PHARMACOKINETICS OF CIPROFLOXACIN IN E.COLI NATURALLY INFECTED CHICKENS

By

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ABSTRACT

Pharmacokinetic properities of ciprofloxacin were investigated in E.coli infected chickens after a single oral and Iv administration of 10 mg/kg b.wt. For oral administration, C_{max} was 0.510 µg /ml at t_{max} 3.0 hours, C_{max} –to– MIC ratio was 16.346 and AUC-to-MIC ratio was 172.01. Also, K_e,$t_{0.5e}$,V_c,CL_B and bioavilability were $0.115h^{-1}$,6.026h, 12.270 L/kg, 1.411 L/kg and 75.9%, respectively. For Iv administration, β, $t_{0.5\beta}$, V_c and CL_B were $0.115h^{-1}$, 6.026h, 3.463 L/Kg and 1.424 L/kg, respectively. Ciprofloxacin dose of 10 mg/kg b.wt. administered orally once daily to E.coli infected chikens would likely achieve the target ratios of C_{max}-to- MIC ratio greater than 10 necessary to minimize bacterial resistence and AUC– to – MIC ratio greater than 125 to maximize clincal and microbiological cures .

INTRODUCTION

Ciprofloxacin is a synthetic antibacterial agent of the fluoroqυinolone class that was developed exclusively for veterinary use. The fluoroquinolones are bactercidal and act principally by inhibition of bacterial DNA-gyrase which is necessary for supercoiling of DNA to provide a suitable spatial arrangement of DNA within the bacterial cell[29]. Fluoroquinolones are active against a wide range of Gram-negative aerobes, a number of Gram-positive bacteria and mycoplasmas at low concentrations compared with other classes of antimicrobial agents (**New, 1987 and Campoli-Richards *et al.*, 1988**).

Results of recent studies have indicated that it is possible to choose the most effective dose and dosing schedule for administration of an antimicrobial on the basis of the pharmacodynamics (drug antibacterial

action versus concentration in serum) of the drug. Investigators testing multiple antimicrobial dosing regimens in animal infection models have determined that bactericidal effect occur by time-dependent or concentration-dependent mechanisms (**Vogeleman *et al.*, 1988 and Leggett *et al.*, 1989**). For antimicrobials (e.g. penicillins, cephalosporines and erythromycin) that kill by time-dependent mechanisms, the pharmacokinetic value that correlates with efficacy is the time that serum concentration remains above the minimum inhibitory concentration for the pathogen (4 to 8 times MIC). For antimicrobials (e.g. aminoglycosides and quinolones) with concentration-dependent killing, the peak serum concentration or the area under the concentration-time curve correlates best with efficacy in treatment.

Various pharmacokinetic aspects of flouroquinolones in normal and experimentelly infected chickens have been studied (**Baggot, 1980 and Atef *et al.*, 1992**) but rare in naturally infected chickens obtained from poultry farms

The objective of this study is to determine the pharmacokinetic properties of ciprofloxacin after adminstration of single dose (10 mg / kg) either by oral or intravenous route in naturally infected chickens with E coli

Also, studying the relation between the pharmacokinetic properties of ciprofloxacin and MIC of the isolated E coli.

MATERIAL AND METHODS

Birds:

Laboratory diagnosis and pharmacokinetic study were conducted on fifteen, naturally infected hubbard broiler chickens of both sexes (35days old –1.5 kg average b. wt.). The chickens obtained from a poultry farm suspected to be infected with colibacillosis which diagnosed as E. coli infection during routine follow up in Animal Health Research Institute (Zagazig). Blood samples were collected from each bird and tested microbiologically (**Bennett *et al.*, 1966 and Rosin *et al.*, 1989**) to sure that they free from any antimicrobial agents.

Laboratory diagnosis:

The chicken are clinically investigated and symptoms were recorded as well as post – mortem examination was carried out and P.M. lesions (**Sojka, 1965**) were recorded. Organ samples were collected from heart blood, liver, spleen, air sacs and pericardial sacs for bacteriological examination using MaConkey agar, MaConkey broth and blood agar, then, incubated at 37°C for 24 hours. Suspected colonies of being E. Coli were subjected to morphological, biochemical as well as serological examination

(Edwards and Ewing, 1962; Sojka, 1965; Kauffman, 1969 and Cruickshank, 1972).

In vitro-sensitivity test and minimum inhibitory concentration:

Of the isolated E.coli was determined using agar plate diffusion[5] and microdilution broth **(Kolmer *et al.*, 1951 and Amsterdam, 1991)** techniques, respectively.

Pharmacokinetics:

Single-dose pharmacokinetic properities of ciproflaxacin were studied in two groups of E.coli naturally infected chickens (n=5/group), one group was given orally ciprofloxacin at rate of (10 mg/kg. b.wt) and the other was given the same dose intravenous. Blood samples were collected at different time intervales as follows 0.1,0.25,0.5,0.75,1,2,3,4,6,8,10,12,and 24 hours after drug administration, centrifuged at 3000 rpm for 5 minutes, then, the serum was separated and stored at-20°C until assayed . Serum concentration of ciprofloxacin was determined by microbiological assay using bacillus subtilis **(Bennett *et al.*, 1966 and Rosin *et al.*, 1989)** ATCC 6633 as the test organism.

Data analysis:

Pharmacokinetic analysis **(Baggot, 1980 and Metzier and Weiner, 1989)** was carried out using one – compartment open model for oral administration and two –compartment open model for Iv administration in to statistical addition analysis **(Rosin *et al.*, 1989)**.

RESULTS

Case investigation :

The diseased birds were examined clinically and respiratory symptoms were observed in the form of rattling sounds, coughing, gasping and ralles. Also the examined birds showed listlessness and off food.

Postmortem examination was carried out on the dead birds which showed the presence of pericarditis, airsaculitis as well as liver, spleen and kidney congestion. Some birds were sccumbed and subjected to bacteriological investigation, samples were collected from heart blood, liver , spleen , air sacs and pericardial sacs, and inoculated or streaked onto MaConky broth, MaConky agar and blood agar . Colonies suspected to be E.coli were subjected to biochemical and serological examination which showed that the isolated colonies were E. coli O2: K1 and O113: K 75 (Table 1).

In vitro – sensitivity test:

Antibiogram was carried out on the E.coli isolated strain against 9 antibiotics including ciprofloxacin which showed the largest inhibition zone of 29 mm (Table 2).

Minimum inhibitory concentration (MIC):

The result of MIC cleared that ciprofloxacin had lowest concentration (0.0312 μg/ml) that enough to inhibit the growth of the isolated field E. coli strains.

Pharmacokinetic properties:

Ciprofloxacin was detected in the serum of E.coli infected chickens for 24 hours after IV and oral administration of 10 mg /kg. b.wt. (Table 3 , Figs. 1 and 2). For compartmental analysis of serum ciprofloxacin concentration over time after IV administration, the data were best described by a two-compartment open model with an α of 1.933h-1 and β equal to 0.115h-1 (Table 4). The estimate of Co was 2.888 ug/ml and the volume of the central compartment (Vc) was computed as 3.463 L/kg . The noncompartmental Iv parameters of CLB ,Vd (area) and Vdss were computed 1.424L/kg, 12.285 L/Kg and 3.026 L/Kg, respectively. Serum concentration of ciprofloxacin after 10mg/kg b.wt. oral dosing were best described as a one – compartment open model. The serum concentration-time curve depicts a slow elemination slope. However, this terminal slope is actually reflective of the slow rate of absorption as the evidence by the tmax of 3.0 hours. The $t_{0.5a}$ was computed as 1.619 h, and the $t_{0.5e}$ was calculated to be 6.026 hours (Table 5) . Bioavilabilty was 75.9%.

DISCUSSION

In designing a dosage regimen for antimicrobial agents, mechanism of bacterial killing and postantibiotic effect (PAE) must be considered (**Vogeleman and Craig, 1986; Vogeleman *et al.*, 1988; Dudly, 1991; Bergeron, 1992; Nightingle, 1993 and Crag, 1993**). Antimicrobials that inhibit DNA gyrase (e.g. quinolones) have concentration-dependent bactericidal activity and induce PAE (**Vogeleman and Craig, 1986; Vogeleman *et al.*, 1988; Bergeron, 1992 and Crag, 1993**). Animal infection model used to study duration of in vivo PAE after exposure to fluoroquinolones used in human beings had suppression of bacterial growth for 1.9 to 7.5 hours in Enterobacteriaceae (**Craig, 1993**). Other factors documented to increase the duration of quinolone PAE are high drug concentration (maximal effect at 5 to 10 times MIC), long exposure time, and presence of neutrophils (**Vogeleman and Craig, 1986; Schentag, 1991 and Craig, 1993**).

In this study, after oral administration of 10mg/kg b.wt. of ciprofloxacin to E.coli naturally infected chickens, peak serum concentration Cmax was 0.510 µg/ml (16.026 times MIC of E.coli isolates)

Also, oral administration of ciprofloxacin characterized by high bioavailability (75.9%), high volume of distribution (12.270 L/kg) and slow elimination rate ($0.115h^{-1}$) which indicated that ciprofloxacin was highly absorbed, easily penetrated all tissues and remain in the body above the MIC of E. coli isolates for 24 hours (C_{24}=0.050 µg/ml).

On the basis of recent trials in human beings (**Forrest *et al.*, 1993 and Kung *et al.*, 1993**) and animal infection studies (**Drusano *et al.*, 1993**), investigators have proposed that the critical break point determining efficacy of quinolones is an AUC– to – MIC ratio which must be not less than 125.

In this study, the recommended dose of ciprofloxacin (10 mg/kg b.wt.), administered once daily would likely result in an AUC – to - MIC ratio greater than 125 (172.019) in E. coli infected chickens.

We conclude that, ciprofloxacin dose of 10 mg/kg b.wt. administered orally once daily to E. coli naturally infected chickens would likely achieve the target ratios of C_{max}-to- MIC greater than 10 necessary to minimize bacterial resistance, and AUC– to – MIC ratio greater than 125 to maximize clinical and microbiological cures .

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Table (1): Biochemical tests for identification of isolated E. coli.

Biochemical test	Result
Indole production	+
Methyl red	+
Nitrate	+
Simmon's citrate	-
Urea hydrolysis	-
H2S production	-
Voges proskauer	-
Carbohydrate fermentation: - Lactose - Glucose - Mannitol	 + - -

Table (2): Susceptibility of isolated E.coli from infected chickens to various antimicrobial agents.

Tested drugs	Disc Potency (µg)	Inhibition zone (mm)
Ciprofloxacin	5	29
Lincospectin	50/100	25
Ceftifour	10	20
Ampicillin	30	16
Gentamycin	10	15
Streptomycin	30	14
Erythromycin	15	10
Oxytetracycline	30	-
Lincomycin	15	-

Table (3): Serum concentration of ciprofloxacin (µg/ml) in E.coli infected chickens after a single oral or intravenous administration of 10 mg/kg b.wt. (X +S.E)

Hours after administration	Serum concentration (µg/ml)	
	Oral	Intravenous
0.10	0.110 + 0.010	2.492 + 0.096
0.25	0.128 + 0.011	2.021 + 0.125
0.50	0.164 + 0.015	1.480 + 0.080
0.75	0.211 + 0.021	1.140 + 0.023
1	0.265 + 0.019	0.895 + 0.065
2	0.439 + 0.023	0.586 + 0.033
3	0.510 + 0.043	0.479 + 0.040
4	0.500 + 0.040	0.427 + 0.035
6	0.399 + 0.032	0.339 + 0.019
8	0.317 + 0.020	0.270 + 0.026
10	0.252 + 0.020	0.214 + 0.016
12	0.200 + 0.016	0.170 + 0.012
24	0.050 + 0.014	0.043 + 0.015

Table (4): Pharmacokinetic variables of ciprofloxacin in E.coli infected chickens after a single IV administration of 10 mg/kg b.wt. (× + S.E.).

Parameter	Unit	Iv Administration
C_o	µg/ml	2.888+0.193
A	µg/ml	2.212+0.035
A	h^{-1}	1.933+0.070
$t_{0.5\alpha}$	h	0.359+0.033
B	µg/ml	0.676+0.029
B	h^{-1}	0.115+0.014
$t_{0.5\beta}$	h	6.026+0.106
K_{12}	h^{-1}	1.096+0.012
K_{21}	h^{-1}	0.541+0.030
K_{el}	h^{-1}	0.411+0.032
V_c	L/kg	3.463+0.021
$V_{d(B)}$	L/kg	14.793+1.103
$V_{d(area)}$	L/kg	12.285+1.098
V_{dss}	L/kg	3.026+0.022
Cl_B	L/kg	1.424+0.091
AUC	µg.h / ml	7.081+0.087

Table (5): Pharmacokinetic variables of ciprofloxacin in E.coli infected chickens following a single oral administration of 10 mg/kg b.wt. (× + S.E)

Parameter	Unit	Oral administration
A	µg/ml	0.718±0.043
K_a	h^{-1}	0.428±0.020
$t_{0.5a}$	h	1.619±0.086
C_{max}	µg/ml	0.510±0.018
C_{max} / MIC		16.346±1.190
C_{24}	µg/ml	0.050±0.002
C_{24}/MIC		1.603±0.098
T_{max}	h	3.00±0.218
B	µg/ml	0.795±0.066
K_e	h^{-1}	0.115±0.011
$t_{0.5e}$	h	6.026±0.041
V_C	L/kg	12.270±0.987
CL_B	L/kg	1.411±0.012
AUC	µg. h/ml	5.367±0.473
AUC/MIC		172.019±12.001

- Co: drug concentration at zero time immediately after IV dosage.
- A and B: Zero - time intercepts of absorption and elimination phase.
- α or Ka : over all absorption rate constant .
- t 0.5 α : absorption half - life .
- β or Ke : Over all elimination rate constant .
- t 0.5 β : elimination half – life.
- Kel: rate constant of elimination.
- K12: rat constant of distribution from central to Peripheral compartment .
- K21: rat constant of distribution from Peripheral to central compartment .
- Vc: apparent volume of central compartment.
- Vd: apparent volume of distribution.
- ClB: body clearance.
- Cmax: maximum drug concentration.
- C24: drug concentration at 24 hours.
- Tmax: time at Cmax.
- Auc: area under the curve.
- MIC: minimum inhibitory concentration.

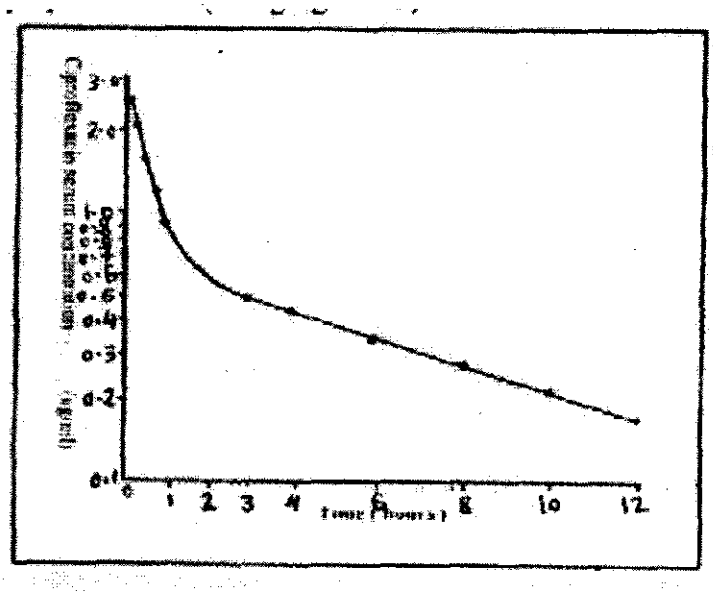

Figure (1): semilogartmic plot of mean +SD (n=5) serum drug concentrations (µg/ml) VS time (hours) in E.coli infected chickens given a single IV dose of ciprofloxacin (10mg/kg b. wt.)

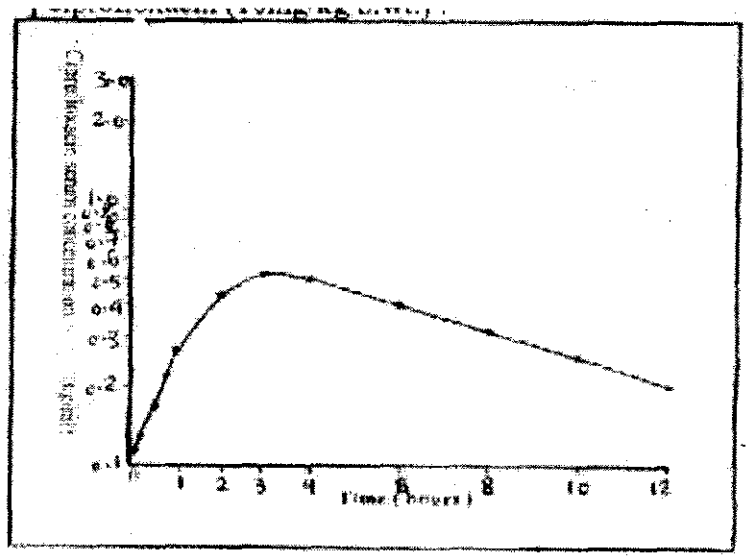

Figure (2): semilogartmic plot of mean ±SD (n=5) serum drug concentrations (µg/ml) VS time (hours) in E.coli infected chickens given a single oral dose of ciprofloxacin (10mg/kg b.wt.).

الملخص العربي

الحركة الدوائية للسبروفلوكساين في الدواجن المصابة بالقولون العصوى

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تم دراسة الحركة الدوائية للسبروفلوكساين فى الدواجن المصابة بالقولون العصوى والتى تم الحصول عليها من احد المزارع التى تعانى من الاصابه بهذا الميكروب وذلك بعد اعطائها جرعه واحده عن طريقى الوريد والفم (١٠مجم/كجم وزن حى).

وقد وجد ان اقل تركيز للسبروفلوكساين يلزم لمنع نمو الميكروب هو ٠,٠٣١٢ ميكروجرام / مل ، كما وجد بعد اعطاء الجرعه العلاجيه عن طريق الفم ان اعلى تركيز للسبروفلوكساين فى السيرم هو ٠,٥١٠ ميكروجرام /مل بعد ٣ ساعات ، فى حين وصل التركيز الى ٠,٠٥٠ ميكروجرام /مل عند ٢٤ ساعة .

وتبين من هذه الدراسة ان فترة نصف العمر للسبروفلوكساين بعد اعطائة بالطريقتين متساوية (٦,٠٢٦ ساعات) وان مدى استفادة الجسم من العقار بعد اعطائة عن طريق الفم هى ٧٥,٩?.

وقد استنتج من هذا البحث ان نسبة اعلى تركيز للسبروفلوكساين فى السيرم الى اقل تركيز يلزم لمنع نمو الميكروب تساوى ١٦,٣٤٦ (أكثر من ١٠ اضعاف) وهذه النسبة كافية لتقليل مقاومة الميكروب للعقار ، وكذلك نسبة المساحه تحت المنحنى الى اقل تركيز يلزم لمنع نمو الميكروب تعادل ١٧٢,٠١٩ (اكثر من ١٢٥ ضعف) وهذه النسبة كافية لزيادة معدلات التماثل للشفاء الظاهرى والقضاء على الاصابة البكتيرية .