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Pharmacokinetics of Cefaclor in Stray Dogs Determined Microbiologically

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Pharmacokinetic of cefaclor were evaluated in healthy stray dogs after oral administration. Serial blood samples were collected in heparinized tubes at pre-scheduled time intervals and analyzed by standard NCCLS microbiological assay against gram negative and gram positive bacterial strains by disc diffusion method. Peaked concentration (12.10 µg/mL) of cefaclor was found to be after 2 h. Average plasma concentration remained above minimum inhibitory concentration (MIC) value from 0.5 to 12.0 h. The values of maximum plasma concentration (C_{max}) were found to be 5.21, 7, 8.20 and 7.67 mg/L and time of peak (T_{max}) 2.65, 1.56, 1.85 and 1.73 h against *Escherichia coli, Staphalococuss aureus, Pasturella multocida* and *Basillus subtilis*, respectively. Absorption half life ($t_{1/2}$) was found to be 1.38, 0.42, 0.67 and 0.43 h against four strains, respectively. Pharmacokinetics was found insignificant ($p < 0.05$) in case of gender. Results showed that oral administration of 375 mg cefaclor as tablet thrice daily might be maintain substantial concentration that prove it to be effective for the treatment of specific infections in dogs.

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

INTRODUCTION

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. 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[1,2] with broad spectrum of activity against gram positive and gram negative bacteria, well tolerated without toxicity and failed to accumulate in the plasma[3]. Orally cefaclor well absorbed and excreted rapidly in the urine[4], 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[5]. 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 *ca.* half life of 2 h[6]. It can be used for the treatment of vulnerable infections in dogs[7-9]. The aim of present study is to estimate the pharmacokinetics of 375 mg cefaclor as a tablet in stray dogs with the help of microbiological assay and to compare the pharmacokinetics in case of gender against gram positive and negative microbes.

EXPERIMENTAL

This research was conducted on seven adult male and seven adult female stray dogs. The ages of dogs ranged between 2 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 (CMS), University of Agriculture, Faisalabad, Pakistan.

Administration of cefaclor: Each of 14 dog received 375 mg a single oral dose of cefaclor (CECLOR®, MR, AGP Ltd). Blood samples were collected prior to drug administration at 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0 and 12.0 h after drug administration. Whole venous (cephalic) blood samples were collected in heparinized tubes, samples were centrifuged at 3000xg and plasma thus separated was stored at -10 °C until further analysis.

Microbial strains: The concentration of cefaclor was tested against a set of microorganisms, including two Gram-positive bacteria: *Staphylococcus aureus* 6736153 APIstaph.tac, *Bacillus subtilis* JS-2004, two Gram-negative bacteria: *Escherichia coli* ATCC 25922 and *Pasteurella 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 was determined by disc diffusion susceptibility tests, performed precisely as described according to NCCLS[10] 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) was poured 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 regression method. The standard curve was constructed from 0.5-80 µg/mL.

Pharmacokinetic parameters: Plasma concentration data for each experimental animal was manipulated by using American Pharmacology Organization (APO) computer software for pharmacokinetic parameters; including maximum plasma concentration (C_{max}), the area under the plasma concentration ($AUC_{0\text{-}12\,h}$), mean residence time (MRT), clearance (CL), volume of distribution (Vd), elimination half life ($t_{1/2}$), absorption rate constant (K_a), lag time and time of peak (T_{max}).

RESULTS AND DISCUSSION

Cefaclor plasma concentrations measured microbiologically up to 12 h after single oral administration in dogs. Concentration peaked by 2 h, showed sharp peaks *versus* time plots (Fig. 1) and gradually declined to 8 h, with lower the limit of quantification at 12 h. The plasma concentration values follow similar plots *versus* time against four microbial strains. Additionally, the plasma concentration curves follow the same trend from 0.5 to 12 h, which was found higher against

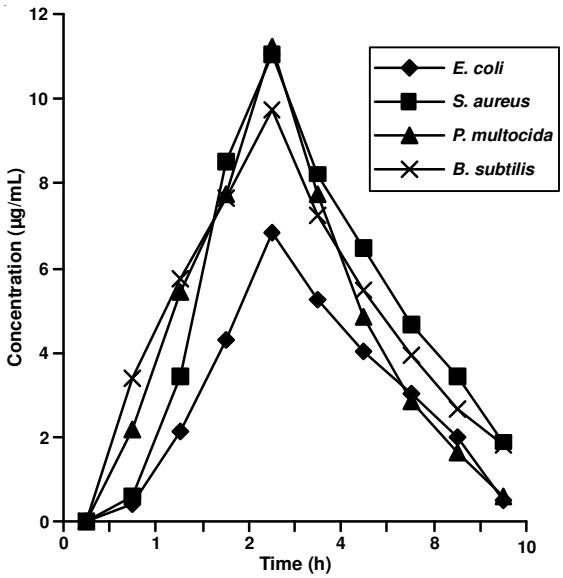

Fig. 1. Cefaclor plasma concentrations (µg/mL) in dogs after a single oral administration of 375 mg tablet measured against four bacterial strains (*E. coli, S. aureus, P. multocida* and *B. subtilis*)

S. aureus, P. multocida and lower for *E. coli*, indicate their better vulnerability to cefaclor. Mean concentrations was observed 6.80 ± 0.91 ranged (5.30-8.66), 11.2 ± 0.47 (11-12.1), 11.0 ± 0.66 (9.90-11.9) and 9.73 ± 0.64 (9.0-11.2) µg/mL against *E. coli, S. aureus, P. multocida* and *B. subtilis*, respectively were attained after 2 h. After 12 h the serum concentration was found to be 0.49 ± 0.31 ranged (0.1-1.3), 0.58 ± 0.32 (0.2-1.26), 1.86 ± 0.49 (1.20-2.76) and 1.82 ± 0.53 (1.0-2.93) µg/mL, respectively against four strain. Serum concentration remains above the MIC value for *S. aureus*, *P. multocida, B. subtilis* and lower against *E. coli* until 12 h. The difference in concentration among strains was found statistically significant ($p < 0.05$). The pharmacokinetic parameters for 14 dogs were summarized in Tables 1-4. The maximum plasma concentration (C_{max}) was found similar for *S. aureus* and *B. subtilis*, significantly

TABLE-1
PHARMACOKINETICS PARAMETERS CALCULATED FOR CEFACLOR AGAINST *Escherichia coli*

Dogs	AUC (h mg/L)	AUC(pex) (h mg/L)	AUC(trz) (h mg/L)	CL (L/h)	Vd (L)	Elimi.$t_{1/2}$ (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)
Male	17.05	16.96	24.20	22.00	48.51	1.53	0.45	4.85	0.45	1.53	0.47	2.68	2.85
Male	25.35	25.07	25.61	14.80	37.90	1.77	0.39	4.47	0.69	1.00	0.46	2.36	4.72
Male	40.90	39.47	39.76	9.20	31.63	2.40	0.39	5.00	0.96	0.72	0.44	2.23	7.06
Male	24.78	24.62	27.14	15.10	35.09	1.61	0.43	5.06	0.43	1.61	0.41	2.73	4.00
Male	29.06	28.10	48.10	12.10	24.10	1.30	0.53	4.23	0.53	1.21	0.48	2.35	5.70
Male	34.95	34.76	34.57	10.70	23.80	1.54	0.45	4.90	0.45	1.54	0.42	2.64	5.80
Male	35.85	34.18	33.87	10.50	39.46	2.62	0.27	5.60	0.74	0.94	0.44	2.61	5.35
Female	31.56	28.68	29.33	11.90	57.44	3.35	0.21	5.10	1.40	0.50	0.42	2.02	4.70
Female	29.93	29.85	31.28	12.50	24.56	1.40	0.51	4.40	0.51	1.36	0.49	2.45	5.62
Female	33.23	33.03	34.03	11.30	25.45	1.56	0.44	4.10	0.44	1.56	0.49	2.74	5.42
Female	45.48	44.77	43.42	8.25	23.05	1.90	0.36	5.10	0.36	1.10	0.38	3.18	5.10
Female	38.45	38.22	37.65	9.75	22.08	1.57	0.44	4.95	0.45	1.56	0.44	2.70	6.27
Female	44.90	44.04	43.04	8.35	24.42	2.03	0.34	6.27	0.34	2.03	0.43	3.35	5.70
Female	36.78	36.69	33.90	10.20	19.69	1.34	0.52	6.27	2.76	2.70	0.37	3.08	4.68
Mean	33.45	32.75	34.71	11.90	31.23	1.85	0.41	5.02	0.75	1.38	0.44	2.65	5.21
SD	7.94	7.79	7.08	3.57	11.18	0.58	0.09	0.66	0.64	0.55	0.04	0.37	1.02

SD = Standard deviation, *P* = *Pasturella, B* = *Basilus,* AUC = Area under the curve, pex = Polyexponential (t= 12), trz = Trapezoidal rule (t= 12), CL = Clearance, Vd = Volume of distribution, Elimi $t_{1/2}$ = Elimination half-life, Abs $t_{1/2}$ = Absorption half-life, Ka_{10} = Rate constant k10, MRT = Mean residence time, ka = Absorption rate constant, L = Lag, T_{max} = peak Time and C_{max} = Peak concentration.

TABLE-2
PHARMACOKINETICS PARAMETERS CALCULATED FOR CEFACLOR AGAINST *Staphalococuss aureus*

Dogs	AUC (h mg/L)	AUC(pex) (h mg/L)	AUC(trz) (h mg/L)	CL (L/h)	Vd (L)	Elimi.$t_{1/2}$ (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)
Male	46.49	41.53	41.37	7.99	43.19	3.76	0.19	6.97	0.90	0.79	0.45	2.61	5.74
Male	51.30	48.25	48.46	7.31	30.26	2.88	0.24	5.55	0.92	0.75	0.32	2.29	7.71
Male	64.16	55.70	54.21	5.85	33.25	3.95	0.18	6.96	1.26	0.55	0.47	2.29	8.18
Male	70.94	63.08	61.12	5.29	27.80	3.64	0.19	6.93	0.81	0.86	0.43	2.78	8.65
Male	47.88	43.16	43.08	7.84	39.17	3.47	0.20	6.12	1.40	0.49	0.41	2.02	6.90
Male	71.06	62.27	60.73	5.28	29.14	3.83	0.18	6.94	1.04	0.66	0.46	2.49	8.90
Male	64.08	57.92	57.14	5.85	29.00	3.43	0.20	6.29	1.07	0.65	0.39	2.31	8.77
Female	59.37	54.44	53.79	6.32	29.26	3.21	0.22	6.01	1.00	0.69	0.47	2.42	8.40
Female	78.98	64.67	62.01	4.75	31.97	4.67	0.15	8.22	1.01	0.69	0.49	2.72	8.43
Female	53.43	49.45	49.25	7.02	31.16	3.08	0.23	5.87	1.04	0.67	0.47	2.35	7.90
Female	85.69	74.03	70.07	4.38	25.25	4.00	0.17	7.80	0.62	1.12	0.49	3.34	9.06
Female	67.70	58.39	56.64	5.54	32.15	4.02	0.17	7.03	1.35	0.51	0.49	2.20	2.63
Female	70.07	61.51	60.25	5.35	29.35	3.30	0.18	7.10	0.87	0.80	0.47	2.70	8.44
Female	73.19	64.66	62.64	5.12	27.51	3.72	0.19	7.02	0.80	0.86	0.46	2.82	8.80
Mean	53.10	45.90	48.00	6.20	33.90	3.60	0.17	6.08	1.53	0.72	0.22	1.56	7.00
SD	16.24	14.17	15.80	1.91	10.67	1.11	0.05	1.84	0.54	0.15	0.08	0.52	2.24

SD = Standard deviation, *P* = *Pasturella, B* = *Basilus,* AUC = Area under the curve, pex = Polyexponential (t= 12), trz = Trapezoidal rule (t= 12), CL = Clearance, Vd = Volume of distribution, Elimi $t_{1/2}$ = Elimination half-life, Abs $t_{1/2}$ = Absorption half-life, Ka_{10} = Rate constant k10, MRT = Mean residence time, ka = Absorption rate constant, L = Lag, T_{max} = peak Time and C_{max} = Peak concentration.

TABLE-3
PHARMACOKINETICS PARAMETERS CALCULATED FOR CEFACLOR AGAINST *Pasturella multocida*

Dogs	AUC (h mg/L)	AUC(pex) (h mg/L)	AUC(trz) (h mg/L)	CL (L/h)	Vd (L)	Elimi.$t_{1/2}$ (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)
Male	46.97	46.87	49.08	7.94	14.98	1.30	0.53	4.15	0.53	1.30	0.40	2.28	9.22
Male	39.58	37.67	38.33	9.47	36.30	2.65	0.26	4.78	1.83	0.38	0.40	1.63	7.48
Male	41.96	41.83	42.57	8.94	18.08	1.40	0.49	3.96	0.60	1.15	0.27	2.10	8.39
Male	51.22	46.55	46.40	7.32	35.94	3.40	0.20	5.99	1.20	0.58	0.25	2.02	7.26
Male	37.16	36.10	37.43	10.10	32.92	2.26	0.31	4.31	1.58	0.44	0.41	1.70	7.67
Male	41.06	38.96	39.49	9.13	35.78	2.71	0.26	4.83	1.82	0.38	0.37	1.62	7.62
Male	45.03	42.34	42.48	8.33	34.33	2.86	0.24	5.04	1.89	0.37	0.39	1.64	8.07
Female	45.62	45.53	46.97	8.22	15.40	1.30	0.53	4.05	0.53	1.30	0.31	2.18	8.96
Female	39.06	38.68	40.03	9.60	24.26	1.75	0.40	3.96	0.80	0.77	0.32	1.95	8.09
Female	42.30	40.55	41.70	8.87	32.38	2.53	0.27	4.62	1.67	0.42	0.37	1.67	8.12
Female	45.03	41.91	43.41	8.33	36.32	3.02	0.23	5.29	1.74	0.40	0.36	1.70	7.60
Female	38.07	37.83	39.29	9.85	22.60	1.59	0.44	3.76	0.87	0.80	0.32	1.91	8.29
Female	48.84	47.15	48.11	7.68	26.68	2.41	0.29	4.65	1.15	0.60	0.31	1.91	8.86
Female	43.40	42.27	43.60	8.64	27.48	2.20	0.31	4.18	1.62	0.43	0.39	1.64	9.19
Mean	43.24	41.73	42.78	8.74	28.10	2.24	0.34	4.54	1.27	0.67	0.35	1.85	8.20
SD	4.14	3.68	3.72	0.83	7.93	0.68	0.12	0.62	0.52	0.35	0.05	0.23	0.65

SD = Standard deviation, *P* = *Pasturella, B* = *Basilus,* AUC = Area under the curve, pex = Polyexponential (t= 12), trz = Trapezoidal rule (t= 12), CL = Clearance, Vd = Volume of distribution, Elimi $t_{1/2}$ = Elimination half-life, Abs $t_{1/2}$ = Absorption half-life, Ka_{10} = Rate constant k10, MRT = Mean residence time, ka = Absorption rate constant, L = Lag, T_{max} = peak Time and C_{max} = Peak concentration.

higher against *P. multocida* and lower for *E. coli*. The value of absorption half life ($t_{1/2}$) found nearly same against *S. aureus, P. multocida* and *B. subtilis* and higher for *E. coli*. The AUC ranged from 33.45-60.84 h mg/L. Clearance (11.9, 6.2, 8.74 and 6.33 L/h for four strain, respectively) was found higher for *E. coli* and lower against *S. aureus* and *B. subtilis*. The level of volume of distribution (Vd) was found insignificant ($p < 0.05$) for all the four bacterial strains. There was an enormous difference in elimination $t_{1/2}$ and ranged from 1.85-4.25 h. The mean residence time (MRT) 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 (K_a) were found similar against *S. aureus*, *P. multocida* and *B. subtilis* and a slightly high against *E. coli*.

Plasma concentrations of cefaclor and the corresponding values of calculated pharmacokinetic parameters showed no significant ($p < 0.05$) differences between gender. Bacterial strains are found to be varied among themselves, *S. aureus* was found to be more susceptible, while *E. coli* least vulnerable to cefaclor. Absorptions and excretion of cefaclor is very rapid and found to be similar. Sourgens *et al.*[11] indicated that cefaclor did not accumulate in the plasma. Cefaclor peak concentrations in serum were attained after 2 h greater then reported[12], 10.6 µg/mL for male healthy volunteer and 7.58 µg/mL for human male volunteer[13]. This variation in concentration may be due to species difference. The serum half life was higher than 1 h in normal subject[14]. This delayed in excretion pointed out more labiality of drug in dogs, which might be due to the chemical degradation of drug. Delayed in excretion as compared to

TABLE-4
PHARMACOKINETICS PARAMETERS CALCULATED FOR CEFACLOR AGAINST *Basillus subtilis*

Dogs	AUC (h mg/L)	AUC(pex) (h mg/L)	AUC(trz) (h mg/L)	CL (L/h)	Vd (L)	Elimi.$t_{1/2}$ (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)
Male	72.84	57.28	56.27	5.14	39.35	5.30	0.13	8.46	1.63	0.42	0.20	1.80	7.64
Male	55.05	47.66	46.85	6.81	39.84	4.05	0.17	6.61	1.94	0.36	0.35	1.62	7.44
Male	82.28	61.92	59.46	4.56	34.40	5.84	0.12	9.39	1.36	0.51	0.23	2.19	7.73
Male	63.15	52.69	51.68	5.93	38.39	4.48	0.15	7.30	2.18	0.32	0.38	1.68	7.98
Male	65.75	53.79	53.16	5.70	39.39	4.79	0.14	7.70	1.80	0.39	0.23	1.75	7.63
Male	75.15	58.89	57.12	5.00	38.24	5.31	0.13	8.43	2.01	0.34	0.27	1.72	8.11
Male	52.75	47.50	47.30	7.10	36.04	3.51	0.20	5.85	2.04	0.34	0.30	1.57	8.10
Female	46.82	41.49	41.10	8.00	43.10	3.73	0.18	6.10	2.37	0.29	0.30	1.47	7.00
Female	60.64	53.18	52.45	6.18	34.82	3.90	0.18	6.85	1.53	0.45	0.21	1.80	8.12
Female	46.22	43.62	44.00	8.11	33.06	2.83	0.25	5.04	1.44	0.48	0.27	1.75	7.89
Female	62.83	54.71	53.19	5.97	34.72	4.03	0.17	6.93	0.99	0.70	0.10	2.24	7.47
Female	53.90	47.65	74.08	6.96	37.86	3.77	0.18	6.32	1.66	0.42	0.28	1.76	7.52
Female	58.24	50.65	49.55	6.44	37.24	4.00	0.17	6.71	1.39	0.50	0.20	0.92	7.49
Female	56.20	49.41	48.36	6.67	37.55	3.90	0.17	6.52	1.29	0.54	0.11	1.90	7.27
Mean	60.84	51.46	52.47	6.33	37.43	4.25	0.17	7.02	1.69	0.43	0.25	1.73	7.67
SD	10.48	5.75	8.03	1.05	2.66	0.81	0.03	1.16	0.39	0.11	0.08	0.31	0.34

SD = Standard deviation, *P* = *Pasturella, B* = *Basilus,* AUC = Area under the curve, pex = Polyexponential (t= 12), trz = Trapezoidal rule (t= 12), CL = Clearance, Vd = Volume of distribution, Elimi $t_{1/2}$ = Elimination half-life, Abs $t_{1/2}$ = Absorption half-life, Ka_{10} = Rate constant k10, MRT = Mean residence time, ka = Absorption rate constant, L = Lag, T_{max} = peak Time and C_{max} = Peak concentration.

absorption indicate the hydrophobic character of cefaclor, which is ratify by the higher elimination $t_{1/2}$ values. 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 Soback *et al.*[15] in lactating cow 3.5 h, Halstead *et al.*[16] reported that in calves 3.1 h. According to Meyer *et al.*[17] the value in foal is 3.26 h. This lower $t_{1/2}$ prolonged drug action and higher $t_{1/2}$ decreases susceptibility of drug. This variation in dogs as compared to cow, calves and foal, may be due to strain difference. The AUC values were found to be comparable[18] for sheep 33.7 µg h/mL.T_{max} values indicate greater potency of cefaclor compared for male volunteers[19], which enable cefaclor more active for longer time in dog. Plasma concentration and pharmacokinetics parameters indicate that cefaclor can be used for treating a wide range of susceptible bacterial infections in dogs.

Mostly pharmacokinetics variable of drug correlate with clinical effectiveness of drug because plasma concentration level remained above the MIC values, so imperative diseases in dogs due to corresponding microbes can be treated with cefaclor. From the results of pharmacokinetics parameters and plasma concentration in present study, it is suggested that oral administrated of cefaclor at the rate of 375 mg tablet orally thrice daily, after every 8 h maintained a sensible concentration in plasma that would prove cefaclor very effective for the treatment of specific infections in dogs.

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