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PHARMACOKINETICS FOR DOSE PROPORTION; ESTIMATION OF 250 MG CEFACLOR TABLET IN MALE VOLUNTEERS

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ABSTRACT... Objective: To estimate dose proportion for male volunteers by calculating pharmacokinetics following oral administration of Cefaclor (CCL) 250mg Tablet and to check the relative susceptibility of four bacterial strains **Design:** Randomized Clinical Trial, case series. **Setting:** Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad. **Period:** From Mar 2006 to Feb 2007. **Materials and methods:** Blood samples were collected for the period of 12 hours in heparinized tubes. Collected samples were centrifuged at 3000x*g* and plasma thus separated was stored at -10˚C until further analysis. The CCL plasma concentration was determined via bioassay using disc diffusion method. Pharmacokinetics parameters were calculated using American Pharmacology Organization (APO) computer software. **Results and conclusion:** Renal Clearance (CL), Volume of distribution (VD), Time of Peak (T_{max}), Maximum plasma concentration (C_{max}), Mean Residence Time (MRT), Absorption half life, Elimination half life & the Area Under plasma Concentration ($AUC_{t0\ to\ t12h}$) showed that the four bacterial strains have different susceptibility against cefaclor and administration of cefaclor at rate of 250 mg as tablet orally thrice daily maintained considerable concentration (>MIC) that prove it to be very effective for the treatment of specific infections in male volunteers.

Key words: Cefaclor, Male Volunteer, Pharmacokinetics, Bioassay.

INTRODUCTION

Cefaclor, 7-[(2-amino-2-phenyl-acetyl) amino]-3-chloro-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid, belonging second generation cephalosporin's[1] and used against various infections[2]. Cefaclor excreted rapidly in the urine, well absorbed without toxicity having broad spectrum of activity against gram positive and gram negative bacteria[3] with peak concentrations in serum 30-60 minutes[4]. It did not degraded in body significantly and excreted with an approximately half life of 2 hours[5].

The present research work was designed to evaluate the pharmacokinetics of 250 mg tablet of CCL in human male volunteer with the help of microbiological assay and to compare the susceptibility of four bacterial strains, namely *Escherichia coli (E. coli), Staphylococcus aureus (S. aureus), Pasteurella multocida (P. multocida)* and *Basillus subtilis (B. subtilis).*

MATERIAL AND METHODS

Experimental subjects

Eighteen healthy male volunteer were enrolled in this study. Informed written consent was obtained from all volunteers. Demographic data of all participants is given in table-I. The average age of volunteer was 28.3 year ranged (21 to 36) where as height was 66.78 inches (64-69) and recorded weight was 59.06 Kg (50-80). On the basis of clinical examination, medical history and laboratory investigations, none of the members revealed any medical liability and involvement in any clinical trials within the three month prior to enrolment in the current investigation. In addition, nobody had received any regular course of drug therapy two month before the present study.

Cefaclor administration and sample collection

Each volunteer received a 250 mg tablet of CCL (CECLOR®, MR, AGP Ltd) with 240 mL of water without infection. Blood samples (5mL) were collected in heparinized glass tubes prior to drug administration and at time interval of 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, 10.0 and 12.0 h. Collected samples were centrifuged at 3000x*g* and separated plasma was stored at -10˚C until further analysis.

Microbial strains

The concentration of CCL was tested against a set of microorganisms, including two Gram-positive bacteria: *Staphylococcus aureus* 6736153-AP Istaph. tac, *Bacillus subtilis* JS-2004 and two Gram-negative bacteria: *Escherichia coli* ATCC 25922 and *Pasteurella multocida* (local isolate). Authentic bacterial strains were obtained from Department of Veterinary Microbiology, University of Agriculture, Faisalabad, Pakistan. Bacterial strains were cultured overnight at 37ºC in Nutrient agar (NA, Oxoid) before bioassay procedure.

Bioassay procedure

The concentration of CCL was determined by disc diffusion susceptibility tests, performed precisely as described by the National Committee for Clinical Laboratory Standards (2002)[6] 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 media (Oxoid, UK). The medium (40 mL) was used for each glass Petri plate (14 cm in diameter). Plasma (100 µL) was loaded per 10 mm disk. Plates were incubated for 16 to 18 h at 37˚C. 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 seeded from 0.2-140 µg/mL in distilled water.

RESULTS

The CCL Plasma concentrations determined microbiologically up to 12h after single oral administration of 250 mg tablet of CCL. Concentration showed sharp peaks versus time plots (Figure 1), gradually declined to 8 h, with lower the limit of quantification at 12 h and maximum concentration was achieved at 1.5 h. The plasma concentration values follow similar plots versus time for four strains.

Additionally, the plasma concentration curves follow the same trend from 0.25 to 12 h, which was found higher against *S. aureus, P. multocida* and lower for *E. coli*, which indicate their vulnerability against CCL. Average plasma concentrations of 9.49 ranged (8.93-9.93), 12.8 (10.7-15.7), 12.1 (10.3-14.3) and 11.7 (9.87-14.1) µg/ml against *E. coli, S. aureus, P. multocida and B. subtilis,* respectively were attained at 1.5 h in male volunteers. At 12 h the serum concentration were found to be 0.86, 0.62, 0.47 and 0.40µg/mL against *E. coli, S. aureus, P. multocida* and *B. subtilis,* respectively. Plasma concentration values were found to be significantly different among microbe studied.

The pharmacokinetic analysis for 18 male volunteer is summarized in table II against four microbes. Average

Fig-1. The CCL plasma concentrations (µg/mL) in male volunteer after a single oral administration of 250 mg tablet measured against four bacterial strains *(E.coli, S. Aureus, P. Mulocida and B. Subtilis.*

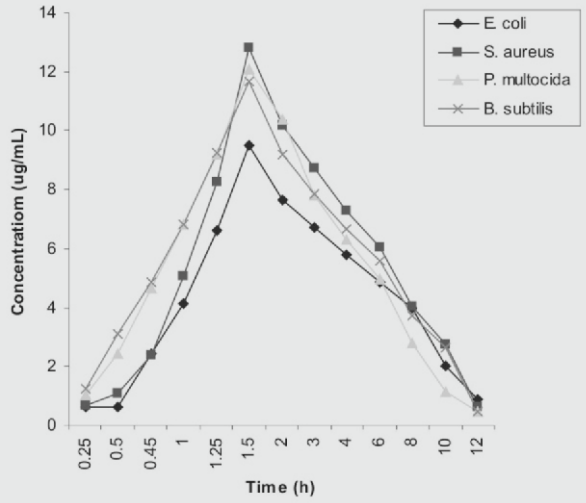

Table-I. Demographic data of eighteen male volunteers subjected to study.

Vol	Age (Year)	W (Kg)	H (Inch)	BP (H/L) mmHg	Tem (F˚)
Mean	28.3	59.06	66.78	115/80	98.3
SD	3.91	8.66	1.4	10.07/4.28	0.30
Max	36	80	69	140/90	99.2
Min	21	50	64	100/75	98.0

Vol = Volunteers (1-18), W = Weight, H = Height, BP (H/L) = Blood pressure (High/Low), Tem = Temperature, Max = Maximum and Min = Minimum

Table- II. Pharamacokinetics parameters calculated for different microbes

	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	Tmax h	Cmax mg/L
Pharmacokinetics parameters calculated for cefaclor against *E.coli*													
Mean	53.82	53.15	51.96	4.63	12.62	1.88	0.36	5.50	0.36	1.88	0.07	2.70	7.32
S.D	01.55	01.48	01.12	0.13	0.30	0.05	0.01	0.15	0.01	0.05	0.00	0.25	0.17
Pharmacokinetics parameters calculated for cefaclor against *S. Aureus*													
Mean	70.47	67.08	64.96	3.57	12.15	2.39	0.64	5.88	0.47	1.57	0.09	2.78	9.04
S.D	05.37	06.11	4.55	0.39	2.99	0.68	1.43	0.55	0.15	0.60	0.01	0.26	0.44
Pharmacokinetics parameters calculated for cefaclor against *P. Multocida*													
Mean	57.17	56.09	55.80	4.38	12.20	1.91	0.39	4.45	0.73	1.08	0.08	2.06	9.93
S.D	03.81	03.76	03.07	0.28	3.44	0.56	0.10	0.41	0.32	0.35	0.02	0.28	0.77
Pharmacokinetics parameters calculated for cefaclor against *B. Subtilis*													
Mean	64.74	62.49	60.45	3.87	11.58	2.09	0.37	4.89	0.68	1.27	0.05	2.20	10.12
S.D	3.48	3.46	3.03	0.21	3.77	0.74	0.11	0.51	0.42	0.41	0.02	0.22	0.68

Abbreviations: Vol = volunteers 1-18, SD = standard deviation, 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

C_{max} calculated for CCL was found similar against *S. aureus, P. multocida,* a little higher against *B. subtilis* and lower against *E. coli.* Elimination $t_{1/2}$ was found to be considerably significant among four strains.

AUC values were ranged from 53.82-70.47 h.mg/L and variation is significant. The CL values were found in following order *E. coli* > *P. multocida* > *B. subtilis* > *S. aureus.* Calculated average VD was found non significant but among the individuals there is a great variation. Absorption $t_{1/2}$ ranged from 1.08-1.91 and MRT 4.45-5.88 h, while that of Lag time level was found non significant. The T_{max} variation was significant in strains as well as among individual volunteers.

DISCUSSION

Plasma CCL concentrations and the corresponding values of calculated pharmacokinetic parameters shows significant differences between four microbial strains *S. aureus* were found to be more susceptible, while *E. coli* found least vulnerable to CCL. Absorption and excretion of CCL is very rapid and finding was in good agreement with previously reported[7]. The CCL peak concentrations was found higher then literature[8]. 10.6 µg/mL for male healthy volunteer[9], 6.05 and 12.8 µg/mL for normal human volunteer at the rate of 250 mg and 500 mg, respectively and 7.58 µg/mL for human male volunteer[10]. The serum half life was found to be greater then reported by Bloch et al[11] 60 min in normal subject. Our findings of the elimination half-life ($t_{1/2}$) were not in agreement as previously reported by Soback et al[12]. 3.5 h in lactating cow, Halstead et al[13]: 3.1 h in calves, Meyer et al[14]. 3.26 h in foal. This variation may be due to species difference.

Our previous study in dogs (Iqbal, 2007)[15] indicated less labiality of CCL for human beings as compared to dogs. The AUC values were found to be grater then reported by Craigmill et al[16].: 33.7h .µg/mL for sheep. The T_{max} value

indicates greater potency and slower clearance of CCL for male volunteers, which enables CCL more active for longer time in serum for the treatment of wide range of susceptible bacterial infection.

The elimination half-life ($t_{1/2}$), absorption half life, T_{max} and C_{max} characteristic put together that CCL is very suitable and important antibacterial agents in use today for human population. The pharmacokinetics variable of drug correlated with clinical efficiency, because the plasma concentration level remain above the MIC value (Soback et al., 0.78 µg/mL for young calves) until 12h after administration. So, of the presence diseases in human being due to corresponding microbes can be treated with CCL. From the results of present study of pharmacokinetics and plasma concentration, it is suggested that oral administrated of CCL at the rate of 250 mg as tablet orally thrice daily maintained reasonable concentration that ensure it to be very effective for the treatment of corresponding infections relevant to studied bacterial strains in human beings.

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"Wise men make proverbs, but fools repeat them."

Samuel Palmer (1805-80)