Review Article

NANOTECHNOLOGY IN DRUG DELIVERY AND THERAPEUTICS

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ABSTRACT

The multidisciplinary approach of nanobiotechnology has opened new vistas for the development of nanoscale drug delivery systems to meet the requirements for new drug moieties. These drug moieties can either be integrated into the matrix or attached to the surface of drug delivery particles. Nanostructures like micelles, liposomes, dendrimers etc. and nanoparticles like solid lipid nanoparticles, polymeric/pegylated nanostructures, metallic nanoparticles etc. have been used to deliver drug at specific sites and reduce side effects on non target organs. Nanoparticles possess larger surface area than volume of the drug. The efficacy of these nanoparticles has been encouraging for gastrointestinal and blood brain barriers. The nanoparticles provide protection against degrading agents and thus prolong the bioavailability of the pharmaceutical agents by controlled drug released systems. Carbon nanotubes (CNT) may be used as carriers for drug delivery as they can easily adapt themselves and enter the nuclei of the cell. The main aim of targeted drug delivery and controlled release is to manage drug pharmacokinetics, non-specific toxicity and biorecognition of systems. A controlled and sustained release of drug is essential in preventing side effects to surrounding or non-targeted cells. Advances in nanobiotechnology are enabling manufacturing nanorobots through nanobioelectronics and biologically inspired devices which could revolutionize the diagnosis and treatment of cancer in near future. Thus, the use of nanoscale devices will help to direct the drug molecules to the target sites and also likely to enhance their acceptability or tolerance by patients.

Keywords: Nanotechnology, noanorobotics, nanoscale drug delivery systems, pharmacokinetics, pharmacodynamics, drug tolerance.

INTRODUCTION

Nanotechnology is defined as the understanding and control of matter at dimensions of roughly 1 to 100 nanometers where unique phenomena enable novel applications. Nanobiotechnolgy is derived from the amalgamation of nanotechnology and biotechnology which gave birth to an emerging sub-discipline nanomedicine - the science of diagnosing, treating and preventing disease with the use of molecular biology combined with nanotechnology. The prefix *nano*- (Greek word *nanos*= "dwarf") means one-billionth (10^{-9}). This recently emerging technology is bestowed upon developing nano-scale drug delivery system by imitating or incorporating biological systems at nanoscale (NNI, 2006).

In 1959, the great physicist Richard Feynman said that "There is plenty of room at the bottom," is widely considered to be the foreshadowing of nanotechnology. Then, the term "Nanotechnology" quickly came into limelight and gained popularity, and almost immediately its meaning shifted to molecular nanotechnology. By 1992, Drexler used "molecular nanotechnology" or "molecular manufacturing" to distinguish his ideas of manufacturing from the simple product to the focused research in this field. A strategy for development of targeted drug delivery system involves the surface functionalization of drug carrier with ligand that is selectively recognized by receptor on the surface of target cell. Thus, the ligand- receptor interaction should be highly selective to allow a more precise targeting of the cells or molecules of interest (Weiss and McClements, 2002). Potential sustained release mechanism either involves diffusion through the carrier matrix or deabsorption of drug to its surface through a combined erosion or diffusion process. Sustained and constant release of a drug involve nanoparticles acting as carrier like polymers that releases the drug at a controlled rate due to diffusion out of the carrier or by degradation of the carrier over the time (Jain, 2005).

Nanoscale technologies are emerging as powerful tools for tissue engineering and drug discovery. In tissue engineering, micro- and nanotechnologies can be used to fabricate biomimetic scaffolds and to control cell–cell, cell–matrix and cell–soluble factor interactions in a reproducible manner and with high temporal and spatial resolution. In drug discovery, miniaturized platforms based on micro- and nanotechnology can be used to precisely control the fluid flow, enable high-throughput screening, and minimize sample or reagent volumes. Input of nanotechnology in drug delivery is a rapidly expanding and one of the most dynamic and fast-growing sectors of the pharmaceutical industry. The high level of innovation at a fast pace in this sector has led to development of various targeted drug delivery nanodevices such as nanorobotics, liposome

technology, micelles, dendrimers and other exotic delivery systems (Chung *et al.,* 2007).

In India, pharmaceutical companies are facing a new challenge of generic competition for drugs whose patent life spans have expired. Repackaging of the same drug with novel drug delivery technology may extend the patent life span of the drug in question. Improving delivery technique that could minimize toxicity and improve efficacy offers great potential benefits to patients and opens up new market for pharmaceutical and drug delivery companies. Liposomal nanoparticle formulation technology is coming up in a big way in the international drug delivery market. The nanoparticles can be tailor-made so far as their size and surface properties are concerned. The core of these carrier particles may be designed either hydrophobic or hydrophilic in nature according to the type of the drug to be entrapped (Fahmy *et al.*, 2007).

Drug delivery carriers

A number of nanoparticles have been exploited for developing delivery carrier. Colloidal drug carrier systems such as micellar solutions, vesicle and liquid crystal dispersions and nanoparticle dispersion consisting of small particles of 10-400nm diameter show great promise as drug delivery systems. The encapsulation materials produced from nanoparticles, have a larger surface area than the volume, small pore size, improved solubility and different structural properties. The dissolution rate nanoparticles is increased significantly as possess very large surface to volume ratios (Kayser *et al.,* 2005). Nanostructures below 100nm in size have the ability to enter cells readily improving both the diffusion and degradation characteristics of the encapsulation material. The drug encapsulation materials like liposomes and polymers protect drugs after they are delivered through the body. The effectiveness of these nanoparticles have been demonstrated to be highly effective in developing mucoadhesive systems, the gastrointestinal tract and for the blood brain barrier (Gessner *et al.*, 2001; Kreuter, 2001).

Micelles

Micelles are spherical nanoparticles, typically 5–100 nm in diameter, that are formed spontaneously upon dissolution of surfactants in water at concentrations that exceed a critical level, known as the “critical micelle concentration” (CMC). This self-assembly process is thermodynamically driven and interactions of the hydrophobic tails surfactants with water are minimized. These self assembled amphiphilic block copolymers allow to entrap drug molecules physically be physically to the core of block copolymer. As a result the contents of the hydrophobic core are effectively protected against hydrolysis and enzymatic degradation (Davis, 1999).

Aqueous two component systems containing a semi purified fraction from Quillaja saponin (Quil—A) and cholesterol prepared by lipid film hydration were reported to form worm like micelle. Polyethylene glycol polypeptide block copolymers (polypeptide hybrid polymers) have been used as polymeric drug and gene delivery system (Osada and Kataoka, 2006). The submicellar nanoparticles eg. NOVAVAX (estrasorb topical estradiol emulsion) which have been commercialized and others are in progress by industries for biological and medical applications. The development of nanocells with beclomethazone dipropionate has improved pharmaceutical and DNA delivery to tumors and CNS (Gaber *et al.,* 2006).

Liposomes

Liposomes are spherical, polymolecular aggregates with a bilayer shell configuration. Depending on the method of preparation, lipid vesicles can be uni- or multi-lamellar, containing one or many bilayer shells, respectively. Liposomes typically vary in size from 20 to a few hundred micrometers. The solubility of the pharmaceutical agent in phospholipids membranes gives the measure of its degree of interaction. On this basis lipid based carriers are designed for oral and parenteral use (Fahr *et al.*, 2006). Anionic surfactants and ethanol can fluidize phospholipids bilayers, thus increasing the depth enabling liposomes penetration into the intracellular pathways of the skin. Cationic lipids are used to develop new delivery models involving cationic liposome/DNA complexes which are more efficient than liposome alone due to interaction between negatively charged DNA molecule and liposomes. Cationic liposomes are commonly used in clinical trials of cancer therapy. Liposomes with nanostructures are also considered as ideal carriers for enhancing topical and transdermal drug delivery (Fang *et al.*, 2006).

A primary advantage of liposomes is their high level of biocompatibility. Liposomes now constitute a mainstream technology for drug delivery system. Clinical approval has been given to liposomal formulations of anticancer drug doxorubicin (Doxil®/Caelyx®/ and Myocet®) and daunorubicin (Daunosome®). Another advantage of liposomes is their ability to transport a diverse array of drugs that can be hydrophilic, lipophilic or amphiphilic. Liposomal vincristine, liposomal paclitaxel and liposomal interleukins have significant anticancer activity with reduced toxicity to many other vital organs. The noncapsulated cisplatin is about 10 -15nm in size but cisplatin encapsulated liposomes with diameters 250nm (nanoliposomes) are more efficiently internalized and induce cell toxicity in time dependent manner (Ramachandran *et al.*, 2006).

Dendrimers

Dendrimers are well-defined, monodisperse, and tree-like polymers having flexibility in terms of their size, shape, branching, length, and surface functionality. Dendrimers are spherical molecules and form synthetic complex with well defined chemical structures. One of

the most appealing aspects of technologies based on the dendrimers is t their size, composition and chemical reactivity that can be controlled easily and precisely. The macromolecule constituents radiate in branching form from the central core, creating an internal cavity as well as sphere of groups that can be tailored as per requirements. The monomers attached to the core (G0), are called first generation monomers (G1) and two second generation monomers (G2) are attached to each first generation monomers. Successive generations will form in this same manner, being two monomers attached to the monomer from the previous generation. The molecular weight of the dendrimer nearly doubles with each additional generation (Tomalia, 2005). Furthermore, terminal groups can be modified to obtain both a charged, and hydrophilic or lipophilic function for the desired biological and drug delivery application. After a critical branch state is reached; dendrimers can not grow anymore because of lack of space. This is called the "starburst effect". Dendrimers can be synthesized via two major strategies: a) divergent methods and b) convergent methods, which differ in their direction of synthesis; either outward from the core or inwardly toward the core, respectively (Hawker and Fréchet, 1990; Tomalia, 2005). Subsequently, a shell is formed on the surface of dendrimer by a chemical reaction of the end groups and the probe is encapsulated yielding a molecular container of nanoscopic dimensions. Opening of the box is achieved by hydrolysis, pH triggered cleavage, or a photochemical reaction of the outer shell liberating the probe. Targeting effectiveness is based on attaching targeting ligands at the external surface of dendrimers while their stability and protection from internal defense system is achieved by conjugation of dendrimers with polyethylene glycol chains. NB-001 and NB-002 an antiherpes and antimycotic dendrimers respectively, have been developed by the University of Michigan (Vine *et al.*, 2006). Dendrimers can act as a particulate system while retaining the properties of a polymer and encapsulate drugs and diagnostic agents in the central core or bound to the surface of the dendrimer by noncovalent or covalent interaction. Dendritic polymers improve pharmacokinetic and pharmacodynamic properties of low molecular weight and protein-based therapeutic agents (Bai *et al.*, 2006). Starburst dendrimers are a unique class of nanoscopic synthetic macromolecules containing a large number of reactive terminal functional groups that have been utilized to covalently couple a large variety of molecules, including proteins. These dendrimer-coupled protein complexes have been exploited in the development of sensitive immunoassays for various clinically significant biochemical markers. (Jain and Asthana, 2007; Singh, 2007). Dendrimers have successfully proved themselves as useful additives in different routes of drug administration because they can render drugs greater water-solubility, bioavailability, and biocompatibility (Cheng *et al.*,2008). Many commercial small-molecule drugs with anticancer, anti-inflammatory and antimicrobial activity have been formulated successfully with dendrimers, such as poly(amidoamine) (PAMAM), poly(propylene imine) (PPI or DAB) and poly (etherhydroxylamine) (PEHAM). Some dendrimers themselves are used in combination therapy in which the dendrimers serve as the drug carrier and simultaneously as an active part of the therapy (Svenson and Chauhan, 2008).

Solid lipid particles

Solid lipid particles were developed as an alternative colloidal carrier system for emulsion, liposome and polymeric nanoparticles for controlled drug delivery system (Kayser *et al.*, 2005; Manjunath *et al.*, 2005). Solid lipid particles are composed of lipids and surfactants and are produced under high pressure homogenization with organic solvents (Kayser *et al.*, 2005). Solid lipid particles have been used in parenteral (Kipp, 2004), pulmonary (Pandey *et al.*, 2005) and dermal application (Dingler *et al.*, 1999) for the treatment of psoriasis. Methotrexate loaded solid lipid nanoparticles can be formulated using cetyl alcohol lipid and surfactant Tween 80 and co-surfactant sodium tauroglucocolate (Shidhaye *et al.,* 2008)

Polymeric nanoparticles

Nanoparticles including nanospheres and nanocapsules of size 10 -200nm are solid state, either amorphous or crystalline. They are able to absorb and/or encapsulate a drug to protect it against chemical and enzymatic degradation. Nanospheres are composed of a matrix system in which the drug is uniformly dispersed and nanocapsulates are described as a polymeric membrane which surrounds the drug in the matrix core. Polymeric nanoparticle drug carriers can be formed both biodegradable and non-biodegradable polymers. In recent years, biodegradable polymeric nanoparticles have been considered as potential drug delivery devices in view of controlled release of drugs in target organs/tissues. Synthetic biodegradable polymer polycyanoacrylate or poly (D, L-lactide) and poly (lactide-co-glycolipid) (PLGA) and some natural polymers like chitosan (Chang and Chen, 2005) gelatin (Farrugia and Groves, 1999) and sodium alginate (Aynie, 1999) have been used as synthetic biodegradable for drug delivery system. These nanoparticles have better therapeutic potential and high stability in the biological fluid than other nanoparticles like liposomes. They are highly specific and achieve higher concentration of drug at target side due to positive change in physiochemical and other properties (Lesniak and Brem, 2004). In these nanoparticles, drug encapsulation and absorption, biodistribution, elimination, and release depend upon polymer composition, hydrophobicity, surface charge, biodegradation and adjuvants (Reis *et al.*, 2006).

Polymeric nanoparticles are suitable for cancer

therapy, delivery of vaccines, contraceptives and delivery of targeted antibiotics. Polyethyleneglycole (PEG) or polyethleneoxide are widely employed in pharmaceutical applications (Riley *et al.,* 1999). The technique of attaching PEG to any drug, peptide, polymer or other compound improves their pharmacokinetics as protein and peptide drugs could be degraded readily by proteolytic enzymes and thus has short half-life. For example, pegylated liposomal doxorubicin has high efficacy in breast cancer treatment. Furthermore, PEG is non toxic and resistant to recognition by the immune system and thus may be used to enhance biological activity of conjugated drugs (Harris and Chess, 2003; Nande *et al.,* 2006).

Metallic Nanoparticles

Metallic nanoparticles have unique optical and chemical properties that make them ideally suited for applications in drug delivery. The silver, gold and magnetic nanoparticles are important carrier for various pharmaceutical preparations. The bactericidal combination of amoxycillin and silver nanoparticles has higher activity against on *E.coli*. An appropriate explanation of this synergistic effect may be the action of silver nanoparticles as a drug carrier for amoxycillin to approach the targeted hydrophobic cell membranes made up of phospholipids and glycoproteins. Silver nanoparticles (16 nm), incorporated into cotton fabrics, exhibited antibacterial activity against *S.aureaus* reducing the bacterial count by 99.9% (Durán *et al.*, 2007). Another application of silver nanoparticles is in wound dressing. Nanocrystalline silver SILCRYST from Nucryst Phamaciuticals is used in anticoat (Vine *et al.*, 2006).

Gold nanoparticles may serve as a model system to explore multipolyvalent interaction of ligand receptor pairs. After conjugation to vancomycin it becomes chemically stable and water soluble having high antibacterial activity against *E.Coli* strain. Recently, a method for fabricating nanoparticles by attaching IgG on to thin surfaces through either electrostatic interaction or covalent bonding has been developed. Such Au-IgG nanoparticles may serve as useful nanoscale probes for exploring the interactions between IgG and pathogens. Also, magnetic nanoparticles containing IgG have been employed as effective affinity probes for selectively concentrating traces of target bacteria from sample solutions.The lowest cell concentration detected for both *Staphylococcus saprophyticus* and *Staphylococcus aureus* in aqueous sample solutions was 3×105 CFU/mL, while the detectable cell concentration for *S. saprophyticus* in a urine sample was 3×107 CFU/mL (Ho *et al.*, 2004).

Carbon nanotubes based drug delivery

A carbon nanotube is composed of a sequence of nanoscale C60 atoms arranged in a long thin cylindrical structure. Nanotubes are related to two other carbon crystal forms - graphite and diamonds. They are often described as looking like rolls of graphite chicken wire, but as member of the fullerene families they are essentially buckyballs expanded from the center into cylinders and thus are also called buckytubes. Many therapeutic agents are proteins and thus can not cross readily the cell membrane to enter into cytoplasm and retaining their biological function. Nanotubes may serve as a new class of generic tools for delivering small peptides and proteins in to cells *in vitro* as well as *in vivo*. Hence, these systems could be utilized for targeting biotechnology drugs such as genes, proteins and peptides. For this purpose various types of nanotubes like carbon nanotubes, magnetic silica nanotubes, cyclic peptide nanotubes and template synthesized nanotubes have been developed. Now template synthesized nanotubes are prepared by the template method. A general approach for preparing nanomaterials involves the synthesis or deposition of the desired material within the cylindrical and monodisperse pore of a nanopore membrane or other solid surface (Martin and Kohli, 2004). Acid oxidized single wall carbon nanotubes (SWCNTs) bind various types of proteins (less than 80 KD) and transport them through the cell membrane. These acid treated carbon nanotubes are stable in water and do not aggregate untreated carbon nanotubes. The uptake mechanism is not fully understood, however proposed mechanisms are endocytosis, phagocytosis, insertion and diffusion through the lipid bilayer of the cell membrane. For application, lipid nanotubes are coated with metallic copper to improve their mechanical strength before loading with antibiotics. This type of material has also been used for controlled release of testosterone in living cells (Goldstein *et al.*, 2001). Cyclic peptide molecule containing 6 and D and L-aminoacid residues have been used as antibiotic against bacterial pathogens which acted preferentially on both gram positive and gram negative bacteria. Carbon nanotubes hold great promise in biotechnology and biomedicine but toxicity studies are still required to establish exposure guidelines and safety regulations. Carbon nanotubes have been proposed as a possible gene delivery vehicle and for use in combination with radiofrequency fields to destroy cancer cells (Singh, 2005).

Nanorobotics

Nanorobots provide miniaturization from microelectronics to nanoelectronics. A nanorobot architecture is based on nanobioelectronics (Cavalcanti *et al.,* 2007) for the future use of nanorobots to combat cancer (Couvreur and Vauthier, 2006; Shantesh and Nagraj, 2006). Important aspects to achieve a successful treatment of cancer include its earlier detection at least before the metastasis and the development of efficient targeted drug delivery to minimize the side effects from chemotherapy. Nanorobots with chemical biosensors in order to find intensity of E-cadherin signals (Janda *et al.*, 2006) can be

used to navigate as blood borne devices for detection of tumor cells in early stages (Cavalcanti *et al.,* 2007; Curtis *et al.*, 2006) and thus can help on extremely important aspects of cancer therapy. A hardware architecture based on nanobioelectronics has been described for the application of nanorobots for cancer therapy (Janda *et al.*, 2006). Thus, nanotechnology is moving fast towards manufacturing of medical nanorobot along with nanoelectronics, RFID (radio frequency identification device) and recent advancements in biotechnology. Integration of computational nanotechnology, 3D prototyping, and real time simulation with relevant interactions for transducers and actuators facilitates in developing medical nanorobots with a great application in drug delivery and treatment of cancers (Cavalcanti *et al.,* 2008).

Production and ppplication

A fairly large number of manufacturing methods are available to produce solid nanoparticles that include nanoprecipitation, solvent evaporation, and spontaneous emulsification followed by solvent diffusion. Several new methods have been developed using mild chemicals and can be easily removed from the final product by methods such as "salting out and electrospraying. Various biological methods of producing nanoparticles and nanostructures have also been explored in recent years (Douglas *et al.*, 2002).

Nanotechnology provides tools and means to precisely graft biological and chemical ligands on to the surface of nanoparticles. The surface modification allows nanoparticle to recognize target cells. The nanoparticles can deliver the drug to the target site that enhance effectiveness and efficiency of the drug molecules using site specific controlled and sustained release mechanism. This capability may allow for better bioavailability and absorption in the gastrointestinal tract. Targeted delivery systems have greater benefits and fewer adverse effects. Ultimately, advances in nanotechnology may lead to multifunctional nanoscale drug delivery systems that can simultaneously detect and recognize the appropriate target, analyze the quantity of the drug to be released that is required by the local and whole body. This is in analogy to "smart drug delivery" or "intelligent therapeutics" which is under investigation in the medical nanotechnology field (Lawrence and Rees, 2000).

Nanotechnology has generated a great deal of interest in the field of oncology due to its potential to selectively deliver and concentrate drugs to tumors while minimizing damage to healthy cells. It has tremendous potential to make an important contribution in diagnosis, imaging and treatment of neoplastic conditions. Nanoparticles like liposomes, dendrimers, carbon nanotubes etc. can be used to target a tumor because of their unique properties at this small scale and thus are unique because of their nanoscaled structure, however, some of the nanoparticles used in cancer therapy are still bigger than many anticancer drugs. Their "large" size can make it difficult for them to evade organs such as the liver, spleen, and lungs, which are constantly clearing foreign materials from the body. In addition, they must be able to take advantage of subtle differences in cells to distinguish between normal and cancerous tissues. Indeed, it is only recently that researchers have begun to successfully engineer nanoparticles that can effectively evade the immune system and actively target tumors. Active tumor targeting of nanoparticles involves attaching molecules called ligands to the outsides of nanoparticles. These ligands can recognize and bind to complementary molecules or receptors found on the surface of tumor cells. Addition of such targeting molecules to a drug delivery nanoparticle, enhances the optimum influx of the anticancer drugs into the tumor cells and, thus, increase the efficacy of the treatment and reduce toxic effects on surrounding normal tissues (Gade *et al.,* 2005; Bondi *et al.*, 2006; Shantesh and Nagraj, 2006).

CONCLUSION

Nanotechnology has shown great potential for improving the effectiveness and efficiency of delivery of drugs to improve human health. It can enhance solubility, facilitate controlled release, improve bioavailability and protect the stability of pharmaceutical compounds. Ultimately, understanding the mechanism of targeted delivery will provide a foundation that will enable drug manufacturers to design smart drug delivery systems capable of ensuring the optimal human health. The nanoparticles could be potential candidates for the development of delivery system for nanopharmaceuticals, however, the safety issues related to toxicity of nanoparticles are needed to be addressed in developing targeted drug delivery system. Emergence of medical nanorobots will essentially be a revolutionizing nanotechnology in near future for diagnosis and treatment of the diseases in man and animals.

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DISPOSITION KINETICS AND DOSAGE REGIMEN OF ACETAMINOPHEN IN CROSS-BRED CALVES ON CO-ADMINISTRATION WITH FLORFENICOL

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ABSTRACT

The disposition kinetics of acetaminophen was investigated in cross-bred calves following single dose intramuscular administration of 50 mg.kg^{-1} along with concurrent administration of florfenicol @ of 20 mg. kg^{-1} body weight, i.v. The concentration of acetaminophen in plasma was estimated spectrophotometrically and the kinetic parameters were calculated by applying one-compartment open model. The peak plasma concentration of acetaminophen was attained at 45 min and the minimum therapeutic plasma concentration was maintained from 2.5 min to 6 h of administration. The absorption half-life and apparent volume of distribution were 0.127 ± 0.019 h and 0.58 ± 0.04 L.kg^{-1}, respectively. The high value of area under the plasma concentration-time curve (332.8 ± 18.3 µg.ml^{-1}.h) indicated that a vast area of the body was covered under drug concentration. The elimination half-life and total body clearance were 2.63 ± 0.085 h and 151.6 ± 8.31 ml.kg^{-1}.h^{-1}, respectively. On the basis of disposition kinetic parameters obtained in the present study, an intramuscular dosage regimen of acetaminophen in cross-bred calves were calculated to be 55 mg.kg^{-1} followed by 45 mg.kg^{-1} at 6 h interval when prescribed with florfenicol in calves.

Keywords: Calves, disposition, dosage regimen, florfenicol, acetaminophen

INTRODUCTION

In veterinary practice, the trend of multiple drug therapy has increased many folds due to several practical complexities in the diagnosis of diseases. Antibacterials and analgesic drugs are used most frequently in multiple prescriptions. It is well documented that concurrent administration of drugs may affect the absorption, distribution, biotransformation and excretion of one or both (Benet *et al.,* 1985). The co-administration of NSAIDs with fluoroquinolones has been associated with pharmacokinetic interactions (Dumka and Srivastava, 2006; Dumka, 2007; Dumka *et al.,* 2007). The disposition kinetics of acetaminophen has been investigated in cross-bred calves (Sharma *et al.,* 1995), buffalo calves (Chaudhary and Srivastava, 1999), dogs (Booth, 1988) and man (Hart and Huskisson, 1984). However, meager information is available on the influence of simultaneously administered antibacterial drugs on the pharmacokinetic behavior of acetaminophen in animals. In view of the paucity of pharmacokinetic data on the interaction of acetaminophen with antibacterials in bovines, this study was undertaken to determine the disposition kinetics and an appropriate dosage regimen for acetaminophen in cross-bred calves after its single intramuscular administration upon co-administration with florfenicol.

MATERIALS AND METHODS

Experimental animals and drug administration

The study was conducted on four healthy male cross-bred calves, 6-12 months old and weighing between 90-120 kg. The animals were housed in the departmental animal shed under the standard conditions of management and were provided seasonal green fodder and water *ad libitum.* Each animal was quarantined for 2 weeks before the start of experiment and was determined to be healthy by regular clinical examination. Acetaminophen (Neomol, Neon Lab. Ltd., Mumbai) was administered intramuscularly in the neck region at the dose rate of 50 mg.kg^{-1} immediately followed by intravenous injection of florfenicol at the dose rate of 20 mg.kg^{-1}. The average day temperature was about 25°C during the experimental period.

Collection of blood samples

Blood samples were withdrawn from the jugular vein into heparinized glass centrifuge tubes before and at 1, 2.5, 5, 7.5, 10, 15, 30, 45 min and 1, 2, 3, 4, 6, 8, 10 h after administration of acetaminophen. Plasma was separated by centrifugation at 1300 g for 15 min at room

temperature and kept at –20°C until analysis, which was done on the following day after collection.

Estimation procedure and pharmacokinetic analysis

The concentration of acetaminophen in plasma samples was estimated by the spectrophotometric method (Omer and Mohammad, 1984). The sensitivity of this method was up to 3 $\mu g.ml^{-1}$. The exact concentration of drug was calculated from the standard curve of acetaminophen prepared simultaneously in plasma from untreated calves. The correlation between concentration of drug and absorbance was linear between 6.25 and 100 $\mu g.ml^{-1}$. The concentration of acetaminophen was also corrected for endogenous interfering substances in the sample by preparing the blank with an untreated (0 min) sample of the same animal. The plasma concentration-time data for each calf was analysed according to the computed least-squares regression technique. The kinetic parameters were calculated manually by applying one-compartment open model (Gibaldi and Perrier, 1982). Based on the pharmacokinetic data, the dosage regimens of acetaminophen were also determined in calves (Baggot, 1977).

RESULTS AND DISCUSSION

Following an intramuscular dose of acetaminophen (50 $mg.kg^{-1}$ body weight) in cross-bred calves subsequently with a single injection of florfenicol (20 $mg.kg^{-1}$, iv), an appreciable concentration of acetaminophen (14.9 ± 1.05 $\mu g.ml^{-1}$) was detected in plasma at 1 min. The peak plasma concentration (94.9 ± 1.32 $\mu g.ml^{-1}$) of acetaminophen was estimated at 45 min, which gradually declined to 6.10 ± 0.49 $\mu g.ml^{-1}$ at 10 h (Table 1). Evaluation of the disposition pattern of acetaminophen indicated that the data can be best fitted to one-compartment open model and was adequately described by the mono-exponential equation : $Cp = Be^{-âô} – Ae^{-Kat}$, where, Cp is the plasma level of acetaminophen at time t, A and B are the extrapolated zero-time intercepts of absorption and elimination phases of the plasma drug concentration-time curves, respectively, Ka and β are the absorption and elimination rate constants, respectively, and e represents the base of natural logarithm. The therapeutic drug concentration of 20 $\mu g.ml^{-1}$ was maintained from 2.5 min to 6 h of administration. The minimum therapeutic plasma concentration of acetaminophen has been reported to be 10-20 $\mu g.ml^{-1}$ (Rawlins *et al.,* 1977). In the present discussion, the higher value of 20 $\mu g.ml^{-1}$ has been considered as the minimum therapeutic concentration of acetaminophen.

Various kinetic determinants which describe the absorption and elimination of acetaminophen after its intramuscular injection, followed by intravenous administration of florfenicol, are given in Table 2. Using convenient dosage intervals, the priming (D) and maintenance (D') doses were calculated from the equations: $D = Cp(min) . Vd(e^{\beta\tau})$ and $D' = Cp(min) .Vd(e^{\beta\tau}–1)$, where $C_p(min)$ is the minimum steady-state plasma level, â is the elimination rate constant and t is dosage interval.

In agreement to the present findings, the disposition pattern of paracetamol was also observed to follow one-compartment open model when administered alone by intramuscular route in calves and buffalo calves

Table 1:
Plasma levels of acetaminophen in cross-bred calves (n=4) given a single intramuscular dose of 50 $mg.kg^{-1}$ body weight along with concurrent intravenous injection of florfenicol (20 $mg.kg^{-1}$).

Time after acetaminophen administration (h)	Animal number				Mean ± SE
	1	2	3	4	
0.017	14.30	12.20	17.10	15.80	14.90 ± 1.05
0.042	27.80	26.80	32.60	25.90	28.30 ± 1.49
0.083	36.30	33.40	41.30	40.20	37.80 ±1.82
0.125	44.80	42.70	49.90	43.70	45.30 ± 1.60
0.167	57.30	55.50	58.40	56.70	57.00 ± 0.60
0.25	64.80	63.20	68.80	66.2	65.80 ± 1.19
0.50	84.10	85.10	89.30	84.30	85.70 ± 1.22
0.75	92.80	93.50	98.70	94.40	94.90 ± 1.32
1	81.10	78.40	86.70	80.00	81.60 ± 1.80
2	52.80	54.80	63.40	55.30	56.60 ± 2.34
3	34.70	42.10	43.50	32.20	38.10 ± 2.76
4	26.30	31.50	34.60	28.10	30.10 ± 1.84
6	20.40	24.80	26.30	20.70	23.10 ± 1.48
8	14.40	14.60	15.20	13.20	14.40 ± 0.42
10	6.30	7.20	6.10	4.80	6.10 ± 0.49

Values given are expressed as $\mu g.ml^{-1}$ of plasma

Table 2:
Disposition kinetic parameters of acetaminophen in cross-bred calves (n=4) given a single intramuscular dose of 50 mg.kg^{-1} body weight along with concurrent intravenous injection of florfenicol (20 mg.kg^{-1}).

Parameter	Unit	Mean±SE
A′	µg.ml^{-1}	82.3 ± 3.78
Ka	h^{-1}	5.81 ± 0.80
$t_{1/2Ka}$	h	0.127 ± 0.019
B	µg.ml^{-1}	92.1 ± 6.87
β	h^{-1}	0.264 ± 0.009
$t_{1/2\beta}$	h	2.63 ± 0.085
AUC	µg.ml^{-1}.h	332.8 ± 18.3
AUMC	µg.ml^{-1}.h^2	1317.9 ± 83.5
$Vd_{(area)}$	L.kg^{-1}	0.58 ± 0.04
$Vd_{(B)}$	L.kg^{-1}	0.55 ± 0.004
$Vd_{(SS)}$	L.kg^{-1}	0.50 ± 0.04
Cl_B	ml.kg^{-1}.h^{-1}	151.6 ± 8.31
MRT	H	3.96 ± 0.12
td	h	3.47 ± 0.11
C_{max}	µg.ml^{-1}	92.4 ±
t_{max}	min	45 ± 0.00

A′ and B = zero time plasma drug concentration intercepts of the regression line of absorption and elimination phases, respectively; Ka and β = the absorption and elimination rate constants, respectively; $t_{1/2Ka}$ = absorption half life; $t_{1/2\beta}$ elimination half life; AUC = area under the plasma concentration-time curve; AUMC = area under the first-moment of the plasma concentration-time curve; $Vd_{(area)}$, $Vd_{(B)}$ and $Vd_{(SS)}$ = volume of distribution based on AUC, elimination phase and steady state plasma level, respectively; Cl_B = total body clearance; MRT = mean residence time; td = duration of therapeutic effect; C_{max} and t_{max} = maximum drug concentration achieved in plasma and the time taken to attain C_{max}, respectively.

(Sidhu *et al.,* 1993; Sharma *et al.,* 1995). A higher peak plasma concentration of acetaminophen was attained following its concomitant administration with florfenicol in the present study as compared to the corresponding level of 78.5 µg.ml^{-1} after administration of paracetamol alone in calves and the minimum therapeutic plasma levels of the drug were maintained for shorter duration (Sharma *et al.,* 1995)**.**

The high value of absorption rate constant (5.81 ± 0.8 h^{-1}) of acetaminophen in calves indicated that the drug was rapidly absorbed from the site of injection into systemic circulation. The corresponding value of Ka was 2.91 ± 0.36 h^{-1} following single dose i.m. administration of paracetamol in cross-bred calves (Sharma *et al.,* 1995). The Vd_{area} of 0.58 ± 0.04 L.kg^{1} obtained in the present study indicated moderate penetration of acetaminophen to various body fluids and tissues. In accordance to the present finding, limited distribution of paracetamol as indicated by low value of Vd_{area} (0.48 L.kg^{-1}), has been reported in calves (Sharma *et al.,* 1995). It has been revealed that because of very high plasma protein binding, the low values of volume of distribution are expected for most NSAIDs. In different studies, low values of Vd_{area} such as 0.14 L.kg^{-1} for tolfenamic acid in man, 0.07 L.kg^{-1} for meloxicam in calves and 0.35 and 0.16 L. kg^{-1} for flunixin in dogs and horses, respectively, have been reported (Pentikainen *et al.,* 1981; Hardie *et al.,* 1985; Lees *et al.,* 1987; Dumka and Srivastava, 2006). The AUC of acetaminophen (332.8 ± 18.3 µg.ml^{-1}.h) in the present study was approximately half the value of AUC (592.3 µg.ml^{-1}.h) observed after administration of paracetamol alone in calves (Sharma *et al.,* 1995). This finding is in accordance to the observation of Dumka and Srivastava (2006) wherein the AUC of meloxicam was reduced to half when administered with levofloxacin in calves.

The lower values of elimination half-life (2.63 ± 0.085 h) and mean residence time (3.96 ± 0.12 h) and higher value of total body clearance (151.6 ± 8.31 ml.kg^{-1}.h^{-1}) in the present study, in comparison to the corresponding values of 4.84 h, 7.14 h and 79.6 ml.kg^{-1}.h^{-1}, respectively, obtained after administration of paracetamol alone in calves (Sharma *et al.,* 1995) indicated that florfenicol increased the elimination of acetaminophen from the body of calves. Accordingly, oxytetracycline has been reported to increase the elimination and Vd_{area} of paracetamol both in febrile and afebrile goats (Manna *et al.,* 1994).

The ultimate aim of the present study was to calculate and modify the dosage regimen of acetaminophen when it is given concomitantly with florfenicol in cross-bred calves. To maintain a minimum therapeutic plasma concentration of 20 µg.ml^{-1}, an appropriate intramuscular dosage regimen of acetaminophen when prescribed with florfenicol in cross-bred calves would be 56.1 mg.kg^{-1} followed by 44.6 mg.kg^{-1} at 6 h intervals or under field conditions the optimal intramuscular dosage regimen of acetaminophen in calves would be 55 mg.kg^{-1}

followed by 45 mg.kg^{-1} at 6 h intervals.

Consistent to the trend observed in the present study, marbofloxacin has been reported to alter the disposition of another NSAID, tolfenamic acid in calves (Sidhu, 2000). Co-administration of marbofloxacin with tolfenamic acid significantly decreased the $t_{½ka}$ and AUC but increased the Cl_B of tolfenamic acid in calves (Sidhu, 2000). Similarly the plasma disposition and dosage regimen of meloxicam was significantly altered by concurrent administration of levofloxacin in calves wherein the values of $t_{½\beta}$, MRT, Cl_B, AUC and td were significantly altered and the dosage interval of meloxicam was reduced to half when administered with levofloxacin in calves (Dumka and Srivastava, 2004; Dumka and Srivastava, 2006). Further, the absorption of paracetamol was decreased and elimination was increased on concomitant administration of cefuroxime in buffalo calves (Chaudhary and Srivastava, 1999).

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