

## Effect of fever on pharmacokinetics and dosage regimen of intramuscularly administered amikacin in goats

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A comparative pharmacokinetic study of amikacin (10 mg/kg intramuscular) by microbiological assay method in normal and experimentally induced febrile goats revealed that the plasma drug concentrations were significantly higher in febrile condition at most of the time intervals. Various pharmacokinetic parameters like  $t_{1/2}$ , AUC, AUMC, MRT and  $V_{d_{area}}$  were significantly higher whereas total body clearance ( $Cl_B$ ) was significantly lower in febrile goats as compared to normal goats. Absorption half-life ( $t_{1/2\ ka}$ ) value differed non-significantly. For maintaining mean therapeutic level of 2 mg/ml, a priming dose ( $D^*$ ) of  $14.73 \pm 2.28$  mg/kg, followed by maintenance dose ( $D_o$ ) of  $13.95 \pm 2.28$  mg/kg at shorter dosage interval ( $\tau$ ) of 8 hr may be useful in case of normal goats whereas lower doses ( $D^*$  of  $13.58 \pm 1.61$  mg/kg followed by  $D_o$  of  $12.65 \pm 1.60$  mg/kg) at longer  $\tau$  of 12 hr may be advised in case of febrile goats.

**Key words:** Amikacin, dosage regimen, goats, intramuscular, pharmacokinetics

### Introduction

Aminoglycosides have assumed an important role in the armamentarium of drugs used for the treatment of serious gram negative infections [7]. Amikacin, an aminoglycoside antimicrobial, is a semisynthetic derivative of kanamycin. It is resistant to almost all the R factor mediated aminoglycoside modifying enzymes [17,21,25] and thus, is used to treat gram negative bacterial infections for which other aminoglycosides are ineffective.

In order to use a drug effectively, it is important to investigate the detailed pharmacokinetics of the drug in a particular species in which the drug is to be used clinically [18]. Fever, which may be associated with many bacterial and viral diseases, changes various physiological parameters viz., heart rate, renal blood flow, hepatic and

total splanchnic blood flow, diuresis, enzyme activities and endocrine function [14], thereby altering the pharmacokinetics of certain drugs [8,9]. Animals suffering from fever may, thus, require a modified dosage regimen. Significant alterations in pharmacokinetics and dosage regimen of minocycline and oxytetracycline-LA [12], sulphadimidine [22] and cefazolin [23] have already been reported in febrile animals. Such reports are, however, lacking for amikacin, particularly in goats after intramuscular (i.m.) administration, the most popular and convenient route of administration.

Objectives of the present investigation were (i) to determine the pharmacokinetic properties of amikacin in normal and febrile goats after i.m. administration, (ii) to determine the dosage regimen in both conditions, (iii) to compare the differences in kinetic parameters and dosage regimen between normal and febrile goats.

### Materials and Methods

#### Experimental animals and drug

The study was conducted on six clinically healthy lactating Indian native goats of non-descript breed between 1.5 to 2 years of age and 20-25 kg. body weight. The animals were maintained on antibiotic free standard diet (in-house made) along with routine grazing and water *ad lib*.

The drug used was Amitax®-an injectible commercial preparation manufactured by Alkem Laboratories Ltd., Mumbai, India which contains amikacin sulphate equivalent to amikacin 250 mg/ml. The drug was injected at the dose rate of 10 mg/kg body weight in each goat by i.m. route first in normal state. After a gap of 4 weeks the drug was administered again to the same goats at the same dose rate by i.m. route after inducing febrile state.

#### Induction of febrile state

Fever was induced by injecting lipopolysaccharide of *Escherichia coli* (055 : B<sub>5</sub>) of Difco Laboratories (Detroit, MI, USA) at the dose rate of 1 µg/kg body weight intravenously. A rise in temperature of 0.7° to 1.2°C was

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**Table 1.** Rectal temperature (°C) of goats after i.v. administration of *E. coli* endotoxin @ 1 µg/kg body weight

Time (h)	Animal No.						Mean ± SEM
	1	2	3	4	5	6	
0	39.2	39.4	39.3	38.9	39.4	39.5	39.28 ± 0.09
0.25	39.6	39.6	39.7	39.0	39.8	39.9	39.60 ± 0.13
0.50	40.0	40.1	40.0	39.2	40.2	40.3	39.97 ± 0.16
0.75	40.2	40.3	40.2	39.8	40.4	40.5	40.23 ± 0.10
1	40.4	40.6	40.5	40.2	40.6	40.6	40.48 ± 0.07
1.5	40.8	40.7	40.7	40.4	40.8	41.0	40.73 ± 0.08
2	41.0	40.9	40.8	40.7	41.2	41.1	40.95 ± 0.08
2.5	41.2	41.1	41.0	41.0	41.3	41.4	41.17 ± 0.07
3	41.5	41.3	41.4	41.2	41.5	41.4	41.38 ± 0.05
3.5	41.6	41.3	41.5	41.4	41.5	41.6	41.48 ± 0.05
4	41.3	41.0	41.4	41.2	41.3	41.5	41.28 ± 0.07
4.5	41.0	40.9	41.2	41.1	41.0	41.3	41.08 ± 0.06
5	40.8	40.9	41.0	40.7	40.7	41.0	40.85 ± 0.06
5.5	40.5	40.7	40.8	40.4	40.6	40.8	40.63 ± 0.07
6	40.3	40.6	40.5	40.1	40.5	40.7	40.45 ± 0.09
6.5	40.2	40.5	40.4	39.8	40.3	40.7	40.32 ± 0.12
7	40.1	40.4	40.1	39.6	40.2	40.6	40.17 ± 0.14
7.5	40.0	40.3	40.0	39.5	40.1	40.3	40.03 ± 0.12
8	39.8	40.2	39.9	39.3	40.0	40.2	39.90 ± 0.14
8.5	39.6	39.9	39.7	39.3	39.8	40.0	39.72 ± 0.10
9	39.5	39.7	39.6	39.1	39.6	39.8	39.55 ± 0.10
9.5	39.2	39.6	39.3	38.9	39.6	39.7	39.38 ± 0.12
10	39.2	39.4	39.3	38.9	39.5	39.6	39.32 ± 0.10

noted 1/2 to 1 hour post injection. Elevation in temperature persisted for about 6-8 hours. Rectal temperature values of goats recorded before and after administration of *E. coli* endotoxin are presented in table 1.

### Collection and storage of blood samples

The samples of blood were collected from the jugular vein at 0.042, 0.083, 0.167, 0.25, 0.333, 0.50, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12 and 24 hours post injection of the drug into sterilized centrifuge tubes containing appropriate amount of sodium oxalate as anticoagulant. Plasma collected prior to drug administration was used for preparation of drug standards of amikacin (Amitax®) obtained from Alkem Laboratories Ltd., Mumbai, India. Plasma was separated after centrifugation at 2000 rpm for 15 minutes. The samples were stored in a refrigerator until assayed.

### Assay procedure

Assay of amikacin in plasma was carried out by microbiological assay technique (cylinder plate diffusion method) [4,11] using *Bacillus subtilis* (ATCC 6633) as the test organism [20]. The standard samples were used simultaneously with test samples in assay plates for

obtaining standard curve. With the aid of standard curve, determination of drug concentrations (including any biologically active metabolite) in test samples were carried out. The sensitivity of assay method was as low as 0.1 µg/ml.

### Calculation of kinetic parameters and dosage regimen

The log plasma drug concentration versus time profile showed a monophasic curve and followed one-compartment open model. Various kinetic parameters were obtained by using least square regression method [10,19]. In one-compartment open model, if the plasma concentration time profile is plotted from the peak concentration onwards on a semilogarithmic scale, a straight line is obtained and the plasma drug level declines according to following equation:

$$C_p = \beta e^{-K_{et}t}$$

Where  $C_p$  = concentration of drug in plasma;  $\beta$  = extrapolated zero time intercept of mono exponential curve;  $K_e$  = over all elimination rate constant;  $t$  = time elapsed after drug administration;  $e$  = base of natural logarithm.

The loading or priming dose ( $D^*$ ) and maintenance dose

( $D_0$ ) were calculated by the following formulae [24]: -

$$D^* = C_p^{\infty} (\text{min}) \cdot Vd_{\text{area}} \cdot (e^{Ke\tau})$$

$$D_0 = C_p^{\infty} \cdot Vd_{\text{area}} \cdot (e^{Ke\tau} - 1)$$

Where  $e$  is the base of natural logarithm,  $Ke$  is the elimination rate constant and  $\tau$  is the dosage interval and  $C_p^{\infty} (\text{min})$  is the minimum inhibitory concentration (MIC) of the drug to be maintained.

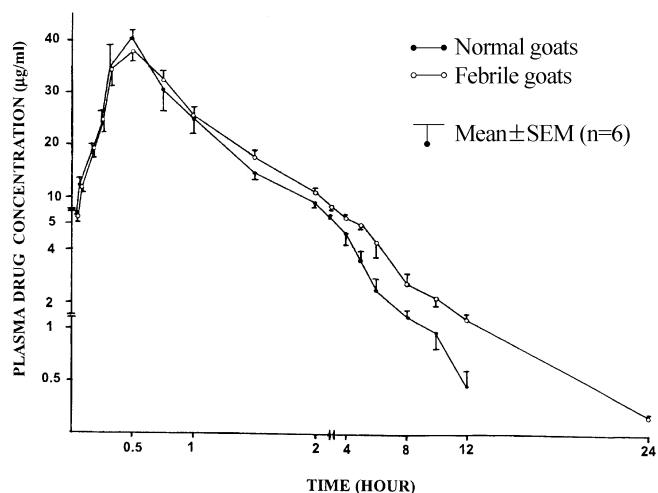
### Statistical analysis

Concentrations of amikacin in plasma at various time intervals, its kinetic parameters and calculated dosage regimen between normal and febrile goats were compared using Students 't' - test [26].

## Results

### Comparison of drug concentrations in plasma

Figure 1 depicts the comparison of amikacin concentrations in plasma of normal and febrile goats at various time intervals. The drug appeared in plasma at 0.042 hr both in normal and febrile goats, but it was detectable up to 12 hr in the plasma of normal goats, whereas, for a prolonged period (24 hr) in the plasma of febrile goats. Significantly higher levels of plasma drug



**Fig. 1.** Showing comparison of concentrations ( $\mu\text{g/ml}$ ) of amikacin in plasma between normal and febrile goats after single intramuscular administration at a dose rate of 10 mg/kg.

concentration were observed in febrile goats from 3 to 24 hr, whereas there was no significant difference in initial period (0.042 to 2 hr). The peak plasma drug concentration was attained at 0.50 hr in both groups of goats. The mean therapeutic concentration (2  $\mu\text{g/ml}$ ) was maintained from

**Table 2.** Comparison of kinetic parameters of amikacin between normal and febrile goats after single intramuscular administration at a dose rate of 10 mg/kg

Kinetic Parameters	Mean $\pm$ SEM (n=6)	
	Normal goats	Febrile goats
<b>Zero time Concentration (<math>\mu\text{g/ml}</math>)</b>		
Absorption ( $A$ )	35.50 $\pm$ 5.91	27.15 $\pm$ 3.50
Elimination ( $\beta$ )	27.45 $\pm$ 1.98	21.92 $\pm$ 0.73*
<b>Rate Constant (<math>\text{h}^{-1}</math>)</b>		
Absorption ( $K_a$ )	11.15 $\pm$ 1.47	12.46 $\pm$ 1.24
Elimination ( $K_e$ )	0.362 $\pm$ 0.020	0.221 $\pm$ 0.009***
<b>Half life (h)</b>		
Absorption ( $t_{1/2} K_a$ )	0.066 $\pm$ 0.007	0.058 $\pm$ 0.005
Elimination ( $t_{1/2}$ )	1.94 $\pm$ 0.10	3.17 $\pm$ 0.13***
<b>Area under curve</b>		
AUC (mg/L.h)	73.18 $\pm$ 5.63	98.18 $\pm$ 5.55*
<b>Area under first moment curve</b>		
AUMC (mg /L.h <sup>2</sup> )	215.8 $\pm$ 25.28	463.1 $\pm$ 42.62***
<b>Mean residential time</b>		
MRT (h)	2.92 $\pm$ 0.14	4.67 $\pm$ 0.19***
<b>Volume distribution</b>		
$Vd_{\text{area}}$ (L/Kg)	0.39 $\pm$ 0.03	0.47 $\pm$ 0.015*
<b>Total body clearance</b>		
$Cl_b$ (ml/Kg/min)	2.34 $\pm$ 0.17	1.72 $\pm$ 0.09**

\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$

0.042 to 6 hr in normal goats whereas in febrile goats it was maintained for a longer duration (0.042 to 10 hr).

### Comparison of kinetic parameters of the drug

Statistical comparison of various pharmacokinetic parameters of the drug between normal and febrile goats is shown in Table 2. The values observed for the extrapolated zero time concentration in elimination phase ( $\beta$ ), elimination rate constant ( $K_e$ ) and total body clearance ( $Cl_B$ ) were significantly lower whereas the values of elimination half life ( $t_{1/2}$ ), area under curve (AUC), area under first moment curve (AUMC), mean residential time (MRT) and volume distribution ( $Vd_{area}$ ) were significantly higher in febrile goats as compared to normal goats. All other parameters were almost similar in both the groups of goats.

### Comparison of dosage regimen of the drug for intramuscular use

Table 3 presents the calculated loading or priming ( $D^*$ ) and maintenance ( $D_o$ ) doses for maintaining minimum inhibitory concentration [ $C_p^\infty$  (min)] of 1 and 2  $\mu\text{g/ml}$  at selected dosage intervals ( $\tau$ ) of 8 and 12 hr between normal and febrile goats. The required  $D^*$  and  $D_o$  are significantly lower in febrile goats as compared to normal goats at  $C_p^\infty$  (min) of 1 and 2  $\mu\text{g/ml}$  at  $\tau$  of both 8 and 12 hr.

### Discussion

The minimum therapeutic concentration of amikacin in plasma ranges from 1-4  $\mu\text{g/ml}$  [15]. For most of the susceptible bacteria, the therapeutic level of amikacin is 1-2  $\mu\text{g/ml}$  [5,20]. Hence, in the present investigation, the dosage regimen of amikacin were calculated at two therapeutic levels (1 and 2  $\mu\text{g/ml}$ ) and at two time intervals

(8 and 12 hr).

The calculated value for the absorption half-life ( $t_{1/2 ka}$ ) obtained in normal goats in the present investigation (Table 2) was very low as compared to those of 0.385 hr in mare [5], approx. 0.214 hr in buffalo calf [27] and 0.244 hr in goats [28] indicating a faster absorption in lactating goats. The values of  $t_{1/2 ka}$  noted in normal and febrile goats differed non-significantly. This finding indicates that the rate of absorption of the drug was more or less similar in both groups of goats. This is supported by similar time of appearance (0.042 hr) and time of achievement of peak concentration (0.50 hr) of amikacin in both normal and febrile goats after i.m. administration.

The mean elimination half life ( $t_{1/2}$ ) of the drug in normal goats calculated in the present study was almost similar to the value of 2.05 hr in lactating goats [1]  $2.02 \pm 0.63$  hr in red tailed hawks [3] and  $2.3 \pm 0.18$  hr in mare [5] but it varied significantly from that of 3 hr in foal [6] and 1.408 hr in goats [28]. Significantly higher  $t_{1/2}$  value was observed in case of febrile goats in the present investigation. Similarly, significantly higher  $t_{1/2}$  value was reported in febrile cross bred bovine calves after intravenous administration of amikacin [24]. Significant increase in  $t_{1/2}$  obtained in febrile goats denotes that amikacin is removed from the body at a slower rate after its i.m. administration in febrile condition as compared to normal state. There are evidences that hepatic and renal dysfunction [30,31] as well as haemodynamic depression [16,29] such as decreased vascular pressure, cardiac output and central venous pressure are caused by endotoxin induced fever. These may lead to decreased renal clearance of drug (which is blood flow dependent) by reducing cardiac output, leading to decrease in renal blood flow [2]. This might have contributed to less excretion of amikacin leading to the longer half-life and persistence of higher

**Table 3.** Comparison of calculated dosage regimen of amikacin for intramuscular route between normal and febrile goats

$C_p^\infty$ (min) ( $\mu\text{g/ml}$ )	$\tau$ (h)	Dose (mg/kg)	Normal goats (n=6)	Febrile goats (n=6)
1	8	$D^*$	$7.36 \pm 1.14$	$2.76 \pm 0.22^{**}$
		$D_o$	$6.97 \pm 1.14$	$2.29 \pm 0.22^{**}$
	12	$D^*$	$33.93 \pm 8.77$	$6.79 \pm 0.80^*$
		$D_o$	$33.54 \pm 8.77$	$6.32 \pm 0.80^*$
2	8	$D^*$	$14.73 \pm 2.28$	$5.53 \pm 0.45^{**}$
		$D_o$	$13.95 \pm 2.28$	$4.59 \pm 0.44^{**}$
	12	$D^*$	$67.87 \pm 17.54$	$13.58 \pm 1.61^*$
		$D_o$	$67.08 \pm 17.55$	$12.65 \pm 1.60^*$

$D^*$ =Loading or priming dose;  $D_o$ =Maintenance dose;  $\tau$ =Dosage interval;  $C_p^\infty$  (min)= Minimum therapeutic concentration in plasma (MIC); \* $P < 0.05$ ; \*\* $P < 0.01$ .

concentration in plasma for most of the time intervals noted with this drug in febrile goats.

Significantly higher values of AUC, AUMC and MRT in febrile goats in the present investigation reflect that the drug remains in the body for comparatively longer duration in febrile condition which is also confirmed by the higher as well as longer duration of plasma drug concentration in febrile goats at most of the time intervals (Figure 1). Similar elevation in the values of AUC, AUMC and MRT was also reported in febrile crossbred bovine calves after i.v. administration of amikacin [24].

The reported values for the volume distribution of  $0.28 \pm 0.03$  L/kg in red tailed hawks [3],  $0.26 \pm 0.029$  L/kg in mare [5] and  $0.201 \pm 0.005$  L/kg in buffalo calf [27] are significantly lower but the value of  $0.58$  L/kg in foal [6] is significantly higher to that of  $0.39 \pm 0.03$  L/kg observed in normal goats in the present study. The  $V_{d_{area}}$  obtained in febrile goats in this investigation ( $0.47 \pm 0.015$  L/kg) was significantly ( $P < 0.05$ ) higher than that obtained in normal state. This indicates that there is extensive penetration of amikacin in various body fluids and tissues during fever.

The total body clearance ( $Cl_b$ ) of the drug in febrile goats was significantly ( $P < 0.01$ ) lower than that of normal goats (Table-2). This finding indicates that in febrile condition total elimination of the drug from the body is lower as compared to normal condition which might have led to longer persistence of the drug in the body under febrile state. This statement is further supported by higher plasma drug concentrations at most of the time intervals in febrile goats (Figure-1). Furthermore, significantly ( $P < 0.001$ ) longer  $t_{1/2}$  and MRT also confirm this fact. Similarly, in cross bred bovine calves, the value of  $Cl_b$  in febrile state ( $0.05 \pm 0.01$  L/kg/hr) was significantly lower as compared to higher value ( $0.09 \pm 0.002$  L/kg/hr) in normal condition after i.v. administration of amikacin [24]. Lower  $Cl_b$  values of  $1.33 \pm 0.11$  ml/kg/min in mare [5] and  $0.752 \pm 0.012$  ml/kg/min in buffalo calves [27] were reported. Variations among species, breed, sex, age and different methods for estimating the kinetic parameters may contribute to the wide discrepancies in kinetic parameters reported by various workers [13].

Table 3 depicts the comparison of calculated dosage regimen of amikacin for i.m. route between normal and febrile goats. For maintaining minimum inhibitory concentration (MIC) of  $1 \mu\text{g/ml}$  in plasma of normal goats, a loading dose ( $D^*$ ) of around  $7.4$  mg/kg followed by maintenance doses ( $D_o$ ) of around  $7.0$  mg/kg at 8 hr interval may be given whereas in case of febrile goats, a  $D^*$  of around  $6.8$  mg/kg followed by  $D_o$  of around  $6.3$  mg/kg at 12 hr interval may be used. Likewise, for maintaining MIC of  $2$  mg/ml in normal goats, a  $D^*$  of around  $14.7$  mg/kg followed by  $D_o$  of around  $14$  mg/kg at 8 hr interval may be given whereas in case of febrile goats, a  $D^*$  of around  $13.6$  mg/kg followed by  $D_o$  of around  $12.6$  mg/kg at 12 hr

interval may be advised for maintaining the same MIC. Similar trend of reduction in doses of amikacin in febrile cow calves for i.v. route has also been reported [24].

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