Pharmacokinetic Study of Tobramycin in Healthy and Febrile Goats

THESIS

SUBMITTED TO THE

RAJENDRA AGRICULTURAL UNIVERSITY

(BIHAR)

In partial fulfilment of the requirements

FOR THE DEGREE OF

Master of Veterinary Science

IN

PHARMACOLOGY & TOXICOLOGY

By

Param Bodh Kumar

Registration No. - M/V. P. T./66/1998-99

Department of Pharmacology & Toxicology

BIHAR VETERINARY COLLEGE

PATNA

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DEDICATED TO

My Respected Parents

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M. V. S.c., Ph.D.
Associate Professor
Deptt. of Pharmacology & Toxicology
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CERTIFICATE – I

This is to certify that the thesis entitled "**PHARMACOKINETIC STUDY OF TOBRAMYCIN IN HEALTHY AND FEBRILE GOATS**" submitted in partial fulfilment of the requirements for the **Degree of Master of Veterinary Science (Veterinary Pharmacology and Toxicology) of the faculty of Post-graduate studies, Rajendra Agricultural University, Bihar**, is the record of bonafide research carried out by **Dr. Param Bodh Kumar** under my supervision and guidance. No part of the thesis has been submitted for any other Degree or Diploma.

It is further certified that such help or information received during the course of this investigation and preparation of the thesis have been duly acknowledged.

(H.N. Jha) 25/01/201
Major Advisor

Endorsed :

25/01/01

(Chariman/Head of the Department)

CERTIFICATE – II

We, the undersigned, members of Advisory Committee of **Dr. Param Bodh Kumar**, a candidate for the degree of **Master of Veterinary Science** with **Major in Veterinary Pharmacology & Toxicology** have gone through the manuscript of the thesis and agree that the thesis entitled "**PHARMACOKINETIC STUDY OF TOBRAMYCIN IN HEALTHY AND FEBRILE GOATS**" may be submitted by **Dr. Param Bodh Kumar** in partial fulfilment of the requirement for the Degree.

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CERTIFICATE – III

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Acknowledgement

I would like to express my deep sense of gratitude and indebtedness to my guide and major advisor Dr. H.N. Jha M.V.Sc., Ph.D., Associate Professor, Department of Veterinary Pharmacology and Toxicology, Bihar Veterinary College, Patna for his valuable guidance, constructive suggestions, close supervision, constant encouragement, keen interest and healthy criticisms during the course of investigation. This painstaking supervision of the manuscript, warrants special mention, without which this research undertaking would not have been completed.

I feel pleasure to express my whole hearted appreciation, hearty and warm thanks to my co-major advisor Dr. C. Jayachandran, Associate professor (pharmacology), Bihar Veterinary College, Patna for his invaluable sacrifice, excellent guidance and immense help during the course of investigation and in the preparation of this manuscript.

I am extremely grateful to the other members of the Advisory Committee, Dr. S.D. Singh, Head of the Deptt. of Pharmacology & Toxicology, Dr. L.N. Prasad Associate Professor, Pathology, Dr. J.N. Singh, Head, Deptt. of Biochemistry & L.P.T. and to my previous member Late Dr. Md. Murtuza, Head, Deptt. of Biochemistry for their constant help, valuable suggestions and prudent guidance for smooth running of the present research work.

My sincere thanks are also due to Dr. M.K. Singh, Ex-Dean and Principal, Bihar Veterinary College, Dr. S.P. Sinha, Ex-Associate professor, Pharmacology & Toxicology, Dr. K.G. Mandal, Sr.Asstt. professor, Animal Breeding & Genetics, Dr. S.R.P. Sinha, Associate professor, Vety. Parasitology, Dr. Satya Deo Kumar, Associate professor-cum-senior scientist, Agronomy, Dr. S.P. Verma, Associate professor, Vety. Medicine, Dr. B.N. Prasad, Head, Deptt. of Vety. Public Health, Dr. B.K. Sinha, Head Microbiology, Dr. S.S. Singh, Head, L.P.M., for their co-operation and showing intense interest in going through the manuscript.

A deep sense of gratitude is expressed to Rajendra Agricultural University, Pusa, Samastipur, Bihar for providing facilities to conduct this investigation.

I am equally obliged to Dr. Mani Mohan, Dean-cum-Principal, Bihar Veterinary College, Patna for extending facilities during the tenure of this investigation.

Gratitude alone fails to convey my feelings which cannot be expressed in words for the affectionate care, thoughtfulness, moral support and encouragement constantly received from all members of my family specially my affectionate father Sri. B.B. Singh, Mother Smt. B. Devi, brother Nihkewal Singh & Sarshabd Kumar, Sister Moheshwary and Puleshwary.

My special thanks to my seniors Dr. Shahid prawez, Dr. Anup Kumar Agrawal, Dr. Uday Kumr, Dr. S.B. Thakur and Dr. S. Mukhiya, for their indispensable help during the course of study.

My warmest thanks are due to my friends Dr. S. Kumar, Dr. D. Oraon, Dr. Zonun Sangi, Dr. S.K. Sahani, Dr. Md. A. Rahaman, Dr. A.K. Rai, Dr. K.D. Yadav, Dr. R.K. Dwibedi, Dr. Raj Shekhar, Dr. Nitesh Kumar, Dr. Mukesh, Dr. P.K. Babbar, Dr. Sanjeev and Junior Colleagues Viz., Dr. B.K. Mukta, Dr. P.K. Manjhi, Dr. Manish Pandey, Dr. I.D. Yadav, Dr. Rupa Lakra, Milli Prasad, Sacchidanand, Sadanand, Amit for their co-operation as well as to the non-teaching staff memebrs of the Pharmacology Department Viz, Sri. Jagan, Mukesh, Sanjay, Nathun, Vijay, Ramashish, O.P. Pandey, and Dr. P.K. Mishra for their assistance.

I am also thankful to Mr. Rajeev Prasad, Mr. Anit Prakash and Mr. Rakesh Kumar of Srishti Computer for their kind support in course of typesetting and printing of this manuscript.

Last but not least, I thank god and my gurudeo Payahari Jee for giving me patience and strength to overcome the difficulties which crossed my way in accomplishment of this endeavour.

Patna

Date :

Param Bodh Kumar

(PARAM BODH KUMAR)

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Introduction

INTRODUCTION

Tobramycin, one of the latest aminoglycoside antibiotics, is relatively safe and effective against organisms which usually cause osteomyelitis, pneumonia, bacterimia, lower respiratory tract infections and chronic urinary tract infections (UTIs) specially in elderly patients. Though tobramycin has similar spectrum of activity as that of gentamicin but it possesses less serious toxicity to cochlear and vestibular parts of auditory nerve and causes less renal tubular damage than other aminoglycosides.

Tobramycin is used in nosocomial infections as opthalmic ointment and solutions. *E. coli* is the most common pathogen involved in UTIs but the role of other gram negative organisms in nosocomial UTIs is increasing day by day. Tobramycin is effective even against gentamicin resistant pseudomonas. Tobramycin exhibits post antibiotic effect in which the drug retains its bactericidal activity in urine for about 48 hr even after stopping the drug. The clinical and bacteriological response is usually prompt and impressive.

In India, rearing of goat is mainly carried out for meat, milk and wool production. It can play a significant role by enhancing the socio-economic status of the common masses as well as increasing the net agrarian economy of the country. Hence, it is essential to provide a better health coverage to this species by achieving the new dimensions through antimicrobial therapy.

Pharmacokinetic studies of antimicrobials are generally carried out to obtain detailed pharmacokinetic data on absorption, distribution in various tissues and body fluids and elimination from the animal body. With the help of pharmacokinetic data, appropriate dosage regimen is derived for effective therapy of the diseases. It is now well established that the kinetic parameters of a drug may change during febrile as well as in other diseased conditions that may necessitate further changes in dosage regimen. Only very few reports on systematic kinetic studies of tobramycin are available in animals and due to this reason, the drug has yet to occupy a suitable place in veterinary practice.

On the basis of the above mentioned facts, the detailed pharmacokinetic studies of tobramycin in healthy and febrile goats were carried out with the following aims and objectives since such information are lacking in animals, particularly in goats.

1. Determination of plasma, milk and urine levels of tobramycin in healthy and febrile goats after i.v. and i.m. administration.
2. Calculation of kinetic parameters of tobramycin in healthy and febrile goats post i.v. and i.m. administration.
3. Calculation of dosage regimen of the drug in healthy and febrile goats.

Review of Literature

REVIEW OF LITERATURE

HISTORY

Tobramycin, one of the latest aminoglycoside antibiotics was introduced into clinical practice in 1970s. Tobramycin is one of several components of an aminoglycoside complex, which is produced by *Streptomyces tenebrarius* (Higgins and Kastners, 1967). It is almost similar in antimicrobial activity and toxicity to gentamicin. Tobramycin is more active against gram negative bacteria mainly *Pseudomonas aeruginosa* .

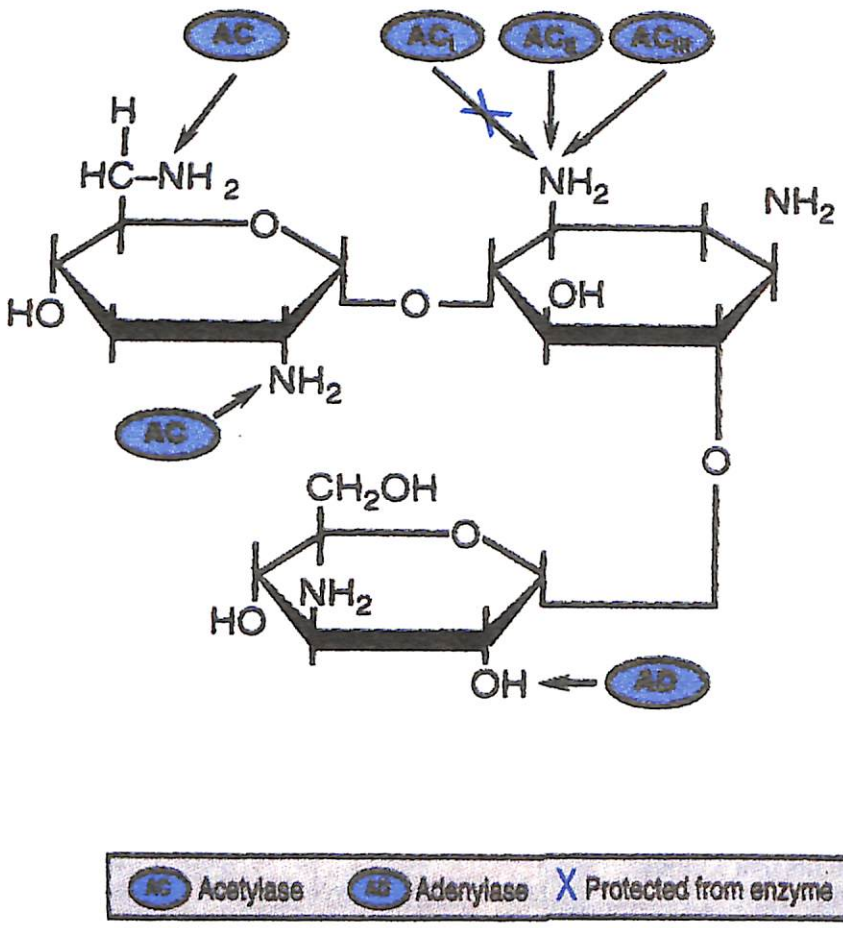
CHEMISTRY

All aminoglycosides contain two or more amino sugars joined in glycosidic linkage to a hexose nucleus, which is usually in central position. This hexose or aminocyclitol is 2-deoxystreptamine in tobramycin. The structure of tobramycin is shown in figure 1.

MECHANISM OF ACTION

Tobramycin is a rapidly acting bactericidal agent. Bacterial killing is concentration dependent; the higher the concentration, the greater the rate at which bacteria are killed (Kapusnik *et al.*, 1988 ; Blaser, 1991). Post-antibiotic effect, that is, residual bactericidal activity persisting after the serum concentration has fallen below the minimum inhibitory concentration is also a characteristic of this antibiotic, and the duration of this effect is concentration dependent.

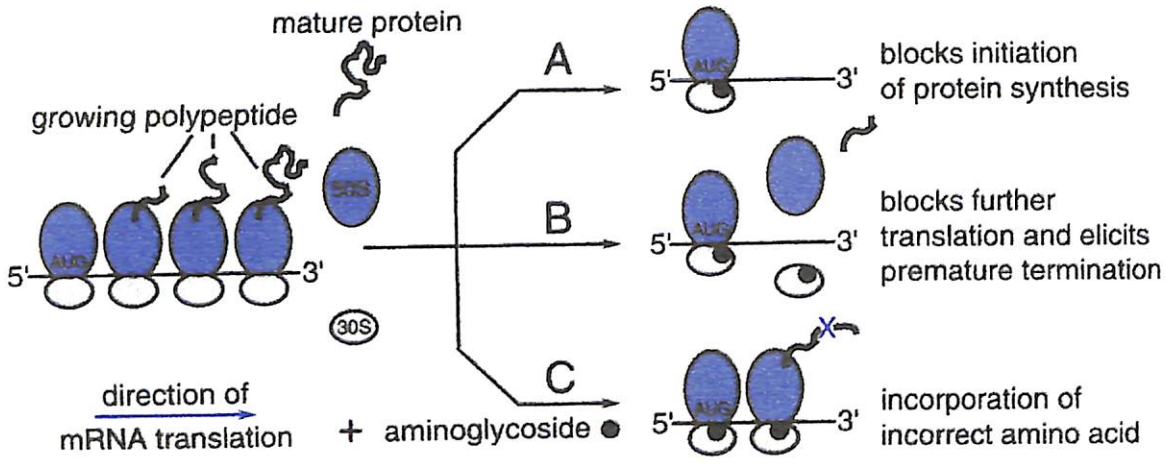
Tobramycin



The symbol 'X' indicates region of molecule that is protected from the designated enzyme.

Tobramycin inhibits protein synthesis and decreases the fidelity of translation of mRNA at the ribosome (Shannon and Phillips, 1982). Tobramycin diffuses through aqueous channels formed by porin proteins in the outer membrane of gram negative bacteria and thereby enter the periplasmic space (Nakae and Nakae, 1982).

The primary site of action of the aminoglycoside is the 30s ribosomal sub unit, which consists of 21 proteins and a single 16s molecule of RNA. However, it also appears to bind to several sites on the 50s ribosomal sub-unit as well. Tobramycin like other aminoglycosides disrupt the normal cycle of ribosomal function by interfering with the initiation of protein synthesis leading to the accumulation of abnormal initiation complexes as shown schematically in figure 2 .



Tobramycin (represented by closed circles) binds to the 30s ribosomal sub-unit and interferes with initiation of protein synthesis by fixing the 30s-50s ribosomal complex at the start codon

(AUG) of mRNA. As 30s-50s complexes downstream complete translation of mRNA and detach the abnormal initiation complexes, accumulate, blocking further translation of message. Tobramycin binding to the 30s sub-unit also causes misreading of mRNA, leading to premature termination of translation with detachment of the ribosomal complex and incompletely synthesized protein, or incorporation of incorrect amino acids. (indicated by the "X"), resulting in the production of abnormal or nonfunctional proteins.

ANTIMICROBIAL ACTIVITY

The antimicrobial activities and pharmacokinetic properties of tobramycin are very similar to those of gentamicin. The antimicrobial activity of tobramycin is reduced significantly by microbes in the anaerobic environment of an abscess, in hyper osmolar acidic urine and so forth (Bryan and Kwan, 1981)

Tobramycin is directed against aerobic gram-negative bacilli. It is more active against *Pseudomonas aeruginosa* and against some strains of *Proteus* species. They are also effective against *Escherichia, Salmonella, Staphylococci, Pasteurellae, Klebsiella* and others; they are less effective against *Streptococci.* In vitro, tobramycin is active against more than 90% strains of *Staphylococcus aureus* and 75% strains of *Staphylococcus epidermidis.* Tobramycin has a broad spectrum of activity and are particularly valuable in treating nosocomial infections (Cross *et al.*, 1983).

Typical minimal activitory concentration of Tobramycin that will inhibit 90%. [MIC $_{90}$] of clinical isolates for several species (adopted from Weidemann B and Atkinson B.A., 1991)

Species of bacteria	MIC $_{90}$ [µg/ml]
Citrobacter freundii	0.5
Enterobacter spp	0.5
Escherichia coli	0.5
Klebsiella pneumoniae	1.0
Proteus mirabilis	0.5
Providencia stuartii	4.0
Pseudomonas aeruginosa	4.0
Serratia spp	16.0
Enterococcus faecalis	32.0
Staphylococcus aureus	0.25

Tobramycin and gentamicin exhibit similar activity against most gram-negative bacilli, although tobramycin is usually more active against *Pseudomonas aeruginosa* and against some strains of *Proteus*. Most gram-negative bacilli (except *Pseudomonas aeruginosa)* are resistant to gentamicin because of plasmid mediated inactivating enzymes will also inactivate tobramycin. However, approximately 50% of *Pseudomonas aeruginosa* that are resistant to gentamicin remain sensitive to tobramycin [symposium, 1976 b]

In contrast to gentamicin, tobramycin shows poor activity in combination with penicillin against *Enterococci* and a large percentage of strains of *Enterococcus faecium* are highly resistant (Moellering *et al.*, 1979) Tobramycin is ineffective against mycobacteria (Gangadharam *et al.*, 1977)

GENERAL PHARMACOKINETIC

An understanding of pharmacokinetics has two valid purposes. The principles of kinetic disposition ensure safety and we find a way to optimize the effective dosing regimen, nevertheless, *in vivo* the efficacy of drug is modulated by disease induced several alterations. Inspite of all the uncertainties, scientific approach to recommend a dose of antibiotic is always based on its detailed pharmacokinetic study. To study the pharmacokinetics of drug, the body is subjected to different compartments. These compartments are mathematical entities and have no physiological meaning (Riegelman *et al.*, 1968).

The main aim of pharmacokinetics is to study the drugs in respect of drug concentration versus time course, their metabolites in various body fluids, tissues and excretion and interpretation of data based on suitable pharmacokinetic model i.e. compartment model (Wagner, 1968). The compartment model is used to determine the behavior and fate of a drug in a biological system, when administered by certain route in a particular dosage form. In

pharmacokinetic studies, compartment is an entity, which has a definite volume and concentration of a drug in that volume at any time. In an open compartment model, the drug is free to move from one compartment to another compartment i.e. blood to tissue and vice-versa.

The one compartment model fits well if the drug is instantaneously distributed in between the blood and tissues. Any change in the concentration of drug in blood shows directly the quantitative change in its tissue levels. Baggot (1974) reported that the rate of elimination of drug from the body is proportional to the concentration of the drug in blood. In one compartment open model, the plasma drug level declines according to the mathematical equation given below

$$C_p = Be^{-\beta t} \qquad \text{Equation (1)}$$

where, C_p = Concentration of drug in plasma.

B = Extrapolated zero time intercept of monoexponential curve.

β = Overall elimination rate constant.

t = Time elapsed after drug administration

e = Base of natural logarithm.

Baggot (1977) reported that one compartment open model is mainly useful in describing the time course of most of drug in plasma after oral/i.m./s.c. administration (extravascular administration).

The pharmacokinetics of most of the drugs after intravenous (i.v.) administration are well described by two compartment open model. Baggot (1974) reported that in two compartment open model the drug is instantaneously and homogeneously distributed to the central compartment such as blood, liver and kidney, and more slowly into the peripheral compartments such as muscles and fat. This indicates that distribution and elimination processes follow the first order kinetics and elimination takes place mainly from central compartment after i.v. administration.

In two-compartment open model the plasma concentration of the drug is expressed according to a biexponential equation as shown below

$$C_p = Ae^{-\alpha t} + Be^{-\beta t} \qquad \text{Equation (II)}$$

where, C_p = Plasma concentration of the drug.

A = Zero time intercept of distribution phase.

B = Zero time intercept of elimination phase.

α = Distribution rate constant.

β = Elimination rate constant

t = Time elapsed following drug administration.

e = Base of natural logarithm.

The values of A, B, α and β are necessary for calculation of other kinetic rate constants such as K_{12}, K_{21} and Kel in two compartment open model. These rate constants indicate the relative contribution in respect of distribution and elimination processes of a drug concentration-time data (Baggot, 1977).

The pharmacokinetics of some drugs may also follow three or multi compartment model. The concentration of drug in plasma after single i.v. administration is expressed by the following triexponential mathematical formula as a function of different time intervals.

$$C_p = Ae^{-\alpha t} + Be^{-\beta t} + Ce^{-\gamma t} \qquad \text{Equation (III)}$$

The additional constants C and γ are calculated by using residual method. These constants may be employed to estimate K_{13} and K_{31} (Gibaldi and Perrier, 1975).

Pharmacokinetic parameters of clinical importance

The clinical application of pharmacokinetic studies are

1. Calculation of various kinetic parameters of drugs following different routes of administration.
2. Estimation of dosage regimen of a drug in a given species of animal.
3. Determination of withdrawal period of drug residues in milk and tissues of food producing animals.

The parameter, bio-availability, is calculated when the drug is administers by extravascular route (oral/i.m./s.c. etc.). The bio-availability indicates the rate of drug absorption as well as the amount of absorption of a drug in biologically active form. The extent of absorption (F). is generally known as bio-availability and is calculated experimentally by the ratio of the area under the plasma concentration time curve after extravascular and extravenous administration (Baggot, 1977; Sams, 1978).

The distribution rate constant (α) and distribution half life ($t_{1/2}\ \alpha$) indicate the rate of distribution (faster or slower) of a drug from plasma to body fluids and tissues following i.v. administration.

Baggot (1977) reported that the overall elimination rate constant (β) is the most important kinetic parameter because it is an essential parameter to determine.

1. The elimination half life ($t_{1/2}\beta$).
2. The volume of distribution by area method (Vd_{area}).
3. The total body clearance of the drug (Cl_B).

In 1971, Gibaldi and Weintraub defined that the elimination half life ($t_{1/2}\beta$) is the time required to reduce the drug concentration in plasma or serum to its half during the elimination phase of the drug concentration time profile. It is inversely proportional to the overall elimination rate constant. Elimination half life is used to calculate the duration of drug action in the body. The

half life of a first order process is independent of the dose as well as the route of administration of the drug. The half life of a drug is highly useful in designing the rational dosage regimen.

The apparent volume of distribution is an important pharmacokinetic parameter used in the kinetic characterization of a drug. It is a hypothetical volume of body fluid that is needed to dissolve the total amount of the drug to attain the same concentration as that found in the blood. Riegelman *et al.* (1968) stated that the calculated value of volume of distribution of a drug is not dependent upon the method used for its calculation if the drug distributes truly according to one compartment open model. The apparent volume of distribution indicates the amount of drug distribution without giving any clue whether the drug is uniformly distributed or restricted to certain tissues (Baggot, 1977). A large volume of distribution (> 1 L/Kg) means wide distribution of drug throughout the body or extensive tissue binding or rapid elimination or combination of all the above. A small volume of distribution means that the drug is restricted to certain fluid compartments or restricted drug distribution, like plasma water, extracellular fluid etc. This is due to the high protein binding or low lipid solubility of a drug.

Another important pharmacokinetic parameter is the total body clearance (Cl_B), which is the sum of the clearance from each eliminating organ, particularly liver and kidney. The half life of a drug is a complex function, which particularly depends upon the

process of distribution, bio-transformation and excretion. On the other hand, the parameter, body clearance is not dependent on the above mentioned processes and indicates the rate of drug removal from the body. Unlike β and $t_{1/2}\beta$ that are hybrid constants and depend upon K_{12}, K_{21} and Kel, the body clearance changes exactly in proportion to Kel (Jusko and Gibaldi, 1972; Rowland *et al.*, 1973). It is expressed that the various constants like A, α, B, β, $t_{1/2}$ α, $t_{1/2}\beta$ and Vd_{area} etc. changes disproportionally with the magnitude of the elimination rate constant (Kel), and hence should not be used individually as a direct or safe measure of a change in drug elimination or distribution (Jusko and Gibaldi, 1972).

Some drugs have tendency to bind with plasma protein mainly with albumin. Binding of drugs with plasma proteins affect.

1. **Drug distribution** : High molecular weight of plasma protein prevents bound drug from diffusing out of capillaries into tissues.

2. **Drug effects** :- Free drug fraction is alone pharmacologically active, since it can penetrate to the region of target organ.

3. **Drug elimination** :- Free drug is alone filtered at the glomerulus and also excreted into saliva, milk etc.

The protein bound drug also acts as a reservoir.

Dose is the amount of drug that is administered through a particular route to produce a particular biological response i.e. to

attain optimum effective concentration of drug in the body fluids. Maintenance of therapeutic concentration of a drug in the body requires the administration of a drug to the extent so that concentration must be above minimum effective level and below a level which produces excessive side effects and toxicities at a particular time interval after the loading dose or initial dose. Thus, the main objective of a multiple dosage regimen is to maintain the plasma concentration of the drug within the limits of the maximum safe concentration and the minimum effective level.

Pharmacokinetics studies in febrile and disease condition

Febrile state is produced by most of the infectious diseases and fever has been reported to change the metabolism and excretion of drugs (Song *et al.*, 1972). Pharmacokinetic profiles or parameters may differ significantly between healthy and febrile animals and thus, change in dosage regimen may be needed in febrile condition.

Changes in volume of distribution of antimicrobials such as penicillin G (Baggot, 1977), trimethoprim (Ledefoged, 1977), rifamycin (Leszezynk, 1979), chloramphenicol (Kume *et al.*, 1986), metranidazole (Mandal *et al.*, 1987), sulphadimidine (Dutta, 1988), cephazolin (Roy, 1991) and norfloxacin (Jha, 1992) have been demonstrated in febrile conditions in animals. The distribution half life ($t_{1/2}\ \alpha$) and elimination half life ($t_{1/2}\beta$) were significantly lower for

sulphadimidine (Dutta, 1988) and nalidixic acid (Patel, 1992) in febrile goats as compared to healthy goats. In febrile dog, the plasma concentration of sulphadimidine was relatively higher and distribution half life was significantly shorter as compared to healthy dog (Riffat *et al.*, 1982). Significantly lower absorption half life ($t_{1/2}Ka$) and longer biological half life ($t_{1/2}\beta$) were observed in febrile goats as compared to healthy goats after intramuscular administration of cephazolin (Roy, 1991). In contrast, Jayachandran (1995) observed that distribution half life ($t_{1/2}\alpha$) and elimination half life ($t_{1/2}\beta$) to be significantly lower and at the same time the total body clearance (Cl_B) for minocycline and oxytetracycline L.A. in febrile goat and buffalo calf was noted to be higher as compared to healthy goat and buffalo calf. Gentamicin, streptomycin and neomycin achieved higher concentrations while penicillin, erythromycin and tylosin concentrations were lower in mastitis milk (Ziv, 1980). Ames *et al.* (1983) observed an increase in biological half life ($t_{1/2}\beta$) and volume of distribution (Vd_{area} and Vd_{ss}) in pneumonic calves as compared to normal calves. They also noted an increase in the concentration of oxytetracycline in the lung tissue of pneumonic calves.

KINETIC PROPERTIES OF TOBRAMYCIN

Pharmacokinetic characters of tobramycin are very similar to those of gentamicin. Doses of tobramycin (1.5mg/kg) are given i.m. or i.v. every 8 hourly. Peak concentrations in plasma are

typically 5 to 8 μg/ml and minimal inhibitory concentrations are 1 to 2 μg/ml. Toxicity is most common at minimal concentration that exceeds 2μg/ml for a prolonged period. The latter observation usually suggests impairment of renal function and requires reduction of dosage.

Tobramycin is poorly absorbed orally and hence used by parentral route. It is absorbed rapidly from i.m. site, and peak concentration is achieved after 30 to 90 minutes.

Tobramycin poorly penetrates blood-brain barrier and only 10% is reached in C.S.F. which may resume upto 25% in meningitis (Strausbaugh *et al.*, .1977). The concentration in bile is around 30%, a minor route of excretion. Its penetration in lung, pleura and synovial fluid is slow but therapeutic concentration achieved after repeated administration. The concentration in renal cortex and lymph of inner ear is high, and hence, chance of renal and ototoxicity occurs. Penetration into respiratory secretion is poor (Levy,1986). It is excreted almost entirely by glomerular filtration and concentration of 50 to 200 μg/ml in urine are achieved. It is excreted almost entirely unchanged during first 24 hr.

Kinetic studies in man and animals :

Pharmacokinetic studies of tobramycin were mainly reported in man but few studies are also done in different species of animals which are stated below :-

Man :- In healthy man, serum half life ($t_{1/2}\beta$) was noted to be 2 hr (Israel *et al.*, 1976) and 92 min (Pechere and Dugal, 1976). The serum half life is prolonged in neonates (4.5 – 8.7 hr). Tobramycin crosses the placenta and is concentrated in the kidney and urine of the fetus. In hemodialyzed patients the half-life was decreased by 6 to 9 folds during dialysis for 6 hr.

In new born, the $t_{1/2}\beta$ was noted to be longer (8.2 to 12.8 hr), volume of distribution (V_d) to be 0.74 to 0.94 L/Kg and total body clearance (Cl_B) to be 0.74 to 1.19 $ml.Kg^{-1}.min^{-1}$ (Nahata *et al.*, 1986).

After i.m. dose (5.0 mg/Kg) of tobramycin in new born infants, peak serum level averaging 2.69 ± 0.70 μg/ml was attained after 30 to 60 minutes, elimination rate decline but absorption rate was similar as that of older children. Average urinary recovery within 8 hr was as low as 26.8% (Yoshioka *et al.*, 1979).

Lode *et al.* (1978) stated that the pharmacokinetics of tobramycin determined after one hour infusion of 1 mg/Kg body wt. The biological half-life varied between 96 and 122 min and it amounted to a mean of 114.2 ± 16.7 min and the apparent volume of distribution could be calculated with 18.1 ± 1.81/100 Kg body wt.

Sheep : Moller *et al.* (1992) estimated $t_{1/2}\beta$ (mean±S.D.) of 1.8±0.3 hr, volume distribution (Vd) of 0.3±0.1 L/Kg and Cl_B of 1.8±0.8 $ml.Kg^{-1}.min^{-1}$ in sheep. The author reported that the $t_{1/2}\beta$ and Vd values are significantly increased during extracorporeal membrane

oxygenation of tobramycin. They found that in control animal and extracorporeal membrane oxygenation animal, the total body clearance was uneffected but in extracorporeal membrane oxygenation animal volume of distribution was noted to be increased.

Camel :- Hadi *et al.* (1994) conducted kinetic study of tobramycin in camel following i.v. and i.m. administration. They noted absorption half life ($t_{1/2}$ ka) of 3.9 ± 0.9 min with a bio-availability of 90.7±14.4% after i.m. administration. The authors noted $t_{1/2}\beta$ of 189 ± 21 and 201 ± 40 min after i.v. and i.m. administration, respectively, Vd_{area} of 245 ± 21 ml/Kg (0.245 ± 0.021 L/Kg) and Cl_B of 0.90 ± 0.10 $ml.Kg^{-1}.min^{1}$. Following i.m. administration of the dose (1.0 mg/Kg), the drug was rapidly absorbed with peak serum concentration of 3.32 ± 0.59 μg/ml at 20-30 min. Based on the study, the authors recommended a dose of 2.5 mg/Kg by i.m. route every 12 hourly to achieve a steady state serum concentration of 4 μg/ml with peak serum concentration approaching but not exceeding 10 μg/ml.

Cat :- Pharmacokinetic study of tobramycin in cat was conducted by Jernigan *et al.* (1988) after i.v., i.m. and s.c. administration at the dose rate of 5 mg/Kg. They noted $t_{1/2}\beta$ of 110.6±32.3 min, volume distribution at steady state (Vd_{SS}) of 0.18 ± 0.003 L/Kg and total body clearance (Cl_B)of 1.69 ± 0.36 $ml.Kg^{-1}.min^{-1}$. The blood urea nitrogen and serum creatinine concentrations were increased 3 weeks after i.v. injection and also 3 weeks after i.m. injection which suggested

possible renal damage. After i.m. and s.c. administration, bio-availabilities of 159.9% and 189.9%, respectively, with no change in elimination rate were noted.

The authors stated that pharmacokinetics of tobramycin is dose dependent. On the basis of the pharmacokinetic values of this study, the average study state serum tobramycin concentration after administration of the drug at the dose rate of 2 mg/Kg every 8 hourly was calculated to be 3 to 4 μg/ml with a peak concentration of 11 μg/ml and lowest concentration of 0.15 μg/ml. These concentrations are antibacterial for many tobramycin sensitive bacteria and may be sub-toxic in cats.

Rabbit :- In rabbit $t_{1/2}\beta$ was observed to be 1.97 hr (Bugnon *et al.*, 1988).

Guinea Pig :- Chung *et al.*(1982) studied many aminoglycoside antibiotics viz., netilimicin, gentamicin and tobramycin. They analysed the pharmacokinetic parameters and the plasma drug concentration time data upto 36 hr after i.v. dose by three compartment open model. The authors reported $t_{1/2}\alpha$ of 0.09 to 0.16 hr, $t_{1/2}\beta$ of 0.88 to 1.01 hr and $t_{1/2}\gamma$ of 7.87 to 8.29 hr, Cl_B of 0.204 $ml.Kg^{-1}.min^{-1}$ in guinea pigs after a single i.v. dose of tobramycin (40 mg/Kg). The maximum perilymph drug concentration of 6.78 μg/ml was achieved at 4 hr. The ratio of area under the curve of perilymph to plasma was lowest for tobramycin (0.57).

Rat :- Lin *et al.* (1994) studied temporal changes of tobramycin and stated that in rest period total clearance of the drug is reduced in respect to active period.

Wasfi (1993) reported the effect of endotoxin on tobramycin pharmacokinetics. Endotoxin significantly decreased tobramycin clearances. The volume of distribution at steady state (Vd_{ss}) is increased in endotoxin treated animals. There were no effect on MRT and $t_{1/2}\beta$ in young age but in old age endotoxin significantly prolonged both MRT and $t_{1/2}\beta$ when compared to normal aged rats.

Nadai *et al.* (1993) investigated the influence of bacterial lipopolysaccharide on the tobramycin kinetic parameters. Lipopolysaccharide delayed the disappearance of tobramycin from plasma in a dose dependent manner. Lipopolysaccharide decreased the central compartment, volume of distribution of tobramycin, but did not influence the steady-state volume of distribution. The glomerular filtration rate was decreased by pretreatment with 250 μg/Kg of lipopolysaccharide and clearance ratio was decreased by 20%; it means that the tubular reabsorption was increased by lipopolysaccharide.

Materials and Methods

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

The present study was conducted on six clinically healthy lactating goats of non-descript breed between 1.5 to 2.0 years of age and 20-25 Kg body weight. The goats were housed in the animal shed with concrete floor. The goats were maintained on standard diet along with routine grazing for at least 4-5 hours a day. Clean water was supplied *ad. lib*. The goats were provided ear tags for proper identification. All the goats were kept under close observation for a week prior to the start of experiment. The preliminary health check up was carried out in each goat prior to the experiment.

EXPERIMENTAL DRUG :

The drug used was tobramycin sulphate (TOBRANEG™)-an injectable commercial preparation manufactured by M.J. Pharmaceuticals Ltd., India and marketed by Eli Lilly Ranbaxy Ltd. which contains tobramycin sulphate I.P. equivalent to 80 mg/2ml. The drug was injected at the dose rate of 2 $mg.kg^{-1}$ body weight in each goat by intravenous (i.v.) as well as intramuscular (i.m.) routes in healthy and febrile states to carry out the present study.

EXPERIMENTAL DESIGN :

The drug tobramycin was studied on a group of six goats. A gap of 15 days was allowed to lapse before administration of the next dose. The drug was administered by i.v. and i.m. routes separately in each healthy goat initially. After inducing febrile state, the drug was administered again by i.v as well as i.m. routes in each goat to find out the variation in distribution of the drug in different biological fluids as well as other pharmacokinetic parameters.

INDUCTION OF FEBRILE STATE :

Three clinically healthy lactating goats were used initially for standardizing the febrile condition. Rectal temperature was noted in each goat at a particular time for three consecutive days. When the temperature was noted to be similar for all the days, the initial trial was carried out. Lipopolysaccharide of *E.coli* (0.55-B5) of Difco Laboratories, U.S.A. was dissolved in sterile distilled water to make a solution of 2 $\mu g.ml^{-1}$. The lipopolysaccharide was injected i.v. at a dose of 0.25, 0.50, 1.0 and 2.0 $\mu g.kg^{-1}$ body weight in three goats at each dose level and rise of temperature was noted every half on hour. A rise of temperature of 1.5 to 2.5^0F was noted after 1/2 to 1 hour post injection at the dose of 1.0 $\mu g.kg^{-1}$ body weight. The temperature was maintained for about 6-8 hours. The drug was administered after the rise of temperature i.e. 1/2-1 hour after the injection of *E.coli* toxin. The temperature was recorded at every 1/2 hour upto 8 hour post injection of drug.

COLLECTION AND STORAGE OF BIOLOGICAL FLUID SAMPLES

The samples of blood, milk and urine were collected pre and post i.v. and i.m. administration of tobramycin in healthy and febrile goats. The samples of blood and milk were collected before and 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 while the urine samples were also collected even beyond 24 hours at 30 and 36 hours post i.v. and i.m. administration of tobramycin for estimating tobramycin levels. The collected samples of biological fluids were kept in a refrigerator until use.

(A) *Blood*

Hairs around the jugular vein on either side of neck of the goats were shaved with shaving blade and the area was cleaned first with soap and water and then with ether. The site of prick was properly sterilized prior to each collection with rectified spirit. Prior to collection of blood samples, appropriate amount of sodium oxalate was taken in sterilized centrifuge tubes. Blood samples were collected in these sterilized centrifuge tubes from jugular vein by venipuncture at the above noted time intervals following drug administration. The blood samples were centrifuged at 2000 rpm. for 15 minutes to separate plasma. The plasma samples thus obtained were stored in a refrigerator. Plasma collected just prior to drug administration was used for preparation of plasma standards of various concentrations of the drug.

(B) *Milk* :

The udders of the goats were washed with soap and water and dried with clean soft towel prior to milk collection. The samples of milk were collected in sterile test tubes by hand milking. The milk samples were collected at various time intervals as noted above following administration of the drug. The samples thus collected were kept in a refrigerator and the drug concentrations were estimated on following days. Milk collected just prior to drug administration was used for preparation of milk standards of known concentrations of the drug.

(C) *Urine* :

On the day of experiment, a sterile Foley's balloon catheter (No.12), lubricated with glycerine was introduced through urethra into the urinary bladder of the experimental goat with the aid of a flexible metal probe. The balloon of the catheter was inflated by injecting 25-30 ml of sterile distilled water through a syringe to keep the catheter in position. The catheter was fixed with a pressure clip to check dripping of urine. After administration of the drug, the urine samples were collected in sterile test tubes at various time intervals as noted above. The samples were kept in a refrigerator and drug concentrations were estimated on following days. Urine collected just prior to drug administration was used for the preparation of urine standards of known concentrations of tobramycin.

ADMINISTRATION OF DRUG

The drug tobramycin sulphate equivalent to tobramycin (40 $mg.ml^{-1}$) was injected at a dose rate of 2 $mg.kg^{-1}$ body weight by i.v. as well as i.m. routes in each healthy (afebrile) and febrile goat.

PROCEDURE ADOPTED FOR THE MICROBIOLOGICAL ASSAY

[I] Sterilization of Glasswares, Needles and Porcelin Assay Cylinders :

All glasswares and porcelin assay cylinders were washed properly with detergent solution in running tap water. These were again rinsed with glass distilled water and finally air dried. Test tubes, centrifuge tubes, vials and vials containing porcelin assay cylinders were plugged with cotton wool. Assay plates, pipettes and syringes were wrapped with paper. All these materials were sterilized in hot air oven at 160^0 C for an hour. For administration of drug, endotoxin of *E.coli* and for collection of blood, sterile disposable needles were used.

[III] Preparation of Media :

(a) <u>*Assay Agar*</u> :

Antibiotic assay media of the following composition was used for microbiological assay of tobramycin in blood, milk and urine after its i.v. and i.m. administration in goats.

Sl. No	INGREDIENTS	GRAMS/LITRE WATER
1.	Peptone	6.0
2.	Tryptone	4.0
3.	Yeast Extract	3.0
4.	Beef Extract	1.5
5.	Dextrose	1.0
6.	Agar	15.0
	Distilled Water	1000 ml.
	Final pH	7.9 ± 0.1

The media was heated to dissolve and the solution was transferred into a conical flask, and pH was adjusted. The mouth of the flask was plugged with non-absorbable cotton wool and wrapped with aluminium foil. Wet sterilization of media was done by autoclaving at 15 pound pressure (121^0 C) for 20 minutes.

(b) *Nutrient Broth* :

Nutrient broth of the following composition was prepared :

Sl. No	INGREDIENTS	GRAMS/LITRE WATER
1.	Sodium chloride	5.0
2.	Peptone	10.0
3.	Beef Extract	10.0
	Distilled water	1000 ml.
	Final pH	7.4 ± 0.1

The media was heated to dissolve completely and pH was adjusted. Sterilization of the broth was done by autoclaving at 15 pound pressure (121^{0}C) for 20 minutes.

[III] *Preparation of Assay Agar Plates* :

20 ml. of autoclaved antibiotic assay media, while in melted condition, was poured gently into each of the sterilized special assay plate (Borosil) with the aid of a sterile measuring cylinder. The plates were kept on a horizontally plane surface to get uniform thickness of media. The plates were left at room temperature for about 1 to 2 hour for solidification of agar. Afterwards the plates were kept inside the incubator at 37^{0}C for 24 hours to ascertain any growth which indicates any microbial contamination. The growth free plates were then wrapped with sterile paper and stored in refrigerator until assay was carried out.

[IV] *Preparation of Test Organism* :

The test organism used for microbiological assay technique of tobramycin was *Escherichia coli* ATCC 25922 which was obtained from National Collection of Industrial Micro-organisms (NCIM), Division of Bio-Chemical Sciences, National Chemical Laboratory, Pune-8. The test organism was grown on the slant of culture tube containing nutrient agar slants at 37^{0}C for overnight. Then it was stored under refrigeration. The organism was transferred weekly to fresh media to maintain its normal activity.

[V] *Preparation of Standards in Biological Samples* :

Tobramycin was diluted in sterile glass distilled water to have different strengths, viz; 160 $\mu g.ml^{-1}$, 80 $\mu g.ml^{-1}$, 40 $\mu g.ml^{-1}$, 20 $\mu g.ml^{-1}$, 10 $\mu g.ml^{-1}$, 5 $\mu g.ml^{-1}$, 2 $\mu g.ml^{-1}$, 1 $\mu g.ml^{-1}$ and 0.5 $\mu g.ml^{-1}$. From each of these solutions, 0.1 ml. was taken with the aid of micropipette and added to sterile vials containing 0.9 ml. of plasma, milk or urine collected prior to drug administration. This yielded drug standards of 16 $\mu g.ml^{-1}$, 8 $\mu g.ml^{-1}$, 4 $\mu g.ml^{-1}$, 2 $\mu g.ml^{-1}$, 1 $\mu g.ml^{-1}$, 0.5 $\mu g.ml^{-1}$, 0.2 $\mu g.ml^{-1}$, 0.1 $\mu g.ml^{-1}$, and 0.05 $\mu g.ml^{-1}$, in the above noted biological samples. These standard samples were stored in refrigerator and used simultaneously with test samples in assay plates for obtaining standard curve. With the aid of standard curve, determination of drug concentrations in test samples were carried out. The concentration of tobramycin was detected as low as 0.05 $\mu g.ml^{-1}$.

[VI] *Assay Procedure* :

The plasma, milk and urine levels of tobramycin were estimated by microbiological assay technique (cylinder plate diffusion method) using *Escherichia Coli* (ATCC 25922) as the test organism.

The test organism was inoculated in sterile nutrient broth and kept under incubation for 2 to 3 hour at 37^0C until the growth was seen (turbid by naked eye). Tobramycin assay plates

were flooded with the broth containing the organism and excess broth was drained out after 10-15 minutes. The plates were dried in the incubator at 37°C for a period of half an hour. Plates were marked for different standards and biological test samples. Sterile porcelin assay cylinders of uniform size were placed against each mark at appropriate distance along the circumference in the inoculated assay plates. 50 microlitres of each of the standard solutions of various strengths as well as test samples of the drug were poured in separate porcelin cylinder in the assay plate. These assay plates were left on horizontally plane surface of the table for about 2 hour and then kept in the incubator at 37°C for overnight to allow the growth of organism. The mean diameters of the bacterial zones of inhibition produced by the standards as well as test samples of the drug were measured. The standard curve was plotted from the measure of zone of inhibition against each concentration of the drug on a semilog scale. With the help of this standard curve and measured zone of inhibition of different test samples, concentrations of drug in test samples were estimated.

CALCULATION OF PHARMACOKINETIC PARAMETERS

Following pharmacokinetic parameters of tobramycin following a single i.v. and i.m. administration were calculated from semilog plot of plasma drug concentration versus time curve. The experimental data was analysed using one compartment (for i.m. route) or two compartment (for i.v. route) open model as described by Gibaldi and Perrier (1975) and Notari (1980).

The concentration of the drug in plasma at any time is obtained by the following formula :-

(i) $C_p = B_e^{-\beta t}$ ---------- (One compartment model)

(ii) $C_p = A_e^{-\alpha t} + B_e^{-\beta t}$ ----------(Two compartment model)

where 'e' is the base of natural logarithm and C_p. is the drug concentration in plasma at time 't'. The description and calculation of the parameters A,B, α and β used in the above formulae and other kinetic parameters are noted below :

(a) A, the zero time concentration of the drug in plasma and α, the regression coefficient (distribution rate constant) for distribution phase were calculated by the method of residual yields (Appendix 1).

(b) B, the zero time concentration of the drug in plasma and β, the regression coefficient (elimination rate constant), for elimination phase were calculated by the method of least squares (Appendix 1).

(c) A, the zero time concentration of the drug in plasma and Ka (absorption rate constant), the regression coefficient for absorption phase after i.m. administration of the drug were calculated by the method of residual yields.

(d) C_o^p, the theoretical zero time plasma concentration of drug:

$C_o^p = A + B$ (Two-compartment model)

(e) Absorption half-life ($t_{1/2}$ Ka), distribution half life ($t_{1/2}$ α) and elimination half-life ($t_{1/2}$ β) were calculated from the following formulae :

$t_{1/2}$ Ka = 0.693/ Ka

$t_{1/2}$ α = 0.693/ α

$t_{1/2}$ β = 0.693/ β

Ka, α and β are described above.

(f) AUC, the total area under the plasma drug concentration time curve ($mg.L^{-1}.h$):

(i) For two compartment model

$$AUC = \frac{A}{\alpha} + \frac{B}{\beta}$$

(ii) For one compartment model

$$AUC = \frac{B}{\beta} - \frac{A}{K_a}$$ (Ritschell, 1976).

(g) AUMC, the total area under the first moment of plasma drug concentration time curve ($mg.L^{-1}.h^2$) :

(i) For two compartment model

$$AUMC = \frac{A}{\alpha^2} + \frac{B}{\beta^2}$$

(ii) For one compartment model

$$AUMC = \frac{B}{\beta^2} - \frac{A}{K_a^2}$$

(h) MRT, mean residential time (h) :

$$MRT = \frac{AUMC}{AUC}$$

(i) K_{21}, rate constant of transfer of drug from peripheral (tissue) compartment to the central (blood) compartment (h^{-1}) :

$$K_{21} = \frac{A.\beta + B.\alpha}{C_o^p}$$

(j) K_{el}, the elimination rate constant of drug from central compartment (h^{-1}) :

$$K_{el} = \frac{\alpha.\beta}{k_{21}}$$

(k) K_{12}, the rate constant of transfer of drug from central to peripheral compartment (h^{-1}) :

$$K_{12} = \alpha + \beta - K_{el} - K_{21}$$

(l) F_c, the fraction of drug available for elimination from central compartment :

$$F_c = \frac{\beta}{K_{el}}$$

(m) $T \approx P$, the approximate tissue to plasma concentration ratio :

$$T \approx P = \frac{K_{12}}{K_{21} - \beta}$$

(n) Vd, the volume of distribution, based on distribution and elimination ($L.Kg^{-1}$) :

$$Vd = \frac{D}{C_o^p}$$

(o) Vd_B, the volume of distribution based on elimination ($L.Kg^{-1}$):

$$Vd_B = \frac{D}{B}$$

(p) Vd_{area}, the volume of distribution based on total area under curve ($L.Kg^{-1}$) :

$$Vd_{area} = \frac{D}{AUC.\beta}$$

(q) Vd_{ss} , the volume of distribution at steady state ($L.Kg^{-1}$):

$$Vd_{ss} = \frac{K_{12} + K_{21}}{K_{21}} . vd$$

(r) Cl_B, the total body clearance ($ml.\ Kg^{-1}.\ min^{-1}$) :

$$Cl_B = Vd_{area} \times \beta$$

CALCULATION OF DOSAGE REGIMEN :

Dosage regimen is generally calculated for an antimicrobial agent to maintain minimum inhibitory concentration (MIC) in plasma at desired dosage intervals. Weidemann and Atkinson (1991) reported the therapeutic plasma level (MIC) of tobramycin to be ≤ 1 to 2 $\mu g.ml^{-1}$. Hence, in the present study, dosage regimen of tobramycin were calculated at 1,2 and 4 $\mu g.ml^{-1}$ levels for the dosage intervals of 6,8 and 12 hours using the following formulae (Saini and Srivastava, 1997) :-

$$D^* = C_p^{\infty}(min).\ Vd_{area}(e^{\beta\gamma})$$

$$D_o = C_p^{\infty}\ (min).\ Vd_{area}.(\ e^{\beta\gamma}-1)$$

Where,

D^*= Loading or priming dose ($mg.kg^{-1}$)

D_o = Maintenance dose ($mg.kg^{-1}$)

C_p^{∞} (min)= Desired minimum plasma concentration ($\mu g.ml^{-1}$)

γ = Dosage interval (h)

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


Result

RESULTS

I. Pharmacokinetic study of Tobramycin following single i.v. administration in healthy Goats

1. *Plasma Levels*

The plasma drug concentration profile at different time intervals following a single i.v.. dose (2 mg/Kg) of tobramycin in healthy goats has been shown in Table-1 and Fig.-1. The mean plasma concentration of the drug at 2.5 min was found to be 9.59 ± 1.75 μg/ml and the value ranged between 5.96 to 18.08 μg/ml. The mean therapeutic concentration (≥ 2 μg/ml) of the drug in plasma was maintained from 2.5 min to around 45 min. The drug was detectable in plasma of all goats upto 6 hr with a mean of 0.17 ± 0.03 μg/ml. The drug was detectable in two out of six goats at 10 hr (0.04 ± 0.01 μg/ml), whereas the drug was not detectable in any goat at 12 hr.

2. *Milk Levels*

Tobramycin was unable to be detected at any of the milk samples collected at different time intervals after i.v. injection at the dose rate of 2 mg/Kg.

3. *Urine Levels*

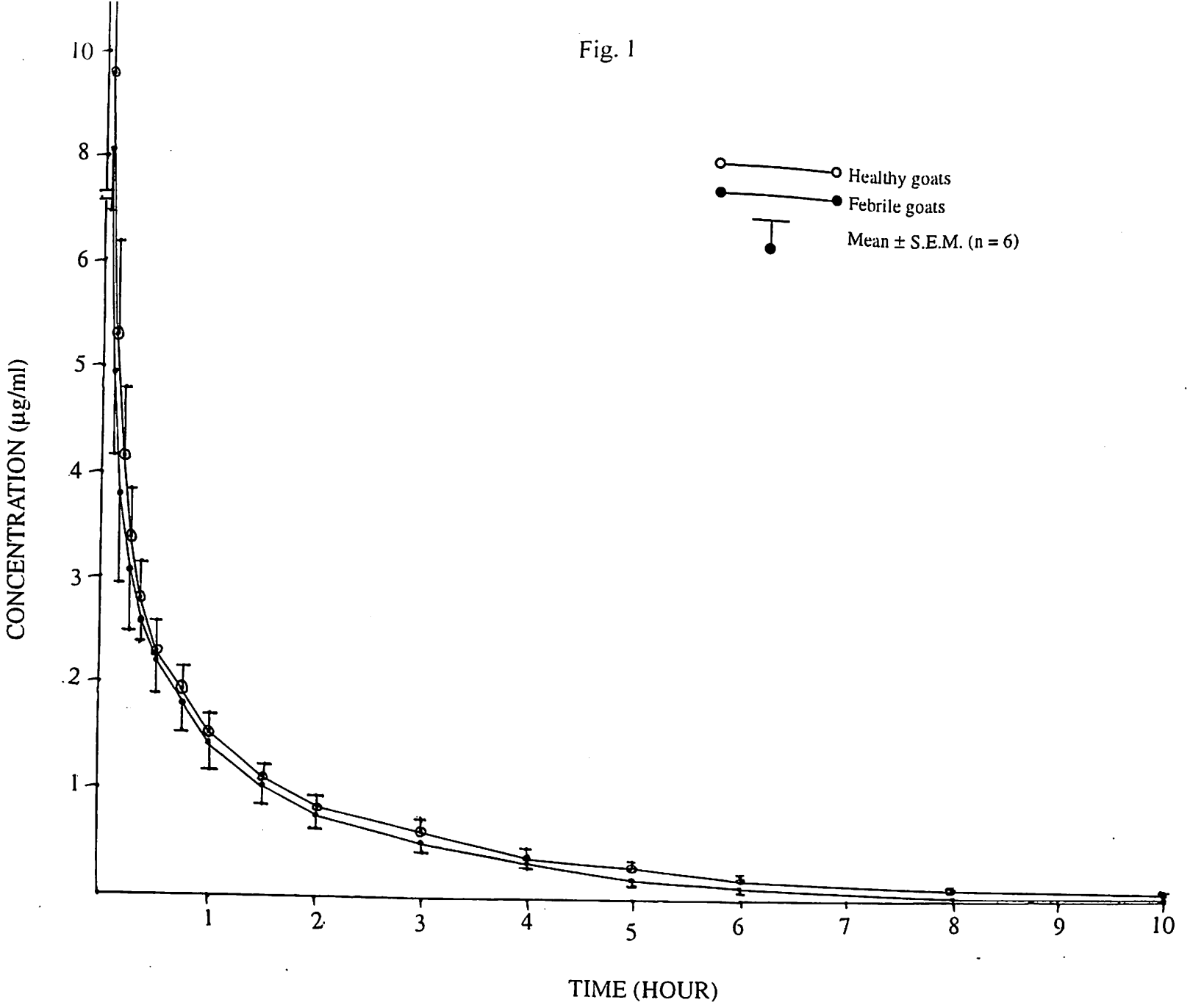
Concentrations of tobramycin in urine at different time intervals following single i.v. dose of 2 mg/Kg are shown in Table-2 and Fig-2. The drug appeared in urine samples of all goats even at 2.5

Table – 1. ***Plasma concentrations (μg/ml) of tobramycin in healthy goats following single i.v. dose of 2 mg/Kg.***

Time	Goat No.						Mean ± S.E.
↓	1	2	3	4	5	6	↓
2.5 min	5.96	18.08	8.44	8.75	8.50	7.86	9.59 ± 1.75
5 min	4.61	9.82	3.97	4.55	4.65	4.15	5.29 ± 0.91
10 min	3.56	7.24	2.73	3.80	3.95	3.65	4.15 ± 0.64
15 min	3.56	5.33	1.87	3.20	3.30	3.10	3.39 ± 0.46
20 min	2.76	3.93	1.28	2.90	3.10	2.75	2.79 ± 0.35
30 min	2.13	2.89	0.88	2.55	2.84	2.45	2.29 ± 0.30
45 min	2.13	2.13	0.88	2.15	2.28	2.10	1.95 ± 0.21
1 hr	1.65	1.57	0.61	1.70	1.92	1.80	1.54 ± 0.19
1.5 hr	1.27	1.16	0.42	1.30	1.52	1.20	1.15 ± 0.15
2 hr	0.99	0.85	0.29	0.90	1.20	0.85	0.85 ± 0.12
3 hr	0.94	0.63	0.19	0.62	0.70	0.55	0.61 ± 0.09
4 hr	0.46	0.46	0.09	0.45	0.48	0.40	0.39 ± 0.06
5 hr	0.27	0.34	0.07	0.28	0.32	0.28	0.26± 0.04
6 hr	0.16	0.25	0.05	0.17	0.25	0.16	0.17 ± 0.03
8 hr	0.06	0.14	N.D.	0.07	0.12	0.06	0.08 ± 0.02
10 hr	N.D.	0.10	-	N.D.	0.06	N.D.	0.04 ± 0.01
12 hr	-	N.D.	-	-	N.D.	-	N.D.
24 hr	-	-	-	-	-	-	

N.D. = Not Detectable

Fig. 1


CONCENTRATION (µg/ml)
10
8
6
5
4
3
2
1
1
2
3
4
5
6
7
8
9
10
TIME (HOUR)
Healthy goats
Febrile goats
Mean ± S.E.M. (n = 6)

min in therapeutic concentration with a mean of 4.18 ± 1.04 μg/ml and the value ranged between 2.80 to 9.36 μg/ml. The mean peak concentration of drug in urine was found to be 231.4 ± 15.06 μg/ml at 45 min and the drug was detected in all six goats at 24 hr with a mean of 0.62 ± 0.06 μg/ml. The therapeutic concentration (≥ 2 μg/ml) of the drug in urine was maintained from 2.5 min to 10 hr in healthy goats.

4. Kinetic Parameters

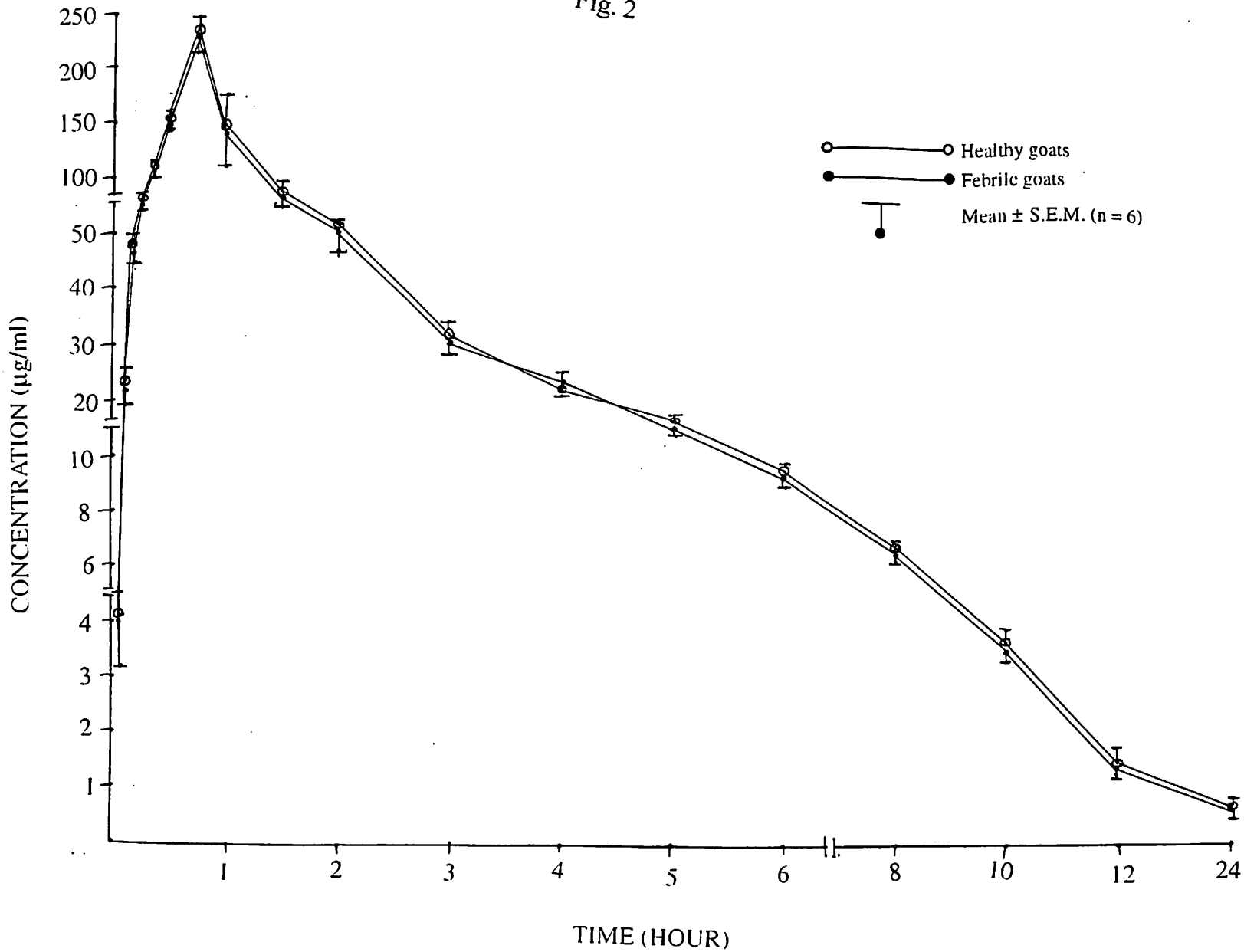
The plasma drug concentration versus time profile plot confirmed a 2-compartment open model for tobramycin(Fig-3). The values of different kinetic parameters calculated by 2-compartment open model have been presented in Table-3.

The mean extrapolated zero time concentration of the drug in plasma during distribution phase (A), elimination phase (B) and the theoretical zero time concentration $\left[C_p^0 = A + B\right]$ were noted to be 6.58 ± 1.46, 2.09 ± 0.35 and 8.68 ± 1.22 $\mu g ml^{-1}$, respectively. The distribution rate constant (α) ranged from 3.321 to 12.139 hr^{-1} with a mean value of 7.009 ± 1.651 hr^{-1}. The elimination rate constant (β) ranged from 0.269 to 0.647 hr^{-1} with a mean value of 0.441 ± 0.053 hr^{-1}. The mean distribution half life ($t_{1/2}\alpha$), elimination half life ($t_{1/2}\beta$) and mean residential time (MRT) of the drug were observed to be 0:13 ± 0.02, 1.69 ± 0.22 and 1.95 ± 0.19 hr, respectively. The average rate of transfer of drug from central to

Table – 2. ***Urine concentrations (μg/ml) of tobramycin in healthy goats following single i.v. dose of 2 mg/Kg.***

Time	Goat No.						Mean ± S.E.
↓	1	2	3	4	5	6	↓
2.5 min	9.36	3.64	2.95	3.20	2.80	3.10	4.18 ± 1.04
5 min	37.98	25.92	18.68	20.20	19.26	18.85	23.47 ± 3.09
10 min	57.49	46.55	43.18	50.55	42.88	48.26	48.15 ± 2.22
15 min	84.28	74.86	66.03	80.28	70.55	78.56	75.76 ± 2.73
20 min	125.5	106.8	101.4	120.9	102.2	115.6	112.1 ± 2.45
30 min	184.3	135.8	121.6	178.6	125.6	165.8	151.9 ± 11.29
45 min	282.2	203.4	229.7	268.5	188.9	215.5	231.4 ± 15.06
1 hr	104.2	264.4	101.6	98.85	205.8	92.64	144.6 ± 29.65
1.5 hr	83.76	98.05	80.46	78.28	110.2	74.32	87.51 ± 5.62
2 hr	56.12	68.92	57.60	52.26	64.86	50.20	58.33 ± 2.96
3 hr	30.69	37.52	31.12	28.20	35.62	26.28	31.57 ± 1.75
4 hr	20.80	27.52	20.59	18.55	26.25	17.24	21.83 ± 1.69
5 hr	15.36	17.86	14.59	13.86	16.94	12.88	15.25 ± 0.77
6 hr	9.66	12.67	8.66	7.58	11.86	6.95	9.55 ± 0.93
8 hr	6.94	8.73	5.05	5.89	7.85	5.12	6.59 ± 0.61
10 hr	3.68	4.30	3.05	3.22	4.10	3.05	3.57 ± 0.22
12 hr	1.26	2.29	0.69	1.05	2.16	1.10	1.43 ± 0.26
24 hr	0.58	1.15	0.47	0.22	1.00	0.28	0.62 ± 0.06

Fig. 2


CONCENTRATION (µg/ml)
250
200
150
100
50
40
30
20
10
8
6
4
3
2
1
1
2
3
4
5
6
8
10
12
24
TIME (HOUR)
Healthy goats
Febrile goats
Mean ± S.E.M. (n = 6)

peripheral (K_{12}), peripheral to central (K_{21}) and elimination from central (K_{el}) compartment were calculated to be 3.423 ± 0.857, 2.353 ± 0.811, and 1.674 ± 0.526 hr^{-1}, respectively. The fraction of drug available for elimination from central compartment (F_c) and approximate tissue to plasma concentration ratio ($T \approx P$) were noted to be 0.33 ± 0.06 and 2.72 ± 0.81, respectively. The value of total area under the curve in plasma (AUC) and the area under first moment curve (AUMC) were found to be 6.18 ± 0.77 $mg.L^{-1}.hr$ and 12.74 ± 2.27 $mg.L^{-1}.hr^2$, respectively. The various values of volume of distribution calculated by different methods are shown in Table-3. The mean value of Vd, Vd_B, Vd_{area} and Vd_{ss} were calculated to be 0.25 ± 0.03, 1.08 ± 0.17, 0.82 ± 0.08, 0.65 ± 0.04 $L.Kg^{-1}$, respectively. The mean value of Cl_B was observed to be 6.08 ± 1.21 $ml.Kg^{-1}.min^{-1}$.

5. *Calculated Dosage Regimen*

Table-4 depicts the calculated dosage regimen of tobramycin for i.v. route in afebrile goats for treating mild to moderate infections $\left[C_p^{\infty} \min = 1\ \mu g/ml\right]$ and severe infections $\left[C_p^{\infty} \min = 2\ \mu g/ml\right]$. For treating mild to moderate infections at the dosage interval (γ) of 6 hr, the loading or priming dose (D*) was calculated to be 16.33 ± 7.69 mg/Kg and maintenance dose (D_0)to be 15.56 ± 7.62 mg/Kg. For treating severe infections at γ of 6 hr, D* and D_0 were calculated to be 32.66 ± 15.37 and 31.12 ± 15.24 mg/Kg, respectively.

Table – 3. ***Pharmacokinetic parameters of tobramycin in healthy goats following single i.v. dose of 2 mg/Kg.***

Parameters	Unit	Goat No.						Mean ± S.E.
↓		1	2	3	4	5	6	↓
A	$\mu g.ml^{-1}$	3.75	11.83	10.53	4.79	4.34	4.26	6.58 ± 1.46
B	$\mu g.\ ml^{-1}$	3.64	1.33	1.22	2.28	1.94	2.17	2.09 ± 0.35
C_p^o	$\mu g.ml^{-1}$	7.39	13.16	11.75	7.08	6.28	6.43	8.68 ± 1.22
α	hr^{-1}	12.139	3.625	12.062	5.623	3.321	5.286	7.009 ± 1.651
$t_{1/2}\alpha$	hr	0.06	0.19	0.06	0.12	0.21	0.13	0.13 ± 0.02
β	hr^{-1}	0.518	0.269	0.647	0.430	0.347	0.437	0.441 ± 0.053
$t_{1/2}\beta$	hr	1.34	2.58	1.07	1.61	1.99	1.56	1.69 ± 0.22
AUC	$mg.L^{-1}.hr$	7.33	8.21	2.76	6.17	6.88	5.78	6.18 ± 0.77
AUMC	$mg.L^{-1}hr^2$	13.59	19.37	2.99	12.52	16.46	11.52	12.74 ± 2.27
MRT	hr	1.85	2.36	1.08	2.03	2.39	1.99	1.95 ± 0.19
K_{12}	hr^{-1}	5:408	1.683	6.618	2.801	1.492	2.536	3.423 ± 0.857
K_{21}	hr^{-1}	6.242	0.608	1.832	2.101	1.265	2.073	2.353 ± 0.811
K_{el}	hr^{-1}	1.007	1.603	4.259	1.151	0.911	1.114	1.674 ± 0.526
F_C	-	0.51	0.17	0.15	0.37	0.38	0.39	0.33 ± 0.06
$T \approx P$	-	0.94	4-96	5.59	1.66	1.63	1.55	2.72 ± 0.81
Vd_C	$L.Kg^{-1}$	0.27	0.15	0.17	0.28	0.32	0.31	0.25 ± 0.03
Vd_B	$L.Kg^{-1}$	0.55	1.5	1.64	0.87	1.03	0.92	1.08 ± 0.17
Vd_{area}	$L.Kg^{-1}$	0.53	0.91	1.12	0.75	0.84	0.79	0.82 ± 0.08
Vd_{SS}	$L.Kg^{-1}$	0.50	0.57	0.78	0.65	0.69	0.69	0.65 ± 0.04
Cl_B	$ml.Kg^{-1}.min^{-1}$	4.50	4.00	12.00	5.33	4.83	5.83	6.08 ± 1.21

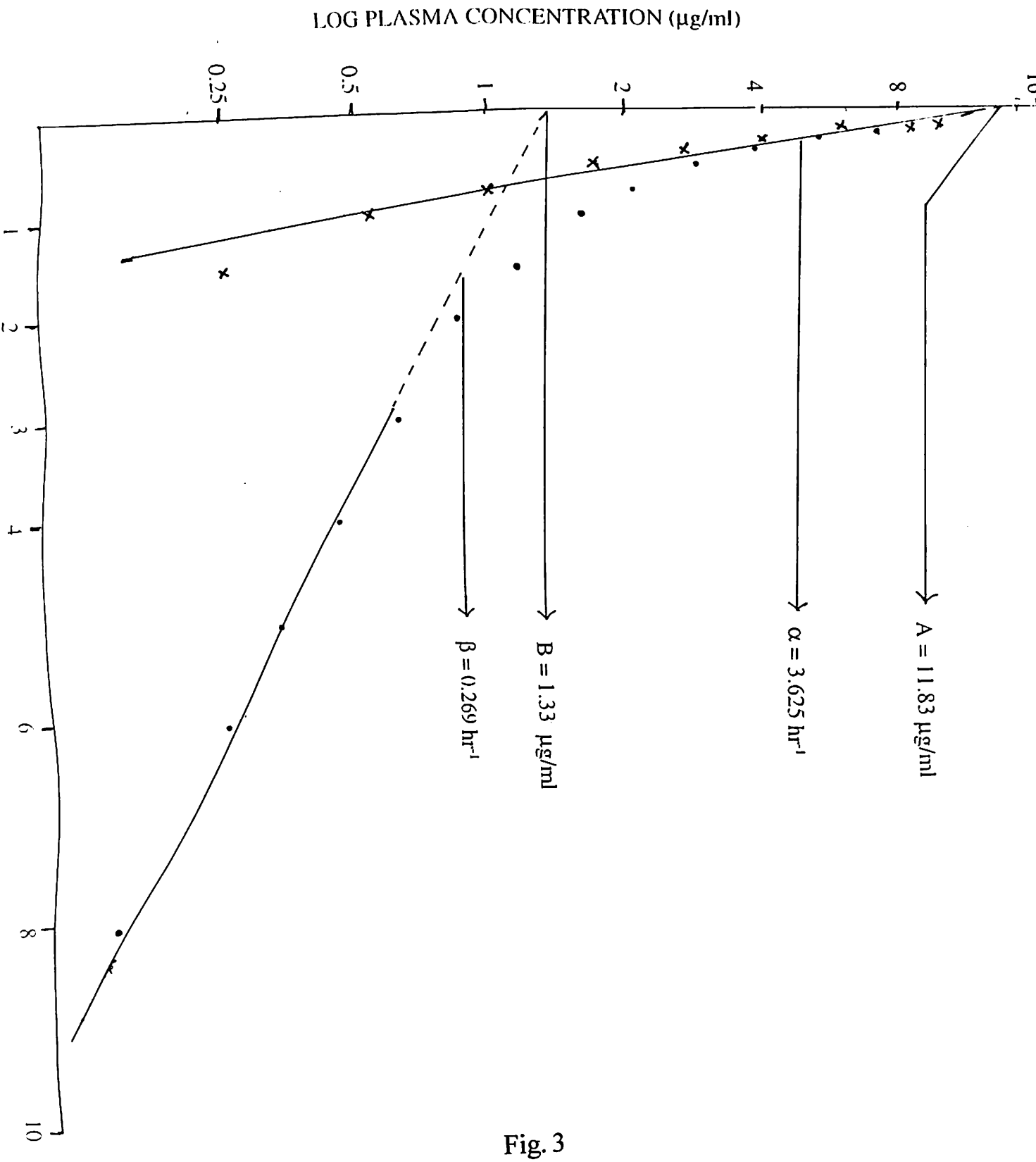

LOG PLASMA CONCENTRATION (µg/ml)
0.25
0.5
1
2
4
8
16
1
2
3
4
6
8
10
β = 0.269 hr⁻¹
B = 1.33 µg/ml
α = 3.625 hr⁻¹
A = 11.83 µg/ml

Fig. 3

Table – 4. ***Dosage regimen of tobramycin for i.v. route in healthy goats.***

↓		Goat No.						Mean ± S.E. ↓
		1	2	3	4	5	6	
C_p^{∞}**min =1.0μg/ml**								
γ = 6hr	D*	11.86	4.57	54.34	9.89	6.44	10.87	16.33 ± 7.69
	D_0	11.33	3.66	53.24	9.15	5.89	10.08	15.56 ± 7.62
γ = 8hr	D*	33.42	7.83	198.21	23.39	13.49	26.06	50.39 ± 29.79
	D_0	32.89	6.92	197.09	22.64	12.65	25.27	49.58 ± 29.74
C_p^{∞}**min =2.0 μg/ml**								
γ = 6hr	D*	23.72	9.16	108.69	19.79	12.87	21.75	32.66 ± 15.37
	D_0	22.66	7.32	106.49	18.29	11.79	20.17	31.12 ± 15.24
γ = 8hr	D*	66.84	15.66	396.42	46.78	26.97	52.11	100.78 ± 59.58
	D_0	65.78	13.84	394.18	45.28	25.29	50.53	99.16 ± 59.48

C_p^{∞} min = Minimum plasma level (μg/ml)

D* = Loading or priming dose (mg/kg)

D_0 = Maintenance dose (mg/kg)

γ = Dosage interval

II. Pharmacokinetic study of Tobramycin following single i.v. administration in febrile goats

1. Plasma Levels

Table-5 and Fig. 1 depict the plasma drug concentration versus time profile at different time intervals following a single i.v. dose (2 mg/Kg) of tobramycin in febrile goats. The mean plasma concentration of drug at 2.5 min was found to be 8.14±1.03 μg/ml and the value ranged between 3.39 to 10.85 μg/ml. The drug was detectable upto 4 hr in all febrile goats with a mean of 0.35±0.06 μg/ml .The drug was present only in five goats at 5 and 6 hr, in four goats at 8 hr in two goats at 10 hr and none at 12 hr. The mean therapeutic concentration (≥ 2 μg/ml) was maintained from 2.5 min to around 45 min.

2. Milk Levels

Tobramycin was not at all detectable in any of the milk samples collected at different time intervals following single i.v. dose of 2 mg/Kg.

3. Urine Levels

Table-6 and Fig-2 reveal the concentrations of tobramycin in urine at different time intervals following single i.v. dose of 2 mg/Kg. The drug appeared in urine samples of all the goats

Table – 5. ***Plasma concentrations (μg/ml) of tobramycin in febrile goats following single i.v. dose of 2 mg/Kg***

Time	Goat No.						Mean ± S.E.
↓	1	2	3	4	5	6	↓
2.5 min	3.39	10.85	8.12	9.26	9.12	8.08	8.14 ± 1.03
5 min	2.26	8.68	3.50	4.98	4.86	4.36	4.77 ± 0.88
10 min	1.50	6.82	2.42	4.05	4.18	3.84	3.80 ± 0.74
15 min	1.40	4.85	1.75	3.66	3.55	3.22	3.07 ± 0.17
20 min	1.30	3.68	1.30	3.15	3.22	2.94	2.59 ± 0.17
30 min	1.20	2.68	1.05	2.76	2.98	2.84	2.25 ± 0.36
45 min	1.00	2.06	0.96	2.38	2.38	2.26	1.84 ± 0.28
1 hr	0.66	1.48	0.80	1.95	2.06	1.95	1.48 ± 0.25
1.5 hr	0.60	1.15	0.52	1.40	1.75	1.28	1.12 ± 0.19
2 hr	0.44	0.96	0.44	1.05	1.16	0.90	0.83 ± 0.13
3 hr	0.34	0.68	0.26	0.68	0.76	0.54	0.54 ± 0.08
4 hr	0.19	0.50	0.15	0.46	0.44	0.36	0.35 ± 0.06
5 hr	N.D.	0.35	0.10	0.24	0.25	0.22	0.19 ± 0.05
6 hr	-	0.25	0.06	0.17	0.18	0.14	0.13 ± 0.04
8 hr	-	0.14	N.D.	0.06	0.08	0.05	0.06 ± 0.02
10 hr	-	0.07	-	N.D.	0.05	N.D.	0.02 ± 0.02
12 hr	-	N.D.	-	-	N.D.	-	
24 hr	-	-	-	-	-	-	

N.D. = Not Detectable

at 2.5 min in therapeutic concentration with a mean of 4.15 ± 0.79 μg/ml and the values ranged between 2.75 to 7.85 μg/ml. The mean peak urine concentration of 228.8 ± 13.51 μg/ml was observed at 45 min and the drug was detectable in therapeutic concentration upto 10 hr in all goats. The drug was detected in all goats upto 24 hr with a mean value of 0.55 ± 0.11 μg/ml.

4. Kinetic Parameters

Kinetic parameters were calculated by 2-compartment open model since the plasma drug concentration versus time profile had shown biphasic curve. Table-7 shows the values of different kinetic parameters calculated by using the above mentioned compartment model.

The mean extrapolated zero time concentration of the drug in plasma during distribution phase (A), elimination phase (B) and the theoretical zero time concentration $\left[C_p^0 = A + B\right]$ were noted to be 5.62 ± 1.8, 2.08 ± 0.38, and 7.53 ±1.29 μg/ml, respectively. The distribution rate constant (α) ranged from 3.979 to 9.035 hr^{-1} with a mean value of 5.987 ± 0.950 hr^{-1} while its elimination rate constant (β) ranged from 0.326 to 0.486 hr^{-1} with a mean value of 0.430 ± 0.025 hr^{-1}.

The mean distribution half life ($t_{1/2}\alpha$), elimination half life ($t_{1/2}\beta$) and mean residential time (MRT) of the drug were observed

Table – 6. ***Urine concentrations (μg/ml) of tobramycin in febrile goats following single i.v. dose of 2 mg/Kg.***

Time	Goat No.						Mean ± S.E.
↓	1	2	3	4	5	6	↓
2.5 min	7.85	4.62	3.00	3.45	2.75	3.22	4.15 ± 0.79
5 min	33.64	26.15	18.60	19.85	18.85	19.15	22.71 ± 2.48
10 min	53.65	48.80	42.25	51.25	41.68	49.06	47.78 ± 1.97
15 min	82.61	76.15	67.12	81.10	69.26	79.44	75.95 ± 2.62
20 min	122.6	107.5	102.6	119.8	104.4	116.2	112.2 ± 3.45
30 min	177.1	136.4	123.2	175.7	126.8	166.4	150.9 ± 10.16
45 min	269.9	200.8	230.2	265.6	190.2	216.2	228.8 ± 13.51
1 hr	102.6	260.6	102.2	97.15	208.6	90.85	143.7 ± 29.58
1.5 hr	82.09	99.12	82.4	74.20	112.2	72.22	87.04 ± 6.35
2 hr	52.43	70.15	56.8	49.86	65.8	49.65	57.45 ± 3.54
3 hr	32.73	38.55	30.22	27.12	34.45	25.58	31.44 ± 1.96
4 hr	23.12	27.85	20.19	18.05	25.05	16.84	21.85 ± 1.73
5 hr	16.25	18.12	14.20	13.16	16.24	11.88	14.98 ± 0.94
6 hr	9.16	13.05	8.15	7.28	10.95	7.05	9.27 ± 0.95
8 hr	6.54	9.10	4.85	5.19	7.15	5.22	6.34 ± 0.66
10 hr	3.28	4.22	2.92	3.14	3.92	3.15	3.44 ± 0.21
12 hr	1.18	2.19	0.78	1.15	2.06	1.20	1.43 ± 0.23
24 hr	0.36	0.85	0.45	0.32	0.92	0.38	0.55 ± 0.11

to be 0.13 ± 0.02, 1.65 ± 0.11, and 1.97 ± 0.10 hr, respectively. The average rate of transfer of drug from central to peripheral (K_{12}), peripheral to central (K_{21}), and elimination from central (K_{el}) compartment were calculated to be 3.097 ± 0.641, 1.899 ± 0.272 and 1.420 ± 0.240 hr^{-1}, respectively. The fraction of drug available for elimination from central compartment (F_C) and approximate tissue to plasma concentration ratio ($T \approx P$) were noted to be 0.33 ± 0.05 and 2.32 ± 0.53, respectively. The value of area under the curve in plasma (AUC) was found to be 5.50 ± 0.85 $mg.L^{-1}.hr$ while area under the first moment curve (AUMC) was 11.04 ± 1.99 $mg.L^{-1}.hr^2$. The various values of volume of distribution calculated by different methods are shown in Table-7. The mean values of Vd_C, Vd_B, Vd_{area} and Vd_{SS} were calculated to be 0.33 ± 0.09, 1.20 ± 0.21, 0.99 ± 0.17, 0.82 ± 0.15 $L.Kg^{-1}$, respectively. The mean value of Cl_B was calculated to be 6.86 ± 1.23 $ml.Kg^{-1}.min^{-1}$.

5. *Calculated Dosage Regimen*

Table-8 depicts the calculated dosage regimen of tobramycin for i.v. route in febrile goats for treating mild to moderate infections $\left[C_p^{\infty} \min = 1 \mu g/ml\right]$ and severe infections $\left[C_p^{\infty} \min = 2 \mu g/ml\right]$ at the dosage interval (γ) of 6 and 8 hr. For treating mild to moderate infections at the dosage interval (γ) of 6 hr, the loading or priming dose (D*) was calculated to be 13.75 ± 3.04 and maintenance dose

Table – 7. ***Pharmacokinetic parameters of tobramycin in febrile goats following single i.v. dose of 2 mg/Kg.***

arameters	Unit	Goat No.						Mean ± S.E.
↓		1	2	3	4	5	6	↓
A	$\mu g.ml^{-1}$	1.56	10.23	6.41	6.61	4.55	4.35	5.62 ± 1.18
B	$\mu g.\ ml^{-1}$	1.05	1.83	1.11	2.87	2.28	3.35	2.08 ± 0.38
C^{o}_{p}	$\mu g.ml^{-1}$	2.61	12.06	7.52	9.47	6.82	6.69	7.53 ± 1.29
α	hr^{-1}	4.148	4.698	9.035	8.842	3.979	5.220	5.987 ± 0.950
$t_{1/2}\alpha$	hr	0.17	0.15	0.08	0.08	0.17	0.13	0.13 ± 0.02
β	hr^{-1}	0.411	0.326	0.486	0.479	0.403	0.476	0.430 ± 0.025
$t_{1/2}\beta$	hr	1.69	2.13	1.43	1.45	1.72	1.46	1.65 ± 0.11
AUC	$mg.L^{-1}.hr$	2.93	7.80	2.99	6.73	6.79	5.77	5.50 ± 0.85
AUMC	$mg.L^{-1}hr^{2}$	6.31	17.72	4.78	12.57	14.30	10.53	11.04 ± 1.99
MRT	hr	2.15	2.27	1.59	1.87	2.11	1.83	1.97 ± 0.10
K_{12}	hr^{-1}	1.754	2.488	5.261	4.905	1.782	2.394	3.097 ± 0.641
K_{21}	hr^{-1}	1.914	0.991	1.748	3.008	1.596	2.141	1.899 ± 0.272
K_{el}	hr^{-1}	0.891	1.545	2.512	1.408	1.004	1.161	1.420 ± 0.240
F_C	-	0.46	0.21	0.19	0.34	0.39	0.41	0.33 ± 0.05
$T \approx P$	-	1.17	3.73	4.16	1.94	1.49	1.44	2.32 ± 0.53
Vd_C	$L.Kg^{-1}$	0.77	0.17	0.26	0.21	0.29	0.29	0.33 ± 0.09
Vd_B	$L.Kg^{-1}$	1.90	1.09	1.8	0.69	0.88	0.85	1.20 ± 0.21
Vd_{area}	$L.Kg^{-1}$	1.66	0.79	1.38	0.62	0.73	0.73	0.99 ± 0.17
Vd_{SS}	$L.Kg^{-1}$	1.48	0.59	1.06	0.55	0.61	0.61	0.82 ± 0.15
Cl_B	$ml.Kg^{-1}.min^{-1}$	10.17	4.33	11.17	4.83	4.83	5.83	6.86 ± 1.23

Table – 8. ***Dosage regimen of tobramycin for i.v. route in febrile goats.***

		Goat No.						Mean ± S.E.
		1	2	3	4	5	6	↓
C_p' min=**1.0μg/ml**								
γ = 6hr	D*	19.55	5.59	25.48	10.98	8.19	12.69	13.75 ± 3.04
	D_0	17.89	4.79	24.10	10.36	7.46	11.97	12.77 ± 2.90
γ = 8hr	D*	44.47	10.72	67.36	28.62	18.34	32.89	33.73 ± 8.25
	D_0	42.81	9.93	65.98	27.99	17.61	32.16	32.75 ± 8.12
C_p' min= **2.0μg/ml**								
γ = 6hr	D*	39.09	11.17	50.97	21.96	16.39	25.39	27.49 ± 6.08
	D_0	35.77	9.59	48.21	20.72	14.93	23.93	25.53 ± 5.80
γ = 8hr	D*	88.94	21.44	134.72	57.23	36.69	65.79	67.47 ± 16.5
	D_0	85.62	19.86	131.96	55.99	35.23	64.33	65.49 ± 16.24

C_p' min = Minimum plasma level (μg/ml)

D* = Loading or priming dose (mg/kg)

D_0 = Maintenance dose (mg/kg)

γ = Dosage interval

(D_0) to be 12.77 ± 2.90 mg/Kg. For treating severe infections at γ at 6 hr, D* and D^0 were calculated to be 27.49 ± 6.08 and 25.53 ± 5.80 mg/Kg, respectively.

III. Comparison of pharmacokinetic of tobramycin between healthy and febrile goats following single i.v. administration.

1. *Plasma Levels* :

Table-9 and Fig-1 show the comparative plasma drug concentrations between healthy and febrile goats following single i.v. dose of tobramycin (2 mg/Kg). The drug was detectable upto 10 hr in both healthy and febrile goats. The drug was present slightly in lower concentrations throughout from 2.5 min to 10 hr in febrile goats though they were statistically insignificant. The therapeutic concentration (≥ 2 μg/ml) was maintained from 2.5 min to around 45 min in both healthy and febrile goats.

2. *Milk Levels* :

Tobramycin was not at all detectable in any of the milk samples collected in both healthy and febrile goats following its i.v. administration.

3. *Urine Levels* :

Table-9 and Fig-2 present the comparative urine concentrations of tobramycin between healthy and febrile goats following its single i.v. dose (2 mg/Kg). Urine concentrations of

Table – 9. ***Comparison of concentrations (μg/ml) of tobramycin in various biological fluids between healthy and febrile goats following single i.v. dose of 2 mg/Kg.***

Time	Healthy Goats (n = 6)		Febrile Goats (n = 6)	
↓	Plasma	Urine	Plasma	Urine
2.5 min	9.59 ± 1.75	4.18 ± 1.04	8.14 ± 1.03	4.15 ± 0.79
5 min	5.29 ± 0.91	23.47 ± 3.09	4.77 ± 0.88	22.71 ± 2.48
10 min	4.15 ± 0.64	48.15 ± 2.22	3.80 ± 0.74	47.78 ± 1.97
15 min	3.39 ± 0.46	75.76 ± 2.73	3.07 ± 0.53	75.95 ± 2.62
20 min	2.79 ± 0.35	112.1 ± 2.45	2.59 ± 0.17	112.19 ± 3.45
30 min	2.29 ± 0.30	151.9 ± 11.29	2.25 ± 0.36	150.9 ± 10.16
45 min	1.95 ± 0.21	231.4 ± 15.06	1.84 ± 0.28	228.8 ± 13.51
1 hr	1.54 ± 0.19	144.6 ± 29.65	1.48 ± 0.25	143.7 ± 29.58
1.5 hr	1.15 ± 0.15	87.51 ± 5.62	1.12 ± 0.19	87.04 ± 6.35
2 hr	0.85 ± 0.12	58.33 ± 2.96	0.83 ± 0.13	57.45 ± 3.54
3 hr	0.61 ± 0.09	31.57 ± 1.75	0.54 ± 0.08	31.44 ± 1.96
4hr	0.39 ± 0.06	21.83 ± 1.69	0.35 ± 0.06	21.85 ± 1.73
5 hr	0.26 ± 0.04	15.25 ± 0.77	0.19 ±0.05	14.98 ± 0.94
6hr	0.17 ± 0.03	9.55 ± 0.93	0.13 ± 0.04	9.27 ± 0.95
8 hr	0.08 ± 0.02	6.59 ± 0.61	0.06 ± 0.02	6.34 ± 0.66
10 hr	0.04 ± 0.01	3.57 ± 0.22	0.02 ± 0.01	3.44 ± 0.21
12 hr	N.D.	1.43 ± 0.26	N.D.	1.43 ± 0.23
24 hr	-	0.62 ± 0.06	-	0.55 ± 0.11

All data are non-significant between healthy vs febrile goats.

N.D. = Non Detectable

tobramycin were noted to differ non-significantly at all time intervals between healthy and febrile goats. Peak urine concentrations were noted at 45 min in both healthy (231.4 ± 15.06 μg/ml) and febrile (228.8 ± 13.51 μg/ml) goats. The mean therapeutic concentration (≥ 2 μg/ml) of the drug was maintained from 2.5 min to > 10 hr in both healthy and febrile goats.

4. *Kinetic Parameters*

The comparative kinetic parameters of tobramycin between healthy and febrile goats following its single i.v. dose of 2 mg/Kg have been presented in Table-10. The value of extrapolated zero time concentration during distribution (A) and elimination (B) phase and theoretical zero time concentration $[C_0^p]$ were noted to be lower in febrile goats as compared to healthy goats but the data were not significant. Similarly, though low value of elimination half life ($t_{1/2}\beta$), area under curve (AUC), area under first moment curve (AUMC) and various micro rate constants namely K_{12}, K_{21}, K_{el} and tissue to plasma concentration ratio ($T \approx P$) were noted to be lower in febrile goats but they were insignificant. Similar values were obtained for distribution half life ($t_{1/2}\alpha$) and fraction of drug available in central compartment (F_C) in both healthy and febrile goats. The various values of volume distribution (Vd_C, Vd_B, Vd_{area}, Vd_{SS}) and total body clearance (Cl_B) were found to be slightly higher in febrile goats as compared to healthy goats but they were non-significant.

Table–10. ***Comparison of pharmacokinetic parameters of tobramycin between healthy and febrile goats following single i.v. dose of 2 mg/Kg.***

Parameter	Unit	Healthy Goat (n = 6)	Febrile Goat (n = 6)
A	$\mu g.ml^{-1}$	6.58 ± 1.46	5.62 ± 1.18
B	$\mu g.\ ml^{-1}$	2.09 ± 0.35	2.08 ± 0.38
C^{o}_{p}	$\mu g.ml^{-1}$	8.68 ± 1.22	7.53 ± 1.29
α	hr^{-1}	7.009 ± 1.651	5.987 ± 0.950
$t_{1/2}\alpha$	hr	0.13 ± 0.02	0.13 ± 0.02
β	hr^{-1}	0.441 ± 0.053	0.430 ± 0.025
$t_{1/2}\beta$	hr	1.69 ± 0.22	1.65 ± 0.11
AUC	$mg.L^{-1}.hr$	6.18 ± 0.77	5.50 ± 0.85
AUMC	$mg.L^{-1}hr^{2}$	12.74 ± 2.27	11.04 ± 1.99
MRT	hr	1.95 ± 0.19	1.97 ± 0.10
K_{12}	hr^{-1}	3.423 ± 0.857	3.097 ± 0.641
K_{21}	hr^{-1}	2.353 ± 0.811	1.899 ± 0.272
K_{el}	hr^{-1}	1.674 ± 0.526	1.420 ±0.240
F_C	-	0.33 ± 0.06	0.33 ± 0.05
$T \approx P$	-	2.72 ± 0.81	2.32 ± 0.53
Vd_C	$L.Kg^{-1}$	0.25 ± 0.03	0.33 ± 0.09
Vd_B	$L.Kg^{-1}$	1.08 ± 0.17	1.20 ± 0.21
Vd_{area}	$L.Kg^{-1}$	0.82 ± 0.08	0.99 ± 0.17
Vd_{SS}	$L.Kg^{-1}$	0.65 ± 0.04	0.82 ± 0.15
Cl_B	$ml.Kg^{-1}.min^{-1}$	6.08 ±1.21	6.86 ±1.23

All data are non – significant between healthy vs febrile goats.

Table-11. ***Comparison of dosage regimen of tobramycin for i.v. route between healthy and febrile goats.***

		Unit	Healthy Goats (n =6)	Febrile Goats (n =6)
$C_p^{'}$ min **=1.0μg/ml**				
γ = 6hr	D*	$mg.kg^{-1}$	16.33 ± 7.69	13.75 ± 3.04
	D_0	$mg.kg^{-1}$	15.56 ± 7.62	12.77 ± 2.90
γ = 8hr	D*	$mg.kg^{-1}$	50.39 ± 29.76	33.73 ± 8.25
	D_0	$mg.kg^{-1}$	49.58 ± 29.74	32.75 ± 8.12
C_p^{∞} min =2.0μg/ml				
γ = 6hr	D*	$mg.kg^{-1}$	32.66 ± 15.37	27.49 ± 6.08
	D_0	$mg.kg^{-1}$	31.12 ± 15.24	25.53 ± 5.8
γ = 8hr	D*	$mg.kg^{-1}$	100.78 ± 59.58	67.47 ± 16.50
	D_0	$mg.kg^{-1}$	99.16 ± 59.48	65.49 ± 16.24

$C_p^{'}$ min = Minimum Inhibitory Concentration (MIC)

D* = Loading dose

D_0 = Maintenance dose

γ = Dosage interval

All data are non-significant between healthy vs febrile goats.

5. *Calculated dosage regimen*

Table – 11 shows the comparison of calculated dosage regimen of tobramycin between afebrile and febrile goats for i.v. route in order to maintain C_p^{∞} min [MIC] of 1 and 2 μg/ml at the selected dosage interval of 6 and 8 hr. The loading (D*) and maintenance (D_0) doses were calculated to be non-significantly lower in febrile goats as compared to healthy goats.

IV. Pharmacokinetic study of Tobramycin following single i.m. administration in healthy goats.

1. *Plasma Levels*

The plasma drug concentration profile at different time intervals following a single i.m. dose (2 mg/Kg) of tobramycin in healthy goats has been shown in Table-12 and Fig-4. The drug appeared in all goats at 2.5 min with a mean value of 0.29 ± 0.03 μg/ml and the value ranged between 0.20 to 0.40 μg/ml. The mean peak plasma concentration of 3.26 ± 0.17 μg/ml was attained at 45 min and the drug persisted upto 10 hr in all goats with a mean concentrationof 0.25 ± 0.02 μg/ml. The drug was present only in four out of six goats at 12 hr with a mean 0.09 ± 0.03 μg/ml and none at 24 hr. The mean therapeutic concentration (≥ 2 μg/ml) of the drug was maintained from 30 min to around 3 hr.

Table-12. ***Plasma concentrations (μg/ml) of tobramycin in healthy goats following single i.m. dose of 2 mg/Kg.***

Time	Goat No.						Mean ± S.E.
↓	1	2	3	4	5	6	↓
2.5 min	0.35	0.40	0.26	0.22	0.20	0.32	0.29 ± 0.03
5 min	0.90	0.96	0.78	0.65	0.62	0.78	0.78 ± 0.05
10 min	1.14	1.22	1.06	0.94	1.05	0.96	1.06 ± 0.04
15 min	1.45	1.68	1.30	1.15	1.28	1.22	1.35 ± 0.08
20 min	1.85	2.04	1.76	1.52	1.66	1.68	1.75 ± 0.07
30 min	2.34	2.88	2.08	1.90	2.15	2.20	2.26 ± 0.14
45 min	3.78	3.60	3.20	3.08	2.62	3.26	3.26 ± 0.17
1 hr	1.45	4.12	2.92	2.88	3.65	2.85	2.98 ± 0.37
1.5 hr	1.14	3.45	2.80	2.68	3.05	2.52	2.61 ± 0.32
2 hr	0.90	2.82	2.24	2.14	2.62	2.40	2.19 ± 0.28
3 hr	0.56	2.52	1.90	1.85	2.25	1.82	1.82 ± 0.28
4 hr	0.35	1.50	1.18	1.06	1.45	1.26	1.13 ± 0.17
5 hr	0.30	1.34	1.02	0.92	1.30	1.00	0.98 ± 0.15
6 hr	0.27	0.88	0.66	0.52	0.78	0.68	0.63 ± 0.09
8 hr	0.21	0.46	0.45	0.37	0.48	0.42	0.39 ± 0.04
10 hr	0.17	0.32	0.26	0.18	0.34	0.25	0.25 ± 0.02
12 hr	N.D.	0.16	0.12	N.D.	0.18	0.12	0.09 ± 0.03
24 hr	-	N.D.	N.D.	-	N.D.	N.D.	N.D.

N.D. = *Not Detectable*

Fig. 4: Mean plasma concentrations of tobramycin in healthy (o——o) and febrile (•——•)goats after single i.m. administration (2 mg/kg)

2. *Milk Levels*

Tobramycin was undetectable in any of the milk samples collected at different time interval after i.m. injection at the dose rate of 2 mg/Kg.

3. *Urine Levels*

The concentrations of tobramycin in urine of healthy goats at different time intervals following single i. m. dose (2 mg/Kg) have been presented in Table-13 and Fig-5. The drug appeared in all goats at 2.5 min with a mean of 1.18 ± 0.09 μg/ml and the value ranged between 0.98 to 1.65 μg/ml. The mean peak urine concentrationof 157.4 ± 9.58 μg/ml was attained at 1.5 hr and the value ranged between 120.2 to 185.4 μg/ml. The drug was detectable upto 24 hr in all goats with a mean concentration of 0.59 ± 0.07 μg/ml. The mean therapeutic concentration (≥ 2 μg/ml) of drug in urine was maintained from 5 min to 10 hr.

4. *Kinetic Parameters*

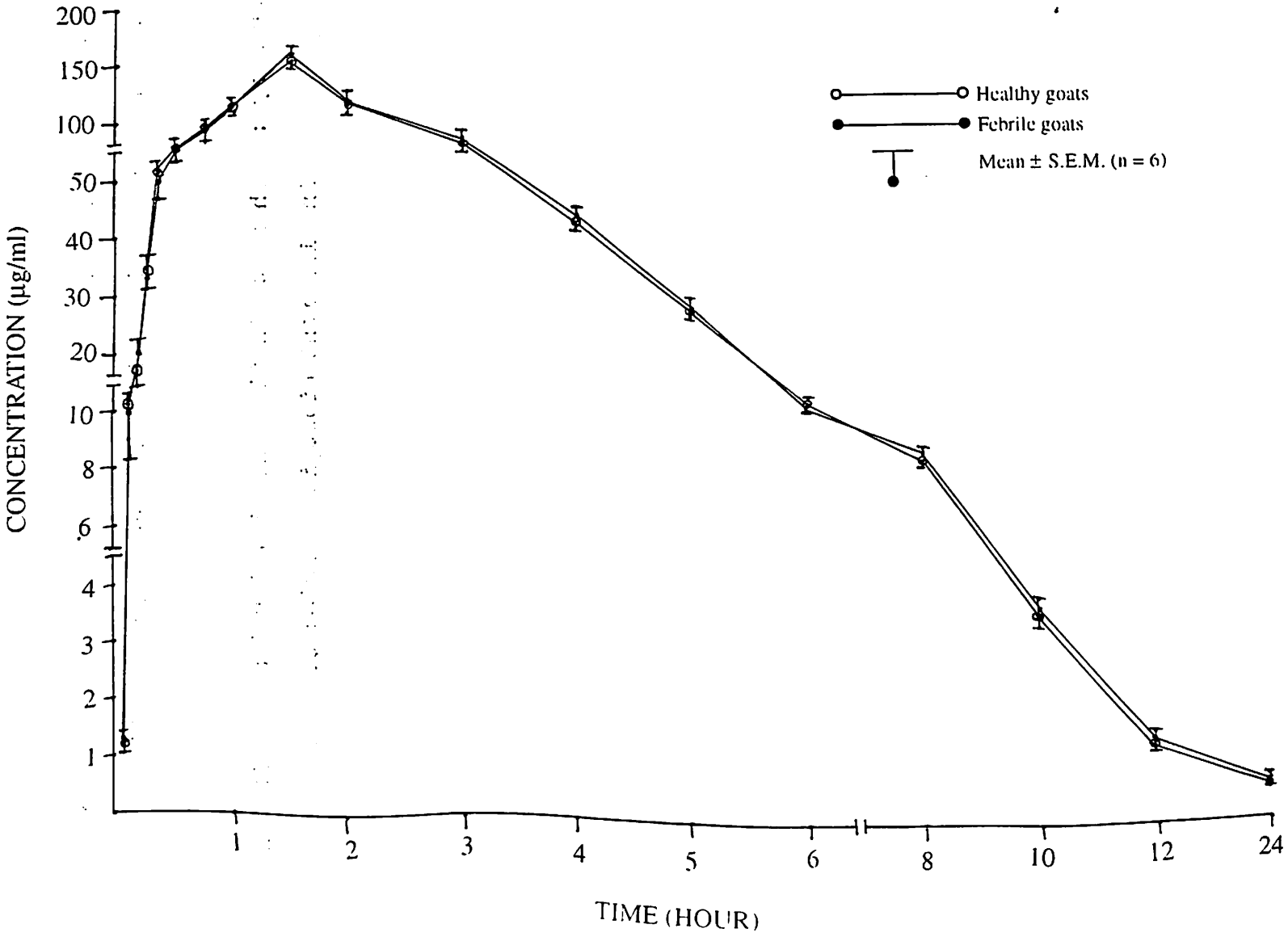
The plasma drug concentration versus time profile showed monophasic curve (Fig-6) and hence, the kinetic parameters were calculated by using one-compartment open model.

The values of different kinetic parameters of the drug following its single i.m. administration have been presented in Table-

Table-13. ***Urine concentrations (μg/ml) of tobramycin in healthy goats following single i.m. dose of 2 mg/Kg.***

Time	Goat No.						Mean ± S.E.
↓	1	2	3	4	5	6	↓
2.5 min	1.65	1.12	1.05	1.20	0.98	1.08	1.18 ± 0.09
5 min	14.98	12.85	10.26	11.85	9.68	10.55	11.69 ± 0.81
10 min	24.90	22.20	18.85	20.25	16.25	19.12	17.82 ± 3.37
15 min	41.96	38.85	30.46	35.68	28.26	31.45	34.44 ± 2.16
20 min	60.85	52.46	48.40	50.22	40.25	50.26	50.41 ± 2.71
30 min	82.20	78.86	75.60	80.55	70.22	77.85	77.55 ± 1.73
45 min	110.8	99.82	89.64	102.2	89.28	90.24	96.99 ± 3.58
1 hr	126.2	110.5	120.2	118.8	100.5	122.5	116.4 ± 3.83
1.5 hr	185.4	140.4	158.5	168.6	120.2	170.5	157.4 ± 9.58
2 hr	102.2	164.2	98.66	100.6	138.8	100.2	117.4 ± 11.27
3 hr	84.68	90.84	80.28	86.86	86.65	82.26	85.26 ± 1.52
4 hr	45.20	48.45	40.25	42.50	44.28	42.46	43.86 ± 1.15
5 hr	28.85	30.12	25.52	27.26	28.56	28.25	28.09 ± 0.64
6 hr	11.20	12.15	9.56	10.48	11.45	10.16	10.83 ± 0.34
8 hr	8.55	9.08	7.42	8.12	8.85	8.12	8.36 ± 0.24
10 hr	3.75	3.96	2.88	3.62	3.88	3.15	3.54 ± 0.18
12 hr	1.32	1.68	1.02	1.15	1.46	1.25	1.31 ± 0.09
24 hr	0.60	0.92	0.44	0.52	0.56	0.48	0.59 ± 0.07

Fig. 5


CONCENTRATION (µg/ml)
200
150
100
50
40
30
20
10
8
6
4
3
2
1
1
2
3
4
5
6
8
10
12
24
TIME (HOUR)
Healthy goats
Febrile goats
Mean ± S.E.M. (n = 6)

14. The mean extrapolated zero time concentration of the drug in plasma during absorption (A) and elimination (B) phases were estimated to be 3.77 ± 0.45 and 3.98 ± 0.48 μg/ml, respectively. The absorption rate constant (Ka) ranged from 1.163 to 6.703 hr^{-1} with a mean value of 2.275 ± 0.887 hr^{-1}, whereas its elimination rate constant (β) ranged from 0.271 to 0.311 hr^{-1} with a mean value of 0.288 ± 0.005 hr^{-1}. The mean absorption half life ($t_{1/2}$ K_a) and elimination half life ($t_{1/2}$ β) of the drug were observed to be 0.44 ± 0.07 and 2.41 ± 0.04 hr, respectively. The mean value of total area under curve in plasma (AUC) was found to be 11.23 ± 1.23 $mg.L^{-1}.hr$. The various values of volume of distribution such as Vd_B, and Vd_{area} were calculated to be 0.56 ± 0.11 and 0.57 ± 0.15 $L.Kg^{-1}$, respectively. The total body clearance (Cl_B) varied from 2.17 to 5.46 $ml.Kg^{-1}.min^{-1}$ with a mean of 3.19 ± 0.48 $ml.Kg^{-1}.min^{-1}$.

5. *Calculated Dosage Regimen*

Table-15 shows the calculated dosage regimen of tobramycin for i.m. route in afebrile goats. For treating mild to moderate infections $\left[C_p^{\infty} \min = 1\ \mu g/ml\right]$ at the desired dosage interval (γ) of 6 hr, the D* and D_0 were calculated to be 3.79 ± 0.56 and 3.12 ± 0.46 mg/Kg, respectively, while at γ of 8 hr D* and D_0 were calculated to be 6.76 ± 1.00 and 6.09 ± 0.91 mg/Kg. For treating severe infections $\left[C_p^{\infty} \min = 2\ \mu g/ml\right]$ at γ of 6 and 8 hr the D* and D_0 are

Table-14. ***Pharmacokinetic parameters of tobramycin in healthy goats following single i.m. dose of 2 mg/Kg.***

arameters	Unit	Goat No.						Mean ± S.E.
↓		1	2	3	4	5	6	↓
A	$\mu g.ml^{-1}$	1.82	5.22	3.83	3.87	4.40	3.89	3.77 ± 0.45
B	$\mu g. ml^{-1}$	1.82	5.35	4.05	4.04	4.61	4.04	3.98 ± 0.48
K_a	hr^{-1}	6.703	1.523	1.446	1.275	1.163	1.545	2.275 ± 0.887
$t_{1/2}ka$	hr	0.10	0.46	0.48	0.54	0.59	0.45	0.44 ± 0.07
β	hr^{-1}	0.285	0.292	0.285	0.311	0.271	0.286	0.288 ± 0.005
$t_{1/2}\beta$	hr	2.43	2.37	2.43	2.23	2.56	2.42	2.41 ± 0.04
AUC	$mg.L^{-1}.hr$	6.12	14.89	11.57	9.97	13.22	11.59	11.23 ± 1.23
AUMC	$mg.L^{-1}hr^2$	22.37	60.49	48.09	39.43	59.50	47.75	46.27 ± 5.78
MRT	hr	3.66	4.06	4.16	3.95	4.50	4.12	4.08 ± 0.11
Vd_B	$L.Kg^{-1}$	1.09	0.37	0.49	0.49	0.43	0.49	0.56 ± 0.11
Vd_{area}	$L.Kg^{-1}$	1.15	0.46	0.61	0.65	0.56	0.60	0.57 ± 0.15
Cl_B	$ml.Kg^{-1}.min^{-1}$	5.46	2.17	2.83	3.33	2.50	2.83	3.19 ± 0.48

Fig. 6

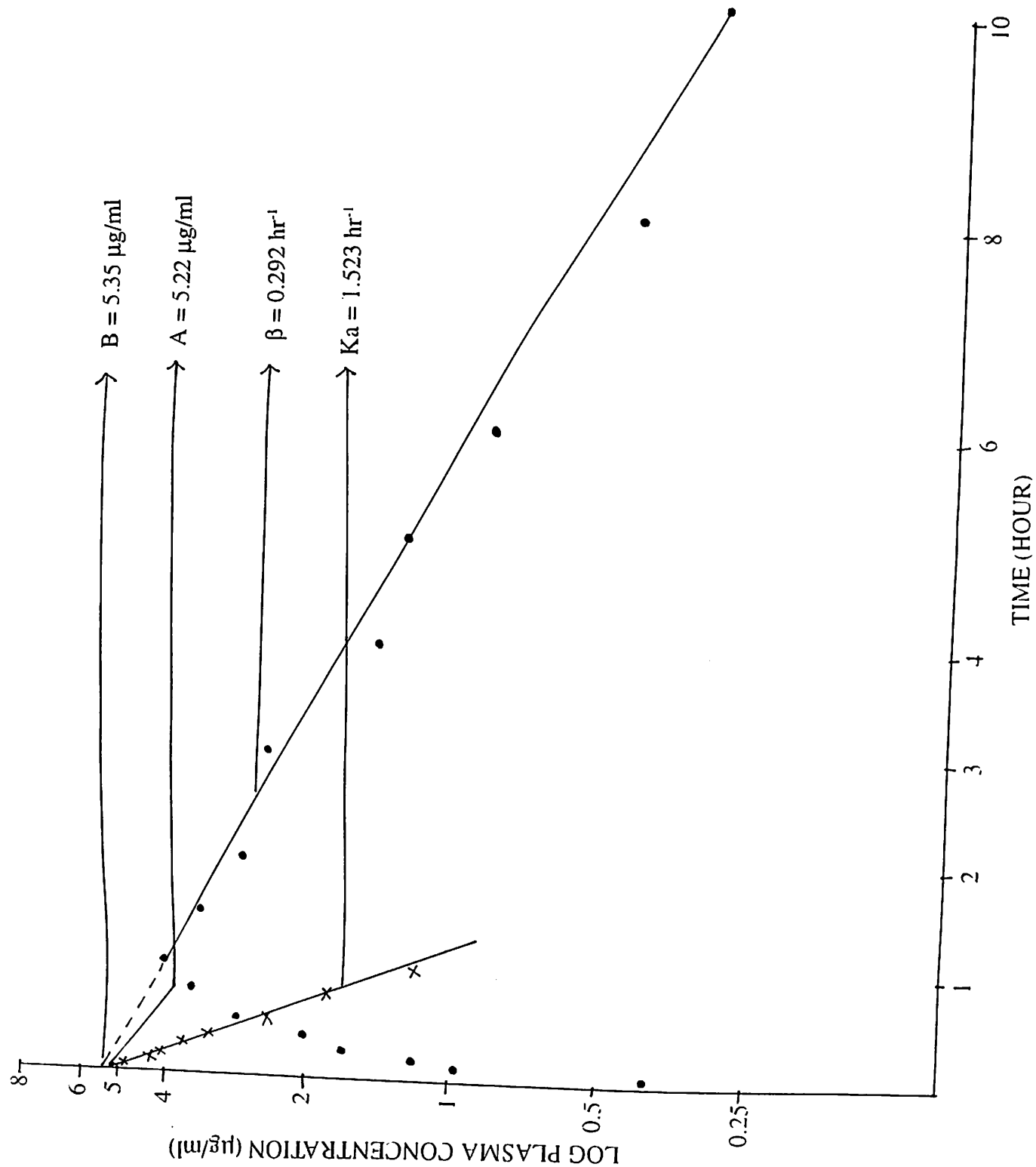

B = 5.35 µg/ml
A = 5.22 µg/ml
β = 0.292 hr⁻¹
Ka = 1.523 hr⁻¹
LOG PLASMA CONCENTRATION (µg/ml)
8
6
5
4
2
1
0.5
0.25
1
2
3
4
6
8
10
TIME (HOUR)

Table-15. ***Dosage regimen of tobramycin for i.m. route in healthy goats.***

		Goat No.						Mean ± S.E.
		1	2	3	4	5	6	↓
$C_{p\ min}$ = **1.0μg/ml**								
γ = 6hr	D*	6.36	2.65	3.37	4.20	2.85	3.34	3.79 ± 0.56
	D_0	5.21	2.19	2.76	3.55	2.28	2.74	3.12 ± 0.46
γ = 8hr	D*	11.24	4.76	5.96	7.82	4.89	5.91	6.76 ± 1.00
	D_0	10.09	4.29	5.35	7.17	4.33	5.31	6.09 ± 0.91
$C_{p\ min}$ =**2.0μg/ml**								
γ = 6hr	D*	12.72	5.30	6.74	8.40	5.69	6.67	7.58 ± 1.12
	D_0	10.42	4.38	5.53	7.10	4.57	5.47	6.24 ± 0.92
γ = 8hr	D*	22.49	9.51	11.93	15.68	9.79	11.83	13.57 ± 2.00
	D_0	20.19	8.59	10.71	14.35	8.67	10.63	12.18 ± 1.82

$C_{p\ min}$ = Minimum plasma level (μg/ml)

D* = Loading or priming dose (mg/kg)

D_0 = Maintenance dose (mg/kg)

γ = Dosage interval

calculated to be 7.58 ± 1.12 and 6.24 ± 0.92 mg/Kg and 13.52 ± 2.00 and 12.18 ± 1.82 mg/Kg.

V. Pharmacokinetic study of Tobramycin following single i.m. administration in febrile goats.

1. Plasma Levels

Table-16 and Fig-4 depict the concentrations of tobramycin in plasma at different time intervals following a single i.m. dose (2 mg/Kg) in febrile goats. The mean plasma concentration of the drug at 2.5 min was found to be 0.33 ± 0.05 μg/ml and the value ranged between 0.22 to 0.54 μg/ml. The drug reached its mean peak concentration of 3.01 ± 0.29 μg/ml at 45 min and the drug persisted up to 8 hr (0.35 ± 0.06 μg/ml) in all febrile goats, in five goats at 10 hr (0.19 ± 0.05 μg/ml), in four goats at 12 hr (0.09 ± 0.02 μg/ml) and none at 24 hr. The mean therapeutic concentration (≥2 μg/ml) of the drug in plasma was maintained from 20 min to 2 hr.

2. Milk Levels

Concentrations of tobramycin in milk samples at different time intervals were not detected in any of the goats after i.m. injection (2 mg/Kg).

3. Urine Levels :

Table 17 and Fig-5 reveal the concentrations of tobramycin in urine of febrile goats at different time intervals

Table-16. ***Plasma concentrations (μg/ml) of tobramycin in febrile goats following single i.m. dose of 2 mg/Kg.***

Time	Goat No.						Mean ± S.E.
↓	1	2	3	4	5	6	↓
2.5 min	0.42	0.54	0.22	0.26	0.25	0.30	0.33 ± 0.05
5 min	0.98	1.12	0.74	0.74	0.72	0.76	0.84 ± 0.07
10 min	1.26	1.54	0.98	0.98	1.15	0.90	1.14 ± 0.09
15 min	1.66	1.86	1.20	1.22	1.35	1.12	1.40 ± 0.12
20 min	2.64	3.06	1.68	1.60	1.76	1.56	2.05 ± 0.26
30 min	3.96	4.18	2.00	1.98	2.26	2.10	2.75 ± 0.42
45 min	1.62	3.54	2.95	3.16	3.55	3.22	3.01 ± 0.29
1 hr	1.22	2.85	3.32	2.82	2.98	2.80	2.67 ± 0.29
1.5 hr	0.95	2.50	2.94	2.60	2.82	2.48	2.38 ± 0.29
2 hr	0.56	1.54	2.65	2.12	2.60	2.38	1.98 ± 0.33
3 hr	0.38	1.30	2.10	1.84	2.05	1.80	1.58 ± 0.27
4 hr	0.32	0.86	1.28	1.02	1.40	1.22	1.01 ± 0.16
5 hr	0.25	0.48	0.96	0.90	1.28	0.98	0.81 ± 0.15
6 hr	0.20	0.30	0.68	0.52	0.80	0.65	0.53 ± 0.09
8 hr	0.16	0.18	0.46	0.38	0.52	0.40	0.35 ± 0.06
10 hr	N.D.	0.09	0.28	0.20	0.35	0.22	0.19 ± 0.05
12 hr	-	N.D.	0.14	0.10	0.16	0.12	0.09 ± 0.02
24 hr	-	-	N.D.	N.D.	N.D.	N.D.	N.D.

N.D. = Not Detectable

Table-17. ***Urine concentrations (μg/ml) of tobramycin in febrile goats following single i.m. dose of 2 mg/Kg.***

Time	Goat No.						Mean ± S.E.
↓	1	2	3	4	5	6	↓
2.5 min	1.52	1.18	1.10	1.28	0.95	1.12	1.19 ± 0.08
5 min	14.12	12.98	10.35	1.92	9.16	11.52	10.01 ± 1.77
10 min	23.95	22.85	18.55	21.05	15.15	19.85	20.23 ± 1.29
15 min	40.92	39.15	30.06	36.12	27.86	31.66	34.29 ± 2.13
20 min	59.80	52.58	47.25	51.22	39.85	50.86	50.26 ± 2.68
30 min	81.88	79.10	74.80	81.50	69.12	78.92	77.55 ± 1.98
45 min	108.8	100.0	89.85	103.3	88.58	90.84	96.89 ± 3.41
1 hr	124.4	111.2	120.0	119.2	99.8	123.2	116.3 ± 3.80
1.5 hr	184.8	141.8	159.0	169.6	121.0	172.6	158.1 ± 9.48
2 hr	101.5	165.0	99.15	99.8	139.2	101.5	117.7 ± 11.39
3 hr	83.86	91.14	81.08	85.66	86.88	83.16	85.29 ± 1.43
4 hr	44.85	49.15	41.05	41.50	44.18	43.46	44.03 ± 1.19
5 hr	27.95	31.18	25.68	28.06	28.26	29.25	28.39 ± 0.73
6 hr	10.92	12.20	9.66	10.88	10.95	10.26	10.81 ± 0.35
8 hr	8.05	9.10	7.82	9.10	7.98	9.12	8.53 ± 0.26
10 hr	3.55	4.06	2.80	3.88	3.90	3.25	3.57 ± 0.19
12 hr	1.28	1.72	0.98	1.25	1.52	1.35	1.35 ± 0.10
24 hr	0.54	0.95	0.46	0.55	0.58	0.52	0.60 ± 0.07

following single i.m. dose 2 mg/Kg. The drug appeared in urine samples of all goats at 2.5 min with a mean of 1.19 ± 0.08 μg/ml and the value ranged between 0.95 to 1.52 μg/ml. The mean peak urine concentration of 158.1 ± 9.48 μg/ml was observed at 1.5 hr. The drug was detectable upto 24 hr (0.60 ± 0.07 μg/ml) in all goats. The mean therapeutic concentration (≥ 2 μg/ml) in urine of febrile goats was maintained from 5 min to > 10 hr.

4. Kinetic Parameters

Table – 18 shows the various kinetic parameters of the drug following its single i.m. dose of 2 mg/Kg in febrile goats. The mean extrapolated zero time concentration of the drug in plasma during absorption phase (A) and elimination phase (B) were estimated to be 3.81 ± 0.35 and 3.79 ± 0.39 μg/ml, respectively. The absorption rate constant (Ka) ranged from 1.383 to 9.666 hr^{-1} with a mean value of 0.320 ± 0.022 hr^{-1} while elimination rate constant (β) varied from 0.264 to 0.405 hr^{-1} with a mean of 0.320 ± 0.022 hr^{-1}. The mean absorption half life ($t_{1/2}Ka$) and elimination half life ($t_{1/2}\beta$) of the drug were calculated to be 0.36 ± 0.08 and 2.21 ± 0.14 hr, respectively. The total area under curve in plasma (AUC) was found to be 10.21 ± 1.23 $mg.L^{-1}.hr$. The various values of volume of distribution such as Vd_B and Vd_{area} were calculated to be 0.58 ± 0.09 and 0.67 ± 0.09 L/Kg, respectively. The total body clearance (Cl_B)

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Table-18. ***Pharmacokinetic parameters of tobramycin in febrile goats following single i.m. dose of 2 mg/Kg.***

Parameters	Unit	Goat No.						Mean ± S.E.
↓		1	2	3	4	5	6	↓
A	$\mu g.ml^{-1}$	2.20	4.54	4.41	3.72	4.10	3.86	3.81 ± 0.35
B	$\mu g.\ ml^{-1}$	1.87	4.29	4.42	3.94	4.26	3.99	3.79 ± 0.39
K_a	hr^{-1}	9.666	3.489	1.465	1.383	1.521	1.444	3.161 ± 1.343
$t_{1/2}ka$	hr	0.07	0.19	0.47	0.50	0.46	0.48	0.36 ± 0.08
β	hr^{-1}	0.370	0.405	0.287	0.304	0.264	0.291	0.320 ± 0.022
$t_{1/2}\beta$	hr	1.87	1.71	2.42	2.28	2.63	2.38	2.21 ± 0.14
AUC	$mg.L^{-1}.hr$	4.83	9.30	12.38	10.25	13.43	11.06	10.21 ± 1.23
AUMC	$mg.L^{-1}hr^2$	13.64	25.81	51.56	40.63	59.31	45.34	39.38 ± 6.9
MRT	hr	2.83	2.77	4.16	3.96	4.42	4.09	3.71 ± 0.29
Vd_B	$L.Kg^{-1}$	1.07	0.47	0.45	0.51	0.47	0.50	0.58 ± 0.29
Vd_{area}	$L.Kg^{-1}$	1.12	0.53	0.56	0.64	0.56	0.62	0.67 ± 0.09
Cl_B	$ml.Kg^{-1}.min^{-1}$	6.91	3.58	2.69	3.24	2.48	3.01	3.65 ± 0.67

Table-19. ***Dosage regimen of tobramycin for i.m. route in febrile goats.***

		Goat No.						Mean ± S.E.
		1	2	3	4	5	6	↓
C_p' min **=1.0μg/ml**								
γ = 6hr	D*	10.31	6.02	3.13	3.97	1.90	3.55	4.82 ± 1.23
	D_0	9.19	5.49	2.57	3.33	1.34	2.93	4.14 ± 1.15
γ = 8hr	D*	21.61	13.53	5.56	7.28	2.86	6.36	9.54 ± 2.81
	D_0	20.49	13.00	5.00	6.64	2.30	5.74	8.86 ± 2.74
C_p' min **=2.0μg/ml**								
γ = 6hr	D*	20.62	12.04	6.27	7.99	3.81	7.11	9.64 ± 2.46
	D_0	18.38	10.98	5.15	6.65	2.69	5.87	8.28 ± 2.30
γ = 8hr	D*	43.23	27.07	11.13	14.57	5.73	12.72	19.08 ± 5.62
	D_0	40.99	26.00	10.01	13.29	4.61	11.48	17.72 ± 5.48

C_p' min = Minimum inhibitory concentration [MIC]

D* = Loading dose

D_0 = Maintenance dose

γ = Dosage interval

varied from 2.48 to 6.91 $ml.Kg^{-1}.min^{-1}$ with its mean value of 3.65 ± 0.67 $ml.Kg^{-1}.min^{-1}$.

5. *Calculated Dosage Regimen*

Table-19 describes the calculated dosage regimen of tobramycin for i.m. route in febrile goats. For treating mild to moderate infections $\left[C_p^{\infty} \min = 1\ \mu g/ml\right]$ at the desired dosage interval (γ) of 6 and 8 hr, the loading dose (D*) and maintenance dose (D_0) were calculated to be 4.82 ± 1.23 and 4.14 ± 1.15 and 9.54 ± 2.81 and 8.86 ± 2.74 mg/Kg. For treating severe infections $\left[C_p^{\infty} \min = 2\ \mu g/ml\right]$ at γ of 6 and 8 hr the D* and D_0 were calculated to be 9.64 ± 2.46 and 8.28 ± 2.30 and 19.08 ± 5.62 and 17.72 ± 5.48 mg/Kg, respectively.

VI. Comparison of Pharmacokinetics of Tobramycin between healthy and febrile goats following i.m. administration.

1. *Plasma Levels*

Comparative plasma concentrations of tobramycin between healthy and febrile goats following single i.m. dose of 2 mg/Kg have been shown in Table-20 and Fig. 4. The drug was detectable upto 12 hr in both healthy and febrile goats with mean concentration 0.09 ± 0.03 and 0.09 ± 0.02 μg/ml, respectively. The plasma drug concentrations were observed to be slightly higher upto 30 min and slightly lower from 45 min to 10 hr in febrile goats as

Table-20. ***Comparison of concentrations (μg/ml) of tobramycin in various biological fluids between healthy and febrile goats following single i.m. dose of 2 mg/Kg.***

Time	Healthy Goats (n = 6)		Febrile Goats (n=6)	
↓	Plasma	Urine	Plasma	Urine
2.5 min	0.29 ± 0.03	1.18 ± 0.09	0.33 ± 0.05	1.19 ± 0.08
5 min	0.78 ± 0.05	11.69 ± 0.81	0.84 ± 0.07	10.01 ± 1.77
10 min	1.06 ± 0.04	17.82 ± 3.37	1.14 ± 0.09	20.23 ± 1.29
15 min	1.35 ± 0.08	34.44 ± 2.16	1.40 ± 0.12	34.29 ± 2.13
20 min	1.75 ± 0.07	50.41 ± 2.71	2.05 ± 0.26	50.26 ± 2.68
30 min	2.26 ± 0.14	77.55 ± 1.73	2.75 ± 0.42	77.55 ± 1.98
45 min	3.26 ± 0.17	96.99 ± 3.58	3.01 ± 0.29	96.89 ± 3.41
1 hr	2.98 ± 0.37	116.4 ± 3.83	2.67 ± 0.29	116.3 ± 3.80
1.5 hr	2.61 ± 0.32	157.4 ± 9.58	2.38 ± 0.29	158.1 ± 9.48
2 hr	2.19 ± 0.28	117.4 ± 11.27	1.98 ± 0.33	117.7± 11.39
3 hr	1.82 ± 0.28	85.26 ± 1.52	1.58 ± 0.27	85.29 ± 1.43
4 hr	1.13 ± 0.17	43.86 ± 1.15	1.01 ± 0.16	44.03 ± 1.19
5 hr	0.98 ± 0.15	28.09 ± 0.64	0.81 ± 0.15	28.39 ± 0.73
6 hr	0.63 ± 0.17	10.83 ± 0.34	0.53 ± 0.09	10.81 ± 0.35
8 hr	0.39 ± 0.04	8.36 ± 0.24	0.35 ± 0.06	8.53 ± 0.26
10 hr	0.25 ± 0.02	3.54 ± 0.18	0.19 ± 0.05	3.57 ± 0.19
12 hr	0.09 ± 0.03	1.31 ± 0.09	0.09 ± 0.02	1.35 ± 0.10
24 hr	N.D.	0.59 ± 0.07	N.D.	0.60 ± 0.07

N.D. = Not Detectable

All data are non-significant between healthy Vs febrile goats.

compared to healthy goats but the data were non-significant. At 12 hr in both healthy and febrile goats the mean plasma drug concentration was similar. The mean therapeutic concentration (≥ 2 μg/ml) was maintained from 20 min to around 3 hr in healthy and 20 min to around 2 hr in febrile goats.

2. ***Milk Levels***

Tobramycin was not at all detected in any of the milk samples collected at different time intervals after i.m. injection at the dose rate of 2 mg/Kg in both healthy and febrile goats.

3. ***Urine Levels***

Table-20 and Fig.-5 reveal the comparative urine concentrations of tobramycin between healthy and febrile goats following its single i.m. dose of 2 mg/Kg. The drug was detectable upto 24 hr in both healthy and febrile goats. The drug concentrations in urine were noted to differ non-significantly between healthy and febrile animals at all time intervals. The mean therapeutic concentration (≥ 2 μg/ml) of the drug was maintained from 5 min to 10 hr in both healthy and febrile goats.

4. ***Kinetic parameters***

Table-21 shows the comparative kinetic parameters of tobramycin between healthy and febrile goats following its single i.m. dose of 2 mg/Kg. Non significant difference in all kinetic parameters were noted between healthy and febrile goats.

Table-21. ***Comparison of pharmacokinetic parameters of tobramycin between healthy and febrile goats following single i.m. dose of 2 mg/Kg.***

Parameter	Unit	Healthy Goats (n = 6)	Febrile Goats (n = 6)
A	$\mu g.ml^{-1}$	3.77 ± 0.45	3.81 ± 0.35
B	$\mu g. ml^{-1}$	3.98 ± 0.48	3.79 ± 0.39
K_u	hr^{-1}	2.275 ± 0.887	3.161 ± 1.343
$t_{1/2}Ka$	hr	0.44 ± 0.07	0.36 ± 0.08
β	hr^{-1}	0.288 ± 0.005	0.320 ± 0.022
$t_{1/2}\beta$	hr	2.41 ± 0.04	2.21 ± 0.14
AUC	$mg.L^{-1}.hr$	11.23 ± 1.23	10.21 ± 1.23
AUMC	$mg.L^{-1}hr^2$	46.27 ± 5,78	39.38 ± 6.9
MRT	hr	4.08 ± 0.11	3.71 ± 0.29
Vd_B	$L.Kg^{-1}$	0.56 ± 0.11	0.58 ± 0.09
Vd_{area}	$L.Kg^{-1}$	0.57 ± 0.15	0.67 ± 0.09
Cl_B	$ml.Kg^{-1}.min^{-1}$	3.19 ± 0.48	3.65 ± 0.67

All data are non – significant between healthy vs febrile goats.

Table-22. ***Comparison of dosage regimen of tobramycin for i.m. route between healthy and febrile goats.***

		Unit	Healthy Goats (n =6)	Febrile Goats (n =6)
C_p' min **=1.0μg/ml**				
γ = 6hr	D*	$mg.kg^{-1}$	3.79 ± 0.56	4.82 ± 1.23
	D_0	$mg.kg^{-1}$	3.12 ± 0.46	4.14 ± 1.15
γ = 8hr	D*	$mg.kg^{-1}$	6.76 ± 1.00	9.54 ± 2.81
	D_0	$mg.kg^{-1}$	6.09 ± 0.91	8.86 ± 2.74
C_p' min = 2.0μg/ml				
γ = 6hr	D*	$mg.kg^{-1}$	7.58 ± 1.12	9.64 ± 2.46
	D_0	$mg.kg^{-1}$	6.24 ± 0.92	8.28 ± 2.3
γ = 8hr	D*	$mg.kg^{-1}$	13.52 ± 2.00	19.08 ± 5.62
	D_0	$mg.kg^{-1}$	12.18 ± 1.82	17.72 ± 5.48

C_p' min = Minimum Inhibitory Concentration [MIC]

D* = Loading dose

D_0 = Maintenance dose

γ = Dosage interval

All data are non-significant between healthy Vs febrile goats.

5. *Calculated dosage regimen :*

Table-22 shows the comparison of calculated dosage regimen of tobramycin between afebrile and febrile goats for i.m. route in order to maintain C_p^{∞} min [MIC] of 1 and 2 μg/ml at the selected dosage interval (γ) of 6 and 8 hr. The loading (D*) and maintenance (D_0) doses were calculated to be non-significantly higher in febrile goats as compared to healthy goats at both the MIC levels and at both the dosage intervals.

□□□□□


Discussion

DISCUSSION

Tobramycin, one of the latest aminoglycosides, is particularly effective against gram negative bacteria. It has less cross-resistance and less serious toxicity to cochlear and vestibular part of auditory nerve and renal tubular damage. Available literature is devoid of any work in goats, particularly in febrile condition. The fundamental appraisal of the therapeutic value of an antibiotic should be based not on the number of "clinical cures", but also the correlation between the antibacterial activity and the concentrations achieved in vivo as failure of drug to respond might erroneously be ascribed to "the ineffective antibiotic" even though the bacterial process is under control or would have been controlled had the drug been given in an adequate dose. Among various factors which determine the efficacy of antibiotics, dosage and route of administration are of much importance. In the present study, an attempt has been made to calculate various kinetic parameters of tobramycin in healthy and febrile goats, which may serve as a guideline to clinicians for effective dose of this drug. Further, based on kinetic data, an attempt has been made to calculate suitable dosage regimen based on maintenance of therapeutic concentration (MIC) at convenient dosage interval for both i.v. and i.m. routes for combating bacterial infections in afebrile and febrile condition.

PHARMACOKINETIC STUDY OF TOBRAMYCIN

A. *Distribution in biological fluids*

Concentrations of tobramycin in plasma after i.v. administration (2 mg/kg) were observed to be slightly lower in febrile goats throughout from 2.5 min to 10 hr, but they were statistically insignificant (Table – 9). Similarly, the mean plasma concentrations of tobramycin at different time interval after i.m. administration (2 mg/kg) were also differed non-significantly only (Table – 20). In contrast to the observations noted in the present study, Agrawal (2000) noted significant increase in concentrations of amikacin (an aminoglycoside) from 15 min to 1 hr and 5 hr to 24 hr in febrile goats as compared to healthy goats after i.v. administration. Similar trend was also noted by Agrawal (2000) after i.m. administration of amikacin. The mean therapeutic concentration (≥ 2 μg/ml) in plasma was maintained from 2.5 min to around 45 min in both healthy and febrile goats after i.v. administration. In case of i.m. administration, the mean therapeutic concentration was maintained for a longer period in healthy goats (20 min to 3 hr) as compared to febrile goats (20 min to 2 hr). Agrawal (2000) noted higher duration of maintenance of mean therapeutic concentration of amikacin (2 μg/ml) from 2.5 min to 5 hr in healthy goats and 2.5 min to > 6 hr in febrile goats after i.v. administration and 2.5 min to 6 hr in health and 2.5 min to 10 hr in febrile goats after i.m. administration of amikacin (10 mg/kg).

In lactating animals, milk is an important route of drug excretion. Many available literatures indicate that drugs are excreted via milk by passive diffusion across the mammary gland membranes. The mechanism of drug diffusion across mammary gland membrane is mostly pH – pKa dependent (Miller *et al.*, 1967; Banerjee *et al.*, 1967; Roy and Banerjee; 1983). All aminoglycosides are polycations and hence crosses cell membrane with difficulty.

In the present study, tobramycin was not at all detectable in any of the milk samples collected in both healthy and febrile goats following its i.v. and i.m. administrations. This is due to the chemical nature of the drug. Agrawal (2000) noted very low concentrations of amikacin in milk of goats following i.v. and i.m. administration though significantly higher drug concentrations in milk were obtained under febrile condition. The above author reported that amikacin never attained its therapeutic concentration in milk in both healthy and febrile condition to goats. Similarly, Jayachandran *et al.* (1987) reporter very low concentrations of streptomycin (an aminoglycoside) in milk that too for a short period (3 to 8 hr) following its i.m. administration. The above noted observation is due to polycation nature of aminoglycosides.

Drugs which are water soluble having low molecular weight and slowly biotransformed by the liver are eliminated by renal excretion. The process of drug excretion via the kidneys may include

any combination of glomerular filtration, active tubular secretion and tubular renal absorption. Tobramycin like other aminoglycosides is expected to be excreted mainly via kidney since it is water soluble and polar compound.

Concentrations of tobramycin in urine were found to differ only non-significantly between healthy and febrile goats after i.v. (Table 9) and i.m. (Table 20) administration when given @2 mg/Kg. The mean therapeutic concentration (> 2 μg/ml) of tobramycin in urine was maintained from 2.5 min to > 10 hr in both healthy and febrile goat. The peak urine concentrations were noted at 45 min in both healthy and febrile goats after i.v. administration. Similarly, concentrations of urine collected at different time intervals did not differ significantly between healthy and febrile animals (Table –20) after i.m. administration. The mean therapeutic concentration (> 2 μg/ml) of the drug was maintained from 5 min to 10 hr in both healthy and febrile goats. The peak urine concentration was noted at 1.5 hr in both healthy and febrile goats.

B. Kinetic Parameters

In mammals, tissue damage, inflammation or invasion of pathogenic micro-organisms induces systemic changes, collectively known as acute phase response (Miert, 1995). Among the changes which together produce this response are fever, increase lassitude, inappetence, inhibition of gastric function, tachycardia, activation of

lymphocytes and changes in metabolism of carbohydrate, lipid and proteins (Alsemgeest, 1994; Groothius *et al.* 1981). Another very important aspect of acute phase response (APR) to infections concerns its effects on drug disposition in diseased states. Among the varied changes which together characterize APR is down regulation of cytochrome P450 associated with drug metabolism and changes in blood flow to various organs such as liver and the kidney (Blatties *et al.*,1988; Mackowiask 1989; Miert 1990).

Experiments in goats have demonstrated that APR in these animals may alter the bioavailability, disposition and metabolite formation of various drugs compared with their pharmacokinetic properties in healthy goats (Knoppert *et al.*, 1988; Lohuis *et al.*,1992). Keeping the above facts in mind, the present experiment was designed to identify the kinetic alternations of tobramycin if any, in goats under febrile condition induced by endotoxin of *E. coli.*

Table – 10 and Table – 21 describe the different kinetic parameters after i.v. and i.m. administration, respectively in healthy and febrile goats.

Tobramycin is absorbed at a similar faster rate under healthy and febrile condition as noted by absorption rate constant (Ka) of 2.275 ± 0.887 hr^{-1} and 3.161 ± 1.343 hr^{-1} and absorption half life ($t_{½}$ ka) of 0.44 ± 0.07 hr (26.4 min) and 0.36 ± 0.08hr (21.6 min) under healthy and febrile condition, respectively. This denotes that

fever may not play any significant role in the alteration of rate of absorption of tobramycine in goats. A faster $t_{1/2}$ Ka of 3.9 ± 0.9 min in camel was noted by Hadi *et al.* (1994). This denotes that the drug is comparatively absorbed slowly in goats as compared to camel.

Tobramycin is distributed at a faster rate in the present study as shown by high distribution rate constant (α) of 7.009 ± 1.651 hr^{-1} and 5.987 ± 0.95 hr^{-1} and a low similar mean distribution half life ($t_{1/2}\alpha$) of 0.13 hr in healthy and febrile goats. (Table- 10) Chung *et al.* (1982) reported similar faster distribution of tobramycin in guinea pig as shown by $t_{1/2}\alpha$ of 0.09 to 0.16 hr.

In the present study, elimination half life ($t_{1/2}$ β) of 1.69 ±0.22 hr and 1.65 ± 0.11 hr after i.v. and 2.41 ± 0.04 hr and 2.21 ± 0.14 hr after i.m. administration in healthy and febrile goats, respectively, were obtained. The above findings reveal that the drug is eliminated at a similar faster rate under healthy and febrile goats. More or less similar $t_{1/2}\beta$ of 1.8 ± 0.3 hr in sheep (Moller *et al.*, 1992), 1.97 hr in rabbit (Bugnon *et al.*, 1988), 110.6 ± 32.3 min (1.83 hr) in cat (Jernigan *et al.*, 1988), and 2 hr (Israel *et al.*, 1976) and 82 min (1.37 hr) in man (Pechere *et al.*, 1976) were noted. However, higher $t_{1/2}\beta$ of 189 ± 21 min (3.15 hr) after i.v. and 201 ± 40 min (3.35 hr) after i.m. administration were noted in camel (Hadi *et al.*, 1994). On the other hand, a low $t_{1/2}\beta$ of 0.88 to 1.01 hr was noted in guinea pig after i.v. administration (Chung *et al.*, 1982). The above findings show

that the drug is expected to be eliminated at a similar rate in sheep, cat, rabbit, man and in goats while it is eliminated slowly in camel and eliminated at a faster rate in guinea pig.

Various values of volume-distribution of tobramycin in healthy and febrile goats are shown in Table – 10 and Table – 21. Non-significant differences were noted among healthy and febrile goats. Notari (1980) stated that for a 2-compartment open model the value of $Vd_{\beta} > Vd_{area} > Vd_{s.s.}$ and Vd. He further mentioned that among these values of volume distribution, only Vd_{area} correctly predicts the amount of drug in the body during elimination phase whereas Vd_{β} over estimates and $Vd_{s.s.}$ and Vd under estimate the amount of drug in the body. In the present study Vd_{area} of 0.82 ±0.08 and 0.99 ± 0.17 $L.Kg^{-1}$ were obtained after i.v. administration and 0.57 ± 0.15 and 0.67 ± 0.09 $L.Kg^{-1}$ after i.m. administration in healthy and febrile goats, respectively. Very low Vd of 0.3 ± 0.1 $L.Kg^{-1}$ in sheep (Moller *et al.*, 1992), Vd_{area} of 245 ± 21 ml. Kg^{-1} (0.245 L. Kg^{-1}) in camel (Hadi *et al.*, 1994) and $Vd_{s.s.}$ of 0.18 ± 0.003 $L.Kg^{-1}$ in cat (Jernigan *et al.*, 1988) were obtained. The values of Vd_{area} in these animals reveal that the drug is expected to be distributed to a lesser extent than in goat. However, more or less similar volume distribution of 0.74 to 0.94 $L.Kg^{-1}$ was noted in man (Nahata *et al.*, 1986) which denotes that the extent of distribution of tobramycin is similar in man and goat. The better distribution of tobramycin in goats is supported by a higher tissue to plasma concentration ratio

($T \approx P$) of 2.72 ± 0.81 and 2.32 ± 0.53 noted in healthy and febrile goats, respectively.

The kinetic parameter, total body clearance (Cl_B), denotes the sum of the clearance of each eliminating organ, particularly liver and kidney. In the present study, Cl_B of 6.08 ± 1.21 and 6.86 ± 1.23 $ml.kg^{-1} min^{-1}$ after i.v. administration and 3.19 ± 0.48 and 3.65 ± 0.67 ml. Kg^{-1} . min^{-1} after i.m. administration were observed in healthy and febrile goats, respectively. However, lower Cl_B of 1.8 ± 0.8 $ml.kg^{-1}. min^{-1}$ in sheep (Möller *et al.*, 1992), 0.90 ± 0.10 ml. $Kg^{-1}. min^{-1}$ in camel (Hadi *et al.*, 1994), 1.69 ± 0.36 ml. min^{-1} in cat (Jernigan *et al.*, 1988), 0.204 ml. $Kg^{-1}. min^{-1}$ in guinea pig (Chung *et al.*, 1982) and 0.74 to 1.19 ml. $Kg^{-1}. min^{-1}$ in man (Nahata *et al.*, 1986) were noted. The above findings as compared to the present study indicate that tobramycin is expected to be removed from the body of goats to a greater extent than that of other species.

Various other kinetic parameters such as AUC, AUMC, MRT, Various micro rate constants (K_{12}, K_{21}, Kel) etc. did not differ significantly between healthy and febrile goats.

C. Dosage Regimen

The success of drug therapy is highly dependent on the design of effective dosage regimen and also route of administration. A properly calculated dosage regimen tries to achieve an optimum concentration of the drug to produce an effective therapeutic response

with minimum adverse side effects. Individual variations in pharmacokinetics make the design of dosage regimen difficult. Therefore, application of pharmacokinetics to calculate dosage regimen must be coordinated with proper clinical evaluation and monitoring.

Minimum inhibitory concentration 90% (MIC 90%) of many bacteria varied from 0.25 to 4 μg/ml (Weidmann and Atkinson, 1991). In the present study, the dosage regimen of tobramycin was calculated based on maintenance of therapeutic concentration $C_p^{\infty}\,min$ Of 1.0 and 2.0 μg/ml. Table – 11 depicts the calculated dosage regimen of tobramycin for i.v. route in afebrile and febrile goats. For treating mild to moderate infection ($C_p^{\infty}\,min = 1.0\ \mu g/ml$) at the convenient dosage interval (γ) of 6 hr, the average loading (D^*) and maintenance (D_0) doses are calculated to be 16.3 and 15.5 mg/kg in afebrile and 13.8 and 12-8 mg/Kg in febrile goats, respectively. Table – 22 depicts the calculated dosage regimen of drug for i.m. route in afebrile and febrile goats. For treating mild to moderate infections a longer dosage interval of 8 hr may be preferred where D^* and D_0 are calculated to be 6.8 and 6 mg/Kg in afebrile goats and 9.5 and 8.9 mg/Kg in febrile goats, respectively. However, for treating severe infections, the D^* and Do are calculated to be 7.6 and 6.2 mg/Kg in afebrile goats and 9.5 and 8.9 mg/Kg in febrile goats at γ of 8 hr. The present study thus clearly showed that tobramycin can preferable be effectively used by

i.m. route where doses are comparatively lower than that of i.v. route. An over all view of the results obtained in this study suggest that tobramycin may be employed effectively for treating various systemic infections in goats preferably by i.m. route at the dosage recommended above. Since the drug maintained its therapeutic concentration (2 μg/ml) > 10 hr in urine of both afebrile and febrile goats after its i.v. and i.m. administration (2 mg/kg), the drug can be effectively used in treating urinary tract infection at the dose rate of 2 mg/Kg every 12 hourly. The drug can not be used in mastitis by systemic route, since the drug is not at all detected in milk at any of the time interval collected after its parental administration both in healthy and febrile condition. Since the drug is not excreted via milk, milk of the treated animals can be consumed safely for human consumption.


Summary

SUMMARY

A detailed pharmacokinetic study of tobramycin was under taken in healthy and febrile goats weighing between 20 to 30 Kg. Kinetic parameters were calculated in healthy and febrile goats based on log plasma drug concentration versus time profile. On the basis of kinetic parameters obtained from the detailed pharmacokinetic study, an appropriate dosage regimen of tobramycin was calculated by taking into account the minimum therapeutic concentration required to inhibit/kill the susceptible bacteria.

1. Following a single i.v. dose of tobramycin (2 mg/kg), the concentrations of the drug in plasma were found to be lower through out from 2.5 min to 10 hr in febrile goats as compared to healthy goats but the data were non-significant. The mean therapeutic concentration in plasma was maintained from 2.5 to around 45 min in both healthy and febrile goats. The drug was not at all detectable in any of the milk samples collected in both healthy and febrile goats. Concentrations of tobramycin in urine were observed to be almost similar in both healthy and febrile goats at all time intervals. Therapeutic concentration of the drug in urine was maintained from 2.5 min to > 10 hr in both healthy and febrile goats.

2. On i.m. administration of the same dose of tobramycin, the plasma drug concentrations were found to slightly higher up to 30 min and slightly lower from 45 min to 10 hr in febrile goats as compared to healthy goats but the data were non-significant. At 12 hr in both healthy and febrile goats the concentration of plasma was similar. The mean therapeutic concentration (≥ 2 μg/ml) was maintained from 20 min to around 3 hr in healthy and 20 min to around 2 hr in febrile goats. Tobramycin was not at all detected in any of the milk samples collected at different time-intervals after i.m. injection (2 mg/kg) in both healthy and febrile goats. However, the drug concentrations in urine were noted to differ only non-significantly between healthy and febrile animals at all time intervals. The mean therapeutic concentration (≥ 2 μg/ml) of the drug was maintained from 5 min to 10 hr in both healthy and febrile goats.

3. The absorption half life ($t_{1/2}$ ka) of 0.44 ± 0.07 hr noted in healthy goats was non-significantly higher as compared to $t_{1/2}$ka of febrile goats (0.36 ± 0.08 hr). The values of distribution half-life ($t_{1/2}\alpha$) were similar in both healthy and febrile goats (0.13 ± 0.02 hr) after i.v. administration of tobramycin. The above noted observation show that the rate of distribution of drug is similar in febrile and healthy goats. The mean elimination half life ($t_{1/2}\beta$) of 1.69 ± 0.22 and 1.65 ± 0.11 hr were obtained after i.v. administration and 2.41 ± 0.04 and 2.21 ± 0.14 hr after i.m.

administration in healthy and febrile goats, respectively. The above findings indicate that the drug is eliminated at a similar rate in febrile goats and healthy goats. Though the total body clearance (Cl_B) is slightly higher in febrile condition (i.v. = 6.86±1.23 ml.Kg^{-1}.min^{-1} and i.m.=3.65 ± 0.67 ml.Kg^{-1}.min^{-1}) as compared to healthy goats (i.v.=6.08±1.21ml.Kg^{-1}.min^{-1} and i.m.=3.19 ±0.48 ml. Kg^{-1}. min^{-1}), the data were insignificant.

4. Rate constant of drug transfer from central to peripheral compartment (K_{12}) was noted to be non-significantly higher in healthy goats (3.423 ± 0.857 hr^{-1}) as compare to febrile goats (3.097 ± 0.641 hr^{-1}). Similarly the rate constant of drug transfer from peripheral to central compartment (K_{21}) differed non-significantly between both healthy and febrile goats after i.v. administration. This has led to non-significantly higher approximate tissue to plasma concentration ratio (T≈P) in healthy goats (2.72 ± 0.81) as compared to febrile goats (2.32 ±0.53). Slightly higher value for rate of elimination of drug from central compartment (Kel) was observed in healthy goats. Fraction of drug available for elimination from central compartment (Fc) was found to be similar in both healthy (0.33 ± 0.06) and febrile goats (0.33 ± 0.05). The area under curve (AUC) was observed in healthy goats (i.v.=6.18±0.77 mg L^{-1}.hr and i.m.=11.23 ± 1.23 mg.L^{-1} hr) non-significantly higher

as compared to febrile goats (i.v.=5.50±0.85 mg.L^{-1} hr and i.m. = 10.21 ± 1.23 mg. L^{-1}.hr)

5. All the calculated values of volume of distribution i.e. (Vd_c,Vd_B, Vd_{area}Vd.s.s) after i.v. administration were found to be insignificantly higher in febrile goats as compared to healthy goats. Similar trend was found after i.m. administration. Vd_{area} of 0.82 ± 0.08 and 0.99 ± 0.17 L.kg^{-1} after i.v. and Vd_{area} of 0.57 ± 0.15 and 0.67 ± 0.09 L.kg^{-1} after i.m. administration were noted in healthy and febrile goats, respectively. These values denote that tobramycin is distributed at a moderate extent in the body of goats.

6. In the present study, loading (D*) and maintenance (Do) doses were calculated to maintain C_p' min (MIC) of 1.0 and 2.0 μg/ml in plasma at desired dosage interval (γ) of 6 and 8 hr. For treating mild to moderate infection(C_p' min =1.0 μg/ml) at γ or 6 hr, D* of 16 mg/Kg and Do of 15 mg/Kg in healthy while D* of 14 mg/Kg and Do of 13 mg/kg in febrile goats may be used for i.v. route. For treating severe infections (C_p' min =2 μg/ml) The D* and Do were calculated to be 33 and 31 mg/kg at γ of 6 hr in afebrile while D* and Do of 27 and 25 mg/kg at γ of 6 hr may be required for treating febrile goats.

For treating mild to moderate infection (C_p' min = 1.0μg / ml) at γ or 8 hr average D* of 7 mg/kg and D_0 of 6 mg/kg in afebrile

while average D* of 10 mg/kg and D_0 of 9 mg/kg at 8 hr (γ) in febrile goats may be used for i.m. route. For treating severe infection (C_p^c min = 2 μg/ml) the average D* and D_0 were calculated to be 14 mg/kg and 12 mg/kg at γ of 8 hr in afebrile while average D* and D_0 of 19 mg/Kg and 18 mg/Kg at γ of 8 hr may be required for treating febrile goats.

7. For the treatment of mastitis, tobramycin may not be used systemically because this drug was not detectable in milk samples after i.v. and i.m. administration of drug in both healthy and febrile condition. However, the drug can be used by intramammary route for treating mastitis caused by susceptible micro-organisms. The early appearance of tobramycin in urine in therapeutic concentration and maintenance of mean therapeutic concentration (> 10 hr after i.v. and i.m.) in both afebrile and febrile goats when given @ 2 mg/kg indicate that tobramycin may be used in the treatment of various bacterial infections of urinary tract at the above dose rate, twice daily.

□□□□□

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Appendix

APPENDIX - I

Calculation of kinetic parameters after i.m. administration of drug :

Kinetic parameters were calculated from the plasma log drug concentration versus time profile. An example is noted below from the data of goat no. - 2 obtained after i.m. injection of tobramycin (2 mg/Kg) in healthy goats. The data showed a monophasic curve and hence, well fitted into one-compartment open model. Elimination phase starts from the time of peak concentration of drug onwards.

No. of observation (n)	Time in hour (X)	X^2	Plasma conc. (Y) (μg/ml)	log (Yx10)	XY
1	1	1	4.12	1.6149	1.6149
2	1.5	2.25	3.45	1.5378	2.3067
3	2	4.0	2.82	1.4502	2.9004
4	3	9.0	2.52	1.4014	4.2042
5	4	16.0	1.50	1.1761	4.7044
6	5	25.0	1.34	1.1271	5.6355
7	6	36.0	0.88	0.9444	5.6664
8	8	64.0	0.46	0.6627	5.3016
9	10	100.0	0.32	0.5051	5.0510
10	12	144.0	0.16	0.2041	2.4492

$\Sigma n = 10$, $\Sigma x = 52.5$, $\Sigma x^2 = 401.25$, $\Sigma y = 10.6238$, $\Sigma xy = 39.8343$,

$\bar{x} = 5.25$ $(\Sigma x)^2 = 2756.25$ $\bar{y} = 1.0624$

$$\text{b, slope of line} = \frac{n.\Sigma xy - \Sigma x.\Sigma y}{n.\Sigma x^2 - (\Sigma x)^2}$$

$$= \frac{10 \times 39.8343 - 52.5 \times 10.6238}{10 \times 401..25 - 2756.25}$$

$$= \frac{398.343 - 557.7495}{4012.5 - 2756.25}$$

$$= \frac{-159.4065}{1256.25}$$

$$= -0.1269$$

β, elimination rate constant = b x -2.303

$$= -0.1269 \times -2.303$$

$$= 0.292\ hr^{-1}$$

B, zero time concentration during elimination phase can be obtained from the formula $\bar{y} = a + b\bar{x}$

Therefore, $a = \bar{y} - b.\bar{x}$

$$= \log 1.0624 - (-0.1269 \times 5.25)$$

$$= \log 1.7286$$

zero time concentration = antilog of 1.7286

$$= 53.530 \approx 53.53\ \mu g/ml$$

Since plasma concentration is multiplied earlier by 10 in the above mentioned calculation, the value of 53.53 μg/ml should be divided by 10 to get the actual żero time concentration. Hence, zero time concentration (B) = 5.353 or 5.35 μg/ml.

Similarly the theoretical plasma concentration (Y) can be calculated by putting the value of the time (X) in the above equation during the time intervals of absorption phase (y = a + b.x).

Subtracting the theoretical values from observed values, a series of residual concentrations were obtained and slope of line in natural log [Absorption constant, Ka] and the zero time intercept (zero time concentration during absorption phase, A) can be calculated as per the method adopted for calculation of B and β. The value are $K_a = 1.523\ hr^{-1}$

A = 5.219 μg/ml

= 5.22 μg/ml

$t_{1/2}$ ka, Absorption half-life

$$t_{1/2}ka = \frac{0.693}{ka} = \frac{0.693}{1.523} = 0.46\ hr$$

$t_{1/2}$ β, Elimination half-life

$$t_{1/2}\beta = \frac{0.693}{\beta} = \frac{0.693}{0.292} = 2.37\ hr$$

AUC, Area under curve

$$AUC = \frac{B}{\beta} - \frac{A}{ka} = \frac{5.35}{0.292} - \frac{5.219}{1.523}$$

$$= 18.32 - 3.43 = 14.89 \text{ mg/lit.hr}$$

Vd_B, the volume of distribution based on elimination

$$Vd_{\beta} = \frac{D}{B} = \frac{2}{5.35} = 0.37 \text{ L/Kg}$$

Vd_{area}, the volume of distribution based on total area under curve

$$Vd_{area} = \frac{D}{(AUC).\beta} = \frac{2}{14.89 \times 0.292} = 0.46 \text{ L/Kg}$$

Cl_B, total body clearance

$$Cl_B = Vd_{area} \times \beta$$

$$= 0.46 \times 0.292$$

$$= 0.13 \text{ L/Kg/hr}$$

$$= 2.17 \text{ ml/Kg/min}$$

APPENDIX - II

Calculation of kinetic parameters after i.v. administration of drug :

Kinetic parameters were calculated from the plasma log drug concentration versus time profile. An example is noted below from the data of goat no. - 2 obtained after i.v. injection of tobramycin (2 mg/Kg) in healthy goats. The data showed a biphasic curve and hence, well fitted into a two-compartment open model. Elimination phase starts 3 hr. onwards.

No. of observation (n)	Time in hour (X)	X^2	Plasma conc. (Y) (μg/ml)	log (Yx10)	XY
1	3	9	0.629	0.7986	2.3958
2	4	16	0.464	0.6665	2.666
3	5	25	0.342	0.5340	2.67
4	6	36	0.252	0.4014	2.4084
5	8	64	0.14	0.1461	1.1688
6	10	100	0.10	00	00
$\Sigma n=6$ $x = 6$ $(\Sigma x)^2=1296$	$\Sigma x=36$	$\Sigma x^2=250$		$\Sigma y=2.5466$ $y = 0.4244$	$\Sigma xy=11.309$

$$\text{b, slope of line} = \frac{n.\Sigma xy - \Sigma x.\Sigma y}{n.\Sigma x^2 - (\Sigma x)^2}$$

where, X = time (hr), Y = drug concentration, n = no. of observation

$$= \frac{6 \times 11.309 - 36 \times 2.5466}{6 \times 250 - 1296}$$

$$= \frac{67.854 - 91.6776}{1500 - 1296}$$

$$= \frac{-23.8236}{204} = -0.1168 \text{ hr}^{-1}$$

β, elimination rate constant = b x -2.303

$$= 0.269 \text{ hr}^{-1}$$

B, zero time concentration during elimination phase can be obtained from the formula $\bar{y} = a + b\bar{x}$

Where, $\bar{y}$ = mean drug concentration

$\bar{x}$ = mean time

b = slope of line

a = zero time concentration

Therefore, $a = \bar{y} - b.\bar{x}$

$$= \log 0.4244 - (-0.1168 \times 6)$$

$$= \log 0.4244 + 0.7008$$

$$= \log 1.1252$$

zero time concentration = antilog of 1.1252

= 13.34 μg/ml

Since plasma concentration is multiplied earlier by 10 in the above mentioned calculation, the value of 13.34 μg/ml should be divided by 10 to get the actual zero time concentration. Hence, zero time concentration (B) = 1.334 μg/ml or 1.33 μg/ml.

Similarly the theoretical plasma concentration (y) can be calculated by putting the value of the time (x) in the above equation during the time intervals of distribution phase ($y = a + b.x$).

Substracting the theoretical values from observed values, a series of residual concentrations were obtained and slope of line in natural log [Distribution constant,α] and the zero time intercept (zero time concentration during absorption phase, A) can be calculated as per the method adopted for calculation of B and β. The value are $\alpha = 3.625\ hr^{-1}$

A = 11.83 μg/ml

C_p^0, theoretical plasma concentration at time zero

$C_p^0 = A + B$

= 11.83 + 1.33 μg/ml

= 13.16 μg/ml

$t_{1/2}\,\alpha$, distribution half-life

$$t_{1/2}\alpha = \frac{0.693}{\alpha} = \frac{0.693}{3.625} = 0.191 \text{ hr}$$

$t_{1/2}\,\beta$, Elimination half-life

$$t_{1/2}\beta = \frac{0.693}{\beta} = \frac{0.693}{0.269} = 2.576 \text{ hr} \simeq 2.58 \text{ hr.}$$

AUC, Area under curve

$$AUC = \frac{A}{\alpha} + \frac{B}{\beta} = \frac{11.83}{3.625} + \frac{1.33}{0.269}$$

$$= 3.263 + 4.944$$

$$= 8.207 \text{ mg/L.hr.} \simeq 8.21 \text{ mg/L.hr.}$$

AUMC, Area under mean curve

$$AUMC = \frac{A}{\alpha^2} + \frac{B}{\beta^2}$$

$$= \frac{11.83}{(3.625)^2} + \frac{1.33}{(0.269)^2}$$

$$= \frac{11.83}{13.14} + \frac{1.33}{0.072}$$

$$= 0.90 + 18.47$$

$$= 19.37 \text{ mg/L.hr}^2$$

$$MRI = \frac{AUMC}{AUC} = \frac{19.37}{8.207} = 2.36$$

k_{21}, rate constant for drug transfer from peripheral to central compartment

$$k_{21} = \frac{A.\beta + B.\alpha}{C_p^0}$$

$$= \frac{11.83 \times 0.269 + 1.33 \times 3.625}{13.16}$$

$$= \frac{3.182 + 4.821}{13.16} = \frac{8.003}{13.16} = 0.608 hr^{-1}$$

Kel, the elimination rate constant of the drug from central compartment

$$Kel = \frac{\alpha.\beta}{K_{21}} = \frac{3.625 \times 0.269}{0.608}$$

$$= \frac{0.975}{0.608} = 1.603\ hr^{-1}$$

K_{12}, rate constant of drug transfer from central to peripheral compartment

$$K_{12} = \alpha + \beta - K_{21} - Kel$$

$$= 3.625 + 0.269 - 0.608 - 1.603$$

$$= 1.683\ hr^{-1}$$

Fc, the fraction of drug available for elimination from central compartment

$$Fc = \frac{\beta}{Kel} = \frac{0.269}{1.603} = 0.168 \simeq 0.17$$

$T \approx P$, approximate tissue to plasma concentration ratio

$$T \approx P = \frac{K_{12}}{K_{21} - \beta} = \frac{1.683}{0.608 - 0.269}$$

$$= \frac{1.683}{0.339} = 4.96$$

Vd_C, the volume of distribution based on distribution and elimination

$$Vd_C = \frac{D}{C_p^0} = \frac{2}{13.16} = 0.152 \text{ L/Kg} \simeq 0.15 \text{ L/Kg.}$$

where D = Dose rate (2 mg/Kg)

Vd_B, the volume of distribution based on elimination.

$$Vd_B = \frac{D}{B} = \frac{2}{1.33} = 1.50 \text{ L/Kg}$$

Vd_{area} the volume of distribution based on total area under curve

$$Vd_{area} = \frac{D}{(AUC).\beta} = \frac{2}{8.207 \times 0.269}$$

$$= 0.906 \text{ L/Kg}$$

$$= 0.91 \text{ L/Kg}$$

$$Vd_{SS} = \frac{K_{12} + K_{21}}{K_{21}} \times Vd$$

$$= \frac{1.683 + 0.608}{0.608} \times 0.152$$

$$= \frac{2.291}{0.608} \times 0.152 \qquad = 0.57 \text{ L/Kg}$$

$Cl_B = Vd_{area} \times \beta$

$$= 0.906 \times 0.269$$

$$= 0.24 \text{ L/Kg/hr}$$

$$= 4.0 \text{ ml/Kg/hr}$$

APPENDIX - III

Dosage regimen of antimicrobial agents are generally calculated to maintain minimum inhibitory concentration (MIC) in plasma at desired dosage interval (γ) using the formulae noted by Baggot (1977) and described by Saini and Srivastava (1997). The data of animal no. - 2 obtained after i.v. injection of tobramycin in healthy goat has been used as an example for calculation of loading (D*) and maintenance doses for maintaining C_p^{∞} min (MIC) of 1.0 and 2.0 μg/ml (for moderate and severe infections) at the dosage interval (γ) of 6 and 8 hr.

Calculation of Loading (D) and maintenance (D_0) Dose*

The loading dose is the initial dose that may be given at the onset of therapy with the aim of achieving target concentration rapidly. The loading (D*) and maintenance (D_0) doses of tobramycin can be calculated by the formulae given below

$$D^* = C_p^{\infty}(\min).Vd_{area}e^{\beta.\gamma}$$

$$D_0 = C_p^{\infty}(\min).Vd_{area}.\left(e^{\beta.\gamma} - 1\right)$$

where, D* = Loading dose, D_0 = Maintenance dose.

$C_p^{\infty}(\min)$ = Minimum therapeutic plasma concentration

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