Pharmacokinetic Study of Amikacin and its Interaction With Diclofenac in Buffalo Calves

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

Binod Kumar Mukta

Registration No.- M/V. P. T./25/1999-2000

Department of Pharmacology & Toxicology

BIHAR VETERINARY COLLEGE

PATNA

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Dedicated to

MY DEAR

PARENTS

Dr. S. D. Singh
M.V.Sc., Ph.D.
Head
Deptt. of Pharmacology & Toxicology
Bihar Veterinary College
Patna – 14 (India)

CERTIFICATE – I

This is to certify that the thesis entitled "**PHARMACOKINETIC STUDY OF AMIKACIN AND ITS INTERACTION WITH DICLOFENAC IN BUFFALO CALVES**" submitted in partial fulfilment of the requirements for the degree of Master of Veterinary Science (Veterinary Pharmacology & Toxicology) of the faculty of Post-Graduate studies, Rajendra Agricultural University, Bihar, Pusa is the record of bonafide research carried out by **Dr. Binod Kumar Mukta** 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.

30/09/02

(S. D. Singh)
Major Advisor

Endorsed :

30/09/02

(Chairman / Head of the Department)

CERTIFICATE - II

We, the undersigned members of the Advisory Committee of **Dr. Binod Kumar Mukta**, 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 AMIKACIN AND ITS INTERACTION WITH DICLOFENAC IN BUFFALO CALVES**" may be submitted by **Dr. Binod Kumar Mukta** in partial fulfilment of the requirements for the degree.

30/09/02

(S. D. Singh)
Chairman
Advisory Committee

Members of the Advisory Committee :

1. **Dr. C. Jayachandran**
   Associate Professor
   Department of Pharmacology & Toxicology

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2. **Dr. J. N. Singh**
   Associate Professor and Head
   Department of Livestock Production & Technology

3. **Dr. Ayodhya Prasad**
   Associate Professor and Head
   Department of Animal Nutrition

A. Prasad. 30.9.02

(Nominee of DRI-cum-Dean, P.G.)

CERTIFICATE - III

This is to certify that the thesis entitled "PHARMACOKINETIC STUDY OF AMIKACIN AND ITS INTERACTION WITH DICLOFENAC IN BUFFALO CALVES" submitted by **Dr. Binod kumar Mukta** in partial fulfilment of the requirements for the degree of Master of Veterinary science (Veterinary Pharmacology & Toxicology) of the faculty of Post-Graduate studies, Rajendra Agricultural University, Bihar, Pusa, was examined and approved on 14-12-2002.

(S. D. Singh)
Chairman
Advisory / Examination
Committee

Members of the Advisory / Examination Committee :

1. **Dr. C. Jayachandran**
2. **Dr. J. N. Singh**
3. **Dr. Ayodhya Prasad**

DRI-cum-Dean, P. G. Studies

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Bmukta
30/09/12

(Binod Kumar Mukta)

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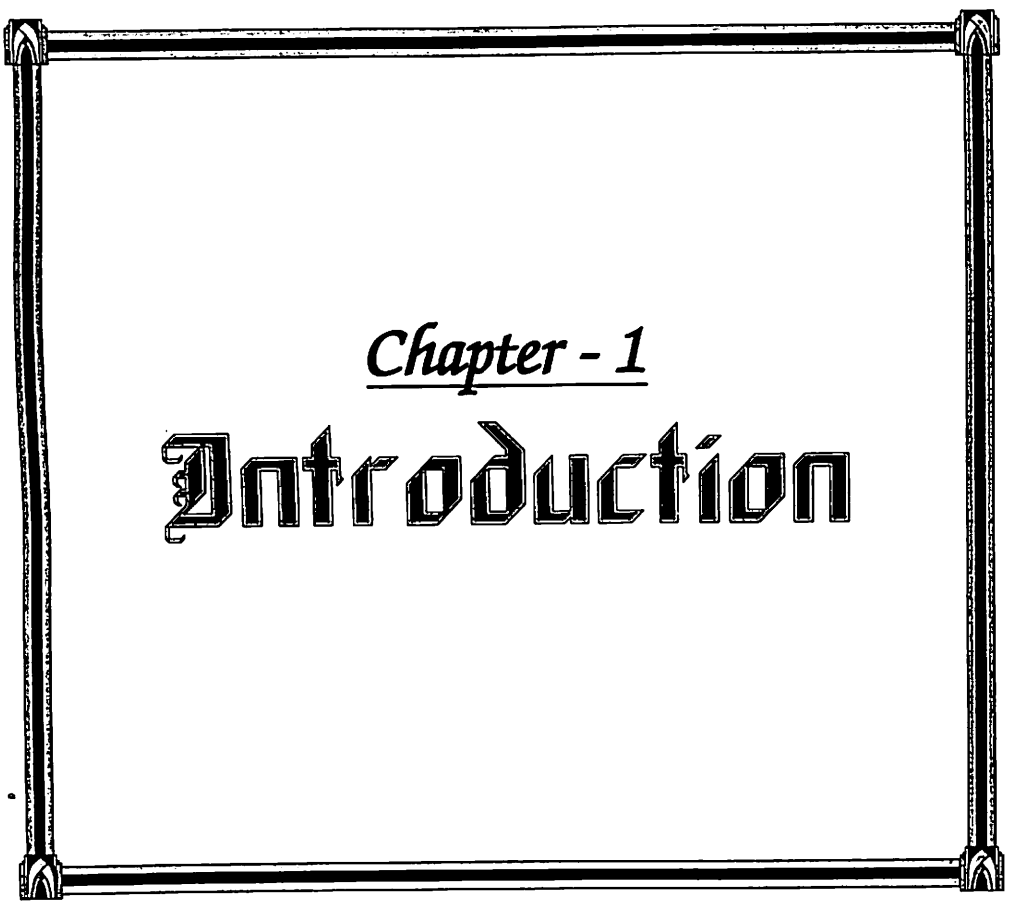


Chapter - 1

Introduction

INTRODUCTION

During the past decade, it has been observed that the effects of many drugs when given concurrently are not necessarily predictable on the basis of knowledge of their effects when given alone. The subjects of drug interactions not only interest the pharmacologists but also highly important to clinical practitioners. Although the original observations about such interactions stemmed from fundamental research, subsequent knowledge of drug interactions, acquired from experiments on animals, has been used to therapeutic advantages in animals as well as humans to enable a physician to minimize or prevent drug toxicity by the dose and dosage schedule.

Antimicrobial agents play a major role in medical and veterinary practices in combating various systemic microbial infections. Systemic microbial infections generally cause pyrexia and / or inflammation associated with pain and hence, non-steroidal anti-inflammatory drugs (NSAIDs) are usually administered along with antimicrobials.

Amikacin is the latest semi-synthetic aminoglycoside antibiotic derived from kanamycin A. The inactivation of other aminoglycoside antibiotics by microbial inactivating enzymes led to the search of amikacin by Kawaguchi and co-workers in 1972, which became very popular in veterinary and medical practices due to its

wide spectrum of activity, excellent disposition characteristics, negligible plasma protein binding, lower minimum therapeutic concentration with least problem of bacterial resistance and cross resistance with other antimicrobial agents as well.

Amikacin is a broad-spectrum bactericidal agent, which is predominantly active against many gram-negative organisms (*Pseudomonas, E. coli, Proteus* spp. *Klebsiella pneumoniae Enterobacter* and *Serratia* etc.) and also against some gram-positive organisms.

Anti-inflammatory agents are generally administered along with antimicrobials in cases of bacterial diseases associated with fever/inflammation. Diclofenac sodium is a newer non steroidal anti-inflammatory drug (NSAID) with a potent analgesic, antipyretic and anti-inflammatory activities along with uricosuric properties. It possess antibacterial activity as well. This powerful prostaglandin synthetase inhibitor affords quick relief of pain in various inflammatory conditions like rheumatoid and osteo arthritis, bursitis, ankylosing spondylitis, dysmenorrhoea and in post traumatic and post operative inflammatory conditions.

Antimicrobials and NSAIDs are frequently used concomitantly and pharmacokinetic interactions between them have been described (Kampmann *et al.*, 1972 ; Carbon *et al.*, 1981, 1984; Sudha Kumari, 1998). In experimental *Staphylococcal* osteomyelitis,

ibuprofen given concomitantly with oxacillin significantly increased antibiotic efficacy but the mechanism of interaction was not studied (Khurana and Deddish, 1986). Joly *et al.* (1988) showed enhancement of the therapeutic effects of cephalosporins (cefotiam, cefmonoxime and ceftriaxone) in experimental endocarditis by altering their pharmacokinetics when simultaneously used with the non steroidal anti-inflammatory drug, diclofenac. No effect of diclofenac on the pharmacokinetics of cloxacillin was shown in man by Nergelious *et al.* (1997). Surya Kumar *et al.* (1995) showed rifampicin pretreatment reduces bioavailability of diclofenac sodium. Influence of enrofloxacin on theopylline steady-state pharmacokinetics in the Beagle dog was demonstrated by Intorre *et al.* (1995).

Buffalo is the chief milk yielding species in India. Buffaloes play an important role in small hold farmer's economy where they form an integral part of agricultural system. They are the important sources of milk, meat and draught power. By considering the huge contribution of buffaloes in nation's economy, its proper and effective health coverage is essential by achieving combined therapy of amikacin with diclofenac sodium.

Before using a drug in therapy, it is essential to study its pharmacokinetic behaviour in detail and on the basis of pharmacokinetic parameters, suitable dosage regimens are derived. Concurrent administration of anti-inflammatory drugs with

antimicrobials may change their disposition characteristics (Joly *et al.* 1998; Sudha kumari, 1998) and thereby changing their dosage regimen. Although pharmacokinetic studies of amikacin have been conducted in many species of animals but on the basis of available literature, it seems that no such work has been conducted in buffalo calves particularly on the interaction of diclofenac sodium with amikacin.

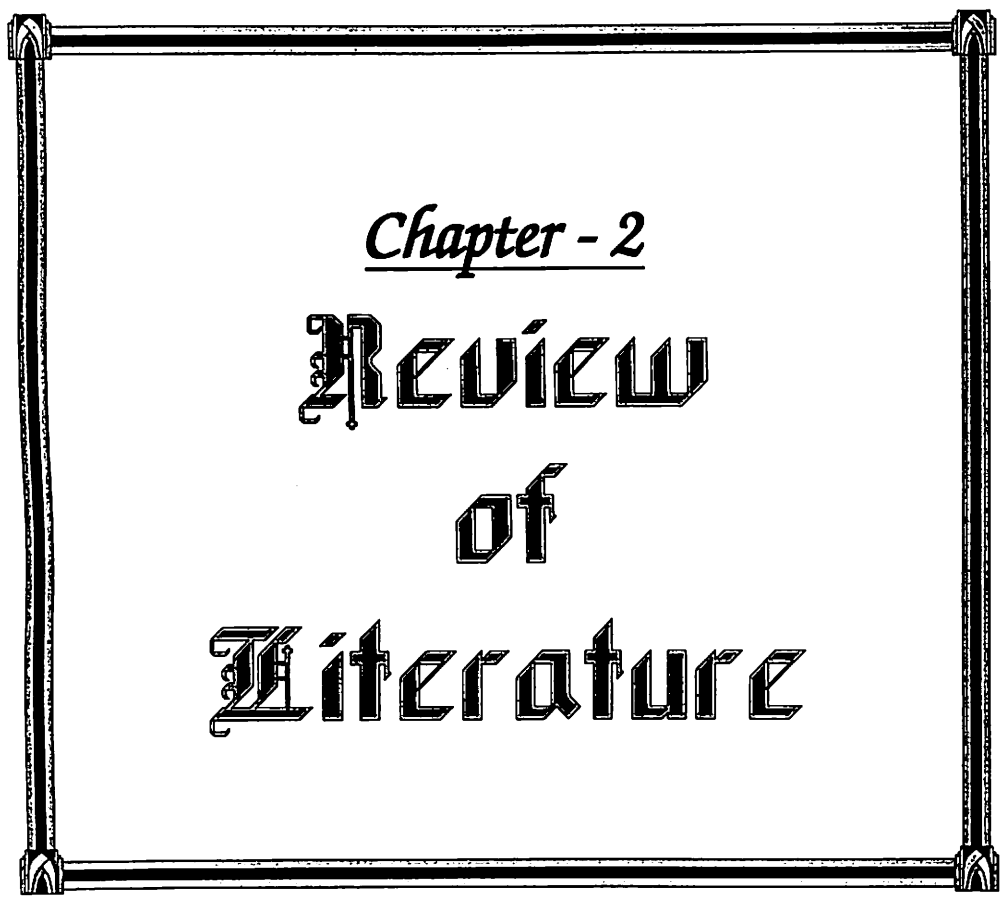
Keeping in view the above mentioned facts, the present study proposes to proceed with the following specific aims and objectives.

(i) Estimation of concentrations of amikacin and diclofenac sodium in biological fluids of buffalo calves following their i.v. administration separately.

(ii) Determination of various pharmacokinetics parameters of amikacin and diclofenac sodium when given alone.

(iii) Calculation of dosage regimen of amikacin when given alone.

(iv) Estimation of concentrations in biological fluids, calculation of kinetic parameters of amikacin and diclofenac sodium and calculation of dosage regimen of amikacin when given in combination by i.v. route to find out the interactions between the two drugs.



Chapter - 2

Review of Literature

REVIEW OF LITERATURE

Aminoglycosides are generally used in clinical practice to treat infections caused mainly by gram-negative bacteria. Recently, many organisms have shown resistance to aminoglycosides such as streptomycin, gentamicin, neomycin, kanamicin, tobramycin etc. The resistance to aminoglycosides mainly occurs by the microbial inactivating enzymes liberated by the microogranisms. This has stimulated to the systemic search of an effective agent to resistant cases of aminologlycoside and in 1972, Kawaguchi and co-workers in Japan produced a semi-synthetic aminoglycoside, amikacin from Kanamycin – A.

AMIKACIN

Amikacin, the latest semi-synthetic aminoglycoside, is clinically used to treat infections caused mainly by gram-negative bacteria like other aminoglycosides. The major advantages of amikacin over the other aminoglycosides are that it is highly resistant to aminoglycoside inactivating enzymes (Ries *et al.*, 1973) and thus used in gentamicin resistant cases, broadest in spectrum among aminoglycosides (active against vast majority of gram-negative organisms) and the minimum therapeutic concentration is two to four folds lower than the other agents of this group.

HISTORY :

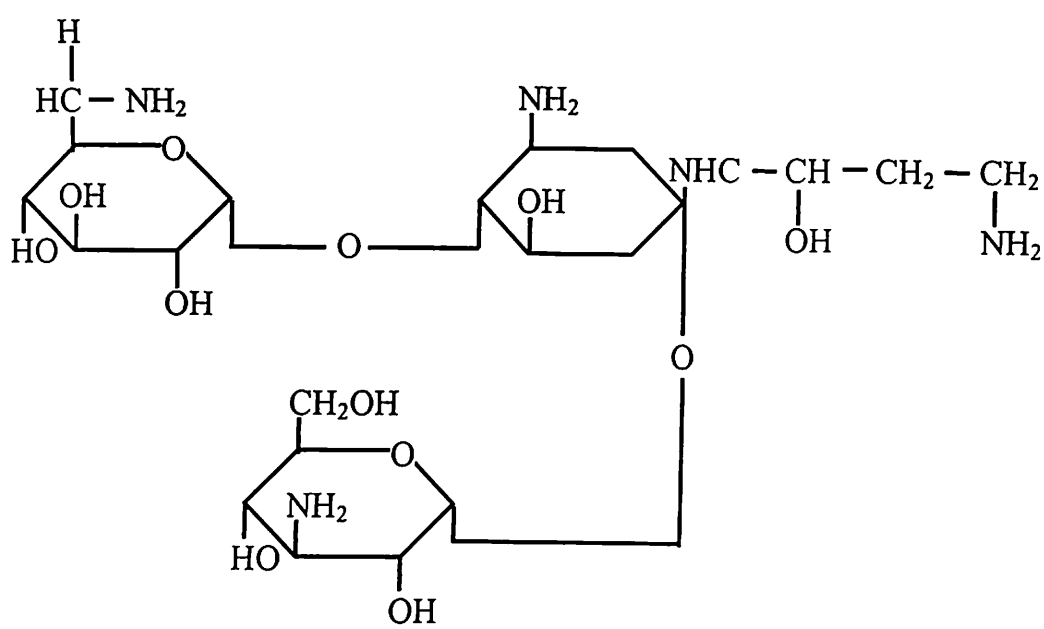
The geneology of aminoglycoside group of antimicrobials began in 1944 with the production of streptomycin from *Streptomyces griseus* by Schatz, Bugie and Waksman for the treatment of infections caused by gram-negative organisms but its clinical usefulness was limited because of emergence of streptomycin resistant gram-negative bacilli. Later on, neomycin was produced by walksman to topical application and local effects in bowel since it leads to severe nephro and oto-toxicity on systemic administration. Umezewa and Co-workers produced another agent kanamycin, from *Streptomyces kanamyceticus* in 1957 but its use is restricted owing to its toxicity and emergence of resistant microorganisms as well. Now a days, it has largely been replaced by the three newer aminoglycosides viz., gentamicin, tobramycin and amikacin.

Inactivation by microbial inactivating enzymes and limited spectrum of activity of these antimicrobial agents were the primary stimulus for the search of better aminoglycosides. Amikacin is a semissynthetic product derived from kanamycin – A. It is water soluble, resistant to most of the aminoglycoside inactivating enzymes unlike other aminoglycosides and its minimum therapeutic concentration is two to four folds lower than other aminoglycosides. It was first approved by the U.S.F.D.A for clinical use in 1976.

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 nucleus or aminocyclitol is 2-deoxystreptamine in the kanamycin family to which amikacin belongs. In this family, two amino sugars are linked to a centrally located 2-deoxystrepamine moiety; one of these is a 3-amino hexose.

Amikacin is the first semi-synthetic aminoglycoside synthesized by acetylation of the 1-amino group of 2-deoxy streptamine residue of kanamycin-A with 2-hydroxy-4-aminobutyric acid. It is a water soluble white crystalline powder, available in the form of its sulphate salt. Molecular formula of amikacin sulphate is $C_{22}H_{43}N_5O_{13}$, $2H_2SO_4$. It is O-3-Amino-3-deoxy-α-D-glucopyrenosyl (1-4)-O-[6-amino-6-deoxy-α-D-glucopyranosyl [1-6]-N^3-(4-amino-L-2-hydroxy butyryl)-2-deoxy-L-streptamine sulphate. The chemical structure of amikacin is given below.



MECHANISM OF ACTION :

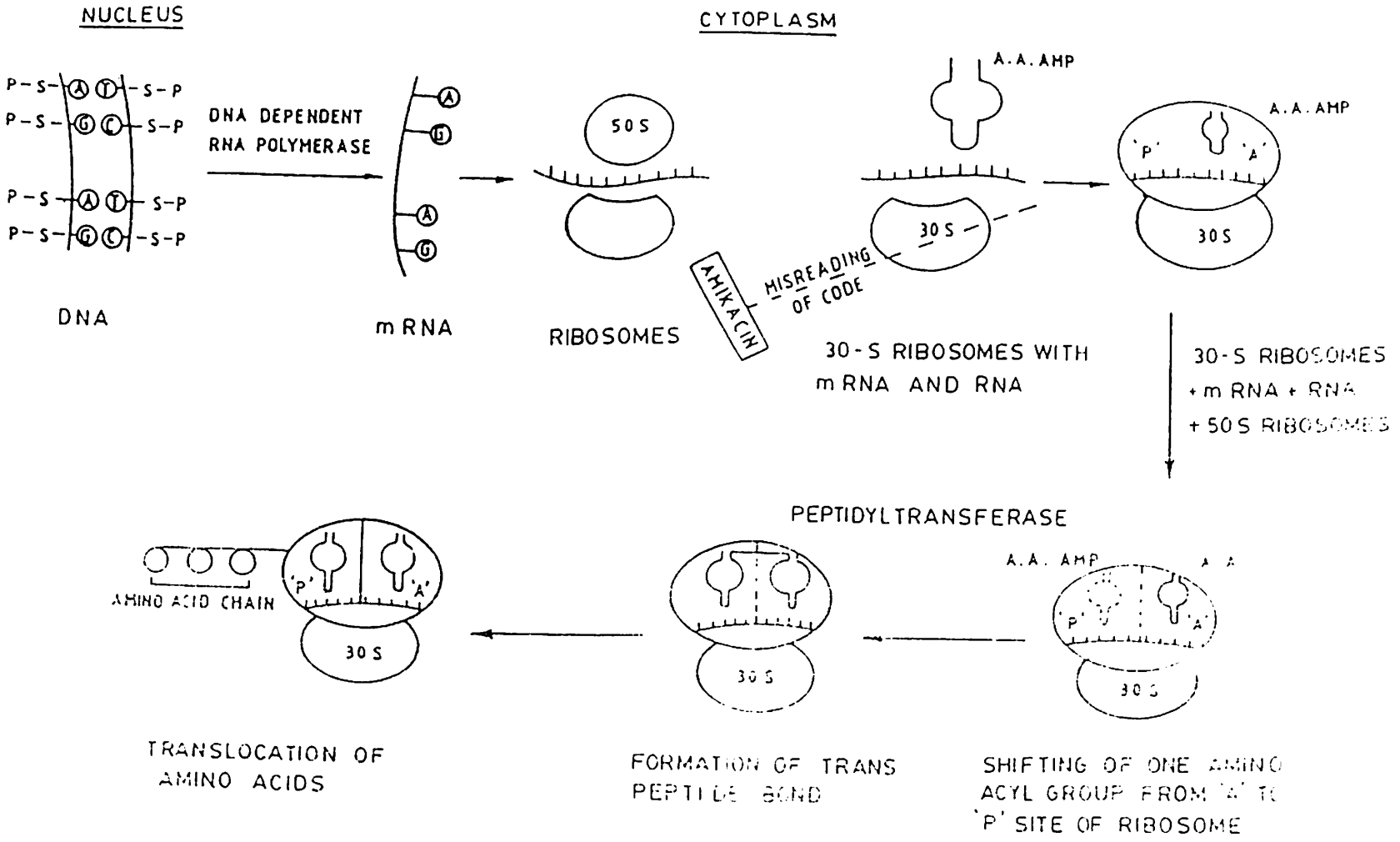
Aminoglycosides are used primarily to treat infections caused by aerobic gram-negative bacteria, in which they interfere with protein synthesis in susceptible microbes. Like other aminoglycosides, amikacin is bactericidal, having high affinity for ribosomal sites and inhibits protein synthesis (Shanon and Phillips, 1982). It is transported across the cell membrane in two steps :

(i) Relatively inefficient and involves binding to energy complex transported through the cell membrane.

(ii) Energy dependent transport which is mainly responsible for the accumulation of the drug in cells.

The primary intracellular site of action of amikacin is 30^s ribosomal subunit. This drug disrupts the normal cycle of ribosomal function partly by interfering with the initiation of protein synthesis leading to the accumulation of abnormal initiation complexes and also its capacity to induce misreading of the m RNA template, thus incorrect growing polypeptide chains (Tai *et al.*, 1978). The mechanism of action of amikacin is depicted in Fig I.

Fig. I.



MODE OF ACTION OF AMIKACIN

Diagramatic representation of protein synthesis in bacterial cell. Broken line indicates site of action of amikacin in its antibacterial action.

Different symbols are as follows :

A = Adenine, G = Guanine, C = Cytosine, T = Thymine, S = Sugar, P = Phosphate, mRNA=Massenger RNA, tRNA=Transfer RNA, 50^S, 30^S = Subunits of Ribosome, 'A' and 'P' = Acceptor and donor sites of 50^S ribosome

ANTIMICROBIAL EFFICACY :

At present, amikacin is potentially and clinically used in medical and veterinary practices to treat mild to severe bacterial infections of respiratory system, skin and uro-genital system. Because of its unique resistance to the aminoglycoside inactivating enzymes, it has a special role in hospitals where gentamicin and tobramycin resistant microorganisms are prevalent. The antibacterial activity of amikacin is primarily directed against aerobic gram-negative bacilli. It has little activity against anaerobic microorganisms or facultative bacteria under anaerobic conditions.

It is very much effective against *Escherichia coli*, *Enterobacter*, *Klebsiella*, *Proteus mirabilis*, *Serratia*, *Staphylococcus aureus*, *Corynebacterium equi*, *Streptococcus zooepidermicus* and *Actinobacillus* species (Huber, 1984). It is also used effectively to treat *Streptococcus fecalis* infection in dogs and mastitis in bovine (Huber, 1984). Amikacin is effective against *Mycobacterium tuberculosis* (99% of strains inhibited by 4 μg/ml) and certain atypical mycobacteria (Gangadharam *et al.*, 1977) and has been used in the treatment of disseminated atypical mycobacterial infections in AIDS patients.

The therapeutic level of amikacin for antibacterial action ranges from 1-4 μg/ml (Leroy *et al.*, 1978). Brown *et al.* (1984) have

reported that the MIC of amikacin for *Corynebacterium equi* (30 isolates), *E. coli* (5 isolates), *Enterobacter cloacae* (1 isolate) is ≤ 2 μg/ml. Orsini *et al.* (1985) determined MIC of amikacin sulphate for six gram-negative pathogens and reported the MIC to be 1 μg/ml for *Serratia rubidaea*, 2 μg/ml for *Klebsiella pneumoniae* (1006), *Pseudomonas stutzeri* and *Pseudomonas aeruginosa* (2) and 4 μg/ml for *E. coli* (998).

The MIC of amikacin that may inhibit 90% (MIC_{90}) of clinical isolates for several species are shown below :

Organism	**MIC_{90} (μg/ml)**
Citrobacter freundii	1
Enterobacter spp.	1
Escherichia coli	1
Klebsiella pneumoniae	1
Proteus mirabilis	2
Providencia stuartii	2
Pseudomonas aeruginosa	2
Serratia spp.	8
Enterococcus faecalis	≤ 64
Staphylococcus aureus	16

Source : Chambers and Sande (1996)

PHARMACOKINETICS OF AMIKACIN :

Amikacin like other aminoglycosides, possesses polycations and therefore, is not adequately absorbed after oral administration (Cox, 1970; Green *et al.*, 1972), poorly penetrates into cerebrospinal fluid and excreted rapidly from kidney (Benet and Sheiner, 1985). The volume of distribution is found to be low in most of the species. The greatest merit with aminoglycosides including amikacin is that there is negligible binding with proteins (Gyelynck *et al.*, 1971; Gordon *et al.*, 1972). Pharmacokinetics of amikacin in different species are given below:-

Cat :

Shille *et al.* (1985) determined the serum concentration of amikacin in healthy adult cats (six male and six female) after administration of 5, 10 and 20 mg/kg body weight of amikacin sulphate by s.c., i.m. and i.v. routes. In a subsequent experiment, the six females were given 10 mg/kg s.c. amikacin and samples of blood, urine and full-thickness uterine wall were taken at 40 and 120 min after treatment. Mean serum concentrations of amikacin peaked between 30 and 45 min after i.m. injection and between 45 and 60 min after s.c. injections. The serum amikacin concentration curves were similar regardless of dose and route of administration except for a slightly longer retention time after the 20 mg/Kg dose given i.m. and s.c. After s.c. injection of 10 mg/kg, the mean uterine concentration of

amikacin at 2 h after treatment was 4.1 μg/g; the concurrent mean serum concentration was 18.6 μg/ml.

Jernigan *et al.*, (1988) studied the detailed kinetics of amikacin in cats. Six mixed breed adult cats were given 5 mg of amikacin sulphate/kg of body weight by rapid i.v., i.m and s.c. routes. The serum concentrations versus time data were analysed using a non compartmental model. The harmonic mean ± pseudo SD of the effective half-life of amikacin was 78.8±19.3 min after i.v. administration, 118.7±14.4 min after i.m. administration and 117.7±12.8 min after s.c. administration. The airthmatic mean ± SD of mean residence time was 118.3±21.7 min, 173.4±19.9 min and 171.17±19.1 min after i.v., i.m. and s.c. drug administration, respectively. The mean apparent volume of distribution at steady state was 0.17±0.02 L/kg and the mean total body clearance was 1.46±0.26 ml/min/kg. Mean bioavailability was 95±20 percent after i.m. administration and 123 ± 33 percent after s.c. administration. A recommended dosage of 10 mg/kg given every 8 h can be expected to provide a therapeutic serum concentration of amikacin with a mean steady state concentration of 14 μg/ml. The s.c. route of administration is preferred, because of rapid absorption, good bioavailability and ease of administration.

Dog :

Baggot *et al.* (1985) determined the elimination kinetics of amikacin after i.v., i.m. and s.c. injection of single doses of amikacin

(5, 10 and 20 mg/kg of body weight) in each of 4 dogs. The pattern of urinary excretion and the cumulative amount excreted unchanged in 24 hours were also determined. Amikacin had a short half-life (approx. 1 h) that was independent of dosage. I.V. injection of 10 mg/kg gave apparent volume of distribution of 226±37 ml/kg and body clearance of 2.64±0.24 ml/min/kg within 6 h, >90 percent of the antibiotic was excreted in the urine, regardless of the route of administration. For dogs with normal renal function, an amikacin dosage of 10 mg/kg (i.m. or s.c.) is recommended every 8 h for treatment of systemic infections, and every 12 h for treatment of urinary tract infections caused by susceptible bacteria.

Birds :

In experiments using 34 African grey parrots, amikacin sulphate was injected i.v. or i.m. at 5, 10 or 20 mg/kg. The elimination half life was approximately 1 h (range 0.9-1.34 h) for all route and dosage combinations. More than 99 percent of the drug was eliminated by 8 h after administration. The apparent bioavailability was 61 to 106 percent after i.m. administration of the drug and was not dose related (Gronwall *et al.*, 1989).

El-Gammal *et al.* (1992) studied the pharmacokinetics of amikacin in healthy mature female chickens (n=6). Single doses of amikacin were injected as i.v. bolus (10 mg/kg) and i.m. (20 mg/kg) into the same birds with a 30 day rest period between treatments.

The i.v. pharmacokinetics could be described by a two-compartment model with distribution phase half-life of 0.150 ± 0.064 h and a terminal phase half life of 1.44 ± 0.34 h. The total body clearance was 1.109 ± 0.017 L/h/kg and the volume of distribution at steady-state was 0.193 ± 0.060 L/kg. Following a single i.m. injection, the peak plasma concentration was 50.79 ± 4.05 μg/ml and occurred at 0.50 ± 0.26 h. The i.m. results provided estimates of an absorption half life of 0.480 ± 0.158 h. The i.m. pharmacokinetics after repeated administration were studied following the 10^{th} dose (20 mg/kg, every 8 h). The maximum plasma concentration was 38.58 ± 6.98 μg/ml and occurred at 0.79 ± 0.37 h. The multiple dosing yielded peak concentrations of 3.26 μg/ml. The recommended amikacin dosage in chickens is 20 mg/kg body weight every 8 h.

The pharmacokinetics of gentamicin sulfate and amikacin sulfate in the cockatiel (*Nymphicus holandicus*) were evaluated utilizing treatment regimens developed in larger parrot species. Serum antibiotic concentrations were determined following twice-daily i.m. treatment with 5 mg gentamicin/kg body weight. Peak values of gentamicin were 4.6 ± 1.45 μg/ml, and trough values were 0.17±0.04 μg/ml. Amikacin administration resulted in peak values of 27.3±6.9 μg/ml and trough concentration of 0.9 ± 0.3 μg/ml. Based on this study, the recommended i.m. dosages regimen for gentamicin in cockatiels is 5 to 10 mg/kg body weight 2 or 3 times per day. An i.m. amikacin dosage of 15 to 20 mg/kg body weight 2 or 3 times per day

was recommended for treatment of infections caused by susceptible bacteria (Ramsay and Vulliet, 1993).

A mean 7.2±0.12 mg/kg dose of amikacin was administered as a single i.v. bolus to 3 emus and serum levels were estimated. The data were best represented by a three compartment model with a mean elimination half-life of 0.87 h, with a longer rate of elimination from the third compartment (6.06 h). Mean model-independent parameters obtained were area under the curve (269.66 μg/ml.h), mean residence time (6.48 h), apparent volume of distribution (0.18 L/kg) and total body drug clearance (0.03 L/h/kg). Mean serum concentrations exceeded a target peak of 32.0 μg/ml for approximately 12 h. Mean serum levels had declined below a target trough of 4 μg/ml at 24 h. It is suggested that a dosing interval of 12 h may be necessary in emus; however, antimicrobial sensitivity of bacterial pathogens, severity of infection and degree of renal function should be considered before determining the frequency of aminoglycoside administration (Helmick *et al.*, 1997).

The pharmacokinetics parameters of amikacin were determined in red-tailed hawks (*Buteo jamaicensis*) following the i.m. administration of a single 20 mg/kg dose. After a rapid absorption phase, mean amikacin serum concentrations peaked at 65 ± 12 μg/ml 30-45 min following injection. The serum amikacin concentration decreased to 2.3 ± 2 μg/ml at 12 h post injection. Amikacin was

eliminated with first order kinetic characteristic of a single compartment model with a half-life of 2.02 ± 0.63 h. The volume of distribution was estimated to be 0.28 ± 0.03 L/kg. The minimum inhibitory concentration (MIC) of amikacin ranged from 0.5 to 8.0 μg/ml (mean = 2.5 μg/ml) for the 42 isolates of gram negative bacteria and and coagulase positive *Staphylococcus* species. The 20 mg/kg dose used in this study resulted in serum concentration at or above the MICs for >12 h for most of the isolates examined. Amikacin administered at 15-20 mg/kg per day either as a single dose or divided into two or three doses, is effective in treating the sensitive pathogens of the red-tailed hawk. (Bloomfield *et al.*, 1997).

Camel :

Single doses of amikacin sulfate, both i.v. (3.75 mg/kg body weight) and i.m. 3.75 mg/kg were given on separate occasions with a 14 day interval to 5 healthy camels. Blood samples were collected at intervals for upto 480 min after drug administration.

The disposition kinetics of amikacin after i.v. administration were best described by a 2-compartme ntopen model. Following a single i.m. dose, a 1–compartment model best described the distribution kinetics. The clearance of amikacin in camels was 0.97 ml/min/kg. The volume of distribution of amikacin in camels was 247 ml/kg body weight. Amikacin was rapidly absorbed following i.m.

administration, reaching a peak concentration of 11.60 μg/ml after 1 h and its bioavailability was close to 100%. It is suggested that i.m. administration is a possible route for amikacin therapy. A once daily dose of 10 mg/kg is suggested for amikacin treatment of camels and this was calculated to result in a maximum serum concentration of about 40 μg/ml It is recommended however, that this dosing rate needs to be tested clinically by a multiple dose study (Wasfi *et al.*, 1999).

Equine :

Six mares were given 5 i.m. injection (at 12 hour intervals between doses) of amikacin sulphate at a dosage of 7 mg/kg of body weight. Serum amikacin concentrations were measured serially throughout the study; synovial, peritoneal, endometrial and urine concentrations were determined after the last injection. Amikacin concentrations in the CSF were measured serially in 3 of the 6 mares; 1 of the 3 mares had septic meningitis. Mean serum amikacin concentrations peaked at 1 to 2 hours after i.m. injection. The highest mean serum concentration was 19.2 μg/ml (1.5 h after the 5^{th} injection). The highest mean synovial concentration was 10.8 μg/ml of 2 hour after the 5^{th} injection the highest mean peritoneal concentrations was 16.2 μg/ml at 3 h after the 5^{th} injection. The mean endometrial amikacin concentration was 2.5 μg. gm^{-1} (1.5 h after the 5^{th} injection).

Amikacin reached a CSF concentration of 0.97 μg/ml in the mare with meningitis, but amikacin was not detected in the CSF of healthy mares. Urine concentrations reached 1458 μg/ml. Pharmacokinetic values were estimated after the first injection (elimination rate constant = 0.31 h^{-1}; half life = 2.3 h apparent volume of distribution = 0.26 L/Kg) and after the 5th injection (elimination rate constant = 0.28 h^{-1}, half life = 2.6 h; apparent volume of distribution = 0.30 L/Kg); significant differences were not observed (Brown *et al.*, 1984).

Orsini *et al.* (1985) studied the pharmacokinetics of amakacin sulphate in horses after intravenous and intramuscular administration of doses of 4.4, 6.6 and 11 mg/kg. Serum, synovial and peritoneal fluid concentrations of the drug were measured. The serum concentrations at 15 min following i.v. injection were 30.3 ± 9.3, 61.2 ± 6.9 and 122.8 ± 7.4 μg/ml, respectively, for the 4.4, 6.6 and 11.0 mg/Kg doses. Mean peak serum concentration was observed at 1 h after the i.m. injections and were 13.3 ± 1.6, 34.0 ± 0.6 and 29.8 ± 3.2 μg/ml, respectively. The half-life of amikacin was 1.44, 1.57 and 1.14 h for the 4.4, 6.6 and 11.0 mg/kg doses, respectively. Based on the minimum inhibitory concentrations for six pathogens (*Klebsiella pneumoniae, E. coli, Serratia rubiadaea, Pseudomonas stutzeri* and two strains of *P. aeruginosa*) and the pharmacokinetic parameters, the recommended dose of amikacin is between 4.4 and 6.6 mg/kg,

twice daily and for the most serious infections, dosing three times a day.

In another study, six healthy foals aged between 2 to 11 days were given a single i.m. injection of amikacin sulphate at a dose of 7 mg/kg. Serum concentrations were measured serially over a 24 hour period. The mean peak serum concentration was 14.7 μg/ml at 0.5 hours. The elimination rate constant was 0.24 h^{-1} the elimination half-life 3.0 hours and the apparent volume of distribution 0.258 L/kg (Brown *et al.*, 1986).

Sheep :

The kinetic disposition of amikacin in Bergamasca sheep was investigated following i.v. and i.m. administration (Carli *et al.*, 1990). The values of $t_{1/2}$ α, $t_{1/2}$ β, Cl_B, AUC and Vd_{area} were found to be 26.2 ± 7.4 min 115.5 min, 0.7 ml/kg/min, 11018 μg/ml.min and 0.2 L/kg, respectively whereas in another study Uppal *et al.* (1998) found these values to be 6.53 ± 1.3 min, 85.40 ± 5.36 min, 2.7 ± 0.13 ml/kg/min, 3712 ± 150 μg/ml.min and 0.335 ± 0.0031 L/kg, respectively after i.v. administration of amikacin. The bioavailability of the drug follwing s.c. administration in sheep was 87% (Carli *et al.*, 1990) and 99.7% (Uppal *et al.*, 1998).

Goat :

Uppal *et al.* (1992) investigated pharmacokinetics of amikacin sulphate in male goats following single i.v. injections of 10

mg/kg. Distribution half-life, elimination half life and apparent volume of distribution values were 15.7 min, 130.1 min and 0.40 L/kg, respectively. The values of total body clearance and AUC were 2.13 ml/kg/min and 4853 μg/ml.min, respectively. At 6 h after the administration of drug, 10.2 percent was in the central and peripheral compartments and about 90 percent had been eliminated.

The pharmacokinetics of amikacin was studied in lactating goats after single i.v. and i.m. administration of 7.5 mg/kg body weight. After i.v. injection, the values of distribution half life, elimination half life and mean residential time were 11.03, 114.81 and 142.96 minutes, respectively. Following i.m. injection, the values of absorption half life, elimination half life and mean residential time were 20.39 122.86 and 205.51 min, respectively. Amikacin was detected only at low concentration in goat's milk 2-6 h after i.v. and i.m. injections. Amikacin urine concentrations were much higher then those of plasma (Abo-el-Sooud, 1999).

Agarwal (2000) studied the pharmacokinetics of amikacin in six lacating goats after a single i.v. and i.m. administration. The author noted absorption half life ($t_{1/2}$ Ka) and distribution half life ($t_{1/2}$ α) of 0.07 ± 0.01 and 0.52 ± 0.02 h, respectively. The elimination half life ($t_{1/2}$ β) and resident time (MRT) of 2.08 ± 0.01 h and 1.73 ± 0.08 h for i.v. route and 1.94 ± 0.10 and 2.92 ± 0.14 h for i.m. route. Vd_{area} of and 0.40 L/kg and Cl_B of and 2.20 ml/Kg/min were noted for both the routes.

Cow Calf :

Following i. v. administration of amikacin in calves, the distribution half-life, elimination half-life total body clearance, volume of distribution and AUC values were reported to be 21.6 min 150.5 min, 1.5 ml/kg/min, 0.35 L/kg and 5512 ± 1759 μg/ml.min, respectively (Carli *et al.*, 1990).

The disposition kinetics, urinary excretion and dosage regimen of amikacin after a single i.v. administration of 10 mg/kg was investigated in cross-bred bovine calves. The elimination half-life and volume of distribution were 3.03 ± 0.27 h and 0.4 ± 0.03 L/kg, respectively. The total body clearance and T ≈ P ratio were 0.09 ± 0.002 L/kg/h and 4.98 ± 0.41, respectively (Saini and Srivastava, 1998).

Buffalo Calf :

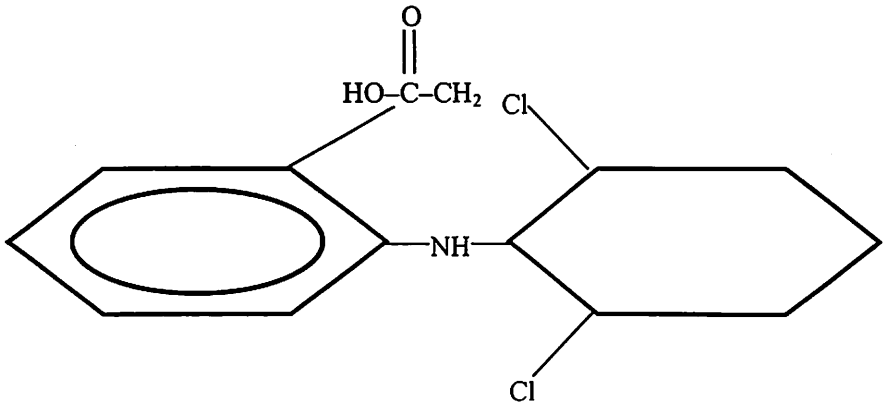
Uppal *et al.* (1998) studied comparative pharmacokinetics of amikacin in buffalo calves following its intramuscular and subcutaneous administration @ 7.5 mg/kg body weight. The values of various pharmacokinetics parameters after i.m. administration like $t_{1/2}\beta$ (185.90 ± 6.43 min), AUC (10504 ± 514 μg/ml.min) Vd_{area} (0.201 ± 0.005 L/kg) and Cl_B (0.752 ± 0.012 ml/min/kg) differed non significantly with the values obtained after s.c. administration. There was a significant difference between the values of $t_{1/2}$ Ka after i.m. administration (12.88 ± 0.86 min) and s.c. administration (23.03 ± 1.11 min). This suggested that the rate of absorption was faster after i.m. than after s.c. route.

DICLOFENAC

Diclofenac is a potent non steroidal anti-inflammatory drug (NSAID), which is widely used in human and veterinary practice. It is the first of a series of phenyl acetic acid derivatives that has been developed as an anti-inflammatory agent. It is also an analgesic compound with good antipyretic and uricouric properties (Maier *et al.*, 1979).

CHEMISTRY

Chemically diclofenac is a phenyl acetic acid derivative. The chemical structure is as follows :



Empirical formula = $C_{14} H_{23} O_2 CI_2 N$

Molecular weight = 307

PHARMACOKINETICS AND METABOLISM :

Diclofenac is rapidly and completely absorbed after oral administration and peak concentrations in plasma are reached within 2 to 3 hours. Administration with food slows the rate but does not alter the extent of absorption. The drug gets completely absorbed following i.m. injection. C_{max} and AUC are dose related in the range of 25-150 mg. It is extensively bound to plasma proteins (99%) and its half life in plasma is 1 to 2 hours. Diclofenac accumulates in synovial fluid after oral administration, that may be the possible reason behind the longer duration of the therapeutic effect than the plasma half life. Diclofenac is metabolized in the liver to 4-hydroxyl diclofenac, the principal metabolite and other hydroxylated forms. The metabolites are excreted in the urine (65%) and bile (35%). Apart from liver, bile and kidney, high levels of diclofenac are found in blood, heart and lungs.

KINETIC STUDIES :

Pharmacokinetic studies on diclofenac were conducted in different species. They are noted as follows.

Man

In man, Willis *et al.* (1979) noted the lag time between dosing and appearance of drug in plasma varied between 1.0 and 4.5 h after oral doses. Peak plasma levels ranged from 1.4 to 3.0 μg/ml. The mean terminal drug half life in plasma was 1.8 h after oral dose and 1.1 h after i.v. dose. He noted availability (oral) 54±2%, urinary

THERAPEUTIC USES :

Diclofenac is used in veterinary practice for treating non descriptive pyrexia, painful conditions due to acute and chronic inflammation, muscular pain, joint pain, neuralgia, soft tissue injuries such as sprain or strain and immobility associated with lamness, arthritis, myositis etc.

MODE OF ACTION :

Diclofenac possesses analgesic, antipyretic and anti-inflammatory properties. It inhibits the cyclo-oxygenase pathways in the metabolism of arachidonic acid and thus exerts its anti-inflammatory action by blocking the synthesis of prostaglandins, prostacycline and thromboxane products. Diclofenac also inhibits the lipo-oxygenese pathway and there by reducing the production of leukotrienes and monohydroxy acids which are associated with the inflammatory processes. It also reduces polymorph chemotaxis and production of lysosomal enzymes and super oxide radicals there by reducing tissue destruction in inflammatory reactions. It also inhibits bradykinin, an important mediator of pain and inflammation. Diclofenac suppresses hyperthermia through its action on the thermo-regulatory centre in hypothalamus. In rats with yeast induced fever, diclofenace reduced body temperature by 1.5^0C in lower doses than did indomethacin, ibuprofen, phenylbutazone, naproxen and aspirin.

excretion less than 1%, bound in plasma more than 99.5%, clearance value of 4.2±0.9 ml/min/kg and volume distribution 0.17±0.11 L/kg. After i.v. injection, plasma levels of diclofenac fell rapidly and were below the limits of detection at 5.5 h post dosing.

Kurowski (1988) noted oral bioavailability of 72.9% with an average lag time of 2.2 h. Peak plasma concentrations amounted to 2.9 μg/ml after 3.1 h as compared to 2.15 μg/ml after 20-30 min following an intramuscular injection of 75 mg. Diclofenac was excreted with an average half life of 1.15 h. The bioavailability of the three i.m. injectable solutions, as calculated from the area under the curve (AUC), did not differ significantly.

Pig

The pharmacokinetics and metabolism of diclofenac was studied in yucatan minipigs after i.v. administration of 25 and 50 mg and after oral administration of 50 mg in a solution of 50 ml buffer, 50 ml water & 200 ml water and the results were compared to historical data in man. The absolute bioavailability after oral administration of 50 ml buffer, 50 ml water and 200 ml water solutions were 107, 97 and 107% respectively as compared to approximately 50% in man. The total plasma clearance in minipigs was five fold slower than in man (57 ± 17 vs 252 ± 54 ml/h/kg).

The volume of distribution of the central compartment (Vdc) was 40% less in man than in pigs (39 vs 67 ml/kg). The terminal half lives of the present drug were similar in pigs (2.4 h) and

man (1.8 h). The rate of oral drug absorption increased in the order of 50 ml aqueous, 200 ml aqueous and 50 ml buffered solutions (Ka = 0.52 ± 0.11, 0.59 ±0.13 and 1.2 ± 0.7 h^{-1}, respectively) as observed by Oberle *et al.* (1994).

Rat

In rat, biliary excretion of the drug (unchanged and conjugated) was detected in bile duct cannulated rats were 27.2 and 31.2 % and only 4.7 and 5.4 % excreted in the bile after i.v. and intra-duodenal administration, respectively. Maximum plasma concentration was reached within 2 min after intraduodenal dosing. Bioavailability in the bile duct cannulated rats was 71% after intraduodenal dose where as in normal animal was 79% after oral dose and 106% after intraduodenal dose (Peris-Ribera *et al.*, 1991).

Buffalo calves

Pharmacokinetics and urinary excretion of diclofenac in buffalo calves was studied by Nitesh Kumar *et al.* (2002). Peak concentrations of 7.04 ± 0.75 and 30.0 ± 6.58 μg/ml were attained at 0.042 and 0.167 h, respectively, in plasma. High distribution half life ($t_{1/2}\,\alpha$) and elimination half life ($t_{1/2}\,\beta$) of 0.34 ±0.08 and 4.06 ± 0.59 h were noted. The shorter $t_{1/2}\,\alpha$ denotes that diclofenac is distributed at a faster rate in buffalo calves. Area under plasma concentration time curve (AUC), mean residential time (MRT) and total body clearance (Cl_B) of 11.24 ± 0.48 μg/l/hr, 4.72 ± 0.85 h and 1.52 ± 0.07 ml/kg/min, respectively were obtained. Vd_{area} of 0.54 ± 0.07 L/kg obtained for

diclofenec denotes good distribution of the drug which is supported by the value of 2.43 ± 0.32 obtained for approximate tissue to plasma concentration (T ≈ P) ratio.

KINETIC INTERACTION OF ANTIMICROBIALS WITH NON STEROIDAL ANTI-INFLAMMATORY, ANALGESIC AND ANTIPYRETIC AGENTS :

Antimicrobials and non steroidal anti-inflammatory, analgesic and antipyretic agents are frequently used concomitantly and pharmacokinetic interactions between them have been described (Joly *et al.*, 1988; Mueller *et al.*, 1993; Manna *et al.*, 1994; Nergelius *et al.*, 1997; Sudha kumari, 1998; Tang *et al.*, 1999 and Varma *et al.*, 2000).

The effect of diclofenac on the pharmacokinetics of the three cephalosporins viz., ceftriaxone, cefotiam and cefmenoxime was studied in rabbits by Joly *et al.*, (1988). Ceftriaxone concentrations at 1, 2, 4, 6, 12 and 24 h and AUC in serum increased significantly ($p<0.05$) when this antimicrobial was administered in conjunction with diclofenac. Diclofenac increased significantly ($p<0.05$) the serum terminal half life ($t_{1/2}$ β) of ceftriaxone and non significantly that of cefotiam but not cefmenoxime.

The mean pharmacokinetic characteristics of cyclosporine were unchanged during coadministration with diclofenac was studied in man by Mueller *et al.*, (1993). A single oral dose of 300 mg cyclosporine was administered alone and on day 8 of multiple oral

dosing interval for diclofenac on day 7 (diclofenac alone) and day 8 (co-administration of diclofenac with cyclosporine). Based on area under the curve (AUC) comparison, lack of a pharmacokinetic interaction was conclusively demonstrated for the extent of cyclosporine absorption. The diclofenac maximum plasma concentration and AUC over a dosing interval were significantly increased during co-administration; however, a straightforward interpretation of the statistical result was confounded by pronounced variability in diclofenac pharmakinetics. The results underscore the need for continued caution when cyclosporine and diclofenac are co-administered.

Modification of the disposition kinetics of paracetamol by oxytetracycline in goats was carried out by Manna *et al.* (1994). They observed that the C_{max} value of paracetamol alone (128.0 ± 8.0 μg/ml) was significantly ($p<0.01$) higher as compared to the combined therapy with oxytetracycline (46.8 ± 3.4 μg/ml) at 0.03 h post i.v. drug administration. Paracetamol persisted in the blood till 2 h and 4 h for alone and combined therapy respectively. The C_P^0 value of paracetamol alone (163.3 ± 9.9 μg/ml) was significantly ($p<0.01$) higher compared to combined therapy (56.0 ± 2.6 μg/ml). The α and $t_{1/2}$ α values of paracetamol alone were higher and lower,respectively, as compared to combined administration. On the other hand $t_{1/2}$ β, Vd, Vd_B, Vd_{area} and Vd_{SS} values of combined therapy was significantly higher ($p<0.02$) form the corresponding values of paracetamol alone.

No effect of diclofenac on the pharamacokinetics of cloxacillin was shown in man by Nergelius *et al.* (1997). Total plasma clearance of cloxacillin was with placebo 219 ± 51 (mean ± S.D.) and with diclofenac 212 ± 39 ml/min/1.73 m^2 (ns); renal clearance was 97 ± 21 and 96 ± 24 ml/min/1.73 m^2, respectively (ns). The terminal $t_{1/2}$ of cloxacillin was 1.03±0.42 h with placebo, and 1.12±0.37 h with diclofenac (ns). Thus, diclofenac does not alter cloxacillin pharmacokinetics.

Pharmacokinetics of enrofloxacin (@ 5 mg/kg) when given alone and in combination with paracetamol (@ 50 mg/kg) by i.v. route in six goats was carried out by Sudha kumari (1998). She observed that the mean therapeutic concentration (0.12 μg/ml) in plasma was maintained up to 10 h for enrofloxacin and 6 h for enrofloxacin with paracetamol. Significantly higher values were obtained for zero time concentration in distribution phase (A) and theoretical zero time concentration (C_P^0 were 19.60 ± 3.92 and 21.52 ± 4.12 μg/ml, respectively, in combined administration as compared to single administration (3.37 ± 0.79 and 5.27 ± 0.96 μg/ml, respectively). Significantly higher elimination rate constant (β) and lower elimination half life ($t_{1/2}$ β) of 0.456 ± 0.067 h^{-1} and 1.70 ± 0.26 h^{-1}, respectively, in combination as compared to single administration (0.270 ± 0.041 h^{-1} and 2.82 ± 0.33 h, respectively). The distribution half life (0.57 ± 0.17 h), AUC (18.90 ± 5.87 mg/L.h), K_{12} (0.251 ± 0.079 h^{-1}), Fc (0.42 ± 0.09), T≈P(1.96 ± 0.48), Vd_{area} (1.10 ± 0.47

L/kg) and Cl_B (9.22 ± 4.73 ml/kg/min) did not show any significant difference when enrofloxacin was given along with paracetamol as compared to enrofloxacin alone (0.60 ± 0.10 h, 9.85 ± 1.38 mg/L.h, 0.436 ± 1.33 h^{-1}, 0.51 ± 0.06, 1.11 ± 0.22, 2.34 ± 0.54 L/kg and 9.40 ± 1.36 ml/kg/min, respectively).

The stimulation of diclofenac metabolism by interaction with quinidine was studied in monkeys by Tang *et al.* (1999). After a dose of diclofenac via portal vein infusion at 0.055 mg/kg/h, steady-state systemic plasma drug concentrations in three male rhesus monkeys were 87, 104 and 32 ng/ml, respectively (control). When diclofenac was co-administered with quinidine (0.25 $mg.Kg^{-1}.h^{-1}$) via the same route, the corresponding plasma diclofenac concentration were 50, 59 and 18 ng/ml, representing 57, 56 and 56% of control values, respectively. In contrast, steady-state systemic diclofenac concentrations in the same three monkeys were elevated to 1.4 to 2.5 times when the monkeys were pretreated with L – 754, 394 (10 mg/kg i.v.), an inhibitor of cytochrome P – 450 (CYP) 3A. Further investigation indicated that the plasma protein binding (>99%) and blood/plasma ratio (0.7) of diclofenac remained unchanged in the presence of quinidine. Therefore, the decreases in plasma concentrations of diclofenac after a combined dose of diclofenac and quinidine are taken to reflect increased hepatic clearance of the drug, presumably resulting from the stimulation of CYP 3A-catalyzed oxidative metabolism. Consistent with this proposed mechanism, a 2-

fold increased in the formation of 5-hydroxy diclofenac derivatives was observed in monkey hepatocyte suspensions containing diclofenac and qunidine. Stimulation of diclofenac metabolism by quinidine was diminished when monkey liver microsomes were pretreated with antibodies against CYP 3A. Subsequent kinetic studies indicated that the K(m) value for the CYP – mediated conversion of diclofenac to its 5-hydroxy derivatives was little changed (75 vs 59 micro M), where as V (max) increased 2.5 fold in the presence of quinidine. These data suggest that the catalytic capacity of monkey hepatic CYP 3A toward diclofenac metabolism is enhanced by quinidine.

Pharmacokinetics of enrofloxacin was studied in five cattle following i.m. administration (5 mg/kg) alone and along with diclofenac sodium (0.8-1.0 mg/kg). Therapeutic concentration (0.1 μg/ml) in plasma was maintained up to 12 and 24 h for enrofloxacin and enrofloxacin along with diclofenac sodium, respectively. The plasma elimination half life (9.2 h), Vd_{area} (17.3 L/kg), T_{max} (2 h), MRT (13.2 h) and body clearance (1.4 L/kg/h) was comparatively significantly higher when enrofloxacin was given along with diclofenac sodium as compared to enrofloxacin alone (5.9 h, 7.1 L/kg, 0.4 h, 6.8 h and 0.82 L/kg/h, respectively). The AUC (3.8 mg/ml.h) and C_{max} (0.2 μg/ml) was significantly lower when enrofloxacin was administered along with diclofenac sodium as compared to enrofloxacin given alone (5 mg/ml/h and 0.82 μg/ml, respectively). Diclofenac sodium significantly ($p<0.1$) reduced the plasma

concentration of ciprofloxacin (as metabolite of enrofloxacin). Based on the pharmacokinetic parameters calculated, an intramuscular dosage regimen of enrofloxacin(Priming dose of 1.8 mg/kg followed by maintenance dose of 1.10 mg/kg every 8 h) to maintain a therapeutic concentration of 0.1 μg/ml is recommended in cattle (Varma et al, 2000).

GENERAL PHARMACOKINETICS

Pharmacokinetics often referred to as disposition kinetics, helps in knowing absorption, distribution, metabolism and excretion of drugs (Dost, 1953). According to Wagner (1968), the aim of pharmacokinetics is to study the time concentration course of drugs and their metabolites in various body fluids, tissues and excreta and interpretation of such date based on suitable pharmacokinetic models (compartment models).

The compartment model is a hypothetical structure which can be used to characterise with reproducibility of behaviour and fate of drugs in a biological system, when administered by a certain route in a particular dosage form. In pharmacokinetic studies, compartment is an entity which has a definite volume and in that concentration of a drug exists at any time. The disposition kinetics of a drug is described either by one compartment or multicompartment open models. Body distributes the drugs in all tissues at widely varying rates and is therefore, designated as open systems. An open

compartment model shows free movement of drug from one compartment to another (i.e. blood to tissue and vice-versa).

One compartment open model :

When the distribution of a drug from central to peripheral compartment is very rapid, the drug is said to follow one compartment open model. Any change in drug concentration the blood reflects directly the quantitative change in its tissue level. Baggot (1974) reported that the rate of drug elimination from the body is proportional to the concentration of the drug in blood.

In one compartment open model, if the plasma concentration time profile is plotted from the peak concentration in onwards on a semilogarithmic scale, a straight line is obtained (Sams, 1978) and the plasma drug level declines according to following equation.

$$Cp = B_e^{-\beta t} \qquad \ldots\ldots \text{Eq. 1.}$$

Where,

Cp = Concentration of the drug in plasma.

B = Extrapolated zero time intercept of mono exponential curve.

β = Overall elimination rate constant.

t = Time elapsed after drug administration.

e = Base of natural logarithm.

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

Two compartment open model :

The pharmacokinetics of most of the drugs following i.v. administration are accurately described by two compartment open model. Baggot (1974) stated that in two compartment open model, the drug distribution is instantaneous and homogeneous into the central compartment (such as blood and other readily accessible tissue like liver and kidney) and more slowly into the peripheral compartment (comprising of less perfused organs and tissue such as muscles and fat). This indicates that distribution and elimination processes follow the first order kinetics and elimination takes place exclusively from central compartment. In two compartment open model, semi logarithmic plot of plasma drug concentration against time shows a biphasic curve. The initial steep decline in plasma drug concentration is mainly due to the distribution of drug from central to peripheral compartment. Once apparent distribution is established, the gradual decline is obtained mainly by irreversible elimination of drug from the central compartment.

The drug concentration in plasma is expressed by the following biexponential mathematical expression as a function of time.

$$Cp = A_e^{-\alpha t} + B_e^{-\beta t} \quad \text{.... Eq.2}$$

Where,

Cp = 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.

e = Base of natural logarithm.

t = Time elapsed after drug administration.

The values of A, B, α and β are essential in calculating other kinetic rate constants (K_{12}, K_{21}, and Kel) in two compartment open model. The values of these rate constants give an idea of relative contribution of distribution and elimination processes to the drug concentration – time data (Baggot, 1977).

Three or multi compartment open model :

The disposition kinetics of drugs may also follow three or multiple compartment when semilogarithmic plot of plasma drug concentration against time shows a triphasic multiphasic curve. The initial sharp decline in plasma concentration against time is due to

distribution of drug from blood to highly perfused tissue compartment (peripheral-I). The gradual decline is because of distribution of drug from central to moderately blood supplied organs (peripheral II). The drug concentration in plasma following single intravenous administration is expressed by the following triexponential mathematical formula as a function of time.

$$C_p = A_e^{\alpha t} + B_e^{-\beta t} + C_e^{-\gamma t} \qquad \text{.....Eq. 3}$$

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

Pharmacokinetics of Clinical Importance :

Clinically, the pharmacokinetics studies consist of

(a) Calculation of various kinetic parameters following different routes of administration.

(b) Estimation of drug dosage regimen in a particular species of animal.

(c) Determination of drug withdrawal period for drug residues in milk and tissues of food producing animals.

Some important pharmacokinetic parameters :

1. Absorption rate constant (Ka) and absorption half-life ($t_{1/2}$ Ka):

These denote the rate of absorption (faster or slower) of a drug from its site after extra vascular (i.m./s.c./oral) administration.

2. Distribution rate constant (α) and distribution half-life ($t_{½}$ α):

These parameters indicate the rate of distribution (faster or slower) of drug from plasma to body fluids and tissues following i.v. administration.

3. Elimination rate constant (β) :

Baggot (1977) and Mercer *et al.* (1977) stated that the over all elimination rate constant (β) is the most essential kinetic parameter since it is employed to determine:

(i) The elimination half-life ($t_{½}$ β)

(ii) The volume of distribution by area method (Vd_{area})

(iii) The total body clearance ($Cl_{β}$)

(iv) The drug withdrawal period for drug residues in milk and tissues of food producing animals.

4. Elimination half-life ($t_{½}$ β) :

Gibaldi and Weintraub (1971) defined that the elimination half life 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. This means that doubling the dose does not double the duration of action of drug but increases it by one half life. It is inversely proportional to the overall elimination rate constant. It 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 of the drug as well as the route of administration. Knowledge of the half-life of a drug is extremely helpful in designing the rational dosage regimen.

5. Volume of distribution :

The apparent volume of distribution is an important pharmacokinetics parameter used in the kinetic characterization of a drug. It is a hypothetical volume of body fluid that would be required 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 is not dependent upon the method used for its calculation, if the drug distributes truly according to one compartment open model. The apparent volumes of distribution indicates the amount of distribution of a drug without providing any clue, whether the drug is uniformly distributed or restricted to certain tissues (Baggot, 1977). A large volume of distribution (>1 L/kg) indicates wide distribution throughout the body or extensive tissue binding or rapid excretion of a drug or combination of all the above. A small volume of distribution indicates that the drug is restricted to certain fluid compartments, like plasma water, extracellular fluid etc. This is due to the high protein binding or low lipid solubility of a drug.

6. Total body clearance (Cl_B) :

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

drug is a complex function which depends upon the process of drug distribution, biotransformation and excretion. The parameter, body clearance, on the other hand is independent of these processes and indicates the rate of drug removal from the body. Unlike β and $t_{½}$ β that are hybrid constants and depend upon K_{12}, K_{21} and Kel, the total body clearance changes exactly in proportion to Kel (Jusko and Gibaldi, 1972; Rowland *et al.*, 1973).

7. Bioavailability :

When a drug is administered intravenously, the peak concentration in blood is attained quickly and the whole administered drug is available for distribution, metabolism and excretion. The peak plasma level following extravescular administration is somewhat delayed and its magnitude decreases. The bioavailability of a drug indicates the rate of drug absorption as well as the amount of absorption of a drug in pharmacologically active form. The extent of absorption (F) is generally known as bioavailability and is calculated experimentally by the ratio of the area under the plasma concentration time curve after extravascular and intravenous administration (Baggot, 1977; Sams, 1978).

8. Protein Binding :

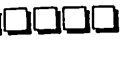
Some drugs have tendency to get bound with plasma protein mainly with albumin. Binding of a drug with plasma protein affects drug distribution (high molecular weight of plasma protein prevents bound drug from diffusing out of capillaries into tissues),

drug effects (free drug fraction is alone pharmacologically active, since it can penetrate to the region of target organ) and 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.

It is reported that the various constants, namely A, α, B, β, $t_{½}$ α, $t_{½}$ β and Vd_{area} etc. change disproportionally with the magnitude of the elimination rate constant from central compartment (Kel) and hence, should not be employed individually as a direct or sole measure of a change in drug elimination or distribution (Jusko and Gibaldi, 1972).

DOSAGE REGIMEN :

Dose is a quantitative term estimating the amount of a drug which must be administrated to produce a particular biological response i.e. to attain optimum effective concentration of a drug in the body fluids. Maintenance of therapeutic concentration of a drug in the body requires the administration of maintenance dose at a particular dose interval after administering the priming or loading dose, so that plasma drug concentration must be above a minimum effective level and below a level producing excessive side effects and toxicity. Thus, the 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 levels.



MATERIALS AND METHODS

In the present study, five clinically healthy female buffalo calves of non-descript breed between 12 to 18 months of age and 102 to 175 kg body weight were used. The buffalo calves were housed in the animal shed with concrete floor. The buffalo calves were maintained on dry fodder, cattle feed and greens. Water was given *ad lib*.

EXPERIMENTAL DESIGN :

Amikacin and diclofenac were administered separately in each of five healthy buffalo calves by intravenous (i.v.) route. An interval of 15 days was allowed to elapse before administration of next dose of the drug. After conducting the kinetic study of these drugs alone, the drugs were administered together in combination by i.v. route to investigate the interaction of these drugs in buffalo calves.

DRUG USED :

Amikacin and diclofenac were used in the present experiment. Alfakim–an injectable commercial preparation containing amikacin sulphate equivalent to amikacin in concentration of 250 mg/ml marketed by Ranbaxy Laboratories Limited, India was used. Diclofenac–an injectable commercial preparation marketed under the trade name of Zobid® by Ambalal Sarabhai Enterprises

Limited, India was used. Each ml of Zobid contains 25 mg of diclofenac sodium.

COLLECTION OF BIOLOGICAL FLUIDS AND THEIR TIMING :

The samples of various biological fluids were collected after i.v. administration of drugs in healthy buffalo calves. The samples of plasma and urine were collected 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 h but samples of urine were collected further upto 48 h (at 30, 36 and 48 h).

(A) Blood

Before collection of blood, the sites around the jugular vein on either side of the neck of the animals were asepticaly prepared. The site was sterilized prior to each collection with rectified spirit. Blood samples were collected in sterilized centrifuge tubes containing appropriate amount of sodium oxalate by vene-puncture with disposable 18 G needles, at various above noted time intervals after drug administration. The blood samples were centrifuged at 3000 rpm for 10 min. for the separation of plasma. The plasma samples were then kept in a refrigerator until assay was carried out. For the preparation of standards, normal plasma prior to drug administration was also collected.

(B) Urine

The urine samples were collected for analysis by introducing a sterile Foley's ballon catheter (No. 12) lubricated with

glycerine through urethra into the urinary bladder of the experimental buffalo calves with the aid of a flexible metal probe. The ballon of the catheter was inflated by injecting 25-30 ml of sterile water through a syringe to keep the catheter in position. The opening of the catheter was blocked with a pressure clip to check dripping of urine. Prior to drug administration, urine sample was collected in a sterile test tube for the preparation of standards. After administration of the drug, the urine samples were collected in sterile test tubes at various above noted time intervals. The samples were kept in a refrigerator and were analysed in successive days.

ADMINISTRATION OF DRUGS :

Alfakim injection containing 250 mg of amikacin per ml was injected at the dose rate of 7.5 mg/kg body weight by i.v. route in each healthy buffalo calf. Zobid injection containing 25 mg of diclofenac sodium per ml was administered at the dose rate of 1 mg/kg body weight by i.v. route in each healthy buffalo calf. After conducting kinetic study of Amikacin and diclofenac by i.v. route separately, both the drugs were administrated together at the above stated dose rate in each animal by i.v. route to know the interaction of the drugs.

ESTIMATION OF AMIKACIN :

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 160^0C for an hour. For administration of drug and for collection of blood, sterile disposable needles were used.

II. Preparation of media :

(a) Assay Agar :

Antibiotic assay media of the following composition was used for microbiological assay of amikacin in blood and urine.

Sl. No.	Ingredients	Gram/litre water
1	Peptone	6.0
2	Tryptone	4.0
3	Yeast extract	3.0
4	Beef extract	1.5
5	Agar	15.0
	Distilled water	1000 ml.
	Final pH	7.9±0.1

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 37^0C for 24 hour to ascertain any growth, which indicates any microbial contamination. The growth free plates were then wrapped with sterile paper and stored in a refrigerator until assay was carried out.

IV. Preparation of test organism :

The test organism used for microbiological assay technique of amikacin was *Bacillus subtilis* ATCC 6633 (Brown *et al.*, 1984; Orsini *et al.*, 1985). 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 37^0C for overnight. Then it was stored under refrigerator. The organism was transferred weekly to fresh media to maintain its normally activity.

V. Preparation of standards in biological samples :

Amikacin was diluted in sterile glass distilled water to have different strengths, viz., 160 μg/ml, 80 μg/ml, 40 μg/ml, 20 μg/ml, 10 μg/ml, 5 μg/ml, 2 μg/ml, 1 μg/ml and 0.5 μg/ml. 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 or urine collected prior to drug administration. This yielded drug standards of 16 μg/ml, 8 μg/ml, 4 μg/ml, 2 μg/ml, 1 μg/ml, 0.5 μg/ml, 0.2 μg/ml, 0.1 μg/ml and 0.05 μg/ml, in the above noted biological samples. These standards 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 amikacin was detected as low as 0.1 μg/ml.

VI. Assay procedure :

The plasma milk and urine levels of amikacin were estimated by microbiological assay technique (cylinder plate diffusion method) using *Bacillus subtilis* (ATCC 6633) as the test organism (Grove, 1955).

The test organism was inoculated in sterile nutrient broth and kept under incubation for 2 to 3 hour at 37^{0}C until the growth was seen (turbid by naked eye). Amikacin 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^{0}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 micolitres of each of the standard solution 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 horizontally on plane surface of the table for about 2 hour and then kept in the incubator at 37^{0}C for overnight to allow the growth of organisms. 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.

ESTIMATION OF DICLOFENAC BY REVERSE PHASE PARTITION CHROMATOGRAPHY BY USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) :

The concentrations of diclofenac sodium in plasma and urine were estimated by HPLC method as described by El-Sayed *et al.* (1988) with slight modification. The details of the procedure are as follows :

Apparatus :

The HPLC equipment used comprised of a HPLC pump, a dual wavelength absorbance detector, a rheodyne manual injector with a 20 µl loop size and a data module (integrator).

Chromatographic separations were performed using C_{18} column (3.9 × 300 mm size).

Chromatographic conditions :

For HPLC analysis of diclofenac in biological samples, the flow rate was 1.5 ml/min, the effluent was monitored at 280 nm, loop size was 20 µl, injection volume was 100 µl, chart speed was 0.25 mm/min and the detector sensitivity was monitored at 2.000 A.U.F.S.

Reagents :

All solvents used were of HPLC grade. All other chemical and reagents were of analytical grade and freshly prepared triple distilled water were used for HPLC analysis.

Mobile phase :

The mobile phase comprised of acetonitrile: water (50: 50% v/v), adjusted of pH 3.3 with glacial acetic acid.

Preparation of standards of diclofenac in biological samples :

Zobid®, an injectable commercial preparation containing diclofenac sodium in concentration of 25 mg/ml was used in the present study. Diclofenac was diluted in triple distilled water to have different strengths viz. 40, 20, 10, 5, 2.5, 1, 0.5, 0.25, and 0.1 µg/ml.

From each standard solution 0.1 ml was added to a centrifuge tube containing 0.9 ml of plasma or urine collected prior to

drug administration. This yielded diclofenac standards of 4, 2, 1, 0.5, 0.25, 0.1, 0.05, 0.025 and 0.01 μg/ml in the above noted biological fluid. Blank plasma/blank urine containing no drug was also prepared. These standards were used simultaneously with test samples for determination of the drug concentrations in the test samples.

Analytical Method :

1. In a clean and dry centrifuged tube 1 ml of plasma samples was taken and 4 ml of acetonitrile was added for precipitation of plasma proteins.
2. The mixture was shaken on a vortex mixer for 1 min and centrifuged for 15 min at 3000 rpm.
3. The supernatant was transferred to a clean tube and evaporated to dryness in a boiling water bath.
4. The residue is reconstituted in 400 μl HPLC eluent (mobile phase) and vortexed for 1 min.
5. An aliquot of this mixture (up to 100 μl) was injected directly into the loop of injector and the integrator print out retention time and area.
6. From various concentrations of standards versus area, standard curve was plotted in a graph paper for diclofenac.
7. Using these standards graph, the area obtained form test plasma and urine samples collected at various time intervals, the concentrations were obtained in test plasma and test urine samples separately.

CALCULATION OF PHARMACOKINETIC PARAMETERS :

The following pharmacokinetics parameters of amikacin were calculated affect its i.v. administration from semilog plot of plasma drug concentration versus time curve. The experimental data was analysed using two compartment (for i.v. route) open model as described by Gibaldi and Perrior (1975) and Notari (1980).

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

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

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

Where 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 I).

(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 squeres (Appendix I).

(c) C_p^0, the theoretical zero theme plasma concentration of drug.

$$C_p^0 = A + B \text{ (two compartment model)}$$

(d) Distribution half life ($t_{1/2}\ \alpha$) and elimination half life ($t_{1/2}\ \beta$) were calculated from the following formula :

$t_{1/2}\ \alpha =$ $0.693/\alpha$

$t_{1/2}\ \beta =$ $0.693/\beta$

α and β are described above.

(e) AUC, the total area under the plasma drug concentration time curve (mg/L.h) :

For two compartment model

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

(f) AUMC, the total area under the first moment of plasma drug concentration time curve (mg/L.h^2) :

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

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

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

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

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

(i) Kel, the elimination rate constant of drug form central compartment (h^{-1}) :

$$Kel = \frac{\alpha.\beta}{K_{21}}$$

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

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

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

$$Fc = \frac{\beta}{Kel}$$

(l) $T \approx P$, the approximate tissue to plasma concentration ratio;

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

(m) Vd_C, the volume of distribution, based on distribution and elimination (L/kg) :

$$Vd_c = \frac{D}{C_p^o}$$

(n) Vd_B, the volume of distribution based on elimination (L/kg).

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

(o) Vd_{area}, the volume of distribution based on total area under curve (L/kg) :

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

(p) Vd_{SS}, the volume of distribution at steady state (L/kg) :

$$Vd_{SS} = \frac{K_{12} + K_{21}}{K_{21}} . Vd_C$$

(q) 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. Leroy *et al.* (1978) reported the therapeutic plasma level (MIC) of amikacin to be 1 – 4 μg/ml. Hence in the present study, dosage regimens of amikacin were calculated at 1, 2 and 4 μg/ml 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,

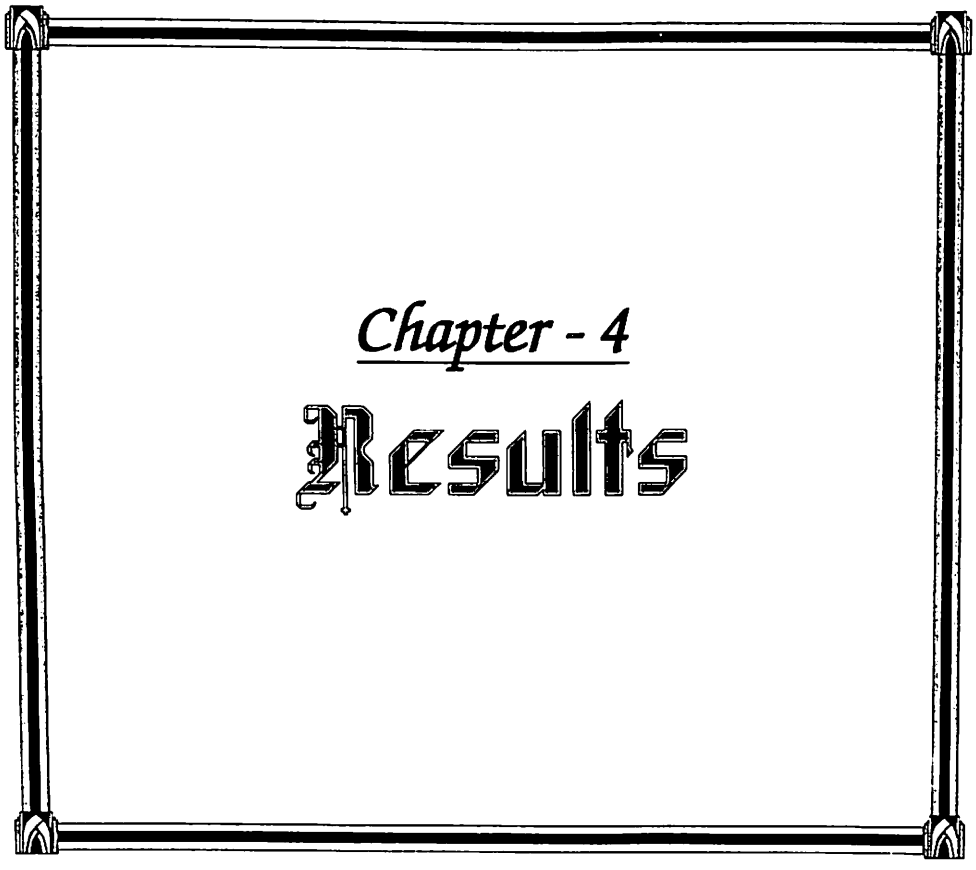
D^* = Loading or priming dose (mg/kg).

D_o = Maintenance dose (mg/kg).

$C_P^{\infty}(min)$ = Desired minimum plasma concentration (μg/ml)

γ = Dosage interval (h)

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



Chapter - 4

Results

RESULTS

I. PHARMACOKINETICS STUDY AFTER SINGLE INTRAVENOUS ADMINISTRATION

(A) Amikacin

1. Plasma levels

Concentrations of amikacin in plasma at various time intervals following its single intravenous administration at the dose rate of 7.5 mg/kg have been shown in Table 1 and Fig. 1. The mean peak plasma concentration of 22.90 ±3.16 μg/ml was attained at 0.083 h. The drug was detectable up to 10 h in all animals with the mean of 1.07 ± 0.14 μg/ml. The mean therapeutic concentration (≥ 2 μg/ml) was maintained up to 6 h. The drug was detectable in 4 out of 5 animals at 12 and 24 h and none at 30 h.

2. Urine levels

Table 2 and Fig. 2 reveal the urine concentrations of amikacin after a single i.v. administration (7.5 mg/kg). The drug appeared in urine of all animals with a mean of 321.8±98.26 μg/ml at 0.083 h. The mean peak urine drug concentration of 1098 ± 74.70 μg/ml was achieved at 0.333h. The drug was detectable up to 30 h in all animals with a mean of 1.32 ±0.41 μg/ml. The mean therapeutic drug concentration of 2 μg/ml was maintained even beyond 24 h.

TABLE - 1

Plasma concentrations (μg/ml) of amikacin in healthy female buffalo calf after a single intravenous dose (7.5 mg/kg)

Time (h)	ANIMAL NUMBER					Mean ± S.E.M
	1	2	3	4	5	
0.083	27.16	11.23	23.58	21.43	26.12	22.90 ± 3.16
0.167	19.61	9.77	20.24	18.00	19.58	17.44 ± 1.95
0.25	16.67	7.39	17.75	15.12	16.50	14.69 ± 1.87
0.333	12.03	6.43	12.98	12.70	13.94	11.62 ± 1.33
0.50	10.22	5.60	11.58	12.50	12.88	10.56 ± 1.32
0.75	8.69	4.87	9.45	10.67	10.15	8.77 ± 1.03
1	7.38	4.80	8.20	9.67	9.00	7.81 ± 0.84
1.5	6.27	4.23	7.18	8.96	8.12	6.95 ± 0.82
2	5.35	3.68	6.20	7.52	6.65	5.88 ± 0.65
3	3.27	3.20	3.75	6.32	3.88	4.08 ± 0.57
4	2.78	2.42	3.20	3.74	3.40	3.11 ± 0.23
5	2.36	2.11	2.80	2.64	2.92	2.57 ± 0.15
6	2.00	1.60	2.35	1.86	2.38	2.04 ± 0.15
8	1.70	0.91	1.80	1.57	1.88	1.57 ± 0.17
10	0.89	0.60	1.25	1.31	1.28	1.07 ± 0.14
12	0.46	N.D.	0.92	0.46	0.95	0.56 ± 0.18
24	0.24	N.D.	0.15	0.23	0.28	0.18 ± 0.05
30	N.D.	N.D.	N.D.	N.D.	N.D.	-

N.D. = Non-detectable

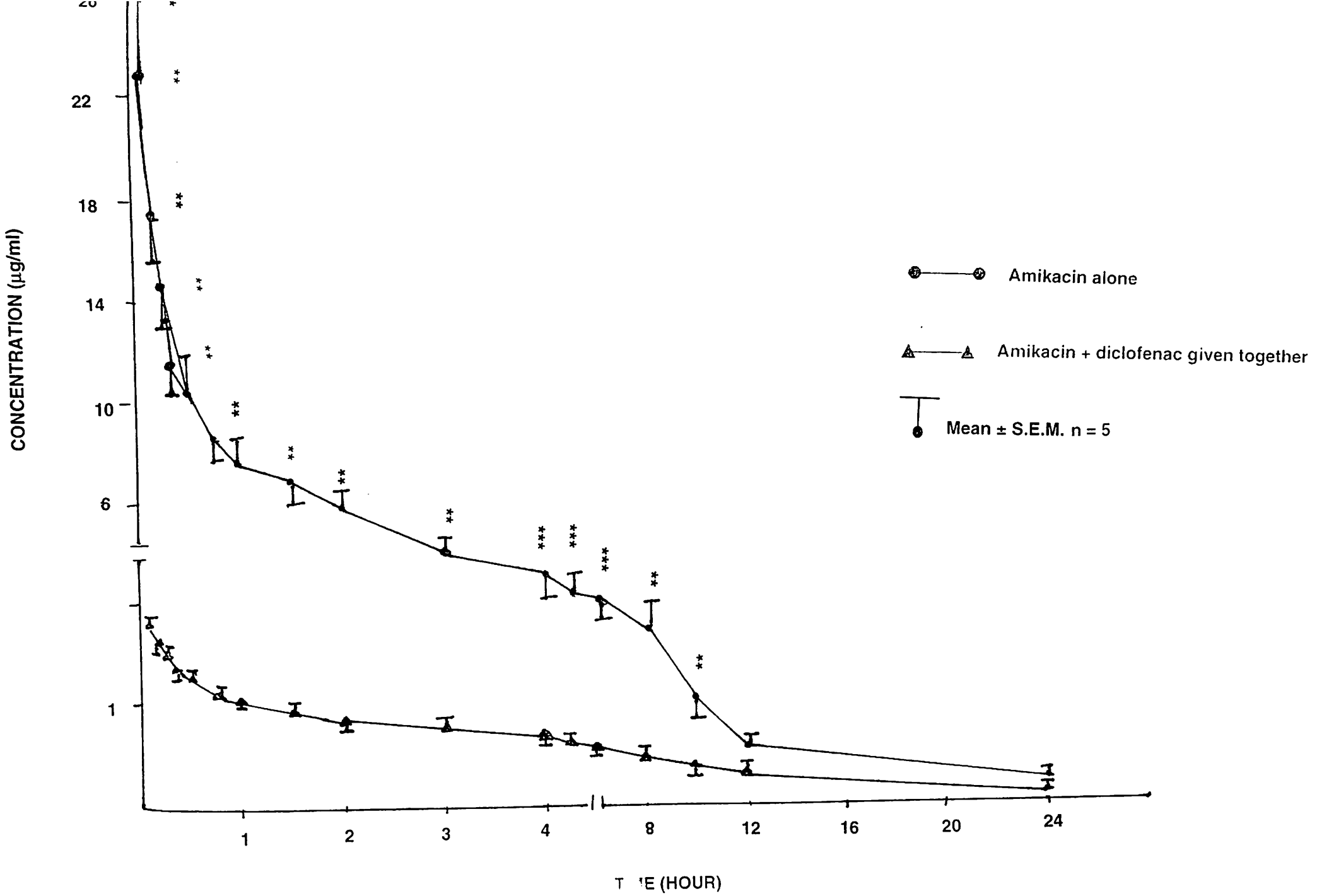

CONCENTRATION (µg/ml)
22
18
14
10
6
1
Amikacin alone
Amikacin + diclofenac given together
Mean ± S.E.M. n = 5
1
2
3
4
8
12
16
20
24
T E (HOUR)

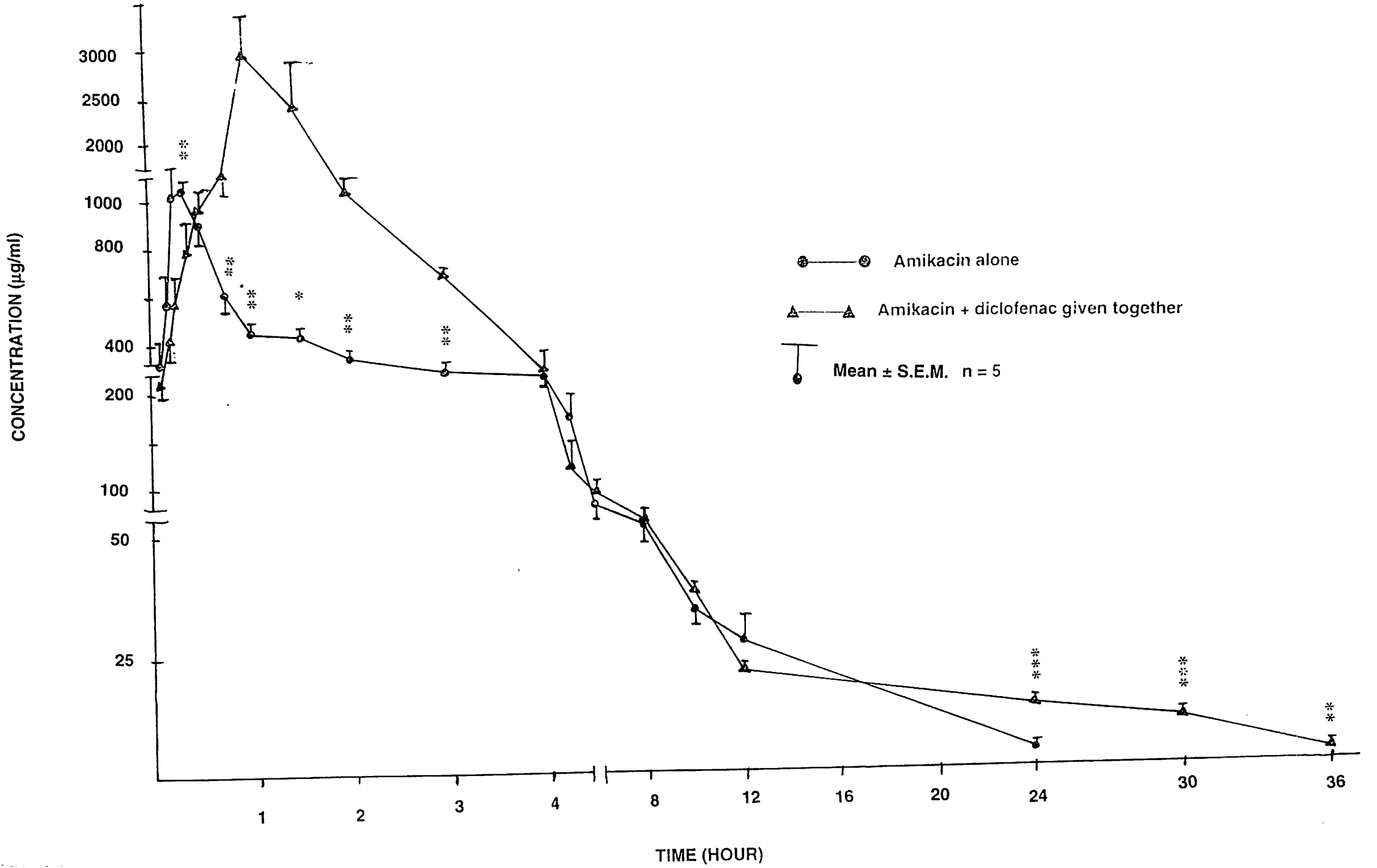
TABLE - 2

Urine concentrations (μg/ml) of amikacin in healthy female buffalo calf after a single intravenous dose (7.5 mg/kg)

Time (h)	ANIMAL NUMBER					Mean ± S.E.M
	1	2	3	4	5	
0.083	610.8	450.5	88.7	333.3	125.5	321.8 ± 98.26
0.167	825.6	780.8	209.4	691.2	355.5	572.5 ± 122.6
0.25	1116	1458	267.3	1884	480.8	1041 ± 300.2
0.333	825.6	1200	1151	1065	1250	1098 ± 74.70
0.50	710.1	1092	707.4	1034	950.8	898.9 ± 80.79
0.75	451.8	750.6	554.7	691.2	614.6	612.6 ± 52.17
1	402.6	510.2	434.7	480.0	428.5	451.2 ± 19.31
1.5	360.5	480.2	401.6	460.8	390.2	418.7 ± 22.41
2	301.2	385.6	369.8	376.8	290.8	344.8 ± 20.16
3	222.8	370.5	289.8	362.2	228.0	294.7 ± 31.58
4	164.8	335.0	227.2	320.0	210.4	251.5 ± 32.76
5	122.0	245.8	139.6	222.2	120.2	170.0 ± 26.62
6	82.4	124.2	33.60	111.1	48.6	79.98 ± 17.41
8	33.4	110.5	20.70	92.60	24.20	56.98 ± 18.81
10	3.34	62.8	12.7	77.1	14.00	33.99 ± 14.97
12	2.85	54.2	7.80	64.3	7.05	27.24 ± 13.19
24	2.47	8.54	1.27	5.36	1.62	3.85 ± 1.37
30	1.00	2.65	0.29	1.80	0.88	1.32 ± 0.41
36	N.D.	N.D.	N.D.	N.D.	N.D.	-

N.D. = Non-detectable

Fig. 2


CONCENTRATION (µg/ml)
3000
2500
2000
1000
800
400
200
100
50
25
1
2
3
4
8
12
16
20
24
30
36
TIME (HOUR)
Amikacin alone
Amikacin + diclofenac given together
Mean ± S.E.M. n = 5

3. *Kinetic parameters*

Plasma drug concentration versus time profile has confirmed a two compartment open model for amikacin as depicted in Fig. 3. Table 3 presents the values of different kinetic parameters calculated by the above noted compartment model.

The mean extrapolated zero time concentration during distribution phase (A), elimination phase (B) and theoretical zero time concentration (C_p^0) were noted to be 13.23 ± 0.70, 5.22 ± 0.38 and 18.41 ± 0.67 μg/ml, respectively. The distribution rate constant (α) ranged from 0.504 to 8.167 h^{-1} with a mean of 2.326 ± 1.467 h^{-1} while its elimination rate constant (β) ranged from 0.127 to 0.232 h^{-1} with a mean value of 0.156 ± 0.020 h^{-1}. The mean distribution half life ($t_{1/2}$ α) and elimination half life ($t_{1/2}$ β) were noted to be 0.75 ± 0.23 and 4.67 ± 0.45 h, respectively. The mean area under curve (AUC) of 48.56 ± 5.84 mg/L.h, area under first moment curve (AUMC) of 256.4 ± 39.72 mg/L.h^2 and mean residential time (MRT) of 5.16 ± 0.30 h were noted in the present study. The average rate of transfer of drug from central to peripheral (K_{12}), peripheral to central (K_{21}) and elimination from central (Kel) compartment were calculated to be 1.220 ± 0.902, 0.851 ± 0.524 h^{-1}, respectively. The fraction of drug available for elimination from central compartment (Fc) and approximate tissue to plasma concentration ratio (T≈P) were noted to be 0.39 ± 0.03 and 1.62 ± 0.21. Various values of volume distribution obtained by different methods are shown in Table 2. A mean Vd_{area} of 1.06 ± 0.06 L/kg was noted. The total body clearance (Cl_B) value ranged from 2.03 to 4.45 with a mean of 2.78 ± 0.44 ml/kg/min.

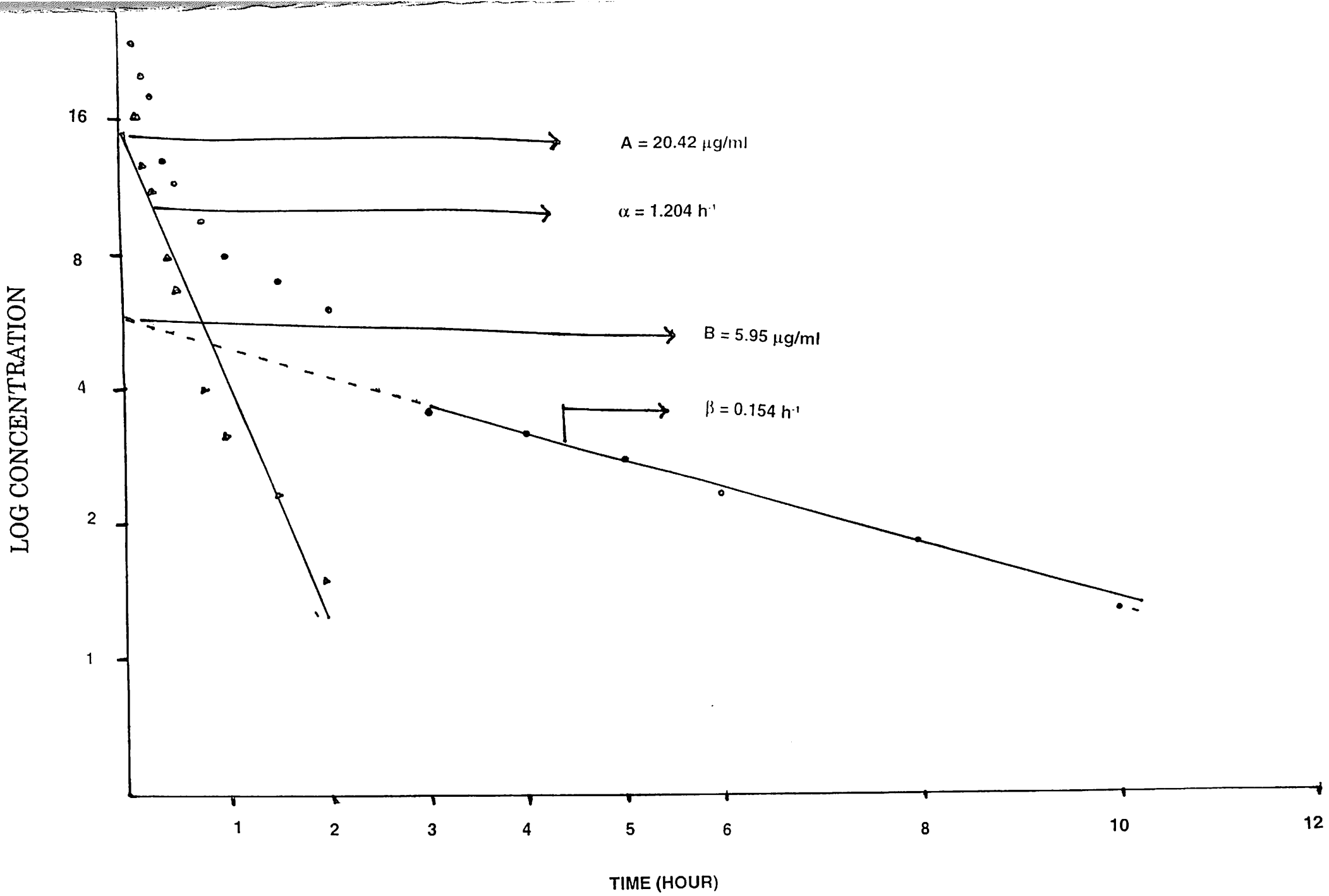

LOG CONCENTRATION
16
8
4
2
1
A = 20.42 µg/ml
α = 1.204 h⁻¹
B = 5.95 µg/ml
β = 0.154 h⁻¹
1
2
3
4
5
6
8
10
12
TIME (HOUR)

TABLE -.3

Kinetic parameters of amikacin in healthy female buffalo calf (calculated by 2-compartment open model) after a single intravenous dose.

Kinetic Parameter	Unit	ANIMAL NUMBER					Mean ± S.E.M
		1	2	3	4	5	
A	µg/ml	15.26	11.91	14.47	11.74	12.78	13.23 ± 0.70
B	µg/ml	4.15	6.17	5.95	4.64	5.20	5.22 ± 0.38
C_P^0	µg/ml	19.21	18.08	20.42	16.38	17.98	18.41 ± 0.67
α	h^{-1}	1.133	8.167	1.204	0.504	0.621	2.326 ± 1.467
β	h^{-1}	0.131	0.232	0.154	0.136	0.127	0.156 ± 0.020
$t_{1/2}\alpha$	h	0.61	0.08	0.58	1.38	1.12	0.75 ± 0.23
$t_{1/2}\beta$	h	5.29	2.98	4.50	5.10	5.46	4.67 ± 0.45
AUC	mg/L.h	45.15	28.05	50.65	57.41	61.52	48.56 ± 5.84
AUMC	$mg/L.h^2$	253.7	114.8	260.8	297.1	355.5	256.4 ± 39.72
MRT	h^{-1}	5.62	4.09	5.15	5.18	5.78	5.16 ± 0.30
K_{12}	h^{-1}	0.489	4.815	0.495	0.114	0.186	1.220 ± 0.902
K_{21}	h^{-1}	0.345	2.940	0.460	0.240	0.270	0.851 ± 0.524
Kel	h^{-1}	0.952	1.925	0.403	0.863	0.292	0.887 ± 0.289
Fc	-	0.30	0.36	0.38	0.48	0.43	0.39 ± 0.03
T ≈ P	-	2.29	1.78	1.62	1.10	1.30	1.62 ± 0.21
Vdc	L/kg	0.39	0.41	0.37	0.46	0.42	0.41 ± 0.02
Vd_B	L/kg	1.81	1.22	1.26	1.62	1.44	1.47 ± 0.11
Vd_{area}	L/kg	1.27	1.15	0.96	0.96	0.96	1.06 ± 0.06
Vd_{SS}	L/kg	0.94	1.08	0.77	0.68	0.71	0.84 ± 0.08
Cl_B	ml/kg/min	2.77	4.45	2.46	2.18	2.03	2.78 ± 0.44

4. *Dosage regimen*

The dosage regimen required maintain the different levels of the therapeutic concentration (C_p^{∞} min = 1, 2 and 4 μg/ml) in plasma for i. v. route in buffalo calves at different selected dosage intervals (γ) of 8 and 12 h are presented in Table 4. For maintaining C_p^{∞} min of 1 μg/ml, the loading doses (D*) were calculated to be 3.95 ± 0.87 and 8.03 ± 2.67 mg/kg, while maintenance doses (D_0) were calculated to be 2.89 ± 0.84 and 6.97 ± 2.64 mg/kg at the dosage interval of 8 and 12h, respectively.

The D*s were calculated to be 7.91 ±1.74 and 16.06 ± 5.33 mg/kg while Do_s were found to be 5.79 ±1.68 and 13.94 ± 5.28 mg /kg at γ of 8 and 12h, respectively, for maintaining C_p^{∞} min of 2 μg/ml. Like wise, to maintain C_p^{∞} min of 4 μg/ml, the D*s were calculated to be 15.82 ±3.47 and 32.11 ± 10.67 mg/kg, while Do_s were found to be 11.50 ± 3.28 and 27.83 ± 10.52 mg/kg at γ of 8 and 12 h.

(B) Diclofenac

The kinetic study of diclofenac in buffalo calves after a single intravenous administration was estimated by HPLC method.

1. *Plasma levels*

Plasma concentrations of diclofenac at various time intervals following single intravenous dose of 1 mg/kg in buffalo

TABLE - 4

Dosage regimen of amikacin in healthy female buffalo calf

C_p^{∞}min (µg/ml)	γ (h)	Dose (mg/kg)	Animal Number					Mean ± S.E.M.
			1	2	3	4	5	
1	8	D*	3.62	7.36	3.29	2.85	2.65	3.95 ± 0.87
		D_0	2.35	6.21	2.33	1.89	1.69	2.89 ± 0.84
	12	D*	6.12	18.61	6.09	4.91	4.41	8.03 ± 2.67
		D_0	4.85	17.46	5.13	3.95	3.45	6.97 ± 2.64
2	8	D*	7.24	14.72	6.58	5.70	5.30	7.91 ± 1.74
		D_0	4.70	12.42	4.66	3.78	3.38	5.79 ± 1.68
	12	D*	12.24	37.22	12.18	9.82	8.82	16.06 ± 5.33
		D_0	9.70	34.92	10.26	7.90	6.90	13.94 ± 5.28
4	8	D*	14.48	29.44	13.16	11.40	10.60	15.82 ± 3.47
		D_0	9.40	24.44	9.32	7.56	6.76	11.50 ± 3.28
	12	D*	24.48	74.44	24.36	19.64	17.64	32.11 ± 10.67
		D_0	19.40	69.64	20.52	15.80	13.80	27.88 ± 10.52

D* = Priming or Loading dose

D_0 = Maintenance dose

γ = Dosage interval

C_p^{∞} min = Minimum therapeutic concentration in plasma (MIC).

calves have been shown in Table 5 and Fig. 4. The mean plasma concentration of the drug at 0.042 h was found to be 7.04 ± 0.75 μg/ml and the value ranged from 4.21 to 8.73 μg/ml. The drug was detectable in three out of five animals at 24 h and the mean plasma concentration was 0.03 ± 0.01 μg/ml.

2. *Urine levels*

The drug concentrations in urine following single intravenous administration of diclofenac (1 mg/kg) have been presented in Table 6 and Fig. 5 . The drug appeared at 0.042 h in two out of five animals with a mean value of 0.14 ± 0.09 $kg.ml^{-1}$ while the drug appeared in all five animals at 0.083 h and was maintained upto 24 h in all animals with a mean value of 1.29 ± 0.18 $\mu g.ml^{-1}$. The drug appeared in three out of five animals at 30 h with a mean value of 0.24 ± 0.10 $\mu g.ml^{-1}$ and appeared in one animal only at 36 h. The mean peak urine concentration of 30.01 ± 6.58 $\mu g.ml^{-1}$ was observed at 0.167 h.

3. *Kinetic parameters*

Plasma drug concentration versus time profile has confirmed the two-compartment open model. Table 7 shows the values of different kinetic parameters calculated by the above noted compartment model.

The mean extrapolated zero time concentration of the drug in plasma during distribution phase (A), elimination phase (B)

TABLE - 5

Plasma concentrations (μg/ml) of diclofenac in buffalo calves following single intravenous dose of 1 mg/kg.

Time (h)	Animal Number					Mean ± S.E.M.
	1	2	3	4	5	
0.042	4.21	8.73	7.63	7.25	7.40	7.04 ± 0.75
0.083	3.02	6.81	6.16	6.20	6.25	5.69 ± 0.68
0.167	1.69	6.47	5.99	5.42	5.45	5.00 ± 0.85
0.25	1.67	5.23	4.75	5.00	5.00	4.33 ± 0.67
0.333	1.50	4.65	4.51	4.32	4.35	3.87 ± 0.59
0.50	1.49	4.06	3.56	3.84	3.42	3.27 ± 0.46
0.75	1.46	3.01	2.54	2.62	2.45	2.42 ± 0.26
1	1.10	2.59	2.08	2.00	1.90	1.93 ± 0.24
1.5	0.93	1.82	1.72	1.50	1.60	1.51 ± 0.16
2	0.89	1.48	1.61	1.35	1.25	1.32 ± 0.12
3	0.71	0.79	1.23	1.15	1.00	0.98 ± 0.10
4	0.69	0.59	1.03	0.90	0.72	0.79 ± 0.08
5	0.56	0.55	0.77	0.70	0.58	0.63 ± 0.04
6	0.42	0.47	0.56	0.65	0.50	0.52 ± 0.04
8	0.36	0.26	0.25	0.50	0.24	0.32 ± 0.05
10	0.30	0.10	0.17	0.22	0.18	0.19 ± 0.03
12	0.20	0.09	0.13	0.12	0.15	0.14 ± 0.02
24	0.06	0.02	N.D.	N.D.	0.07	0.03 ± 0.01

N.D. = Non-detectable

Fig. 4

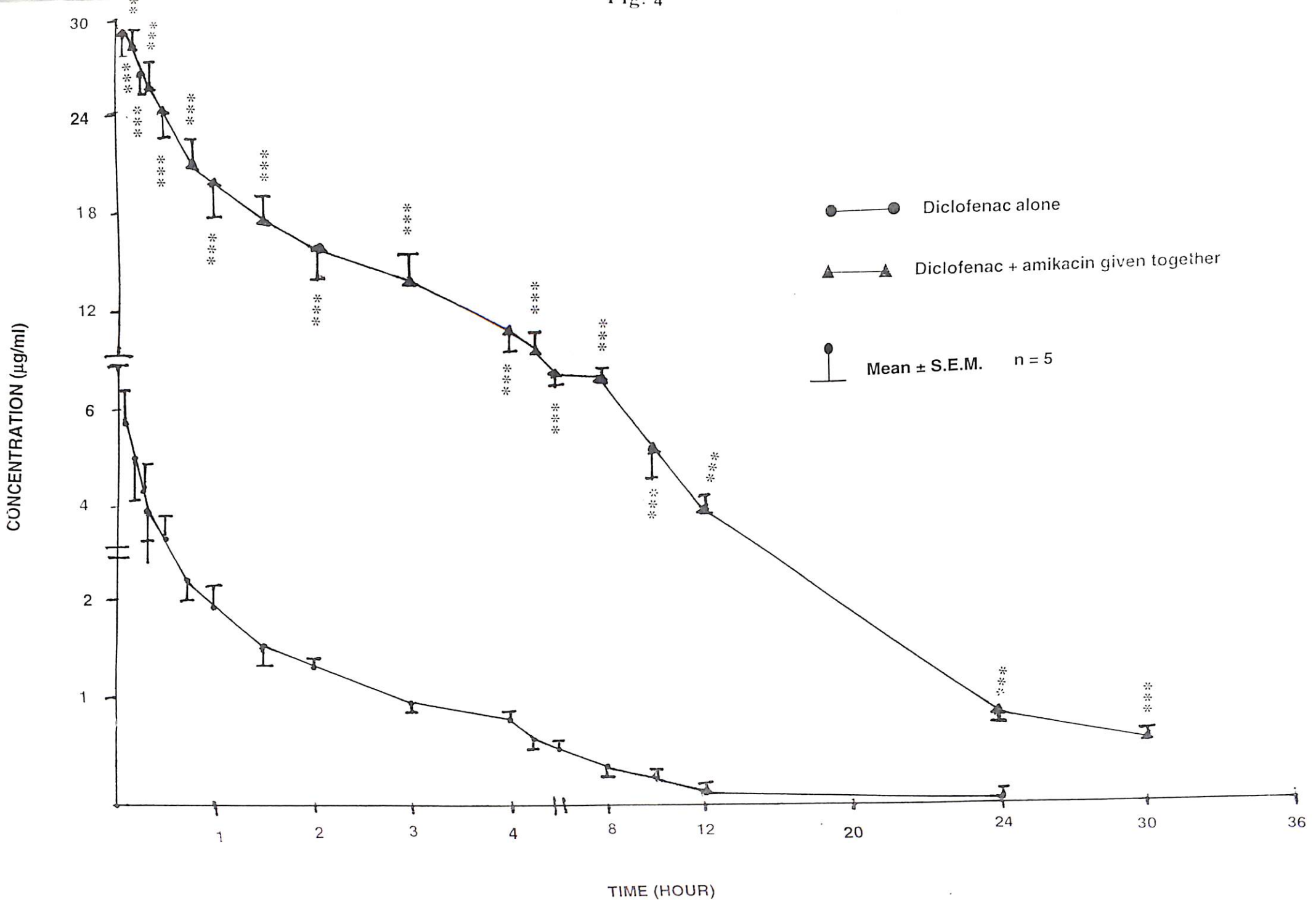

30
24
18
12
6
4
2
1
CONCENTRATION (µg/ml)
Diclofenac alone
Diclofenac + amikacin given together
Mean ± S.E.M.
n = 5
1
2
3
4
8
12
20
24
30
36
TIME (HOUR)

TABLE - 6

Urine concentrations (μg/ml) of diclofenac in buffalo calves following single intravenous dose of 1 mg/kg.

Time (h)	Animal Number					Mean ± S.E.M.
	1	2	3	4	5	
0.042	N. D.	N. D.	N. D.	0.45	0.26	0.14 ± 0.09
0.083	0.50	2.52	8.24	4.82	2.28	3.67 ± 1.33
0.167	52.36	25.65	18.96	36.55	16.54	30.01 ± 6.58
0.25	28.33	35.50	23.82	25.16	24.25	27.41 ± 2.17
0.333	25.48	28.60	27.09	22.45	31.62	27.05 ± 1.53
0.50	20.98	22.85	22.73	18.54	24.15	21.85 ± 0.97
0.75	17.03	17.68	18.46	16.28	18.54	17.60 ± 0.43
1	15.49	15.55	17.50	12.45	16.12	15.42 ± 0.83
1.5	13.44	13.86	16.25	11.68	14.15	13.88 ± 0.73
2	10.83	11.22	15.87	10.52	12.88	12.26 ± 0.99
3	9.82	10.15	15.07	9.82	10.45	11.06 ± 1.01
4	5.74	8.82	10.14	7.65	9.12	8.29 ± 0.75
5	5.26	4.56	8.41	5.25	6.62	6.02 ± 0.68
6	4.48	4.10	7.80	4.64	4.00	5.00 ± 0.71
8	3.73	3.80	7.28	3.92	2.80	4.31 ± 0.77
10	3.04	3.10	6.44	2.84	2.10	3.50 ± 0.76
12	2.06	2.16	4.33	2.42	1.58	2.51 ± 0.47
24	1.51	1.22	0.98	1.85	0.88	1.29 ± 0.18
30	0.29	0.38	N. D.	0.52	N. D.	0.24 ± 0.10
36	0.19	N. D.	-	N. D.	-	0.04 ± 0.04
48	N. D.	-	-	-	-	-

N.D. = Non-detectable

Fig. 5

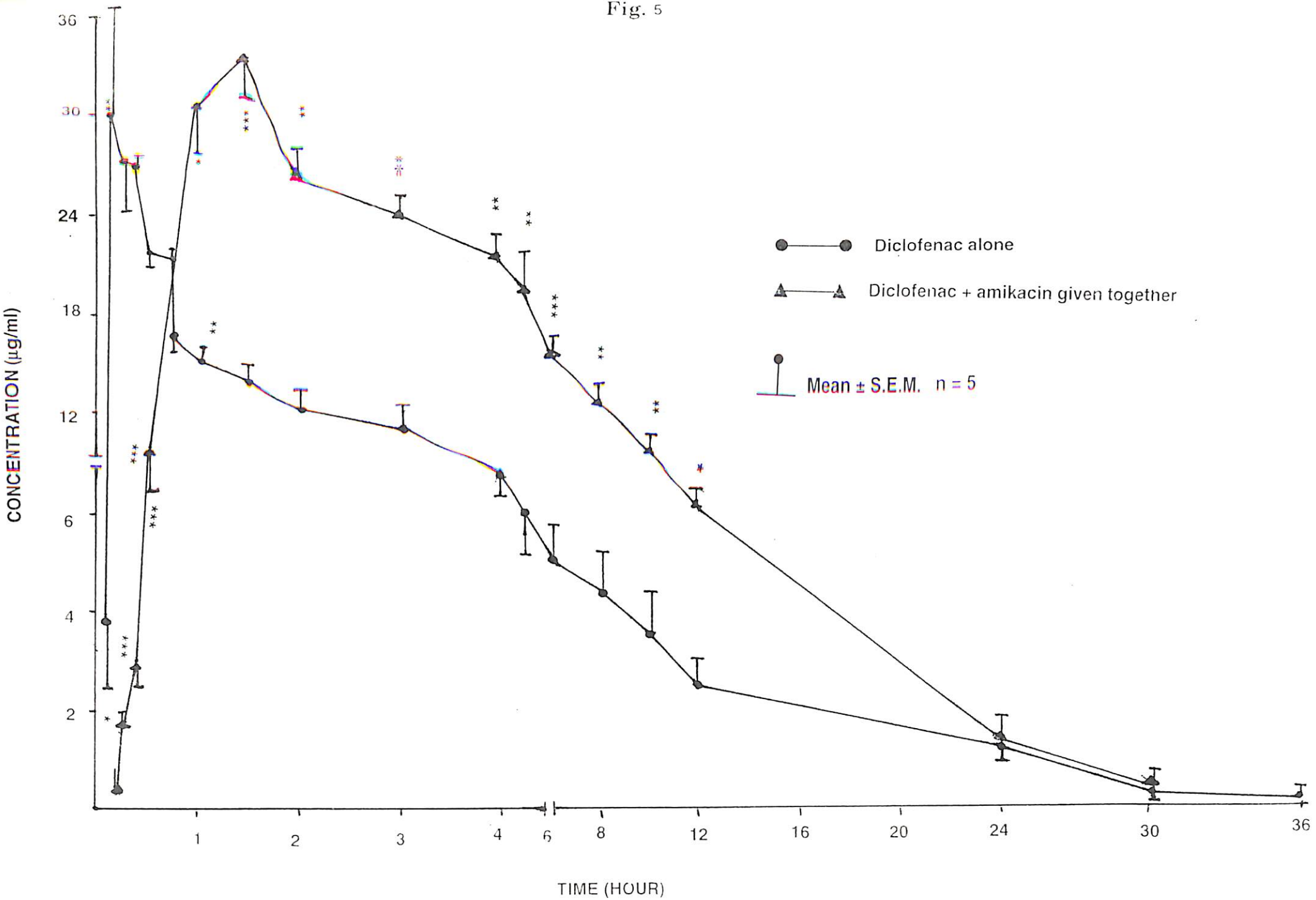

CONCENTRATION (µg/ml)
36
30
24
18
12
6
4
2
1
2
3
4
6
8
12
16
20
24
30
36
TIME (HOUR)
Diclofenac alone
Diclofenac + amikacin given together
Mean ± S.E.M. n = 5

TABLE - 7

Kinetic parameters of diclofenac in buffalo calves following single intravenous dose of 1 mg/kg.

Parameter (unit)	Animal Number					Mean ± S.E.M.
	1	2	3	4	5	
A ($\mu g.\ ml^{-1}$)	1.70	5.98	9.27	6.07	5.66	5.74 ± 1.20
B ($\mu g.\ ml^{-1}$)	1.03	1.12	2.71	2.29	1.09	1.65 ± 0.35
C_p^0 ($\mu g.\ ml^{-1}$)	2.73	7.10	11.98	8.36	6.75	7.38 ± 1.49
α (h^{-1})	2.19	1.18	5.70	3.18	1.56	2.76 ± 0.81
$t_{½}\ \alpha$ (h)	0.32	0.59	0.12	0.22	0.44	0.34 ± 0.08
β (h^{-1})	0.12	0.18	0.27	0.23	0.13	0.19 ± 0.03
$t_{½}\ \beta$ (h)	5.62	3.84	2.59	3.02	5.22	4.06 ± 0.59
AUC ($mg.L^{-1}.h$)	9.36	11.29	11.66	11.87	12.01	11.24 ± 0.48
AUMC ($mg.\ L^{-1}.h^2$)	71.88	38.86	37.46	43.89	66.82	51.78 ± 7.30
MRT (h)	7.68	3.44	3.21	3.70	5.56	4.72 ± 0.85
K_{12} (h^{-1})	1.12	0.40	3.44	1.67	0.77	1.48 ± 0.53
K_{21} (h^{-1})	0.90	0.34	1.50	1.04	0.36	0.83 ± 0.22
Kel (h^{-1})	0.29	0.62	1.03	0.70	0.56	0.64 ± 0.12
Fc	0.41	0.29	0.26	0.33	0.23	0.30 ± 0.03
$T \approx P$	1.44	2.50	2.80	2.06	3.35	2.43± 0.32
Vdc ($L.kg^{-1}$)	0.37	0.14	0.08	0.12	0.15	0.17 ± 0.05
Vd_B ($L.kg^{-1}$)	0.97	0.89	0.37	0.44	0.92	0.72 ± 0.13
Vd_{area} ($L.kg^{-1}$)	0.89	0.49	0.32	0.37	0.64	0.54 ± 0.10
Vd_{SS} ($L.kg^{-1}$)	0.83	0.30	0.26	0.31	0.47	0.43 ± 0.10
Cl_B ($ml.\ kg^{-1}.min^{-1}$)	1.78	1.50	1.50	1.50	1.34	1.52 ± 0.07

and theoretical zero time concentration (C_p^0 = A+B) were noted to be 5.74 ± 1.20, 1.65 ± 0.35 and 7.38 ± 1.49 μg/ml, respectively. The distribution rate constant (α) ranged from 1.18 to 5.70 h^{-1} with a mean value of 2.76 ± 0.81 h^{-1} while its elimination rate constant (β) ranged from 0.12 to 0.27 h^{-1} with a mean value of 0.19± 0.03 h^{-1}. The mean distribution half life ($t_{1/2}$ α) and elimination half life ($t_{1/2}$ β) values of the drug were observed to be 0.34±0.08 and 4.06 ± 0.59 h, respectively. The average rate of transfer of drug from central to peripheral (K_{12}), peripheral to central (K_{21}) and elimination from central (Kel) compartment were calculated to be 1.48 ± 0.53, 0.83 ±0.22 and 0.64 ± 0.12 h^{-1}, respectively. The fraction of drug available for elimination from central compartment (Fc) and approximate tissue to plasma concentration ratio (T≈P) were noted to be 0.30 ± 0.03 and 2.43 ± 0.32 . The value of area under curve in plasma (AUC) and area under first moment curve (AUMC) were found to 11.24 ± 0.48 mg/L.h and 51.78 ± 7.30 mg/L.h^2 with a mean residential time (MRT) of 4.72 ± 0.85 h. The various values of volume of distribution calculated by different methods are shown in Table 7. The mean value of Vd_{area} was calculated to be 0.54 ± 0.10 L/kg. The total body clearance (Cl_B) ranged from 1.34 to 1.78 with a mean value of 1.52 ± 0.07 ml/kg/min.

II. PHARMACOKINETICS STUDIES OF DRUGS AFTER COMBINED ADMINISTRATION OF AMIKACIN AND DICLOFENAC

(A) Amikacin

1. Plasma levels

Plasma concentrations of amikacin at various time intervals following combined intra venous administration of amikacin (7.5 mg /kg) and diclofenac (1 mg/kg) have been shown in Table 8 and Fig. 1. The drug was present at 0.083 h with a mean of 1.81 ± 0.21 μg/ml and was detectable in plasma sample of all the buffalo calves up to 12 h with a mean value of 0.27 ±0.03 μg/ml. The drug was detectable in 2 out of 5 animals at 24 h (0.06 ±0.04 μg/ml) and none of the animals at 30 h.

2. Urine levels

Urine concentrations of amikacin at various time intervals following combined i. v. administration of amikacin (7.5 mg/kg) and diclofenac (1 mg/kg) have been presented in Table 9 and Fig 2. The drug appeared in all animals at 0.083 h with the mean of 258.1 ± 52.53 μg/ml. The drug attained its peak concentration of 2480 ± 386.2 μg/ml at 1 h. The drug was detectable in all animals up to 36 h (1.98 ± 0.25 μg/ml) and none at 48 h. The therapeutic concentration of 2 μg/ml was maintained around 36 h.

TABLE - 8

Plasma concentrations (μg/ml) of amikacin in buffalo calves following combination administration of amikacin (7.5 mg/kg) and diclofenac (1 mg/kg) after i. v. administration.

Time (h)	ANIMAL NUMBER					Mean ± S.E.M
	1	2	3	4	5	
0.083	1.64	2.05	1.52	1.32	2.52	1.81 ± 0.21
0.167	1.45	1.82	1.40	1.28	2.20	1.63 ± 0.17
0.25	1.28	1.60	1.35	1.20	2.12	1.51 ± 0.17
0.333	1.13	1.52	1.20	1.08	1.90	1.37 ± 0.15
0.50	1.00	1.40	1.15	0.96	1.75	1.25 ± 0.15
0.75	0.89	1.26	1.08	0.92	1.40	1.11 ± 0.10
1	0.83	1.26	1.02	0.88	1.32	1.06 ± 0.10
1.5	0.78	1.08	0.95	0.80	1.10	0.94 ± 0.07
2	0.74	0.92	0.86	0.70	1.00	0.84 ± 0.06
3	0.69	0.88	0.80	0.62	0.92	0.78 ± 0.06
4	0.61	0.76	0.68	0.54	0.86	0.69 ± 0.06
5	0.54	0.68	0.58	0.50	0.78	0.62 ± 0.05
6	0.48	0.60	0.46	0.45	0.76	0.55 ± 0.06
8	0.42	0.48	0.42	0.35	0.54	0.44 ± 0.03
10	0.29	0.40	0.30	0.26	0.50	0.35 ± 0.04
12	0.23	0.32	0.20	0.22	0.36	0.27 ± 0.03
24	N.D.	0.10	N.D.	N.D.	0.18	0.06 ± 0.04

N.D. = Non-detectable

TABLE - 9

Urine concentrations (μg/ml) of amikacin in buffalo calf following combined administration of amikacin (7.5 mg/kg) and diclofenace (1 mg/kg) after i.v. administration

Time (h)	ANIMAL NUMBER					MEAN ± S.E.M.
	1	2	3	4	5	
0.083	96.6	320.5	250.8	410.0	212.5	258.1 ± 52.53
0.167	166.8	495.0	460.4	528.6	410.8	412.3 ± 64.40
0.25	183.5	680.8	648.2	720.8	625.5	571.8 ± 98.38
0.333	290.2	912.4	875.0	988.6	860.6	785.4 ± 125.8
0.50	498.0	1200	1178	1290	1155	1064 ± 143.4
0.75	580.6	1455	1375	1555	1288	1251 ± 173.2
1	1014	2950	2878	3150	2406	2480 ± 386.2
1.5	3062	1180	1162	1290	3100	1959 ± 458.4
2	1280	915.6	889.4	1025	1298	1082 ± 87.72
3	424.8	480.4	462.6	492.8	480.8	468.3 ± 11.89
4	208.2	280.6	275.5	290.6	280.6	267.1 ± 14.93
5	104.5	124.5	115.8	128.4	122.2	119.1 ± 4.18
6	88.12	95.55	90.28	99.98	92.26	93.24 ± 2.08
8	58.38	62.24	58.44	65.52	60.12	60.94 ± 1.34
10	30.68	32.26	30.16	36.50	34.55	32.83 ± 1.19
12	19.85	20.15	18.55	24.15	22.20	20.98 ± 0.99
24	12.24	13.34	12.20	14.55	11.85	12.84 ± 0.50
30	9.67	10.24	8.65	10.20	8.65	9.48 ± 0.35
36	1.84	2.10	1.45	2.88	1.64	1.98 ± 0.25

3. *Kinetic Parameters*

Plasma drug concentration versus time profile has confirmed a two compartment open model, and hence, the kinetic parameters were calculated by using the formulae of the above noted compartment model.

Table 10 presents the values of different kinetic parameters of amikacin after its combined administration with diclofenac. The mean extrapolated zero time concentration of the drug in plasma during distribution phase (A), elimination phase (B) and the theoretical zero time concentration- (C_p^0 = A+B) were noted to be 0.87 ± 0.21, 1.07 ± 0.06 and 1.97 ± 0.23 μg/ml, respectively. The distribution rate constant (α) ranged from 1.377 to 5.250 h^{-1} with the mean value of 2.885 ± 0.813 h^{-1} where as its elimination rate constant ranged from 0.082 to 0.124 h with a mean of 0.112 ±0.010 h^{-1}.

The mean distribution half life ($t_{1/2}$ α) and elimination half life ($t_{½}$ β) were observed to be 0.32 ± 0.08 and 6.37 ± 0.60 h. The value of area under curve in plasma (AUC), the area under first moment curve (AUMC) and mean residential time (MRT) were found to be 10.25 ± 1.34 mg/L.h, 100.9 ± 25.04 mg/L. h^2 and 9.35 ± 1.01 h, respectively. The average rate of transfer of drug from central to peripheral (K_{12}), peripheral to central (K_{21}) and elimination from central (Kel) compartment were calculated to be 1.082 ± 0.426,

TABLE - 10

Kinetic parameters of amikacin in buffalo calf following combined administration of amikacin (7.5 mg/kg) and diclofenac (1 mg/kg) after i.v. administration.

Parameter (Unit)	ANIMAL NUMBER					Mean ± S.E.M
	1	2	3	4	5	
A (μg/ml)	1.13	0.80	0.49	0.41	1.52	0.87 ± 0.21
B (μg/ml)	0.97	1.13	1.18	0.89	1.16	1.07 ± 0.06
C_P^0 (μg/ml)	2.10	1.93	1.67	1.30	2.68	1.94 ± 0.23
α (h^{-1})	5.250	1.377	4.429	1.464	1.903	2.885 ± 0.813
$t_{1/2}\alpha$ (h)	0.13	0.50	0.14	0.47	0.36	0.32 ± 0.08
β (h^{-1})	0.118	0.102	0.142	0.118	0.082	0.112 ± 0.010
$t_{1/2}\beta$ (h)	5.87	6.79	4.88	5.87	8.45	6.37 ± 0.60
AUC (mg/L.h)	8.44	11.65	8.42	7.82	14.91	10.25 ± 1.34
AUMC (mg/L.h^2)	69.70	113.4	59.02	68.65	193.7	100.9 ± 25.04
MRT (h)	8.26	9.73	7.01	8.78	12.99	9.35 ± 1.01
K_{12} (h^{-1})	2.631	0.466	1.203	0.175	0.937	1.082 ± 0.426
K_{21} (h^{-1})	2.480	0.848	3.170	1.039	0.869	1.681 ± 0.481
Kel (h^{-1})	0.249	0.165	0.198	0.166	0.179	0.191 ± 0.016
Fc	0.47	0.62	0.17	0.71	0.46	0.59 ± 0.06
T ≈ P	1.05	0.62	0.39	0.19	1.19	0.69 ± 0.19
Vdc (L/kg)	3.57	3.89	4.49	5.77	2.80	4.10 ± 0.50
Vd_B (L/kg)	7.73	6.64	6.36	8.43	6.47	7.13 ± 0.41
Vd_{area} (L/kg)	7.53	6.30	6.28	8.15	6.15	6.88 ± 0.40
Vd_{SS} (L/kg)	7.35	6.03	6.19	1.01	2.62	4.64 ± 1.20
Cl_B (ml/kg/min)	14.81	10.71	14.86	16.03	8.41	12.96 ± 1.45

1.681 ± 0.481 and 0.191 ± 0.016 h^{-1}, respectively. The fraction of drug available for elimination from central compartment (Fc) and approximate tissue to plasma concentration ratio (T≈P) were noted to be 0.59 ± 0.06 and 0.69 ± 0.19. The various values of volume distribution calculated by different methods are shown in Table 10. The mean value of Vd_{area} was calculated to be 6.88 ± 0.40 L/kg. The total body clearance (Cl_B) ranged from 8.41 to 16.03 ml/kg/min with an average of 12.96 ± 1.45 ml/kg/min.

4. Dosage regimen

Table 11 presents the dosage regimen of amikacin following combined administration of this drug (7.5 mg/kg i.v.) and diclofenac (1 mg/kg, i.v) in buffalo calves. For maintaining C_P^{∞} min of 1 μg/ml, the loading doses (D*s) were calculated to be 17.15 ± 1.78 and 27.32 ± 3.65 mg/kg while maintenance doses (D_0s) were calculated to be 10.27 ± 1.51 and 20.44 ± 3.42 mg/kg at the selected dosage intervals (γ) of 8 and 12h, respectively. Similarly, for maintaining C_P^{∞} min of 2 μg/ml. The D*s were noted to be 34.31 ± 3.57 and 54.64 ± 7.30 mg/kg, while D_0s were noted to be 20.54 ±3.03 mg/kg and 40.87 ± 6.84 mg/kg at γ of 8 and 12 h, respectively. For maintaining C_P^{∞} min of 4 μg/ml, the calculated D*s and D_0s were noted to be 68.62 ±7.14 & 109.3 ± 14.59 and 41.09 ± 6.0+6 & 81.74 ± 13.68 mg/kg, respectively, at γ of 8 & 12 h.

TABLE - 11

Dosage regimen of amikacin in buffalo calf following administration of amikacin (7.5 mg/kg) and diclofenac (1 mg/kg) after i. v. administration.

C_p^{∞} min (µg/ml)	γ (h)	Dose (mg/kg)	Animal Number					Mean ± S.E.M.
			1	2	3	4	5	
1	8	D*	19.35	14.25	19.56	20.95	11.66	17.15 ± 1.78
		D_0	11.82	7.95	13.28	12.80	5.51	10.27 ± 1.51
	12	D*	31.02	21.42	34.51	33.58	16.06	27.32 ± 3.65
		D_0	23.49	15.12	28.33	25.43	9.91	20.44 ± 3.42
2	8	D*	38.70	28.50	39.12	41.90	23.32	34.31 ± 3.57
		D_0	23.64	15.90	26.56	25.60	11.02	20.54 ± 3.03
	12	D*	62.04	42.84	69.02	67.16	32.12	54.64 ± 7.30
		D_0	46.98	30.24	56.46	50.86	19.82	40.87 ± 6.84
4	8	D*	77.40	57.00	78.24	83.80	46.64	68.62 ± 7.14
		D_0	47.28	31.80	53.12	51.20	22.04	41.09 ± 6.06
	12	D*	124.1	85.68	138.01	134.3	64.24	109.3 ± 14.59
		D_0	93.96	60.48	112.9	101.7	39.64	81.74 ± 13.68

D* = Priming or Loading dose

D_0 = Maintenance dose

γ = Dosage interval

C_p^{∞} min = Minimum therapeutic concentration in plasma (MIC).

(B) Diclofenac

1. Plasma levels

Concentrations of diclofenace in plasma after combined i.v administration amikacin (7.5 mg/kg) and diclofenace (1 mg/kg) are presented in Table 12 and Fig. 4. The drug appeared with a mean concentration of 29.36 ± 1.64 μg/ml at 0.083 h. The drug was present in all animals up to 30 h with a mean of 0.37 ± 0.03 μg/ml. There after, the drug was not detected in any of the samples.

2. Urine levels

Table 13 and Fig. 5 depict the urine concentrations of diclofenac in buffalo calves following combined i. v. administration of amikacin (7.5 mg/kg) and diclofenac (1 mg/kg). The drug did not appear at 0.083 (first sample) in any of the animals; however, the drug appeared in four out of five animals at 0.167 h with a mean of 0.36 ±0.11 μg/ml. The drug reached its peak urine concentration of 34.47 ± 1.53 μg/ml at 1.5 h. Thereafter, the drug declined with time and was present in all animals up to 12 h. The drug was present in 3 out of 5 animals at 24 h (1.41 ± 0.16 μg/ml) and 30 h (0.33 ± 0.16 μg/ml). The drug was not detected in any of the samples at 36 h.

3. Kinetic parameters

The plasma drug concentration versus time profile had shown a biphasic pattern following combined i. v. administration of amikacin and diclofenac and hence, kinetic parameters were derived by using the methods of 2 compartment open model.

TABLE - 12

Plasma concentrations (μg/ml) of diclofenac in buffalo calves following combined administration amikacin (7.5 mg/kg) and diclofenac (1 mg/kg) after intravenous administration.

Time (h)	ANIMAL NUMBER					Mean ± S.E.M
	1	2	3	4	5	
0.083	26.61	27.10	34.00	26.45	32.65	29.36 ± 1.64
0.167	26.17	26.86	32.15	25.96	30.14	28.25 ± 1.23
0.25	24.82	24.95	30.08	24.10	28.54	26.50 ±1.18
0.333	23.78	24.18	29.52	22.06	27.92	25.49 ±1.39
0.50	22.94	23.08	27.68	20.00	26.80	24.10 ± 1.40
0.75	18.15	19.05	25.16	17.86	24.65	20.97 ± 1.62
1	17.71	18.22	24.08	15.14	23.86	19.80 ± 1.78
1.5	15.87	17.05	21.54	12.76	20.24	17.49 ± 1.57
2	15.16	16.12	19.22	10.85	18.06	15.88 ± 1.45
3	15.02	14.22	16.10	8.10	15.62	13.81 ± 1.46
4	11.69	11.15	13.12	7.26	12.55	11.15 ± 1.03
5	10.69	9.12	11.05	6.85	11.24	9.79 ± 0.82
6	9.21	8.20	10.00	6.12	9.50	8.61 ± 0.69
8	7.35	5.85	7.72	5.24	7.42	6.72 ± 0.49
10	5.10	4.62	6.06	4.16	6.12	5.21 ± 0.39
12	4.06	3.50	4.52	3.46	4.28	3.96 ± 0.21
24	0.83	0.75	0.85	1.12	0.96	0.90 ± 0.06
30	0.34	0.28	0.36	0.45	0.40	0.37 ± 0.03

TABLE - 13

Urine concentrations (μg/ml) of diclofenac in buffalo calves following combined administration of amikacin (7.5 mg/kg) and diclofenac (1 mg/kg) after intravenous administration

Time (h)	ANIMAL NUMBER					Mean ± S.E.M
	1	2	3	4	5	
0.083	N.D.	N.D.	N.D.	N.D.	N.D.	
0.167	N.D.	0.52	0.26	0.58	0.45	0.36 ± 0.11
0.25	1.53	1.85	1.25	2.10	1.65	1.68 ± 0.14
0.333	2.68	3.12	2.14	3.58	3.12	2.93 ± 0.24
0.50	8.24	10.85	7.56	11.12	10.56	9.67 ± 0.73
0.75	20.21	22.25	18.85	24.16	22.24	21.54 ± 0.92
1	30.03	38.65	25.50	27.15	34.12	31.09 ± 2.39
1.5	36.62	32.26	34.42	38.85	30.22	34.47 ± 1.53
2	27.63	25.15	24.22	29.16	26.68	26.57 ± 0.88
3	26.97	22.26	20.50	27.25	24.22	24.24 ± 1.31
4	24.73	20.10	18.86	25.12	19.65	21.69 ± 1.34
5	23.76	18.64	16.12	22.22	16.80	19.51 ± 1.50
6	16.82	15.12	14.50	16.50	14.85	15.56 ± 0.46
8	14.05	12.54	1.58	14.65	11.96	12.96 ± 0.60
10	10.98	9.08	8.80	11.15	9.12	9.83 ± 0.51
12	7.11	6.51	4.10	8.12	5.50	6.27 ± 0.69
24	N.D.	2.08	N.D.	3.10	1.85	1.41 ± 0.61
30	N.D.	0.42	N.D.	0.86	0.35	0.33 ± 0.16

N.D. = Non-detectable

Table 14 presents the different values of kinetic parameters calculated by the above noted compartment model. The extrapolated zero time concentration during distribution phase (A), elimination phase (B) and theoretical zero time concentration (C_p^0) were noted to be 11.61 ±1.70, 18.91 ± 1.98 and 30.53 ±1.07 μg/ml. The distribution rate constant (α) and elimination rate constant (β) were noted to be 1.02 ± 0.13 and 0.13 ± 0.01 h^{-1}. Distribution half life ($t_{1/2}$ α) ranged from 0.48 to 0.87 h with a mean of 0.73 ± 0.08 h where as the elimination half life ranged from 4.95 to 6.73 h with an average of 5.46 ± 0.33 h. Area under curve (AUC), area under first moment curve (AUMC) and mean residential time (MRT) were calculated to be 157.9 ± 9.85 mg/L.h, 1168 ± 52.85 mg/L.h^2 and 7.48 ± 0.46 h. The average rate constant of drug transfer from central to peripheral (K_{12}), peripheral to central (K_{21}) and elimination from central (Kel) compartment were observed to be 0.27 ± 0.07, 0.68 ± 0.11 and 0.19 ± 0.01 h^{-1}. The mean values of 0.67 ± 0.06 and 0.55 ± 0.16 were noted for fraction of drug available for elimination from central compartment (FC) and approximate tissue to plasma concentration ratio (T≈P), respectively. Table 14 presents the various values of volume distribution calculated by different methods. Vd_{area} of 0.38 ±0.05 L/kg was calculated. The total body clearance ranged from 0.67 to 1.00 ml/kg/min with a mean of 0.80 ±0.06 ml/kg/min.

TABLE - 14

Kinetic parameters of diclofenac in buffalo calves following combined administration of amikacin (7.5 mg/kg) and diclofenac (1 mg/kg) after intravenous administration.

Parameter (Unit)	ANIMAL NUMBER					Mean ± S.E.M
	1	2	3	4	5	
A (μg/ml)	6.90	13.98	10.43	16.72	10.04	11.61 ± 1.70
B (μg/ml)	20.80	18.19	22.57	11.53	21.47	18.91 ± 1.98
C_P^0 (μg/ml)	27.70	32.17	33	28.25	31.51	30.53 ± 1.07
α (h^{-1})	1.45	0.81	0.81	1.21	0.80	1.02 ± 0.13
$t_{1/2}$ α (h)	0.48	0.86	0.86	0.57	0.87	0.73 ± 0.08
β (h^{-1})	0.14	0.14	0.13	0.10	0.13	0.13 ± 0.01
$t_{1/2}$ β (h)	4.95	4.95	5.33	6.73	5.33	5.46 ± 0.33
AUC (mg/L.h)	157.1	150.0	181.3	125.8	175.2	157.9 ± 9.85
AUMC (mg/L.h^2)	1128	994.0	1277	1164	1278	1168 ± 52.85
MRT (h)	7.18	6.63	7.04	9.26	7.30	7.48 ± 0.46
K_{12} (h^{-1})	0.29	0.22	0.16	0.54	0.16	0.27 ± 0.07
K_{21} (h^{-1})	1.12	0.52	0.60	0.55	0.59	0.68 ± 0.11
Kel (h^{-1})	0.18	0.21	0.18	0.22	0.18	0.19 ± 0.01
Fc	0.77	0.64	0.74	0.46	0.74	0.67 ± 0.06
T ≈ P	0.29	0.57	0.36	1.18	0.37	0.55 ± 0.16
Vdc (L/kg)	0.27	0.23	0.22	0.27	0.24	0.25 ± 0.01
Vd_B (L/kg)	0.36	0.41	0.33	0.65	0.35	0.42 ± 0.06
Vd_{area} (L/kg)	0.35	0.36	0.30	0.58	0.32	0.38 ± 0.05
Vd_{SS} (L/kg)	0.34	0.33	0.28	0.52	0.31	0.36 ± 0.04
Cl_B (ml/kg/min)	0.79	0.83	0.67	1.00	0.70	0.80 ± 0.06

III. COMPARISON OF PHARMACOKINETICS OF AMIKACIN WHEN GIVEN ALONE AND WHEN GIVEN TOGETHER WITH DICLOFENAC BY I. V. ADMINISTRATION

1. Plasma levels

Comparison of plasma concentrations of amikacin (7.5 mg/kg), when given alone and when given together with diclofenac (1 mg/kg) after i.v. administration are shown in Table 15 and Fig. 1. The concentrations of amikacin were found to be significantly lower in case of its combined administration with diclofenac as compared to its single administration from 0.083 to 10 h. Only non significant difference was noted at 12 and 24 h. The therapeutic concentration (2 μg/ml) was maintained up to 6 h when amikacin given alone, while therapeutic concentration was not at all attained in case of its combined administration with diclofenac. The drug present up to 24 h in both groups.

2. Urine levels

Table 15 and Fig. 2 reveal the urine concentrations of amikacin when given alone and when given together with diclofenac. Initially from 0.083 to 0.333 h, the urine levels of amikacin in combined administration group was noted to be lower non significantly but it was significantly ($p<0.01$) lower at 0.333 h as compared to its alone administration. Thereafter, increases in urine

TABLE - 15

Comparison of plasma and urine concentrations (μg/ml) of amikacin (7.5 mg/kg) when given alone and when given together with diclofenac (1 mg/kg) in buffalo calves following intravenous administration.

TIME (h)	Amikacin given alone		Amikacin + diclofenac give together	
	Plasma	Urine	Plasma	Urine
0.083	22.90 ± 3.16	321.8 ± 98.26	1.81 ± 0.21 **	258.1 ± 52.53 NS
0.167	17.44 ± 1.95	572.5 ± 122.6	1.63 ± 0.17 **	412.3 ± 64.40 NS
0.25	14.69 ± 1.87	1041 ± 300.2	1.51 ± 0.17 **	571.8 ± 98.38 NS
0.333	11.62 ± 1.33	1098 ± 74.70	1.37 ± 0.15 **	785.4 ± 125.8 **
0.50	10.52 ± 1.32	898.9 ± 80.79	1.25 ± 0.15 **	1064 ± 143.4 NS
75	8.77 ± 1.03	612.6 ± 52.17	1.11 ± 0.10 **	1251 ± 173.2 **
1	7.81 ± 0.84	451.2 ± 19.31	1.06 ± 0.10 **	2480 ± 386.2 **
1.5	6.95 ± 0.82	418.7 ± 22.41	0.94 ± 0.07 **	1959 ± 458.4*
2	5.88 ± 0.65	344.8 ± 20.16	0.84 ± 0.06 **	1082 ± 87.72 **
3	4.08 ± 0.57	294.7 ± 31.58	0.78 ± 0.06 **	468.3 ± 11.89 **
4	3.11 ± 0.23	251.5 ± 32.76	0.69 ± 0.06 **	267.1 ± 14.93 NS
5	2.57 ± 0.15	170 ± 26.62	0.62 ± 0.05 ***	119.1 ± 4.18 NS
6	2.04 ± 0.15	79.98 ± 17.41	0.55 ± 0.06 ***	93.24 ± 2.08 NS
8	1.57 ± 0.17	56.98 ± 18.81	0.44 ± 0.03 **	60.94 ± 1.34 NS
10	1.07 ± 0.14	33.99 ± 14.97	0.35 ± 0.04 **	32.83 ± 1.19 NS
12	0.56 ± 0.18	27.24 ± 13.19	0.27 ± 0.03 NS	20.98 ± 0.99 NS
24	0.18 ± 0.05	3.85 ± 1.37	0.06 ± 0.04 NS	12.84 ± 0.50 ***
30	N.D.	N.D.	N.D.	9.48 ± 0.35 ***
36	N.D.	N.D.	N.D.	1.98 ± 0.25 **

N.D. Non-detectable, NS Non-significant, ** $p < 0.01$, *** $p < 0.001$.

levels were noted and it was significant from 0.75 to 3 h and from 24 to 36 h in combined administration as compared to single administration. The drug attained its peak level in urine at 0.333 h (1098 ± 74.70 μg/ml) when given as a single administration as compared to peak urine concentration of 2480 ± 386.2 μg/ml at 1h incase of its combined administration with diclofenac. The mean therapeutic concentration in urine (2 μg/ml) was maintained up to 24 h in case of single administration where as around 36 h in case of combined administration.

3. *Kinetic Parameters*

Kinetic parameters of amikacin when it was given alone (7.5 mg/kg) and when given together with diclofenac (1 mg/kg) following i. v. administration in buffalo calves are presented in Table 16. The values of extrapolated zero time concentration during distribution phase (A), elimination phase (B) and theoretical zero time concentration (C_p^0) were noted to be highly significantly ($p<0.001$) lower in case of combined administration as compared to single administration of amikacin. Similarly, area under curve (AUC) and area under first moment curve (AMUC) were also noted to be significantly lower in combined administration as compared to single administration of amikacin. The mean residential time (MRT),

fraction of drug available for elimination from central compartment (Fc) and various values of volume distribution were noted to be significantly higher in case of combined administration of amikacin with diclofenac as compared to single administration of amikacin (Table 16). The value of rate constant of drug elimination from central compartment (Kel) and approximate tissue to plasma concentration ratio (T ≈ P) were calculated to be significantly lower in case of combined administration as compare to single administration of amikacin. The other kinetic parameters like distribution rate constant (α), distribution half life ($t_{1/2}$ α), elimination rate constant (β), elimination half life ($t_{1/2}$ β) etc. did not differ significantly between both the groups (Table 16).

4. Dosage regimen

The comparison of calculated dosage regimen of amikacin when given alone and given together with diclofenac in buffalo calves following i.v. administration is shown in Table 17. The calculated loading (D*) and maintenance (D_0) doses for maintaining (C_p^{∞} min) of 1,2 and 4 μg/ml at the selected doses interval (γ) were noted to be significantly higher in combined administration as compared to single administration of amikacin.

TABLE - 16

Comparison of kinetic parameters of amikacin when it was given alone (4 mg/kg) are when given together with diclofenac (1 mg/kg) in buffalo calves following i. v. administration.

Parameter (Unit)	Amikacin given alone	Amikacin+diclofenac combined administration
A (μg/ml)	13.23 ± 0.70	0.87 ± 0.21 ***
B (μg/ml)	5.22 ± 0.38	1.07 ± 0.06 ***
C_P^0 (μg/ml)	18.41 ± 0.67	1.94 ± 0.23 ***
α (h^{-1})	2.326 ± 1.467	2.885 ± 0.813 NS
$t_{1/2}\alpha$ (h)	0.75 ± 0.23	0.32 ± 0.08 NS
β (h^{-1})	0.156 ± 0.020	0.112 ± 0.010 NS
$t_{1/2}\beta$ (h)	4.67 ± 0.45	6.37 ± 0.60 NS
AUC (mg/L.h)	48.56 ± 5.84	10.25 ± 1.34 **
AUMC (mg/L.h^2)	256.4 ± 39.72	100.9 ± 25.04 *
MRT (h)	5.16 ± 0.30	9.35 ± 1.01 **
K_{12} (h^{-1})	1.220 ± 0.902	1.082 ± 0.426 NS
K_{21} (h^{-1})	0.851 ± 0.524	1.681 ± 0.481 NS
Kel (h^{-1})	1.006 ± 0.262	0.191 ± 0.016 *
Fc	0.39 ± 0.03	0.59 ± 0.06 **
T ≈ P	1.62 ± 0.21	0.69 ± 0.19 **
Vdc (L/kg)	0.41 ± 0.02	4.10 ± 0.50 **
Vd_B (L/kg)	1.47 ± 0.11	7.13 ± 0.41 ***
Vd_{area} (L/kg)	1.06 ± 0.06	6.88 ± 0.40 ***
Vd_{SS} (L/kg)	0.84 ± 0.08	4.64 ± 1.20 *
Cl_B (ml/kg/min)	2.78 ± 1.45	3.56 ± 2.81 NS

NS Non-significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

TABLE – 17

Comparison of calculated dosage regimen of amikacin when given alone and when given together with diclofenac in buffalo calves following intravenous administration.

C_P^{∞} min (μg/ml)	γ (h)	Dose (mg/kg)	Amikacin given alone	Amikacin + diclofenac given together
1	8	D *	3.95 ± 0.87	17.15 ± 1.78 **
		D_0	2.89 ± 0.84	10.27 ± 1.51 *
	12	D*	8.03 ± 2.67	27.32 ± 3.65 *
		D_0	6.97 ± 2.64	20.44 ± 3.42 *
2	8	D*	7.91 ± 1.74	34.31 ± 3.57 **
		D_0	5.79 ± 1.68	20.54 ± 3.03 *
	12	D*	16.06 ± 5.33	54.64 ± 7.30 *
		D_0	13.94 ± 5.28	40.87 ± 6.84 *
4	8	D*	15.82 ± 3.47	68.62 ± 7.14 **
		D_0	11.50 ± 3.28	41.09 ± 6.06 *
	12	D*	32.11 ± 10.67	109.3 ± 14.59 *
		D_0	27.83 ± 10.52	81.74 ± 13.68 *

* $p < 0.05$, ** $p < 0.01$.

C_P^{∞} min = Minimum therapeutic concentration in plasma (Minimum inhibitory concentration)

D* = Loading dose

D_0 = Maintenance dose

γ = Dosage interval

IV. COMPARISON OF PHARMACOKINETICS OF DICLOFENAC WHEN GIVEN ALONE AND WHEN GIVEN TOGETHER WITH AMIKACIN BY I.V. ADMINISTRATION

1. Plasma levels

Plasma concentration of diclofenac when given alone (1 mg/kg) and when given together with amikacin (7.5 mg/kg) after i.v. administration in buffalo calves are presented in Table 18 and Fig. 4. The drug concentrations were noted to be highly significantly ($p<0.001$) higher through out the various time intervals as compared to its single administration.

2. Urine levels

Table 18 and Fig. 5 depict the comparison of urine concentrations of diclofenac when given alone and when given together with amikacin. The urine levels were noted to be significantly lower initially up to 0.50 h and significantly higher for longer period (0.75 to 12 h) in case of combined administration as compared to single i.v. administration of diclofenac. No significant difference was observed at 24, 30 and 36 h between both the groups.

3. Kinetic parameters

Kinetic parameters of diclofenac when given alone and when given together with amikacin after i.v. administration are shown in Table 19. The values of extrapolated zero time concentration during distribution phase (A), elimination phase (B)

TABLE - 18

Comparison of plasma and urine concentrations (μg/ml) of diclofenac when given alone (1 mg/kg) and when given together with amikacin (7.5 mg/kg) in buffalo calves after intravenous administration

TIME (h)	Diclofenac given alone		Diclofenac + amikacin given together	
	Plasma	Urine	Plasma	Urine
0.083	5.69 ± 0.68	3.67 ± 1.33	29.36 ± 1.64 ***	N.D *
0.167	5.00 ± 0.85	30.01 ± 6.58	28.25 ± 1.23 ***	0.36 ± 0.11 **
0.25	4.33 ± 0.67	27.41 ± 2.16	26.50 ± 1.18 ***	1.68 ± 0.14 ***
0.333	3.87 ± 0.59	27.05 ± 1.56	25.49 ± 1.39 ***	2.93 ± 0.24***
0.50	3.27 ± 0.46	21.85 ± 0.97	24.10 ± 1.40 ***	9.67 ± 0.73 ***
75	2.42 ± 0.26	17.60 ± 0.43	20.97 ± 1.62 ***	21.54 ± 0.92 *
1	1.93 ± 0.24	15.42 ± 0.83	19.80 ± 1.78 ***	31.09 ± 2.39 **
1.5	1.51 ± 0.16	13.88 ± 0.73	17:49 ± 1.57 ***	34.47 ± 1.53 ***
2	1.32 ± 0.12	12.26 ± 0.99	15.88 ± 1.45 ***	26.57 ± 0.88 **
3	0.98 ± 0.10	11.06 ± 1.01	13.81 ± 1.46 ***	24.24 ± 1.31 **
4	0.79 ± 0.08	8.29 ± 0.75	11.15 ± 1.03 ***	21.69 ± 1.34 **
5	0.63 ± 0.04	6.02 ± 0.68	9.79 ± 0.82 ***	19.51 ± 1.50 **
6	0.52 ± 0.04	5.00 ± 0.71	8.61 ± 0.69 ***	15.56 ± 0.46 ***
8	0.32 ± 0.05	4.31 ± 0.77	6.72 ± 0.49 ***	12.96 ± 0.60 **
10	0.19 ± 0.03	3.50 ± 0.76	5.21 ± 0.39 ***	9.83 ± 0.51 **
12	0.14 ± 0.02	2.51 ± 0.47	3.96 ± 0.21 ***	6.27 ± 0.69 *
24	0.03 ± 0.01	1.29 ± 0.18	0.90 ± 0.06 ***	1.41 ± 0.61 NS
30	N.D.	0.24 ± 0.10	0.37 ± 0.03 ***	0.33 ± 0.16 NS
36	N.D.	0.04 ± 0.04	N.D.	N.D.

N.D. = Non-detectable

NS Non-significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

TABLE - 19

Comparison of kinetic parameters of diclofenac when given alone (1 mg/kg) and when given together with amikacin (7.5 mg/kg) in buffalo calves following intravenous administration.

Parameter (Unit)	Diclofenac given alone	Diclofenac + Amikacin given together
A (μg/ml)	5.74 ±1.20	11.61 ± 1.7 *
B (μg/ml)	1.65 ± 0.35	18.91 ± 1.98 **
C_P^0 (μg/ml)	7.38 ± 1.49	30.53 ± 1.07 ***
α (h^{-1})	2.76 ± 0.89	1.02 ± 0.13 ^{NS}
$t_{1/2}$ α (h)	0.34 ± 0.08	0.73 ± 0.08 *
β (h^{-1})	0.19 ± 0.03	0.13 ± 0.01 ^{NS}
$t_{1/2}$ β (h)	4.06 ± 0.59	5.46 ± 0.33 ^{NS}
AUC (mg/L.h)	11.24 ± 0.48	158.0 ± 9.85 ***
AUMC(mg/L.h^2)	51.78 ± 7.30	1168 ± 52.85 ***
MRT (h)	4.72 ± 0.85	7.48 ± 0.46 ^{NS}
K_{12} (h^{-1})	1.48 ± 0.53	0.27 ± 0.07 ^{NS}
K_{21} (h^{-1})	0.83 ± 0.22	0.68 ± 0.11 ^{NS}
Kel (h^{-1})	0.64 ± 0.12	0.195 ± 0.010 *
Fc	0.30 ± 0.03	0.67 ± 0.06 **
T ≈ P	2.43 ± 0.32	0.55 ± 0.16 **
Vdc (L/kg)	0.17 ± 0.05	0.25 ± 0.01 ^{NS}
Vd_B (L/kg)	0.72 ± 0.13	0.42 ± 0.06 ^{NS}
Vd_{area} (L/kg)	0.54 ± 0.10	0.38 ± 0.05 ^{NS}
Vd_{SS} (L/kg)	0.43 ± 0.10	0.36 ± 0.04 ^{NS}
CI_B (ml/kg/min)	1.52 ± 0.07	0.80 ± 0.06 ***

^{NS}Non-significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

and theoretical zero time concentration were found to be significantly higher in combined administration of the drugs as compared to single administration of diclofenac. Similarly, area under curve (AUC) and area under first moment curve (AUMC) were noted to be significantly higher in animals of combined administration as compared to single administration of diclofenac. Significantly higher value of fraction of drug available for elimination from central compartment (FC) was noted in case of combined administration of diclofenac with amikacin. The rate constant of drug elimination from central compartment (Kel), the approximate tissue to plasma concentration ratio (T≈P) and total body clearance (Cl_B) values were noted to be significantly lower in combined administration as compare to single administration of diclofenac. Though the value of distribution rate constant (α) did not differ significantly between both the groups, the value of distribution half life ($t_{1/2}$ α) was noted to be significantly higher in combined administration as compare to single administration of diclofenac. Rest of the kinetics parameters did not differ significantly between both the groups.

DISCUSSION

Amikacin, the latest semisynthetic aminoglycoside is clinically used due to its advantage over other aminoglycosides since it is highly resistant to aminoglycoside inactivating enzymes and thus used in gentamicin resistant cases. Further, it is broad spectrum among aminoglycosides and the minimum therapeutic concentration is 2 to 4 folds lower than other agents of this group.

Diclofenac, a potent NSAID having analgesic and antipyretic properties is usually employed in treating inflammatory conditions associated with pyrexia in animals. It seems that kinetic study of diclofenac has not been carried out so far in buffalo calves. Antimicrobial agents are concurrently used along with diclofenac for treating microbial infections as well as to treat inflammatory and febrile conditions. Though pharmacokinetic interactions between antimicrobials and NSAIDs were studied in animals, available literatures show little studies on interaction between amikacin and diclofenac in animals, particularly in buffalo calves. Therefore, the present study was undertaken to know the kinetic interactions of amikacin with diclofenac in buffalo calves.

I. KINETIC STUDY OF AMIKACIN

(a) Distribution in plasma

Concentrations of amikacin in plasma at all time intervals (except at 12 and 24 h where non significant difference) in

case of its (7.5 mg/kg i.v.) combined administration with diclofenac (1 mg/kg i.v.) were found to be significantly lower as compared to its single i.v. administration (Table 15 and Fig. 1). This has led to significant changes in various kinetic parameters when amikacin was administered concurrently with diclofenac (Table 16). The mean therapeutic concentration in plasma (≥ 2 μg/ml) was maintained up to 6 h when amikacin was given alone but it was not at all attained when amikacin was given in combination with diclofenac.

(b) Urinary Excretion

Concentration of amikacin in urine was noted to be significantly lower at 0.333 h only where as its concentrations were found to be significantly higher at most of the time intervals (0.75 to 3 h and 24 to 36 h) when amikacin was given concurrently with diclofenac by i.v. route as compared to its single i.v. administration. The change in urinary pattern when amikacin was given concurrently with diclofenac may also contributed to the change in various kinetic parameters. The therapeutic concentration of amikacin in urine (≥2 μg/ml) was maintained up to 24 h when it was given alone as compared to a longer period of around 36 h when it was given in combination with diclofenac.

(c) Kinetics parameters

In the present study, highly significantly ($p<0.001$) lower values of extrapolated zero time concentration during distribution phase (A), elimination phase (B) and theoretical zero time

concentration (C_P^0) were noted when amikacin was administered in combination with diclofenac as compared to single i.v. administration of amikacin (Table 16). The lower values obtained for the above parameters in combined administration of amikacin with diclofenac is due to the consistent lower plasma concentrations of amikacin obtained at most of the time intervals during combined administration (Table 15).

The distribution rate constant (α) of 2.326 ± 1.467 h^{-1} and distribution half life ($t_{1/2}\ \alpha$) of 0.75 ± 0.23 h were obtained for amikacin when administered alone. These values did not differ significantly in buffalo calves when amikacin was administered along with diclofenac which denote that similar rate of distribution of the drug occurred in both groups. The values of α were reported to be higher i.e. 4.16 ± 1.84, 3.66 ± 2.07 & 4.74 ± 1.06 h^{-1} in horse after i.v. administration of amikacin at the dose rate of 4.4, 6.6 & 11.0 mg/kg body weight respectively (Orsini *et al.*, 1985), 3.77 h^{-1} in lactating goats (Abo-el-Sooud, 1999) and 4.62 h^{-1} in chicken (El-Gammal *et al.*, 1992) and 1.925 h^{-1} in calf (Carli *et al.*, 1985) were reported. Lower values of $t_{1/2}\ \alpha$ i.e. 0.17, 0.24 and 0.16 h in horse after i.v. administration of amikacin at the dose rate of 4.4, 6.6 and 11.0 mg/kg body weight, respectively, were noted. Similarly, lower values of $t_{1/2}\ \alpha$ were also reported in lactating goats (0.184 h) by Abo-el-Sooud (1999) and in chicken (0.150 ± 0.064 h) by El-Gammal *et al.* (1992). More or less similar $t_{1/2}\ \alpha$ of 0.251 h in male goat (Uppal *et al.*, 1992) and 0.36 h in calf (Carli *et al.*, 1990) were observed.

The mean elimination rate constant (β) of 0.156 ± 0.020 h^{-1} and elimination half life $t_{1/2}$ β of 4.67 ± 0.45 h were noted for amikacin after its single i.v. administration (Table 16). The values did not differ significantly, when amikacin was administered in conjunction with diclofenac in buffalo calves. The $t_{1/2}$ β observed in the present investigation is found to be higher in contrast to the reports of Jernigan *et al.* (1988) in cat (1.31 ± 0.32 h), Baggot *et al.* (1985) as well as Gronwall *et al.* (1989) in African grey parrots (approx. 1 h), Orisini *et al.* in horse (approx. 1.5 h), Uppal *et al.* (1998) in sheep (1.42 ± 0.09) Abo-el-Sooud (1999) in lactating goats (1.91 h) Carli *et al.* (1990) in calf (2.5 h) and Saini and Srivastava (1988) in cross-bred bovine calves (3.09 ± 0.27 h). The difference in the observed elimination half life value in the present study in buffalo calf as compared to other species may be due to differences in physiological and biochemical factors such as metabolism, process of excretion etc.

The value of area under curve (AUC) and area under first moment curve (AUMC) were noted to be 48.56 ± 5.84 mg/L.h and 256.4 ± 39.72 $mg/L.h^2$, respectively after i.v. administration of amikacin alone. When this drug was combined with diclofenac, significant decrease in the values of AUC (10.25 ± 1.34 mg/L.h) and AUMC (100.9 ± 25.04 $mg/L.h^2$ were noted. The decrease in these values may be due to lower plasma levels of amikacin obtained after its combined administration with diclofenac. Significant increase in

mean residential time (MRT) of 9.35 ± 1.01 h was obtained when amikacin was administered concurrently with diclofenac as compared to MRT value of 5.16 ± 0.30 h when the drug was given alone. As compared to the value of MRT obtained in the present study in buffalo calf, lower MRT of 1.97 ± 0.36 and 2.89 ± 0.333 h in cats after i.v. and i.m. administration, respectively (Jernigan *et al.*, 1988) and 2.38 and 3.42 h after i.v. and i.m. administration in healthy lactating goats (Abo-El-Sooud, 1998) and 2.92 ± 0.14 h in lactating goats after i.m. administration (Agrawal *et al.*, 2001) were reported.

The rate constant of drug transfer from central to peripheral (K_{12}) and peripheral to central (K_{21}) compartment were noted to be 1.220 ± 0.902 and 0.851 ± 0.524 h^{-1}, respectively when amikacin was given alone. These values did not differ significantly when amikacin was given simultaneously with diclofenac. More or less similar K_{12} (1.20 ± 0.18 h^{-1}) and K_{21} (0.90 ± 0.12 h^{-1}) were noted in male goats (Uppal *et al.*, 1992), whereas lower K_{12} and K_{21} values of 0.270 ± 0.027 h^{-1} and 0.497 ± 0.022 h^{-1} were observed in female goats (Agrawal, 2000). However, the rate constant for drug elimination from central compartment (Kel) noted when amikacin was given alone (1.006 ± 0.262 h^{-1}) is significantly higher as compared to the value of 0.191 ± 0.016 h noted when amikacin was given in conjunction with diclofenac. Kel value of 0.092 ± 0.051 h^{-1} was noted in lactating goats (Agrawal, 2000).

The kinetic parameter, fraction of drug available for elimination from central compartment (Fc) when amikacin was given alone (0.39 ± 0.03) is significantly lower as compared to the value of 0.59 ± 0.06 noted when amikacin was given in combination with diclofenac. More or less similar value of 0.37 ± 0.02 in female goats (Agrawal, 2000) and 0.32 ± 0.02 in male goats (Uppal *et al.*, 1992) were reported. Approximate tissue to plasma concentration ratio (T ≈ P) of 1.62 ± 0.21 noted when amikacin was administered alone is significantly higher than that of 0.69 ± 0.19 noted when it was simultaneously administered with diclofenac. Similar T ≈ P value of 1.72 ± 0.17 was noted in female goats (Agrawal, 2000) while a slightly higher value of 2.10 ± 0.21 was noted in male goats (Uppal *et al.*, 1992).

The various values of volume distribution of amikacin are significantly higher when the drug was given in combination in the diclofenac as compared to the values observed when it was given alone (Table 16). Notari (1980) stated that for a two compartment open model, the value of $Vd_B > Vd_{area} > Vd_{SS}$ and Vd_C. He further mentioned that among these values of volume distribution, only Vd_{area} correctly predicts during elimination phase where as Vd_B over estimates and Vd_{SS} and Vd_C under estimate the amount of drug in the body. Vd_{area} of 1.06 ± 0.06 L/kg was estimated when amikacin was given alone as compared to a significantly ($p < 0.001$) higher Vd_{area} of 6.88 ± 0.40 L/kg when it was administered along with diclofenac in

the present study. This high Vd_{area} may be expected due to its higher distribution in tissues of the buffalo calves when amikacin was given in combination with diclofenac. But in fact, the approximate tissue to plasma concentration ratio is found to be significantly ($p<0.01$) lower in case of amikacin when administered with diclofenac as compared to its single administration. A high $Vd_{area}>1$ may also be due to its higher excretion, tissue and protein bindings apart from higher distribution in tissues (Baggot, 1977). In the present study, amikacin was excreted in significantly very high concentrations from 0.75 to 3 h and 24 to 36 h in urine when amikacin was given in combination with diclofenac as compared to its single administration (Table 15). Hence, it is assumed that amikacin may not be distributed in higher amounts in tissues of buffalo as noted by $T \approx P$ values but rather the high Vd_{area} may be due to its higher excretion when the drug was given in combination with diclofenac. The changes in tissue binding was not undertaken in the present study which may also possible contribute to the higher Vd_{area} obtained when amikacin was given in combination with diclofenac. Lower Vd_{area} of 0.17 ± 0.02 L/kg in cats (Jernigan *et al.*, 1988), 0.226 ± 0.037 L/kg in dog (Baggot *et al.*, 1985), 0.193 ± 0.060 L/kg in chicken (El-Gammal *et al.*, 1992), 0.247 L/kg in camel (Wasfi *et al.*, 1999), 0.335 ± 0.003 L/kg in sheep (Uppal *et al.*, 1992) 0.040 L/kg in male goats & 0.35 L/kg in calf (Carlie *et al.*, 1990) and 0.40 ± 0.03 L/kg in cross bred bovine calves (Saini and Srivastava, 1998) after i.v. administration of amikacin. After i.m.

administration Vd_{area} of 0.30 L/kg in mare (Brown *et al.*, 1984), 0.2-0.3 L/kg in sheep (Carli *et al.*, 1990) and 0.39 ± 0.03 L/kg in goat (Agrawal *et al.*, 2001) were reported. The above facts show that amikacin may be extensively penetrated in various body tissues of buffalo calves as compared to other species.

The total body clearance (Cl_B) values of 2.78 ± 1.45 and 3.56 ± 2.81 ml/kg/min were obtained when amikacin was given alone and when it was given in conjunction with diclofenac, respectively. This finding may indicate that amikacin may equally removed from the body of buffalo calves when it was given either alone or in combination with diclofenac. More or less similar Cl_B values of 2.64 ± 0.24 ml/kg/min in dog (Baggot *et al.*, 1985) and 2.71 ± 0.13 ml/kg/min in sheep (Uppal *et al.*, 1998) were reported.

(D) Dosage regimen

The therapeutic plasma level (C_p^{∞} min) of amikacin was reported to be 1 to 4 μg/ml (Leroy *et al.*, 1978). In the present study, calculation of dosage regiment of amikacin was carried out at three different therapeutic levels (1, 2 and 4 μg/ml) with a view to combat mild, moderate and severe infections, respectively. For treating mild infection (C_p^{∞} min = 1 μg/ml), a loading dose (D*) of 8.03 ± 2.64 mg/kg and maintenance dose (D_0) of 6.97 ± 2.64 mg/kg may be advised at the dosage interval (γ) of 12 h when amikacin was given alone. When amikacin was given in combination for the same therapeutic level (C_p^{∞} min = 1 μg/ml) and similar dosage intervals (γ)

of 12 h, significantly higher doses of D* and D_0 may be needed which may cause severe toxicity. Hence, a shorter γ of 8 h may be recommended in this case where D* and D_0 were calculated to be 17.15 ± 1.78 and 10.27 ± 1.51 mg/kg, respectively.

For combating moderate infections (C_p^∞ min = 2 μg/ml) D* of 7.91 ± 1.74 mg/kg and D_0 5.79 ± 1.68 mg/kg, respectively were calculated at a shorter γ of 8 h. A significantly higher D* and D_0 were calculated for the same C_p^∞ min and γ, which may cause severe toxicity in animals. Hence, the drug may not be recommended in combination with diclofenac for moderate infections.

Similarly, for treating severe infections (C_p^∞ min=4 μg/ml) D* of 15.82 ± 3.47 mg/kg and D_0 of 11.50 ± 3.28 mg/kg at γ of 8 h can be used when amikacin was given alone. But when combined with diclofenac, the calculated, D* and D_0 are quite high which may cause toxicity. Hence the combination of amikacin with diclofenac may not be preferred for clinical use.

II. KINETIC STUDY OF DICLOFENAC

Kinetic studies of diclofenac in animals are very little and studies in man (Willis *et al.*, 1979 ; Kurowski, 1988), pig (Oberle *et al.*, 1997 and rat (Peris-Ribera *et al.*, 1991) were reported.

(a) Distribution in plasma

Concentrations of diclofenac in plasma were found to be highly significantly ($p<0.001$) higher at all time intervals (0.083 to 30 h) in buffalo calves when the drug was administered in

combination with amikacin (Table 18 and Fig. 4). Available literature shows that no kinetic study including plasma level of diclofenac was carried out in domestic animals so far.

(b) Urinary Excretion

Concentrations of diclofenac were noted to be significantly lower initially from 0.083 to 0.50 h and significantly higher from 0.75 to 12 h when it was given together with amikacin. However, no significant difference was noted from 24 to 36 h between both the groups. Peak concentration in urine was noted earlier at 0.167 h in case of administration of diclofenac alone as compared to 1.5 h noted in case of combined administration of diclofenac with amikacin by i.v. route. Urinary excretion less than 1% in man was observed by Willis *et al.* (1979).

(c) Kinetic parameters

A significantly ($p<0.05$) higher value for the extrapolated zero time concentration during distribution (A), elimination (B) phase and theoretical zero time concentration (C_p^0) were obtained for diclofenac when administered in combination with amikacin as compared to its single administration by i.v. route (Table 19). In contrast, Nitesh Kumar (2002) observed lower values of A, B and C_p^0 when diclofenac was administered along with enrofloxacin as compared to its single i.v. administration.

The distribution rate constant (α) did not differ significantly when the drug was given alone or in combination with diclofenac but significantly higher distribution half life ($t_{1/2}$ α) of 0.73 ± 0.08 h was noted when given in combination with diclofenac as compared to its single administration ($t_{1/2}$ α = 0.34 ± 0.08 h). This denotes that diclofenac is distributed comparatively at slower rate when combined with amikacin.

The elimination rate constant (β) of 0.19 ± 0.03 h^{-1} and elimination half life ($t_{1/2}$ β) of 4.06 ± 0.59 h were calculated after single. i.v. administration of diclofenac. These values did not differ significantly when diclofenac was given in combination with i.v. route. The $t_{1/2}$ β value of 1.1 h in man after i.v. administration of diclofenac (Willis *et al.*, 1979) and 1.15 h in man after i.m. injection of diclofenac (Kurowski, 1988) were noted to be very low than the value obtained in buffalo calves in the present study. Similarly, lower $t_{1/2}$ β in pigs (2.4 h) and man (1.8 h) were noted by Oberle *et al.* (1994).

The values of area under curve (AUC) of 157.98 ± 9.85 mg/L.h and area under first moment curve (AUMC) of 1168 ± 52.85 mg/L.h^2 were found to be highly significantly ($p<0.001$) higher when diclofenac was given along with amikacin as compared to AUC and AUMC values of 11.24 ± 0.48 mg/L.h and 51.78 ± 7.30 mg/L.h^2 obtained after single i.v. administration of diclofenac.

A significantly ($p<0.01$) higher value of fraction of drug available for elimination from central compartment (Fc) of 0.67 ± 0.06 (67%) was obtained when the drug was given in combination with amikacin as compared to Fc value of 0.30 ± 0.03 (30%) when diclofenac was given alone. This may be the reason that diclofenac was eliminated at higher quantities when it was given in combination with diclofenac since higher percentage of drug was available for elimination in the central compartment when the drug was given in combination with amikacin. The approximate tissue to plasma concentration ratio ($T \approx P$) was calculated to be significantly lower ($p< 0.01$) in case of combined administration of diclofenac with amikacin (0.55 ± 0.16) as compared to its single administration (2.43 ± 0.32). The above fact may suggest that diclofenac may not be distributed in good amount in peripheral tissues when combined with amikacin. This is further supported by lower values of volume distribution (Vd_B, Vd_{area} & Vd_{SS}) obtained when diclofenac was given along with diclofenac as compared to its single administration though there was no significant difference in the data between both the groups. Vd_{area} of 0.54 ± 0.10 L/kg was noted for single administration of diclofenac by i.v. route. A very low value of volume distribution of 0.17 ± 0.11 L/kg in man was noted by Willis *et al.* (1979).

The total body clearance (Cl_B) was noted to be highly significantly ($p<0.001$) lower in case of combined administration with

amikacin (0.80 ± 0.06 ml/kg/min) as compared to its single administration (1.52 ± 0.07 ml/kg/min). A high Cl_B value of 4.2 ± 0.9 ml/kg/min in man (Willis *et al.*, 1979) was observed. The Cl_B in mini-pigs was five folds slower than in man (57 ± 17 ml/kg/h = 0.95 ± 0.28 ml/kg/min versus 252 ± 54 ml/kg/h = 4.2 ± 0.9 ml/kg/min) as noted by Oberle *et al.* (1994). The values obtained by Oberle *et al.* (1994) in mini-pig was more or less similar to buffalo calves noted in the presented study.

III. KINETIC INTERACTION BETWEEN AMIKACIN AND DICLOFENAC :

The distribution of amikacin and diclofenac in plasma and urine as well as different kinetic parameters have been described above when given alone or in combination following i.v. administration. Definite kinetic interactions between the drugs occurred in buffalo calves and the salient features are described below.

The results of the present study clearly establishes that diclofenac influences the kinetics of amikacin, which are noted below. The plasma drug concentrations of amikacin were found to be significantly lower when it was given in combination with diclofenac as compared to its alone administration. This has resulted in significantly lower values of extrapolated zero time concentration during distribution (A) and elimination (B) phase as well as

theoretical zero time concentration. Further, it has resulted in significantly lower value of area under curve (AUC) and area under first moment curve (AUMC), significantly higher values of mean residential time (MRT), fraction of drug available for elimination from central compartment (Fc) and various values of volume distribution were found when amikacin was given in combination with diclofenac. However, lower value of rate constant of drug elimination (Kel) and approximate tissue to plasma concentration ration ($T \approx P$) were observed when the drug was given in combination with diclofenac as compared to its single administration. There is no influence of diclofenac over the distribution as well as elimination of amikacin as shown by insignificant difference in the values of α, $t_{1/2}$ β, as well as Cl_B between both the groups.

The calculated loading (D*) and maintenance (D_0) doses were found to be significantly higher when amikacin was given together with diclofenac at various therapeutic levels (C_p^{∞} min of 1, 2 and 4 μg/ml) and at various dose intervals (γ of 8 and 12 h). The study shows that there may not be any advantage of combination therapy of amikacin with this analgesic, antipyretic and anti-inflammatory agent. Further, if it is combined with diclofenac, higher doses of amikacin may be required which may in turn possibly lead to toxicity in animals.

Unlike amikacin, plasma concentrations of diclofenac were found to be highly significantly ($p < 0.001$) higher when it was given in combination with amikacin as compared to its single administration. This has led to significantly higher value of zero time concentration during distribution (A), elimination (B) phase as well as theoretical zero time concentration (C_p^0). Due to maintenance of higher drug concentrations in plasma, the values of area under curve (AUC) and area under first moment curve (AUMC) were found to be highly significantly ($p< 0.001$) higher when diclofenac was given together with amikacin as compared to its single administration.

The value of rate constant of drug elimination from central compartment (Kel) tissue to plasma concentration ratio ($T \approx P$) and total body clearance were found to be significantly lower while fraction of drug available for elimination from central compartment was found to be significantly higher when diclofenac was given in combination with amikacin as compared to its single administration.

Chapter - 6

Summary

SUMMARY

A detailed pharmacokinetic study of amikacin and diclofenac when given alone and their interactions when given together in combination was carried out in buffalo calves post i.v. administration. Concentrations of the drugs in plasma and urine as well as various kinetic parameters were calculated by using two compartment open model when given alone or when given together. Attempts were made to calculate the rational dosages regimen of amikacin on the basis of kinetic data and maintenance of therapeutic concentrations (MICs) in plasma. The following findings were obtained.

1. Following combined i.v. administration of amikacin (7.5 mg/kg) with diclofenac (1 mg/kg), plasma concentrations of amikacin were found to be significantly lower at all time intervals as compared to single i.v. administration of amikacin. In case of urine, significantly lower concentration was obtained at 0.333 h while significantly higher concentrations were noted from 0.75 to 3 h and 24 to 36 h in combined administration of amikacin with diclofenac as compared to its single administration.

   Following combined i.v. administration of diclofenac with amikacin, concentrations of diclofenac in plasma were found to be significantly higher at all time intervals (0.083 to 0.30 h) as compared to single i.v. administration of diclofenac. In case of

urine samples, the drug concentrations were lower initially (0.083 to 0.50 h) and significantly higher thereafter (0.75 to 12 h). Non significant difference was observed at 24, 30 and 36 h between both the groups (Table 18).

2. The values of extrapolated zero time concentration during distribution (A) and elimination (B) phase and theoretical zero time concentration (C_p^0) of amikacin were found to be highly significantly ($p<0.001$) lower in case of combined administration of amikacin with diclofenac as compared to single administration of amikacin alone. This is due to the significantly lower concentrations of amikacin in plasma of buffalo calves when these two drugs are gives together. This has led to significantly lower values of area under curve (AUC) and area under first moment curve (AUMC), when amikacin was given in combination with diclofenac as compared to its single i.v. administration.

3. There was no significant difference in the values of distribution rate constant (α), distribution half life ($t_{1/2}\ \alpha$), elimination rate constant (β) and elimination half life ($t_{1/2}\ \beta$) when these two drugs are given together. The above findings denote that similar rate of distribution and elimination may occur when amikacin was given alone or in combination with diclofenac.

4. Significantly higher value of mean residential time (MRT) of 9.35 ± 1.01 obtained for amikacin when it was given together with

diclofenac as compared to MRT value of 5.16 ± 0.30 h when amikacin was given alone denotes that amikacin remained for a longer period in the body of buffalo calves when it was given together with diclofenac.

5. Significantly higher value of 0.59 ± 0.06 was obtained for the parameter fraction of drug available for elimination from central compartment (Fc) when amikacin was given together with diclofenac as compared Fc value of 0.39 ± 0.03 when amikacin was given alone. This may indicate that more percentage of drug (59%) was available for elimination when these drugs are given together as compared to its single i.v. administration. The value of approximate tissue to plasma concentration ratio (T ≈ P) was found to be significantly lower when these drugs are given together as compared to single i.v. administration of amikacin.

6. Various values of volume of distribution were found to be significantly higher for amikacin after combined i.v. administration with diclofenac as compared to its single i.v. administration. Vd_{area} of 6.88 ± 0.40 L/kg was noted for amikacin when it was given together with diclofenac as compared to Vd_{area} of 1.06 ± 0.06 L/kg when amikacin was given alone. This may lead to the inference that amikacin may be distributed to a greater amount when it was given together with diclofenac. But a significantly lower value of tissue to plasma concentration ratio

($T \approx P$) was obtained when these two drugs are given together. A high Vd_{area} may denote apart from wide distribution it may also be due to higher tissue binding as well as higher amount of urinary excretion of the drug. Hence, it may be assumed that the high Vd_{area} obtained when amikacin was given together may be due to its higher excretion as noted in the present study for a long period (0.75 to 3 h and 24 to 36 h).

7. For treating mild systemic infection (C_P^{∞} min = 1 μg/ml) loading (D*) and maintenance (D_0) doses of 8 and 7 mg/kg at dosage interval (γ) of 12 h may be used when amikacin was given alone while higher D* and D_0 of 17 and 10 mg/kg may be needed when amikacin was given in combination with diclofenac. For treating moderate (C_P^{∞}min = 2 μg/ml) and severe (C_P^{∞} min = 4 μg/ml) infections, D* of 8 and 16 mg/kg and D_0 of 6 and 11.5 mg/kg at γ of 8 h, respectively are needed when amikacin was given alone, while very high D* and D_0 are needed when amikacin was administered with diclofenac which may cause possible severe toxicity in buffalo calves. Hence, for treating moderate and severe infections, amikacin can be used alone but not along with diclofenac simultaneously.

8. When these two drugs are used together, concentrations of diclofenac in plasma were found to be significantly higher at all time intervals as compared to single i.v. administration of

diclofenac when given alone. In case of urine, concentration of diclofenac were found to be significantly lower initially for a shorter time (0.083 to 0.5 h) while significantly higher concentrations of the drug were noted thereafter for a longer period (0.75 to 12 h) as compared to its single i.v. administration.

9. The extrapolated zero time concentration during distribution (A), elimination (B) phase and theoretical zero time concentration (C_p^0) were found to be significantly higher when diclofenac was given together with diclofenac as compared to its alone administration. Similarly, high values of area under cuve (AUC) and area under first moment curve (AUMC) were noted when these drugs are given together as compared to its alone administration. The above noted changes may be due to higher maintenance of plasma concentrations of diclofenac when both the drugs are given together. There is no significant change in rate of distribution as well as elimination when these two drugs are given together as compared to its alone administration as shown by non significant difference in the values of α, β and $t_{1/2}$ β {only significant ($p<0.05$) difference in $t_{1/2}$ α value}. Non significant differences were noted among various values of volume distribution, Mean residential time (MRT) and rate constant of drug transfer from central to peripheral (K_{12}) and peripheral to central (K_{21}) compartment.

The present study established that both amikacin and diclofenac interact with one another and cause many changes in their kinetic behaviour. The study further points out that the combination of amikacin with diclofenac may not be much advantageous since higher doses of amikacin may be required for treating moderate to severe infection, which may cause toxicity in animals.

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Appendix

APPENDIX - I

CALCULATION OF KINETIC PARAMETERS

Kinetic parameters were calculated from log plasma drug concentration versus time profile. An example is noted below from the data of buffalo calf no. 3 obtained after a single i.v. injection of amikacin (7.5 mg/kg). The data showed a biphasic curve and hence, fits well into a two compartment open model.

Sl. No.	Time (h) x	x^2	Plasma Drug Concentration (y) (μg/ml)	Log y	xy
1	4	16	3.20	0.5051	2.0204
2	5	25	2.80	0.4472	2.2360
3	6	36	2.35	0.3711	2.2266
4	8	64	1.80	0.2553	2.0424
5	10	100	1.25	0.0969	0.9690
6	12	144	0.92	-0.0362	-0.4345
7	24	576	0.15	-0.8239	-19.7736
$\sum n = 7$	$\sum x = 69$ $\bar{x} = 9.8570$	$\Sigma x^2 = 961$		$\Sigma \log y =$ 0.8155 $\bar{y} = 0.1165$	-10.7137

b, Slope of line $= \frac{n.\sum x.y - \sum x.\sum y}{n.\sum x^2 - (Ex)^2}$

$$= \frac{(7x - 10.7173) - (69 \times 0.8155)}{(7 \times 961) - (69 \times 69)}$$

$$= \frac{-75.0211 - 56.2695}{6727 - 4761}$$

$$= \frac{-131.2906}{1966} = -0.0668$$

β, elimination rate contant = b x (-2.303)

= -0.0668 x –2.303

$=0.154\ h^{-1}$

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

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

$\bar{x}$ = mean time

b = slope of line

a = zero time concentration

Therefore,

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

= 0.1165 - (668 x 9.8571)

= log 0.7750

Zero time concentration (B) = antilog of 0.7750 = 5.95 μg/ml

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

Subtracting the theoretical values from observed values, a series of residual concentrations were obtained and slope of line in natural log (distribution rate constant, α) and the zero time intercept (zero time concentration during distribution phases, A) can be calculated as per method adopted for calculation of B and β. The value of A is 14.47 μg/ml and α is 1.204 h^{-1}. C_p^0, the theoretical plasma concentration at time zero during distribution phase

$$C_p^0 = A+B$$

$$= 14.47 + 5.95$$

$$= 20.42\ \mu g/ml$$

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

$$t_{1/2}\alpha = \frac{0.693}{\alpha} = \frac{0.693}{1.204} = 0.58h$$

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

$$t_{1/2}\beta = \frac{0.693}{\beta} = \frac{0.693}{0.154} = 4.50\ h$$

AUC, area under curve

$$AUC = \frac{A}{\alpha} + \frac{B}{\beta} = \frac{14.47}{1.204} + \frac{5.95}{0.154} = 12.02 + 38.63,$$

$$= 50.65\ mg/L.h$$

AUMC, area under first moment curve

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

$$= \frac{14.47}{(1.204)^2} + \frac{5.95}{(0.154)^2}$$

$$= \frac{14.47}{1.4496} + \frac{5.95}{0.0237}$$

$$= 9.98 + 250.82$$

$$= 260.8 \text{ mg/ L. h}^2$$

MRT, mean residential time

$$MRT = \frac{AUMC}{AUC} = \frac{260.8}{50.65}$$

$$= 5.15 \text{ h}$$

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

$$K_{21} = \frac{A.\beta + B.\alpha}{C_P^o}$$

$$= \frac{14.47x.154 + 5.95x1.204}{20.42}$$

$$= \frac{2.228 + 7.164}{20.42} = \frac{9.392}{20.42}$$

$$= 0.460 \text{ h}^{-1}$$

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

$$Kel = \frac{\alpha.\beta}{K_{21}} = \frac{1.204x0.154}{0.460}$$

$$= \frac{0.1854}{0.460}$$

$$= 0.403$$

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

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

$$= 1.204 + 0.154 - 0.460 - 0.403$$

$$= 0.495$$

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

$$Fc = \frac{\beta}{Kel}$$

$$= \frac{0.154}{0.403} = 0.38$$

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

$$T \approx P = \frac{K_{12}}{K_{21} - \beta} = \frac{0.495}{0.460 - 0.154}$$

$$= \frac{0.495}{0.306} = 1.62$$

Vdc, volume of distribution based on both distribution and elimination

$$Vdc = \frac{D}{C_p^o} = \frac{7.5}{20.42} = 0.37 L./kg$$

where, D = Dose (7.5 mg/kg)

Vd_B, the volume of distribution based on elimination

$$Vd_B = \frac{D}{B} = \frac{7.5}{5.95} = 1.26 L/kg$$

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

$$Vd_{area} = \frac{D}{AUC.\beta} = \frac{7.5}{50.65x.154} = \frac{7.5}{7.800} = 0.96 \quad L/kg$$

Vdss, the volume of distribution at steady state

$$Vdss = \frac{K_{12} + K_{21}}{K_{21}} xVdc = \frac{0.495 + 0.460}{0.460} x0.37 = 2.076x0.37 = 0.77 L/kg$$

Cl_B, the total body clearance

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

$$= 0.96 \times 0.154$$

$$= 0.1478 \text{ L/kg/h}$$

$$= 2.46 \text{ mg/kg/min}$$