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HPLC ANALYSIS OF CIPROFLOXACIN IN BLOOD AND URINE OF MALE VOLUNTEERS AFTER ORAL ADMINISTRATION

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ABSTRACT

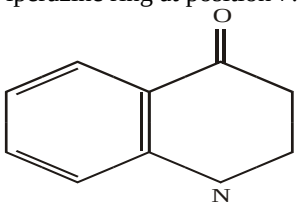
The disposition kinetics, renal clearance and urinary excretion of ciprofloxacin in blood and urine were analysed by HPLC from 12 healthy male after oral dose of 500mg tablet. Urinary excretion was determined and disposition kinetic parameters were calculated by using one compartment open model. After single oral administration of ciprofloxacin the average AUC was 11.77 ± 0.92 h.mg/L, clearance(45.6 ± 4.30 L/h), distribution (86.9 ± 10.8 L), elimination half life (0.82 ± 0.08h), and MRT 0.59 ± 0.075h. Absorption rate constant was 3.54 ± 0.26h, with its half life 1.37 ± 0.19 and lag time 0.382 ± 0.01h. Correlation between renal clearance and diuresis was positive and 30.4% of administered drug was excreted in urine. The study supported the needs for the comprehensive evaluation of drug under indigenous conditions to obtain disposition kinetics renal clearance and urinary excretion on which the rationa1 dose regimes of drug could be based.

Key Words: HPLC, Ciprofloxacin, Blood Urine, Male volunteers, Oral administration.

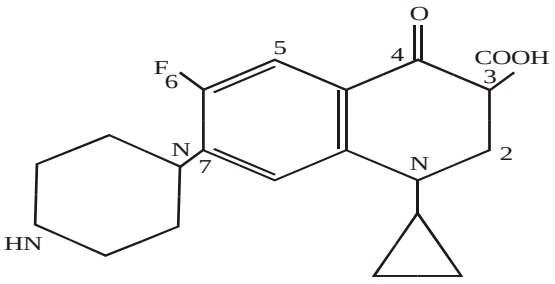
INTRODUCTION

Ciprofloxacin is a synthetic third generation quinoline. It has been studied for its vitro activity against a wide range of gram negative and gram positive organisms. Ciprofloxacin one of the most potent antibacterial drugs currently available is a fluoroquinolone. It is available as the monohydrochloride salt of cyclo propyl 6 fluoro 1, 4-dihydro 4 oxo-1-priperazinyl 3-quinoline carboxylic acid. It has a chemical structure modified from that of the basic bicyclic quinolone ring. The following modifications account for the drugs potent antimicrobiol activity.

(i) Cyclopropane ring at position 1.
(ii) Fluorinated carbon at position 6.
(iii) Piperazine ring at position 7.



Quinolone ring



Ciprofloxacin

It has a bactericidal mode of action. This action is achieved through inhibition of DNA gyrase, an essential component of the bacterial DNA replication system. Inhibition of the alpha subunit of the DNA gyrase blocks the resealing of the mink of the DNA strands induced by this alpha subunit, leading to the degradation of the DNA by exconuceases. This bactericidal activity persists not only during the multiplication phase, but also during the resting phase of the bacterium. Ciprofloxacin retained some of its bactericidal activity after inhibition of RNA and protein synthesis by rifamin and chloromphenicol respectively. The observations suggest that ciprofloxacin may possess two bactericidal mechanisms. One mechanism resulting from the inhibition of DNA gyrase and a second mechanism may be independent of RNA and protein synthesis. It has been used for the treatment of respiratory tract infections, urinary tract infections, bone and joint infections, infectious diarrhea and typhoid fever. The absolute bioavailability of ciprofloxacin is approximately 70%. The volume of distribution ranges from 1.74 to 5.0 L/kg. Non-renal clearance amounts 33% of the elimination of ciprofloxacin. Glomenular filtration and tubular secretion account for 66% of the total serum clearance. The terminal disposition half life ($t_{½}$) is about 3 to 4 hours. The present project was designed to evaluate ciprofloxacin under local environment by studying disposition. Kinetics, renal clearance and urinary excretion in male volunteers after oral administration of 500 mg tablets.

MATERIAL AND METHODS

Twelve healthy volunteers participated in the study. Their ages ranged from 20 to 25 years and body weight 59 to 72 kg. The volunteers were judged to be in good health before the study on the basis of medical history and physical examination. No other medications were permitted one week prior to and during the study.

A commercial preparation of ciprofloxacin was obtained as film coated tablets by the name of proflox (ciprofloxacin U.S.P. 500 mg) from Feroz Sons Laboratories Ltd.

After an overnight fasting control blood and urine samples were collected from all volunteers. Each volunteer was given a 500 mg tablet of ciprofloxacin with 250 ml of water. The volunteers were given breakfast two hours following drug administration.

Blood (5 ml) from vein of each volunteer was drawn in heparinized centrifuge tubes at 30, 60, 120, 150, 180, 240, 300, 480 and 720 minutes following oral administration of ciprofloxacin tablet. The blood samples were centrifuged at 4000rpm for 10 min and plasma was separated and stored in a freezer at 5^{o}C till further analysis.

The urine samples of each volunteer were taken at 45, 75, 105, 135, 165, 240, 360, 480 and 720 min following oral administration of ciprofloxacin tablet.

Unchanged ciprofloxacin in plasma and urine was determined by isocratic, reversed phase HPLC with ultraviolet detection. The plasma concentration time data was analyzed by one compartmental model using computer programme MW, PHARM Version 3.02.

The results were tabulated and statistical calculations were done according to the standard method. The results are given as average ± SEM. The correlation between diuresis, renal clearance, plasma concentration and time was determined by means of regression/correlation analysis (Steel and Torrie, 1992).

RESULTS AND DISCUSSION

The values of disposition kinetic parameters of ciprofloxacin following oral administration of 500 mg, tablets to each of the 12 healthy male volunteers are given in Table 1. The area under curve in the volunteers ranged between 5.90 to 16.54 h.mg/L with mean ± SE 11.77 ± 0.92 h.mg/L. the geometric mean AUC value obtained by Shah *et al*. (1999) was 12h mg/L. The clearance of ciprofloxacin in the volunteers ranged between 30.2 to 84.7 L/h with mean ± SE 45.6 ± 4.30 L/h. The value earlier reported was 36 to 41 L/h (Nix et al, 1992). The V_d in the volunteers ranged between 37.7 to 143.5 L mean ±SE 86.9 ± 108.

Table 1. Disposition kinetics parameters of ciprofloxacin following oral administration of 500 mg tablets to each of the 12 healthy male volunteers.

Parameters	Volunteers												Ave	± SE
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Area under the Curve (AUC)[h.mg/l]	9.895	11.84	11.39	11.65	12.14	10.87	14.83	16.51	10.51	5.901	9.198	16.54	11.77	0.92
AUC polyexponential (t=6.74)	9.624	11.77	11.24	11.24	11.87	10.07	14.82	16.2	10.44	5.879	8.695	13.93	11.31	0.84
AUC trapezoidal rule (t=6.74)	8.972	11.98	11.05	11.6	11.08	10.11	17.56	16.53	10.54	6.08	8.478	12.9	11.41	0.96
Clearance (CL) [l/h]	50.53	42.24	43.88	42.93	41.18	45.99	33.7	30.29	47.58	84.73	54.36	30.23	45.6	4.30
Volume of distribution [l]	65.19	62.62	47.03	97.1	82.81	134.4	37.74	57.32	72.45	116.9	143.5	126.3	86.9	10.8
Elimination half-life [h]	0.894	1.029	0.751	0.525	1.399	0.406	0.776	0.535	1.055	0.966	0.725	0.798	0.82	0.08
Rate constant k10 [l/h]	0.775	0.675	0.933	0.442	0.497	0.342	0.983	0.529	0.657	0.725	0.379	0.239	0.59	0.07
Mean Residence Time (MRT) [h]	2.938	3.37	2.557	3.416	4.388	3.89	2.656	3.137	3.435	3.101	4.014	5.617	3.54	0.26
Absorption rate constant (ka) [l/h]	0.776	0.673	0.923	1.32	0.495	1.706	0.893	1.295	0.657	0.718	0.956	0.868	0.94	0.10
Absorption half-life [h]	0.894	1.028	0.742	1.568	1.391	2.025	0.776	1.312	1.056	0.957	1.83	2.896	1.37	0.19
Lag-time [h]	0.359	0.402	0.402	0.397	0.358	0.382	0.416	0.472	0.39	0.327	0.329	0.288	0.38	0.01
Time to peak Tmax [h]	1.648	1.885	1.48	1.643	3.372	1.56	1.536	1.641	1.912	1.714	1.932	2.336	1.80	0.09
Peak concentration (Cmax) [mg/l]	2.822	2.935	3.89	2.968	2.217	2.486	4.874	4.702	2.539	1.565	1.898	2.425	2.94	0.31

The elimination half life in the volunteers ranged between 0.41 to 1.40h with mean ± SE 0.82 ± 0.08h. Catachpole *et al*. (1994) reported elimination half life of 4.5 h after 500 mg oral dose. The rate constant (K_{10}) in the volunteers ranged between 0.24 to 0.93 L/h with mean ± SE of 3.54 ± 0.26 h. The absorption rate constant K(a) of ciprofloxacin in the volunteers ranged between 0.5 to 1.71 L/h with mean ± SE of 0.94 ± 0.10 L/h. The absorption half life of ciprofloxacin in the volunteers ranged between 0.74 to 2.90 h with mean ± SE of 1.37 ± 0.19h.

The ciprofloxacin concentration in urine samples of 12 volunteers at different time intervals are given in Table 2. The pH of blood and urine, diuresis and renal clearance are given in Table 3. Average ± SE values for PH of blood and urine in male volunteers were 7.678 ± 0.058 and 6.037 ± 0.185. The average ± SE value renal clearance was 1.744 ± 0.330 and ranged from 0.368 to 3.901 ml min-1 kg-1 body weight. A calculation of renal clearance value from data of Kamberi *et al*. (1999) revealed a value of 16.31 ± 2.67 L/h. This value is several times higher than the values observed in the present study. This difference may be related with the lower G.F.R under indigenous conditions. The contradictions may be due to indigenous conditions because of geographical influences which may produce dissimilarities in biochemical, physiological and pharmacological parameters and urinary excretion (Nawaz *et al*., 1988).

Table 2. Concentration (µg/ml) of ciprofloxacin in urine of male volunteers after its oral administration as analyzed by HPLC.

S. No.	Volunteers	Time (minutes)								
		45	**75**	**105**	**135**	**165**	**240**	**360**	**480**	**720**
1	V_1	322.42	472.10	644.21	458.23	426.92	63.52	264.68	179.23	91.98
2	V_2	283.99	107.52	87.61	36.84	34.41	36.16	53.94	51.45	69.78
3	V_3	32.90	107.56	543.54	140.00	172.51	90.82	82.65	196.17	176.98
4	V_4	206.72	142.52	30.42	27.83	65.72	110.86	97.19	149.88	41.41
5	V_5	228.53	39.34	68.33	169.65	238.24	125.30	551.92	126.08	154.10
6	V_6	132.27	234.43	33.75	98.07	184.10	147.39	227.41	74.52	47.86
7	V_7	5.22	59.09	66.86	186.64	444.56	146.15	637.12	90.80	29.90
8	V_8	0.27	342.96	186.74	-	0.27	549.24	91.68	24.97	161.82
9	V_9	264.95	259.25	150.55	40.58	73.22	30.66	276.08	107.32	77.25
10	V_{10}	31.00	284.44	315.07	37.33	569.56	448.29	509.94	395.42	57.61
11	V_{11}	31.32	935.41	309.80	573.76	107.20	59.80	1.43	0.11	4.56
12	V_{12}	239.48	66.88	46.29	33.55	23.74	24.87	21.67	45.74	32.86
	Mean	148.25	254.29	206.93	163.86	195.04	152.76	234.64	120.14	78.84
	S.E.	35.24	72.59	59.70	53.46	54.44	48.65	63.72	30.50	16.34

Table 3. Renal Clearance of ciprofloxacin in male volunteers after oral dose of 500 mg tablets.

S. No.	Volunteers	Weight (kg)	Diuresis ($ml.min^{-1}kg^{-1}$)	pH		Concentration of ciprofloxacin (µg/ml)		Clearance ($ml.min^{-1}kg^{-1}$)
				Urine	**Blood**	**Blood**	**Urine**	
1	V_1	68	0.004	7.425	7.747	1.781	324.81	3.302
2	V_2	71	0.010	5.292	7.749	2.008	86.601	3.901
3	V_3	57	0.009	6.086	7.755	2.329	171.459	0.843
4	V_4	59	0.016	6.580	7.792	1.901	96.95	2.291
5	V_5	62	0.004	5.919	7.770	1.762	189.05	0.946
6	V_6	68	0.005	6.063	7.767	1.774	131.089	0.547
7	V_7	72	0.021	5.467	7.656	2.777	185.149	0.358
8	V_8	63	0.011	6.788	7.678	2.635	169.744	2.729
9	V_9	63	0.024	6.178	7.056	1.648	142.208	2.321
10	V_{10}	72	0.008	5.733	7.689	1.069	294.295	1.340
11	V_{11}	64	0.013	5.611	7.706	1.559	224.808	0.936
12	V_{12}	66	0.024	5.306	7.769	1.826	59.453	1.398
	Mean	65.42	0.0124	6.037	7.678	1.922	172.80	1.744
	S.E.	1.427	0.002	0.185	0.058	0.136	23.027	0.330

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