We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900
Open access books available

185,000
International authors and editors

200M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



19

Nanotechnology Based Targeted Drug Delivery: Current Status and Future Prospects for Drug Development

Sadhna Sharma and Amandeep Singh
Department of Biochemistry,
Postgraduate Institute of Medical Education and Research, Chandigarh,
India

1. Introduction

Nanotechnology mediated delivery of therapeutic agents is one of the rapidly emerging fields today that has gained significant commercial and academic attention. It is a promising approach to alleviate the drawbacks of conventional therapy and major limitations associated with drug development like poor water solubility, low bioavailability, drug toxicity etc. Nano-scale drug-delivery systems can be devised to tune and regulate release pharmacokinetics, pharmacodynamics, solubility, immunocompatibility, cellular uptake, biodistribution and to minimize toxic side effects, thus enhancing therapeutic index of traditional pharmaceuticals (Emerich & Thanos, 2007). They can be used to deliver both small-molecule drugs and various classes of biomacromolecules such as peptides, proteins, plasmid DNA and synthetic oligodeoxynucleotides. Nanoparticle mediated drug delivery, thus has the potential to contribute significantly in the drug development process which has relied on conventional formulation strategies that are often inadequate. An underlying concept in drug development process is to establish a link between in vitro potency, physicochemical properties and absorption, distribution, metabolism, excretion and toxicity characteristics of a drug candidate which is often cited as a major contributing factor in the failure of drug development. While the nanoparticle mediated sustained release of drugs offers an obvious therapeutic advantage, the targeted delivery of drugs in the body is required to prevent the release of therapeutics at non-specific sites and unwanted side-effects. The conjugation of targeting moieties with drug-loaded nanoparticles can be used for receptor-mediated and targeted delivery. Such targeted nanoparticles have the characteristics of a perfect drug delivery system that tends to maximize the therapeutic activity while minimizing the toxic side effects of drugs.

2. Nanotechnology mediated drug delivery systems

Drug delivery systems are defined as supramolecular assemblies incorporating agents intended to treat a disease. They are intended to overcome the shortcomings of the conventional drugs, such as unfavorable pharmacokinetics, poor solubility, instability, high toxicity, drug resistance and low cellular uptake. Since the discovery of liposomes (Bangham &

Horne, 1964), there has been extensive research towards the development of new drug delivery systems. Liposomes and emulsions dominated the drug delivery field for some period. With the renewed interest in nanotechnology, new nano-sized formulations and nanomaterials have been developed. These new materials include polymeric nanoparticles, solid lipid nanoparticles, liposomes, nanoemulsions, cyclodextrins and dendrimers.

Polymeric nanoparticles: Nanoparticles are solid, colloidal particles consisting of macromolecular substances varying in size from 10 to 1000 nanometers. A drug can be dissolved, entrapped, adsorbed, attached or encapsulated into a nanoparticle. Depending on the method of preparation, nanospheres or nanocapsules can be developed with different properties and different release characteristics for the encapsulated therapeutic agent. For nearly three decades, polymeric nanoparticles have been studied extensively because of their unique and valuable physicochemical and biological properties. Indeed, nanoparticles can protect the drug from degradation (physical stability during storage and in biological fluids), enhance its transport and distribution (possibility of drug targeting by modification of surface charge with inserted ligands, such as antibodies, surfactants, polymers and others) and prolong its release; hence, the plasma half-life of the drug entrapped can be improved (Allemann et al., 1993). As some nanoparticle characteristics such as particle size and surface charge can be modulated by modifying some process parameters, they can be used in various applications involving different routes of administration. Although polymers are the most widely used materials nanoparticles consist of a variety of materials, including polymers, proteins and lipids. The polymers used include natural and synthetic materials and the main characteristics required are biodegradability and biocompatibility. In general, synthetic polymers (polyesters and their copolymers polyacrylates and polycaprolactones) offer greater advantages than natural ones (albumin, gelatin, alginate, collagen and chitosan) because they can be tailored to have a wider range of properties. The advantage of using polymeric nanoparticles as colloidal carriers for advanced drug delivery is mainly their small size, which allows nanoparticles to penetrate even small capillaries and be taken up within cells, allowing efficient drug accumulation at targeted sites in the body. Also, the biodegradable polymers used for their preparation allow for sustained drug release at the targeted site over a period of days or even weeks after administration (Vinogradov et al., 2002). Biodegradable polymer nanoparticles have been extensively investigated as therapeutic carriers (Moghimi et al., 2001). Polymeric nanoparticles have been formulated to encapsulate either hydrophilic or hydrophobic small drug molecules, as well as macromolecules such as proteins and nucleic acids (Perez et al., 2001). The release of encapsulated drugs occurs at a controlled rate in a time or environment dependent manner. More importantly, the rate of drug release can be controlled by modification of the polymer side chain, development of novel polymers or synthesis of copolymers (Wang et al., 2008). In general, these biodegradable polymer systems can provide drug levels at an optimum range over a longer period of time than other drug delivery methods, thus increasing the efficacy of the drug and maximizing patient compliance, while enhancing the ability to use highly toxic, poorly soluble or relatively unstable drugs. Poly(d,l-lactic acid), poly(d,l-glycolic acid), poly(ε-caprolactone) and their copolymers at various molar ratios diblocked or multiblocked with polyethylene glycol (PEG) are the most commonly used biodegradable polymers (Wang et al., 2008). For instance, poly lactide-co-glycolide (PLGA) encapsulated antibiotics have been investigated for the treatment of tuberculosis using murine models (Pandey & Khuller, 2006). Nanoparticles being compact are well suited to traverse cellular

membranes to mediate drug or gene delivery. It is also expected that due to small size and high surface/volume ratio, nanoparticles will be less susceptible to reticuloendothelial system clearance and will have better penetration into tissues and cells, when used in vivo (Nimesh et al., 2006). Thus, PLGA has generated tremendous interest due to its excellent biocompatibility, biodegradability, and mechanical strength.

Solid Lipid Nanoparticles: Solid lipid nanoparticles (SLNs) are nanocrystalline structures made of fatty acids that are solid or semisolid at room temperature (Jenning et al., 2002). A wide variety of high melting-point lipids and methods can be used to prepare and stabilize the SLNs (Muller et al., 2000). Besides, their surface characteristics can be altered by coating with hydrophilic molecules which tends to improve plasma stability, biodistribution and subsequent bioavailability of drugs entrapped (Uner & Yener, 2007). Sustained drug release and site specificity for drug delivery can be achieved by altering the properties of SLNs, such as their lipid composition, size, and surface charge. SLNs offer several advantages such as relative ease of production, sterilization, and scale-up, without the use of organic solvents, low-cost excipients, and biocompatibility. As compared to nanoemulsions which are liquid-lipid encapsulations of the drug, SLNs containing the lipid in the solid state impart greater drug stability and better control over drug-release kinetics (Mallipeddi & Rohan, 2010).

Liposomes: Liposomes are lipid vesicles consisting of phospholipid bilayers. They are spherical vesicles that contain a bilayered membrane structure composed of natural or synthetic amphiphilic lipid molecules (Zhang & Granick, 2006; Torchilin, 2005). Their biocompatible and biodegradable composition, as well as their unique ability to encapsulate both hydrophilic and hydrophobic therapeutic agents, makes liposomes excellent therapeutic carriers. They have an aqueous core which can be used to encapsulate hydrophilic drugs while hydrophobic and amphiphilic drugs can be solubilized within the phospholipid bilayers. Liposomes are of three types, i.e. small unilamellar vesicles, large unilamellar vesicles, and multilamellar vesicles. Liposomes in their native form are taken up by the reticuloendothelial system and are quickly cleared from the circulation. This property has been exploited for the macrophage delivery of antiretrovirals. Since liposomes are typically constructed from naturally occurring phospholipids, they tend to pose a lower risk of eliciting unwanted toxic or antigenic reactions when used as drug carriers. Liposomes can also be coated with biocompatible moieties such as PEG to prolong their circulation half-life (Torchilin, 2005). The polymer coating of the liposomes can also be engineered to carry a functional group, which can be used for targeting ligand conjugation. Liposomes have been used widely as pharmaceutical carriers in the past decade, with 11 formulations approved for clinical use and many more in clinical development. Some of the commonly used therapeutics include liposomal amphotericin, liposomal doxorubicin and liposomal daunorubicin (Wang et al., 2008).

Dendrimers: Dendrimers are a versatile class of regularly-branched macromolecules with unique structural and topologic features that are 2.5 – 10 nm in size (Svenson & Tomalia, 2005). They consist of repeatedly branched polymeric macromolecules with numerous arms extending from a center, resulting in a nearly-perfect three-dimensional geometric pattern. Small size, narrow molecular weight distribution, and relative ease of incorporation of targeting ligands make them attractive candidates for drug delivery. Dendrimers have minimal polydispersity and high functionality. Similar to polymers, they are obtained by attaching several monomeric units, but unlike the conventional polymers, they have a highly branched three-dimensional architecture. Dendrimers are characterized by the

presence of three different topologic sites, i.e., a polyfunctional core, interior layers, and multivalent surface (du Toit et al., 2010). The polyfunctional core, surrounded by extensive branching has the ability to encapsulate several chemical moieties. The core may be surrounded by several layers of highly branched repeating units such as polyethers, porphyrins, polyamidoamines, polyphenyls, and polyamino acids. The properties of the dendrimers are predominantly based on the multivalent surface, which has several functional groups that interact with the external environment. The precise physicochemical properties of dendrimers can be controlled during synthesis by controlling the core groups, the extent of branching, and the nature and/or number of functional groups on the surface (Svenson & Tomalia, 2005). They are synthesized from either synthetic or natural building blocks such as amino acids, sugars and nucleotides. Their characteristics as carriers of therapeutics include nanoscale spherical architecture, narrow polydispersity, multifunctional surface chemistry and large surface area. Many dendrimer families have been reported (Bosman et al., 1999) and amongst them, polyamidoamine (PAMAM) and poly(propylenemine) (PPI) families have been most widely used for biomedical applications. The specific molecular structure of dendrimers enables them to carry various drugs through their multivalent surfaces by covalent conjugation or electrostatic adsorption. Alternatively, dendrimers can be loaded with drugs, by using the cavities in their cores through hydrophobic interaction, hydrogen bonding or chemical linkage. Their surface can be engineered to provide precise spacing of surface molecules and to conjugate targeting molecules. Other remarkable properties of dendrimers include the availability of terminal surface groups which can be customized for bioconjugation of drugs, signaling groups or targeting moieties. They possess unique surfaces that may be designed with functional groups to augment or resist trans-cellular, epithelial or vascular biopermeability. Their surface groups can be modified to optimize biodistribution receptor mediated targeting, therapy dosage or controlled release of drug from the interior space (Tomalia et al., 2007)

3. Nanotechnology and cancer

3.1 Limitations of the current chemotherapeutic agents

Cancer is one of the leading causes of morbidity and mortality globally (World Health Organization, 2009). The conventional treatments for cancer include the use of chemotherapeutic drugs, radiotherapy and interventional surgery. Breast cancer is the most common type of malignancy diagnosed in women and almost one third of all cancers diagnosed in women are breast cancer (Jemal et al., 2008). The main objectives of the treatment strategies are to prolong the survival and improve the quality of life. Despite availability of few new drugs (Newman & Singletary,2007; Guarneri & Conte, 2004), breast cancer treatment is still unsatisfactory. Amongst active drugs, Taxanes (paclitaxel and docetaxel) (Miele et al., 2009) have proved to be fundamental in the treatment of advanced and early-stage breast cancer. Paclitaxel has demonstrated significant antitumor activity in clinical trials against a broad range of cancers (Singla et al., 2002). These drugs, however, do have a few limitations. The main limitation is their highly hydrophobic nature. Owing to this, lipid-based solvents (mixture of Cremophor and ethanol) or surfactants like polysorbate 80 (Tween® 80) are used as a vehicle for taxanes. Cremophor EL® (CrEL) is a non-ionic surfactant polyoxyethylated castor oil (Rowinsky et al., 1990). Polyoxyethylated castor oil is toxic itself as it can leach plasticizers from standard intravenous tubing releasing

di (2-ethylhexyl) phthalate (DEHP). It stimulates the release of histamine with consequent well-described hypersensitivity reactions, including anaphylaxis in patients (Rowinsky & Donehower, 1995). Besides, intravenous administration of the current Cremophor EL-based formulation in a non-aqueous vehicle may lead to some serious side effects in some patients such as hypersensitivity, neurotoxicity, nephrotoxicity & hyperlipidemia (Gelderblom et al., 2001). Polysorbate 80 is also associated with hypersensitivity reactions, although less frequently than CrEL. Polysorbate 80 may cause irreversible sensory and motor neuropathies and may alter the membrane fluidity (Vaishampayan et al., 2001). More importantly, CrEL and polysorbate 80 may limit tumor penetration as polar micelles of CrEL–paclitaxel in the plasma compartment entraps the drug and can lead to non-linear pharmacokinetics due to decreased drug clearance as well as volume of distribution. Most of other current chemotherapeutic agents in the market are low molecular weight agents with high pharmacokinetic volume of distribution both of which may contribute to their cytotoxicity. Because of their low molecular weight, they are readily excreted from the body, hence requiring a higher concentration that may be toxic. The most important fact is that most of these drugs lack specificity and cause significant damage to normal tissues, eventually leading to serious unwanted side effects such as bone marrow suppression, alopecia, and the sloughing of the gut epithelial cells (Lou & Prestwich, 2002). The use of nanocarriers can help alleviate these problems and allow for the preparation of low water soluble cancer medications. The nanoscale dimension of these carriers enables the drug to accumulate in the tumor mass by passively crossing fenestrations in the diseased vasculature and avoiding the perfusion of normal tissues. These nanoparticles have the potential to cross the inter-endothelial junctions and diffuse within the extravascular compartment, addressing all the possible therapies in a more specific manner. In addition, such carriers can be optimized and modified to target the tumor cells particularly. This helps to deliver the drug specifically to neoplastic tissues, sparing the normal ones, thereby reducing systemic toxicity. The modifications include chemical binding of specific moieties or ligands on these nanocarriers. Tumor-specific high affinity ligand like folate (Farokhzad et al., 2006) enhance the interaction of nanoparticles with tumor cells, greatly improving biodistribution and bioavailability of the concerned drug. Perhaps the most important and vast utilization of nanotechnology mediated drug delivery has been in cancer chemotherapy and presently, approximately 150 drugs in development for cancer treatment are based on nanotechnology (Jain, 2010).

3.2 Nanodrug delivery systems for anti-cancer agents

A large number of researchers have used different approaches and techniques for formulating nanoparticles for anti-cancer agents. Some of these studies along with their prominent findings are mentioned here. Paclitaxel has been the focus of many drug delivery approaches to alleviate the side effects of its conventional formulation. Several approaches have been employed till date, and one of the most successful of them is Albumin-bound paclitaxel (ABI-007, Abraxane®; Abraxis BioScience and AstraZeneca). Albumin has a number of biological characteristics that make it an attractive drug vehicle in oncology. It is a natural carrier of endogenous hydrophobic molecules such as vitamins, hormones and other water-insoluble plasma substances (Hawkins et al., 2008). Moreover, albumin seems to help endothelial transcytosis of protein-bound and unbound plasma constituents through

binding to a cell-surface (John et al., 2003; Minshall et al., 2003). Besides, osteonectin, also known as secreted protein acid rich in cysteine (SPARC) has been shown to bind albumin because of a sequence homology with gp60. SPARC, as caveolin-1, is often present in some neoplasms (breast, lung, and prostate cancer), leading to the accumulation of albumin in some tumors and thus facilitating intra-tumor accumulation of albumin-bound drugs (Hawkins et al., 2008). Albumin-bound paclitaxel ABI-007 is a nanovector application for breast cancer. It represents one of the strategies developed to overcome the solvent-related problems of paclitaxel and it has been recently approved by the US Food and Drug (FDA) Administration for pre-treated metastatic breast cancer patients. ABI-007 is a novel, albumin-bound, 130-nm particle formulation of paclitaxel, free from any kind of solvent (Miele et al., 2009). It is used as a colloidal suspension derived from the lyophilized formulation of paclitaxel and human serum albumin diluted in saline. Albumin tends to stabilize the drug particle and prevents any risk of capillary obstruction and does not require any specific infusion systems or steroid/antihistamine premedication before the infusion (Desai et al., 2006). Preclinical studies, conducted in athymic mice with human breast cancer demonstrated that ABI-007 has a higher penetration into tumor cells with an increased anti-tumor activity, compared with an equal dose of standard paclitaxel (Desai et al., 2006). A phase I clinical trial on patients with solid tumors and breast cancer showed a maximum tolerated dose of ABI-007 about 70% higher than that of CrEL paclitaxel formulation. ABI-007 was administered intravenously with no premedication, in shorter infusion periods and with a standard infusion device. The toxicities observed were sensory neuropathy, stomatitis, and ocular toxicity. None of the patients experienced hypersensitivity reactions. Moreover, the pharmacokinetic parameters showed a linear trend (Ibrahim et al., 2002). A consequent phase II trial confirmed that ABI-007 has significant antitumor activity in patients with metastatic breast cancer, with a good overall response rate and less side effects (Ibrahim, 2005). A micellar nanoparticle formulation of paclitaxel (NK105) was also developed to reduce its toxicity and increase the antitumor activity of paclitaxel (Hamaguchi et al., 2005). Paclitaxel was incorporated into the inner core of the micelle system by physical entrapment through hydrophobic interactions between the drug and the block copolymers for paclitaxel. When compared to free paclitaxel, NK105 increased plasma AUC by approximately 90-fold together with a 25-fold higher tumor AUC. NK105 showed potent antitumor activity against a human colorectal cancer cell line HT-29 xenograft compared with paclitaxel owing to its enhanced accumulation in the tumor and its sustained release from micellar nanoparticles. Neurotoxicity was significantly decreased with NK105 as evidenced by both histopathological and physiological assessments. Although these current vehicles employed hold promise to replace the Cremophor EL-based vehicle for paclitaxel delivery, their role to overcome multi-drug resitance (MDR) of tumor cells to paclitaxel still remains unsolved. Therefore, another challenge is to develop a new delivery system that consists of aqueous-based vehicles and possesses ability to overcome the MDR of tumor cells for paclitaxel delivery. Poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol) (PEO-block-PPO-block-PEO) micelles have been commonly used for solubilization of hydrophobic drugs (Kabanov et al., 2002). It is found that Pluronics could interact with MDR cancer cells resulting in drastic sensitization of these tumors with respect to doxorubicin and other anticancer agents (Minko et al., 2005). In addition, inclusion of paclitaxel in liposomal formulations (LEP-ETU) has proved to be a good approach to improve the drug's antitumor efficacy (Zhang et al., 2005). Endostatin, a

20 kDa internal fragment of the carboxy terminus of collagen XVIII, has the potential to inhibit the growth of a variety of human tumors by inhibiting neovascularization (Zhuo et al., 2010). However, most available endostatins are either unstable or expensive, which limits their clinical application. Endostar, a novel recombinant human endostatin, has been expressed and purified in *E.coli*. It has been approved by the Chinese State Food and Drug Administration for the treatment of non-small cell lung cancer in 2005 and has a broad spectrum of activity against solid tumors. Endostar has been shown to inhibit endothelial cell proliferation, migration, and vessel formation (Zhuo et al., 2010). Nanoparticles containing endostar were formulated from modified (PEG-PLGA) and they could maintain adequate concentrations of endostar in plasma and tumor, thereby improving its antitumor effect. Compared with endostar, endostar-loaded PEG-PLGA nanoparticles had a longer elimination half-life and lower peak concentration, caused slower growth of tumor cell xenografts, and prolonged tumor doubling time. The nanoparticles changed the pharmacokinetic characteristics of endostar in mice and rabbits, thereby enhancing anticancer activity. Endostar-loaded PEG-PLGA nanoparticles were observed to have a better anticancer effect than conventional endostar (Sanyuan et al., 2010). CPX-1 is another novel liposome-encapsulated formulation of irinotecan and floxuridine designed to prolong in vitro optimized synergistic molar ratios of both drugs following infusion. Phase I studies in patients with advanced solid tumors showed that CPX-1 was well tolerated, and had significant antitumor activity (Batist et al., 2009). MCC-465 is an immunoliposome-encapsulated doxorubicin which is tagged with polyethylene glycol (PEG) and the F(ab) fragment of human mAb GAH (goat anti-human), which positively reacts to >90% of cancerous stomach tissues but negatively to all normal tissues. In preclinical studies, MCC-465 showed superior cytotoxic activity against several human stomach cancer cells compared with doxorubicin or doxorubicin- incorporated PEG liposomes. A phase I clinical trial showed that MCC-465 was well tolerated (Matsumura et al., 2004). Polymeric micelles can be utilized to increase the accumulation of drugs in tumor tissues utilizing the enhanced permeability and retention (EPR) effect and to incorporate various kinds of drugs into the inner core by chemical conjugation or physical entrapment with relatively high stability. There are several anticancer drug-incorporated micelle carrier systems under clinical evaluation, these include a CDDP (cisplatin)-incorporated micelle, NC-6004, and Paclitaxel incorporated micelle, NK105 for stomach cancer. Phase I studies of polymer doxorubicin (PK1) showed signs of activity coupled with five-fold decreased anthracycline toxicity in chemotherapy-refractory patients. Phase II studies were conducted using a similar material in patients with breast cancer, non-small cell lung cancer and colorectal cancer (Seymour et al., 2009). The results showed an increased efficacy with limited side effects, supporting the concept that polymer-bound drugs can improve anticancer activity. The anti-tumor activity of SP1049C, a novel P-glycoprotein targeting micellar formulation of doxorubicin consisting of doxorubicin and two non-ionic block copolymers, has been evaluated in patients with advanced adenocarcinomas of the esophagus and gastroesophageal junction and showed good tolerability (Valle et al., 2010). These results thus demonstrate superior antitumor activity of SP1049C compared with doxorubicin in a standard formulation. Phase III clinical trials are now in progress (Jain, 2010). In a study, 5-fluorouracil (5-FU) loaded and polyethylene glycol-poly(γ-benzyl-L-glutamate (PEG-PBLG) nanoparticles (5-FU/PEG-PBLG) were formulated. These nanoparticles exhibited favorable pharmacokinetic characteristics, including sustained drug release, prolonged drug half-life, and increased tissue retention. In vivo, 5-FU/PEG-PBLG nanoparticles had good anti-tumor activity against colon cancer xenografts and oral squamous cell carcinoma xenografts. The results

imply that PEG-PBLG nanoparticle delivery system for 5-FU may be able to effectively reduce adverse side effects of 5-FU therapy and improve the therapeutic index of 5-FU (Su et al., 2008).

Dendrimers have been extensively used for delivering anti-cancer drugs. Polyamindoamine (PAMAM) dendrimers have been used to formulate doxorubicin conjugates which led to significantly increased nuclear accumulation of doxorubicin from the PAMAM-hyd-DOX conjugates and thus exhibited higher cytotoxicity to tumor (Kwon, 2003). Polyester-based dendrimer–PEO–doxorubicin conjugate was observed to substantially inhibit the progression of DOX-insensitive C-26 tumor subcutaneously implanted in BALB/c mice. This dendrimer–PEO–doxorubicin conjugate also showed the ability to eliminate the tumors at certain doses and was found to be equally effective to a liposomal formulation of doxorubicin (Martin, 1998). PAMAM dendrimers have also been conjugated to cisplatin to form a fairly water soluble nanoformulation with the ability to release cisplatin slowly in vitro. This formulation showed superior activity over conventional cisplatin when injected intraperitoneally into mice bearing B16F10 tumor cells. Also, when administered intravenously to treat a subcutaneous B16F10 melanoma, the dendrimer-cisplatin displayed additional antitumor activity whereas cisplatin was inactive (Nishiyama & Kataoka, 2006). In another study, dendrimer-based stealth nanoparticles were designed to encapsulate anastrozole, which is a drug used to treat breast cancer after surgery and for metastases in both pre and post-menopausal women. It was demonstrated that stealth nanoparticles composed of a PAMAM dendrimers core and a poly-ethylene glycol (PEG) layer could encapsulate anastrozole, hence causing improved water solubility of anastrozole. A sustained release of anastrozole was achieved, implicating an increased therapeutic index (Sarkar, 2008).

3.3 Tumor-specific targeting with nanocarriers

Tumors have unique features, which make them distinct from normal tissues. These include leaky tumor blood vessels and defective lymphatic drainage, that promote the delivery and retention of particles, a phenomenon recognized as the enhanced permeability and retention (EPR) effect. Nanoformulation can more easily enter and accumulate within tumor cells. This implicates that higher doses of the drug can be delivered, increasing its anticancer effects while decreasing the side effects associated with systematic chemotherapy. However, there are many variable factors, such as clearance of nanoparticles in the circulation by kidneys and uptake by reticuloendothelial cells, that affect the amount of anticancer nanoparticles retained in the tumor. One way to overcome some of these variables is targeted drug delivery. Targeted delivery of therapeutic agents to cancer has important implications for detection, diagnosis and therapy of cancer. Biomarkers that differentiate cancerous tissue from normal tissues can be used as targets for this purpose.

3.4 Ligands employed for tumor-specific targeting

Folate is nonimmunogenic and folate nanoparticles are rapidly internalized by receptor-bearing cancer cells (Sudimack & Lee, 2000) in a manner that bypasses cancer cell multi-drug-efflux pumps (Goren et al.. 2000). The folate receptor is expressed on human ovarian, endometrial, colorectal and lung cancers but is largely absent from normal tissues (Sudimack & Lee 2000). Folate receptor, a cell membrane associated glycosylphosphatidylinositol anchored glycoprotein involved in human growth and

development, cell division and DNA synthesis, has been explored to target therapeutics in cancer cells due to its over expression on malignant cancer cells. Binding of folic acid to folate receptor (FR-α and FR-β) initiates receptor-mediated endocytosis and internalization of folic acid. Most human tissues lack the folate receptor, except the placenta, choroids plexus, lungs, and kidneys; however, cellular activation and proliferation leads to over expression of high-affinity folate receptors in many cancers. Thus, folate-mediated targeting has been used to deliver protein toxins, low-molecular weight chemotherapeutic agents, liposomes containing chemotherapeutic drugs and immunotherapeutic agents to cancer cells (Xiang, 2008). Many studies have been carried out to prove the enhancement of anticancer activity via folate mediated targeting. Folate-conjugated nanoparticles have been used on human cervical carcinoma cells and found no cellular uptake of folate-conjugated nanoparticles in A549 cells which lacks folate receptor (Zhang, 2010). It was demonstrated that uptake of folic acid conjugated doxorubicin by HeLa cells showed greater cytotoxicity compared to non-folate-mediated nanoparticles (Zhang, 2010). Another characterized ligand to be exploited for targeting tumor cells is transferrin which plays an essential role in iron homeostasis and cell growth. Inherent characteristic of some cancer cells is over expression of transferrin receptor. However, high expression of transferrin receptor is seen in hypothalamus and medulla oblongata compared to other parts of brain and many in vivo studies showed that transferrin increases brain delivery of nanoparticles (Hänninen et al., 2009). Uptake of transferrin into cells is mediated by transferrin receptors which are cell membrane associated glycoproteins. Binding of transferrin to transferrin receptor initiates receptor mediated endocytosis and internalization of transferrin. Whereas in presence of inhibitors, transferrin mediated nanoparticles interact with the cells in a specific manner and enter the cells via the caveolae pathway (Chang et al., 2009). Many studies have been carried out to prove the enhancement of anticancer activity via transferrin mediated targeting. The anticancer activity of transferrin conjugated solid lipid nanoparticles of curcumin on MCF-7 breast cancer cells has also been studied and results showed that the cell uptake and cytotoxicity increased considerably with transferrin conjugated solid lipid nanoparticles compared to curcumin solution. Transferrin conjugated nanoparticles enhance the antitumor activity via active target mechanism and also contributes to the photo stability and sustain release of drug (Mulik, 2010).

Another attractive molecular target is vasoactive intestinal peptide receptors (VIP-R). In vitro studies using human breast cancer tissues and cells have shown the presence of high densities of VIP receptors, with high affinity and specificity for VIP. It is well known that angiogenesis is vital for tumor growth (Naumov et al., 2006).Studies in breast cancer patients have showed that angiogenesis positively correlates with the degree of metastasis, tumor recurrence and shorter survival rates, thus demonstrating the value of angiogenesis as a prognostic cancer marker (Weidner et al., 1992; Weidner et al., 1992). There is an up regulation of angiogenic cytokines and growth factors, most notably the vascular endothelial cell growth factor (VEGF) and angiopoietin (Ang) families, as well as integrins (Desgrosellier & Cheresh, 2010). It is hence not surprising that these molecules are often targeted in both experimental and clinical cancer settings. Development of anti-angiogenesis therapy is based on either drugs that prevent the formation of new blood vessels supplying to the tumor (e.g. TNP-470, endostatin, angiostatin), or drugs that damage existing blood vessels (e.g. combretastatin) (Folkman, 2003). Specifically targeting tumor vasculature significantly lowers the side effects associated with the drug. It has been shown that

polymer-conjugated angiogenesis inhibitor TNP-470 (caplostatin) accumulates selectively in the tumor vessels by the EPR effect and inhibits hyperpermeability of tumor blood vessels (Satchi-Fainero et al., 2005; Satchi-Fainero et al., 2004). Nanoparticle-conjugated chemotherapeutic agents such as doxorubicin (Chaudhuri et al., 2010) and angiogenic small molecule inhibitors (Harfouche et al., 2009) can preferentially home into tumors by the EPR effect, resulting in selective vascular shutdown and inhibition of tumor growth. It should be noted that EPR alone is not always sufficient in targeting the tumor sites and hence is often used in conjunction with active targeting. This combination ensures that nanoparticles are retained in the tumor tissues following their extravasation from leaky vessels. Active targeting of tumor tissues is achieved by chemically arraying ligands on the surface of nanoparticles that can recognize and selectively bind to receptors specifically expressed on tumor cells and vessels. The high surface area to volume ratio of the nanoparticles leads to high local density of ligands for targeting. Nanoparticle mediated active targeting of the tumor vasculature in anti-angiogenic therapy has been achieved by targeting the VEGF receptors (VEGFRs), avb3 integrins, and other angiogenic factors. Integrin avb3 has been the most widely used targeting moiety on nanovectors due to its pleitropic up regulation in a variety of tumors (Anderson et al., 2000; Park et al., 2004), some of which have been successfully translated into several clinical trials (Desgrosellier & Cheresh, 2010). Tumor-homing peptides have been used to target abraxane, a clinically approved paclitaxel-albumin nanoparticle to tumors in mice. The targeting was accomplished with two peptides, CREKA, and LyP-1 (CGQKRTRGC). LyP-1-abraxane produced a statistically highly significant inhibition of tumor growth compared to untargeted abraxane. CREKA (cysteine-arginine-glutamic acid-lysine-alanine) is a pentapeptide that binds to clotted plasma proteins and homes to tumors because interstitial tissue of tumors (Dvorak et al., 1985) and the vessels wall contain clotted plasma proteins, while the vessels in normal tissues do not. LyP-1 is a cyclic 9-amino-acid peptide (Cys-Gly-Gln-Lys-Arg-Thr-Arg-Gly-Cys) that provided the first demonstration that lymphatic vessels in tumors can differ molecularly from normal lymphatics (Laakkonen et al., 2002). A protein known as p32 or gC1qR receptor (Ghebrehiwet et al., 1992) is the target molecule for the LyP-1 peptide and, in addition to overexpression in tumors, it also exhibits aberrant cell surface expression in tumor lymphatics, tumor cells, and, a subset of myeloid cells which contributes to the tumor specificity of LyP-1 homing (Fogal et al., 2008). The results showed that synthetic particles coated with LyP-1 extravasate and spread into tumor tissue.

Various other polymeric nanoparticles have been used for targeted delivery of cancer therapeutics. PLGA copolymers have been extensively used in the field of cancer research, owing to their biodegradability and bio-compatibility, resulting in their FDA approval. In a study targeting the MAPK signaling pathway, the use of PLGA copolymer for chemically conjugating PD98059, a selective MAPK inhibitor has been reported (Basu et al., 2009). The resulting nanoparticles selectively resulted in melanoma regression in a mouse model. In a novel strategy, temporal targeting of tumor cells and the tumor vasculature was achieved using a nanoscale delivery system that comprised of a core PLGA nanoparticle encapsulated within a (PEG)-linked lipid envelop (Sengupta et al., 2005). PEGylation of a molecule renders the latter non-toxic and non-immunogenic, and is an FDA approved method (Veronese & Pasut, 2005). PLGA nanoparticles have also been utilized for delivering natural products like curcumin, thought to have anti-cancer effects. Curcumin-loaded PLGA nanoparticles were reported to successfully suppress tumor necrosis factor (TNF)-regulated expression of VEGF, culminating in reduced tumor metastasis (Anand et al., 2010). In a

study, chitosan nanoparticles have shown significant inhibition of tumor growth and induction of tumor necrosis in a mouse hepatocellular carcinoma xenograft model (Xu et al., 2009). The anti-tumor activity of these nanoparticles was found to be related with their anti-angiogenic activity, which was linked to significant reduction in the levels of VEGFR-2 expression and subsequent blockage of VEGF-induced endothelial cell activation. In a study, doxorubicin-loaded solid lipid nanoparticles on MCF-7/ADR cells (doxorubicin-resistant breast cancer cell line) showed that doxorubicin-loaded solid lipid nanoparticles efficiently enhanced apoptotic cell death through the higher accumulation of doxorubicin in MCF-7/ADR cells in comparison with free doxorubicin (Kang et al., 2010). Doxorubicin, when conjugated with polymeric dextrans of various molecular weights, its cytotoxicity was significantly higher than free doxorubicin when studied on human carcinoma KB-3-1 cells and its multidrug-resistant subclone KB-V-1 cells (Lam et al., 2000). Similarly, it has been demonstrated that paclitaxel nanocrystal formulation using D-α-tocopheryl polyethylene glycol 1000 succinate have significant advantages over Taxol in achieving better therapeutic effect in Taxol-resistant cancer cells both in vitro and in vivo (Liu, 2010).

4. Nanodelivery of therapeutics to central nervous system (CNS)

The blood-brain barrier (BBB) is one of the stringent and efficient barriers present in human body. BBB allows only a restricted exchange of compounds between the plasma and CNS, which include hydrophilic molecules, small proteins, and charged molecules. This barrier consists of a layer of endothelial cells connected by tight junctions, which circumferentially surround the entire cell margin at the brain capillaries (Butte et al., 1990). The luminal blood–brain barrier (BBB) is comprised of tight junction bound endothelia that serve to retard brain entry of most high molecular weight and/or hydrophilic therapeutics. Principal mechanisms involved in limited uptake of drugs by BBB include: a) absence of paracellular openings, b) lack of pinocytocis and c) significant protein mediated efflux. The deficiency in pinocytic vesicles and the high metabolic capacity of cerebral endothelial cells (Reese & Karnovsky, 1967) also contribute to limiting the exchange of anticancer agents between the plasma and the CNS. Furthermore, the cerebral endothelium has a high level of ATP-binding cassette (ABC) transporters such as P-glycoprotein involved in drug efflux mechanisms (Golden & Pollack, 2003). Thus the BBB prevents the uptake of all large-molecule and more than98% of pharmaceutical small-molecule drugs (Pardridge, 2001). Only small (<5000Da), lipid-soluble, electrically neutral molecules and weak bases are able to diffuse passively across the BBB (Abraham et al., 1994). Therefore, significant research is dedicated to develop methods and technologies to circumvent the BBB for brain drug delivery (Smith, 2003).Previous technologies for brain delivery of drugs (i.e. BBB circumvention) includes drug or BBB manipulation. Manipulation of the BBB chiefly consists of temporary disruption of tight junctions to allow paracellular movement of the molecule from plasma to brain. This methodology has indeed proven to be efficacious (Kroll et al., 1998; Remsen et al., 2000), yet there are concerns regarding significant toxicity of free CNS drug (Remsen et al., 1995; Fortin et al., 2000). These physiological characteristics of the BBB hence offer a substantial hinderance for delivery of drugs to the CNS. Theoretically, there are two strategies to overcome this: either the barrier integrity can be altered or drug characteristics can be altered. However, interventional methods do have their drawbacks.
Such non-specific opening of the barrier by either mechanism allows the entry of toxins and unwanted molecules, potentially resulting in significant damage (Greig, 1989). The primary

disadvantage is the requirement of extremely invasive neurosurgery, thus limiting their potential. Besides, diffusion of the drug from the injection site may occur. Owing to such risks associated with altering of the BBB physiology, modifying the drugs or their mode of delivery is a much better option. Nanoparticle mediated drug delivery may be superior to both of these techniques, since no manipulation of the barrier or the drug is necessary. Furthermore, native carriers and receptors expressed at the BBB can be used for targeted delivery. Such native carriers as lipoproteins can deliver hydrophilic and large compounds across the barrier. Nanoparticles may cross the BBB either by passive diffusion or receptor-mediated endocytosis. One significant benefit of tumor therapy with nanoparticles as a drug carrier is the prolong of mean residence time in the body. Whereas this benefit may increase the exposure of the tumor to the chemotherapeutic agent, it also prolongs the exposure of the remainder of the body to the drug potentially increasing toxicity. Using high-affinity ligands for these transporters along with nanoparticles can lead to site-directed delivery of drugs. Increased uptake of polysaccharide nanoparticles cross-linked with phosphate (anionic) and quaternary ammonium (cationic) ligands, with a surrounding lipid bilayer has been demonstrated (Fenart et al., 1999). It was observed that lipid bilayer containing dipalmitoyl phosphatidyl choline and cholesterol coating on the charged nanoparticles leads to a 3–4 fold increase in brain uptake. In addition, the nanoparticles remained intact as they crossed the BBB, without altering BBB integrity at the same time. Another drug, amitriptyline, when adsorbed onto polybutylcyano-acrylate nanoparticles, using polysorbate-80 as a surfactant, led to a 10-fold increase in its levels in brain (Schroder et al., 1998). This was attributed to an increased of the plasma concentration of the drug resulting in a larger gradient at the BBB and thus greater concentrations of the drug entering the brain by passive diffusion (Alyautidin et al., 1995). Cellular endocytosis has been suggested to be the transport mechanism of polybutyl-cyanoacrylate nanoparticles coated with polysorbate-80 across the BBB, when the nanoparticles were not coated with surfactants, the particles remained in the blood vessels (Kreuter et al., 1995).It is postulated that apolipoprotein-E (apo-E) adsorbs onto nanoparticles coated with polysorbates thereby causing endocytosis at the BBB (Kreuter, 2001). A number of studies have been done to improve the brain drug distribution of anesthetic agents such as dalagrin, kytorphin, and the neuromuscular blocking agent tubocurarine. These anesthetics show therapeutic effects only when given directly to the brain, as they do not cross the BBB appreciably from the plasma. Tubocurarine (a myoparalytic, quaternary ammonium compound) when adsorbed onto polybutylcyanoacrylate particles coated with polysorbate-80 was efficiently transported at BBB. Otherwise, Tubocurarine, when given intravenously, is a found in negligible concentrations in the cerebrospinal fluid and does not affect spontaneous and evoked bioelectric activity of the brain. On the other hand, with peripherally administered nanoparticles, seizure electroencephalograph patterns were observed (Alyautdin et al., 1998). In addition, most of the chemotherapeutic drugs used for brain tumours are polar molecules and do not readily penetrate the BBB. This is further complicated by the need to maximize time and exposure concentration of the chemotherapeutic agent to the cancer cells. However, when these two factors are maximized to provide therapeutic efficacy, plasma concentrations are high, resulting in significant systemic toxicity. Nanoparticles as chemotherapeutic carriers have been studied as a solution to these issues (Lockman, 2002). In the case of brain tumors, however, the proliferation and invasion of tumoral cells generally cause a local disruption of the BBB (Gururangan & Friedman, 2002). Cancer cells produce various mediators such as arachidonic acid, leukotrienes, prostaglandin E and

thromboxane B2, thus increasing the permeability of the capillary endothelium (Wahl et al., 1993). Moreover, the tumor secretes proangiogenic factors including a basic fibroblast growth factor and a vascular endothelial growth factor inducing the formation of new blood vessels in the tumor (Folkman, 1995). These capillaries, characterized by frequent fenestrations, also improve the permeability of the blood–tumor interface and consequently the penetration of drugs. But the disruption of the BBB does not occur in the healthy tissue surrounding the main tumor and thus the desired anticancer agents cannot reach the adjacent tumors located in the normal tissue. The choroid plexus forms a second barrier separating the blood from the cerebrospinal fluid (CSF) (Wolburg et al., 2001). The blood–CSF barrier is functionally and morphologically different from the BBB. The choroid epithelial cells form tight junctions and are responsible for the barrier function. These cells show a low resistance in comparison with the endothelial cells of the BBB (Saito & Wright, 1983). The capillary endothelium in the choroid plexus is fenestrated, allowing the diffusion of small molecules (Pappas & Tennyson, 1962). Despite its permeability the blood–CSF barrier does not significantly increase the penetration of drugs into the brain, its surface being 1000-fold smaller than the surface area of the BBB (Pardridge, 1997). Active targeting of the BBB represents a promising non-invasive strategy for improving drug delivery to brain tumors. It consists in using the various influx transport systems expressed within the cerebral endothelial, including carrier-mediated transport, receptor-mediated endocytosis and adsorptive-mediated endocytosis. These transport systems are usually overexpressed on tumors. More than 20 transporters have been identified, all highly expressed on the cerebral capillaries of the BBB. Amogst them, GLUT1 transporter is of significant importance. It promotes the transport of D-glucose from the blood to the brain and mediates the passage of substances exhibiting similar structures, including 2-deoxyglucose, galactose, mannose, and glucose analogs through the BBB (Pardridge,1995). Its capacity to transport glucose through the BBB is considerably higher than other nutrient transporters (Tsuji, 2005). Besides, the GLUT1 transporter is differentially regulated in human brain tumors, for example it is overexpressed in cerebral hemangioblastoma but under expressed in glioblastoma multiforme (Tsukamoto et al., 1996). Usually the predominant glucose transporter in high-grade gliomas is the GLUT3 isoform, which is also expressed on neurons in the healthy brain (Boado et al., 1994). Thus, considering their affinity for the GLUT1 transporter, mannose derivatives were incorporated on the surface of liposomes. Mannose-liposomes prepared from p-aminophenyl, a mannoside were able to cross the BBB via the glucose transporter, to finally reach the mouse brain (Umezawa & Eto, 1988). The choline transporter consists of an anionic-binding area which interacts with positively charged quaternary ammonium groups or simple cations (Lockman, 2002). It plays a major role in the brain uptake of choline, acting as a precursor for the neurotransmitter acetylcholine and as an essential component of membrane phospholipids (phosphatidylcholine) (Allen & Smith, 2001). Moreover, the choline transporter also interacts with other quaternary ammoniums such as carnitine (Cornford et al., 1978) and thiamine (Kang et al., 1990). No saturation of this carrier was observed under physiological concentration, allowing the transport of other components without affecting the choline delivery to the brain (Allen & Smith, 2001). Besides, the concentration of choline containing components is increased in brain tumors (Tedeschi et al., 1997), suggesting a high choline transport activity in cerebral cancerous cells. The nanoparticles coated with choline were able to cross an in vitro model of the BBB. Their passage through the endothelial cell monolayer was three or four fold higher than that of uncoated nanoparticles, without any modification of paracellular

permeability (Fenart et al., 1999). In another instance, nanoparticles were coated with thiamine. Endogenous serum/blood ligands such as insulin and tranferrin have gained much attention (Pardridge, 2002). Folic acid also represents a promising site-specific ligand for brain targeting. The main advantage of these endogenous ligands is their high affinity for both brain and tumoral cells. Moreover, they are biocompatible and non-immunogenic (Vyas & Sihorkar, 2000). Transferrin is a monomeric glycoprotein that can transport one or two iron atoms (Daniels et al., 2006). Transferrin receptor is overexpressed on the brain capillary endothelium (Jefferies et al., 1984) and at the surface of proliferating cells such as brain tumor cells (Hall, 1991). In contrast, a low level of transferrin receptor is observed on normal tissues. However, transferrin receptor can be saturated under physiologic conditions due to a high endogenous plasma concentration of transferrin. The useful properties of transferrin have been exploited for the delivery of various drugs to the brain. Transferrin has been used as an endogenous cellular transport system for the delivery of diphteria toxin (CRM 107) to malignant brain tumors (Laske et al., 1999). Diphtheria toxin conjugated with transferrin produced tumor responses without any systemic toxicity in patients with cerebral tumors refractory to conventional therapy. In another study, beta-endorphin peptides were successfully delivered to the brain after conjugation with cationized albumin (Pardridge et al., 1990). Ligands such as peptidomimetic monoclonal antibodies (MAbs) have been developed, which can bind to the endothelium (Pardridge, 1999). The MAb known as OX26 recognizes an extracellular domain on the transferrin receptor, distinct from the transferrin binding site and thus does not interfere with endogenous transferrin binding. Other studies have shown targeting of OX26 on the brain capillary endothelial cells and its ability to reach the cerebral parenchyma (Pardridge et al., 1991). This antibody has also been used as a neurodiagnostic agent for the early detection of brain cancers (Kurihara & Pardridge, 1999). Transferrin has been coupled to pegylated liposomes and a significant increase of the brain uptake for transferrin-PEG-liposomes in comparison with PEG-liposomes was observed (Hatakeyama et al., 2004). Doxorubicin, an antineoplastic agent, was encapsulated in liposomes coupled to transferrin (Eavarone et al., 2000). In vitro studies revealed a four-fold increase of pegylated transferrin-liposome uptake by glioma cells in comparison with non-targeted liposomes. Transferrin-liposomes used for the delivery of antimetabolic drug 5-fluorouracil (5-FU) to the brain were also investigated (Soni et al., 2005). In vivo experiments revealed that their accumulation was higher than that of non-modified liposomes. The cytotoxicity against cancer cells of doxorubicin packaged within this targeted micellar system was significantly improved (Lai et al., 2005). Folates such as folic acid and 50-methyltetrahydrofolic acid (MTFA) are also transported across the cell membranes (Zhao et al., 1997). The folate receptor is expressed in a limited number of normal tissues such as the thyroid, kidney, choroid plexus (Ross et al., 1994) and the BBB (Wu et al., 1999) It has been identified as a tumor marker due to its overexpression in a large number of tumors such as ovarian carcinomas and brain tumors (Weitman et al., 1992). In addition, immediately after binding with its ligand, the folate receptor is internalized in an early endosome and after a conformational change at acidic pH, the folate molecule is released (Lee et al., 1996). The folate receptor expressed at the BBB has been postulated to mediate the transport of MTFA and folic acid through the BBB (Wu & Pardridge, 1999). Folate-conjugated nanocarriers have been used to selectively target the cells expressing the folate receptor. Enhanced uptake of doxorubicin-loaded folic acid liposomes into C6 glioma has been demonstrated. The amount of doxorubicin internalized into these tumoral cells was sufficient to limit cell growth (Saul et al., 2003). Furthermore, this preferential binding

of folic acid-PEG-liposomes was observed through in vitro and in vivo experiments for cancer cells expressing high levels of FR such as murine lung carcinoma, human epidermal carcinoma and lymphoma (Shmeeda et al., 2006). Folate-coupled copolymeric micelles have been widely used for the tumor-specific drug delivery (Nishiyama & Kataoka, 2006). Doxorubicin-loaded folic acid-PEG-PLGA micelles showed a significant accumulation of drugs in the tumor tissue in mice (Yoo & Park, 2004). Paclitaxel-loaded PCL/MPEG micelles decorated with folic acid exhibited a higher cytotoxic effect on cancer cells such as MCF-7 and HeLa cells (Park et al., 2005). Folate targeting was also developed from PEG poly (cyanoacrylate) nanoparticles (Stella et al., 2003). In addition to BBB functional permeation limiting characteristics, brain microvasculature endothelia also presents an electrostatic barrier at physiologic pH. The negative electrostatic charge is created by surface expression and adhesion of the glycocalyx residues: proteoglycans, sulfated mucopolysaccharides, and sulfated and sialic acid-containing glycoproteins and glycolipids (Poduslo & Curran, 1996). This anionic nature of the edndothelium repels anionic molecules (Vorbrodt et al., 1990) and cationic molecules have been shown to occupy anionic areas at the BBB endothelium (Nagy et al., 1983) and increase BBB permeability via presumed tight junction disruption (Hardebo & Kahrstrom, 1985). Transport of cationized albumins and cationized immunoglobulins to the cerebral parenchyma was hence significantly improved in comparison with native proteins (Pardridge et al., 1990). Similar electrostatic interactions between nanoparticles and BBB endothelia have been demonstrated. Cationized NPs have an increased brain distribution compared to anionic and neutral NPs, owing to this interaction (Fenart et al.., 1999). Such cationic NPs have been shown to have immediate toxic effects at brain microvasculature endothelium (Lockman et al., 2004). Anionic sites are located on the luminal surface of brain capillaries due to the sialic acid residues of glycoproteins (Vorbrodt, 1989). The active targeting of drugs has been used for cationized albumin (Pardridge et al., 1987; Kumagai et al., 1987) and evaluated in isolated brain capillaries and in rat brain. In comparison with native protein, it was noted that there was an enhanced uptake of positively charged albumin by the brain capillaries. In vivo studies in rats on cationized albumin transport through the BBB were also carried out (Triguero et al., 1990). About 15% of the cationized protein detected in the whole brain was located in the post-capillary extracellular space. Cationization was shown to improve the accumulation of the protein in brain tissues (Pardridge et al., 1990). Cationized heterologous proteins have more immunogenic properties than homologous proteins (Muckerheide et al., 1987). In another study, it was demonstrated that the beta-endorphin, a non-transportable chimeric peptide, when covalently coupled to cationized albumin was able to reach the cerebral parenchyma (Pardridge et al., 1990). Cationized bovin serum albumin (CBSA) has been conjugated to pegylated liposomes and these liposomes were specifically taken up when in contact with isolated brain capillary endothelial cells (BCEC) and a monolayer of porcine BCEC (Thole et al., 2002). These results showed the ability of CBSA nanoparticles to pass through the BBB to reach the cerebral parenchyma. The coating of nanoparticles using hydrophilic surfactants has proved promising for the delivery of drugs to the brain. However, their targeted effect depends on the chemical structure, physicochemical and biochemical parameters of the surfactant. Only a few polysorbates have been reported to interact with the brain endothelium (Kreuter et al., 1997). Another approach uses the adsorption of plasma proteins such as apolipoproteins (apo) on the surface of coated nanoparticles after intravenous administration. Because apoE is involved in the transport of low-density lipoprotein to the brain nanocarriers coated with polysorbate mimic LDL after apoE adsorption. This protein

is expressed at a high level in brain tumors such as astrocytomas and glioblastomas (Murakami et al., 1988). The effects of nanoparticles made of PBCA coated with polysorbates such as polysorbate 80 have been widely investigated (Kreuter et al., 1995). Polysorbate 80-coated PBCA nanoparticles were taken up into human and bovine endothelial cells rapidly and in an amount 20-fold higher than with conventional nanocarriers (Ramge et al., 2000). The pharmacokinetic behavior of doxorubicin packaged within coated PBCA nanoparticles was significantly enhanced after intravenous injection in healthy rats. This formulation allowed a considerable accumulation of the drug in the brain (Gulyaev et al., 1999). The therapeutic potential of doxorubicin-loaded PBCA nanoparticles coated with polysorbate 80 was evaluated for the treatment of glioblastoma intracranially implanted in rats (Steiniger et al., 2004; Gelperina et al., 2002). Antitumor efficiency, based on the increase of the median survival time as compared to doxorubicin, was improved with coated nanoparticles in comparison with uncoated nanocarriers. Coating in a hydrophilic surfactant have been applied to more biocompatible nanocarriers such as lipid colloidal systems for drug delivery to the brain. The SLN surface was coated in various hydrophilic surfactants (Goppert & Muller, 2005) and polysorbate-coated in SLN showed a specific adsorption of plasma proteins such as apoE. Polysorbate 80-coated atovaquone-loaded SLN were used for the treatment of toxoplasmic encephalitis (Scholer et al., 2001). The role of polysorbate 80 in the brain targeting of PLA nanoparticles was also investigated (Sun et al., 2004). In another study, dipalmitoylated apoE-derived peptides, characterized by a high lipid affinity, were anchored on liposomes (Sauer et al., 2006) and taken up within BCEC. Doxorubicin is a polar molecule that does not normally cross the BBB. When doxorubicin adsorbed on polybutylcyanoacrylate nanoparticles with polysorbate-80 as a surfactant was given intravenously, therapeutic concentrations of doxorubicin could be achieved (Gulyaev et al., 1999). Besides, nanoparticles containing doxorubicin administered intravenously to rats led to a significant cure of glioblastomas. Another lipophilic anticancer drug camphotericin when adsorbed on solid lipid nanoparticles led to an increased bioavailability of the drug in brain (Yang et al., 1999). Nanoparticle mediated brain drug delivery has also been used successfully for dalargin (Kreuter et al., 1995), the hydrophilic antitrypanosomal drug diminazene diaceturate (Olbrich et al., 2004) and paclitaxel (Feng et al., 2004; Koziara et al., 2004).

5. Nanotechnology and pulmonary drug-delivery systems

Pulmonary delivery of chemotherapeutic entities is one of the highly desired aspects of drug delivery and the application of polymeric nanoparticles to the pulmonary routes is widely recognized now. The lungs offer a non-invasive route for the delivery of various dugs as they demonstrate relatively high permeability to hydrophilic macromolecules and express relatively low peptidase/protease activity (Wall, 1995). The lungs are an attractive target for drug delivery as they provide high systemic bioavailability, avoid first-pass metabolism, enhance the onset of therapeutic action and provide huge surface area (Yang et al., 2008, Patton & Byron, 2007). It should be noted that if the lungs are to be considered for the systemic delivery, a high percentage of the dose must be delivered to the lungs and the site of deposition should be as peripheral as possible (Colthorpe et al.., 1992). An approach to improve the pulmonary delivery of drugs would be to produce much smaller drug particles, as they offer high penetration and deposition of the aerosol (Burch et al., 1986). Nanocarrier systems in pulmonary drug delivery have the potential to achieve relatively uniform distribution of drug dose among the alveoli. They can also help to achieve

enhanced solubility of the drug than its own aqueous solubility while maintaining the sustained-release of drug which consequently reduces the dosing frequency, with improved patient compliance (Bailey & Berkland, 2009). Due to their biocompatibility, surface modification capability and sustained-release properties, polymeric nanoparticles are intensively studied using various important drugs. The pulmonary drugs include anti-asthmatic drugs (Stark et al., 2007), antituberculosis drugs (Pandey et al., 2003; Zahoor et al., 2005), pulmonary hypertension drugs (Kimura, 2009), and anticancer drugs (Azarmi et al., 2006). However, there are some obstacles to the successful delivery of drugs to the lungs. These include degradation by the proteases in the lung, which tends to reduce their overall bioavailability, the limitations posed by barrier between capillary blood and alveolar air that eventually hinders direct exposure of the drugs to lungs. To overcome these limitations, the design (size, shape, and aerodynamic properties) of the dosage forms (nanocarriers) is a rational option. Nanoparticle dispersions consisting of small particles of 10–400 nm diameter show great promise as carriers in pulmonary drug delivery systems. Drugs can be trapped in the core of a micelle and transported at concentrations even greater than their intrinsic water solubility. In addition, a hydrophilic shell can form around the micelle, effectively protecting the contents and it may prevent recognition by the reticuloendothelial system and prevent early elimination from the bloodstream (Smola et al., 2008). Such polymeric micelles are able to evade the mononuclear phagocytic system due to their bulky hydrophilic outer shell and lead to a sustained release of the drug (Marsh et al., 2003). In this direction, beclomethasone dipropionate loaded polymeric micelles were designed which were directly administrable to the lung in nanoparticle sizes in inhalation dosage form intended to be an effective means of treating asthma and chronic pulmonary obstructive disease. Among the various drug delivery approaches for lungs, liposomes are one of the most extensively investigated systems for controlled delivery of drug to the lung (Zeng et al., 1995). Liposomes seem particularly appropriate for delivery of therapeutic agent to lung, as these vesicles can be prepared from compounds endogenous to the lungs such as the components of lung surfactant and these properties make liposomes attractive candidates as drug delivery vehicles (Justo & Moraes, 2003). The first pharmaceutical liposomal products in market include the synthetic lung surfactant Alveofact® for pulmonary instillation for the treatment of respiratory distress syndrome (Muller et al., 2000). Typically, liposomal formulations have been delivered to the lung in the liquid state, and nebulizers have been used extensively for the aerosol delivery of liposomes in the liquid state (Schreier et al., 1993). Liposomal drug formulations for aerosol delivery have their own potential advantages, including aqueous compatibility, sustained pulmonary release to maintain therapeutic drug levels and facilitated intra-cellular delivery particularly to alveolar macrophages (Schreier et al. 1993). Perhaps more importantly, liposomes may prevent local irritation and reduce toxicity both locally and systematically (Gonzalez-Rothi & Schreier 1995). Increased potency with reduced toxicity is characteristic of many drug-liposomal formulations (Cullis et al. 1989). Liposomal aerosols have proven to be non-toxic in acute human and animal studies (Waldrep et al., 1997). These results suggest that drug-liposome aerosols are more effective for delivery, deposition and retention of water-insoluble, hydrophobic, lipophilic compounds in contrast to water soluble compounds (Taylor & Farr, 1993). In another study, non-phospholipid vesicles loaded with beclomethasone dipropionate were fabricated with non-ionic surfactant, polysorbate 20 (Terzano et al., 2005). Levonorgestrel encapsulated liposomes were instilled intratracheally in rats and were

compared with the plain drug suspension. The results clearly demonstrated the superiority of pulmonary drug delivery with regards to maintenance of effective therapeutic concentration of the levonorgestrel in the plasma over a longer period and also to reduce frequency of dosing and systemic side effects associated with oral administration of levonorgestrel (Shahiwala & Misra, 2004). Much interest has also been focused on cationic liposomes for pulmonary delivery which have additional advantages like evasion from complement inactivation after in vivo administration (Densmore, 2006). Moreover, liposomes conjugated with cell-penetrating peptides are recognized as potential nanocarrier systems for intracellular delivery of macromolecules to the lung. Liposomes modified with cell-penetrating peptides, antennapedia, the HIV-1 transcriptional activator, and octaarginine have been reported to enhance the cellular uptake of liposomes to airway cells (Cryan et al., 2006). Liposomes of EYPC-cholesterol (CHOL) incorporating dexamethasone palmitate (DEXP) were studied (Benameur et al., 1995), the DEXP incorporated into the liposomes kept its biological activity. It has been shown that a 30 minutes after the instillation the pulmonary concentration of glucocorticoids was twice higher when the drug is encapsulated into liposomes compared to the solubilized drug (Suntres & Shek, 1998). Particles composed of biocompatible and bio-degradable polymers have also been studied for the targeting of drugs by pulmonary route (Zeng et al. 1995; Li et al., 2001). Synthetic polymers are much more frequently used than natural polymers. Solid lipid nanoparticles (SLN) combine the advantages of the biocompatibility of lipids and the possibility of industrial scale up of nanoparticles. The advantages of drug release from SLNs in the lung are controlled drug release profile, a faster in vivo degradation compared to particles made from PLA or PLGA. In addition, SLNs proved to possess a higher tolerability in the lungs compared to particles made from some polymeric materials (Muller et al., 2000) Besides, toxicological profile of SLNs when using physiological lipids, is expected to be better than that of polymer-based systems, because physiological lipids have little or no cytotoxicicity.(Muller et al., 1997) It is feasible that aqueous suspensions and perhaps dry powder formulations of SLN can be used for pulmonary inhalation aerosol administration of drugs using nebulizers and dry powder inhalers (Muller et al., 2000). Several studies have been published on the pulmonary applications of SLNs as local delivery carriers for small molecules (Pandey & Khuller, 2005) or as systemic delivery carriers for macromolecules (Liu et al., 2008). Drugs like prednisolone, diazepam and camptotecin have been incorporated into SLN for pulmonary applications (Muller et al. 2000). Pandey and Khuller studied the chemotherapeutic potential of SLNs incorporating rifampicin, isoniazid and pyrazinamide against experimental tuberculosis and observed the slow and sustained-release of drugs from the SLNs in vitro and in vivo (Pandey & Khuller, 2005). Novel nebulizer-compatible SLNs containing insulin have been examined for pulmonary delivery (Liu, 2008). In this case, SLNs were successful as a pulmonary carrier system for insulin. Deposition and clearance of SLNs after inhalation of aerosolized insoluble particles showed that after deposition, inhaled material began to translocate to regional lymph nodes (Videira et al., 2006) indicating that inhalation can be an effective route to deliver drug-containing lipid particles to the lymphatic systems and lipid particles can be used as potential drug carriers for lung cancer therapy (Videira, 2006). Dendrimers have also been assessed for pulmonary delivery. In a study, low molecular weight heparin (LMWH)–dendrimer complex was formulated using various PAMAM dendrimers, then evaluated for safety and the efficacy in preventing deep vein thrombosis, concluding that cationic dendrimers can be used as pulmonary delivery carriers for a relatively large molecular weight anionic drug (Bai, 2007).

Later, pegylated dendrimers (mPEG–dendrimer) were formulated to increase the pulmonary absorption and circulation time of the drug, with significant positive results showing increased half-life and absorption of the drug. These results also implicated that LMWH loaded in the mPEG–dendrimer could potentially be used as noninvasive delivery system for the treatment of thromboembolic disorder (Bai, 2009). Nanoparticles based on lecithin have also shown promising deposition profile for hydro-fluoroalkanes (HFAs) (Dickinson et al. 2001). Liposomes functionalized with lecithins have shown to improve their binding to human alveolar cells (Abu-Dahab et al., 2001). Pulmospheres™ have been successfully formulated using phospholipids to be dispersed into HFAs and have been demonstrated to release uniform amounts of drugs when aerosolized (Dellamary et al. 2000). Anticancer drug 9-nitrocamptothecin (9NC) has been encapsulated into DLPC-liposomes, which prevented the loss of drug by albumin and the amount of effective 9NC contained in the liposomes was 10–50 times lower than that used by other routes of administration (Knight et al. 2000). The greater therapeutic effectiveness is a result of rapid absorption in the respiratory tract and more specifically, in the pulmonary tissues and penetration into the organ and tumor sites. One of the highly desired objectives of pulmonary drug delivery is the targeted, specific delivery to the alveolar macrophages. Targeting drugs to alveolar macrophages has the distinct advantage of delivering high concentrations of drug to a cell that plays a central role in the progression of disease (tuberculosis) and in immune responses. Microspheres have been shown to target alveolar macrophages without eliciting a pulmonary inflammatory response in vitro (Ng et al. 1998), and were non-toxic. Lectins are non-immunological glycoproteins that have the capacity to recognize and bind to glycoproteins exposed at the epithelial cell surface. Mucoadhesive nanoparticles, coated with mucoadhesive polymers such as poly(acrylic acid) or chitosan demonstrated a slower elimination rate, indicating that chitosan-nanospheres adhere to the mucus in the trachea and in the lung tissues as a result of the mucoadhesive properties of chitosan (Takeuchi et al., 2001). Perhaps the most important application of drug delivery for pulmonary disease has been the chemotherapeutics of tuberculosis. Tuberculosis treatment is lengthy and often leads to poor patient compliance. Poly-lactide-co-glycolide (PLGA), alginate and solid lipid nanoparticles nanoparticles have been successfully used to achieve a significant sustained release in vivo. Not only were the drugs available in the plasma and tissues of experimental animals for a longer time, less frequent dosing with nanoparticle loaded drugs was equally effective as free drugs. These drug loaded nanoparticles were even effective at much lower concentrations than free drugs and were completely non-toxic (Ahmad & Khuller, 2008; Ahmad et al., 2006; Sharma et al., 2004; Ahmad et al., 2007).

6. Nanoparticle mediated antiretroviral therapy

Acquired immunodeficiency syndrome (AIDS) is one of the biggest global threats today. Despite standard therapy, the disease is still far from being under control. The current clinical therapy, known as 'highly active antiretroviral treatment' (HAART), has made significant contribution towards reducing mortality (Richman et al., 2009). HAART, however, is not as effective, owing to a few drawbacks. First and foremost, these drugs are unable to eliminate human immunodeficiency virus (HIV) from resting CD4+T cells in the blood (Chun et al., 2007). Most of the drugs under HAART have various limitations. Didanosine has poor stability in the gastric environment and low bioavailability owing to hepatic first pass. Zidovudine has a short half-life, variable bioavailability and

hematological toxicity. Tenofovir can cause renal toxicity, including acute renal failure, Fanconi syndrome and proteinuria (Cihlar & Ray, 2010). Efavirenz has a very low solubility, low absorption and limited biodistribution. Etravirine also has low solubility (Sosnik et al., 2009). The protease inhibitors (saquinavir, indinavir, ritonavir, lopinavir, nelfinavir, amprenavir, fosamprenavir, atazanavir, tipranavir and darunavir) too have a poor oral bioavailability (Hochman, 2000) and limited penetration into the lymphatic system and CNS (Li & Chan, 1999). In addition, other associated problems such as adverse drug effects, poor drug regimen compliance and drug interactions are associated with antiretroviral therapy (Richman et al., 2009). Nanotechnology based drug delivery has the potential to overcome nearly all of the shortcomings mentioned above. Nanoparticles can provide a target specific and sustained release of these drugs, thus improving their bioavailabilty and associated side effects. In this direction, poly (isohexyl cyanate) nanoparticles of zidovudine have been synthesized for targeting the lymphoid tissue in the gastrointestinal tract. Use of this carrier system, when compared with aqueous drug solution resulted in higher of the drug levels in the Peyer's patches. In another study, polyhexylcyanoacrylate nanoparticles were employed for the delivery of zidovudine (Lobenberg et al., 1998), thus improving its bioavailability. In a distinct experiment, PLGA nanoparticles containing multiple antiretroviral drugs, i.e ritonavir, lopinavir, and efavirenz were formulated and results showed that drugs could be detected in peripheral blood mononuclear cells in vitro for 28 days (Destache et al., 2009). In a study with zidovudine-loaded poly(isohexyl cyanate) nanoparticles, zidovudine was accumulated in the cells of the reticuloendothelial system (Lobenberg et al., 1998). Poly(epsilon-caprolactone) nanoparticles loaded with saquinavir were also successfully used for targeting the phagocytic mononuclear system by modifying the surface of the nanoparticles (Shah & Amiji, 2006). Results showed that the intracellular drug concentrations were found to be higher with encapsulated saquinavir compared with free drug solution. In separate experiments, stavudine, zidovudine and lamivudine have been entrapped in polybutylcyanoacrylate (PBCA) and methylmethacrylate-sulfopropylmethacrylate (MMA-SPM) nanoparticles for brain targeting. The permeability of zidovudine and lamivudine was 8–20 fold higher and 10–18 fold higher, respectively, with PBCA nanoparticles and MMA-SPM nanoparticles led to a 2-fold increase in the BBB permeability of both drugs (Kuo, 2005). In a similar experiment, stavudine, delavirdine, and saquinavir were delivered as PBCA and MMA-SPM nanoparticles and their delivery to the brain was studied. The results showed that the permeability of all three drugs increased about 12–16 fold with PBCA nanoparticles and 3–7-fold with MMA-SPM nanoparticles (Kuo & Su, 2006). Dendrimers have also been used to deliver antiretroviral drugs. Tuftsin-conjugated poly(propyleneimine) dendrimers loadedwith efavirenz was evaluated for targeted delivery to macrophages. These dendrimer formulations showed reduced cytotoxicity compared with nonconjugated poly(propyleneimine) dendrimers in vitro and enhanced cellular uptake by mononuclear phagocytic cells, with greater anti-HIV activity in vitro (Dutta et al., 2008). SLNs have also been used for antiretroviral drugs with success. SLNs loaded with stavudine, delavirdine, and saquinavir have been evaluated for their ability to cross the BBB in vitro using human brain microvascular endothelial cells. The permeability of the drugs was improved 4–11 fold when incorporated into SLNs (Kuo & Su, 2007). Similarly, SLNs incorporating atazanavir with Pluronic F68 as an emulsifier were evaluated. In vitro studies using hCMEC/D3, a human brain microvessel endothelial cell line, showed a higher uptake of the drug when delivered in SLN form, as compared with free atazanavir (Chattopadhyay et al., 2008). Regarding liposomal formulations, stavudine

loaded into mannosylated and galactosylated liposomes exhibited greater cellular uptake by cells of the mononuclear phagocytic system and greater accumulation in organs of the reticuloendothelial system as compared with free drug solution or even non-modified liposomes (Garg et al., 2006). PLGA nanoparticles containing ritonavir, lopinavir and efavirenz led to an increased uptake of the drugs by macrophages (Destache et al., 2009). Quite similarly, PHCA nanoparticles containing zidovudine showed a higher drug concentration in the organs of the reticuloendothelial system. An interesting finding was the higher levels of zidovudine in the brain when the nanoparticles were coated with polysorbate 80 (Bender et al., 1994). Further studies evaluated PBCA and MMSPM nanoparticles for brain targeting of zidovudine and lamivudine. The permeability of both the drugs to BBB was found to be significantly increased (Kuo & Chen, 2006). In a similar study, stavudine, delaviridine and saquinavir loaded PBCA and MMSPM nanoparticles coated with PS-80 and SLNs showed a higher drug permeability to brain (Kuo & Su, 2007). In an important finding, researchers observed a significant enhancement of brain localization of zidovudine when it was delivered by transferrin-anchored PEGylated albumin nanoparticles (Mishra et al., 2006). In another study, PLGA nanoparticles loaded ritonavir, lopinavir and efavirenz showed a sustained release for 28 days and anti-HIV inhibition was comparable to that of free drugs. Besides, PPI dendrimer-based nanocontainers have been used for targeting of efavirenz macrophages. The haemolytic activity and cytotoxicity of PPI dendrimer was found to be very high and there was a significant increase in cellular uptake of efavirenz by macrophages (Dutta et al., 2007).

7. Conclusion

Nanotechnology provides a wide range of techniques and strategies that can optimize the delivery of pharmaceutical agents. Nano-carrier mediated delivery offers sustained release of drugs in the body as well as protecting them from premature in-vivo degradation or clearance, subsequently increasing the bioavailability and therapeutic potential. By shielding the drug in nanoparticles, the otherwise toxic effects of the drug can be reduced. Most importantly, site-specific delivery of drugs allows increased local concentrations of the drugs and significantly lowers the undesirable systemic toxicity. Nano-carriers have another unprecedented potential that they can allow for new patent opportunities in the case of dugs with expired patents. Thus, nanotechnology can be applied at all stages of drug development, from formulations for optimal delivery to therapeutic applications in clinical trials.

8. References

Abraham, M.H.; Chadha, H.S. & Mitchell, R.C. (1994). Factors that influence the distribution of solutes between blood and brain, *Journal of Pharmaceutical Sciences,* Vol.83, No.9, pp. 1257–1268

Abu-Dahab, R.; Schäfer, U.F. & Lehr, C.M. (2001). Lectin-functionalized liposomes for pulmonary drug delivery: effect of nebulization on stability and bioadhesion, *European Journal of Pharmaceutical Sciences,* Vol.14, pp. 37–46

Ahmad, Z. & Khuller, G.K. (2008). Alginate-based sustained release drug delivery systems for tuberculosis, *Expert Opinion in Drug Delivery*, Vol.5, No.12, pp. 1323-1334

Ahmad, Z.; Pandey, R.; Sharma. S. & Khuller, G.K. (2006). Alginate nanoparticles as antituberculosis drug carriers: formulation development, pharmacokinetics and therapeutic potential, *Indian Journal of Chest Diseases and Allied Sciences*, Vol.48, No.3, pp. 171-176

Ahmad, Z.; Sharma, S. & Khuller, G.K. (2007). Chemotherapeutic evaluation of alginate nanoparticle-encapsulated azole antifungal and antitubercular drugs against murine tuberculosis, *Nanomedicine,* Vol.3, No.3, pp. 239-243

Allemann, E.; Gurny, R. & Doelker E. (1993). Drug loaded nanoparticles preparation methods and drug targeting issues, *European Journal of Pharmaceutics and Biopharmaceutics*, Vol.39, pp. 173-191

Allen, D.D. & Smith, Q.R. (2001). Characterization of the blood–brain barrier choline transporter using the in situ rat brain perfusion technique, *Journal of Neurochemistry,* Vol.76, No.4, pp. 1032–1041

Alyautdin, R.N.; Tezikov, B.E.; Ramge, P.; Kharkevich, D.A.; Begley, D.J. & Kreuter, J. (1998). Significant entry of tubocurarine into the brain of rats by adsorption to polysorbate-80 coated polybutylcyanoacrylate nanoparticles: an in situ brain perfusion study, *Journal of Microencapsulation*, Vol.15, No.1, pp. 67–74

Alyautidin, R.N.; Gother, D. & Petrov, V. (1995). Analgesic activity of the hexapeptide dalagrin adsorbed on the surface of polysorbate-80 coated polybutylcyano-acrylate nanoparticles, *European Journal of Pharmaceutics and Biopharmaceutics*, Vol.41, pp. 44–48

Anand, P.; Nair, H.B.; Sung, B.; Kunnumakkara, A.B.; Yadav, V.R.; Tekmal, R.R. & Aggarwal, B.B. (2010). Design of curcumin-loaded PLGA nanoparticles formulation with enhanced cellular uptake, and increased bioactivity in vitro and superior bioavailability *in vivo*, *Biochemical Pharmacology*, Vol.79, pp. 330-338

Antohe, F.; Dobrila, L.; Heltianu, C.; Simionescu, N. & Simionescu, M. (1993). Albumin-binding proteins function in the receptor-mediated binding and transcytosis of albumin across cultured endothelial cells, *European Journal of Cell Biology*, Vol.60, pp. 268–275

Antohe, F.; Heltianu, C. & Simionescu, M. (1991). Albumin-binding proteins of endothelial cells: Immunocytochemical detection of the 18 kDa peptide, *European Journal of Cell Biology*, Vol.56, pp. 34–42

Azarmi, S.; Tao, X.; Chen, H.; Wang, Z.; Finlay, W.H.; Löbenberg, R.; Roa, W.H. (2006). Formulation and cytotoxicity of doxorubicin nanoparticles carried by dry powder aerosol particles, *International Journal of Pharmacology,* Vol.319, pp. 155–161

Bai, S. & Ahsan, F. (2009). Synthesis and evaluation of pegylated dendrimeric nanocarrier for pulmonary delivery of low molecular weight heparin, *Pharmaceutical Research,* Vol.26, pp. 539–548

Bai, S.; Thomas, C. & Ahsan, F. (2007). Dendrimers as a carrier for pulmonary delivery of enoxaparin, a low-molecular weight heparin, *Journal of Pharmaceutical Sciences*, Vol.96, pp. 2090–2106

Bailey MM, Berkland CJ. (2009). Nanoparticle formulations in pulmonary drug delivery, *Medicine Research Reviews*, Vol.29, pp. 196–212

Bangham, A.D. & Horne, R.W. (1964). Negative Staining of Phospholipids + Their Structural Modification by-Surface Active Agents as Observed in Electron Microscope, *Journal of Molecular Biology,* Vol.8, pp. 660-668

Basu, S.; Harfouche, R.; Soni, S.; Chimote, G.; Mashelkar, R.A. & Sengupta, S. (2009). Nanoparticle-mediated targeting of MAPK signaling predisposes tumor to chemotherapy, *Proceedings of the National Academy of Sciences USA,* Vol.106, pp. 7957-7961

Batist, G.; Gelmon, K.A.; Chi, K.N.; Miller, W.H. Jr.; Chia, S.K.; Mayer, L.D.; Swenson, C.E.; Janoff, A.S. & Louie, A.C. (2009). Safety, pharmacokinetics, and efficacy of CPX-1 liposome injection in patients with advanced solid tumors, *Clinical Cancer Research*, Vol.15, pp. 692-700

Benameur, H.; Latour, N.; Schandene, L.; Van Vooren, J.P.; Flamion, B.; Legros, F.J. (1995). Liposome-incorporated dexamethasone palmitate inhibits in-vitro lymphocyte response to mitogen, *Journal of Pharmaceutics and Pharmacology*, Vol.47, pp. 812–817

Bender, A.; Schfer, V.; Steffan, A.M.; Royer, C.; Kreuter, J.; Rübsamen-Waigmann, H. & von Briesen, H. (1994). Inhibition of HIV in vitro by antiviral drug-targeting using nanoparticles, *Research Virology,* Vol.145, pp. 215-220

Boado, R.J.; Black, K.L. & Pardridge, W.M. (1994). Gene expression of GLUT3 and GLUT1 glucose transporters in human brain tumors, *Molecular Brain Research*, Vol.27, No.1, pp. 51–57

Bosman, A.W.; Janssen, H.M. & Meijer, E.W. (1999). About dendrimers: Structure, physical properties, and applications, *Chemical Reviews*, Vol.99, No.7, pp. 1665–1688

Burch, W.M.; Sullivan, P.J. & McLaren. C.J. (1986). Technegas - A new ventilation agent for lung scanning, *Nuclear Medicine Communications*, Vol.7, pp. 865-871

Butte, A.M.; Jones, H.C. &Abbot, N.J. (1990). Electrical resistance across the blood-brain barrier in anaesthetized rats: a developmental study, *Journal of Physiology*, Vol.429, pp. 47–62

Chang, J.; Jallouli, Y.; Kroubi, M.; Yuan, X.; Feng, W.; Kang, C.; Pu, P. & Betbeder, D. (2009). Characterization of endocytosis of transferrin-coated PLGA nanoparticles by the blood-brain barrier, *International Journal of Pharmaceutics*, Vol.379, No.2, pp. 285-292

Chattopadhyay, N.; Zastre, J.; Wong, H.L.; Wu, X.Y. & Bendayan, R. (2008). Solid lipid nanoparticles enhance the delivery of the HIV protease inhibitor, atazanavir, by a human brain endothelial cell line, *Pharmaceutical Research,* Vol.25, No.10, pp.2262–2271

Chaudhuri, P.; Harfouche, R.; Soni, S.; Hentschel, D.M. & Sengupta, S. (2010). Shape effect of carbon nanovectors on angiogenesis, *ACS Nano,* Vol.4, pp. 574-582

Choy, H. (1999). Taxanes in combined-modality therapy for solid tumors, *Oncology (Williston Park),* Vol.13, pp. 23–38

Chun, T.W.; Justement, J.S.; Moir, S.; Hallahan, C.W.; Maenza, J.; Mullins, J.I.; Collier, A.C.; Corey, L. & Fauci, A.S. (2007). Decay of the HIV reservoir in patients receiving antiretroviral therapy for extended periods: implications for eradication of virus, *Journal of Infectious Diseases,* Vol.195, pp. 1762-1764

Cihlar, T. & Ray, A.S. (2010). Nucleoside and nucleotide HIV reverse transcriptase inhibitors: 25 years after zidovudine, *Antiviral Research,* Vol.85, pp. 39-58

Cornford, E.M.; Braun, L.D. & Oldendorf, W.H. (1978). Carrier mediated blood–brain barrier transport of choline and certain choline analogs, *Journal of Neurochemistry*, Vol.30, No.2, pp. 299–308

Crown, J. & O'Leary, M. (2000). The taxanes: An update, *Lancet,* Vol.355, pp. 1176–1178

Cryan, S.A.; Devocelle, M.; Moran, P.J.; Hickey, A.J. & Kelly JG. (2006). Increased intracellular targeting to airway cells using octaarginine-coated liposomes: In vitro assessment of their suitability for inhalation, *Molecular Pharmacology*, Vol.3, pp. 104–112

Cullis, P.R.; Mayer, L.D. & Bally, M.B. (1989). Generating and loading of liposomal systems for drug delivery systems, *Advanced Drug Delivery Reviews*, Vol.3, pp. 267–282

Daniels, T.R.; Delgado, T.; Helguera, G. & Penichet, M.L. (2006). The transferrin receptor part II: targeted delivery of therapeutic agents into cancer cells, *Clinical Immunology,* Vol.121, No.2, pp. 159–176

Dellamary, L.A.; Tarara, T.E.; Smith, D.J.; Woelk, C.H.; Adractas, A.; Costello, M.L.; Gill, H. & Weers, J.G. (2000). Hollow porous particles in metered dose inhalers, *Pharmaceutical Research,* Vol.17, pp. 168–174

Densmore, C.L. (2006). Advances in noninvasive pulmonary gene therapy, *Current Drug Delivery*, Vol.3, pp. 55–63

Desai, N.; Trieu, V.; Yao, Z.; Louie, L.; Ci, S.; Yang, A.; Tao, C.; De, T.; Beals, B.; Dykes, D.; Noker, P.; Yao, R.; Labao, E.; Hawkins, M. & Soon-Shiong, P. (2006). Increased antitumor activity, intratumor paclitaxel concentrations, and endothelial cell transport of Cremophor-free, albumin-bound paclitaxel, ABI-007, compared with Cremophor-based paclitaxel, *Clinical Cancer Research*, Vol.12, pp. 1317–1324

Desgrosellier, J.S. & Cheresh, D.A. (2010). Integrins in cancer: biological implications and therapeutic opportunities, *Nature Review Cancer*, Vol.10, pp. 9-22

Destache, C.J.; Belgum, T.; Christensen, K.; Shibata, A.; Sharma, A. & Dash, A. (2009). Combination antiretroviral drugs in PLGA nanoparticle for HIV-1, *BMC Infectetious Diseases*, Vol.9, pp.198

Dickinson, P.A.; Howells, S.W. & Kellaway, I.W. (2001). Novel Nanoparticles for Pulmonary Drug Administration, *Journal of Drug Targeting,* Vol.9, No.4, pp. 295-302

du Toit, L.C.; Pillay, V. & Choonara, Y.E. (2010). Nano-microbicides: Challenges in drug delivery, patient ethics and intellectual property in the war against HIV/AIDS, *Advanced Drug Delivery Reviews,* Vol.62, No.4–5, pp. 532–546

Dutta, T.; Agashe, H.B.; Garg, M.; Balakrishnan, P.; Kabra, M. & Jain, N.K. (2007). Poly (propyleneimine) dendrimer based nanocontainers for targeting of efavirenz to human monocytes/macrophages *in vitro*, *Journal of Drug Targeting*, Vol.15, No.1, pp. 89-98

Dutta, T.; Garg, M. & Jain, N.K. (2008). Targeting of efavirenz loaded tuftsin conjugated poly(propyleneimine) dendrimers to HIV infected macrophages *in vitro, European Journal of Pharmaceutical Sciences*, Vol.34, No.2–3, pp.181–189

Dvorak, H.F.; Senger, D.R.; Dvorak, A.M.; Harvey, V.S. & McDonagh, J. (1985). Regulation of extravascular coagulation by microvascular permeability, *Science*, Vol.227, pp. 1059–1061

Eavarone, D.A.; Yu, X. & Bellamkonda, R.V. (2000). Targeted drug delivery to C6 glioma by transferrin-coupled liposomes, *Journal of Biomedical Materials Research*, Vol.51, No.1, pp. 10–14

Emerich, D.F. & Thanos, C.G. (2007). Targeted nanoparticle-based drug delivery and diagnosis. *Journal of Drug Targeting*, Vol.15, pp. 163-183

Farokhzad, O.C.; Cheng, J.;Teply, B.A.; Sherifi, I.; Jon, S.; Kantoff, P.W.; Richie, J.P. & Langer, R. (2006). Targeted nanoparticle-aptamer bioconjugates for cancer chemotherapy *in vivo, Proceedings of the National Academy of Sciences USA,* Vol.103, pp. 315– 6320

Farr, C.P.; Taylor, S.G.; Smith, G. & Wyatt, D. (1992). The pharmacokinetics of pulmonary-delivered insulin: A comparison of intratracheal and aerosol administration to the rabbit, *Pharmaceutical Research*, Vol.9, pp. 764-768

Fenart, L.; Casanova, A.; Dehouck, B.; Duhem, C.; Slupek, S.; Cecchelli, R. & Betbeder, D. (1999). Evaluation of effect of charge and lipid coating on ability of 60 nm nanoparticles to cross an in vitro model of the blood-brain barrier, *Journal of Pharmacology and Experimental Therapeutics,* Vol.291, No.3, pp. 1017–1022

Fogal, V.; Zhang, L. & Ruoslahti, E. (2008). Mitochondrial/ Cell surface protein p32/gC1qR as a molecular target in tumor cells and tumor stroma, *Cancer Research,* In press

Folkman, J. (1995). Angiogenesis in cancer, vascular, rheumatoid and other disease, *Nature Medicine*, Vol.1, No.1, pp. 27–31

Folkman, J. (2003). Fundamental concepts of the angiogenic process, *Current Molecular Medicine*, Vol.3, pp. 643-651

Fortin, D.; McCormick, C.I.; Remsen, L.G.; Nixon, R. & Neuwelt, E.A. (2000). Unexpected neurotoxicity of etoposide phosphate administered in combination with other chemotherapeutic agents after blood–brain barrier modification to enhance delivery, using propofol for general anesthesia, in a rat model, *Neurosurgery*, Vol.47, pp. 199–207

Garg, M.; Asthana, A.; Agashe, H.B.; Agrawal, G.P. & Jain, N.K. (2006). Stavudine-loaded mannosylated liposomes: In-vitro anti-HIV-I activity, tissue distribution and pharmacokinetics, *Journal of Pharmaceutics and Pharmacology,* Vol.58, pp. 605–616

Gelderblom, H.; Verweij, J.; Nooter, K. & Sparreboom, A. (2001). Cremophor EL: The drawbacks and advantages of vehicle selection for drug formulation, *European Journal of Cancer,* Vol.37, pp. 1590–1598

Gelperina, S.E.; Khalansky, A.S.; Skidan, I.N.; Smirnova, Z.S.; Bobruskin, A.I.; Severin, S.E.; Turowski, B.; Zanella, F.E. & Kreuter, J. (2002). Toxicological studies of doxorubicin bound to polysorbate 80-coated poly(butyl cyanoacrylate) nanoparticles in healthy rats and rats with intracranial glioblastoma, *Toxicological Letters*, Vol.126, No.2, pp. 131–141

Ghebrehiwet, B.; Lim, B.L.; Peerschke, E.I.; Willis, A.C. & Reid, K.B. (1994). Isolation, cDNA cloning, and overexpression of a 33-kD cell surface glycoprotein that binds to the globular "heads" of C1q, *Journal of Experimental Medicine*, Vol.179, pp. 1809–1821

Golden, P.L. & Pollack, G.M. (2003). Blood–brain barrier efflux transport, *Journal of Pharmaceutical Sciences,* Vol.92, No.9, pp. 1739–1753

Gonzalez-Rothi, R.J. & Schreier, H. (1995). Pulmonary delivery of liposome-encapsulated drugs in asthma therapy, *Clinical Immunotherapy,* Vol.4, pp. 331–337

Goppert, T.M. & Muller, R.H. (2005). Polysorbate-stabilized solid lipid nanoparticles as colloidal carriers for intravenous targeting of drugs to the brain: comparison of plasma protein adsorption patterns, *Journal of Drug Targeting*, Vol.13, No.3, pp. 179–187

Goren, D.; Horwitz, A.T.; Tzemach, D.; Tarshish, M.; Zalipsky, S. & Gabizon, A. (2000). Nuclear delivery of doxorubicin via folate-targeted liposomes with bypass of multidrug-resistance efflux pump, *Clinical Cancer Research*, Vol.6, pp. 1949–1957

Gradishar, W.J.; Tjulandin, S.; Davidson, N.; Shaw, H.; Desai, N.; Bhar, P.; Hawkins, M. & O'Shaughnessy, J. (2005). Superior efficacy of albumin-bound paclitaxel, ABI-007, compared with polyethylated castor oil-based paclitaxel in women with metastatic breast cancer: results of a phase III trial, *Journal of Clinical Oncology,* Vol.23, pp. 7794-7803

Greig, N.H. (1989). Drug delivery to the brain by blood–brain barrier circumvention and drug modification. In: Implications of the Blood–Brain Barrier and its Manipulation; Neuwelt, E.A., Ed.; Plenum Press: New York, pp. 311–367

Guarneri, V. & Conte, P.F. (2004). The curability of breast cancer and the treatment of advanced disease, *European Journal of Nuclear Medicine and Molecular Imaging,* Vol.31, pp. S149–S161

Gulyaev, A.E.; Gelperina, S.E.; Skidan, I.N.; Antropov, A.S.; Kivman, G.Y. & Kreuter, J. (1999). Significant transport of doxorubicin into the brain with polysorbate-80 coated nanoparticles, *Pharmaceutical Research*, Vol.16, pp. 1564–1569

Gururangan, S. & Friedman, H.S. (2002). Innovations in design and delivery of chemotherapy for brain tumors, *Neuroimaging Clinics of North America,* Vol.12, No.4, pp. 583–597

Hall, W.A. (1991). Transferrin receptor on glioblastoma multiforme, *Journal of Neurosurgery*, Vol.74, No.2, pp. 313–314

Hamaguchi, T.; Matusmura, Y.; Suzuki, M.; Shimizu, K.; Goda, R.; Nakamura, I.; Nakatomi, I.; Yokoyama, M.; Kataoka, K. & Kakizoe, T.(2005). NK105, a paclitaxel-incorporating micellar nanoparticle formulation, can extend in vivo antitumor activity and reduce the neurotoxicity of paclitaxel, *British Journal of Cancer*, Vol.92, pp. 1240–1246

Hänninen, M.M.; Haapasalo, J.; Haapasalo, H.; Fleming, RE.; Britton, R.S.; Bacon, B.R. & Parkkila, S. (2009). Expression of iron-related genes in human brain and brain tumors, *BMC Neuroscience,* Vol.10, pp. 36

Hardebo, J.E. & Kahrstrom, J. (1985). Endothelial negative surface charge areas and blood–brain barrier function, *Acta Physiologica Scandinavica,* Vol.125, pp. 495–499

Harfouche, R.; Basu, S.; Soni, S.; Hentschel, D.M.; Mashelkar, R.A. & Sengupta, S. (2009). Nanoparticle-mediated targeting of phosphatidylinositol-3-kinase signaling inhibits angiogenesis, *Angiogenesis*, Vol.12, No.4, pp. 325-38

Hatakeyama, H.; Akita, H.; Maruyama, K.; Suhara, T. & Harashima, H. (2004). Factors governing the in vivo tissue uptake of transferrin-coupled polyethylene glycol liposomes *in vivo*, *International Journal of Pharmacology,* Vol.281, No.1–2, pp. 25–33

Hawkins, M.J.; Soon-Shiong, P. & Desai, N. (2008). Protein nanoparticles as drug carriers in clinical medicine, *Advanced Drug Delivery Reviews,* Vol.60, pp. 876–885

Hochman, J.H.; Chiba, M.; Nishime, J.; Yamazaki, M. & Lin, A.H. (2000). Influence of P-glycoprotein on the transport and metabolism of indinavir in Caco-2 cells expressing cytochrome P-450 3A4, *Journal of Pharmacological and Experimental Therapeutics,* Vol.292, pp. 310-318

Hu, S. & Zhang, Y. (2010). Endostar-loaded Peg-PLgA nanoparticles: in vitro and in vivo evaluation, *International Journal of Nanomedicine*, Vol.5, pp. 1039–1048

Ibrahim, N.K.; Desai, N.; Legha, S.; Soon-Shiong, P.; Theriault, R.L.; Rivera, E.; Esmaeli, B.; Ring, S.E.; Bedikian, A.; Hortobagyi, G.N. & Ellerhorst, J.A. (2002). Phase I and

pharmacokinetic study of ABI-007, a Cremophor-free, protein-stabilized, nanoparticle formulation of paclitaxel, *Clinical Cancer Research*, Vol.8, pp. 1038–1044

Ibrahim, N.K.; Samuels, B.; Page, R.; Doval, D.; Patel, K.M.; Rao, S.C.; Nair, M.K.; Bhar, P.; Desai, N. & Hortobagyi, G.N. (2005). Multicenter phase II trial of ABI-007, an albumin- bound paclitaxel, in women with metastatic breast cancer, *Journal of Clinical Oncology,* Vol.23, No.25, pp. 6019–6026

Jain, K.K. (2010). Advances in the field of nanooncology, *BMC Medicine*, Vol.8, pp. 83

Jefferies, W.A.; Brandon, M.R.; Hunt, S.V.; Williams, A.F.; Gatter, K.C. & Mason, D.Y. (1984). Transferrin receptor on endothelium of brain capillaries, *Nature*, Vol.312, No.5990, pp. 162–163

Jemal, A.; Siegel, R. & Ward E. (2008) Cancer statistics. *CA: A Cancer Journal for Clinicians,* Vol.58, No.2, pp. 71–96

Jenning, V.; Lippacher, A. & Gohla, SH. (2002) Medium scale production of solid lipid nanoparticles (SLN) by high pressure homogenization, *Journal of Microencapsulation,* Vol.19, pp. 1–10

John, T.A.; Vogel, S.M.; Tiruppathi, C.; Malik, A.B. & Minshall, R.D. (2003). Quantitative analysis of albumin uptake and transport in the rat microvessel endothelial monolayer, *Lung Cellular and Molecular Physiology*, Vol.284, pp. L187–L196

Justo, O.R. & Moraes, A.M. (2003). Incorporation of antibiotics in liposomes designed for tuberculosis therapy by inhalation, *Drug Delivery*, Vol.10, pp. 201–207

Kabanov, A.V.; Batrakova, E.V. & Alakhov, V.Y. (2002). Pluronic block copolymers as novel polymer therapeutics for drug and gene delivery, *Journal of Control Release,* Vol.82, pp. 189–212

Kang, K.W.; Chun, M.K.; Kim, O.K.; Subedi, R.K.; Ahn, S.G.; Yoon, J.H. & Choi, H.K. (2010). Doxorubicin-loaded solid lipid nanoparticles to overcome multidrug resistance in cancer therapy, *Nanomedicine Nanotechnology Biology and Medicine*, Vol.6, No.2, pp. 210-213

Kang, Y.S.; Terasaki, T.; Ohnishi, T. & Tsuji, A. (1990). In vivo and in vitro evidence for a common carrier mediated transport of choline and basic drugs through the blood–brain barrier, *Journal of Pharmacobiodyn,* Vol.13, No.6, pp. 353–360

Kimura, S.; Egashira, K.; Chen, L.; Nakano, K.; Iwata, E.; Miyagawa, M.; Tsujimoto, H.; Hara, K.; Morishita, R.; Sueishi, K.; Tominaga, R. & Sunagawa, K. (2009). Nanoparticle-mediated delivery of nuclear factor {kappa}B decoy into lungs ameliorates monocrotaline-induced pulmonary arterial hypertension, *Hypertension, 2009 May;53(5):877-83*

Knight, V.; Koshkina, N.; Waldrep, C.; Giovanella, B.C.; Kleinerman, E. & Gilbert, B. (2000). Anti-cancer activity of 9-nitrocamptothecin liposome aerosol in mice. *Transactions of the American Clinical and Climatological Association*, Vol.11, pp. 135–145

Koziara, J.M.; Lockman, P.R.; Allen, D.D. & Mumper, R.J. (2004). Paclitaxel nanoparticles for the potential treatment of brain tumors, *Journal of Controlled Release*, Vol.99, pp. 259–269

Kreuter, J. (2001). Nanoparticulate systems for brain delivery of drugs, *Advanced Drug Delivery Reviews*, Vol.47, pp. 65–81

Kreuter, J.; Alyautdin, R.N.; Kharkevich, D.A. & Ivanov, A.A. (1995). Passage of peptides through the blood–brain barrier with colloidal polymer particles (nanoparticles), *Brain Research*, Vol.674, No.1, pp. 171–174

Kreuter, J.; Petrov, V.E.; Kharkevich, D.A. & Alyautdin, R.N. (1997). Influence of the type of surfactant on the analgesic effects induced by the peptide dalargin after its delivery across the blood–brain barrier using surfactant-coated nanoparticles, *Journal of Controlled Release*, Vol.49, No.1, pp. 81–87

Kumagai, A.K.; Eisenberg, J.B. & Pardridge, W.M. (1987). Absorptive-mediated endocytosis of cationized albumin and a b-endorphin-cationized albumin chimeric peptide by isolated brain capillaries. Model system of blood–brain barrier transport, *Journal of Biological Chemistry*, Vol.262, No.31, pp. 15214–15219

Kuo, Y.C. & Chen, H.H. (2006). Effect of nanoparticulate polybutylcyanoacrylate and methylmethacrylate-sulfopropylmethacrylate on the permeability of zidovudine and lamivudine across the in vitro blood-brain barrier, *International Journal of Pharmaceutics*, Vol.327, pp. 160-169

Kuo, Y.C. & Su, F.L. (2007). Transport of stavudine, delavirdine, and saquinavir across the blood-brain barrier by polybutylcyanoacrylate, methylmethacrylate-sulfopropylmethacrylate, and solid lipid nanoparticles, *Journal of Pharmaceutics,* Vol.340, No.1–2, pp. 143–152

Kuo, Y.C. (2005). Loading efficiency of stavudine on polybutylcyanoacrylate and methylmethacrylate-sulfopropylmethacrylate copolymer nanoparticles, *International Journal of Pharmaceutics,* Vol.290, No.1–2, pp.161–172

Kurihara, A. & Pardridge, W.M. (1999). Imaging brain tumors by targeting peptide radiopharmaceuticals through the blood–brain barrier, *Cancer Research*, Vol.59, No.24, pp. 6159–6163

Laakkonen, P.; Porkka, K.; Hoffman, J.A. & Ruoslahti, E. (2002). A tumor-homing peptide with a targeting specificity related to lymphatic vessels, *Nature Medicine*, Vol.8, pp. 751–755

Lai, S.K.; Fu, J.; Man, S.T. & Hanes, J. (2005). Doxorubicin-loaded transferrin-targeted polymeric micelles rapidly enter cancer cells and accumulate near the cell nucleus. AIChE Annual Meeting, Conference Proceedings, pp. 14370

Lam, W.; Leung, C.H.; Chan, H.L. & Fong, W.F. (2000). Toxicity and DNA binding of dextran-doxorubicin conjugates in multidrug-resistant KB-V1 cells: optimization of dextran size, *Anticancer Drugs*, Vol.11, pp. 377-384

Laske, D.W.; Youle, R.J. & Oldfield, E.H. (1997). Tumor regression with regional distribution of the targeted toxin TF-CRM107 in patients with malignant brain tumors, *Nature Medicine*, Vol.3, No.12, pp. 1362–1368

Lee, R.J.; Wang, S. & Low, P.S. (1996). Measurement of endosome pH following folate receptor-mediated endocytosis, *Biochimca Biophysca Acta,* Vol.1312, No.3, pp. 237–242

Li, F.Q.; Hu, J.H.; Lu, B.; Yao, H. & Zhang, W.G. (2001). Ciprofloxacin-loaded bovine serum albumin microspheres: preparation and drug-release *in vitro*, *Journal of Microencapsulation,* Vol.18, pp. 825–829

Li, S.; Wang, A.; Jiang, W. & Guan, Z. (2008). Pharmacokinetic characteristics and anticancer effects of 5-Fluorouracil loaded nanoparticles, *BMC Cancer*, Vol.8, pp. 103

Li, X. & Chan, K.W. (1999). Transport, metabolism and elimination mechanisms of anti-HIV agents, *Advanced Drug Delivery Reviews,* Vol.39, pp. 81-103

Liu, J.; Gong, T.; Fu, H.; Wang, C.; Wang, X.; Chen, Q.; Zhang, Q.; He, Q. & Zhang, Z. (2008). Solid lipid nanoparticles for pulmonary delivery of insulin, *International Journal of Pharmaceutics*, Vol.356, pp. 333–344

Liu, Y.; Huang, L. & Liu, F. (2010). Paclitaxel Nanocrystals for Overcoming Multidrug Resistance in Cancer. *Molecular Pharmaceutics,* Vol.7, No.3, pp. 863-869

Lobenberg, R.; Maas, J. & Kreuter, J. (1998). Improved body distribution of 14C-labelled AZT bound to nanoparticles in rats determined by radioluminography, *Journal of Drug Targeting*, Vol.5, No.3, pp. 171–179

Lockman, P.R., Mumper, R.J., Khan, M.A. & Allen, D.D. (2002). Nanoparticle technology for drug delivery across the blood–brain barrier, *Drug Development and Industrial Pharmacy,* Vol.28, pp. 1–13

Lockman, P.R.; Koziara, J.M.; Mumper, R.J. & Allen, D.D. (2004). Nanoparticle surface charges alter blood-brain barrier integrity and permeability, *Journal of Drug Targeting,* Vol.12, No.9-10, pp. 635-641

Mallipeddi, R.; & Rohan, L.C. (2010). Progress in antiretroviral drug delivery using nanotechnology, *International Journal of Nanomedicine,* Vol.5, pp. 533–547

Marsh, D.; Bartucci, R. & Sportelli, L. (2003). Lipid membranes with grafted polymers: physicochemical aspects, *Biochimica Biophysica Acta – Biomembranes,* Vol.1615, pp. 33–59

Martin, F.J. (1998). Clinical pharmacology and antitumor efficacy of DOXIL (pegylated liposomal doxorubicin). In: Lasic DD, Papahadjopoulos D, editors. Medical Applications of Liposomes, New York: Elsevier Science BV, pp. 635–688

Matsumura, Y.; Gotoh, M.; Muro, K.; Yamada, Y.; Shirao, K.; Shimada, Y.; Okuwa, M.; Matsumoto. S.; Miyata, Y.; Ohkura, H.; Chin, K.; Baba, S.; Yamao, T.; Kannami, A.; Takamatsu, Y.; Ito, K. & Takahashi, K. (2004). Phase I and pharmacokinetic study of MCC-465, a doxorubicin (DXR) encapsulated in PEG immunoliposome, in patients with metastatic stomach cancer, *Annals of Oncology,* Vol.15, pp. 517-525

Miele, E.; Spinelli, G.P.; Miele, E.; Tomao, F. & Tomao, S. (2009). Albumin-bound formulation of paclitaxel (Abraxane® ABI-007) in the treatment of breast cancer, *International Journal of Nanomedicine,* Vol.4, pp. 99–105

Minko, T.; Batrakova, E.V.; Li, S.; Li, Y.; Pakunlu, R.I.; Alakhov, V.Y. & Kabanov, A.V. (2005). Pluronic block copolymers alter apoptotic signal transduction of doxorubicin in drug-resistant cancer cells, *Journal of Control Release,* Vol.105, pp. 269–278

Minshall, R.D.; Sessa, W.C. & Stan, R.V. (2003). Caveolin regulation of endothelial function, *Lung Cellular and Molecular Physiology,* Vol.285, pp. L1179–L1183

Mishra. V.; Mahor, S.; Rawat, A.; Gupta, P.N.; Dubey, P.; Khatri, K. & Vyas, S.P. (2006). Targeted brain delivery of AZT via transferrin anchored pegylated albumin nanoparticles, *Journal of Drug Targeting*, Vol.14, pp. 45-53

Moghimi, S.M.; Hunter, A.C. & Murray, J.C. (2001). Long-circulating and target-specific nanoparticles: theory to practice, *Pharmacological Reviews,* Vol.53, No.2, pp. 283-318

Moorthi, C.; Manavalan, R. & Kathiresan, K. (2011). Nanotherapeutics to Overcome Conventional Cancer Chemotherapy Limitations, *Journal of Pharmacy and Pharmaceutical Sciences,* Vol.14, No.1, pp. 67 - 77

Muckerheide, A.; Apple, R.J.; Pesce, A.J. & Michael, J.G. (1987). Cationization of protein antigens. I. Alteration of immunogenic properties, *Journal of Immunology*, Vol.138, No.3, pp. 833–837

Muller, R.H.; Mader, K. & Gohla, S. (2000). Solid lipid nanoparticles (SLN) for controlled drug delivery – a review of the state of the art, *European Journal of Pharmaceutics Biopharmaceutics,* Vol.50, pp. 161–177

Murakami, M.; Ushio, Y.; Morino, Y.; Ohta T. & Matsukado Y. (1988). Immunohistochemical localization of apolipoprotein E in human glial neoplasms, *Journal of Clinical Investigation,* Vol.82, No.1, pp. 177–188

Nagy, Z., Peters, H. & Huttner, I. (1983). Charge-related alterations of the cerebral endothelium, *Laboratory Investigation*, Vol.49, pp. 662–671

National Comprehensive Cancer Network, Clinical Practice Guidelines in Oncology: Breast Cancer v2, (2008). Available at http://www.nccn.org/professionals/physician_gls/default.asp

Naumov, G.N.; Akslen, L.A. & Folkman J. (2006). Role of angiogenesis in human tumor dormancy: animal models of the angiogenic switch, *Cell Cycle*, Vol.5, pp. 1779-1787

Newman, L.A. & Singletary, S.E. (2007). Overview of adjuvant systemic therapy in early stage breast cancer, *Surgical Clinics of North America,* Vol.87, No.2, pp. 499–509

Ng, K.; Stringer, K.A. & Cohen, Z. (1998). Alveolar macrophage cell line is not activated by exposure to polymeric microspheres, International Journal of Pharmaceutics, Vol.170, pp. 41–49

Nimesh, S.; Kumar, R. & Chandra, R. (2006). Novel polyallylamine- dextran sulfate-DNA nanoplexes: Highly efficient non-viral vector for gene delivery, *International Journal of Pharmaceutics,* Vol.320, No.12, pp. 143-149

Nishiyama, N. & Kataoka, K. (2006). Current state, achievements, and future prospects of polymeric micelles as nanocarriers for drug and gene delivery, *Pharmacological Therapeutics*, Vol.112, No.3, pp. 630–648

Olbrich, C.; Gessner, A.; Schroder, W.; Kayser, O. & Muller, R.H. (2004). Lipid-drug conjugate nanoparticles of the hydrophilic drug diminazene-cytotoxicity testing and mouse serum adsorption, *Journal of Controlled Release*, Vol.96, pp. 425–435

Pandey, R. & Khuller, G.K. (2005). Solid lipid particle-based inhalable sustained drug delivery system against experimental tuberculosis, *Tuberculosis (Edinb),* Vol.85, pp. 227–234

Pandey, R. & Khuller, G.K. (2006). Nanotechnology based drug delivery system(s) for the management of tuberculosis, *Indian Journal of Experimental Biology,* Vol.44, No.5, pp. 357-366

Pandey, R.; Sharma, A.; Zahoor, A.; Sharma, S.; Khuller, G.K. & Prasad, B. (2003). Poly(DL-lactide-co-glycolide) nanoparticle-based inhalable sustained drug delivery system for experimental tuberculosis, *Journal of Antimicrobial Chemotherapy*, Vol.52, pp. 981–986

Pappas, G.D. & Tennyson, V.M. (1962). An electron microscopic study of the passage of colloidal particles from the blood vessels of the ciliary processes and choroid plexus of the rabbit, *Journal of Cell Biology*, Vol.15, pp. 227–239

Pardridge, W.M. (1995). Transport of small molecules through the blood–brain barrier: biology and methodology, *Advanced Drug Delivery Reviews*, Vol.15, No.1–3, pp. 5–36

Pardridge, W.M. (1997). Drug delivery to the brain, *Journal of Cerebral Blood Flow & Metabolism,* Vol.17, No.7, pp. 713–731

Pardridge, W.M. (1999). Non-invasive drug delivery to the human brain using endogenous blood–brain barrier transport systems, *Pharmaceutical Science & Technology Today,* Vol.2, No.2, pp. 49–59

Pardridge, W.M. (2001). BBB-genomics: creating new openings for brain–drug targeting, *Drug Discovery Today*, Vol.6, No.8, pp. 381–383

Pardridge, W.M. (2002). Drug and gene targeting to the brain with molecular Trojan horses, *Nature Reviews Drug Discovery*, Vol.1, No.2, pp. 131–139

Pardridge, W.M.; Buciak, J.L. & Friden, P.M. (1991). Selective transport of an anti-transferrin receptor antibody through the blood–brain barrier *in vivo, Journal of Pharmacology and Experimental Therapeutics,* Vol.259, No.1, pp. 66–70

Pardridge, W.M.; Eisenberg, J. & Yang, J. (1987). Human blood–brain barrier transferrin receptor, *Metabolism,* Vol.36, No.9, pp. 892–895

Pardridge, W.M.; Triguero, D. 7 Buciak, J.L. (1990). Beta-endorphin chimeric peptides: transport through the blood–brain barrier in vivo and cleavage of disulfide linkage by brain, *Endocrinology,* Vol.126, No.2, pp. 977–984

Park, E.K.; Kim, S.Y.; Lee, S.B. & Lee, Y.M. (2005). Folate-conjugated methoxy poly(ethylene glycol)/poly(E-caprolactone) amphiphilic block copolymeric micelles for tumor-targeted drug delivery, *Journal of Controlled Release,* Vol.109, No.1–3, pp. 158–168

Park, J.H.; Kwon, S.; Nam, J.O.; Park, R.W.; Chung, H.; Seo, S.B.; Kim, I.S.; Kwon, I.C. & Jeong, S.Y. (2004). Self-assembled nanoparticles based on glycol chitosan bearing 5beta-cholanic acid for RGD peptide delivery, *Journal of Control Release*, Vol.95, pp. 579-588

Patton, J.S. & Byron, P.R. (2007). Inhaling medicines: delivering drugs to the body through the lungs, *Nature Reviews Drug Discovery*, Vol.6, pp. 67–74

Perez, C.; Sanchez, A.; Putnam, D.; Ting, D.; Langer, R. & Alonso, M.J. (2001). Poly(lactic acid)-poly(ethylene glycol) nanoparticles as new carriers for the delivery of plasmid DNA, *Journal of Controlled Release,* Vol.75, No.1-2, pp. 211-224

Poduslo, J.F. & Curran, G.L. (1996). Polyamine modification increases the permeability of proteins at the blood–nerve and blood–brain barriers, *Journal of Neurochemistry*, Vol.66, pp. 1599–1609

Ramge, P.; Unger, R.E. & Oltrogge, J.B. (2000). Polysorbate-80 coating enhances uptake of polybutylcyanoacrylate (PBCA)-nanoparticles by human, bovine, and murine primary brain capillary endothelial cells, *European Journal of Neurology,* Vol.12, pp. 1935–1940

Reese, T.S. & Karnovsky, M.J. (1967). Fine structural localization of a blood–brain barrier to exogenous peroxidase, *Journal of Cell Biology*, Vol.34, No.1, pp. 207–217

Remsen, L.G.; Trail, P.A.; Hellstrom, I.; Hellstrom, K.E. & Neuwelt, E.A. (2000). Enhanced delivery improves the efficacy of a tumor-specific doxorubicin immunoconjugate in a human brain tumor xenograft model, *Neurosurgery*, Vol.46, pp. 704–709

Richman, D.D.; Margolis, D.M.; Delaney, M.; Greene, W.C.; Hazuda, D. & Pomerantz, R.J. (2009). The challenge of finding a cure for HIV infection, *Science,* Vol.323, pp. 1304-1307

Ross, J.F.; Chaudhuri, P.K. & Ratnam, M. (1994). Differential regulation of folate receptor isoforms in normal and malignant tissues in vivo and in established cell lines. Physiologic and clinical implications, *Cancer*, Vol.73, No.9, pp. 2432–2443

Rowinsky, E.K.; Cazenave, L.A. & Donehower, R.C. (1990). Taxol: a novel investigational antimicrotubule agent, *Journal of the National Cancer Institute,* Vol.82, pp. 1247–1259

Saari, S.M.; Vidgren, M.T.; Koskinen, M.O.; Turjanmaa, V.M.; Waldrep, J.C. & Nieminen, M.M. (1998). Regional lung deposition and clearance of 99mTc-labeled beclomethasone-DLPC liposomes in mild and severe asthma, *Chest*, Vol.113, No.6, pp. 1573-1579

Saito, Y. & Wright, E.M. (1983). Bicarbonate transport across the frog choroid plexus and its control by cyclic nucleotides, *Journal of Physiology,* Vol.336, pp. 635–648

Sarkar, K. & Yang, H. (2008). Encapsulation and Extended Release of Anti-CancerAnastrozole by Stealth Nanoparticles, *Drug Delivery*, Vol.15, pp. 343–346

Satchi-Fainaro, R.; Mamluk, R.; Wang, L.; Short, S.M.; Nagy, J.A.; Feng, D.; Dvorak, A.M.; Dvorak, H.F.; Puder, M.; Mukhopadhyay, D.; Folkman, J. (2005). Inhibition of vessel permeability by TNP-470 and its polymer conjugate, caplostatin, *Cancer Cell*, Vol.7, pp. 251-261

Sauer, I.; Nikolenko, H.; Keller, S.; Abu Ajaj, K.; Bienert, M. & Dathe, M. (2006). Dipalmitoylation of a cellular uptake-mediating apolipoprotein E-derived peptide as a promising modification for stable anchorage in liposomal drug carriers, *Biochimca Biophysica Acta-Biomembranes*, Vol.1758, No.4, pp. 552–561

Saul, J.M.; Annapragada, A.; Natarajan, J.V. & Bellamkonda, R.V. (2003). Controlled targeting of liposomal doxorubicin via the folate receptor *in vitro*, *Journal of Controlled Release,* Vol.92, No.1–2, pp. 49–67

Schöler, N.; Krause, K.; Kayser, O.; Müller, R.H.; Borner, K.; Hahn, H. & Liesenfeld, O. (2001). Atovaquone nanosuspensions show excellent therapeutic effect in a new murine model of reactivated toxoplasmosis, *Antimicrobial Agents and Chemotherapy*, Vol.45, No.6, pp. 1771–1779

Schroder, U.; Sommerfeld, P.; Ulrich, S. & Sabel, B.A. (1998). Nanoparticle technology for delivery of drugs across the blood-brain barrier, *Journal of Pharmaceutical Sciences*, Vol.87, pp. 1305–1307

Seymour, L.W.; Ferry, D.R.; Kerr, D.J.; Rea, D.; Whitlock, M.; Poyner, R.; Boivin, C.; Hesslewood, S.; Twelves, C.; Blackie, R.; Schatzlein, A.; Jodrell, D.; Bissett, D.; Calvert, H.; Lind, M.; Robbins, A.; Burtles, S.; Duncan, R. & Cassidy, J. (2009). Phase II studies of polymer-doxorubicin (PK1, FCE28068) in the treatment of breast, lung and colorectal cancer, *International Journal of Oncology*, Vol.34, pp. 1629-1636

Shah, L. & Amiji, M. (2006). Intracellular delivery of saquinavir in biodegradable polymeric nanoparticles for HIV/AIDS, *Pharmaceutical Research,* Vol.23, No.11, pp. 2638–2645

Shahiwala, A. & Misra, A. (2004). Pulmonary absorption of liposomal levonorgestrel, *AAPS PharmSciTech*, Vol.5, No.1, E13

Sharma, A.; Pandey, R.; Sharma, S. & Khuller, G.K. (2004). Chemotherapeutic efficacy of pol(DL-lactide-co-glycolide) nanoparticle encapsulated antitubercular drugs at sub-therapeutic dose against experimental tuberculosis, *International Journal of Antimicrobial Agents,* Vol.24, No.6, pp. 599-604

Shmeeda, H.; Mak, L.; Tzemach, D.; Astrahan, P.; Tarshish, M. & Gabizon, A. (2006). Intracellular uptake and intracavitary targeting of folate-conjugated liposomes in a mouse lymphoma model with up-regulated folate receptors, *Molecular Cancer Therapeutics*, Vol.5, No.4, pp. 818–824

Singla, A.K.; Garg, A. & Aggarwal, D. (2002). Paclitaxel ant its formulations, *International Journal of Pharmaceutics*, Vol.235, pp. 179-192

Smith, Q.R. (2003). A review of blood–brain barrier transport techniques, *Methods in Molecular Medicine*, Vol.89, pp. 193–208

Smola, M.; Vandamme, T. & Sokolowski, A. (2008). Nanocarriers as pulmonary drug delivery systems to treat and to diagnose respiratory and non respiratory diseases, *International Journal of Nanomedicine*, Vol.3, No.1, pp. 1–19

Soni, V.; Kohli, D.V. & Jain, S.K. (2005). Transferrin coupled liposomes as drug delivery carriers for brain targeting of 5-florouracil, *Journal of Drug Targeting*, Vol.13, No.4, pp. 245–250

Sosnik, A.; Chiappetta, D.A. & Carcaboso, A.M. (2009). Drug delivery systems in HIV pharmacotherapy: what has been done and the challenges standing ahead, *Journal of Controlled Release*, Vol.138, pp. 2-15

Stark, B.; Debbage, P.; Andreae, F.; Mosgoeller, W. & Prassl, R. (2007). Association of vasoactive intestinal peptide with polymer-grafted liposomes: structural aspects for pulmonary delivery, *Biochim Biophys Acta*, Vol.1768, pp. 705–714

Steiniger, S.C.; Kreuter, J.; Khalansky, A.S.; Skidan, I.N.; Bobruskin, A.I.; Smirnova, Z.S.; Severin, S.E.; Uhl, R.; Kock, M.; Geiger, K.D. & Gelperina, S.E. (2004). Chemotherapy of glioblastoma in rats using doxorubicin-loaded nanoparticles, *International Journal of Cancer*, Vol.109, No.5, pp.759–767

Stella, B.; Arpicco, S.; Peracchia, M.T.; Desmaële, D.; Hoebeke, J.; Renoir, M.; D'Angelo, J.; Cattel, L. & Couvreur, P. (2000). Design of folic acid-conjugated nanoparticles for drug targeting, *Journal of Pharmaceutical Science*, Vol.89, No.11, pp. 1452–1464

Sudimack, J. & Lee, R.J. (2000). Targeted drug delivery via the folate receptor, *Advanced Drug Delivery Reviews*, Vol.41, pp. 147–162

Sun, W.; Xie, C.; Wang, H. & Hu, Y. (2004). Specific role of polysorbate 80 coating on the targeting of nanoparticles to the brain, *Biomaterials,* Vol.25, No.15, pp. 3065–3071

Suntres, Z.E. & Shek, P.N. (1998). Liposomes promote pulmonary glucocorticoid delivery, *Journal of Drug Targeting*, Vol.6, pp. 175–182

Svenson, S. & Tomalia, D.A. (2005). Dendrimers in biomedical applications–reflections on the field, *Advanced Drug Delivery Reviews,* Vol.57, No.15, pp. 2106-2129

Takeuchi, H.; Yamamoto, H. & Kawashima, Y. (2001). Mucoadhesive nanoparticulate systems for peptide drug delivery, *Advanced Drug Delivery Reviews,* Vol.47, pp. 39–54

Taylor, K.M.G. & Farr, S.J. (1993). Liposomes for drug delivery to the respiratory tract, *Drug Development and Industrial Pharmacy*, Vol.19, pp. 123–142

Tedeschi, G.; Lundbom, N.; Raman, R.; Bonavita, S.; Duyn, J.H.; Alger, J.R. & Di Chiro, G. (1997). Increased choline signal coinciding with malignant degeneration of cerebral gliomas: a serial proton magnetic resonance spectroscopy imaging study, *Journal of Neurosurgery,* Vol.87, No.4, pp. 516–524

Terzano, C.; Allegra, L.; Alhaique, F.; Marianecci, C. & Carafa, M. (2005). Non phospholipids vesicles for pulmonary glucocorticoid delivery, *European Journal of Pharmaceutics and Biopharmaceutics*, Vol.59, pp. 57–62

Thole, M.; Nobmanna, S.; Huwyler, J.; Bartmann, A. & Fricker G. (2002). Uptake of cationzied albumin coupled liposomes by cultured porcine brain microvessel endothelial cells and intact brain capillaries, *Journal of Drug Targeting*, Vol.10, No.4, pp. 337–344

Tomalia, D.A; Reyna, L.A. & Svenson, S. (2007). Dendrimers as multi-purpose nanodevices for oncology drug delivery and diagnostic imaging, *Biochemical Society Transactions*, Vol.35, (Pt 1), pp. 61–67

Torchilin, V.P. (2005). Recent advances with liposomes as pharmaceutical carriers, *Nature Reviews Drug Discovery,* Vol.4, pp. 145-159

Triguero, D.; Buciak, J. & Pardridge, W.M. (1990). Capillary depletion method for quantification of blood–brain barrier transport of circulating peptides and plasma proteins, *Journal of Neurochemistry*, Vol.54, No.6, pp. 1882–1888

Tsukamoto, H.; Boado, R.J. & Pardridge, W.M. (1996).Differential expression in glioblastoma multiforme and cerebral hemangioblastoma of cytoplasmic proteins that bind two different domains within the 30-untranslated region of the human glucose transporter 1 (GLUT1) messenger RNA, *Journal of Clinical Investigation,* Vol.97, No.12, pp. 2823–2832

Umezawa, F. & Eto, Y. (1988). Liposome targeting to mouse brain: mannose as a recognition marker, *Biochemical and Biophysical Research Communications,* Vol.153, No.3, pp. 1038–1044

Uner, M. & Yener, G. (2007). Importance of solid lipid nanoparticles (SLN) in various administration routes and future perspectives. *International Journal of Nanomedicine,* Vol.2, No.3, pp. 289-300

Vaishampayan, U.; van Zuylen, L.; Verweij, J. & Sparreboom, A. (2001). Role of formulation vehicles in taxane pharmacology, *Investigational New Drugs,* Vol.19, pp. 125–141

Valle, J.W.; Armstrong, A.; Newman, C.; Alakhov, V.; Pietrzynski, G.; Brewer, J.; Campbell, S.; Corrie, P.; Rowinsky, E.K. & Ranson, M.: (2010). A phase 2 study of SP1049C, doxorubicin in P-glycoprotein-targeting pluronics, in patients with advanced adenocarcinoma of the esophagus and gastroesophageal junction, *Investigational New Drugs,* 2010. [Epub ahead of print]

Veronese, F.M. & Pasut, G. (2005). PEGylation, successful approach to drug delivery, *Drug Discovery Today*, Vol.10, pp. 1451-1458

Videira, M.A.; Gano, L.; Santos, C.; Neves, M. & Almeida, A.J. (2006). Lymphatic uptake of lipid nanoparticles following endotracheal administration, *Journal of Microencapsulation*, Vol.23, pp. 855–862

Vinogradov, S.V.; Bronich, T.K. & Kabanov, A.V. (2002). Nanosized cationic hydrogels for drug delivery: preparation properties and interactions with cells, *Advanced Drug Delivery Reviews,* Vol.54, pp. 135-147

Vorbrodt, A.W., Lossinsky, A.S., Dobrogowska, D.H. & Wisniewski, H.M. (1990). Sequential appearance of anionic domains in the developing blood–brain barrier, *Brain Research. Developmental Brain Research*, Vol.52, pp. 31–37

Vyas, S.P. & Sihorkar, V. (2000). Endogenous carriers and ligands in non-immunogenic site-specific drug delivery, *Advanced Drug Delivery Reviews*, Vol.43, No.2–3, pp. 101–164

Wahl, M.; Schilling, L.; Unterberg A. & Baethmann A. (1993). Mediators of vascular and parenchymal mechanisms in secondary brain damage, *Acta Neurochir Suppl*, Vol.57, pp. 64–72

Waldrep, J.C.; Gilbert, B.E.; Knight, C.M.; Black, M.B.; Scherer, P.W.; Knight, V. & Eschenbacher, W. (1997). Pulmonary delivery of beclomethasone liposome aerosol in volunteers, *Chest*, Vol.111, pp. 316–323

Wall, D.A. (1995). Pulmonary absorption of peptides and proteins, *Drug Delivery*, Vol.2, pp. 1-20

Wang, A.Z.; Gu, F.; Zhang, L.; Chan, J.M.; Radovic-Moreno ,A.; Shaikh, M.R. & Farokhzad, O.C. (2008). Biofunctionalized targeted nanoparticles for therapeutic applications, *Expert Opinion on Biological Therapy*, Vol.8, No.8, pp. 1063-1070

Wang, Y.; Yu, L.; Han, L.; Sha, X. & Fang, X. (2007). Difunctional Pluronic copolymer micelles for paclitaxel delivery: Synergistic effect of folate-mediated targeting and Pluronic-mediated overcoming multidrug resistance in tumor cell lines, *International Journal of Pharmaceutics*, Vol.337, pp. 63–73

Weidner, N.; Folkman, J.; Pozza, F.; Bevilacqua, P.; Allred, E.N.; Moore, D.H.; Meli, S. & Gasparini, G. (1992). Tumor angiogenesis: a new significant and independentprognostic indicator in early-stage breast carcinoma, *Journal of the National Cancer Institute*, Vol.84, pp. 1875-1887

Weidner, N.; Semple, J.P.; Welch, W.R. & Folkman J. (1991). Tumor angiogenesis and metastasis– correlation in invasive breast carcinoma, *The New England Journal of Medicine*, Vol.324, pp. 1-8

Weitman, S.D.; Lark, R.H.; Coney, L.R.; Fort, D.W.; Frasca, V.; Zurawski, V.R. Jr & Kamen, B.A. (1992). Distribution of the folate receptor GP38 in normal and malignant cell lines and tissues, *Cancer Research*, Vol.52, No.12, pp. 3396–3401

Wiedenmann, N.; Valdecanas, D.; Hunter, N.; Hyde, S.; Buchholz, T.A.; Milas, L. & Mason, K.A. (2007). 130-nm albumin-bound paclitaxel enhances tumor radiocurability and therapeutic gain, *Clinical Cancer Research*, Vol.13, No.6, pp. 1868–1874

Wolburg, H.; Wolburg-Buchholz, K.; Liebner, S. & Engelhardt B. (2001). Claudin-1, claudin-2 and claudin-11 are present in tight junctions of choroid plexus epithelium of the mouse, *Neuroscience Letters*, Vol.307, No.2, pp. 77–80

World Health Organization. Cancer. (2009). http://www.who.int/mediacentre/factsheets/fs297/en/index.html.

Wu, D. & Pardridge, W.M. (1999). Blood–brain barrier transport of reduced folic acid, *Pharmaceutical Research*, Vol.16, No.3, pp. 415–419

Xiang, G.; Wu, J.; Lu, Y.; Liu, Z. & Lee, R.J. (2008). Synthesis and Evaluation of a Novel Ligand for Folate-mediated Targeting liposomes, *International Journal of Pharmacology,* Vol.356, No.1-2, pp. 29-36

Xu, Y.; Wen, Z. & Xu, Z. (2009). Chitosan nanoparticles inhibit the growth of human hepatocellular carcinoma xenografts through an antiangiogenic mechanism, *Anticancer Research*, Vol.29, pp. 5103-5109

Yang, C.S.; Lu, F.L. & Cai, Y. (1999). Body distribution in mice of intravenously injected camphotothericin solid lipid nanoparticles and targeting eect on the brain, *Journal of Controlled Release*, Vol.59, pp. 299–307

Yang, W.; Peters, J.I. & Williams, R.O. (2008). Inhaled nanoparticles–a current review, *International Journal of Pharmacology,* Vol.356, pp. 239–247

Yoo, H.S. & Park, T.G. (2004). Folate receptor targeted biodegradable polymeric doxorubicin micelles, *Journal of Controlled Release,* Vol.96, No.2, pp. 273–283

Zahoor, A.; Sharma, S. & Khuller, G.K. (2005). Inhalable alginate nanoparticles as antitubercular drug carriers against experimental tuberculosis, *International Journal of Antimicrobial Agents,* Vol.26, pp. 298–303

Zeng, X.M.; Martin, G.P. & Marriott, C. (1995). Preparation and in vitro evaluation of tetrandrine-entrapped albumin microspheres as an inhaled drag delivery system, *European Journal of Pharmaceutical Sciences*, Vol.3, pp. 87–93

Zhang, C.; Zhao, L.; Dong, Y.; Zhang, X.; Lin, J. & Chen, Z. (2010). Folate-mediated poly (3-hydroxybutyrate-co-3-hydroxyoctanoate) nanoparticles for targeting drug delivery. *European Journal of Pharmaceutics and Biopharmaceutics,* Vol.76, No.1, pp. 10-16

Zhang, J.A.; Anyarambhatla, G.; Ma, L.; Ugwu, S.; Xuan, T.; Sardone, T. & Ahmad, I. (2005). Development and characterization of a novel Cremophor® EL free liposome-based paclitaxel (LEP-ETU) formulation, *European Journal of Pharmaceutics and Biopharmaceutics*, Vol.59, No.1, pp. 177-187

Zhang, L. & Granick, S. (2006). How to stabilize phospholipid liposome (using nanoparticles), *Nano Letters,* Vol.6, pp. 694-698

Zhao, R.; Seither, R.; Brigle, K.E.; Sharina, I.G.; Wang, P.J. & Goldman, I.D. (1997). Impact of overexpression of the reduced folate carrier (RFC1), an anion exchanger, on concentrative transport in murine L1210 leukemia cells, *Journal of Biological Chemistry*, Vol.272, No.34, pp. 21207–21212

Zhuo, W.; Luo, C.; Wang, X.; Song, X.; Fu, Y. & Luo, Y. (2010). Endostatin inhibits tumour lymphangiogenesis and lymphatic metastasis via cell surface nucleolin on lymphangiogenic endothelial cells, *Journal of Pathology*, Vol.222, No.3, pp. 249–260



Drug Discovery and Development - Present and Future

Edited by Dr. Izet Kapetanović

ISBN 978-953-307-615-7
Hard cover, 528 pages
Publisher InTech
Published online 16, December, 2011
Published in print edition December, 2011

Drug discovery and development process aims to make available medications that are safe and effective in improving the length and quality of life and relieving pain and suffering. However, the process is very complex, time consuming, resource intensive, requiring multi-disciplinary expertise and innovative approaches. There is a growing urgency to identify and develop more effective, efficient, and expedient ways to bring safe and effective products to the market. The drug discovery and development process relies on the utilization of relevant and robust tools, methods, models, and validated biomarkers that are predictive of clinical effects in terms of diagnosis, prevention, therapy, and prognosis. There is a growing emphasis on translational research, a bidirectional bench to the bedside approach, in an effort to improve the process efficiency and the need for further innovations. The authors in the book discuss the current and evolving state of drug discovery and development.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Sadhna Sharma and Amandeep Singh (2011). Nanotechnology Based Targeted Drug Delivery: Current Status and Future Prospects for Drug Development, Drug Discovery and Development - Present and Future, Dr. Izet Kapetanović (Ed.), ISBN: 978-953-307-615-7, InTech, Available from: http://www.intechopen.com/books/drug-discovery-and-development-present-and-future/nanotechnology-based-targeted-drug-delivery-current-status-and-future-prospects-for-drug-development

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2011 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the Creative Commons Attribution 3.0 License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen