Curative Radioimmunotherapy of Human Mammary Carcinoma Xenografts with Iodine-131-Labeled Monoclonal Antibodies

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The radioiodinated monoclonal antibody BW 495/36 showed an exceptionally high uptake and long residence time in human ductal mammary carcinoma xenografts in nude mice. There was a mean tumor uptake of 82%/g 24 hr p.i., decreasing with a biologic half-life of ~ 6 days, to 15%/g by Day 16. The tumor-to-blood ratio increased from 2.8 to 21.4 and the percentage of the whole-body retention recovered in the tumor from 47% to 80% during the same time interval. The therapeutic efficiency of two injections of 7.4 MBq ^{131}I-BW 495/36 was evaluated by comparing the tumor size with that in mice injected with either the same amount of the unlabeled MoAb, the same radioactivity of an ^{131}I-labeled nonspecific MoAb, or with saline only. The high tumor accumulation of ^{131}I-BW 495/36 led to a total tumor dose of 77 Gy resulting in a mean reduction in tumor diameter of 50%, corresponding to a reduction in tumor volume of 88% within 42 days p.i. Unlabeled MoAb had no effect on tumor growth compared with controls, whereas ^{131}I nonspecific antibody caused a slight inhibition of tumor growth. Histologic tumor sections showed large areas of necrosis and a pronounced vacuolation of the tumor cell cytoplasm between Days 7 and 30 p.i. By Day 42 all remaining tissue in the tumor was identified as mouse connective tissue.

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Radiolabeled monoclonal antibodies (MoAb) reactive with tumor associated antigens have been successfully used for localization of tumors by scintigraphic imaging. In addition to the diagnostic use there are promising indications that MoAbs labeled with a suitable radioisotope may be used for radioimmunotherapy (*1–5*). The uptake of activity by the tumor, however, is often too low, and the tumor residence time too short to achieve radiation absorbed doses high enough to destroy the entire viable tumor tissue. Thus most clinical trials using radiolabeled monoclonal antibodies for radioimmunotherapy have only resulted in palliative, and not curative effects (*6–13*). A MoAb that demonstrates high tumor binding and a long retention in tumor tissue would therefore be well suited for therapeutic studies.

In our institute more than 20 different radiolabeled MoAbs have been investigated in biokinetic studies in nude mice using various human tumor xenografts. Of these, the iodine-131- (^{131}I) labeled MoAb BW 495/36 showed an unexpected high tumor uptake and a long biologic half-life in human mammary carcinoma xenograft, indicating its potential for use in radioimmunotherapy.

In the following report we describe the results of experiments aimed for evaluating the specific therapeutic effect of iodinated BW 495/36 using human mammary carcinoma xenografts in nude mice. Tumor response was measured following administration of (a) the specific ^{131}I-labeled MoAb and compared with the effect of (b) the same amount of unlabeled MoAb, (c) with the same amount of radioactivity attached to a nonspecific MoAb, and (d) pure saline.

MATERIALS AND METHODS

Tumor Xenografts

Human carcinoma xenografts were established from primary tissue taken from a patient with a solid ductal mammary

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carcinoma, and passaged in 4-wk-old athymic mice by subcutaneous implantation of 5-mm tissue slices. The tumors grew to a weight of ~ 200 mg by 2 mo after implantation. These tumors were used for further serial propagation in nude mice. Mice bearing tumors with a mean weight of 300 mg were chosen for the present study. Every passage was checked to confirm that the implanted tumor tissue was still of human origin and comparable with the primary tissue by immunohistochemical staining using an anti-mouse IgG-antibody and an anti-human IgG-antibody (Amersham Buchler, Braunschweig, FRG) (*14*).

Monoclonal Antibody BW 495/36

The monoclonal antibody BW 495/36 (Behring, Marburg, FRG), a murine IgG3, showed homogenous binding to almost all cells of human pancreatic, lung, colon, and mammary carcinoma using immunoperoxidase staining. SDS-polyacrylamide-gel-electrophoresis following immunoprecipitation has shown that the monoclonal antibody is directed against an epitope localized on a 200.000 D glycoprotein.

The intact MoAb was labeled with ^{131}I to a specific activity of 110 MBq/mg with a labeling yield of 92% using the conventional Iodogen method either commercially (Hoechst, Frankfurt, FRG) or at our institute. The immunoreactivity after labeling was 85% to 90%.

In order to be able to compare the specific effect of ^{131}I-BW 495/36 with that of a nonspecific antibody, a monoclonal IgG3 anti-hepatitis virus antibody was labeled with the same specific activity.

Biodistribution Studies

Athymic mice bearing 300 mg human mammary tumor xenografts were injected intravenously with 1.85 MBq of the intact antibody BW 495/36. Thyroid uptake of free iodine was blocked by adding perchlorate (IRENAT) to the drinking water 2 days before injection and throughout the experiment. The biodistribution data were obtained by killing and dissecting animals in groups of six mice at 1, 2, 4, 6, 9, 13, and 16 days after antibody injection. Tumor, blood, liver, spleen, kidney, lungs, femur, and muscle were removed and weighed. The activity in the tissue was measured in a well-counter and expressed as percent injected dose per gram of tissue. The mean and s.d. was calculated for every time point.

The whole-body clearance of the injected antibodies was determined for every animal by whole-body counting using a specially constructed small animal whole-body counter. The whole-body activity of the animals was measured at different times up to 16 days and compared with the whole-body activity measured immediately after the injection (100% retention). The total activity uptake in the tumors with a mean weight of 300 mg was measured in a well-counter and expressed in percent of the injected dose. In addition, tumor uptake was expressed as the percentage of the whole-body activity recovered in the tumor (activity in the tumor / whole-body activity × 100 [%]).

Radioimmunotherapy Studies

Nude mice bearing human mammary carcinoma xenografts were divided into four groups. The animals received two injections, 1 wk apart, each containing the following.

Group a: 100 μg of the ^{131}I-labeled BW 495/36 antibody, with a total activity of 7.4 MBq;

Group b: 100 μg of unlabeled BW 495/36 antibody;

Group c: 100 μg of ^{131}I-labeled nonspecific anti-hepatitis antibody with a total activity of 7.4 MBq; and

Group d: pure saline.

In all animal groups the tumor size was measured twice a week with a caliper in two perpendicular axes. Tumor growth was spherical and only the changes in tumor diameter with time after the first activity injection are recorded in the tumor growth curves.

Scintigraphic Imaging

Scintigraphic images were obtained from anesthetized mice in posterior views immediately after injection of the first therapeutic dose and at different times thereafter using a gamma camera equipped with a 2-mm aperture pinhole collimator. The collimator-to-animal distance was fixed as 5.5 cm for all acquisitions. The image data were stored in a 64 × 64 pixel matrix using a digital computer. The images shown (Fig. 2) are displayed without background subtraction and computer smoothing.

Image Analysis and Dose Calculation

To calculate the absorbed dose delivered to the tumor, a region of interest was selected for the tumor to integrate the counts present in the tumor at different times after the injection of the two therapeutic doses. After calibrating the camera system the obtained values were used to calculate the time-activity curve. Background activity was determined also over time by selection of a region identical in size, outside the animal. These values were used to correct the values of the tumor region.

The radiation absorbed dose for the tumor region was calculated using the Medical Internal Radiation Dose (MIRD) formalism (*15–17*). For the nonpenetrating component the radiation absorbed dose in the target region depends on the cumulated activity and the physical properties of the radionuclide. The cumulated activity in the tumor was determined from the scintigrams as mentioned above. From the mean energy of the beta radiation of ^{131}I and the tumor mass the radiation absorbed dose in the tumor was calculated assuming that all the beta-decay energy was absorbed. The contribution of the penetrating part of the radiation to the tumor was negligible in the dose calculation.

Histologic Examination

In order to evaluate the effect of the radiation absorbed dose on the tumor tissue, mice were killed at different times after start of treatment. Tumor and normal tissue were removed and 5-μm histologic sections stained with hematoxylin and eosin were examined. In some animals blood was taken from the retrobulbar venous plexus for leukocyte and platelet counts at different times after the activity injection. Bone marrow smears taken from animals 3 mo after beginning the treatment were used to evaluate possible radiotoxicity.

RESULTS

Biodistribution of ^{131}I-BW 495/36

The biodistribution data at different times after injection of 1.85 MBq BW 495/36 are shown in Table 1. The specific localization of the antibody in the tumor tissue resulted in an extremely high uptake of 82% injected dose per gram 24 hr after injection. The activity concentration declined to 51%/g by Day 6 and to

TABLE 1
Biodistribution and Tumor-to-Blood Ratio of ^{131}I-Labeled Monoclonal Antibody BW 495/36 in Nude Mice Bearing Human Mammary Carcinoma Xenografts Depending on Time p.i. (% inj. dose/g; mean ± s.d., n = 6)

Days p.i.	1	2	4	6	9	13	16
Organ							
Tumor	82 ± 16	73 ± 21	56 ± 16	51 ± 18	36 ± 9	24 ± 8	15 ± 5
Blood	29 ± 8.4	22 ± 7.2	10 ± 3.0	7.1 ± 2.8	4.0 ± 1.9	2.1 ± 1.1	0.7 ± 0.2
Liver	7.1 ± 1.8	5.2 ± 1.4	2.1 ± 0.3	1.6 ± 0.4	0.85 ± 0.22	0.63 ± 0.14	0.22 ± 0.11
Spleen	6.4 ± 2.2	4.2 ± 1.7	2.1 ± 0.9	2.0 ± 0.7	0.78 ± 0.15	0.67 ± 0.18	0.23 ± 0.09
Kidney	8.5 ± 2.6	5.5 ± 1.9	2.4 ± 0.6	2.2 ± 0.7	0.74 ± 0.26	0.71 ± 0.24	0.21 ± 0.07
Lungs	15 ± 4	11 ± 3	4.8 ± 1.3	4.3 ± 0.9	1.8 ± 0.6	1.7 ± 0.5	0.43 ± 0.18
Femur	3.7 ± 1.2	3.4 ± 1.2	1.2 ± 0.4	1.1 ± 0.5	0.68 ± 0.14	0.64 ± 0.16	0.15 ± 0.04
Muscle	2.4 ± 0.7	1.5 ± 0.4	0.7 ± 0.22	0.7 ± 0.26	0.34 ± 0.11	0.31 ± 0.17	0.06 ± 0.02
T/B	2.8	3.3	5.6	7.2	9.0	11.4	21.4

15%/g by Day 16 p.i. The biologic tumor half-life was calculated from the activity concentration in the tumor tissue and found to be ~ 6 days. No other tissue showed specific uptake of the antibody so that tumor-to-tissue ratios were high and increased with time after activity injection. The high activity concentration in the blood of 29%/g at 24 hr i.p. declined to 0.7%/g by 16 days p.i. The tumor-to-blood ratio therefore increased from 2.8 at 24 hr to 21.4 by Day 16 p.i. The radioactivity in the whole body decreased in the early phase with a biologic half-life of 30 hr. Between Days 9 and Days 16 p.i. the whole-body excretion was almost entirely determined by the clearance from the tumor tissue.

In Table 2, the values referring to the percentage of the whole-body activity recovered in the tumor are listed together with the values obtained for the whole-body retention and the activity accumulated in the tumor. The whole-body retention decreased from 53% of the injected dose 24 hr p.i. to 24% by Day 6 and 5.6% by Day 16. The activity retained in the tumor declined from 25% to 15% and 4.5% within the same time intervals. The percentage of the whole-body activity recovered in the tumor increased from 47% 24 hr p.i. to 63% on Day 6 and 80% by Day 16 after injection of the labeled MoAb.

TABLE 2
Iodine-131-Labeled Monoclonal Antibody BW 495/36 Retained in Whole Body and Tumor Depending on Time p.i.*

Days p.i.	Whole body	Tumor	Tumor/wb × 100 (%)
1	53	25	47
2	42	22	52
4	31	17	55
6	24	15	63
9	16	11	69
13	9.2	7.1	77
16	5.6	4.5	80

* Expressed in % inj. dose and percentage of the whole-body activity recovered in the tumor (mean, n = 6).

Radioimmunotherapy Studies

Figure 1 shows the average tumor diameter in the four experimental groups at different times after injection expressed as a percentage of the values at the beginning of the study. The specific therapeutic effect of the high tumor uptake of ^{131}I-labeled BW 495/36 resulted in an inhibition of tumor growth immediately after activity administration. There was a mean decrease in tumor diameter of 50% between Days 14 and 42 p.i. (Curve a). Animals injected with the ^{131}I-labeled nonspecific antihepatitis MoAb also showed an inhibition of tumor growth but the effect was much less pronounced resulting in a slowing down of the growth rate rather than a reduction in tumor size. In this group there was a 40% increase in tumor diameter by Day 42 compared with the initial value (Curve c). Tumor growth in animals injected with unlabeled MoAb BW 495/36 (Curve b) was not significantly different than that in the untreated control group (Curve d), both showing an increase in tumor diameter of ~ 80% by Day 42.

Scintigraphic images from nude mice bearing human tumor xenografts injected twice with 7.4 MBq ^{131}I-BW 495/36 obtained at 24 hr and 7, 15, 18, 25, and 31 days after the first activity injection are shown in Figure 2. These digital computer images demonstrate the specific localization of the antibody in the tumor. On Day 1 after injection the radioactivity was primarily detected in the area of the heart and lungs (Fig. 2A). At later time intervals, with decreasing blood-pool activity and increasing tumor-to-blood ratios, the tumor became more clearly delineated (Fig. 2b). On Day 14 after the first antibody administration virtually only the tumor was visible in the scintigrams (Fig. 2C). The retention of radioactivity in the tumor compared to the whole-body retention declined in correlation with the decreasing tumor diameter between Days 16 and 42 (Fig. 2D and E) indicating that with a decrease in tumor diameter the activity is released from the tumor tissue. By Day 32 p.i. the tumor could not be detected on the scintigram even when the tumor diameter was still measurable (Fig. 2F).

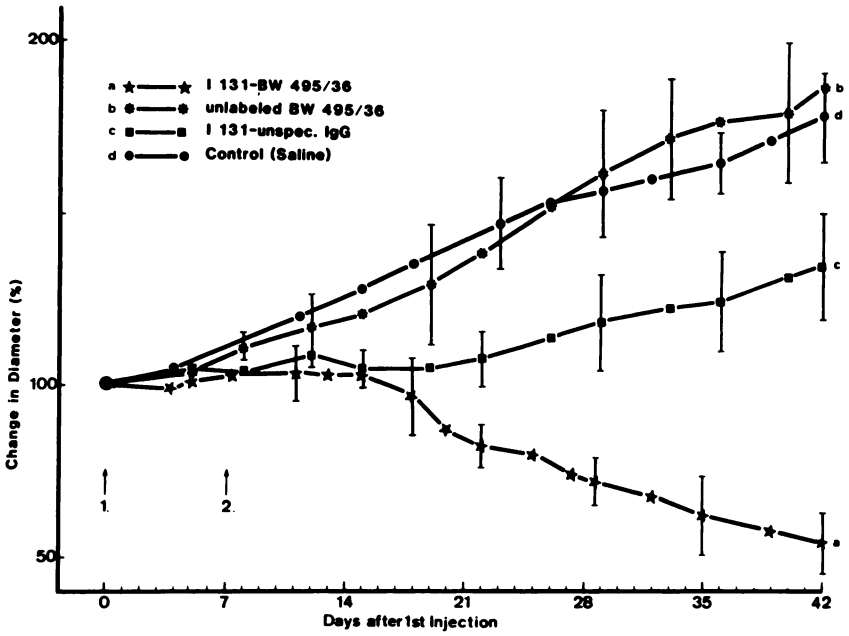

FIGURE 1
Changes in tumor diameter of human mammary carcinoma xenografts (%) depending on time after the first injection of: (a) ^{131}I-BW 495/36 MoAb; (b) unlabeled BW 495/36 MoAb; (c) ^{131}I nonspecific IgG MoAb; (d) saline (control).

Radiation Absorbed Dose

The radiation absorbed dose delivered to the tumor xenografts after two injections of 7.4 MBq ^{131}I-BW 495/36 was calculated to be 77 Gy. Figure 3 shows the activity per mass of tumor tissue versus time after the first activity injection. The increase in activity on Day 7 results from the second injection. The activity retained per gram of tumor showed a faster decline after Day 16 corresponding to the time when the tumor decreased in size.

Histologic Examinations

The tumor response to the delivered dose was evaluated by comparing histologic tumor sections before and after radioimmunotherapy. Figure 4A shows a 5-μm human mammary tumor xenograft from an untreated nude mouse. Even after ten passages the tumor appears as a solid carcinoma of human origin comparable to the primary tumor tissue. Although the uptake of the MoAb was extraordinarily high the vascularization was comparable to that in other tumor xenografts.

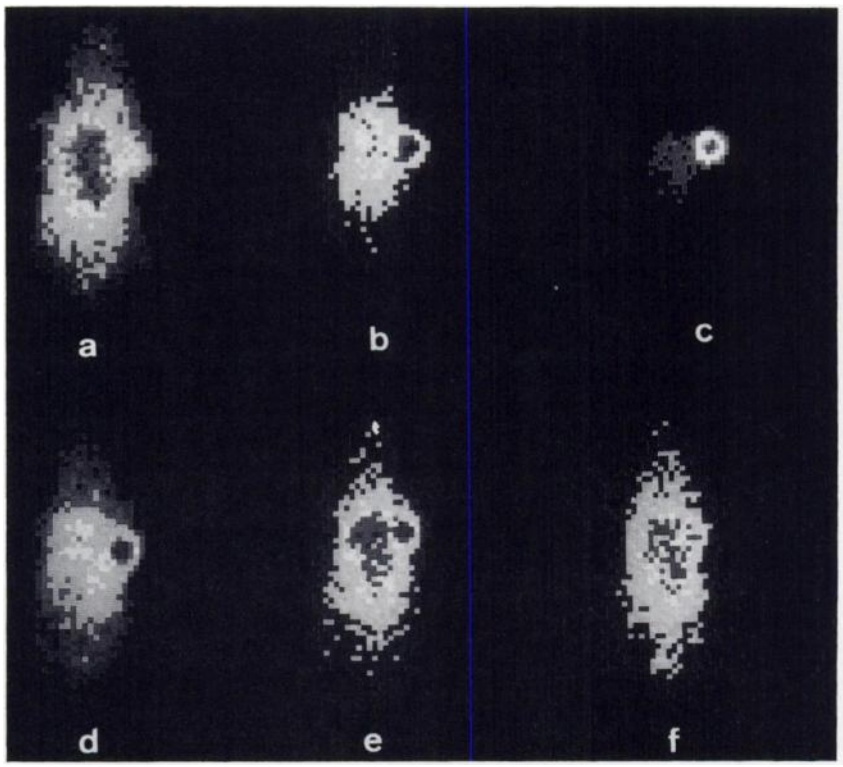

FIGURE 2
Scintigrams of a nude mouse xenografted with a human mammary carcinoma in the right flank. Images were obtained with a pinhole collimator on Day 1(a), 7(b), 15(c), 18(d), 25(e), and 31(f) after the first injection of ^{131}I-BW 495/36 MoAb.

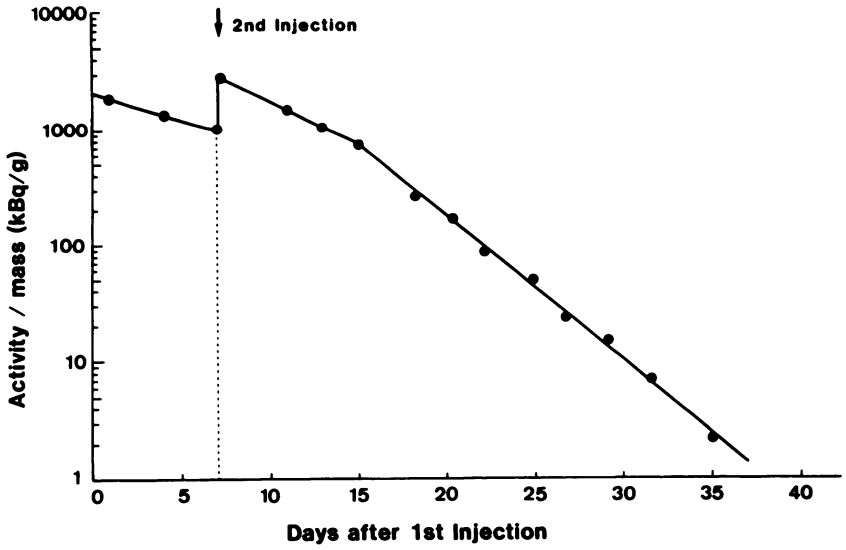

FIGURE 3
Cumulated activity per gram of tumor at different times after the first injection of ^{131}I-BW 495/36 MoAb.

Small amounts of connective tissue invaded the tumor (arrow). The connective tissue was identified as being of murine origin by immunohistochemical staining using anti-mouse antibodies.

Histologic tumor sections from animals injected twice with 7.4 MBq ^{131}I-labeled BW 495/36 antibody obtained on Day 32 after injection show large areas of necrotic tissue and regressively altered tumor cells with pronounced vacuolation of the cytoplasm (Fig. 4B). In addition, there is a large amount of mouse connective tissue. By Day 42 the remaining "tumor" tissue was seen to be composed entirely of mouse connective tissue.

The leukocyte and platelet counts in treated animals showed a decrease of < 30% within 14 days after the first activity administration with a gradual improvement thereafter. By Day 42 p.i. there was no significant difference in cell counts compared with the control animals. Bone marrow smears taken from selected animals 3 mo after radioimmunotherapy did not show any sign of radiotoxicity.

DISCUSSION

Until now only a few clinical studies have been performed using radiolabeled monoclonal antibodies for tumor therapy, and in these only palliative effects were achieved. One curative tumor response was reported by Beierwaltes (*18*) in a patient with a malignant melanoma after treatment with iodinated polyclonal antibodies, but the author himself was not sure if this effect was a result of the radiation absorbed dose delivered to the tumor following specific accumulation of the radioactivity attached to the antibody. Some animal studies have demonstrated impressive effects on tumor growth after administration of labeled MoAbs (*19–21*) but complete tumor remission is rare even in experimental studies.

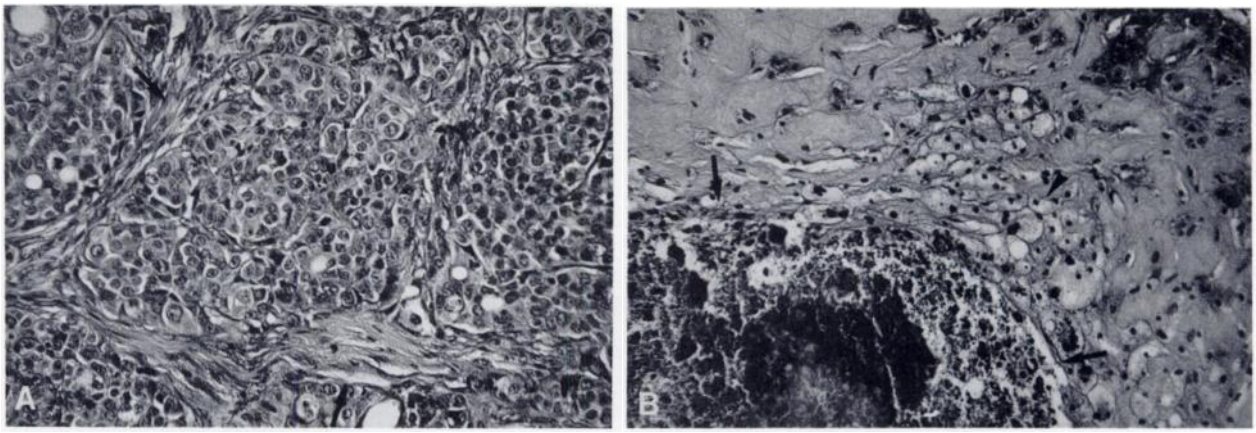

FIGURE 4
A: Histologic section of a solid human ductal mammary carcinoma after ten passages in nude mice showing mouse connective tissue invading the human tumor tissue (arrow). B: Histologic section of the same human mammary carcinoma xenograft as shown in Figure 4A on Day 32 after start of the radioimmunotherapy with ^{131}I-BW 495/36. In addition to mouse connective tissue there are large areas of necrosis (arrow) and some regressively altered tumor cells.

Important factors for successful application of radioactive labeled monoclonal antibodies in radioimmunotherapy are the uptake in the tumor tissue in relation to normal tissue, and the biologic half-life in the tumor tissue. These factors determine whether the radiation absorbed dose delivered to the tumor is sufficient for tumor devitalization. DeNardo (*22*) suggested, that in order to apply MoAbs successfully in radioimmunotherapy the MoAbs used should have a prolonged residence time in the tumor, compared with normal tissue, leading to tumor-to-background ratios of more than 10 within a few days after the administration of the labeled MoAb.

The ^{131}I-labeled monoclonal antibody BW 495/36, showed an exceptional high accumulation in the tumor tissue, although vascularization was not different from that in other tumor xenografts. The tumor uptake was far higher than similar values reported in the literature for radiolabeled monoclonal antibodies in tumor xenografts which ranged between 10%/g and 31%/g of tissue at 48 hr p.i. (*23–26*). The amount of activity in the tumor rose from 7% of the total activity in the animal at Day 1 to only 33 at Day 14 p.i..

The biologic half-life of the activity in the tumor of ~ 6 days showed to be also very high. Usually half-lives reported in tumor tissue are only ~ 4 days (*27*). For optimal dose delivery in radioimmunotherapy the physical half-life of the radionuclide used for labeling the MoAb should match the biologic half-life of the radioactivity in the tumor tissue (*28,29*). Thus ^{131}I is a suitable isotope for labeling the intact MoAb BW 495/36 for therapy studies.

The injection of 14.8 MBq ^{131}I-BW 495/36 resulted in a significant inhibition of tumor growth compared with the untreated control animals. The tumor diameter of the treated animals declined to 50% of the initial value 42 days after beginning the therapy. This decrease corresponds to a decline in volume of 87.5%. The nonspecific monoclonal antibody, labeled in the same way only resulted in an inhibition of tumor growth and not in a reduction in tumor size.

IgG2a and IgG3 have been reported to show a poor antibody dependent cytotoxicity resulting in an inhibition in tumor growth by unlabeled antibodies (*30*). This was not observed in our experiments. Unlabeled BW 495/36, an IgG3, had no effect on tumor growth compared with untreated animals.

The total dose delivered to the tumor by the ^{131}I-labeled BW 495/36 was 77 Gy within 38 days, 30 Gy were delivered within 7 days after the first injection and 47 Gy within the following days. Vaughan (*31*) has postulated that 60 Gy given in 1 wk are necessary for tumor sterilization. The same author postulated that the minimum requirement needed to achieve tumor destruction with a survivable whole-body dose was at least a tenfold increase in activity per g of tumor compared with normal tissue. Only the latter postulation was achieved in our experiments. A uniform distribution of the injected activity in the body of a nude mouse with a weight of 25 g would result in an activity concentration of 4%/g of tissue. An activity concentration of 80%/g at 24 hr p.i., as achieved in our experiments, is a 20-fold increase in specific tumor uptake. As a result of the long tumor residence time of the activity there was still a tenfold increase of tumor uptake on Day 10 p.i.

The long tumor retention of ^{131}I-labeled MoAb in the tumor xenograft indicates that there is a low biologic turnover of the antigen-antibody complex (*32*) and a low rate of in vivo deiodination. The deiodination, however, does depend to some extent on the labeling method (*33,34*).

The histologic sections showed that after therapy the entire remaining "tumor" tissue, 12.5% of the original tumor volume, consisted solely of mouse connective tissue. This volume corresponds approximately to the volume of the stroma in tumors of untreated mice. Thus remaining tumor volume does not necessarily mean that there is still viable tumor tissue.

When using an animal model for biodistribution and therapy studies, it is important to know whether the morphologic characteristics of the xenografted human carcinoma remain unchanged during passages in the animal or whether the tumor tissue is altered in its biologic structure or is even replaced by murine mesenchymal tissue (*35*). This problem is not always considered but we have shown here that the origin of the xenografted tissue, human or mouse, can be easily identified using specific anti-human and anti-murine antibodies.

The present results are very encouraging even when accepting that there are considerable limitations in extrapolating experimental results obtained in animal models to the human situation. The results indicate that antibodies exist that have a high specifity and affinity to antigens on human tumor cells. Such antibodies have a clear potential for use in curative clinical treatment of human tumors. By combining the advantages of the high uptake of the injected dose by the tumor, and labeling with radionuclides tailor-made for the tumor morphology (*36,37*) it should be possible to achieve tumor eradication with tolerable side effects.

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