Tumour localisation with a radioactively labelled reshaped human monoclonal antibody

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Summary A genetically reshaped human IgG1 monoclonal antibody (Hu2PLAP) with anti-tumour specificity, was radiolabelled with Indium-111 by chelation with a new macrocyclic compound (DOTA) which allows the production of stable radioimmunoconjugates for *in vivo* application. This was used to image seven patients with malignant disease, of whom two had been previously exposed to mouse monoclonal antibodies and had developed human anti-mouse antibodies (HAMA). Successful tumour localisation was seen in the four patients with active disease and antigen positive tumours. No patient showed any antibody responses against Hu2PLAP, but three out of six patients tested showed an immune response against the macrocycle DOTA.

Reshaped human monoclonal antibodies with anti-tumour specificity may facilitate repeated administrations of radioactive antibodies, thus allowing new possibilities, both in the diagnosis and treatment of cancer.

It has been previously shown that successful tumour localisation, and sometimes therapy can be achieved by the use of radioactively labelled murine monoclonal antibodies (Epenetos *et al.*, 1982; Epenetos *et al.*, 1984; Epenetos *et al.*, 1987). Two major problems, however, have been identified that do not allow for the full potential of antibodies to be realised. Firstly, murine antibodies when administered into humans, often act as immunogens themselves, leading to the development of antiglobulin responses (Courtenay-Luck *et al.*, 1988). This limits further treatment with murine antibodies.

Secondly, conjugation of antibodies with radioisotopes has often produced unstable radioimmunoconjugates resulting in the release of radioisotope *in vivo*; this can reduce the anti-tumour efficacy of such radioimmunoconjugates and increase toxicity to normal organs such as bone marrow (Stewart *et al.*, 1990). The problem of antiglobulin responses should be reduced or eliminated by the use of human antibodies. The reshaped human antibody (Hu2PLAP) with anti-tumour specificity has been constructed by transplanting the hypervariable regions otherwise known as complementarity determining regions (CDRs) of the mouse monoclonal antibody H17E2 (Travers & Bodmer, 1984) onto human immunoglobulin framework regions (Riechmann *et al.*, 1988; Verhoeyen *et al.*, 1990). Human IgG1 was chosen because, if shown to target successfully to tumours, it can potentially be used therapeutically due to its great activity in complement lysis and cell mediated killing. The problem of instability of radioimmunoconjugates has been overcome for some metallic radionuclides by the use of a new bifunctional macrocyclic chelating agent known as DOTA (Moi *et al.*, 1988). DOTA has been shown in previous *in vitro* (Moi *et al.*, 1988; Snook *et al.*, 1990; McCall *et al.*, 1990) and in preclinical (Snook *et al.*, 1990; McCall *et al.*, 1990; Deshpande *et al.*, 1990) and clinical (Meares *et al.*, 1990) *in vivo* studies to result in the production of stable radioimmunoconjugates. Radioimmunoconjugates incorporating DOTA and radiolabelled with yttrium-90 have been investigated therapeutically in patients with ovarian cancer (Hird *et al.*, 1990).

Here we describe a study of imaging and pharmacokinetics of a reshaped human antibody Hu2PLAP-DOTA-^{111}In conjugate in patients with and without previous human antimouse globulin responses, and compared its kinetics with the original murine H17E2. This is the first report of *in vivo* targetting using a fully reshaped human monoclonal antibody with anti-tumour specificity and stably radiolabelled using a novel macrocycle for clinical application.

Patients, materials and methods

Patients

The seven patients were aged between 36 and 65 years, and had undergone prior surgery and chemotherapy for their malignant disease. One patient (K.S.) was in complete remission at the time of the antibody scan. Two patients (M.F. and J.E.) had raised serum levels of human antimouse antibody prior to receiving Hu2PLAP antibody. This followed previous exposure to murine antibodies; M.F. had received HMFG1-DTPA-^{90}Y intraperitoneally as an attempted form of treatment of extensive intraperitoneal disease. J.E. had previously undergone H17E2-DOTA-^{111}In radioimmunoscintigraphy and had, also developed anti-DOTA antibody responses.

Humanised monoclonal antibodies

Transfectoma cells (cells transfected with a certain gene) were prepared containing the genes for Hu2PLAP (Verhoyen *et al.*, 1990). Purified Hu2PLAP was prepared from transfectoma supernatants derived from hollow fibre cell culture using protein-A and PLAP affinity chromatographies. Immunoreactivity of this material was greater than 80% as judged by analytical PLAP affinity chromatography.

H17E2 is a murine IgG1 which reacts with human placental alkaline phosphatase (Travers & Bodmer, 1984). Immunohistologically it reacts strongly against a wide range of neoplasms including those of the testis, ovary and cervix, and negatively against healthy tissues except term placenta (Epenetos *et al.*, 1984). It has been shown to localise *in vivo* to tumour lesions in patients with ovarian, testicular and other cancers (Epenetos *et al.*, 1985).

Radiolabelling

The antibody Hu2PLAP was coupled via 2-iminothiolane (2IT) to bromoacetamido-benzyl-DOTA (2-p-nitrobenzyl-

1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid) (Snook et al., 1990). DOTA coupled antibody was subsequently labelled with ^{111}In (Amersham Int. UK) to a specific activity of 2 mCi mg^{-1}.

ELISA for anti-DOTA, anti-mouse and anti-human antibodies

Ninety-six well, round bottomed microtiter plates were coated overnight with 37°C with 2 µg ml^{-1} HSA (Human Serum Albumin), HSA-2IT and HSA-2IT-DOTA in bicarbonate buffer, pH 9.6. Control wells were treated with bicarbonate buffer alone in order to determine non-specific binding of serum Ig. Then serial dilutions in PBS/0.05% Tween (10-fold) of each patient's serum were applied on the plates and incubated at 37°C for 2 h in a humidified chamber. After three washes in PBS/0.05% Tween the plates were incubated with a species-specific second reagent (sheep anti-human Ig, 1:1000 dilution in PBS/0.05% Tween), at 37°C for 1 h. Then the plates were washed three times in PBS/0.05% Tween and incubated in the dark at room temperature with 100 µl of ABTS substrate, and the absorbance at 405 nm was determined by a multiscan plate reader.

In the above described ELISA method each patient's serum was tested against: (i) no antigen to determine any non-specific binding on the plates, (ii) HSA in order to determine if there is a pre-existing response against HSA, (iii) HSA-2IT, in order to test for the development of antibodies against the 2IT linker and (iv) HSA-2IT-DOTA, in order to test the response against the macrocycle DOTA. Some control wells were incubated with PBS/Tween instead of serum and then received only the second layer reagent in order to determine any cross reactivity between the above antigens and the second layer reagent. The background binding of serum on the plates was always between 0–20% and there were no detectable antibodies against HSA and HSA-2IT. Most of the patients had ≤5% background binding, but two of these had 15–20%, a rare finding, thought to be due to high immunoglobulin serum content.

The ELISA methods used for measuring anti-mouse (Courtenay-Luck et al., 1986) and anti-human (Courtenay-Luck et al., 1987) antibody responses have been described previously. The development of antibodies to Hu2PLAP was estimated in two different aspects: (i) Anti-idiotypic antibodies; and (ii) Anti-constant region antibodies.

(i) The development of anti-idiotypic (anti-variable region antibodies) was estimated after coating 96 well round bottomed ELISA plates with 5 µg ml^{-1} in bicarbonate buffer, pH 9.6, of H17E2, AUA1, HMFG1 which are of the same isotype IgG1, but with different binding specificities. The assay was performed as described in the ELISA for anti-DOTA antibodies. Development of significantly higher response against H17E2 having identical binding sites with Hu2PLAP (because this is grafted with the CDRs of murine H17E2 antibody), than AUA1 and HMFG1 would suggest the development of anti-variable region antibodies.

(ii) The development of anti-constant region antibodies was tested as previously described (Courtenay-Luck et al., 1986) provided that the Hu2PLAP antibody has the same degree of glycosylation (on the Fc portion) as the other murine monoclonal antibodies (H17E2, HMFG1, AUA1). We assumed this to be the case as it also produced by a NSO myeloma cell lines (transfectoma).

Imaging

220–833 µg Hu2PLAP-DOTA-In^{111} (specific activity 2 mCi mg^{-1}) was administered intravenously to each patient. Images of the whole body and abdomen were obtained immediately after injection and again at 48 and 96 h later.

Pharmacokinetics

Blood samples were obtained immediately after injection, at 1 h, and then at the time of subsequent scans.

Urine was collected continuously from the time of the injection to the time of the last scan.

Results

Toxicity

No toxicity was seen. The procedure was well tolerated by all the patients.

Images

Tumour localisation was seen in four patients (M.F., M.D., N.N., P.H.) with active disease (Figure 1). Three patients (K.S., J.E., S.L.) showed no evidence of tumour localisation (Table I). Of these three patients, one patient (K.S.) had no evidence of disease clinically, or on CT scan and her serum CA 125 was within normal limits. One patient (J.E.) cleared the antibody rapidly due to pre-existing anti-DOTA antibodies, and no images could be obtained. The other patient (S.L.) had antigen negative tumour.

Antibody responses

Anti-DOTA antibodies were found in the sera of three out of six patients tested following administration of the antibody. Two patients (M.F., J.E.) had human anti-mouse antibodies (HAMA) due to prior exposure to mouse monoclonal antibodies. One of these (J.E.) also had anti-DOTA antibodies due to prior exposure to a murine antibody conjugated to DOTA. A further two patients developed anti-DOTA anti-
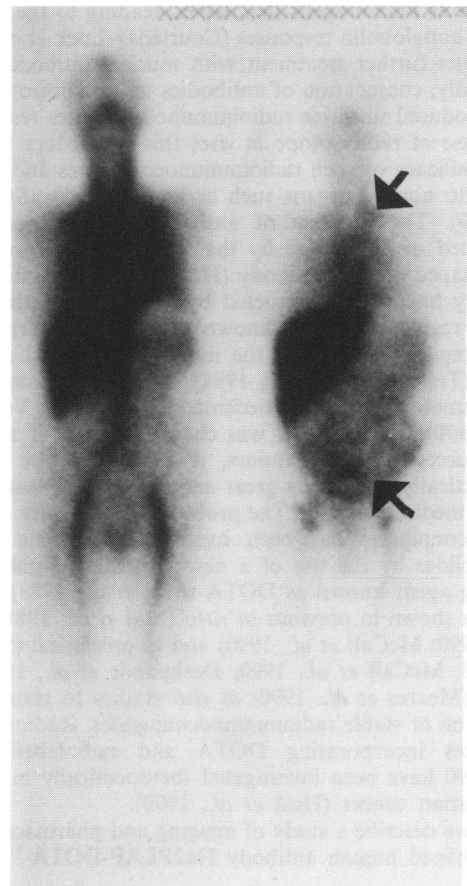

Figure 1 The whole body antibody scans using Hu2PLAP-DOTA-In-111 in a patient with extensive ovarian carcinoma. This woman had high levels of human anti mouse antibody prior to administration of the humanised H17E2. The images have been taken immediately and 2 days post injection. Arrows point to tumour areas.

bodies following administration of Hu2PLAP-DOTA-[111]In (S.L., M.D.). None of the controls or the patients had any pre-existing anti-DOTA antibodies. No antibody responses against the human monoclonal antibody Hu2PLAP were seen (Table I).

It was not possible to quantitate polyclonal serum responses. IgM antibodies are of lower affinity than IgG and therefore any attempt to purify the anti-DOTA or HAMA responses, with antigen (HSA-DOTA or murine Ig) coated Sepharose CNBr beads, would be more likely to lead to isolation of IgG.

Pharmacokinetics of Hu2PLAP-DOTA-[111]In

Patients M.F. and J.E. had pre-existing HAMA responses (Table I), hence, excluding these patients, Hu2PLAP-DOTA-^{111}In cleared from blood with a mean $T_{1.2}\beta = 73.1 \pm 30.2$ h ($n = 5$); this is significantly longer ($P < 0.05$, Student's t-test) than that of H17E2-DOTA- [111]In $T_{1/2}\beta = 27.2 \pm 5.9$ ($n = 3$) (Hird, unpublished data). At 96 h the mean cumulative urine excretion for these patients was $11 \pm 8\%$ ($n = 4$) compared to $14 \pm 5\%$ ($n = 3$) for the murine equivalent (Hird, unpublished data). Patient J.E. had only 27% of the administered activity remaining in her blood at 1 h post-injection, the remainder cleared with a $T_{1/2}\beta = 39$ h giving a cumulative urine excretion (at 96 h) of 31%. For patient M.F. these values were $T_{1/2}\beta = 47$ h and 66% respectively.

Discussion

This study shows that the genetically reshaped human IgG1 Hu2PLAP monoclonal antibody with anti-tumour specificity, when conjugated with the macrocycle DOTA and radiolabelled with Indium-111, can successfully localise to tumours in patients with antigen positive malignant disease such as ovarian cancer. No toxicity was observed. It was of interest that the reshaped human IgG1 antibody had a longer half life than the original murine H17E2 antibody. However, this was considerably shorter than normal human IgG1 antibody. It is possible, that following chelation with the macrocycle DOTA and labelling with ^{111}In, the antibody does not behave any more as normal human IgG1. Furthermore, Hu2PLAP is produced in a mouse cell line and is probably glycosylated differently than it would be in a 'normal' human cell.

None of the patients produced any serologically detectable anti-Hu2PLAP or other anti-human globulin responses, even though two patients had a high human anti-mouse antibody response due to prior exposure to murine antibody. Two patients, however, developed an anti-immunoconjugate response, but this was found to be directed against the macrocycle DOTA rather than against the reshaped human antibody. The immunogenicity of the DOTA and other similar compounds is currently being investigated in order to design new and non-immunogenic chelates (Kosmas et al., submitted).

The long-term advantages of reshaped human IgG1 Hu2PLAP or other antibody used both for imaging and therapy will be assessed only in larger trials, but this data provide considerable encouragement to this approach.

Table I Details of imaging of the patients who received Hu2PLAP-DOTA-In-111, their disease status at the time of injection, and their immune responses against murine (HAMA), reshaped Hu2PLAP antibodies and the macrocycle DOTA

Patient's number	Abbrev.	Disease	Status	Pre-existing responses DOTA	HAMA	Dose of Hu2PLAP (µg)	Responses a Hu2PLAP	a DOTA	Tumour localisation
1	M.F.	OvCa Stage III	Active	–	+	220	–	–	+
2	J.E.	OvCa Stage Ia recurrent	–	+	+	833	–	+	–
3	N.N.	Stomach Ca	Active disease	–	–	250	–	–	+
4	S.L.	OvCa Stage III	Ag Neg tumour	–	–	550	–	+	–
5	W.S.	OvCa Stage I	NED	–	–	500	–	NT	–
6	M.D.	OvCa Stage IIIc	Active disease	–	–	765	–	+	+
7	P.H.	Breast	Active disease	–	–	765	–	–	+

HAMA: human antimouse murine antibodies; DOTA: 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid; NT: not tested; HSA: human serum albumin; ELISA: enzyme linked immunosorbent assay; PLAP: placental alkaline phosphatase; 2IT: 2 iminothiolane hydrochloride; OvCa: ovarian carcinoma; NED: no evidence of disease.

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