Eur J Cancer, Vol. 27, No. 11, pp. 1430–1436, 1991.
Printed in Great Britain

0277–5379/91 $3.00 + 0.00

Tumour Localisation with ^{131}I-Labelled Human IgM Monoclonal Antibody 16.88 in Advanced Colorectal Cancer Patients

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Human IgM monoclonal antibody 16.88 recognised an intracellular antigen strongly expressed in colorectal cancer tissue in 51% of our patients. Tumour localisation was carried out with 185 MBq ^{131}I-16.88 (8 mg) in 20 of these patients with advanced disease. In 16 patients (80%) immunoscintigraphy was positive in at least one organ site with disease. Of all sites, 55% could be visualised. In general, lesions < 3 cm could not be detected. Sequential immunoscintigrams of liver metastases showed variable patterns. Initial "cold" lesions corresponded to liver metastases with poor blood supply as indicated by ^{99m}Tc-sulphur-colloid and ^{99m}Tc-HMPAO scintigraphy, respectively. The mean (S.D.) biological half-life (whole body clearance of radioactivity) was 37.6 (5.0) h. A second infusion of ^{131}I-16.88 with the addition of high doses of unlabelled 16.88 could be done safely, but did not result in better visualisation of tumour lesions or affect radioactivity clearance from the body.
Eur J Cancer, Vol. 27, No. 11, pp. 1430–1436, 1991.

INTRODUCTION

A VARIETY of monoclonal antibodies (Mabs) of predominantly murine origin have been developed which are reactive with colorectal cancer cells. The high degree of tumour specificity of some of these antibodies has led to their clinical use after radiolabelling for detection of colorectal cancer lesions. Among others, successful visualisation of malignant sites has been reported for Mabs B72.3 [1], 17-1A [2, 3], 19-9 [3], and those directed against the carcinoembryonic antigen (CEA) [4–6]. Clinical trials on the administration of high doses of radiolabelled antibodies in the treatment of colorectal cancer are still in early phases of development.

The patient's antibody response to foreign immunoglobulins is a considerable limitation for repeated tumour localisation or radioimmunotherapy to be effective. Several attempts have been made to reduce the potential immunogenicity of Mabs in order to make multiple injections of radiolabelled antibodies a feasible approach. In this respect, mouse/human chimeric antibodies have been synthesised, which are composed of the variable region of the mouse Mab and the constant region of a human IgG immunoglobulin as described for B72.3 [7] and for an antibody against CEA [8]. Less immunogenicity was observed in patients for the chimeric Mab 17-1A reported by LoBuglio *et al.* [9], but the antibody response was not completely absent. Human Mab technology has only progressed slowly, because of the difficulty to design reliable methods for generation as well as detection systems to select the antibody of choice [10]. Autoantibody-producing cells of patients with colorectal cancer [11], patients with breast cancer [12] and leukaemia patients [13] have been isolated and could be generated continuously. Such Mabs of human origin may eventually prove to be the best candidates for radioimmunotherapy.

The human Mab 16.88 was produced by Haspel *et al.* [11] and is an IgM immunoglobulin shown to specifically localise in colorectal cancer lesions in 9/12 patients studied at the National Cancer Institute, USA [14]. No patient developed an antibody response to 16.88, regardless of the fact that patients received additional weekly doses up to 200 mg of unlabelled protein. In the study presented, we report the results of tumour localisation with ^{131}I-16.88 in 20 colorectal cancer patients. At the time of a second infusion of ^{131}I-16.88 the effects of high doses of unlabelled antibody (200–1000 mg) were analysed to determine a possible improvement of immunoscintigraphic images. The pharmacokinetics and the lack of a human anti-16.88 response of the preparation are described in a separate report.

PATIENTS AND METHODS

Patients

20 patients with histologically confirmed advanced colorectal cancer were included in the study. The patients were eligible for the trial if their tumour tissue was reactive with Mab 16.88. Chemotherapy or radiotherapy could not be administered for at least 3 weeks prior to study entry. Adequate renal (creatinine < 125 μmol/l) and hepatic (bilirubin < 25 μmol/l) function and a WHO performance status ≤ 2 were required. All patients had to give their oral informed consent. Organ sites involved with tumour and the extent of disease were recorded by appropriate radiological studies including plain X-rays and computed tomography (CT) in all patients. Patients' characteristics are listed in Table 1.

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Revised 10 July 1991; accepted 18 July 1991.

Table 1. Patients' characteristics and ^{131}I-16.88 localisation

Patient (age, sex)	16.88 reactivity	Tumour sites (*n*)	Size (cm)	Scintigram (*n*)
I-1 (42,F)	3+	Local recurrence	> 3	+
I-2 (62,F)	3+	Retroperitoneal nodes	2 × 2 × 2.5	−
I-3 (67,M)	2+/3+	Primary (rectum)	CT neg	+
		Liver (9)	1 to 5 × 2.5	+ (7)
I-4 (23,M)	3+	Primary (rectum)	> 3	+
		Liver (3)	2.5/4/5	+ (2)
		Peritoneum (1)	2 × 2 × 3	−
		Mediastinal nodes	1.5 × 4 × 6	+
		Supraclavicular nodes (3)	1/1/1	−
I-5 (34,M)	2+/3+	Liver (3)	3/4/5 × 6	+ (3)
II-1 (52,F)	4+	Liver (multiple)	> 3	+
		Peritoneum (multiple)	CT neg	−
II-2 (53,M)	3+	Lung (multiple)	≤ 2	−
		Mediastinal nodes	> 3	+
		Brain (2)	2.5/3	+ (2)
II-3 (49,F)	3+	Local recurrence	> 3	+
		Liver (2)	1/2.5	−
		Lung (multiple)	≤ 2	−
II-4 (59,F)	3+	Liver (multiple)	> 3	+
		Lung (multiple)	≤ 1	−
II-5 (57,M)	3+	Peritoneum (4)	2.5/4/6/10	+ (2)†
		Retroperitoneal nodes	2 × 2 × 3.5	−
III-1 (60,M)	3+	Liver (3)	2/3/4	+ (2)
		Lung (1)	0.5	−
		Retroperitoneal nodes	2 × 2 × 6	−
III-2 (52,M)	3+	Liver (diffuse left lobe)	> 3	−
		Lumbal vertebra	4	+
III-3 (49,M)	3+	Liver (4)	1.5/1.5/2/2	−
III-4 (68,M)	2+/3+	Liver (3)	0.5/0.5/2	−
III-5 (68,M)	3+	Liver (1 + cysts)	5	+ (1)†
IV-1* (67,M)	3+	Liver (5)	2/2/2/4/4 × 4.5	+ (2)
		Lung (multiple)	≤ 1	−
IV-2 (41,F)	2+/3+	Inguinal nodes	5.5 × 6 × 10	+
IV-3 (65,M)	2+/3+	Liver (diffuse)	> 3	−
		Lung (3)	0.5/0.5/1.5	−
IV-4 (68,M)	3+/4+	Liver (3)	1.5/2/3	+ (1)
		Peritoneum (sigmoid)	CT neg	+
IV-5 (65,M)	4+	Liver (2)	2.5/3.5	+ (1)†

* Infusions with an interval of 4 months.
† Better visualisation at second infusion.

Monoclonal antibody (Mab)

Human Mab 16.88 was provided by the Biotechnology Research Institute (Rockville, Maryland). The antibody development and preparation have been described previously [11]. Briefly, peripheral blood mononuclear cells were obtained from a patient participating in a vaccination programme and immunised with irradiated autologous colorectal cancer cells admixed with Bacillus Calmette-Guérin (BCG) [15]. After selection of Mab 16.88, the antibody was produced on a large scale in hollow fibre bioreactors and purified by a combination of salt precipitation, gel filtration and ion exchange chromatography. Purity by sodium dodecylsulphate polyacrylamide gel electrophoresis was greater than 95%. Mab 16.88 is an IgM antibody derived from an Epstein–Barr virus transformed human B-cell line. The tumour specificity for 16.88 has been demonstrated using biotinylated Mab in formalin-fixed tissue sections [11]. The antibody reactivity with normal human tissues was absent or only weakly present. Recent investigations have indicated that 16.88 reacts with an altered form of cytokeratin and crossreacts weakly with normal cytokeratins 8 and 19 [16]. These are cytoplasmic, not membrane-bound, antigens.

Immunohistochemistry

Immunohistochemical analysis of formalin-fixed tumour tissue sections of patients with advanced colorectal cancer for reactivity with Mab 16.88 was carried out in the following way. Each specimen was tested at five antibody concentrations ranging from 0.05–1.2 μg/ml. An irrelevant human IgM was used as a non-specific binding control reagent. Also, a positive control of previously tested and reactive tumour tissue was included. Staining was visualised with a polyclonal goat anti-human IgM horseradish peroxidase-conjugated antibody (Dakopatts, Glostrup, Demark) and the chromogenic enzyme substrate 3, 3′-diaminobenzidine. Patients were candidates for entry into the study, if most tumour areas showed a moderate (3+) to

strong (4+) reactivity at an antibody concentration of 0.3 μg/ml of 16.88.

Radiolabelling

The purified Mab 16.88 was labelled with ^{131}I via the iodogen method by Mallinckrodt Diagnostica (Petten, The Netherlands) 24 h before use. After 10 min incubation, free ^{131}I was removed by a Sephadex G-10 column (Pharmacia, Uppsala). The mixture was then filtered through an 0.22 μm filter to sterilise the product. The immunoreactivity was determined on glutaraldehyde-fixed WiDr colon cancer cells according to the method of Lindmo *et al.* [17]. The final product contained less than 1% free ^{131}I, had a mean specific activity of 50 MBq/mg (range 30–63 MBq/mg) and a mean immunoreactivity of 90.6% (range 72.9–107.0%). The final product was stable for at least 48 h at 4°C. Endotoxin levels were below 350 EU per total volume of radiolabelled antibody and sterility of each preparation was confirmed according to the guidelines of the USA Pharmacopeia.

Study design

Prior to each dose of antibody, the patients were tested for hypersensitivity to Mab 16.88 by injecting 0.1 mg unlabelled antibody in 0.1 ml NaCl 0.9% intradermally. Routine clinical analyses to evaluate hematopoietic, hepatic and renal functions were performed before injection and were repeated weekly for 4 weeks. Thyroid function was monitored for 2 months.

Approximately 185 MBq (range 148–292 MBq) of ^{131}I-16.88 was prepared with addition of unlabelled 16.88 to a total protein dose of 8 mg. The antibody preparation was diluted in 1% human serum albumin in NaCl 0.9% to a total volume of 60 ml. The infusion was carried out over a 2 h period under closely monitoring of the blood pressure and the pulse rate of the patient. In three groups of 5 patients each, a second infusion was carried out 14 days after the first and mixed with 200, 500 or 1000 mg unlabelled 16.88.

For suppression of thyroid uptake of ^{131}I the first 5 patients received sodium perchlorate 400 mg twice daily for 8 days and starting one day prior to the infusion. The other patients received potassium iodide 135 mg twice daily for 10 days and starting 4 days prior to ^{131}I-16.88 administration. The latter regimen appeared to result in a better thyroid blockade as visualised on the immunoscintigrams.

Tumour localisation

Radioimmunoscintigraphy was carried out within 2 h after Mab administration (day 0) and at days 2, 5 and 7. Scintigrams were obtained by standardised techniques using a large field-of-view gamma camera (Gemini 700; General Electric) fitted with a high-energy collimator. Anterior and posterior views of the chest and the abdomen/pelvis, respectively were obtained using the 364 keV photopeak of ^{131}I and a 20% window. Patient's position was recorded to assure reproducibility for each subsequent acquisition. The immunoscintigraphic images were interpreted as positive when focal areas of increased uptake not corresponding to sites of physiological uptake (blood pool, thyroid and bladder) or dehalogenation (stomach) could be detected. The results were compared with both physical and radiological findings.

Table 2. Positive organ sites with ^{131}I-16.88

Organ site	Patients	Images
Primary tumour/recurrence	4	4
Liver	15	10
Lung	6	
Nodes		
Neck	1	
Mediastinum	2	2
Retroperitoneum	3	
Groin	1	1
Peritoneal wall	4	2
Brain	1	1
Bone	1	1
Total	38	21

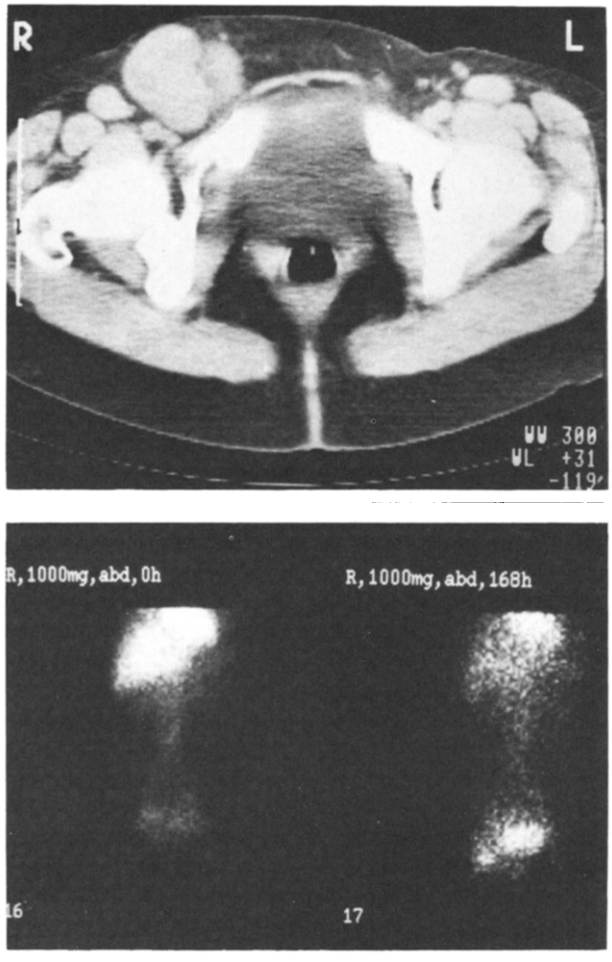

Fig. 1. Patient IV-2 had lymph node metastases palpable in the right groin as visualised on CT (upper); an immunoscintigram at day 7 (anterior view) shows uptake of radioactivity at the right side of the urinary bladder (lower).

In a number of patients with liver metastases ^{99m}Tc-sulphur-colloid (75 MBq) and ^{99m}Tc-HMPAO (hexamethylpropyleneamine oxime) (370 MBq) scintigrams were obtained to visualise filling defects caused by replacement of the reticulo-endothelial system or by areas of poor blood supply, respectively. In both instances, anterior, posterior and lateral views were made with the same gamma camera and collimator (setting at 140 keV and a 20% window). The images were compared with those obtained at immunoscintigraphy to interpret the findings.

Total body radioactivity was measured at the time points of radioimmunoscintigraphy with an uncollimated small field-of-view gamma camera (Cardiac, Siemens) at a defined (4 m) distance from the patient for 1 min both anteriorly and posteriorly. The biological half-life of ^{131}I was calculated for each

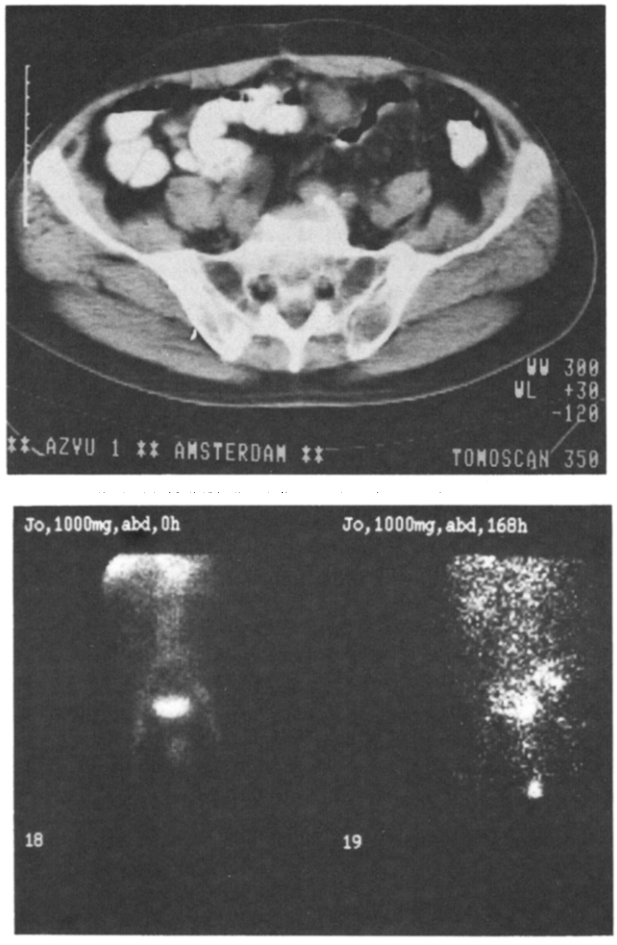

Fig. 2. Patient IV-4 had a positive area at the left side of the urinary bladder on the immunoscintigram (anterior view) at day 7 (lower); CT at that time did not reveal abnormalities (upper).

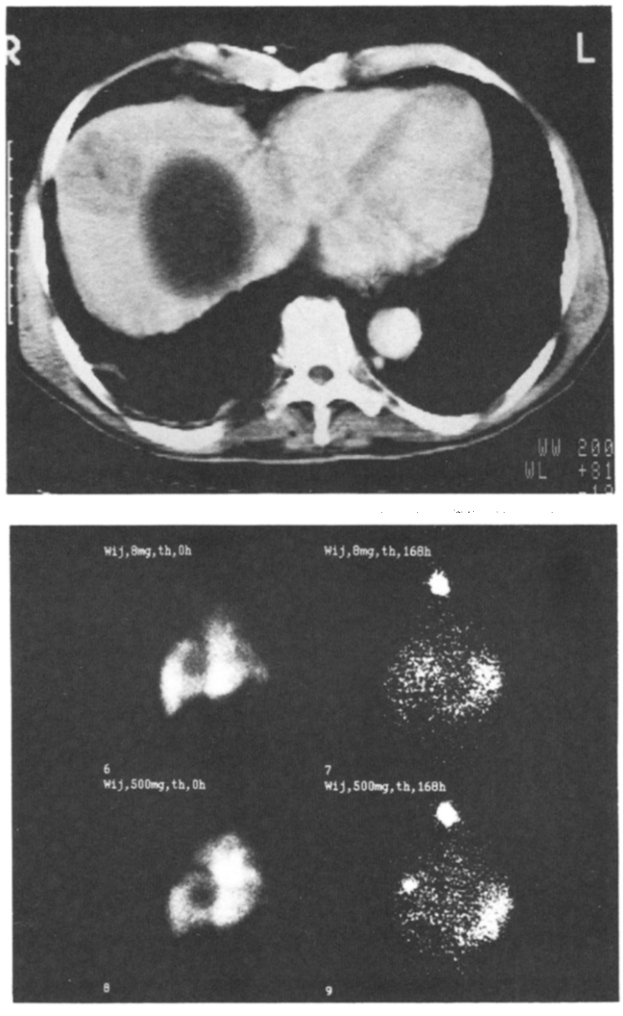

Fig. 3. Patient III-5 had cysts in the liver. Adjacent to the large cyst in the right liver lobe was a metastasis with a diameter of 5 cm (upper); immunoscintigraphy of the liver area at day 7 showed uptake in the cyst at the time of the first infusion with ^{131}I-16.88 (8 mg), whereas after the second infusion (with addition of 500 mg unlabelled 16.88) clear visualisation was obtained of the metastasis (lower). Immunoscintigrams at day 7 also show uptake in the thyroid and in the stomach.

infusion correcting the geometrical mean whole body counts for decay, administered dose and machine drift, and fitting the patient's data by use of a least squares fit to a monoexponential function.

RESULTS

Immunohistochemistry

From 94 patients with advanced colorectal cancer and potentially eligible for study entry, formalin-fixed tumour tissue specimens were examined for reactivity with Mab 16.88. At an antibody concentration of 0.3 μg/ml of 16.88 tumour staining intensity was (−) in 19.5%, (+) in 21%, (2+) in 8.5%, (3+) in 42.5% and (4+) in 8.5% of patients. Staining was homogeneously distributed within tumour tissue and in a number of specimens focal areas of higher intensity could be detected. Only patients with (3+), (4+) as well as some patients with (2+)/(3+) focal areas of staining intensity were selected for the study.

Tumour localisation

Of 20 patients with advanced colorectal cancer reactive with Mab 16.88, 16 (80%) showed a positive image with ^{131}I-16.88 in at least one organ site. Optimal contrast between tumour lesions and background activity was obtained at days 5 and 7. Table 1 shows the various organ sites involved and where possible, the number and the size of the lesions is indicated as measured by palpation, plain X-rays or CT. Lesions < 3 cm were infrequently visualised. In Table 2 the number of positive organ sites is listed, resulting in a percentage of 55%. Examples of positive immunoscintigrams are given in Figs 1 and 2. Large retroperitoneal nodes extending along the aortic artery were missed, probably because of the prolonged persistence of blood pool activity. In a few patients images were obtained of lesions not detected by CT or previously known. For example, in patient IV-4 (Fig. 2) the peritoneal metastasis present in the sigmoid area could not be revealed by CT. The immunoscintigrams, however, clearly showed a positive image at the left side of the abdomen. The presence of a peritoneal mass was confirmed at laparotomy 4 weeks after the ^{131}I-16.88 tumour localisation. Patient II-2 presented with headache and vomiting 10 days after the first infusion with ^{131}I-16.88. An immunoscintigram of the head at day 13 as well as at day 7 after the second infusion was indicative for ^{131}I-uptake in 2 areas. CT of the brain confirmed two metastatic lesions.

Abnormal areas of ^{131}I-accumulation occurred in patient II-1 in the right kidney from hydronephrosis as well as in the left part of the chest from a pleural effusion which developed shortly after the first ^{131}I-16.88 infusion. Patient I-4 also had localisation

Table 3. Scintigraphic patterns of liver metastases

Patient	Immunoscintigram*	Cold areas	
		^{99m}Tc-colloid	^{99m}Tc-HMPAO
I-3	cold d0, hot d5 + 7	5	8
I-4	homogenous d0, hot d5 + 7	1	1
I-5	cold d0, hot d5 + 7	3	3
II-1	cold d0, cold and hot d5 + 7	ND	ND
II-3	homogeneous	ND	ND
II-4	cold d0, cold and hot d7	ND	ND
III-1	homogeneous d0, hot d5 + 7	ND	ND
III-2	cold d0, homogeneous d5 + 7	ND	3
III-3	homogeneous	ND	1
III-4	homogeneous	Normal	1
III-5	cold d0, hot d5 + 7†	4	4
IV-1	homogeneous d0, hot d5 + 7	3	3
IV-3	cold d0, homogeneous d5 + 7	4	3
IV-4	homogeneous d0, hot d5 + 7	Normal	1
IV-5	cold d0, hot d5 + 7	2	2

* Obtained at days 0, 2, 5 and 7.
† at second infusion.
ND = not determined.

of ^{131}I in a hydronephrotic right kidney. Apart from metastatic disease patient III-5 (Fig. 3) was known to have cysts in the liver. The cyst with the largest diameter filled in with radioactivity and was best detectable at day 7. In this patient at the time of the second infusion the metastatic site visualised in contrast to the cyst. In a number of patients radioactivity was detectable in the gastric area at day 2. Occasionally, the stomach could still be visualised at later days, such as in patient III-5 (Fig. 3).

Liver scintigraphy

Sequential immunoscintigrams of liver metastases showed variable patterns (Table 3). Initially, these lesions were frequently visualised as "cold" areas and filled in with radioactivity at later time points to become "hot" or isodense areas within the liver. To obtain better insight in the destruction to the liver parenchyma as well as in the perfusion of the metastatic lesions, ^{99m}Tc-sulphur-colloid and ^{99m}Tc-HMPAO scintigrams were acquired. Invariably, the "cold" spots detected by both techniques corresponded with areas of liver metastases. Fig. 4 (patients I-3 and I-5) shows examples of such findings. In 3 patients ^{99m}Tc-HMPAO indicated more abnormalities than the conventional liver-colloid scan.

Excess unlabelled 16.88

The addition of unlabelled 16.88 at a dose of 200 mg, 500 mg and 1000 mg to groups of 5 patients each (groups II, III and IV) infrequently produced better visualisation of tumour lesions. Improved images were obtained of peritoneal lesions in patient II-5 and of liver metastases in patients III-5 (Fig. 3) and IV-5. Patient IV-1 received a second infusion only 4 months after the first. At that time, CT showed progressive liver metastases, but immunoscintigraphy did not reveal better images. Generally, the scintigrams obtained at the first and second infusion were similar for ^{131}I-uptake in tumour lesions.

Total body radioactivity

Total body radioactivity measured at the time points of immunoscintigraphy indicated a mean (S.D.) biological half-life

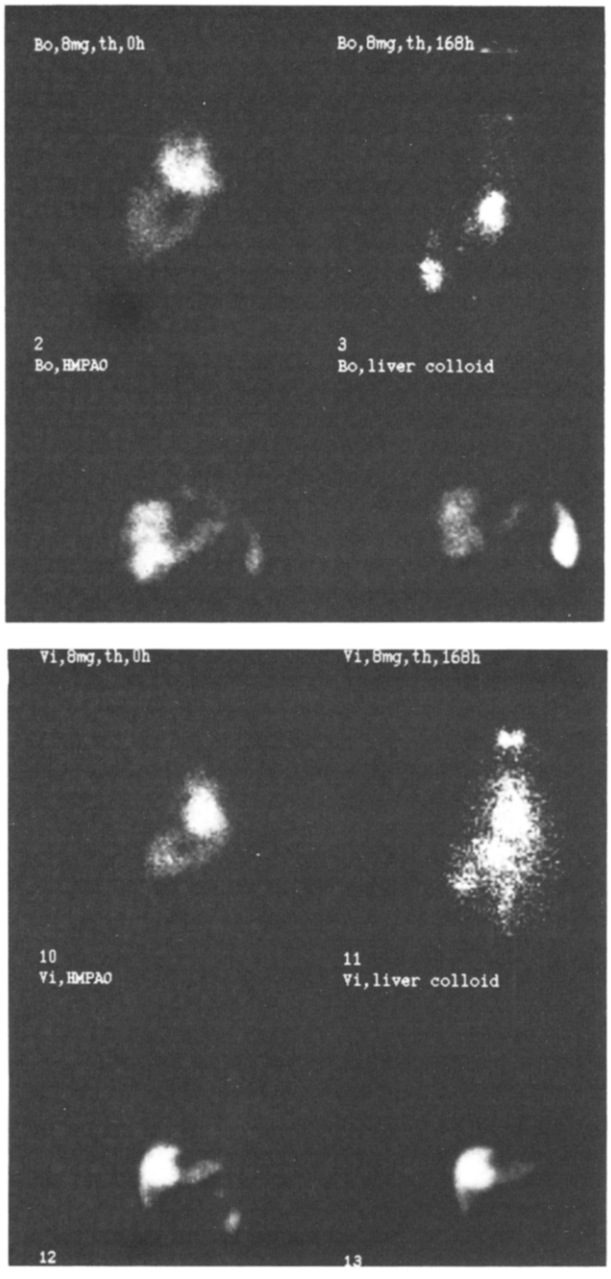

Fig. 4. Patients I-3 (upper) and I-5 (lower) were known with various liver metastases. Initially at day 0, in both patients immunoscintigraphy showed "cold" lesions after ^{131}I-16.88 infusion which filled up with radioactivity to "hot" areas (shown at day 7). Apart from the metastases in patient I-5 uptake is still visible in the heart region at day 7. Liver images obtained with ^{99m}Tc-HMPAO and ^{99m}Tc-colloid confirmed metastatic sites with poor blood supply.

for ^{131}I of 37.6 (5.0) h at the time of the first infusion (Table 4). The mean half-life was not different from that at the time of the additional unlabelled 16.88. Also between groups of patients ^{131}I-clearance was found to result in similar mean half-lives.

Toxicity

In none of the patients hypersensitivity to Mab 16.88 was detected by skin testing. Nonetheless, in 1 patient (III-4) the second infusion caused an erythematous rash with urticaria subsiding 2 h after the infusion had been discontinued. A repeated skin test was also negative, suggesting that a non-

Table 4. Biological half-life for whole body radioactivity

Patient group	First infusion (h) 185 MBq ^{131}I/8 mg 16.88	Second infusion (h) 186 MBq ^{131}I/excess 16.88
I	38.5 (3.9)	
II	38.4 (2.4)	41.3 (5.6) (200 mg)
III	36.1 (8.8)	39.7 (7.0) (500 mg)
IV	37.4 (4.0)	41.7 (4.9) (1000 mg)

Mean (S.D.)

specific reaction to the foreign protein had incited the allergic symptoms. In the other patients the infusion of 16.88 did not cause any side effects. Uptake of ^{131}I in the thyroid of patients on potassium iodide protection did not exceed 1.0% of the dose administered as measured with conventional uptake techniques. Thyroid function monitored up to 2 months after the first infusion did not show a decrease in hormone levels.

DISCUSSION

Mab 16.88 is one of the first human antibodies reactive with a tumour-associated antigen to show preferential localisation in colorectal cancer. Concomitant administration of excess unlabelled 16.88 can be performed safely, but has neither shown improvement in tumour visualisation on immunoscintigrams nor influence on total body radioactivity kinetics.

Steis *et al.* [14] recently reported the results obtained with ^{131}I-labelled Mab 16.88 and another human IgM Mab 28A32 in the localisation of colorectal cancer lesions. For 16.88, 56% of patients had tumour tissue strongly reactive with the antibody (3+ to 4+). Tumour uptake of ^{131}I-16.88 was observed in 9/12 patients (75%) and in 60% of tumour lesions > 2 cm. These percentages closely correspond to our findings. Lesions ≤ 2 cm were detectable in only 6% of cases, whereas we also rarely found visible uptake of radioactivity in small lesions.

An IgM antibody recognising an intracellular antigen as well as the isotope ^{131}I are not considered the best tools for radioimmunolocalisation. With Mabs of murine origin, tumour localisation in colorectal cancer patients has improved with fragments of complete IgG conjugated to ^{123}I, ^{99m}Tc or ^{111}In, radionuclides enabling single-photon emission CT. For the anti-CEA antibody NP-4, this approach resulted in an overall sensitivity on a tumour site basis for ^{123}I-F(ab')$_2$ and for ^{99m}Tc-F(ab) of 95.9% and 94.9%, respectively [4]. In 19 patients with rising CEA levels and CT findings negative for recurrent disease, a positive immunoscintigram upon administration of the ^{111}In-labelled anti-CEA antibody 2CE-025 correctly indicated the presence of tumour sites [5]. In this respect, the F(ab')$_2$ fragments of the intact IgG Mab 2CE-025 were found more useful in the detection of lesions, resulting in an overall sensitivity of 79.4% and 32%, respectively [18]. In a multicentre tumour localisation trial of ^{111}In-labelled F(ab')$_2$ fragments of the anti-CEA antibody F023C5, early detection of tumour recurrences appeared to be a common finding [6]. Invariably, these studies have described visualisation of tumour lesions within 24 h after administration of the conjugate. In spite of these favorable data obtained with fragments of murine anti-CEA Mabs, our findings with human IgM ^{131}I-16.88 are not discouraging as immunoscintigrams were positive in 80% of patients and in 55% of organ sites. These percentages are well within the range of those obtained with ^{131}I-labelled murine complete IgG antibodies.

Radioimmunolocalisation has been usually performed with Mabs directed against tumour-associated antigens localised on the cell membrane. However, this characteristic is not a prerequisite for successful tumour detection as illustrated by 16.88. The cytoplasmic antigen may have come available by local release from necrotic tumour cells. Also, other Mabs recognising intracellular antigens have been reported of potential use for tumour localisation. As an example, the murine antibody TNT-1 reactive with nuclear histones was shown to concentrate primarily at the centre of tumours where binding is facilitated by degenerating cells [19]. At high doses, ^{131}I-labelled TNT-1 induced a remarkable therapeutic response in an experimental human cervical cancer model [20].

Detection of tumour lesions may be improved with higher doses of Mab. In melanoma patients fixed doses of ^{111}In-labelled murine Mab coinjected with unlabelled specific antibody (up to 100 mg) resulted in improved detection of known metastatic lesions proportionally to the total dose [21, 22]. With the higher doses, a prolonged serum half-life for the radiolabelled conjugate was found as well as a reduced uptake in non-specific sites, such as spleen, liver and bone marrow. Our attempts to saturate non-specific binding sites by adding excess unlabelled 16.88 did not result in improved immunoscintigraphic images or prolonged ^{131}I-16.88 retention. Possibly, such improvement may only be expected if non-specific uptake is high as may be the case for ^{111}In-labelled murine Mabs.

Effective Mab delivery to tumours is hampered by the heterogeneous blood supply within tumour tissue, the transport across the microvascular wall, and the interstitial distance to the tumour cells [23]. In this respect, a macromolecule such as 16.88 may encounter considerable difficulties to reach its target. Poor blood supply to liver metastases was observed with ^{99m}Tc-HMPAO in our patients. HMPAO is concentrated and excreted by the normal liver and shows high activity on radioscintigraphy [24]. Apart from insight in the vascularisation of tumour tissue, this technique may therefore give additional information on the extent of the destruction of liver parenchyma caused by tumour infiltration.

An extensive search for human IgG or IgM antibodies against the human IgM Mab of more specifically against 16.88 in our patients was negative as determined in three different assays. Also, in none of the patients investigated by the group of Steis *et al.* [14] could an anti-human Mab response be detected. The tumour-localising properties and the absence of immunogenicity of 16.88 are encouraging and indicate possibilities for therapeutic applications of human Mabs.

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Acknowledgement—This study was supported by the Praeventiefonds.

Eur J Cancer, Vol. 27, No. 11, pp. 1436–1440, 1991.
Printed in Great Britain

0277–5379/91 $3.00 + 0.00

Increased Levels of Mitochondrial DNA in an Etoposide-resistant Human Monocytic Leukaemia Cell Line (THP-1/E)

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Electron microscopic observations of THP-1/E (an etoposide-resistant human monocytic leukaemia cell line) showed a remarkable change of mitochondrial structure. Mitochondria were swollen and cristae were relatively intact. There was no difference in the activity of cytochrome oxidase, an enzyme which contains three subunits coded by mitochondrial DNA (mtDNA) between THP-1/E and THP-1 (the parent cell of THP-1/E). No measurable quantitative change of mitochondrial RNA was observed, but the level of mtDNA in THP-1/E was increased by a factor of about 4 compared with that of mtDNA in THP-1. These results suggest that, on acquisition of resistance to etoposide, some factors affect mitochondria, change its morphology and amplify its DNA.
Eur J Cancer, Vol. 27, No. 11, pp. 1436–1440, 1991.

INTRODUCTION

UNDER PATHOLOGICAL conditions, mitochondria show changes in size, shape and number of cristae [1]. These ultrastructural changes are associated with biochemical alterations as exemplified by defects in the pathway of substrate oxidation and ion transport systems, deficiency in enzyme levels or cytochrome content. Whether the mitochondrial abnormalities are a cause or consequence of a pathological condition cannot always be easily determined.

Mammalian mitochondrial DNA (mtDNA) is a closed circular double-stranded molecule consisting of 16 kilobase pairs. It codes for two ribosomal RNA genes, 22 transfer RNA genes, and 13 protein-coding genes such as cytochrome b, subunits of cytochrome oxidase, ATPase and complex I of the respiratory chain [2, 3]. Unlike nuclear DNA, mtDNA is not in association with histones, and its repair mechanism has not yet been elucidated [4].

We have recently established an etoposide-resistant leukaemia