PHARMACOKINETIC STUDY OF AMIKACIN AND ITS INTERACTION WITH PARACETAMOL IN BUFFALO CALVES

THESIS

SUBMITTED TO THE

RAJENDRA AGRICULTURAL UNIVERSITY

PUSA (SAMASTIPUR) BIHAR

(FACULTY OF POST-GRADUATE STUDIES)

In partial fulfilment of the requirement

FOR THE DEGREE OF

Master of Veterinary Science

IN

VETERINARY PHARMACOLOGY AND TOXICOLOGY

By

Mukesh Kumar

Registration No. - M/VPT/67/2000-2001

Department of Veterinary Pharmacology and Toxicology

BIHAR VETERINARY COLLEGE

PATNA (BIHAR)

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DEPARTMENT OF VETERINARY PHARMACOLOGY
AND TOXICOLOGY
BIHAR VETERINARY COLLEGE
PATNA (BIHAR)

2003

My reverent dedication to
adorable patriots

A working knowledge of pharmacodynamics and pharmacokinetics is essential for the maximum benefit to be obtained from the therapeutic agents available. This should be leading to a recognition of the vital importance of Veterinary Pharmacology and Therapeutics within the Veterinary curriculum.

G. C. Brander
and
D. M. Pugh

DEPARTMENT OF PHARMACOLOGY AND TOXICOLOGY
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Dr. S. D. Singh
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Associate Professor & Head
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CERTIFICATE - I

This is to certify that the thesis entitled **"PHARMACOKINETIC STUDY OF AMIKACIN AND ITS INTERACTION WITH PARACETAMOL IN BUFFALO CALVES"** submitted in partial fulfillment of the requirement for the degree of "**Master of Veterinary Science (Veterinary Pharmacology & Toxicology)"** of the faculty of Post-Graduate Studies, Rajendra Agricultural University, Bihar, is the record of bonafide research carried out by **DR. MUKESH KUMAR**, under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

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

[Dr. S. D. Singh]
Major Advisor

Endorsed :

(Chairman / Head of the Department)

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CERTIFICATE - II

We, the undersigned, members of the Advisory Committee of **DR. MUKESH KUMAR**, a candidate for the degree of Master of Veterinary Science with Major in **Veterinary Pharmacology & Toxicology**, have gone through the manuscript of the thesis and agree that the thesis entitled "**PHARMACOKINETIC STUDY OF AMIKACIN AND ITS INTERACTION WITH PARACETAMOL IN BUFFALO CALVES**" may be submitted by **DR. MUKESH KUMAR** in partial fulfillment of the requirements for the degree.

10/12/03

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CERTIFICATE - III

This is to certify that the thesis entitled **"PHARMACOKINETIC STUDY OF AMIKACIN AND ITS INTERACTION WITH PARACETAMOL IN BUFFALO CALVES"** submitted by **DR. MUKESH KUMAR**, in partial fulfillment of the requirement for the degree of **Master of Veterinary Science (Veterinary Pharmacology & Toxicology)** of the faculty of Post-Graduate Studies, Rajendra Agricultural University, Bihar was examined and approved on ...22/03/04.....

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[DRI-cum-Dean, P.G. Studies]

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Patna

Date : 10/12/2003

Mukesh Kumar

(MUKESH KUMAR)

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Chapter - 1

Introduction

INTRODUCTION

Antimicrobials are the recent tools in the armamentarium of modern clinicians to combat the local and systemic infections both in medical and veterinary practices. It is also a fact that clinicians are facing the problems of microbial resistance and suprainfections apart from different side effects, which are mainly due to improper selection of antimicrobials and their irrational dosing. Optimal and judicious selection of antimicrobial agents for therapy of infectious diseases requires clinical judgment and detailed knowledge of pharmacological factors.

Amikacin, a latest member of aminoglycoside group of antimicrobials, is a semisynthetic derivative of Kanamycin - A. The inactivation of other aminoglycosides by microbial enzymes led to the synthesis of amikacin by Kawaguchi and co-workers in 1972. In contrast to the parent compound, which was most toxic with least antimicrobial efficacy, amikacin is generally more active and least toxic. The outstanding feature of amikacin is its resistance against bacterial aminoglycoside inactivating enzymes (Ries *et al.*, 1973). Thus, it is widely used to treat various gram negative bacterial infections particularly against gentamicin-resistant microorganisms

(Sande and Mandell, 1980). It is a bactericidal agent and possesses widest spectrum of activity among its group (Chambers and Sande, 1996). It has gained wide acceptance both in medical and veterinary practices due to its wide spectrum of activities, excellent disposition characteristics, negligible plasma protein binding (Brown and Riviere, 1991), lower minimum therapeutic concentration (Ziv, 1977), least problem of bacterial resistance as well as cross resistance with other antimicrobials and high distribution in extracellular space (Brown and Riviere, 1991).

Anitmicrobials have a major role in combating various systemic microbial infections. Systemic infections are usually associated with pyrexia and/or inflammation accompanied by pain. Hence, antimicrobials are frequently and concomitantly used along with non-steroidal antiinflammatory drugs (NSAIDs) to overcome this usual problem.

Paracetamol, a potent NSAID, having strong antipyretic with analgesic and antiinflammatory properties is commonly used in treating pyrexia both in human and animals. Paracetamol (Acetaminophen, N-acetyl-p-aminophenol), the active metabolite of phenacetin, a so called coal tar analgesic was first used in medicine by von Mering in 1893. Its special features are: (a) doesn't stimulate respiration (b) doesn't disturb the acid-base balances (c) no effect on

cardiovascular system (d) doesn't increase cellular metabolism (e) gastric irritation is insignificant in contrast to other analgesics and antipyretics and (f) it doesn't affect platelet function as well as clotting factors. It is particularly valuable in patients where aspirin is contraindicated as in peptic ulcer or in patients with prolonged bleeding time.

Antimirobials and NSAIDs are being frequently and concomitantly used and pharmacokinetic interactions between them have been described by various workers (Kampmann *et al.*, 1972; Carbon *et al.*, 1981, 1984). Joly *et al.* (1988) described the enhancement of the therapeutic effects of cephalosporins (cefotiam, cefmenoxime and cefriaxone) in experimental endocarditis in rabbits by altering their pharmacokinetics. In experimental *staphylococcal* osteomyelitis, ibuprofen given concomitantly with oxacillin significantly increased the antibiotic efficacy (Khurana and Deddish, 1986). Manna *et al.* (1994) described altered disposition kinetics of paracetamol by oxytetracyclin in goats.

Buffalo, a multipurpose Asian animal, is utilized for dairy, drought and meat purposes. The Indian subcontinent is the home tract of world dairy buffalo. India is ranking first in world with 42% of world's buffalo population (83.5 million). It alone contributes about 45% of total milk produced in India and known as "milk

machine of India". An average buffalo cow has been considered four times as productive as indigenous cow. The buffalo fits well in poor countries like India where resources are meagre and thus, it contributes to the upliftment of poor farmers of this country. Keeping in view of the major contribution of buffalo in our national economy and huge unemployment avenue, it becomes more imperative to provide necessary health coverage with the help of effective antimicrobials and other commonly used drugs like antipyretics.

The subject of drug interaction interests pharmacologists and now it becomes very much important for clinicians. In order to use drugs effectively, it is important to investigate the detailed pharmacokinetics of the drug in the same species and in similar climate in which the drug is to be used clinically (Nawaz *et al.*, 1980). Pharmacokinetic studies of antimicrobials and NSAIDs are carried out in healthy animals to obtain the detailed pharmacokinetic data. From these data, appropriate dosage regimen of a drug is derived for effective treatment of the diseases, when administered alone. It is well established through the study of many workers that the kinetic parameters of a drug may change during combination therapy resulting into sharp changes in dosage regimen.

Although pharmacokinetic studies of amikacin have been carried out in different species of animals including buffalo calves, but

studies on the interaction of amikacin with paracetamol in animals, particularly in buffalo calves are scarce. Keeping in view of the aforesaid facts, the present investigation has been carried out in buffalo calves with following objectives:

(i) Estimation of concentrations of amikacin and paracetamol at different time intervals in the body fluids following parenteral administrations when given alone in buffalo calf.

(ii) Determination of kinetic parameters of amikacin and paracetamol when given alone.

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

(iv) Estimation of concentrations in biological fluids calculation of kinetic parameters of amikacin & paracetamol and calculation of dosage regimen of amikacin when these two drugs are given together to know the interaction of the drugs after their intravenous administrations.

The results of this investigation would help clinicians in a long way in making the suitable recommendation of combination therapy of amikacin with paracetamol for the effective treatment of various microbial infections associated with pyrexia in buffalo calf.

Chapter - 2

Review of Literature

REVIEW OF LITERATURE

Aminoglycoside group of antibiotics possesses common chemical structure containing amino sugars joined by a glycosidic linkage. These antibiotics are mostly active against gram negative bacteria, but presently many microorganisms have shown resistance against various aminoglycoside antibiotics such as streptomycin, gentamicin, neomycin, kanamycin, tobramycin etc. The resistance to these aminoglycosides are due to different enzymes liberated by microorganism, like aminoglycoside phosphotransferase, amino-glycoside acetyltransferase and aminoglycoside nucleotidyl-transferase which attack different parts of aminoglycoside molecule and destroy their antibacterial effects. This problem was solved when Kawaguchi and co-workers in 1972 introduced a new semisynthetic aminoglycoside - amikacin which was derived from kanamycin-A and previously it was named as BB-K8.

AMIKACIN

Amikacin, a latest member of aminoglycoside group of antimicrobials, is mostly used against aerobic gram negative infections. The outstanding feature of amikacin is its resistance to most of the bacterial aminoglycoside inactivating enzymes (Ries *et al.*, 1973). It has broadest spectrum of activity among aminoglycosides

and requires two to four folds lower minimum therapeutic concentrations than other antimicrobials of this group, which lessens the toxicity without affecting the therapeutic efficacy.

It has low plasma protein binding (Brown and Riviere, 1991). Saini and Srivastava (1998) reported that binding of amikacin to calves plasma protein was 6.3% and plasma therapeutic concentration remained upto 8 h after single i.v. dose. Distribution of amikacin is high in extracellular space (Brown and Riviere, 1991; Lancini *et al.*, 1995) and therapeutic concentrations are attained in kidney, urinary tract, lungs, pericardium, peirtonium and synovial fluid (Chambers and Sande, 1996).

History

The geneology of aminoglycoside group of antimicrobials began in 1944 with the production of strepotomycin from *Streptomyces griseus* by Schatz, Bugie and Waksman. It was found to be effective against gram-negative organisms. Later on, neomycin was introduced by Waksman and Lechevier in 1949 and used for topical applications and in local gastro-intestinal tract infections since it has severe nephro and oto-toxicity on systemic administration. Umezawa and co-coworkers in 1957 produced another aminoglycoside antibiotic, kanamycin from *Streptomyces kanamyceticus*. Recently, three newer aminoglycoside *viz.*, gentamicin, tobramycin and amikacin have largely replaced it.

Inactivation of most of the aminoglycoside antibiotics by microbial inactivating enzyme and narrow spectrum of their activity led to the search of newer semisynthetic aminoglycoside antibiotic i.e. "Amikacin" and it was first approved by USFDA in 1976 for its clinical use.

Chemistry

All aminoglycoside antibiotics have a common chemical structure containing two or more amino sugars joined in glycosidic linkage to a hexose nucleus, which is usually in central position. Amikacin belongs to kanamycin family, which has a hexose nucleus aminocyclitol named 2-deoxystreptamine. In this family, two amino sugars are linked to a centrally located 2-deoxystereptamine moiety, one of these is 3-amino hexose.

Amikacin, a derivative of kamamycin acylated with L (-) γ amino – α- hydroxybutyric acid at the C-1 amino group of the 2-deoxystreptamine residue of kanamycin – A. It is a water soluble, white crystalline powder having melting point of 203-204°C and available in the form of its sulphate salt. Molecular formula of amikacin sulfate is $C_{22}\ H_{43}\ N_5\ O_{13} - 2\ H_2SO_4$. Its chemical formula is O-3- Amino-3-deoxy-α-D-glucopyranosyl (1-4)-O-[6-amino-6-dexoy-α-D-glycopyerosyl (1-6)]-N_3-(4-amino-L-2-hydroxy butyryl)-2-dexoy-L-streptamine sulphate. The chemical structure of amikacin is given in Fig I.

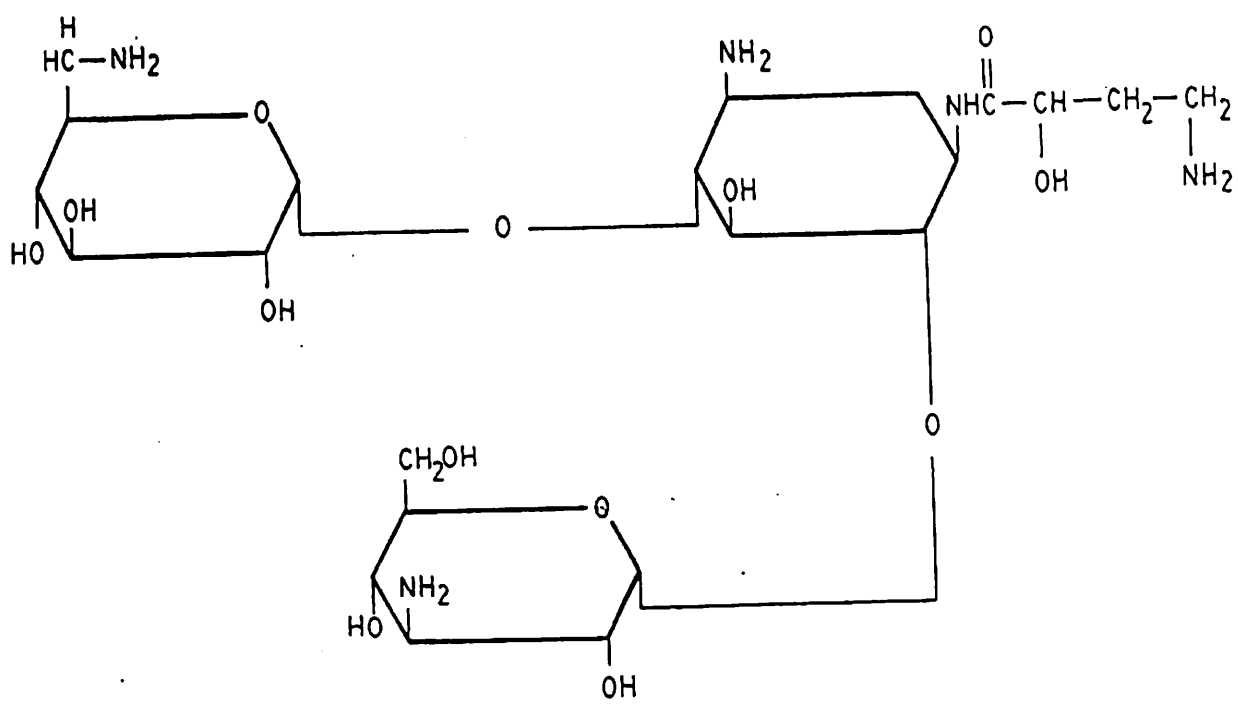


Fig. I. : Chemical Structure of Amikacin

Mechanism of action

Aminoglycosides are mostly used against gram negative infections in which they interfere with protein synthesis. Like other aminoglycosides, amikacin is a bactericidal agent having high affinity for ribosomal sites and inhibits protein synthesis (Shanon and Phillips, 1982). It is transported across the cell membrane in two steps :-

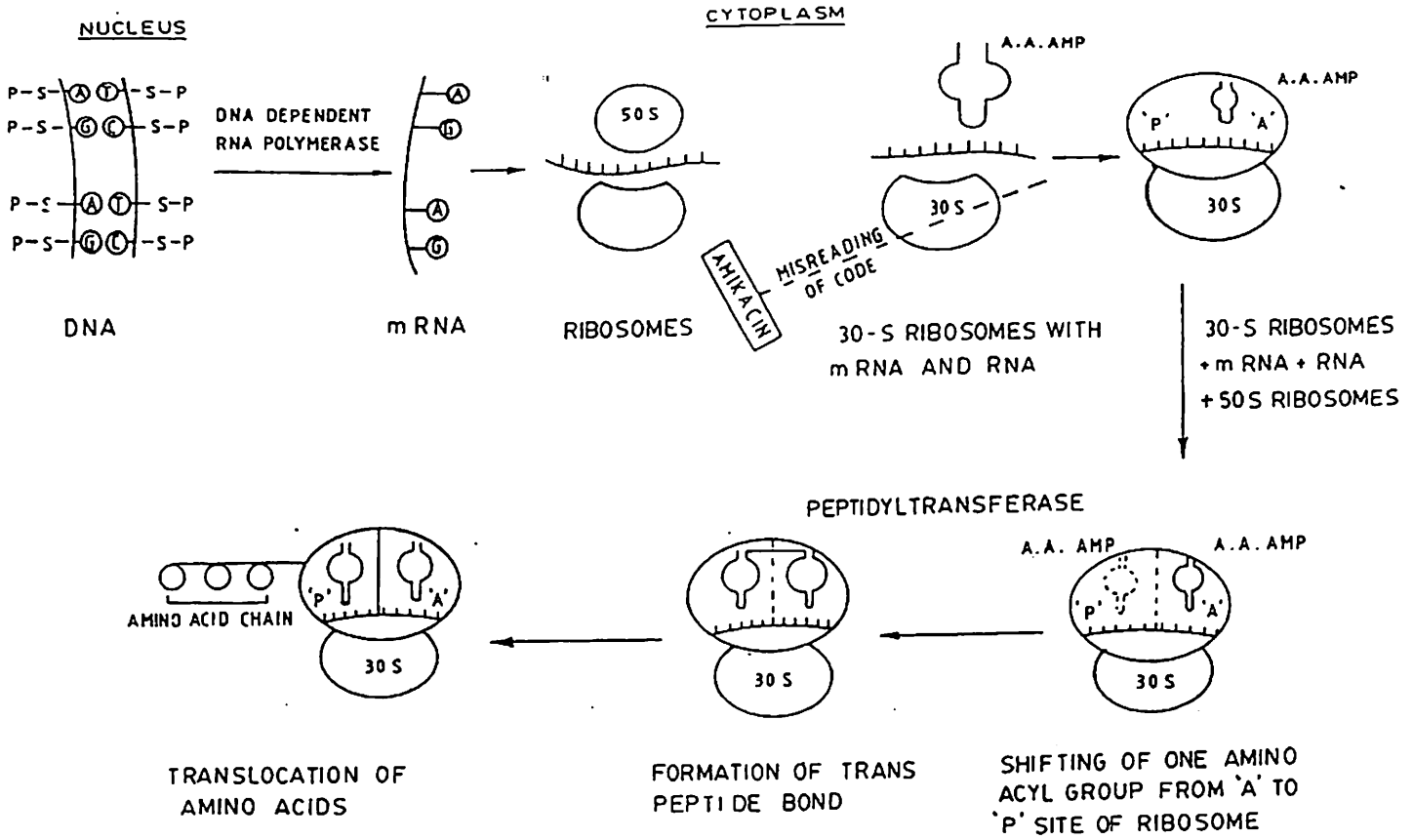
a. *Energy dependent phase I* - Relatively inefficient and involves binding to energy complex transported through the cell membrane (Bryan and Kwan, 1981; Mates *et al.*, 1983).

b. *Energy dependent phase II* - Energy dependent transport which is mainly responsible for accumulation of the drug in cells (Busse *et al.*, 1992).

Amikacin combines with 30s ribosome and induces misreading of m RNA template and also interferes with the initiation of protein synthesis leading to accumulation of abnormal initiation complex (Tai *et al.*, 1978). The mechanism of action of amikacin is shown in fig II.

Antimicrobial efficacy

Amikacin, a bactericidal agent at present is widely used in medical and veterinary practices to treat mild to severe bacterial infections of respiratory system, skin and urogenital system. Amikacin is a preferred agent to treat serious nosocomial gram negative bacillary infections in hospital where resistance of microorganisms to gentamicin and tobramycin has become a significant problem. It has little activity against anaerobic microorganisms or facultative bacteria under anaerobic conditions.



MODE OF ACTION OF AMIKACIN

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

Fig. II : Mode of action of Amikacin

Amikacin has unique resistance to aminoglycoside inactivating enzymes and it is active against the vast majority of aerobic gram-negative bacilli. It is much more effective against *Escherichia coli, Enterobacter, Klebsiella, Proteus mirabilis, Actinobacillus* species, *corynebacterium equi, Streptococcus zooepidermicus, Staphylococcus aureus* and *Serratia* (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 also been used in the treatment of disseminated atypical mycobacterial infections in AIDS patients.

The therapeutic plasma levels of amikacin for antibacterial action range from 1-4 μg/ml (Leroy *et al.*, 1978). Minimum inhibitory concentration of amikcain for *Corynebacterium equi* (30 isolates), *Escherichia coli* (5 isolates), *Enterobacter cloacae* (1 isolate) is less than or equal to 2 μg/ml (Brown *et al.*, 1984). Orisini *et al.* (1985) reported the MIC of amikacin 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).

Typical a minimum inhibitory concentrations 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 pneumonia	1
Proteus mirabilis	2
Providencia stuartii	2
Pseudomonas aeruginosa	2
Serratia spp.	8
Enterobacter faecalis	≥ 64
Staphylococcus faecalis	16

Sources : Chamber and Sande (1996)

Pharmacokinetics of amikacin

Like other aminoglycosides, amikacin possesses polycations and therefore, it is not adequately absorbed after oral administration (Cox, 1970; Breen *et al.*, 1972), poorly penetrates into cerebrospinal fluid, (Strausbaugh *et al.*, 1997) and excreted rapidly from kidney (Benet and Sheiner, 1985). In most of the species, volume

of distribution is found to be low (Prescott and Baggot, 1988) - characteristics of all aminoglycosides. It is reported that aminoglycoside group of antimicrobials have negligible binding with proteins (Gyselynck *et al.*, 1971; Gordan *et al.*, 1972). Pharmacokinetics of amikacin in different species are given below :-

Cat

Jernigan *et al.* (1988) studied the kinetics of amikacin in cats. Six mixed-breed adult cats were given 5 mg/kg body weight of amikacin sulphate by rapid i.v., i.m., and s.c. routes. The serum concentrations time data were analysed using non-compartmental model. The harmnic mean ± pseudo SD of the effective half-life ($t_{1/2}$ β) of amikacin was 78.8 ± 19.3 minutes after i.v. administration, 118.7 ± 14.4 min. after i.m. administration and 117.7 ± 12.8 min after s.c. administration. The arithmetic mean ± SD of mean resident time (MRT) was 118.3 ± 21.7 min, 173.4 ± 19.9 min, and 171.7 ± 19.1 min. after i.v., i.m., and S.C. routes of drug administration, respectively. The mean apparent volume of distribution at steady state (Vd_{SS}) was 0.17 ± 0.02 L/kg, and the mean total body clearance (Cl_B) was 1.46 ±0.26 ml/min/kg. Mean bio-availability (F) was 95 ± 20 percent after i.m. administration and 123 ± 33% after s.c. route of drug 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 its rapid absorption, good bio-availability, and ease of administration.

Dog

After i.v., i.m. and s.c. injections of single dose of amikacin (5, 10 and 20 mg/kg of body weight) in each of four dogs, the elimination kinetics of amikacin were determined by Baggot *et al.* (1985). The pattern of urinary excretion and cumulative amount excreted unchanged in 24 h is independent of dosage. Amikacin had a short half life (approx. 1 h) that was independent of dosage. Intravenous injection of 10 mg/kg gave apparent volume of distribution (Vd_B) of 226 ± 37 ml/kg and body clearance (Cl_B) of 2.64 ± 0.24 ml/min/kg. Within 6 h, more than 90% of the antibiotic was excreted in the urine, regardless of the route of administration. For dogs with normal renal function, amikacin of 10 mg/kg (intramuscular or subcutaneous) is recommended every 8 h for treatment of systemic infections and every 12 h for the treatment of urinary tract infections caused by susceptible bacteria.

Birds

The pharmacokinetics of amikacin in healthy mature female chickens (n = 6) was studied by El- Gammal *et al.* (1992).

Single dose of amikacin was injected as i.v. bolus (10 mg/kg) and i.m. (20 mg/kg) in the same bird after 30 days interval. The i.v. pharmacokinetics could be described by a two compartment model with distribution phase half-life ($t_{1/2}$ α) of 0.150 ± 0.064 h and a terminal phase half life ($t_{1/2}$ β) of 1.44 ± 0.34 h. The total body clearance (Cl_B) was 1.109 ± 0.017 L/kg/h and the volume of distribution at steady state (Vd_{SS}) was 0.193 ± 0.60 L/kg. Following a single i.m. injection, the peak plasma concentration was 50.79 ± 4.05 μg/ml and occurred at 0.50 ± 0.2 h. Simultaneous modeling of i.v. and i.m. results provided estimates of an absorption half life ($t_{1/2}$ Ka) of 0.480 ± 0.158 h. The i.m. pharmacokinetics after repeated administration were studied following the 10th dose (20 mg/kg, every 8h). The maximum plasma concentration was 38.58 ± 6.98 μg/ml and occurred at 0.79 ± 0.37 h. The multiple dosing yielded peak concentration of 39 μg/ml and trough concentration of 3.26 μg/ml. The recommended amikacin dosage in chickens is 20 mg/kg body weight every 8 h.

Ramsay *et al.* (1993) studied the pharmacokinetics of gentamicin sulfate and amikacin sulfate in the cockatiel (*Nymphicus hollandicus*). They were evaluated by utilizing treatment regimens developed in larger parrot species. Serum antibiotic concentrations were determined following twice daily i.m. treatment with 5 mg

gentamicin per kg body weight and 15 mg amikacin per kg body weight. Peak value of gentamicin was 4.6 ± 1.45 μg/ml and trough concentration was 0.17 ± 0.04 μg/ml. Based on this study, the recommended i.m. dose regimen for gentamicin in cockatiel is 5 to 10 mg/kg body weight 2 or 3 times per day. An 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.

The pharmacokinetic parameters of amikacin in red-tailed hawks (*Buteo jamaicensis*) following i.m. administration of a single 20 mg/kg dose were studied by Bloomfield *et al.* (1997). After a rapid absorption phase, mean amikacin serum concentration peaked (65 ± 12 μg/ml) at 30-45 min following injection. The serum amikacin concentration decreased to 2.3 ± 0.2 μg/ml at 12 h post injection. Amikacin was eliminated with first order kinetics, characteristic of 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 μg/ml (mean=2.5 μg/ml) for 42 isolates of gram-negative bacteria and coagulase positive *Staphylococcus* species. The 20 mg/kg dose used in this study resulted in serum concentration or above the MICs for > 12 h for most of isolates examined. Amikacin administrations 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 red-tailed hawk.

Camel

Wasfi *et al.* (1999) studied disposition kinetics of amikacin sulfate after i.v. (3.75 mg/kg body weight) and i.m. (3.75 mg/kg body weight) administration on separate occasions with intervals of 14 days in 5 healthy camels. Blood samples were collected at intervals for upto 480 min after drug administration. 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 bio-availability was close to 100%. It is suggested that i.m. administration is a possible route for amikacin therapy. A daily dose of 10 mg/kg was suggested for amikacin treatment to result in a maximum serum concentration of about 40 μg/ml. It was recommended however, that this dosing rate should be tested clinically by a multiple dose study.

Equine

Six mares were given 5 i.m. injection (at 12 h intervals between doses) of amikacin sulphate at a dose rate 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. Mean serum amikacin concentration peaked at 1 to 2 hours after i.m. injection.

The highest mean serum concentration was 19.2 μg/ml (1.5 hours after the 5th injection). The peak urine concentration of 1458 μg/ml was reached. Pharmacokinetic values were estimated after 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 between both the groups (Brown *et al.*, 1984)

The pharmacokinetics of amikacin sulphate were studied in 12 horses after i.v. and i.m. administration of doses of 4.4, 6.6 and 11 mg/kg. Serum (cs), synovial (csf) and peritoneal (cpf) fluid concentrations of the drug were measured. The serum concentrations at 15 min. following i.v. injections were 30.3 ± 0.3, 61.2 ± 6.9 and 122.8 ± 7.4 μg/ml, respectively, for the 4.4, 6.6 and 11.0 mg/kg doses. The mean peak cs values occurred 1 h after 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 mg/kg doses, respectively. Based on minimum inhibitory concentration for six pathogens (*Klebsiella pneumoniae, E. coli, Serretia rubidaea, Pseudomonas stutzeri* & 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 serious infections, dosing three times a day (Orsini *et al.*, 1985).

Brown *et al.* (1986) carried out pharmacokinetics study in six healthy foals aged between 2 to 11 days. Amikacin sulphate was administered i.m. at the dose rate of 7 mg/kg. Serum concentrations were measured serially over 24 h. The mean peak serum concentration was 14.7 μg/ml at 0.5 h. The elimination rate constant (β)of 0.24 h^{-1}, the elimination half life ($t_{1/2}$ β) of 3.0 h and the apparent volume of distribution of 0.58 L/kg were recorded.

Sheep

The disposition kinetics of amikacin in Bergamasca sheep was investigated following i.v. and i.m. administration by Carli *et al.* (1990) and following i.v. administration by Uppal *et al.* (1998). The values of distribution half life ($t_{1/2}$ α), elimination half life ($t_{1/2}$ β), total body clearance (Cl_B), area under curve (AUC) and volume of distribution 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 by Carli *et al.* (1990) following i.v., administration whereas 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 ± 0.031 L/kg, respectively, were noted by Uppal *et al.* (1998) after i.v. administration of amikacin. The bio-availability of the drug following s.c. administration in sheep was 87% (Carli *et al.* 1990) and 99.7 % (Uppal *et al.*, 1998).

Goat

Uppal *et al.* (1992) investigated the pharmacokinetics of amikacin sulphate in male goats following i.v. injection of 10 mg/kg. The values for $t_{1/2}\,\alpha$, $t_{1/2}\,\beta$, Cl_B, AUC and Vd_{area} values were noted to be 15.7 min, 130.1 min, 2.13 ml/kg/min, 4853 μg/ml.min and 0.40 L/kg, respectively. At 6 h after the drug administration, about 90% had been eliminated.

Abo-el-Sooud (1999) studied the pharmacokinetics of amikacin 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 $t_{1/2}\,\alpha$, $t_{1/2}\,\beta$ and MRT were 11.03, 114.81 and 142.96 min, respectively. Following i.m. injection, the values of $t_{1/2}$ Ka, $t_{1/2}\,\beta$ and MRT 20.39, 122.86 and 205.5 min, respectively. Amikacin was hardly detected in goats milk 2.6 h after i.v. and i.m. injections. Amikacin urine concentrations were found much higher than those of plasma.

The pharmacokinetic behaviuor of amikacin was studied in 35 goats after i.m. administration @ 5 mg/kg (Errecalde *et al.*, 2000). Plasma concentration of amikacin was determined by using microbiological test with *Bacillus subtilis* BGA as the test microorganism. Blood samples were obtained at 5, 10, 15, 20, 25 and

30 min and 1, 2, 3, 4, 6, 8, 10 and 12 h after drug administration. The average plasma level (C_P max) elimination half life ($t_{1/2}$ β), total body clearance (Cl_B) and distribution volume (Vd/f) were found to be 29.7 ± 5.1 μg/ml at 32.4 min, 3.09 ± 0.6 h, 0.66 ± 0.1 ml/min/kg, 0.162 ± 0.03 L/kg, respectively. The drug was quickly absorbed from the injection site, reaching effective concentrations exceeding minimum inhibitory concentration for at last 10 h for most pathogens of veterinary interest. Intramuscular administration of 4-5 mg/kg can be considered satisfactory for the treatment of diseases caused by these microorganisms.

Agrawal *et al.* (2002) studied the comparative pharmacokinetic of amikacin (10 mg/kg i.m.) by microbiological assay method in normal and experimentally induced febrile goats (n=6) revealed that the plasma drug concentrations were significantly higher in febrile condition at most of the time intervals. Various pharmacokinetic parameters like $t_{1/2}$ β, AUC, MRT and Vd_{area} were significantly higher, whereas Cl_B, was significantly lower in febrile goats as compared to normal goats. Various pharmacokinetic parameters like $t_{1/2}$ α, $t_{1/2}$ β, AUC, MRT, Vd_{area} and Cl_B were found to be 0.52 ± 0.02 h, 2.08 ± 0.01 h, 75.95 ± 3.94 mg/L.h, 1.73 ± 0.08 h,

0.40 ± 0.02 L/kg and 2.20 ± 0.14 ml/kg/min, respectively in healthy goats. For maintaining mean therapeutic level of 2 μg/ml, a priming dose (D*) of 11.44 ± 0.59 mg/kg followed by maintenance dose (D_o) of 10.63 ± 0.55 mg/kg at shorter dosage interval of 8 h may be useful in normal goats, where as lower doses (D*) of 9.25 ± 0.47 mg/kg followed by D_o of 8.00 ± 0.37 mg/kg at longer dosage interval of 12 h may be advised in case of febrile goats.

Cow calf

The pharmacokinetics of amikacin sulphate were investigated in five calves following i.v. and i.m. administration (7.5 mg/kg). The initial concentration (87.05 μg/ml), distribution half life (21.6 min), elimination half life (150.5 min), the apparent volume distribution (350 ml/kg), the area under curve (5512 μg/ml.min) and the clearance (1.5 ml/min/kg) were noted after i.v. administration. Following i.m. administration, the peak time (45 min) and the area under curve (5458 μg/ml.min.) were significantly different to that of i.v. administration. No significant difference was observed in the terminal half life values, suggesting that elimination rate was independent of route of administration. The drug in aqueous solution showed a good bi-availability of about 0.99 (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 6 cross-bred calves. At 1 min, the concentration of amikacin in plasma was 116.9 ± 3.16 μg/ml and the minimum therapeutic concentration was maintained upto 8 h. The elimination half life and volume of distribution were 3.09 ± 0.27 h and 0.40 ± 0.03 L/kg, respectively. The Cl_B and T/P ratio were 0.09 ± 0.002 L/kg/h and 4.98 ± 0.41, respectively. Approximately 50% of the total dose of amikacin was recovered in urine within 24 h after administration. The drug concentrations ranging from 5 to 150 μg/ml was bound to plasma proteins to the extent of 6.32 ± 0.42% A satisfactory i.v. dosage regimen of amikacin in bovine calves would be 13 mg/kg followed by 12 mg/kg at 12 h intervals (Saini *et al.*, 1998).

Errecalde *et al.* (2001) studied the pharmacokinetics of amikacin in twelve Holando-Argentino (Argentine Friesian) calves divided into two groups of six, which were administrated amikacin at the same dose rate 5 mg/kg by i.v. and i.m. route to determine single-dose pharmacokinetics of the drug and also to propose i.v. and/or i.m. amikacin dosage regimen against gram-negative bacterial infections. Blood samples were taken before and after the drug administration at 0.08, 0.16, 0.25, 0.33, 0.41, 0.5, 1, 2, 3, 4, 6, 8, 10, and 12 h and serum

amikacin concentrations were determined. The average disposition kinetics curves of amikacin after i.v. and i.m. administration indicated that the elimination process is independent from these routes of administration. The findings also suggest that i.m. serum concentrations were similar to those obtained for i.v. administration at the elimination phase. Values for the parameters obtained by i.v. and i.m. routes did not differ significantly ($p > 0.05$). The systemic bioavailability for i.m. route was 99%. The values of various kinetic parameters *viz.*, $t_{1/2}$ β (6.2 h), AUC (143.8 μg/ml.h), MRT (8.5 h), Cl_B (0.6 ml/kg/min) and Vd_{SS} (0.29 L/kg) were obtained following i.v. administration of amikacin. The dosage regimen of amikacin after i.v. or i.m administration was proposed to be 3 mg/kg (D*) and 2 mg/kg (Do) at dosage interval of 12 h.

Buffalo calf

Uppal *et al.* (1998) studied the comparative pharmacokinetics of amikacin in buffalo calves. A single dose of 7.5 mg/kg body weight of amikacin was given by i.m. or s.c. routes to the same group of 6 buffalo calves at an interval of three weeks. The plasma levels of drug were measured at 10 intervals (from 5-360 min) after injection, and various pharmacokinetic values were recorded.

The values of various kinetic parameters after i.m. administration was $t_{1/2}$ β (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 (12.88 ± 0.86 min) and t_{max} (52.97 ± 2.10 min) following i.m. administration and s.c. administration ($t_{1/2}$ Ka = 23.03 ± 1.11 min; t_{max} = 80.50 ± 2.508 min). The findings suggested that the rate of absorption was faster after i.m. than s.c. route.

Mukta (2002) studied the pharmacokinetics of amikacin in five buffalo calves following intravenous administration of the drug at the dose rate of 7.5 mg/kg. The values of various kinetic parameters like $t_{1/2}$ α (0.75±0.23h), $t_{1/2}$ β (4.67 ± 0.45h), MRT (5.16 ± 0.30 h), Kel (1.006 ± 0.262 h^{-1}), Vd_{area} (1.06 ± 0.06 L/kg) and Cl_B (2.78 ± 1.45) ml/kg/min) were obtained when it was administered alone through i.v. route.

Some of the important kinetic parameters of amikacin obtained in different species of animals are shown in Table I.

Table I : IMPORTANT KINETIC PARAMETERS OF AMIKACIN IN DIFFERENT SPECIES

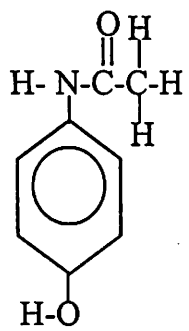
Species	Absorption half life, $t_{1/2}$ Ka (h)	Distribution half life, $t_{1/2}$ α (h)	Elimination half-life, $t_{1/2}$ β (h)	Volume of distribution (L/Kg)	Total body clearance, Cl_B (ml/kg/min)	Dose (mg/kg)	Route of administr-ation	Reference
1. Cat			1.31 ± 0.32	0.17 ± 0.02	1.46 ± 0.26	5	i.v.	Jernigan *et al.* (1988)
2. Dog			Approx. 1.0	0.226 ± 0.037	2.64 ± 0.24	10	i.v.	Baggot *et al.* (1985)
3. Birds								
(i) Chicken		0.150 ± 0.064	1.44 ± 0.34	0.193 ± 0.060	1.811 ± 0.283	10	i.v.	El-Gammal *et al.* (1992)
(ii)Red-tailed hawk's			2.02 ± 0.63	0.28 ± 0.03		20	i.m.	Bloomfield *et al.* (1997)
4. Camel				0.247	0.97	3.75	i.v./i.m.	Wasfi *et al.* (1999)
5. Equine								
(i) Mare	0.38		2.3 ± 0.18	0.26 ± 0.029	1.33 ± 0.11	7	i.m.	Brown *et al.* (1984)
(ii) Horse		0.17	1.44	0.198 ± 0.052	1.49 ± 0.39	4.4	i.v.	
		0.24	1.57	0.174 ± 0.028	1.28 ± 0.19	6.6	i.v.	Orsini *et al.* (1985)
		0.16	1.14	0.138 ± 0.018	1.41 ± 0.22	11.0	i.v.	
(iii) Foal			3.0	0.58		7	i.m.	Brown *et al.* (1986)
6. Sheep		0.436 ± 0.12	1.92	0.20	0.70	7.5	i.v.	Carl *et al.* (1990)
		0.109 ± 0.02	1.42 ± 0.89	0.335 ± 0.003	2.71 ± 0.13	10	i.v.	Uppal *et al.* (1988)
7. Goat		0.26 ± 0.03	2.16 ± 0.13	0.40 ± 0.04	2.13 ± 0.18	10	i.v.	Uppal *et al.* (1992)
		0.18	1.91			7.5	i.v.	Abo-el-Sooud (1999)
			3.09 ± 0.6	0.162 ± 0.03	0.66 ± 0.1	5	i.m.	Errecalde *et al.* (2000)
		0.52 ± 0.02	2.08 ± 0.01	0.40 ± 0.02	2.20 ± 0.14	10	i.v.	Agrawal *et al.* (2002)
8. (i) Cow calf		0.36 ± 0.05	2.51	0.35	1.50	7.5	i.v.	Carli *et al.* (1990)
(ii) Cross-bred calf			3.09 ± 0.27	0.40 ± 0.03	1.50 ± 0.03	10	i.v.	Saini *et al.* (1998)
(iii) Holando-Argentino calf			6.2	0.29	0.6	5	i.v.	Errecalde *et al.* (2001)
9. Buffalo calf	0.214 ± 0.14		3.1 ± 0.107	0.201 ± 0.005	0.752 ± 0.012	7.5	i.m.	Uppal *et al.* (1998)
	3.83 ± 0.18		3.24 ± 0.83	0.214 ± 0.006	0.756 ± 0.13	7.5	s.c.	Uppal *et al.* (1998)
		0.75 ± 0.23	4.67 ± 0.45	1.06 ± 0.06	2.78 ± 1.45	7.5	i.v.	Mukta (2002)

PARACETAMOL

Paracetamol is a potent antipyretic classified under nonsteroidal antiinflammatory drugs (NSAIDs). It is a para-aminophenol derivative. Paracetamol is well tolerated and lacks many of the side effects of aspirin.

Chemistry

Chemically, paracetamol is a para-acetaminophenol. The chemical structure is as follows:-



Chemical formula = N-acetyl-p-amino phenol

Emperical formula = $C_8H_{15}O_2N$

Molecular weight = 157

History

Acetanilide is a parent member of para-aminophenol group of drugs which was introduced into medicine 1866 by Cahn and Hepp. Due to its excessive toxicity, a new agent of para–aminophenol derivatives, phenacetin (Acetophenetidine) was introduced into therapy in 1887. Paracetamol (acetaminophen) was first used in

medicine by von Mering in 1893. It has gained popularity since 1949, after it was recognized as a major active metabolite of both acetanilide and phenacetin.

Pharmacological properties

Paracetamol is an effective analgesic–antipyretic but only a weak antiinflammatory agent. Although classified as a NSAID, its mechanism does not involve inhibition of cyclooxygenase. Paracetamol is a weak inhibitor of cyclooxygenase in the presence of high concentrations of peroxides that are found in inflammatory lesion, and hence, fails to exert antiinflammatory action (Marshall *et al.*, 1987; Hanel and Lands, 1982). Like other NSAIDs, acetaminophen does not inhibit neutrophil activation (Abramson and Weissmann, 1989). The minimum therapeutic concentrations of paracetamol was noted to be 10-20 μg/ml (Rawling *et al.*, 1977).

Therapeutic activity

Paracetamol is a suitable substitute for aspirin for analgesic or antipyretic uses. It is valuable for patients in whom aspirin is contraindicated (*e.g.*, those with peptic ulcer) or those patients who have prolonged bleeding time caused by aspirin. It is one of the most commonly used 'over the counter' analgesics for headache, musculoskeletal pain, dysmenorrhoea *etc.* where anti-inflammatory action is not required. It is one of the best drugs to be used as antipyretic in man and animals.

Kinetic study

Microbial infections are most commonly associated with pyrexia. Hence, antipyretics like paracetamol are commonly used concurrently with antimicrobials. Pharmacokinetic studies of paracetamol were conducted in different species of animals. They are as follows: -

Goat

Modification of the disposition kinetics of paracetamol by oxytetracyclin and endotoxin induced fever in goats were studied by Manna *et al.* (1994). The theoretical zero time concentration (C_p^o =A+B) of paracetamol alone (163.3 ± 9.9 μg/ml) was significantly lower ($p<0.01$) as compared to combined administration with oxytetracyclin (56.0 ± 2.6 μg/ml) post i.v. dose of 50 mg/kg. The distribution rate constant (α) of 6.93 h^{-1} ($t_{1/2}$ α = 0.10 h) and elimination rate constant (β) of 1.30 h^{-1} ($t_{1/2}$ β = 0.53 h) were obtained when paracetamol (50 mg/kg) was administered alone in goat.

Sudha Kumari (1998) studied the pharmacokinetics of paracetamol in 6 lactating goats following single i.v. administration of paracetamol (50 mg/kg). Peak plasma concentration of 29.92 ± 3.33 μg/ml was attained at 5 min. when given alone. The values of different kinetic parameters *viz.*, $t_{1/2}$ α (0.24 ± 0.04 h), β (0.196 ± 0.008 h^{-1}), $t_{1/2}$ β (3.56 ± 0.13 h), AUC (62.49 ± 14.43 mg/L.h), Kel (0.563 ± 0.117 h^{-1}), T ≈P (1.99 ± 0.64), Vd_{area} (5.48 ± 1.40 L/kg) and

Cl_B (17.37 ± 4.24 ml/kg/min) were noted following single i.v. administration of paracetamol.

Crossbred calf

Pharmacokinetic study of paracetamol was done in crossbred calves following single i.m. administration of paracetamol (50 mg/kg) by Sharma *et al.* (1995). They noted absorption rate constant (Ka) of 2.91 ± 0.36 h^{-1} and absorption half life ($t_{1/2}$ Ka) of 0.251 ± 0.035 h. They further noted elimination rate constant (β) of 0.167 ± 0.03 h^{-1} and elimination half life ($t_{1/2}$ β) of 4.84 ± 1.26 h. They also obtained volume distribution (Vd_{area}) of 0.48 ± 0.11 L/kg and total body clearance (Cl_B) of 79.6 ± 22.7 ml/kg/h.

Buffalo Calf

Plasma levels, disposition kinetics and dosage regimen of paracetamol (PCM) were investigated in 6 buffalo calves following single i.m. administration (50 mg/kg). The absorption half-life ($t_{1/2}$ Ka) and elimination half life ($t_{1/2}$ β) were 0.47 ± 0.40 and 8.69 ± 0.83 h, respectively. The volume of distribution and total body clearance were 1.30 ± 0.26 L/kg and 113.1 ± 39.8 ml/kg/h, respectively. A satisfactory i.m. dosage regimen of PCM in buffalo species would be 47 mg/kg followed by 23 mg/kg body weight at 8 h intervals (Sidhu *et al.*, 1993).

Some of the important kinetic parameters of paracetamol obtained in different species of animals are shown in Table II.

Table II : IMPORTANT KINETIC PARAMETERS OF PARACETAMOL IN DIFFERENT SPECIES

Species	Absorption half-life, $t_{1/2}$ Ka (h)	Distribution half-life, $t_{1/2}\alpha$,(h)	Elimination half-life, $t_{1/2}\beta$ (h)	Volume of distribution (L/kg)	Total body clearance (ml/kg/min)	Dose (mg/kg)	Route of administration	Reference
Buffalo calf	0.47±0.04	-	8.69±.0.83	1.22±0.23	113.1 ± 39.8	50	i.m.	Sidhu *et al.* (1993)
Cross bred calf	0.251±0.03 5	-	4.84±1.26	0.48±0.11	79.6 ± 22.7	50	i.m.	Sharma *et.al.* (1995)
Goat	--	0.10	0.53	--	--	50	i.v.	Manna *et al.* (1994)
	0.32 ± 0.04	0.24±0.04	3.56±0.13	5.48±1.40	17.37 ± 1.46	50	i.v	Sudha Kumari (1998)

KINETIC INTERACTIONS OF ANTIMICROBIALS WITH NSAIDs

Antimicrobials and non steroidal analgesics, antipyretics and antiinflammatory drugs (NSAIDs) are used frequently to combat infections associated with pyrexia and pharmacokinetic interactions between them have been described (Joly *et al.*, 1988; Manna *et al.*, 1994; Sharma *et al.*, 1997; Sudha Kumari, 1998; Tang *et al.*, 1999; Varma *et al.*, 2002; Chaudhary *et al.*, 2002; Mukta, 2002; Nitesh Kumar *et al.*, 2003).

Joly *et al.*, (1988) studied the effects of non-steroidal antiinflammatory drug, diclofenac, in rabbit on the kinetics of three cephalosporins *viz.*, Cefotiam, cefmenoxime and ceftriaxone. They compared the antibacterial effect of these antibiotics, given alone or with diclofenac, in experimental endocarditis. Diclofenac significantly increased ($p < 0.05$) the area under the curve in tissue cage fluid of ceftriaxone and cefotiam treated animals, and the terminal half life of ceftriaxone in their sera (3.45 ± 4 vs 2.8 ± 0.5 h). Diclofenac reduced urinary excretion of cefotiam only. Cefmenoxime pharmacokinetics remained unchanged by diclofenac. The alteration of ceftriaxone kinetics appeared to be due to non-renal mechanism and could suggest reduction of biliary excretion. In *Esch. coli* endocarditis, diclofenac enhanced the concentration ($p < 0.05$) of cefotiam (23 ± 16

vs 8.9 ± 5 μg/g) and ceftriaxone (13.2 ± 3 vs 8.5 ± 4 μg/g) in infected vegetation, but not that of cefmenoxime. The antibacterial effect of cefriaxone increased with diclofenac (5.5 ± 1 vs 7.2 ± 1 log^{10} colony forming unit/g of vegetation). *In vitro*, neither protein binding to rabbit serum proteins nor intrinsic activity on the *E. coli* strain of each antibiotic was modified by diclofenac. These results suggest that antiinflammatory drugs could increase antibiotic efficacy by altering their pharmacokinetics.

Pharmacokinetic study of paracetamol alone and along with oxytetracyclin in goats was carried but by Manna *et al.* (1994). They observed that the C_{max} value of paracetamol alone as 128.0 ± 8.0 μg/ml at 0.03 h post i.v. drug administration. Paracetamol persisted in the blood till 2 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 as 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 were significantly higher ($p < 0.02$) from the corresponding values of paracetamol alone.

Influence of paracetamol on disposition kinetics of cefotaxime in crossbred calves was studied by Sharma *et al.*, (1997).

First, paracetamol was injected intramuscularly (50 mg/kg) followed by cefotaxime into jugular vein @ 10 mg/kg. The calculated value of C_p^0 (77.8 ± 14 μg/ml) of cefotaxime administered alone was higher as compared to combined administration. The higher values of distribution rate constant indicated rapid distribution into the various body fluids and tissue compartments in case of combined administration. The calculated apparent value of distribution (5.02 ± 0.56 L/kg) and total body clearance (0.65 ± 0.5 L/kg/h) of cefotaxime given with paracetamol are quite identical with their respective values obtained following its alone administration. The suitable dosage schedule of cefotaxime given with paracetamol is 10 mg/kg followed by 8 mg/kg repeated at 12 h intervals by i.m. route.

Modification of disposition kinetics of enrofloxacin at the rate of 5 mg/kg when given alone and along with paracetamol at the dose rate of 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 when given alone and 6 h for enrofloxacin when given along with paracetamol. Significantly higher values were obtained for zero time concentration in distribution phase (A) and theoretical zero time concentration (C_p°) which 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, 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±0.10h, 9.85 ± 1.38mg/L.h, 0.436 ± 0.133 h^{-1}, 0.51 ± 0.06, 1.11± 0.22, 2.34 ± 0.54L/kg and 9.40 ± 1.36 ml/kg/min, respectively).

Pharmacokinetic interaction of quinidine with diclofenac was studied in rhesus monkey by Tang *et al.* (1999). Following infusion of diclofenac *via* portal vein 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 co-administered with quinidine (0.25 mg/kg) *via* the same route, the corresponding plasma diclofenac concentrations were 50, 59 and 18 ng/ml, representing 57, 56 and 56% 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 clearances 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 quinidine. Stimulation of diclofenac metabolism of 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 diclofenace 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 towards diclofenac metabolism is enhanced by quinidine.

Alternation of disposition kinetics of enrofloxacin was studied in five cattle following i.m. administration alone and along with diclofenac sodium (0.8-1.0 mg/kg) by Varma *et al.* (2002). Therapeutic concentration (0.1 μg/ml) in plasma was maintained upto 12 and 24 h for enrofloxacin alone 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 for enrofloxacin when given along with diclofenac sodium as compared to its alone administration (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/L.h) and C_{max} (0.2 μg/ml) were significantly lower when enrofloxacin was administered along with diclofenac sodium as compared to its alone administration (5 mg/L.h and 0.82 μg/ml, respectively). Diclofenac sodium significantly ($p < 0.1$) reduced the plasma concentrations of ciprofloxacin (a metabolite of enrofloxacin). Based on the calculated pharmacokinetic parameters, an intramuscular dosage regimen of enrofloxacin (priming dose of 1.8 mg/kg following by maintenance dose of 1.10 mg/kg every 8h) to maintain a therapeutic concentration of 0.1 μg/ml is recommended in cattle.

The pharmacokinetics of cefuroxime administered with paracetamol was investigated in buffalo calves. Cefuroxime was administered by single intravenous dose of 10 mg/kg and paracetamol was given by single intramuscular dose of 50 mg/kg. Pharmacokinetics of cefuroxime was described by two compartment open model. The highest concentration of cefuroxime was 80.9 ± 6.40 μg/ml at 1 min, which was decreased to 0.97 ± 0.07 μg/ml at 8 hr after its administration. The values of distribution half life, ($t_{1/2}$ α)

elimination half life ($t_{1/2}\beta$) and Vd_{area} were 0.101 ± 0.009 h, 1.91 ± 0.07 h and 0.55 ± 0.12 L/kg, respectively. The dosage regimen of cefuroxime when administered with paracetamol was calculated to be 10 mg/kg body weight repeated at 8 h intervals (Chaudhary *et al.*, 2002).

Mukta (2002) studied the kinetic interaction of amikacin with diclofenac in five female buffalo calves following i.v. administration of amikacin (7.5 mg/kg) alone and along with diclofenac (1 mg/kg i.v). The plasma drug concentrations were found to be significantly lower when it was given in combination with diclofenac as compared to its alone administration. Lower values of AUC, AUMC, Kel and T ≈ P were noted when amikacin was given in combination with diclofenac (10.25 ± 1.34 μg/ml.h, 100.9 ± 25.04, μg/ml.h^2, 0.191 ± 0.016 h^{-1}, & 0.69 ± 0.19) as compared its alone to administration. However, higher values of MRT and Vd_{area} were observed in case of co-administration (9.35 ± 1.01 h and 6.88 ± 0.40 L/kg). There is no effect of diclofenac over the α, $t_{1/2}\beta$ and Cl_B on amikacin were noted between both groups. The calculated loading (D*) and maintenance (D_o) 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).

Kinetic interaction between enrofloxacin and diclofenac was studied in buffalo calf following intravenous administration by Nitesh *et al.* (2003). The various kinetic parameters of enrofloxacin obtained when given alone and with diclofenac did not differ significantly implying that diclofenac did not have any particular influence over the distribution, elimination and metabolic processes of enrofloxacin in buffalo calves. No significant difference in dosage regimen was observed between both the groups. It may lead to conclusion that enrofloxacin can be used safely and effectively with diclofenac.

GENERAL PHARMACOKINETICS

Pharmacokinetics is well known as disposition kinetics, which helps in knowing absorption, distribution, metabolism and excretion of drugs (Dost, 1953). The main objectives of pharmacokinetics of drugs are to study in respect of drug concentrations *versus* time course, their metabolites in various body fluids, tissues and excretion and interpretation of such data based on suitable pharmacokinetic compartment models (Wagner, 1968).

The compartment model is a hypothetical structure and is used to characterise with reproducibility of behaviour and fate of a drug in a biological system, when administered by certain route in a particular dosage form. In pharmacokinetic studies, compartment is an entity, which has definite volume and in that concentration a drug exists at any time.

The pharmacokinetics of a drug is described either by one compartment, two compartment or multi compartment open model. In an open compartment model, body distributes the drug in all tissues at widely varying rates. in an open compartment model, the drug moves freely from one compartment to another (i.e. from blood to tissues and *vice versa*).

One compartment open model

The drug is said to follow one compartment open model when the distribution of drug is instantaneous between central and peripheral compartment. Any change in drug concentration in blood reflects directly the quantitative change in its tissue level. The rate of drug elimination from the body is directly proportional to concentration of drug in blood (Baggot, 1974). When the plasma concentration time profile is plotted from the peak concentration onwards on a semilogarithmic scale, a straight line is obtained in case of one compartment open model (Sams, 1978). The plasma drug levels decline according to the following equation.

$$Cp = Be^{-\beta t} \quad \text{.....Eq.1}$$

where ,

Cp = Concentration of 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 intravenous administration is accurately described by two compartment open model. Baggot (1974) described that in two compartment open model, the drug distribution is quick and homogenous into the central compartment (blood and other readily accessible tissues like liver and kidney) and comparatively more slowly into peripheral compartment (less perfused organ and tissue such as muscle and fat). This clearly shows that elimination mainly takes place from central compartment and distribution and elimination processes follow the first order kinetic. Semilogarithmic plot of plasma log drug concentration against time shows a biphasic curve in two compartment open model. This biphasic curve shows initial steep decline in plasma drug concentration, which is mainly due to distribution of drug from central to peripheral compartment

and the gradual decline is obtained in later phase 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.

$$C_p = Ae^{-\alpha t} + Be^{-\beta t} \quad \text{.....Eq. 2}$$

where,

C_p = Plasma concentration of the drug

A = Zero time intercept of distribution phase

B = Zero time intercept of elimination phase

α = Distribution rate constant

β = Elimination rate constant

t = Time elapsed after drug administration

e = Base of natural logarithm

The values of A, B, α and β are essential in calculating the kinetic rate constants (K_{12}, K_{21} and Kel) in two compartment open model. The value 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 multiple compartment open model

The disposition kinetics of some drugs may also follow three or multi compartment. A semilogarithmic plot of plasma drug

concentration against time shows triphasic or multiphasic curve. The initial steep decline in plasma drug concentration against time is due to distribution of drug from blood to highly perfused tissue known as peripheral – I. After that, gradual decline is due to distribution of a drug from central to less perfused tissue known as peripheral - II. The drug concentration in plasma following single i.v. administration is expressed by following triexponential mathematical formula as a function of time.

$$Cp = Ae^{-\alpha t} + Be^{-\beta t} + Ce^{-\gamma t} \quad \text{.....Eq. 3}$$

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

Clinical importance of pharmacokinetic study

Clinically, the pharmacokinetic 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 its extravascular (i/m/s.c./oral) administration.

2. *Distribution rate constant (α) and distribution half life ($t_{1/2}$ 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)*

The rate of elimination from the body for most of drugs follows first order of reaction process and is a hybrid or composite constant describing a rate of fall to which more than one process is contributing.

Baggot (1977) and Mercer *et al.* (1977) stated that elimination rate constant is the most essential kinetic parameters and is employed to determine: -

a. The elimination half-life ($t_{1/2}$ β)

b. The total body clearance (Cl_B)

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

d. The drug withdrawal period for drug residues in milk and tissues of food producing animals.

4. *Elimination or biological half life ($t_{1/2}$ 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 clearly shows 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. The elimination half life follows the first order process and hence is not dependent on the dose of the drug as well of route of administration. It is used to calculate the duration of drug action in the body and designing the rational dosage regimen.

5. *Volume of distribution*

The apparent volume of distribution 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. The apparent volume of distribution indicates the amount of distribution of a drug without providing any clue whether the dug 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 is due to high protein binding or low lipid solubility of a drug and indicates that the drug is restricted to certain fluid compartment, like plasma water, extracellular fluids *etc*. This is due to high protein binding or low solubility of drug.

6. Total body clearance (Cl_B)

Total body clearance is another important pharmacokinetic parameter, which is the sum of the clearance of each eliminating organ, particularly liver and kidney. The half life and total body clearance of a drug are different in the sense that half life depends upon the process of drug distribution, biotransformation and excretion, whereas Cl_B is independent of these processes and indicates the rate of drug removal from the body. Unlike β and $t_{1/2}$ 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

The extent of absorption (F) is generally known as bioavailability. Bioavailability of a drug indicates the rate of drug

absorption as well as the amount of absorption of a drug in pharmacologically active from. It is a measure of the fraction of administered dose of a drug that reaches the systemic circulation in the unchanged from. It is calculated experimentally by the ratio of the area under 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, drug effects and drug elimination. The protein bound drug also acts as a reservoir.

It is reported that the various constant, namely A, α, B, β, $t_{1/2}\,\alpha$, $t_{1/2}\,\beta$ 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 administered 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 a maintenance dose at a particular dose interval after administering the priming dose, so that plasma drug concentration must be above a minimum effective level and below a level producing excessive side effect 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 minimum effective levels.

Chapter - 3

Materials and Methods

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 105 to 180 kg body weight were used. The buffalo calves were kept in well maintained animal shed with concrete floor. The animals were maintained on dry fodder, cattle feed and greens along with routine grazing. Water was given *ad lib*.

EXPERIMENTAL DESIGN

Amikacin and paracetamol were administered by intravenous (i.v.) route separately in five healthy buffalo calves. Before administration of next dose of the drug, an interval of 21 days was allowed to elapse. After analysing kinetic parameters of these drugs separately, the drugs (amikacin and paracetamol) were administered concurrently by i.v. route to investigate the interaction of these drug in buffalo calves.

DRUGS USED

In the present investigation, amikacin and paracetamol were used. Amikacin, an injectable veterinary preparation containing amikacin sulphate equivalent to amikacin in concentration of 250 mg/ml marketed under the trade name of Vetacin® (25%) by Indo

Biocare Pvt. Ltd., India was used. Paracetamol an injectable commercial preparation marketed under the trade name of Paracetol-Vet® (15%) by Sarabhai Zydus Animal Health Limited, India was used. Paracetol–Vet contain paracetamol in a concentration of 150 mg/ml.

COLLECTION OF BIOLOGICAL FLUIDS AND THEIR TIMING

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

(A) **Blood**

Prior to blood collection, the sites around the jugular vein on either side of the neck of the animals were aseptically prepared. The sites were shaved and the area was cleaned with rectified spirit before each collection of blood. Blood samples were collected in sterilized centrifuge tubes containing sufficient amount of sodium oxalate by venepuncture with disposable 18G needles at various intervals of time as noted above. The blood samples were centrifuged at 3000 rpm for 10 min for the separation of plasma. The separated 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 collected.

(B) **Urine**

The urine samples were taken at different time intervals after the drug administration in sterile test tubes from 0.042 to 48 h. Urine samples were collected by introducing a sterile Foley's balloon 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. Prior to introducing the catheter it was lubricated with xylocaine 2% cream to desensitize the urinary passage. The balloon of catheter was inflated by injecting 25-30 ml of sterile water though a syringe to keep the catheter in position. The opening of the catheter was closed with a pressure clip to check dripping of urine. Before the drug administration, urine sample was colleted in a sterile test tube for the preparation of standards. The samples were kept in a refrigerator and analysed on successive days.

ADMINISTRATION OF DRUGS

Amikacin injection containing 250 mg of amikacin sulfate per ml was administered through i.v. route @ 7.5 mg/kg in each buffalo calf. Paracetamol (150 mg/ml) was injected by i.v. route at the dose rate of 40 mg/kg in each healthy buffalo calf. After conducting kinetic study of the above two drugs when administered alone, both the drugs were administered concurrently at the above stated dose rate in each animal through i.v. route to know the kinetic interactions of the drugs.

ESTIMATION OF AMIKACIN

I. Sterilization of needles, glass wares and porcelain assay cylinders

Materials like glasswares and porcelain assay cylinders were washed properly with detergent solution in running tap water. After that, these were rinsed with glass distilled water and finally air dried. Test tubes, centrifuge tubes, vials and vials containing porcelain assay cylinders were plugged with cotton wool. Assay plates, pipettes and syringes were wrapped with papers. All these materials were then kept in hot air oven for an hour at 160^0C. For blood collection and administration of drugs, sterile disposable needles were used.

II. Preparation of media

(a) Assay agar

Following ingredients were used for microbiological assay of amikacin in plasma 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

The different constituents were added in proper amount and were mixed thoroughly in a conical flask and pH was adjusted. The mouth of the flask was plugged tightly with cotton wool and wrapped with aluminium foil. Wet sterilization was done by autoclaving at 15 pound pressure (121^{0}C) for 20 minutes.

(b) Nutrients broth

Nutrient broth for culturing the microogranism with following composition was prepared :

Sl. No.	Ingredients	Grams/litre water
1	Sodium chloride	5.0
2	Peptone	10.0
3	Beef extract	10.0
	Distilled water	1000 ml
	Final pH	7.4 ± 0.1

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

III. Preparation of assay agar plates

Accurately 20 ml of autoclaved antibiotic assay media, while in melted state, was poured slowly into each of the sterilized

special assay plate (Borocil) with the help of a sterile measuring cylinder. For getting uniform thickness of media, the plates were kept on a horizontal plain surface. For solidification of agar, the plates were left at room temperature for about 1-2 hour. To ascertain any microbial contamination, they were then incubated at 37^{0}C for 24 hour. 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

Bacillus subtilis (ATCC 6633) was the test organism used for the microbiological assay technique of amikacin (Brown *et al.*, 1984; Orsini *et al.*, 1985). It was obtained from National Collection of Industrial Microorganism (NCIM), Division of Bio-chemical Sciences, National Chemical Laboratory, Pune-8.

Slant in culture tubes was made after transferring antibiotic assay media in hot melted condition. After solidification, plugged with cotton wool and wrapped with silver foil, they were kept in incubation at 37^{0}C for 24 hour to test contamination. Then uncontaminated tubes were transferred to a refrigerator till for further use. The test organism was grown by transferring the microorganism on the slant of culture tube and kept in incubation at 37^{0}C for overnight. Then it was stored in a refrigerator. The organism was weekly transferred to fresh slant to maintain its normal activity.

V. Preparation of standards in biological samples

Amikacin was diluted in sterile glass distilled water to have different strengths, *viz.*, 80, 40, 20, 10, 5, 2, 1 and 0.5 μg/ml. From each of the these solution, 0.1 ml was taken with the help of micropipette and added to sterile vials containing 0.9 ml of plasma or urine collected prior to drug administration. This yielded drug concentrations of 8, 4, 2, 1, 0.5, 0.2 and 0.1 μg/ml in the above noted biological samples. These standard samples were stored in a refrigerator and used simultaneously with test samples in assay plates for obtaining standard curve. Determinations of drug concentrations in test samples were carried out with the aid of standard curve. The concentration of amikacin was detected as low as 0.1 μg/ml.

VI. Assay procedure

The plasma 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 incubated in sterile nutrient broth and kept under incubation for 2 to 3 hour at 37^0C until the growth was seen (turbid by naked eye). Amikacin assay plates were flooded with the broth containing the organism. Excess broth after 10-15

minutes was drained out. The plates were then dried in the incubator at 37°C for a period of half an hour. Plates were marked against different standards and biological samples. Sterile porcelain assay cylinders of uniform size were placed against each mark at appropriate distance along the circumference in the incubated assay plates. 50 µl of each of the standard solution of various strengths as well as test samples of the drug was poured in separate porcelain cylinder in the assay plates. These assay plates were then left horizontally on plane surface of the table for about 2 hour and then kept in incubator at 37°C for overnight to allow the growth of organism. The mean diameters of the bacterial zone 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 PARACETAMOL BY SPECTROPHOTOMETRIC METHOD

The concentrations of paracetamol in plasma and urine were estimated by spectrophotometric method (Archer and Richardson, 1980; Omar and Mohammad, 1984). The details of procedure are as follows: -

Reagents

1. Trichloro acetic acid (CCl_3 COOH) 10%
2. Hydrochloric acid (HCl) 6 M
3. Sodium nitrite 10% ($NaNO_2$) freshly prepared
4. Ammonium sulfamate 15%
5. Sodium hydroxide 25% (NaOH)

Preparation of standards of paracetamol in biological fluids

The paracetamol was diluted in glass distilled water to different strengths *viz.*, 1000, 500, 250, 100, 50, 25 and 10 µg/ml. From each standard solution 0.1 ml was added to sterile vial containing 0.9 ml of normal plasma or normal urine collected prior to drug administration. This yielded standards of 100, 50, 25, 10, 5, 25 and 1 µg/ml in the above noted biological fluids. These standards were used simultaneously with test samples for the determination of drug concentrations in biological fluids.

Procedure

1. In a glass test tube, 1 ml of biological fluid was taken and 2 ml of trichloroacetic acid (10%) was added and thoroughly mixed for 30 sec. The content was then filtered through Whatman filter paper No. 1.
2. Whole filtrate was taken into 25 ml test tube containing 1 ml of 6 M HCl and 2 ml of freshly prepared sodium nitrite (10%) solution. The contents were mixed properly and allowed to stand exactly for 2 min. at room temperature.

3. Thereafter 2 ml of ammonium sulfamate (15%) solution was added drop by drop into the tubes and thoroughly mixed for 1 min.

4. Then 2.5 ml of sodium hydroxide (25%) solution was added and mixed for 15 sec. and allow to stand for 2 min at room temperature.

5. The optical density of the resultant colour developed was measured at 430 mμ in a spectrophotometer against blank.

6. The exact concentration of paracetamol was calculated with help of standard curve simultaneously prepared in biological fluids collected prior to drug administration.

7. Blank was simultaneously prepared in the same way using 1 ml of biological fluid taken prior to drug administration in place of test sample.

CALCULATION OF PHARMACOKINETIC PARAMETERS

The following pharmacokinetic parameters of amikacin and paracetamol were calculated after 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 Perrier (1975) and Notari (1980).

The concentration of the drug in plasma at any time 't' is obtained by following farmula

$C_P = Ae^{-\alpha t} + Be^{-\beta t}$.....(two compartment open model)

where , Cp = drug concentration of the drug in plasma at time 't'

e = base of natural logarithm.

The description of different kinetic parameters are as follows:

a. A, the zero time concentration of the drug in plasma during distribution phase (μg/ml).

b. α, the regression coefficient (distribution rate constant) for distribution phase was calculated by the method of residual yield (calculated in Appendix I)

c. B, the zero time concentration of the drug in plasma during elimination phase (μg/ml)

d. β, the regression coefficient (elimination rate constant) for the elimination phase was calculated by the method of least squares (shows in Appendix I).

e. C_p° (A+B), the theoretical zero time concentration of the drug in plasma (μg/ml)

f. $t_{1/2}\,\alpha$, distribution half life (h)

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

g. $t_{1/2}\beta$, elimination half life (h)

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

h. AUC, the total area under plasma drug concentration time curve ($mg/L.h^2$)

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

i. AUMC, the total area under the first moment of plasma drug concentration curve (mg/L.h)

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

j. MRT, mean residential time (h)

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

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

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

l. Kel, the elimination rate constant of drug from central comportment (h^{-1})

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

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

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

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

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

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

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

p. Vdc, the volume of distribution, based on distribution and elimination (L/kg)

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

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

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

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

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

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

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

t. Cl_B, the total body clearance (ml/kg/min)

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

CALCULATION OF DOSAGE REGIMEN

Dosage regimen is mainly 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. Keeping in view of the above fact, dosage regimen of amikacin were calculated at 1,2 and 4 μg/ml levels for the dosage intervals of 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^{∞} (min) = desired minimum plasma concentration (μg/ml)

γ = dosage interval (h)

e = base of natural logarithm

β = elimination rate constant (h^{-1})

Vd_{area} = volume of distribution based on total area under curve (L/kg)

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

STATISTICAL ANALYSIS

Concentrations of amikacin and paracetamol in plasma and urine at various time intervals, their kinetic parameters and the calculated dosage regimen of amikacin in buffalo calves when given alone and when given together were compared using paired 't'-test (Snedecor and Cochran, 1967).

Chapter - 4

Results

RESULTS

I. PHARMACOKINETIC STUDY AFTER SINGLE INTRAVENOUS ADMINISTRATION

(A) KINETIC STUDY OF AMIKACIN

1. *Plasma Levels*

The plasma drug concentration profile at different time intervals after single intravenous (i.v.) dose of 7.5 mg/kg of amikacin sulfate in female buffalo calves has been shown in Table 1 and Fig. 1. The mean plasma drug concentration of the drug at 0.042 h was found to be 30.01± 0.44 μg/ml and the value ranged from 28.52 to 31.09 μg/ml. The mean therapeutic concentration (≥ 2 μg/ml) was maintained upto 5 h. The drug was detectable in all buffalo calves upto 12 h with the mean plasma concentration of 0.58 ± 0.11 μg/ml. The drug was detectable in three out of five animals upto 24 h and the mean concentration was noted to be 0.14 ± 0.06 μg/ml.

2. *Urine levels*

Table 2 and Fig. 2 present urine concentrations of amikacin (7.5 mg/kg) post i.v. administration. The drug appeared in effective concentration of 2 μg/ml at 0.042 h and was maintained upto 36 h. At 0.042 h, the mean drug concentration in urine was 2.53 ± 0.44 μg/ml. The mean peak urine concentration of 1220 ± 99.64 μg/ml was observed at 1 h. The drug was detectable in all animals upto 36 h with a mean of 2.03 ± 0.30 μg/ml. The drug was present in three out of five animals with a mean of 0.19 ± 0.09 μg/ml at 48 h.

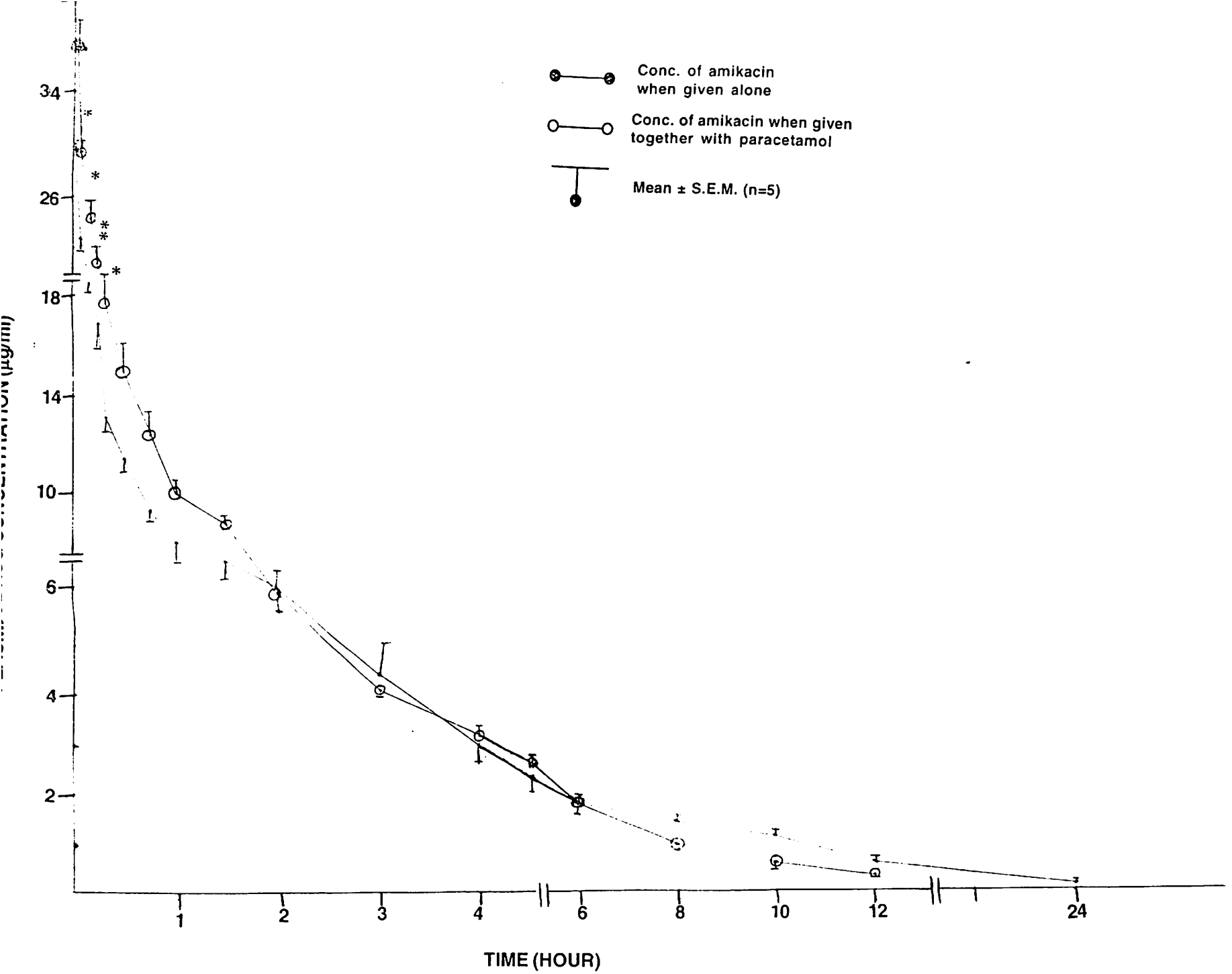

Conc. of amikacin when given alone
Conc. of amikacin when given together with paracetamol
Mean ± S.E.M. (n=5)
34
26
18
14
10
6
4
2
1
2
3
4
6
8
10
12
24
TIME (HOUR)

Table – 1

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

Time (h)	Animal Number					Mean ± S.E.M.
	1	2	3	4	5	
0.042	31.09	28.52	30.25	29.65	30.55	30.01 ± 0.44
0.083	23.29	20.20	23.18	21.23	26.05	22.79 ± 1.00
0.167	20.16	17.80	20.14	17.85	19.50	19.09 ± 0.53
0.25	20.16	14.85	17.55	15.00	16.25	16.76 ± 0.98
0.333	15.10	12.20	12.28	12.50	13.54	13.12 ± 0.55
0.50	9.79	11.85	11.38	11.85	12.28	11.43 ± 0.43
0.75	7.34	10.15	9.15	10.05	10.05	9.35 ± 0.53
1	4.76	9.15	8.00	9.12	9.12	8.03 ± 0.85
1.5	4.12	8.10	7.12	8.66	8.10	7.22 ± 0.81
2	3.56	7.12	6.15	7.14	6.65	6.12 ± 0.67
3	3.08	6.02	3.45	6.20	3.68	4.48 ± 0.67
4	1.73	3.24	3.10	3.82	3.24	3.03 ± 0.35
5	1.60	2.12	2.60	2.60	2.90	2.36 ± 0.23
6	1.49	1.36	2.15	1.82	2.32	1.83 ± 0.18
8	1.12	1.27	1.50	1.55	1.85	1.46 ± 0.13
10	0.84	1.15	0.95	1.30	1.26	1.10 ± 0.09
12	0.73	0.40	0.36	0.48	0.92	0.58 ± 0.11
24	N.D.	0.22	N.D.	0.24	0.26	0.14 ± 0.06

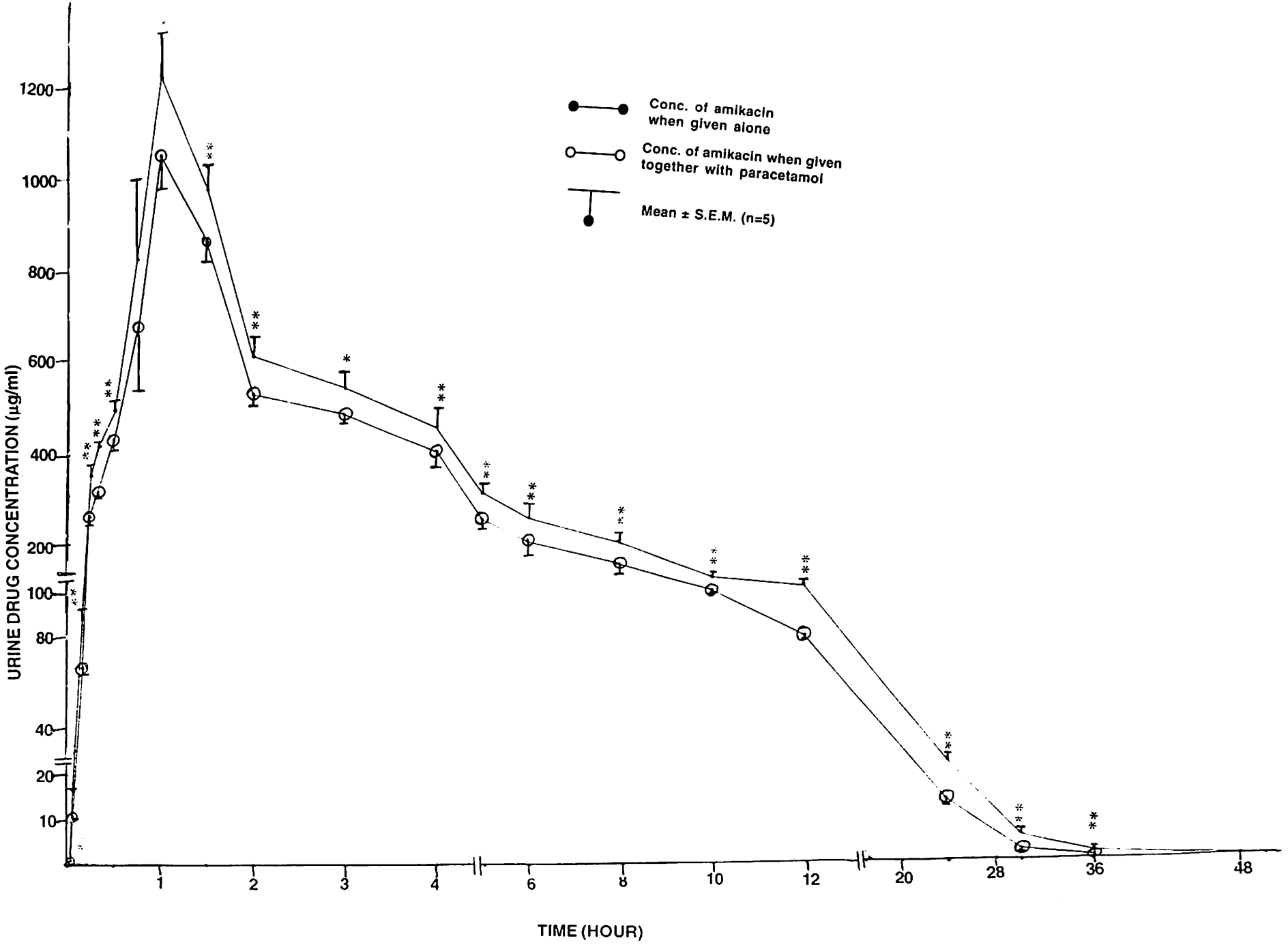
N.D. = Non-detectable

Table – 2

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

Time (h)	Animal Number					Mean ± S.E.M.
	1	2	3	4	5	
0.042	1.82	3.85	2.25	1.48	3.25	2.53 ± 0.44
0.083	15.70	18.82	16.85	12.55	16.85	16.15 ± 1.03
0.167	86.78	95.68	92.24	75.88	90.24	88.16 ± 3.39
0.25	362.2	388.2	388.5	268.4	368.6	355.2 ± 22.32
0.333	413.1	425.4	452.2	385.6	412.8	417.8 ± 10.78
0.50	571.1	554.2	512.6	412.2	548.6	499.7 ± 26.46
0.75	537.3	1285	608.2	488.5	1215	826.8 ± 174.2
1	1349	1002	1506	1250	992.6	1220 ± 99.64
1.5	1037	878.5	1112	998.6	850.4	975.3 ± 48.99
2	699.0	558.4	702.8	612.4	512.6	617.0 ± 37.71
3	612.9	506.0	624.6	528.2	490.4	552.4 ± 27.79
4	537.4	448.2	542.5	490.6	312.2	466 .2 ± 42.15
5	358.4	288.6	368.2	306.2	265.6	317.4 ± 19.87
6	314.1	192.8	320.4	280.2	188.5	259.2 ± 28.82
8	241.5	175.6	248.5	205.5	162.2	206.7 ± 17.19
10	142.7	120.4	150.6	118.5	112.8	129.0 ± 7.42
12	125.1	96.45	132.4	98.85	90.45	108.7 ± 8.39
24	26.57	18.62	28.62	20.12	15.52	21.89 ± 2.47
30	6.26	4.12	7.85	5.15	4.02	5.48 ± 0.72
36	2.49	1.50	2.88	2.02	1.25	2.03 ± 0.30
48	N.D.	N.D.	0.52	0.26	0.20	0.19 ± 0.09

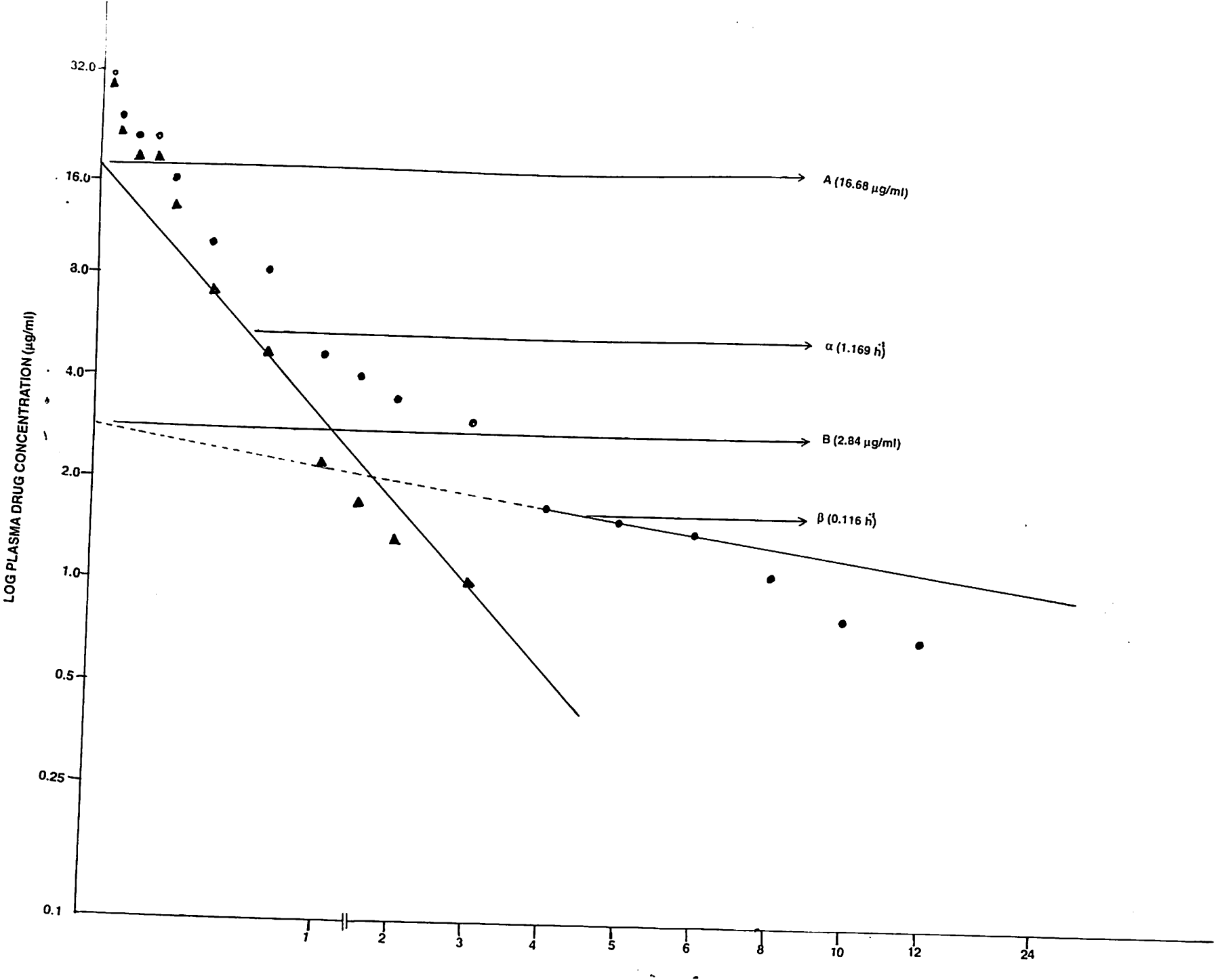
N.D. = Non-detectable


Conc. of amikacin when given alone
Conc. of amikacin when given together with paracetamol
Mean ± S.E.M. (n=5)
URINE DRUG CONCENTRATION (µg/ml)
1200
1000
800
600
400
200
100
80
40
20
10
1
2
3
4
6
8
10
12
20
28
36
48
TIME (HOUR)

3. *Kinetic parameters*

The plasma drug concentration *versus* time profile depicts the two compartment open model as shown in Fig. 3. Table 3 shows the values of various kinetic parameters calculated on the basis of the above mentioned compartment model.

The mean extrapolated zero time concentration of the drug in plasma during distribution phase (A), elimination phase (B) and theoretical zero time concentration (C_p^o) were noted to be 14.50 ± 0.90, 4.87 ± 0.94 and 19.37 ± 0.81 μg/ml, respectively. The distribution rate constant (α) varied from 0.556 to 1.543 h^{-1} with a mean value of 0.979 ± 0.185 h^{-1}, while its elimination rate constant (β) ranged from 0.116 to 0.237 h^{-1} with a mean value of 0.148 ± 0.022 h^{-1}. The mean distribution half life ($t_{½}\alpha$) and mean elimination half life ($1_{½}\beta$) of the drug were observed to be 0.83 ± 0.16 and 4.90 ± 0.53 h, respectively. In the present investigation, the mean area under curve (AUC) of 49.36 ± 3.54 mg/L.h mean area under first moment curve (AUMC) of 252.4 ± 30.00 mg/L.h^2 and average mean resident time (MRT) of 5.08 ± 0.41 h were noted. The average rate of transfer of drug from central to peripheral (K_{12}), peripheral to central (K_{21}) and elimination on from central (Kel) compartment were noted to be 0.357 ± 0.086 h^{-1}, 0.368 ±0.101h^{-1} and 0.403± 0.039h^{-1}, respectively. The fraction of drug available for elimination from central


LOG PLASMA DRUG CONCENTRATION (µg/ml)
32.0
16.0
8.0
4.0
2.0
1.0
0.5
0.25
0.1
A (16.68 µg/ml)
α (1.169 h⁻¹)
B (2.84 µg/ml)
β (0.116 h⁻¹)
1
2
3
4
5
6
8
10
12
24

compartment (Fc) and approximate tissue to plasma concentration ratio (T≈P) were noted to be 0.37 ± 0.04 and 1.87 ± 0.41, respectively. The various values of volume of distribution calculated by different method are shown in Table 3. The mean value of Vd_{area} was calculated to be 1.01 ± 0.07 L/kg. The total body clearance (Cl_B) ranged from 2.22 to 3.20. ml/kg/min with a mean of 2.59 ± 0.19 ml/kg/min.

4. ***Dosage regimen***

Table 4 shows the dosage regimen needed to maintain various levels of 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 . For maintaining C_p^{∞} min of 1μg/ml, the loading doses (D*s) were calculated to be 3.34 ± 0.39 and 6.46 ± 1.56 mg/kg while maintenance doses (D_os) were calculated to be 2.34 ± 0.46 and 5.45 ± 1.63 mg/kg, at of 8 h and 12 h, respectively.

For maintaining C_p^{∞} min of 2 μg/ml, the D*s were calculated to be 6.70 ± 0.79 and 12.93 ± 3.13 mg/kg, while D_os were found to be 4.67 ± 0.93 and 10.93 ± 3.26 mg/kg at γ of 8 and 12 h, respectively. Likewise, the D*s were calculated to be 13.40 ± 1.58 and 25.86 ± 625 mg/kg while D_os were found to be 9.35 ± 1.86 and 21.81 ± 6.53 mg/kg at (γ) of 8 and 12 h for maintaining C_p^{∞} min of 4 μg/ml.

Table – 3

Kinetic parameters of amikacin in healthy buffalo calves (calculated by 2 – compartment open model)after a single intravenous dose (7.5 mg/kg)

Kinetic Parameter	Unit	Animal Number					Mean ± S.E.M.
		1	2	3	4	5	
A	µg/ml	16.68	13.83	12.34	13.09	16.39	14.50 ± 0.90
B	µg/ml	2.84	3.57	8.30	4.55	5.10	4.87 ± 0.94
C_p^o	µg/ml	19.70	17.40	20.64	17.64	21.49	19.37 ± 0.81
α	h^{-1}	1.169	0.556	1.543	0.594	1.032	0.979 ± 0.185
$t_{1/2}\alpha$	h	0.59	1.25	0.45	1.17	0.67	0.83 ± 0.16
β	h^{-1}	0.116	0.127	0.237	0.133	0.128	0.148 ± 0.022
$t_{1/2}\beta$	h	5.97	5.46	2.92	5.21	5.40	4.90 ± 0.53
AUC	mg/L.h	38.89	52.99	43.02	56.24	55.56	49.36 ± 3.54
AUMC	$mg/L.h^2$	223.38	266.08	152.97	294.32	325.46	252.4 ± 30.00
MRT	h	5.74	5.02	3.56	5.23	5.85	5.08 ± 0.41
K_{12}	h^{-1}	0.512	0.140	0.539	0.161	0.431	0.357 ± 0.086
K_{21}	h^{-1}	0.268	0.215	0.762	0.252	0.343	0.368 ± 0.101
Kel	h^{-1}	0.507	0.328	0.479	0.314	0.386	0.403 ± 0.039
Fc	-	0.23	0.39	0.49	0.42	0.33	0.37 ± 0.04
$T \approx P$	-	3.37	1.59	1.03	1.35	2.01	1.87 ± 0.41
Vd_c	L/kg	0.38	0.43	0.36	0.43	0.35	0.39 ± 0.02
Vd_B	L/kg	2.64	2.10	0.90	1.65	1.47	1.75 ± 0.23
Vd_{area}	L/kg	1.16	1.12	0.74	1.00	1.05	1.01 ± 0.07
Vd_{ss}	L/kg	1.11	0.71	0.62	0.69	0.79	0.78 ± 0.09
Cl_B	ml/kg/min	3.20	2.37	2.90	2.22	2.25	2.59 ± 0.19

Table – 4

Dosage regimen of amikacin in healthy buffalo calves

C_p^{∞} min (μg/ml)	γ (h)	Dose (mg/kg)	Animal Number					Mean ± S.E.M.
			1	2	3	4	5	
1	8	D*	2.93	3.09	4.93	2.89	2.92	3.34 ± 0.39
		D_o	1.77	1.97	4.19	1.90	1.87	2.34 ± 0.46
	12	D*	4.66	5.14	12.71	4.93	4.88	6.46 ± 1.56
		D_o	3.50	4.02	11.97	3.93	3.83	5.45 ± 1.63
2	8	D*	5.85	6.18	9.86	5.78	5.84	6.70 ± 0.79
		D_o	3.53	3.94	8.38	3.78	3.74	4.67 ± 0.93
	12	D*	9.33	10.28	25.43	9.86	9.77	12.93 ± 3.13
		D_o	7.01	8.04	23.95	7.86	7.77	10.93 ± 3.26
4	8	D*	11.69	12.37	19.71	11.56	11.67	13.40 ± 1.58
		D_o	7.05	7.89	16.75	7.56	7.48	9.35 ± 1.86
	12	D*	18.65	20.56	50.85	19.72	19.53	25.86 ± 6.25
		D_o	14.01	16.08	47.89	15.72	15.33	21.81 ± 6.53

D* = **Priming or loading dose**

D_o = **Maintenance dose**

γ = **Dosage interval**

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

(B) KINETIC STUDY OF PARACETAMOL

1. Plasma levels

The plasma drug concentration profile at different time intervals after single i.v. dose of 40 mg/kg of paracetamol in buffalo calves has been shown in Table 5 and Fig. 4. At 0.042 h, the mean plasma concentration of the drug was found to be 20.23 ± 0.84 μg/ml. The drug was detectable in four out of five animals at 2 h with the mean concentration of 1.87 ± 0.48 μg/ml.

2. Urine Levels

Table 6 and Fig. 5 present the drug concentrations in urine after single i.v. administration of paracetamol in buffalo calves when given at the dose rate of 40 mg/kg. In all five animals, the drug appeared within 0.042 h. with a mean of 12.01 ± 1.38 μg/ml and it was maintained upto 48 h. with a mean value of 10.90 ± 1.14 μg/ml. The mean peak urine concentration of 2022 ± 118.8 μg/ml was attained at 1.5 h. The drug was detectable in all animals up to 48 h.

3. Kinetic Parameters

The 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.

Fig. 4

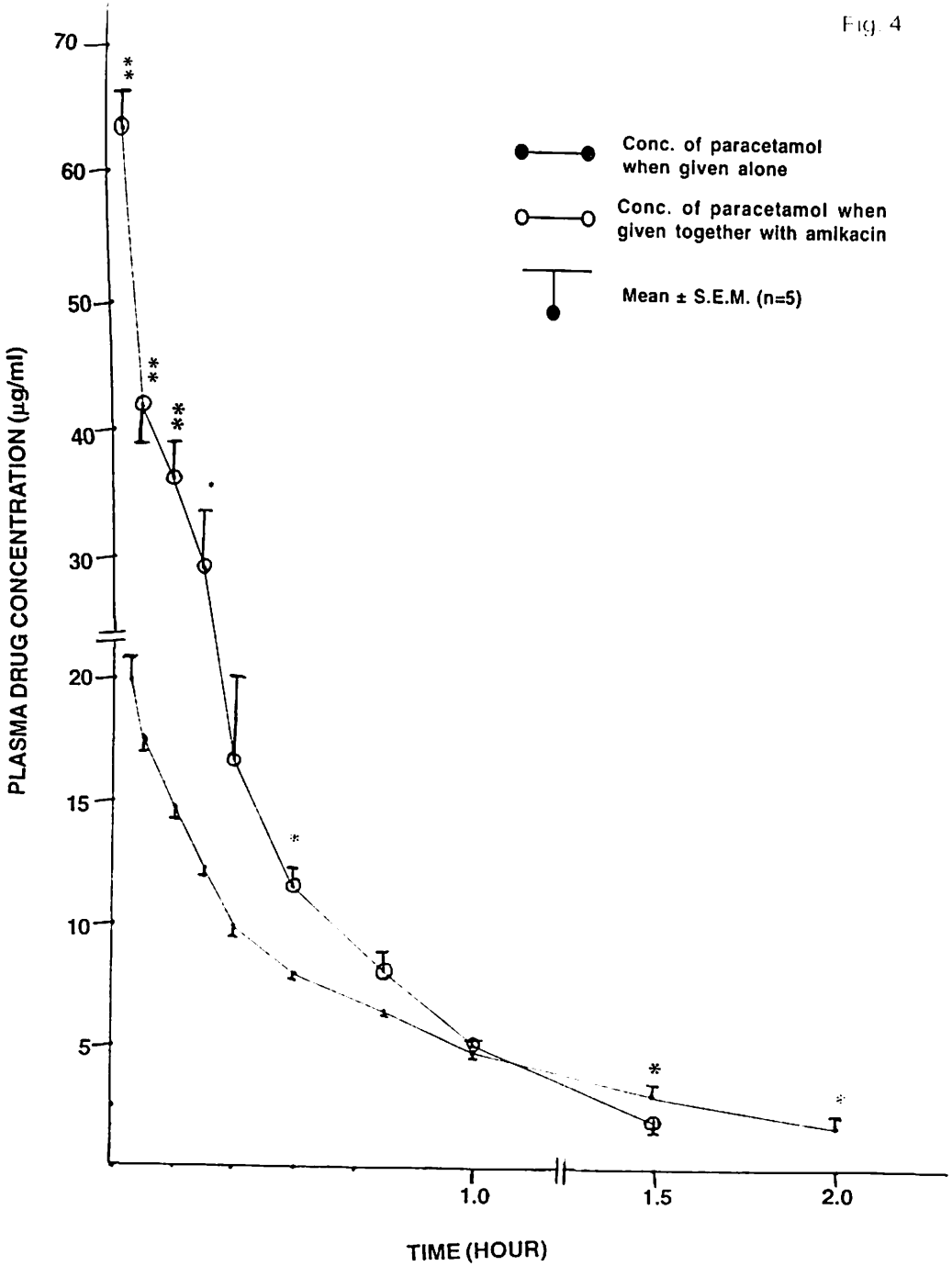

70
60
50
40
30
20
15
10
5
PLASMA DRUG CONCENTRATION (µg/ml)
Conc. of paracetamol when given alone
Conc. of paracetamol when given together with amikacin
Mean ± S.E.M. (n=5)
1.0
1.5
2.0
TIME (HOUR)

Table – 5

Plasma concentrations (μg/ml) of paracetamol in healthy buffalo calves following single intravenous dose of 40 mg/kg

Time (h)	Animal Number					Mean ± S.E.M.
	1	2	3	4	5	
0.042	17.95	22.50	20.25	21.62	18.82	20.23 ± 0.84
0.083	15.93	18.20	18.68	19.00	15.85	17.53 ± 0.68
0.167	13.92	15.00	15.04	15.85	14.12	14.78 ± 0.35
0.25	12.02	12.65	12.00	12.88	11.85	12.28 ± 0.20
0.333	9.88	11.20	8.88	9.02	10.65	9.93 ± 0.45
0.50	7.86	8.25	7.80	7.45	9.00	8.07 ± 0.26
0.75	6.84	6.55	6.66	6.12	6.60	6.55 ± 0.12
1	3.82	4.38	5.15	5.25	5.42	4.80 ± 0.30
1.5	1.80	2.76	2.80	4.02	4.12	3.10 ± 0.44
2	N.D.	2.05	2.10	2.54	2.65	1.87 ± 0.48
3	-	N.D.	N.D.	N.D.	N.D.	-

N.D. = Non detectable

Table – 6

Urine concentrations (µg/ml) of paracetamol in healthy buffalo calves following single intravenous dose of 40 mg/kg

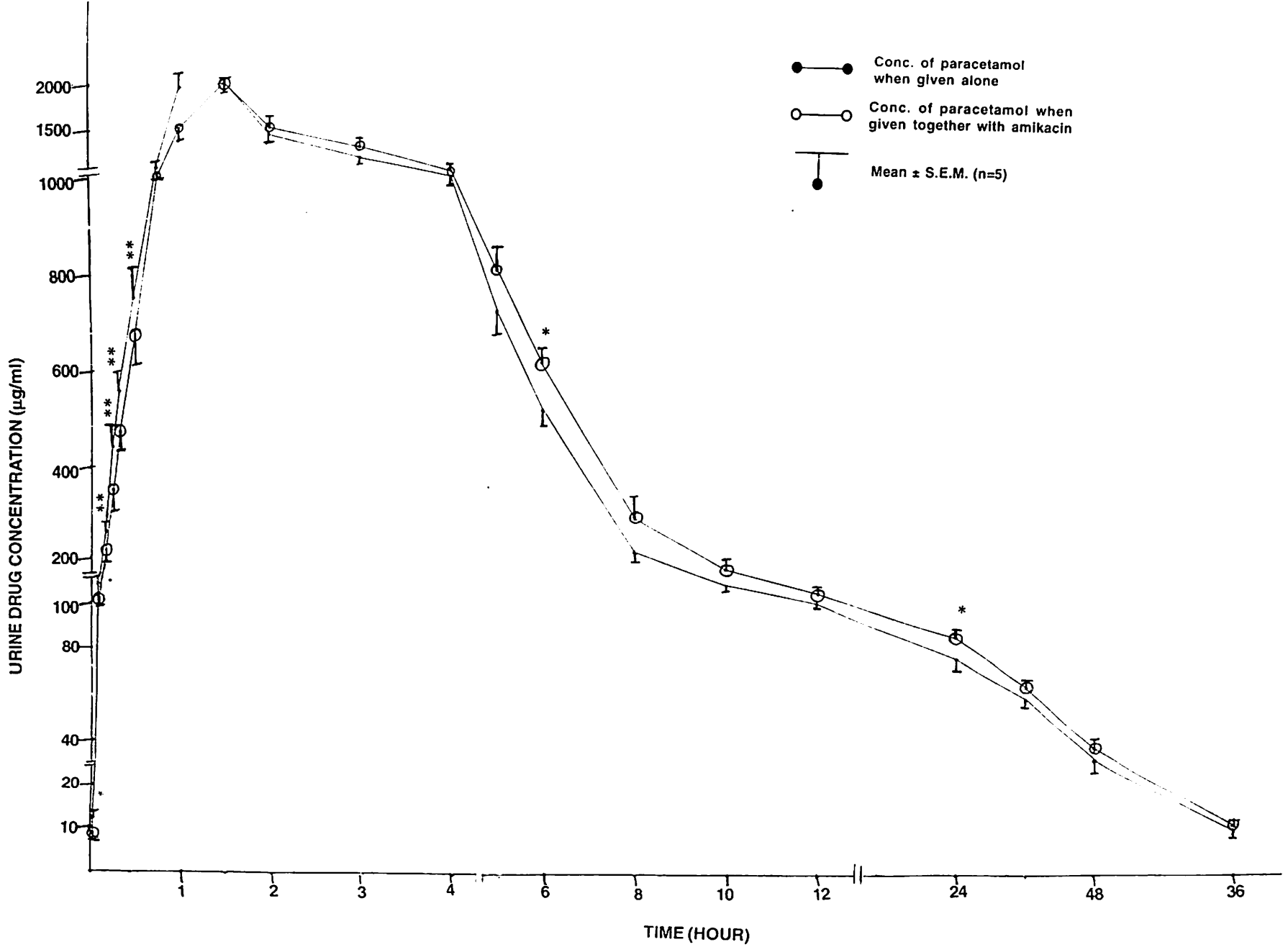
Time (h)	Animal Number					Mean ± S.E.M.
	1	2	3	4	5	
0.042	12.28	9.85	15.60	8.12	14.26	12.01 ± 1.38
0.083	189.6	110.4	192.2	105.8	109.6	141.5 ± 20.18
0.167	291.0	220.6	312.4	208.2	244.8	255.4 ± 20.08
0.25	522.4	388.4	570.5	326.6	395.2	440.6 ± 45.47
0.333	575.5	495.2	683.2	455.8	588.4	559.6 ± 39.55
0.50	727.5	688.5	1002	640.2	705.8	752.8 ± 63.94
0.75	1037	912.8	1455	884.8	1286	1115 ± 110.6
1	1249	2255	2650	1226	2456	1967 ± 304.4
1.5	2480	1889	2015	1812	1918	2022 ± 118.8
2	2056	1605	1414	1126	1218	1484 ± 165.2
3	1759	1106	1212	1004	1056	1227 ± 137.3
4	1552	912.4	1012	815.6	902.2	1039 ± 132.0
5	786.0	658.2	810.8	580.4	788.6	724.8 ± 44.99
6	579.8	465.6	605.2	425.2	524.2	520.0 ± 33.76
8	225.2	168.4	268.4	240.4	170.5	214.6 ± 19.69
10	162.4	124.2	180.2	145.6	110.4	144.6 ± 12.59
12	112.6	93.82	120.5	98.60	94.60	104.0 ± 5.33
24	83.80	68.44	90.80	68.50	70.25	76.36 ± 4.61
30	63.60	50.50	70.40	52.50	56.44	58.69 ± 3.69
36	42.50	32.20	44.80	20.40	24.36	32.85 ± 4.81
48	10.28	9.15	12.26	8.25	14.58	10.90 ± 1.14

The mean extrapolated zero time concentration of the drug in plasma during distribution phase (A), elimination phase (B) and theoretical zero time concentration (C_p^o = A+B) were noted to be 9.51 ± 1.94, 13.11 ±1.08 and 22.62 ± 1.00 μg/ml, respectively. The distribution rate constant (α) varied from 5.064 to 12.490 h^{-1} with a mean value of 7.148 ± 1.361 h^{-1} while elimination rate constant (β) ranged from 0.715 to 1.479 h^{-1} with a mean value of 0.965 ± 0.136 h^{-1}. The mean value of distribution half life ($t_{1/2}$ α) and elimination half life ($t_{1/2}$ β) of the drug were observed to be 0.11 ± 0.01 and 0.77 ± 0.09 h, respectively. The average rate of transfer of drug from central to peripheral (K_{12}), peripheral to central (K_{21}) and elimination from central compartment (Kel) were calculated to be 1.672 ± 0.142, 4.970 ± 1.540 and 1.471 ± 0.089 h^{-1}, respectively. The fraction of drug available for elimination from central compartment (Fc) and approximate tissue to plasma ratio (T≈P) were noted to be 0.65 ± 0.06 and 0.59 ± 0.13, respectively. The mean value of area under curve (AUC), area under first moment curve (AUMC) and mean residential time (MRT) were found to be 15.59 ± 1.07 mg/L.h, 16.09 ± 2.56 $mg/L.h^2$ and 1.00 ± 0.10 h, respectively. The different values of volume of distribution calculated by different methods are depicted in Table 7. The mean value of Vd_{area} was calculated to be 2.79 ± 0.14 L/kg. The total body clearance (Cl_B) ranged from 37.09 to 56.20 ml/kg/min with a mean value of 43.67 ± 3.39 ml/kg/min.

Table – 7

Kinetic parameters of paracetamol in healthy buffalo calves following single intravenous dose of 40 mg/kg

Kinetic Parameter	Unit	Animal Number					Mean ± S.E.M.
		1	2	3	4	5	
A	μg/ml	2.58	11.34	11.85	13.61	8.18	9.51 ± 1.94
B	μg/ml	17.26	12.60	12.39	10.93	12.37	13.11 ± 1.08
C_p^o	μg/ml	19.84	23.94	24.24	24.54	20.55	22.53 ± 1.00
α	h^{-1}	12.490	5.457	6.561	5.064	6.171	7.148 ± 1.361
$t_{1/2}\alpha$	h	0.06	0.13	0.11	0.14	0.11	0.11 ± 0.01
β	h^{-1}	1.479	0.952	0.914	0.715	0.767	0.965 ± 0.136
$t_{1/2}\beta$	h	0.47	0.73	0.76	0.97	0.90	0.77 ± 0.09
AUC	Mg/L.h	11.88	15.32	15.37	17.97	17.44	15.59 ± 1.07
AUMC	$Mg/L.h^2$	7.91	14.30	15.12	21.90	21.22	16.09 ± 2.56
MRT	h	0.67	0.93	0.98	1.22	1.22	1.00 ± 0.10
K_{12}	h^{-1}	1.241	1.523	2.097	1.761	1.740	1.672 ± 0.142
K_{21}	h^{-1}	11.060	3.323	3.800	2.653	4.021	4.970 ± 1.540
Kel	h^{-1}	1.671	1.563	1.577	1.365	1.178	1.471 ± 0.089
Fc	-	0.89	0.60	0.58	0.52	0.65	0.65 ± 0.06
$T \approx P$	-	0.13	0.64	0.73	0.91	0.54	0.59 ± 0.13
Vd_c	L/kg	2.02	1.67	1.65	1.63	1.95	1.78 ± 0.08
Vd_B	L/kg	2.32	3.17	3.23	3.66	3.23	3.12 ± 0.22
Vd_{area}	L/kg	2.28	2.74	2.85	3.11	2.99	2.79 ± 0.14
Vd_{ss}	L/kg	2.25	2.44	2.56	1.66	2.79	2.34 ± 0.19
Cl_B	ml/kg/min	56.20	43.47	43.33	37.09	38.22	43.67 ± 3.39


Conc. of paracetamol when given alone
Conc. of paracetamol when given together with amikacin
Mean ± S.E.M. (n=5)
URINE DRUG CONCENTRATION (µg/ml)
2000
1500
1000
800
600
400
200
100
80
40
20
10
1
2
3
4
6
8
10
12
24
48
36
TIME (HOUR)

II. PHARMACOKINETIC STUDIES OF DRUGS AFTER COMBINED ADMINISTRATION OF AMIKACIN AND PARACETAMOL

(A) AMIKACIN

1. Plasma levels

Table 8 and Fig. 1 depict the plasma concentrations of amikacin at various time intervals after combined i.v. administration of amikacin (7.5 mg/kg) and paracetamol (40 mg/kg). At 0.042 h, the drug was present with a man value of 38.42 ± 2.25 μg/ml. The drug was detectable in plasma samples of all animals upto 12 h with a mean concentration of 0.34 ± 0.06 μg/ml. The mean therapeutic concentration (≥2 μg/ml) of the drug in plasma was maintained from 0.042 to 5 h.

2. Urine levels

Table 9 and Fig. 2 present the concentrations of amikacin in urine at various time intervals following combined i.v. administration of amikacin (7.5 mg/kg) and paracetamol (40 mg/kg). The drug was detectable in all five animals at 0.042 h with a mean value of 1.39 ± 0.31 μg/ml. The drug appeared in effective concentration from 0.083 to 30h. The mean peak urine concentration of 1051 ± 73.46 μg/ml was observed at 1 h. The drug was present in all animals upto 36 h with a mean value of 0.96 ± 0.31 μg/ml and in 2 out of 5 animals with a mean of 0.07 ± 0.05 μg/ml.

Table – 8

Plasma concentrations (μg/ml) of amikacin in healthy buffalo calves following combined administration of amikacin (7.5 mg/kg) and paracetamol (40 mg/kg)

Time (h)	Animal Number					Mean ± S.E.M.
	1	2	3	4	5	
0.042	44.74	38.50	41.62	35.25	32.00	38.42 ± 2.25
0.083	33.24	29.12	30.12	28.12	27.52	29.62 ± 1.01
0.167	28.65	21.55	27.55	22.00	21.15	24.18 ± 1.62
0.25	24.69	18.24	22.45	19.88	17.95	20.64 ± 1.29
0.333	21.28	14.75	19.68	17.16	15.50	17.67 ± 1.24
0.50	18.85	12.00	16.06	15.14	12.68	14.95 ± 1.23
0.75	15.81	10.20	12.92	12.98	10.10	12.40 ± 1.06
1	11.75	9.22	9.55	10.50	9.12	10.03 ± 0.49
1.5	10.13	8.16	8.05	8.88	8.08	8.66 ± 0.39
2	5.59	4.95	7.08	7.88	4.78	6.06 ± 0.61
3	4.15	3.80	4.05	4.00	4.14	4.12 ± 0.12
4	3.82	2.70	3.15	3.25	3.12	3.21 ± 0.18
5	3.08	2.22	2.72	2.55	2.65	2.64 ± 0.14
6	1.70	1.50	1.70	1.78	2.40	1.82 ± 0.15
8	1.27	0.95	0.92	0.86	1.00	1.00 ± 0.07
10	0.94	0.52	0.65	0.55	0.46	0.62 ± 0.09
12	0.60	0.28	0.32	0.24	0.26	0.34 ± 0.06
24	N.D.	N.D.	N.D.	N.D.	N.D.	-

N.D. = Non Detectable

Table – 9

Urine concentrations (μg/ml) of amikacin in healthy buffalo calves following combined i.v. administration of amikacin (7.5 mg/kg) and paracetamol (40 mg/kg)

Time (h)	Animal Number					Mean ± S.E.M.
	1	2	3	4	5	
0.042	0.84	2.15	1.16	0.68	2.12	1.39 ± 0.31
0.083	10.78	10.12	10.25	9.66	12.15	10.59 ± 0.43
0.167	53.75	75.26	72.42	65.14	70.42	67.39 ± 3.79
0.25	212.4	278.2	302.2	206.2	308.2	261.4 ± 21.84
0.333	313.2	312.2	365.4	286.5	322.4	319.9 ± 12.84
0.50	392.0	484.0	409.5	388.4	480.6	430.9 ± 21.29
0.75	435.2	1015	506.4	408.6	1025	681.6 ± 139.0
1	1240	898.2	1208	1005	902.4	1051 ± 73.46
1.5	925.5	768.4	952.5	928.2	758.2	866.6 ± 42.45
2	585.2	505.2	604.6	502.4	480.2	535.5 ± 24.82
3	506.8	470.5	568.8	468.5	460.4	495.0 ± 20.11
4	488.6	408.4	468.8	422.2	282.5	414.0 ± 36.00
5	302.2	206.2	302.6	285.5	216.5	262.5 ± 21.21
6	284.8	152.8	280.6	208.2	128.2	210.9 ± 32.05
8	201.5	125.6	218.5	175.4	102.2	164.6 ± 22.13
10	112.8	98.20	110.6	98.20	82.55	100.5 ± 5.36
12	97.8	76.66	82.60	78.25	70.54	81.17 ± 4.59
24	20.2	10.12	20.00	12.20	10.52	14.61 ± 2.27
30	3.12	2.15	4.28	1.22	2.12	2.58 ± 0.52
36	1.28	0.75	1.98	0.16	0.65	0.96 ± 0.31
48	N.D.	N.D.	0.22	N.D.	0.14	0.07 ± 0.05

N.D. = Non Detectable

3. Kinetic parameters

The plasma drug concentration *versus* time profile has confirmed the 2 compartment open model and hence, the kinetic parameters were calculated by using the formulae of the above noted compartment model.

Table 10 shows the values of different kinetic parameters of amikacin after combined administration with paracetamol. 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^o = A+B) were noted to be 19.22 ± 2.29, 9.93 ± 0.56 and 29.15 ± 1.78 μg/ml, respectively. The distribution rate constant (α) ranged from 1.270 to 2.459 h^{-1} with a mean value of 1.788 ± 0.219 h^{-1} while its elimination rate constant (β) ranged from 0.225 to 0.316 h^{-1} with a mean value of 0.283 ± 0.016 h^{-1}. The average distribution half life ($t_{1/2}$ α) and elimination half life (($t_{1/2}$ β) were observed to be 0.37 ± 0.04 and 2.48 ± 0.16 h, respectively. The mean value of area under curve (AUC), the area under first moment curve (AUMC) and mean residential time (MRT) were found to be 46.66 ± 2.79 mg/L.h, 132.9 ± 12.08 mg/L.h^2 and 2.85 ± 0.10 h, respectively. The average rate of transfer of drug from central to peripheral (K_{12}), peripheral to central (K_{21}) and elimination from central compartment (Kel) were found to be 0.635 ± 0.124, 0.801 ±

0.091 and 0.631 ± 0.037 h^{-1}, respectively. The fraction of drug available for elimination from central compartment (Fc) and approximate tissue to plasma concentration ratio (T≈P) were found to be 0.47 ± 0.05 and 1.26 ± 0.19, respectively. The various values of volume of distribution calculated by different methods are shown in Table 10. The mean value of Vd_{area} was noted to be 0.58 ± 0.03 L/kg. The total body clearance (Cl_B) ranged from 2.21 to 3.16 ml/kg/min with an average of 2.72 ± 0.16 ml/kg/min.

4. Dosage regimen

The dosage regimen of amikacin following combined administration with paracetamol in buffalo calves are shown in Table 11. Loading doses (D*s) were calculated to be 6.70 ± 0.84 and 21.59 ± 3.56 mg/kg while maintenance doses (Dos) were calculated to be 6.04 ± 0.82 and 20.93 ± 3.55 mg/kg for maintaining C_P^{∞} min of 1 μg/ml at the selected dosage intervals (γ) of 8 and 12 h, respectively. Similarly, for maintaining C_P^{∞} min of 2 μg/ml, the Dos, were found to be 13.41 ± 1.68 and 43.19 ± 7.13 mg/kg while Dos were noted to be 12.07 ± 1.65 and 41.85 ± 7.09 mg/kg at γ of 8 and 12 h, respectively. For maintaining C_P^{∞} min of 4 μg/ml, the calculated D*s and Dos were found to be 26.81 ± 3.36, 86.37 ± 14.25 and 24.14 ± 3.29, 83.70 ± 14.19 mg/kg respectively at γ of 8 and 12 h.

Table – 10

Kinetic parameters of amikacin in healthy buffalo calves following combined i.v. administration of amikacin (7.5 mg/kg) and paracetamol (40 mg/kg)

Parameter	Unit	Animal Number					Mean ± S.E.M.
		1	2	3	4	5	
A	µg/ml	26.69	21.29	19.99	13.75	14.79	19.22 ± 2.29
B	µg/ml	8.44	8.78	10.37	11.27	10.77	9.93 ± 0.56
C_p^o	µg/ml	34.73	30.08	30.36	25.02	25.55	29.15 ± 1.78
α	h^{-1}	1.419	2.459	1.691	1.270	2.100	1.788 ± 0.219
$t_{1/2}\alpha$	h	0.49	0.28	0.41	0.32	0.33	0.37 ± 0.04
β	h^{-1}	0.225	0.285	0.288	0.316	0.303	0.283 ± 0.016
$t_{1/2}\beta$	h	3.08	2.44	2.40	2.19	2.29	2.48 ± 0.16
AUC	mg/L.h	56.02	39.52	47.77	46.44	42.54	46.66 ± 2.79
AUMC	$mg/L.h^2$	179.6	111.9	131.6	121.1	120.4	132.9 ± 12.08
MRT	h	3.21	2.83	2.76	2.60	2.83	2.85 ± 0.10
K_{12}	h^{-1}	0.509	1.043	0.577	0.301	0.744	0.635 ± 0.124
K_{21}	h^{-1}	0.515	0.919	0.767	0.746	1.060	0.801 ± 0.091
Kel	h^{-1}	0.620	0.762	0.636	0.539	0.600	0.631 ± 0.037
Fc	-	0.36	0.37	0.45	0.59	0.50	0.47 ± 0.05
$T \approx P$	-	1.76	1.64	1.21	0.70	0.98	1.26 ± 0.19
Vd_c	L/kg	0.22	0.25	0.25	0.29	0.29	0.26 ± 0.01
Vd_B	L/kg	0.29	0.85	0.72	0.67	0.69	0.64 ± 0.09
Vd_{area}	L/kg	0.59	0.67	0.54	0.51	0.58	0.58 ± 0.03
Vd_{ss}	L/kg	0.43	0.53	0.43	0.42	0.50	0.46 ± 0.02
Cl_B	ml/kg/min	2.21	3.16	2.59	2.69	2.93	2.72 ± 0.16

Table – 11

Dosage regimen of amikacin in healthy buffalo calves following combined i.v. administration of amikacin and paracetamol

C_p^{∞} min (μg/ml)	γ (h)	Dose (mg/kg)	Animal Number					Mean ± S.E.M.
			1	2	3	4	5	
1	8	D*	3.57	6.55	7.20	8.39	7.79	6.70 ± 0.84
		D_o	2.98	5.88	6.49	7.73	7.10	6.04 ± 0.82
	12	D*	8.78	20.48	22.82	29.72	26.18	21.59 ± 3.56
		D_o	8.19	19.81	22.09	29.05	25.49	20.93 ± 3.55
2	8	D*	7.14	13.11	14.42	16.79	15.58	13.41 ± 1.68
		D_o	5.96	11.77	12.98	15.45	14.20	12.07 ± 1.65
	12	D*	17.56	40.95	45.63	59.43	52.36	43.19 ± 7.13
		D_o	16.38	39.61	44.19	58.09	50.98	41.85 ± 7.09
4	8	D*	14.28	26.21	28.83	33.58	31.16	26.81 ± 3.36
		D_o	11.92	23.53	25.95	30.90	28.40	24.14 ± 3.29
	12	D*	35.12	81.9	91.27	118.86	104.70	86.37 ± 14.25
		D_o	32.76	79.22	88.39	116.18	101.95	83.70 ± 14.19

D* = Priming or loading dose

D_o = Maintenance dose

γ = Dosage interval

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

(B) PARACETAMOL

1. Plasma levels

Table 12 and Fig 4 present the plasma concentrations of paracetamol (40 mg/kg i.v.) when administered in combination with amikacin (7.5 mg/kg i.v.) in buffalo calves. The average plasma concentration of the drug at 0.042 h was calculated to be 63.25 ± 3.59 μg/ml. The drug was present in four out of five animal with a mean of 9.03 ± 0.59 μg/ml at 1.5 h.

2. Urine levels

Urine concentrations of paracetamol after combined i.v. administration of amikacin (7.5 mg/kg) and paracetamol (40 mg/kg) are presented in Table 13 and Fig. 5. A mean urine concentration of 8.91 ±0.79 μg/ml was obtained at 0.042 h. The mean peak concentration of 2013 ± 143.9 μg/ml was observed at 1.5 h. The drug was present in all animals upto 48 h with a mean value of 12.25 ± 0.94 μg/ml.

3. Kinetic Parameters

The plasma drug concentration *versus* time profile of paracetamol had confirmed biphasic pattern following combined i.v. administration of amikacin and paracetamol and hence, kinetic parameters were calculated by using two compartment open model methods (Table 14).

Table – 12

Plasma concentrations (μg/ml) of paracetamol in healthy buffalo calves following combined i.v. administration of amikacin (7.5 mg/kg) and paracetamol (40 mg/kg)

Time (h)	Animal Number					Mean ± S.E.M.
	1	2	3	4	5	
0.042	69.40	58.25	64.26	72.00	52.36	63.25 ± 3.59
0.083	44.32	37.15	43.85	48.65	35.15	41.82 ± 2.48
0.167	40.46	32.00	36.12	44.22	27.46	36.05 ± 2.97
0.25	38.53	25.88	18.20	40.05	21.96	28.92 ± 4.40
0.333	13.45	11.88	15.60	30.68	12.00	16.72 ± 3.35
0.50	11.52	10.02	12.30	14.05	10.15	11.61 ± 0.75
0.75	7.67	7.26	8.05	11.00	6.86	8.17 ± 0.74
1	4.77	4.28	5.28	6.42	4.55	5.06 ± 0.38
1.5	N.D.	1.75	2.35	3.55	2.50	2.03 ± 0.59
2.0	-	N.D.	N.D.	N.D.	N.D.	-

N.D. = Non Detectable

Table – 13

Urine concentrations (μg/ml) of paracetamol in healthy buffalo calves following combined i.v. administration of amikacin (7.5 mg/kg) and paracetamol (40 mg/kg)

Time (h)	Animal Number					Mean ± S.E.M.
	1	2	3	4	5	
0.042	9.15	8.68	10.25	6.00	10.46	8.91 ± 0.79
0.083	120.5	98.85	168.7	75.50	89.68	110.7 ± 16.25
0.167	240.2	188.6	292.2	145.6	198.8	213.1 ± 24.85
0.25	412.8	354.5	470.8	238.4	259.4	347.2 ± 44.26
0.333	476.4	416.6	612.2	375.2	486.5	473.4 ± 40.19
0.50	610.2	620.2	945.5	600.2	604.2	676.1 ± 67.44
0.75	995.6	970.8	1256	788.4	988.0	1040 ± 54.21
1	1208	1155	2180	1014	2056	1523 ± 245.9
1.5	2475	2158	1912	1615	1906	2013 ± 143.9
2	2002	2006	1208	1114	1268	1519 ± 199.3
3	1760	1686	1012	1008	1152	1324 ± 165.5
4	1508	1100	985.5	720.2	1004	1064 ± 127.8
5	802.4	921.2	825.5	652.6	848.4	810.0 ± 44.11
6	584.2	665.2	685.6	529.2	608.8	614.6 ± 28.15
8	228.0	475.4	286.2	278.8	188.5	291.4 ± 49.32
10	164.5	165.4	255.6	168.4	120.2	174.8 ± 22.07
12	114.2	103.8	190.5	119.8	105.6	126.8 ± 16.19
24	85.4	75.56	98.65	84.62	84.25	85.69 ± 3.70
30	65.2	52.6	74.48	68.15	60.24	64.13 ± 3.69
36	44.8	34.55	48.42	32.08	25.56	37.08 ± 4.19
48	10.76	11.84	12.85	10.25	15.54	12.25 ± 0.94

Table – 14

Kinetic parameters of paracetamol in healthy buffalo calves following combined administration of amikacin (7.5 mg/kg) and paracetamol (40 mg/kg)

Parameter	Unit	Animal Number					Mean ± S.E.M.
		1	2	3	4	5	
A	µg/ml	39.58	35.49	44.13	40.18	36.91	39.26 ± 1.49
B	µg/ml	24.05	22.79	27.36	29.18	19.30	24.54 ± 1.74
C_p^o	µg/ml	63.63	58.28	71.49	69.36	56.22	63.79 ± 2.98
α	h^{-1}	2.810	5.039	6.796	3.422	6.285	4.870 ± 0.777
$t_{1/2}\alpha$	h	0.25	0.14	0.10	0.20	0.11	0.16 ± 0.03
β	h^{-1}	1.579	1.679	1.637	1.421	1.382	1.539 ± 0.059
$t_{1/2}\beta$	h	0.44	0.41	0.42	0.49	0.50	0.45 ± 0.02
AUC	mg/L.h	29.32	20.61	23.21	32.28	19.84	25.05 ± 2.46
AUMC	$Mg/L.h^2$	14.66	9.48	11.16	17.89	11.04	12.85 ± 1.52
MRT	h	0.50	0.46	0.48	0.55	0.56	0.51 ± 0.19
K_{12}	h^{-1}	0.175	0.898	1.741	0.431	1.768	1.003 ± 0.328
K_{21}	h^{-1}	2.044	2.994	3.611	2.263	3.066	2.796 ± 0.285
Kel	h^{-1}	2.171	2.827	3.081	2.149	2.839	2.613 ± 0.191
Fc	-	0.73	0.59	0.53	0.66	0.49	0.60 ± 0.04
$T \approx P$	-	0.38	0.68	0.88	0.51	1.05	0.70 ± 0.12
Vd_c	L/kg	0.63	0.69	0.56	0.58	0.71	0.63 ± 0.03
Vd_B	L/kg	1.66	1.75	1.46	1.37	2.07	1.66 ± 0.12
Vd_{area}	L/kg	0.86	1.16	1.05	0.87	1.46	1.08 ± 0.11
Vd_{ss}	L/kg	0.68	0.89	0.83	0.69	1.12	0.84 ± 0.08
Cl_B	ml/kg/min	22.67	32.35	28.65	20.64	33.58	27.58 ± 2.57

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 39.26 ± 1.49, 24.54 ± 1.74 and 63.79 ± 2.98 μg/ml, respectively. The distribution rate constant (α) varied from 2.810 to 6.796 h^{-1} with an average value of 4.870 ± 0.777 h^{-1} while its elimination rate constant (β) ranged from 1.382 to 1.679 h^{-1} with a mean value of 1.539 ± 0.059 h^{-1}. The mean distribution half life ($t_{1/2}$ α) and elimination half life ($t_{1/2}$ β) were calculated to be 0.16 ± 0.03 and 0.45 ± 0.02 h, respectively. The mean value of area under curve (AUC), area under first moment curve (AUMC) and mean residential time (MRT) were observed to be 25.05 ± 2.46 mg/L.h, 12.85 ± 1.52 mg/L.h^2 and 0.51 ± 0.19 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 observed to be 1.003 ± 0.328, 2.796 ± 0.285 and 2.613 ± 0.191 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 0.60 ± 0.04 and 0.70 ± 0.12, respectively. Table 14 shows the various values of volume of distribution calculated by different methods. A mean value of 1.08 ± 0.11 L/kg was estimated for Vdarea. The total body clearance (Cl_B) ranged from 20.64 to 33.58 ml/kg/min with a mean value of 27.58 ± 2.57 ml/kg/min.

III. COMPARISON OF PHARMACOKINETICS OF AMIKACIN WHEN GIVEN ALONE AND WHEN GIVEN TOGETHER WITH PARACETAMOL IN BUFFALO CALVES BY I.V. ROUTE

1. Plasma levels

Comparative study of plasma concentrations of amikacin (7.5 mg/kg) when given alone and when given together with paracetamol (40 mg/kg) after i.v. administration are presented in Table 15 and Fig. 1. Concentrations of amikacin were found to be significantly higher initially (0.042 to 0.333 h) in case of combined administration with paracetamol as compared to its single i.v. administration. Thereafter non significant differences were noted upto 24 h (except significant low drug concentration was noted at 10 h in combined administration). The minimum therapeutic concentration of 2 μg/ml in both the cases were maintained upto 5 h. The drug was detectable upto 12 h in case of combined administration where as upto 24 h in case of single administration.

2. Urine levels

Table 15 and Fig. 2 represent the urine concentrations of amikacin when given alone and when given together with paracetamol. Throughout the time from 2.5 min to 48 h (except 0.75 h and 48 h) significantly lower concentrations were maintained in case

Table – 15

Comparison of plasma and urine concentrations (μg/ml) of amikacin (7.5 mg/kg) when given alone and when given together with paracetamol (40 mg/kg) in healthy buffalo calves following intravenous administration.

Time (h)	Amikacin given alone		Amikacin + paracetamol given together	
	Plasma	Urine	Plasma	Urine
0.042	30.01 ± 0.44	2.53 ± 0.44	38.42 ± 2.25*	1.39 ± 0.31**
0.083	22.79 ± 1.00	16.15 ± 1.03	29.62 ± 1.01*	10.59 ± 0.43**
0.167	19.09 ± 0.53	88.16 ± 3.39	24.18 ± 1.62*	67.39 ± 3.79**
0.25	16.76 ± 0.98	355.2 ± 22.32	20.64 ± 1.29**	261.4 ± 21.89**
0.333	13.12 ± 0.55	417.8 ± 10.78	17.67 ± 1.24*	319.9 ± 12.84**
0.50	11.43 ± 0.43	499.7 ± 26.46	14.95 ± 1.23 NS	430.9 ± 21.29**
0.75	9.35 ± 0.53	826.8 ± 174.2	12.40 ± 1.06 NS	681.6 ± 139.0 NS
1	8.03 ± 0.85	1220 ± 99.64	10.03 ± 0.49 NS	1051 ± 73.46*
1.5	7.22 ± 0.81	975.3 ± 48.99	8.66 ± 0.39 NS	866.6 ± 42.45**
2	6.12 ± 0.67	617.0 ± 37.71	6.06 ± 0.61 NS	535.5 ± 24.82**
3	4.48 ± 0.67	552.4 ± 27.79	4.12 ± 0.12 NS	495.0 ± 20.11*
4	3.03 ± 0.35	466.2 ± 42.15	3.21 ± 0.18 NS	414.0 ± 36.00**
5	2.36 ± 0.23	317.4 ± 19.87	2.64 ± 0.14 NS	262.6 ± 21.21**
6	1.83 ± 0.18	259.2 ± 28.82	1.82 ± 0.15 NS	210.9 ± 32.05**
8	1.46 ± 0.13	206.7 ± 17.19	1.00 ± 0.07 NS	164.6 ± 22.13**
10	1.1 ± 0.09	129.0 ± 7.42	0.62 ± 0.09 NS	100.5 ± 5.36**
12	0.58 ± 0.11	108.7 ± 8.39	0.34 ± 0.06 NS	81.17 ± 4.59**
24	0.14 ± 0.01	21.89 ± 2.47	0.0 ± 0 NS	14.61 ± 2.27**
30	--	5.48 ± 0.72	--	2.58 ± 0.52**
36	--	2.03 ± 0.30	--	0.96 ± 0.31**
48	--	0.19 ± 0.09	--	0.07 ± 0.05 NS

NS = Non significant, * p < 0.05, ** p < 0.01.

of combined administration as compared to single administration. In both the cases, amikacin attained its peak concentration in urine at 1 h (1220 ± 99.64 μg/ml in case of single administration and 1051 ± 73.46 μg/ml in case of combined administration with paracetamol). The mean therapeutic concentration in urine (2 μg/ml) was maintained upto 36 h in case of single administration whereas upto 30 h in case of combined administration.

3. Kinetic Parameters

Table 16 shows the different kinetic parameters of amikacin when it was given alone (7.5 mg/kg) and when given together with paracetamol (40 mg/kg) after i.v. administration in buffalo calves. The value of extrapolated zero time concentration during elimination pahse (B) and theoretical zero time concentration (C_p^o) were calculated to be highly significantly ($p < 0.01$) higher in case of combined administration as compared to single administration of amikacin. The elimination half life ($t_{1/2}$ β) as well as mean residential time (MRT), Vd_C and Vd_{area} were noted to be highly significantly ($p < 0.01$) lower in combined administration as compared to single administration of amikacin. Similarly, area under first moment curve (AUMC), Vd_B and Vdss show significantly

($p < 0.05$) lower values in case of combined administration with paracetamol as compared to single administration of amikacin. The values of elimination rate constant (β), rate constant of drug transfer from peripheral to central compartment (K_{21}) and rate constant of drug elimination from central compartment (Kel) were estimated to be significantly higher in combined administration with paracetamol as compared to single administration of amikacin. The values of other kinetic parameters like extrapolated zero time concentration during distribution phase (B), distribution rate constant (α), distribution half life ($t_{1/2}$ α) *etc.* did not differ significantly between both the groups.

4. Dosage regimen

The comparative calculated dosage regimen of amikacin when given alone and when given together with paracetamol in buffalo calves following i.v. administration is presented in Table 17. The calculated loading doses (D*s) and maintenance doses (Dos) for maintaining C_p^{∞} min of 1,2 and 4 μg/ml at the selected dosage interval (γ) of 8 to 12 h 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 given alone (7.5 mg/kg) and when given together with paracetamol (40 mg/kg) in healthy buffalo calves following intravenous administration

Parameter	Unit	Amikacin given alone	Amikacin + paracetamol combined administration
A	µg/ml	14.50 ± 0.90	19.22 ± 2.29^{NS}
B	µg/ml	4.87 ± 0.94	9.93 ± 0.56**
C_p^o	µg/ml	19.37 ± 0.81	29.15 ± 1.78**
α	h^{-1}	0.979 ± 0.185	1.788 ± 0.219^{NS}
$t_{1/2}\alpha$	h	0.83 ± 0.16	0.37 ± 0.04^{NS}
β	h^{-1}	0.148 ± 0.022	0.283 ± 0.016**
$t_{1/2}\beta$	h	4.90 ± 0.53	2.48 ± 0.16**
AUC	mg/L.h	49.36 ± 3.54	46.46 ± 2.79^{NS}
AUMC	$mg/L.h^2$	252.4 ± 30.00	132.9 ± 12.08*
MRT	h	5.08 ± 0.41	2.85 ± 0.10**
K_{12}	h^{-1}	0.356 ± 0.086	0.635 ± 0.124^{NS}
K_{21}	h^{-1}	0.368 ± 0.101	0.801 ± 0.091*
Kel	h^{-1}	0.403 ± 0.039	0.631 ± 0.037*
Fc	-	0.37 ± 0.04	0.47 ± 0.05^{NS}
$T \approx P$	-	1.87 ± 0.41	1.26 ± 0.19^{NS}
Vd_c	L/kg	0.39 ± 0.02	0.26 ± 0.01**
Vd_B	L/kg	1.75 ± 0.23	0.64 ± 0.09*
Vd_{area}	L/kg	1.01 ± 0.07	0.58 ± 0.03**
Vd_{ss}	L/kg	0.78 ± 0.09	0.46 ± 0.02*
Cl_B	ml/kg/min	2.59 ± 0.19	2.72 ± 0.16^{NS}

NS = Non significant, * p < 0.05, ** p < 0.01.

Table – 17

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

C_p^{∞} min (μg/ml)	γ (h)	Dose (mg/kg)	Amikacin given alone	Amikacin + paracetamol given together
1	8	D*	3.34 ± 0.39	6.70 ± 0.84*
		Do	2.34 ± 0.46	6.04 ± 0.82*
	12	D*	6.46 ± 1.56	21.59 ± 0.36*
		D_o	5.45 ± 1.63	20.93 ± 3.55*
2	8	D*	6.70 ± 0.79	13.41 ± 1.68*
		D_o	4.67 ± 0.93	12.07 ± 1.65*
	12	D*	12.93 ± 3.13	43.19 ± 7.13*
		D_o	10.93 ± 3.26	41.85 ± 7.09*
4	8	D*	13.4 ± 1.58	26.81 ± 3.36*
		D_o	9.35 ± 1.86	24.14 ± 3.29**
	12	D*	25.86 ± 6.25	86.37 ± 14.25*
		D_o	21.81 ± 6.53	83.7 ± 14.19*

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

D* = Priming or loading dose

D_o = Maintenance dose

γ = Dosage interval

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

IV. COMPARISON OF KINETICS OF PARACETAMOL WHEN GIVEN ALONE AND WHEN GIVEN TOGETHER WITH AMIKACIN BY I.V. ADMINISTRATION

1. Plasma levels

Concentrations of paracetamol in plasma when given alone (40 mg/kg) and when given together with amikacin (7.5 mg/kg) after i.v. administration in buffalo calves are shown in Table 18 and Fig 4. The drug levels were noted to be significantly higher upto 0.5 h. in case of combined administration of paracetamol with amikacin as compared to its alone administration. Paracetamol was detectable upto 2 h in case of its single administration and upto 1.5 h in case of its combined administration with amikacin.

2. Urine levels

Table 18 and Fig. 5 present the comparative values of urine concentrations of paracetamol when given alone and when given together with amikacin. The drug levels were noted to be significantly lower upto 0.5 h in case of its combined administration with amikacin as compared to its alone administration. No significant difference was noted between both the groups (except at 6 h and 24 h where significantly higher concentrations of paracetamol were noted when given together with amikacin compared its single administration).

Table – 18

Comparison of plasma and urine concentrations (μg/ml) of paracetamol when given alone (40 mg/kg) and when given together with amikacin (7.5 mg/kg) in healthy buffalo calves following intravenous administration

Time (h)	Paracetamol given alone		Paracetamol + amikacin given together	
	Plasma	Urine	Plasma	Urine
0.042	20.23 ± 0.84	12.02 ± 1.38	63.25 ± 3.59**	8.91 ± 0.79*
0.083	17.53 ± 0.68	141.5 ± 20.18	41.82 ± 2.48**	110.7 ± 16.25*
0.167	14.78 ± 0.35	255.4 ± 20.08	36.05 ± 2.97**	213.1 ± 24.85**
0.25	12.28 ± 0.20	440.6 ± 45.47	28.92 ± 4.40*	347.2 ± 44.26**
0.333	9.93 ± 0.45	559.6 ± 39.55	16.72 ± 3.55^{NS}	473.4 ± 40.19**
0.50	8.07 ± 0.26	752.8 ± 63.94	11.61 ± 0.75*	676.1 ± 67.44**
0.75	6.55 ± 0.12	1115 ± 110.6	8.17 ± 0.74^{NS}	1040 ± 54.21^{NS}
1	4.80 ± 0.30	1967 ± 304.4	5.06 ± 0.38^{NS}	1523 ± 245.9^{NS}
1.5	3.10 ± 0.44	2022 ± 118.8	2.03 ± 0.39*	2013 ± 143.9^{NS}
2	1.87 ± 0.48	1484 ± 165.2	0.00 ± 0.00*	1519 ± 199.3^{NS}
3	--	1227 ± 137.3	--	1324 ± 165.5^{NS}
4	--	1039 ± 132.0	--	1064 ± 127.8^{NS}
5	--	724.8 ± 44.99	--	810.0 ± 44.11^{NS}
6	--	520.0 ± 33.76	--	614.6 ± 28.15*
8	--	214.6 ± 19.69	--	291.4 ± 49.32^{NS}
10	--	144.6 ± 12.59	--	174.8 ± 22.07^{NS}
12	--	104.0 ± 5.33	--	126.8 ± 16.19^{NS}
24	--	76.36 ± 4.61	--	85.69 ± 3.70*
30	--	58.69 ± 3.69	--	64.13 ± 3.69^{NS}
36	--	32.85 ± 4.81	--	37.08 ± 4.19^{NS}
48	--	10.90 ± 1.14	--	12.25 ± 0.94^{NS}

NS = Non significant, *p < 0.05, **p < 0.01.

Table – 19

Comparison of kinetic parameters of paracetamol when given alone (40 mg/kg) and when given together with amikacin (7.5 mg/kg) in healthy buffalo calves following intravenous administration

Parameter	Unit	Paracetamol given alone	Paracetamol + Amikacin given together
A	µg/ml	9.51 ± 1.94	39.26 ± 1.49**
B	µg/ml	13.11 ± 1.08	24.54 ± 1.74**
C_p^o	µg/ml	22.62 ± 1.00	63.79 ± 2.98**
α	h^{-1}	7.148 ± 1.361	4.870 ± 0.777^{NS}
$t_{1/2}\alpha$	h	0.11 ± 0.01	0.16 ± 0.03^{NS}
β	h^{-1}	0.965 ± 0.136	1.539 ± 0.059**
$t_{1/2}\beta$	h	0.77 ± 0.09	0.45 ± 0.02**
AUC	mg/L.h	15.59 ± 1.07	25.05 ± 2.46*
AUMC	$mg/L.h^2$	16.09 ± 2.56	12.85 ± 1.52^{NS}
MRT	h	1.00 ± 0.10	0.51 ± 0.19**
K_{12}	h^{-1}	1.672 ± 0.142	1.003 ± 0.328^{NS}
K_{21}	h^{-1}	4.970 ± 1.540	2.796 ± 0.285^{NS}
Kel	h^{-1}	1.471 ± 0.089	2.613 ± 0.191**
Fc	-	0.65 ± 0.06	0.60 ± 0.04^{NS}
$T \approx P$	-	0.59 ± 0.13	0.70 ± 0.12^{NS}
Vd_c	L/kg	1.78 ± 0.08	0.63 ± 0.03**
Vd_B	L/kg	3.12 ± 0.22	1.66 ± 0.12**
Vd_{area}	L/kg	2.79 ± 0.14	1.08 ± 0.11**
Vd_{ss}	L/kg	2.34 ± 0.19	0.84 ± 0.08**
Cl_B	ml/kg/min	43.67 ± 3.39	27.58 ± 2.57*

NS = Non significant, *p < 0.05, **p < 0.01.

3. Kinetic Parameters

Table 19 depicts the kinetic parameters of paracetamol when given alone and when given together with amikacin after i.v. administration. The values of extrapolated zero time concentration during distribution phase (A), elimination phase (B) and theoretical zero time concentration (C_p^o) elimination rate constant (β) and rate of drug elimination from central compartment (Kel) were found to be significantly higher ($p < 0.01$) in case of combined administration as compared to single administration of paracetamol. Similarly, area under curve (AUC) was significantly higher ($p < 0.05$) in case of combined administration with amikacin as compared to its single administration.

Highly significantly lower ($p < 0.01$) values of elimination half life ($t_{1/2}$ β), various values of volume of distribution including Vd_{area}, and mean residential time (MRT) were noted whereas significantly lower ($p < 0.05$) value of total body clearance (Cl_B) was noted in case of combined administration with amikacin as compared to single administration of paracetamol. Various other kinetic parameters like distribution rate constant (α), distribution half life ($t_{1/2}$ α), tissue to plasma concentration ratio (T≈P) *etc.* did not differ significantly between both the groups.

Chapter - 5

Discussion

DISCUSSION

Amikacin, a recent member of aminoglycoside group of anitimicrobials, possesses many advantages such as least problem of bacterial resistance and cross resistance with other antimicrobial agents and most broad spectrum among the aminoglycosides (Chambers and Sande, 1996) with excellent disposition characteristics. Further its minimum therapeutic concentration is two to four folds lower (Ziv, 1977) with negligible plasma protein binding (Brown and Riviere, 1991) and distribution is high in extracellular space (Brown and Riviere, 1991). Although pharmacokinetic studies of amikacin were carried out in various species but little work has been done so far in buffalo calf. Paracetamol, a member of NSAIDs, possesses potent antipyretic property along with analgesic and antiinflammatory activities, which is frequently used to treat pyrexia in animals. Antimicrobials are often used along with antipyretic agents such as paracetamol for treating microbial infections associated with fever. Though pharmacokinetic interactions between antimicrobial agents and NSAIDs were studied in different species of animals but available

literature show that very little studies were done on interactions between amikacin and paracetamol in animals, particularly in buffalo calf. Therefore, keeping in view of aforesaid facts, the present study was undertaken to know the interactions of amikacin with paracetamol in buffalo calves.

I. PHARMACOKINETIC STUDY OF AMIKACIN IN BUFFALO CALVES

(A) Distribution in plasma

Concentrations of amikacin in plasma were significantly higher upto 0.333 h in a case of its (7.5mg/kg i.v.) combined administration with paracetamol (40 mg/kg i.v.) as compared to its single i.v. administration (Table 15 and fig 1). This fact denotes that paracetamol influences in altering plasma levels of amikacin, particularly in buffalo calves. In contrast to the present study, Mukta (2002) noted lower plasma drug concentrations of amikacin when it (7.5 mg/kg i.v.) was given in combination with diclofenac (1 mg/kg i.v.) as compared to its single administration. The mean therapeutic concentration of amikacin in plasma ($\geq$ 2 μg/ml) was maintained upto 5 h in both the cases in present study whereas Mukta (2002) noted that the mean therapeutic concentrations was maintained upto 6 h

when amikacin was given alone but it was not at all attained when amikacin was given along with diclofenac. The differences in plasma concentration of amikacin in buffalo calf by the above worker as compared to the present study may be due to the administration of different NSAID (paracetamol) used instead of diclofenac.

(B) Urinary excretion

Concentrations of amikacin in urine were noted to be significantly ($p < 0.01$)) lower at most of time intervals (0.042 to 36 h) when amikacin was given along with paracetamol in buffalo calves by i.v. route as compared to its single i.v. administration. The change in urinary pattern when amikacin was given along with paracetamol may also contribute to the change in various kinetic parameters. The therapeutic concentration of amikacin in urine ($\geq$ 2 μg/ml) was maintained upto 30 h in case of co-administration with paracetamol whereas upto a longer period of 36 h in case of alone administration (Table 15 and Fig. 2).

(C) Kinetic parameter

Various kinetic parameters of amikacin obtained when given alone (7.5 mg/kg i.v.) and when given along with paracetamol (40 mg/kg i.v.) differed significantly which indicate that paracetamol may influence over distribution, elimination and metabolic processes

of amikacin in buffalo calves. The present investigation on the comparison of kinetic parameters when the drug was given alone and when given together with paracetamol (Table 16) clearly established that paracetamol may influence the various physiological, biochemical and metabolic processes of amikacin in buffalo calves. Mukta (2002) also showed various changes in kinetic parameters such as area under plasma drug concentration time curve (AUC), rate of transfer of drug from central to peripheral compartment (K_{12}), Vd_{area} and approximate tissue to plasma ratio ($T \approx P$) when amikacin was given alone (7.5 mg/kg i.v.) as compared to its combined administration with diclofenac (1 mg/kg i.v).

In the present study, higher values of extrapolated zero time concentration during distribution phase (A), highly significantly ($p < 0.01$) higher values during elimination phase (B) and theoretical zero time concentration (C_p^o) were noted when amikacin was administered along with paracetamol as compared to its single i.v. administration (Table 16). The higher values obtained for the above parameters in combined administration of amikacin with paracetamol for amikacin may be due to consistent higher plasma concentrations and lower urinary excretion of amikacin obtained at most of the time intervals as compared to its single administration.

The distribution rate constant (α) of 0.979 ± 0.185 h^{-1} and distribution half life ($t_{1/2}$ α) of 0.83 ± 0.16 h were noted for amikacin when administered alone. The values didn't differ significantly in buffalo calves in case of combined administration of amikacin with paracetamol which denote that similar rate of distribution of the drug occurred in both the groups of animals. The values of α were reported to be higher i.e. 4.16 ± 1.84, 3.66 ± 2.07 and 4.74 ± 1.06 h^{-1} 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 (Orsini *et al.,* 1985), 3.77 h^{-1} in lactating goats (Abo-el-sooud, 1999), 4.62 h in chicken (El-Gammal *et al.,* 1992), 1.337 h^{-1}in lactating goat (Agrawal *et al.,* 2002) and 1.925 h^{-1}in calf (*Carli et al.,* 1990). Lower values of $t_{1/2}$ α of 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 (Orsini *et al.*,1985). Similarly, lower values of $t_{1/2}$ α of 0.184 h in lactating goat (Abo-el-sooud, 1999), 0.44 h in sheep (Carli *et al.,* 1990), 0.19 h in sheep (Uppal *et al.* 1998), 0.52 h in healthy goat (Agrawal *et al.,* 2002) and 0.36h in cow calf (Carli *et al.,* 1990) were reported. More or less similar value of $t_{1/2}$ α of 0.75 h was noted in buffalo calf (Mukta, 2002).

The elimination rate constant (β) of 0.148 ± 0.022 h^{-1} and elimination half life ($t_{1/2}$ β) of 4.90 ± 0.53 h were noted for amikacin

following its single i.v administration in the present study. Significantly higher β of 0.283 ± 0.116 h^{-1} and lower $t_{1/2}$ β of 2.48 ± 0.16 h were obtained when amikacin (7.5 mg/kg i.v.) was given in combination with paracetamol (40 mg/kg i.v.) in the present work. This indicates that paracetamol may influence the quicker elimination of amikacin. This fact is further supported by significantly higher value of rate constant of the drug for elimination from central compartment (Kel) and significantly lower mean resident time (MRT) in case of combined administration of the drug as compared to its single administration (Table 16). 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 (1.3 ± 0.32 h), Baggot *et al.* (1998) in dog (approx. 1h), Orsini *et al.* (1985) in horse (1.14 h), Brown *et al.* (1984) in mare (1.44 h), El- Gammal *et al.* (1992) in chickens (1.44h), Carli *et al.* (1990) in sheep (1.92 h), Uppal *et al.* (1998) in sheep (1.42 h), Abo-el-sooud (1999) in lactating goat (1.91 h), Carli *et al.* (1990) in calf (2.51 h), Saini *et al.* (1998) in cross bred bovine (3.09 h) and Uppal *et al.* (1998) in buffalo calf (3.1h). In contrast, higher value of $t_{1/2}$ β was obtained by Errecalde *et al.* (2001) in Holando-Argentino calf (6.2 h). The differences in the elimination half life in the present investigation in buffalo calf as compared to other species may be due to variation in physiological and biochemical factors such as metabolism, process of excretion *etc.*

The rate constant of drug transfer from central to peripheral (K_{12}) and peripheral to central (K_{21}) compartment were noted to be 0.356 ± 0.086 h^{-1}, and 0.368 ± 0.101 h^{-1}, respectively, when amikacin was given alone. In case of combined administration with paracetamol, the value of K_{12} (0.635 ± 0.124 h^{-1}) differed only non significantly whereas K_{21} (0.801 ± 0.091 h^{-1}) differed significantly ($p < 0.05$). More or less similar values of K_{12} (0.270 ± 0.27h^{-1}) and K_{21} (0.497 ± 0.22 h^{-1}) were observed in female goat (Agrawal *et al.*, 2002) whereas higher values of K_{12} (1.20 ± 0.18 h^{-1}) and K_{21} (0.90 ± 0.12 h^{-1}) were noted in male goat (Uppal *et al.*, 1992).

The value of area under curve (AUC), area under first moment curve (AUMC) and mean resident time (MRT) were noted to be 49.36 ± 3.54 mg/L.h, 252.4 ± 30.0 mg/L.h^2 and 5.08 ± 0.41h, respectively, following i.v. administration of amikacin alone. When the drug administered along with paracetamol, non significant difference was noted for AUC (46.46 ± 2.79 mg/L.h) whereas significantly lower values were noted for AUMC (132.9 ± 12.08 mg/ L.h^2) and MRT (2.85 ± 0.10 h). Mukta (2002) noted more or less similer value of AUC (48.56 ± 5.84 mg/L.h) for amikacin when given alone @ 7.5 mg/kg i.v. As compared to the value of MRT obtained in the present study in buffalo calf, lower MRT of 1.97 ± 0.36 h in cat after i.v. administration (Jernigan *et al.*, 1988), 2.38 h after i.v.

administration in healthy lactating goat (Abo-El-Sooud, 1999) and 2.92 ± 0.14 h in lactating goat after i.m administration (Agrawal *et al.*, 2002) were reported.

The kinetic parameter, fraction of drug available for elimination from central compartment (Fc) when amikacin was given alone (0.37 ± 0.04) differed non significantly (0.470 ± 0.05) when it was given in combination with paracetamol. Approximately similar values were noted by Agrawal *et al.* (2002) in lactating female goat (0.37 ± 0.02) and Uppal *et al.*, (1992) in male goat (0.32 ± 0.02) whereas comparatively higher value was noted by Mukta (2002) in female buffalo calf (0.59 ± 0.03). Approximate tissue to plasma concentration ratio (T≈P) of 1.87 ± 0.41 noted when amikacin was given alone which differed non significantly when it was simultaneously administered with paracetamol (1.26 ± 0.19). More or less similar T≈P values were noted by Agarwal *et al.* (2002) in female lactating goat (1.72 ± 0.17), Uppal *et al.* (1992) in male goat (2.10 ± 0.21) and Mukta (2002) in female buffalo calf (1.62 ± 0.21).

The various values of volume distribution of amikacin were observed to be significantly lower when amikacin was given in combination with paracetamol as compared to its alone administration (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 also mentioned that among these values of volume of distribution only Vd_{area} correctly predicts the amount of drug in the body during elimination phase whereas Vd_B overestimates and Vd_{SS} & Vd_C underestimate the amount of drug in the body. In the present investigation, Vd_{area} of 1.01 ± 0.07 L/kg was estimated when amikacin was given alone as compared to significantly ($p < 0.01$) lower Vd_{area} of 0.58 ± 0.03 L/kg when it was co-administered with paracetamol. A large volume (> 1 L/kg) indicates wide distribution through out the body or extensive tissue binding or rapid excretion of drug or combination of all the above (Baggot, 1977). Since no significant difference in approx. tissue to plasma ratio (T≈P) was noted for amikacin when it was given alone or given together with paracetamol (Table 16), significantly higher Vd_{area} (1.01 ± 0.07 L/kg) was obtained for amikacin when given alone may be due to its rapid and significantly higher amount of excretion at all time intervals (0.042 to 36 h). Lower Vd_{area} of 0.17 ± 0.02 L/kg in cat (Jernigon *et al.*, 1988), 0.226 ± 0.037 L/kg in dog (Baggot *et al.*, 1985), 0.193 ± 0.06 L/kg in chicken (El-Gammal *et al.*, 1992), 0.247 L/kg in camel (Wasfi *et al.*, 1993), 0.335 ± 0.003 L/kg in sheep (Uppal *et al.*, 1992), 0.040 L/kg in male goat (Uppal *et al.*, 1992), 0.35 L/kg in calf (Carli *et al.*, 1990), 0.40 ± 0.03L/kg in crossbred bovine calves (Saini and Srivastava, 1998) and 0.40 ± 0.02 L/kg in female goat (Agrawal *et al.*, 2002), after i.v. administration of amikacin. Following i.m.

administration of amikacin Vd_{area} of 0.26 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.*, 2002) were reported. Comparatively similar value (1.06 ± 0.06 L/kg) was obtained by Mukta (2002) following i.v. administration in buffalo calf. The above facts show that amikacin may be extensively penetrated in various body fluids and tissues of buffalo calf as compared to other species. The above fact is further supported by the value of 1.87 ± 0.41 for approx. tissue to plasma concentration ratio (T≈P) obtained for amikacin when given alone in the present study.

The total body clearance (Cl_B) value of 2.59 ± 0.19 ml/kg/min and 2.72 ± 0.16 ml/kg/min were obtained when amikacin was given alone and when it was given in combination with paracetamol, respectively, and they differed only non significantly. This investigation indicates that amikacin may be equally removed from the body of buffalo calves either when it was given alone or in combination with paracetamol. Quite close values were obtained by Baggot *et al.* (1985) in dog (2.64 ± 0.24 ml/kg/min), Uppal *et al.* (1998) in sheep (2.71 ± 0.13 ml/kg/min), Agrawal *et al.* (2002) in female goat (2.20 ± 0.14 ml/kg/min) and Mukta (2002) in buffalo calf (2.78 ± 1.45 ml/kg/min) following single i.v. administration of amikacin. Comparatively lower values of Cl_B were observed by Jernigan *et al.* (1988) in cat (1.46 ± 0.26 ml/kg/min), Orsini *et al.* (1985) in horse

(1.41 ± 0.22 ml/kg/min), Carli *et al.* (1990) in Bergamasca sheep (0.70 ml/kg/min), Carli *et al.* (1990) in calf (1.50 ml/kg/min), Saini *et al.* (1998) in calf (1.50 ± 0.03 ml/kg/min) and Uppal *et al.* (1998) in buffalo calf (0.75 ± 0.01 ml/kg/min) following i.v. administration. Wasfi *et al.* (1999) observed a higher value of Cl_B (3.75 ml/kg/min) in camel.

(D) Dosage Regimen

Leroy *et al.* (1978) reported therapeutic plasma level (C_p^{∞}min) of amikacin to be 1-4 μg/ml. In the present investigation, calculation of dosage regimen 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. Significantly higher loading (D^*) and maintenance (D_o) doses for amikacin were calculated in case of combined administration of amikacin with paracetamol at all the above mentioned therapeutic concentrations and at different dosage intervals (8 and 12 h) as compared to its single administration.

For treating mild infections (C_p^{∞}min = 1 μg/ml), a loading dose (D^*) of 6.46 ± 1.56 mg/kg and maintenance dose (D^*) of 5.45 ± 1.63 mg/kg may be advised at the dosage interval (γ) of 12 h when amikacin was given alone. Amikacin when given in combination at the same therapeutic level (C_p^{∞}min = 1 μg/ml) and similar dosage

interval (γ) of 12 h, significantly ($p < 0.05$) higher doses of D^* (21.59 $\pm$0.36 mg/kg) and D_o (20.93 $\pm$ 3.55 mg/kg) may be needed which may cause toxicity Hence, a shorter dosage interval (γ) of 8 h may be recommended in this case where D^* and D_o were calculated to be 6.70 $\pm$ 0.84 mg/kg and 6.04 $\pm$ 0.82 mg/kg, respectively.

For treating moderate infections ($C_p^{\infty}min$ = 2 μg/ml), D^* of 12.93 $\pm$ 3.13 mg/kg and D_o of 10.93 $\pm$ 3.26 mg/kg at γ of 12 h can be effectively used when amikacin given alone while the dosage calculated at he same γ of 12 h are too high which may cause toxicity in animals and hence D^* of 13.41 $\pm$ 1.68 mg/kg and D_o of 12.07 $\pm$ 1.65 mg/kg at shorter γ of 8 h when amikacin given along with paracetamol.

In case of treating severs infections ($C_p^{\infty}min$ = 4 μg/ml), D^* of 13.40 $\pm$ 1.58 mg/kg and D_o of 9.35 $\pm$ 1.86 mg/kg at γ of 8 h can be used when amikacin given alone. The D^* and D_o of amikacin for γ of 12 h are calculated to be higher when amikacin given alone which may case toxicity and hence, can not be recommended. Similarly, when amikacin to be administered along with paracetamol, the calculated dosage regimen at both dosage intervals (8 and 12 h) are very high and hence, amikacin can not be recommended to be used in combination with paracetamol in case of severe infections.

II. KINETIC STUDY OF PARACETAMOL

Kinetic studies of paracetamol in animals are very few. However, few reports are available in goat (Manna *et al.*, 1994; Sudha Kumari, 1998) and buffalo calf (Sidhu *et al.*, 1993).

(A) Distribution in plasma

Concentrations of paracetamol in plasma were found to be significantly higher almost at all time intervals (0.042 to 0.50 h) in case of its combined administration with amikacin except 0.333, 0.75 and 1 h where non significant differences were noted (Table 18 and Fig. 4). Similarly, Sudha Kumari (1998) noted maintenance of higher concentrations of paracetamol in plasma at all time interval in goats following its combined i.v administration with enrofloxacin.

(B) Urinary excretion

Concentrations of paracetamol in urine were noted to be significantly lower from 0.042 to 0.50 h when the drug was administered along with amikacin i.v. in buffalo calf as compared to its alone administration. However, no significant difference was noted from 0.75 h to 48 h except at 6 and 24 h where significantly higher concentrations were noted when paracetamol given along with amikacin. Peak concentration of 2013 ± 143.9 μg/ml (combined administration with a amikacin) and 2022 ± 118.8 μg/ml (single administration of paracetamol) were noted at 1.5 h (Table 18 Fig. 2).

(C) Kinetic parameters

Highly significantly ($p < 0.01$) higher value for the extrapolated zero time concentration during distribution phase (A), elimination phase (B) and theoretical zero time concentration (C_p^0) were obtained for paracetamol, when given along with amikacin as compared to its alone administration by i.v. route (Table 19). Manna *et al.* (1995) showed higher C_p° value of 163.3 ± 9.9 μg/ml in goat whereas lower C_p° value of 29.26 ± 3.64 μg/ml in lactating female goats by Sudha Kumari (1998).

The distribution rate constant (α) of 7.148 ± 13.61 h^{-1} and distribution half life ($t_{1/2}$ α) of 0.11 ± 0.01 h were calculated for paracetamol when given alone by i.v route. These values did not differ significantly in buffalo calves when administered by i.v route in combination with amikacin (Table 19). This indicates that the rate of distribution of paracetamol may not be influenced by amikacin. Comparatively higher values of $t_{1/2}$ α were noted by Sidhu *et al.* (1993) in buffalo calf (0.47 ± 0.04 h) and Sudha Kumari (1998) in lactating goat (0.24 ± 0.04 h). More or less similar value of $t_{1/2}$ α was noted by Manna *et al.* (1994) in Black Bengal goat (0.10 h).The shorter $t_{1/2}$ α denotes that the drug is comparatively distributed at a faster rate in buffalo calf.

The elimination rate constant (β) of 0.965 ± 0.136 h^{-1} and elimination half life ($t_{½}\beta$) of 0.77 ± 0.09 h were estimated after single i.v administration of paracetamol. Highly significantly ($p < 0.01$) increased β (1.539 ± 0.059h^{-1}) and decreased $t_{1/2}$ β (0.45 ± 0.02 h) values were noted for paracetamol when it was given in combination with amikacin by i.v. route. The decreased $t_{1/2}$ β observed post combined i.v. administration of paracetamol and amikacin indicates faster removal of the drug from the body of the animal as compared to single administration of the drug. This is further supported by highly significantly higher value of rate constant of drug elimination from central compartment (Kel) obtained in buffalo calves post combined i.v. administration of paracetamol and amikacin. More or less similar $t_{1/2}$ β values were obtained by Manna *et al.* (1994) in Black Bengal goat (0.53 h) and Sidhu *et al.* (1993) in buffalo calves (0.47 ± 0.40 h). Comparatively higher $t_{1/2}$ β values were noted by Sharma *et al.* (1995) in cross bred calf (4.84 ± 1.26 h) and by Sudha Kumari (1998) in lactating female goat (3.56 ± 0.13 h).

The values of rate of transfer of drug from central to peripheral (K_{12}) and peripheral to central (K_{21}) compartment did not differ significantly between both the groups Similarly some other kinetic parameters *viz.*, area under first moment curve (AUMC), fraction of drug available for elimination from central compartment

(F_c) and tissue to plasma concentration (T≈P) which did not differ significantly between buffalo calves of both the groups (Table 19).

The various values of volume of distribution were highly significantly ($p < 0.01$) lower in buffalo calves when paracetamol was given in combination with amikacin as compared to its single i.v. administration (Table 19). Vd_{area} of 2.79 ± 0.14 L/kg was noted for single administration of paracetamol by i.v. route whereas it was noted to be 1.08 ± 0.11 L/kg in case of combined i.v. administration with amikacin. A lower Vd_{area} of 1.22 ± 0.23 L/kg in buffalo calf (Sidhu *et al.*, 1993) and very low Vd_{area} of 0.48 ± 0.11 L/kg in cross bred calf (Sharma *et al.*, 1995) were noted following i.m. administration of paracetamol. A higher Vd_{area} value of 5.48 ± 1.40 L/kg noted in case of single i.v. administration in goat (Sudha Kumari, 1998). Vd_{area} > 1 L/kg obtained in the present study of paracetamol denotes good distribution of the drug in body fluids and tissues of buffalo calf.

The total body clearance (Cl_B) value was significantly lower in case of combined administration of paracetamol with amikacin (27.58 ± 2.57 ml/kg/min) as compared to its alone administration (43.67 ± 3.39 ml/kg/min). A very high Cl_B value denotes that paracetamol may be eliminated quickly from the body of buffalo calves. Low Cl_B values of 1.88 ± 0.66 ml/kg/min in buffalo calf

(Sidhu *et al.*, 1993), crossbred calf (1.33 ± 0.38 ml/kg/min) by Sharma *et al.* (1995) and 17.37 ± 1.46 ml/kg/min in goat (Sudha Kumari, 1998) were obtained following i.v. administration of paracetamol.

III. KINETIC INTERACTION BETWEEN AMIKACIN AND PARACETAMOL

The distribution of amikacin and paracetamol in plasma and urine as well as various kinetic parameters and also dosage regimen of amikacin have been described above when given alone or in combination following i.v. administration in buffalo calf. Definite kinetic infractions between the drugs occurred in buffalo calves and the salient features are described below: -

The study reveals that distribution of amikacin as well as paracetamol was not at all affected when these drugs were given together as compared to their single administration as shown by α and $t_{1/2}$ α values. Similarly, Sudha Kumari (1998) also observed no influence of paracetamol on the rate of distribution of enrofloxacin. Mukta (2002) also noted no influence of diclofenac (NSAID) on amikacin on the rate of distribution in buffalo calves when given in combination.

Amikacin was eliminated faster when given with paracetamol in the present study as noted by lower $t_{1/2}$ β of 2.48 ± 0.16 h in case of combined administration while a higher $t_{1/2}$ β of 4.9 ±

0.53 h was obtained when amikacin was given alone (Table 16). Paracetamol also eliminated faster when given in combination with amikacin ($t_{1/2}$ β = 0.45 ± 0.02 h) as compared to its alone administration ($t_{1/2}$ β = 0.77 ± 0.09 h). Sudha Kumri (1998) also showed similar faster elimination of enrofloxacin and paracetamol when these drugs are given in combination through i.v. route. Choudhary and Srivastava (1999) observed lower $t_{1/2}$ β of 2.63 h when administered cefuroxime with paracetamol as compared to its alone administration. In contrast, Mukta (2002) observed no effect of diclofenac in the elimination of amikacin as shown by non significant difference in $t_{1/2}$ β value. The quicker elimination of amikacin as well as paracetamol when given together is further supported by significantly higher Kel values and significantly lower MRT values of both the drugs when given in combination as compared to their single administration (Table 16 and Table 19).

The rate and amount of distribution of amikacin and paracetamol in various tissues and body fluid may not differ much as noted by non significant differences in the values of α, $t_{1/2}$ α, K_{12}, K_{21} and T≈P between single and combined administration (except significantly higher K_{21} value was obtained for amikacin when given with paracetamol). Various values of volume of distribution were noted to be significantly higher for amikacin as well as paracetamol

when given in combination as compared to single administration. High values of Vd_{area} were obtained for amikacin (1.01 ± 0.07 L/kg) and paracetamol (2.79 ± 0.14 L/kg) when given alone as compared to their combined administration (0.58 ± 0.03 L/kg and 1.08 ± 0.11 L/kg, respectively). High Vd_{area} values in case of single administration for these drugs may not be due to their better distribution as compared to combined administration since these drugs are excreted in greater amount when given alone and T≈P values did not differ significantly. Baggot (1977) stated that a large volume of distribution of a drug indicates wide urine distribution throughout the body or extensive tissue binding or rapid excretion or combination of all the above. In the present study, rapid and higher excretion of both the drug occurred when given alone as compared to their combined administration (Table 15 and Table 18). The above facts indicate that both amikacin and paracetamol may equally distribute in the body of buffalo calves when given alone and when given together in combination.

The dosage regimen of amikacin when given alone were calculated to be significantly lower as compared to its combined administration (Table 17). It may be due to significantly lower value obtained for $t_{1/2}$ β and Vd_{area} in case of combined administration as compared to their alone administration. For treating mild

(Cp^{∞} min = 1 μg/ml) and moderate infections (Cp^{∞} min = 2 μg/ml), loading (D*) and maintenance (D_0) doses of 6.46 ± 1.56 & 5.45 ± 1.63 and 12.93 ± 3.13 & 10.93 ± 3.26 mg /kg, respectively may be used at γ of 12 h in case of single administration. In case of combined administration with paracetamol, D* and D_0 of 6.70 ± 0.84 & 6.04 ± 0.82 and 13.41 ± 1.68 & 12.07 ± 1.65 mg/kg, respectively, at shorter γ of 8 h may be used. In case of severe infections (Cp^{∞} min = 4 μg/ml), D* and D_0 of 13.40 ± 1.58 & 9.35 ± 1.86 mg /kg may be recommended at shorter γ of 8 h while the required dosage when given with paracetamol were calculated to be too high and cannot be recommended since it may lead to severe toxicity in animals.

In case of urine, therapeutic concentration (≥ 2 μg/ml) of amikacin was maintained upto 36 and 30 h when given alone and when given together with paracetamol, respectively. Considering the above fact and by taking into account of the lag phase of bacteria amikacin may be recommended @ 7.5 mg/kg at 48 h interval when given alone and at shorter interval of 36 h when given together with paracetamol.

□□□□□

Chapter - 6

Summary

SUMMARY

A detailed pharmacokinetic study of amikacin and paracetamol when given alone and their interactions when given together intravenously (i.v.) was carried out in buffalo calves weighing between 105-180 kg. Concentrations of the drug in plasma and urine were estimated and various kinetic parameters were calculated by using two compartment open model when the drugs are given alone or when given together in combination. Attempts were made to calculate the rational dosage regimen of amikacin on the basis of obtained kinetic data, maintenance of therapeutic concentrations (MICs) and also taking into account of the lag phase of bacteria. Based on the above objectives, the following findings were obtained: -

1. After combined i.v. administration of amikacin (7.5 mg/kg) with paracetamol (40 mg/kg), plasma concentrations of amikacin were found to be significantly higher initially (0.042 to 0.333 h) as compared to its single i.v. administration of amikacin. In case of urine, significantly lower concentrations were noted at almost all time intervals (0.042 to 36 h) following combined administration of amikacin with paracetamol as compared to its

alone administration. The therapeutic concentration (≥ 2 μg/ml) of amikacin in plasma and urine was maintained from 0.042 to 5 h and 0.083 to 30 h, respectively, when administered along with paracetamol whereas, it was maintained upto to 0.042 to 5 h and 0.042 to 36 h in plasma and urine, respectively, after single administration of amikacin, (Table 15).

Following i.v. administration of paracetamol with amikacin, concentrations of paracetamol in plasma were found to be significantly higher initially (0.042 to 0.25 h) as compared to single administration of paracetamol. In case of urine, when paracetamol administered along with amikacin, significantly lower concentrations of paracetamol were maintained from 0.042 to 0.5 h and thereafter non significant differences were noted at almost all time intervals as compared to its single i.v. administration.

2. The values of extrapolated zero time concentration during elimination phase (B) and the theoretical zero time concentration (C_p^o) of amikacin were found to be highly significantly ($p < 0.01$) higher in case of combined i.v. administration of amikacin with paracetamol as compared to that of single administration. This is due to significantly higher concentrations of amikacin in plasma of buffalo calves initially

when these two drugs were given together as compared to its single administratoin.

3. There was no significant difference in the values of distribution rate constant (α), distribution half-life ($t_{1/2}\ \alpha$), rate constant of the drug from central to peripheral compartment (K_{12}), fraction of drug available for elimination from central compartment (Fc), approximate tissue to plasma concentration ratio ($T \approx P$) and total body clearance (Cl_B) between both the groups. The above findings denote that similar rate of distribution and equal amount of distribution and removal of amikacin from the body buffalo calves in both the groups (Table 16).

4. Highly significantly higher value of elimination rate constant (β) of $0.283 \pm 0.116\ h^{-1}$ and lower elimination half life ($t_{1/2}\ \beta$) of 2.48 ± 0.16 h were noted when amikacin was given along with paracetamol as compared to its single administration (β of $0.148 \pm 0.022\ h^{-1}$ and $t_{1/2}\ \beta$ of 4.90 ± 0.53 h). This indicates that paracetamol may influence the quicker elimination of amikacin. The above fact is further supported by significantly ($p < 0.01$) higher value of rate constant of drug available for elimination from central compartment (Kel) obtained in case of combined i.v. administration of amikacin with paracetamol to that of its single administration.

5. The value of area under curve (AUC) did not differ significantly in case of combined administration of amikacin with paracetamol as compared to its alone administration. Other parameters like area under first moment curve (AUMC) and mean resident time (MRT) were significantly lower when the drugs were given together. Lower MRT value in case of combined administration denotes that amikacin may be retained for a shorter period in the body of buffalo calves as compared to its single administration (Table 16).

6. Various values of volume of distribution were found to be highly significantly ($p < 0.01$) lower for amikacin after its combined i.v. administration with paracetamol as compared to its alone administration. Vd_{area} of 0.59 ± 0.03 L/Kg was noted for amikacin given together with paracetamol as compared to Vd_{area} of 1.01 ± 0.07 L/kg when given alone. This may lead to the inference that amikacin may be distributed to a lesser extent when it was given together with paracetamol. But approximate tissue to plasma concentration ratio (T≈P) showed non-significant difference between both the groups. A high Vd_{area} may denote that apart from wide distribution, it may also be due to extensive tissue binding as well as rapid of urinary

excretion (Baggot 1977). Hence, it may be assumed that a low Vd_{area} obtained when amikacin was given together with paracetamol may be due to its lower excretion in urine as noted in the present study throughout the time intervals (0.042 h to 36 h).

7. For treating mild systemic infections (C_p^{∞} min = 1 μg/ml), a loading (D*) and maintenance dose of around 6.5 and 5.5 mg/kg at dosage interval (γ) of 12 h may be used when amikacin given alone while very high D* and D_o of 21.6 and 21.0 mg/kg are required when given along with paracetamol that may cause toxicity. Hence, of shortage dosage interval of 8 h can be used for which the D* and D_o of 6.7 and 6.0 mg/kg may be required in case of combined administration with paracetamol. For treating moderate infections (C_p^{∞} min = 2 μg/ml), D* of 12.9 mg/kg and D_o of 10.9 mg/kg at γ of 12 h are needed when amikacin given alone while D* of 13.41 mg/kg and D_o of 12.07 mg/kg at shorter γ of 8 h may be used in case of combined administration with paracetamol. In case severe systemic infections (C_p^{∞} min =4 μg/ml), D* of 13.4 mg/kg and D_o of 9.4 mg/kg at γ of 8 h may be recommended when amikacin given alone while very high D* and D_o are needed when amikacin administered along with paracetamol which may cause possible

severe toxicity in buffalo calves. Hence, for treating mild and moderate infection amikacin may be used alone or along with paracetamol whereas in case of severe infection it can be preferably used alone.

8. The extrapolated zero time concentration during distribution (A), elimination (B) phase and theoretical zero time concentration (C_p^o) were found to be significantly higher when paracetamol was given along with amikacin as compared to its alone administration. These findings may be due to significantly higher plasma concentrations of paracetamol obtained initially when given along with amikacin. No significant difference was noted in case of distribution rate constant (α), distribution half life ($t_{1/2}\ \alpha$), rate of drug transfer from central to peripheral (K_{12}) and peripheral to central (K_{21}) compartment, fraction of drug available for elimination from central compartment (Fc) and approximate tissue to plasma concentration ratio (T≈P) between both the group. Significantly higher elimination rate constant (β) and lower elimination half life ($t_{1/2}\ \beta$) for paracetamol were noted in case of combined i.v. administration of paracetamol with amikacin as compared to it alone administration. This finding denotes quicker elimination of the drug in case of combined administration. This is further supported by significantly lower mean resident time (MRT) and

higher rate of elimination from central compartment (Kel) in case of co-administration of the drug with amikacin as compared to its alone administration. Various values of volume of distribution were noted to be highly significantly lower in case of combined administration as compared to that of alone administration. Total body clearance value (Cl_B) was significantly lower in case of combined administration of paracetamol with amikacin for paracetamol as compared to that of its alone administration. This finding may indicate the lesser removal of paracetamol from the body of buffalo calves when given along with amikacin as compared to its single administration.

9. Amikacin is a drug of choice in human medicine for treating gentamicin resistant organisms causing urinary tract infections apart from its other uses. Early appearance of amikacin in urine in both the cases in therapeutic concentrations and for longer duration after i.v. administration indicates clearly that this drug may be used in the treatment of various bacterial infections of urinary tract particularly resistant to other aminoglycosides. In the present study, the therapeutic concentration ($\geq$ 2 μg/ml) was maintained upto around 36 and 30 h when given alone and when given together with paracetamol, respectively. Hence by taking into account of lag phase of bacteria, amikacin may be recommended @ 7.5 mg/kg

at 48 h interval when given alone and at 36 h interval when given together with paracetamol for treating urinary tract infections caused by drug sensitive microorganism. This can be used in septicemia and other systemic infection at the dosage regimen stated above for treating infections caused by drug susceptible bacteria.

The present study clearly establishes that both amikacin and paracetamol interacts with each other. This has lead to definite change in various kinetic parameters and dosage regimen of amikacin. The study points out that the combination of amikacin with paracetamol necessitates higher doses of amikacin for treating severe infections that may cause severe toxicity. Hence, the combination of amikacin with paracetamol may not be advantageous in severe infections but may be given together in case of mild and moderate systemic infections. However, the combination of amikacin with paracetamol can be used successfully in case of urinary tract infections associated with febrile condition in animals, particularly in buffalo. Kinetic interactions between other effective newly introduced NSAIDs with antimicrobials should be studied for effective treatment of infections accompanied by fever and inflammatory conditions 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 noted below from the data of buffalo calf no. 2 obtained after single i.v. injection of amikacin (7.5 mg/kg). The data showed a biphasic curve and hence, fits well into two compartment open model. Here, elimination phase starts from 4 h.

Sl.No.	Time in h (X)	X^2	Plasma drug concentration in μg/ml (Y)	Log Y	XY
1.	4	16	3.24	0.511	2.044
2.	5	25	2.12	0.326	1.63
3.	6	36	1.36	0.133	0.798
4.	8	64	1.27	0.104	0.832
5.	10	100	1.15	0.061	0.610
6.	12	144	0.40	-0.398	-4.776
7.	24	576	0.22	-0.658	-15.792

$n = 9$, $\Sigma X = 69$, $\Sigma X^2 = 961$, $\Sigma Y = 0.079$, $\Sigma XY = 14.654$ $\bar{X} = 9.8519$

$(\Sigma X)^2 = 4761$ $\bar{Y} = 0.0113$

where , X = time; Y = drug concentration ; n = number of samples

$$\text{b, slope of line} = \frac{n.\Sigma XY - \Sigma X.\Sigma Y}{n.\Sigma X^2 - (\Sigma X)^2} = \frac{7\times -14.654 - 69\times 0.079}{7\times 961 - (69)^2}$$

$$= \frac{-102.578 - 5.451}{6727 - 4761} = \frac{-108.029}{1966} = -0.0549$$

β, elimination rate constant $= b\times (-2.303)$

$= -0.0549\times -2.303$

$= 0.127\ h^{-1}$

B, zero time concentration during elimination can be obtained from the formula $\bar{Y} = a + b.\ \bar{X}$

Where, $\bar{Y}$ = mean log drug concentration

$\bar{X}$ = mean time

b = slope of line

a = zero time concentration

Therefore,

$a = \bar{Y} - b.\ \bar{X}$

$= \log 0.0113 - (0.0549)\times 9.857$

$= \log 0.0113 + 0.541 = \log 0.5523$

zero time concentration (B) = antilog of 0.5523

$= 3.57\ \mu g/ml$

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

Subtracting the theoretical value from observed values, a series of residual concentrations were obtained and slope of line in natural log (distribution rate constant, α) and 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 13.832 μg/ml and α is 0.556 h^{-1}.

The theoretical plasma concentration at zero time,

C_p^o = A + B = 13.832 + 3.57

= 17.402 μg/ml

Distribution half life, t½ α

$$t_{½}\,\alpha = \frac{0.693}{\alpha} = \frac{0.693}{0.556} = 1.25\,h$$

Elimination half life, t½ β

$$t_{½}\,\beta = \frac{0.693}{\beta} = \frac{0.693}{0.127} = 5.46\ h$$

Area under curve, AUC

$$AUC = \frac{A}{\alpha} + \frac{B}{\beta} = \frac{13.832}{0.556} + \frac{3.57}{0.127} = 24.8776 + 28.110$$

= 52.99 mg/L.h

Area under first moment curve, plasma drug concentration time curve, AUMC

$$AUMC = \frac{A}{\alpha^2} + \frac{B}{\beta^2} = \frac{13.832}{(0.556)^2} + \frac{3.57}{(0.127)^2}$$

= 44.744+221.340 = 266.08 mg/L. h^2

Mean residential time, MRT

$$MRT = \frac{AUMC}{AUC} = \frac{266.08}{52.99}$$

$$= 5.02 \text{ h.}$$

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

$$K_{21} = \frac{A.\beta + B.\alpha}{Cp^0} = \frac{13.832 \times 0.127 + 3.57 \times 0.556}{17.402}$$

$$= \frac{1.7566 + 1.9849}{17.402} = 0.215 \text{ h}^{-1}$$

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

$$Kel = \frac{\alpha \times \beta}{K_{21}} = \frac{0.556 \times 0.127}{0.215}$$

$$= 0.328 \text{ h}^{-1}$$

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

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

$$= 0.556 + 0.127 - 0.328 - 0.215$$

$$= 0.14 \text{ h}^{-1}$$

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

$$Fc = \frac{\beta}{Kel} = \frac{0.127}{0.328} = 0.39$$

Approximate tissue to plasma concentration ratio, T≈P

$$T \approx P = \frac{K_{12}}{K_{21} - \beta} = \frac{0.14}{0.215 - 0.127}$$

$$= \frac{0.14}{0.088} = 1.59$$

Volume of distribution based on both distribution and elimination, Vdc

$$Vd_c = \frac{D}{C_p^o} = \frac{7.5}{17.402}$$

$$= 0.43 \text{ L/kg}$$

Volume of distribution based on elimination

$$Vd_B = \frac{D}{B} = \frac{7.5}{3.57}$$

$$= 2.10 \text{ L/kg}$$

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

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

$$= \frac{7.5}{52.99 \times 0.127} = \frac{7.5}{6.729}$$

$$= 1.12 \text{ L/kg}$$

Volume of distribution at steady state, Vd_{ss}

$$Vd_{ss} = \frac{K_{12} + K_{21}}{K_{21}} \times Vdc$$

$$= \left(\frac{0.14 + 0.215}{0.215}\right) 0.43$$

$$= \left(\frac{0.355}{0.215}\right) 0.43 = 0.71 \text{ L/kg}$$

Total body clearance, Cl_B

$$Cl_B = Vdarea \times \beta$$

$$= 1.12 \times 0.127 = 0.142 \text{ L/kg/h}$$

$$= 2.37 \text{ ml/kg/min.}$$

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APPENDIX - II

CALCULATION OF DOSAGE REGIMEN

Dosage regimen of antimicrobial agents are generally calculated to maintain the minimum inhibitory concentration (MIC) in plasma at desired dosage interval (γ) using formula noted by Baggot (1977) and described by Saini and Srivastava (1997).

The data of animal no. 2 obtained after a single i.v. injection of amikacin in healthy buffalo calf has been used as an example for calculation of dosage regimen for maintaining C_p° min (MIC) of 1 μg/ml at the dosage interval of 8 and 12 h.

Calculation of loading (D*) and maintenance dose (D_o)

The loading dose (D*) is the initial dose that may be given at the onset to reach the target concentration rapidly. The maintenance (D_o) dose is the dose given at particular dosage interval (γ) for maintaining C_p^{∞} min (MIC) during the course of treatment. The loading (D*) and maintenance (D_o) doses of amikacin can be calculated by the formula given below:-

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

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

Where,

D^* = Loading dose

D_o = maintenance dose

C_p^{∞} (min) = minimum therapeutic plasma drug concentration

Vd_{area} = The volume of distribution based on total area under the plasma drug concentration versus time curve.

β = Elimination rate constant

γ = Dosage interval

e = base of natural logarithm

The loading and maintenance doses of amikacin are repeated at different time intervals (8 and 12h) to maintain the minimum plasma concentration of 1 μg/ml. Hence by considering 1 μg/ml as the minimum therapeutic concentration (C_p^{∞} min = MIC) at dosage interval of 8h in animal No. 2 after i.v. administration of the drug, D* and D_0 were calculated as shown below:-

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

$= 1 \times 1.12 \times e^{0.127 \times 8}$

$= 1 \times 12 \times 2.76$

$= 3.09$ mg/kg

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

$= 1 \times 1.12 (e^{0.127 \times 8}-1)$

$= 1.12 \times (2.76-1)$

$= 1.12 \times 1.76$

$= 1.97$ mg/kg