DISPOSITION KINETICS OF DIFLOXACIN IN NORMAL AND EXPERIMENTALLY INFECTED CHICKENS WITH *MYCOPLASMA GALLISEPTICUM*

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ABSTRACT

The pharmacokinetic properties of difloxacin were investigated following intravenous, intramuscular and oral administration of 10 mg/kg body weight, in non-infected and experimentally mycoplasma infected chickens. Serum concentrations of difloxacin were assayed microbiologically after intravenous, intramuscular and oral administrations. difl-oxacin residues were detected in chicken tissues following repeated oral administrations in non-infected and infected chickens.

Following a single intravenous injection, the serum difloxacin level was best approximated to follow a two-compartment open model. The elimination half-life (t0.5β) was 4.58 + 0.008 h. The volume of distribution at steady-state (Vdss) was 2.12 + 0.07 L/kg and the mean resid-ence time (MRT) was 3.91 + 0.07 h.

Following a single oral administration of 10 mg/kg b.wt difloxacin, the recorded results revealed that serum level of difloxacin was higher in infected chickens than non infected chickens. Serum concentrations peaked one hour post-drug administration with half lifes of absorption (t0.5ab) of 0.48 + 0.02 and 0.37 + 0.02 hour in non infected and mycoplasma infected chickens, respectively. The extent of serum protein binding of difloxacin in non-infected chickens was 15.44 + 1.24%. Systemic bioavailability after oral and intramuscular administration of difloxacin in non-infected chickens was 75.87 and 93.03%, respectively.

Following oral and intramuscular administration of 10 mg difloxacin/kg b.wt once daily for 5 consecutive days in infected and non infected chic-kens, the drug could be detected in all tested tissue up to 48 hours after last oral and intramuscular dose in non infected and infected chickens.

INTRODUCTION

Difloxacin is a new aryl-fluoroquinolone antimicrobial agent which is structurally similar to norfloxacin except for a P-fluorophenyl substituent at position 1 and a methyl group in piperazine ring ***(Stamm et al., 1986)***. It has been found to be very potent *in vitro* against wide variety of gram-positive and gram negative bacteria ***(Stamm et al., 1986)***. Difloxacin has an extended spectrum of activity against anaerobia, Chlamydia and Rickettsia due to the presence of four moieties at position 1 ***(Brown, 1996).***

Mycoplasmosis is ore of the major problems affecting poultry production.It causes chronic respiratory disease in chickens and is responsible for economic losses in the poultry industry due to reduction of weight gain and condemnation of carcasses at slaughter ***(Yoder, 1991).***

The efficacy of various chemotherapeutic agents in treatment and control of mycoplasmosis in poultry has been investigated ***(Domermuth and Johnson,1955;Newnham,1963;Gale et al.,1967 and Yoder, 1984).*** Difloxacin was equally effective as enrofloxacin in treating respiratory symptoms of chickens experimentally inoculated with *Mycoplasma gallisepticum* (MG) ***(Kempf et al., 1998)***. However, no available literatures are related to the effect of infection on difloxacin kinetics.

The present work was therefore designated to describe the influence of experimental infection with *Mycoplasma gallisepticum* on disposition kinetics of difloxacin in broiler chickens, as well as to determine the withdrawal period by detection of its residues in chicken tissues.

MATERIALS AND METHODS

Difloxacin:

It was obtained from Fort Dodge Animal Health Holland under the trade name dicural as 10% solution (each ml of dicural solution contain 100 mg difloxacin as hydrochloride).

Chickens:

Sixty Hubbard broiler chickens of 6 weeks of age were used in the present study. All chickens were examined by clinical and laboratory methods according to ***Jordan and Kulasegarum (1968)*** to be sure that they were free from Mycoplasma. The birds were raised under good hygienic measures, and fed on a balanced ration free from therapeutic agents. Water and food were free from antibacterial additives and water was available ad libitum. The chickens were weighed and the mean weights were ranged from 1600 to 1900 grams.

Organisms:

Tested organism:

E.coli(ATCC 11229)was obtained from Department of Bacteriology, Animal Health Research Institute, Dokki, Giza.

Challenge organism:

Mycoplasma gallisepticum (R-Strain) was obtained from Department of Bacteriology, Animal Health Research Institute, Dokki, Giza. *Mycoplasma gallisepticum* was isolated from a broiler flock and was confirmed to be *Mycoplasma gallisepticum* by growth inhibition test ***(Clyde, 1964)*.**

Grouping of chickens and experimental design:

1. Pharmacokinetic studies of difloxacin in non-infected chickens:

Thirty apparently healthy chickens were chosen randomly to study the pharamcokinetics of difloxacin. These chickens were divided into two equal groups. Each chicken in both groups was injected intravenously with difloxacin in the left wing vein in a single dose of 10 mg/kg b.wt ***(Kempf et al., 1998)***. These chickens were left for 15 days after intravenous injection to insure complete excretion of the drug from their bodies, then the chickens in the first group were administered difloxacin orally once daily for five consecutive days. Also, the chickens

in the second group were injected with difloxacin intramuscularly in the thigh muscle in a single dose of 10 mg/kg b.wt once daily for five consecutive days.

2. Pharmacokinetic studies of difloxacin in infected chickens:

Thirty chickens experimentally infected with *Mycoplasma gallisepticum* strain were used in two equal groups. The first group was administered the drug in a single oral dose of 10 mg/kg b.wt for five successive days. The second group was injected with the difloxacin intramuscularly in a dose of 10 mg/kg b.wt. once daily for 5 consecutive days.

Experimental infection:

Chickens were inoculated intratracheally with 0.1 ml (containing 10 CFU) *Mycoplasma gallisepticum* field isolate (FI). The infected chickens were left till the clinical symptoms appear in form of sneezing, cough and nasal discharge.

Sampling:

1. Blood samples: One ml of blood was taken from the right wing vein of each chicken after the single intravenous, oral and intramuscular injection of difloxacin in non-infected and infected chickens. Blood samples were collected at 5, 10, 15, 30 min., 1, 2, 4, 8, 12 and 24 hours. After each oral and intramuscular dose of difloxacin for 5 consecutive days in non-infected and infected chickens, the samples were collected daily at 1/2, 1, 2, 4 hours.

2. Tissue samples: After the end of 5^{th} day of repeated oral and intramuscular administration of difloxacin in non-infected and infected groups, three chickens were slaughtered at 24 hours following the last dose, then daily for four successive days. From each slaughtered chicken, heart, lung, liver, kidney, spleen, breast, thigh muscles, gizzard, and skin were taken for drug assay.

Preparation of samples:

The blood samples were allowed to clot and clear serum was separated by centrifugation at 3000 r.p.m. for 15 minutes. Two grams were mixed with 2 ml sterilized buffer and minced in porcelain mortar. The mixtures were centrifuged at 3000 r.p.m. for 15 minutes. The supernatants were transferred to sterilized test tubes for microbiological assay.

Analytical procedure:

Estimation of difloxacin concentration in the collected samples were carried out according to the method of ***Arret et al. (1971)*** using *E. coli* (ATCC 11229) as a tested organism. The serum protein binding of difloxacin was assayed by the method described by ***Lorian (1980).***

Pharmacokinetic analysis:

For estimating the pharmacokinetic parameters,the two-compartments open model after intravenous injection was applied ***(Ritschel, 1973 and Baggot, 1978)***. Mean and standard error of the obtained data were calculated according to ***Berly and Lindgren (1990)***.The pharmacokinetic parameters of difloxacin in experimentally mycoplasma infected chickens were compared with that recorded in non-infected chickens by students "t" test.

RESULTS

Following intravenous injection of 10 mg difloxacin/kg b.wt in non-infected chickens, the serum concentration-time data were described by the two compartments open model (Fig.1).The drug was rapidly distributed and slowly eliminated with half-lifes of distribution and elimination of 0.30 $\pm$ 0.01 and 4.58 $\pm$ 0.008 h respectively (Table 1). Difloxacin was highly distributed to the tissues with Vdss of 2.12 $\pm$ 0.07 L/kg and eliminated from the body by a total body clearance of 1.52 $\pm$ 0.07 ml/kg/min.

Following single oral administration of 10 mg difloxacin/kg b.wt. in non infected and mycoplasma infected chickens, the drug reached its maximum concentrations (T_{max}) at 1.60 ± 0.08 and 1.49 ± 0.05 hours in serum of non infected and infected chickens, respectively, with mean serum concentration (C_{max}) of 2.78 ± 0.08 µg/ml and 2.97 ± 0.134 µg/ml in non-infected and infected chickens, respectively (Tables 2). The absorption half-lives ($t_{0.5ab}$) were 0.48 ± 0.02 and 0.37 ± 0.02 hours and the elimination half-lives($t_{0.05\beta}$) were 5.25 ± 0.12 and 6.99 ± 0.09 hours in non infected and infected chickens, respectively. The results showed a significant increase in serum concentrations of difloxacin and its half-life of elimination in infected chickens compared to non-infected chickens.

Following a single intramuscular injection of 10 mg difloxacin/kg b.wt in non infected chickens, the absorption half life ($t_{0.5ab}$) was 0.23 ± 0.006 hours. Difloxacin reaches its maximum concentration (C_{max}, 3.80 ± 0.09 µg/ml) at maximum time (T_{max}) 1.04 ± 0.02 hours. It is eliminated from the body with elimination half life ($t_{0.5\beta}$) of 4.36 ± 0.09 hours (Table, 3)

Following oral administration of 10 mg difloxacin/kg b.wt once daily for 5 consecutive days in non infected and mycoplasma infected chickens.

The results revealed that the drug was efficiently distributed in all tested tissues with the highest concentration in kidney (2.1 ± 0.11 µg/gm and 3.20 ± 0.04 µg/gm), liver (1.82 ± 0.01 µg/gm and 3.30 ± 0.03 µg/gm) and lung (1.44 ± 0.03 µg/gm and 3.35 ± 0.05 µg/gm) in non infected and infected chickens, respectively after 24 hours from the last oral dose.

The results showed a significant increase of drug concentrations in most tissue and organs in infected chickens compared to those in non-infected chickens (Table 4).

Difloxacin was detected in all tested tissues up to 48 hours after the oral dose in non-infected and infected chickens. After 120 hours following last oral administration, the drug was detected only in liver and kidneys in non-infected chickens while in experimentally infected chickens, the drug was detected in heart, lung, liver, kidneys and gizzard.

The systemic bioavailability was 75.87% and 93.03% on oral and intramuscular administration in non infected chickens, respectively. The serum protein binding of difloxacin was 15.44%.

Table (1): Pharmacokinetic parameters of difloxacin following a single intravenous injection of 10 mg/kg b.wt in non infected chicken (n = 10).

Parameters	**Unit**	$\overline{X}$ **± S.E.**
C^o	µg/ml	23.56 ± 1.16
A	µg/ml	20.78 ± 1.12
α	h^{-1}	2.92 ± 0.09
$t_{0.5(\alpha)}$	h	0.30 ± 0.01
V_c	L/kg	0.53 ± 0.03
$Vd_{(area)}$	L/kg	3.15 ± 0.09
$Vd_{(ss)}$	L/kg	2.12 ± 0.07
$Vd_{(B)}$	L/kg	4.74 ± 0.17
K_{12}	h^{-1}	1.47 ± 0.07
K_{21}	h^{-1}	0.49 ± 0.02
B	µg/ml	2.71 ± 0.1
β	h^{-1}	0.19 ± 0.003
$t_{0.5\,\beta}$	h	4.58 ± 0.008
Cl_{tot}	ml/kg/min	1.52 ± 0.07
AUC	µg/ml/h	27.72 ± 0.75
MRT	h	3.91 ± 0.07

Table (2): Pharmacokinetic parameters of difloxacin following a single oral administration of 10 mg/kg b.wt in non-infected and experimentally infected chickens with Mycoplasma gallisepticum strain (n = 10).

Parameter	Unit	Chickens	
		Non-infected	Infected
B.Wt	Kg	1.84±0.02	1.67±0.01***
A	μg/ml	3.87±0.16	4.24±0.21
$K_{(ab)}$	h^{-1}	1.82±0.08	2.73±0.15***
$t_{0.5(ab)}$	h	0.48±0.02	0.37±0.02**
B	μg/ml	3.20±0.11	3.17±0.15
K_{el}	h^{-1}	0.17±0.03	0.14±0.003
$t_{0.5\ (\beta)}$	h	5.25±0.12	6.99±0.09***
$C_{max\ calc}$	μg/ml	2.78±0.08	2.97±0.13
$T_{max\ calc}$	h	1.60±0.08	1.49±0.05
AUC	μg/ml/h	21.06±0.79	25.38±1.12**
MRT	h	7.99±0.27	10.68±0.40***

**** P < 0.01** ***** P < 0.001**

Table (3): Pharmacokinetic parameters of difloxacin following a single intramusuclar injection of 10mg/kg b.wt in non infected chicken previously given the same dose intravenously (n = 10).

Parameters	Unit	$\overline{X}$ ± S.E.
B.Wt	Kg	1.66 ± 0.03
A	μg/ml	6.32 ± 0.2
$K_{(ab)}$	h^{-1}	3.71 ± 0.09
$t_{0.5(ab)}$	h	0.228 ± 0.006
B	μg/ml	4.47 ± 0.12
K_{el}	h^{-1}	0.20 ± 0.003
$t_{0.5\ (\beta)}$	h	4.36 ± 0.09
$C_{max\ calc}$	μg/ml	3.80 ± 0.09
$T_{max\ calc}$	h	1.04 ± 0.02
AUC	μg/ml/h	25.56 ± 0.45
Cl_{tot}	ml/kg/min	0.73 ± 0.02
I.B.D.	h	24.75 ± 0.46
Vd	L/kg	4.12 ± 0.12
MRT	h	6.21 ± 0.17

Table (4): Tissue concentrations of difloxacin (μg/gm) following oral administration of 10 mg/kg b.wt once daily for 5 consecutive days in non-infected and experimentally infected chicken with Mycoplasma gallisepticum strain (n = 3).

Tissue	Time of slaughter following last dose of administration					
	24 h		48 h		72 h	
	N	I	N	I	N	I
Heart	0.72±0.04	1.42±0.04***	0.29±0.02	0.75±0.05***	0.06±0.002	0.25±0.01***
Lung	1.44±0.03	3.35±0.05***	0.48±0.03	2.01±0.03***	0.34±0.02	1.56±0.05***
Liver	1.82±0.01	3.30±0.03***	1.06±0.3	1.99±0.03***	0.78±0.03	1.74±0.03***
Kidney	2.1±0.11	3.2±0.04***	1.03±0.04	2.35±0.04***	0.90±0.08	1.78±0.04***
Spleen	0.37±0.01	0.42±0.05	0.16±0.01	0.31±0.02**	-	0.12±0.01
Breast Ms	0.32±0.02	1.2±0.01***	0.98±0.03	0.64±0.03**	-	0.38±0.01
Thigh Ms	0.25±0.02	0.85±0.03***	0.07±0.001	0.68±0.04***	-	0.19±0.002
Gizzard	1.04±0.04	1.45±0.01***	0.69±0.03	1.12±0.01***	0.08±0.001	0.68±0.01***
Skin	-	0.12±0.001	-	0.06±0.001		-

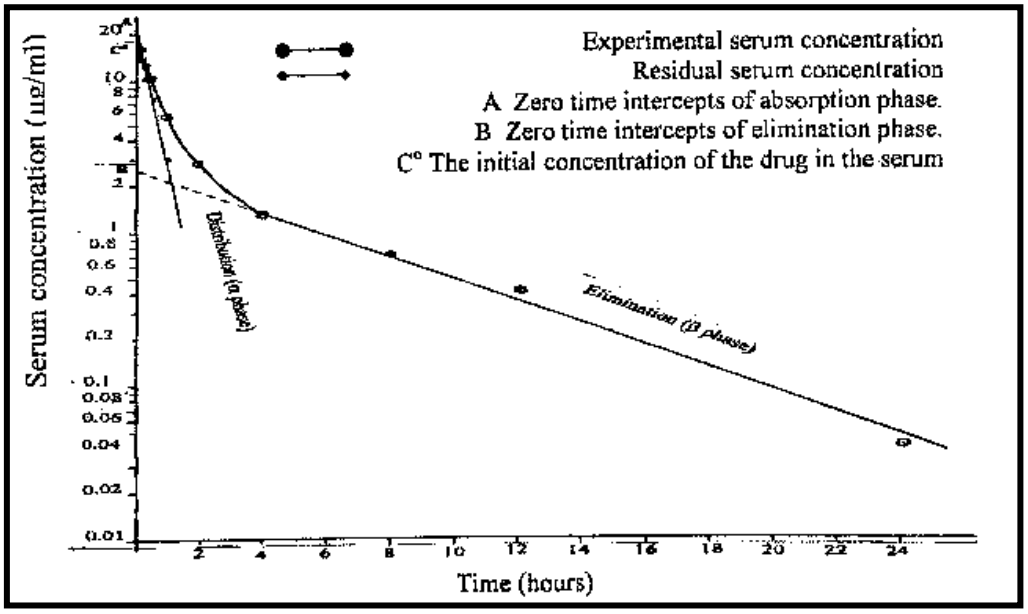

Fig. (1): Semilogarithic graph depicting the time course of difloxacin in serum following a single intravenous injection of 10 mg/kg b. wt. in non-infected chickens.

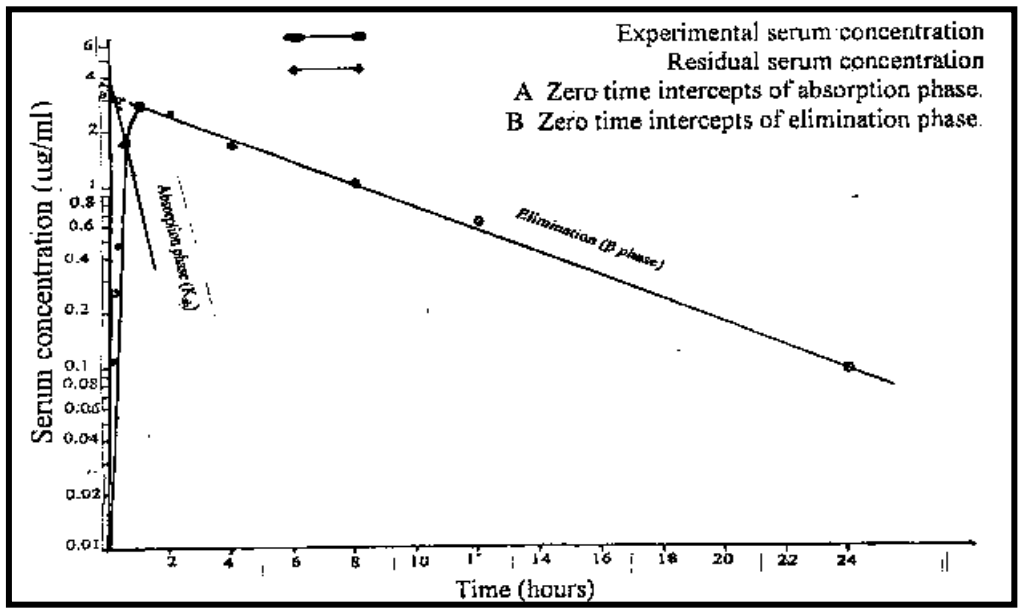

Fig. (2): Semilogarithic graph depicting the time course of difloxacin in serum following a single oral administration of 10 mg/kg b.wt.in non-infected chickens peviously given the same dose by a single intravenous injection.

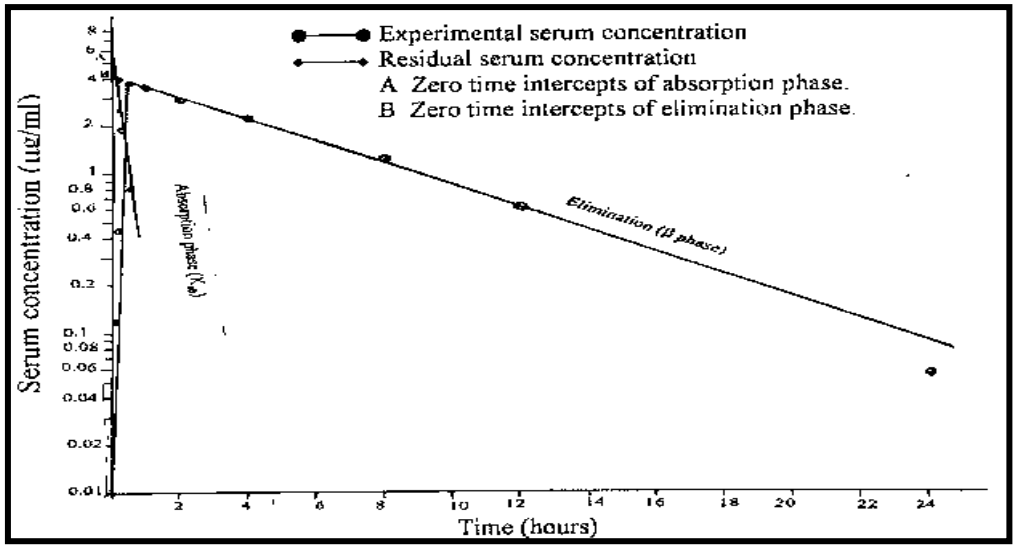

Fig. (3): Semilogarithic graph depicting the time course of difloxacin in serum following a single intravenous injection of 10mg/kg b.wt.in non-infected chickens peviously given the same dose by a single intravenous injection.

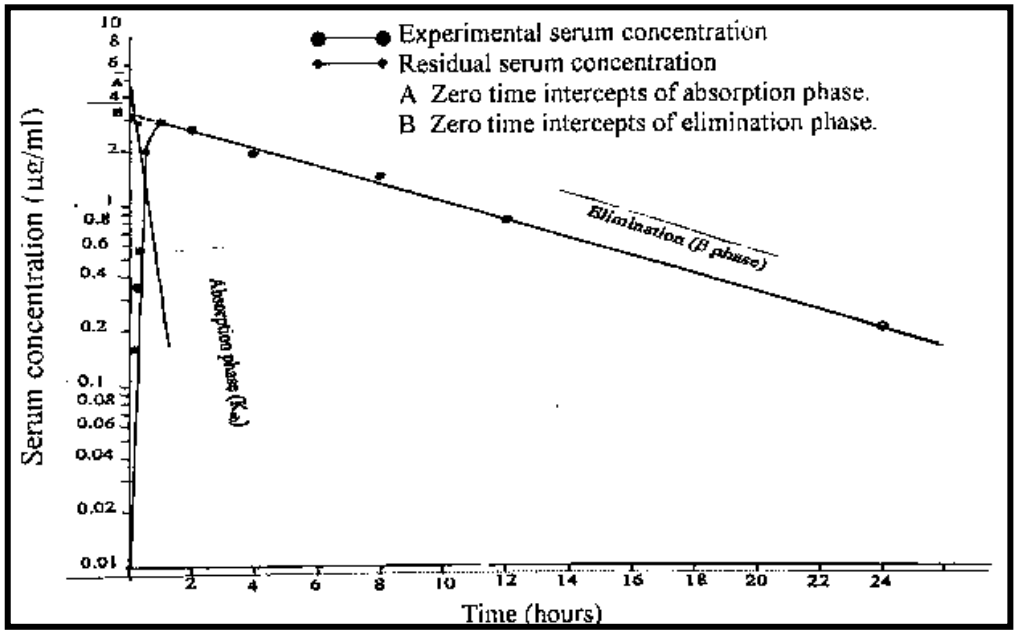

Fig. (4): Semilogarithic graph depicting the time course of difloxacin in serum following a single oral administration of 10mg/kg b.wt.in non-infected chickens.

DISCUSSION

In the present study, serum pharmacokinetic variables and tissue residues of difloxacin in normal chickens were investigated after single intravenous, oral and intramuscular, as well as repeated oral of 10 mg/kg b.wt difloxacin.

Our findings showed that the pharmacokinetic profile of difloxacin after intravenous injection in chickens in a single dose of 10 mg/kg was described by a two compartment open model.This finding is in agreement with the result previously recorded for difloxacin in pigs and chickens ***(Inui et al., 1998)*** in goats ***(Atef et al., 2002)*** and for enrofloxacin in chickens ***(Abd El-Aziz et al., 1997)*** and ciprofloxacin in chickens***(Atta and Sharif, 1997)***.

Difloxacin was rapidly distributed with distribution half-life ($t_{0.5\alpha}$) of 0.30 ± 0.01 hours and slowly eliminated with elimination half-life ($t_{0.5\beta}$) of 4.58 ± 0.008 hours. The obtained result of distribution half-life is nearly consistent with those reported in goats ($t_{0.5\alpha}$ = 0.22 ± 0.2 h) by ***Atef et al. (2002)***. However, it is shorter than the values reported for chickens (0.66 h), by ***Inui et al. (2002)***; pig (0.50 h), by ***Inui et al. (1998)*** and ciprofloxacin in chickens (30.49 ± 5.68) min., by ***Atta and Sharif (1997)***. The elimination half life ($t_{0.5\beta}$ = 4.58 ± 0.008 h) was similar to that reported in chickens (4.10 h), by ***Inui et a. (1998)*** and that of enrofloxacin in chickens (3.81 ± 0.24 h), by ***Abd El-Aziz et al. (1997)*** and was shorter than values reported for pigs (7.92 h), by ***Inui et al. (1998)***, goats (6.3 ± 0.11 h), by ***Atef et al. (2002)***.

The volume of distribution at steady-state (Vd_{ss}) is an accurate indication for the diffusion of the drug in the body tissues ***(Galinsky and Svenson ,1995)***. The Vd_{ss} was large (2.12±0.07 L/kg),this value is nearly consistent with those previously investigated for enrofloxacin in chickens (2.40 ± 0.16 L/kg), by ***Abd El-Aziz et al. (1997)***, and difloxacin in chickens(3.06 L/kg),by ***Inui et al. (1998)***. On the other hand, this value differ greatly from that found in goats (1.1 ± 0.012 L/kg) by ***Atef et al. (2002)***, pigs (1.70 L/kg), by ***Inui et al. (1998)*** and that for ciprofloxacin in chickens (1.132 ± 0.04), by ***Atta and Sharif (1997)***.

Difloxacin was cleared from the blood of non infected chickens at a lower rate of 1.52 ± 0.078 ml/kg/min. Comparable values were provided by **Atef *et al.* (2002)** 0.13 ± 0.0001 L/kg/h in goats, these differences may be related to the species variation.

Following oral administration of 10 mg difloxacin/kg b.wt as a single dose in non infected chicken, difloxacin produced a maximum serum level of 2.78 ± 0.08 µg/ml. This concentration exceeded the minimum inhibitory concentration (MIC) for various sensitive bacteria. These MIC values ranged from 0.03 top 0.25 µg/ml (**Stamm *et al.*, 1986)**. This value was similar with that reported for man with a dose of 200 mg(2.17 µg/ml),

by ***Granneman et al. (1986)***. However, this value differs greatly from that found in mice (22.4 µg/ml), by ***Fernandes et al. (1986)*** in chickens (0.7 µg/ml), by ***Dijkstra (1997)***, also in chickens (0.96 µg/ml) and pigs (3.61 µg/ml) by ***Inui et al. (1998)*** and in dogs (1.11 µg/ml), by **Heinen (2002)**. These difference may be attributed to dosage quantities, method of oral dosing and interspecies variation.

The maximum serum concentration (C_{max}) was achieved after about 1.60 $\pm$ 0.08 hours, which indicates rapid absorption of difloxacin. This result was nearly similar to 1.40 hours, 1.25 hours reported in chickens and pigs, respectively by ***Inui (1998)***.

The elimination half-life ($t_{0.5\beta}$) in the present investigation after oral administration of 10 mg difloxacin/kg b.wt. in normal chickens was 5.25 $\pm$ 0.12 hour.

This result is related to that reported for enrofloaxacin in chickens (4.29 $\pm$ 0.097) by ***Abd El-Aziz et al. (1997)***. On the other hand, this value was lower than that reported in chickens (7.35 hours) and pigs (11.8 hours) by ***Inui et al. (1998)*** and that reported in dogs (6.9 hours) by ***Heinen (2002)***. This difference is common in kinetic investigations and is often related to specific interspecies variations or in handling of samples ***(Haddad et al., 1985)***.

Following oral administration of 10 mg difloxacin/kg b.wt once daily for 5 consecutive days in non infected chickens, the serum concentration of the drug was gradually increased by time. Similar results were recorded for ciprofloxacin in chickens by ***Ebrahim (1999)*** and for danofloxacin in chickens by ***El-Ragaby (1999)*** and for enrofloxacin in chickens by ***El-Refaey (2000)***.

Following single intramuscular injection of 10 mg difloxacin/kg b.wt in normal chickens, the maximum serum concentration of 3.80 $\pm$ 0.09 µg/ml was achieved after about one hour (1.04 $\pm$ 0.02 hour) which indicated a rapid absorption after intramuscular injection. A similar result

(C_{max} = 3.76 ± 0.22 µg/ml) was recorded in goat ***(Atef et al., 2002)*** and related results were reported for enrofloxacin in chickens by ***Abd El-Aziz et al. (1997) and El-Refaey (2000)***.

A single oral administration of difloxacin 10 mg/kg b.wt in experimentally infected chickens with *Mycoplasma gallisepticum* produced a higher maximum blood level (2.97 ± 0.13 µg/ml) than that in normal chickens (2.78 ± 0.08 µg/ml). Similar results were reported for ciprofloxacin in chickens by ***Ebrahim (1999).*** The maximum serum concentration of difloxacin in infected chickens was achieved at 1.49 ± 0.05 hours. This was shorter than the value reported in normal chickens which indicated that the rate of drug absorption increased in infected chickens than non-infected chickens.

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