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Review Article

Polymer-Drug Conjugates: Recent Achievements.

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ABSTRACT

Polymer-drug conjugates have demonstrated several advantages over the corresponding parent drugs, including fewer side effects, enhanced therapeutic efficacy, ease of drug administration, and improved patient compliance. Polymer-drug conjugates are nano-sized hybrid constructs that covalently combine a bioactive agent with a polymer to ensure not only its efficient delivery to the required intracellular compartment but also its availability within a specific period of time. Polymer-drug conjugates such as HMPA Copolymer-Doxorubicin (PK1), HMPA Copolymer-Doxorubicin-Galactosamine (PK2), HMPA Copolymer-Camptothecin, HMPA Copolymer-Platinate (AP5346), PEG-Camptothecin (Pegamotecan) and PEG-SN38 (EZN-2208) have main role in treatment of a wide variety of human pathologies, from diabetes, heart failure, and brain stroke. Future generation of polymer-drug conjugates will have to meet a number of challenges, including the development of novel polymers with modulated rates of degradation, versatile conjugation chemistry allowing site-specific attachment of targeting moieties.

Key words: Polymer-drug conjugates, Polymer, Prodrugs.

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INTRODUCTION

Many people's are aware about 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 polyethyleneglycol (PEG)-Lasparaginase (Oncaspar) and PEGylated-recombinant methionyl human granulocyte colony stimulating factor (G-CSF) (Neulasta) in the USA [1], coupled with the transfer of the *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymer–doxorubicin conjugate (PK1, FCE28068 into clinical trials in Europe, 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. 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 series of PEG and polyglutamic acid conjugates. Gaikwad et al., 2007 suggested that unconjugated form of leuprolide, LHRH agonist may be clinically effective in OCD, resulted dose dependently attenuated marble-burying behavior in mice has been used to model anxiety disorders viz. Obsessive–compulsive disorder (OCD) [2]. But, the LHRH-conjugate such as CPT-PEG-LHRH accumulated preferentially in tumour tissue which occurred passive targeting in tumors; resulted in greatest reduction of tumour size [3].

Advancement In Polymer-Drug Conjugates

Polymer-drug conjugates are nano-sized hybrid constructs that covalently combine a bioactive agent with a polymer to ensure not only its efficient delivery to the required intracellular compartment but also its availability within a specific period of time. It has already been demonstrated that polymer-drug conjugation promotes tumor targeting by the enhanced permeability and retention effect and, at the cellular level following endocytic capture, allows lysosomotrophic drug delivery. Consequently, polymer-drug conjugates have the potential to improve the therapy of common drug-resistant solid tumors by reducing toxicity and improving activity in chemotherapy-refractory patients. These multicomponent constructs have been already transferred to clinics as anticancer agents, either as single agents or as elements of combinations. The promising results arising from clinical trials with polymer-bound chemotherapy have laid a firm foundation for more sophisticated second-generation constructs delivering newly emerging target-directed treatments (eg, cell cycle or apoptosis modulators) [4] and polymer-drug combinations [5]. The use of polymer-drug conjugates in combination therapy is seen as an important opportunity to enhance disease response rates. The macromolecular prodrugs comprise a minimum of three components, as shown 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 [6]. 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 , 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). 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). 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.

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', carboplatin-'like' 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. 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 ^{125}I-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. 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 [7]. This hypothesis is supported by the following evidences- (a) 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. (b) An increase in circulating natural killer (NK) cell numbers and anticancer antibodies is seen in animals treated with conjugate. (c) Increased NK and lymphokine activated killer (LAK) cells have been seen in breast cancer patients treated with HPMA copolymer-Dox-IgG

Clinical Status of Polymer Drug Conjugates

The first synthetic polymer – anticancer conjugate such as HPMA copolymer- Gly-Phe-Leu-Gly-doxorubicin (PK1, FCE28068 entered in clinical trial in 1994. It has a molecular mass of ~30 000 Da and a doxorubicin content of ~8.5 wt% This peptidyl linker was designed to be hydrolysed by thiol-dependent proteases (particularly cathepsin B) after lysosomotropic delivery.

Polymer-Drug Conjugates Carrying Classical Anticancer Agents

Over the past decade 11 polymer-drug conjugates have entered clinical trials as intravenously administered anticancer agents. Most of these conjugates are known to exert their antitumor activity by inducing apoptosis through the traditional anticancer drug attached. However, certain chemotherapeutic agents, such as doxorubicin (Dox), paclitaxel (PTX), or camptothecin (CPT), work by initially acting nonspecifically by either damaging DNA (Dox and CPT) or disrupting the cytoskeleton (PTX) [8]. The N-(2-hydroxypropyl)methacrylamide (HPMA) copolymer-Dox conjugate PK1 (also named FCE28068) was the first synthetic polymer conjugate to enter phase I in 1994. The molecular mechanism of action of PK1 in comparison to free Dox has been studied thoroughly by different research groups, but a consensus has not yet been reached. The kinetic complexities of in vitro experiments involving these macromolecules make design and interpretation of such studies particularly challenging. Conjugates and free drugs have such different cellular pharmacokinetics and, moreover, all conjugates contain 1.0 to 0.01% free drug. Consequently, some studies have suggested that PK1 acts by a strong activation of apoptosis signaling pathways [9], while others have suggested that the primary mechanism of cell death induced by PK1 is necrosis. On the other hand, there is growing evidence that the early antitumor activity in vivo occurs via cytotoxic or cytostatic drug action but that the secondary immunostimulatory action of circulating low levels of conjugate augments this effect. Since PK1, 5 more HPMA copolymer conjugates containing established chemotherapy, such as PTX, platinates, or CPT, and 2 HPMA copolymer-derived gamma camera imaging agents have also progressed to clinical testing. Conjugates based on other polymeric carriers, such as poly(ethylene glycol) (PEG), poly(glutamic acid) (PGA), or polysaccharides, are also now found in clinics. Currently, polyglutamate-PTX (CT-2103 or Xyotax) is the most clinically advanced polymer-anticancer conjugate. This PGA conjugate was first designed by Li et al [10] Xyotax is expected to be the first polymer-anticancer conjugate to enter the market for the treatment of NSCLC in women. In this conjugate, PTX is linked to the carrier via an ester bond. This type of linkage had proved unsuccessful for HPMA copolymer CPT and HPMA PTX, since it led to premature drug release by blood esterases. However, the presence of a different polymeric carrier (PGA as opposed to HPMA

copolymer), as well as the high drug loading (~37% wt/wt), resulted in a stabilization of the linker, probably because of the conformation adopted in solution. Indeed, it was shown that the main drug release occurred subsequent to polymer degradation by the lysosomal enzyme cathepsin B. Morphological analysis and biochemical characterization have demonstrated that both PGA-PTX conjugate and free PTX possess similar abilities to induce apoptosis and that p53 did not appear to play a significant role in drug-induced cell death with either compound. It has also been demonstrated that both agents induced a characteristic G(2)/M arrest in the cell cycle, consistent with the disturbance of microtubule polymerization as their mechanism of action.

Another PGA conjugate, a PGA-CPT conjugate (CT-2106), has also entered phase I/II trials in patients with advanced malignancies. Stable disease was seen in 6 of the 24 treated patients, and the conjugate was well tolerated. CPT is a classical anticancer agent that induces cell death by converting DNA topoisomerase I into a DNA-damaging agent; formation of covalent and nonreversible topoisomerase I-DNA complexes during DNA replication results in strand breaks and subsequent induction of apoptosis. The conjugation of CPT to a polymeric carrier through an appropriate linker clearly enhances its anticancer efficacy, as demonstrated by PGA-CPT (CT-2106), PEG-CPT (pegamotecan, EZ-246),[11] and cyclodextrin-CPT (IT-101) conjugates. IT-101 is a conjugate of camptothecin and a linear cyclodextrin-based polymer (CDP).

Polymer-Drug Conjugates Carrying Novel Target-Directed Anticancer Therapy

On the basis of cancer's molecular mechanism there is evolved discovery of newly emerging target-directed anticancer agents such as tumor-selective apoptosis-inducing agents, modulators of the cell cycle, signal transduction inhibitors, and anti-angiogenic drugs. The first polymeric anti-angiogenic conjugate, HPMA copolymer-fumagillol (TNP-470), caplostatin has shown considerable promise in preclinical studies. TNP-470 is known to produce apoptosis in vascular endothelial cells, which has been proved by TUNEL experiments. Drug conjugation prevents TNP-470 from crossing the blood-brain barrier, thus preventing its inherent neurotoxicity. A recent study showed the eradication of human colon carcinoma in mice when caplostatin was combined with the monoclonal antibody bevacizumab (Avastin). Mitra et al recently described a novel polymer-peptide conjugate, HPMA copolymer-RGD4C-Tc-99m conjugate, capable of targeting tumor angiogenic vessels and delivering adequate radiotherapy to arrest tumor growth. In a xenograft model of human prostate carcinoma, this targeted conjugate showed significant tumor accumulation. Additionally, a histopathological examination revealed increased apoptosis in the treated tumors with no acute signs of radiation-induced toxicity to other organs. Other similar examples are the PEGylated cyclic arginine-glycine-aspartic acid (RGD) radiotracers (64-Cu-

DOTAPEG-RGD and ^{125}I-RGD-mPEG).

Focusing on tumor-selective apoptosis-inducing agents, it is well known that the Bcl-2 protein plays a key role in the mitochondrial-dependent apoptosis pathway and is therefore considered an interesting therapeutic target in tumor pathogenesis. Several low-molecular-weight Bcl-2 inhibitors have already been identified, but their efficacy in vivo has been very poor, mainly because of solubility and cell membrane permeability problems.

Conjugation to a hydrophilic polymeric carrier could overcome these drawbacks. Recently, the first bioconjugate of this type has been developed, HPMA copolymer-HA14-1 conjugate. The conjugate's in vivo studies demonstrated a much greater efficacy than free drug's. After intraperitoneal administration, HA14-1 conjugates were capable of suppressing tumor growth by 50%. Whereas activated caspase-9 protein was detected in tumors treated with the bioconjugate, none was found in either normal organs or tumors treated with a control polymer. Reactive oxygen species (ROS) are potentially harmful byproducts of normal cellular metabolism that directly affect cellular functions and survival. It has also been reported that ROS induce apoptosis of many tumor cells in vitro via the activation of the caspase cascade. Therefore, targeting tumor cells by inducing oxidative stress, or targeting the cells that sensitize the tumor to oxidative insults, has been considered another interesting approach in cancer therapy. The synthesis of the PEG–zinc protoporphyrin (ZnPP) conjugate, a specific heme oxygenase (HO) inhibitor, was developed with this objective in mind. PEG-ZnPP is found to induce cytotoxic effects by itself, as it makes cells more vulnerable to toxic insults. In some studies, PEG-ZnPP treatment produced a tumor-selective suppression of HO activity as well as an induction of apoptosis, possibly by increasing oxidative stress.

Other Polymer-Drug Conjugates In the Clinic

1. HMPA Copolymer-Doxorubicin (PK1)

Doxorubicin is an anthracycline cytotoxic agent widely used in the treatment of solid tumors, lymphoma, and leukemia. The DLTs of doxorubicin include bone marrow suppression, mucositis, and cardiotoxicity. In an attempt to reduce its toxicity, doxorubicin was conjugated to a water-soluble synthetic polymer, *N*-(2-hydroxypropyl) methacrylamide (HMPA)/methacylic acid copolymers through a tetrapeptide linker (Gly-Phe-Leu-Gly). The tetrapeptide linker allowed selective release of the active drug in the tumors through the action of lysosomal enzymes. The conjugate, known as PK1, was found to be more potent than free doxorubicin in antitumor activity in preclinical animal studies. PK1 was the first synthetic polymer-anticancer drug conjugate that entered clinical trials (in 1994). Data from Phase II trials for the treatment of breast, NSCLC, and colon cancers were presented in 2002 and showed positive responses in six of 63 patients, with manageable side effects. The subject of development history, mechanism of action, and pharmacology for PK1 has been reviewed extensively.

2. HMPA Copolymer-Doxorubicin-Galactosamine (PK2)

PK2 is a 27-kDa HMPA copolymer derivatized with 6.5% mol/wt, <2% free doxorubicin, and 2 % mol/wt galactose, with efficient targeting of the asialoglycoprotein receptor selectively expressed on hepatocytes and in hepatomas. In preclinical murine models, 80% of an administered dose targeted the liver, associated with high anticancer activity. PK2 remained the only targeted polymeric conjugate to be tested clinically to date. In Phase I/II trial, the maximum tolerated dose of PK2 was 160 mg/m^2, with neutropenia being the DLT. Hepatic targeting was confirmed by planar imaging and single photon emission computed tomography (SPECT) with ^{123}I-labeled PK2 co-injected with unlabeled

PK2. The majority of conjugate was present in normal liver (16.9% after 24 h), with lower accumulations within the hepatic tumors (3.2%).

3. HMPA Copolymer-Camptothecin (MAG-CPT, PNU166148) and HMPA Copolymer-Paclitaxel (PNU166945)

The camptothecins (CPTs) are a family of synthetic and semisynthetic analogues of 20(*S*)-camptothecin that exhibit a broad range of anticancer activity by inhibiting topoisomerase-1 activity. Two properties of CPT compounds limit their therapeutic efficacy in humans: instability of the lactone form because of preferential binding of the carboxylate to serum albumin, and a lack of aqueous solubility. Conjugation of CPT to water-soluble polymeric carriers has in general resulted in improved stability of the lactone ring and increased aqueous solubility. In HPMA-CPT (MAG-CPT, PNU166148), CPT is attached to the copolymer at the C-20 hydroxyl group of CPT through an ester linkage. A Phase I study in a total of 23 patients showed DLTs of myelosuppression, neutropaenic sepsis, and diarrhoea. The maximum tolerated dose and dose recommended for further clinical study was 200 mg equivalent CPT/m^2. The plasma half-life of both MAG-CPT and released CPT was extended to > 6 days, indicating that the kinetics of free CPT was release rate dependent. Results presented in an earlier clinical study showed serious bladder toxicity which became dose limiting. Patients with higher plasma AUC values had the more severe symptoms of renal toxicity. In HPMA copolymer-paclitaxel (PNU166945), paclitaxel was linked to the same tetrapeptide linker used to create PK1 and PK2. In a Phase I clinical study in a small patient cohort (12 patients), one patient with advanced breast cancer had a partial response. PNU166945 showed toxicity consistent with commonly observed toxicities associated with paclitaxel. One patient developed grade 3 neurotoxicity. Dose escalation was discontinued prematurely due to concerns about potential clinical neurotoxicity.

4. HMPA Copolymer-Platinate (AP5346)

The platinum-based compounds, cisplatin and carboplatin, are standard treatment regimens for a wide range of cancers. The DLTs of these drugs include nephrotoxicity, neurotoxicity, and myelosuppression. 1,2-Diaminocyclohexane (DACH) has received increasing attention in recent years because of its activity against cisplatin-resistant cancer cells. In AP5346, DACH-platinum was bound to HPMA via a pH-sensitive chelate. A methacrylamide monomer substituted with a triglycine aminomalonate group provided the primary binding site for the DACH-platinum moiety. The conjugate contained approximately 10% platinum by weight and had a molecular weight of 25 kDa, which was designed to allow glomerular filtration while also being large enough to benefit from an EPR effect. While negligible in neutral solutions, release of the DACH-platinum moiety was increased at low pH so that platinum release was favored in environments such as the extracellular space of hypoxic tumors and the intracellular lysosomal compartment. In a clinical Phase I study, AP5346 was administered as a 1-hour intravenous infusion on days 1, 8, and 15 of a 28-day cycle. Twenty-six patients received 41 cycles. Antitumor activity included two partial responses in metastatic melanoma and ovarian cancer and an additional CA-125 normalization in a suspected ovarian cancer. AP5346 administered weekly for 3 weeks out of every 4 weeks was tolerated up to a dose of 640 mg Pt/m^2 on the first cycle. The pharmacokinetics of AP5346 indicated a prolonged half-life (mean terminal half-life $t_{1/2}$ = 72

h) and evidence of antitumor activity. Additional human clinical trials are planned for this agent.

5. PEG-Camptothecin (Pegamotecan) and PEG-SN38 (EZN-2208)

Pegamotecan was developed by Enzon Pharmaceuticals, Inc. The conjugate consists of two CPT molecules conjugated to a 40-kDa PEG using an alaninate ester linkage. Free CPT must be cleaved from the PEG to be pharmacologically active. The hydroxyl group (-OH) at the 20-position of CPT is the active portion of the molecule responsible for the conformational changes between the active lactone and relatively inactive carboxylate forms. The 20-OH of CPT in Pegamotecan, is blocked by the alaninate linker, which stabilizes the CPT molecule into its active lactone conformation. The MTD of Pegamotecan when administered weekly for 3 of 4 weeks was 3,240 mg/m^2, with neutropenia being its DLT. Other grade 3 and 4 toxic effects were anemia, thrombocytopenia, fatigue, prolonged partial thromboplastin time, hemorrhagic cystitis, dysuria, and urinary frequency. Of the 27 patients enrolled, two patients had unconfirmed partial responses. The main limitation of PEG as drug carrier is the presence of only two reactive groups per polymer chain, which leads to an intrinsically low drug payload. To overcome this limitation, the construction of a dendron structure at the PEG's end chain has been proposed. Enzon is currently developing a conjugate of SN38, an active metabolite of CPT, with a 40-kDa PEG containing four arms. EZN-2208 has shown activity in a panel of human tumor xenografts. A Phase I study of EZN-2208 to evaluate the safety and tolerability of intravenous EZN-2208 in patients with advanced solid tumors or lymphoma is currently enrolling participants.

CONCLUSION

Polymer-drug conjugates have enormous potential for researchers and clinicians. From macromolecular prodrugs of established anticancer agents, the applications of polymer-drug conjugates have expanded dramatically in recent years. Apoptosis as a molecular target allows the design of second-generation polymer conjugates for the treatment of a wide variety of human pathologies, from diabetes, heart failure, and brain stroke to diseases such as cancer. Results from early clinical trials of about a dozen polymer-drug conjugates have demonstrated several advantages over the corresponding parent drugs, including fewer side effects, enhanced therapeutic efficacy, ease of drug administration, and improved patient compliance. Xyotax™ (PG-TXL) has become the first polymer-drug conjugate for the delivery of cytotoxic chemotherapeutic agents to advance to clinical Phase III trials. Future generation of polymer-drug conjugates will have to meet a number of challenges, including the development of novel polymers with modulated rates of degradation, versatile conjugation chemistry allowing site-specific attachment of targeting moieties, and polymerization methods that allow accurate control of polymer molecular weights and molecular weight distributions.

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