Antibody-directed enzyme prodrug therapy (ADEPT) for treatment of major solid tumour disease


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Introduction

Antibody-directed enzyme prodrug therapy (ADEPT) is a two-step, antibody-based, targeting strategy for the treatment of cancer [1–4]. The first step involves the administration of a tumour-selective antibody linked to an enzyme. After tumour localization of the antibody–enzyme conjugate and clearance of the conjugate from blood and normal tissues, the second step involves administration of an inactive prodrug. The targeted enzyme converts the prodrug locally at the tumour site into a potent cytotoxic drug that can kill the tumour cells.

Each enzyme molecule delivered by the antibody to the tumour can convert many prodrug molecules into drug molecules. Thus, potency limitations of directly targeting cytotoxic drug molecules to a tumour with an antibody, caused by the fact that only a limited number of drug molecules can be directly attached to an antibody without compromising antibody binding [5], are overcome by ADEPT. Another advantage of ADEPT is that a small-molecular-mass cytotoxic drug is generated locally at the tumour site and outside the cell. If appropriately designed, the drug should be capable of diffusing and killing both antigen-negative tumour cells within the tumour and tumour cells not accessed by the high-molecular-mass antibody–enzyme conjugate.

ADEPT systems

A number of different ADEPT systems have been shown to result in anti-tumour activity that exceeds that of conventional cytotoxic drugs in human tumour xenograft models (Table 1).

Use of mammalian enzymes such as alkaline phosphatase and β-glucuronidase for ADEPT suffers from the disadvantage that endogenous enzyme may turn over prodrug at non-tumour sites, leading to enhanced toxicity. This is particularly true for alkaline phosphatase, as demonstrated by the fact the etoposide phosphate has anti-tumour activity in its own right and is

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ADEPT systems resulting in anti-tumour activity in tumour xenografts

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Prodrug</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaline phosphatase</td>
<td>Etoposide phosphate</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Mitomycin C phosphate</td>
<td>5</td>
</tr>
<tr>
<td>Phenol mustard phosphate</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>β-Glucuronidase</td>
<td>Adriamycin-glucuronide</td>
<td>7</td>
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<tr>
<td>β-Lactamase</td>
<td>Mustard-cephalosporin</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Vinca alkaloid-cephalosporin</td>
<td>9</td>
</tr>
<tr>
<td>Carboxypeptidase G2</td>
<td>Mustard-glutamate</td>
<td>1</td>
</tr>
</tbody>
</table>

Table I

Enzyme Prodrug
Alkaline phosphatase Etoposide phosphate 2
Mitomycin C phosphate 5
Phenol mustard phosphate 6
β-Glucuronidase Adriamycin-glucuronide 7
β-Lactamase Mustard-cephalosporin 8
Vinca alkaloid-cephalosporin 9
Carboxypeptidase G2 Mustard-glutamate 1

rapidly ($t_{1/2} < 5$ min) converted to etoposide in plasma in the absence of any antibody–enzyme conjugate [10]. Using bacterial enzymes such as β-lactamase and carboxypeptidase G2 (CPG2) has the advantage that turnover by endogenous enzyme is avoided. However, enzymes have the drawback that they are immunogenic and this will limit the number of courses of ADEPT therapy that can be administered.

We have focused on the bacterial enzyme CPG2 and mustard alkylating agent prodrugs. CPG2 catalyses the hydrolysis of folates to pter- oates and L-glutamic acid [11]. Mustard alkylating agents were chosen as the drug component for a number of reasons. Firstly, they can kill both proliferating and quiescent cells. This is important since a large percentage of tumour cells in major solid tumours are quiescent and the number of courses of ADEPT therapy with an antibody–CPG2 conjugate will be limited by its immunogenicity. Thus, the drug released needs to kill both the quiescent and proliferating tumour populations to have major anti-tumour activity. Secondly, only low levels of drug resistance are reported to develop after exposure of tumour cells to alkylating agents [12]. Thirdly, the chemical half-life of the drug can be optimized so that the drug has sufficient time to diffuse and kill tumour cells throughout the tumour but becomes rapidly inactivated if it escapes into the peripheral circulation.

Benzoic acid mustard prodrugs

The original prodrug developed for CPG2 was 4-[N,N-bis (2-chloroethyl) amino] benzoyl-L-glutamic acid [1]. In combination with the F (ab')₂ fragment of the anti-CTA antibody, A5B7 and CPG2. In LoVo (D. C. Blakey, unpublished work) and LS174T colorectal tumour xenografts [14] the combination of F (ab')₂A5B7–CPG2 and CMDA also gave significant growth delays that exceeded the activity of 5-fluouracil (5-FU) given at its maximum tolerated dose. However, major tumour regressions were not seen with this CMDA ADEPT system in these tumour models.

A conjugate clearing system was developed consisting of an anti-CPG2 monoclonal antibody conjugated to galactose residues (SB43–gal). Administering this clearing system resulted in peripheral conjugate being removed to the liver. This allowed the CMDA prodrug to be administered earlier after F (ab')₂A5B7–CPG2 conju-
gate administration when tumour levels of the conjugate were higher in the LS174T tumour xenograft model, and this led to improved anti-tumour activity [15].

A pilot-scale clinical trial of the F(\text{ab}')\text{2}_A5B7–CPG2 conjugate in combination with the SB43–gal clearing conjugate and the CMDA prodrug has been carried out in patients with advanced, drug-resistant, colorectal cancer [16]. In the absence of conjugate there was no evidence of activation of the CMDA prodrug to active drug, confirming the lack of mammalian enzymes with equivalent enzymic activity to CPG2. Partial or mixed tumour responses occurred in five out of eight patients who were assessable for tumour response. All patients developed antibodies to both the mouse F(\text{ab}')_2 antibody and CPG2 components of the conjugate. Cyclosporin delayed development of host antibodies and allowed up to three cycles of ADEPT to be given. Dose-limiting toxicity was myelosuppression and active drug was detected in the bloodstream.

These encouraging clinical results support the potential of ADEPT to improve cancer therapy. However, three limitations of the CMDA ADEPT system are that: (1) relatively high conjugate and prodrug doses are required for therapy since the drug generated is only modestly cytotoxic; (2) the active drug released by CPG2 from CMDA has a relatively long biological half-life (17 min); thus drug generated at the tumour has time to escape into the peripheral circulation and enhance myelotoxicity; and (3) the use of a clearing system to achieve significant anti-tumour activity increases the complexity of ADEPT. To overcome these problems we have developed improved prodrugs based on more potent mustard drugs which, in combination with the F(\text{ab}')_2A5B7–CPG2 conjugate, give excellent anti-tumour activity in tumour xenograft models.

**Improved mustard prodrugs for CPG2**

Glutamate prodrugs of both phenol mustard and aniline mustard incorporating a carbamate and urea linkage respectively between the mustard and glutamate moieties were prepared and surprisingly were found to be good substrates for CPG2 (\(K_a = 1-10 \mu M, k_{cat} = 10-40 \text{ s}^{-1}\)). The active drugs generated by CPG2 from these prodrugs were 50- to 100-fold more potent than the drug released from CMDA [17]. Both the aniline mustard prodrug [18] and the phenol mustard prodrug [19] administered 72 h after the F(\text{ab}')_2A5B7–CPG2 to athymic mice bearing established LoVo colorectal tumour xenografts resulted in tumour regressions and 15-20 day growth delays at doses of prodrug that gave only approximately 6% body weight loss.

The efficacy of the phenol mustard prodrug has been further enhanced by the use of bisiodo-mustard arms instead of bischloro-mustard arms in the original prodrug. This prodrug (ZD2767P) is a good substrate for CPG2 (\(K_a < 3 \mu M, k_{cat} = 30 \text{ s}^{-1}\)) and the active drug released has an IC\(_{50}\) versus LoVo colorectal tumour cells in vitro (1 h incubation) of approximately 0.3 \(\mu M\). It is approximately 300-fold more cytotoxic than the drug released by CMDA. In addition, it has a short chemical half-life, which should promote its retention at the tumour site [20]. Administration of ZD2767P (3 \times 70 mg/kg given as three intraperitoneal injections over 2 h) 72 h after administration of F(\text{ab}')_2A5B7–CPG2 conjugate (2.5 mg/kg i.v.) to athymic mice bearing established LoVo colorectal tumour xenografts resulted in approximately 50% of the tumours undergoing complete regressions, tumour growth delays >30 days and little toxicity as judged by body weight loss (6–7%) [21]. In contrast, ZD2767 prodrug alone had no anti-tumour activity, and in combination with a control conjugate [F(\text{ab}')_2MOPC–CPG2] that does not bind to the LoVo tumour cells only resulted in tumour growth delays of <5 days. These results confirm the specificity of this ADEPT approach [21]. These anti-tumour effects of ZD2767P in this
colorectal tumour xenograft model were achieved without the need to use a clearing antibody conjugate to remove residual antibody–enzyme conjugate from the bloodstream.

The ADEPT system (Figure 2) consisting of ZD2767P and F(ab')2A5B7–CPG2 (ZD2767C) has been given the development number ZD2767 and is currently in preclinical development for treatment of CEA expressing major solid tumours such as colorectal cancer.


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