ORIGINAL ARTICLE

Determination of the presence and pharmacokinetic profile of ciprofloxacin by TLC and HPLC method respectively in broiler chicken after single oral administration

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The objective of this study was to determine the presence of ciprofloxacin and pharmacokinetics of ciprofloxacin in blood plasma of broiler chicken after single oral administration at feeding state. Ciprofloxacin was administered orally at 10 mg kg^{-1} to each of the 10 broiler chickens at feeding state. Presence of ciprofloxacin was determined by TLC method and pharmacokinetics by HPLC method. Peak plasma concentration (C_{max}) of ciprofloxacin, $0.26 \pm 0.03 \text{ mg l}^{-1}$ was achieved at $4.40 \pm 0.1 \text{ h}$ (T_{max}). Biological half-life of ciprofloxacin was $5.25 \pm 0.02 \text{ h}$. Area under the curve (AUC) was $13.397 \pm 0.13 \text{ mg ml}^{-1} \text{ h}$, elimination rate constant was $0.13 \pm 0.02 \text{ h}^{-1}$, volume of distribution was $0.194 \pm 0.04 \text{ l kg}^{-1}$ and bioavailability was $49 \pm 0.48\%$. On the basis of the results of the present study, we conclude that, feeding state may have a vital effect on the pharmacokinetic profile of ciprofloxacin in broiler blood plasma.

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INTRODUCTION

Poultry industry is the fastest growing segments of animal industry. It is the raising of domesticated birds such as chickens, turkeys, ducks and geese, for the purpose of farming meat or eggs for food. Poultry are farmed in great numbers with chickens being the most numerous. More than 50 billion chickens are raised annually as a source of food, for both their meat and their eggs.

Antibiotics are widely used by the poultry industry and poultry veterinarians to increase growth and feed efficiency and reduce mortality. Antibiotic usage has facilitated the efficient production of poultry, allowing the consumer to purchase, at a reasonable cost, high quality meat and eggs. Although these uses benefit all involved, unfortunately, consumer perceptions are that edible poultry tissues are contaminated with harmful concentrations of drug residues.¹

Fluoroquinolones (FQs) are antibacterial agents related to nalidixic acid. FQs are used in both human and veterinary medicine to treat a variety of infections.² FQs are broad-spectrum antibiotics that have an important role in treatment of serious bacterial infections in human as well as poultry.³ The FQs have been valuable in veterinary medicine and they have extensive application in clinical practices because of favorable bioavailability and pharmacokinetic profile.⁴ All FQs are bactericidal and act against the same bacterial target: the bacterial

DNA gyrase (type II topoisomerase) and topoisomerase IV. DNA gyrase is the major target in Gram-negative bacteria and topoisomerase IV is the major target for gram-positive bacteria.⁵ Ciprofloxacin (INN) was first patented in 1983 by Bayer A.G. and subsequently approved by the U.S. Food and Drug Administration (FDA) in 1987. Ciprofloxacin is a synthetic and second generation of antibiotic of the fluoroquinolone drug class. It is one of the FQs that have been used to treat various infectious diseases in poultry and animals.⁶ It is a major, active metabolite of enrofloxacin in different species and is formed by the deethylation of enrofloxacin. Ciprofloxacin was the first of the two compounds to be developed and is only approved for use in humans.² Its spectrum of activity includes most strains of bacterial pathogens responsible for respiratory, urinary tract, gastrointestinal and abdominal infections. It has 12 FDA-approved human uses and other veterinary uses, but it is often used for unapproved uses (off-label).

The present study was designed to determine the presence of ciprofloxacin and pharmacokinetics of ciprofloxacin in broiler chicken's blood plasma after single oral administration at feeding state. There are some literatures that conducted their study on pharmacokinetics of ciprofloxacin.^{7–9} But there are no literatures on the presence of ciprofloxacin and pharmacokinetics of ciprofloxacin in blood plasma of broiler chicken after single oral administration at

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feeding state. Hence, we carried out this research which is important for health concern.

MATERIALS AND METHODS

Animals

Ten broiler chickens were used for this study. Animals were maintained from 16 weeks of age in conventional cages with an automated system for temperature, humidity and ventilation control. The birds had *ad libitum* access to water and no medicated feed. The ration was formulated by an avian expert. Experimental animals were kept in conditions in compliance with animal welfare guidelines approved by the Bioethics Committee of the Chittagong Veterinary and Animal Sciences University, Chittagong, Bangladesh. Throughout the experiment, an avian medicine veterinarian monitored the hens. The institutional and international guide for the care and use of laboratory animals was followed.

Animal dosing and sample collection

Ciprofloxacin (Neofloxin, 50 mg ml⁻¹, Beximco Pharmaceuticals Ltd, Dhaka, Bangladesh) was administered 10 mg kg⁻¹ orally to each broiler chicken. Following administration, blood samples were collected intermittently via a 26-G butterfly syringe and needle. Blood samples were collected before treatment and after 1, 2, 3, 4, 5, 6, 7 and 8 h of treatment. Plasma was separated from each sample by centrifuging at 3500 r.p.m. (2460 g) for 15 min at 4 °C. About 400 µl plasma was separated from each sample through a micropipette. A volume of 100 µl cold methanol was then added to each plasma sample. This mixture was then finally mixed by vortex machine. Finally, the supernatant plasma sample was collected from each sample and transferred to an ependorf tube. The extracted plasma was stored at -70 °C until further experiment.¹⁰

TLC analysis

To detect the presence of ciprofloxacin in each plasma sample, TLC was run. TLC Silica plates with 0.25 mm. thickness (Merck, Darmstadt, Germany), were activated in 120 °C for 2 h before use.¹¹ For comparison of extracted sample with routinely used standard antibiotics, were prepared by dissolving of 0.1 g of ciprofloxacin standard (Globe Pharmaceuticals, Noakhali, Bangladesh) in 4 ml methanol. About 50 µl of methanol-dissolved deposits were pointed on silica plates. Treated plates were transferred to the TLC tank containing acetone-methanol (1:1) as the mobile phase. Solvent front and solute front (standard and sample) were run through the TLC plate according to their affinity to stationary phase. After reaching of the solution front to end of the plates, chromatograms were observed under UV light at 256 nm. By calculating retention factor value, the presence of ciprofloxacin was determined in each sample.¹²

HPLC analysis

The concentration of ciprofloxacin in each plasma sample was evaluated in plasma using ultra HPLC. A volume of 200 µl of internal standard solution of ciprofloxacin (concentration 1 mg ml⁻¹) was retained in a 15 ml glass centrifuge tube using an amendable pipette, followed by 1 ml of plasma sample containing ciprofloxacin. Then 100 µl of concentrated o-phosphoric acid was added to the plasma sample. After shaking the mixture briefly (about 5 s) on a vortex mixer, 2 ml of acetonitrile was added. The centrifuge tube was plugged and then shaken at high speed on a vortex mixer for about 10 s. After centrifuging the mixture for 5 min at 4000 r.p.m. and 4 °C, the supernatant was transferred into another 15 ml glass centrifuge tube and 3 ml of dichloromethane was added. This mixture was shaken on a vortex mixer for about 10 s at high speed and then centrifuged for 5 min at 4000 r.p.m. (2840 g) and 4 °C. For chromatography, the upper, aqueous layer was transferred into an auto sampler vial, using a micropipette. The aqueous plasma extracts were investigated for ciprofloxacin using the following HPLC conditions: Chromatographic system: Mobile phase: isocratic: phosphate buffer: acetonitrile (50:50, v/v); flow rate 1.4 ml min⁻¹; wave length 229 nm; injection volume 150 µl. Each sample showed its corresponding chromatogram. From each chromatogram, the amount of plasma drug concentration was finally determined.²

Data analysis

The peak plasma concentrations (C_{max}), time to reach peak plasma concentration (T_{max}) were determined from the area under the concentration versus time curve. Elimination rate constant (k_{el}) was determined from the slope of AUC. AUC was determined from the trapezoidal rule. Biological half-life was determined from the elimination rate constant. Bioavailability was determined from peak plasma concentration.

RESULTS

Presence of ciprofloxacin in blood plasma sample

Blood plasma samples were analyzed by TLC method with response to standard ciprofloxacin. Each plasma sample showed characteristic retention factor, which are listed in Table 1.

From this table, we see that the average retention factor of each plasma sample and standard ciprofloxacin is almost same (0.54). Hence, we can say that each broiler blood plasma sample contains ciprofloxacin after single oral administration.

Table 1 Retention factor (R_f) of each plasma sample containing ciprofloxacin

Sample	Retention factor (R_f)
Sample 1	0.52
Sample 2	0.51
Sample 3	0.57
Sample 4	0.60
Sample 5	0.55
Sample 6	0.54
Sample 7	0.54
Sample 8	0.52
Average	0.54
Standard	0.54

Figure 1 Area under the plasma concentration versus time curve (AUC).

Table 2 Calculated pharmacokinetic data

Pharmacokinetic data	Value
C_{max}	$0.26 \pm 0.03 \text{ mg l}^{-1}$
T_{max}	$4.40 \pm 0.1 \text{ h}$
k_{el}	$0.13 \pm 0.02 \text{ h}^{-1}$
$T_{1/2}$	$5.25 \pm 0.02 \text{ h}$
AUC	$13.397 \pm 0.13 \text{ mg ml}^{-1} \text{ h}^{-1}$
V_d	$0.194 \pm 0.04 \text{ l kg}^{-1}$
Bioavailability	$49 \pm 0.48\%$

Abbreviation: AUC, area under the curve.

Table 3 Pharmacokinetics of ciprofloxacin in different species

Animal	Dose (mg kg ⁻¹)	Route	T _{1/2} (h)	V _d (area) l kg ⁻¹	AUC (μg h ⁻¹ ml ⁻¹)	C _{max}	T _{max}	F
Goats	4	i.v.	1.83	2.27	5.44	n/a	n/a	n/a
Goats	5	i.m.	2.5 ± 0.76	n/a	n/a	1.26 ± 0.25	0.20 ± 0.05	68.5
Sheep	7.5	i.v.	1.21 ± 0.07	1.89 ± 0.15	7.02 ± 0.53	n/a	n/a	n/a
Cows	10	i.v.	2.16 ± 0.29	2.84 ± 0.23	3.31 ± 0.38	n/a	n/a	n/a
Calves	2.8 ± 0.11	po	8.0 ± 1.4	n/a	2.1 ± 0.9	0.27 ± 0.13	3.0	53.14
Pigs	3.3	po	3.1	n/a	1.16	0.17	2	37.3
Horses	5	i.v.	2.63	3.45 ± 0.72	4.83 ± 1.08	n/a	n/a	n/a
Dogs	2.5	i.v.	3.0 ± 0.64	4.88 ± 0.68	2.30 ± 0.84	n/a	n/a	n/a
Dogs	5.0	i.v.	2.16 ± 0.78	3.06 ± 0.75	5.23 ± 1.90	n/a	n/a	n/a
Dogs	10.0	i.v.	2.55 ± 0.63	2.96 ± 0.43	12.93 ± 4.66	n/a	n/a	n/a
Dogs	10.0	po	4.90 ± 0.63	n/a	12.67 ± 1.56	1.55 ± 0.26	2.58 ± 0.18	n/a

Abbreviations: AUC, area under the curve; n/a, not available.

Determination of bioavailability and maximum plasma concentration, T_{max}, area under the concentration versus time curve (AUC), biological half-life of ciprofloxacin after single oral administration

Blood plasma samples were analyzed by ultra HPLC, after ensuring the presence of ciprofloxacin in each sample by TLC method. Each plasma sample showed corresponding chromatogram with response to standard ciprofloxacin which indicated the concentration of ciprofloxacin in each sample. Area under the plasma concentration versus time curve (AUC) is shown in Figure 1. The calculated pharmacokinetic data are presented in the Table 2. From this result it is shown that, peak plasma concentration (C_{max}) of ciprofloxacin, 0.26 ± 0.03 mg l⁻¹ was achieved at 4.40 ± 0.1 h (T_{max}). Biological half-life of ciprofloxacin was 5.25 ± 0.02 h. AUC was 13.397 ± 0.13 mg ml⁻¹ h⁻¹, elimination rate constant was 0.13 ± 0.02 h⁻¹, volume of distribution was 0.194 ± 0.04 l kg⁻¹ and bioavailability was 49 ± 0.48%.

DISCUSSION

During the last 20 years, various FQs such as enrofloxacin, danofloxacin, sarafloxacin, ciprofloxacin and marbofloxacin have been carefully investigated for veterinary application in the treatment of various bacterial infections. There are many papers published on the pharmacokinetics of these antibacterial agents in various animal species.¹³ Although the pharmacokinetics of ciprofloxacin after oral administration in broiler has been described in some literature,⁷⁻⁹ there is no previous literature on the TLC analysis of plasma sample containing ciprofloxacin after oral administration in broiler chickens; besides, there is no previous study to determine the concentration of ciprofloxacin for per hour interval. From Table 1, we see that the average retention factor of each plasma sample and standard ciprofloxacin is almost same (0.54). After TLC analysis, we tried to find out some pharmacokinetic data in the sample. In Table 3, we have shown pharmacokinetic parameter of ciprofloxacin in different species.¹⁴ The plasma half-life, t_{1/2}, after oral administration in broiler was estimated to be 5.25 ± 0.02 h, much higher than i.v. administration of ciprofloxacin and enrofloxacin^{15,16} and oral administration of sarafloxacin,¹³ but apparently shorter than oral administration of danofloxacin and enrofloxacin.^{17,18} This value is almost same to po administration of ofloxacin in broiler. Volume of distribution was 0.194 ± 0.04 l kg⁻¹, which was much lower than danofloxacin, enrofloxacin and ofloxacin.^{17,19} This small volume of distribution indicates that ciprofloxacin was not well distributed into tissue after po administration in broiler. Maximum concentration,

0.26 ± 0.03 mg l⁻¹, after po administration of ciprofloxacin in this study was observed at 4.40 ± 0.1 h. which is much higher than sarafloxacin, enrofloxacin, danofloxacin^{13,17,19} and ciprofloxacin.^{8,20} This higher value indicates that ciprofloxacin reached slowly to its peak concentration than sarafloxacin, enrofloxacin, danofloxacin and ofloxacin after po administration in broiler. Elimination rate constant (k_{el}) was calculated at 0.13 ± 0.02 h⁻¹. The bioavailability was 49 ± 0.48% which is lower than norfloxacin,²¹ ciprofloxacin,^{8,20} enrofloxacin, danofloxacin,¹⁸ ofloxacin^{19,22,23} and sarafloxacin¹³ after oral administration. This low bioavailability may be due to the result of feeding state. Area under the plasma concentration versus time curve (AUC) was estimated to be 13.397 ± 0.13 mg ml⁻¹ h⁻¹, was higher than sarafloxacin,¹³ danofloxacin, enrofloxacin¹⁸ and ciprofloxacin.²⁰

CONCLUSION

The pharmacokinetic study of ciprofloxacin in blood plasma of broiler chickens suggests that after a single oral administration of ciprofloxacin at 10 mg kg⁻¹, it reaches the peak plasma concentration (0.26 ± 0.03 mg l⁻¹) at 4.40 ± 0.1 h and a half-life of 5.25 ± 0.02 h. It also suggests that the feeding state may also affect the pharmacokinetic profile of ciprofloxacin in broiler blood plasma.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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