Polymer–drug conjugates, PDEPT and PELT: basic principles for design and transfer from the laboratory to clinic

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Abstract

There are now at least seven polymer–drug conjugates that have entered phase I/II clinical trial as anticancer agents. These include N-(2-hydroxypropyl)methacrylamide (HPMA) copolymer–doxorubicin (PK1, FCE28068), HPMA copolymer–paclitaxel (PNU 166945), HPMA copolymer–camptothecin, PEG–camptothecin, polyglutamic acid–paclitaxel, an HPMA copolymer–platinate (AP5280) and also an HPMA copolymer–doxorubicin conjugate bearing additionally galactosamine (PK2, FCE28069). The galactosamine is used as a means to target the conjugate to liver for the treatment of primary and secondary liver cancer. Promising early clinical results with lysosomotropic conjugates has stimulated significant interest in this field. Ongoing research is developing (1) conjugates containing drugs that could otherwise not progress due to poor solubility or uncontrollable toxicity; (2) conjugates of agents directed against novel targets; and (3) two-step combinations such as polymer-directed enzyme prodrug therapy (PDEPT) and polymer–enzyme liposome therapy (PELT) that can cause explosive liberation of drug from either polymeric prodrugs or liposomes within the tumour interstitium. Moreover, bioresponsive polymer-based constructs able to promote endosomal escape and thus intracytoplasmic delivery of macromolecular drugs (peptides, proteins and oligonucleotides) are also under study. © 2001 Elsevier Science B.V. All rights reserved.

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1. Background

Conjugation of anti-tumour agents to hydrophilic polymers [1] provides the opportunity to solubilise poorly water soluble drugs, improve tumour targeting and reduce drug toxicity. Over the last two decades our increased understanding of the biological factors most relevant to the design such conjugates (reviewed extensively in Refs. [2–5]) has enabled the design of polymer therapeutics sufficiently interesting to justify clinical testing.

Several ‘simple’ polymer–drug conjugates have now entered Phase I/II clinical trial (Fig. 1). N-(2-hydroxypropyl)methacrylamide (HPMA) copolymer doxorubicin (PK1, FCE28068) [6], HPMA copolymer–paclitaxel (PNU 166945) [7], HPMA copolymer–camptothecin [8], PEG–camptothecin [9], polyglutamic acid paclitaxel [10] and an HPMA copolymer–platinate [11]. In addition, one compound also bearing a site-specific targeting residue, HPMA copolymer doxorubicin–galactosamine (PK2, FCE28069) (Fig. 2), is undergoing Phase I/II testing...
Fig. 1. Polymer–drug conjugates undergoing clinical testing (a) PK1 (FCE 28068), (b) HPMA copolymer–paclitaxel, (c) HPMA copolymer–camptothecin, (d) HPMA copolymer–platin, (e) polyglutamic acid–paclitaxel and (f) PEG–camptothecin.
[12] and this conjugate was developed as potential treatment for primary hepatocellular carcinoma or secondary liver disease.

Early clinical experience with PK1 has shown a good correlation between the pharmacokinetics and the toxicological profile seen in man, and the earlier observations made in our pre-clinical studies [6,13]. Most importantly the novel polymer backbone, HPMA, did not show evidence of toxicity/immunogenicity in man. Not only does this validate the basic hypothesis of the original concept, but it also supports the fundamental rationale for the design of the pre-clinical methods used to optimise the first compounds.

Phase I results obtained with HPMA copolymer-Gly-Phe-Leu-Gly-doxorubicin (PK1) [6] showed that, when given once every 3 weeks, PK1 displayed greatly reduced toxicity compared to free doxorubi-
cin, and also had activity in chemotherapy refractory patients. The maximum tolerated dose (MTD) of PK1 was 320 mg/m² (doxorubicin-equivalent), 4–5 times higher than the usual clinical dose of free doxorubicin (60–80 mg/m²). Clinical dose limiting toxicities were typical of the anthracyclines (bone marrow suppression and mucositis) with no polymer-related toxicity and interestingly there was no evidence of the PK1-related cardiotoxicity (despite individual cumulative doses of up to 1680 mg/m² doxorubicin-equivalent). PK1 is currently undergoing Phase II evaluation for treatment of breast, colon and non-small cell lung cancer.

Although it is still early days in the clinical development of polymer–anticancer conjugates the observed profile of PK1 looks favourable compared to that of other anthracycline delivery systems (Table 1). This, together with the fact that several of these complex, multicomponent polymer pro-drugs have now successfully been scaled-up to industrial specification bodes well for the future development of this new class of anticancer compounds.

2. Polymer–drug conjugates: important aspects for design

The important characteristics of polymer-anticancer conjugates are listed in Table 2 and these points have been discussed at length elsewhere [2–5].

2.1. The polymer

It is essential that the polymer used is neither inherently toxic nor immunogenic. If the polymer is non-degradable in main chain the carrier must have a molecular weight sufficiently low to allow renal elimination (i.e. less than 30–40 kDa) and thus prevent progressive accumulation in the body.

2.2. The polymer–drug linker

Unless the drug bound to the polymeric chain is membrane active, a non-biodegradable polymer–drug linker will yield an inactive conjugate. Prefer-
ably the linker chosen should be stable in the circulation but amenable to specific enzymatic or hydrolytic cleavage intratumourally [2]. To allow drug delivery via the lysosomotropic route we originally designed peptidyl linkages that would allow cleavage by the lysosomal cysteine proteases (e.g. Gly-Phe-Leu-Gly-Dox of PK1) [20]. Coincidentally (not part of the original hypothesis for the design of such linkers), it is now known that high levels of cysteine proteases in human tumours correlate with poor prognosis. This would suggest that polymeric pro-drugs containing peptidyl spacers could be more rapidly activated within particularly aggressive tumours. Interestingly the HPMA copolymer platinates undergoing clinical testing (Fig. 1) allow drug liberation by simple hydrolysis, although both the paclitaxel and platinate conjugates also contain the enzymatically cleavable Gly-Phe-Leu-Gly linker. In contrast the HPMA copolymer-Gly-C<sub>e</sub>-Gly-camptothecin would be expected to liberate drug by hydrolytic release only. As it emerges, the clinical profile of these conjugates their comparative therapeutic index should be a useful indicator of the relative importance of these different mechanisms of drug liberation.

Recent studies with HPMA copolymer-platinates showed a good correlation between in vitro platinum release rates and in vivo therapeutic index in a B16F10 model [11] (Fig. 3).

### 2.3. Polymer–drug conjugate pharmacokinetics

Unlike low molecular weight antitumour agents which penetrate cells readily, the cellular uptake of polymeric pro-drugs is restricted to the endocytic route, i.e. lysosomotropic delivery (Fig. 4). It is this change in pharmacokinetics that limits rapid drug access to the normal sites of toxicity, thus reducing toxicity and offering opportunities for passive and active tumour targeting [2]. It is important to remember that the altered cellular pharmacokinetics makes impossible any meaningful comparison of the antitumour activity of polymer conjugates and free drug in vitro pharmacology screening is not possible. We advocate selection on the basis of: (i) linker release kinetics; (ii) ability to demonstrate tumour targeting by the advanced permeability and retention (EPR) effect [21] in vivo; and (iii) ability to demonstrate improved therapeutic activity (compared to the parent drug) in appropriately defined mouse and xenograft models.

The increased plasma residence of polymer conjugates (compared to free drug) encourages passive accumulation within solid tumour tissue due to

![Graph](image-url)

Fig. 3. Hydrolytic release of Pt from HPMA copolymer platinates in vitro measured in phosphate buffered saline pH 7.4 and antitumour activity and toxicity in a s.c. B16F10 tumour model. Note that the HPMA Gly-Phe-Leu-Gly-en-Pt conjugate requires enzymatic activation. (See Ref. [11] for full details.)
Fig. 4. Schematic representative of EPR-mediated tumour targeting and lysosomotropic delivery.
increased vascular permeability and the lack of an effective tumour lymphatic drainage. This phenomenon, (the so-called EPR effect) described by Maeda and colleagues [21] is responsible for substantially elevated tumour levels of HPMA copolymer–doxorubicin [22] and HPMA copolymer platinites [11] seen when compared to those measured following administration of free drug (Fig. 5). As HPMA copolymers of molecular weight of up to ~800 000 Da all showed equal ability to localise in two tumour models [23] we suggested that the tolerance limits for extravasation of hydrophilic polymers is quite large (up to 30 nm diameter). EPR-mediated targeting of PK1 produced tumour drug levels of up to 20% dose/g in a variety of murine and xenograft tumour models, but the extent of targeting varied from one tumour model to another (~10-fold) [24]. It was also noted that tumour targeting (% dose/g) frequently declined with increasing tumour size [24], but this in itself may be helpful if small micrometastases are the best sites for selective deposition. Importantly for the first time these studies also indicated that there may be a much higher variability in the levels of activating enzyme (~200-fold) than seen for the EPR-mediated targeting (~5- to 10-fold) [25].

Factors influencing EPR-mediated tumour-targeting of polymer–drug conjugates are listed in Table 3. Plasma residence time (and hence plasma concentration) is the primary driving force for continued tumour accumulation. Hence long circulating liposomes have a greater accumulation with longer times than PK1 [27]. However, long circulation can also lead to loss of selectivity as normal tissue exposure is often increased. This leads to the lower clinical doses of liposomal anthracyclines that can be safely given (typically 40–60 mg/m² doxorubicin-equivalent) (Table 1). Although the tumour doxorubicin (s.c. B16F10 model) levels seen following administration of Doxil® showed a 4-fold increase in tumour ACU compared PK1 [27], the fact that the polymer conjugate can be safely administered to patients at a much higher clinical dose would suggest that greater ‘tumour targeting’ may be possible.

3. Strategies for the development of second generation polymer therapeutics

Although now well established in animal models, the magnitude of the EPR effect in relation to polymer therapeutics targeting to human tumours must still be established in the clinical setting. Although critical gamma camera imaging has visualised polymer conjugates within solid tumours in man [6,35] to date the imaging has been poor. A better understanding of the extent of tumour capture in man will only come from improved clinical imaging (better gamma camera probes or PET) and/or traditional HPLC measurement of drug levels in tumour
Table 3
Factors effecting the EPR effect

| Vehicle related                  | Plasma residence time (Influenced by molecular weight) | Seymour et al. [23]  
Noguchi et al. [26]  
Sat et al. [27]  
Yuan et al. [28,29]  
Gianasi et al. [11]  
Malik et al. [30]  
| Liposome/particle size          | Tumour size  
Tumour type  
Microenvironment | Duncan and Sat [31]  
Sat et al. [24]  
Hobbs et al. [32]  
| Polymer architecture            | Radiation  
Bradykinin antagonist, cyclooxygenase inhibitor and NO scavenger | Li et al. [33]  
Wu et al. [34] |

after administration of polymer-drug conjugates. None-the-less, the increasing depth of the pre-clinical data and clinical observations using liposomal formulations support the significance of the EPR effect as a means of tumour targeting.

Our current research is exploring several different therapeutic strategies that can capitalise of improved tumour targeting by the EPR effect and these are shown in Fig. 6.

3.1. Lysosomotropic delivery

The above-mentioned anticancer polymer–drug conjugates in clinical trial were all initially designed to capitalise either on lysosomotropic drug delivery or the ability of hydrophilic polymers to solubilise poorly soluble drugs. Opportunities abound for the synthesis of novel anticancer agents (already in routine clinical use such as doxorubicin, paclitaxel, camptothecin, platinates) with new, more potent drugs that have novel mechanisms of action. Additionally, polymer conjugation may provide an opportunity to ‘rescue’ promising new agents that have failed in early clinical development due to poor solubility or unacceptable toxicity. For example, we have recently described a family of HPMA copolymer-6-(3-aminopropyl)-ellipticine (APE) conjugates [36] (Fig. 7). Compared to free APE, HPMA copolymer–APE conjugates displayed improved solubility, reduced haemolytic activity, and could be designed to release APE in the presence of lysosomal enzymes. Preliminary experiments showed that HPMA copolymer–APE displays antitumour activity in vivo against s.c. B16F10 melanoma.

3.2. Intracytoplasmic delivery

As the basic understanding of the genetic basis of cancer is unravelled many novel antitumour opportunities emerge. However many of the new approaches, e.g. gene and antisense therapeutics, antitumour proteins and drugs that are peptidyl mimetics share the common problem of restricted tumour targeting and moreover limited intracytoplasmic access. To assist delivery of these compounds we are developing bioresponsive polyamidoamine polymers that are membrane active within the endosomal compartment and promote cytosolic access [37,38].

3.3. Delivery of drug extracellular using PDEPT or PELT

Should a slow rate of endocytic internalisation in certain tumour types effectively prohibit the use of lysosomotropic or endosomotropic systems it is necessary to develop approaches that can deliver drugs more rapidly, extracellularly. The combination approaches polymer-directed enzyme polymer prodrug therapy (PDEPT) [39] and polymer-directed enzyme liposome therapy (PELT) [40] have been designed to facilitate explosive release of drug from either polymer drug conjugates (PDEPT) or liposo-
al formulations (PELT) that lie inactive in the tumour interstitium (Fig. 6).

3.4. Membrane active polymer conjugates

A fourth approach we are exploring (which is still in its infancy) is the concept of using polymers as a platform for targeted delivery of antitumour agents that are active at the level of the plasma membrane. These conjugates too should be active in the tumour interstitium and would not require endocytic internalisation for activation. In this context HPMA copolymer conjugates have been prepared to contain membrane active peptides (e.g. melittin) [41]. Melittin conjugates displayed greatly reduced haemolytic activity (Hb₅₀ and IC₅₀ both ~6 μg/ml) [41]. The melittin conjugate was retained longer in the circulation than free melittin and showed less liver accumulation than the cationic, hepatotropic, free melittin. This led to significantly improved tumour uptake of the conjugate at the 4 h [41]. The evidently diminished in vivo toxicity and potential for improved tumour targeting of the HPMA copolymer–melittin conjugate suggest that this too may be an interesting approach.

4. Conclusions

Although liposomal formulations and antibody–drug conjugates are widely explored as systems for improved targeting of anticancer agents during the last two decades, the first synthetic polymer-drug conjugate to enter Phase I did so as recently as 1994.
parent drugs (usually a 3 weekly cycle). However, as the pharmacokinetics and toxicological profile (especially for PK1 [6]) is so different form that of the parent compound, and the pre-clinical pharmacology/pharmacokinetic studies suggest the benefit of multiple daily doses. It is essential that clinical dosing schedule be optimised for administration of polymer–drug conjugates if they are to realise full potential.

Pre-clinically, it is clear that routine in vitro cytotoxicity screening (in its present form) is not a suitable method to select the ‘lead’ polymer–drug conjugates for further evaluation. The results obtained give false positive results with conjugates that contain free drug (all do to some extent) and conjugates that are unstable, these offload drug rapidly into the culture medium. Exciting times are ahead and the polymer approach potentially provides a universal ‘platform’ that can be used for tumour selective delivery of many different kinds of anti-tumour agent (Fig. 6).

Fig. 7. Structure of HPMA copolymer–APE.

Since that time there has been considerable interest in this approach and a further six conjugates are now in early clinical development. As mentioned recently [42] considerable ‘patience’ (and perseverance) coupled with the collaborative efforts of chemistry, biology, medicine (not least in both academia and industry) was required to bring the concept of polymer–protein conjugates into clinical trial. Undoubtedly, transfer of the first polymer–protein conjugates to market as anticancer therapies (Oncaspar® and SMANCS) has played an important role in establishing the practicality of polymer therapeutics in cancer therapy. This, couple with the promising start for polymer–drug conjugates in early clinical trials suggests an interesting future for polymer therapeutics in cancer therapy.

Several words of caution! First, it is disappointing that the early clinical studies have not moved more quickly towards dose-schedule optimisation in respect of the pharmacokinetics (and toxicological profile) of polymer conjugates. Inevitably the first studies followed the standard dose schedule of the

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