Polymer–drug conjugates: towards a novel approach for the treatment of endocrine-related cancer

R Duncan¹, M J Vicent*¹, F Greco¹,² and R I Nicholson²

¹Centre for Polymer Therapeutics and ²Tenovus Centre for Cancer Research, Welsh School of Pharmacy, Cardiff University, Redwood Building, King Edward VII Avenue, Cardiff CF10 3XF, UK

(Requests for offprints should be addressed to R Duncan; Email: DuncanR@cf.ac.uk)

*(M J Vicent is now at Centro de Investigación Príncipe Felipe, FVIB, Medicinal Chemistry Unit, Av Autopista del Saler 16, E-46013 Valencia, Spain)

Abstract

The last decade has seen successful clinical application of polymer–protein conjugates (e.g. Oncaspar, Neulasta) and promising results in clinical trials with polymer–anticancer drug conjugates. This, together with the realisation that nanomedicines may play an important future role in cancer diagnosis and treatment, has increased interest in this emerging field. More than 10 anticancer conjugates have now entered clinical development. Phase I/II clinical trials involving N-(2-hydroxypropyl)methacrylamide (HPMA) copolymer-doxorubicin (PK1; FCE28068) showed a four- to fivefold reduction in anthracycline-related toxicity, and, despite cumulative doses up to 1680 mg/m² (doxorubicin equivalent), no cardiotoxicity was observed. Antitumour activity in chemotherapy-resistant/refractory patients (including breast cancer) was also seen at doxorubicin doses of 80–320 mg/m², consistent with tumour targeting by the enhanced permeability (EPR) effect. Hints, preclinical and clinical, that polymer anthracycline conjugation can bypass multidrug resistance (MDR) reinforce our hope that polymer drugs will prove useful in improving treatment of endocrine-related cancers. These promising early clinical results open the possibility of using the water-soluble polymers as platforms for delivery of a cocktail of pendant drugs. In particular, we have recently described the first conjugates to combine endocrine therapy and chemotherapy. Their markedly enhanced in vitro activity encourages further development of such novel, polymer-based combination therapies. This review briefly describes the current status of polymer therapeutics as anticancer agents, and discusses the opportunities for design of second-generation, polymer-based combination therapy, including the cocktail of agents that will be needed to treat resistant metastatic cancer.

Endocrine-Related Cancer (2005) 12 S189–S199

Introduction

The search continues for more selective therapies that will destroy either tumour cells or angiogenic tumour vasculature without harming normal tissue. Although the last two decades have seen successes, including introduction of new chemotherapy (e.g. taxanes (Jordan & Wilson 2004), tamoxifen (Jordan 2003) second- and third-generation aromatase inhibitors (Brodie & Njar 2000), Herceptin (Harries & Smith 2002), Gleevec (Atkins & Gershell 2002) and Avastin (Ferrara et al. 2004)), improvement in terms of tumour response or increased patient survival has largely relied on a combination of these new agents with existing chemotherapy, leading to incremental benefit in survival. Emergence of drug resistance remains a significant problem in the treatment of breast and prostate cancer. In the case of breast cancer, arrival of the selective oestrogen receptor (ER) antagonist
tamoxifen contributed to a 28% reduction in mortality at 5 years (Jordan 2003). Even so, the prognosis for patients with metastatic breast cancer is still poor, the survival rate at 5 years being under 20%. The mixed antagonist/agonist activity of tamoxifen and the acquired resistance that can develop in the long term limit its therapeutic potential (Coleman 1999, Cummings 2002). To circumvent this problem, there has been growing interest in the use of aromatase inhibitors (Brodie & Njar 2000, Lønning 2004), and recent clinical trials indicate that letrozole and anastrozole are more effective in treating ER-positive breast cancer than tamoxifen (Goss & Strasser 2002, Howell et al. 2005). The challenge of finding ‘breakthrough’ therapeutics able significantly to prolong the survival of patients with resistant metastatic breast and prostate cancer remains.

Two distinct research approaches are being pursued in the hunt for improved therapy. First, and by far the largest area reviewed in the literature (Chabner & Roberts 2005), is the use of low-molecular-weight chemotherapy and the search for novel, tumour-specific molecular targets. Of particular interest in relation to endocrine-related cancer are agents designed to interrupt the signal transduction pathways and/or stimulate apoptosis, such as epidermal growth factor receptor inhibitors (Atalay et al. 2003, Haran 2004), tyrosine kinase inhibitors (Daub et al. 2004, Singer et al. 2004) and modulators of apoptosis (Igney & Krammer 2002). In theory, new targets should allow design of ‘perfect’ drug molecules with exquisite therapeutic activity and no side effects. In reality, with the exception of Gleevac, which is used for the treatment of chronic myelogenous leukaemia and gastrointestinal tumours, this has proved difficult to achieve, and even in this case acquired resistance is a problem. With the explosion of molecular mechanism information from genomics and proteomics research, global oncology research is largely focusing on the search for the ‘perfect’, low-molecular-weight anticancer agent. Approaches include screening of natural product molecules, and modelling-driven synthesis of synthetic low-molecular-weight drugs. Recent successes with monoclonal antibodies have also popularised the search for natural macromolecules, including antibodies, proteins and oligonucleotides, that might have the required antitumour biological activity. Evolving in parallel, and, indeed, as a complementary approach, is the design of novel drug delivery systems (DDS) as cancer treatments (recently reviewed in Duncan 2005b). DDS have been designed for controlled release of endocrine therapy, such as Zoladex and Leupron depot, formulations which have proved so important in the treatment of endocrine-related cancers, for local delivery of chemotherapy (e.g. Gliadel for treatment of glioblastoma multiforme), and to improve tumour drug targeting (e.g. the antibody conjugate Mylotarg and the polymer conjugate Xyotax). In the context of cancer therapeutics, the current and potential contribution of DDS is often overlooked (Chabner & Roberts 2005). Over the last 10–15 years, systemically administered DDS and monoclonal antibody therapeutics have come of age. Entry of a growing number of products into routine clinical use is giving credibility to this field (reviewed in Duncan 2003b, 2005b) (Table 1). These nanosized hybrid systems often combine a drug, protein or antibody with a polymer or polymer-coated liposome and they can rightly be viewed as the first ‘nanomedicines’ (Fig. 1) (Allen 2002, Duncan 2003a, Torchillin 2005). Although the contribution of DDS as cancer therapeutics is still overlooked by many, there is a growing realisation that nanotechnology, as applied to medicine, has the potential to bring significant advances in the diagnosis and treatment of cancer (Ferrari 2005). See also the following reports for an introduction to this field: Editorial 2003 ‘Nanomedicine: grounds for optimism’. Lancet, 362; 673; NIH Roadmap for Nanomedicines, May 2004 http://nihroadmap.nih.gov/; Commission of the European Communities Communication: Towards a European Strategy for Nanotechnology, Brussels, COM 338, May 2004; UK; the European Science Foundation’s ‘Forward Look on Nanomedicines’, February 2005 (http://www.esf.org/newsrelease/83/SPB23Nanomedicine.pdf) and the NIH/NCI ‘Cancer Nanotechnology Plan’ July 2004 (http://nano.cancer.gov/alliance_cancer_nanotechnology_plan.pdf). In 2003, an amazing milestone was reached when the US Federal Drug Administration approved more biotech products (defined in the broadest sense and including DDS) as new medicines than more conventional, low-molecular-weight drugs.

**Polymer conjugates: rationale for design**

Although many are aware of the emergence of liposomal and antibody-based products, there is still little appreciation of the growing list of polymer therapeutics used as medicines. Commercialisation of polymer–protein conjugates (such as polyethylene glycol (PEG)-Lasparaginase (Onaspar) and PEGylated-recombinant methionyl human granulocyte colony stimulating factor (G-CSF) (Neulasta) in the USA (Harris & Chess 2003), coupled with the transfer of the N-(2-hydroxypropyl)methacrylamide (HPMA)
copolymer–doxorubicin conjugate (PK1, FCE28068) into clinical trials in Europe in 1994 (Duncan 2003b, 2005a), has been the breakthrough that led to the exponential growth of interest in this field. The term ‘polymer therapeutics’ describes several distinct classes of agent, including polymeric drugs, polymer–drug conjugates, polymer–protein conjugates, polymeric micelles to which drug is covalently bound, and the multicomponent polyplexes that are now being developed as non-viral vectors (reviewed in Duncan 2003b, 2005a). They are all considered ‘new chemical entities’ by regulatory authorities, more like therapeutic antibodies and their conjugates than DDS, which simply non-covalently entrap their drug payload. Over the last decade, more than 10 water-soluble polymer-drug conjugates (sometimes best visualised as macromolecular prodrugs) have entered phase I/II clinical trials as i.v. administered anticancer agents. These include six conjugates based on N-(2-hydroxypropyl)methacrylamide (HPMA) copolymers and, more recently, a

Table 1 Examples of antibodies and drug delivery systems used in cancer therapy

<table>
<thead>
<tr>
<th>Product</th>
<th>Status</th>
<th>Payload</th>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Therapeutic antibodies</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rituxan</td>
<td>Market</td>
<td>–</td>
<td>Non-Hodgkin’s Lymphoma CD20 +ve</td>
</tr>
<tr>
<td>Herceptin</td>
<td>Market</td>
<td>–</td>
<td>HER2 +ve breast cancer</td>
</tr>
<tr>
<td>Campath</td>
<td>Market</td>
<td>–</td>
<td>B-cell Chronic Lymphocytic</td>
</tr>
<tr>
<td>Avastin</td>
<td>Market</td>
<td>–</td>
<td>Leukaemia</td>
</tr>
<tr>
<td><strong>Antibody-drug conjugates</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mylotarg</td>
<td>Market</td>
<td>calicheamicin</td>
<td>Acute Myeloid Leukaemia CD33 +ve</td>
</tr>
<tr>
<td><strong>Radioimmunotherapeutics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tositumomab</td>
<td>Market</td>
<td>[131]I iodide</td>
<td>Non-Hodgkin’s Lymphoma</td>
</tr>
<tr>
<td>Zevalin</td>
<td>Market</td>
<td>[90]Y tritium</td>
<td>Non-Hodgkin’s Lymphoma</td>
</tr>
<tr>
<td><strong>Liposomes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daunoxome</td>
<td>Market</td>
<td>daunorubicin</td>
<td>Kaposi’s Sarcoma</td>
</tr>
<tr>
<td>Doxil/ Caelyx</td>
<td>Market</td>
<td>doxorubicin</td>
<td>Kaposi’s Sarcoma, Ovarian cancer</td>
</tr>
<tr>
<td>Depocyt-lipidic formulation</td>
<td>Market</td>
<td>cytarabine</td>
<td>Intrathecal therapy of lymphomatous meningitis</td>
</tr>
<tr>
<td><strong>Polymer-protein conjugates</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zinostatin Stimalmer (SMANCS)</td>
<td>Market</td>
<td>neocarzinostatin</td>
<td>Local administration – hepatocellular carcinoma</td>
</tr>
<tr>
<td>Oncaspar PEG-L-asparaginase</td>
<td>Market</td>
<td>asparaginase</td>
<td>Acute Lymphoblastic Leukaemia</td>
</tr>
<tr>
<td>PEG-intron</td>
<td>Market</td>
<td>α-interferon 2b</td>
<td>Hepatitis C, also in clinical development in cancer, multiple sclerosis, HIV/AIDS</td>
</tr>
<tr>
<td>PEG-α-interferon 2b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neulasta</td>
<td>Market</td>
<td>GCSF</td>
<td>Neutropenia associated with cancer chemotherapy</td>
</tr>
<tr>
<td>PEG-GCSF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Polymer-drug conjugates</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CT-2103, XYOTAX</td>
<td>Phase II/III</td>
<td>paclitaxel</td>
<td>Particularly lung and ovarian cancer</td>
</tr>
<tr>
<td>Polyglutamate-paclitaxel</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PK1; FCE28068</td>
<td>Phase II</td>
<td>doxorubicin</td>
<td>Particularly lung and breast cancer</td>
</tr>
<tr>
<td>HPMA copolymer-doxorubicin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PK2; FCE28069</td>
<td>Phase I/II</td>
<td>doxorubicin</td>
<td>Hepatocellular carcinoma</td>
</tr>
<tr>
<td>HPMA copolymer-doxorubicin-galactosamine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>APS280</td>
<td>Phase II</td>
<td>platinate</td>
<td>Cancer</td>
</tr>
<tr>
<td>HPMA copolymer platinate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>APS346</td>
<td>Phase I</td>
<td>platinate</td>
<td>Cancer</td>
</tr>
<tr>
<td>HPMA copolymer platinate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CT-2106 Polyglutamate-camptothecin</td>
<td>Phase I</td>
<td>camptothecin</td>
<td>Cancer</td>
</tr>
<tr>
<td>PROTHECAN TM</td>
<td>Phase II</td>
<td>camptothecin</td>
<td>Cancer</td>
</tr>
<tr>
<td>PEG-camptothecin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Polymeric micelles</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NK11 PEG-aspartic acid-doxorubicin micelle</td>
<td>Phase I</td>
<td>doxorubicin</td>
<td>Cancer</td>
</tr>
</tbody>
</table>

Table 1 Examples of antibodies and drug delivery systems used in cancer therapy

www.endocrinology-journals.org S191
A series of PEG and polyglutamic acid conjugates (Table 1). The evolution of this field has been reviewed in Duncan (2003a, b, 2005a, b).

Polymer-drug conjugates: rationale for design

The concept of polymer–anticancer conjugates was first proposed in 1975 (Ringsdorf 1975), and the biological rationale for their design (Duncan & Kopecek 1984, Duncan 1992) and current understanding of the mechanism of action is well documented (Duncan 2003b, 2005a). Briefly, these macromolecular prodrugs comprise a minimum of three components, as shown schematically in Fig. 2a): a natural or synthetic, water-soluble polymeric carrier (usually of 10 000–100 000 Da), a biodegradable polymer–drug linkage (often a peptidyl or ester linkage) and a bioactive antitumour agent. Not surprisingly, the first conjugates synthesised in the 1970s and early 1980s incorporated the most important anticancer agents of that era, particularly anthracycline antibiotics (daunorubicin and doxorubicin), alkylating agents (cyclophosphamide and melphalan) and antimetabolites (methotrexate and 5-fluorouracil).

Normally polymer–drug conjugates achieve tumour-specific targeting by the enhanced permeability and retention (EPR) effect (Matsumura & Maeda 1986). Hyperpermeable angiogenic tumour vessels allow preferential extravasation of circulating macromolecules and liposomes, and once in the interstitium they are retained there by lack of intratumoural lymphatic drainage. This leads to significant tumour targeting (>10-100-fold compared to free drug) and levels up to 20% dose/g have been reported for HPMA copolymer–doxorubicin conjugates, depending on tumour size. Both polymer- and tumour-related characteristics govern the extent of EPR-mediated targeting. Smaller tumours exhibit the highest concentration of polymer–drug. Using HPMA copolymer fractions in the range 10 000–800 000 Da as probes (Seymour et al. 1995, Noguchi et al. 1998), we found that tumour uptake of polymers (usual molecular diameter 5–20 nm) had broad size tolerance and good intratumoural penetration compared with that reported for liposomes and nanoparticles. Conjugates have also been synthesised to contain ligands that might promote receptor-mediated targeting (including antibodies, peptides and saccharides) (reviewed in Duncan 2005a, b). Although this is an attractive possibility, and proof of concept can easily be verified...
in vitro, so far only one such conjugate has progressed into phase I trial, and this was HPMA copolymer-
doxorubicin-galactosamine, which was designed as a
treatment for hepatocellular carcinoma or secondary
liver disease (PK2, FCE28069) (Seymour et al. 2002).

The growing database of in vitro, in vivo and clinical
data allows reappraisal of our current understanding
of the mechanism of action of HPMA copolymer-
anticancer conjugates (Duncan 2005a). The mechanism
of action is complex, and it is clear that many
factors act in concert to produce the antitumour effect
observed in vivo (Fig. 2b). Drug pharmacokinetics are
profoundly changed after polymer conjugation. After
i.v. administration, the conjugate is initially retained in
the vascular compartment, so that drug $T_{1/2}$ is increased
and the levels of free drug detected in plasma are very
low (>100–1000 times less than seen for the conjugated
drug). Preclinical rodent pharmacokinetic studies and
clinical pharmacokinetics correlate well (reviewed in
Duncan 2003b, 2005a). The plasma half-life of HPMA
copolymer anticancer conjugates is typically 1–6 h, and
elimination occurs predominantly via the kidney; over
50% of conjugated drug is excreted within 24 h. Only
in the case of the hepatocyte-targeted conjugate PK2
does hepatobiliary elimination play a major role after
liver targeting. This altered biodistribution reduces
drug access to potential sites of toxicity, including the
heart and bone marrow. This, together with the
enhanced elimination of inactive, conjugated drug via
the kidney (when the polymer–drug linker is stable),
explains the significant reduction in toxicity of antic-
cancer conjugates such as PK1 and PK2 in man (Vasey

Figure 2 Schematic diagram showing the structure of polymer–drug conjugates (panel a) and the mechanisms of action of polymer conjugates (panel b).
et al. 1999, Seymour et al. 2002). Gamma camera imaging has shown some evidence to support EPR-mediated tumour localisation in patients (Vasey et al. 1999). However, when colorectal cancer patients were given HPMA copolymer–camptothecin (MAG-CPT) 24 h before surgical removal of the tumour, the levels of conjugate measured in tumour tissue did not show preferential localisation compared to normal tissue (Sarapa et al. 2003). Further studies are needed in the clinical setting to clarify the clinical significance of the EPR effect in human tumours of different tissue origin and the extent of targeting at different stages of tumour development (primary, metastatic, postsurgery, etc.). Not least, we need to understand more about the effect of different dosing protocols and combinations with drug and/or radiotherapy on clinically related, EPR-mediated targeting.

Observations made in preclinical and clinical studies underline the need for careful design of the polymer drug linker so that it is stable in transit and degraded at a suitable rate intratumourally (reviewed in Duncan 2003b, 2005a). With HPMA copolymer conjugates, the lysosomally degradable peptidyl linkers (activated by thiol-dependant proteases) have shown the most promise. Hydrolytically labile terminal ester bonds have also been used to prepare conjugates of paclitaxel and camptothecin, and pH-sensitive hydrazone or cis-aconityl linkers are also currently being explored preclinically. A variety of terminal ligands have been used to synthesise HPMA copolymer-platinates with cisplatin-'like' (Gianasi et al. 1999), carboplatin-'like' (Gianasi et al. 2002) and oxaliplatin-'like' structure. Whichever linking chemistry is used, it is important to note that there is a clear influence of drug loading on conjugate conformation in solution. This in turn governs drug release rate and consequently therapeutic index. High loading with hydrophobic drugs can reduce the rate of prodrug activation, and solution conformation determines rates of both hydrolytic and enzymatic degradation.

Not only does drug conjugation affect whole-body pharmacokinetics, but it also changes fate at the cellular level. While many low-molecular-weight compounds enter tumour cells rapidly (within minutes) by passage across the plasma membrane, polymer conjugates are taken into cells much more slowly by endocytosis (reviewed in Duncan 2005a,b). This frequently makes comparative in vitro screening of activity almost meaningless. Conjugates containing free drug as a contaminant or that rapidly off-load drug in the tissue culture medium appear most potent. These conjugates, however, are often the least likely to exhibit a good therapeutic index in vivo. Endocytic internalisation of conjugates has been verified with a variety of cell lines, using 125I-labelled probes, HPLC assay of drug, and both epifluorescence and confocal microscopy. This route of cellular entry appears to enable agents to bypass efflux pump-mediated MDR.

There is growing evidence to support an immunostimulatory action of HPMA copolymer anticancer conjugates (reviewed in Duncan 2005a,b). Rihova has postulated that the early antitumour activity in vivo occurs via cytotoxic or cytostatic action, but that secondary immunostimulatory action of circulating low levels of conjugate supplement this effect (Rihova et al. 2003). This hypothesis is supported by the following evidence:

1. It is observed that pretreatment of animals with immunosuppressive agents (such as doxorubicin and cyclosporine A) accelerates the growth of subsequently implanted tumour, whereas pretreatment with HPMA copolymer-doxorubicin does not.
2. An increase in circulating natural killer (NK) cell numbers and anticancer antibodies is seen in animals treated with conjugate.
3. Increased NK and lymphokine activated killer (LAK) cells have been seen in breast cancer patients treated with HPMA copolymer-Dox-IgG (Rihova et al. 2003).

**Clinical status of polymer–drug conjugates as single agents**

In 1994, the first synthetic polymer–anticancer conjugate entered clinical trial. This was HPMA copolymer-Gly-Phe-Leu-Gly-doxorubicin (PK1, FCE28068). It has a molecular mass of ~30,000 Da and a doxorubicin content of ~8.5 wt% (Vasey et al. 1999). This peptidyl linker was designed to be hydrolysed by thiol-dependent proteases (particularly cathepsin B) after lysosomotropic delivery. In phase I trials, PK1 was administered as a short infusion every 3 weeks, and it had a maximum tolerated dose of 320 mg/m² (doxorubicin equivalent) (Vasey et al. 1999). This is approximately fourfold higher than the normal safe clinical dose of doxorubicin, and a much higher anthracycline dose than can be safely given in liposomal form. The FCE28068 dose-limiting toxicities were typical of the anthracyclines, including febrile neutropenia and mucositis. Despite cumulative doses up to 1680 mg/m² (doxorubicin equivalent), no cardiotoxicity was observed. Antitumour activity was seen in patients considered chemotherapy resistant/refractory (including breast cancer) and at lower doxorubicin doses
(80–180 mg/m²). Activity at the lower dose was consistent with EPR-mediated targeting.

Despite a large number of research studies exploring ligand-targeted polymer conjugates (reviewed in Duncan 2005a), PK2 (FCE28069) is still the only targeted conjugate to be tested clinically (Seymour et al. 2002). It was designed to recognise the hepatocyte asialoglycoprotein receptor and has been explored as a treatment for hepatocellular carcinoma. In phase I/II, the maximum tolerated dose of FCE28069 was 160 mg/m² (doxorubicin equivalent). Gamma camera imaging confirmed that most of the conjugate localised to liver. The majority of conjugate was present in normal liver (after 24 h, 16.9% dose) with lower accumulations within hepatic tumour (3.2% dose). However, it was estimated that this hepatoma-associated drug was still 12–50 fold higher than could be achieved with administration of free doxorubicin. Antitumour activity was seen in patients with primary hepatocellular carcinoma in this study.

Clinical studies with an HPMA copolymer-paclitaxel conjugate (PNU166945) and HPMA copolymer-camptothecin (MAG-CPT; PNU 166148) were disappointing. In both cases, this was probably due to lack of ester linker stability during transport in the circulation and/or renal elimination. HPMA copolymer-paclitaxel showed toxicity consistent with commonly observed paclitaxel toxicities: flu-like symptoms, mild nausea and vomiting, mild haematological toxicity was seen in patients with primary hepatocellular carcinoma in this study.

Clinical studies with an HPMA copolymer-paclitaxel conjugate (PNU166945) and HPMA copolymer-camptothecin (MAG-CPT; PNU 166148) were disappointing. In both cases, this was probably due to lack of ester linker stability during transport in the circulation and/or renal elimination. HPMA copolymer-paclitaxel showed toxicity consistent with commonly observed paclitaxel toxicities: flu-like symptoms, mild nausea and vomiting, mild haematological toxicity and neuropathy (Meerum Terwogt et al. 2001). Neurotoxicity grade 2 occurred in two patients at a dose of 140 mg/m² (although grade 1 was pre-existing on their entry), and one patient at 196 mg/m² had grade 3 neuropathy after the fourth cycle. Although no dose limiting toxicities (DLTs) were reported, dose escalation was discontinued prematurely due to concerns of potential clinical neurotoxicity. In this small patient cohort, antitumour activity was also observed. A paclitaxel-refractory breast cancer patient showed remission of skin metastasis after two courses at 100 mg/m² (paclitaxel equivalent). Two other patients had stable disease at a dose of 140 mg/m². PNU166148 (MAG-CPT) containing Gly-C6-Glycamptothecin showed severe and unpredictable cystitis in phase I clinical trials, and cumulative bladder toxicity was dose limiting. No objective clinical responses were seen; however, one patient with renal cell carcinoma had tumour shrinkage and a colon patient had stable disease (Schoemaker et al. 2002).

HPMA copolymer platinates (Rademaker-Lakhai et al. 2004), polymeric micelles containing doxorubicin and paclitaxel (Nakanishi et al. 2001), and PEG-camptothecin and paclitaxel conjugates (Greenwald et al. 2003) are also undergoing early clinical evaluation. However, the polymer conjugate most advanced in clinical development is a polyglutamate-paclitaxel conjugate (Li et al. 1998, Auzenne et al. 2002) called Xyotax, which is being developed by Cell Therapeutics (Seattle, WA, USA). An extensive phase II/III evaluation is under way, focusing on non small cell lung cancer (NSCLC) patients being treated with Xyotax as either a single agent (compared with paclitaxel, gemcitabine or vinorelbine) or in combination with carboplatin. Earlier phase I/II studies have reported very interesting activity in NSCLC and also relapsed ovarian cancer. Several phase III clinical trials are currently concluding (see latest abstracts at American Society for Clinical Oncology 2005, www.asci.org), and the Gynecologic Oncology Group (GOG) in the USA has recently initiated a phase III clinical trial involving Xyotax in ovarian cancer patients.

**Novel polymeric anticancer agents and polymer–drug combinations**

Conjugates tested clinically so far have incorporated only established chemotherapeutic agents, including doxorubicin, paclitaxel, camptothecins and platinates. Clinical proof of the concept is now paving the way for synthesis of second-generation conjugates containing experimental chemotherapy and novel polymer-based combinations. The approaches under investigation are shown schematically in Fig. 3, and all are based on the premise that the EPR effect will promote tumour selective delivery of polymeric anticancer conjugates to tumour tissue in humans (acting as a gateway for both passive and, in future, receptor-mediated targeting). Recently synthesised conjugates designed for lyso-somotropic delivery contain novel antitumour agents, such as compounds that have failed in early clinical development due to unacceptable toxicity (e.g. ellipticines (Searle et al. 2001) and TNP-470 (Satchi-Fainaro et al. 2004)), or interesting novel natural product antitumour agents (e.g. geldanamycin derivatives (Nishiyama et al. 2003), 1,5-diazaanthaquinones (Vicent et al. 2004a) and wortmannin (Varticovski et al. 2001)). In all cases, HPMA copolymers of traditional structure (molecular weight characteristics and a Gly-Phe-Leu Gly linker) have been used as a platform, with the terminal linker dependent on compound chemistry. HPMA copolymer-TNP470 (Satchi-Fainaro et al. 2004) is the first polymer-based antiangiogenic agent, and it shows considerable promise in vivo.

While many are beginning to explore novel polymer architectures, including dendrimers as carriers of
anticancer agents (Malik et al. 1999), the most pressing need is design of new biodegradable polymeric carriers that can be used at relatively high molecular weight to promote greater EPR-mediated targeting and then safely eliminated. Our recent studies have explored dextrin, a natural polymer degraded by amylase (Hreczuk-Hirst et al. 2001), and pendant chain functionalised polyacetals that display pH-dependent degradation after internalisation into the endosomal or lysosomal compartment (Tomlinson et al. 2002, 2003). These polymers can incorporate the drug (e.g. doxorubicin) via pendant linkage or as a component of the polymer main chain (Vicent et al. 2004b).

In the context of endocrine-related cancer, we have recently described diethylstilboestrol (DES) conjugate with DES as a component of the polymer backbone. Although this relatively old agent was used as a model compound, a new concept of anticancer drug delivery was established (Vicent et al. 2004b). These polymeric produgs incorporate DES into the polymer main chain in such a way that after endocytic internalisation the conjugates undergo pH-dependent degradation (much faster rates of DES release are seen at acidic pH) to liberate principally the bioactive trans-DES form. Polyacetal DES showed enhanced in vitro cytotoxicity compared to free DES, indicating potential for

Figure 3 Schematic diagram showing the polymer anticancer drugs, polymer–drug conjugates and polymer–drug combinations currently under study.
further evaluation in vivo where EPR-mediated targeting can be exploited to deliver higher tumour DES concentrations selectively.

Two-step anticancer treatments, such as polymer enzyme polymer prodrug therapy (PDEPT), have also been developed with the advantage of a burst of drug release in the tumour interstitium. In this case, a polymer–enzyme conjugate is prepared (PEGylated enzymes are already in routine clinical use (Table 1)) that will hydrolyse selectively a polymer drug linker within the tumour interstitium. The concept of PDEPT was exemplified first by an HPMA copolymer cathepsin B–PK1 combination (Satchi et al. 2001), and subsequently a non-mammalian enzyme linker combination HPMA copolymer-β-lactamase combined with HPMA-copolymer-glycine-glycine-cephalosporin-doxorubicin (Satchi-Fainaro et al. 2003). We have also shown that HPMA copolymer-bound phospholipases can also be used to modulate drug liberation from liposomes (Duncan et al. 2001). This strategy has been called polymer-enzyme liposome therapy (PELT).

Polymer conjugates containing endocrine and chemotherapy combinations

The polymeric carrier provides an ideal platform for delivery of a cocktail of drugs simultaneously. We have recently reported the first endocrine-chemotherapy combination in the form of the model compound HPMA-copolymer-aminoglutethimide-doxorubicin (Vicent et al. 2005). Here it was hypothesised that combination of endocrine therapy and chemotherapy by simultaneous attachment to the same polymer would bring significant advantages. The combination can be administered as a single dose, leading to the benefits of manufacture of a single conjugate and improved patience compliance. After EPR-mediated targeting, arrival of both pendant drugs within the tumour cells at the same time is guaranteed. It also provides the opportunity to tailor polymer–drug linkers to impart different rates of drug release for each compound, allowing agents to act synergistically. In in vitro experiments using MCF-7 cells and an aromatase-transfected cell line MCF-7Ca, it was found that conjugates containing both drugs (aminoglutethimide and doxorubicin) showed markedly enhanced cytotoxicity compared to PK1 (the conjugate that has already shown activity in breast cancer patients clinically), while mixtures of polymer conjugates containing only aminoglutethimide or only doxorubicin did not show synergistic benefit. These observations underline the possibility of designing polymer–drug combinations for improved treatment of breast and prostate cancer.

Conclusions

Enormous progress is being made in understanding the molecular basis of endocrine-related cancer, and this brings great potential to design improved therapeutic strategies (Sledge & Miller 2003). Although the pharmaceutical industry prefers to develop small-molecule anticancer agents that can be administered to patients orally (very convenient to use), macromolecular drugs and delivery systems, including antibodies, proteins and polymer conjugates, are establishing their niche in modern chemotherapy. It is now universally agreed that combination approaches, perhaps involving small molecules (endocrine and chemotherapy), macromolecular drugs such as antibodies, and DDS, including polymer therapeutics, will probably be required to increase therapeutic index and circumvent all routes to resistance.

Acknowledgements

There is no conflict of interest that would prejudice the impartiality of this review. It should be noted that R D’s group at Keele University (UK) has been involved in the design and clinical development of FCE28068 and FCE28069. She was employed by Pharmacia, Milan while PNU166945 and PNU166148 were being designed. Her group at the London School of Pharmacy designed the HPMA copolymer platinates that were licensed to Access Pharmaceuticals, Inc., and have subsequently entered clinical testing. Many thanks to Wendy Meeson for editorial assistance with the manuscript.

References


Auzenne E, Donato NJ, Leroux E, Price RE, Farquhar D & Klostergaard J 2002 Superior therapeutic profile of poly-1-glutamic acid-paclitaxel copolymer compared with taxol in xenogeneic compartmental models of
Duncan R, Gac-Breton S, Keane R, Musila R, Sat YN, Satchi R & Searle F 2001 Polymer-drug conjugates, PDEPT and PELT: basic principles for design and transfer from the laboratory to the clinic. Journal of Controlled Release 74 135–146.