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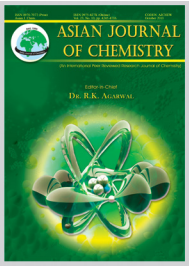

Biokinetics Study of 1000-mg Cefaclor Tablet in Stray Dogs

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Pharmacokinetic of cefaclor (1000 mg tablet) were assessed after single oral administration in stray dogs. Serial blood samples were collected in heparinized tubes at pre-scheduled time intervals and analyzed by microbiological assay against gram negative and gram positive bacterial strains by disc diffusion method. The concentration data obtained from bioassay were subjected for biokinetics. Peaked concentration of cefaclor was reached after 2 h in dog blood plasma. Average plasma concentration remained above minimum inhibitory concentration (MIC) value from 0.5 to 10.0 h. The values of maximum plasma concentration (C_{max}) were found to be 18, 19.80, 21.10 and 20.17 mg/L and time of peak (T_{max}) was found to 2.80, 1.35, 1.99 and 1.95 h against *Escherichia coli, Staphalococuss aureus, Pasturella multocida* and *Basillus subtilis*, respectively. Absorption half life ($t_{1/2}$) was ranged from 1.30-1.96 h against four strains. The difference in biokinetics parameter was found non-significant ($p < 0.05$) in case of gender. It is concluded that the oral administration of 1000 mg cefaclor as tablet twice a day may maintain significant concentration in dogs and effective for the treatment of infections in dogs due to above mentioned *microbes*.

Key Words: Biokinetics, Cefaclor, Stray dogs, NCCLS, Disc diffusion, Bioassay.

INTRODUCTION

At present hundreds of antibiotics are available, active against a number of microbial diseases, but few have been proven safe and effective and also the safer one may become inactive with the passage of time due to resistance mechanism in microbes. The reason is that the microbes have the ability to develop the resistance against the antibiotic and their pharmacokinetics and dynamics studies are necessary in parallel manner[1-7]. Antibiotics called the miracle drugs, because before their discovery the medicine era was very worsen. For thousands of years, people have used many types of plants, fungi, lichen and other chemicals to try healing infections without knowing their actual working. Medicine was more of an experimental practice. Antibiotics are one of the most frequently prescribed medications at present time and used to cure many diseases such as for killing or stopping the growth of bacteria such as bacterial meningitis, neurosyphilis, endocarditis, burn wounds, skin infections, respiratory and urinary tract infections, pneumonia, anthrax, Lyme disease, bronchitis, diarrhea diseases, abdominal infections, severe acne, gastrointestinal tract infections, blood poisoning, TB, infections and many more[8,9].

Cefaclor (CCL), 7-[(2-amino-2-phenyl-acetyl)amino]-3-chloro-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, is a second generation, semisynthetic cephalosporin. Cefaclor well absorbed orally and excreted rapidly in the urine, more active than the other oral cephalosporin and cefaclor peak concentrations in serum attained within 0.5-1.0 h. Food intake reduces the rate, but not the extent of absorption[2,10]. It is widely used as a life saving antibiotic for the treatment of urinary tract, lower and upper respiratory tract, skin structure, gastrointestinal infections, hypersensitivity[11,12], with broad spectrum of activity against gram positive and gram negative bacteria, well tolerated without toxicity and failed to accumulate in the plasma. Cefaclor does not metabolize significantly, partially degrades and excreted unchanged in the urine. The serum half life after oral administration is 0.5-0.7 h with an half life of 2 h. It can be used for the treatment of vulnerable infections in dogs[13,14]. The aim of present study is to estimate the pharmacokinetics of 1000 mg cefaclor as a tablet in stray dogs with the help of microbiological assay and to compare the pharmacokinetics against gram positive and negative microbes. The effect on biokinetics has also been evaluated in case of gender.

EXPERIMENTAL

Demographic data of the doges: For measurement of biokinetics in doges, seven adult male and seven adult female stray dogs was assigned in this study. The ages of dogs ranged between 2.5 to 3 years and weight was 19 to 21 Kg, where as height of dog ranged from 20 to 25 inches. Dogs were selected and look after for a week at dog house, Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad, Pakistan.

Administration of cefaclor: After proper pre-medication check up at dog house, Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad, Pakistan and each of 14 dogs (male and female) was given 1000 mg a single oral dose of cefaclor (CECLOR®, MR, AGP Ltd). Blood samples were collected prior to drug administration (t = 0) and at 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0 and 12.0 h after drug administration. The cephalic blood samples were collected in heparinized tubes and centrifuged at 3000 xg and plasma thus separated was stored at -10 ºC until further analysis.

Microbial strains: The concentration of cefaclor was tested against a set of microorganisms, including two Gram positive bacteria: *Staphylococcus aureus (S. aureus)* 6736153 APIstaph.tac, *Bacillus subtilis (B. subtilis)* JS-2004, two Gram-negative bacteria: *Escherichia coli (E. coli)* ATCC 25922 and *Pasteurella multocida (P. multocida)* local isolate. The bacterial strains were obtained from Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan. Purity and identity were verified by the Department of Veterinary Microbiology, University of Agriculture, Faisalabad, Pakistan. Bacterial strains were cultured overnight at 37 ºC in nutrient agar (NA, Oxoid).

Bioassay procedure: The concentration of cefaclor in blood samples was determined by disc diffusion method, performed precisely as described by NCCLS against *E. coli, S. aureus, P. multocida* and *B. subtilis*. Cefaclor standard disks (Wicks No. 319329, Beckman, U. S. A) and medium (dehydrated powder) were obtained from suppliers of culture medium (Oxoid, UK). Each of the petri plate (14 cm in diameter) impregnated with 40 mL medium. Plasma (100 µL) was loaded per 10 mm disk. Inoculated plates were incubated for 16 to 18 h at 37 ºC. Zones of inhibition were measured with zone reader in mm. All determinations were performed in triplicate and the results were averaged. The concentration of drug in plasma was measured over time by standard curve method.

Pharmacokinetic parameters: Plasma blood concentration data for each subject was manipulated by using APO computer software for biokinetics parameters; including maximum plasma concentration (C_{max}), the area under the plasma concentration ($AUC_{0\text{-}10\,h}$), mean residence time (MRT), clearance (CL), volume of distribution (V_d), elimination half life ($t_{1/2}$), absorption half life ($t_{1/2}$) absorption rate constant (Ka), lag time and time of peak (T_{max}).

RESULTS AND DISCUSSION

Cefaclor plasma concentrations measured microbiologically up to 10 h after single oral administration of 1000 mg in stray dogs. Peaked of cefaclor concentration was reached after 2 h (Fig. 1). It showed sharp peaks *versus* time in plot and gradually declined to 10 h, but higher the limit of quantification after 10 h. After 10 h the cefaclor concentration in blood was found to be 1.3, 1.8, 2.3 and 4.7 µg/mL, respectively against *E. coli, S. aureus, P. multocida* and *B. subtilis*. The plasma cefaclor concentration followed similar plots *versus* time against four microbial strains. Additionally, the plasma concentration curves showed the same trend from 0.5 to 10 h against four strains and was found higher against *B. subtilis, P. multocida* and lower for *E. coli*, indicate their better vulnerability to cefaclor. The mean concentrations of cefaclor in dogs blood plasma was observed 6.37, 9.37, 13.18 and 25.51 µg/mL against *E. coli, S. aureus, P. multocida* and *B. subtilis*, respectively after 2 h of administration. Serum cefaclor concentration remains above the MIC (≤ 2 µg/mL reported in literature) value for *S. aureus, P. multocida, B. subtilis* and *E. coli* until 10 h. The difference in concentration among strains was found statistically significant ($p < 0.05$).

The pharmacokinetic parameter for 14 dogs is given in Table-1. The maximum plasma concentration (C_{max}) was found nearly similar for *P. multocida* and *B. subtilis*, marginally lower against *E. coli* and *P. multocida*. The mean value of absorption half life ($t_{1/2}$) found nearly same against *S. aureus, P. multocida, B. subtilis* and *E. coli* (ranged form 1.30-1.96 h). The average AUC value was found 53.15, 56.5, 48.17 and 62.45 h mg/L, while clearance was found to be 15.49, 13.90, 13.89 and 13 L/h, respectively against *E. coli, S. aureus, P. multocida* and *B. subtilis*. The level of volume of distribution (V_d) was found considerably different at $p < 0.05$ for four bacterial strains and decreasing order was found as; *S. aureus* > *E. coli* > *B. subtilis* > *P. multocida*. The difference in elimination $t_{1/2}$ was found non-significant and higher for *B. subtilis*. The mean residence time was also found to be significant ($p < 0.05$) among four bacterial strains. There was no variation in the values of lag time. The values of time of peak (T_{max}) and absorption rate constant (Ka) were found similar against *S. aureus, P. multocida* and *B. subtilis*, while higher for *E. coli*. Plasma concentrations of cefaclor and the corresponding values of calculated biokinetics parameters showed no significant ($p < 0.05$) differences between male and female dogs. The blood plasma cefaclor and biokinetics parameter was found similar in male and female. According to Ashfaq[8] the biokinetics/pharmacokinetics parameters are directly related to the per body weight of the subject, the non-significant difference between genders might be due to same body weight. The sensitivity of bacterial strains was found to be different; *B. subtilis* was found to be more susceptible followed by *P. multocida, S. aureus* and *E. coli*. Absorptions and excretion of cefaclor was very rapid and same as reported in literature, which indicate that the cefaclor did not accumulate in the plasma. Cefaclor peak concentrations in serum were attained after 2 h, which is greater than reported[12], 10.6 µg/mL and 7.58 µg/mL for human male volunteers[14] and this variation in concentration might be due to species difference. The serum half life calculated for dogs was higher than reported for human beings[10]. This difference may be again due species difference. This delayed in excretion pointed out more labiality of drug in dogs, which may cause the chemical degradation of drug in dog's body. Delayed in excretion as compared to absorption indicate the hydrophobic character of cefaclor ratify by the higher elimination $t_{1/2}$

TABLE-1
PHARMACOKINETICS PARAMETERS CALCULATED FOR CEFACLOR AGAINST FOUR BACTERIAL STRAINS

	n[a]	AUC (hmg/L)	AUC (pex) (hmg/L)	AUC (trz) (hmg/L)	CL (L/h)	V_d (L)	Elim.$t_{1/2}$ (h)	K_{10} (L/h)	MRT (h)	Ka (L/h)	Abs $t_{1/2}$ (h)	L. time (h)	T_{max} (h)	C_{max} (mg/L)
E. coli	14													
Mean		53.15	52.90	55.10	15.49	37.09	2.20	0.61	8.40	0.95	1.96	0.55	2.80	18.0
±SD		3.10	3.31	3.95	2.15	6.91	0.54	0.09	1.25	0.16	0.08	0.02	0.24	2.0
S. Aureus	14													
Mean		56.5	51.46	54.15	13.90	39.5	2.45	0.45	8.85	1.15	1.60	0.31	1.35	19.80
±SD		4.10	5.80	9.76	2.25	11.33	0.81	0.07	1.42	0.33	0.27	0.06	0.41	1.95
P. Multocida	14													
Mean		48.17	43.56	53.80	13.89	31.16	2.30	0.75	6.75	1.21	1.55	0.40	1.99	21.10
±SD		4.70	3.57	3.10	1.98	7.77	0.86	0.11	1.30	0.56	0.49	0.03	0.62	2.50
B. Subtilis	14													
Mean		62.45	55.30	56.1	13.0	36.78	3.0	0.59	9.97	1.14	1.30	0.30	1.95	20.17
±SD		9.33	5.70	6.90	2.0	3.20	1.55	0.06	2.13	0.29	0.17	0.07	0.50	0.90

[a]Mean of fourteen values; SD = Standard deviation, P = *Pasturella*, B = *Basilus*, AUC = Area under the curve, pex = Polyexponential (t= 12), trz = Trapezoidal rule (t = 12), CL = Clearance, V^d = Volume of distribution, Elimi $t_{1/2}$ = Elimination half-life, Abs $t_{1/2}$ = Absorption half-life, Ka10 = Rateconstant k10, MRT = Mean residence time, k_a = Absorption rate constant, L = Lag, T_{max} = peak time and C_{max} = Peak concentration

values *versus* absorption. The elimination half life ($t_{1/2}$) following oral administration found greater against *B. subtilis* and *S. aureus* and lower for *E. coli* and *P. multocida* as reported by Iqbal[2] 3.5 h in lactating cow and 3.1 h in calves[12]. This lower $t_{1/2}$ prolonged duration enable the drug more active in doge as compared to human. The AUC values were found to be comparable with sheep 33.7 µg h/mL. The average T_{max} values indicate greater potency of cefaclor in dogs as compared to male volunteers[8] which enable cefaclor more active for longer time in dog. Plasma concentration and biokinetics parameters indicate that cefaclor can be used for treating susceptible bacterial infections in dogs[14] due to microbes studied in this study.

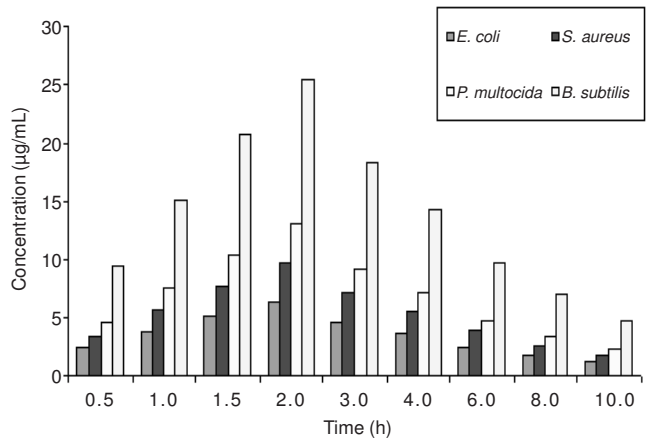

Fig. 1. Cefaclor concentration (µg/mL) in dog blood samples measured after single oral administration of 1000 mg tablet against four microbial strains

In summary, the biokinetics parameters of cefaclor correlate with clinical efficacy because plasma cefaclor concentration level remained above the MIC values until 10 h in high concentration and the decisive diseases in dogs due to these microbes can be treated with cefaclor antibiotic agent. From the results of pharmacokinetics parameters and plasma concentration, it is suggested that oral administrated of cefaclor @ 1000 mg orally twice a day maintained a rational concentration in plasma that would prove it a effective agent for infection treatment in dogs due to *S. aureus, E. coli, B. subtilis* and *P. multocida*.

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