Localisation and toxicity study of a vindesine-anti-CEA conjugate in patients with advanced cancer

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Summary  Safety of administration of a vindesine (VDS)-anti-CEA conjugate and its ability to localise after radiolabelling were investigated in patients with advanced metastatic carcinoma (4 colorectal and 4 ovarian). For imaging, patients received between 230 and 520 μg of 131I labelled antibody. In 5, localisation of conjugate was demonstrated, in another it was equivocal and in 2 patients, undetectable. For assessment of safety each patient also received a single dose of conjugate increasing from 1.2 to 42 mg antibody linked to 24 to 1800 μg VDS. The in vitro activity of the anti-CEA antibody and its ability to localise in vivo were preserved after conjugation. There was no obvious toxicity or hypersensitivity attributable to either the radiolocalisation or escalated doses of conjugate in any of the patients. The feasibility of the preparation and administration to patients of a vindesine-antibody conjugate has been demonstrated.

The concept of targeting drugs on to malignant cells proposed by Ehrlich (1900) offers a potential improvement over one of the major limitations of cancer chemotherapy, viz. the limited selectivity of drugs for cancer cells. The use of antibodies as carriers was tested experimentally in an L1210 leukaemia (Mathé et al., 1958) and clinically in malignant melanoma (Ghose et al., 1972).

Despite reports of some success with this approach it has not been widely adopted. This has been partly due to the inability to demonstrate tumour-specific antigens as targets in human tumours; however, in order to increase the therapeutic index of a drug, differential expression of the target by the tumour compared to normal tissue may be sufficient. The well characterised tumour-associated antigens, carcinoembryonic antigen (CEA) and alpha fetoprotein (AFP), offer such potential targets in man. Another reason for caution with this approach has been scepticism about the in vivo stability of the drug and antibody conjugate. It was demonstrated in the mouse EL4 lymphoma model, that drug and antibody were interactive and more effective than a conjugate of the two (Davies & O'Neill, 1973). This led to a pilot study of drug and antibody interaction in patients with resected bronchogenic carcinoma (Newman et al., 1977) and to studies of a variety of drug and antibody conjugates and intermediate carriers (Ghose & Blair, 1978; Lee & Hwang, 1979; Dullens & De Weger, 1980, and Rowland, 1982).

The demonstration of enhanced toxicity of vincristine for a CEA-secreting lung cancer cell line in the presence of anti-CEA-immunoglobulin (Ig) (Johnson et al., 1980) encouraged us to investigate direct conjugation of vinca alkaloids to antibody. The toxicity of vindesine for a CEA-secreting cell line was found to be greatly increased when conjugated to an anti-CEA-Ig (Johnson et al., 1981). The aims of the present study were to investigate the ability to localise and safety of administration of this conjugate in patients with advanced metastatic adenocarcinomas refractory to established forms of treatment.

Materials and methods

Patients

Eight patients with advanced metastatic carcinoma refractory to previous treatment were entered into this study; all gave informed consent. Patients selected had tumour types likely to express CEA and to localise anti-CEA-antibodies (Goldenberg et al., 1978a; Dykes et al., 1980; Van Nagell et al., 1980). Four had disseminated ovarian carcinomas and 4 had disseminated colorectal carcinomas. Before injection of radio-labelled antibody, patients were tested for immediate and delayed hypersensitivity to sheep Ig (0.1 ml of 1 mg ml⁻¹). When there was a delay of more than a week between the first dose of conjugate and the next dose sensitivity testing was repeated. To block

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thyroid uptake of $^{131}$I, potassium iodide tablets (180 mg day $^{-1}$) were given, beginning 1–3 days before administration of the iodinated conjugate/antibody and continuing for 8–12 days thereafter.

Patients were admitted to hospital 1–3 days prior to the study for complete physical examination, baseline laboratory investigations and hypersensitivity testing. Venous blood was sampled for full blood count, urea and electrolytes, biochemical profile and GTs. Twenty-four hour creatinine clearances were also performed. Temperature, blood pressure and pulse were recorded half-hourly and for 4–6 h immediately after infusion of conjugate and every 4 h thereafter. Subjective toxicity was monitored daily by the attending physician (JRJ) and routine follow-up investigations performed every 1–2 days for the first 10–14 days. Particular attention was paid to evidence of hypersensitivity reactions and neurological status on clinical examination. Patients were then followed up in the clinic at weekly intervals for a minimum of 1 month, and in most cases 2–3 months.

**Antibody**

Immunoglobulin (Ig) was prepared from sheep anti-CEA serum by ammonium sulphate precipitation and was provided by Dr. A.R. Bradwell, Immunodiagnostics Research Laboratory, University of Birmingham, U.K. The antibody had been absorbed with normal liver, colon, lung and spleen. In fused rocket immunoelectrophoresis it did not recognise the CEA cross-reacting determinants shared with the non-specific cross-reacting antigen (NCA). Localisation of this antibody to human gastrointestinal tumour deposits has been reported (Dykes et al., 1980). In our hands it localised on sections of a formalin-fixed CEA-secreting colonic carcinoma in an indirect immunoperoxidase test at a titre of 1/80,000. However, in the immunocytochemical tests there was still residual antibody activity to shared NCA determinants as demonstrated by staining of chronic myeloid leukaemia cells and splenic myeloid cells (Ford et al., 1981).

**Conjugate**

Vindesine (VDS)-anti-CEA Ig conjugates were prepared at Lilly Research Centre Ltd. from desacetylvincaleucoblastine acid hydrazide under aseptic conditions by a modification of the procedure described for vindesine—BSA (Conrad et al., 1979) and purified by gel filtration. Four batches were prepared with initial conjugation ratios of 4.1, 5.4, 4.3 and 11 moles vindesine per mole IgG. An iodinated aliquot of Batch I was used to scan patients 1 and 2. Iodinated Batch II was used to scan patients 3–6. Patients 7 and 8 were scanned with an iodinated aliquot of the sheep anti-CEA Ig used to prepare Batch IV.

For assessment of safety, patients received doses of 1.2–42 mg conjugate, containing 24–1800 µg VDS, injected i.v. in 100 ml of 1% human serum albumin in saline (HSA-saline) over a 30–60 min period. All conjugates were 0.22 µm filtered before dilution in sterile, HSA-saline.

**Radiolabelling of conjugate/antibody**

Aliquots of batches of conjugate (or antibody) were iodinated with $^{131}$I using a modified chloramine-T method (Garvey et al., 1977). Free iodine was removed on a Sephadex G-25 column which was eluted with HSA-saline. Fractions containing the protein peak as determined by $\gamma$-counting were pooled and sterile filtered through a 0.22 µm filter. Radiolabelled conjugate (or antibody) was injected i.v. in 100 ml of sterile HSA-saline over a 30–60 min period.

All solutions were tested for pyrogenicity and sterility. Iodinated conjugates were tested at 6 µg Ig kg $^{-1}$ body weight in rabbits and uniodinated conjugates were tested from 45–198 µg Ig kg $^{-1}$ body weight depending on the dose to be administered to patients. All batches were negative.

**Scanning**

Between 230–520 µg Ig conjugate (6–14 µg of VDS) containing 541–1014 µCi protein bound $^{131}$I was administered to patients 1–6. Patients 7 and 8 each received 471 µg of unconjugated Ig containing 1056 µCi $^{131}$I.

Each patient received i.v. $^{99m}$Tc-pertechnetate (500 µCi) 30 min before each scan, and $^{99m}$Tc-labelled human serum albumin (500 µCi) 5 min before each scan. These distribute similarly to free iodide and radiolabelled antibody respectively in the blood pool. Images of the chest and abdomen were obtained with a gamma camera (Searle LFOV with medium energy collimator) initially at 4, 24 and 48 h after injection of iodinated material. The 4h scans were discontinued for patients 3–8. The camera was linked to a DEC PDP11/40 computer with a dual isotope facility and a colour scale visual display unit. The data were stored and displayed in a 64 x 64 matrix. After normalising over the cardiac area, subtraction of the technetium component from the iodine component was performed to visualise areas of selective uptake of conjugate.

**Measurement of anti-CEA activity**

A modified enzyme-linked immunosorbent assay
(ELISA) (Woodhouse et al., 1982) was used to measure anti-CEA activity of antibody, conjugate and iodinated conjugate. Briefly, disposable cuvettes were coated with purified CEA, washed, blocked by incubation with BSA, washed and the test sample added, before incubation at 35°C for 3 h. Following a further washing, rabbit anti-sheep IgG horseradish peroxidase conjugate (Nordic, U.K.) was added. The cuvettes were incubated at 35°C for 3 h, washed and then ABTS (2,2-azino-di-(3-ethylbenzthiazoline sulphonic acid) (Sigma)) was added. Absorbance at 405 nm was measured using a Gilford PR-50 processor-reader.

Measurement of serum CEA levels

CEA measurements were performed by Dr. P. Gosling, East Birmingham Hospital, using a modified double antibody technique (Booth et al., 1973). Serum samples were perchloric acid-extracted before assay.

Results

The dosages of radiolabelled conjugate/antibody and uniodinated conjugate received by each patient are given in Table I.

Details of the patients in this study and the scanning results are summarised in Table II. Patients 1–6 received radiolabelled conjugate, followed within 4–54 days by unlabelled conjugate. Localisation of radioactivity which equated with clinically detectable disease was seen in patients 1, 2, 3, 4 & 6. The localisation picture for patient 5 was equivocal. Figures 1 and 2 illustrate localisation images. Figure 1 is the 48 h subtraction scan for patient 2 who had a large abdomino-pelvic mass which was confirmed on CT scans. Localisation of isotope occurred in the mass and in the right kidney. Subsequent investigation of this patient by intravenous pyelography indicated a right hydronephrosis due to compression of the right ureter by the tumour. Impaired excretion apparently resulted in an accumulation of isotope in the right kidney.

Figure 2 is the 48 h subtraction scan for patient 6 who had an extensive pelvic tumour mass and central palpable abdominal masses. Ultrasound examination confirmed the presence of enlarged para-aortic and coeliac axis nodes. Localisation of isotope coinciding with these, and the pelvic mass at the primary site, can be seen in Figure 2.

For practical reasons, the last 2 patients received uniodinated conjugate and then radiolabelled unconjugated antibody. There was no evidence of localisation in these patients (see Discussion).

The relative anti-CEA activities of the non-iodinated conjugates in ELISA compared to the original antibody, when tested within 14 days of conjugation, were: Batch I, 98%; Batch II, 100%; Batch III, 80%. In the case of Batch IV it was not possible to obtain a relative anti-CEA value. After iodination the values were: Batch I, 48%; Batch II, 72%. Batches III and IV were not iodinated and the radiolabelled antibody used for patients 7 and 8 had 70% activity.

With the exception of patient 6, all had raised (>15 ng ml⁻¹) pretreatment serum CEA levels (Table 2). We noted no significant decline in CEA levels in any of the patients after the radiolocalising dose. One day after administration of 11.06 mg VDS-Ig conjugate to patient 3, there was a fall from 31–17 ng ml⁻¹ which was sustained for 3 days. Similarly, for patient 6, who developed a raised CEA level of 37 ng ml⁻¹ from a pre-treatment value

| Patient | VDS µg | Ig µg | ¹³¹I µCi | VDS µg | Ig mg |
|---------|-------|------|---------|--------|------|
| 1       | 6     | 300  | 996     | 24.5   | 1.2  |
| 2       | 6     | 300  | 541     | 30.3   | 1.5  |
| 3       | 6.25  | 230  | 1014    | 300.8  | 11.06|
| 4       | 6.5   | 240  | 581     | 300.8  | 11.06|
| 5       | 14.1  | 520  | 633     | 722    | 33.4 |
| 6       | 14.1  | 520  | 633     | 722    | 33.4 |
| 7       | —     | 471  | 1056    | 924.4  | 20.9 |
| 8       | —     | 471  | 1056    | 1849‡  | 41.8‡|

*protein-associated radioactivity.
‡dose given was within this estimated range—see Discussion.
Table II  Summary of clinical localisation data

| Case | Origin of primary                  | Summary of disease at entry                                                                 | Pre-treatment serum CEA level (ng ml\(^{-1}\)) | Scan localisation findings                        |
|------|------------------------------------|---------------------------------------------------------------------------------------------|-----------------------------------------------|-------------------------------------------------|
| 1    | Colon, Duke’s C, well-differentiated adenocarcinoma | Widespread intra pulmonary metastases; pelvic and hepatic metastases | >3,550                                        | Uniform liver (4 h); two small abdominal areas (48 h) |
| 2    | Ovarian, FIGO IV mucinous cystadenocarcinoma | Palpable abdominopelvic mass; left axillary nodes; left cervical nodes | 375                                           | Liver (4 h; 24 h); left axillary; right kidney; abdomino-pelvic mass (48 h) |
| 3    | Ovarian, FIGO III moderately well-differentiated adenocarcinoma | Mass in right groin; large left pelvic mass | 29                                            | Central lower abdomen (48 h)                      |
| 4    | Ovarian, FIGO III-IV adenocarcinoma | Left malignant pleural effusion; malignant peritoneal seedlings | 23                                            | Left chest (48 h); scattered areas in abdomen and pelvis (48 h) |
| 5    | Ovarian, FIGO III papillary cystadenocarcinoma | Pelvic recurrence | 32                                            | Medial to upper part of stomach scattered abdominal areas (48 h) |
| 6    | Recto-sigmoid, Duke’s C, well-differentiated adenocarcinoma | Extensive pelvic mass (biopsy proven adenocarcinoma) and central palpable abdominal masses | 13                                            | Central abdominal and pelvic localisation (48 h) |
| 7    | Rectum, Duke’s C, mucinous adenocarcinoma | Perineal recurrence biopsy proven | 43                                            | *No localisation                                 |
| 8    | Caecum, Duke’s C, adenocarcinoma | Retroperitoneal tumour, biopsy proven | 749                                           | *No localisation                                 |

*See text for details.

of 13 ng ml\(^{-1}\), one day after receiving 33.4 mg VDS-Ig conjugate this level fell to 22 ng ml\(^{-1}\). Five days later the CEA level began to increase. In the other patients no change was observed.

None of the patients had immediate or delayed hypersensitivity reactions to normal sheep Ig, either before the first or second dose of conjugate, nor any reaction to the conjugate. The period between conjugation and administration of the localising dose was 6–20 days; between conjugation and administration of escalated dose was 3–25 days, and the time between localising and escalated doses was 4–54 days. Patients 7 and 8 received unconjugated antibody 8 and 6 days respectively before they received conjugate.

In none of the 8 patients was there any toxicity or derangement of biochemical, renal or liver function which had not been present at entry into the investigation and which could be attributed to the administration of conjugate. Liver function became increasingly abnormal during follow-up in patient 3. Patient 6 developed obstructive jaundice
Figure 1 48h abdominal subtraction scan for Patient 2, showing accumulation of isotope in the right kidney (k) and in the abdomino-pelvic tumour mass (→). Accumulation of isotope, in the form of free iodide, can be seen in the stomach at the top of the scan.

Figure 2 48h abdominal subtraction scan for Patient 6, showing accumulation of isotope in the pelvic tumour mass (p) and in the central abdominal masses (→). Accumulation of isotope, in the form of free iodide, can be seen in the stomach at the top of the scan.
and deranged liver function between the first and second administration of conjugate which was due to enlarged metastatic lymph nodes in the porta hepatis and para-aortic areas. Progressive gastrointestinal obstruction was noted in patients 1 and 3 and was attributable to neoplastic adhesions which were confirmed at laparotomy for patient 1. Progressive pelvic recurrence was noted in patient 5 during the study. Five of the patients were anemic (3, 4, 5, 7 and 8) and only in the last patient was this progressive. Two patients (4 and 8) also had a short-lived thrombocytosis. In no case was the abnormal renal function in patients 1, 3, 4 and 5 further impaired by administration of conjugate. Also, the cis-platinum-related peripheral sensory neuropathy in patient 4 apparently improved during the investigation.

Three days after receiving radiolabelled conjugate patient 1 underwent sigmoidoscopy and biopsies of tumour and normal rectal tissue were obtained. These were weighed and the radioactivity measured in a γ-counter. The ratio of normal: tumour (N:T) counts was 1:1.2. The tissues were then macerated with a scalpel blade, weighed, washed 3 x with RPMI 1640 and the counts remeasured. The N:T ratio was 1:4.6. One week after receiving radiolabelled conjugate this patient had a laparotomy and was found to have numerous adhesions and liver secondaries. A colostomy was performed and biopsies taken of “normal” and metastatic hepatic tissue. Following the same procedure as before the N:T ratio was 1:0.6.

Discussion

One of the aims of this study was to determine whether radio-localisation of 131I-labelled VDS-anti-CEA could be achieved in human tumours. This was clearly demonstrated in 4/8 patients (2, 3, 4 & 6). Overall, the scanning results for Patient 1 were also consistent with localisation. However, the uniform uptake in the liver at 4 h may have been due to liver secondaries, or, alternatively, to deposition of anti-CEA/CEA immune complexes (pre-treatment CEA level of > 3,550 ng ml⁻¹). The latter possibility is strengthened by the N:T ratio of radioactivity in the liver of 1:0.6. The two isolated abdominal areas showing localisation at 48 h equated with the neoplastic adhesions seen at laparotomy. The pelvic and pulmonary metastases did not show localisation. In patient 5 localisation was equivocal. Patients 7 and 8 were given conjugate first, then scanned with radiolabelled unconjugated antibody, and neither showed convincing localisation. There are a number of possible explanations for this. Firstly, that the patients’ tumours were not producing CEA. The pretreatment serum CEA levels of 43 and 749 ng ml⁻¹ would argue against this. Secondly, avascularity could have reduced access of the conjugate to the tumour, resulting in false negativity as suggested by others (Dykes et al., 1980). However, we favour the third possibility which is that the CEA binding sites had been saturated by the administration of conjugate prior to receiving the radiolocalising dose. Both patients received up to 42 mg of conjugated Ig 8 and 6 days respectively before their radiolocalising dose (Table 1).

Our results suggest that drug conjugation has destroyed neither the activity of the anti-CEA antibody in vitro, nor its ability to localise in vivo. Most of the antibody activity was retained after conjugation as demonstrated by ELISA and the doses of conjugate required for localisation were similar to doses of unconjugated antibody reported by others (Goldenberg et al., 1978a; Dykes et al., 1980; Mach et al., 1980). Furthermore, there was an N:T radioactivity ratio of 1:4.6 at 3 days in a biopsy of the colonic tumour from patient 1.

There was no obvious toxicity or hypersensitivity attributable to administration of either radiolocalising or escalated doses of conjugate in any of the eight patients. There were abnormalities, e.g. in liver function, during the course of the study, but none could be directly attributed to the conjugate. Most could be explained by the fact that the patients had advanced disease and had previously undertaken several different treatment programmes. The maximum dose of conjugated drug we administered was 0.9-1.8 mg on a single occasion, which is less than the conventional therapeutic dose of vindesine of 3-4 mg m⁻2 every 1-2 weeks (Yap et al., 1981; Cobleigh et al., 1981), or 4-5 mg m⁻2 every 2 weeks (Valdivieso et al., 1981b). However, since the conjugate had been shown to be ~25 times as potent as free VDS against lung cancer cells in vitro (Johnson et al., 1981; Rowland et al., 1982a), we felt justified in taking a cautious approach when investigating it in patients.

Overall there was no significant decrease in circulating CEA levels due to the administration of either dose of conjugate. Whilst Patients 3 and 6 did show decreases (31-17 ng ml⁻¹ and 37-22 ng ml⁻¹ respectively), fluctuations similar to these were noted at other times and did not appear to be treatment related.

Whilst we have clearly demonstrated the feasibility of this approach a number of problems were noted. The most important was aggregation of Batches III and IV, forcing us to change our plan of investigation. For Batch III 80% relative anti-CEA activity was used to calculate the amount of conjugate given. However, for Batch IV it was not
possible to do this. We estimate the dose given was within the 50–100% range. The reason for
aggregation remains unexplained at present. A
possible factor may have been the higher
conjugation ratio of 11:1 achieved with Batch IV. A
single batch of conjugate was not used for the entire
study in order to minimise the time between
conjugation and administration of both
radiolocalising and escalated doses, since a loss of
anti-CEA activity of the conjugate had been noted
in vitro after storage at 4°C for more than 31 days
(Johnson et al., 1981). The longest period between
conjugation and administration of conjugate in the
present study was 25 days.

CEA was chosen as a model for this investigation
because, by immunocytochemistry, it has been
shown to be expressed by 62% of colonic carcinomas (Goldenberg et al., 1978b), 62% of
gastric carcinomas (Lee et al., 1978), 63% of
invasive cancers of the cervix (Van Nagell et al.,
1979), and 82% of lung cancers (Ford et al., 1981).
Also, 100% of primary and 67% of metastatic
ovarian carcinoma sites have been shown to localise
anti-CEA antibodies in vivo (Van Nagell et al., 1980)
and successes have been achieved with other
tumours (Goldenberg et al., 1978a; Dykes et al.,
1980; Mach et al., 1980), although, as in this study,
not all tumour sites in a patient and not all patients
have shown localisation. CEA, therefore, is a
potential target applicable to a variety of human
cancers.

Vindesine is active in small cell anaplastic
carcinoma of the lung (Osterlind et al., 1981),
colorectal (Valdivieso et al., 1981a) and breast
 carcinomas (Yap et al., 1981; Cobleigh et al., 1981).
Since it is feasible to make conjugates of vindesine
with antibody, VDS-anti-CEA conjugates are
candidates for clinical evaluation of possible
therapeutic effect. Questions that also require
resolution and which are the subject of
investigation are whether the conjugate is stable in
vivo and whether administration of conjugate
actually results in increased tissue levels of drug in
the target tissue. We are exploring this potential
further in vitro and in vivo using polyclonal and
monoclonal VDS-antibody conjugates (Rowland et
al., 1982ab). Clinical use of monoclonal conjugates
should be acceptable in view of the reports of
successful administration of monoclonal antibodies
to patients (Nadler et al., 1980; Miller & Levy,
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