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Original Article

PHARMACOKINETICS AND BIOAVAILABILITY OF TYLVALOSIN AFTER ORAL, INTRAMUSCULAR AND INTRAVENOUS ADMINISTRATION IN TURKEYS

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ABSTRACT

Objective: Tylvalosin, is a new macrolide antibiotic, it is highly effective against a range of important diseases in many different veterinary species specially pigs and poultry. The pharmacokinetics and bioavailability of Tylvalosin were investigated in healthy turkeys.

Methods: Tylvalosin was orally, intramuscularly, and intravenously administrated to turkeys in a single dose of (25 mg/kg b.w.), and the blood samples were analyzed by using a microbiological assay method.

Results: After intravenous injection, the serum concentration-time curves were best described by a two compartment-open model. The distribution and elimination half–life $t_{0.5\,\alpha}$ and $t_{0.5\,\beta}$ were (0.076±0.0014 h, 0.788±0.107 h), respectively. The volume of distribution Vd_{ss} was (1.155±0.183 L/kg), with body clearance $Cl_{(B)}$ of (1.489±0.143 ml/kg/h). Following oral administration, Tylvalosin was absorbed with $t_{0.5\,ab}$ (0.283±0.012 h) and eliminated, with $t_{0.5\,el}$ (5.309±0.542 h). The peak serum concentration was (0.637±0.018 µg/ml) at T_{max} of (1.293±0.024 h). Following intramuscular administration, Tylvalosin was absorbed with $t_{0.5\,ab}$ (0.076±0.003 h) and eliminated with $t_{0.5\,el}$ (0.467±0.058 h). The peak serum concentration was (1.446±0.121 µg/ml) at T_{max} of (0.282±0.008 h). The systemic bioavailability of Tylvalosin following oral administration was 33.84% and 14.06% after intramuscular administration.

Conclusion: These results indicate that, after oral and intramuscular administration, Tylvalosin was rapidly absorbed and distributed to tissues of turkeys. However, repeated doses are necessary to maintain Tylvalosin serum concentration above the MIC for most susceptible microorganisms.

Keywords: Tylvalosin, Pharmacokinetics, Bioavailability, Oral, Intramuscular, Intravenous, Turkeys.

INTRODUCTION

In turkeys, bacterial infections of the respiratory tract are more common and frequently result in economic losses due to increased mortality and feed conversion rates, reduced growth and high medical costs [1]. Macrolides are widely used antibiotics in veterinary medicine due to their ability to accumulate in the respiratory tract [2].

Tylvalosin is a new macrolide antibiotic. It is previously known as (acetyl isovaleryl Tylosin). It derived from fermentation of factor A Tylosin with *Streptomyces thermotolerans*. This fermentation results in the acetylation of the highly active 16-member lactone ring [3].

Tylvalosin has antibacterial activity against Gram-positive (e.g. *Staphylococcus, Micrococcus, Microbacterium, Bacillus, Coryne-bacterium, Aerococcus, Arthrobacter and Streptococcus, Campylobacter, Enterococcus and Clostridia*) and some Gram-negative organisms and against *mycoplasma* by way of inhibition of protein synthesis in the bacteria cell by reversibly binding to the 50S ribosome subunit. It was not active against most of the gram-negative strains (including *Escherichia coli, Serratia, Klebsiella, Proteus, Salmonella, Shigella and Pseudomonas*). It is highly effective against a range of important diseases in many different veterinary species especially pigs and poultry [4]. It indicated for prevention and treatment of Mycoplasmosis (*Mycoplasma gallisepticum, M. synoviae* and other *Mycoplasma* species), *Ornithobacterium rhinotracheale* and for diseases associated with *Clostridium perfringens* in chickens, replacement pullets and turkeys. In addition to its direct antimicrobial effect, Tylvalosin exhibits anti-inflammatory property [5].

The pharmacokinetics (PK) behavior of Tylvalosin has been studied in chickens after oral administration [6]. However, few pharmacokinetics studies of macrolides in turkeys and no data are available for Tylvalosin pharmacokinetics in turkeys, so the aim of the work was to study the pharmacokinetic aspects of Tylvalosin and its bioavailability after oral, intramuscular and intravenous administration in turkeys.

MATERIALS AND METHODS

Drug

Aivlosin ® is a veterinary medicinal product containing the macrolide antibiotic Tylvalosin (previous name: acetyl iso-valeryl tylosin). The drug obtained from (ECO Animal Health, London, UK), it is a water-soluble powder and contains 625 mg/g Tylvalosin (as Tylvalosin tartrate) as the active substance.

Turkeys

The study was carried out on 15 turkeys with an average body weight from 1.500 to 1.900 kg and 2 mo old. These birds were obtained from a governmental turkey's farm in Beni-Suef Governorate. The turkeys were fed on commercial balanced ration and water *ad-libitum*. They were treated in accordance with the Guidelines for Animal Experimentation (of the Ethics Review Committee) of the Faculty of Veterinary Medicine, Beni-Suef University. Turkeys were left without treatment for 15 d before the experiment for acclimatization and ensuring complete clearance of any antibacterial drugs.

Experimental design

Fifteen clinically healthy turkeys were given Tylvalosin 25 mg/kg b.w. according to (ECO Animal Health company instruction) and classified into three groups (5 each), the 1st group was given a single intravenous dose (through wing vein), the 2nd group given a single intramuscular dose (thigh muscle) and the 3rd group given a single oral dose (intra-crop route). A crossover design was used, with two weeks washout period between each route. Blood samples (1.5 ml each) were taken from the wing vein just after 5, 10, 15, 30 min, 1, 2, 4, 6, 8, 12 and 24 h post drug administration. All blood samples were left to clot for 30 min, centrifuged at 3000 r. p. m. for 15 min and the

obtained clear serum was transferred to eppendorff 's tubes and kept in the deep freeze (-20 ^{o}C) till assayed.

Assay for tylvalosin

Tylvalosin concentrations in serum were determined by microbiological assay method [7] and *Micrococcus luteus* ATCC 9341 as tested organism [8]. Standard curves of Tylvalosin in turkeys were linear over the range of 0.195-50 µg/ml. The diameter of the inhibition zones (mm) were linear when plotted against of concentration of Tylvalosin (µg/ml) with a correlation coefficient of 0.998 in normal turkey's serum, 0.994 in distilled water. Estimation of protein binding tendency of Tylvalosin was carried by preparing a standard solution in distilled water and also in normal antibiotic free turkey's serum at concentrations of Tylvalosin 0.195, 0.39, 0.78, (reference concentration) 1.56, 3.125, 6.25, 12.5, 25, 50 µg/ml. The difference in the diameter of inhibition between the solutions of the drugs in the distilled water (buffer) and that in the serum of the turkeys was used to calculate the percentage of protein binding of the tested antibacterial according to the following equation [9].

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

Pharmacokinetic analysis

Serum concentrations of Tylvalosin versus time curve were generated, and best fitted by the aid of computer poly-exponential curve stripping program, (R-Strip Micromath, software, USA). Data from each chicken was fitted individually, and the pharmacokinetic variables were computed by the aid of the software programs. The hybrid rate constants of the distribution and elimination phase (α and β), and the first order absorption and elimination rate constants (K_{ab} and K_{el}) and corresponding extrapolated zero time intercepts (A and B), absorption, distribution and elimination half-lives ($t_{0.5\,ab}$, $t_{0.5\,\alpha}$, $t_{0.5\,\beta}$, $t_{0.5\,el}$), transfer rate constants (K_{12} and K_{21}). The area under the curve from zero to infinite time (AUC 0-œ), mean residence time (MRT), maximum serum concentration (C_{max}) and time to be achieved (T_{max}) were calculated. The other pharmacokinetic parameters as total body clearance $Cl_{(B)}$, the volume of the central compartment (Vc), the volume of distribution at steady state (Vd_{ss}) and the bioavailability (F) were calculated by standard methods [10]. The results were expressed as mean±SE and the obtained data statistically using Student "t" test as described by [11].

RESULTS

After intravenous injection

The mean serum concentration of Tylvalosin was measured in healthy turkeys following a single intravenous dose of 25 mg/kg b.w. was (63.33±5.84) and presented in (table 1). The serum drug concentrations declined in a biphasic pattern that can be described by a two-compartment open model (fig. 1). The pharmacokinetic analysis of serum concentration versus time plot after single intravenous injection of Tylvalosin was presented in (table 2). It was shown that the drug was rapidly distributed with a distribution half-life ($t_{0.5\,\alpha}$) of 0.076±0.0014 min. The mean elimination half-life ($t_{0.5\,\beta}$) was 0.788±0.107 h and the total body clearance of the drug (CL_B) was 1.489±0.143 ml/kg/h. The apparent volume of distribution (Vc) of Tylvalosin in the central compartment showed a significant low value (0.406±0.04 L/kg) as compared with the apparent volume of distribution of the peripheral compartment (Vd_B) of (2.56±0.40 L/kg) and the total volume of distribution at the steady state (Vd_{ss}) (1.155±0.183 L/kg).

After oral administration

The mean serum concentrations of Tylvalosin at different time intervals following a single oral administration of 25 mg/kg b.w. in turkeys were presented in (table 1) and depicted in (fig. 1). The drug was firstly detected (0.2362±0.016 µg/ml) after 10 min. The pharmacokinetic parameters of Tylvalosin following its oral administration are tabulated in (table 3). The peak concentration (C_{max}) was (0.637±0.018 µg/ml) and the calculated value of T_{max} was (1.293±0.024 h). The drug was absorbed from healthy turkey gut with absorption half-life ($t_{0.5\,ab}$) of 0.283±0.012 hour and eliminated with a mean half-life ($t_{0.5\,el}$) of 5.309±0.542 hour. The calculated bioavailability (F %) of Tylvalosin following its single oral administration of 25 mg/kg b.w. in turkey was (33.84%±1.864 %).

After intramuscular injection

The mean serum concentrations of Tylvalosin at different time intervals following a single intramuscular injection of 25 mg/kg b.w. in turkeys were presented in (table 1), and depicted in (fig. 1). The drug was firstly detected (0.665±0.055 µg/ml) after 5 min. The pharmacokinetic parameters of Tylvalosin following its intramuscular injection were tabulated in (table 3). Tylvalosin was rapidly absorbed after intramuscular administration with absorption half-life ($t_{0.5\,ab}$) 0.076±0.003 h. Peak serum concentration (C_{max}) was (1.446±0.121 µg/ml) achieved after maximum time (T_{max}) of (0.282±0.008 h) post administration. The drug was highly eliminated from blood with a mean half-life ($t_{0.5\,el}$) of (0.467±0.0.58 h). The systemic bioavailability of Tylvalosin after intramuscular injection was (14.06±0.69 %).

Protein binding percent

The extent of serum protein binding was (12.33±0.489) %.

DISCUSSION

Tylvalosin is a recently approved macrolide antibiotic used in veterinary medicine with proven efficacy for controlling respiratory infection in poultry. To identify the disposition of Tylvalosin in turkeys, a pharmacokinetic study of Tylvalosin after oral, intramuscular and intravenous administration was performed.

After a single intravenous injection of Tylvalosin (25 mg/kg b.w.), the serum concentration-time curve was best described by a two–compartment open model [12].

The drug was rapidly distributed with a distribution half-life of ($t_{0.5\,\alpha}$) of (0.076 h). The rapid distribution of Tylvalosin may be due to increased tissue distribution, similar to that reported for tylosin phosphate and tylosin tartrate in chickens (0.07, 0.09 h), respectively [13].

Tylvalosin was distributed in the central compartment with a volume of distribution (Vc = average of 0.406 L/kg) and volume of distribution at steady state (Vd_{ss} =1.155 L/kg), this increase of Vd_{ss} over Vc indicated that the peripheral compartment is the major compartment for Tylvalosin distribution at steady state [14]. Our result of Vd_{ss} was similar to the result of tylosin phosphate and tylosin tartrate in chickens (1.09, 0.94 L/kg), respectively [13] and in pigs (1.4 L/kg) [15], while it was slightly higher than those previously reported for broiler chickens (0.69 L/kg) [16]. Tissue macrolide concentrations (peripheral compartment) are consistently higher than serum (central) concentrations, and peak organ tissue concentrations may be as much as five to 10 times serum concentrations [17]. This difference in the kinetic parameters is due to changes in the chemical structure of Tylvalosin than tylosin. *In vitro* studies have clearly shown Tylvalosin to enter and accumulate inside several cell types, including gut epithelial cells, whereas tylosin penetration in all cell types is relatively poor [18].

The volume of distribution at steady-state Vd_{ss}, Vd_B and Vd_{area} recorded in our study were more than unity (>one L/kg) following intravenous dosage in turkeys, indicating more clearly that the drug was widely distributed in the extravascular tissues than in serum. These results were supported by [19, 20].

Tylvalosin was rapidly eliminated with an elimination half-life of ($t_{0.5\,\beta}$) of (0.788 h), our result was nearly similar with finding reported for tylosin in broiler chickens (0.52 h) [16], but it was slightly lower than reported in chickens (1.04-1.16 h) [13], (1.99-2.67 h) [21] and (0.9) in dog [22].

The rate of clearance in the present study ($CL_{(B)}$ =1.489 ml/kg/h) was similar to those reported for tylosin phosphate, tylosin tartrate in chickens (1.71-1.61 ml/kg/h), [13] but higher than reported in chickens (5.30 ml/kg/min=0.088 ml/kg/h) [16]. Differences in the kinetic parameters are relatively common and frequently related to interspecies variation, age, breed, health status of the animals and/or the assay method used [23].

Following a single oral administration of Tylvalosin (25 mg/kg b.w.) in normal turkeys, the drug was detected in serum 10 min post administration 0.236 µg/ml. It was continued to increase gradually thereafter to reach its maximum concentration (C_{max}) 0.637 µg/ml at T_{max} of 1.293 h post-administration. This result (C_{max}) was lower than recorded for Tylvalosin in chickens (1.64 µg/ml) by [6]. The lower C_{max} may be due to the presence of the microbial flora in the crop, which could inactivate macrolides [24].

The present study showed that Tylvalosin was rapidly absorbed and widely distributed to tissues indicated by low absorption rate constant (k_{ab}) and absorption half-life ($t_{0.5\,ab}$) 0.283 h, this result was similar to that obtained in chickens (0.175, 0.471 h) when used flexible and rigid catheter, respectively [6, 4]. The pharmacokinetics of Tylvalosin in chickens showed a high variation in the absorption profile not only between individuals but also within individuals when used on separate occasions [6]. The absorption may be decreased a consequence of the presence of food in the crop or due to the presence of Lactobacillus flora in the crop [25]. The capacity of Lactobacillus to inactivate macrolides has been reported [24] and could explain the differences observed when Tylvalosin is deposited into the crop, in which the flora is almost 100% composed of *Lactobacillus.*

Our finding showed that the elimination half-life $t_{0.5\,el}$ of Tylvalosin was 5.309 h. This finding was higher than reported in chickens (2.474 h) [6] and (1 to 1.45 h) in poultry [4]. This difference may be related to the changes in the chemical structure of Tylvalosin than tylosin, due to the addition of the isovaleryl group [26].

Following a single intramuscular administration of Tylvalosin (25 mg/kg b.w.), the drug was detected in serum 5 min post administration 0.666 µg/ml. It was continued to increase gradually thereafter to reach its maximum concentration (C_{max}) 1.446 µg/ml at 15 min. post-administration, this result was similar to that reported for rabbit after intramuscular administration of tylosin tartrate (0.96-1.25 µg/ml) [27] and with subcutaneous administration of tylosin in chickens (1.3 µg/ml) [28].

After intramuscular administration, Tylvalosin was absorbed very rapidly with a T_{max} of (0.282), whereas T_{max} after the oral administration was delayed (1.293). This result was similar to finding obtained after subcutaneous injection of gamithromycin a macrolide antibiotic in chickens [29]. Tylvalosin was rapidly absorbed after intramuscular administration with absorption half-life ($t_{0.5\,ab}$) 0.076 h, this result was similar to that obtained with tylosin in chickens (0.170 h) [30].

Our finding showed that the elimination half-life ($t_{0.5\,el}$) of Tylvalosin after IM injection was (0.467 h), this finding was lower than reported with tylosin in the calf (2.24 h), buffalo (2.40 h) and sheep (2.3-6 h) [31]. This difference may be attributed to the anatomical and physiological differences between these species as well as to the difference in the chemical structures of Tylvalosin. The systemic bioavailability of Tylvalosin after oral administration in normal turkeys was 33.84 %.; this result was similar to that reported with tylosin in chickens (30.7-34%) [16], but higher than that reported in chickens (25%) [13], and lower than reported in chickens (35.41-40.56 %) [21].

Table 1: Mean serum concentrations of Tylvalosin (µg/ml) in turkeys following a single intravenous, intramuscular and oral administration of 25 mg/kg b.w. (mean±SE)

Time of sampling (h)	Mean serum concentration of Tylvalosin after		
	Intravenous	Intramuscular	Oral
0.083	34.35±3.12	0.666±0.05	ND
0.176	21.48±2.26	0.873±0.078	0.236±0.016
0.25	12.485±1.074	1.864±0.168	0.312±0.001
0.5	7.904±0.827	1.05±0.077	0.408±0.001
1	3.54±0.768	0.479±0.03	0.747±0.028
2	1.833±0.77	0.337±0.011	0.551±0.019
4	0.667±0.089	0.232±0.015	0.442±0.015
6	0.223±0.01	0.178±0.012	0.358±0.019
8	ND	ND	0.277±0.015
12	ND	ND	0.187±0.011
24	ND	ND	ND

(n=10), ND**:** not detected

Table 2: Pharmacokinetic parameters of Tylvalosin in turkeys following a single intravenous, of 25 mg/kg b.w. (mean±SE)

Pharmacokinetic parameters	Units	Intravenous injection
Cp^o	µg/ml	63.33±5. 84
A	µg/ml	52.74±6.03
α	min	9.137±0.177
$t_{0.5\,\alpha}$	h	0.076±0.0014
B	µg/ml	10.588±1.721
β	h^{-1}	0.947±0.163
$t_{0.5\,\beta}$	h	0.788±0.107
K_{12}	h^{-1}	4.03±0.373
K_{21}	h^{-1}	2.33±0.338
K_{el}	h^{-1}	3.72±0.369
Vc	L/kg	0.406±0.04
Vd_B	L/kg	2.555±0.40
Vd_{area}	L/kg	1.662±0.243
Vd_{ss}	L/kg	1.155±0.183
$Cl_{(B)}$	ml/kg/h	1.489±0.143
AUC	µg/ml/h	17.37±1.97
MRT	h	0.793.73±0.125
AUMC	µg/ml/h	14.189±3.50

(n=10) , $C_p°$ concentration at zero time (immediately after single IV injection), **A, B** zero-time intercepts of the biphasic disposition curve. **α, β** hybrid rate constants representing the slopes of distribution and elimination phases, respectively. $\mathbf{k_{12}}$ first-order constant for transfer from central to the peripheral compartment; $\mathbf{k_{21}}$ first-order constant for transfer from peripheral to the central compartment; $\mathbf{K_{el}}$ elimination rate constant. $t_{0.5\,\alpha}$ distribution half-life; $\mathbf{t_{0.5\,\beta}}$ elimination half-life; **Vc** apparent volume of the central compartment; $\mathbf{Vd_{ss}}$ volume of distribution at steady state; $\mathbf{Vd_B}$ volume of distribution calculated by extrapolation method; $\mathbf{Vd_{area}}$ volume of distribution calculated by area method. **MRT** mean residence time; **AUC** area under serum concentration-time curve; AUMC area under moment curve; $\mathbf{Cl_{(B)}}$ total body clearance.

The systemic bioavailability of Tylvalosin after intramuscular administration was 14.06%, this result similar to that reported with tylosin phosphate after oral administration in chickens (13.74%) and lower than that recorded for tylosin tartrate in chicken (25.8%) after oral administration [13] and in camels (41%) after IM administration [32]**.** The difference in the AUC may be due to the differences in the achieved bioavailability.

The *in-vitro* protein-binding tendency of Tylvalosin in serum was (12.33%). This finding provides evidence that Tylvalosin was not extensively bound to serum protein in turkeys, and it might explain the high diffusion of Tylvalosin in tissues of turkeys and high value of the volume of distribution.

Previous result recorded that tylosin has low binding to serum proteins [33, 13].

Table 3: Pharmacokinetic parameters of Tylvalosin in turkeys following a single intramuscular and oral administration of 25 mg/kg b.w. (mean±SE)

Pharmacokinetic parameter	Units	Intramuscular	Oral
A	µg/ml	2.55±0.313	0.801±0.042
K_{ab}	h^{-1}	9.107±0.335	2.46±0.107
$t_{0.5\ ab}$	h	0.076±0.003	0.283±0.012
B	µg/ml	2.55±0.313	0.801±0.042
K_{el}	h^{-1}	1.55±0.190	0.135±0.014
$t_{0.5\ el}$	h	0.467±0.058	5.309±0.542
T_{max}	h	0.282±0.008	1.293±0.024
C_{max}	µg/ml	1.446±0.121	0.637±0.018
AUC	µg/h/ml	2.44±0.120	5.878±0.324
MRT	h	0.784±0.083	8.064±0.768
F	%	14.06±0.69	33.84±1.86

(n=10)

k_{ab} first-order absorption rate constant; K_{el} elimination rate constant; C_{max} maximum serum concentration; T_{max} time to peak serum concentration; $t_{0.5\ ab}$ absorption half-life; $t_{0.5\ el}$ elimination half-life; MRT mean residence time; AUC area under serum concentration-time curve; F fraction of drug absorbed systemically after oral or intramuscular administration.

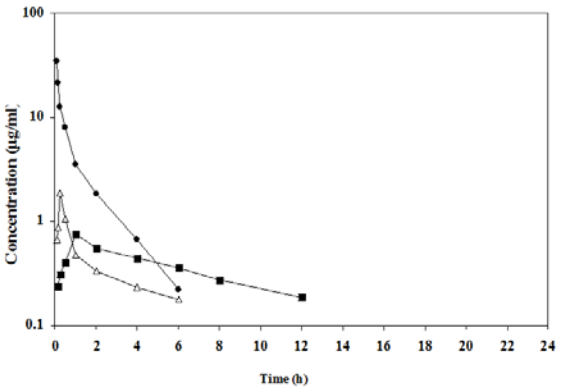

Fig. 1: Semilogarithmic plot depicting the time-course of tylvalocin in serum of turkeys after a single intravenous (·) intramuscular (▲) and oral administration (■) of 25 mg/kg b. w

CONCLUSION

These data allow concluding that Tylvalosin was highly absorbed and distributed to tissues and rapidly eliminated from the body of turkeys after oral and intramuscular administration. However, repeated doses are necessary to maintain Tylvalosin serum concentration above the MIC for most susceptible microorganisms.

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CONFLICT OF INTERESTS

The author declares that there are no conflicts of interest associated with this work.

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