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Recent Progress in Drug Delivery Systems for Anticancer Agents

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Recent progress in understanding the molecular basis of cancer brought out new materials such as oligonucleotides, genes, peptides and proteins as a source of new anticancer agents. Due to their macromolecular properties, however, new strategies of delivery for them are required to achieve their full therapeutic efficacy in clinical setting. Development of improved dosage forms of currently marketed anticancer drugs can also enhance their therapeutic values. Currently developed delivery systems for anticancer agents include colloidal systems (liposomes, emulsions, nanoparticles and micelles), polymer implants and polymer conjugates. These delivery systems have been able to provide enhanced therapeutic activity and reduced toxicity of anticancer agents mainly by altering their pharmacokinetics and biodistribution. Furthermore, the identification of cell-specific receptor/antigens on cancer cells have brought the development of ligand- or antibody-bearing delivery systems which can be targeted to cancer cells by specific binding to receptors or antigens. They have exhibited specific and selective delivery of anticancer agents to cancer. As a consequence of extensive research, clinical development of anticancer agents utilizing various delivery systems is undergoing worldwide. New technologies and multidisciplinary expertise to develop advanced drug delivery systems, applicable to a wide range of anticancer agents, may eventually lead to an effective cancer therapy in the future.

Key words: Drug delivery system, Anticancer Agents, Targeted delivery system

INTRODUCTION

While extensive efforts have been made for the treatment of patients with cancer, cancer is still the first or second leading cause of death in many countries. In addition to the surgical removal and radiation therapy, cancer therapies use a wide range of chemotherapeutic agents. However, in many cases, the effectiveness of cancer therapy is limited by rapid removal of anticancer agents from the circulation by metabolism or excretion, inability to access and penetrate the target cancer cells/tissues, or nonspecific uptake by sensitive normal cells and tissues.

During the past few decades, great strides have been made in understanding the molecular basis of cancer. They brought out new materials such as oligonucleotides, genes, peptides and proteins as a source of potential new cancer therapeutics. The intrinsically large size and extreme hydrophilicity/hydophobicity of most of these new therapeutic agents, however, often limits their *in vivo* utility, due to undesirable biodistribution and poor delivery to the intended sites. Therefore, delivery systems adequate for these new therapeutic materials should be developed to achieve their full efficya in clinical setting. In addition to, development of improved dosage forms of currently marketed anticancer drugs may be able to enhance their therapeutic values. Extensive efforts in DDS field already led to the commercialization of new formulations of some of anticancer agents (Table 1). This article will review the current status of development of delivery systems and the challenges for developing improved drug delivery systems (DDS) for anticancer agents in the future.

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Physiological Considerations In Delivery of Anitcancer Agents

Generally, ideal DDS should be able to direct drugs

Table I. Status of clinical development of anticancer drugs using DDS technology

Preferred name	Trade name	DDS	Company	Status	Action
Zinostatin stimalamer	SMANCS	Polymer conjugate	Yamanouchi; Kayaky; Pola	Marketed	
Carmustine Wafer Implants	Gliadel®				
Depofoam Cytarabine	Depocyt™	Lipid vesicle	Skyepharma; Pharmacia; Paladin; Chiron	Marketed	Antimetabolite; nucleoside analogue
DDS, fluorouracil		Collagen-based system	Matrix; Dompe; Savage; Altana	Marketed	Antimetabolite
Liposomal doxorubicin	Doxil™	Liposome	Alza; Schering Plough	Marketed	Antibiotic; anthracycline; DNA intercalator
Triptorelin pamoate	Trelstar™	Biodegradable microgranule	Debio Recherche	Registered	Gonadotropin inhibitor
HIT diclofenac		Gel matrix	Skyepharma; Meditech Res.; Bioglan; Faulding	Registered	NSAID; analgesic
Liposomal doxorubicin	Myocet™	Liposome	Liposome Company; Elan; Pfizer	Registered	Antibiotic; anthracycline; DNA intercalator

Table II. Comparision of colloidal drug delivery systems for anticancer agents

Property	Liposomes	(Lipid) Emulsions	SLN	Polymeric nanoparticle
Systemic toxicity	Low	Low	Low	Realtively high
Cytoxocity	Low	Low	Low	Realtively high
Residues from organic solvents	depending on the manufacturing method	No	No	Ralatively high
Large scale production	Possible	Possible	Possible	?
Autoclaving	Possible	Possible	Possible	?
Controlled release	Sometimes possible	No	Possible	Possible
Stability in solution	Low	High	High	High
Examples of applications in chemotherapy	Paclitaxel (Ceruti, 2000) Doxorubicin (Hong, 2001) Methotrexate (Kim and Han, 1995) Cytarabine (Koller-Luwe, 1999) Daunorobucin(Forsen, 1997) Vincristin (Kanter, 1994) Cysplatin(Harrington, 2000)	Paclitaxel (Lundberg, 1997; Constantinides, 2000) Doxorubicin (Yi, 1998) Methotrexate (Trotta, 1996) Epirubicin (Ichida, 1994)	Paclitaxel (Miglietta, 2000) Doxorubicin (Fundaro, 2000) Camptothecin (Yang, 1999)	Camptothecin (Ertl, 1999) Bleomycin (Sludden, 2000) Paclitaxel (le Visage, 2000) Tamoxifen (Brigger, 2000) 5-Fluorouracil (Chandy, 2000)

exclusively to their desired sites of action, with minimal toxic exposure to sensitive non-target tissues. The delivery system itself should be pharmacologically inactive with minimal toxicity and biodegradable to be able to meta-bolized and cleared from the circulation once its carrier function has been completed.

The vascular permeability of cancer tissue is a significant physiological factor that should be considered in designing delivery systems in terms of carriers ability to extravasate and gain access to target tissue. Neovasculature formed in cancer tissues tends to be poorly formed, with gaps between the endothelial cells. This abnormal blood vessel structure allows the ready extravasation of particulate (<100 nm diameter) and their entrapment in the surrounding tissue matrix. In this regard, colloidal delivery systems with small diameter (<100 nm) have been extensively exploited for passive targeting to solid tumors by many investigators, as will be discussed in following section.

Colloidal Delivery Systems

Encapsulation of therapeutic agents inside of carriers with colloidal size is one of the most attractive strategies in cancer therapy (Kim *et al.*, 2001). Colloidal delivery systems have achieved improved therapeutic indices primarily through physical means: 1) by retaining anticancer drugs within carrier while in circulation, thus minimizing uptake by sensitive normal tissues 2) by selectively extravasating into target tissues.

Colloidal delivery systems include liposomes, (micro) emulsions, solid lipid nanoparticles, polymeric particles and polymeric micelles (Kim *et al.*, 1994). The characteristics of various colloidal delivery systems were compared as shown in Table 2.

Liposomes

Over the years, liposomes have gained extensive attention as carriers for a wide range of drugs in the category of colloidal carrier systems. It is mainly due to their

biodegradability and flexibility in structure, which allows the easy manipulation of *in vivo* fate of drugs by changing the composition of liposomes (Kim and Han, 1995; Zou *et al.*, 1995). Liposomal encapsulation has shown to reduce toxicity of conventional as well as newly introduced anticancer agents such as antisense deoxynucleotides (Koncoh *et al.*, 2000), while retaining or even enhancing their efficacy (van Slooten *et al.*, 2001).

The rapid recognition and clearance of liposomes themselves, however, limited the usefulness of liposomes as drug carriers, by the reticuloendothelial system (RES) from blood stream. Recently, the inclusion of glucuronate (Oki 1999) and polyethylene glycol (Torchilin, 1998) in liposomal bilayers has shown to reduced RES uptake and thus prolonged duration of liposomes in the circulatory system (Woodle, 1998). liposomes having a long-circulating character showed further advantage for passive targeting to tumor tissues by extravasation to more leaky vasculature in tumor tissues (Gabizon *et al.*, 1997).

Two decades of research in liposome field led to the development and commercialization of liposomal daunorobicin, cytarabine and doxorubicin as shown in Table 1.

Currently, a number of clinical trials are undergoing worldwide with liposomal formulations of drugs (paclitaxel (Pharmacia corporation), lurtotecan (NX 211) (Gilead Sciences), platinum (Aroplatin™, Aronex Pharmaceuticals), vincristine (OncoTCS™, INEX Pharmaceutical Corp.), genes (tgDCC-E1A, Targeted Genetics; Allovectin™, Vical), antisense molecules and immunomodulating molecules such as interleukin, interferon gamma, muramyltripeptide (AC⁻ Jenner Biotherapies), vaccines (Prostatecancer Vaccine™; Novasome®, Novanex Inc.) and proteins (DNA repair enzyme (Dimericine™, AGI Dermatics Inc.).

Emulsions/Solid lipid nanoparticles

Emulsions which have oil core, in contrast to the aqueous core of liposomes (Fig. 1), can provide formulations for poorly water-soluble agents with improved therapeutic efficacy and reduced toxicity (Higashi *et al.*, 1999). In addition to the chemotherapeutic drugs as listed in Table 2, emulsion systems were employed for the incorporation of protoporphyrin IX (De Rosa *et al.*, 2000), prostaglandin (Fukushima *et al.*, 2000) and 1,25-dihydroxyvitamin D3 (Finlay *et al.*, 2000). Emulsified perfluboron-based contrast agent for imaging to detect solid tumors, known as Imavist™, has recently received approvable status from the FDA in United States.

Clinical trials are undergoing with emulsion formulation of taxol (S-8184®, Sonus Pharmaceutical). Lipid-coated microbubbles, named Filmix®, could be employed effectively for cancer diagnosis by enhanced ultrasound-image and MRI contrast, cavitation-therapy of tumors, and especially for targeted-delivery of anticancer drugs to tumors.

Recently, solid lipid nanoparticles that have solid lipid core in contrast the oil core of emulsions (Fig. 1) have been given interest as a novel delivery system. Their major advantage as a delivery system is the possibility of freeze-drying, autoclaving and large-scale production. They can also be loaded with poorly water-soluble anticancer agents very efficiently. Furthermore, owing to the solid core, they can provide controlled release of drugs for extended period. They have been employed for the delivery of anticancer agents as shown in Table 2.

Polymeric micro/nanospheres

The biological properties of polymeric micro/nanospheres can be easily controlled by modification of physicochemical properties of polymers. Micro/nanospheres prepared from polymers such as poly(lactide-co-glycolide) (PLGA), polyvinylpyrrolidone (PVP) and poly (ε-caprolactone) (PCL) are one of the most extensively investigated delivery systems. These delivery systems allowed the entrapped anticancer agents to be controlled released form the polymers and provided greater or longer *in vitro* and *in vivo* antitumor activity compared with free drugs.

Polymeric micro/nanoparticles have been employed for many kinds of chemotherapeutic drugs (Table 1) as well as newly-introduced anticancer agents such as cytokines (Egilmez *et al.*, 2000; Maheshwari *et al.*, 2000), retinoic

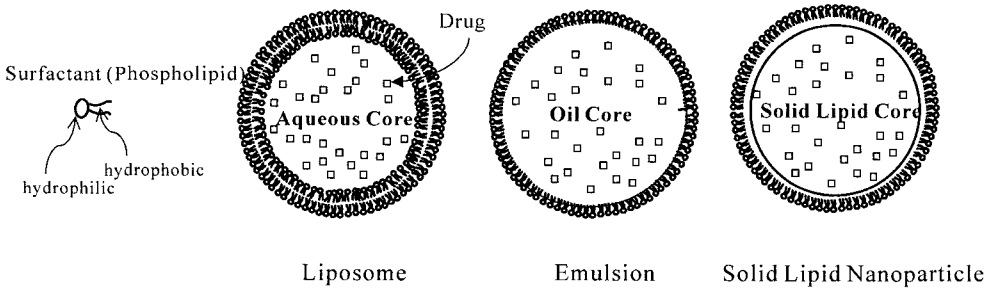

Fig. 1. Schematic diagram of polymeric micelle formation from diblock polymers

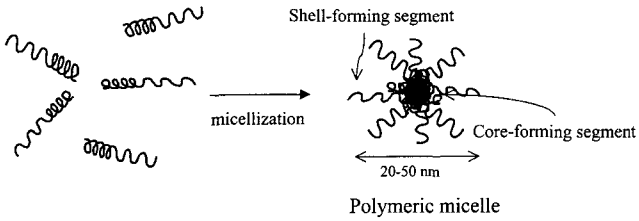

Fig. 2. Comparison of structure of various colloidal delivery systems

acid (Choi *et al.*, 2001) and oligonucleotide (Delie *et al.*, 2001).

Clinical trials are undergoing with microsphere-encapsulated Her-2/neu vaccine in patients with breast, ovarian and lung cancer. Polymerized nanoparticles such as Targesome® (Targesome Inc.) could enhance the targeted delivery of integrin antibody (Vitaxin™, Medimmune Inc.) to blood vessels.

Dendrimers are a new class of polymers characterized by their highly branched, tree-like structures (Bielinska *et al.*, 1996). The ability to act as unimolecular micelles of dendrimers suggests a drug carrier function with the surface groups available for targeting, and thus self-assembling dendrimeric drug delivery systems may selectively accumulate in tumor tissues.

Polymeric micelle

Biodegradable polymeric micelles containing drugs in the core region can be prepared from di-block copolymers (Fig. 2). Polymeric micelles are considerably more stable than surfactant micelles and can solubilize substantial amounts of hydrophobic compounds in their inner core. Drugs can be coupled to (Yoo and Park, 2001) or physically incorporated within the hydrophobic core of polymeric micelles (Kataoka *et al.*, 2000) (Fig. 2). Due to their hydrophilic shell and small size, they sometimes exhibit prolonged circulation times *in vivo* and can accumulate in tumoral tissues (Pechar *et al.*, 2000). Poly (DL-lactide)-block-methoxy polyethylene glycol diblock copolymers and N-lauryl-carboxymethylchitosan were used as micellar carriers for solubilization of paclitaxel (Zhang *et al.*, 1996; Miwa *et al.*, 1998).

Implanted systems

Implantation of anticancer drugs, in general, can provide prolonged release of drugs from the implants in local area, thus reducing systemic toxicity. Implanted delivery system with cross-linked gelatin as the biodegradable matrix material could improve the targeting of doxorubicin as well as sustain the rate of release of the drug to the bone cancer (Fan and Dash, 2001). Another example is polymer bis (p-carboxyphenoxy) propane-sebacic acid implant for cispatin (Yapp *et al.*, 1998). Implantation of drugs in ethylne-vinyl acetate polymer matrix for the chemotherapy of brain tumor reduced the systemic toxicity after bolus injection of drug (Wang *et al.*, 1999). Biodegradable polymer implant of paclitaxel (Paclimer™, Guilford Tec.) and Leuprolide (LeuPromaxx™) could provide site-specific and controlled delivery of drugs, and they are at the stage of clinical trials.

Gel-type delivery systems allow the drug to be injected as a liquid, but then solidify at body temperature and thereby release drugs slowly for extended periods. Gel formulation of cispatin/epinephrine (Intradose™, Matrix Pharmaceutical) could retain high concentrations of potent chemotherapeutic drug inside tumors for extended periods and have recently being filed for New Drug Application. Intratumoral injection of gel formulation of fluorourcail/epinephrine (Smith *et al.*, 1999) and paclitaxel (Oncogel™, Macromed) could also provide a localized depot which keeps the drug at the tumor site for an extended period of time.

Drug-conjugated delivery systems

Polymer conjugates

Drug-polymer conjugates are potential candidates for the preferential uptake/delivery of anticancer agents to tumor tissues (Ciaolfa *et al.*, 2000) through the enhanced permeability and retention through tumor vasculature. Generally, drugs should be liberated from polymers to exhibit their antitumor activity, but in some cases drug conjugates themselves are pharmacologicall active, depending on the characateristics of conjugation and polymers. Incorporating acid-sensitive bonds between the drug and the polymer ensures the effective release of polymer-bound drug at the tumor site.

Various biodegradable polymers have been evaluated for conjugation. They include copolymers of polylactic and polyglycolic acids, polylactic acid, poly(orthoesters), polyanhydrides, poly(E-caprolactone) and polyurethanes.

Hydrophilic and biocompatible N-(2-hydoxypropyl)methacrylamide (HPMA) copolymer conjugate with anticancer drugs such as taxol, doxorubicin and camptothecin, enhanced their therapeutic efficacy while reducing toxicity of drugs in animal models (Ciaolfa *et al.*, 2000; Minko *et al.*, 2000). The higher anticancer efficacy of HPMA copolymer conjugate with anticancer drugs even *in vitro* suggests a different mechanism of action from free drugs (Minko *et al.*, 2000). The decrease in toxicity of conjugated drugs may be mainly attributed to the change in body distribution (Noguchi *et al.*, 1998).

Polyethyleneglycol (PEG) conjugation with anticancer proteins or small molecules also enhanced the therapeutic value of those anticancer agents by offering extended circulation life, lower toxicity, increased drug stability and solubility (Conover *et al.*, 1999; Pechar *et al.*, 2000). PEG-

Table II. Clinical development of anticancer drugs using targeted delivery systems

Technology	Applied drug	Company	Target	Means for targeting	Status
TAPET (Tumor Amp-lified Protein expression technology)	Anticancer drugs	Vion Pharm.	Tumor tissue	Use of genetically altered strain of salmonella bacteria as a vehicle	Clinical trial
Skeleta Targeted Radio-therapy (holidurn-166)	Radiotherapy	NeoRx Corp.	Bone and marrow	Bindign of Holidium to tumor in the bone	Clinical trial
Cerepo.t™ (lobradi nil)	Carboplatin	Alkermes	Brain cancer	Transient increase in the permeability of the blood-brain barrier	Clinical trial
Folatescan	Diagnostic imaging agent	Endocyte	Ovarian cancer	Folate ligand for folate receptor	Clinical trial
Dox-HER2/neu	Doxorubicin		Breast cancer	Antibody for Her2/neu antigen	Clinical trial
HuN90'-DM1	Tumor activated prodrug		Small cell lung cancer	Antibody for CD56 antigen	Clinical trial
LP 23)'®		Avanir Pharm.	melanoma	Antibody for melanoma-specific antigen	Clinical trial
AntiCD20 chimeric Ab conjugate to yttirium 90	Radiation therapy		Non-Hodgkins lymphoma	Antibody for CD20 antigen	Clinical trial
Trova:T M	Gene vaccine	Biomedica	Colorectal cancer	TAA (Tumor-associated antigen)	Clinical trial
MTC	Doxorubicin	FeRx Inc.	Liver cancer	Magnetic targeted carrier	Clinical trial
Drug-Penetratin conjugate	Anticancer drug	Cyclacel Limited	nucleus	Penetratin	Clinical trial

attached camptothecin (PROTHECAN™, Enzon), paclitaxel (PEG-paclitaxel, Enzon), interferon (PEGINTRON, Enzon) and G-CSF (Roche Holding) are examples of PEG-enhanced version undergoing clinical trials.

Dextran conjugation selectively accumulated platinum complex (II) (Ichinose *et al.*, 2000) and epidermal growth factor (Bue *et al.*, 2000) in the cancer tissue.

Polyglutamate conjugates of paclitaxel and camptothecin are under the clinical trial by the name of PG-TXL™ and PG-CPT™ by Cell Therapeutics.

Conjugation of water-insoluble cancer chemotherapeutic drugs to macromolecular polymers SMANCS™ (Table 1) could also improve the therapeutic indices due to accumulation of the polymer-drug conjugate in tumor tissue. Pyran copolymer-drug conjugate showed a marked reduction in bone marrow toxicity normally associated with the use of drug.

Protein conjugates

Albumin-, globulin- and other protein-based drug carrier systems have been developed in the field of chemotherapy to improve the passive tumor targeting properties of anti-cancer drugs, by their macromolecular sizes. The anticancer efficacy of methotrexate, doxorubicin and invasion-inhibiting factor 2 were significantly enhanced by albumin conjugation (Lim *et al.*, 1996; Han *et al.*, 1999). Antibody- or transferrin- conjugated drugs have also been developed for targeted delivery to antigen-presenting cancer cells, as will be discussed in following section.

Combined delivery systems

Recently, combinations of different delivery systems have been attempted to further improve the therapeutic value of anticancer agents. Use of nanoparticle, temperature-sensitive liposomes or HPMA copolymer with mild hyperthermia could considerably increase the delivery of drug to the cancer cells (Kong *et al.*, 2000). Photodynamic therapy together with HPMA copolymer-doxorubicin conjugates also enhanced the efficacy of doxorubicin *in vivo* (Lu *et al.*, 1999).

GDEPT (Gene directed enzyme/prodrug therapy) is one of the novel approach to selectively eradicate cancer cells by delivering a gene that encodes an enzyme which is able to convert prodrug into a potent cytotoxin (Greco and Dachs, 2001). Enzyme/Prodrug combination such as Herpes simplex 1 virus thymidine kinase/ganciclovir and cytosine deaminase/5-fluorocytosine combination is under clinical trial now.

Nonviral or viral gene delivery systems, recently reviewed by us (Kim *et al.*, 2001), exhibited higher DNA transfection efficiency when combined with each other. Examples are virus vector in solid-state vehicles (Siemens *et al.*, 2000), polymer-coated virus to evade neutralizing antibodies (Fisher *et al.*, 2001), Virosome (virus and liposome) (Kaneda, 2000) and Eepstein-barr virus-based plasmid vectors combined with polyamidoamine dendrimer (Harada *et al.*, 2000). Delivery of plasmid DNA adsorbed on cationic microparticles led to enhanced target gene expression and antigen presentation by dendritic cells (Denis-Mize *et al.*, 2000).

Targeted delivery systems

Targeted delivery of anticancer drugs to tumor cells/tissues can improve the therapeutic index of drugs by minimizing the toxic effects of them on healthy cells/tissues. Cell- or tissue- specific receptor/antigens can provide

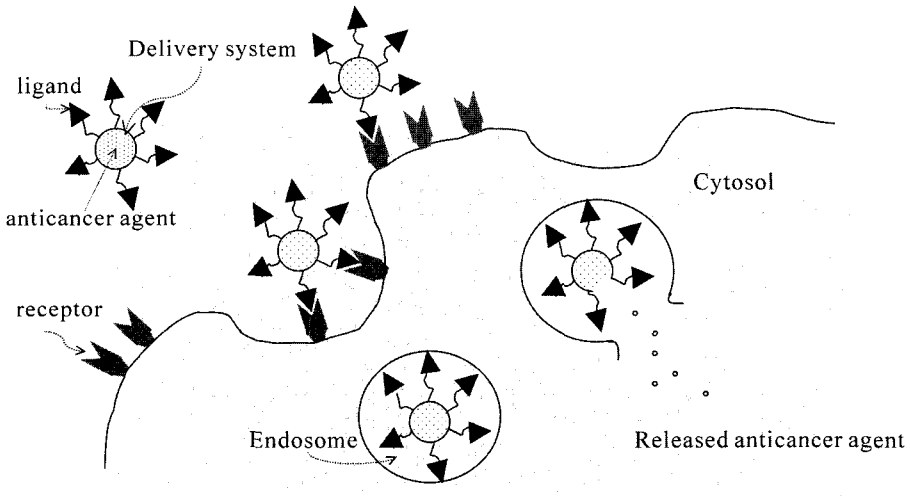

Fig. 3. Principal modes of receptor-mediated uptake (endocytosis) of anticancer agents incoporated in carriers bearing ligands. Upon bi nding to surface recptors, ligands-attached carriers are internalized into cells mainly by endocytosis, resulting in the formation of end osomes. Sometimes the receptors are recycled to the cell surface. To exhibit anticancer effect in specific intracellular organelles such as nucleus, c arrier-entrapped drugs should be released ouf from endosomes prior to the degradation of internalized materials.

a useful target for targeted delivery of anticancer drugs. Clinical developments of anticancer drugs utilizing targeted delivery systems are now at initial stages by diverse pharamceutical companies. Small selections of them are listed in Table 3. For targeted delivery, drugs should be directly conjugated to ligands/antibodies or should be incorporated in carriers bearing these ligands/antibodies (Kim *et al.*, 1993). In order to achieve high tumor to normal tissue ratios, low molecular weight conjugations are preferred.

Tissue-targeted delivery

The brain-blood barrier appears to be a major obstacle for delivery of anticancer drugs to brain. Approaches to overcome the barrier can be divided into two categories: those that attempt to increase drug delivery of systemically administered drugs by employing brain-specific delivery systems (Robert *et al.*, 2000) and those that attempt to increase drug delivery by local administration of polymer implanted drugs into the cerebrospinal fluid or directly into the tumor (Emerich *et al.*, 2000). Recently, delivery systems using drug-loaded nanoparticles, which mimic low density lipoprotein (LDL) particles, have proven to greatly enhance delivery to brain by interaction with the LDL receptor, leading to their preferential uptake by the endothelial cells (Kreuter, 2001).

High molecular weight substances can freely pass through the walls of the lymphatic capillary. Therefore, various colloidal systems have been investigated for lymphatic targeting delivery systems. For example, PIBCA (polyisobutylcyanoacrylate) nanocapsules showed enhanced accumulation of drug in the lymph node.

Liposomal aerosol formulations of interleukin-2 (Skubitz and Anderson, 2000), 9-Nitrocamptothecin (Koshkina *et al.*, 2000) and genes offered significant advantage over existing methods in the treatment of lung cancer/metastasis.

Galactose-related compounds as ligands for asiaglycoprotein receptors abundant on hepatocyte have been a useful tool for hepatocyte/liver targeting of anticancer drugs. HPMA copolymer bearing both doxorubicin and galactose is at the stage of clinical trial (Julyan *et al.*, 1999).

Cell-targeted delivery

One of the significant weaknesses of current cancer treatments is that the anticancer agents kill normal healthy cells as well as cancer cells. Understanding of molecular basis of cancer identified that some plasma membrane receptors and antigens are unique to certain cancer cells. Cell-specific targeting, therefore, may be achieved using DDS bearing a monoclonal antibody or a receptor-specific ligand.

Upon ligation to targeted receptor, both drug carriers and the engaged receptors are often internalized by receptor-mediated endocytosis/phagocytosis as described in Fig. 3.

Cancer cell-specific receptors/antigens that have been applied as targets for cancer therapy by many investigators include tuftsin receptor on macrophage plasma membrane, folate receptors (Leamon and Low, 2001), transferrin receptors (Sato *et al.*, 2000), HER2/NEU receptors, anti-

B-cell lymphoma monoclonal antibody, LL2, (Lundberg, 1999) vasoactive intestinal peptide receptors (Miller, 2000), CD 33, CD 45, CD 20, CD 13 (Curnis *et al.*, 2000), melanoma-specific antigens (MAGE) (Reed *et al.*, 1997), somatostatin receptor (Huang *et al.*, 2000), neurotensin (Martinez-Fong *et al.*, 1999) and prostate-specific antigen (PSA) (Wong *et al.*, 2001). Ligands/antibodies for receptor/ antigens were conjugated to anticancer agent itself (Ward *et al.*, 2000) or attached to the surface of carriers containing anticancer agents (Sugano *et al.*, 2000).

Intracellular delivery

For maximal therapeutic effect, anticancer drugs must safely reach not only its target cell but also the appropriate location within the cell. Most of new therapeutic agents developed from logical design based on the structure and function of their targets have their action sites in the cytosol or nucleus of cells. Targeting of drugs to designated intracellular locations relies upon their release from the carrier and subsequent passage across the biological membranes. An understanding of differences in membrane function, properties and structure among cellular organelles is essential for successful subcellular targeting.

a) Cytosolic delivery

Most ligands and colloidal/macro- molecular carriers are taken up by endocytosis/ phago- cytosis and it often leads to the degradation of internalized material. Therefore, getting the drug across the plasma membrane to cytosol is one of the biggest rate-limiting steps for macromolecular drugs.

Several strategies have been employed for the safe and efficient delivery of biologically active macromolecules to the cytosol by destabilizing/permeabilizing the endosome/ lysosome membrane (Gruenberg and Maxfield, 1995). Such delivery strategy employed the bacterial pore-forming proteins such as streptolysisn (Giles *et al.*, 1998), endosomolytic peptides from influenza hemagglutin (Plank *et al*, 1994) or pH-sensitive synthetic peptides (Wyman *et al.*, 1997). pH-sensitive delivery systems that can liberate anticancer agents inside pH-lower endosomes have also showed potential for cytosolic delivery of anticancer agents. They include HPMA copolymer conjugate to anticancer drug *via* pH-sensitive linkage (Etrych *et al.*, 2001), polymeric micelles (Leroux *et al.*, 2001) and pH-sensitive liposomes (Straubinger, 1993). Combination of delivery strategies by incorporating bacterial hemolysin (Listeriolysin O) together with macromolecular drugs inside ph-sensitive liposomes (Provoda and Lee, 2000), has shown to be a novel strategy, which allowed the escape of endocytosed material into the cytosol. Another strategy used photosensitizer together with cationic lipid gene delivery system for the purpose of improving cytosolic delivery (Prasmickaite *et al.*, 2001).

b) Nuclear delivery

Plasmid DNA/targeted proteins should be delivered intact to a cell nucleus to be effective in many cases. From recent information on nucleocytoplasmic transport and the identification of a family of nuclear import and export receptors, a strategy to tag proteins and DNA with a classical nuclear localization signal came out (Rosenkranz *et al.*, 2000). Use of the cellular trafficking properties of the herpesvirus-1 structural protein VP22 (Elliott and OHare, 1997) and yeast transcription factor GAL4 (Chan *et al.*, 1998) has also been shown to be effective for the nuclear delivery of transcriptional factors, cell cycle control regulators, tumor suppressor proteins and genes/DNA vaccines.

Reverse Targeting: Avoidance of MDR

Resistance of malignant tumors to chemotherapeutic agents remains the major cause of failure in cancer therapy. A membrane glycoprotein, termed P-glycoprotein, function as an energy-dependent efflux pump to remove cytotoxic agents from the resistant cells. The elucidation of function of P-glycoprotein and mechanism of multi drug resistance have had impact on the understanding of MDR in cancers.

Colloidal/macromolecular DDS such as liposomes (Warren, 1992), polymer conjugates (štastny *et al.*, 1999), polymeric micelles (Alakhov *et al.*, 1996) and nanospheres (Cuvier *et al.*, 1992) or vehicles with targeted ligand (Goren *et al.*, 2000) have shown potential in overcoming the MDR, although the mechanism is not clear yet. Currently, polymer platinate (AP5280™, Acess Pharmaceuticals) which allows the drug to bypass the drugresistance mechanism, is at the stage of clinical trials.

CONCLUSION

Wide range of delivery systems have been employed for developing DDS for anticancer agents to enhance their therapeutic values. Recent drastic progress in this field can be attributed to the great strides made in understanding the molecular basis of cancer as well as advances in development of DDS. Future research in this field will be expanded even to subcellular and molecular targeting to maximize the therapeutic efficacy of anticancer agents. For example, mitochondria may emerge as a new target of cancer therapeutics, since recent studies indicate that mitochondrial function is particularly important in programmed cell death, molecular metabolism, energy production and calcium signaling.

Whatever form the new anticancer therapeutics take, methods of delivery and tumor targeting will play an

important role in enhancing the therapeutic efficacy of existing drugs as well as development-stage drugs. The value that drug delivery adds in cancer therapy can be improved safety, efficacy, convenience and patient compliance. New technologies and multidisciplinary expertise to develop advanced drug delivery systems, applicable to a wide range of anticancer agents, will improve economic value as well as medical value, and may eventually lead to the effective cancer therapy in the near future.

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