L/h)	3.15 ± 0.48^a	0.58 ± 0.11^b	0.26 ± 0.05^b
AUC (h.µg/mL)	4.19 ± 0.74^a	37.24 ± 1.53^b	152.40 ± 13.83^c
Vd (L/kg)	2.12 ± 0.14^a	0.79 ± 0.10^b	0.58 ± 0.02^b

Mean ± SEM^{abc}; significantly different ($p < 0.05$). C_{max}: maximum drug concentration in serum; T_{max}: time of maximum drug concentration in serum; Ka: absorption rate constant; Ke: elimination rate constant; $T_{1/2}$ka: absorption half-life; $T_{1/2}$Ke: elimination half-life; SS_c: steady state concentration, T_{ss}: time to attain steady state, CL: Total clearance; AUC: area under curve; Vd: volume of distribution

thus indicating a fast absorption. The semi-log plot of the serum concentration versus time of the three doses of CFZ showed a very fast absorption, distribution and elimination phases and best described by a double compartment model with biphasic elimination.

The dose-dependent serum CFZ concentrations is attributed to the fact that increase in drug dose will lead to increase in the serum concentration of CFZ, however, this did not translate to dose-dependent T_{max} as there was no significant difference in the T_{max} of the three CFZ doses used. Absorption rate constants obtained in the current study was equally dose-dependent and this could be a reflection of the drug doses. There was significantly ($p < 0.05$) higher elimination rate constant in group A dogs, but there was no significant difference in the elimination half-life of the drug in the three groups. The implication of this finding is that the elimination half-life of CFZ is not significantly affected by the administered dose.

It has been established that 4 – 5 elimination half-lives are required to attain steady state drug concentration [15]. In the current study, at steady state, 93.73, 92.47 and 93.75% of the administered doses of CFZ in groups A, B and C respectively, had been eliminated. Therefore, at this point the dose of the drug should be repeated. Hence, based on the calculated $T_{1/2}$Ke for the three CFZ doses, the time to attain steady state were 3.0, 4.0 and 4.3 h for groups A, B and C respectively. According to Hoffman la Roche [2], the MIC_{50} of most susceptible bacteria including *Bacteroides spp* and *Staphylococcus spp* range from 2 – 4 µg/mL while the MIC_{90} range from 4 – 16 µg/mL. Therefore, based on the determined steady state concentrations for the three CFZ doses, the therapeutic dose of CFZ in dogs for susceptible organisms should range from 25 – 50 mg/kg approximately every 4 h. This is a reflection of very short $T_{1/2}$Ke of CFZ in dogs. The higher CL of CFZ in dogs in group A is a reflection of the higher rate of elimination of the drug in that group. The area under the concentration-time curve (AUC) is the integral of the drug concentration-time curve [14]. The AUC reflects the actual body exposure to the drug after administration of a dose of the drug [14]. It is dependent on the rate of elimination and dose administered and inversely proportional to the drug clearance [14].

In this study the AUC was dose-dependent. This suggests that the body exposure to the drug is lower in group A compared to groups B and C. Clinically this implies that 12.5mg/kg of CFZ will exhibit a reduced toxicity and efficacy when compared to 25 and 50mg/kg. Also, the dose-dependent AUC observed implies that the potential for toxicity of CFZ increases as the dose increases.

The low Vd observed in all the administered doses of CFZ in this study is a reflection of the high-water solubility of the drug. However, the recorded Vd of CFZ is an indication that the drug was distributed into the interstitial fluid compartment in dogs.

CONCLUSION

CFZ undergoes rapid absorption, distribution and elimination in dogs. The very short $T_{1/2}$Ke of CFZ in dogs requires that it should be administered at 4 h interval, thereby making it an inconvenient prescription for out-patient dogs. Therefore, it is here recommended that CFZ use in dogs be restricted to cases where bacterial culture and antibiotic susceptibility test has indicated its use and patients should be admitted as in-patients for strict adherence to prescription.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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