Pharmacokinetics and biodistribution of a new anti-episialin monoclonal antibody 139H2 in ovarian-cancer-bearing nude mice

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Summary. The new murine anti-episialin monoclonal antibody (mAb) 139H2 has been selected for its strong reactivity with a series of human ovarian cancer xenografts. In the present report we describe the characteristics of mAb 139H2 investigated in vitro as well as in vivo. Scatchard plot analysis using the human ovarian cancer cell line NIH:OVCAR-3 showed an affinity constant of 1×10^8 M⁻¹ and the expression of 7×10^6 antigenic sites/cell. Reactivity with OVCAR-3 xenograft tissue was intense, localized at the cell membrane, heterogeneously distributed, and mainly detectable at the apical site of the cell. Administration of radiolabelled mAb 139H2 to nude mice bearing s.c. OVCAR-3 xenografts showed specific uptake in the tumour up to 9% of the injected dose/g. The maximum uptake in the tumour was retained for 3.5 days and mAb 139H2 cleared from the tumour with a half-life of 5.5 days. The half-life in blood was 50 h and no antibody-antigen complex formation could be detected. Poor uptake and no retention in episialin-negative WiDr colon cancer xenografts demonstrated specificity. Administration of an excess of an unlabelled irrelevant mAb did not influence the uptake in the OVCAR-3 xenografts or in other tissues. In contrast, tumour uptake decreased after addition of 300 µg or more unlabelled mAb 139H2 to a tracer dose of radiolabelled mAb 139H2. The uptake of mAb 139H2 in OVCAR-3 xenografts appeared inversely related to the tumour size.

Key words: Monoclonal antibody – Ovarian cancer – Xenografts

Introduction

The specific tumour-localizing properties of monoclonal antibodies (mAbs) have been proven in a variety of tumour

types. For ovarian cancer, mAbs OC125 [5] and OV-TL3 [13] among others were shown to be useful for immunoscintigraphy. mAbs reactive with mucin-like antigens belong to another category of antibodies with potential value for the detection of tumour lesions in ovarian cancer patients [19]. Factors that may influence the results of immunoscintigraphy are the specificity of the mAb for cancer cells, the affinity, the immunoglobulin class and isotype of the antibody, as well as the localization and the possible release of the target antigen [3]. Other important variables are the radionuclide and labelling method [2]. The ideal mAb should be of the IgG class and recognize an antigen that is specific for tumour cells, and this antigen should be present at high density on the cell membrane and not released into the circulation. Also, different subtypes of the tumour involved should express the antigen.

In a previous study, we have tested the reactivity of a large number of mAbs directed against carcinoma cells using a panel of human ovarian cancer xenografts [14]. The xenografts were shown to retain the antigenic expression of the original human tumour. One of the best reactive mAbs was 139H2, which recognizes the epithelial sialomucin, designated episialin [10]. In order to determine the potential value of mAb 139H2 for in vivo diagnosis of ovarian cancer, we evaluated first its in vitro cell-binding characteristics. Thereafter, we determined the pharmacokinetics and biodistribution after intravenous administration of radiolabelled 139H2 to nude mice bearing subcutaneous human ovarian cancer xenografts. Tumour tissue uptake of radiolabelled mAb 139H2 was related to tumour size as well as to the amount of simultaneously injected unlabelled mAb 139H2.

Materials and methods

Monoclonal antibodies. Ascitic fluid containing mAb 139H2 was kindly provided by Dr. J. Hilkens (Netherlands Cancer Institute, Amsterdam). The production and biochemical characterization of mAb 139H2 have been reported earlier [10]. This antibody is of the IgG1 isotype and binds to a protein determinant of episialin, also designated CA 15-3. Purification was performed by affinity chromatography using Affi-Gel protein-A MAPS II (Bio-Rad Laboratories, Utrecht, NL). mAb A2C6 was used as

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a control antibody and was kindly provided by Dr. S. O. Warnaar (Centocor Inc., Leiden, NL). This antibody is also of the IgG1 isotype and reacts with the hepatitis B surface antigen [23].

Cell lines. The NIH:OVCAR-3 (OVCAR-3) human ovarian cancer cell line was kindly donated by Dr. T. C. Hamilton (Fox Chase Cancer Center, Philadelphia, Pa.). WiDr is a human colon cancer cell line described by Noguchi et al. [16]. Both cell lines were grown as monolayers in Dulbecco's modified Eagle's medium obtained from Flow (Amsterdam, NL), supplemented with heat-inactivated 10% fetal calf serum. In an immunoperoxidase assay of cytospin preparations of OVCAR-3 cells, 139H2 was shown to stain these cells strongly, whereas A2C6 was negative. None of the antibodies reacted with WiDr cells.

Xenografts. Xenografts were established from cells grown in vitro and injected s.c. in both flanks of female, 8- to 10-week-old NMRI/Cpb (nu/nu) mice (Harlan/Cpb, Zeist, NL). OVCAR-3 xenografts show a poorly to moderately differentiated serous adenocarcinoma pattern, while that of WiDr is a poorly differentiated adenocarcinoma. The tumour volume doubling times are respectively 10 and 8 days. Serial transfer was carried out by implanting fragments of solid tumour tissue with a diameter of 2–3 mm s.c. through a small skin incision in subsequent recipients. Tumour volume was measured in three dimensions and expressed by the equation $\text{length} \times \text{width} \times \text{height} \times 0.5$ in mm^3 . Light microscopy was performed in each passage to confirm the histological subtype and the degree of differentiation. Binding of mAbs to target cells was determined in an indirect immunoperoxidase assay as has been described earlier [14].

Radiolabelling. mAbs 139H2 and A2C6 were labelled with either ^{125}I or ^{131}I by the iodogen method [9]. Free iodine was removed by an anion-exchange resin suspension in phosphate-buffered saline (PBS) containing 1% bovine serum albumin (BSA) (AG1-X8, Bio-Rad, Utrecht, NL). The percentage of radioactive iodine bound to the mAb, determined by trichloroacetic acid precipitation, was always >95%. The specific activity varied between 2 mCi/mg and 5 mCi/mg.

Immunoreactivity and affinity. After radiolabelling of mAbs 139H2 and A2C6, the immunoreactive fraction was determined on OVCAR-3 cells according to Lindmo et al. [11]. The immunoreactive fraction of mAb 139H2 was consistently between 65% and 75%, while mAb A2C6 was not reactive. The affinity constant of mAb 139H2 on OVCAR-3 cells was determined by incubating a fixed amount of radiolabelled mAb mixed with unlabelled mAb over a concentration range of 1–150 $\mu\text{g}/\text{ml}$. The cell-bound radioactivity was measured in a gamma counter. The affinity constant was calculated from a Scatchard plot of specifically bound mAb versus bound over free mAb.

Pharmacokinetics and biodistribution. In nude mice, thyroid uptake of iodine was blocked by addition of potassium iodide to the drinking water (0.1%) from 3 days before until the end of the study. Animals bearing OVCAR-3 or WiDr xenografts with a mean tumour volume of 400 mm^3 were injected with a tracer dose (7–15 μCi) of a mixture of ^{125}I -labelled control mAb and ^{131}I -labelled mAb 139H2 and sacrificed at various times: 1, 3, 15, 30, 48, 72, 96, 168 and 240 h after injection. For each time point three mice were used. Blood was collected from mice under ether anaesthesia. Thereafter, normal tissues and tumours were rinsed in saline and dried to minimize blood residues. Blood and all tissues were weighed and the radioactivity was measured in a two-channel gamma counter with automatic correction for spillover of both radionuclides in the channels. The results were expressed as the percentage of injected dose per gram. To correct for radioactive decay, a standard solution of the injected material in PBS+1% BSA was prepared and counted simultaneously with the tissues on each day studied. The proportion of radioactivity associated with protein in serum was determined by precipitation with 10% trichloroacetic acid. As a measure of the specificity of the antibody uptake in tumours, the localization index was used:

$$\frac{\% \text{ ID/g specific mAb (tissue)} / \% \text{ ID/g control mAb (tissue)}}{\% \text{ ID/g specific mAb (blood)} / \% \text{ ID/g control mAb (blood)}}$$

where ID = injected dose.

In separate experiments the influence of excess unlabelled mAb on antibody uptake in tumours was investigated in groups of three mice each. The amount of unlabelled antibody, either mAb 139H2 or the control mAb A2C6, added to the tracer dose of radiolabelled mAb 139H2 varied between 50 μg and 1000 μg .

Antigen-antibody complexes. Mouse serum, collected at various times, after injection of radiolabelled 139H2 was analysed by fast protein liquid chromatography (FPLC) using a Superose 6 column (Pharmacia, Uppsala, Sweden) with PBS as eluent for the presence of antigen-antibody complexes.

Immunoscintigraphy. Mice with OVCAR-3 tumours were injected i.v. with 100 μCi ^{131}I -mAb 139H2 and scintigraphy was performed on days 1, 2, 3, and 7 after antibody administration. Posterior imaging was carried out with a gamma camera with a large field of view (Ohio-Nuclear, General Electric) equipped with a high-energy collimator. Immunoscintigrams were taken with a minimum of 60 000 accumulated counts. On each image, regions of interest were drawn for whole body, heart and tumour, and the radioactivity was expressed as the percentage of the total image radioactivity of the whole body at day 0.

Results

Reactivity of mAbs

A Scatchard analysis was performed of radiolabelled mAb 139H2 on OVCAR-3 cells. The affinity constant was $1.0 \times 10^8 \text{ M}^{-1}$ ($\pm 0.7 \times 10^8$) and the OVCAR-3 cells expressed 7.5×10^6 ($\pm 3.3 \times 10^6$) antigenic sites per cell. No reactivity was detected with mAb 139H2 on WiDr cells and with mAb A2C6 on OVCAR-3 and WiDr cells. The same mAbs were also tested for reactivity on sections of OVCAR-3 and WiDr xenografts by immunoperoxidase staining. For mAb 139H2 the staining was intense, but heterogeneously distributed. The binding was mainly detectable at the apical cell membrane of the cells in the better differentiated areas of the tumour. WiDr tissue sections were negative for mAb 139H2, as were both xenografts for mAb A2C6.

Pharmacokinetics and biodistribution

In vivo pharmacokinetic analysis and tissue distribution were performed in nude mice bearing OVCAR-3 or WiDr tumours. Groups of three mice each were injected with ^{131}I -mAb 139H2 and ^{125}I -mAb A2C6. Figure 1A, B shows the pharmacokinetics of ^{131}I -mAb 139H2 and ^{125}I mAb A2C6 in blood, tumours and liver of OVCAR-3-bearing mice. The half-life of both mAbs 139H2 and A2C6 in the blood was approximately 50 h. The amount of free iodine in the serum, measured at each time point, was negligible. The maximum percentage injected dose/g ^{131}I -mAb 139H2 in tumour tissue was 9% and this level was retained for 3.5 days. Thereafter, the half-life in the tumours of ^{131}I -mAb 139H2 was 5.5 days. In contrast, the maximum percentage injected dose/g in the tumour for the control antibody was 2.3% and no retention was observed. Uptake in the liver was much lower than in tumours. Other tissues showed an equal or lower uptake as compared to that in the liver (Table 1). Figure 2 illustrates the specificity of the localization of ^{131}I -mAb 139H2 in the OVCAR-3 xeno-

Fig. 1 a–c. Pharmacokinetics of radiolabelled mAbs in blood (●), tumour (■), and liver (▲) of nude mice bearing OVCAR-3 or WiDr xenografts. a, OVCAR-3 and ^{131}I -139H2; b, OVCAR-3 and ^{125}I -A2C6; c, WiDr and ^{131}I -139H2. SE <10%

grafts. The localization index increased to 5.6 1 week after injection. The analogous experiment performed in mice bearing WiDr xenografts showed that no specific localization was observed with ^{131}I -mAb 139H2 (Fig. 1C).

Complex formation in serum

As previously reported [14] OVCAR-3-bearing mice express low serum levels of CA 15-3. Therefore, serum of mice injected with ^{131}I -mAb 139H2 was analysed by FPLC at various times. An example is shown in Fig. 3. At no time after injection were we able to demonstrate com-

Fig. 2. Localization index for mAb 139H2 in OVCAR-3 xenografts after injection of a mixture of a tracer dose of ^{131}I -139H2 and ^{125}I -A2C6 at 1, 15, 30, 48, 72, 96, 168, and 240 h after injection

Fig. 3. Size-exclusion fast protein liquid chromatography profiles of ^{131}I -139H2 in serum of OVCAR-3-bearing nude mice at 24 (—), 72 (---), and 168 (...) h after injection

plexation of the injected antibody with the high-molecular-mass episialin antigen (molecular mass >400 kDa).

Excess of unlabelled mAbs

In OVCAR-3-bearing mice, tumour tissue uptake of a tracer dose of 2 μg ^{125}I -mAb 139H2 was measured upon simultaneous administration of 50 μg or 300 μg unlabelled 139H2 or unlabelled control mAb A2C6. A control group was injected with ^{125}I -139H2 only. Mice were sacrificed 3 days after administration of the mAb. Addition of 50 μg 139H2 did not affect the uptake in tumours and other tissues, but with 300 μg the uptake in the tumours decreased with a factor of 1.8 (Table 2A). This relative decrease in tumour uptake was confirmed by the co-administration of 1000 μg unlabelled mAb 139H2 (factor 1.9, Table 2B). Except for tumour tissue, uptake in blood and

Table 1. Biodistribution of ^{131}I -139H2 and ^{125}I -A2C6 in OVCAR-3-bearing nude mice

Tissue	Percentage of injected dose/g tissue $\pm$ SE							
	1 h ^a	3 h	15 h	30 h	48 h	72 h	96 h	168 h
^{131}I-139H2								
Tumour	1.31 $\pm$ 0.09	2.36 $\pm$ 0.28	6.87 $\pm$ 0.90	5.95 $\pm$ 0.60	7.22 $\pm$ 0.44	6.10 $\pm$ 0.80	9.00 $\pm$ 1.86	2.95 $\pm$ 0.26
Blood	19.69 $\pm$ 1.74	18.20 $\pm$ 0.37	12.51 $\pm$ 0.27	8.90 $\pm$ 0.65	7.75 $\pm$ 0.72	5.14 $\pm$ 0.92	6.19 $\pm$ 0.99	1.26 $\pm$ 0.11
Liver	4.28 $\pm$ 0.21	3.77 $\pm$ 0.42	2.46 $\pm$ 0.14	1.72 $\pm$ 0.14	1.55 $\pm$ 0.20	1.09 $\pm$ 0.15	1.04 $\pm$ 0.16	0.26 $\pm$ 0.03
Spleen	3.96 $\pm$ 0.20	3.50 $\pm$ 0.12	2.73 $\pm$ 0.11	1.73 $\pm$ 0.26	1.61 $\pm$ 0.18	1.16 $\pm$ 0.22	1.19 $\pm$ 0.18	0.26 $\pm$ 0.03
Heart	2.51 $\pm$ 0.02	2.74 $\pm$ 0.14	2.42 $\pm$ 0.13	1.52 $\pm$ 0.12	1.57 $\pm$ 0.20	0.96 $\pm$ 0.15	1.08 $\pm$ 0.18	0.23 $\pm$ 0.01
Kidney	4.41 $\pm$ 0.35	4.01 $\pm$ 0.21	2.74 $\pm$ 0.14	2.07 $\pm$ 0.11	1.82 $\pm$ 0.23	1.22 $\pm$ 0.22	1.34 $\pm$ 0.16	0.28 $\pm$ 0.02
Colon	0.88 $\pm$ 0.02	1.65 $\pm$ 0.15	1.02 $\pm$ 0.06	0.85 $\pm$ 0.03	0.74 $\pm$ 0.09	0.45 $\pm$ 0.06	0.48 $\pm$ 0.07	0.11 $\pm$ 0.02
Ileum	2.09 $\pm$ 0.14	2.29 $\pm$ 0.17	1.32 $\pm$ 0.07	1.26 $\pm$ 0.004	0.95 $\pm$ 0.16	0.75 $\pm$ 0.17	0.78 $\pm$ 0.06	0.15 $\pm$ 0.01
Stomach	1.49 $\pm$ 0.11	2.07 $\pm$ 0.06	1.33 $\pm$ 0.02	1.50 $\pm$ 0.05	0.91 $\pm$ 0.12	0.58 $\pm$ 0.08	0.60 $\pm$ 0.11	0.16 $\pm$ 0.01
Muscle	0.49 $\pm$ 0.06	0.46 $\pm$ 0.04	0.62 $\pm$ 0.01	0.59 $\pm$ 0.06	0.52 $\pm$ 0.07	0.31 $\pm$ 0.06	0.28 $\pm$ 0.05	0.09 $\pm$ 0.01
Femur	1.60 $\pm$ 0.06	1.74 $\pm$ 0.20	1.28 $\pm$ 0.01	1.01 $\pm$ 0.09	0.94 $\pm$ 0.12	0.57 $\pm$ 0.08	0.57 $\pm$ 0.10	0.15 $\pm$ 0.01
^{125}I-A2C6								
Tumour	1.26 $\pm$ 0.07	1.66 $\pm$ 0.17	2.33 $\pm$ 0.18	1.74 $\pm$ 0.22	1.72 $\pm$ 0.22	1.47 $\pm$ 0.27	1.39 $\pm$ 0.17	0.90 $\pm$ 0.09
Blood	17.37 $\pm$ 1.38	14.24 $\pm$ 0.90	8.85 $\pm$ 0.43	6.61 $\pm$ 0.41	5.21 $\pm$ 0.50	4.18 $\pm$ 0.15	4.02 $\pm$ 0.30	2.17 $\pm$ 0.16
Liver	6.85 $\pm$ 0.62	6.62 $\pm$ 0.82	3.44 $\pm$ 0.41	1.62 $\pm$ 0.21	1.41 $\pm$ 0.12	1.12 $\pm$ 0.10	0.83 $\pm$ 0.05	0.49 $\pm$ 0.04
Spleen	6.13 $\pm$ 0.59	8.62 $\pm$ 1.96	5.75 $\pm$ 0.97	1.77 $\pm$ 0.34	1.82 $\pm$ 0.05	1.21 $\pm$ 0.06	1.02 $\pm$ 0.09	0.52 $\pm$ 0.06
Heart	2.28 $\pm$ 0.04	2.24 $\pm$ 0.15	1.83 $\pm$ 0.06	1.12 $\pm$ 0.06	1.07 $\pm$ 0.13	0.82 $\pm$ 0.06	0.69 $\pm$ 0.06	0.42 $\pm$ 0.01
Kidney	4.43 $\pm$ 0.28	3.79 $\pm$ 0.16	2.28 $\pm$ 0.12	1.59 $\pm$ 0.09	1.32 $\pm$ 0.14	1.06 $\pm$ 0.07	0.93 $\pm$ 0.04	0.49 $\pm$ 0.02
Colon	0.95 $\pm$ 0.01	1.45 $\pm$ 0.16	0.85 $\pm$ 0.08	0.65 $\pm$ 0.04	0.55 $\pm$ 0.07	0.40 $\pm$ 0.04	0.34 $\pm$ 0.02	0.19 $\pm$ 0.03
Ileum	2.23 $\pm$ 0.16	2.53 $\pm$ 0.05	1.55 $\pm$ 0.21	1.08 $\pm$ 0.06	0.75 $\pm$ 0.10	0.69 $\pm$ 0.07	0.61 $\pm$ 0.02	0.28 $\pm$ 0.02
Stomach	2.65 $\pm$ 0.25	3.47 $\pm$ 0.17	1.04 $\pm$ 0.03	1.22 $\pm$ 0.06	0.64 $\pm$ 0.08	0.47 $\pm$ 0.03	0.40 $\pm$ 0.03	0.24 $\pm$ 0.02
Muscle	0.50 $\pm$ 0.05	0.43 $\pm$ 0.04	0.49 $\pm$ 0.02	0.47 $\pm$ 0.04	0.38 $\pm$ 0.05	0.27 $\pm$ 0.01	0.19 $\pm$ 0.02	0.17 $\pm$ 0.01
Femur	1.69 $\pm$ 0.06	1.78 $\pm$ 0.16	1.17 $\pm$ 0.04	0.88 $\pm$ 0.07	0.68 $\pm$ 0.08	0.52 $\pm$ 0.03	0.36 $\pm$ 0.02	0.26 $\pm$ 0.02

^a Time in hours after injection of radiolabelled mAb

Table 2. Biodistribution of ^{125}I -139H2 in OVCAR-3-bearing nude mice with or without the addition of unlabelled 139H2 or A2C6 on day 3 after injection

Tissue	Percentage of the injected dose/g tissue (SE <15%)									
	A			B		C				
	¹²⁵ I-139H2 alone	¹²⁵ I-139H2 + 139H2	¹²⁵ I-139H2 + 139H2	¹²⁵ I-139H2 alone	¹²⁵ I-139H2 + 139H2 1000 µg	¹²⁵ I-139H2 alone	¹²⁵ I-139H2 + unlabelled A2C6			
		50 µg	300 µg				50 µg	300 µg	50 µg 2 h before	300 µg 2 h before
Tumour	7.5	5.7	4.2	13.1	6.9	8.1	10.1	7.4	8.2	8.2
Blood	7.6	5.9	6.6	12.7	9.5	6.5	8.1	8.8	7.3	8.6
Liver	1.4	1.1	1.4	2.5	2.0	1.7	1.5	1.4	1.4	1.3
Spleen	1.6	1.2	1.4	2.8	1.8	1.5	1.4	1.4	1.2	1.6
Lung	2.3	1.7	2.1	4.1	3.0	2.6	2.3	2.3	2.3	2.7
Heart	1.4	1.3	1.3	2.6	2.1	1.8	1.3	1.5	1.7	1.5
Colon	0.8	0.6	0.6	1.1	0.9	0.8	0.5	0.7	0.5	0.8
Ileum	1.4	0.7	0.8	1.2	0.9	1.2	0.7	0.8	0.7	0.8
Stomach	0.9	0.7	0.9	1.3	1.0	1.0	1.0	0.8	0.8	0.9
Muscle	0.4	0.4	0.4	0.8	1.0	0.5	0.6	0.5	0.5	0.6
Femur	0.8	0.7	0.8	1.6	3.1	1.0	0.8	0.7	0.7	0.8

normal tissues was relatively unchanged. In contrast, the addition of 50 μg or 300 μg unlabelled non-specific mAb A2C6 did not result in a reduced uptake of ^{125}I -139H2 in OVCAR-3 xenografts (Table 2C). In a separate experiment, two groups of mice received unlabelled non-specific mAb A2C6 (respectively 50 μg and 300 μg) 2 h prior to the administration of the radiolabelled mAb 139H2. Biodistribution did not reveal important differences in tissue

uptake as compared to mice injected with ^{125}I -mAb 139H2 alone (Table 2C).

Tumor size and uptake of mAb 139H2

A possible relationship between tumour size and mAb uptake in tumour tissue was investigated 3 days after ad-

Fig. 4. Relationship between OVCAR-3 tumour size and tumour uptake expressed as the percentage of the injected dose/g tumour of ^{131}I -139H2 ($\blacktriangle$) and ^{125}I -A2C6 ($\circ$)

ministration of $2\text{ }\mu\text{g}$ ^{131}I -mAb 139H2 in OVCAR-3-bearing nude mice. At that time tumours varied in weight from 0.043 g to 1.1 g. As shown in Fig. 4, the percentage injected dose per gram tumour tissue was inversely related to the tumour size. Using linear regression, a negative regression slope was found ($r = -6.26$). This value deviated significantly from zero ($P = 0.0056$). The tumour uptake of the radiolabelled control antibody A2C6 was not dependent on tumour size ($r = -1.11$, $P = 0.28$).

Immunoscintigraphy of OVCAR-3-bearing mice

Tumour-bearing mice were injected with $100\text{ }\mu\text{Ci}$ ^{131}I -mAb 139H2 and whole-body scintigrams were obtained at 1, 2, 3, and 7 days after injection. Figure 5 illustrates the decrease of whole-body radioactivity over time and the preferential uptake of mAb 139H2 at the s.c. tumour sites, which is also shown in Fig. 6A for the radioactivity of the various regions of interest drawn on the immunoscintigrams. At day 8 the mice were sacrificed and biodistribution was performed (Fig. 6B). In xenografts, uptake was higher as compared to blood and normal organs. Data of biodistribution agreed well with the distribution of radioactivity visualized on the immunoscintigram at day 7.

Discussion

The new murine anti-episialin mAb 139H2 was demonstrated earlier to be strongly reactive with a series of human ovarian cancer xenografts. In OVCAR-3-bearing nude mice, we now showed that with mAb 139H2 specific tumour localization can be obtained with a long retention of the mAb at the maximum level. Although the target antigen is released into the circulation, no complex formation was detected. Co-administration of an excess of an irrelevant mAb did not influence the uptake of mAb 139H2 in the OVCAR-3 xenografts, but doses of $300\text{ }\mu\text{g}$ or more unlabelled mAb 139H2 reduced the tumour uptake of a tracer dose of radiolabelled mAb 139H2. The uptake of

Fig. 5. Immunoscintigrams of a nude mouse bearing s.c. OVCAR-3 xenografts, injected with $100\text{ }\mu\text{Ci}$ ^{131}I -139H2, at 1 (a), 2 (b), 3 (c), and 7 (d) days after injection. p, Proximal; d, distal; h, heart region; x, xenograft

Fig. 6 a, b. Comparison of immunoscintigraphic data and the results of biodistribution in OVCAR-3-bearing nude mouse injected with 100 μ Ci 131 I-139H2. The percentage of the total image radioactivity of the whole body at day 0 (percentage of injected dose) for the whole body, heart, tumour right, and tumour left regions at 1, 2, 3, and 7 days after injection are depicted in **a**. The percentage of the injected dose/g for the various tissues obtained at day 8 after injection is depicted in **b**.

mAb 139H2 in OVCAR-3 xenografts was inversely related to the tumour size.

Antigenic differences between normal and malignant cells have been the focus of attention to develop mAbs with specificity for cancer. Various mAbs have been generated against mucin-like antigens on carcinoma cells [22]. These antigens are complex glycoproteins with apparent molecular masses greater than 250 kDa and are known to be released into the circulation. Episialin is a major sialylated mucin present at the apical cell surface of most types of glandular epithelia. An increase in episialin expression is often found in carcinoma cells, where the glycoprotein is detected both intracellularly and on the entire cell surface, including the membranes lining the adjacent cells. mAbs directed against episialin are in clinical use for ovarian cancer, either for immunoscintigraphy or for serological monitoring of disease.

Radiolabelled mAbs HMFG-1 and HMFG-2 have been successfully applied for tumour detection in ovarian cancer [12]. Another antibody extensively studied in ovarian cancer is mAb OC125, reactive with the CA125 antigen.

CA125 is not a mucin, but also appears as a high-molecular-mass, highly glycosylated glycoprotein complex at the cell surface [6]. mAb OC125 has been primarily used for the serological follow-up of ovarian cancer patients [1]. The various anti-episialin mAbs in clinical use do not react consistently with all histological subtypes of ovarian cancer. mAb 139H2 is a newer murine anti-episialin antibody that appears to have a higher relative reactivity on epithelial tumours than 19 other mAbs directed against episialin or related antigens [22]. In addition, mAb 139H2 was also found to react better with each of our human ovarian cancer xenografts tested than either HMFG-1, HMFG-2, or OC125 [14].

Circulating antigen may negatively influence tumour localization with radiolabelled antibodies [3]. Contrary to this hypothesis, immunoscintigraphy with mAbs HMFG-1, HMFG-2, and OC125 resulted in visualization of tumour lesions, and high tumour-to-non-tumour ratios were achieved [5, 12]. The concentration of circulating episialin is dependent on the total tumour burden. In OVCAR-3-bearing nude mice, serum episialin levels were low and no immune complexes could be demonstrated.

The inverse relationship we found between tumour size and uptake of specific antibody in tumour tissue corresponds to previous observations in other human tumour xenografts [8, 20]. Like Hagan et al. [8], we did not find such a relationship for the uptake of the irrelevant mAb. The effect of tumour burden on antibody uptake in tumours has also been demonstrated in cancer patients. Scheinberg et al. [18] demonstrated a sixfold higher tumour uptake of 131 I-labelled mAb in non-Hodgkin's lymphoma patients with minimal disease as compared to those with multiple tumour sites.

Various investigators have studied the possible reduction of the uptake of radiolabelled mAbs in normal tissues by excess unlabelled antibodies, thereby increasing the amount of radiolabelled specific mAb available for the tumour lesions. Indeed, with the use of indium-111-labelled mAbs a relatively higher tumour tissue uptake was obtained in the presence of increasing doses of unlabelled specific antibody [4, 17]. Clinical studies with indium-111-labelled mAbs confirmed a lower uptake in liver and bone upon co-administration of unlabelled mAb [4, 15]. In animal tumour models [7, 21] using iodine-labelled antibodies, such a relationship was not always found. In our model we even observed a decreased tumour uptake with doses of 300 μ g or more unlabelled mAb 139H2, while no differences were observed for the uptake in normal tissues. Unlabelled control antibody did not influence the biodistribution of radiolabelled mAb 139H2, which also illustrates the specificity of tumour localization for mAb 139H2. Hence, the addition of an excess of unlabelled specific mAb to the iodine-labelled antibody may be of disadvantage for immunoscintigraphy.

In conclusion, the strong reactivity with all subtypes of ovarian cancer and the good binding characteristics of the new anti-episialin mAb 139H2 in vitro as well as in vivo may indicate this antibody as promising for radioimmunoscintigraphy in ovarian cancer patients. As an extension of our study we are at present investigating the potential usefulness of mAb 139H2 for radioimmunotherapy.

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