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Pharmacokinetics of Toltrazuril after Oral Administrations in Broilers

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Abstract : A study on the oral bioavailability and pharmacokinetics of an anticoccidal agent, toltrazuril, was conducted in broilers following a single oral doses of 10 mg/kg body weight (BW) or 40 mg/kg BW. The concentrations of toltrazuril in plasma were determined by a high-performance liquid chromatography/mass spectrometry. Plasma concentration–time data after single oral administration were analyzed by a non-compartmental analysis. Toltrazuril was very well-absorbed through the gastrointestinal tract with C_{max} of 18.04±5.80 μg/mL and 47.15±9.40 μg/mL at 4.33±1.51 h and 3.67±1.15 h after oral dose of 10 mg/kg and 40 mg/kg in broilers, respectively. A comparison between 10 mg/kg and 40 mg/kg dose groups showed that t_{max} were similar while C_{max} and area under curve (AUC) increased with increasing dose.

Key words **:** pharmacokinetics, toltrazuril, broiler

Introduction

Toltrazuril, 1-methyl-3-[3-methyl-4-[4-(trifluoromethylsulfanyl)phenoxy]phenyl]-1, 3, 5-triazinane-2, 4, 6-trione, is a symmetrical triazinetrione compound. It has broad-spectrum anticoccidial and antiprotozoal activity (7,10,11,12). In addition, it is active against both asexual and sexual stages of coccidia by inhibiting nuclear division of schizonts and microgamonts and the wall-forming bodies of macrogamonts (5,7,8,12). It is widely used in calves, piglets, chicken and turkeys for the prevention and treatment of coccidiosis, by administraiton via drinking water (2,3,10,11,12). Toltrazuril is usually administered as an oral suspension at a single oral dose of 20 mg/kg body weight (BW) in the first week of life of piglets, or 15 mg/kg BW in the calves (2,3,12).

For the efficient and safe therapy, pharmacodynamic and pharmacokinetic information for drug are required. Vermeulen *et al.* reported that 17% of the total of therapeutic drugs used in veterinary medicine is administered to poultry. However information on avian pharmacokinetic profiles of therapeutic drugs is still scarce (15). Moreover, although tolrazuril is effective against many coccidial species of chickens, pigeons, calves, dogs, cats, pigs and mice (1,4-7,9,11-13), the pharmacokinetic profiles of toltrazuril in broilers were not fully investigated. Thus, the objective of this study was to investigate the pharmacokinetics of toltrazuril after a single oral administration in broilers.

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Materials and methods

Chemicals

Toltrazuril (99.4% assay purity) as the analytical standard was purchased from Sigma (Missouri, USA). HPLC grade methanol and acetonitrile were purchased from Mallinckrodt Baker (New Jersey, USA). Other analytical grade chemicals were purchased from Sigma (Missouri, USA).

Experimental design

Twenty male broiler chickens (1.09 ± 0.25 kg) were obtained from a commercial farm in Korea. Before the experiment the animals were acclimatized for 1 week. The birds were monitored daily and no clinical signs of disease were observed. The room temperature ranged between 20 and 22°C and the relative humidity was maintained at 50-70%. A dark period was given between 0 : 00 h and 6 : 00 h. Water and commercial feed were available *ad libitum*. The birds were given a single oral administration directly into the crop using gavages at the dosage of 10 mg/kg body weight and 40 mg/kg BW of toltrazuril, respectively. Blood samples (0.5 mL) were drawn from the left brachial vein at 0.5, 1, 2, 4, 6, 8, 12, 24, 36, 48, 72, 120 and 168 h after the oral administration. The samples were centrifuged at 1500 *g* for 10 min to obtain plasma and stored at −70°C until analysis.

Analytical methods

Samples were analyzed on an Agilent 1100 series LC/MSD system. Separation was achieved on Zorbax XDB-C_{18} reverse phase column (5 μm 4.6 mm × 150 mm, Agilent, USA) and equilibrated with 30% solution A (10 mM ammonium acetate, pH 3.5) and 70% solution B (acetonitrile). The instru-

ment was tuned and optimized for the transmission of the nominal negative ion of toltrazuril. Mass spectrometer was performed using the negative ion mode and the selected ion monitoring (SIM), detecting m/z 424 with peak width of 0.07 and a dwell time of 197 ms. The limit of quantitation (LOQ) of toltrazuril was 10 ng/mL, the inter-day and intra-day precision (coefficient of variation, CV; %) was both below 15% and calibrations were linear (correlation coefficient, r; $r >$ 0.999) from 5 ng/mL to 50 μg/mL.

Pharmacokinetic analysis

The data analysis was performed by the non-compartmental analysis using a combined linear trapezoidal rule approach (WinNonlin 5.2, Pharsight, USA). Peak plasma concentrations (C_{max}) of the drug and times to reach peak concentration (t_{max}) were determined from the individual plasma concentration-time curves. Lambda z is a first-order rate constant associated with the terminal (log linear) segment of the curve. It was estimated by the linear regression of the terminal data points. The terminal elimination half-life ($t_{1/2\lambda z}$) was calculated by $t_{1/2\lambda z} = 0.693/\lambda_z$. The area under the plasma concentration-time curves (AUC) was calculated by the method of trapezoids. The area under the first moment curve (AUMC) was calculated as the product of time and drug concentration-time. Mean residence time (MRT) was calculated from MRT = AUC/AUMC. The total body clearance (Cl/F) was calculated from Cl/F = Dose/AUC, and the volume of distribution (V_z/F) was calculated using V_z/F=MRT*Cl.

Results and discussion

The mass spectromic analysis for the determination of toltrazuril was rapid with a high degree of reproducibility and successfully applied to the pharmacokinetic study of toltrazuril in broilers. The plasma concentrations below LOQ were not used for the pharmacokinetic analysis. Non-compartmental methods based on statistical moment theory do not require the assumption of a specific compartmental model for either drug or metabolite (16). In fact, these methods can be applied to virtually any compartmental model for the system (body) and can be applied to virtually any pharmacokinetic data. The moment analysis allows calculation of the major pharmacokinetic parameters without encountering the numerous problems involved in curve fitting. Therefore, the results of the present study were analyzed by use of non-compartmental methods.

Toltrazuril is active against both asexual and sexual stages of coccidia by inhibiting nuclear division of schizonts and microgamonts and the wall-forming bodies of macrogamonts. It may also be useful in the treatment of neonatal porcine coccidosis, equine protozoal myeloencephalitis and canine hepatozoonosis. No adverse effects associated with toltrazuril including abdominal pain, nausea, vomiting, abdominal cramps and diarrheas were observed in any bird after drug administrations. Plasma toltrazuril concentrations after oral administration are presented graphically as semi-logarithmic time vs. plasma concentrations (Fig 1).

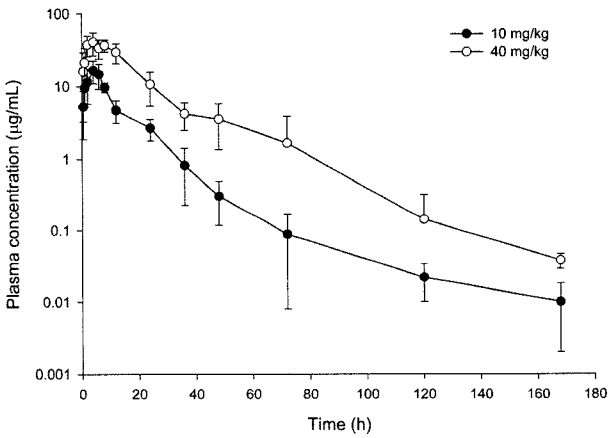

Fig 1. Semilogarithmic plot of toltrazuril plasma concentration vs. time after a single oral administration at the dose rate of 10 mg/kg BW(●) and 40 mg/kg BW(○) in broilers.

Table 1. Pharmacokincetic parameters determined after oral administration of toltrazuril at the dose rate of 10 mg/kg BW and 40 mg/kg BW to broilers

Parameters (unit)	Doses of oral administration	
	10 mg/kg	40 mg/kg
λ_z (1/h)	0.07±0.03	0.07±0.03
$T_{1/2\lambda z}$ (h)	11.40±4.68	11.64±4.08
AUC (μg · h/mL)	209.46±44.89	869.98±226.90
t_{max} (h)	4.33±1.51	3.67±1.51
C_{max} (μg/mL)	18.04±5.80	47.15±9.40
MRT (h)	12.15±3.16	18.14±6.10
V_z/F (mL/kg)	802.75±365.00	193.24±51.21
Cl/F (mL/kg/h)	49.35±9.14	12.21±3.36

Values are the mean ± SD from broilers after i.v. administrations. λ_z, the first order rate constant associated with the terminal portion of the curve; $t_{1/2\lambda}$, terminal half-life, t_{max}, time of maximum observed concentration; C_{max}, maximum observed concentration; MRT, mean residence time; V_z/F, the volume of distribution based on the terminal phase to the fraction of dose absorbed; Cl/F, total body clearance to the fraction of dose absorbed.

Toltrazuril was very well-absorbed through the gastrointestinal tract with C_{max} of 18.04 ± 5.80 μg/mL and 47.15 ± 9.40 μg/mL at 4.33 ± 1.51 h and 3.67 ± 1.15 h after oral dose of 10 mg/kg BW and 40 mg/kg BW in broilers, respectively (Table 1). A comparison between 10 mg/kg BW and 40 mg/kg dose groups showed that t_{max} were similar while C_{max} and AUC increased with increasing dose. In European Medicines Agency (EMEA) reports, the C_{max} of 33.41 μg/mL was observed 120 h following oral administration of ^{14}C-toltrazuril at a dose of 15 mg/kg BW in calves (3). In addition, plasma concentration of toltrazuril in piglets increased from 3.0 μg/mL at 3 hours after dosing, to a maximum of 13.0 μg/mL at 2-day post dosing after a single oral administration of 15 mg/kg BW of ^{14}C-toltrazuril (2). Tobin *et al.* reported peak plasma concentration (C_{max}, 4.5 μg/mL) occurred at 24 h after single oral administration of tolatrazuril at 10 mg/kg BW in horses (14).

Following EMEA reports, ^{14}C-toltrazuril in calves and piglets was slowly eliminated in both of urine and feces with 154 h and 148 h of elimination half-life, respectively (2,3). In horses, after the oral administration of single doses of toltrazuril, the apparent plasma half-life was approximately 55 h (14). In non-radiolabelled pharmacokinetics in piglet of a single oral administration of 20 mg/kg BW, the mean terminal half-life of toltrazuril was 72 h (3). However, in the present study, the terminal elimination half-lives (11.40 ± 4.68 h and 11.64 ± 4.08 h) after oral administration of toltrazuril at 10 mg/kg BW and 40 mg/kg BW in broilers showed much shorter than other species.

In conclusion, the pharmacokinetic data obtained in this study indicate toltrazuril to be a qualified candidate for the treatment of coccidial infections in broilers. The high oral bioavailability and slow elimination of the drug are excellent properties from a therapeutic point of view. However, further critical studies on the therapeutic window, potential for therapeutic efficacy, and potential for adverse reactions in this group of agents are clearly required.

Acknowledgement

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톨투라주릴의 육계에서의 약물동태학적 연구

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요 약 : 본 연구는 육계에 톨투라주릴을 체중당 10 mg 및 40 mg 용량으로 경구 투여한 후 톨투라주릴의 약물동태학적 분석을 실시하였다. 혈장내의 톨투라주릴의 정량은 액체크로마토그래프/질량분석기를 사용하였으며, 경구 투여후 혈장 농도-시간 자료는 non-compartmental analysis를 이용하여 분석하였다. 톨투라주릴을 10 mg/kg 및 40 mg/kg 용량으로 각각 경구투여 후 혈중최고농도(18.04±5.50 μg/mL 및 47.15±9.40 μg/mL)는 4.33±1.51 h 및 3.67±1.51 h에 나타났다. 소실반감기는 11.40±4.68 h 및 11.64±4.08 h으로 각각 나타났다. 톨투라주릴의 경구투여 후 혈중최고농도 및 곡선하면적은 용량이 증가함에 따라 증가하였다.

주요어 : 톨투라주릴, 약물동태학, 육계