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DRUG TARGETTING FOR 7 NEUROBLASTOMA PATIENTS USING HUMAN POLYCLONAL ANTIBODIES

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ABSTRACT

Allogeneic antibody directed against neuroblastoma tumour cells was used as a carrier of daunorubicin and chlorambucil. 7 children with neuroblastoma were treated by drug targetting alone, achieving three cures and one long term good response. Antibodies were bound to target cells to give a clinically safe treatment with a good quality of life. Better antibody production and a wider panel of drugs are needed to improve results.

KEYWORDS

Drug targetting; neuroblastoma; chemotherapy; antitumour antibody; immunotherapy.

INTRODUCTION

Despite the recent progress in chemotherapy the prognosis for neuroblastoma remains poor and only some 7% of Evans Stage IV children will survive. Recently we have been interested in exploring the therapeutic potential of drug-targetting (10) a technique which has evolved from the original use of chlorambucil adsorbed to xenogeneic antibodies (2) to the use of toxins covalently coupled to relatively specific monoclonal antibodies (1,3,11,12). Westminster Hospital's clinical experience of drug-targetting, has been mainly with cases of advanced neuroblastoma over the age of two years (4,7). In this report we summarise our clinical results using allogeneic antibodies as carriers of chlorambucil (CHL) or daunorubicin (DNR).

ANTIBODY PRODUCTION

Allogeneic IgG was raised in haploidentical well-informed volunteers inoculated with irradiated neuroblastoma cells, following an ethically approved immunisation schedule (10). Specific antibody was detected on day 14 by cytofluorimetry. Figure 1 shows that the plasma before immunisation evinced only a low degree of autofluorescence with the neuroblastoma cells used for the immunisation. The stronger intensity of the fluorescence on day 14 indicates the presence of antibody binding to the tumour cells. This antibody did not bind to the lymphocytes of the tumour donor, so excluding the presence of anti-HLA antibodies. Purified gamma globulins were prepared from litre batches of plasma using the cold ethanol method (10). The yield of the purification was IgG 57%, IgM 60%, IgA below 7%, Albumin below 2%. The high IgM recovery can be attributed to minor errors in the composition of the first precipitation buffer.

CONJUGATION

After testing for sterility and pyrogenicity, the antibodies were covalently coupled to DNR or CHL (8) by the carbodiimide method, to give 3 moles DNR

bound per mole antibody. Residual free drug, (70%), was removed by dialysis. The coupling did not impair the ability of the antibodies to bind to neuroblastoma cells, as shown in figure 1 where the intrinsic fluorescence of DNR contributes 7.6%; the non-specific fluorescence was 0.27% for cells alone, 0.96% for normal human serum, 1.23% for cells + DNR and 6.41% for cells + IgG/DNR conjugate without FTIC 2nd antibody. Fluorescence increased to 68.9% for cells + IgG + 2nd FTIC Ab and to 76.5% for cells + IgG/DNR conjugate + 2nd FTIC Ab. All conjugates contained IgG polymers on SDS-PAGE but, most importantly, the drug-antibody conjugates retained the capacity to kill neuroblastoma cells (8).

PATIENTS & PROTOCOL

Patients were chosen, as in our previous reports (4), from children affected by neuroblastoma, see table 1. Each intravenous dose was 1 mg DNR/Kg/dose or 0.5 mg CHL/Kg/dose, coupled to gamma globulin, usually at a frequency of two DNR and/or one CHL dose per week. The treatment was continued up to 1 year, reaching a total DNR dose of 35-50 mg/Kg of body weight, over double that permitted for free DNR.

The results are shown in Table I. Catecholamine reduction was observed in all 7 patients (6). The non-stage IV children present no evidence of disease three years after diagnosis. Masses were reduced in 3 out of 4 stage IV patients, according to X-ray, CT scan and ultrasound measurements. Bone deposits healed in 2 out of 7 children. In one case a bilateral chylothorax was resolved using intrapleural conjugate injections (9).

Side effects were originally confined to minor bone marrow depression in the first 5 patients, attributed to unbound drug. For the last two patients the conjugates were dialysed before injection, to remove the free drug, and no bone marrow depression occurred. No heart toxicity was seen, either clinically or by ECG and ultrasound analyses, despite the high total doses of DNR achieved. No serious immunological and/or toxic effects were observed.

PRELIMINARY LOCALISATION

The ability of the conjugates to bind to tumour cells 'in vivo' is seen in figure 2, which shows immunoperoxidase staining of a malignant cell from an infiltrated bone marrow aspirate from patient 1, using anti-human IgG. The suggested presence of anti-tumour antibody doesn't elucidate the origin of the human IgG. Preliminary experiments of radioimmunolocalisation autoradiography using nude mouse xenografts and 'in vivo' on patients, also indicate that the 125-I-antibodies are indeed able to localise to the tumour cells.

DISCUSSION

The original use of xenogeneic antibodies to deliver toxic substances to tumours, (Mathe (5) and Ghose (2)), employed 'one-shot' doses hoping to eliminate the tumours in the hosts and have, in general, failed to produce cures. Xenogeneic antibodies usually produce a host response with subsequent neutralisation of the treatment. To avoid such problems our work has used human antibodies aiming for long-term drug targetting.

Previous reports (4,6,7) have presented our results in other neuroblastoma patients. Here we confirm that allogeneic antibodies can be used to localise DNR and CHL onto tumour cells in human patients, producing measurable tumour effects and minimal side effects (e.g. no hair loss was observed). Furthermore we showed that 3 drug molecules bound per molecule of antibody preserves the ability of that antibody to bind to the target, figure 1, and kill. The prolonged response, as indicated by the continued fall in VMA for 10 days after a single dose in contrast to a rise again after only 3 days with free drug, indicates that conjugate therapy may not be cell cycle dependent. Higher binding ratios produce protein denaturation, affecting turnover and antigenicity. Even our low drug antibody ratios form protein polymers. Since these may stimulate absorption into the cell (possibly by phagocytosis) we intend to evaluate the role of these aggregates before trying new coupling methods. More clinical evidence of localisation is needed but the 3 cures achieved in patients 2,3 and 4 and the good response of

patient 7 show that allogeneic antibody can be used successfully for drug targetting on neuroblastoma. A wider panel of antitumour agents and a better antibody production technique will benefit the patients.

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TABLE I: Patients details

Pts.	age	sex	stage	'in vitro' killing	clin.resp.	survival since diagnosis (mo)
1	2	m	IV	+	P	4.
2	2	f	III*	+	NED	46.+
3	7	f	III*	+	NED	44.+
4	2	f	II*	NT	NED	32.+
5	5	f	IV	NT	P	12.
6	8	m	IV	NT	N	3.
7	4	f	IV	+	G	14.+

*= Lymph node deposits and macroscopic residual tumour after surgery.
clin.resp:- Good: over 50%, Partial: below 50%, None: below 25%.
NED= no evidence of disease

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