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Research paper

Bioavailability, pharmacokinetics and tissue residues of cephradine (Atocef Forte®) in healthy and colisepticemic broiler chickens

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Abstract

The pharmacokinetics (after single intravenous and oral dose) and tissue residues (orally and daily for five days) of cephradine (20 mg/kg b.wt.) were investigated in healthy and experimentally *E.coli* infected broiler chickens. Following single intravenous injection to healthy chickens, cephradine obeyed a two compartments open model and the elimination half-life ($t_{1/2\beta}$), volume of distribution (V_{dss}) and total body clearance (CL_{tot}) of cephradine were 2.93 h, 321.5 ml/kg and 0.08 L/h/kg, respectively. Following single oral administration of cephradine to healthy chickens, the peak serum concentration (C_{max}) of it was 26.7 µg/mL and achieved (T_{max}) at 2.41 h. The oral bioavailability of cephradine was 87.7%. Cephradine was assayed in kidney, liver, heart, gizzard, spleen, breast muscle, thigh muscle and skin after 24, 48, 72, 96 and 120 h after last dose. On conclusion, cephradine is a good choice for treatment of colisepticemia in chickens due to its higher oral bioavailability and distribution.

Keywords: *Pharmacokinetics; Tissue Residues; Cephradine; Chickens; Bioavailability.*

1. Introduction

Cehpradine is a first generation cephalosporin antibiotic. Its activity includes Gram-positive bacteria and Gram-negative cocci, actinomyces and spirochaetes. Cephradine has the advantage of being active against penicillinase-producing staphylococci although not against methicillin-resistant strains or penicillin resistance *Streptococcus pneumonia* among Gram-negative bacteria. Cephradine has activity against some entero bacteria, including strains of *E coli, Klebsiella pneumonia, proteus mirabilis,* Salmonella and Shegella species. It is also active against influenza, moraxella and Neisseria species (Martindale, 1993). Cephradine is stable to gastric acid when given orally (Griffith and Black, 1970); peak blood levels are reached within one hour.It is widely distributed in tissues, and high concentrations are found in all organs, especially liver and kidney. Cephradine is reported to be about 15 to 20 % protein bound (Wise, 1990) and excreted unchanged in urine in the range of 69 to 100 % (Griffith, 1983 and Wise, 1990).Approximately 26 % are excreted through glomerular filtration, and about 33 % are execrated by tubular secretion (Foord et al, 1969). One percent of the drug is recovered in the bile (Wise, 1990). Cephradine half-life is reported to range from 0.6 to 1.8 hours (Wise, 1990).

The pharmacokinetics of cephradine were investigated in mice, rats, dogs (Weliky et al., 1974), human (Rattie et al., 1976; Roberts et al., 1981), foals (Henry et al., 1992), goats (El-Sayed et al., 1994) and chickens (El-Sayed et al., 2016).

To our knowledge, only a few studies about disposition of cephardine in broilers are available. Therefore, the present work was undertaken to study the pharmacokinetics and bioavailability of cephradine after IV injection and oral administration (in drinking water) in healthy broiler chickens. Also, residues of cephradine in chicken's tissues were studied in healthy and *E.coli* infected chickens.

2. Material and methods

2.1. Drug

Cephradine was used within this study under the trade name (Atocef Forte®). It is dispensed as a water-solublepowder. Each 100 gm of powder contains 20 gm cephradine base. It is produced by ATCO Pharma for Pharmaceutical Industries Co., Egypt. Pure cephradine powder for IV injection was obtained from the company.

2.2. Experimental birds

Forty five clinically Healthy Hubbard chickens (30 days old and weighing 1.60 – 1.85 kg) were used in the pharmacokinetics and tissues residues studies. Chickens were of both sexes and purchased from a local poultry farm. They were kept individually in thoroughly clean and disinfected cages within a well ventilated room with a suitable temperature and humidity according to their age. The birds were fed *ad libitum* with a commercial standard ration free of antibiotics, coccidiostats, and growth promoters before starting the experiment to ensure a complete clearance of any anti-bacterial substances from their bodies. Water was provided *ad libitum*. The experiments were performed in accordance with the guidelines set by the Ethical Committee of Faculty of Veterinary Medicine, Benha University, Egypt.

2.3. Experimental design

Chickens were divided into 3 groups:
Group (1): Five healthy chickens were injected cephradine at a dose level of 20 mg/kg b. wt. by IV route into the right wing vein. These chickens were left for 15 days after the IV injection to ensure complete elimination of cephradine from their bodies and then administered the same dose by oral route (in drinking water) to determine the bioavailability of cephradine in healthy chickens.
Group (2): twenty healthy chickens were administered Atocef Forte® orally (1 gm/Liter drinking water, corresponding to 20 mg cephradine/kg b. wt. once daily for five consecutive days, to determine tissue residues of cephradine in healthy chickens.
Group (3): twenty experimentally *E.coli* infected chickens were administered Atocef Forte® orally in the same way as in group (2) to determine tissue residues of cephradine in infected chickens.

2.4. Experimental infection

E.coli strain O_{78} serotype of poultry origin was obtained from Poultry Department, Animal Health Research Institute, Dokki, Giza, Egypt. The preparation of infecting dose was performed according to Shen et al. (2002), where it was 0.1ml from a concentration of 1×10^6CFU/ml. Chickens in group (3) were infected by subcutaneous injection of the infective dose. This group was left three days after infection until symptoms were observed (chickens suffering from severe diarrhaea, lack of appetite and ruffled feathers).

2.5. Collection of samples

2.5.1. Blood samples

About one milliliter of blood was withdrawn from the left wing vein at 0.083, 0.17, 0.42, 0.5, 1, 2, 4, 6, 8, 12, 24 h after single IV and oral administration (in drinking water) of cephradine. Blood samples were collected in sterilized tubes and allowed to clot. The samples were centrifuged at 1000 *g* for 5 minutes and then serum samples were collected and stored at -20°C until analysis.

2.5.2. Tissue samples

At the end of fifth day of repeated oral administration (1 gm/liter drinking water) of Atocef Forte®, three chickens were slaughtered from group (2) and group (3). From each slaughtered chicken, samples of kidney, liver, heart, gizzard, spleen, breast muscle, thigh muscle and skin were taken for assay of residues of cephradine at 24, 48, 72, 96, 120 h after last dosing.

2.6. Analytical procedure

Cephradine was assayed in chicken's serum and distilled water by using microbiological method using *Echerichia coli* ATCC 25922 as test organisms for cephradine (Arret et al., 1971). The test organism was obtained from Department of Microbiology, Animal Health Research Institute, Dokki, Giza, Egypt. Three plates were used for each sample. One well in each plate was filled with reference concentration (12.5 µg/ml of cephradine in distilled water or normal chicken's serum). The plates were incubated at 37˚C for 24 h then the diameter of inhibitory zones was measured. The average diameter of inhibition zone of the samples was corrected by using the diameter of the reference concentration. From the standard curve, the concentration corresponding to the correct values of the zone diameter were obtained.
For assay of tissue samples, two grams of tissue were homogenized by automatic homogenizer with 2 ml of distilled water. Mixtures were centrifuged at 1000 g for 10 minutes and the supernatant fluid of each sample was obtained and directly assayed microbiologically for cephradine concentration.

2.7. Pharmacokinetic analysis

Pharmacokinetics parameters were calculated by Winnonlin program, and other parameters according to (Ritchel, 1973; Baggot, 1978 a,b).

2.8. Statistical analysis

The obtained data were expressed as mean ± S.E. This data were analyzed statistically using Student *t*-test to express the differences between healthy and infected groups (Snedecor and Cokran, 1980).

3. Results

Following a single IV injection of 20 mg pure cephradine/kg b. wt. in healthy chickens, cephradine could be detected therapeutically for 12 h post injection. The serum concentration-time curve of cephradine following IV injection showed that cephradine obeyed a two compartments open model. The disposition kinetics of cephradine following a single IV and oral administration (in drinking water) were recorded in table (1) and showed in figure (1).
Tissue samples from liver, kidney, spleen, heart, breast muscle, thigh muscle, skin and fats were taken for assaying of residues of cephradine at 24, 48, 72, 96 and 120 h after the last oral dose of the drug from healthy chickens were compared to those in *E.coli* infected chickens (Table 2).

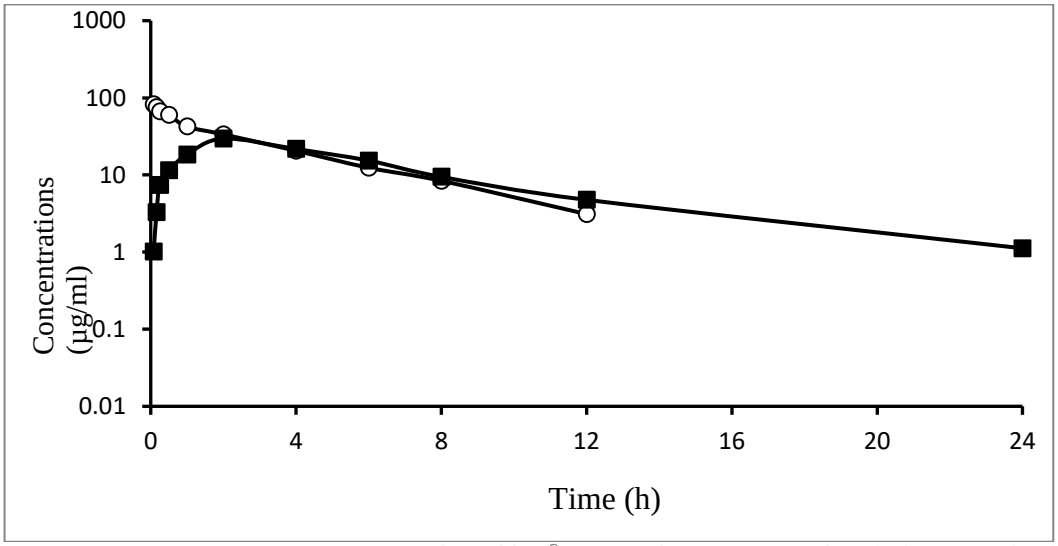

Fig. 1: Semi-logarithmic graph depicting the time-concentration of atocef forte® in serum of broiler chickens after asingle IV (○) and oral (■) administration (1 g/ liter drinking water) of 20 mg cephradine/kg b.wt. (n=5).

Table 1: Mean ± SE serum pharmacokinetic parameters of atocef forte® in healthy chickens following a single IV and oral administration (in drinking water) of 20 mg cephradine/kg b.wt. (n=5).

Parameter	Unit	IV	Oral (in drinking water)
α (k_{ab})	h^{-1}	2.45 ± 0.37	0.58±0.01
$t_{1/2\alpha}$ ($t_{1/2ab}$)	h	0.28 ± 0.03	1.18 ± 0.02
β (k_{el})	h^{-1}	0.236 ± 0.005	0.13 ± 0.006
$t_{1/2\beta}$ ($t_{1/2el}$)	h	2.93 ± 0.11	5.31 ± 0.27
AUC	µg ml^{-1} h^{-1}	243.7 ± 23.7	213.1 ± 17.4
AUMC	µg ml^{-1} h^{-2}	954.07±29.8	1580.5±36.8
MRT	h	3.91±0.21	8.60±0.47
V_{dss}	l kg^{-1}	321.5±14.8	—
Cl_{tot}	l kg^{-1} h^{-1}	0.08±0.004	—
C_{max}	µg ml^{-1}	—	26.7 ± 2.65
t_{max}	h	—	2.41 ± 0.12
F	%	—	87.6±8.41

α; β hybrid rate constant representing the slope of distribution and elimination phase after IV injection; K_{ab}; K_{el} absorbtion and elimination rate constant after oral administratin; $t_{1/2(\alpha)}$ distribution half-life after IV injection; $t_{1/2(ab)}$ absorption half-life after oral administration; $t_{1/2(\beta)}$ elimination half-life after IV injection; $t_{1/2(el)}$ elimination half-life after oral administration; AUC area under concentration-time curve; AUMC area under moment curve; MRT mean residence time; V_{dss} volume of distribution at steady state; Cl_{tot} total body clearance. C_{max} maximum serum concentration; T_{max} time to peak serum concentration; F fraction of drug absorbed systemically after oral adminstration.

Table 2: Tissue concentrations (Mean ± SE) of atocef forte® (µg/g) in healthy (H) and experimentally *E.coli* infected chickens (I) during repeated oral administration (1 g/liter) of 20 mg cephradine/kg b.wt.once daily for 5 consecutive days (n=3).

Tissues	After 24 h		After 48 h		After 72 h		After 96 h		After 120 h	
	H	I	H	I	H	I	H	I	H	I
Heart	-	-	-	-	-	-	-	-	-	-
Liver	24.9± 1.98	14.78±0.71	18.87± 1.46	10.01± 1.51	11.32± 0.18	5.87±0.12	4.23± 0.15	2.14±0.21	-	-
Spleen	-	-	-	-	-	-	-	-	-	-
Kidney	22.1± 1.44	5.97± 1.13	3.54± 0.32	1.39± 0.11	1.28± 0.10		-	-	-	-
Fat	-	-	-	-	-	-	-	-	-	-
Skin	-	-	-	-	-	-	-	-	-	-
Breast muscle	6.45± 0.89	3.65± 0.46	2.26± 0.31	1.47± 0.21	-	-	-	-	-	-
Thigh muscle	5.12± 0.49	3.46± 0.41	2.28± 0.27	1.31± 0.19	-	-	-	-	-	-

4. Discussion

In the present investigation, IV injection of cephradine at a dose level of 20 mg/kg b. wt. in healthy chickens showed that, the disposition best fitted by a two compartments open model. The obtained result was consistent with that reported for cephradine in human (Rattie et al., 1976) and goats (El-Sayed et al. 1994).

In this study, the V_{dss} for cephradine was 321.53 mL/kg, suggesting a higher penetration through biological membranes and tissue after IV injection in broiler chickens. The obtained V_{dss}value was higher than the data of cephradine(142.4 mlL/kg)in goats (El-Sayed et al. 1994). On the other hand, volume of distribution was lower than those recorded for cephradine in human 17.4 L/kg (Roberts et al. 1981) and higher than those recorded for ceftriaxone in dogs (0.217 L/kg; Rebuelto et al. 2002), ceftiofur in calves 0.134 L/kg; El-Gendy et al. 2007), cefquinome in ducks, goats and chickens 0.41, 0.51, 0.49 L/kg, respectively (Yuan et al. 2011; Dumka et al. 2013; Xie et al. 2013).

The elimination half-life ($t_{1/2(\beta)}$) of cephradine following a single IV injection of 20 mg/kg b. wt. was equal to 2.93 h. This observation agreed with the data (2.41 h) reported after IV administration of cefpirome in cow calves (Patel et al. 2013).On contrast, this obtained value was longer than that (1.02 h) recorded for ceftriaxone in cows (Kumar et al., 2010). In addition, it was shorter than that (4.00 h) reported in cephradine in goats (El-Sayed et al., 1994).

The rate of total body clearance (CL_{tot}) of cephradine following IV injection was 0.08 L/kg/h. This value was close to the value reported for ceftiofur (0.051 L/kg/h) in cows (Tohamy et al. 2008).

Following a single oral administration of cephradine (in drinking water) at a dose of 20 mg/kg b. wt., maximal serum concentration (C_{max}) was 26.71 µg/ml achieved at (t_{max}) 2.41 h. These values were higher than those recorded for cefquinome in rabbits where C_{max} was 8.12 µg/ml and t_{max} was 1.01 h (Shalaby et al. 2014).

The bioavailability of cephradine in healthy chickens, which estimated the rate and extent of the dose, entered the systemic circulation after oral administration was 87.7%. This percent indicated a good absorption of cephradine after oral administration. This value was similar to that recorded for cefotaxime in Muscovy ducks 79.6% (Aboubakr, 2016).

Repeated oral administration of 20 mg cephradine/kg b. wt. every 24 h (in drinking water) for five consecutive days in healthy and experimentally *E.coli* infected chickens revealed that, cephradine could be detected in liver, kidney, muscles (breast & thigh). This result slightly agreed with that recorded after oral administration to rats, cephradine was distributed widely throughout the body tissues, with the greatest concentrations in the kidneys and liver and cephradine concentrations in the kidneys and livers were about 8 and 3 times higher, respectively, than those in plasma (Weliky et al. 1974). Also, oral administration of cephradine in broiler chickens twice daily for five consecutive days revealed that, treated chickens must not be slaughtered before 6 days from last dose of repeated administration of cephradine (Elsayed et al., 2016).

5. Conclusions

The oral bioavailability of cephradine is excellent, so it is recommended to be used against colisepticemic infection. Treated chickens must not be slaughtered before 4 days from last dose of repeated administration of cephradine (Atocef Forte®) to withdraw the drug residues from all tissues of treated chickens.

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