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Pharmacokinetics and bioavailability of azithromycin following intramuscular and oral administrations in broiler chickens

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ABSTRACT

The pharmacokinetics azithromycin were investigated in broiler chickens after intravenous (i.v.), intramuscular (i.m.) and oral (p.o.) administrations to estimate an appropriate dosage regimen of azithromycin. Moreover, to determine the bioavailability after the extravascular routes and the serum protein binding capacity with azithromycin's molecules. Three equal groups of 5 chickens each were given a single dose of 20 mg/kg body weight (bw) of azithromycin via i.v., i.m. and p.o. administrations. Serum concentrations of azithromycin were determined by a modified agar diffusion bioassay using *Bacillus subtilis* ATCC 6633 as the test organism. Following compartmental analysis, a three-compartment open model best described the concentration-time data of azithromycin after i.v. administration. The total body clearance (Cl_{tot}) was 0.77 L/kg/h the volume of distribution at steady-state (V_{dss}) was 47.75 L/kg and the value of the elimination half-life ($t_{1/2l3}$) was 31.91 h. After i.m. administration, the elimination half-life ($t_{1/2el}$) and mean residence time (MRT) were significantly higher (38.95 h and 47.16) than after p.o. route (31.50 h and 39.93 h), respectively. Azithromycin was bound to the extent of 24.42 % to serum protein of chickens. The absolute bioavailabilities were 95.17 and 83.52 % after i.m. and p.o. administrations, respectively. Based on the fortunate pharmacokinetic characteristics, a single dose of azithromycin at 20 mg/kg (bw) via i.m. and p.o. administrations every 72 h for susceptible bacterial infections in chickens is greatly recommended.

KEYWORDS

Pharmacokinetics;
Azithromycin;
Bioavailability;
Chickens.

INTRODUCTION

Clinical pharmacology of therapeutic agents in avian species is an area of research that is needed to insure proper dosing and treatment. Azithromycin is classified as an azalide, a subclass of macrolide antimicrobials[1] with a broad spectrum of activity in vitro against many potential bacterial pathogens including spirochetes, anaerobes, and *Chlamydia trachomatis*[2]. Additionally, azithromycin have *in vitro* activity against enteric bacterial pathogens, including C*ampylobacter* spp. and enteropathogenic/enterotoxigenic *Escherichia coli*, *Shigella* spp., and *Salmonella* spp[3,4]. Despite macrolides generally being considered bacteriostatic, azithromycin has established *in vitro* bactericidal activity against a variety of intracellular pathogens and has

been used for treatment of toxoplasmosis, borreliosis, cryptosporidiosis, chlamydophilosis, and mycobacteriosis (*Mycobacterium avium complex*) in humans[5,6]. Azithromycin is also much more stable in an acid environment than erythromycin[7] and it has wider distribution and a longer elimination half-life[8]. Pharmacokinetics of azithromycin has been studied in humans[9], experimental animals several animal[10], cats[11], foals[12], goats[13], dairy cows[14] and rabbits[15]. The properties of azithromycin suggest that it may be useful in the treatment of various infectious diseases in chickens. Treatment with azithromycin potentially offers the advantage of less frequent administration over a shorter duration because its synergism with serum components and intracellular enzymes, increasing antibiotic uptake by phagocytes and efficacy of intracellular bactericidal enzymes[16]. Because of limited data on the use of azithromycin in avian medicine, Because of limited data on the use of azithromycin in avian medicine, this study was designed to determine the pharmacokinetics of azithromycin in broiler chickens after i.v., i.m. and p.o. administrations to estimate an appropriate dosage regimen of azithromycin. Moreover, to investigate the bioavailability after the extravascular routes and to determine the serum protein binding capacity with azithromycin's molecules.

MATERIALS AND METHODS

Drug

Azithromycin for injection (Zithromax®, Pfizer Labs, New York, NY 10017, USA), is supplied in lyophilized form in a 10-mL vial equivalent to 500 mg of azithromycin for i.v. administration. Reconstitution, according to label directions, results in approximately 5 mL of Zithromax for injection with each mL containing azithromycin dihydrate equivalent to 100 mg of azithromycin.

Chickens

Eighty female broiler chickens (Hubbard breed), 40–45 days old, weighing between 2 and 2.5 kg, were obtained 2 weeks before the start of the study. During acclimatization (at least 2 weeks before starting the experiment to ensure the complete withdrawal of any residual drugs) and subsequent treatment periods, all chickens had free access to water and antibacterial-free food. The animal house temperature was maintained at 22±2^{0}C and humidity at 40–55%. The study was approved by the Animal Care and Use Committee at the Faculty of Veterinary Medicine, Cairo University.

EXPERIMENTAL DESIGN

Chickens were individually weighed before drug administration and doses were calculated precisely for each bird. Fifteen chickens were allocated to three equal groups of 5 each. Birds in all groups were given a single dose of azithromycin at 20 mg/kg through i.v., into the left brachial vein, i.m. administration through pectoral muscles, respectively and orally via a gavage tube.. All chickens had free access to water and food during experiment.

Blood samples (1–1.5 mL) were collected from brachial and cutaneous ulnar veins at time 0 (pretreatment) and at 5, 15 and 30 min and 1, 2, 4, 6, 8, 12, 24, 48, 72 and 96 h after drug administration. The samples were left to clot at room temperature then centrifuged at 1500*g* for 15 min to obtain clear serum and were kept frozen at -70^{0}C until analyzed.

Analytical procedure

Serum concentrations of azithromycin were measured by using a modified agar diffusion bioassay[17] using *Bacillus subtilis* ATCC 6633 as test organism and Mueller–hinton agar (Difco, Detroit, MI, USA). Five wells, 6 mm in diameter, were made in a standard Petri-dish plate (120 mm) containing 25 mL inoculated agar. Wells were filled with tested serum and tissue extracts samples or azithromycin standard. Zones of inhibition were measured after 18 h of incubation at 37^{0}C and the concentrations of azithromycin were calculated from the standard curve. Standard curves of azithromycin were prepared in antibacterial-free chicken's serum by the appropriate serial dilution. Standard curves were derived using azithromycin concentrations ranging from 0.039 to 10 µg/mL. A mean zone diameter (derived from four or five measurements) was used to calculate each drug concentration. Separate calibration standard curves for azithromycin were prepared in serum from control chickens, on the same day. Calibration graphs were constructed by plotting the mean diameters of the inhibition zones against the logarithm of azithromycin concentrations. The semilogarithmic

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plots of the inhibition zone diameters vs standard azithromycin concentrations in serum were linear from 0.039 to 10 µg/mL and had coefficients of determination of 0.99 ± 0.15. Assay validation was performed by analyzing replicates of blank serum fortified with azithromycin at four levels of concentration depending on the route. The intra-assay coefficient of variation was 4.3%. The inter-assay precision of the assay was evaluated by processing 6 replicates aliquots of drug-free chicken serum samples containing the four levels of azithromycin concentrations on different days. The inter-assay coefficient of variation was 8 %. The limit of detection was 0.01 µg/mL and the limit of quantification was 0.02 µg/mL.

In vitro serum protein binding

The extent of protein-binding was determined *in vitro* using the method of Craig and Suh[18] which is based on the diffusion of the free antibiotic into the agar medium. The drug was dissolved in phosphate buffer (pH 6.2) and antibiotic-free chicken's serum at concentrations of 0.3125, 0.625, 1.25, 2.5 and 5 µg/mL. The differences in the diameter of the inhibition zone between the solutions of the drugs in the buffer and serum were calculated. The percentage of protein bound fraction was calculated according to the following equation:

$$\text{Protein binding \%} = \frac{\text{Zone of inhibition in buffer} - \text{Zone of inhibition in serum}}{\text{Zone of inhibition in buffer}} \times 100$$

Pharmacokinetic analysis

Serum concentrations of azithromycin after i.v., i.m. and p.o. administrations were subjected to a compartmental analysis using a nonlinear least-squares regression analysis with the help of a computerized curve-stripping software package (R Strip; Version 5.0; Micromath Scientific Software, Salt Lake City, UT, USA). Data were examined by sequential weighted nonlinear regression. Monoexponential, biexponential and triexponential equations were fitted to individual serum concentration–time data. The data were analyzed on an individual chicken basis using a weighting of 1/ concentration. Akaike's Information Criterion (AIC)[19], coefficient of determination, residual sum of squares and analysis of residuals plots were used to select the best equation that define serum concentration-time data for each chicken. The distribution and elimination half-lives ($t_{1/2\alpha}$ and $t_{1/2\beta}$), the volume of distribution at steady state (V_{dss}) were calculated according to standard equations[20]. The total body clearance was calculated as Cl_{tot} = Dose/ AUC. The following parameters were calculated by non-compartmental methods (based on statistical moment theory): area under the concentration–time curves (AUC), area under the first moment curve (AUMC); mean residence time (MRT); Mean absorption time (MAT) was calculated as $MAT = MRT_{i.m.\ or\ P.o.} - MRT_{i.v.}$ and bioavailability (F), where: F = [mean $AUC_{i.m.\ or\ p.o.}$ / mean $AUC_{i.v.}$] X 100.

Statistical analysis

The statistical analysis was performed using the SPSS® 10.0 software package (SAS, Cary, NC, USA). Results are presented as arithmetic mean ± standard errors (SE). The nonparametric Wilcoxon test was used to compare the parameters obtained in healthy and diseased chickens following each route of administration. Means were considered significantly different at $p < 0.05$ and $P < 0.001$.

RESULTS

Following compartmental analysis, a three-compartment open model best described the concentration-time data of azithromycin after i.v. administration. The mean (±SE) serum concentrations of azithromycin at the times of sample collection after i.v. injection is plotted in Figure 1. The mean (±SE) pharmacokinetic parameters based on compartmental pharmacokinetic analysis and non-compartmental methods are presented in TABLE 1.

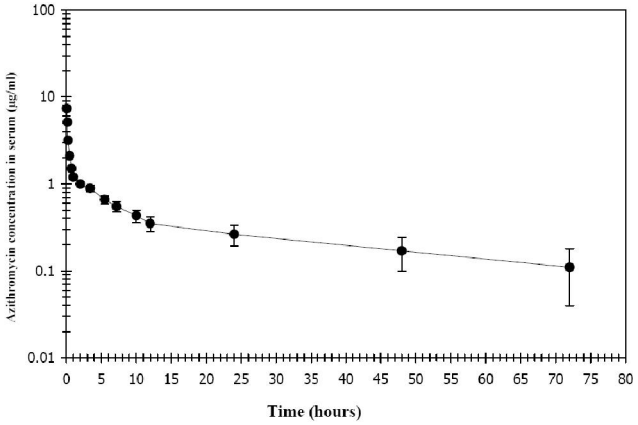

Figure 1 : Mean ± SE serum concentrations of azithromycin in chickens after i.v. injection of 20 mg/kg b.w. (n=5).

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The total body clearance (Cl_{tot}) was 0.77 L/kg/h the volume of distribution at steady-state (V_{dss}) was 47.75 L/kg and the value of the elimination half-life ($t_{1/2\lambda3}$) was 31.91 h. The mean (±SE) serum concentrations of azithromycin at the times of sample collection after i.m. and p.o. administrations are plotted in Figure 2. An open two-compartment model with first order absorption best fitted the data obtained after i.m. and p.o. administrations of azithromycin to broiler chickens. The mean (±SE) pharmacokinetic parameters based on compartmental pharmacokinetic analysis and non-compartmental methods are presented in TABLE 2.

TABLE 1 : Mean ± SD serum pharmacokinetic parameters of azithromycin in broiler chickens following i.v. administration at a dose rate of 20 mg/kg (bw) (n=5).

Parameters	Unit	i.v.
λ_1	h^{-1}	6.43 ± 1.86
$t_{1/2\lambda1}$	h	0.107 ± 0.01
λ_2	h^{-1}	0.23 ± 0.01
$t_{1/2\lambda2}$	h	3.05 ± 0.68
λ_3	h^{-1}	0.022 ± 0.09
$t_{1/2\lambda3}$	h	31.91 ± 3.50
K_{el}	h^{-1}	0.43 ± 0.13
V_d	L/kg	34.83 ± 5.20
V_{dss}	L/kg	47.75 ± 5.21
Cl_{tot}	L/h/kg	0.77 ± 0.17
$AUC_{0-?}$	µg•h/mL	26.10 ± 2.51
AUMC	µg•h^2/mL	933.50 ± 68.88
MRT	h	35.77 ± 3.45

$t_{1/2\lambda1}$: the disposition half-life associated with the initial slope (λ_1) of a semi-logarithmic concentration–time curve; $t_{1/2\lambda2}$: the disposition half-life associated with the second slope (λ_2); $t_{1/2\lambda3}$: the elimination half-life associated with the terminal slope (λ_3) of a semi-logarithmic concentration–time curve; k_{el}: elimination rate constant; V_d the apparent volumes calculated by the area method; V_{dss}: volume of distribution; Cl_{tot}: total body clearance; AUC: area under the curve by the trapezoidal integral; AUMC: area under moment curve by the trapezoidal integral; MRT: mean residence time.

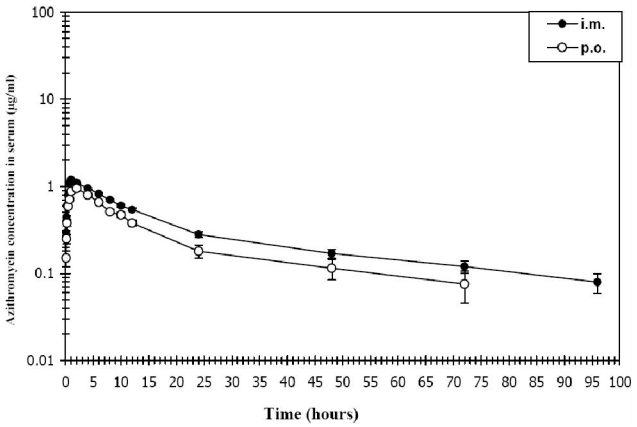

Figure 2 : Mean ± SE serum concentrations of azithromycin in chickens after i.m. and p.o. administrations of 20 mg/kg b.w. (n=5).

TABLE 2 : Mean ± SD serum pharmacokinetic parameters of azithromycin in broiler chickens following i.m. and p.o. administrations at a dose rate of 20 mg/kg (bw) (n=5).

Parameters	Unit	i.m.	p.o.	Level of significance
k_{ab}	h^{-1}	2.37 ± 0.33	1.21 ± 0.18	P < 0.05
$t_{1/2ab}$	h	0.29 ± 0.05	0.57 ± 0.08	P < 0.05
k_{el}	h^{-1}	0.018 ± 0.05	0.022 ± 0.01	NS
$t_{1/2el}$	h	38.95 ± 2.15	31.50 ± 1.40	P < 0.05
$AUC_{0-?}$	µg•h/mL	24.84 ± 1.22	21.80 ± 1.10	NS
AUMC	µg•h^2/mL	967.52 ± 35.20	686.70 ± 25.25	P < 0.001
MRT	h	47.16 ± 3.31	39.93 ± 2.43	P < 0.05
MAT	h	11.39 ± 1.10	4.16 ± 0.72	P < 0.001
C_{max}	µg/mL	1.20 ± 0.11	0.95 ± 0.13	NS
T_{max}	h	1.37 ± 0.22	1.91 ± 0.31	NS
Cl/F	L/kg/h	0.81 ± 0.15	0.91 ± 0.17	NS
F	%	95.17 ± 2.55	83.52 ± 1.24	P < 0.001

AUC: area under the curve by the trapezoidal integral; AUMC: area under moment curve by the trapezoidal integral; MRT: mean residence time. k_{ab}: absorption rate constant; $t_{½ab}$: absorption half-life; k_{el}: elimination rate constant; $t_{½el}$: elimination half-life; MAT: mean absorption time; C_{max}: maximum serum concentration; T_{max}: time to peak concentration; Cl/F total body clearance: F(%), bioavailability. Values after i.m. were significantly different from corresponding values following p.o.

After i.m. administration, the elimination half-life ($t_{1/2el}$), mean residence time (MRT) and maximum plasma concentration (C_{max}) were higher (38.95 h, 47.16 h and 1.20 µg/mL) than after p.o. route (31.50 h, 39.93 h and 0.95 µg/mL), respectively. The Wilcoxon Rank Sum test and the Student's t-test performed on pharmacokinetic parameters after i.m. and p.o. administrations revealed significant differences between both routes.

In vitro protein binding percent of azithromycin in serum chickens ranged from 17.78 to 30.37% at azithromycin concentrations ranged between 5 to 0.3125 µg/mL with an average of 24.42% TABLE 3.

The absolute bioavailabilities were 95.17 and 83.52 % after i.m. and p.o. administrations, respectively.

DISCUSSION

This study used a microbiological assay method to estimate the pharmacokinetics and azithromycin con-

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TABLE 3 : *In vitro* protein binding percent of azithromycin in chicken's serum

Concentrations (μg/mL)	Average corrected values of inhibition zones (mm)		
	Serum	Phosphate buffer	Protein binding %
5	22.5 ± 1.12	18.5 ± 0.79	17.78
2.5	20.80± 0.12	16.22 ± 0.15	22.02
1.25	17.30 ± 0.33	13.00 ± 0.29	24.85
0.625	16.20 ± 0.72	11.81 ± 0.22	27.10
0.3125	15.80± 0.33	11.00 ± 0.12	30.37
Mean ± S.E.			24.42 ± 2.95

centrations in broiler chickens. The bioassay did not distinguish between the active metabolites and the parent compound. Because the metabolites are mostly microbiologically inactive[21], their presence may not necessarily interfere with the determination of a therapeutic dosage regimen. The metabolism of azithromycin in the rat, cat, dog, and human has been described[11,22]. Comparison between bioassay and HPLC methods of analysis of azithromycin have also been reported by Riedel *et al*.[23], showing no antimicrobial activity from azithromycin metabolites. In human, up to 10 metabolites of azithromycin have been identified and all were microbiologically inactive[24,25].

Approximately 75% of drug-related material in excreta was unchanged azithromycin, indicating the major xenobiotic component is the unaltered compound. When ^{14}C azithromycin labeled in the 9a-N-methyl position was administered to the dog and rat by i.v. administration, approximately 96 and 88%, respectively, of the dose was recovered in excreta, two third in feces and one-third in urine, by 7 days postdose[21]. The pharmacokinetic profile of azithromycin is characterized by low serum drug concentrations but high and persistent tissue concentrations[4,6,11].

The azithromycin plasma concentration vs time data after i.v. administration were best fitted to a three-compartment open model. This conclusion is in agreement with that found in previous studies of azithromycin carried out in dogs and rats[21] and goats[13].

The half-life of azithromycin after i.v. administration was about 31.91 ± 3.50 h, a high value similar to those described by Cárceles *et al*.[13] in goats of 32 h, Hunter *et al*.[11] in cats of 35 h and Shepard and Falkner[22] in dogs of 30 h. Shorter half-lives have been described in foals of 20 h[26] and 16 h[12]. The polyphasic plasma pharmacokinetics of azithromycin in chickens is consistent with a drug being distributed rapidly and extensively in tissue and then redistributed slowly from tissue, thereby producing high tissue levels and modest but prolonged serum levels. Consequently the prolonged half-life is attributed to rate-limiting slow release of azithromycin from tissue into serum, accompanied by excretion and metabolism. Azithromycin had a very large volume of distribution (V_{dss} = 47.75 L/kg) indicating that it is widely distributed in tissues and then slowly redistributed as previously reported in cats (23 L/kg), goats (35 L/kg), rabbits (41.50 L/kg), in humans (23-31 L/kg), rats (84 L/kg), and[11,13,15,21,22]. This large volume of distribution can be attributed to high tissue and intracellular concentrations as it has been demonstrated previously[11,12,26]. An open two-compartment model with first order absorption best fitted the data obtained after i.m. and p.o. administrations of azithromycin to broiler chickens. This is a common phenomenon for drugs whose disposition after i.v. administration fits a three-compartment model, because if the value of the absorption rate constant is the same or lower than the largest disposition rate constant (λ_1), this phase will not appear in the extravascular curves and the disposition of the drug is best interpreted according to an open two-compartment model[20]. Mean residence time (MRT) reflects the difference in persistence of the drug in the body after i.v. and i.m. administrations. The significantly prolonged MRT after i.m. administration compared to the i.v. administration, the clearances being similar, was due to the influence of the absorption phase. Similar results have been reported in goats[13].

In our study, azithromycin i.m. absorption was high with a mean systemic availability of 95.17%, very similar to the value reported in rabbits 97.7%[15] and goats of 92%[13]. The short T_{max} (1.37 h) and average bioavailability of nearly 100% support a rapid and complete absorption of the drug from the i.m. injection site, in contrast with p.o. administration. Bioavailability following p.o. administration in broiler chickens (83.52%) is different than that reported in ball pythons (77%), in humans (37%), in rats (46%), in cats (58%), in foals (39%) and in dogs (97%)[6,9-12,22]. It may be important to take into count the possibility of enterohepatic recycling for azithromycin. It is known that some macrolides undergo recycling after oral administration.

Following p.o. administration, azithromycin is rap-

idly absorbed and widely distributed into animal tissues, including peripheral blood polymorph-nuclear leukocytes[1]. Studies suggest that azithromycin is delivered to sites of infection by leukocytes as part of the body's normal response to infection and is then released in response to phagocytosis[1], which partly explains its high concentrations in areas of inflammation and infection.

The serum protein binding of azithromycin in chickens is concentration dependent, ranging from 17.78 to 30.37% at azithromycin concentrations ranged between 5 to 0.3125 μg/mL with an average of 24.42%. In this respect, Shepard and Falkner[22] stated that the protein binding of azithromycin and erythromycin is low in mouse serum (7.2 and 19%, respectively, at a drug concentration of 0.5 μg/mL) and is saturated at a concentration of 0.5 μg of azithromycin per mL. The serum protein binding of azithromycin in man declined from about 50% at 0.02 mg/l to 12% at 0.5 mg/l. Tissue concentrations of azithromycin were much higher than serum concentrations[9].

CONCLUSION

Based on the fortunate pharmacokinetic characteristics which are slow elimination and extensive distribution obtained from the study, a single dose of azithromycin at 20 mg/kg (bw) i.m. and p.o. every 72 h for susceptible bacterial infections in chickens as determined by sensitivity is recommended. The results of this study may not only benefit chickens but also could have clinical applications for other related avian species.

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