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Immunoscintigraphy and Pharmacokinetics of Indium-111-labeled ZME-018 Monoclonal Antibody in Patients with Malignant Melanoma

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Immunoscintigraphic and pharmacokinetic characteristics of ^{111}In-labeled ZME-018 monoclonal antibody were examined in 8 patients with malignant melanoma. Each patient received a single intravenous infusion of 20 mg of ZME-018, coupled to 3 mCi of ^{111}In without any acute toxicity. Scintigrams were taken 1, 3, and 6 days after the administration, and blood and urine samples were also taken frequently. Rapid clearance of some radioactivity was seen in early urine samples in the form of ^{111}In DTPA, but after 1 day, urinary excretion of radioactivity was slow and steady, with an average of 2.5% of the injected dose excreted per day. The scans demonstrated that there was blood retention of radioactivity in the heart and great vessels 1 day after infusion and considerable clearance from the blood pool occurred by 3 days. However, ^{111}In was deposited in the liver, spleen and bone for up to 6 days. The optimal time for imaging appeared to be at 3 days. Nineteen out of 26 known lesions or 6 out of 8 patients were positive. There were 21 lesions detected that were not suspected during the work-up the patient. Five patients developed human anti-mouse antibody in the serum by 3 weeks. These results suggest that immunoscintigraphy with ^{111}In-labeled ZME-018 antibody is safe and useful for the detection of metastatic lesions in a selected group of patients with malignant melanoma.

Key words: Monoclonal antibody, ZME-018 — Malignant melanoma — Immunoscintigraphy — Indium-111

The production of monoclonal antibodies (MoAb)[*5] reactive with cancer-associated antigens and the development of effective methods for labeling MoAbs with radionuclides have generated considerable interest in the imaging and therapy of various cancers.[1-8] Many radiolabeled MoAbs that are reactive with malignant melanoma are now available for clinical evaluation. One such MoAb, ZME-018, is a murine IgG_{2a} that is directed against the high-molecular-weight melanoma surface antigen gp 240, a glycoprotein of 240,000 molecular weight.[9] This antigen has limited heterogeneity of expression in metastatic lesions,[10] is not modulated by antibody interaction,[11] and is present in the blood to only a limited degree even when patients have widespread metastasis.[12] ZME-018 antibody can be coupled with ^{111}In by using DTPA as a bifunctional chelating agent. Scintigraphic imaging after intravenous administration of ^{111}In-labeled ZME-018 demonstrated clear uptake in melanoma lesions.[8] In this report, we present our data on (a) the efficacy of immunoscintigraphy using ^{111}In-labeled ZME-018, (b) the pharmacokinetics of ^{111}In-labeled ZME-018; and (c) the measurement of HAMA in patients' serum.

MATERIALS AND METHODS

Preparation of ^{111}In-labeled ZME-180 The ZME-018 antibody was provided by Hybritech, Inc. (La Jolla, CA) via Teijin, Ltd. (Osaka) in a purified, sterile and pyrogen-free preparation. Two different forms of this antibody were supplied separately: one as 19 mg of chemically unmodified protein, at a concentration of 1.0 mg/ml with normal human serum albumin in an aqueous solution (1.1 mg/

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[*5] The abbreviations used are: MoAb, monoclonal antibody; DTPA, diethylenetriaminepentaacetic acid; HPLC, high-performance liquid chromatography; HAMA, human anti-mouse antibody; ELISA, enzyme-linked immunoassay.

ml), pH 8.2, and the other as 1.0 mg of DTPA-coupled ZME-018 at a concentration of 0.5 mg/ml along with 0.55 mg/ml of human serum albumin. ZME-018 coupling with DTPA was performed using a modification of a technique described by Krejcarek and Tucker.[13]

Three mCi of ^{111}In Cl_3 (Nihon Mediphysics, Takarazuka) was mixed with citrate buffer. This was added to the DTPA-coupled ZME-018 in a reaction vial. After 30 min of incubation, neutralizing buffer containing excess DTPA was added to the reaction solution to scavenge any unincorporated ^{111}In. The radiochemical purity of ^{111}In-labeled ZME-018 was assessed prior to administration to each patient using both Sephadex G-50 gel chromatography and paper chromatography (Toyo filter paper No 51B in acetone:water=1:1).

Patients Eight patients with histologically proven malignant melanoma were included in this study. Prior to MoAb infusion, all patients were examined clinically and had a chest X-ray and electrocardiogram as well as assessment of urinalysis, hematological and biochemical profiles, including liver function tests. A skin test was performed prior to MoAb infusion. Informed consent was obtained from all patients and the procedure was appoved by our institution's ethical committee.

Antibody Administration Each patient received a single 1 hr infusion of approximately 100 ml of saline containing 20 mg of ZME-018 MoAb (1 mg of ^{111}In-labeled and 19 mg of unmodified ZME-018). Vital signs were monitored at 15 min intervals during infusion and for 1 hr after administration. Hematological and biochemical assessments and urinalysis were performed after 3 and 7 days to monitor toxicity. Anterior and posterior whole-body imagings were obtained 1, 3 and 6 days after administration of ^{111}In ZME-018 using a gamma-camera equipped with a medium energy collimator (Hitachi Medico Inc., Tokyo). Regions of interest were also scanned using a gamma camera with computer-assisted storage. Background subtraction techniques were not employed. Scintigraphic results were divided into three groups; strongly positive (lesions were detected from 1 day), positive (lesions were unclear at day 1, but obvious at day 3), and negative.

Pharmacokinetic Study Blood samples were collected at the end of infusion and 3, 6, 24, 48, 72, 96, 120 and 144 hr after the administration of ^{111}In-labeled ZME-018. A small aliquot of injected solution was kept to serve as a standard and isotope decay control. Urine specimens were also obtained at multiple time points to determine the radioactivity released into the urine.

HPLC with a TSK G3000 SW size exclusion column (Toyo Soda, Tokyo) was used for the analysis of the injected sample, serum at 24 hr after infusion and 0–6 hr urine.[14] Both radioactivity and absorbance at 280 nm were monitored. Gel and paper chromatographic analyses were also performed on the same samples and 6–14 and 14–38 hr urine. Because of the low level of radioactivity remaining, paper chromatography and HPLC analysis could not be applied for the later serum or urine samples.

Measurement of Human Anti-mouse Antibody Serum was collected at various time intervals and frozen at $-20°$. HAMA was measured using the ELISA technique.[15] ZME-018 was immobilized on 96-well polystyrene plates and kept at 4°. Serum samples (100 μl) at various dilutions was added and incubated for 1 hr. The plates were washed three times and 100 μl of 1:2,000 dilution of goat anti-human IgG coupled with alkaline phoshatase was added to each well for 1 hr at room temperature. Plates were washed three times and 200 μl of *p*-nitrophenyl phosphate solution (0.6 mg/ml) was added for 30 min. The optical density at a wavelength of 405 nm was read. The results were expressed as dilution titers of the sample which showed the same intensity as the negative controls.

RESULTS

Toxicity The skin test was negative for all patients and no side effects were observed in any patient during or following the studies. Allergic symptoms such as fever, chill, rash or anaphylactic reactions were not seen. Likewise, there were no significant changes in hematological findings, biochemical profile or urinalysis.

Pharmacokinetics of ^{111}In-labeled ZME-018 Radiochemical purity of injected materials, defined as the fraction of activity present as labeled antibody was 89.3 ± 1.75 (mean $\pm$ SD) by gel and paper chromatography. Radioactivity not coupled to MoAb was confirmed to be an ^{111}In-DTPA chelate form by chromatographic analysis (Figs. 1 and 2).

Figure 3 shows the blood disappearance curve. ^{111}In clearance from the blood could be described by an open two-compartment mathematical model. However, the injected material is considered to contain about 10% of unbound ^{111}In-DTPA chelate form.[16] ^{111}In-DTPA is known to be excreted rapidly in the urine, influencing the alpha phase. The half-time of blood clearance in the beta phase was calculated as 42.63 ± 7.28 hr (mean $\pm$ SD), and the volume of distribution was 5.57 ± 1.27 liter (mean $\pm$ SD). During the first 6 hr following administration of the ZME-018 anti-

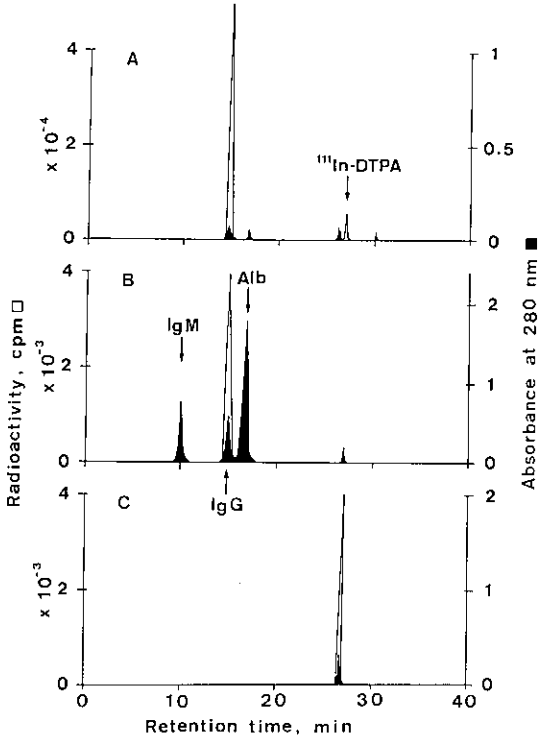

Fig. 1. HPLC patterns of original sample (A), serum obtained at 24 hr (B), and urine collected over 0–6 hr (C). Scan speed was 1 ml/min and scanning was continued for 40 min for each material. The original sample contained murine IgG and human albumin, which were detected by measuring the absorbance at 280 nm. The first peak of radioactivity in the original sample corresponded to the IgG fraction and the second peak was an ^{111}In-DTPA chelate. Serum at 24 hr contained only one peak of radioactivity corresponding to the IgG fraction, since In-DTPA was rapidly released into the urine.

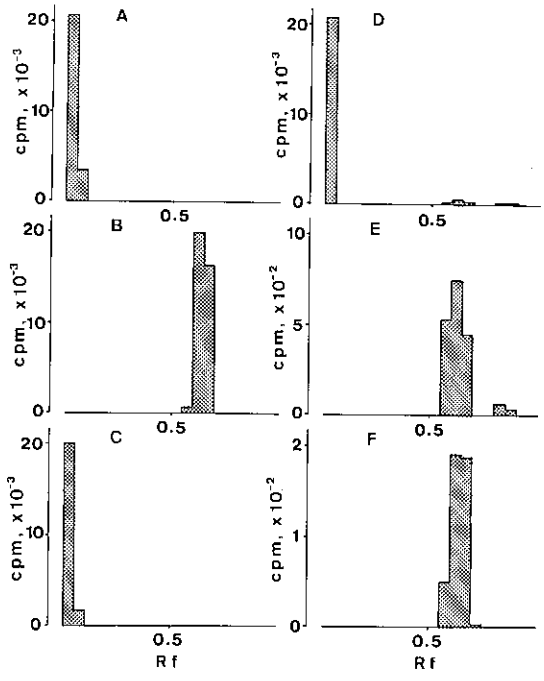

Fig. 2. Paper chromatographic analysis of ^{111}In Cl_3 (A), ^{111}In DTPA (B), ^{111}In-coupled MoAb (C), original sample (D), urine during 0–6 hr (E), and urine during 2–3 days (F). ^{111}In Cl_3, and ^{111}In-coupled MoAb remained at the starting point, whereas ^{111}In DTPA chelate migrated to Rf=0.6.

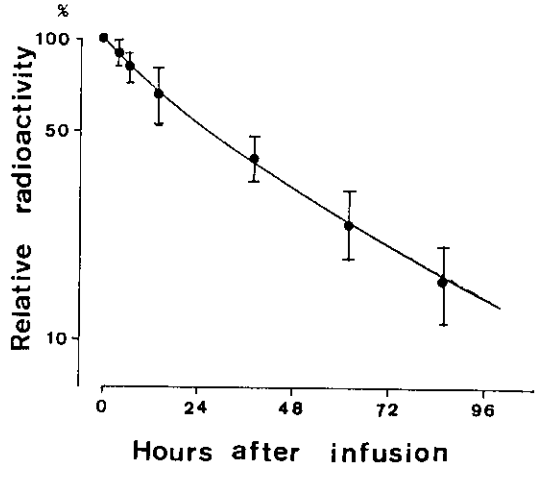

Fig. 3. Blood clearance of radioactivity from the end of infusion to 4 days. Blood radioactivity was measured by serial blood sampling and counting for radioactivity. Data were expressed as mean values and SD for 8 patients.

body, 6.4% of ^{111}In was released into urine (Fig. 4), but after 1 day, urinary excretion of radioactivity was slow and steady, with an average of 2.5% of the injected dose excreted per day. Values were similar for all patients. HPLC and paper chromatography revealed that ^{111}In in the serum was attached to the IgG fraction, and the radioactivity excreted in the urine was associated with a low-molecular-weight material or an ^{111}In-DTPA chelate form (Figs. 1 and 2).

Scintigraphy of ^{111}In-labeled ZME-018

Figure 5 shows representative total-body scans of a 22-year-old male patient with a

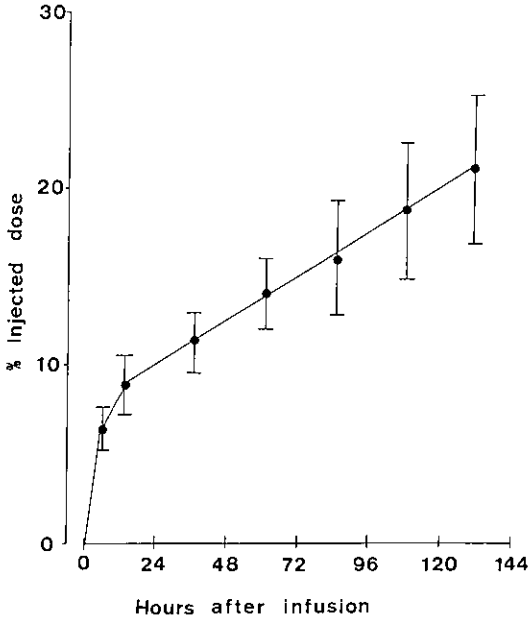

Fig. 4. Cumulative urinary excretion of radioactivity. Values shown are means±SD for 8 patients. During the initial 6 hr, 6.4% of ^{111}In was excreted into urine. However, from 1 day after the administration, about 2.5% of ^{111}In was steadily released into the urine per day.

subcutaneous tumor in the head. At 24 hr after the infusion of ^{111}I-labeled ZME-018, there was considerable distribution of the isotope in the great vessels and heart, with nonspecific uptake occurring in the liver and spleen. After 72 hr, considerable clearance of radioactivity was observed from the blood pool and ^{111}In deposited in the liver and spleen. At 6 days, imaging of bone increased. The scans obtained at 1, 3 and 6 days clearly demonstrated a significant amount of radioactivity in the tumor. However, significant uptake occurs in the liver and spleen.

Figure 6 is the scan of a 76-year-old man with a subcutaneous tumor in his thigh. The 24-hr scan was equivocal but the tumor became obvious at 3 days. A portion of the tumor was counted in a well type gamma-counter 9 days after administration and contained 0.005% of the injected dose of ^{111}In per gram. This was 5 times higher than the concentration in the blood. However, the resected tumor of case 8 retained 0.05% of the injected dose per gram at 7 days after the infusion.

Table I shows a summary of the imaging results. Overall, 19 out of 26 lesions (73%)

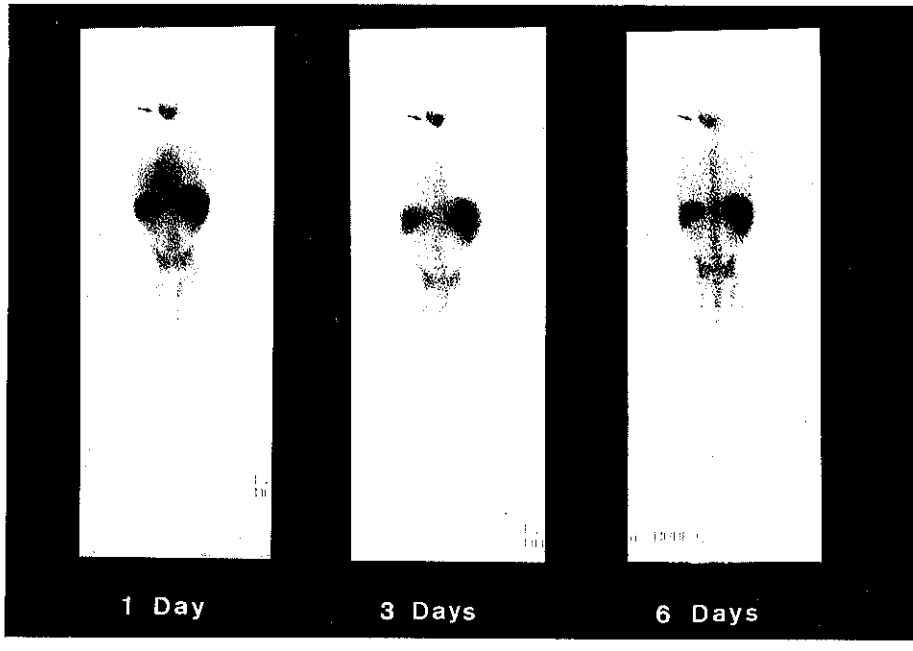

Fig. 5. Posterior view of total-body scan of a 22-year-old male (case 1) 1, 3 and 6 days after administration of ^{111}In ZME-018. Tumor of the head (arrow) was confirmed to be malignant melanoma by surgery.

could be imaged. In a 65-year-old man with widespread metastases (case 3), many previously unknown lesions (21 sites) were revealed by the immunoscintigraphy (Fig. 7). The previously unknown lesions were subcutaneous lesions not found by palpation and intraperitoneal lymph nodes undetectable by computed tomography. The smallest tumor detected was a subcutaneous nodule 1.0 cm in diameter in case 3 seen 3 days after infusion. Lesions less than 1.0 cm in diameter were not imaged. In cases 2, 5 and 6, lesions became visible at 3 days which could not be seen even retrospectively at 1 day, and were defined as positive (+). In cases 1, 3 and 8, lesions were clearly seen 1 day after administration and these cases were defined as strongly positive (++). In six patients, tumor samples were examined immunohistochemically for expres-

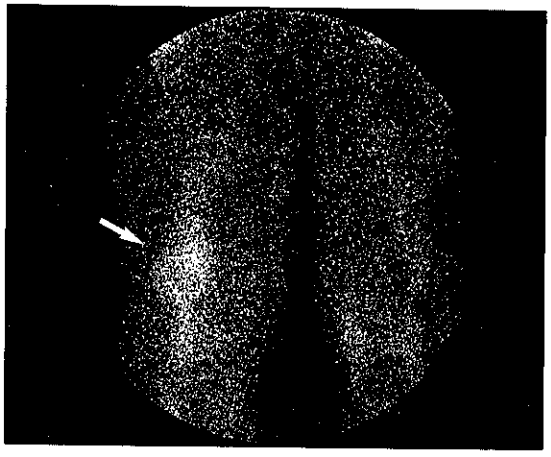

Fig. 6. Scan of the thigh of a 76-year-old male 3 days (case 2) after administration of ^{111}In-labeled ZME-018. Tumor contained 0.005% of injected ^{111}In/g tissue.

Table I. Results of Immunoscintigraphy Using ^{111}In-labeled ZME-018 and Immunohistochemistry

Patient No.	Age Sex / Location	Scintigraphy[a]	Immunohistochemistry[b]
1.	22 male Subcutis	++ (1/1)	+
2.	76 male Lymph node	+ (2/2)	+
3.	65 male Multiple metastases	++ (13/18)[c]	+
4.	58 female Skin	− (0/1)	−
5.	71 female Eye lid	+ (1/1)	ND[d]
6.	66 male Bone	+ (1/1)	ND
7.	72 female Skin	− (0/1)	−
8.	65 male Skin	++ (1/1)	+
	Total	(19/26)	

a) Numbers in parentheses represent number of tumors imaged/previously diagnosed number of tumors.
b) Tissues were snap-frozen, and cryostat sections were made. Immunohistochemistry was performed with indirect immunofluorescence[17] using ZME-018 at a concentration of 0.07–20 ng/ml.
c) Twenty-one previously unknown lesions were detected by immunoscintigraphy. They were subcutaneous and intraperitoneal lesions.
d) Not done.

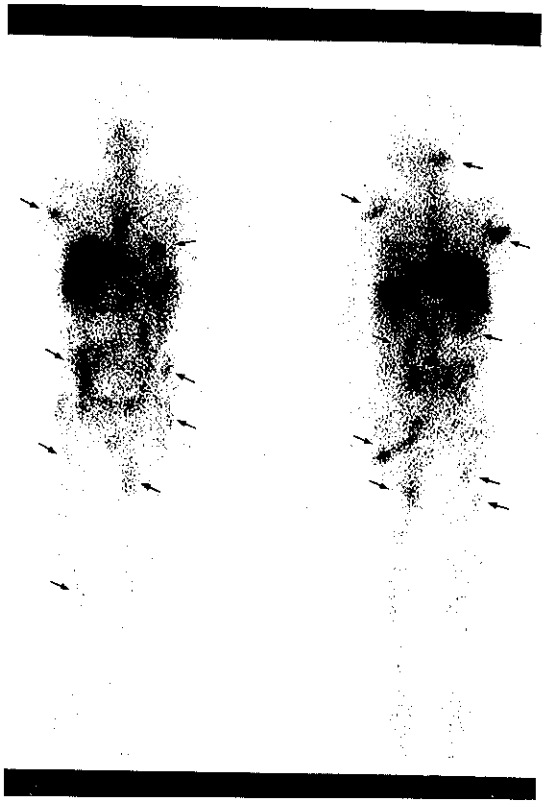

Fig. 7. Anterior and posterior views of total-body scans of a 65-year-old male (case 3) 6 days after administration of ^{111}In-labeled ZME-018. Multiple uptake was observed (arrows).

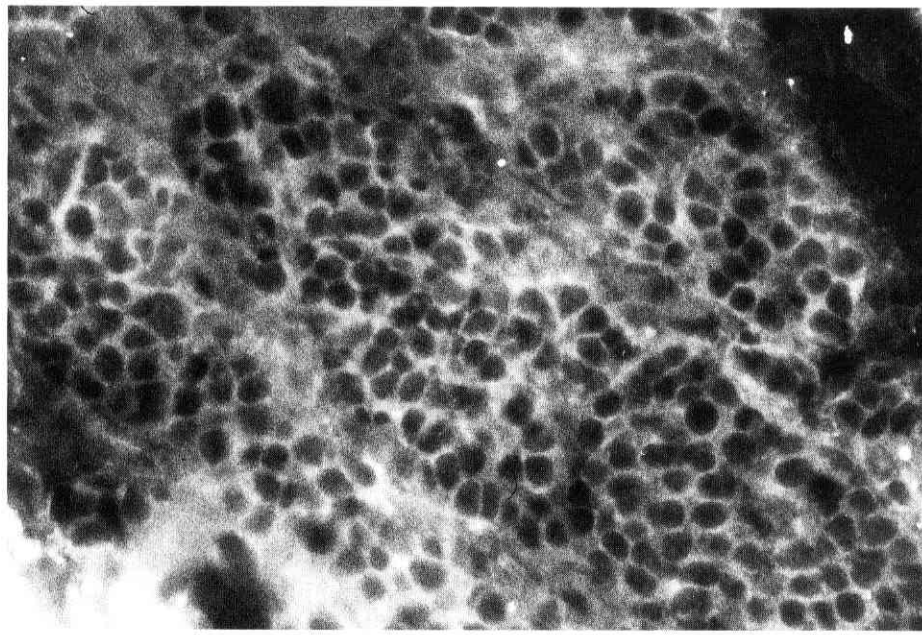

Fig. 8. A snap-frozen section of malignant melanoma tissue of case 3 was immunostained with ZME-018. Positive staining of the tumor cell surface was noticed. ×200.

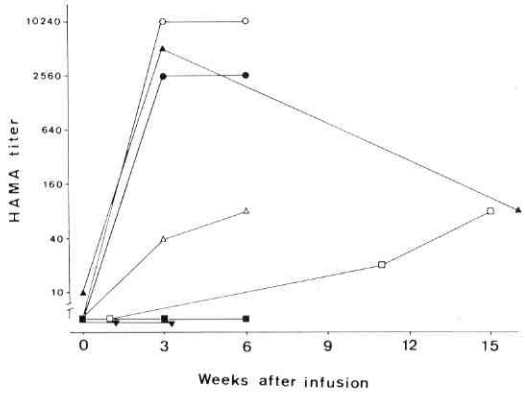

Fig. 9. Serum HAMA titer. Five out of 7 patients had positive values 3 weeks after administration of ^{111}In-labeled ZME-018. A low titer of HAMA was detectable prior to infusion in case 4, the patient who had previously received ^{111}In-labeled MoAb.

sion of the antigen recognized by ZME-018 by the previously described method.[17] Figure 8 represents the immunohistochemical result of case 3, showing prominent staining of the tumor cell surface. The scintigraphic results showed a good correlation with antigenic expression on tumor tissues determined by the immunohistochemical analysis.

Human Anti-mouse Antibody Response

Seven patients had serum samples drawn before and after ^{111}In-labeled ZME-018 infusion to determine whether HAMA was produced. A positive response was seen in 5 out of 7 patients at 3 weeks after infusion (Fig. 9) and remained up to 15 weeks. A low titer of HAMA was detected in the serum of case 4 prior to ZME-018 infusion. This patient had had an ^{111}In labeled 96.5 anti-melanoma antibody infusion 1 year earlier.[6, 7] However, the skin test was negative and no toxicity was observed during or following the studies.

Discussion

This study demonstrated the safety and efficacy of immunoscintigraphy using ^{111}In-labeled ZME-018 in patients with malignant melanoma. Seventy-three percent of previously known lesions and 21 previously unknown tumors or 6 out of 8 patients were positive in the immunoscintigraphy. The optimal time for imaging appeared to be at 72 hr,

since some tumors were not detectable at 24 hr but became obvious at 72 hr. ^{111}In was selected for the labeling of MoAb in this study, although ^{131}I has also been used as a radionuclide for immunoscintigraphy. ^{111}In has many advantages over ^{131}I for imaging; (a) a suitable half life (2.8 days), (b) it is a pure gamma emitter with a suitable gamma energy and abundance of gamma emission, and (c) easy labeling of MoAb just before use in a kit form employing DTPA as a chelating agent.[13] Furthermore, radioiodinated MoAb is susceptible to dehalogenation, and release of radioiodine from MoAb is observed *in vivo*, resulting in (d) a higher tumor uptake of ^{111}In- labeled MoAb than radioiodinated MoAb.[18] However, a high nonspecific uptake of ^{111}In also occurs in the liver, spleen, gastrointestinal tract and bone marrow, as seen in the present studies. The mechanism of high liver uptake is not fully understood, and this activity can obscure abdominal lesions.

^{67}Ga scintigraphy is a method used routinely these days for detecting malignant melanoma. Seven out of 8 patients were also investigated by ^{67}Ga scintigraphy. The sensitivity of the ^{67}Ga scan was similar to that of the ^{111}In ZME-018 scan (data not shown). However, ^{67}Ga accumulates not only in various malignant tumors including malignant melanoma but also in benign lesions such as abscess, sarcoidosis and tuberculosis.[19] The major drawback of the ^{67}Ga scan is its nonspecificity. The major benefit of MoAb imaging is its pre-determined specificity. Therefore, observation of ^{111}In MoAb uptake provides a different kind of information from that of ^{67}Ga accumulation.

Recently several investigators have reported that more tumors were detectable with increasing MoAb dose,[7, 8] but the mechanism by which such a phenomenon occurs is not clear. In the present study, each patient received 1 mg (3 mCi) of ^{111}In-labeled ZME-018 plus 19 mg of unlabeled, unconjugated ZME-018 (20 mg total antibody). Prolonged isotope retention *in vivo* in plasma has been obtained with escalating MoAb dose, and tumor accumulation of radiolabeled MoAb may be related to the integral of blood radioactivity over time, that is, the area under the curve.[8] It should be noted that ^{111}In-labeled ZME-018 did not detect all melanoma lesions. The correlation between immunoscintigraphy and immunohistochemistry indicates that one of the reasons why we could not detect some tumors was because they lacked a high-molecular-weight antigen recognized by ZME-018. Although numerous factors, such as blood supply, low penetration of MoAb into interstitial tissues and so on, affect the tumor uptake, the presence of an antigen on the tumor appears to be crucial for tumor targeting of antibody-conjugates.

In all patients, rapid elimination of radioactivity from the circulation was seen within the first 6 hr. Chromatographic analysis of urine samples demonstrated in the early samples the presence of labeled DTPA, which was generated by the addition of excess DTPA in the injected samples to scavenge any unincorporated ionic ^{111}In. However, ^{111}In in the serum was attached to IgG and after one day, urinary excretion was slow and steady, with an average of 2.5% of the injected dose excreted per day.[14]

We have employed intact whole IgG, but some workers have used $F(ab')_2$ fragments.[2, 4] Nonspecific uptake caused by the Fc receptor should be avoided, and rapid blood clearance of fragments results in a higher tumor-to-blood ratio.[18] However, in animal studies performed by our group, high accumulation of ^{111}In in the liver, spleen and kidney was still observed even by employing antibody fragments instead of intact IgG.[18]

Although data regarding HAMA were limited, 5 out of 7 patients developed HAMA reaction within 3 weeks after the administration of ZME-018. It is important to know that when patients with a high titer of HAMA receive murine MoAb infusion, murine MoAb and HAMA form complexes which result in a rapid blood clearance of radioactivity, high liver uptake, and low tumor uptake of murine MoAb.[20] This problem may be overcome by the application of human type MoAb or genetically engineered murine/human chimeric MoAb.[21, 22] Although we did not differentiate the subpopulations of HAMA, a part of HAMA may be the antibody against the idiotypic portion of ZME-018, which was reported to have significant implications for immunotherapy using anti-tumor MoAb.[23, 24]

We obtained a promising result in immunoscintigraphy using ^{111}In-labeled ZME-

018 in selected cases of malignant melanoma, and further clinical evaluation is warranted. More specific MoAbs reactive with all patients with malignant melanoma would certainly improve the sensitivity and specificity of the diagnosis of melanoma lesions. Furthermore the procedure using radiolabeled antitumor MoAbs is applicable to the diagnosis as well as to the therapy of other malignancies.[25, 26]

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