PHARMACOKINETICS OF CIPROFLOXACIN IN NORMAL RABBITS AND CHANGES OBSERVED IN INDUCED DEHYDRATED STATE

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ABSTRACT

The pharmacokinetics of ciprofloxacin following oral administration of a single dose of 20mg/Kg body weight was investigated in normal rabbits and changes were observed in water-deprived rabbits. High performance liquid chromatographic method was employed for the determination of plasma concentration of ciprofloxacin. The mean plasma concentration and AUC (0-t) and AUC (0-inf) were significantly different between normal and dehydrated rabbits ($P<0.05$), but the absorption rate, distribution rate, and elimination rate did not show any statistically significant difference. The results reflect a need for monitoring toxicity of ciprofloxacin in the water-deprived condition.

Keywords: Pharmacokinetics, ciprofloxacin, plasma concentration, dehydrated rabbits.

INTRODUCTION

Ciprofloxacin (CFX) is a fluoroquinolone derivative with outstanding antibacterial activity against gram-negative and some gram-positive bacteria as well as on some Chlamydia and Mycoplasma, and many mycobacterium species (Neu, 1987; Campoli-Richards *et al*., 1988 and Hyatt *et al*., 1994).

Its action takes place via the inhibition of the bacterial DNA gyrase which is an essential enzyme for DNA replication and synthesis. In animals Quinolones, especially CFX, exhibit favorable pharmacokinetic properties, their apparent volume of distribution suggested substantial tissue penetration (Abd El-Aty *et al*., 2005; Albarellos *et al*., 2004).

Dehydration is the condition that results from the excessive loss of body fluid, occurring during severe bleeding, sweating, and polyurea, excessive exudation from raw areas, diarrhea or vomiting. The Clinical and the physiological consequences of dehydration are a function of the nature of the body. Dehydration may be absolute, hypernatremic, relative or of voluntary nature. The most prominent manifestation of water deprivation is loss of body weight and decrease in both blood and plasma volumes (Aarseth *et al*., 1972). As water deprivation continues, plasma osmotic pressure, hematocrit value and plasma protein concentration increases but the acid base status and pH remains essentially constant. Dehydration usually disturbs the plasma electrolytes and attributed to physiological and biochemical changes which can significantly modify the pharmacokinetic profile of drugs. The past studies revealed that in dehydration, absorption, half life, volume of distribution and total body clearance of different drugs like gentamycin, chloramphenicol, oxytetracycline, erythromycin, salicylates, mefenamic acid and piroxicam were changed (Lecompte *et al*., 1981; Ahmad *et al*., 1972; Baker *et al*., 1983; Elsheikh *et al*., 1998) The changes in pharmacokinetic profile may result in altered sensitivity and toxicity of drugs requiring a new basis of drug selection and dosage modification. In present study the influence of dehydration on the pharmacokinetic behavior of CFX was investigated.

MATERIALS AND METHODS

Animals

Twelve healthy white albino rabbits of either sex ranging in body weight from 1-1.2 kg were used. All the animals were maintained under similar conditions. The animals were fed with fresh green fodder and black gram in the morning and evening, while water was provided freely as much they required.

Protocol of study

Pharmacokinetics of CFX was studied after administration of an oral dose in normal and dehydrated rabbits. The dehydration condition was produced in the normal rabbits during a wash out period of 10 days. Dehydration was induced by keeping the rabbits off water but not food. Body weight of rabbits was recorded daily as an indication of dehydration. The rabbits with a significant decrease in body weight (10%) and significant increase in packed cell volume were considered dehydrated.

Drug administration

For the determination of pharmacokinetics of CFX in normal and water deprived rabbits, the drug was administered as a single dose of 20mg/kg of body weight (CFX tablet of Bayer Pharmaceutical) prepared in

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Table 1: Weight and packed cell volume of normal and dehydrated rabbits and their % difference

Rabbit #	Weight (kg)			Packed Cell Volume		
	Normal	Dehydrated	% Diff.	Normal	Dehydrated	% Diff.
1	1.00	0.90	10	32.20	38.45	6
2	1.05	0.95	10	33.18	37.95	5
3	1.10	1.00	10	32.10	39.15	7
4	1.10	1.00	10	34.18	38.20	4
5	1.25	0.95	30	36.70	43.01	6
6	1.05	1.00	05	29.80	34.90	5
7	1.00	0.90	10	32.40	39.50	7
8	1.15	1.00	15	35.70	46.20	10
9	1.10	0.95	10	31.85	39.90	8
10	1.00	0.90	10	33.45	40.25	8
11	1.15	1.00	15	37.50	42.65	5
12	1.20	1.00	20	39.50	48.75	9

solution form. The preparation was administered through the feeding tube orally.

Sampling procedure

The blood samples were collected through the juggler vein of the rabbits, which were held in wooden cages, in heparinized glass centrifuge tubes with the aid of sterilized disposable plastic syringes just before and at 10, 20, 40, 60, 90, 120, 240, 480, and 720 min after the drug administration. The blood samples were centrifuged at 3000 rpm for 10 minutes to separate the plasma for analysis.

Drug analysis

The concentration of CFX in plasma was determined by the high performance liquid chromatographic procedure as described by Kordick *et al* (1997) with some modified sample preparation technique and few other variables suitably adjusted in the lab. Estimation of concentration was carried out by interpolating CFX peak areas on a calibration curve of spiked the blank plasma over the range assayed.

Sample preparation

After separating the plasma from blood sample, an equal amount of 5% perchloric acid was added for protein precipitation, vortexed for two minutes then centrifuged at 2000 rpm for 10 minutes. The aliquot was separated for injecting into the HPLC system

Pharmacokinetic analysis

Pharmacokinetic analysis was performed by non compartmental approach along with compartmental approach as well using two compartment model, using a soft wear Pharmacolysis® (Ahmad, 2006) provided by Pharma professional services.

STATISTICAL ANALYSIS

All values are expressed as the mean± standard deviation (SD) of twelve animals. The pharmacokinetic parameters obtained for the normal and dehydrated rabbits, after a single oral dose of 20mg/kg body weight were compared using a Paired t test, considering a probability of $P<0.05$ to be significant.

RESULTS AND DISCUSSION

Dehydration

Table 1 reports the weights of rabbits in normal and dehydrated conditions along with the packed cell volumes of the rabbits in normal and dehydrated. The table also reports the percent difference by dehydration. Present study showed a significant loss in body weight after deprivation of water in rabbits, which was more than 10% in dehydrated rabbits. Body weight loss becomes clinically important if it is more than 5% (Pickering *et al.*, 1959).

Plasma levels

After oral administration of 20 mg CFX/Kg body weight to normal and dehydrated rabbits blood samples were collected and analyzed for CFX plasma levels. Mean ± SEM plasma levels of CFX at different sampling times are given in table 2. This Table also reports the ratio of dehydrated levels with that of normal rabbits along with the percent change in treatment condition.

The profiles of Mean ± SEM plasma levels of CFX are shown in fig. 1. The maximum plasma concentration of 0.813 ± 0.009 µg/ml and 0.752 ± 0.007 µg/ml was attained in normal and dehydrated rabbits respectively, after 0.67 hr. From the mean plasma concentration data, a

Table 2: Time Vs. Plasma concentrations [Mean (12) ± SEM] of ciprofloxacin in normal and treatment (dehydrated) states following oral administration of a single dose of 20mg/Kg body weight, along with the Ratio of Normal and Treatment (T/N) (dehydrated/normal)

Plasma Levels (µg/ml)						
Time (hr)	Normal		Dehydrated			
	Average	SEM	Average	SEM	Ratio	% Diff.
0.17	0.643	0.01	0.612	0.005	0.95	-4.82
0.33	0.758	0.01	0.693	0.007	0.91	-8.58
0.67	0.813	0.009	0.752	0.007	0.92	-7.50
1.00	0.670	0.011	0.596	0.008	0.89	-11.04
1.50	0.586	0.009	0.523	0.006	0.89	-10.75
2.00	0.393	0.008	0.319	0.006	0.81	-18.83
4.00	0.268	0.007	0.237	0.005	0.88	-11.57
8.00	0.168	0.005	0.132	0.004	0.79	-21.43
12.00	0.121	0.004	0.1	0.003	0.83	-17.36

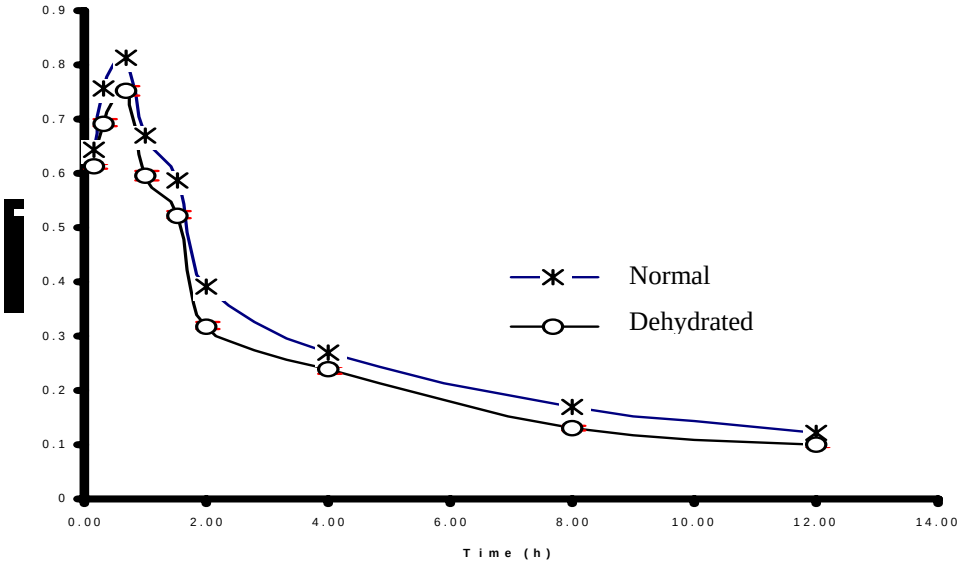

Fig. 1: Plasma concentration-time curves [Mean (n=12) ± SEM] of ciprofloxacin in rabbits in normal and treatment (dehydrated) states following oral administration of a single dose of 20 mg/kg body weight.

consistent decrease in the levels at all sampling times was observed in dehydration. The reduction in the drug plasma concentration in dehydrated rabbits may be due to higher osmolarity, interfering with rapid absorption of drug from the gastrointestinal tract. This observation is in conformity with previous reported works. Oukesso and Toutain also reported a decrease in ampicillin trihydrate plasma concentration due to water deprivation. The plasma concentration of erthromycin, mefenamic acid and piroxicam yielding a significantly lower plasma concentration after an oral administration of drug (Ahmad *et al*., 1992; Ahmad *et al*., 1996 and Qamar *et al*., 1999).

Pharmacokinetic parameters

The plasma levels versus time data of ciprofloxacin after oral administration of 20mg/kg body weight best fitted the two compartment open model. The derived pharmacokinetic parameters in both of the condition are given in table 3.

In the dehydrated rabbits statistically significant reduction ($P<0.05$) was observed in total area under the plasma concentration versus time curve upto the last sampling time AUC (0-t) and upto infinity (estimated) AUC (0-inf). The reduction may be attributed to the higher osmolarity, interfering with rapid absorption of drug from the

Table 3: Pharmacokinetic parameters [Mean ± SEM (n=12)] of ciprofloxacin in rabbits following a single oral dose of 20 mg/kg body weight in normal and treatment (dehydrated) states along with the ratio of dehydrated and normal (D/N) (dehydrated/normal)

Parameter	Unit	Normal	Dehydrated	Ratio	% Diff.	t-value	p
Absorption Rate Constant	$[h^{-1}]$	4.36±0.23	5.45 ± 0.44	1.25	25	2.521	ns
Absorption Half-Life	*[h]*	0.16±0.01	0.14 ± 0.01	0.875	-12.5	0.326	ns
Distribution Rate Constant	*[h-1]*	0.92±0.10	0.70 ± 0.04	0.761	-23.913	1.128	ns
Distribution Half-Life	*[h]*	0.83±0.08	1.02 ± 0.06	1.229	22.89	0.959	ns
Elimination Rate Constant	$[h^{-1}]$	0.08±0.01	0.08 ± 0.01	1	0	0.107	ns
Elimination Half-Life	*[h]*	9.56±1.68	10.23 ± 1.03	0.829	-17.099	1.933	ns
AUC(0-t) (obs. area)	$[h.mg.l^{-1}]$	3.3 ± 0.05	2.82 ± 0.03	0.855	-14.545	3.338	**
AUC (0-inf.) (area)	$[h.mg.l^{-1}]$	5.61±0.72	4.32 ± 0.17	0.77	-22.995	2.601	**
MRT (area)	*[h]*	4.08±0.03	3.94 ± 0.04	0.966	-3.431	1.046	ns
C(max)(calc)	$[mg.l^{-1}]$	0.26±0.04	0.2 ± 0.02	0.769	-23.077	0.433	ns
T(max)(calculated)	*[h]*	0.98±0.07	0.84±0.05	0.857	-14.286	0.772	ns
Lag Time	*[h]*	0.23±0.02	0.19 ± 0.02	0.826	-17.391	0.349	Ns

NULL Hypothesis that treatments (normal and dehydrated) are equal was tested by paired t-test. Those found significantly different ($p<0.05$) are denoted by '**' and those found non-significantly different are denoted by 'ns'.

gastrointestinal tract in dehydrated rabbits or due to less affinity in aqueous compartment of the drug. Previous studies in rat and rabbits also concluded with similar results (Baker *et al*., 1983 and Bukhari *et al*., 1999).

No statistical changes in absorption rate constant, distribution rate constant, absorption half life and elimination rate constant was demonstrated, which is in harmony with the earlier studies as oxytetracycline hydrochloride effect on dehydrated goats (Elsheikh *et al*., 1998) and the effect of dehydration on the pharmacokinetic of mefnemic acid (Qamar *et al*., 1999).

Oukessou and Toutain (1992) reported a low systemic availability of ampicillin and change in its pharmacokinetic parameter during water deprivation, which was linked to adaptation of renal function as assessed PAH (para-aminohippuric acid) clearance. The same factors may also contribute the pharmacokinetics of ciprofloxacin in normal and water deprived rabbits.

The clinical significance of the above findings can be associated to the dosing of ciprofloxacin, when utilizing this drug on a long term basis. Steady state of ciprofloxacin in dehydrated rabbit will be the same when given in equivalent doses, consequently this requires due concern in monitoring patients on prolonged therapy.

So concluded that additional pharmacokinetic study of ciprofloxacin in dehydrated condition might be recommended for studying the pharmacokinetic of this drug in humans.

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