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PHARMACOKINETICS, BIOAVILABILITY AND TISSUE RESIDUES OF AMIKACIN IN BROILER CHICKENS

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ABSTRACT

The disposition kinetics and serum availability of amikacin in broiler chickens after single intravenous (IV) and intramuscular (IM) administrations of 10 mg/kg body weight were investigated. The concentrations of the drug in the serum were measured using microbiological assay on samples collected at frequent intervals after drug administration. Following intravenous injection, the serum concentration-time curves were best described by a two compartment open model. The elimination half-life ($t_{1/2\beta}$), volume of distribution at steady state (V_{dss}) and total body clearance (Cl_{tot}) of amikacin were 4.48 h, 501.03 ml/kg and 0.08 L/h/kg, respectively. After intramuscular injection of amikacin at the same dose the peak serum concentrations (C_{max}) were 15.25 µg/ml and were obtained at 1.89 h (t_{max}), the elimination half-life ($t_{1/2el}$) were 5.23 h and absorbtion half-life ($t_{1/2ab}$) were 0.75 h. The systemic bioavailability was 95.20%. Amikacin was detected in liver and kidney for 5 days post a single IM injection and withdrawal time was 6 days.

KEYWORDS: Amikacin, Pharmacokinetics, Residues, Broiler chickens.

1. INTRODUCTION

Amikacin, an aminoglycoside antibiotic, is recommended in the treatment of several bacterial infections such as *Escherichia coli, Klebsiella, Enterobacter, Staphylococcus aureus, Proteus mirabilis, Serratia, Mycobacterium, Corynebacterium equi, Streptococcus zooepidermicus* and *Actinobacillus* in various animal species.[1] The primary adverse effects associated with systemic administration of aminoglycoside antimicrobials are nephrotoxicity and ototoxicity.[2] Aminoglycoside antimicrobials accumulate in the renal tubular cells via pinocytosiswhich produce renal tubular injury.[3]

The disposition kinetics of amikacin has been investigated in dogs[4-5], horses[6-7-8], calves and sheep[9], cats [10], chickens[11], goats[12-13-14], camels[15], foals[16] and sheep.[17]

During the preapproval process for veterinary drugs, studies are conducted to determine safe and efficient protocols for use. This process also includes specific studies on the characteristics of drug incorporation in the target animal including absorption, distribution, metabolism, storage and excretion.[18-19]. One of the most important results of these studies is the identification of the edible tissue in which the concentration of residues persists for the longest period of time at the highest concentration. This tissue, called the target tissue, is then used to monitor the safety of edible tissues from food producing animals.[20] Based on these determinations, it will be determined if the edible tissues of the animal are safe for human consumption.

The aim of this study is to perform the pharmacokinetic profile of amikacin (10 mg/kg b.wt.) following single intravenous (IV) and intramuscular (IM) administrations in broiler chickens. Also, tissue residues following a single IM injection of amikacin (10 mg/kg b.wt.) were investigated in broiler chickens.

2. MATERIAL AND METHODS

2.1. Drug (Amikacin)

Amikacin (Amigasol®), as injectable solution containing amikacin sulphate at a concentration of 250 mg/ml). It was manufactured by (ATCO Pharma Trading Co.), Egypt,

2.2. Broiler Chickens and Experimental Design

A total number of sixty clinically healthy Hubbard broiler chickens chickens, 40 days of age, weighing between 1.8 and 1.95 kg, were obtained 2 weeks before the start of this study. During acclimatization (2 weeks before starting the experiment to ensure the complete withdrawal of any residual drugs), chickens were fed antibacterial-free, balanced, commercial rations and drinking water was freely available. The birds were housed in a cage. The study was approved by the Animal Care and Use Committee at the Faculty of Veterinary Medicine.

The chicken was divided in two main experiments

2.2.1. Pharmacokinetics and bioavailability study

Ten broiler chickens were individually weighed before drug administration and doses were calculated precisely. The chickens were given a single IV dose of amikacin at a dose of 10

mg/kg into the left wing vein. After 30 days, the same chickens were given the same dose of amikacin by IM route through the thigh muscle at same dose.

2.2.2. Tissue residue study

Fifty broiler chickens were given amikacin at a single dose of 10 mg/kg b.wt. by IM route. Five broiler chickens were slaughtered everyday post amikacin injection for 10 days. Blood and tissue samples (liver, kidney, lung, heart and thigh & pectoral muscles) were taken and stored at -20 ^{o}C pending assay.

2.3. Blood and tissue samples

Blood samples were obtained from the right wing vein (1 ml) and collected in test tubes immediately before and at 0.08, 0.16, 0.25, 0.5, 1, 2, 4, 8, 12 and 24 hours after a single IV or IM administration and blood samples were obtained also every day following the last dose of repeated oral administration. Samples were centrifuged at 3000 rpm for 10 minutes and the obtained sera were used for the estimation of concentration. Blood and tissue samples (liver, kidney, lung and muscles) were taken and stored at -20 ^{o}C pending assay. The serum and tissue samples were stored at −20˚C until analysis, and the assay was performed within a week of obtainment.

2.4. Analytical procedures

The concentration of amikacin in serum samples was estimated by a standard microbiological assay using *Bacillus subtilis* ATCC 6633 as test micro-organism.[21] The medium was prepared by dissolving 9.5 g Mueller–Hinton agar in 250 ml distilled water in a 0.5 l flat-bottomed flask, which was autoclaved for 20 min. After cooling to 50°C in a water bath, 0.4 ml of the diluted suspension of reference organism was added to the media. Six wells, 8 mm in diameter were cut at equal distances in standard Petri dishes containing 25 ml seeded agar. The wells were filled with 100 µl of either the test samples or amikacin standards. The plates were kept at room temperature for 2 h before being incubated at 37°C for 18 h. Zones of inhibition were measured using micrometers, and the amikacin concentrations in the test samples were calculated from the standard curve. Negative control samples showed no bacterial inhibition, indicating no intrinsic antibacterial activity of the samples.

For assay of tissue samples, two grams of tissue were homogenized by automatic homogenizer with 2 ml of distilled water. Mixtures were centrifuged at 3000 r.p.m. for 10

minutes and supernatant fluid of each sample was obtained and directly assayed microbiologically for amikacin concentration.

2.5. Pharmacokinetic analysis

Pharmacokinetic parameters were determined for each individual chicken. Serum concentrations of amikacin after a single IV and IM administrations were subjected to a compartmental and non-compartmental analysis using computerized program, WinNonlin 4.1 (Pharsight, Mountain View CA, USA). The priming and maintenance doses were calculated according to formula mentioned by.[22]

3. RESULTS

Following a single IV injection of 10 mg kg^{-1}.b.wt in normal broiler chickens, amikacin could be detected therapeutically for 24 hours. The serum concentration – time curve of amikacin following IV injection showed that amikacin obeyed a two compartments open model. The disposition kinetics of amikacin following a single IV and IM administration were recorded in **table (1)** and showed **(Figure 1)**. Calculated IM dosage regimen of amikacin required to maintain specified serum amikacin concentration in broiler chickens was recorded in **table (2)**. Serum and tissues concentrations of amikacin (µg/ml or µg/gm) following IM injection at a dose of 10 mg/kg b.wt. in broiler chickens were mentioned in **table (3)**.

Table: 1. Mean ± SE serum pharmacokinetic parameters of amikacin in broiler chickens following IV and IM injections of 10 mg/kg b.wt. (n=10).

Parameter	Unit	IV	IM
α (k_{ab})	h^{-1}	3.14 ± 0.22	0.91 ± 0.03
$t_{1/2\alpha}$ ($t_{1/2ab}$)	h	0.22 ± 0.01	0.75 ± 0.02
β (k_{el})	h^{-1}	0.15 ± 0.01	0.13 ± 0.003
$t_{1/2\beta}$ ($t_{1/2el}$)	h	4.48 ± 0.20	5.23 ± 0.13
AUC	µg ml^{-1} h^{-1}	119.12 ± 7.01	112.64 ± 3.52
AUMC	µg ml^{-1} h^{-2}	712.36 ± 64.09	760.10 ± 32.53
MRT	h	5.93 ± 0.18	6.73 ± 0.09
MAT	h	—	0.80 ± 0.11
Vd_{ss}	ml kg^{-1}	501.03 ± 11.98	—
Cl_{tot}	l kg^{-1} h^{-1}	0.08 ± 0.001	—
C_{max}	µg ml^{-1}	—	15.25 ± 0.20
t_{max}	h	—	1.89 ± 0.01
F	%	—	95.20 ± 6.74

α; **β** hybrid rate constant representing the slope of distribution and elimination phase after IV injection; **K_{ab}; K_{el}** absorbtion and elimination rate constant after IM injection; **$t_{1/2(\alpha)}$** distribution half-life after IV injection; **$t_{1/2(ab)}$** absorption half-life after IM injection; **$t_{1/2(\beta)}$**

elimination half-life after IV injection; $t_{1/2(el)}$ elimination half-life after IM injection; **AUC** area under serum concentration-time curve; **AUMC** area under moment curve; **MRT** mean residence time; **MAT** mean absorption time; Vd_{ss} volume of distribution at steady state; **Cl** total body clearance. C_{max} maximum serum concentration; T_{max} time to peak serum concentration; **F** fraction of drug absorbed systemically after IM injection.

Table 2: Calculated IM dosage regimen of amikacin required to maintain specified serum amikacin concentration in broiler chickens.

Desired serum concentration (µg/ml)	Dosage interval (h)	Priming doses (mg/kg)	Maintenance doses (mg/kg)
0.5	24	7.24	6.92
1	24	14.49	13.85
2	24	28.98	27.70

Table 3: Mean ± SE serum and tissues concentrations of amikacin (µg/ml or µg/gm) following IM injection at a dose of 10 mg/kg b.wt. in broiler chickens.

	Time after injection (Days)									
Tissues	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Serum	0.46± 0.002	0.11± 0.001	ND	ND	ND	ND	ND	ND	ND	ND
Kidney	16.4±0.87	9.72± 0.62	4.51±0.51	2.01±0.13	0.63±0.004	ND	ND	ND	ND	ND
Liver	9.32± 0.67	6.82± 0.47	2.73±0.17	1.38±0.09	0.13±0.001	ND	ND	ND	ND	ND
Lung	5.29± 0.43	2.03± 0.18	0.32±0.003	ND	ND	ND	ND	ND	ND	ND
Thigh M	3.19± 0.24	1.02± 0.09	ND	ND	ND	ND	ND	ND	ND	ND
Breast M	2.36± 0.18	0.65± 0.03	ND	ND	ND	ND	ND	ND	ND	ND

ND = Not Detected

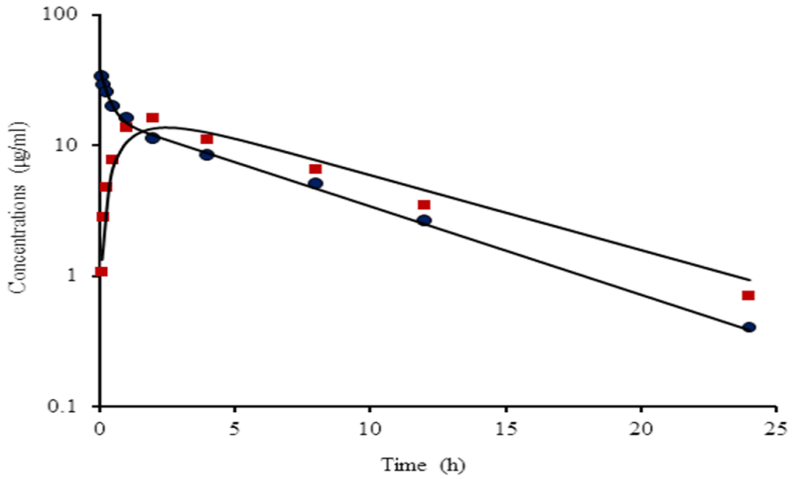

Figure 1: Semi-Logarithmic graph depicting the time-concentration of amikacin in serum of broiler chickens after a single IV (○) and IM (■) administration of 10 mg/kg b.wt. (n=10).

4. DISCUSSION

Intravenous injection of 10 mg amikacin/kg b.wt. in healthy broiler chickens, showed that the drug disposition best fitted a two-compartments- open model. The obtained result was consistent with those reported for amikacin in broiler chicken.[11] Elimination half-life ($t_{0.5\beta}$) of amikacin in chickens following IV administration was 4.48 h. This observation was longer than the data reported for amikacin in calves 3.09 h.[23] and sheep 1.64 h[17], gentamicin in chickens 2.93 h[24] and shorter than amikacin (5.07 and 5.2 h) in foals.[16] Variation in biology, method and environmental conditions, each of these variables influenced the pharmacokinetic parameters between species.[25]

Amikacin showed a relatively good Vd_{ss} (501.03 ml kg^{-1}) in chickens. The obtained value was higher to that recorded for amikacin in broiler chickens (0.19 L kg^{-1}[11] and in sheep (0.18 L kg^{-1}[17] and lower than value recorded for grntamicin in chickens 0.77 L kg^{-1}.[24] The total body clearance (CL_{tot}) of amikacin determined in the present study (0.08 L $kg^{-1}h^{-1}$) was higher to that reported for lactating goats 0.05 L $kg^{-1}h^{-1}$[12] but much lower than that reported for dogs (0.24 L $kg^{-1}h^{-1}$[26] and humans 0.25 L $kg^{-1}h^{-1}$.[27]

Following IM administration, amikacin was rapidly and efficiently absorbed as the absorption half-life ($t_{0.5ab}$: 0.75 h). The obtained value was higher than amikacin in broiler chickens 0.48 h[11] and lower than amikacin (0.06 h) in goats.[13] The elimination half-life ($t_{0.5el}$: 5.23 h) was longer to amikacin in calves 3.09 h[23] and in sheep 1.29 h.[17]

Maximal plasma concentration (C_{max}) was 15.25 µg/ml achived at (T_{max}) 1.89 h. These values were nearly similar to amikacin (16.97 µg/ml) achived at 1 h in sheep.[17] The (C_{max}) obtained in this study was lower than those reported for amikacin (50.79 µg/ml) in broiler chickens.[11]

The bioavailability of amikacin in chickens was 95.20 %. This value referred to an excellent absorption of amikacin following IM injection. This agrees with results reported in lactating goats 98.27%;[12] and camels 96.5%.[15]

The ultimate objective of the present study was to determine a satisfactory IM dosage regimen of amikacin in broiler chickens. The minimum inhibitory concentration (MIC) of micro-organisms from isolates of chickens origin which are susceptible to amikacin, have not yet been determined. However, an MIC of 0.5-5 µg/ml has been reported for many animal species[15] The comparison of calculated dosage regimen of amikacin for IM route in broiler

chickens. For maintaining minimum inhibitory concentration (MIC) of 1 µg/ml in plasma of broiler chickens, a priming dose (D*) of 14.49 mg/kg followed by maintenance doses (D) of 13.85 mg/kg at 24 hr interval may be given. Likewise, for maintaining MIC of 2 µg/ml in chickens, a D* of 28.98 mg/kg followed by D of 27.70 mg/kg at 24 hr interval may be given.

Following a single IM injection of amikacin in broiler chickens, amikacin was detected in kidney and liver for 6 days. After a single IM injection of gentamicin, tissue concentrations were significantly higher in chicken injected gentamicin through pectoral muscles than those injected in thigh muscles and highest concentrations were in kidney and liver.[28] No gentamicin residues were detected in tissues after 24 h except in kidney of chickens received gentamicin through pectoral injection; it was detected until 48 h post-dosing. The accumulation of gentamicin it may be due to a slow release from tissues containing high concentrations.[29] Such high concentration may be achieved by active uptake by the proximal tubules and other body tissues.[30]

5. CONCLUSION

In conclusion, the absence of general adverse reactions in broiler chickens in the study, and the favourable pharmacokinetic properties (high bioavailability) and very good volume of distribution of amikacin administered IM at a dose of 10 mg/kg suggest the possibility of its safe and effective clinical use in broiler chickens. The withdrawal time following a single IM injection of amikacin is 6 days.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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