Regression of Established Breast Carcinoma Xenografts with Antibody-directed Enzyme Prodrug Therapy against c-erbB2 p185

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ABSTRACT

The enzyme carboxypeptidase G2 (CPG2) was conjugated to the rat IgG2a monoclonal antibody (mAb) ICR12, which recognizes the external domain of the human c-erbB2 protooncogene product. The conjugate retained antigen-binding and enzyme activity. Radiolabeled conjugate localized efficiently and stably to MDA MB 361 breast carcinoma xenografts, which overexpress the c-erbB2 gene product p185. Radiotracer determinations and plasma enzyme activity studies in nude mice gave conjugate blood clearance rate half-lives of approximately 4 days. In separate antibody-directed enzyme prodrug therapy regimes, one dose of the 4-[(2-chloroethyl)(2-mesyloxyethyl)amino]benzoyl-L-glutamic acid prodrug was administered to nude mice bearing established MDA MB 361 tumors (mean volume, 170—260 mm²). In mice which had received ICR12-CPG2 12—14 days previously, sustained dose-dependent tumor stasis or regressions were effected, which in some cases persisted throughout observation periods of up to 90 days. In control mice which had received the isotype-matched irrelevant mAb ICR16-CPG2 conjugate, tumors grew progressively, as did those in mice treated with prodrug alone, or treated simultaneously with ICR12-CPG2 and prodrug at the maximum tolerated dose. Control chemotherapy with conventional drugs proved toxic and induced only minimal growth delays.

INTRODUCTION

ADEPT has been proposed as a two-phase approach to targeted chemotherapy of human cancer (1). Briefly, in the first phase a monoclonal antibody conjugated to a nonendogenous enzyme is administered, and time is allowed for localization to tumors and clearance from the blood and other tissues. The second component is a nontoxic prodrug which is converted to a cytotoxic drug by the action of the targeted enzyme localized at the tumor site. Theoretically, such an approach should enhance the therapeutic index of chemotherapeutic agents by minimizing systemic toxicity and maximizing drug concentrations in tumor. In addition, unlike other antibody-targeting strategies (such as delivery of chemioimmunoconjugates, immunotoxins, or activation of host effector mechanisms) this approach is not limited by tumor antigenic heterogeneity or the need to internalize conjugates or recruit ancillary effectors (2).

Many different enzyme prodrug systems have been designed, each with their own advantages and disadvantages (recently reviewed in Ref. 3). We have used the bacterial enzyme CPG2 (which has no mammalian homologue) to activate a glutamic acid prodrug derivative of a benzoic acid mustard (4). Previous ADEPT experiments have demonstrated the efficacy of mAb-CPG2-activated prodrug activation in choriocarcinoma (2, 5) and colorectal tumor models (6). The advantages of using mustard-alkylating agents as opposed to other types of drugs is that their cytotoxicity is not cell-cycle specific and is dose related, and their use is not generally associated with the development of induced resistance (7). In ADEPT experiments with other systems, an anti-colorectal carcinoma mAb conjugated to alkaline phosphatase has been used with etoposide phosphate as prodrug to effect tumor regressions (8). The same enzyme has also been proved beneficial when conjugated to a mAb in a lung xenograft model, using the prodrugs mitomycin-phosphate (9) or phenol mustard-phosphate (10). The enzyme β-lactamase conjugated to mAbs in colon carcinoma models was effective with a vinca alkaloid-cephalosporin prodrug (11). Likewise, in a colorectal xenograft, a mAb fusion protein of the enzyme β-glucuronidase in combination with a doxorubicin-glucuronide prodrug had some efficacy (12). To date, no group has published on the ADEPT system in a breast xenograft model.

The choice of antigenic target and of the corresponding mAb used to target the enzyme is obviously of paramount importance. The c-erbB2 protooncogene is amplified and/or overexpressed in 20—30% of many types of epithelial tumors, including those of breast, ovary, bladder, stomach, lung, and cervix; in many cases, this has been linked with poor prognosis (13—18). The cell surface location of the gene product (p185) and its relatively low expression on normal adult tissues (19) makes antibody-guided therapy of such tumors an attractive proposition.

We have described previously the production and characterization of a panel of rat monoclonal antibodies directed against the external domain of human c-erbB2 p185 (20, 21). Of these, mAb ICR12 was outstanding in its ability to localize stably and specifically to human tumor xenografts overexpressing c-erbB2, 22, 23; a phase I trial of radiolabeled mAb has proved its ability to localize in primary and secondary tumors in breast cancer patients (24). In addition, unlike many of the murine antibodies described (such as 4D5 and TA1), ICR12 does not readily internalize or down-modulate the target antigen but remains stably bound to the surface of cells for prolonged periods (24). These properties suggested that ICR12 would be a good candidate for delivering enzymes capable of activating prodrugs in ADEPT protocols; the present study was designed to investigate the feasibility of this approach.

MATERIALS AND METHODS

Cell Lines and Xenografts. The human breast carcinoma cell line MDA MB 361 (which overexpresses the c-erbB2 protooncogene) and the squamous carcinoma cell line LICR-LON-HN5 (which overexpresses EGFR), were maintained in Dulbecco’s modified Eagle’s medium containing 10% heat-inactivated fetal calf serum and the antibiotics penicillin, streptomycin, and neomycin. Xenograft tumors were established in female outbred athymic mice 5 weeks of age by the s.c. inoculation of 5 × 10⁶ cells. Subsequent passages of tumors were initiated from trocar fragments for up to 10 generations, during which time the continued overexpression of the c-erbB2 oncprotein was confirmed by immunohistochemical examination of randomly selected tumor samples. Each mouse received two trocar fragments implanted s.c. at the midpoint of each flank under hypnorm-hynovel anesthesia. The animals were housed according to British Home Office and Institutional guidelines in filter boxes in Maximiser laminar flow cabinets and fed sterilized food and water. All procedures were carried out in class 1 laminar flow hoods using sterile equipment and reagents.

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2 To whom requests for reprints should be addressed.

3 The abbreviations used are: ADEPT, antibody-directed enzyme prodrug therapy; CPG2, carboxypeptidase G2; CMDA, 4-[(2-chloroethyl)(2-mesyloxyethyl)amino]benzoyl-L-glutamic acid prodrug; DMSO, dimethyl sulfoxide; EGFR, epidermal growth factor receptor; mAb, monoclonal antibody; PBS, phosphate-buffered saline.
Prodrug. The CMDA prodrug was synthesized and characterized as described previously (25, 26).

Monoclonal Antibodies and Immunoconjugates. ICR12 is a rat IgG2a mAb raised against the external domain of the human c-erbB2 protooncogene product p185 using the breast carcinoma cell line BT474 as immunogen (20). ICR16, an isotype-matched rat mAb directed against the external domain of the related EGFR was used as an irrelevant mAb control (27). mAbs were purified from hybridoma culture supernatants by salt precipitation and ion-exchange chromatography as described previously (20). All preparations were dialyzed extensively against PBS, filter-sterilized, and stored frozen until use.

Preparation of Monoclonal Antibody-Enzyme Conjugates. CPG2 (Division of Biotechnology, Centre for Applied Microbiology Research, Porton Down, UK; specific activity, 456 units/mg) was conjugated to ICR12 or ICR16 using a modification of the method described previously (28). A 5-fold molar excess of 2-iminothiolane (Sigma Chemical Co., Poole, UK; 2.2 mg/ml in DMSO; 100 μl) was added to the mAb (10 mg/ml). CPG2 (11 mg/ml; 2.5 ml in PBS was reacted with a 5-fold molar excess of N-succinimidyl-4- (p-maleimidophenyl) butyric acid (Sigma; 6.7 mg/ml in DMSO; 100 μl). The mixtures were left for 30 min. Excess 2-iminothiolane or N-succinimidyl-4- (maleimidophenyl) butyric acid was removed on Sephadex G25 PD10 columns and the modified proteins (1.5 mg/ml in PBS) were mixed and left for 12 h at 4°C. Solid N-ethylmaleimide (1 mg; 30 min) was added to terminate the reaction; then 2-mercaptoethanol (5 μl) was added to cap off reactive thiol and maleimide residues.

The conjugate was purified on a Superdex G200 column in PBS on a fast protein liquid chromatography system (Pharmacia). Nonreducing sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed on the fractions using 4–15% gradient Phastgels (data not shown). Peak fractions corresponding in molecular weight to both M, 233,000 and 316,000 conjugates (1:1 and 2:1, respectively) were pooled, concentrated, and rerun under the same conditions. Analysis of fast protein liquid chromatography traces indicated a typical ratio of 60% (1:1) and 40% (2:1) conjugate. This equates to an overall ratio of approximately 1.3 CPG2 molecules/IgG. The final samples were filter sterilized and stored frozen until use. Two batches of ICR12-CPG2 and one batch of ICR16-CPG2 were assayed in the experiments described in this paper.

Radiolabeling of Antibodies and Immunoconjugates. 125I (NaI; specific activity, 100 mCi/mM) was obtained from ICN Biomedicals, Inc. and was used to radiolabel monoclonal antibodies or immunoconjugates to a specific activity of 1 μCi/μg using the iodogen method (29). Potency of the labeled ICR12-CPG2 conjugate was determined by Scatchard analysis on MDA MB 361 target cells in comparison with radiolabeled ICR12.

Quality Control of Immunoconjugates. Prior to their use in vivo, antibody-enzyme conjugates were tested to ensure that the coupling procedure had not compromised antigen-binding or enzymatic activity. Briefly, the conjugates were assessed for their ability to bind target cells in a competitive radioimmunoassay in comparison with unconjugated antibodies, and immunoreactivity was assessed as described previously (20) using Sepharose 4B beads coated with an excess of antigen (p185, or EGFR for the control antibody). Enzyme activity of the conjugates was determined by a spectrophotometric assay as described previously (5).

In Vitro Cytotoxicity Assays. Sensitivity of the target cells MDA MB 361 to the CMDA prodrug and its CPG2-generated active derivative was tested in vitro in a modification of the method described previously (4). Briefly, MDA MB 361 carcinoma cells were seeded onto 96-well microtiter plates at 5 × 103 cells/well and allowed to adhere overnight in a humidified CO2 incubator. Prodrug was dissolved in DMSO immediately prior to use, diluted in Dulbecco’s modified Eagle’s medium, adjusted to pH 7.4, and added to the wells at varying concentrations between 1 μM and 1 mM in triplicate wells. CPG2 (final concentration, 6 units/ml) was added to test wells in parallel with each dose of prodrug to achieve activation. The treatment was repeated twice more at 24-h intervals. Controls consisted of untreated cells or those receiving equivalent doses of DMSO or enzyme alone. Cell viability was assessed by trypan blue 7 days. Viable cells were fixed by a 15-min incubation with 20 μl glutaraldehyde; nonviable cells were washed off and the remainder were stained by addition of 100 μl 0.05% methylene blue. After 30 min, the cells were washed and air dried, and the stain was solubilized with 0.33 N HCl. The absorbance was read at 620 nm on a Titertek Multiscan, and the results were expressed as percent age of control cell values.

In Vivo Biodistribution of ICR12-CPG2 Conjugates. Two different batches (A and B) of antibody-enzyme conjugate were labeled with 125I as described above, and approximately 10–12 μg of antibody (18–20 μg total protein; 0.83 and 0.66 units of enzyme activity, respectively) were injected i.v. into mice bearing MDA MB 361 breast carcinoma xenografts 3–4 mm in diameter. Groups of 2–6 mice were bled at intervals and at various times up to 14 days; randomly selected mice were killed by CO2 asphyxiation and the radioactivity in weighed samples of whole blood, normal tissues, and tumors was determined in a Packard autogamma spectrometer. Results were expressed as the percentage of injected dose/g of tissue, and as tumor:normal tissue ratios.

In Vivo ADEPT Therapy of Established Breast Carcinoma Xenografts. Groups of mice were implanted with MDA MB 361 breast carcinoma xenografts bilaterally in the flanks as described above. After 14–16 days of growth, when the tumors had achieved a mean diameter of 3–4 mm, mice were randomized to some (in the pilot experiment) or all of the following treatment groups: (a) untreated controls; (b) ICR12-CPG2 conjugate alone; (c–e) prodrug alone at 600, 900, and 1200 mg/kg; (f–h) ICR12-CPG2 conjugate followed later by administration of prodrug at 600, 900, and 1200 mg/kg; (i) irrelevant ICR16-CPG2 conjugate followed later by administration of prodrug at 1200 mg/kg; and (j) ICR12-CPG2 conjugate plus concomitant administration of prodrug at the maximum tolerated dose (systemically activated drug, non-pretargeted).

The conjugates were thawed and enzyme activity was determined prior to use. Mice were inoculated i.v. via an exposed jugular vein under hypnorm-hypnovel anesthesia at a standard dose of 50 units of CPG2/animal, which was associated with approximately 300–400 μg of ICR12 and 300 μg of ICR16 mAb, respectively. Control (group a) animals received i.v. inoculations of an equivalent volume of PBS under anesthesia. Group j mice received no treatment at this time.

A further group of “sentinel” animals was also inoculated with conjugate containing 50 units of enzyme activity. These mice were bled at intervals, and enzyme levels were determined on fresh and/or frozen plasma samples.

Prior to CMDA prodrug administration in the second experiment, mice were given a short course of antibiotics to prevent any possible untoward activation of prodrug by caecal microflora, which has been observed previously (30). Briefly, the mice received fresh benzylpenicillin (0.5 g/liter) in their drinking water for 5 days, combined with streptomycin (2.5 mg/ml) on days 5 and 1. Prodrug was prepared immediately prior to use by dissolving in DMSO and then 1.26% bicarbonate solution (1:20), and was administered i.p. to groups c–e and f–h. Mice were weighed, and the volumes injected were adjusted to give accurate individual doses on a mg/kg basis. Control animals received i.p. injections of vehicle at the maximum volume used. A pilot experiment had determined that the maximum tolerated dose of prodrug that could be administered concurrently with ICR12-CPG2 conjugate was 400 mg/kg. Accordingly, group j mice were anesthetized and inoculated i.v. with ICR12-CPG2 from the same batch used in the other groups, and within 30 min also received prodrug i.p. at 400 mg/kg. All mice were observed daily, and body weights and tumor measurements were recorded at frequent intervals. Tumor volume was calculated according to the formula:

\[ V = \frac{4}{3} \pi (d_1 + d_2)^3 \]

where \(d_1\) and \(d_2\) = perpendicular diameters.

Response of Established MDA MB 361 Xenografts to Conventional Chemotherapeutic Agents. In order to compare the efficacy of the ADEPT protocol with conventional chemotherapy, mice were transplanted with xenograft MDA MB 361 tumors as before, and after 26–28 days of growth, when tumors reached 6–7 mm in diameter (matching those at the time of prodrug administration), the animals were randomized to receive one of a variety of drug treatments. The current standard adjuvant treatment for breast cancer is a combination of cyclophosphamide + methotrexate + 5-fluorouracil, given as several courses comprising both bolus and infusion administration. Clearly, such a protocol is impractical in small immunocompromised rodents, and the differential drug sensitivity of the two species (particularly to antifolates) renders the design of an equivalent therapeutic regime difficult. We attempted two schedules of combined therapy comprising cyclophosphamide + methotrexate + 5-fluorouracil at 200/40/63 mg/kg and 130/20/50 mg/kg administered i.p. as a single bolus, but both proved highly toxic and led to deaths of...
RESULTS

**In Vitro Activity of Conjugates.** The immunoreactivity of radiolabeled ICR12-CPG2 conjugates was estimated to be between 70 and 76%, which compares favorably with the value obtained for ICR12 alone (79%). Scatchard analysis using MDA MB 361 cell monolayers comparing binding of ICR12 with ICR12-CPG2 (batch B) is shown in Fig. 1. Unlabeled ICR12-CPG2 was found to compete with radiolabeled ICR12 for binding to target antigen as effectively as ICR12 alone at equivalent molar ratios of antibody (data not shown).

MDA MB 361 target cells were tested for their susceptibility to the cytotoxic effects of prodrug and the activated derivative produced by the action of CPG2 *in vitro*; the results are illustrated in Fig. 2. Minimal cytotoxicity was evident with doses of prodrug up to 800 μM, whereas significant cell killing was obtained at all doses down to 100 μM with concomitant addition of CPG2 and prodrug.

**Blood Clearance of Radiolabeled ICR12-CPG2 Conjugates and Plasma Enzyme Determinations from Sentinel Mice.** The blood clearance of two different radiiodinated ICR12-CPG2 conjugates is illustrated in Fig. 3, and compared with the rate of loss of enzyme activity of unlabeled conjugates in plasma samples from sentinel mice. In spite of the fact that the unlabeled conjugate was administered at therapy levels approximately 30 times the antibody dose used in the radiotracer studies (300–400 μg versus 10–12 μg), the rate of clearance in both assays was in most cases in good agreement, yielding half-life estimations of approximately 4 days.

In the pilot experiment (conjugate A), each plasma enzyme determination was from a single mouse, which may explain the one spurious point (■) at day 12. Once it became evident that 50 μl of plasma would provide sufficient material for assay, we were able to take small repeated blood samples from groups of mice, and for conjugate B (mean of 5 mice/point), the results show excellent accord with the parallel radiotracer study. Plasma samples which were snap frozen and thawed gave results that were not significantly different from those obtained with fresh material, and blood samples from normal mice gave negative results in all cases.

**Tumor and Normal Tissue Distribution of Radiolabeled ICR12-CPG2 Conjugates.** The biodistribution of ICR12-CPG2 conjugate B assayed at 7, 11, and 14 days following administration is shown in Fig. 4. Tumor localization at 7 days was >10% of the injected dose/g and by 14 days significant levels remained tumor associated (2.42% i.d./g), despite the fact that tumor volume had increased 2–3-fold during this period. Tumor to normal tissue ratios are illustrated in Fig. 5, and ranged between 1.7 (blood) and 14.1 (gut) at day 14 when prodrug was administered in the parallel ADEPT therapy experiment. The results obtained with ICR12-CPG2 conjugate A were almost identical (data not shown).
**In Vivo ADEPT Treatment of Established MDA MB 361 Breast Carcinoma Xenografts.** A pilot experiment was performed with conjugate A and a full therapy experiment with conjugate B. In the first experiment, on day 0, tumors were implanted, on day 16, ICR12-CPG2 conjugate A was administered i.v. to 3 groups of mice, and on day 28 prodrug was administered i.p. at 600, 900, and 1200 mg/kg to these animals and 3 parallel groups of mice which had not received conjugate. Two further groups of mice received vehicle inoculations only or ICR12-CPG2 conjugate only and served as controls. There were 3–5 mice each bearing 2 tumors in each group. The mean tumor volume at the time of prodrug treatment was 262 ± 41 mm³.

All of the mice treated with ICR12-CPG2 conjugate followed by 1200 mg/kg prodrug died within days of treatment, indicating that systemic activation of prodrug by conjugate remaining in the blood 12 days after administration was sufficient to induce toxicity (plasma enzyme levels, 0.59 units/ml based on measurements in sentinel mice). All remaining groups of mice which received prodrug (with or without conjugate) sustained transient body weight losses of between 5 and 12%. For this reason in the second experiment, a prophylactic antibiotic course of treatment was introduced.

In all treated with conjugate followed by the two lower doses of prodrug, tumors regressed and reached a nadir of 28–30% of their initial volumes at 22 days post-drug injection, as shown in Fig. 6. At 35 days, most tumors began to regrow; the ADEPT treatments were shown to have induced growth delays of 50–55 days, and the experiment was terminated 64 days following prodrug administration (day 92 of tumor growth). All control groups were killed when tumors reached 10–12 mm mean diameter (500–700 mm³ volume), which occurred at days 41–43 of tumor growth (13–15 days post-prodrug).

There was no significant effect on tumor growth of ICR12-CPG2 conjugate following day 28 when mean tumor volumes were 170 ± 26 mm³. Assays on a batch B or ICR16-CPG2) were administered on day 14 of tumor growth, and prodrug (and conjugate in the case of group j mice) on day 28 when mean tumor volumes were 170 ± 26 mm³. Tumors in mice treated for about 12 days and then regrew more slowly than controls, giving a growth delay of the order of 30–35 days. Tumors in mice treated with conjugate plus 600 mg/kg prodrug remained static for about 12 days and then regrew more slowly than controls, giving a growth delay of the order of 30–35 days. Tumors in mice treated with ADEPT protocols incorporating 900 and 1200 mg/kg prodrug regressed to 24 and 4% of initial volumes, respectively. In the latter group no progressive growth of residual nodules was seen during a posttreatment observation period of 90 days; in the former group slow resumption of growth occurred after 65 days but tumors did not achieve their pretreatment volumes until 85 days post-prodrug. Toxicity as evidenced by body weight loss was minimal (maximum 6%).

ICR12-CPG2 or prodrug alone at 3 dose levels (as in the pilot experiment) again had no significant effects on tumor growth (data omitted for clarity). Treatment of mice with an irrelevant antibody-enzyme conjugate (ICR16-CPG2) prior to administration of the highest drug dose produced a growth delay of only 7 days. Cleavage of prodrug to maximum tolerated levels of active drug in the systemic circulation by concomitant administration of ICR12-CPG2 also yielded tumor growth delays of only 7–9 days, and 2 of 5 mice died 2 days after treatment.
**REGRESSION OF TUMORS WITH ADEPT AGAINST c-erbB2**

**In Vivo Chemotherapy of Established MDA MB 361 Breast Carcinoma Xenografts.** Table 1 shows the outcome of attempts to inhibit the growth of established MDA MB 361 breast carcinoma xenografts using a variety of drugs and dosage regimes. All treatments were associated with severe toxicity, and in no cases did the tumor growth delay induced exceed 6 days.

**DISCUSSION**

Other authors have utilized different enzymes to activate distinct prodrug systems in ADEPT (3). Notably, antitumor effects were observed when alkaline phosphatase-antibody conjugates were used in combination with phosphorylated etoposide and mitomycin C prodrugs (8). This group has also reported encouraging data with antibody-enzyme conjugates of cytosine deaminase, penicillin V amidase, therapeutic agents as shown. Therapeutic effects were assessed by mean tumor growth delay induced exceed 6 days.

**Table 1 Effect of conventional therapeutic agents on nude mice bearing established MDA MB 361 breast carcinoma xenografts**

Mice bearing established tumor xenografts were dosed with single or combined therapeutic agents as shown. Therapeutic effects were assessed by mean tumor growth delay and toxicity by weight loss and death.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dosage mg/kg</th>
<th>Wt loss (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Deaths</th>
<th>Tumor Growth Delay (d)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxorubicin</td>
<td>4.0 (&lt;x&gt;5)</td>
<td>22</td>
<td>2/6</td>
<td>6</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>3.5 (&lt;x&gt;5)</td>
<td>15</td>
<td>1/7</td>
<td>4</td>
</tr>
<tr>
<td>Cis-platinum</td>
<td>5.8 (&lt;x&gt;2)</td>
<td>23</td>
<td>1/7</td>
<td>4</td>
</tr>
<tr>
<td>Cis-platinum</td>
<td>4.5 (&lt;x&gt;2)</td>
<td>14</td>
<td>0/7</td>
<td>1</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>40 (&lt;x&gt;2)</td>
<td>8</td>
<td>0/7</td>
<td>0</td>
</tr>
<tr>
<td>CMF</td>
<td>200/40/63 (&lt;x&gt;1)</td>
<td>9/9</td>
<td>NM&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>CMF</td>
<td>130/20/50 (&lt;x&gt;1)</td>
<td>12/12</td>
<td>NM&lt;sup&gt;c&lt;/sup&gt;</td>
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<sup>a</sup> Maximum body weight loss as % of initial weight.  
<sup>b</sup> Difference in time (d) taken for mean test and control tumor volumes to increase 3 fold.  
<sup>c</sup> Not measurable.

Fig. 7. Growth of MDA MB 361 breast carcinoma xenografts in mice treated with ICR12-CPG2 (conjugate B) ADEPT. Key to treatment groups: I, vehicle controls (a); , ICR12-CPG2 ADEPT with prodrug at 600 mg/kg (f); , ICR12-CPG2 ADEPT with prodrug at 900 mg/kg (g); , ICR12-CPG2 ADEPT with prodrug at 1200 mg/kg (b); *, irrelevant ICR16-CPG2 ADEPT with prodrug at 1200 mg/kg (i); , ICR12-CPG2 plus concomitantly administered prodrug at 400 mg/kg (j). Letters in parentheses refer to treatment groups described in text.

DISCUSSION

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Other authors have utilized different enzymes to activate distinct prodrug systems in ADEPT (3). Notably, antitumor effects were observed when alkaline phosphatase-antibody conjugates were used in combination with phosphorylated etoposide and mitomycin C prodrugs (8). This group has also reported encouraging data with antibody-enzyme conjugates of cytosine deaminase, penicillin V amidase, therapeutic agents as shown. Therapeutic effects were assessed by mean tumor growth delay induced exceed 6 days.
activation in blood without the need for "clearing" agents, such as those described by Sharma et al. (47). Our protocol is in accord with the pharmacokinetic model of Yuan et al. (48), which predicts that the longer the time delay between the antibody-enzyme conjugate and the prodrg administration, the higher the therapeutic index. An alternative protocol currently being explored utilizes antibody fragments linked to CPG2 to reduce the time delay between conjugate and prodrg administration.

Previous experiments with radioiodinated ICR12 had indicated that dehalogenation of the protein was not a significant problem (22, 23); the present study using radiolabeled ICR12-CPG2 conjugates also suggested that loss of the label was minimal. Because of this, we found that radiotracer estimates of blood clearance of conjugate agreed closely with direct measurements of plasma enzyme activity. Toxicity was evident in the first study where the highest dose of prodrg was administered 12 days following conjugate but not in the main study when administration was delayed until day 14 (CPG2 = 0.35 units/ml). These figures provide a guide to the levels of enzyme required for safe administration of prodrg and suggest that in this system, concurrent radiotracer studies could be used to predict the optimum interval between the first and second phases of treatment.

In summary, an ADEPT protocol utilizing CPGG2 targeted to c-erbB2-overexpressing tumors with rat mAb ICR12 showed greatly improved antitumor activity compared with conventional chemotherapeutic regimes, and with less associated toxicity. The results highlight the importance of careful selection of antibody for the precise biological properties required. Many previously described murine monoclonal antibodies recognizing p185 are rapidly internalized and catabolized, and their binding leads to down-regulation of the target antigen (43, 49), properties at variance with those ideally required for ADEPT but suitable for some other applications as described above. It is critical that generalizations regarding the potential applications of new mAbs directed against a particular target are not drawn from studies on existing individual antibodies, since it is becoming clear that the epitope recognized, the isotype, and other as yet undefined characteristics of each mAb will all contribute to the results obtained (50).

In conclusion, c-erbB2 represents an appropriate target for ADEPT treatment, using the ICR12 mAb in combination with the CPG2 enzyme and the CMDA prodrg. The results of the ADEPT experiments described herein suggest that these components are ideally suited for a clinical trial in breast carcinoma patients that overexpress c-erbB2. A clinical trial using the CMDA prodrg in colorectal carcinoma patients with the antitumourheinogenic antigen mAb ASB7, conjugated to CPG2, is in progress with promising results (51, 52).

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REFERENCES


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REGRESSION OF TUMORS WITH ADEPT AGAINST \textit{c-erbB-2}


Regression of Established Breast Carcinoma Xenografts with Antibody-directed Enzyme Prodrug Therapy against c- erbB2 p185

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