Dept. of Pharmacology,
Fac. Vet. Med. Moshtolor, Benha Univ. Egypt.
Head of Dept. Prof. Dr. M.G. El-Sayed.

PHARMACOKINETICS OF APRAMYCIN IN NORMAL AND PNEUMONIC CALVES

(With 4 Tables & 2 Fig.)

By

M.G.A. EL-SAYED; M.I. ABD EL-AZIZ*
and M.H. EL-GAMEL

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حركية عقار الأبراميسين فى العجول السليمه والمصابه بالالتهاب الرئوى

مسعد السيد ، مجدى عبد العزيز
محمد الجمل

تم استخدام حركية عقار الأبراميسين . وقد تم تحديد تركيز العقار فى كل من مصل الدم والبول فى العجول السليمه والمصابه بعد الحقن العضلى بالطريقه الميكروبيولوجيه . وقد وجد أم منحنى تركيز العقار بالنسبه للزمن بعد الحقن الوريدى بجرعة ٢٠ مجم/ كجم من وزن العجل أوضح أن الأبراميسين يتحرك فى منطقتين داخل جسم الحيوان وأن فترة نصف عمر إفراز العقار هى ٥٣ ر٢ + ٠٢ ر٠ ساعه . وبعد الحقن العضلى بجرعه واحده (٢٠ مجم/ كجم من وزن العجل) وصل أعلى تركيز للأبراميسين فى الدم بعد ساعتين وأن فترة نصف عمر إمتصاص العقار كانت ٦٣٧ ر٠ ، ٤٨٧ ر٠ ساعه لكل من العجول السليمه والمصابه على الترتيب . وبعد الحقن العضلى المتكرر لعقار الأبراميسين بجرعه ٢٠ مجم/ كجم من وزن العجل مرتين يومياً لمدة خمسة أيام متتاليه وجد أن أعلى تركيز للعقار بالدم وصل بعد ساعتين وفترة نصف عمر افرازه من الجسم كانت تتراوح من ٢٤ ر٢ إلى ١٨ ر٣ ساعه للعجول السليمه ومن ٣١ ر٢ إلى ٧٨ ر٢ ساعه فى العجول المصابه بالالتهاب الرئوى . وقد وجد أن معدل استفادة الجسم من عقار الأبراميسين كانت ٩٨ ر٦١ + ١٩ ر٢ ٪ ونسبة اتحاد معملياً مع بروتينات الدم كانت ٢٩ ر٢ + ٠٢ ر٠ ٪ .

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*: Dept. of Pharmacology, Fac. Vet. Med. Kafr El-Shiekh, Tanta Univ. Egypt.

SUMMARY

Four clinically normal and four pneumonic Holstein Friesian calves were used to study the pharmacokinetic profile of apramycin. Apramycin concentrations in serum and urine were assayed microbiologically after intramuscular injection in normal and pneumonic calves. Following a single intravenous dose of apramycin (20 mg/kg) the blood concentration time-curve indicated a two compartment open model with an elimination half value ($t_{0.5}\beta$) of 2.53±0.02 hours. Following a single intramuscular dose of apramycin (20 mg/kg) the highest serum concentration occurred 2 hours post-injection with absorbtion half lives ($t_{0.5}a\beta$) 0.637 and 0.487 hour for normal and pneumonic calves, respectively. The elimination half lives ($t_{0.5}\beta$) were 2.44 hours in normal and 2.315 hours in pneumonic calves. After intra- muscular injection of apramycin at a dose of 20 mg/kg. twice aday for five consecutive days, the highest serum concentration occurred 2 hours after each dose with elimination half lives ($t_{0.5}\beta$) ranging from 2.24 hours to 3.18 hours in normal and from 2.31 hours to 2.78 hours in pneumonic calves. The mean systemic bioavailability was 61.98±2.19% and the in-vitro protein biling percent of apramicin was 2.29±0.2%

Keywords: Pharmacokinetics, Apramycin, Normal, Pneumonic, Calves

INTRODUCTION

Apramycin is an aminoglycoside antibiotic, which is produced by a strain of *Streptomyces tenebrarius (O CONNOR et al., 1963).* It is effective against *Escherichia coli, Samonella* and other gram-negative enterobacteria *(PANKHURST et al., 1975)* Apramycin has been evaluated as a treatment for naturally occuring enteritis and pneumo-enteritis in young calves *PANKHURST et al.; 1975).* Although the disposition kinetics of this drug has been studied in normal calves *(ZIV et al., 1985; SHIKHA, 1987),* there is no information about

pharmacokinetics in pneumonic calves following systemic administration. Thus, the aim of the present work was to investigate the pharmacokinetics of apramycin in normal and pneumonic calves to establish a proper maintaince dosage regimen in pneumonic calves. Also clearance of apramycin following repeated intramuscular administration in normal and peumonic calves was studied.

MATERLALS AND METHODS

Drug:

Apramycin (Aprolan)(R) was obtained as solution from Lilly company, Italy.

Animals:

4 clinically normal and 4 pneumonic Holstein Friesian female calves (from Veterinary Service of the Egyptian Army, West El-Nobaria Dairy Stations, Alexanderia, Egypt) weighing from 50-70 kg each were used. The animals were fed milk and starter feed ration.

The normal calves were injected intravenously with 20 mg of apramycin/kg. After 15 days, these calves were injected with 20 mg/kg of apramycin base, 2 times daily for 5 consecutive days. The first dose was represented as a single intramuscular dose to calculate the bioavailabity of the drug.

The pneumonic calves were injected with 20 mg/kg of apramycin base, 2 times daily for 5 consecutive days.

Blood samples after first dose were collected from the jugular vein at 5, 10 minutes, 0.25, 0.5, 1, 2, 4, 8, 12 hours. Blood samples were collected every day at 0.25, 0.5, 1, 2, 4, 8, 12 hour. The blood samples were allowed to clot and serum was separated by centrifugation. Urine samples were collected by metal urinary catheter at 0.5, 1, 2, 4, 8, 12 hours. The samples taken at 0.25 hour were discarded. Serum and urine samples were stored at-20 C^0 until assayed.

Analytical Procedure:

Estimation of apramycin in blood and urine was carried out by the microbiological assay described by *WALTON (1978), using Bacillus Subtilus* ATCC 6633 as the test organism. Protein binding percent of apramycin in calf serum was assyed by the method described by *LORIAN (1975)*. Estimation of creatinine in serum or urine and creatinine clearance were applied according to method described by *HENRY (1974)*.

Pharmacokinetic Analysis:

Pharmacokinetic parameters were calculated according to *RITSCHEL(1973)*. The obtained data were analyzed statistically and the results are represented as mean ± S.E. students t test was performed according to *SNEDECOR(1967)*.

RESULTS

Following asingle intramuscular injection of apramycin in a dose of 20 mg/kg (i.e.,after the first dose), serum concentrations of apramycin were significantly lower in pneumonic than in normal calves and were detected 2 hours post-injection (Fig.1a and 1b). The pharmacokinetic parameters in table (1) revealed that most of the recorded data in pneumonic calves were significantly changed compared to those from normal calves. peak urine concentrations of apramycin were hight (138.25±11.49 and 117.25±11.49 and 117.25±2.01 ug/ml in normal and pneumonic clves, respectively) and reached 4 hours post-intramuscular injection.

Following a single intravenous injection of 20 mg/kg of apramycin in normal calves, the drug obeyed a two-compartment open model (Fig 2). The calculated kinetic parameters revealed a rapid distribution (V d[area]) and the steady state (Vdss) were 153 and 159.2 ml/kg, respectively. The elimination half-life of the drug (t0.5B) was 2.5.±0.023 ml/kg/min. The mean systemic bioavailability was 61.98±2.19% (Table 2). The serum concentration of apramycin in calves after repested intramuscular administration of 20 mg/kg., two times daily for 5 consecutive days showed ahigher level of the drug which ranged from 61.5±1.09 to 66.25±0.96 and from 46.5±1.14 to 54±0.935 ug/ml in normal and pneumonic calves, respectively. The serum level of the drug in pneumonic calves was significanlty decreased compared to those in normal calves at all sampling times on the fifth day (Fig 1a and 1b).

The kinetic paramycin after repeated intramuscular injection in normal and pneumonic calves (Table 3) indicated no apparent changes between normal and pneumonic calves.

The ratios between apramycin clearance and creatinine clearance were less than one,as shown in Table (4). The protein binding percent of apramycin in calf serum was 2.29±0.20%

DISCUSSION

The present investigation revealed that the serum concentration of apramycin in both normal and pneumonic calves through 12 hours exceeded the minimal inhibitory concentration (0.78±1.56 ug/ml) required for *Salmonella, E.coli, p.multocida* and *K.pneumoniae* (*Kondrack and Pejsak (1985); Ziv et al. (1985) and Chaslus* et al.*(1986)*the results showed that the drug after a single intramuscular injection of 20 mg/kg reached its highest concentration (61.5±1.09 ug/ml and 46.5±1.14 yg/ml) in normal and pneumonic calves,respectively,at 2 hours post-injection. The findings agreed with values reported for normal calves (Ziv et al. 1985).

Serum concentrations of apramycin was higher in normal calves than those in pneumonic calves. On the other hand, pashov *(1988)* reported that the blood concentrations of apramycin injected intramuscularly 20 mg/kg. in normal and E.coli infected sheep,calves,and pigs were higher than normal. The apramycin concentrations in blood of pneumonic calves following a single and repeated intramuscular injections were significanlty lower than those in normal calves. The lower level could be explained by the higher capability of the drug to pnetrate the diseased tissues (Baggot, 1980) the same finding was also recorder by Burrows *(1980)* in mastitic cows,*Kosters* et al. *(1984)* in infected pigeons, *Atef* et al. *(1986)* in chickens and *El-Sayed* et al *(1989)* in endometric cows

The biological half life ($t_{0.5}\beta$) determined in the present study following intramuscular injection in normal and pneumonic calves (2.44 and 2.32 hours respectively) was consistent with that reported value in calves (2 hours)(*Shikha; 1987)* in turkeys (2-5 hours)(*Freidln* et al. *(1985)* and inconsistent with those reported value in calves (4.3 hours)(*Ziv* et al.*; 1985)* and (3.17-4.14 hours),(*Pashov* et al., *1988)* and in turkeys (5.88 days) (*Romvary* et al .,*1991).*

Following a single intravenous injection of 20 mg/kg of apramycin in normal calves, the time-concentration curve revealed a two compartment open model. The Vc in the present study (0.097 litre/kg) was smaller than recorded in calves (0.34 litre/kg)by *Ziv* et al *(1985).* This might be attributed to the low lipid solubility of aperamycin, and restricted distribution within the body. The values of k_{12} and k_{21} (1.0 and 1.8 h-1) in normal calves were inconsistent with other reported values by *Ziv* et al. *(1985)* and *Shikha (1987)* in calves. The fraction of apramycin bound to serum proteins of

calves (2.29±0.2%) was lower than that recorded for other aminoglycosides like gentamycin (16% *El-sayed* et al., *1989*), and streptomycin (30%, *Brander* et al., *1987*).

The apramycin clearance in normal calves as compared to creatinine clearance indicated that glomerular filteration seemed to be the main pathway of elimination with variable rates of tubular reabsorption. The apramycin clearance/ creatinine clearance ratio in the present study (0.01 to 0.035) in normal calves with alkaline urine was less than one. This result indicated that apramycin was eliminated in urine of calves mainly by glomerular filteration with a significant amount reabsorbed back to the blood. Delayed excretion of apramycin could be explained on this basis, as other aminoglycoside antibiotics of apramycin accumulation in renal tissue (*Nicholas* et al., *1988*). In conclusion the results show that the use of apramycin 2 times daily for 5 consecutive days seems to be the suitable regimen for treatment of respiratory diseases.

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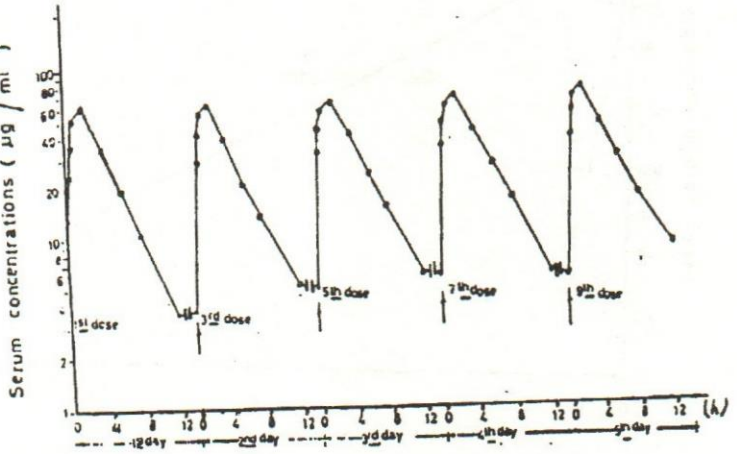

Fig (1,a)
Semilogarithmic graph depicting the time course of apramycin in serum of normal calves during intramuscular injection of 20 mg/kg b.wt. two times daily for five days (n = 4).

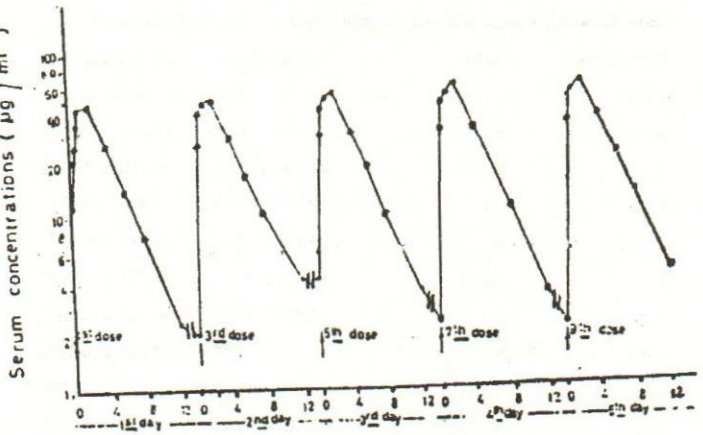

Fig (1,b)
Semilogarithmic graph depicting the time course of apramycin in serum of pneumonic calves during intramuscular injections of 20 mg/kg b.wt. two times daily for five days (n = 4).

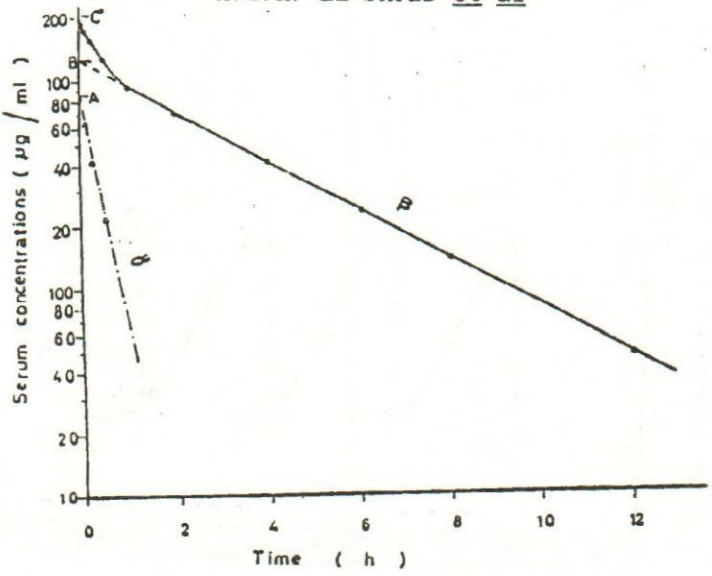

Fig (2): Semilogarithmic graph depicting the time course of apramycin in serum of normal calves following a single intravenous injection of 20 mg/kg b.wt. in calves previously given the same dose by intramuscular injection (n = 4).

TABLE 1 : Pharmacokinetic parameters of apramycin in normal and pneumonic calves following a single intramuscular injection of 20 mg/kg B.Wt. (n=4).

Parameters	Units	Normal calves	Pneumonic calves
B.Wt.	Kg	52.500 ± 2.165	52.500 ± 1.2500
A	µg/ml	97.500 ± 2.250	77.750 ± 3.8700**
K_{ab}	h-1	1.097 ± 0.060	1.380 ± 0.08*
$t_{0.5(ab)}$	h	0.637 ± 0.032	0.487 ± 0.018
B	µg/ml	113.250 ± 3.78	86.750 ± 4.080***
K_{el}	h-1	0.285 ± 0.0082	0.302 ± 0.011
$t_{0.5\beta}$	h	2.440 ± 0.0723	2.315 ± 0.098
C_{max}	µg/ml	70.320 ± 1.5700	52.180 ± 0.259***
t_{max}	h	1.670 ± 0.059	1.580 ± 0.0259
C^{-}_{max}	µg/ml	67 320 ± 1.64	58.820 ± 1.335***
C^{-}_{min}	µgml	3.410 ± 0.179	2.400 ± 0.273**

* :p <0.05

** p <0.01

*** p<0.001

TABLE 11 : Pharmacokinetic parameters of apramycin in normal calves following a single intravenous injection of 20 mg/kg B.Wt. in calves previously given the same dose by a single intramuscular injection (n=4).

Parameters	-Units	- X ± S. E.
C°	µg/ml	208.500 ± 12.17
A	µg/ml	84.900 ± 13.94
α	h^{-1}	3.26 ± 0.398
$t_{0.5(\alpha)}$	h^{-1}	0.91 ± 0.007
V_{1c}	ml/kg	97.13 ± 5.190
$V_{d\beta}$	ml/kg	162.00 ± 4.33
$V_{d\beta}$	ml/kg	152.90 ± 4.2
$V_{d\ area}$	ml/kg	153.00 ± 4.00
V_{dss}	ml/kg	159.22 ± 3.72
K_{12}	h-1	0.98 ± 0.02
K_{21}	h-1	1.80 ± 0.158
B	µg/ml	123.75 ± 3.34
β	h-1	0.275 ± 0.0025
$t_{0.5\beta}$	h	2.53 ± 0.02
K_{13}	h-1	0.44 ± 0.035
$Cl_{tot.}$	ml/kg/min.	0.447 ± 0.023
Bioavailability	%	61.98 ± 2.19

TABLE 111: Pharmacokinetic parameters of apramycin in normal and pneumonic calves during repeated intramuscular injection of 20 mg/kg B.Wt. 2 times daily for 5 consecutive days.

Parameters	Unit	1 st dose		3 rd dose		5 th dose		7 th dose		9 th dose	
		N	P	N	P	N	P	N	P	N	P
B	ug/ml	113.25 ± 3.78	96.75 ± 4.08	101± 2.8	94 ± 3.58	100 ± 3.79	93 ± 4.03	103.5± 3.19	94.87± 1.89	101 ± 2.62	91.25± 3.2
Kel	h^{-1}	0.285 ± 0.008	0.302 ± 0.001	0.265± 0.007	0.27± 0.009	0.297± 0.008	08080± 0.014	0.240± 0.0093	0.270*± 0.006	0.220± 0.016	0.250± 0.012
$t_{0.5(\beta)}$	h	2.440 ± 0.0723	2.315 ± 0.098	2.625± 0.070	2.56± 0.084	2.81± 0.100	2.500± 0.135	2.89 ± 0.112	2.550**± 0.057	3.180± 0.230	2.780± 0.152
C_{max}	ug/ml	70.32 ± 1.57	52.18***± 0.26	67.00 ± 1.75	57.67**± 1.50	68.84± 2.58	64.87± 2.82	68.53± 1.43	66.15± 0.99	70.34± 0.404	64.14**± 1.55
t_{max}	ug/ml	1.67 ± 0.059	1.58 ± 0.011	1.94 ± 0.038	1.37*± 0.045	1.51± 0.074	1.29*± 0.067	1.66± 0.027	1.34**± 0.059	1.67± 0.058	1.39± 0.112

N: Normal

P: Pneumonic

* : $P < 0.05$
** : $P < 0.01$
*** : $P < 0.001$

TABLE 1V: Apramycin clearance/creatinine clearance in normal and pneumonic calves following repeated intramuscular injection of 20 mg/kg B.Wt. 2 times daily for five consecutive days. (n=4).

Time after administration (h)	1 st day		2 nd day		3 rd day		4 th day		5 th day	
	N	P	N	P	N	P	N	P	N	P
0.5	0.0173±	0.0130±	0.0150±	0.0120±	0.0140±	0.0118±	0.0145±	0.0113±	0.0286±	0.0100±
	0.0022	0.00074	0.0028	0.00124	0.0019	0.00085	0.00079	0.00032	0.015	0.00051
1	0.0115±	0.0142±	0.0120±	0.0148±	0.0132±	0.0143±	0.0140±	0.0129±	0.0131±	0.0139±
	0.0008	0.0012	0.001	0.00095	0.00099	0.00078	0.00062	0.0010	0.00098	0.00052
2	0.0134±	0.0164±	0.0120±	0.0192±	0.0145±	0.0169±	0.0149±	0.0151±	0.0145±	0.0164±
	0.002	0.00046	0.0012	0.00281	0.00051	0.00082	0.00072	0.0006	0.00095	0.00055
4	0.0313±	0.0371±	0.0276±	0.0353±*	0.0314±	0.0315±	0.0245±	0.0306±	0.0269±	0.0293±
	0.0044	0.0011	0.0027	0.00139	0.0048	0.0023	0.0022	0.0016	0.001	0.0026
6	0.0283±	0.0380±**	0.0299±	0.0316±	0.0300±	0.0339±	0.0234±	0.0319±	0.0274±	0.0291±
	0.0019	0.002	0.0022	0.00075	0.0049	0.00071	0.0010	0.0016	0.0040	0.0017
8	0.0255±	0.0361±	0.0250±	0.0265±	0.0232±	0.03115±	0.0198±*	0.0282±	0.0186±	0.0238±
	0.00163	0.00372	0.0079	0.0019	0.002	0.00218	0.0006	0.0037	0.0024	0.0032
12	0.0268±	0.0348±	0.0264±	0.0257±	0.0241±	0.0335±	0.0269±	0.0400±	0.0210±	0.0354±
	0.0031	0.00144	0.0064	0.00395	0.0039	0.0045	0.00271	0.0083	0.0038	0.004

N : Normal calves * : $P < 0.05$

P : Pneumonic calves ** : $P < 0.01$