Pharmacokinetics of Amikacin after Repetitive Intravenous Administration in Healthy Goats

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ABSTRACT

Pharmacokinetic of amikacin was carried out in clinically healthy female goats of Sirohi breed following multiple once daily dose (@ 10 mg/kg bwt I/V) for five days. Concentrations of amikacin in blood plasma were estimated by microbiological assay technique and various kinetic parameters were calculated using two compartment open model. The minimum therapeutic concentration ($\geq$ 1.0 µg/ml) was maintained up to 12 h in both 1st and 5th day of drug administration. The drug was detectable up to 24 h. Significantly higher plasma concentrations of the drug appeared at 0.042, 0.83, 0.50, 0.75, 2, 4, 8, 12 h except 0.166, 0.25, 1.0, 1.5, 6, 24 h on 5th day as compared to 1st day of drug administration. Following multiple once daily I/V administration, the values of the extrapolated zero time concentration of the drug during distribution phase (A), theoretical zero time concentration ($C_p{}^o$), mean residential time (MRT) and elimination of drug from central compartment (Kel) remained non-significant, while significantly lower value of elimination rate constant (β), significantly increased value of elimination phases (B), area under curve (AUC), area under first moment curve (AUMC) and total body clearance (Cl_B)were observed in 5th day as compared to 1st day of amikacin administration. From these kinetic parameters, the loading (D*) and maintenance (D⁰) doses of 07.02 ± 0.36 and 05.91 ± 0.15 mg/kg bwt I/V, respectively were calculated for maintaining the therapeutic concentration ($C_p{}^\infty$ min = MIC) of 1.0 µg/ml at the dosage interval of 12 h.

HIGHLIGHTS

⦿ Pharmacokinetics of amikacin after repetitive intravenous administration in goats.
⦿ The elimination half-life ($t_{1/2}\beta$) of amikacin was 4.75h.
⦿ The loading doses (D*) of amikacin was 07.02 mg/kg bwt I/V.

Aminoglycosides are a group of antibiotics primarily used to treat a wide spectrum of microbial infections (Houghton *et al.,* 2010). Unfortunately, as it is the case with all other antibiotics, bacteria developed several mechanisms of resistance which challenges the utility of these antibiotics. The enzymes which catalyzes inactivation of aminoglycosides are aminoglycoside acetyl-transferases, aminoglycoside nucleotidyl-transferases or aminoglycoside phosphor-transferases. These enzymes are the leading cause of the rapid increase

and dissemination of resistance among earlier vulnerable organisms (Lin *et al.*, 2015). In order to counter and or restrict these enzymes, semisynthetic aminoglycosides has been developed (Kondo and Hotta, 1999). Amikacin, one of the most important semisynthetic aminoglycosides, was synthesized by acylation of the kanamycin A (Ramirez and Tolmasky, 2017). It can be used alone or in combination with other antibiotics, to treat a variety of serious infections caused by aerobic gram-negative bacteria, as well as *Mycobacteria and Nocardia* (Tamma *et al.,* 2012; Yuan, 2015). This antibiotic is also essential in the treatment of life-threatening infections (Tayman *et al.,* 2011; Pacifici and Marchini, 2017). Due to its property of being refractory to most of the aminoglycoside modifying enzymes, amikacin has been successfully used to treat the aminoglycoside resistant infections (Marsot *et al.*, 2017; Pacifici and Marchini, 2017). Since, amikacin exhibits serious adverse effects (ototoxicity and nephrotoxicity) common to aminoglycosides, the dosage regimen of drug to maximize therapeutic outcomes and minimize adverse consequences is of great importance (White *et al.*, 2015). Hence, the present study was undertaken to investigate whether the dosage regimen of amikacin calculated from single I/V administration actually maintains the minimum inhibitory concentration (MIC) at the end of every dosage interval during repetitive administration or not.

MATERIALS AND METHODS

Experimental animals

The experiment was performed in four clinically healthy female goats of Sirohi breed between 1 to 2 years of age and 15 to 25 kg body weight. The experimental animals were maintained in the College of Veterinary Science and Animal Husbandry, Rewa (M.P.) under uniform managemental conditions for 3 weeks. The animals were dewormed before the commencement of the study. During the entire period of experiment, animals were subjected to regular clinical examination, and maintained on dry as well as green fodder, concentrate and a routine grazing for at least 4 to 5 hours a day. Clean potable drinking water was provided *ad libitum*. All the animals were apparently healthy during the study. The experimental protocol for general procedure and use of animals for conducting the present study has been reviewed and approved by the Institutional Animal Ethics Committee (IAEC), College of Veterinary Science & AH, Rewa, Madhya Pradesh, India,

Chemicals

Injectable commercial preparation containing amikacin equivalent to 250 mg/ml (Amidac India) was used in the present investigation. Antibiotic media no. 1 and 11 were procured from HiMedia laboratories Pvt. Ltd., Mumbai.

Test organism

Escherichia coli (ATCC 25922) as test organism was used for estimation of concentration of the drugs in plasma by microbiological assay technique obtained from the national collection of industrial micro-organism (NCIM) Division of Bio-chemical sciences, National Chemical Laboratory, Pune.

Dosage and administration of drugs

Amikacin was administered at the dose rate of 10 mg/kg bwt I/V in each of four healthy goats once daily for five consecutive days (Saini and Srivastava, 1998).

Collection of blood samples

Blood samples (approx.1 ml) were withdrawn from jugular vein into heparinized glass centrifuge tubes on days 1 and 5 of treatment: at 0, 2.5, 5, 10, 15, 20, 30, 45 min and 1, 1.5, 2, 3, 4, 6, 8, 10, 12 and 24 h after administration of the drug. On days 2, 3 and 4 blood samples were taken 1 and 6 h after drug administration. Plasma was separated by centrifugation at 3,000 rpm for 15 min at room temperature and kept at -4ºC until analysis, which was usually done within two days of collection of the samples.

Estimation of amikacin

The concentration of amikacin in plasma was estimated by a rapid, specific microbiological assay technique using *Escherichia coli* as the test organism (Paul *et al.,* 1971).

Bioassay technique

Punch bioassay technique, which is the modified method of standard cylinder plate bioassay technique was used to

estimate the concentration of amikacin in plasma. In this technique, only a seed layer with bacteria suspension was poured on assay plates and the wells were prepared on assay plates (Arret *et al*., 1971).

Pharmacokinetic analysis

The plasma concentration-time profile of amikacin was used to determine the pharmacokinetic profile for each animal. The gathered data was further subjected to two compartment open model and kinetic parameters were calculated on the basis of Gibaldi and Perrier (1982).

Calculation of dosage regimen

The dosage regimen for maintaining minimal therapeutic concentration in plasma at the desired dosage intervals (τ) was calculated using the following equations (Baggot, 1977).

$$D^* = C_P^{\infty} \text{ min. } Vd_{area} \cdot (e^{\beta.\tau})$$

$$D^0 = C_P^{\infty} \text{ min. } Vd_{area} \cdot (e^{\beta.\tau}-1)$$

Where,

D^* = Priming or Loading dose

D^0 = Maintenance dose

C_P^{∞} (min) = Desired minimum plasma concentration

τ = Dosage interval

e = Base of natural logarithm

β and Vd_{area} was obtained from kinetic study.

Statistical Analysis

Comparison of concentrations of the drugs in plasma and various kinetic parameters of amikacin on first and last doses after multiple I/V administration in goats was compared by using paired 't' test (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

Concentrations of amikacin in plasma at various time intervals following multiple I/V injection at the dose rate of 10 mg/kg bwt have been shown in Fig. 1. The plot (semi

logarithm) of plasma levels of amikacin as a function of time after its multiple once daily I/V dose exhibited two distinct phases on 1st and 5th day of drug administration and the data obtained were adequately described by two compartment open model, in the present study. The two-compartment open model after I/V administration of amikacin has been reported in calves (Saini and Shrivastava, 1998) camel (Wasfi *et al*., 1999), lactating goat (Abo el sooud, 1999), dogs (Baggot *et al.,* 1985) and goats (Uppal *et al*., 1992). Though Zhou *et al.* (1997) exhibited first compartment open model in mice.
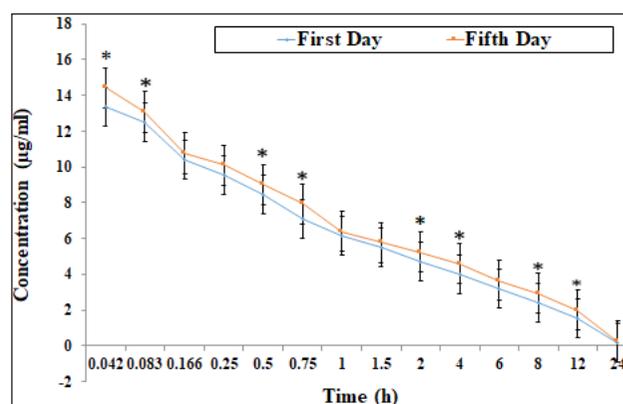
Fig. 1: Comparative plasma concentration of amikacin on 1st and 5th day after I/V administration in healthy goats;*Significant difference (p≤0.05).

The mean peak plasma concentration at 2.5 min was 13.37 ± 0.69 µg/ml on 1st day and 14.42 ± 0.73 µg/ml on 5th day following multiple once daily I/V dose of amikacin in healthy goat. Amikacin was detected up to 24 h with a mean plasma concentration 00.19 ± 0.02 on 1st day and 00.24 ± 0.02 µg/ml on 5th day. The minimum therapeutic concentration (≥1.0 µg/ml) of amikacin was maintained up to 12 h in both 1st and 5th day after drug administration. The significantly higher plasma concentrations of the drug appeared at 0.042, 0.83, 0.50, 0.75, 2, 4, 8, 12 h except 0.166, 0.25, 1.0, 1.5, 6, 24 h on 5th day as compared to 1st day of drug administration. It might be due to cumulative effect of drug after repetitive administration at particular interval of time till 5th day. The study conducted by Bhat and Kumar (2019) found that the higher mean peak plasma concentration at 2.5 min was 24.69 ± 0.011 µg/ml on 1st day and 25.56 ± 0.097 µg/ml on 5th day after a multiple once daily I/V dose (10 mg/kg bwt) of amikacin in healthy cow calves and drug was detected up to 10 h with a mean

Table 1: Comparison of pharmacokinetic parameters of amikacin after I/V administration in healthy goats between 1st and 5th day (Mean±SE)

Parameter (Unit)	1st day	5th day
A (µg/ml)	4.82±1.28	3.74±1.13
B (µg/ml)	7.39±0.54	8.46±0.91*
C_p^o (µg/ml)	12.22±0.46	12.22±0.47
β (h^{-1})	0.15±0.00	0.14±0.01
α (h^{-1})	2.09±0.44	1.38±0.55*
$t_{1/2}\alpha$ (h)	0.40±0.11	1.04±0.49
$t_{1/2}\beta$ (h)	4.75±0.12	4.88±0.21
AUC (µg/ml.h)	55.20±2.91	63.93±3.68*
AUMC (µg/ml.h^2)	370.55±20.85	450.05±25.62*
MRT (h)	6.65±0.09	6.98±0.17
K_{12} (h^{-1})	0.76±0.28	0.45±0.25*
K_{21} (h^{-1})	1.25±0.15	0.87±0.28*
Kel (h^{-1})	0.24±0.02	0.20±0.02
Vd_{area} (L/kg)	1.25±0.09	1.10±0.23
Cl_B (ml/kg/h)	181.35±9.40	161.81±4.99*

Zero-time concentration during distribution phase (A); Zero-time concentration during elimination phases (B); Theoretical zero time concentration (C_p^o); Elimination rate constant (β); Distribution rate constant (α); Distribution half-life ($t_{1/2}\alpha$); Elimination half-life ($t_{1/2}\beta$); area under curve (AUC); Area under first moment curve (AUMC); Mean residence time (MRT); Elimination of drug from central compartment (Kel); Volume of distribution (Vd); Total body clearance (Cl_B); Rate of transfer of drug from central to peripheral compartment (K_{12}) and from peripheral compartment to central (K_{21}); *Significant difference (p≤0.05).

Table 2: Comparative dosage regimens for amikacin after I/V administration in healthy goats (Mean±S.E.)

CP$^\infty$min (µg/ml)	τ (h)	Dose	Amikacin (mg/kg bwt)
1	12	D	07.02±0.36
		D^0	05.91±0.15
2	12	D	14.32±0.48
		D^0	11.83±0.31

CP$^\infty$min = Minimum therapeutic concentration in plasma (MIC); τ (h)=Dosage interval; D* = Loading or priming dose; D^o = Maintenance dose.

plasma concentration of 0.08 ± 0.002 µg/ml on 1st day and 0.09 ± 0.002 µg/ml on 5th day. Another study conducted by Orsini *et al.* (1985) indicated that doses of amikacin at the rate of 4.40, 6.60 and 11.00 mg/kg bwt showed the concentrations at the level of 30.30 ± 0.30, 61.20 ± 6.90 and 122.80 ± 7.40 µg/ml, respectively at 15 min following I/V administration in horse.

The distribution half-life ($t_{1/2}\alpha$) of amikacin in goat following multiple once daily I/V administration in the present study was 0.40 ± 0.11 h on 1st day and while 1.04 ± 0.49 h on 5th day. Bhat and Kumar (2019) reported $t_{1/2}\alpha$ of amikacin in cow calves was 0.182 ± 0.002 h on 1st day and 0.181 ± 0.005 h on 5th day following multiple once daily I/V administration. The value of $t_{1/2}\alpha$ reported in goat

to be 0.24 h (Uppal *et al.,* 1997), 0.36 h in calves and 0.43 h in sheep (Carli *et al.,* 1990).

The elimination half-life ($t_{1/2}\beta$) is the time taken for plasma concentration in the body to be reduced by its half (50 %). Half-life provides a good indicator of time which is required to reach steady state after initiation of dosage regimen. The $t_{1/2}\beta$ of amikacin in goat following multiple once daily I/V administration in the present study was 4.75 ± 0.12 h on 1st day and while 4.88 ± 0.21 h on 5th day. Bhat and Kumar (2019) found the $t_{1/2}\beta$ of amikacin to be 1.83 ± 0.012 h on 1st day and 1.91 ± 0.010 h on 5th day in cow calves, following multiple once daily I/V administration. The $t_{1/2}\beta$ of amikacin is more or less similar to 3.09 ± 0.27 h in bovine calves (Saini and Shrivastava, 1998), in

lactating sheep 1.64 ± 0.06 h (Haritova, 2004) and 2.16 ± 0.45 h for goats (Uppal *et al.,* 1992).

The high values of AUC and AUMC reflect that most of the body area is covered with the drug concentrations. The AUC values of amikacin in goat after multiple once daily I/V administration in the present study was 55.20 ± 2.91 μg/ml.h on 1st day and 63.93 ± 3.68 μg/ml.h on 5th day. Bhat and Kumar (2019) found the AUC values to be lower i.e. 13.3 ± 0.051 μg/ml.h on 1st day and 13.67 ± 0.042 μg/ml.h on 5th day after multiple once daily I/V administration of amikacin. The higher AUC values has been recorded in goats 73.18 μg/ml.h (Agrawal *et al.*, 2001), lactating sheep 94.09 ± 6.95 μg/ml.h (Haritova, 2004) and Greyhounds dogs 79.97 μg/ml.h (Kukanich and Coetzee, 2007). Similarly, the AUMC values of amikacin in goat following multiple once daily I/V administration in the present study was 370.55 ± 20.85 μg/ml.h^2 on 1st day and 450.05 ± 25.62 μg/ml.h^2 on 5th day. Bhat and Kumar (2019) reported lower AUMC values 22.7 ± 0.266 μg/ml.h^2 on 1st day and 24.57 ± 0.229 μg/ml.h^2 on 5th day after multiple once daily I/V administration of amikacin in cow calves.

The MRT values of amikacin in goat following multiple once daily I/V administration in the present study was 6.65 ± 0.09h on 1st day and 6.98 ± 0.17 h on 5st day. But lower MRT value of amikacin was reported in cow calves following multiple once daily I/V administration i.e. 1.70 ± 0.02 h on 1st day and 1.82 ± 0.012 h on 5th day (Bhat and Kumar, 2019). Similar MRT values observed in goat 4.67 ± 0.19 h (Agrawal *et al.*, 2001) and oryx 2.27 h (Kathryn *et al.,* 1995).

The values for volume of distribution (Vd$_{area}$) of amikacin in goat following multiple once daily I/V administration in the present study was 1.25 ± 0.09 L/kg on 1st day and 1.10 ± 0.23 L/kg on 5th day. The higher Vd$_{area}$ was observed in cow calves following multiple once daily I/V administration i.e. 1.99 ± 0.007 L/kg on 1st day and 2.02 ± 0.007 L/kg on 5th day (Bhat and Kumar, 2019). However, lower Vd$_{area}$ was found in bovine calves 0.40 ± 0.03 L/kg (Saini and Shrivastava, 1998), in human 0.27 ± 0.04 L/kg (Bauer and Blouin, 1983) and Beagle dog 234.00 ml/kg (Kukanich and Coetzee, 2007). This reflects good penetration of amikacin into various body fluids and tissues of goat and bovine calves. A very high value of Vd$_{area}$ obtained in the present study may be attributed to

wide distribution of amikacin in the body because of its polar organic base nature (Carli *et al.,*1990).

The total body clearance (Cl$_B$) values of amikacin in goat following multiple once daily I/V administration in the present study was 181.35 ± 9.40 ml/kg/h on 1st day and 161.81 ± 4.99 ml/kg/h on 5th day. The higher Cl$_B$ values of amikacin observed in cow calves following multiple once daily I/V administration was 754.66 ± 2.68 ml/kg/h on 1st day and 732.994 ± 2.187 ml/kg/h on 5th day (Bhat and Kumar, 2019). The lower Cl$_B$ values noted after I/M administration in goats was 2.34 ± 0.17 ml/kg/min (Agrawal *et al.*, 2001) and in camel as 0.97 ml/kg/min (Wasfi *et al.,* 1999). The Cl$_B$ values in normal condition in cow calves was 0.09 ± 0.002 L/kg/h which is higher than that of febrile condition 0.05 ± 0.01 L/kg/h after I/V administration of amikacin (Saini and Shrivastava, 1997). This difference in the values of Cl$_B$ amongst various species of the animals indicated respective difference in their glomerular filtration rates of amikacin, which is polar organic base, hence weakly bound to serum proteins and is excreted unchanged into the urine by glomerular filtration (Carli *et al.,* 1990).

The ultimate objective of the study of disposition kinetics is to determine an appropriate dosage regimen of amikacin. The dosage regimen for any antimicrobial agent is calculated to maintain the minimum therapeutic concentration (C$_p^\infty$min = MIC) throughout the course of infection. An average plasma concentration of 1.0-4.0 μg/ml has been reported to be the minimum therapeutic concentration (MIC$_{90}$) of amikacin against most gram positive, gram negative and atypical bacteria (Leroy *et al.,*1978; Agrawal *et al.,* 2001). Amikacin possessed excellent antibacterial activity (MIC for 90% of tested strains i.e. MIC$_{90}$<2.0 μg/ml) against most common gram-negative aerobic pathogens, including *E. coli*, *K. pneumonia*, *Enterobacter* spp., *Brucella* spp. (Shaffer *et al.*, 1953), and Mycobacteria (Suter, 1952). Thus, in the present study, the dosage regimen was derived at MIC of 1.0 and 2.0 μg/ml for amikacin at dosage interval of 12 h (Table 02). Conclusively, the calculated dosage regimens of amikacin for C$_p^\infty$min = 1.0 μg/ml were 07.02 ± 0.36 mg/kg bwt (D*) and 05.91 ± 0.15 mg/kg bwt (D^0) and for C$_p^\infty$min = 2.0 μg/ml were 14.32 ± 0.48 mg/kg bwt (D*) and 11.83 ± 0.31 mg/kg bwt (D^0) respectively at 12 h dosage intervals (τ).

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