

Advances in Drug Delivery for Cancer Therapy

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Introduction

Bolus iv injection is the standard method for the administration of anti-cancer therapeutics. Whereas iv injection of soluble drugs provides a simple and clinically feasible approach, it has a number of disadvantages including inferior drug pharmacokinetics, inability to target drugs to tumours, dose-limiting systemic toxicity and side-effects associated with solvents used for drug solubilisation. To this end, a number of drug delivery

technologies have been developed in the past two decades to improve the pharmacokinetic profiles, reduce the toxicity and improve the bioavailability of anti-cancer therapeutics (Table 1). These strategies can be divided into two major categories based on the chemistry of the carriers used, i.e. lipid- and polymer-based delivery technologies. This article will summarise and update the recent developments in these areas.

Method	Advantage	Disadvantage	References
Lipid-Based Emulsions	Easy to prepare and use Increased bioavailability compared to free drug Ability to solubilise hydrophobic drugs	Unstable No targeting Difficult to use with biological macromolecules due to denaturation	Marahao <i>et al.</i> , 2002 Constantinides <i>et al.</i> , 2000 Homma <i>et al.</i> , 2004 Wang <i>et al.</i> , 2002 Rodrigues <i>et al.</i> , 2005
Conventional Liposomes	Stable Increased bioavailability Can be used to deliver biologicals Preferential accumulation in tumours	Rapid clearance by the RES No specific targeting Inefficient drug release at target	Torchilin, 2005
Long-circulating Liposomes	Same as conventional liposomes Significantly longer life	No specific targeting Inefficient drug release at target	Torchilin, 2005 Prestidge <i>et al.</i> , 2005
Modified Long-circulating Liposomes (Immuno-Liposomes, pH-sensitive Liposomes, etc.)	Same as long-circulating liposomes plus the ability to target tumours and superior drug release at tumour site	More difficult to prepare Possibly more immunogenic Limited target availability Still in development	Torchilin, 2005 Andresen <i>et al.</i> , 2005 Sugano <i>et al.</i> , 2000
Polymer Conjugates	Easy to prepare and use Enhanced bioavailability compared to free drug	Inability to target RES-clearance still a problem Cannot be used with biologicals	Vicent <i>et al.</i> , 2005 Langer, 2004 Ulbrich and Subr, 2004 Satchi-Fainaro <i>et al.</i> , 2004
Micelles	Stable RES-clearance minimised Can be targeted pH-sensitive versions possible	Limited to hydrophobic drugs Possible immunogenicity	Lukyanov and Torchilin, 2004 Gillies and Frechet, 2005 Rapoport, 2003 Matsumura <i>et al.</i> , 2004 Kim <i>et al.</i> , 2004
Biodegradable Polymer Devices	Sustained, long-term local release Can be used for delivery of biologicals Injectable micro/nanoparticle versions Available for local and systemic delivery Potential for oral delivery Potential for targeting (nanoparticles)	Wafer versions require surgical implantation Currently limited to local therapy No systemic targeting yet	Harper <i>et al.</i> , 1999; Fournier <i>et al.</i> , 2003 Liu <i>et al.</i> , 2003 Raza <i>et al.</i> , 2005 Dong and Feng, 2005 Farokhzad <i>et al.</i> , 2004 Gommersall <i>et al.</i> , 2004 Sinha and Trehan, 2003 Golumbek <i>et al.</i> , 1993 Egilmez <i>et al.</i> , 2000 Sabel <i>et al.</i> , 2004 Hill <i>et al.</i> , 2002

Table 1 – A summary of current cancer drug delivery technologies.

Lipid-based Carriers

Lipid-based carriers include simple emulsions of oil, lipid and/or fat molecules or the more advanced liposomal carriers. Lipid-based emulsions represent a simple technology where drugs are emulsified with a mixture of lipids and fatty acids to prolong their circulation time and enhance bioavailability. The additional benefit of these formulations is the ability to solubilise hydrophobic drugs. Lipid-based emulsions of carmustine, paclitaxel and doxorubicin demonstrate improved drug pharmacokinetics and reduced toxicity (Maranhao *et al.*, 2002 Constantinides *et al.*, 2000 and Homma *et al.*, 2004). The serum half-lives and tumour-targeting ability of emulsions can be enhanced by addition of PEG-lipids and by use of cholesterol-rich formulations that bind to LDL receptors on tumours, respectively (Wang *et al.*, 2002 and Rodrigues *et al.*, 2005). While the major advantage of emulsions is simplicity of preparation, their short-term stability (24–72 hours) is a significant drawback.

Liposomes, which are lipid bi-layer vesicles into which drugs are encapsulated, represent a more elegant, stable and effective drug delivery technology (Torchilin, 2005). The benefit of liposomal carriers over free drug is clinically well-established (Torchilin, 2005), i.e. reduced toxicity, preferential accumulation in tumours and increased bioavailability. The main disadvantage of the standard liposome formulations is their rapid clearance from circulation due to uptake by the reticuloendothelial system (RES), primarily in the liver. To circumvent this problem, long-circulating liposomes with polyethylene glycol (PEG) molecules attached to their surfaces have been developed. The PEG molecules form a protective layer over the surface, reducing opsonin binding to liposomes, thus prolonging their serum half-life. Long-circulating PEG-liposomal formulations of doxorubicin (doxil) have been used successfully in patients with metastatic breast cancer, head and neck cancer, ovarian cancer and unresectable hepatocellular carcinoma (Torchilin, 2005). As a result, numerous long-circulating liposomal formulations of chemotherapeutic drugs have been developed and are currently in clinical trials (Prestidge *et al.*, 2005). Current research in long-circulating liposomes focuses on attaching PEG molecules in a removable fashion (i.e. when exposed to the low pH of the tumour microenvironment) to enhance cellular uptake at the target site (Torchilin, 2005 and Andresen *et al.*, 2005).

Incorporation of tumour-targeting molecules to the surface of liposomes can significantly enhance intra-tumoral accumulation. The primary targeting moieties that have been used for this purpose are antibodies that recognise tumour- or neovasculature-associated antigens (Torchilin, 2005 and Andresen *et al.*, 2005). The antibodies are covalently attached either directly to the surface of the liposome or preferably, to the end of the PEG molecule without affecting the integrity of the liposome. Several studies have shown that these 'immunoliposomes' can target drugs to tumours more effectively than standard formulations (Torchilin, 2005 and Andresen *et al.*, 2005). Use of (Fab')₂ fragments of antibodies can reduce the

visibility of such complexes to the immune system and enhance their extravasation into tumours (Sugano *et al.*, 2000). In addition to targeting, immunoliposomes provide the advantage that binding of the complex to a membrane antigen usually results in the internalisation, leading to enhanced cytotoxicity (Torchilin, 2005, Andresen *et al.*, 2005 and Sugano *et al.*, 2000). Other targeting agents that have been used include folate and transferrin, the receptors of which are over expressed on many tumours. Finally, RGD peptides have been used to target $\alpha v \beta 3$ integrin expressed on the endothelial cells of tumour neovasculature (Torchilin, 2005 and Andresen *et al.*, 2005).

Another important parameter is the ability to release the encapsulated drug within the cell following uptake. To this end, pH sensitive liposomes, which destabilise upon encountering the low pH environment of the endosomes/lysosomes and release their contents into the cell, have been designed (Torchilin, 2005 and Andresen *et al.*, 2005). Other variations on this theme include the use of loco-regional photoexcitation, heat or enzymatic action to destabilise the liposome and achieve drug release within the tumour microenvironment (Andresen *et al.*, 2005).

Use of biologicals in cancer therapy is becoming increasingly more common and liposomes have been used for peptide, protein and nucleic acid delivery (Torchilin, 2005). In addition to improving bioavailability and reducing toxicity, encapsulation also provides protection from neutralising antibody responses in the case of biological macromolecules. A number of proteins including enzymes, growth factors, hormones and cytokines have been successfully encapsulated into liposomes and have been tested in pre-clinical studies (Torchilin, 2005). The most common application of liposomal biologicals in cancer has been in the area of immunotherapy (Torchilin, 2005). Encapsulation of cytokines, i.e. IL-1, IL-2, IL-6, TNF α , GM-CSF and IFN γ results in dramatically improved serum half-life and therapeutic efficacy, either as non-specific systemic immune stimulators or as tumour vaccine adjuvants (Ten Hagen, 2005).

Polymer Conjugates and Controlled-release Polymers

Polymers represent an alternative carrier technology to lipids for improving the bioavailability of anti-cancer agents (Moses *et al.*, 2003). Polymer-based delivery technologies can be categorised into two groups based on the method used for drug modification, i.e. polymer-drug conjugates and drug-loaded polymeric vesicles. The former approach requires covalent attachment of polymers to the drug, whereas the latter involves the ability to entrap drugs inside a polymer vesicle, i.e. micelles, injectable particles or other implantable devices.

Conjugation of polymers to chemotherapeutics results in a significant reduction in toxicity, enhanced serum half-life, intra-tumoral accumulation and superior anti-tumour activity compared to free drug (Vicent *et al.*, 2005). N-(2-hydroxypropyl)-methacrylamide (HPMA) copolymer, PEG, poly-L-glutamic acid (PGA) or polysaccharide derivatives

of doxorubicin, camptothecin and paclitaxel have been described with several of these reagents advancing to clinical trials (Vincent *et al.*, 2005 and Langer, 2004). Modifications of these conjugates include pH- or enzyme-sensitive variants that release the conjugated drug upon encountering the low-pH environment of the lysosome or via enzymatic digestion by lysosomal enzymes following endosome-lysosome fusion (Ulbrich and Subr, 2004). A recent non-chemotherapeutic application of polymer conjugation technology involves the development of capostatin, an HPMA copolymer conjugate of the anti-angiogenic molecule TNP-470, for anti-angiogenic therapy of cancer (Satchi-Fainaro *et al.*, 2004).

Micelles are nano-sized supramolecular assemblies of amphiphilic block copolymers that self-assemble in aqueous solution. These nano-containers are capable of encapsulating hydrophobic drugs in their core either as free drug or via conjugation to the hydrophobic segment of the polymer chain. Due to their small size, micelles can avoid uptake by the RES resulting in prolonged circulation and preferential accumulation within tumours, and have been successfully used to deliver doxorubicin, paclitaxel and camptothecin to tumour cells (Lukyanov and Torchilin, 2004). More advanced versions involve immuno-micelles, thermo-responsive micelles, ultra-sound sensitive formulations and pH-responsive assemblies for targeting and intra-tumoral/cellular release of the encapsulated drug (Lukyanov and Torchilin, 2004, Gillies and Frechet, 2005 and Rapoport *et al.*, 2003). Two Phase I trials of Doxorubicin- and paclitaxel-encapsulated micelle formulations in cancer patients were recently reported (Matsumura *et al.*, 2004 and Kim *et al.*, 2004).

Biodegradable polymer-based controlled-release devices represent the third category of polymeric carriers. These formulations are composed of natural polymers (cellulose, chitin or chitosan) or polymeric chains of naturally occurring monomers (lactic acid and glycolic acid), formed into a matrix with complex interconnecting pores through which the encapsulated drugs can diffuse (Moses *et al.*, 2003). Pore structure and degradation profile can be controlled by modulating the polymer composition/encapsulation method to achieve the desired rate and duration of drug release (Moses *et al.*, 2003). Such polymers can be manufactured as implantable devices such as rods, rings, wafers, or as injectable micro/nanoparticles.

Chemotherapeutic agents such as doxorubicin, paclitaxel and 5-fluorouracil have been encapsulated into biodegradable poly-lactic-co-glycolide injectable microparticles for sustained intratumoral delivery and were effective in suppressing tumour growth in pre-clinical studies (Harper *et al.*, 1999, Fournier *et al.*, 2003 and Liu *et al.*, 2003). The most successful application of controlled-release chemotherapeutics has been in the treatment of brain tumours, which are difficult to treat systemically due to the blood brain barrier (Raza *et al.*, 2005). In clinical studies, carmustine-encapsulated poly(carboxyphenoxy propane) co-sebacic acid (PCPP:SA) polymer wafers (Gliadel™), implanted into the post-surgical tumour cavities of malignant glioma patients, increased long-term

survival by 50%, resulting in US FDA approval (Raza *et al.*, 2005). Efficacy testing of Gliadel in newly diagnosed brain tumours as first line therapy is currently underway (Raza *et al.*, 2005). Other chemotherapeutic agents including Taxol, cyclophosphamide, 5-fluorouracil, doxorubicin and platinum drugs have been encapsulated into PCPP:SA wafers and are also being tested in patients (Raza *et al.*, 2005). While polymer matrix-based formulations are currently administered either by direct injection (in the case of microspheres) or by surgical implantation (wafers), oral administration represents a non-invasive systemic delivery route. To this end, oral polymer formulations of chemotherapeutics are currently an active area of investigation (Dong and Feng, 2005). Finally, a recently reported innovative strategy involves the use of tumour-targetable aptamer-conjugated nanospheres administered by iv injection (Farokhzad *et al.*, 2004).

Use of biodegradable micro/nanoparticles for delivery of biologically active macromolecules can provide a potentially more effective alternative to liposomes as polymer particles are more stable and provide longer-term release of cancer therapeutics *in vivo*. Injectable polymer microspheres have been successfully used to deliver luteinising hormone-releasing hormone analogs such as leuprolide acetate (nonapeptides) for the treatment of advanced prostate cancer (Gommersall *et al.*, 2002). These injectable depot formulations, which are currently marketed under the brand names Lupron®, Zolodex® and Decapeptyl®, can provide extended drug release (up to three months as opposed to daily injections of free drug) and are being used to treat patients worldwide with advanced prostate cancer.

Use of biodegradable polymer microspheres for delivery of macromolecules has been more difficult due to the labile nature of proteins and their incompatibility with the organic solvents that are used during encapsulation (Sinha and Trehan, 2003). However, recent advances in encapsulation technology have now made it possible to encapsulate proteins efficiently while preserving bioactivity (Sinha and Trehan, 2003). The utility of protein-encapsulated microspheres in cancer therapy has been demonstrated in studies where intra-tumoral release of cytokines such as IL-12, GM-CSF and TNF α from biodegradable PLA microspheres resulted in the development of potent anti-tumour immune responses (Golumbek *et al.*, 1993, Egilmez *et al.*, 2000 and Sabel *et al.*, 2004). Further studies established that a single intra-tumoral injection of IL-12 + GM-CSF-loaded PLA microspheres not only promoted the regression of established primary tumours and induced long-term systemic anti-tumour immunity, but also resulted in the complete eradication of disseminated disease in a metastatic murine tumour model (Hill *et al.*, 2002). These findings establish that biodegradable polymeric cytokine adjuvants provide a simple yet more clinically feasible alternative to gene-modification for the sustained delivery of immune-modulatory proteins in cancer therapy.

Conclusions

The developments outlined above clearly demonstrate that the use of advanced pharmaceutical carrier technologies in cancer therapy has progressed well beyond the conceptual/experimental stages with a number of successful products already on the market, and many more either in clinical trials or in the developmental pipeline. In addition, highly novel new applications, especially in the delivery of biological macromolecules which were off-limits until a decade ago, are emerging rapidly. It is expected that the application of such technologies to cancer therapy, an area where biology, chemistry, physics and pharmaceutical sciences intersect, will revolutionise current treatments.

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