USE OF ^{131}I-CEA ANTIBODY AS A TUMOR SCANNING AGENT

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Tumor scanning can be accomplished successfully in an animal model by the use of radioiodinated antibodies specific for the carcinoembryonic antigen produced by the tumor. In this tumor model (GW-39), the radiolabeled antibodies showed tumor localization superior to that of ^{111}In-bleomycin, ^{67}Ga-citrate, and control ^{131}I-labeled normal IgG. Although ^{111}In-bleomycin did show a relatively high tumor uptake, tumor-to-intestine and tumor-to-liver ratios for this agent were approximately 1:1. Relatively slow clearance of blood background (presumably due to incidental labeling of nonspecific IgG) produces a long delay before optimal tumor-to-background ratios are achieved. For this reason, further purification of specific CEA antibodies would be desirable. Efforts are currently under way to apply the above findings to the identification of tumors in human subjects.

Carcinoembryonic antigen (CEA), a glycoprotein found in human colonic tumors, was isolated and described by Gold and Freedman in 1965 (*1,2*). The molecular weight of CEA is approximately 200,000. Its chemical structure has been extensively investigated (*3,4*). The antigen has been detected in gut tissue during the first two trimesters of fetal life (*2*). It may also be present to a variable extent in malignant tissue of other than colonic origin (*5*). Although it was originally thought that CEA was a tumor-specific antigen, there is now indirect evidence of its presence in minute amounts in nonmalignant tissues, including the normal colon (*6,7*).

Cytological studies with fluorescine (*8*) and ferritin-conjugated antisera (*9*) indicate that in colonic tumor cells CEA is localized in the glycocalyx exterior to the cell membrane. The antigen is also present in the systemic circulation of patients with various tumors in high enough amounts to allow its detection by radioimmunoassay (*10*). The purpose of our preliminary investigation was to determine whether radioiodinated CEA antibody is taken up specifically by CEA-containing tumor tissue and how the localization of this specific antibody compares with that of other localizing agents.

METHODS

IgG fractions from samples with high anti-CEA titer were obtained from Primus and Hansen of Hoffmann-LaRoche, Inc. The immunoglobulin was of rabbit (#351) and goat (Ro-183) origin. Control IgG was obtained from nonimmunized goats and from commercial sources (rabbit IgG from Miles Laboratories, Kankakee, Ill., Lot #28; goat IgG from Miles Laboratories, Kankakee, Ill., Lot #15). No further attempt was made to purify the CEA antibody preparations which contained relatively large amounts of nonspecific IgG.

The antibody preparations as well as the control normal IgG were iodinated with "carrier-free" ^{131}I (New England Nuclear and Amersham/Searle) using the chloramine-T method (*11*). Following iodination, the immunoglobulins were separated from the unreacted iodide by filtration through Sephadex G75 column. The specific activity of the labeled antibody varied from approximately 60 to 80 mCi/mg protein for the imaging studies and 4 to 8 mCi/mg protein for the quantitative studies.

The tumor model used for these experiments was a GW-39 colonic tumor of human origin and transplantable in golden Syrian hamsters. This tumor, known to produce CEA (*12*), was developed and previously described by Goldenberg (*13*).

For imaging studies, 60- to 80-gm hamsters bear-

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ing 2- to 5-gm tumors located subcutaneously on the lateral aspect of the thigh were used. Imaging was performed with an Anger scintillation camera equipped with a pinhole collimator. Animals were injected intravenously with 200–300 μCi of ^{131}I-labeled anti-CEA IgG or ^{131}I-labeled normal IgG from either nonimmunized rabbit or goat. Serial determinations of tumor localization of radioiodinated antibody and normal IgG were made at 24-hr intervals for up to 144 hr. The animals were given Lugol's solution in their drinking water.

Quantitative studies comparing localization of CEA antibody in tumor to that of $^{111}InCl_3$ (Medi+Physics), ^{111}In-bleomycin (Medi+Physics), ^{67}Ga-citrate (Medi+Physics), and ^{131}I-normal IgG (Hoffmann-LaRoche, Inc.) were also performed. All tumors weighed between 1 and 2 gm. Between 100 and 200 μCi of ^{131}I-CEA antibody, $^{111}InCl_3$, ^{111}In-bleomycin, ^{67}Ga-citrate, and ^{131}I-normal IgG were given by intracardiac injection in 0.3- to 0.4-ml volumes.

At the "optimum" scanning time for each agent, animals were anesthetized with nembutal and sacrificed by exsanguination through cardiac puncture. The optimum scanning times were determined by previous experience gained in imaging the various agents and were for ^{131}I-CEA antibody and ^{131}I-normal IgG, 6 days (144 hr); for ^{111}In-bleomycin, 24 hr; for $^{111}InCl_3$ and ^{67}Ga-citrate, 48 hr. Tissues were removed immediately after sacrifice to tightly capped containers of known weight and counted in a scintillation counter along with appropriate aliquots of an injection standard.

RESULTS

Localization of ^{131}I-CEA antibody in the tumor site was clearly demonstrated on the scan images within 48 hr following injection (Fig. 1). The tumor activity remained relatively constant after 48 hr. Blood background was relatively high during the first 48 hr and decreased slowly so that images with a high tumor-to-background ratio were not obtained until approximately 6 days after injection. Some tumor localization was also noted in the animals injected with ^{131}I-normal IgG (Fig. 2). However, this activity faded on the views taken 6 days following injection.

The quantitative localization of the radionuclides in various tissue are shown in Tables 1A and B. Tumor-to-tissue ratios of labeled CEA and other radionuclides for selected organs are listed in Table 2. Tumor-to-intestine ratios of approximately 6:1 were achieved with the ^{131}I-CEA antibody preparation compared with a ratio of approximately 3.5:1 for normal IgG and considerably lower ratios for

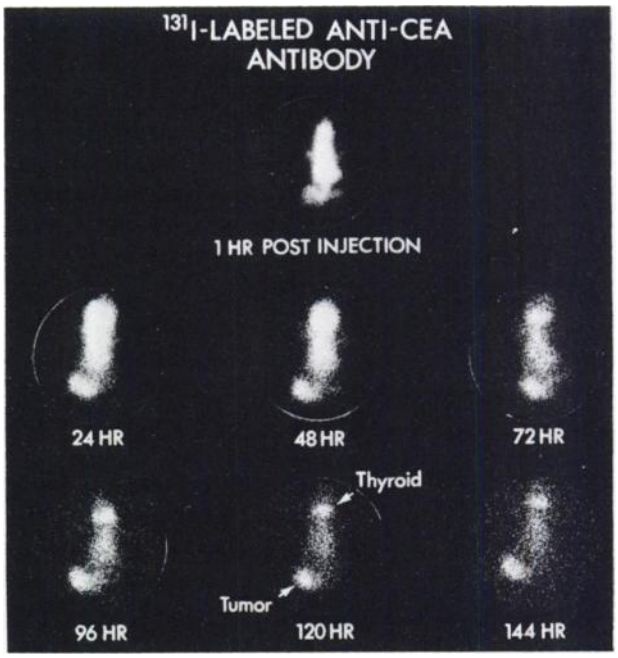

FIG. 1. Localization of ^{131}I-CEA antibody (rabbit) in GW-39 tumor implanted in thigh of golden Syrian hamster. Note relatively constant tumor activity after 24 hr and slow clearance of background activity. Thyroid localization presumably occurred due to failure to include Lugol's solution in animals' water supply before administration of labeled antibody. Images obtained with Pho/Gamma HP camera and pinhole collimator at 11 cm.

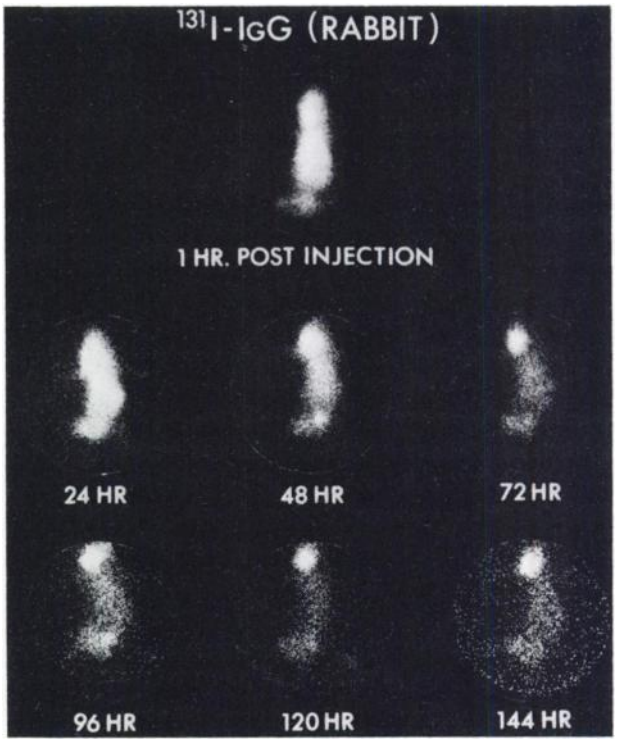

FIG. 2. Localization of control ^{131}I-normal IgG (Miles Laboratories, Kankakee, Ill., Lot #28 rabbit) in hamster with tumor implant similar to that in Fig. 1. Note faint visualization of tumor which fades with decrease in background activity. Activity in neck is primarily due to slight extravasation of radionuclide during direct injection into superior vena cava. Images obtained with Pho/Gamma HP camera and pinhole collimator at 11 cm.

TABLE 1A. DISTRIBUTION OF RADIONUCLIDES IN TISSUES OF HAMSTERS IMPLANTED 10 DAYS PREVIOUSLY WITH GW-39 TUMOR*

	% injected dose/organ (±s.e.)				
Tissue	^{131}I-CEA antibody (6 days)	^{131}I-normal IgG (6 days)	^{111}In-bleomycin (24 hr)‡	^{111}InCl$_3$ (48 hr)	^{67}Ga-citrate (48 hr)
Tumor	2.30 ± 0.28	0.65 ± 0.06	1.36 ± 0.30	3.54 ± 0.51	0.99 ± 0.28
Intestine†	1.33 ± 0.18	1.01 ± 0.05	6.05 ± 1.15	9.78 ± 1.19	4.44 ± 0.99
Liver	0.84 ± 0.12	0.63 ± 0.06	3.36 ± 0.44	13.84 ± 1.29	5.04 ± 0.96
Kidney	0.31 ± 0.05	0.23 ± 0.03	3.77 ± 0.66	10.89 ± 3.13	1.33 ± 0.24
Brain	0.03 ± 0.004	0.02 ± 0.003	0.07 ± 0.02	0.15 ± 0.04	0.08 ± 0.05
Femur	0.01 ± 0.005	0.01 ± 0.003	0.11 ± 0.02	0.30 ± 0.07	0.44 ± 0.02
Heart	0.25 ± 0.02	0.26 ± 0.11	0.26 ± 0.04	0.44 ± 0.14	0.09 ± 0.04
Lung	0.40 ± 0.04	0.65 ± 0.15	0.68 ± 0.19	0.97 ± 0.29	0.41 ± 0.16
Pancreas	0.08 ± 0.02	0.08 ± 0.02	0.11 ± 0.02	0.16 ± 0.03	0.01 ± 0.00
Spleen	0.02 ± 0.002	0.02 ± 0.00	0.10 ± 0.03	0.30 ± 0.05	0.09 ± 0.03
Stomach†	0.70 ± 0.16	0.38 ± 0.06	0.90 ± 0.13	1.04 ± 0.22	0.93 ± 0.22
Thyroid	0.14 ± 0.08	0.11 ± 0.04	0.01 ± 0.00	0.01 ± 0.00	0.00
Carcass	20.92	17.25	18.14	25.77	28.59
Excretion	68.82	76.14	60.39	29.84	50.01
Recovered (%)	98.63	98.91	97.78	98.44	93.45

* Four animals per group, average numbers. All data based on counts that were twice background or greater.
† With contents.
‡ Times after injection.

TABLE 1B. DISTRIBUTION OF RADIONUCLIDES IN TISSUES OF HAMSTERS IMPLANTED 10 DAYS PREVIOUSLY WITH GW-39 TUMOR*

	% injected dose/gm (±s.e.)				
Tissue	^{131}I-CEA antibody (6 days)	^{131}I-normal IgG (6 days)	^{111}In-bleomycin (24 hr)	^{111}InCl$_3$ (48 hr)	^{67}Ga-citrate (48 hr)
Tumor	1.20 ± 0.21	0.56 ± 0.05	0.91 ± 0.13	2.28 ± 0.46	0.72 ± 0.20
Intestine	0.20 ± 0.04	0.16 ± 0.04	0.99 ± 0.13	1.21 ± 0.25	0.65 ± 0.17
Liver	0.25 ± 0.04	0.19 ± 0.02	0.96 ± 0.12	3.68 ± 0.47	1.55 ± 0.39
Kidney	0.35 ± 0.05	0.22 ± 0.04	4.30 ± 0.61	15.26 ± 4.17	1.50 ± 0.28
Blood (whole)	1.47 ± 0.19	0.97 ± 10.16	0.78 ± 0.26	0.54 ± 0.22	0.17 ± 0.08
Brain	0.03 ± 0.004	0.02 ± 0.003	0.08 ± 0.03	0.17 ± 0.05	0.08 ± 0.05
Femur	0.11 ± 0.02	0.08 ± 0.01	0.72 ± 0.14	1.57 ± 0.28	1.93 ± 0.07
Heart	0.60 ± 0.06	0.45 ± 0.11	0.63 ± 0.14	0.99 ± 0.09	0.20 ± 0.07
Lung	0.65 ± 0.08	0.69 ± 0.10	1.17 ± 0.34	1.37 ± 0.59	0.53 ± 0.19
Pancreas	0.24 ± 0.04	0.84 ± 0.03	0.30 ± 0.02	0.46 ± 0.11	0.02 ± 0.00
Spleen	0.26 ± 0.04	0.18 ± 0.01	1.25 ± 0.23	2.84 ± 0.55	0.94 ± 0.23
Stomach	0.42 ± 0.12	0.24 ± 0.05	0.45 ± 0.04	0.62 ± 0.22	0.52 ± 0.15
Thyroid	27.46 ± 12.7	49.47 ± 15.82	4.02 ± 1.05	2.37 ± 0.34	0.00
Muscle	0.14 ± 0.01	0.08 ± 0.01	0.16 ± 0.02	0.28 ± 0.07	0.03 ± 0.01
Skin	0.22 ± 0.03	0.18 ± 0.02	0.39 ± 0.06	0.70 ± 0.18	0.26 ± 0.17

* Four animals per group, average numbers. All data based on counts that were twice background or greater.

the other agents. Tumor-to-liver ratios were also favorable for the labeled CEA antibody as compared with other agents, suggesting its usefulness in detecting CEA-containing hepatic metastases. Tumor-to-blood ratios were relatively poor for ^{131}I-CEA antibody, probably due to the relatively high content of nonspecific labeled IgG in the preparation.

DISCUSSION

Most tumor scanning agents currently available are insensitive and certainly nonspecific for many tumor types. Gallium-67-citrate, for example, localizes in inflammatory tissue as well as in tumors. Furthermore, ^{67}Ga localization can be demonstrated in less than 50% of digestive tissue tumors (*14,15*).

The use of radiolabeled antibodies that are specific for tumor antigen is an attractive concept for tumor scanning. The radiolabeled antibody is potentially tumor-specific since tumor antigen is not usually found in inflammatory tissues. Also, as more tumor-

TABLE 2. TUMOR/TISSUE RATIOS FOR RADIONUCLIDES* IN GW-39 TUMOR HAMSTER HOST (±s.e.)†

Tissue	Radiopharmaceutical				
	^{131}I-CEA antibody (6 days)	^{131}I-normal IgG (6 days)	^{111}In-bleomycin (24 hr)	$^{111}InCl_3$ (48 hr)	^{67}Ga-citrate (48 hr)
Intestine	6.40 ± 1.40	3.57 ± 0.65	0.96 ± 0.19	2.07 ± 0.45	1.55 ± 0.61
Liver	4.82 ± 0.43	3.03 ± 0.28	0.96 ± 0.13	0.61 ± 0.05	0.67 ± 0.27
Kidney	3.24 ± 0.38	2.66 ± 0.29	0.20 ± 0.03	0.21 ± 0.10	0.56 ± 0.17
Blood	0.81 ± 0.07	0.59 ± 0.04	1.37 ± 0.28	7.01 ± 3.51	9.62 ± 4.83

* Avg. of ratios for individual animals.
† Four animals per group.

specific antigens are isolated and identified, it will be possible to produce scanning agents which are specific for various tumor types.

The application of radiolabeled antibody preparations to tumor scanning was investigated by Spar and associates in 1959 (*16*). In 1966 McCardle, et al reported on an effort at tumor scanning and treatment with ^{131}I-labeled antibodies to human fibrinogen (*17*). This effort was only partially successful but did demonstrate localization in about 50% of a variety of primary and metastatic tumors. It also established, together with the earlier work of Spar (*18*) and Dewey (*19*), that small quantities of radiolabeled antibodies produced in animals against human serum constituents could be administered safely to man. In the 50 patients studied by McCardle, et al, none had a clinical reaction to the radiolabeled antibody and only two patients showed unusually rapid excretion (one on the 14th day postinjection and one earlier), suggesting immunologic recognition of the injected antibody.

Primus and associates have previously described the specific localization of radiolabeled CEA antibody in Goldenberg's GW-39 hamster tumor model (*20*). Similar observations were made by Mahaley (*21*) in a glioma tumor model. Goldenberg has also observed ^{131}I-labeled CEA antibody tumor localization in his GW-39 tumor model (*22*). Our observation supports not only the fact that ^{131}I-CEA antibody localizes preferentially in tumors which produce CEA but also the fact that this agent is potentially superior to other tumor-scanning agents available for this purpose.

A major deficiency of our present CEA-antibody preparation is the relatively high content of nonspecific IgG. It is unlikely that animals immunized with CEA will produce CEA-antibody titers above those available in the preparations used in these studies. In fact, titers present in these animals are uniquely high. The CEA antibody constituted about 35% of the total immunoglobulin pool of the goat donor Ro-183 (*20*). Our studies suggest that the nonspecific component produced a high blood-pool background which clears slowly and hampers the usefulness of the preparation for tumor scanning. Purification of the CEA antibody by methods such as affinity chromatography could improve tumor-to-blood ratios and consequently the tumor scanning potential of this antibody. Another possible problem in the use of this agent for tumor scanning in humans is the effect of circulating antigen. The animals used for these experiments have relatively low circulating CEA blood titers. Human subjects with CEA-producing tumors, however, have relatively high concentrations of circulating CEA capable of competing with the tumor for the injected antibody. This competitive action would probably lessen tumor localization but may enhance hepatic and renal clearance of the label. The net effect on tumor identification by scanning is unknown and will require clinical investigation.

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