COMPARISON OF $^{131}$I-LABELLED ANTI-EPISIALIN 139H2 WITH CISPLATIN, CYCLOPHOSPHAMIDE OR EXTERNAL-BEAM RADIATION FOR ANTI-TUMOR EFFICACY IN HUMAN OVARIAN CANCER XENOGRAFTS

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Three human ovarian cancer xenografts of different origin and grown s.c. in nude mice as well-established tumors were studied for their sensitivity to cisplatin (CDDP), cyclophosphamide (CTX), $^{131}$I-labelled anti-episialin monoclonal antibody (MAB) 139H2, or external-beam radiotherapy. The maximum tolerated dose of CDDP given weekly i.v. × 2 induced a tumor growth inhibition (GI) of 77.5% and 85.1% of the serous xenografts Ov.Ri(C) and OVCAR-3, respectively. The mucinous xenografts Ov.Pe was relatively resistant to CDDP. The maximum tolerated dose of CTX, given i.p. × 2 with a 2-week interval, induced a GI between 52.9% and 59.7% for each of the 3 xenografts. Radioimmunotherapy with 500–750 μCi $^{131}$I-specific MAB 139H2, administered i.v. × 2 with a 2-week interval, was more effective than CDDP or CTX. The 500 μCi $^{131}$I-MAB 139H2 schedule induced 100% GI in Ov.Ri(C) xenografts and all tumors were cured. The same schedule was slightly less effective in OVCAR-3 xenografts, but complete tumor regressions could still be obtained. Ov.Pe xenografts were least sensitive to radioimmunotherapy. The 2 injections of 500 μCi $^{131}$I-control MAB gave only transient growth inhibition of OVCAR-3 and Ov.Pe tumors, but gave complete regressions of Ov.Ri(C) xenografts. Biodistribution using tracer doses of $^{131}$I-MAB 139H2 and $^{125}$I-control MAB showed different degrees of specificity for MAB 139H2 in the 3 xenografts. Radiation doses absorbed in Ov.Ri(C), OVCAR-3 and Ov.Pe xenografts per 10 μCi injected dose were 30, 41 and 29 cGy respectively. Treatment with 10 Gy external-beam radiation suggested that the effects of radioimmunotherapy in each tumor line were related to the intrinsic radiosensitivity of the xenografts.

Ovarian cancer is the leading cause of death among women with a gynecological malignancy. Unfortunately, most patients present in FIGO stage III or IV. Nowadays, best treatment results in these patients can be obtained with cytoreductive surgery followed by combination chemotherapy. Chemotherapy schedules include at least a platinum compound and an alkylating agent, such as the combination of cisplatin [cis-diamminochloroplatinum(II), CDDP] and cyclophosphamide (CTX). However, the 5-year survival rate of only 20% (Fraumeni et al., 1989) clearly shows a need for new treatment modalities.

Radio-labelled monoclonal antibody (MAB) conjugates may offer a new modality to improve treatment results in ovarian cancer patients. The specific tumor-localizing properties of MABs have been shown in a variety of tumor types, including ovarian cancer. Experimental therapy with high doses of radio-labelled MABs has demonstrated effective inhibition of tumor growth in a few human tumor xenograft models (Wessels, 1990). Several clinical trials with radioimmunotherapy of ovarian cancer and melanoma have been carried out (Stewart et al., 1989; Divgi and Larson, 1989). Encouraging therapeutic responses have already been obtained with high doses of $^{131}$I-labelled MABs in lymphoma patients (DeNardo et al., 1988; Press et al., 1989; Goldenberg et al., 1991).

From a number of MABs reactive with ovarian carcinomas, we selected MAB 139H2 because of its favorable reaction pattern in a panel of human ovarian cancer xenografts (Molthoff et al., 1991a). MAB 139H2 is a new monoclonal antibody, which binds to a protein epitope of the sialylated mucin episialin, also designated as MAM-6 or CA 15-3. Episialin is primarily present at the apical cell membrane of most types of glandular epithelia (Hilken et al., 1989). In carcinoma cells, episialin expression is often elevated and the antigen can be detected on the entire cell membrane as well as intracellularly. The antibody was shown to react with the majority of ovarian carcinomas. We have demonstrated specific uptake and retention in tumor tissue of MAB 139H2 using OVCAR-3 xenografts grown in nude mice (Molthoff et al., 1991b).

In the present study, we examined 3 human ovarian cancer xenografts of different histological sub-type and growth rate for their response to maximum tolerated doses of CDDP and CTX and high doses of $^{131}$I-MAB 139H2. We analyzed the difference in growth inhibition by determination of the pharmacokinetics and biodistribution of the radio labelled conjugate in each of the 3 tumor lines. In addition, we determined the intrinsic radiosensitivity of the xenografts by external-beam radiation.

MATERIAL AND METHODS

Xenografts

The human ovarian cancer xenografts Ov.Ri(C), OVCAR-3 and Ov.Pe have been described previously (Molthoff et al., 1991a). Some important characteristics are summarized in Table I. The xenografts were transferred by implanting fragments of solid tumor tissue with a diameter of 2 to 3 mm s.c. through a small skin incision in both flanks of female, 8- to 10-week-old NMRI/Cpb (nu/nu) mice (Harlan, Zeist, NL). Tumors were measured weekly or twice a week in 3 dimensions and the volume was calculated by the equation length × width × height × 0.5 in mm$^3$.

Monoclonal antibodies

MAB 139H2 is of the IgG1 isotype and binds to a protein determinant of episialin, also designated as MAM-6 or CA 15-3 (Hilken et al., 1988; Hilken et al., 1989). Ascitic fluid containing MAB 139H2 was kindly provided by Dr. J. Hilken (Netherlands Cancer Institute, Amsterdam, NL). Purification of the antibody was performed by affinity chromatography using Affi-Gel Protein-A MAPS II (Bio-Rad, Utrecht, NL). MAB 2C7 is also of the IgG1 isotype and was used as a control antibody. MAB 2C7 reacts with human α-glucocerebroside (Barneveld et al., 1983) and was kindly provided by Dr. J.M. Tager (University of Amsterdam, Amsterdam, NL). With the indirect immunoperoxidase technique, absence of staining with MAB 2C7 in tissue sections (cyrostat and formalin-fixed, paraffin-embedded) of Ov.Ri(C), OVCAR-3 and Ov.Pe was shown.

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Radiolabelling

MABs 139H2 and 2C7 were labelled with either 125I or 131I by the iodoxen method (Haisma et al., 1986). Free iodine was removed by an anion-exchange resin suspension in PBS containing 1% BSA (AG1-X8, Bio-Rad). The percentage of radioactive iodine bound to the MAB was determined by TCA precipitation and was always >95%. The specific activities of the iodinated MABs ranged from 2 to 5 mCi per mg protein. After radiolabelling of MABs 139H2 and 2C7, the immunoreactive fraction was determined on OVCAR-3 cells according to Lindmo et al. (1984). The immunoreactive fraction of MAB 139H2 was consistently between 65 and 75%, while MAB 2C7 was not reactive.

Treatment studies

Treatment and control groups consisted of 5 or 6 tumor-bearing animals each. Experiments were started when tumors had reached a volume between 50 and 150 mm3 (= day 0). During the therapy experiments, mice were weighed twice a week and examined visually for toxic effects. Tumors were measured for a period of 2 to 3 months or until tumors had become so large (2000 mm3) that the animals were killed. In case of tumor regression, mice were followed up for 9 months. Tumor volumes were calculated relative to the initial volume, and the mean values of the relative tumor volumes was used to draw Figure 1. The effect of treatment was expressed as the percentage of growth inhibition (100 – the ratio of the mean relative volume of treated tumors over that of control tumors multiplied by 100). Complete regression was defined as the total disappearance of the tumor without regrowth within the next 2 months. Deaths within 2 weeks after the final treatment were considered toxic deaths, and the animals concerned were excluded from the study.

Cytostatic agents. CDDP (Platinol; Bristol Myers-Squibb, Weesp, NL) was provided as a 0.5 mg/ml solution. CTX (Endoxan; Asta, Bielefeld, Germany) was dissolved in 0.9% NaCl. The maximum tolerated dose schedule for CDDP was 5 mg/kg i.v. on days 0 and 7, and that for CTX was 150 mg/kg i.p. on days 0 and 14. At this schedule, the mean weight loss of the mice was approximately 10% in the first week of treatment.

Monoclonal antibodies. In tumor-bearing animals, thyroid uptakes of iodine were blocked by addition of potassium iodide to the drinking water (0.1%) from 3 days before until the end of the study. Animals were injected i.v. in the eye plexus at days 0 and 14 with either 131I-MAb 139H2, the irrelevant 131I-MAb or NaCl 0.9%.

External-beam radiation. Tumor-bearing nude mice were anesthetized and the tumors were irradiated locally with 10 Gy (250 kV, 15 mA) at a dose rate/min of 3.2 Gy using a 0.4 mm thorium filter.

Pharmacokinetics and biodistribution

Tumor-bearing mice were injected with a tracer dose (between 7 and 15 μCi) of a mixture of 125I-labelled control MAb and 131I-labelled MAB 139H2 and killed at various time points after injection. 3 mice at each time point. Blood was collected from mice under ether anesthesia. Normal tissues and tumors were dissected and rinsed in saline and dried to minimize blood residues. Blood and all tissues were weighed and the radioactivity was measured in a 2-channel gamma counter with automatic correction for spillover of both radionuclides in each channel. To correct for radioactive decay, a standard solution of the injected material in PBS + 1% BSA was prepared and counted simultaneously with the tissues at each time point. The results were expressed as the percentage of injected dose per gram (% ID/g).

Radiation dose measurements and calculations

Whole-body radiation dose was measured using a dose calibrator VDC-101 (Veenstra, Ext, NL). The absorbed dose for the whole body was then calculated using the trapezoid integration method for the area under the curve (AUC) (Badger et al., 1985). These doses were expressed in cGy by multiplying the integrated mCi/hr/mouse by the g.cGy/mCi.h factor for 131I (0.3985) published by the Medical Internal Radiation Dose committee (Dillman, 1969). For the tracer-dose experiments, approximate radiation doses to various tissues were calculated from the uptake data of the conjugate in each tissue, assuming uniform distribution of the radionucide within the organs. The gamma-radiation dose has been neglected because of low absorbed fractions in the small organs of the mouse. The initial concentration of radiolabelled MAB 139H2 in each organ was assumed to be 0 μCi/g.

RESULTS

Treatment with cytostatic agents

The efficacy of CDDP and CTX treatment in nude mice bearing s.c. Ov.Ri(C), OVCAR-3 or Ov.Pc human ovarian

<table>
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<tr>
<th>Xenograft</th>
<th>Histology</th>
<th>TD1 days</th>
<th>Reactivity</th>
<th>Mouse serum CA 15-3 in U/ml</th>
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<tr>
<td>Ov.Ri(C)</td>
<td>Moderately differentiated serous</td>
<td>11</td>
<td>+</td>
<td>16 (8.0-33.0)3</td>
</tr>
<tr>
<td>OVCAR-3</td>
<td>Poorly to moderately differentiated serous</td>
<td>10</td>
<td>+</td>
<td>2 (0.4-3.2)3</td>
</tr>
<tr>
<td>Ov.Pc</td>
<td>Moderately differentiated mucinous</td>
<td>8</td>
<td>ND4</td>
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1 Tumor doubling time. 2 Reactivity of MAb 139H2 with xenograft tissue. 3 Mean (range). 4 ND, not detectable.

<p>| TABLE II – THE EFFECT OF VARIOUS TREATMENT MODALITIES ON THE GROWTH OF Ov.Ri(C), OVCAR-3 AND Ov.Pc XENOGRAGTS |
|---------------------------------------------------|---------------------------------------------------|---------------------------------------------------|</p>
<table>
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<tr>
<th>Agent</th>
<th>Dose</th>
<th>Days of treatment</th>
<th>Maximum % tumor growth inhibition1</th>
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<th>OVCAR-3</th>
<th>Ov.Pc</th>
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<td>CDDP</td>
<td>5 mg/kg</td>
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<td>CTX</td>
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<td>0, 14</td>
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<td>59.7 (0/10)</td>
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<td>0, 14</td>
<td>100.0 (11/11)</td>
<td>97.6 (2/8)</td>
<td>80.7 (0/12)</td>
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<td>700 μCi</td>
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<td>0, 14</td>
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<td>Control MAb</td>
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<td>100.0 (6/6)</td>
<td>82.4 (0/13)</td>
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</table>

1 Maximum tumor growth inhibition reached for CDDP and CTX at day 26 to 30, for 131I-MAb at day 62 to 64 and for external-beam radiation at day 43 to 53; in parenthesis, the number of tumors in complete regression over the total number of treated tumors. 5 nt, not tested.

Ov.Ri(C), OVCAR-3 or Ov.Pc human ovarian
cancer xenografts is visualized in Figure 1. The OVCAR-3 tumor line was most sensitive to CDDP; the drug induced a maximum tumor growth inhibition of 85.1% (Table II). The mucinous tumor line Ov.Pe was found to be relatively resistant, resulting in a growth inhibition of 36.6%. Treatment with CTX induced a growth inhibition of Ov.Ri(C), OVCAR-3 and Ov.Pe xenografts of 52.9%, 59.7%, and 57.5%, respectively. CDDP appeared to be more effective than CTX in the serous xenografts.

Radioimmunotherapy

Treatment with $^{131}$I-labelled MAb 139H2 and the control MAb 2C7 was performed with doses in the range of 500–750 μCi $^{131}$I. These doses did not induce weight loss or visible toxicity, with the exception of 2 OVCAR-3-bearing mice treated with 2 injections of 750 μCi $^{131}$I-MAb 139H2. These mice died 2½ weeks after the second injection and their deaths were considered toxic. The therapeutic results are shown in Figure 1 and the percentages of tumor growth inhibition are

**Figure 1** - Treatment results obtained with cisplatin (upper graphs), cyclophosphamide (middle graphs), radioimmunotherapy and external-beam radiation (lower graphs) in nude mice bearing human ovarian cancer xenografts Ov.Ri(C), OVCAR-3 or Ov.Pe. Cisplatin (▼) 5 mg/kg i.v. on days 0 and 7; cyclophosphamide (●) 150 mg/kg i.p. on days 0 and 14; radioimmunotherapy i.v. on days 0 and 14: $^{131}$I-MAb 139H2 500 (▲), 700 or 750 (OVCAR-3) (●) μCi/mouse, $^{131}$I-MAb 2C7 500 μCi/mouse (□); external beam: 1 × 10 Gy (○) on day 0. In all graphs, the upper curves represent tumor growth in control animals (○). Vertical bars represent SE.
summarized in Table II. In mice bearing the serous tumors Ov.Ri(C) or OVCAR-3, 500–750 μCi $^{131}$I-MAb 139H2 induced long-lasting complete regression. In particular, Ov.Ri(C) xenografts were very sensitive to the treatment: 500 or 700 μCi $^{131}$I-MAb 139H2 induced a growth inhibition of 100%. After 9 months, tumors were still in complete regression, thus, cures had been obtained. The percentage of growth inhibition in OVCAR-3 xenografts was slightly less than that in Ov.Ri(C) tumors, while the mucinous tumor line Ov.Pe was least sensitive to $^{131}$I-MAb 139H2 treatment. Although the control
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MAb gave some growth inhibition of OVCAR-3 and Ov.Pc tumors and had a considerable effect on Ov.Ri(C) tumors. Comparison of overall growth inhibition and the number of complete regressions indicated tumor specificity for MAb 139H2. Also, our results shown that in the 3 tumor lines radioimmunotherapy was superior to maximum tolerated doses of CDDP or CTX.

The difference in efficacy of radioimmunotherapy in the 3 tumor lines could be due to differences in pharmacokinetics, biodistribution, or intrinsic radiosensitivity of the tumors. Our investigations on each of these variables are described below.

Pharmacokinetics and biodistribution of radio-labelled MAb

Figure 2 illustrates the pharmacokinetics of a tracer dose of $^{131}$I-MAb 139H2 and $^{125}$I-control MAb in Ov.Ri(C)-, OVCAR-3- and Ov.Pc-bearing mice in blood, xenografts and liver. The half-life of $^{131}$I-MAb 139H2 in blood was respectively 52 hr, 50 hr and 45 hr. The maximum percentage of the injected dose of $^{131}$I-MAb 139H2/g tumor tissue was 7.1%, 11.1% and 6.5% for Ov.Ri(C)-, OVCAR-3- and Ov.Pc-bearing mice respectively. This concentration of MAb was retained in maximum level in the tumors for 6, 5, 3.5 and 6 days, respectively. In contrast, no retention of the control MAb was observed in the tumors. The uptake of $^{131}$I-MAb 139H2 in the liver was much lower than in tumors and blood. Other tissues showed equal or lower uptake as compared with that in the liver (not shown).

Tumor/non-tumor ratios of $^{131}$I-MAb 139H2 are listed in Table III. In all animals, ratios increased over time and were highest in OVCAR-3-bearing mice. The tumor/blood ratios in Ov.Ri(C)- and Ov.Pc-bearing mice reached a value of approximately 1. The results obtained from the pharmacokinetic and biodistribution experiments demonstrated minor differences between the 3 xenografts and did not account for the variation in response observed with high doses of $^{131}$I-MAb 139H2.

Dosimetry

Figure 3 shows the clearance pattern of whole-body radioactivity of mice injected with high doses of $^{131}$I-MAb 139H2 or $^{131}$I-control MAb over a period of 2 weeks after the first injection. The effective whole-body half-life of $^{131}$I-MAb 139H2 in Ov.Ri(C)-, OVCAR-3- and Ov.Pc-bearing mice was 65, 70 and 72 hr, respectively, while for $^{131}$I-MAb 2C7 it was 51, 80 and 49 hr. Results from radiation dosimetry are given in Table IV. Whole-body radiation doses absorbed for 2 injections of 500 $\mu$Ci $^{131}$I-MAb 139H2 or $^{131}$I-control MAb were not much different, while MAb 139H2 showed better growth inhibition of the tumors. The whole-body pharmacokinetics and dosimetric data did not explain the differential efficacy of radioimmunotherapy in the 3 tumor lines.

Table V summarizes the radiation-absorbed doses for tumors and organs calculated from the tracer dose biodistribution data. The doses were expressed in cGy per 10 $\mu$Ci injected dose for $^{131}$I-labelled MAb. Tumor uptake of $^{131}$I-MAb 139H2 and Ov.Pc xenographs received respectively 30, 41 and 29 cGy after injection of the specific MAb. The control MAb these doses were 24, 12 and 27 cGy. Radiation doses for the blood ranged from 45 to 60 cGy and other organs received less than 14 cGy. Radiation-absorbed doses for MAb 139H2 and 2C7 in Ov.Pc xenographs were similar. This explains that in Ov.Pc xenographs the therapeutic effects of equal doses of $^{131}$I-MAb 139H2 and $^{131}$I-MAb 2C7 were only slightly different, in contrast to the effects obtained in the serious tumors.

External-beam radiation

The intrinsic radiosensitivity of the 3 tumor lines was investigated by external-beam radiation. Local irradiation of Ov.Ri(C), OVCAR-3 or Ov.Pc xenographs with 10 Gy resulted in growth inhibition of 100%, 82.4% and 77.0% respectively (Table II, Fig. 1). Complete regression of all Ov.Ri(C) tumors was achieved. Ov.Pc xenographs were least sensitive, while OVCAR-3 xenographs showed intermediate sensitivity to external-beam radiation. No complete regression could be induced in these 2 tumor lines. From the results of external-beam radiotherapy, ranking of efficacy could be made: Ov.Ri(C) > OVCAR-3 > Ov.Pc.

DISCUSSION

We have reported on the specific uptake and the therapeutic potential of MAb 139H2 in the human ovarian cancer xenografts OVCAR-3 grown in nude mice (Molthoff et al., 1991b, 1992). We have now demonstrated remarkable therapeutic effects of high doses of $^{131}$I-labelled antibody 139H2 in 3 different human ovarian cancer xenografts. Best effects were obtained with 500 to 750 $\mu$Ci $^{131}$I-MAb 139H2 administered twice with a 2-week interval in mice bearing tumors of the serous sub-type. This treatment resulted in complete tumor regression. The efficacy of radioimmunotherapy with $^{131}$I-MAb 139H2 was superior to chemotherapy using maximum tolerated doses of the cytostatic agents CDDP and CTX. Local treatment of the 3 xenografts with 10 Gy external-beam radiation demonstrated a radiosensitivity profile similar to that of radioimmunotherapy.

The retention of the chemosensitivity and the histology of origin in human tumors transplanted to nude mice has been shown in a number of studies (Winograd et al., 1987). Therefore human tumor models are being used to predict the activity of new anti-cancer agents to be applied in patients. Our panel of human ovarian cancer xenografts has indeed predictive capacity, as previously demonstrated by therapeutic results acquired with conventional and new drugs (Boven et al., 1985; Boven, 1988). Growth inhibition obtained upon treatment with CTX was nearly similar in the 3 tumor lines used in the present study. CDDP treatment showed greatest efficacy in the serious xenografts. The mucinous Ov.Pc tumors were minimally respon-

<table>
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<tr>
<th>Blood</th>
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$^{1}$Hours after injection.
FIGURE 3 – Whole-body radioactivity of mice injected with $^{131}$I-MAb 139H2 or $^{131}$I-control MAb in nude mice bearing Ov.Ri(C), OVCAR-3 and Ov.Pe xenografts. Results are expressed as the mean relative dose, which is the dose in $\mu$Ci injected at a given day/the dose in $\mu$Ci injected at the start of treatment. ●, 500 $\mu$Ci $^{131}$I-MAb 139H2; ▲, 700 or 750 (OVCAR-3) $\mu$Ci $^{131}$I-MAb 139H2; ○, 500 $\mu$Ci $^{131}$I-MAb 2C7.
Table V - Whole-Body Radiation-Absorbed Dose

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<th>Dose (µCi)</th>
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<th>Schedule</th>
<th>eGy¹</th>
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<td>1000</td>
<td>Day 0, 14</td>
<td>561</td>
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<tr>
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<tr>
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<td>1500</td>
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</tr>
<tr>
<td>131I-control MAb</td>
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<td>Day 0, 14</td>
<td>432</td>
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¹Whole-body absorbed dose in eGy, calculated over days 0 to 14. — nt, not tested.

Table V - Cumulative Radiation Absorbed Dose of a Tracer Dose of 131I-Mab 139H2

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</tr>
<tr>
<td>OVCAR-3</td>
<td>30</td>
<td>24</td>
<td>41</td>
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<tr>
<td>Ov.Pe</td>
<td>30</td>
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¹Absorbed dose in eGy per 10 µCi injected dose, calculated over days 0 to 7.

sive to CDDP treatment, which corresponded to the resistance to this drug in the patient (Boven et al., 1985). The serous Ov.Ri(C) tumor line was derived from a patient who responded well to combination chemotherapy, including CTX and CDDP. Thus the differences in histology and chemosensitivity between the tumor lines selected were representative of characteristics found in ovarian cancer patients.

The effects of radioimmunotherapy were different for the 3 xenografts. Treatment with 131I-labelled MAb 139H2 was most effective in the serous tumors types. Ov.Ri(C) xenografts especially were very sensitive and complete eradication of all tumors could be achieved. The mucinous tumor line Ov.Pe was least sensitive. Specificity for MAb 139H2 could be demonstrated in OVCAR-3 tumors, where 2 x 500 µCi 131I-MAb 139H2, but not equal doses of 131I-control MAb, induced considerable growth inhibition and complete regression in 2 mice. Toxicity due to 131I-MAb was minor, since only at the highest dose level of 2 x 750 µCi did weight loss occur, with subsequent death in 2 animals. The differences in sensitivity to 131I-MAb 139H2 could not be explained by dissimilar biodistribution and pharmacokinetics of MAb 139H2 in the respective tumor lines or by variations in whole-body kinetics or whole-body radiation doses.

The different efficacy for radioimmunotherapy observed in our study could be attributed to variations in response to irradiation, another characteristic of ovarian cancer. Roftad and Brustad (1985) have shown that the radiosensitivity of human melanoma cells is retained if they are grown as xenografts. Also, Schwachhoefer et al. (1990) demonstrated in 4 human tumor lines of different origin that the pattern of radio-responsiveness agreed well with the observations in the respective patients. Indeed, we could confirm a variation in response to 10 Gy external-beam radiation. Ov.Ri(C) xenografts responded best to this treatment modality, whereas Ov.Pe xenografts were least responsive. The differential effects of radioimmunotherapy clearly reflected the intrinsic radiosensitivity of each of the tumor lines. As a consequence, in the sensitive Ov.Ri(C) xenografts anti-tumor effects were to be expected from high doses of 131I-control MAb.

Radioimmunotherapy has been compared with external-beam radiation in some animal tumor models. Wessels et al. (1989) and Knox et al. (1990) reported better efficacy for radio-labelled MABs than for dose-equivalent external-beam radiation as demonstrated in a human renal-cell-cancer model and a murine B-cell lymphoma, respectively. In contrast, Buchsbaum et al. (1990) observed fewer effects from radio-labelled MABs than from comparable doses of external radiation in the LS174T human colon-cancer model. In earlier experiments with LS174T xenografts, Neacy et al. (1986) have shown the superiority of radioimmunotherapy. The differences in observations may be explained by biological factors, such as the uptake of the radio-labelled MAB and the radiosensitivity of the tumor type involved, or by factors related to the various methods used to determine the radiation-absorbed dose.

For radioimmunotherapy to be effective, the dose rate of the prolonged radiation should be above a certain threshold to prevent sub-lethal damage repair. Therefore the total radiation dose from radioimmunotherapy may be quite different from that of external-beam high-dose-rate radiation to obtain the same biological effect. In our experiments, upon extrapolation of the radiation dose absorbed in tumor tissue from a tracer dose of 131I-MAb 139H2 to a therapeutic dose of 2 x 500 µCi, we calculated the xenografts to have received a total dose of 30 Gy, 41 Gy and 29 Gy for Ov.Ri(C), OVCAR-3 and Ov.Pe, respectively. Tumor growth inhibition by 2 x 500 µCi 131I-MAb 139H2 was more pronounced in OVCAR-3 and Ov.Pe xenografts than that obtained with external-beam radiation at a dose of 10 Gy. Our results suggest that part of the dose delivered by radioimmunotherapy had no impact on the ultimate biological effect. These findings are in agreement with the dosimetric values calculated by Buchsbaum et al. (1990) in the LS174T human colon-cancer model.

The various methods used by a number of investigators to determine the radiation-absorbed dose from radiolabelled MABs in tumor tissue may limit comparisons as well as give poor insight into correct values. As an example, Buchsbaum et al. (1990) used biodistribution data, whereas Doorn et al. (1986) implanted thermoluminescent dosimeters in the LS174T xenografts and reached contrary conclusions with respect to dose-equivalent external-beam radiation. In earlier experiments, we have shown differences in dosimetric values obtained from biodistribution data of a therapeutic dose vs. a tracer dose of 131I-MAb 139H2 (Molthoff et al., 1991c). Future experiments in animal tumor models should focus on the optimal methodology for acquiring accurate dosimetry from radiolabelled MABs.

In our human tumor model, radioimmunotherapy with MAb 139H2 was very effective. Significant tumor growth inhibition in experimental systems has also been reported by other investigators (Wessels, 1990). In patients, however, the percentage of injected dose of MAB per gram tumor tissue can be 1,000-fold lower than that in mice, although the efficiency of antibody localization is variable. In contrast to experimental studies, tumor lesions in patients are often large and contain poorly vascularized, oxygen-depleted areas. Pharmacology and, as a consequence, side-effects of high doses of radiolabelled MABs may differ between patients and animals. Therefore it may be expected that therapeutic effects from radiolabelled MABs are reduced in the clinical situation (Begent and Pedley, 1991). However, in relation to mice, considerably higher doses of radionuclides can be administered to patients, which may increase the radiation-absorbed dose in tumor tissue. In order to facilitate tumor-tissue uptake in ovarian cancer patients, radiolabelled MABs can be administered into the intrapero-
neal cavity. Epenetos et al. (1987) administered up to 140 mCi of $^{131}$I-labelled antibodies i.p. to ovarian-cancer patients and observed minimal toxicity. Complete responses were reported by Stewart et al. (1989) in 3 of 6 ovarian cancer patients treated i.p. with high doses of $^{131}$I-labelled MAb.

Although human tumor xenografts appear to over-predict the effectiveness of radiolabelled MAbs in patients, treatment with $^{131}$I-MAb 139H2 was shown to induce complete tumor regression. This antibody is therefore a good candidate for investigating specific uptake in tumor tissue in ovarian cancer patients.

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REFERENCES


