PRELIMINARY NOTE

Localization of I-131-Labeled Tumor-Specific Monoclonal Antibody in the Tumor-Bearing BALB/c Mouse

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One of the most frequent indications for patient imaging is the need to establish the presence or absence of malignant disease. Considerable effort has been invested in attempting to develop tumor-specific radiopharmaceuticals. We report the localization of an iodine-131-labeled, hybridoma-derived monoclonal antibody in the MH-15 teratocarcinoma-bearing BALB/c mouse. Tumor-to-muscle and tumor-to-blood ratios of 150:1 and 15:1, respectively, were observed at 5 days after administration of 15 μCi of labeled antibody. The relationship between the optimal imaging time and localization kinetics is discussed.

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One of the most frequent indications for patient imaging in nuclear medicine is the need to establish the presence or absence of malignant disease. As a consequence, considerable effort has been invested in attempting to develop tumor-specific radiopharmaceuticals. The bases for localization of some radiopharmaceuticals currently in use include: alteration of blood-brain barrier; increase in regional blood flow; and nontumor-specific intracellular localization (e.g., gallium). Recently, however, antitumor antibodies to human tumors have been labeled and studied in a few limited situations.

The use of specific antibodies for tumor detection and treatment has been suggested and achieved for some time (*1,2*), but progress has been hampered by two major difficulties associated with the use of tumor-specific labeled antibodies for tumor imaging in malignant disease. The first has been the general unavailability of suitable antibodies to give sufficient target-to-nontarget contrast for imaging; the second is the difficulty of reproducible preparation and purification of antitumor antibodies.

Wright et al. (*3*) have summarized developments to 1978 and projected the future possibilities for the use of immunospecific globulins derived from foreign species in the diagnosis and therapy of cancer. Belitsky et al. have successfully imaged both primary tumors and metastases of renal carcinomas in man using I-131 IgG from absorbed goat antisera, although they noted difficulties caused by the presence of background due to a large excess of nonspecific radiolabeled antibody (*4,5*). Goldenberg et al. have used an iodine-131-labeled affinity-purified goat antibody to carcinoembryonic antigen (CEA) for scintigraphy in humans. In order to minimize some of the background caused by excess circulating antibody and antibody-antigen complexes, they also used a second radiolabeled system ($^{99m}TcO_4^-$ and Tc-99m serum albumin). The distribution of the second (nonspecific) circulating radiolabel could then be subtracted from that of the radiolabeled tumor-specific antibody to provide a correction for unbound or metabolized antibody and for circulating antigen-antibody complexes. Images of several different human tumors were obtained (*2*). The background-subtraction technique has been applied previously in other settings (*6*).

The recent development of monoclonal tumor-specific antibodies gives promise of a simpler and more incisive approach (*7–11*). Such antibodies, derived from lymphocyte hybridomas (*12*), are in principle homogeneous,

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require relatively little labor for purification, and can be prepared reproducibly in large quantities. Additionally, no adsorption or specific affinity purification should be required.

Moreover, if background subtraction is considered desirable (particularly in the first few days following administration), it should be possible to use a monoclonal nonselected antibody of the same class as that of the tumor-specific antibody for background subtraction. If both antibodies (specific and nonspecific) were similarly labeled with different radionuclides of the same element, metabolism of the two should be similar except for binding to tumors.

This report describes the uptake kinetics of an iodine-131-labeled, hybridoma-derived, monoclonal antibody over a 5-day period in (MH-15) teratocarcinoma-bearing BALB/c mice. Comparison is made with an I-123-labeled indifferent antibody of the same immunoglobulin class. The relationship between imaging and localization kinetics is discussed.

MATERIALS AND METHODS

Tumor preparation. The MH-15 teratocarcinoma cell line, derived from a BALB/c teratocarcinoma (*11*) and the P3/X63-Ag8 (hereafter, P3) BALB/c myeloma cell line (*12*) were grown in vitro in Dulbecco's modified Eagle's medium supplemented with 2 m*M* glutamine and 15% heat-inactivated fetal bovine serum. Tumors were produced by intramuscular injection of about 1 million teratocarcinoma cells in one thigh of female BALB/c mice. Where mice were used for imaging, P3 myeloma cells were injected into the other thigh as a control tumor. Tumor-bearing animals were subsequently injected with I-131-labeled teratocarcinoma-specific antibody (*13*).

Antibody preparation. Monoclonal antibody against stage-specific embryonic antigen 1 (SSEA-1) (*9,11*) is an IgM,κ, that reacts with several human and mouse teratocarcinoma cell lines. The antibody was isolated from the ascitic fluid of hybridoma-bearing BALB/c mice by collecting the void volume after gel exclusion chromatography of ascitic fluid on Sephadex G-200. That the void volume consists almost exclusively of IgM was confirmed by electrophoresis. However, we cannot exclude contamination of monoclonal antibody by a small amount of general mouse IgM. The antibody was iodinated (*14*) and unbound iodine removed by gel exclusion chromatography on G-25 Sephadex in Dulbecco's phosphate-buffered saline. Labeling efficiency was determined chromatographically (Whatman 3MM and 85% methanol). Iodine-131 was obtained commercially as NaI. Typical specific activities of labeled antibodies were 10–30 μCi I-131/μg. For kinetic studies 15 μCi were used, and 150 μCi were used for tumor-specific antibody imaging. This relatively large amount of activity on a W/W basis was used because of the difficulty of imaging even anesthetized mice for more than a short time. In each case the antibody preparation was dissolved in 0.2 ml phosphate-buffered saline and injected into the tail vein.

Imaging. Scintigraphy was done with a gamma camera, using a medium-energy parallel-hole collimator. Scintigraphic data were recorded and analyzed on a laboratory computer and displayed as a linear color-

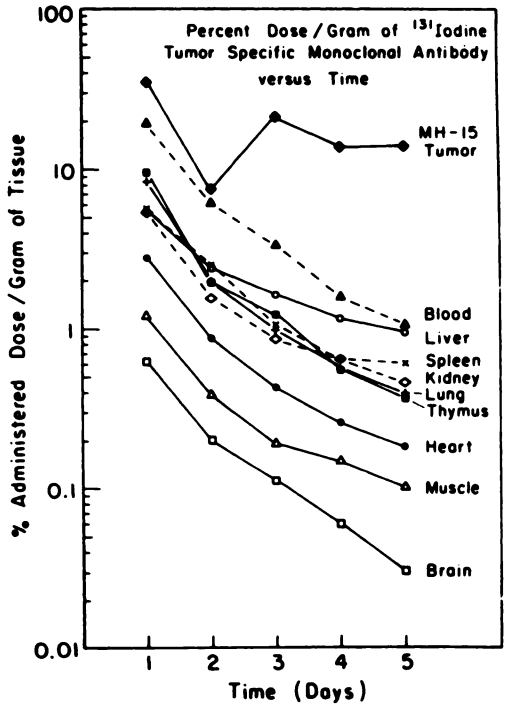

FIG. 1. Time course in various tissues for I-131-labeled, tumor-specific, hybridoma-derived, monoclonal antibody (% dose/g tissue).

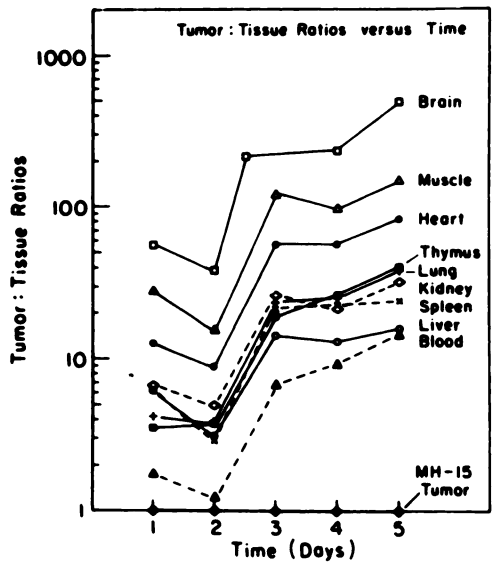

FIG. 2. Time course of tumor-to-tissue ratios for I-131-labeled antibody.

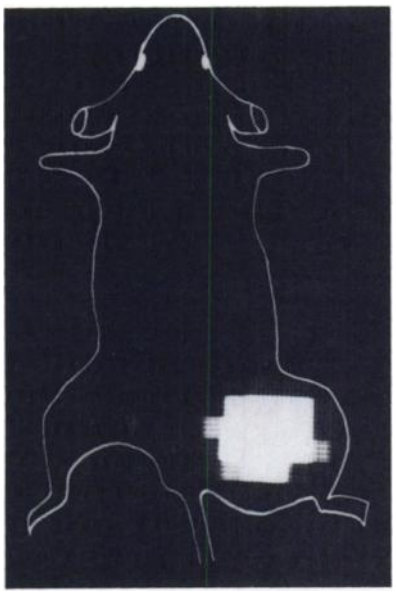

FIG. 3. Image of BALB/c mouse at 5 days after administration of 150 μCi of I-131 antibody (α-SSEA-1). Right thigh contains MH-15 teratocarcinoma (25 mm); left thigh contains a P3/X63-Ag8 myeloma (10 mm) of smaller size used as control.

scaled television image (converted to a less informative gray-scale image for Fig. 3). Images were recorded from the I-131 peak (364 keV) at the time of injection, at 4–5 hr, and at 1, 2, 3, 4, and 5 days after injection of the radiolabeled antibody. Mice were anesthetized for imaging by intraperitoneal injection of pentobarbital sodium. Lugol's solution, 2 drops per 100 ml of drinking water, was used to inhibit the thyroid accumulation of free iodine.

Uptake kinetics. Following administration of labeled antibodies, the mice were serially killed by cervical dislocation, daily for 5 consecutive days. Tissues were removed, washed in heparinized saline where applicable, blotted dry, weighed, and counted by well scintillation spectrometry.

Tissue-specific activities are expressed as mean ($\bar{X}$) percentage administered dose per gram. N equals two, four, four, four, and five mice on days 1–5, respectively. Tabular data—including calculation of standard error of the mean and estimated standard error of the ratio—are available from the authors on request.

RESULTS

Data obtained from serial sacrifice over a 5-day span revealed a similar pattern of elimination from each of the tissues examined, except that of the MH-15 tumor (Fig. 1). Between days 2–5, the effective eliminations approximate first-order kinetics for most tissues, including blood, but not for the MH-15 tumor. On a percentage dose per gram basis, relative tissue uptake over the 5 days is in the following descending order: MH-15 tumor > blood > liver > (spleen, lung, kidney, thymus) > heart > muscle > brain.

Activity in the MH-15 (antigenic) tumor appears to follow blood kinetics on days 1 and 2, and then levels off at about 15% of the administered dose per gram of tissue. Although tumor-to-blood specific activities on a % dose/g basis remain within a factor of 2 on the first 2 days, by the third day the tumor-to-blood ratio is about 7, and is 15 on day 5 (Fig. 2). Tumor-to-muscle ratios increase from 28 on day 1 to 150 on day 5. In fact, tumor-to-tissue ratios were higher on day 5 than on day 1 by a factor of 3 to 11 times.

Figure 3 is a picture of a single mouse bearing both a P3 myeloma and an MH-15 teratocarcinoma. This mouse, injected with only the I-131-labeled antibody (anti-SSEA-1) as described, had received a previous thyroid-blocking dose of Lugol's solution. Five days after injection the mouse was imaged. The uptake ratio of

TABLE 1. EXPERIMENTAL SCANS WITH RADIOLABELED IMMUNOGLOBULIN*

Author	System	Ag specificity	Days	Tumor/muscle ratio
Goldenberg	Hamster cheek pouch, human colonic cancer	CEA	6	7.5
Hoffer	Hamster cheek pouch, human colonic cancer	CEA	6	6.4†
Mach	Nude mouse, human colonic cancer	CEA	3	8
Order	Syngeneic ovarian cancer	Tumor-associated antigen (IgG)	10	7.6
Order	Syngeneic ovarian cancer	Fab (IgG)	2	1.8
Ballou	Mouse teratocarcinoma	SSEA-1	2	20
Ballou	Mouse teratocarcinoma	SSEA-1	5	148

* After Ref. 3, Wright et al.
† Tumor-to-intestine ratio.

MH-15 tumor (antigenic) in this mouse to the P3 tumor (nonantigenic) was 26:1. This ratio is about the same as those for MH-15 tumor-to-liver (22:1) and tumor-to-spleen (30:1). The P3 accumulation is therefore similar to that of the heavily vascularized tissues. Tumor-to-liver (16:1) and tumor-to-spleen (25:1) ratios in mice bearing only the MH-15 tumor were found to be about the same order of magnitude in a separate set of experiments.

DISCUSSION

Our work described herein and elsewhere (*13*) suggests the feasibility of hybridoma-derived monoclonal antitumor antibodies for tumor imaging. Target-to-nontarget ratios are relatively high, particularly if one waits longer than the first 48 hr. Following administration, the labeled specific antitumor antibody was eliminated from the various nontumor tissues, presumably as a function of the perfusion kinetics. Our findings have remained consistent with numerous animals and with several subsequent labelings.

Observation of our tumor-to-tissue ratios, particularly tumor-to-blood, suggests the necessity for a background-subtraction technique at least during the first 48 hr. In several other sets of experiments (*13* and unreported) we have successfully applied a background-subtraction technique using I-123-labeled circulating nonspecific antibody. Although the half-life of the I-123 becomes a limiting factor, an excellent image may be obtained at 48 hr. We have also used Tc-99m-labeled nonspecific circulating antibody, obtaining fair to adequate images. However, the 6-hr half-life of Tc-99m becomes a major constraint.

The specificity of our hybridoma-derived monoclonal antitumor antibody is particularly noteworthy as a potential diagnostic tool in light of previously successful efforts (Table 1) with whole immunoglobulin fractions.

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ERRATUM

In "Status of Gallium-67 in Tumor Detection" by Paul Hoffer, on page 396, right column, last paragraph, lines 11 and 12 should read: "Reports from other investigators, however, have been somewhat less enthusiastic." (*J Nucl Med* 21:394-398, 1980)