GENTAMICIN DISPOSITION IN NORMAL AND MASTITIC COWS

BY

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ABSTRACT

Blood and milk concentrations of gentamicin and the extents of its penetration into milk were determined in normal and mastitic cows. The disposition kinetics following intravenous (I.V.), i.m. (i.m.) and intramammary administrations were investigated. The serum concentration-time curve of gentamicin in normal cows following a single intravenous injection of 5 mg kg^{-1} b.wt. was best described by a three compartments- open model.

Blood gentamicin concentration in normal and mastitic cows following repeated 1.m. and intramammary administrat-ions of 5 mg kg^{-1} b.wt. twice daily for 5 days regimen were peaked at 1 and 4 hours post-each dose,respectively. The bioavailability of gentamicin after i.m. and intramammary administration were 81.24 and 13.82%, respectively. The milk gentamicin concentrations following i.m. administration were lower than the concurrent blood concentrations during 5-days regimen. The *in-vitro* protein binding percent of gentamicin in normal cow's serum was 16.04%.

It was concluded that, i.m. administration of 5 mg gentamicin kg^{-1}. b.wt. twice daily for 5 consecutive days produce maximal and minimal drug levels at steady-state exceeded the MIC for gram- negative bacteria isolated from cattle . Gentamicin failed to treat the mastitic cows following multiple i.m. administrations due to its poor solubility in non- polar solvents and its lower oil / water partition co-efficient .

INTRODUCTION

Bovine mastitis is recognized worldwide as the most costly diseases affecting dairy cattle and induces great economic losses to dairy industry (Morschkel and Kitchen, 1982). Local and parenteral treatment with antibiotics are recommended in all cases of mastitis to control or prevent the development of systemic reaction and to assist in the treatment of the udder infection (Ziv and Sulman, 1973).

Gentamicin is a broad-spectrum aminoglycoside antibiotic,it is effective against most microorganisms associated with infections of the bovine udder and reproductive tract (Hennessey *et al.*,1971).Its mechanism of action involves irreversible inhibition of bacterial, 305 ribosomal subunit and therefore , impaired protein synthesis (Conzelman ,1980).

Pharmacokinetic variables of gentamicin have been widely studied in humanbeings and several domestic species (Lackey *et al.*,1996). There are species –and age- related differences in drug kinetics (Riviere and Coppoc,1981; Dorrestein *et al.*,1984 and Clarke *et al.*,1992).

Disposition kinetics of gentamicin have been varied after parenteral administration in humanbeings (Siber *et al.,* 1975), horses (Pedersoli *et al.*,1980), rabbits (Halkin *et al.*,1981), dogs (Riviere and Coppoc,1981), birds (Bird *et al.*,1983), ponies (Haddad *et al.*,1985), sheep (Brown *et al.,*1986),cats (Jernigan *et al.,*1988), and buffalo (Garg *et al.*, 1991) .

Despite the pharmacokinetic data of gentamicin available for several large animal species, no such parameters have been established for mastitic cows.

Thus, the objective of the present work was undertaken to estimate the pharmacokinetic profiles of gentamicin following i.m. and intramammary administrations in mastitic cows. Also,to study the mechanism of penetration of the drug into milk following systemic administration. Systemic availability of gentamicin was also investigated following both routes.

MATERIALS AND METHODS

Gentamicin :

Gentamicin is a broad spectrum aminoglycoside antibiotic. It is dispensed in ampoules (2 ml) as gentamicin sulphate, Each one milliliter contains 40 mg gentamicin base. It is available under trade name Rigaminol from Chemical Industries Development (CID), Giza, Egypt.

Cows :

Sixteen Friesian lactating cows yielding 17-20 liters of milk daily and weighing from 555 to 590 kg., were used in this work. Cows were fed on barseem, commercially dairy ration and water *ad-lib.*Cows were classified into four equal groups (4 cows each). Cows of the first and third groups were clinically healthy, but those of the second and fourth groups were suffered from acute mastitis. All exhibited the acute stage of infection showing clinical symptoms as swelling, hotness, painful and abnormal mammary secretion (watery, yellow watery to serous fluid, milk contained some blood, flakes of pus, coagulated materials, clots ,or flakes barely visible by naked eye). Some cows in this study had manifested systemic reactions including pyrexia (body temperature ?39.5 °C), tachycardia, lethargy or decreased rumen motility and anorexia. Cows of the first and third groups were injected intravenously with 5 mg gentamicin $kg.^{-1}$b.wt. as a single dose, then 15 days later, they were administered the same dose i.m.ly and intramammary twice daily for 5 consecutive days, respectively. Acute mastitic cows of the second and fourth groups were administered i.m.ly and intramammary 5 mg gentamicin $kg.^{-1}$b.wt. twice daily for 5 consecutive days, respectively.

Sampling:

Blood samples :

Serial blood samples (about 8ml) were taken from the right jugular vein at 0.083, 0.25, 0.50,1,2,4,6,8 and 12 hours after intravenous injection and 1^{st} dose of i.m. or imtrammary administration. Then blood samples were taken at 0.5,1,2,4,6,8 and 12 hours after the 3^{rd},5^{th},7^{th} and 9^{th} i.m. or intramammary administrations. Blood samples were allowed to clot and serum was separated by centrifugation, removed, and stored at – 20° C until assayed.

Milk samples :

Milk samples were collected by hand stripping. The udder was emptied before drug administration and milk samples were taken at 0.5,1,2,4,6,8 and 12 hours following the 1st, 5th and 9th i.m. or imtramammary administration. Milk samples were allowed to clot (milk : trichloroacetic acid 15% v/v), centrifuged and the skim milk was removed and stored at –20°C until assayed.

Analytical procedure :

Zurich *et al.* (1997) described a rapid diffusion assay for the quantitative determination of gentamicin in small volumes of body fluid by using *Staphylococcus epidermidis* and nutrient agar II as the culture media. The microbial suspension *Staphylococcus epidermidis* was prepared according to Arret *et al.*(1971) to obtain a density of 10^7 spore/ml by using Mcfarland and nephelometer barium sulphate standard (Edwin *et al.*,1980).

For estimating the protein binding of gentamicin, concentrations of 0.5,1,5,20 and 50g gentamicin per milliliter phosphate buffer pH 8(94.6 ml of 0.067 M Na_2HPO_4 and 5.4 ml of 0.067 M KH_2PO_4) and normal cow's serum were used. This estimation was based on the fact that the free unbound parts of antibiotic only capable to diffuse through agar. The equation of **Lorian (1975)** was used to calculate the percentage of protein binding of gentamicin from the differences in the diameter of zones of inhibition between the tested drug in phosphate buffer and those of serum.

$$\text{Protein binding\%} = \frac{\text{Zone of inhinbition in Phosphate buffer (mm)} - \text{Zone of inhibition in normal cow's serum (mm)}}{\text{Zone of inhibition in phosphate buffer (mm)}}$$

The pharmacokinetic parameters were calculated according to Ritchel (1973) and Baggot (1978 a and b). All studied parameters were listed as mean (standard error. The obtained data were analysed statistically according to Snedecor and Cochran (1989).

RESULTS

Following a single intravenous injection of 5 mg gentamicin $kg.^{-1}$ b. wt.in normal cows in G_1 and G_2, the drug could be detected in blood till 12 hours. The blood gentamicin concentration-time curve showed that the drug obeyed a three compartments-open model (Table1 and Figs.1&2). Repeated i.m. and intramammary administrations of 5 mg gentamicin kg^{-1}.b.wt. twice daily for five consecutive days produced peak drug level on 1 and 4 hours post-each dose, respectively . These concentrations were significantly decreased in mastitic cows than in normal ones following both routes (Tables 2 & 3 and Figures 3,4,5 & 6). The disposition kinetics of gentamicin during repeated i.m. and intramammary administration in normal and mastitic cows are recorded in Table (4). The systemic bioavailability of gentamicin following i.m. and intramammary administration in normal cows were 81.24 and

13.82%, respectively (Table 4). The mean peak milk concentrations of gentamicin were determined on 4 and 1 hours post i.m. and intramammary administration, respectively (Table 5). The *in –vitro* protein binding percent of gentamicin in normal cow's serum was 16.04% (Table 6).

DISCUSSION

Following a single intravenous injection of 5 mg gentamicin $kg.^{-1}$ b.wt. in normal cows, the drug could be detected in blood till 12 hours. Serum gentamicin concentrations-time curve after intravenous injection showed that the drug obeyed a-3 compartments open model; a central compartment which represented by the blood and rapid equilibrating tissues (lung-liver-kidney and spleen) and another two slower equilibrating tissues . This result is similar to that recorded in rabbits (Huang *et al.*,1979), dogs (Riviere and Carver,1984), sheep (Brown *et al.*,1985) and newborn piglets(Giroux *et al.*,1995). Pedersoli *et al.*(1980) and Haddad *et al.*(1986) described gentamicin disposition after intravenous administration by a-two compartments open model in horses and cattle, respectively . Zurich *et al.*(1997) and Tomas *et al.*(1998) recorded a-one compartment open model in analysing gentamicin disposition after a single intravenous injection to horses. Vozeh *et al.*(1989) described the latter model (1-compartment model) with a simple model which fails to adequately describe the plasma concentration-time profile in certain patients after multiple dosing in human beings with renal function impairment . The differences in getnamicin disposition might be attributed to age (Riviere and Coppoc,1981; Clarke *et al.*,1985 and Cummimg *et al.*,1989), sex (Finco *et al.*,1981), dose (Brown *et al.*,1986), diet (Oukesson and Toutain,1992 and Behrend *et al.*,1994) and healthy state of each animal (Frazier *et al.*,1988 and Jernigan *et al.*,1988). Values of K12 & K21 and K13 & K31 are the first rate constant for the transfer of drug from the central to peripheral compartments and from peripheral to central ones, respectively . The passage of gentamicin from the central to the 1^{st} and 2^{nd} compartments were equals; k12= 0.57 h^{-1} and K13 = 0.05 h^{-1}, while its passage from 1^{st} compartment to the central one (K21 = 0.20 h^{-1}) was slower than its passage from the 2^{nd} compartment (k31= 0.90 h^{-1}) to the central one.

The distribution and elimination half-lives and volume of distribution of gentamicin following intravenous administration in normal cows showed nearly similar values as with other ruminants and camelid species (Brown *et al.*,1985 ; Haddad *et al.*,1986 ; Jernigan *et al.*,1988 and Wasfi *et al.*,1992). The small volume of distribution (below 1.00 $ml.kg^{-1}$ and rapid clearance of gentamicin might be related to the highly polar, lipophobic nature of the drug; it crosses membranes slowly and remains principally in the extracellular fluid from which it cleared rapidly by glomerular filtration (Jernigan *and Wilson.*,1988). Gyselynck *et al.* (1971) and Brown *et al.*(1985) attributed the smaller volume of distribution of gentamicin to its low lipid solubility, higher percentage of body fat content in cows or to the large fraction of the total body weight.

Serum gentamicin concentrations were peaked at 1 and 4 hours post each-i.m. and intramammary adminisitrations , respectively, For 5 days regimen. The drug could be detected therapeutically till 12 hours post administration by both routes in a level that exceeds the minimum inhibitory concentration (0.20 (g ml^{-1}) for gram

negative bacteria isolated from cattle (Ziv *et al.*,1982). The lower drug concentrations in mastitic cows than those in normal ones might be attributed to the high penetrating power of the drug to the diseased tissues as observed by Kosters *et al.*(1984) in infected pigeons. Pennington and Renyolds (1975) concluded that fever led to decrease in the gentamicin concentrations in feverish man and dogs.

During multiple i.m. and intramammary dosage regimen , the equilibrating organs (kidneys) accumulated gentamicin and there may be an increase in peak drug concentrations as a consequence of deep tissue accumulation (Haddad *et al.*,1985).The maximal [C^{-}_{max}] and minimal [C^{-}_{min}] blood gentamicin levels at steady-state following i.m. administration in normal and mastitic cows indicated that a dosage of 5 mg kg^{-1} b.wt. twice daily for 5 consecutive days would provide effective and safe concentrations that would presumably be highly effective against most gram negative aerobes (Conzelman,1980 and Haddad *et al.*,1986). Factors such as site of injection, regional blood flow, and prior injection in the same site may slow or accelerate the absorption of i.m. or imtramammary gentamicin.

The variable absorption rate would influence the time at which the peak serum concentration occurs as well as its magnitude. These considerations are important for drugs with short half-life such as gentamicin (Haddad *et al.*,1985). Thus, intravenous rather than i.m. injection might provide more consistent peak blood levels which are needed to treat severe gram negative infections in cows. The intravenous route is being used for human patient in whom precise peak blood concentrations of gentamicin are needed (Siber *et al.*,1975 ; Federspil *et al.*,1976 and Schentag *et al.*,1978) . The biological half- life [^{t} 0.5(a)] of gentamicin following i.m. and intramammary administration in normal and mastitic cows were higher when compared with those recorded in human beings (1 h.) by Siber *et al.,(*1975) , horses (2.54 h.) by Pedersoli *et al.*,(1980) , sheep (1.43 h.) by wilson *et al.,(*1981), ponies (1.82 h.) by Haddad *et al.,(*1985) and cows (1.83 h.) by Haddad *et al.,(*1986) . The present differences are common in kinetic investigations and often are related to specific interspecies variations in the handling of the drug , the method of drug analysis and the healthy status of the animals (Haddad *et al.*,1985) .

The obtained results showed a lower bioavailability percent of gentamicin after intramammary administration (13.82 %) than following i.m. injection (81.24 %) . The latter value is nearly similar to that reported after i.m. injection of 5 mg-kg^{-1} bwt in cows (90%) by Haddad *et al.,(*1986) .

The milk concentrations of gentamicin following i.m. administrations were lower than the concurrent blood concentration at all times of sampling during the 5-days regimen . Atef *et al.*,(1986) attributed the lower gentamicin concentrations in milk of goats to the limited extent of penetration of the drug through the udder which could be related to its extremely poor solubility in non- polar solvents and to its lower oil to water partition coefficients . For these reasons, gentamicin failed to treat the mastitic cows after repeated systemic (i.m) administrations . Gentamicin residues in milk are violative not only for milk consumption , but also for processing of cheese and other dairy products .

Gentamicin was bound to serum proteins of normal cows to 16.04 % . This value is lower than those obtained (20 to 25 %) by Wilson *et al.*,(1983).

In conclusion, *i.m. administration of 5 mg gentamicin kg^{-1}. b.wt. twice daily for 5 consecutive days produce maximal and minimal drug level at steady- state exceeded the MIC for gram- negative bacteria isolated from cattle . Gentamicin failed to treat the mastitic cows following multiple i.m. administrations due to its poor solubility in non- polar solvents and its lower oil / water partition co-efficients .*

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Table (1) : Pharmacokinetic parameters of gentamicin following a single intravenous injection of 5 mg kg-1 b.wt. in normal lacating cows (n = 4):

Parameter	Unit	G1 (Before intramuscular)	G2 (Before intramammary)
C°p	Ug/ml	52.44±5.71	64.72±3.11
A	Ug/ml	28.97±2.70	39.77±2.54
∝	h^{-1}	0.82±0.04	1.08±0.06
$T_{0.5 (\alpha)}$	H	0.85±0.08	0.64±0.07
B	Ug/ml	17.40±2.37	19.28±1.48
β	h^{-1}	0.59±0.04	0.88±0.05
$T_{0.5 (\beta)}$	H	1.17±0.16	0.79±0.02
C	Ug/ml	6.07±0.28	6.75±0.12
δ	h^{-1}	0.11±0.005	0.09±0.005
$T_{0.5 (\delta)}$	H	6.93±0.37	7.7±0.54
V1C	ml/kg	195.51±6.49	177.26±3.74
V2	ml/kg	143.60±2.99	220.19±3.92
V3	ml/kg	192.04±3.84	184.29±5.61
Vdss	ml/kg	531.15±6.35	581.74±6.45
K12	h^{-1}	0.46±0.03	0.57±0.03
K21	h^{-1}	0.21±0.07	0.20±0.05
K13	h^{-1}	0.06±0.008	0.05±0.003
K31	h^{-1}	1.10±0.06	0.90±0.02
K10	h^{-1}	0.55±0.007	0.46±0.006
AUC	Ug/ml/h	10.34±0.86	10.52±1.29

C°p= Drug concentration in the plasma at zero time immediately after a single intravenous injection (ug/ml)

A&B = Zero-time serum drug concentration intercepts of basic intravenous disposition curve.The coefficient

B is based on the terminal exponential phase.

J&a= Hyprid rate constants of biphasic intravenous disposition. They are related to the slopes of distribution and elimination phases respectively, of biexponential drug disposition curve (h^{-1}).

Kab = Apparent first order absorption rate constant (h^{-1}).

$t_{0.05 (J)}$ = Distribution half-life (h)

$t_{0.5\ a}$ = Elimination half-life (h).

C = Zero time serum concentration inte rcepts triphasic intravenous disposition curve (ug/ml).

ა = The elimination rate constant in the three compartment model.

$t_{0.05 (ა)}$ = Elimination half-life in three compartment model(h)

V1C = The apparent volume of central compartment (ml/kg).

V2 = The apparent volume of distribution which was calculated by the extrapolation method (ml/kg).

V3 = The apparent volume of the second peripheral compartment in three compartment model (ml/kg).

Vdss = The apparent volume of distribution which was calculated by stea dy-state method (ml/kg).

K12 = First-order transfere rate constant for drug distribution from the central to peripheral compartment (h^{-1}).

K21 =First-order transfere rate constant for drug distribution from the peripheral to central compartment (h^{-1}).

K13 = Rate constant for distribution from the central to second peripheral compartment in three compartment
model.

K31 = Rate constant for distribution from the second peripheral to the central compartment in three compartment model.

K10 = Elimination rate constant.

AUC = Total area under the serum drug concentration versus time curve fro m t= 0 to t = ? after administration
of a single dose.

Table (2): Mean ($X^{?}$±S.E.) serum concentrations (ug/ml) of gentamicin in normal (N) and mastitic (M) cows following repeated intramuscular administration of 5 mg kg^{-1} b.wt. twice daily for five consecutive days (n=4) :

Dose / Time after administration (h)	1st day (1st dose)		2nd day (3rd dose)		3rd day (5th dose)		4th day (7th dose)		5th day (9th dose)	
	N	M	N	M	N	M	N	M	N	M
0.083	0.36±0.007	0.37±0.008	1.24±0.002	0.61±0.06***	2.64±0.009	1.39±0.007**	3.78±0.05	3.54±0.06*	6.84±0.03	4.75±0.06***
0.25	1.89±0.008	1.11±0.03***	3.65±0.06	2.85±0.02***	5.67±0.07	4.63±0.05***	8.42±0.13	7.28±0.08***	14.71±0.17	11.64±0.13***
0.5	7.29±0.04	6.89±0.08**	9.34±0.08	7.70±0.13***	11.43±0.32	9.30±0.13***	15.36±0.22	12.17±0.27***	18.94±0.79	15.16±0.27
1	12.67±0.35	11.07±0.17**	15.16±0.16	13.60±0.24**	16.30±1.13	15.73±0.27	22.67±0.78	20.37±0.18*	24.91±2.38	23.67±1.35
2	8.96±0.28	8.14±0.12*	10.44±0.25	9.99±0.15	12.93±0.26	12.30±0.52	17.05±0.99	16.48±0.26	20.05±0.16	18.50±0.16***
4	4.66±0.16	3.65±0.03***	5.89±0.81	5.49±0.09	7.51±0.08	6.66±0.03***	11.33±0.28	10.75±0.17	14.86±0.58	11.28±0.83
6	2.45±0.09	2.17±0.05*	2.97±0.06	2.86±0.08	4.65±0.07	3.63±0.06***	7.50±0.25	6.30±0.03**	10.00±0.37	7.48±0.29**
8	1.45±0.08	1.18±0.003**	1.74±0.003	1.61±0.06*	2.65±0.07	2.20±0.02***	4.73±0.03	3.61±0.02***	6.96±0.83	3.85±0.05**
12	0.37±0.006	0.33±0.001***	0.44±0.008	0.38±0.07	0.96±0.006	0.44±0.03***	1.79±0.007	1.14±0.05***	3.36±0.06	1.47±0.02***

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

Table (3): Mean (X^2±S.E.) serum concentrations (ug/ml) of gentamicin in normal (N) and mastitic (M) cows following repeated intramammary administration of 5 mg kg^{-1} b.wt. twice daily for five consecutive days (n=4) :

Dose	1st day (1st dose)		2nd day (3rd dose)		3rd day (5th dose)		4th day (7th dose)		5th day (9th dose)	
Time after administration (h)	N	M	N	M	N	M	N	M	N	M
0.083	0.06±0.002	0.015±0.005***	0.11±0.008	0.073±0.001**	0.27±0.06	0.11±0.002*	0.58±0.05	0.22±0.06**	1.08±0.17	0.41±0.07**
0.25	0.17±0.005	0.08±0.003***	0.38±0.004	0.16±0.03***	0.83±0.04	0.27±0.02***	1.32±0.16	0.40±0.09**	1.75±0.20	0.66±0.08**
0.50	0.62±0.03	0.11±0.03***	0.87±0.03	0.22±0.05***	1.25±0.19	0.35±0.06**	1.66±0.29	0.56±0.09**	2.11±0.15	0.85±0.08***
1	1.20±0.16	0.21±0.09	1.39±0.08	0.37±0.09***	1.75±0.09	0.43±0.08***	2.17±0.18	0.75±0.04***	2.48±0.18	1.23±0.06***
2	1.42±0.19	0.40±0.06**	1.63±0.06	0.58±0.06***	2.15±0.28	0.67±0.04**	2.48±0.36	1.17±0.08**	2.79±0.21	1.59±0.18**
4	1.63±0.08	0.55±0.05***	2.23±0.09	0.86±0.08***	2.52±0.09	0.99±0.09***	2.71±0.25	1.48±0.09**	3.08±0.22	2.62±0.16
6	1.17±0.03	0.43±0.02***	1.51±0.05	0.61±0.06***	1.75±0.35	0.84±0.09*	1.97±0.18	1.21±0.08**	2.35±0.16	2.21±0.15
8	0.77±0.001	0.32±0.02***	1.05±0.03	0.45±0.06***	1.31±0.17	0.76±0.05*	1.49±0.16	0.96±0.05*	1.73±0.27	1.73±0.22
12	0.42±0.004	0.19±0.09***	0.56±0.08	0.27±0.03*	0.68±0.06	0.44±0.06*	0.77±0.07	0.67±0.03	1.03±0.05	1.21±0.16

* P< 0.05 ** P< 0.01 *** P < 0.001

Table (4) : Pharmacokinetics of gentamicin in normal (N) and mastitic (M) cows following repeated intramuscular and intramammary administration of 5 mg kg^{-1} b.wt. twice daily for five consecutive days (n=4) :

Parameter	Unit	Intramuscular						Intramammary					
		1st day (1st dose)		3 rd day (5th dose)		5 th day (9 th dose)		1st day (1st dose)		3rd day (5th dose)		5th day (9th dose)	
		N	M	N	M	N	M	N	M	N	M	N	M
A	Ug/ml	20.58±0.14	17.86±0.52**	23.02±0.58	25.19±0.98*	23.08±0.93	28.34±1.61*	2.95±0.09	1.00±0.06***	4.27±0.03	1.29±0.06***	3.82±0.06	3.40±0.06**
K_{ab}	h^{-1}	2.65±0.06	2.88±0.06*	2.68±0.06	1.88±0.08***	2.76±0.04	1.48±0.09***	1.00±0.08	0.28±0.003***	0.72±0.006	0.44±0.05**	0.68±0.007	0.36±0.05***
$T_{0.5\,ab}$	h	0.27±0.008	0.24±0.070***	0.26±0.008	0.37±0.005***	0.25±0.008	0.47±0.002***	0.69±0.03	2.48±0.07***	0.96±0.08	1.58±0.09**	1.01±0.08	1.93±0.07***
T_{max}	h	0.92±0.06	0.86±0.07***	0.96±0.004	1.13±0.008***	1.06±0.15	1.43±0.05*	2.18±0.04	4.95±0.02***	2.62±0.06	3.28±0.09***	2.92±0.06	4.92±0.03**
C_{max}	Ug/ml	12.83±0.28	11.18±0.14**	16.58±0.56	16.50±0.97	24.57±0.16	21.17±1.04*	2.01±0.05	0.48±0.03***	3.03±0.08	0.80±0.03***	3.55±0.003	2.38±0.07***
C_{max}	Ug/ml	13.87±0.17	11.44±0.28***	17.94±0.17	19.64±0.26**	27.35±1.07	23.06±1.13*	2.30±0.06	0.59±0.09***	3.71±0.05	3.28±0.04***	4.33±0.04	3.35±0.04***
C_{min}	Ug/ml	0.37±0.009	0.30±0.003***	0.92±0.06	0.56±0.08**	3.95±0.16	1.34±0.05***	0.48±0.08	0.22±0.07*	0.75±0.04	0.80±0.06	1.22±0.05	1.65±0.03***
B	Ug/ml	17.15±0.67	14.65±0.59*	21.29±0.87	23.65±0.79	29.71±1.08	30.65±1.17	2.83±0.03	0.95±0.05***	4.75±0.03	1.55±0.08***	5.31±0.09	3.89±0.08***
Kel	h^{-1}	0.32±0.003	0.32±0.06	0.26±0.003	0.32±0.005***	0.18±0.01	0.26±0.006***	0.16±0.008	0.14±0.003*	0.17±0.008	0.20±0.06	0.14±0.002	0.10±0.006***
$T_{0.5\,(\beta)}$	h	2.17±0.05	2.17±0.05	2.67±0.05	2.17±0.04***	3.85±0.03	2.67±0.15***	4.33±0.06	4.95±0.08***	4.08±0.05	3.47±0.07***	4.95±0.04	6.95±0.09***
Cl_{tot}	Ml/kg/min	1.55±0.006	1.82±0.007***	1.02±0.004	1.13±0.02**	0.50±0.003	0.71±0.008***	4.71±0.08	12.28±0.19***	2.98±0.04	10.75±0.18***	2.19±0.08	2.14±0.003
AUC	Ug/ml/h	8.66±0.08	4.05±0.04***	-	-	-	-	1.47±0.03	0.82±0.04***	-	-	-	-
B	%	81.24± 3.11						13.82±1.56					

* P< 0.05 ** P< 0.01 *** P < 0.001

A&B = Zero-time serum drug concentration intercepts of basic intravenous disposition curve.The coefficient B is based on the terminal exponential phase.

Kab = Apparent first order absorption rate constant (h^{-1}).

$t_{0.05\ ab}$ = The absorption half-life (h).

$t_{max.}$= The time at which the maximum concentration of drug was reached after extravascular administration (h)

$C'_{max.}$ = Maximum serum concentration of drug in blood after extravascular administration (ug/ml). C-max.= Maximum serum concentration at steady state during multiple dose regimen (ug/ml).

C′-min. = Minimal serum concentration at steady state during a multiple dose regimen (ug/ml).

K_{el} = First-order transfer rate constant for disappearance of drug from central compartment (h-1).

$t_{0.5\ \beta}$ = Elimination half-life (h).

$Cl_{tot.}$ = The total clearance of a drug which represents the sum of all clearance processes in the body (ml/kg/min.).

AUC = Total area under the serum drug concentration versus time curve from t= 0 to t = ? after administration of a single dose.

Table (5) : Mean ($X^2 \pm$S.E.) milk concentrations (ug/ml) of gentamicin in normal (N) and mastitic (M) cows following repeated intramuscular and intramammary administration of 5 mg kg^{-1} b.wt. twice daily for five consecutive days (n=4) :

Parameter	Intramuscular						Intramammary					
	1st day (1st dose)		3 rd day (5th dose)		5 th day (9 th dose)		1st day (1st dose)		3rd day (5th dose)		5th day (9th dose)	
	N	M	N	M	N	M	N	M	N	M	N	M
0.5	0.016± 0.008	0.010± 0.002	0.08± 0.008	0.02± 0.004***	0.33± 0.07	0.12± 0.003*	0.73± 0.008	0.41± 0.05***	0.92± 0.006	0.81± 0.008***	1.32± 0.04	0.96± 0.008***
1	0.04± 0.006	0.17± 0.006*	0.13± 0.006	0.07± 0.006***	0.54± 0.09	0.29± 0.004*	3.17± 0.002	1.97± 0.05**	4.65± 0.03	3.93± 0.004***	4.96± 0.07	4.11± 0.05***
2	0.09± 0.005	0.03± 0.002***	0.21± 0.008	0.13± 0.002***	0.63± 0.04	0.37± 0.02***	2.63± 0.002	2.12± 0.001***	3.58± 0.007	3.06± 0.006***	3.87± 0.03	3.14± 0.08***
4	0.46± 0.07	0.18± 0.007**	0.73± 0.08	0.24± 0.005**	0.77± 0.06	0.45± 0.06**	1.96± 0.003	1.62± 0.001***	2.83± 0.05	2.39± 0.007***	3.35± 0.16	3.19± 0.19
6	0.33± 0.06	0.12± 0.05*	0.56± 0.06	0.37± 0.02*	1.08± 0.08	0.79± 0.03*	1.19± 0.09	0.96± 0.003*	1.78± 0.03	1.15± 0.02***	2.41± 0.09	2.07± 0.08*
8	0.28± 0.03	0.09± 0.002***	0.35± 0.06	0.18± 0.009*	0.87± 0.06	0.43± 0.06**	0.92±0 .08	0.63± 0.002**	1.37± 0.09	1.07± 0.006*	1.77± 0.12	1.16± 0.06**
12	0.12± 0.006	0.03± 0.009***	0.22± 0.05	0.07± 0.008	0.49± 0.03	0.31± 0.02***	0.65± 0.005	0.34± 0.07**	0.99± 0.003	0.62± 0.007***	1.14± 0.003	1.07± 0.008***

* P< 0.05 ** P< 0.01 *** P < 0.001

Table (6) : The in- vitro protein binding % of gentamicin in normal cow's serum

Concentrations (ىg/ml)	Average corrected values of inhibition zones (mm)		***Average protein***
	Phosphate buffer pH 8	Normal cow's serum	Binding %
0.5	16.22	13.79	14.98
1.0	19.34	16.08	16.86
5.0	25.11	20.50	18.36
20.0	31.08	26.53	14.64
50.0	34.00	28.77	15.38
$\bar{X}$± S.E.			16.04±0.69%

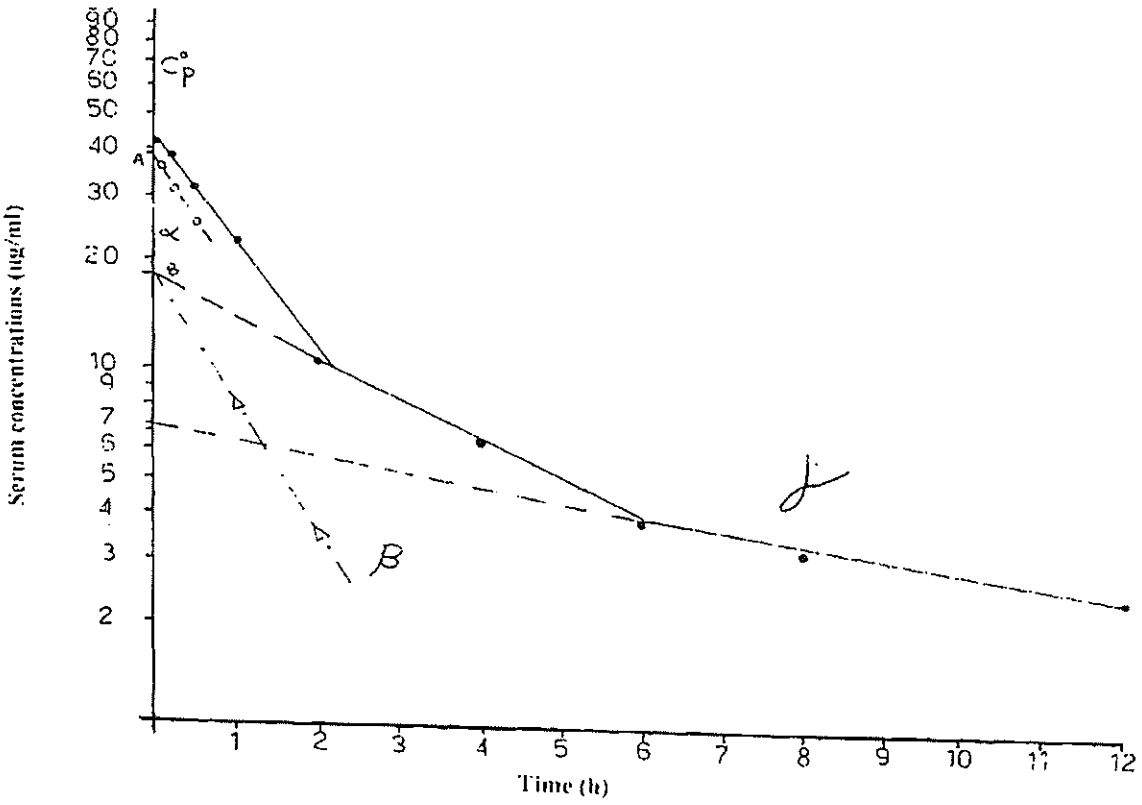

Fig.(1): Semilogarithmic graph depicting the time course of gentamicin in serum of normal cows (G1) following intravenous administration of 5 mg kg^{-1} b.wt. (n=4).

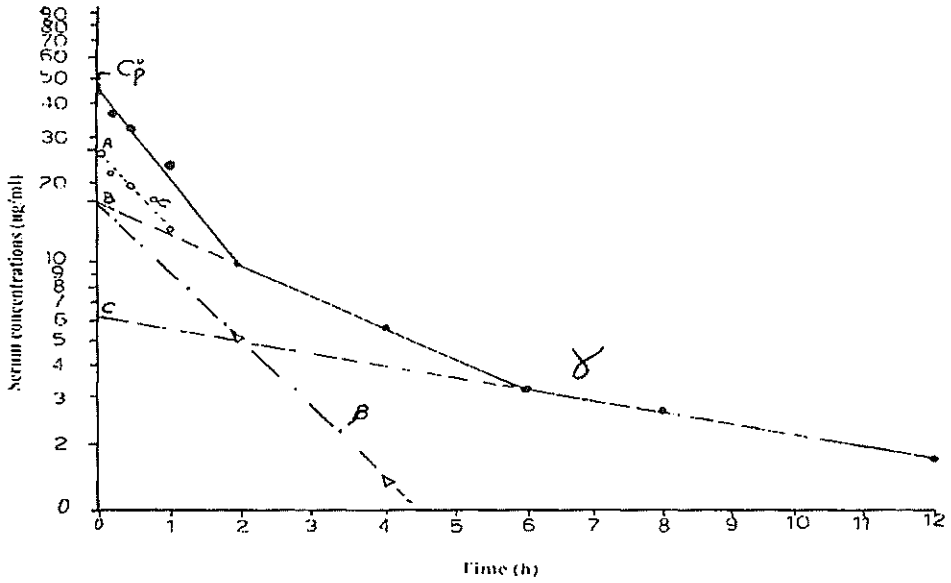

Fig.(2): Semilogarithmic graph depicting the time course of gentamicin in serum of normal cows (G2) following intravenous administration of 5 mg kg^{-1} b.wt. (n=4).

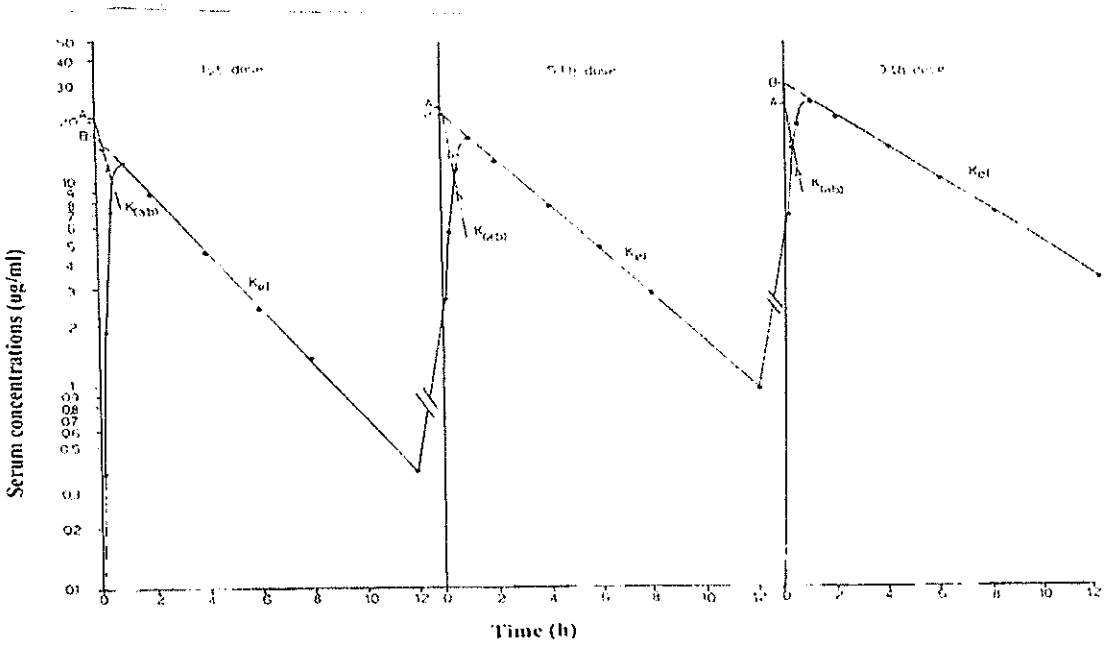

Fig.(3): Semilogarithmic graph depicting the time course of gentamicin in serum of normal cows during repeated i.m. administration of 5 mg kg^{-1} b.wt. twice daily for 5 consecutive days (n=4).

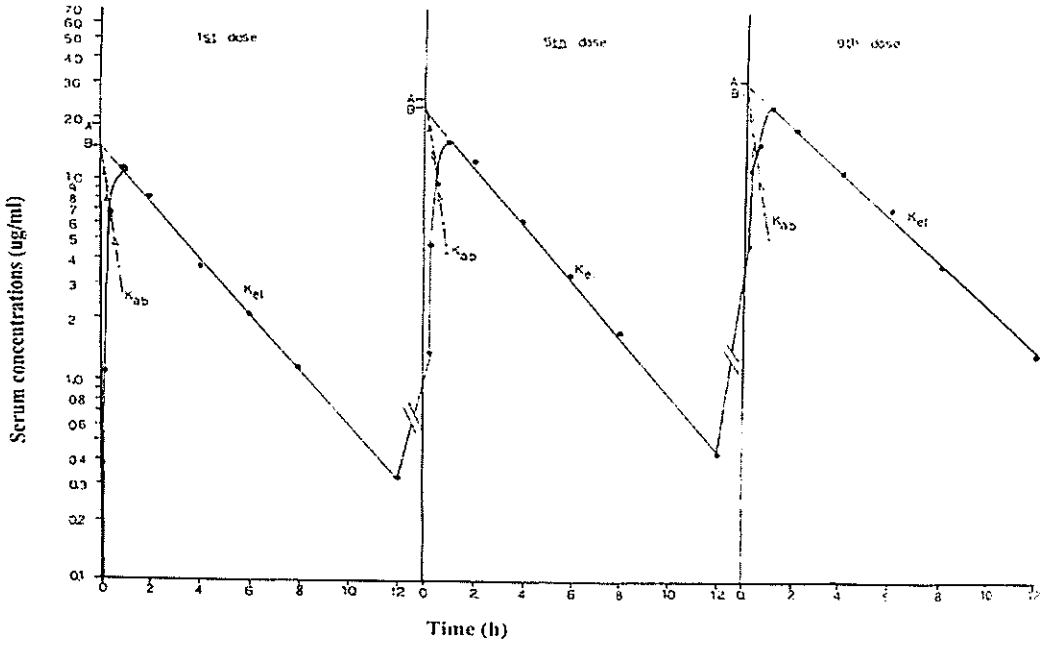

Fig.(4): Semilogarithmic graph depicting the time course of gentamicin in serum of normal cows during repeated intramammary administration of 5 mg kg^{-1} b.wt. twice daily for 5 consecutive days (n=4).

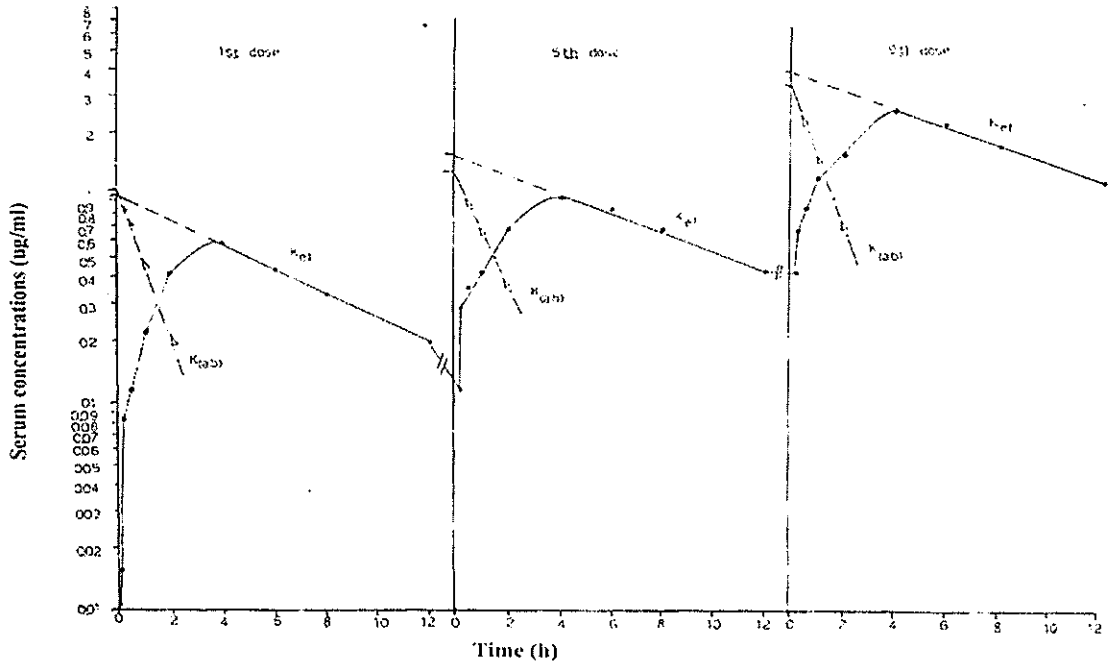

Fig.(5): Semilogarithmic graph depicting the time course of gentamicin in serum of mastitic cows during repeated i.m. administration of 5 mg kg^{-1} b.wt. twice daily for 5 consecutive days (n=4).

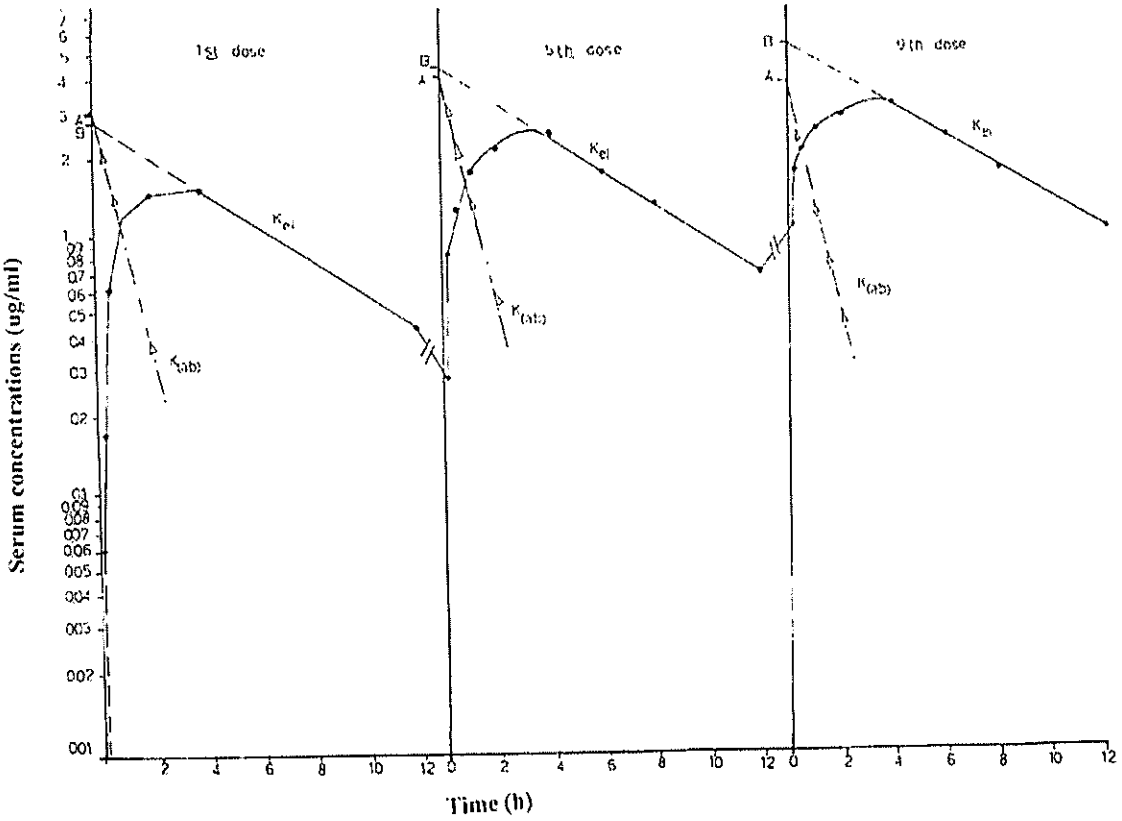

Fig.(6): Semilogarithmic graph depicting the time course of gentamicin in serum of mastitic cows during repeated intramammary administration of 5 mg kg^{-1} b.wt. twice daily for 5 consecutive days (n=4).

الملخص العربى

حركية الجنتاميسين فى الأبقار المصابة بالتهاب الضرع

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فى هذا العمل تم دراسة حركية عقار الجنتاميسين على الأبقار السليمة والأخرى المصابة بالتهاب الضرع ، وقد استخدم فى هذه الدراسة ١٦ بقرة فريزيان حلاب وقد قسمت إلى أربع مجموعات متساوية (٤ بقرات فى كل مجموعة). المجموعتان الأولى والثالثة سليمة إكلينيكياً ، وقد أعطيت عن طريق الوريد ٥ مجم جنتاميسين/ كجم من وزن الجسم . ثم تركت ١٥ يوم ، وبعد ذلك أعطيت نفس الجرعة عن طريق العضل وداخل الضرع مرتين يومياً لمدة ٥ أيام متتالية على الترتيب و المجموعتان الثانية والرابعة كانت مصابة بالتهاب حاد بالضرع . وقد أعطيت عن طريق العضل وداخل الضرع – على الترتيب – ٥ مجم جنتاميسين/ كجم من وزن الجسم مرتين يومياً لمدة ٥ أيام متتالية.

وقد أوضحت النتائج الآتى: أوضح منحنى تركيز عقار الجنتاميسن فى دم الأبقار مقابل الزمن بعد الحقن الوريدى أن الدواء قد سلك حركيته فى الجسم مسلك ثلاثى الحجرات. وبعد الحقن العضلى وداخل الضرع بـ ٥ مجم جنتاميسين / كجم من وزن الجسم مرتين يومياً لمدة ٥ أيام متتالية سجل الدواء أعلى منسوب له فى الدم بعد ساعة وأربع ساعات بعد كل إعطاء على الترتيب. كان معدل الاستفادة الحيوية (الإتاحة الحيوية) من عقار الجنتاميسين هو ٨١،٢٤، ١٣،٨٢ % بعد الحقن العضلى وداخل الضرع – على الترتيب. بعد الحقن العضلى المتكرر وجد أن منسوب عقار الجنتاميسين فى اللبن كان أقل من تركيزه فى الدم فى جميع العينات المقاسة. كانت نسبة إتحاد عقار الجنتاميسين مع بروتينات دم الأبقار السليمة معملياً هو ١٦،٠٤ %.

مما سبق نستنتج أن إعطاء الجنتاميسين عن طريق الحقن العضلى بجرعة ٥ مجم/ كجم من وزن الجسم مرتين يوميا لمدة ٥ أيام متتالية قد أعطى منسوب له فى الدم فى جميع العينات المقاسه يفوق أقل تركيز للدواء مثبط للبكتريا المعزولة من الأبقار. ومن جهة أخرى وجد أن الجنتاميسين بعد الحقن العضلى المتكرر قد فشل فى علاج إلتهاب الضرع وذلك يرجع إلى أن الجنتاميسين شحيح الذوبان فى الفوسفوليبيد للغشاء الفاصل بين الدم واللبن مما يعوق عبوره إلى اللبن ليعالج التهاب الضرع