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Review Article

Drug delivery to tumours: recent strategies

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Abstract

Despite several advancements in chemotherapy, the real therapy of cancer still remains a challenge. The development of new anti-cancer drugs for the treatment of cancer has not kept pace with the progress in cancer therapy, because of the nonspecific drug distribution resulting in low tumour concentrations and systemic toxicity. The main hindrance for the distribution of anti-cancer agents to the tumour site is the highly disorganized tumour vasculature, high blood viscosity in the tumour, and high interstitial pressure within the tumour tissue. Recently, several approaches such as drug modifications and development of new carrier systems for anti-cancer agents have been attempted to enhance their tumour reach. Approaches such as drug delivery through enhanced permeability and retention (EPR) effect have resulted in a significant improvement in concentration in tumours, while approaches such as drug-carrier implants and microparticles have resulted in improvement in local chemotherapy of cancer. This review discusses different strategies employed for the delivery of anti-cancer agents to tumours, such as through EPR effect, local chemotherapeutic approaches using drug delivery systems, and special strategies such as receptor-mediated delivery, pH-based carriers, application of ultrasound and delivery to resistant tumour cells and brain using nanoparticles.

Introduction

Cancer is defined by two of its characteristic features – the uncontrolled cell growth not regulated by external signals and the capacity to invade tissues, metastasize and colonize at distant sites. Cancers are of two types: those of epithelial origin are known as carcinomas, and those of non-epithelial (mesenchymal) origin are called sarcomas (Fenton & Longo 1998).

Despite several modes of therapy, such as chemotherapy, immunotherapy and radiotherapy, the therapy of cancer still remains a challenge. Systemic chemotherapy is the main treatment available for disseminated malignant disease. Progress in drug therapy has resulted in the treatment of curative chemotherapy regimens for several tumours. Chemotherapy, whether curative or palliative, requires multiple cycles of treatment. Chemotherapeutic agents exhibit a dose–response effect and the cell kill is proportional to the drug exposure (Fenton & Longo 1998). Normal cells are also susceptible to the cytotoxic effects of chemotherapeutic agents and exhibit a dose–response effect, but the response curve is shifted relative to that of malignant cells (Fenton & Longo 1998). This difference represents the therapeutic index; the usefulness of many chemotherapeutic drugs is limited by the fact that they have a narrow therapeutic index. Proliferative normal tissues, such as bone marrow and gastrointestinal mucosa, are generally the most susceptible to chemotherapy-induced toxicity. The ability of chemotherapy to eradicate tumour cells without causing lethal host toxicity depends on drug selectivity. Although cytokinetics are important, other differences between normal and tumour cells in cellular processes, such as metabolic pathways and susceptibility to programmed cell death (Kerr et al 1972), also contribute. Chemotherapy has been widely adopted for the treatment of cancer and as a result several chemotherapeutic agents have been introduced with potential anti-cancer activity. However, due to the non-specificity of these drugs, chronic administration leads to severe systemic toxicity (Son et al 2003). In an attempt to improve therapy with these anti-cancer agents and reduce the associated toxicity, drug delivery systems have been introduced that can deliver these drugs at the site of interest (Poupaert & Couvreur, 2003).

Several drug delivery systems were introduced, namely liposomes, microparticles, polymeric conjugates, micelles and nanoparticles, to facilitate effective chemotherapy

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with anti-cancer agents. The introduction of doxorubicin long-circulating liposomes in the market for cancer therapy has brought a renewed interest in the field of targeted drug delivery to cancers.

Recently, several new approaches have been adopted for delivering anti-cancer agents to the tumour tissues.

Enhanced permeability and retention (EPR) effect

Tumours exhibit a unique character, namely enhanced permeability and retention (EPR) effect, greatly different from the normal tissues. The EPR effect renders tumour tissues to display several distinctive characteristics, such as hypervasculature, defective vascular architecture and a deficient lymphatic drainage system, which lead macromolecules to be accumulated preferentially and to be retained to a greater extent in tumour tissues than in normal tissues (Maeda et al 2000).

Many anti-cancer drugs exert their action by binding to macromolecules (Au et al 2001). Conjugation of low-molecular-weight drugs to polymeric carriers passively targets the chemotherapeutic agent to solid tumours, since high-molecular-weight soluble polymeric conjugates are frequently found to be tumoritropic (Maeda & Matsumura 1989).

Polymeric drugs, such as SMANCS or SMA (polystyrene-co-maleic acid-half-butylate) copolymer conjugated with neocarzinostatin, which has a mass of 16 kDa, with albumin-binding and lipophilic characteristics, have been used experimentally in many patients with liver and other cancers. This drug was approved in 1994 in Japan for use in hepatoma treatment (Maeda et al 1979). Another drug, L-asparaginase conjugated with poly(ethylene glycol), developed by Enzon, was approved as an orphan drug in 1994 in the USA for use in lymphoma and leukaemia treatment (Maeda et al 2001).

Nomura et al (1998) studied the therapeutic effects of macromolecular prodrugs of mitomycin C and mitomycin C–dextran conjugates after intratumoral administration in rats bearing Walker 256 carcinosarcoma (Table 1). Conjugation of mitomycin C with dextran has significantly enhanced the tumour retention of mitomycin C. Among cationic and anionic conjugates, the cationic conjugate showed relatively high tumour retention properties. Ulbrich et al (2003) synthesized PHPMA poly(2-hydroxy propyl methacrylate) conjugates, wherein the doxorubicin is attached to the HPMA (*N*-(2-hydroxy propyl) methacrylate) backbone either via a spacer containing a hydrazone bond or a cisaconitic acid residue. The conjugates were stable in blood at pH 7.4, but degraded hydrolytically and released doxorubicin in the mildly acidic environment of the target cells (pH $\sim$ 5–6; e.g., in endosomes or lysosomes). The copolymer drug was also conjugated with antibody. The in-vivo anti-tumour activity of hydrazone conjugates containing doxorubicin was significantly enhanced (EL4 T-cell lymphoma bearing mice) compared with the free doxorubicin. Antibody-targeted conjugates with doxorubicin bound via a hydrazone bond exhibited even more pronounced anti-tumor activity, indicating the significance of the chemical nature of the conjugates in the tumour delivery. The use of PEG conjugates in the delivery of BH3 and LHRH peptides has been reported by Dharap et al (2003).

Caiolfa et al (2000) synthesized soluble copolymers of *N*-(2-hydroxy propyl) methacrylate loaded with camptothecin by covalent linkage at its α-hydroxyl group to the polymers through a Gly-Phe-Leu-Gly-spacer. These conjugates, upon intravenous administration to HT-498 human CNS tumour-bearing mice, led to 90% tumour inhibition and some complete tumour regressions and no toxic deaths. Son et al (2003) prepared doxorubicin-conjugated glycol-chitosan nanoaggregates with a diameter of 250–300 nm. These nanoaggregates, upon intravenous administration in human mesothelioma cell 1145 tumour-bearing rats, showed sustained blood levels up to 8 days, implying their sustained circulation property. The concentration of nanoaggregates in tumour tissue increased gradually with time by the EPR effect, and the tumour size was suppressed over 10 days post injection. Nakanishi et al (2001) synthesized a polymeric micelle carrier system consisting of polyethylene glycol and conjugated doxorubicin–polyaspartic acid, having a particle size of 40 nm diameter. The anti-tumour activity was tested in P338 tumour-implanted mice. The micelle carrier showed higher concentrations of doxorubicin in plasma and tumour tissue after intravenous administration, indicating the accumulation of carrier by the EPR effect. For in-depth information on the application of micelles for delivering anti-cancer drugs, readers are directed to a recent review by Garrec et al (2004).

Nanoparticles are one of the delivery systems recently attempted for drug delivery to tumours. Chawla & Amiji (2002, 2003) used poly(ε-caprolactone) nanoparticles as drug carrier for the delivery of tamoxifen (indicated in breast cancer). The in-vitro cytotoxicity of drug-loaded nanoparticles was performed by incubating with MCF-7 breast cancer cells. The study revealed that a fraction of nanoparticles were taken up by the cells through non-specific endocytosis. The nanoparticles were found concentrated mainly in the perinuclear region of the cell after 1 h of incubation. Fonseca et al (2002) used poly(D,L-lactide-co-glycolide) nanoparticles for delivering paclitaxel to tumours. The in-vitro anti-tumour activity of the drug-loaded nanoparticles was assessed in NCI-H69 cells (human small-cell lung carcinoma cell line). The cell uptake of nanoparticles was found to be significantly higher than that of the free paclitaxel. Also the drug-free nanoparticles did not show considerable cytotoxicity on the cells. Williams et al (2003) produced irinotecan-loaded lipid nanoparticles by a hot homogenization technique. The anti-tumour effect of drug-loaded nanoparticles was determined after intravenous injection in an HT-29 (human adenocarcinoma of colon) mice tumour model and showed prolonged blood levels up to 24 h. The nanoparticles also had a higher anti-tumour activity at low doses and less dosing frequency compared with the free irinotecan.

Prodrugs of *N*,*N*-di-(2-chloroethyl)-4-phenylene diamine (PDM) based on soluble poly[*N*-(2-hydroxyethyl)-L-

Table 1 Different strategies adopted for drug delivery to tumours

Strategy	Delivery system	Reference
Enhanced permeability and retention (EPR) effect	Polymeric conjugate	Maeda & Matsumura 1979
	Polymeric conjugate	Maeda et al 2001
	Polymeric conjugate	Ulbrich et al 2003
	Polymeric conjugate	Dharap et al 2003
	Polymeric conjugate	Caiolfa et al 2000
	Nanoaggregates	Son et al 2003
	Polymeric micelles	Nakanishi et al 2001
	Nanoparticles	Fonseca et al 2002
	Lipid nanoparticles	Williams et al 2003
	Polymeric conjugate	Soyez et al 1999
	Microemulsion	Junping et al 2003
	Micelles	Greish et al 2004
Local chemotherapy	Microspheres	Tamura et al 2002
	Liposomes	Sadzuka et al 2002
	Implant	Vogelhuber et al 2002
	Solid lipid nanoparticles	Reddy et al 2004a
	Macromolecular prodrug	Nomura et al 1998
	Solid lipid nanoparticles	Reddy et al 2005
Receptor-mediated delivery	Folate-conjugated polymeric mixed micelles	Lee et al 2003
	Folate-coated solid lipid nanoparticles	Oyewumi & Mumper 2003
	Folate-conjugated polymer conjugate	Paranjpe et al 2004
Antibody-conjugated delivery	Lipid-drug carrier	Lundberg et al 2004
	Liposomes	Lopes de Menezes et al 1998
	Liposomes	Lukyanov et al 2004
pH-sensitive drug carriers	Nanoparticles	Han et al 2003
	Nanoparticles	Na et al 2003
	Polymeric conjugate	Bulmus et al 2003
Ultrasound	Polymeric micelles	Rapoport et al 2003
	Micelles	Husseini et al 2003
Poly alkylcyanoacrylate nanoparticles	Nanoparticles	Vauthier et al 2003
	Nanoparticles	de Verdiere et al 1997
	Nanoparticles	Hu et al 1996
Magnetic carriers	Microsphere/nanoparticle	Safarik & Safarikova 2002
	Microsphere/nanoparticle	Fricker 2001
	Liposomes	De Cuyper & Joniau 1988
	Liposomes	Babincova et al 2000, 2002
	Liposomes	Kubo et al 2000
	Liposomes	Kubo 2001
Magnetic Fluid Hyperthermia	Superparamagnetic nanoparticles	Jordan et al 1996, 1997
	Superparamagnetic nanoparticles	Johanssen et al 2005
	Superparamagnetic nanoparticles	Josef et al 2002

glutamine] (PHEG) were synthesized by Soyez et al (1999) as tumour-targeted drugs. These materials were designed to exploit the enhanced permeability of tumour vasculature, combining a passive tumour tropism with systemic liberation of free PDM. This derivative, upon intravenous administration, showed low systemic toxicity and better anti-tumour activity against a C26 colorectal carcinoma tumour model, compared with no activity for the free drug.

Junping et al (2003) reported injectable microemulsions of vincristine as drug carriers for anti-cancer agents for tumour delivery through the EPR effect. The microemulsion contained poly(ethylene glycol) (PEG)-lipid and cholesterol as surfactants, and the vitamin E solution of oleic acid and drug as an oil phase. The vincristine-loaded microemulsion showed a significantly higher tumour-weight inhibition rate (67%) compared with the free vincristine (42%) in a solid M5076 tumour mice model.

Greish et al (2004) used copolymer of styrene-maleic acid (SMA) to construct micelles containing doxorubicin through a hydrophobic interaction between the styrene moiety of SMA and doxorubicin. The micelles thus obtained had a high solubility in water and a constant in-vitro doxorubicin release rate of about 3–4% per day. The SMA–doxorubicin micelle preparation was less (36–70%) cytotoxic to the SW480 human colon cancer cell line in-vitro compared with free doxorubicin. In-vivo assay of SMA–doxorubicin in ddY mice bearing S-180 tumour revealed a potent anti-cancer effect with no remarkable toxicity up to a dose of $100\,mg\,kg^{-1}$ of free doxorubicin equivalent. The drug concentration in tumour after administration of SMA–doxorubicin was

13 times higher than that after the free drug. This result can be attributed to the enhanced permeability and retention (EPR) effect of macromolecular drugs observed in solid tumours.

Local chemotherapy

Apart from the EPR effect, another strategy employed to enhance the tumour delivery of anti-cancer agents is local chemotherapy. Local delivery of chemotherapeutic drugs has long been recognized as one potential method of delivering high drug doses at the target site(s) while minimizing systemic exposure (Almond et al 2003). In this type of delivery, the drugs or drug delivery systems are administered at the local site of the tumour to facilitate more effective exposure of cancer cells to drugs and hence greater anti-tumour activity. This is true especially in the case of inoperable accumulation of malignant ascites caused by peritoneal carcinomatosis (Tamura et al 2002) and in some other types of cancers. Theoretically it should be possible to carry out intraperitoneal chemotherapy by increasing local drug exposure, resulting in less systemic toxicity. However, administration of drugs alone does not result in reduction in systemic toxicity (Casper et al 1983; Lopez et al 1985). One possible way of avoiding the systemic toxicity associated with drug while maintaining the higher concentrations of drug in the local regions of tumours is their incorporation into drug carriers, such as liposomes (Sadzuka et al 2002), microparticles (Tamura et al 2002) or implants (Vogelhuber et al 2002). Sadzuka et al (2000) studied the anti-tumour effect of liposomes after intraperitoneal administration in Ehrlich ascites abdominal tumours. Liposomes were prepared using different lipids such as L-α-dimyristoyl phosphatidyl choline (DMPC), L-α-distearoyl phosphatidyl choline (DSPC) and dimyristoyl phosphatidyl glycerol (DMPG). The positively charged liposomes were prepared from DSPC–cholesterol–doxorubicin. The liposomal size and composition was found to significantly influence the liposomal disposition from the abdominal cavity after intraperitoneal administration. The liposomes significantly prolonged the survival time of the tumour-bearing mice compared with the free doxorubicin. Tamura et al (2002) evaluated the anti-tumour effect of cisplatin-loaded poly (lactic acid) microspheres in various types of tumours implanted peritoneally. The cisplatin-loaded microspheres showed effective anti-tumour activity (tumour-growth-inhibition rate of 70.3%) against Li-7 (human liver cancer) xenografts transplanted into the peritoneal cavity of mice. The microspheres also resulted in an increased life span of 47.2%, where as the cisplatin solution was found to be ineffective, with a very low tumour-inhibition rate and survival. Similarly, the microspheres also enhanced the mean survival time of mice with Li-7 xenografts transplanted into the spleen compared with the cisplatin solution.

Recently, Reddy et al (2004a) reported an enhanced tumour concentration of doxorubicin-loaded poly(butyl cyanoacrylate) nanoparticles administered subcutaneously in Dalton's lymphoma tumour-bearing mice. Owing to the surface hydrophobicity and negative charge, the nanoparticles underwent lymphatic absorption and concentrated in the lymphoma.

Vogelhuber et al (2002) produced implants of BCNU (anti-cancer agent) loaded into polymer containing a 1:1 (w/w) mixture of poly(1,3-bis[*p*-carboxyphenoxypropane]-co-sebacic acid) 20:80 and poly(D,L-lactide-co-glycolide)50 and implanted subcutaneously at the tumour site in a U-87 MG glioblastoma mice model and also injected BCNU solution intraperitoneally. Subcutaneous implantation of BCNU implants resulted in significant tumour regression, while BCNU solution did not have significant effect on the tumour growth, indicating the effectiveness of BCNU implants in local chemotherapy. On the other hand, subcutaneous injection of high doses of BCNU resulted in a 16-fold size reduction compared with sham operated mice. The combination of BCNU and paclitaxel led to complete remission in some mice. The studies indicate the importance of administration route and the possibility of combination chemotherapy in effective tumour therapy. Seong et al (2003) prepared BCNU wafer by compressing BCNU-loaded poly(D,L-lactide-co-glycolide) microparticles. In-vitro anti-tumour activity of these wafers was performed after incubation with XF498 human CNS cancer cells. The drug-loaded wafers showed prolonged cytotoxicity over 1 month, while the cytotoxicity of BCNU powder disappeared after 12 h, suggesting that the wafer formulation facilitates prolonged release of BCNU in-vivo and results in effective treatment of malignant glioma. Gliadel Wafer (Polifeprosan 20 with carmustine (BCNU)) manufactured by Guilford Pharmaceuticals (USA) is the chemotherapeutic implant approved by the FDA in 1996 for the treatment of a specific brain tumour called a high-grade malignant glioma (recurrent glioblastoma multiforme). Gliadel Wafers, when implanted in the cavity remaining after the surgical removal of tumour, slowly deliver BCNU directly to the tumour site.

When the drug is directly injected into a tumour, such as by intratumoral injection, or by direct instillation into peritumoral space, such as in intravesical therapy of superficial bladder cancer and in intraperitoneal dialysis of ovarian cancer, the drug transport to tumour cells is primarily by diffusion through interstitial space (Markman et al 1995; Nativ et al 1997; Markman 1998; Song et al 1997). Nomura et al (1998) reported the pharmacokinetics and therapeutic effects of the macromolecular prodrugs of mitomycin C (MMC), MMC–dextran conjugates (MMC-D), after intratumoral injection in rats bearing a Walker 256 carcinosarcoma. MMC immediately disappeared from the tumour preparation following direct intratumoral injection, while cationic and anionic MMC-D were retained in the tumour longer, demonstrating that the intratumoral clearance of MMC can be greatly retarded by dextran conjugation.

Reddy et al (2004b) studied the biodistribution and tumour retention properties of ^{99m}Tc-labelled etoposide-loaded tripalmitin nanoparticles (ETPL) after intratumoral administration in Dalton's lymphoma tumour-bearing mice. The disposition of nanoparticles from the tumour site was significantly lower than etoposide, exhibiting a 14-fold

greater tumour retention even 48 h post injection. A greater anti-tumour effect can be expected to result due to the increased exposure of tumour cells to etoposide and increased cell uptake of nanoparticles by endocytosis.

Different routes of administration may result in varying effects on the biodistribution pattern of drug carriers. Allen et al (1993) compared the biodistribution pattern of liposomes administered through different routes and reported wide differences in the tissue distribution. Thus, the route of administration appears to be an important parameter in the delivery of anti-cancer agents to tumours. Reddy et al (2005) administered etoposide-loaded solid lipid (ETP) nanoparticles by three different routes, intravenous, intraperitoneal and subcutaneous, and the tumour distribution was studied in Dalton's lymphoma tumour-bearing mice. Dalton's lymphoma is a T-cell lymphoma and is grown in the hind leg of mice after subcutaneous implantation of tumour cells. For effective penetration into the lymphatic interstitium, the carrier systems need to possess smaller size, hydrophobicity and negative surface charge (Porter 1997). The smaller size of the drug carriers has also been reported to facilitate the infiltration into tumour interstitium through the leaky tumour vasculature (Moghimi 2001). The ETP nanoparticles administered through subcutaneous injection at 2 cm below the tumour region showed greater tumour distribution to the Dalton's lymphoma solid tumour implanted in the right hind limb of mice. The order of tumour distribution of nanoparticles was subcutaneous > intraperitoneal > intravenous route. The very low distribution of ETP nanoparticles after intravenous injection was attributed to the barriers hindering the transport of drug and particles into solid tumour, such as highly disorganized tumour vasculature, high viscosity of blood within the tumour and abnormally high pressure in the interstitial matrix retarding the molecular transport across the vessel walls and into the interstitium (Jain 1994).

Special strategies

Apart from various technological approaches, some special strategies, such as receptor-mediated drug delivery and tumour-specific antibody conjugates, have been adopted for enhancing the tumour delivery of anti-cancer agents. Lee et al (2003) developed adriamycin-loaded pH-sensitive polymeric mixed micelles composed of poly(L-histidine) poly(His; MW 5000)/PEG (Mn 2000) and poly(lactic acid) (PLLA) (Mn 3000)/PEG (Mn 2000) block copolymers with or without folate conjugation by a diafiltration technique. Folate-receptor mediated internalization and cytotoxicity of micelles was investigated using MCF-7 cells in-vitro. The polyHis/PEG micelles showed accelerated release of adriamycin as the pH decreased from 8.0. Blending of PLLA/PEG block copolymer with polyHis/PEG to form mixed micelles, and their conjugation with folic acid, resulted in effective tumour-cell kill by micelles due to accelerated drug release and folate-receptor-mediated tumour uptake. Wang & Low (1998) also used a folate-mediated targeting mechanism for targeting of anti-cancer agents, imaging agents and nucleic acids to tumour cells (Figure 1). Oyewumi & Mumper (2003) prepared folate-ligand-coated gadolinium-loaded lipid nanoparticles from emulsifying wax or Brij 72. The uptake of nanoparticles by KB cells (human nasopharyngeal epidermal carcinoma cell line) significantly increased after folate conjugation and the uptake increased with an increase in folate-ligand concentration, indicating the occupation of a greater number of folate receptors by the nanoparticles at high folate concentrations, leading to more effective endocytosis of nanoparticles.

Paranjpe et al (2004) synthesized a novel camptothecin bioconjugate with a linear PEG and the amino acid glycine as the spacer and linker, respectively. Folic acid was used as the targeting ligand to take advantage of folate-receptor-mediated endocytosis. The bioconjugate

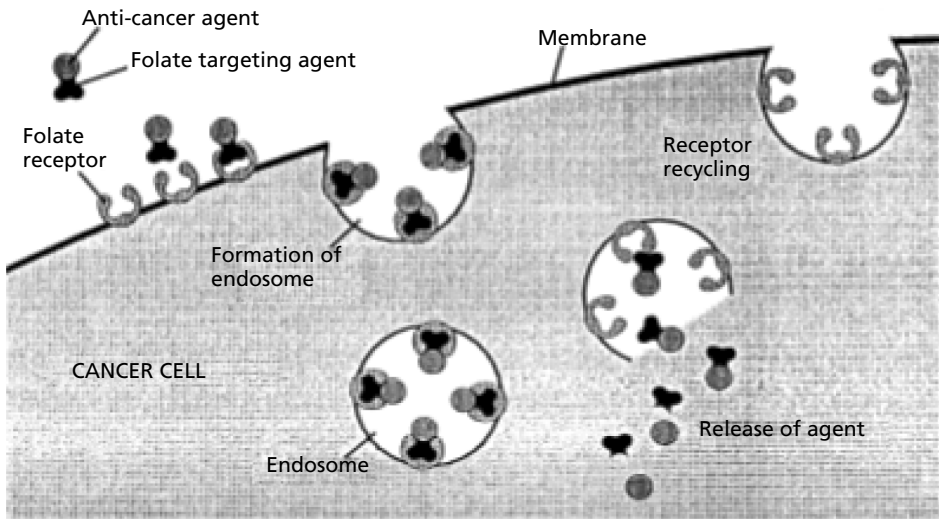

Figure 1 Representative mechanism of folate-receptor-mediated endocytosis pathway using folate-targeting ligands (reproduced with permission from Wang & Low (1998)).

was evaluated in-vitro for specific targeting to folate-receptor-expressing KB cells. The bioconjugate exhibited significantly higher efficacy in comparison with camptothecin. A control conjugate without PEG demonstrated no improvement in efficacy over untargeted camptothecin, emphasizing the importance of the spacer between the anti-cancer compound and targeting moiety.

The ability to selectively target anti-cancer agents via specific ligands against antigens expressed on malignant cells could greatly improve the therapeutic indices of drugs. Lundberg et al (2004) developed sterically stabilized lipid drug carriers (emulsion and liposomes) and covalently attached the anti-CD74 antibody to the surface of the carrier using a PEG-based hetero-bifunctional coupling agent. During a 24-h in-vitro incubation with the target Raji B-lymphoma cells, about 30% of the complexes were found to be associated with the cells. The results indicated that the antibody-conjugated drug carrier was selectively targeted to B-cells and showed selective toxicity of the incorporated drug. On similar lines, several studies with PEG-liposomes targeted with tumour-cell-specific monoclonal antibodies have shown improved therapeutic activity over non-targeted formulation (Lopes de Menezes et al 1998).

Lukyanov et al (2004) modified the commercially available doxorubicin-loaded long-circulating liposomes (Doxil; Alza Pharmaceuticals) with the monoclonal nucleosome (NS)-specific 2C5 antibody (mAb 2C5) that recognizes a broad variety of tumours via the tumour cell surface-bound NSs. For incorporation into liposomes, mAb 2C5 was modified with poly(ethylene glycol)-phosphatidyl ethanolamine conjugate (PEG-PE) with the free PEG terminus activated with the *p*-nitrophenylcarbonyl group (pNP-PEG-PE). 2C5-targeted Doxil liposomes acquired the ability to recognize NSs and specifically bound to various tumour cells. Doxorubicin-loaded long-circulating liposomes modified with the mAb 2C5 showed a greater cell-kill effect in-vitro than non-targeted doxorubicin-loaded liposomes.

Drug delivery based on tumour pH

The tumour extracellular pH is a consistently distinguishing phenotype of most solid tumours from surrounding normal tissues. The measured pH values of most solid tumours in patients using invasive microelectrodes are in the range 5.7–7.8, with a mean value of 7.0 (Ojugo et al 1999). The acidity of the tumour interstitial fluid is mainly attributed, if not entirely, to the higher rate of aerobic and anaerobic glycolysis in cancer cells than in normal cells (Stubbs et al 2000). Such acidic extracellular pH prompted the establishment of pH-sensitive anti-cancer drug delivery systems. Recently, Han et al (2003) introduced new pH-sensitive functional group (a weak acid of sulfonamide) containing polymers. The water-soluble polymers modified with sulfonamide self-assembled nanoparticles showed enhanced drug release and interaction with, and into, cells at tumour pH (Na et al 2003).

Cytoplasmic delivery of enzyme-susceptible biomolecular drugs is one of the major limitations in many therapeutic strategies. Synthetic, pH-sensitive polymers have also been investigated and showed enhanced endosomal delivery of biomolecular therapeutics (Lackey et al 1999, 2002; Murthy et al 2003). Most of the studies (Vinogradov et al 1998; Richardson et al 1999) were focused on cationic amino polymers. Murthy et al (1999) prepared pH-sensitive polymers that mimicked viral peptides and contained a combination of acidic –COOH groups and hydrophobic alkyl groups. This combination was used for cytoplasmic delivery applications, because such polymer compositions can be varied to cause disruption of lipid membranes at specific pHs. When these polymers are protonated at endosomal pH, they increase in hydrophobicity, leading to enhanced endosomal membrane disruption. A novel pH-responsive polymeric carrier was synthesized by Bulmus et al (2003) for the enhanced cytoplasmic delivery of enzyme-susceptible drugs, such as antisense oligonucleotides, proteins and peptides. A novel functionalized monomer, pyridyl disulfide acrylate, was synthesized and incorporated into an amphiphilic copolymer consisting of methacrylic acid and butyl acrylate, which resulted in a glutathione- and pH-sensitive, membrane-disruptive terpolymer with functional groups that allow thiol-containing molecules to be readily conjugated. The polymer had two key actions: firstly, pH-dependent, endosomal membrane disruption and escape into the cytoplasm; and secondly, this is followed by reaction of disulfide-conjugated drug with glutathione, a normal constituent of the cytoplasm of cells, causing release of the drug from the polymer.

Drug delivery by ultrasound

Drug delivery to tumours using ultrasound has been the recent mechanism adopted by Rapaport et al (2003). They developed a new drug modality based on drug encapsulation into polymeric micelles followed by a controlled release at the tumour site triggered by ultrasound focused on the tumour. Ultrasound not only released the drug from micelles, but also enhanced the local uptake of both free and encapsulated drug by tumour cells, thus providing effective drug targeting. Ultrasound also promotes extravasation of drug-loaded carriers into the tumour interstitium. The in-vivo results revealed that application of a low-frequency ultrasound (20–70 kHz) significantly reduced the tumour size when compared with insonated controls. A similar approach was reported by Husseini et al (2000), involving the encapsulation and release of doxorubicin from micelles through ultrasound.

Drug delivery to resistant tumour cells

Several types of cancer show resistance to conventional chemotherapy. Cancer-cell resistance is considered to be one of the major reasons for failure of chemotherapy for the majority of cancer patients (Ambudkar et al 1999; Silverman 1999). Some tumours are intrinsically resistant to treatment, whereas others acquire resistance with exposure to structurally unrelated drugs (Vauthier et al 2003).

The resistance mechanism can have different origins. In tumour tissue, it can be either directly linked to specific mechanisms developed by the tumour cells or it can be connected to the physiology of the tumour tissue, including a poor vasculature and unsuitable physicochemical conditions (Krishna & Mayer 2000; Hobbs et al 1998). Outside the tumour tissue, resistance to chemotherapy can be due to the more general problem of the distribution of a drug relative to its targeted tissue (Moghimi et al 2001). This phenomenon of multidrug resistance (MDR) is the result of overexpression of membrane-bound proteins that efflux drugs from the cells, thus decreasing the intracellular concentration of the drugs (Kartner et al 1985). Two proteins, in particular P-glycoprotein (P-gp) and MDR-associated protein (MRP2), are responsible for MDR associated with a variety of cancers (Stouch & Gudmundsson 2002). BCRP (breast cancer resistant protein) is another type of protein that appears to play a major role in the MDR phenotype of a specific human breast cancer (Doyle et al 1998). Resistance to cancer chemotherapy involves both altered drug activity at the designated target and modified intra-tumour pharmacokinetics (e.g. uptake and metabolism). The membrane transporter P-gp plays a major role in pharmacokinetic resistance by preventing sufficient intracellular accumulation of several anti-cancer agents (Figure 2), while inhibiting P-gp has great potential to restore the effectiveness of chemotherapeutics (Walker et al 2004). Doxorubicin is a well-known P-gp substrate (Vauthier et al 2003). Resistant cells treated with doxorubicin-loaded poly(alkyl cyanoacrylate) nanoparticles showed much higher sensitivity to the drug relative to the free doxorubicin (de Verdiere et al 1997). Degradation of the carrier was shown to play a key role in the mechanism of action. The poly(cyanoacrylic acid) resulting from the nanoparticle degradation can form an ion-pair with doxorubicin (Pepin et al 1997). The mechanism was reported to be based on adhesion of nanoparticles to cell surfaces followed by release of doxorubicin and nanoparticle degradation products that combine as an ion-pair able to cross the cell membrane without being recognized by P-gp (Hu et al 1996; Vauthier et al 2003). Soma et al (2000) suggested co-encapsulating doxorubicin and ciclosporin within the same nanoparticles, with ciclosporin being at the surface. Ciclosporin is a chemosensitizing compound that can bind to P-gp and can inhibit the pump efflux activity. The combination has been shown to effectively inhibit the growth rate of resistant cells. Other strategies proposed to regulate P-gp expression have involved using ribozymes (Kobayashi et al 2001) or oligonucleotides (Juliano et al 1999). However, because of their poor solubility in biological fluids, their intracellular diffusion is very poor.

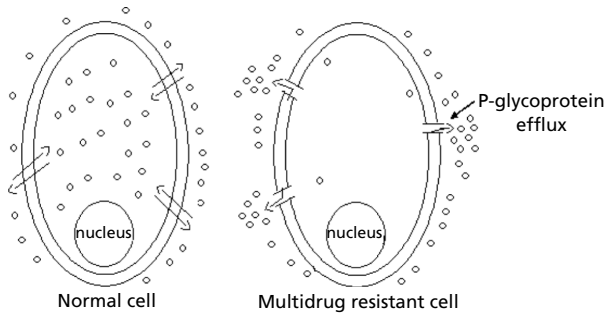

Figure 2 Schematic representation of transport of anti-cancer agent in normal cells and multidrug resistant cells. In the multidrug resistant cells the active drug efflux by P-glycoprotein can be observed.

Patients with primary brain tumours and brain metastases have a very poor prognosis. Low responses to chemotherapy are mainly attributed to impermeability of the blood–brain barrier to anti-cancer agents. Paclitaxel, an anti-cancer agent, has a low blood–brain barrier permeability and serious side effects. Lack of paclitaxel brain uptake is thought to be associated with the P-gp efflux transporter. In a recent work by Koziara et al (2004), paclitaxel was entrapped in novel cetyl alcohol/polysorbate nanoparticles. The paclitaxel nanoparticle cytotoxicity profile was monitored using two different cell lines, U-118 and HCT-15. Brain uptake of paclitaxel nanoparticles was evaluated using an in-situ rat brain perfusion model. Paclitaxel-loaded nanoparticles significantly increased the brain uptake of paclitaxel and its toxicity toward P-gp-expressing tumour cells. It was hypothesized that paclitaxel nanoparticles could mask paclitaxel characteristics and thus limit its binding to P-gp, which consequently would lead to higher brain and tumour cell uptake of the otherwise effluxed drug.

Tumour targeting using magnetic carriers

Tumour targeting using magnetic carriers has been attempted to increase the efficacy and reduce the systemic toxicity associated with chemotherapy. This involves incorporation of anti-cancer agent in biocompatible magnetic nanoparticles or microspheres (Safarik & Safarikova 2002), and may result in more effective chemotherapy due to enhancement of the drug concentration at the tumour site, limiting the associated systemic drug toxicity. FeRx Inc. (San Diego, USA) developed Magnetic Targeted Carriers (MTCs) for site-specific targeting, tissue retention and sustained release of drugs. MTCs are composed of iron particles and activated carbon. MTCs (1–2 μm in size) can adsorb and desorb drugs such as doxorubicin. Drug delivery using MTCs includes insertion of a catheter into an arterial feed to the tumour, followed by the application of a powerful magnetic field to cause the MTC-doxorubicin (in phase III clinical trial) to extravasate through the capillary bed into the targeted tissue. The particles remain trapped in the tumour, from which the drug is released at a controlled rate (Fricker 2001).

Magnetoliposomes

Magnetoliposomes are prepared by entrapment of ferrofluids within the core of liposomes (De Cuyper & Joniau 1988). Several groups of researchers have investigated the application of magnetoliposomes for site-specific targeting (Babincova et al 2000), cell-sorting (Margolis et al 1983) and as magnetic resonance contrast-enhancing agents (Bulte et al 1999).

Babincova et al (Babincova et al 2002) developed magnetoliposomes encapsulated with doxorubicin for site-specific chemotherapy in response to an externally applied AC magnetic field. The results revealed that specifically heating the magnetoliposomes to 42°C resulted in a massive release of encapsulated doxorubicin. Another in-vivo study for site-specific targeting using magnetoliposomes incorporated with doxorubicin showed that administration of magnetic liposomes under an applied external magnetic force produced an approximately 4-fold higher maximum doxorubicin concentration in the tumour compared with doxorubicin solution. These results suggest that systemic chemotherapy could effectively control the primary tumour without significant side effects, due to the targeting of magnetic doxorubicin liposomes (Kubo et al 2000, 2001).

Magnetic fluid hyperthermia

Magnetic fluid hyperthermia (MFH) is a new technique for interstitial hyperthermia or thermoablation based on AC magnetic field-induced excitation of biocompatible superparamagnetic nanoparticles. Magnetic fluids have been investigated as potential hyperthermia-causing agents due to their high specific absorption rate. Hyperthermia is a promising approach for cancer treatment, which uses AC magnetic fields to heat target areas (cancer tissue) containing magnetic fluids. To study the biological effects of AC magnetic field excited ferrofluids, both in-vitro and in-vivo studies have been carried out in cancer cell lines and spontaneously induced tumours in animal models. The results of these studies indicated that magnetic fluid hyperthermia is able to reduce the viability of cancer cells, thereby indicating the potential of this therapy (Jordan et al 1997).

Johannsen et al (2005) evaluated the potential of MFH as a minimally invasive treatment for prostate cancer by carrying out a systematic analysis of the effects of MFH in the orthotopic Dunning R3327 tumour model of the rat. Rats received two MFH treatments following a single intratumoral injection of a magnetic fluid. Treatments were carried out on days 10 and 12 after tumour induction using an AC magnetic field applicator system operating at a frequency of 100 kHz and a variable field strength (0–18 kA m^{-1}). The rats were sacrificed after day 20, and the tumour weights were determined and compared with controls. The results indicated that the MFH led to a significant growth inhibition in an orthotopic model of the aggressive MatLyLu tumour variant.

Jordan et al (1996) investigated the cellular uptake and biological effects of biocompatible magnetic fluids excited by an AC magnetic field on human carcinoma cells in-vitro. One of the fluids tested was a dextran magnetite, which possesses very low cytotoxicity. The results indicated that there is a sensitizer effect of ferrofluids at 43 °C probably caused by free ferric ions, which induce oxidative stress, and there was no cytotoxic effect of intracellular dextran magnetite particles excited with AC magnetic field.

In a recent report by Josef et al (2002), a patient with advanced hepatocellular carcinoma was treated with an intravenous infusion of pegylated liposomal doxorubicin (PLD, Caelyx) in combination with ultrasound hyperthermia of the liver.

Future perspectives

Despite extensive research in the field of drug delivery to tumours using delivery systems, only a few products have found their way onto the market. A better understanding of physiological barriers and biochemical mechanisms of cancer, which a drug has to face to reach the cancer site, enables drug delivery researchers to develop a successful delivery system for cancer therapy. Sterically stabilized liposomes, such as Doxil and Daunosome, and Gliadel Wafers, are a few recent examples of the products that have reached the market for cancer therapy. For injectable delivery systems several factors, such as stability, particle size, sterility and injectability, are important for effective delivery and therapeutic efficacy. Many a time, the delivery systems, such as solid lipid nanoparticles, show an increase in particle size upon storage, which may alter the biodistribution and therapeutic efficacy of the incorporated drug.

For polymeric nanoparticles, large-scale manufacturing is most critical and many of the reported techniques use organic solvents, which is not advisable for large scale production. Residual solvents in the nanoparticles may lead to toxic effects upon administration. Hence, complete evaporation of the organic solvents should be ensured in the final formulation and levels should be below the prescribed limits. The stabilizers used in the preparation of nanoparticles need to be biocompatible and nontoxic. Also, the batch-to-batch uniformity of particle size and stability should be ensured. Solid lipid nanoparticles possess the advantage of feasibility of large-scale production. However, the nanoparticles prepared from glyceride lipids possess the problems of recrystallization, particle growth and expulsion of drug from the particle matrix leading to a decrease in the amount of the incorporated drug on storage. Hence, selection of the type of lipid is essential to produce nanoparticles with better stability. Storage of nanoparticles in a dry form can improve the long-term stability. Spray drying is one of the better techniques to recover nanoparticles from aqueous dispersions, and is also feasible for large-scale manufacturing purposes.

For rapidly growing tumours, the extensive angiogenesis to meet the nutritional requirements of newly formed cells can be taken advantage of in delivering macromolecular drugs and colloidal systems. This can be achieved through the EPR effect. For such delivery, the systems should possess long blood circulation time, stability in the circulation and slow drug release until it reaches the tumour. Polymer–drug conjugation is another means of delivering low-molecular-weight anti-cancer agents specifically to tumour cells. These conjugates allow passive targeting of tumours by the EPR effect and they can be further conjugated to bio-responsive linkers or receptor-specific ligands, such as folic acid, to enhance the cell specificity. In the case of MDR cancers, some special strategies, such as co-incorporation of P-gp inhibitors

into the delivery systems, or a prodrug approach can be used. Drug delivery to some types of cancers, such as lymphatic carcinomas, by conventional means is very difficult since in lymphomas, the lymph nodes are the affected regions of the lymphatic system. The lymph nodes are not present superficially, and are deep seated in the body. The strategies applied to improve the delivery of anti-cancer agents to other types of tumours, such as EPR effect, may not result in high tumour concentrations in the case of lymphomas. As lymphomas are more richly supplied by lymph than blood, drug carriers administered intravenously may not have better access to the lymphomas, resulting in poor drug concentrations in lymph nodes. In such cases, the delivery route can be subcutaneous or intraperitoneal, as these routes have relatively better lymphatic access. Nanoparticles with hydrophobicity, high molecular weight, negative charge and small size can be effectively delivered to the lymphatic system through the above routes.

Anti-cancer agents can be delivered specifically to the tumours through low-density lipoprotein (LDL) receptors. LDL, a normal blood constituent, is the body's principal means of delivering cholesterol to tissues. Cancer cells need large amounts of cholesterol because of the rapid formation of new membranes. Thus LDL could be used as a carrier for anti-cancer agents, wherein the drugs can be delivered specifically to cancer cells. Different strategies can be adopted to deliver the drugs, such as chemically linking the drugs to LDL or incorporation of drugs into the delivery systems made from cholesterol derivatives.

An important drawback is the non-availability of appropriate cancer models for evaluation of the anti-cancer agent-loaded drug delivery systems. The need exists for the development of appropriate models for individual types of cancer, as the physiology may differ for all cancers. Studies should be more focused on direct evaluation in animal models than in cell cultures, as many of the physiological processes are different in the above two cases. Results obtained by testing in appropriate cancer models would lead to better conclusions.

Conclusion

The recent strategies adopted for delivering anti-cancer agents have been discussed. Drug delivery to tumours taking advantage of leaky tumour vasculature has been proved to be beneficial in enhancing the tumour drug concentration. Receptor-mediated drug delivery to tumours has resulted in successful enhancement of drug concentrations in tumours and effective tumour therapy. Local chemotherapy through drug carriers has been found to be the potential method of delivering a high drug dose at the target site leading to greater anti-tumour activity, while minimizing the systemic drug exposure. Direct administration of drugs or drug delivery systems into the tumour results in significantly high tumour concentrations. However, this approach may not be possible for remote tumours. Despite extensive research in the area of drug delivery to tumours, only very few products have successfully reached the market. Hence the focus of the research in this area needs to be carried out with an industrial viewpoint to develop commercially successful products for effective cancer therapy.

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