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Pharmacology

Pharmacokinetic and Bioavailability of Apramycin in Broiler Chickens.

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ABSTRACT

The pharmacokinetics inquiries of apramycin were evaluated after receiving a single intravenous (IV) and single oral (PO) dosage in healthy broiler chickens. The serum concentration was measured using high-performance liquid chromatography (HPLC). The kinetic parameters showed that apramycin suggested a two compartments open model following a single intravenous administration of 10 mg/kg b.wt, with distribution half-life ($t_{0.5\alpha}$) of 0.24 ± 0.04h, elimination half-life ($t_{0.5\beta}$) was 5.45 ± 0.47h, steady state volume f distribution (V_{dss}) was 0.970 ± 0.049 L/kg and 0.083 ± 0.003 L/kg/hr for total body clearance (CL_{tot}). After administering a single oral dose of 25 mg apramycin/kg b.wt, the kinetic parameters revealed that the drug was rapidly absorbed by the chickens alimentary canal. This was evidenced by the absorption half-life ($t_{0.5\ (ab)}$) of 1.51± 0.09 hours, Maximum level of serum concentrations C_{max} was 3.255 ± 0.03 µg/ml that was achieved at maximum time T_{max} 2.59± 0.03h and the elimination half-life {$t_{0.5\ (\beta)}$} was 1.93± 0.15 h. The absolute systemic bioavailability (F %) was 11.60±1.2 % indicating a poor absorption of apramycin following oral administration. It was concluded that apramycin is advised for treating enteric infections in chickens.

Keywords: Apramycin, Bioavailability, Broiler chickens, and kinetics.

INTRODUCTION

Apramycin is a broad-range aminocyclitol antibiotic. It acts bactericidal and it inhibits protein synthesis by binding irreversibly to the 30S ribosomal subunit. It is mostly used to treat systemic and intestinal infections. It works well against different Gram-negative bacteria. Apramycin is applied to treat sepsis caused by *E. coli* in chickens, collibacillosis in lambs, bacterial enteritis in pigs, and collibacillosis and salmonellosis in calves. It is not permitted to use apramycin on laying birds, cattle, or sheep that produce milk for human use (Hesham et al., 2019). The disposition kinetics of apramycin has been studied in different animal species; in calves (Ziv et al., 1985), in turkey (Freidlin et al., 1985), in sheep, rabbits, pigeons, chickens and ewes (EL-Sayed et al., 2018), in goats, lactating cows, Japanese quail and broilers (Cracknell et al., 1986). The purpose of this investigation was to look at the kinetics of apramycin and its bioavailability after a single intravenous (10 mg/kg b.wt) and single orally administered (25 mg/kg b.wt) in healthy chickens.

MATERIALS AND METHODS

Drug

Apramycin, its trade name isApracure®, it was obtained from WAKI Pharma for pharmaceutical industries. Each 100 gm contains 86.5gm apramycin sulphate equivalent to 59.5gm apramycin base. It is used for oral administration.

Birds

Ten clinically healthy chickens of 2 weeks age, a random selection of chickens weighing 1200 g was chosen from a farm in Egypt. These chickens were provided with a well-balanced diet free from antibiotics. Prior to the commencement of the experiment, a two-week observation period was conducted to ensure that there were no traces of drug residues present in the chickens' bodily fluids or tissues.

Experimental design

Before apramycin is administered, the dose must be determined so each chicken was weighed individually. Each chicken was injected with 10 mg/kg b.wt of apramycin as a single therapeutic dosage (EL- Sayed et al., 2018) in the vein of the left wing. 1ml of blood samples were obtained from each bird's right wing at 0.08, 0.17, 0.25, 0.5, 1, 2, 4, 8, 12, and 24 hours following intravenous injection, to assess the bioavailability of apramycin in typical hens, 25 mg/kg b.wt of apramycin was given orally to these chickens 15 days subsequent to intravenous administration to guarantee their complete removal from their body. The ration was not offered for twelve hours prior to oral apramycin administration and was made available 5 hours later. 1ml of blood samples were obtained from each bird's right wing at 0.25, 0.33, 0.5, 1, 2, 4, 6, 8, and 12 hours. A blood sample arrangement was made in a sloppy position to separate sera samples at room temperature next centrifuged for 15 minutes at 3000 rpm. Serum samples obtained from the experiment were kept in sterile Eppendorf tubes at a temperature of -20°C up to further analysis.

Analytical method

Apramycin serum concentrations were assessed using high-performance liquid chromatography(HPLC).Half mL of serum samples was used and treated with 1.0 mL 10% trichloroacetic acid (containing 0.04 mM Na_2EDTA, w/v).The sample was vortexed for two minutes and then centrifuged for ten minutes at 4˚C at 9500 ×g. The clear supernatant was poured into 5 milliliter centrifuge tubes, the extraction process was repeated then applied twice supernatant for the PE procedure (Dai et al., 2017). In Solid-phase extraction, methanol (1 mL) with water (2 mL) were used to condition the (Oasis MCX 30 mg, 1 cc, Waters) solution. For Chromatographic circumstances, HPLC was achieved using Agilent 1200 with a reversed-phase column (C_{18}, 4.6 ×250 mm i.d., 5 µm, Agilent, USA). The mobile phase for separation was acetonitrile water (v/v, 77:23). A quantification limit (LOQ) of 0.05 µg/mL and a detection limit (LOD) of 0.015 µg/mL were confirmed by the validation of the apramycin assaying.

Pharmacokinetic analysis:

Pharmacokinetic analysis of the data was achieved using a two-compartment open model data were analyzed using Win N online 2.1 software (Pharsight analysis by the USA). The pharmacokinetic parameters were calculated according to Baggot (1978).

Statistical analysis:

The data were calculated as mean ± standard error.

All statistical analysis was performed using the following formulas, in accordance with Snedecor (1969)

RESULTS

The average serum concentrations of apramycin were measured after a single intravenous dose of 10 mg/kg b.wt, and a single oral dose of 25 mg/kg b.wt. The results of this analysis were presented in Table 1 and Figure 1. The kinetic parameters of apramycin after single intravenous dose of 10 mg and oral dose of 25 mg/kg b.wt were presented in Table 2 and 3, respectively.

After I.V. administration, apramycin was rapidly distributed with a distribution half-life ($t_{0.5\ (\alpha)}$) of0.24 ± 0.04 h, steady-state distribution volume (V_{dss}) was 0.970 ± 0.049 L/kgand elimination half-life ($t_{0.5\ (\beta)}$) was 5.45 ± 0.47h. The rate of total body clearance [CL_{tot}] was 0.083 ± 0.003 L/kg/hr. After oral administrated, the peak of serum concentration C_{max} (3.255 ± 0.03µg/ml) attained at a time of maximum concentration (T_{max}) of 2.59± 0.03 h, absorption half-life {$t_{0.5\ (ab)}$} was 1.51± 0.09 h, and the elimination half-life {$t_{0.5\ (\beta)}$} was 1.93± 0.15h. Systemic absolute bioavailability (F %) was 11.60±1.2%reflectinga poor absorption of apramycin following oral administration.

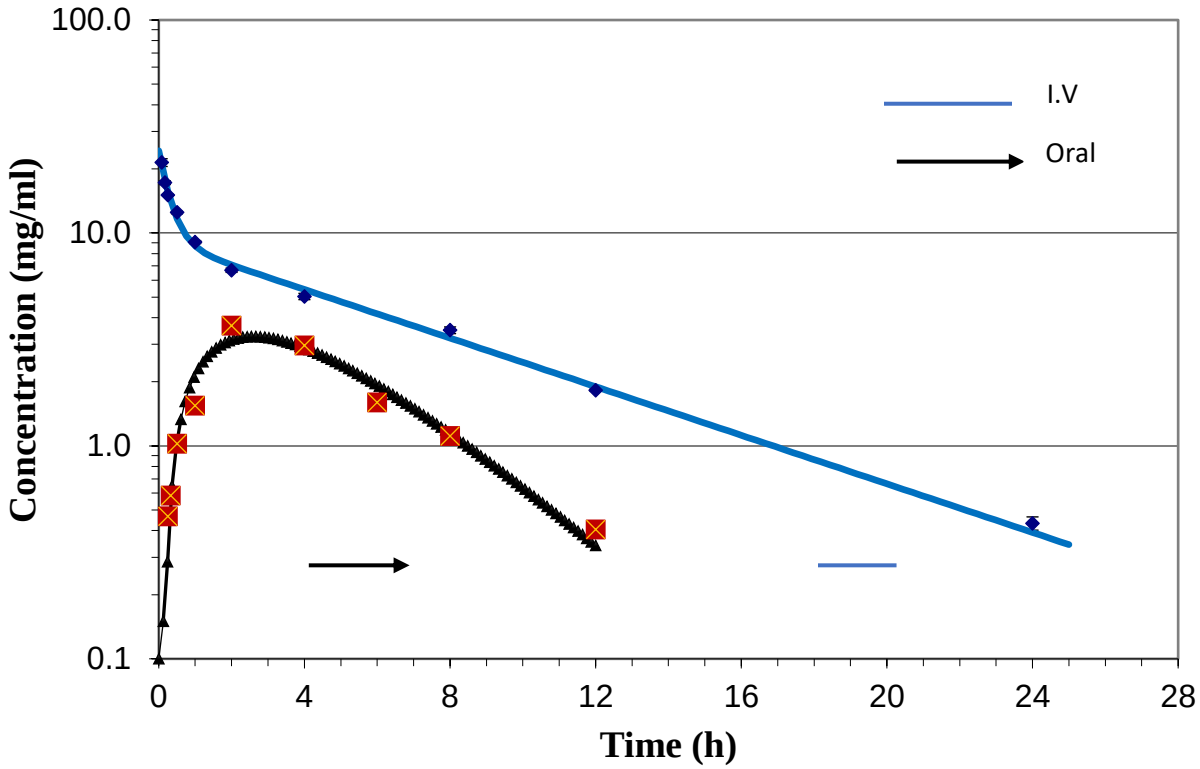

Fig. (1): Semi-logarithmic chart presenting serum time-concentration of apramycin in broiler chickens' sera following single intravenous (10mg/kg b.wt) and oral (25 mg/kg b.wt) administration (n = 6).

Table 1. Serum concentrations of apramycin (µg/ml) in normal broiler chickens following a single I.V. dose of10 mg/kg b.wt and a single 25 mg/kg b.wt oral dose. (n=6)

Apramycin concentrations (µg/ml serum)			
Intravenous		**Oral administration**	
Time	**I.V ±S.E.**	**Time**	**P.O ±S.E.**
0.08 h	21.41± 0.903	0.25h	0.467± 0.018
0.17 h	17.213± 0.339	0.33h	0.585± 0.020
0.25 h	15.080± 0.137	0.5h	1.022± 0.029
0.5 h	12.470± 0.175	1h	1.547± 0.023
1 h	9.058± 0.150	2h	3.667± 0.043
2 h	6.685± 0.157	4h	2.960± 0.026
4 h	5.033± 0.173	6h	1.598± 0.036
8h	3.492± 0.126	8h	1.113± 0.032
12h	1.827± 0.024	12h	0.405± 0.012
24h	0.433± 0.031	----------	----------

Table 2. Mean ± SE serum pharmacokinetic values after a single intravenous (10 mg/kg b.wt) administration of apramycin in healthy chickens. (n = 6).

Parameter	**Units**	**I.V ±SE**
Cp°	$\mu g/ml^{-1}$	25.128 ± 1.941
A	µg/ml	15.98 ± 1.47
α	h^{-1}	3.50 ± 0.68
t0.5 (α)	h	0.24 ± 0.04
B	µg/ml	9.15 ± 0.59
β	h^{-1}	0.13 ± 0.01
t0.5 (β)	h	5.45 ± 0.47
K12	h^{-1}	1.93 ± 0.42
K21	h^{-1}	1.36 ± 0.25
K_{el}	h^{-1}	0.34 ± 0.04
AUC	µg/ml.h	75.15 ± 3.00
AUMC	$\mu g/ml.h^{-2}$	559.93 ±62.20
Cl_{tot}	L/Kg/h	0.083 ± 0.003
MRT	H	7.344± 0.595
Vss	L/kg	0.970 ± 0.049
Vd_{area}	L/kg	0.409 ±0.027

Table 3. Mean ± SE serum pharmacokinetic parameters of apramycin in healthy chickens following single oral administration (P.O) of 25 mg/kg b.wt (n = 6).

Parameter	**Units**	**P.O ±S.E**
C_{max}	$\mu g/ml^{-1}$	3.255 ± 0.03
T_{max}	h	2.59± 0.03
t0.5 (ab)	h	1.51± 0.09
t0.5 (el)	h	1.93± 0.15
Kab	h^{-1}	0.47 ± 0.03
Kel	h^{-1}	0.37 ± 0.03
AUC	$\mu g/ml/h^{-1}$	21.81± 0.52
F	%	11.60±1.2

DISCUSSION

In The present study, an intravenous injection of 10 mg apramycin per kg body weight in healthy chickens showed that the drug best fit with a two-compartment open model. These results were in contrast to those previously reported for apramycin when it was administered to healthy chickens at a dose of 75 mg/kg body weight (Afifi et al., 1997), and with those formerly accepted in normal chicken (EL-Sayed et al., 2018) and (Elbadawy & Aboubakr,2017). Apramycin was rapidly distributed with short half–life after I.V. injection as indicated by the value of $t_{1/2\alpha}$ (0.24 ± 0.04h). This outcome is very similar to that reported of apramycin in goats at 0.1±0.04h (Dinev et al., 2009), in calves at 0.47h (Ziv et al., 1985), streptomycin in camel at 0.156 h (Hadi et al., 1998). Longer distribution half-life was observed for apramycin in chicken (1.50 ±0.20 h) (Afifi et al., 1997), and4.56±0.079 h (EL-Sayed et al., 2018).

The half-life of the drug's elimination, or ($t_{0.5\beta}$) was 5.45 ± 0.47 in grill chickens. This observation was greater than the following: 2.15±0.01 h (Elbadawy & Aboubakr, 2017), 0.999±0.006 h (EL-Sayed et al., 2018), 2.10 h (Afifi et al., 1997), 0.83±0.03 h (Lashev 1998), and 1.32±0.09 h (goats). Similar to the data on amikacin in grill chickens (4.48 h) (Elbadawy et al., 2017), calves (4.4 h) (Ziv et al., 1985), and foals (5.07 and 5.2 h) (Bucki et al., 2004). In Turkey, the result was 2.62 ± 0.13 h (Haritova et al., 2004), whereas in Japanese quails, it was 0.50 ± 0.02 h (Lashev and Mihailov, 1994), and in camels, it was 3.35 h (Hadi et al., 1998).

The steady-state distribution's volume ({V_{dss}}) in the current investigation was 0.970 ± 0.049 L/kg. The somewhat lower V_{dss} values suggested that the medication was less widely dispersed in extravascular tissues. This result is less than the data reported for chicken 1.46L/kg (EL-Sayed et al., 2018) and in chicken (4.82 ± 0.08 L/kg) (Afifi et al., 1997). The volume of distribution, on the other hand, was greater than that reported for apramycin in chicken (0.246±0.024 L/kg) (Lashev, 1998), adult chicken (0.182±0.021 L/kg) (Apramycin), rabbits (0.284±0.035 L/kg), sheep (0,167±0.008 L/kg), pigeons (0.077±0.001 L/kg) (LASHEV et al., 1992), and calves (0.71 L/kg) (Ziv et al., 1985).

Apramycin moved rapidly from the central compartment to the peripheral compartment. (K12 = 1.93 ± 0.42 h^{-1}) rather than moving from the peripheral to the central compartment (K21 =1.36 ± 0.25 h^{-1}). Conversely, these data were lower than that explained for apramycin in broiler chickens (K12 = 3.89 ± 0.0251 h^{-1}) and (K21 =1.44 ± 0.151 h^{-1}) (EL-Sayed et al., 2018), and in goat (K12 = 4.124±1.432 h^{-1}) and (K21 =

2.215±0.487 h^{-1}) (Dinev et al., 2009). However, these values were greater than the data provided for apramycin in chickens (K12 = 0.01 h^{1}) and (K21 = 0.39 h^{-1}) (Afifi et al., 1997), in calves (K12 = 0.037 h^{-1}) and (K21 = 0.031 h^{-1}) (Ziv et al., 1985).

The rate of apramycin's whole body clearance (CLtot) after IV was (0.083 ± 0.003 L/kg/hr.) This result is about as reliable as the apramycin values (0.078± 0.010 L/kg/hr) found in broiler chickens (LASHEV et al., 1992). The outcome attained was lower than those documented for apramycin in chicken (0.245±0.024 L/kg/hr) (Lashev 1998), apramycin lactating cow (0.729 L/kg/hr) (Ziv et al., 1995), in Japanese quails (0.186± 0.007 L/kg/hr) (Lashev and Mihailov, 1994), in chickens (1.88± 0.05 L/kg/hr) (Afifi et al., 1997), and in chickens (0.257±0.002 L/kg/hr) (EL-Sayed et al., 2018). The assay methods used, the time between blood samples, the age and health of the animal, and other variables are frequently linked to these very regular contrasts. (Haddad et al., 1985).

After 15 days a similar chicken which administered a 10 mg/kg b.wt intravenous injection of apramycin, taken apramycin 25 mg/kg b.wt. Following a single oral dosage, the drug's mean recorded peak serum level in the current study was C_{max} (3.255 ± 0.03µg/ml) achieved at (t_{max}) (2.59± 0.03hours). The got result almost like those recorded in chicken C_{max} (2.00± 0.87µg/ml) at T_{max} (1.59+0.55h) (Lashev, 1998), and higher than those stated of apramycin in chicken (C_{max}) (0.712 ± 0.002 µg/ml) and (t_{max}) (0.524 ±0.006 hours) (EL-Sayed et al., 2018), in chicken (C_{max}) (0.75±0.03µg/ml) at (t_{max}) (0.18±0.011h) (Elbadawy & Aboubakr, 2017), in chicken (C_{max}) (0.790 µg/ml) and reached at (tmax) (0.200 h) (Afifi et al., 1997), tobramycin in chicken (C_{max}) (0,106±00,28µg/ml) and (t_{max}) (1,83±0,31 h) (Lashev et al., 2005), Neomycin in pig (C_{max}) (0.11 ± 0.07 µg/ml) (t_{max}) (1.92 ± 0.97h) (Liu et al., 2021).

The drug was a poorly absorbed half-life ($T_{0.5\ (ab)}$) of 1.51± 0.09 h. These values were greater than the apramycin recorded in chicken (0.10 ±0.001 h) (Afifi et al., 1997), in Japanese quails (0.21 ± 0.09 h) (Lashev and Mihailov, 1994), in chicken 0.11±0.001h (Elbadawy & Aboubakr, 2017), these values were similar to those recorded for tobramycin in chicken (1.67±0.15h) (Lashev et al., 2005).

Elimination half-life for apramycin ($T_{0.5\ (el)}$) was (1.93± 0.15 h). The value is similar to apramycin in chicken (1.90 ± 0.164 h) (EL. Sayed et al., 2018), (1.22± 0.01h) (Afifi et al., 1997), in Japanese quails 2.31± 0.38h (Lashev and Mihailov, 1994), in chicken 1.22±0.01 h (Elbadawy & Aboubakr, 2017) and lower than those stated of neomycin in pigs 12.43 ± 7.63 h (Liu et al., 2021).

The determined AUC was found to be 21.81± 0.52 µg/h/ml. Also, these values were higher than apramycin in chicken (2.54±0.023 µg/h/ml) (EL-Sayed et al., 2018), (0.83±0.02 µg/h/ml) (Elbadawy & Aboubakr, 2017), tobramycin in chicken (0.393±0.102 µg/h/ml) (Lashev et al., 2005), (0.81 µg/h/ml) (Afifi et al., 1997), in Japanese quails 1.53±0.23 µg/h/ml (Lashev and Mihailov, 1994). Following oral administration, the systemic bioavailability of apramycin after its single oral dose of 25 mg/kg b.wt. in control chickens was (11.60±1.2%), this value indicates that apramycin typically does not absorb effectively from the gut. Drug retention in the gastrointestinal tract is advantageous for treating gastrointestinal infections. The result was higher than apramycin in chicken (1.31%) (EL-Sayed et al., 2018) in chicken (2.03%) (Afifi et al., 1997), 2.5 % (Elbadawy & Aboubakr,2017), in Japanese quails 0.56% (Lashev and Mihailov, 1994), in chicken (3.77%) (Lashev, 1998), However, the got data was lower than that seen for different species when given by

ways other than the oral method such as in calves (61.98%) (EL-Sayed et al., 1994), and in turkey roosters (107.61 ± 33.56%), gentamycin in turkey roosters (97.2 ± 31.41 %) (Haritova et al., 2004, amikacin in lactating ewes (98.27%) (Abo el-Sooud, 1999), amikacin in cats (95± 20%) (Jernigan et al., 1988), gentamycin in chicken (79%) (Abu-Basha et al., 2007), and neomycin in sheep (74-85%) (Errecalde et al., 1990).

CONCLUSIONS

Apramycin has a restricted oral bioavailability of about 11.60 % in chicken, indicating a poor oral absorption, so it is advised to be used for treating enteric infections caused by *E. coli*, *Salmonella*, and other sensitive bacterial species.

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