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Research Paper

Pharmacokinetics and tissue residue of toltrazuril in broiler chickens

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Abstract

The pharmacokinetics and tissue residue of toltrazuril (Baycox®, Bayer Animal Health) were investigated in broiler chickens after repeated oral administrations of 7 mg/kg b.wt once daily for two consecutive days. Chickens were subjected to clinical diagnosis to ensure that each one is free from any Eimeria was received a standard broiler feed free from any medications. The results revealed that following repeated oral administration; toltrazuril peaked in blood with [C_{max}] of 6.56 and 6.70 µg/ml at [T_{max}] of 2.56 and 2.52 hours and the elimination half-life [$t_{1/2\beta}$] of 14.92 and 16.19 hours and the mean residence times (MRT) were 21.33 and 23.14 hours after first and second doses, respectively. The tissue level concentrations were highest in the kidney and decreased in the following order: liver > lung > heart > muscle > skin and fat. No toltrazuril residues were detected in tissues and plasma after 8 days.

Keywords: *Broiler; Disposition; Pharmacokinestics; Tissue Residue; Toltrazuril.*

1. Introduction

Intestinal coccidiosis, caused by various species of Eimeria, is an economically important infectious disease of poultry and reared livestock throughout the world caused by Eimeria tenella, the most pathogenic strain of coccidium is usually located in cecum and leading to cecal coccidiosis [1], [2]. There have been applied several drugs to minimize the possible negative effects of infection [3].
During commercial broiler chicken production, chickens are continually exposed to coccidial oocysts found in litter [9]. Moreover, broiler chickens will have multiple stages of coccidial development simultaneously. After clinical signs of coccidiosis appear in a broiler chickens facility, it may be too late to therapeutically control the infection in all birds unless the therapeutic anti-coccidial is against multiple stages of development [1].
The aim of the present study is to estimate the concentrations of toltrazuril and investigate the pharmacokinetics and tissue residue of toltrazuril following a repeated oral administration (for 2 consecutive days) in broiler chickens.

2. Materials and methods

2.1. Birds

Forty five white leghorn broiler chickens (one month old) were used in this study. Chickens were subjected to clinical diagnosis to ensure that each one is free from any Eimeria. They were kept individually in cages, within a ventilated, heated room (20ºC), and 14 hours of day light. They received a standard broiler feed free from any medications and water *ad. Libitum*. The protocols for the bird studies were approved by the Institutional Animal Care and Use Committee (IACUC) of Cairo University.

2.2. Drug

Baycox® (Bayer Animal Health): It is available as 500 ml or 100 ml oral solution in high density polyethylene bottles. Each one ml contains 25 mg toltrazuril base (2.5%).

2.3. Experimental design

Chicks were received and raised till one month old with normal vaccination and medication programs. All Chickens were received toltrazuril 7 mg/kg b.wt orally (intra-crop administered) with Baycox® for 2 consecutive days.

2.4. Pharmacokinetics and tissue residue studies

Blood samples (one ml) were collected from the right-wing vein from 10 chickens before medication and at 0.25, 0.5, 1, 2, 4, 6, 8, 12 and 24 hour after the first and second doses. Blood samples were allowed to clot at room temperature and then centrifuged at 3000 r.p.m. /10 minutes. The resulting serum was stored at -20ºC until analysis. 24 hours following the second dose, three chickens were slaughtered on daily bases for 15 days. Tissue samples (lung, heart, liver, kidney, breast muscle, thigh muscle, skin and fat) were taken and stored at -20 ºC until analysis.

2.5. Toltrazuril analysis

Serum and tissue concentrations of toltrazuril were determined using a high performance liquid chromatographic (HPLC) method. The HPLC method and extraction procedures were used from previously published methods [10, 11]. Frozen serum samples were thawed at room temperature and 100 µl of the sample was mixed with 400 µl of ethyl acetate and vortex mixed for 5 min and then centrifuged at 1,000 x g for 10 min at room temperature. The organic layer was transferred to a clean tube and evaporated under a steam of nitrogen at 40 ºC. The residue was re-constituted with 100 µl of acetonitrile and vortex mixed for 5 min. This was filtered through a 0.22 µm syringe filter to remove the insoluble proteins, and the clear filtrate was transferred into an auto sampler vial and 50 µl was injected into the HPLC system.

For the tissue samples, three ml of acetonitrile were added to one gram of the obtained tissue sample and homogenized in a porcelain mortar by the aid of sterile sand. The homogenate was left in the refrigerator overnight then centrifuged. The supernatant was collected and subjected to the same manner in serum samples.

The HPLC system consisted of a Beckman System Gold® 126 solvent module, a Beckman model 508 auto sampler, a Beckman model, 168 detector (Beckman Coulter) and a reversed phase C18 (3 µm, 125 X 4.6 mm) Column (Phenomenex, Germany) with an isocratic mobile phase which consists of Water HPLC and acetonitrile HPLC (20:80 v/v). The mobile phase was eluted at a flow rate of 1 mL/min and detected at a UV wave length of 254 nm.

2.6. Toltrazuril calibration curve

A standard calibration curve was prepared by adding one ml of toltrazuril base (25 mg/ml) to nine ml chicken serum or acetonitrile. This was further diluted to produce standards of 0.5, 1, 2.5, 5, 10, 25, 50 and 100 µg toltrazuril/ml. These standards were extracted and analyzed in the same manner as unknown samples. The HPLC method for toltrazuril determination in chicken serum was validated by assessing linearity, precision, accuracy and recovery. Two sets of quality control samples (5, 20 and 80 µg toltrazuril/ml) were prepared and analyzed at the beginning and at the end of each assay to determine the intra- and inter-assay coefficients of variation (CV). The calibration curves were linear over the range of 0.5-100 µg/ml g/ml, and the correlation coefficients (r^2) were >0.999. Analytical recovery of toltrazuril, calculated by comparing the peak height ratios for serum samples and aqueous samples, ranging 82 - 87%. The inter-assay CV was 3, 3 and 5%, while the intra-assay CV were 4, 5 and 8%, respectively at toltrazuril concentrations of 5, 20 and 80µg/ml.

2.7. Pharmacokinetic and statistical analysis

The pharmacokinetic parameters of toltrazuril were calculated by using a non-compartmental software program (WinNonlin® software, version 5.2, Pharsight Corporation, NC, USA). The area under serum concentration–time curve (AUC) was calculated using the trapezoidal rule with extrapolation to infinity. The maximum concentration (C_{max}) and the corresponding peak time (t_{max}) were determined by the inspection of the individual drug serum concentration–time profiles. The slope of the terminal phase of the time–concentration curve was determined by linear regression and converted to an elimination half-life ($t_{1/2\beta}$) by multiplying the reciprocal by 0.693.

Data were expressed as X ± SE. and were statistically analyzed using analysis of variance. Mean comparisons were performed using Tukey's test. The differences were considered significant when $p<0.05$. These calculations were performed using Prism 5.0 (GraphPad).

3. Results

The mean serum concentration-time curve of toltrazuril following repeated oral administration at a dose of 7mg/kg b.wt is plotted and presented graphically in Figure 1. The pharmacokinetic parameters of toltrazuril are shown in Table 1. Following a repeated oral administration for two consecutive days, the mean $AUC_{0-\infty}$ was 131.65±4.45 and 144.23±6.34 µg/ml/h, and the elimination half-life ($t_{1/2\beta}$) was 14.92±1.28 and 16.19±1.60 hours and the mean residence times ($MRT_{0-\infty}$) were 21.33±1.90 and 23.14±1.66 hours following administration of the first and second doses, respectively. The maximum serum concentration (C_{max}) was 6.56±1.26 and 6.70±0.60 µg/ml reached at T_{max} of 2.56±0.16 and 2.59±0.35 hours following administration of the first and second doses, respectively. Tissue residue profiles of toltrazuril are incorporated in Table 2. The tissue level concentrations were highest in the kidney and decreased in the following order: liver > lung > heart > muscle > skin and fat. No toltrazuril residues were detected in tissues and plasma after 8 days following repeated oral administration.

Figure Legend:

Fig 1 the serum concentration-time profile of toltrazuril (7 mg/kg bw) after repeated oral administration for 2 consecutive days in broiler chickens. Maximum serum concentration (C_{max}) was 6.56 and 6.70 µg/ml reached at T_{max} of 2.56 and 2.59 hours following administration of the first and second doses, respectively.

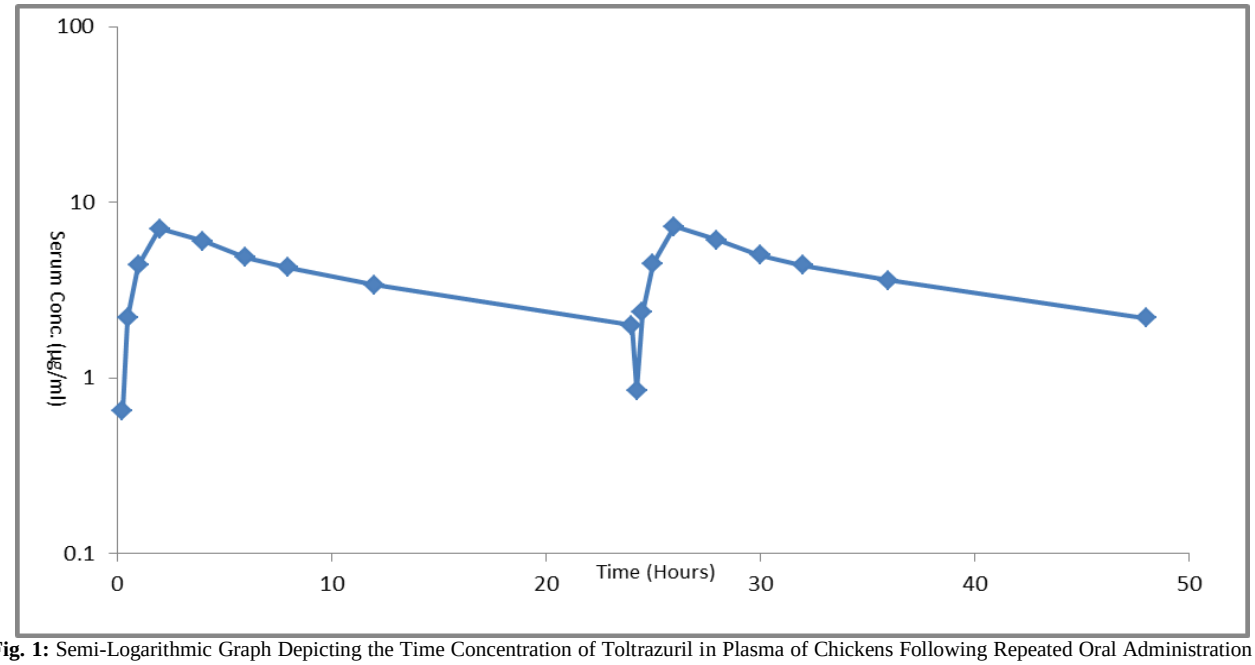

Fig. 1: Semi-Logarithmic Graph Depicting the Time Concentration of Toltrazuril in Plasma of Chickens Following Repeated Oral Administration of 7 Mg/Kg B.Wt. (N=10).

Table 1: Pharmacokinetics of Toltrazuril in Chickens after Repeated Oral Administration of 7 Mg / Kg.B.Wt. (N=10), Mean ± S.E.

Parameter	Unit	First dose	Second dose
K_{el}	h-1	0.046±0.008	0.042±0.007
$t_{1/2\beta}$	h	14.92±1.28	16.19±1.60
$C_{max\ (Calculated)}$	µg/ml	6.56±1.26	6.70±0.60
$T_{max\ (Calculated)}$	h	2.56±0.16	2.59±0.35
AUC_{0-24}	µg. h/ml	88.58±1.60	92.82±1.45
$AUC_{0-\infty}$	µg. h/ml	131.65±4.45	144.23±6.34
MRT_{0-24}	h	9.56±1.22	9.72±0.98
$MRT_{0-\infty}$	h	21.33±1.90	23.14±1.66

C_{max} = maximal concentration; T_{max} = when the maximal serum concentration is reached; AUC_{0-t} = area under serum concentration–time curve; $t_{1/2\beta}$ = Elimination half-life; MRT = mean residence time; K_{el} = first-order elimination rate constant.

Table 2: Tissue Concentration of Toltrazuril (µg/g) in Different Tissues after Repeated Oral Administration of 7 Mg / Kg.B.Wt. (N=45), Mean ± S.E.

	Lung	Heart	Liver	Kidney	Thigh muscle	Breast muscle	Fat and skin
Days after the 2nd dose	Toltrazuril concentrations (µg/g)						
1	4.50±0.02	4.40±0.09	4.70±0.03	5.89±0.07	3.45±0.02	2.60±0.07	1.90±0.02
2	3.20±0.03	3.11±0.01	3.95±0.01	3.13±0.06	1.11±0.09	1.22±0.01	1.10±0.07
3	2.88±0.03	2.84±0.09	2.15±0.03	2.22±0.02	0.64±0.01	0.61±0.07	0.77±0.06

4	1.20±0.02	1.25±0.06	1.25±0.02	1.47±0.06	0.31±0.07	0.14±0.03	0.22±0.07
5	0.60±0.04	0.50±0.04	0.79±0.06	0.88±0.02	N.D	N.D	N.D
6	0.47±0.06	0.25±0.08	0.40±0.08	0.47±0.05	N.D	N.D	N.D
7	0.20±0.05	N.D	0.22±0.05	0.25±0.06	N.D	N.D	N.D
8	N.D	N.D	N.D	N.D	N.D	N.D	N.D
9	N.D	N.D	N.D	N.D	N.D	N.D	N.D
10	N.D	N.D	N.D	N.D	N.D	N.D	N.D
11	N.D	N.D	N.D	N.D	N.D	N.D	N.D
12	N.D	N.D	N.D	N.D	N.D	N.D	N.D
13	N.D	N.D	N.D	N.D	N.D	N.D	N.D
14	N.D	N.D	N.D	N.D	N.D	N.D	N.D
15	N.D	N.D	N.D	N.D	N.D	N.D	N.D

N.D = Not detected.
24 hours following the second dose, three chickens were slaughtered on daily bases for 15 days.

4. Discussion

The prudent use of toltrazuril in veterinary medicine, especially in poultry production is strongly required to preserve the safety and efficacy of toltrazuril in the future. Therefore, its kinetics characteristic should be considered to select the dosage regimens which induce the maximum efficacy and minimize the development of resistance.
In the present study, the mean C_{max} of toltrazuril calculated after repeated oral administrations were 6.56 and 6.70 µg/ml attained at 2.56 and 2.59 h after the first and second dose, respectively. However, different results were obtained in broiler chickens at dose of 10 mg/kg (16.4 µg/ml attained at 5.0 h) and at dose of 20 mg/kg (25.2 µg/ml attained at 4.7 h) [12], in rabbits at dose of 10 mg/kg (30.2 µg/ml attained at 20.0 h) [10], in rats (25.0 µg/ml, 20 mg/kg, [13] and in calves at dose of 15 mg/kg (33.41µg/ml) [13] Similar findings were obtained in piglets at dose of 20 mg/kg (7.5 µg/ml) [14], in horses at dose of 10 mg/kg (4.5 µg/ml) [15]. The apparent elimination half-life of toltrazuril was 14.92 and 16.14 h after the first and second dose, respectively. This finding was different to the elimination half-life of 55 h after oral dosing at 10 mg/kg to horses [15]. However, terminal elimination half-life of broilers was observed 11.4 h [16]. In European Medicines Agency (EMEA) reports, the elimination half-lives of rats, pigs and calves were observed 23.0, 148.2 and 154.0 hours following oral administration of ^{14}C-toltrazuril at a dose of 20 mg/kg in rats and pigs, and 15 mg/kg in calves [13], [14]. These clearly indicate species-specific differences in terms of absorption and elimination characteristics of toltrazuril following oral administration. The gastro-intestinal absorption of toltrazuril depends on lipophilic characteristics and dissociation rate and it is expected to be well absorbed following oral administration [17]. The physiological pH in the avian stomach is low, particularly at the gizzard which is considered as a powerful triturating machine and facilitates the disintegration of solid oral dosage forms [18]. These properties could contribute to enhance toltrazuril solubility and its absorption processes in broilers compared to the other species.
Tissue residue (µg/g) of toltrazuril following repeated oral administration revealed wide distribution of the drug in the tested tissues (brain, lung, heart, liver, muscle, skin and fat). The tissue concentrations of toltrazuril were highest in the kidney and decreased in the following order: liver > lung > heart > muscle > skin and fat. No toltrazuril residues were detected in tissues and plasma after eight days following repeated oral administration. Similar findings were found in chicken and porcine tissues [19] and skin and fat of chicken and pig [20].

5. Conclusion

Toltrazuril was absorbed very well through gastro-intestinal tract and slowly eliminated after repeated oral administration at a dose of 7 mg/kg in broiler chickens. Toltrazuril residue levels reached a maximum 7 days after the second dose in all tissue samples.

6. Conflict of Interests Statement

The authors declare that there is no conflict of interests regarding the publication of this paper.

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