Colon Carcinoma Immunoscintigraphy by Monoclonal Anti-CEA Antibody Labeled with Gallium-67-Aminooxyacetyldeferroxamine

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Previous experimental results in nude mice showing that radiolabeling the monoclonal antibody anti-CEA 35 with ^{67}Ga-aminooxyacetyldeferroxamine could give better tumor localization than radioiodination prompted us to initiate the present clinical study. The ^{67}Ga-labeled antibody anti-CEA 35 (185 MBq, 0.7–1.7 mg) was injected preoperatively into 14 patients for colorectal carcinoma imaging. The same antibody labeled with ^{125}I (3.7 MBq, 0.25 mg) was injected simultaneously to compare the ^{67}Ga and ^{125}I dose recoveries in surgical specimens. Twelve of 14 primary tumors gave a positive ^{67}Ga scintigraph. The mean %ID/g recovered in all tumors 3–9 days after injection was significantly higher for ^{67}Ga (0.019%) than for ^{125}I (0.005%) ($p < 0.001$, paired t test). The tumor-to-normal tissue ratios were generally higher for ^{67}Ga, with the exception of liver. We conclude that ^{67}Ga-aminooxyacetyldeferroxamine improved immunoscintigraphy outside the liver, particularly in the pelvic region. We also show that deferroxamine infusion accelerates the excretion of ^{67}Ga in eight patients and propose that this could lead to further improvement of immunoscintigraphy.

J Nucl Med 1992; 33:1766–1773

In a previous comparative study performed in the nude-mouse model for immunoscintigraphy, we showed that the radiolabeling of the anti-CEA monoclonal antibody (Mab) 35 (*1*) with ^{67}Ga-aminooxyacetyldeferroxamine gave equivalent and sometimes better results than radioiodination (*2*). The advantages of the aminooxyacetyldeferroxamine over earlier methods for labeling antibody with ^{67}Ga (for instance, the cross-linking of the antibody and deferroxamine by glutaraldehyde) (*3*) are thought to derive from a better preservation of the protein structure and from the attachment of the radiolabel to selected sites of the antibody (the oxidized carbohydrate).

Received Dec. 31, 1991; revision accepted May 21, 1992.

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The physical half-life of ^{67}Ga, 3.25 days, may be more favorable than that of ^{123}I (13.3 hr) or ^{99m}Tc (6 hr) because the localization of the antibody in patients is expected to take more than 24 hr. A derivative of another chelator, DTPA, has been attached to the carbohydrate of the antibody for labeling with ^{111}In, with reported decrease of the liver uptake in nude-mouse experiments relative to results obtained by random labeling of the protein's side chains (*4*).

In this clinical study, we chose to compare the ^{67}Ga-labeled antibody to the same antibody labeled conventionally with ^{125}I. The direct comparison of the recoveries of the two radionuclides in surgical specimens obtained from patients who had received an injection of both antibody preparations allowed us to demonstrate the advantage of the new immunoconjugate in a series of 14 patients.

MATERIALS AND METHODS

Patients

The study protocol was approved by the Ethics Committee of the department of surgery of the Geneva University Hospital. Patients presenting to the clinic of Digestive Surgery, having been programmed for elective resection of a primary colorectal cancer, were enrolled after giving informed consent. Patients were not included if they had an history of allergic disease. There were eight male and six female patients aged from 43 to 80 yr (median 67.5 yr, Table 1).

To minimize the irradiation dose to the thyroid, cold iodine was given orally for five days (10 drops of Lugol solution, 3 times daily), starting the day before the antibody injection. An antihistaminic (terfenadin, Teldane®, Merrell Dow, 60 mg) was given orally 30 min before the antibody injection. Gallium-67-Mab (185 MBq) was diluted in 5 ml saline and injected it slowly by the intravenous route; the injected volume was precisely measured. Iodine-125-Mab (3.7 MBq) was diluted and injected in the same way. The two antibody injections were performed immediately in sequence, the ^{67}Ga-labeled antibody either preceding or following the iodinated antibody. A 1-ml aliquot of 1/1000 dilutions of the injected solutions were kept back at the time of injection; they were used as standards when determining the

radioactivity recovery values in the blood and urine samples and in surgical specimens.

The first six patients did not receive free deferroxamine. The other eight patients received an intravenous infusion of deferroxamine (Desferal®, Ciba-Geigy, Switzerland): 1 g/24 hr during the last two or three days before surgery in Patients 7–10, and 50 mg/kg/24 hr throughout between the time of the antibody injection and in Patients 11–14. Blood samples were drawn 10 and 30 min and 4, 24, 48 and 72 hr after the antibody injection. The patients underwent surgery three to nine days after the injection. One patient had two tumors (hepatic flexure and transverse), and in one patient the pre-operative diagnosis of tumor of the cecum (which did not depend on the studies reported here) was not confirmed at surgery. The sizes, histological differentiation, locations and Duke's stages of the tumors are indicated in Table 1. Patients 5 and 6 had liver metastasis and a serum CEA above 100 ng/ml. The serum CEA of 10 other patients was <8 ng/ml; the serum CEA values for two other patients were not available.

Radiolabeling

Gallium-67-chloride was purchased from Mallinckrodt Diagnostica (Petten, Holland) and [^{125}I]sodium iodide from the Paul Scherrer Institute (Würenlingen, Switzerland). The conjugation of the anti-CEA Mab 35 with the chelator deferroxamine was essentially as previously described (*2*), with the following modifications to permit the preparation of a large batch (70 mg starting antibody): gel filtration of oxidized antibody and later of Mab defferoxamine was performed on a column of Sephadex G50 fine grade (Pharmacia, Uppsala, Sweden) eluted with 10 m*M* $Na-H_2PO_4$, which was also 0.1 *M* in NaCl, and adjusted to pH 7.0 with NaOH. The conjugate was stored frozen at −80°C in aliquots of 5 mg (1 ml).

Gallium-67-deferroxamine-Mab was prepared routinely without problems. In order to produce material of high specific activity, radiolabeling conditions were modified as follows. We diluted 0.9–1.1 GBq ^{67}Ga in 0.8 *M* HCl (original concentration, 5.5–18.2 GBq/ml) to a final volume of 175 μl with 0.8 *M* HCl, then mixed it with 75 μl 4.65 *M* ammonium acetate. A 5 mg aliquot of Mab-deferroxamine was immediately added and allowed to react for 2 hr at room temperature. Then 50 μl 1 m*M* freshly prepared unlabeled gallium was added and allowed to react for 5 min (the unlabeled gallium solution was prepared by diluting a stock solution, 0.2 *M* $GaCl_3$ in 0.8 *M* HCl, to 1.43 m*M* with 0.8 *M* HCl, then diluting to 1 m*M* with 4.65 *M* ammonium acetate). The labeled antibody was then isolated by gel filtration on the Superose 12 column, eluting with Dulbecco's phosphate-buffered saline without calcium and magnesium, pH 7.2. In order to keep the protein concentration high, the tail of the protein peak was not collected.

The collected material was passed through a sterile 0.22 μm filter (Millipore, Bedford, MA) under a sterile hood. Between 307 and 740 MBq, ^{67}Ga (31%–77%) was recovered in the antibody peak, which contained between 2.8 and 3.5 mg protein in a volume of 1.8–2.0 ml. The specific radioactivity was between 110 and 270 MBq (3.0 and 7.3 mCi)/mg. Analysis by gel filtration and native PAGE showed less than 2% aggregate before injection. Pyrogens were assayed by the Limulus test (Pyrogent® Whittaker Bioproducts, Inc, Walkersville, MD) (*5*) and sterility controls were performed after every radiolabeling. All samples were found to be sterile and to conform to the standards for the preparation of saline solutions for infusion.

For radioiodination, 1 mg of anti-CEA Mab 35 was labeled with 18.5 MBq ^{125}I using 0.03 mg iodogen (Pierce Chemicals, Rockford, IL) (*6*), yielding a specific activity of about 14.8 MBq/mg protein. The labeled antibodies were separated from free iodine by chromatography on a Sephadex G25 column (Phar-

TABLE 1
Patient's Summary and Scintigraphic Results

Patient no.	Age (yr)	Sex	Tumor sites	Dukes stage, differentiation, tumor size*	Operation delay†	Deferroxamine infusion	Scintigraphy‡	
							Planar	SPECT
1	75	F	(None)	—	6	—	+	+
2	62	F	Sigmoid	A, G1, 2.5 × 5	7	—	−	−
3	43	M	Left flexure	C, G1, 10 × 10	7	—	+	+
4	62	F	Cecum	B, G1, 6 × 10	6	—	+	+
5	65	F	Sigmoid, liver	D, G1, 4 × 4.5	5	—	−, −	+, −
6	56	M	Left flex., liver, nodes	D, G2, 4 × 6.5	7	—	−, −, +	−, −, +
7	77	M	Rectum	C, G2, 5.5 × 7.5	6	§	+	+
8	77	M	Rectum	B, G1, 4.3 × 6.2	6	§	+	+
9	80	M	Rectum	B, G2, 5 × 4	6	§	+	+
10	77	M	Rectum	B, G2, 5 × 6	8	§	+	+
11	80	F	Cecum	B, G3, 3.5 × 7	9	¶	+	+
12	70	M	Right flex., transv. colon	B, G2, 8 × 8, 3 × 4	6	¶	+, +	+, +
13	48	M	Left flexure	B, G2, 5 × 7	3	¶	+	+
14	60	F	Descending colon	B, G2, 5 × 6	3	¶	+	+

* G1, G2 and G3 for high, intermediate and low differentiation; size in cm.
† Time from injection to operation, days.
‡ Tumor sites seen (+) or not seen (−).
§ deferroxamine perfusion for 2–3 days before surgery.
¶ deferroxamine continuously from Mab injection to surgery.

macia) equilibrated in pyrogen-free 0.15 *M* saline, and further sterilized by filtration under a sterile hood through a 0.22 μm Millipore filter. Controls by filtration through a Sephadex G200 column always showed less than 1% aggregate.

The immunoreactivity of all ^{67}Ga- and ^{125}I-labeled conjugates was determined in vitro by a direct binding assay (*7*) to CEA immobilized on Sepharose-CNBr (Pharmacia). Nonspecific binding was determined similarly using Sepharose-CNBr coupled to an irrelevant protein. There was no significant difference between the mean specific bindings of the ^{67}Ga-deferroxamine-Mab and ^{125}I-Mab, which had been labeled in two different laboratories. The mean and standard deviations of the specific bindings of the ^{67}Ga-deferroxamine-Mab and ^{125}I-Mab were 64.2% (13.6) and 67.7% (10.3), respectively.

Analysis of Blood Samples

The chemical form of ^{67}Ga in serum samples was analyzed by gel filtration on Superose 12 in Dulbecco's phosphate buffered saline without calcium and magnesium, pH 7.4, and by electrophoresis on polyacrylamide gels in the absence of sodium dodecyl sulfate (native PAGE). For control purposes, samples of ^{67}Ga-Df-Mab were diluted into 0.1% BSA (or into normal human serum) and analysed in the same way.

Scintigraphic Studies

A Toshiba gamma camera (model GCA-901A) was used with a medium-energy, medium-resolution collimator with a field of view of 50 by 35 cm. For planar scintigraphy, 10^6 counts were collected on a 128 × 128 matrix using 20% symmetrical windows centered on two gamma peaks of ^{67}Ga (93 and 185 keV). Four overlapping views (2 anterior and 2 posterior) covering the thorax, abdomen and pelvis were taken on the day of the antibody injection and 1, 2 and 3 or 5 days later (with the exception of the two patients who underwent surgery on Day 3). Single-photon emission computed tomography (SPECT) was performed for all patients on Days 1, 2 and 3 or 5 by acquiring 60 views of 30 seconds (128 × 128 matrix) covering the abdomen and the pelvis. About 50 transaxial slices of 0.8 cm thickness (2 pixels) and coronal slices were reconstructed.

Surgical Specimens

Two or three samples from the tumor and one sample each from blood, normal mucosa, subcutaneous fat and skin were obtained in all cases. Samples from the liver were obtained in eight cases. The specimens were weighed, fixed in 2 ml fixative and kept in closed tubes until counting and processing for immunoperoxidase examination. To minimize the spillover of ^{67}Ga counts in the ^{125}I window, ^{67}Ga ($T_{½}$ 3.25 days) was counted first and ^{125}I ($T_{½}$ 60 days) approximately 2 wk later. The count rates were corrected for a 11.7% spillover of the ^{67}Ga counts in the ^{125}I window. The standards were then used to calculate the percentage of the injected dose per gram (%ID/g). The tumor-to-normal-tissue ratios are the values of mean radioactivity per gram in the tumor samples divided by the values of radioactivity per gram in the normal tissue samples.

Statistical Analysis

The percent of the ^{67}Ga and ^{125}I ID/g values were compared for each sample by the Student's paired t-test (Table 2). Figures 3–5 show the arithmetic means and standard deviations of these values per organ. The mean tumor-to-normal-tissue ratios and standard deviations shown in Figure 6 were calculated from the logarithm of the individual tumor-to-normal-tissue ratios.

RESULTS

All antibody injections were tolerated without any side effects. Prior to the scintigraphic study each of the 14 patients had been suspected or positively diagnosed as having a single tumor, but upon surgery one patient was found to be tumor free. Of the 13 remaining suspected tumors, 11 were clearly delineated by the anti-CEA antibody radiolabeled with ^{67}Ga. In addition, in one patient, an unsuspected additional primary tumor was detected by this means and confirmed at surgery. Table 1 summarizes the major characteristics of the 14 patients and the scintigraphic results. Eight patients received an intravenous infusion of deferroxamine, four patients during the last 2–3 days preceding surgery and four patients from the beginning of the antibody injection until surgery.

The mean ^{67}Ga levels in the initial blood samples (10 and 30 min) were higher than those of ^{125}I, but the biological half-lives of the two preparations in blood were very similar, as shown in Figure 1. Assuming a single exponential decay of the antibody in blood, the mean biological half-lives were 37.6 hr for ^{67}Ga and 42.4 hr for ^{125}I. In eleven patients, serum samples were analysed by gel filtration. The results showed that in the two patients with high serum CEA concentration (Patients 5 and 6), the ^{67}Ga was circulating exclusively as a species of high molecular weight, consistent with the complexed antigen/antibody. In seven patients, no high molecular weight material was found. Of the other two patients, one showed approximately equivalent amounts of monomeric antibody and of high molecular weight material, and the other showed mainly monomeric antibody with only a small proportion of high molecular weight material. In the two last patients, the ratio shifted in time towards the high molecular weight species.

The administration of free deferroxamine has been shown previously to increase the excretion of ^{67}Ga after an injection of ^{67}Ga-citrate in experimental models (*8,9*). The effect of deferroxamine on ^{67}Ga excretion was evalu-

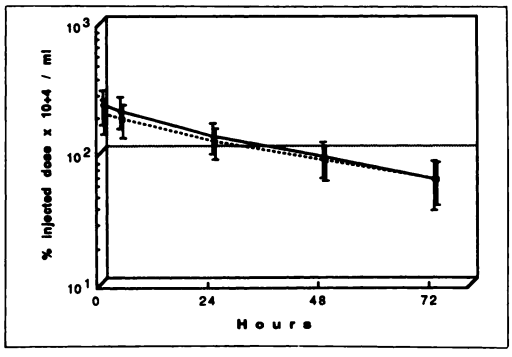

FIGURE 1. Time-activity curve of ^{67}Ga and ^{125}I in blood. The means and standard deviations of the injected dose recoveries of ^{67}Ga (continuous line) and ^{125}I (dotted line) per ml blood are shown. Time 0 is the time of the injection. Patients who had surgery 3 days after the injection are not included. The figures of the vertical axis are % of the injected dose per gram multiplied by 10^4 (i.e. 10^{-6} ID/g).

ated in this series of patients by comparing ^{67}Ga urinary excretion with and without free deferroxamine infusion. To this end, we measured the ^{67}Ga excretion in the first 48-hr urine collection after the injection and compared four patients receiving deferroxamine (Patients 11–14) with four patients without deferroxamine infusion at that time (Patients 7–10). We found a significant increase of the excretion of ^{67}Ga with deferroxamine infusion, from 2.33% (s.d. = 0.58) to 7.77% (s.d. = 1.55) of the injected dose per 48 hr ($p = 0.001$).

The optimal imaging time was on Day 1 or Day 2 after the antibody injection. In addition to the tumors, the blood pool, liver, spleen and skeletal structures were easily identified in all images. An important advantage of ^{67}Ga-labeled antibody over an antibody labeled with radioiodine or technetium was less radioactivity excretion in the urine. This facilitated greatly the interpretation of the pelvic region. Also of interest was the observation that good tumor images could be obtained by planar scintigraphy and by SPECT up to five days after the injection of 185 MBq of ^{67}Ga-labeled Mab. This was due to the convenient 3.25 days physical half-life of ^{67}Ga and to the longer retention of the ^{67}Ga-labeled Mab in the tumor. The latter advantage over the ^{125}I-labeled Mab was confirmed by the study of the recovery of radioactivity in the various tissue samples.

Due to its diffuse and changing pattern, the large bowel content of ^{67}Ga, which occurred to a variable extent in most patients, did not present much difficulty of interpretation. In Patient 1, however, a nontumoral lesion of the cecum gave rise to a circumscribed and relatively stable focus, which was falsely interpreted as a tumor. This patient suffered a nontumoral pathology of the cecum, which had been suspected of being a tumor before the operation. No final diagnosis could be obtained in this case.

In patients who did not receive deferroxamine infusion before scintigraphy, seven of nine primary tumors were visualized, six by both planar scintigraphy and SPECT and one by SPECT alone. If we take together the primary tumors and the known secondary tumor deposits in liver or lymph nodes, 8 of 12 tumor sites were visualized in this subgroup of patients (Table 1). Figures 2A (planar) and 2a (SPECT) show the images of a cecum carcinoma obtained one day after injection in Patient 4. There is no detectable nonspecific ^{67}Ga localization other than in liver and spleen. Figures 2B and 2b show a carcinoma of the rectum as a central focus anterior to the sacrum, 40 hr after the antibody injection in Patient 9. There is no interference from the urinary bladder since the tracer excretion in the urine is low. Had the tracer accumulated in the bladder, it probably would have interfered with the detection of the tumor in this case. The two linear images on the anterior side of the tumor on the transaxial sections are the iliac arteries (Fig. 2b).

One of the two false-negative images of a primary tumor

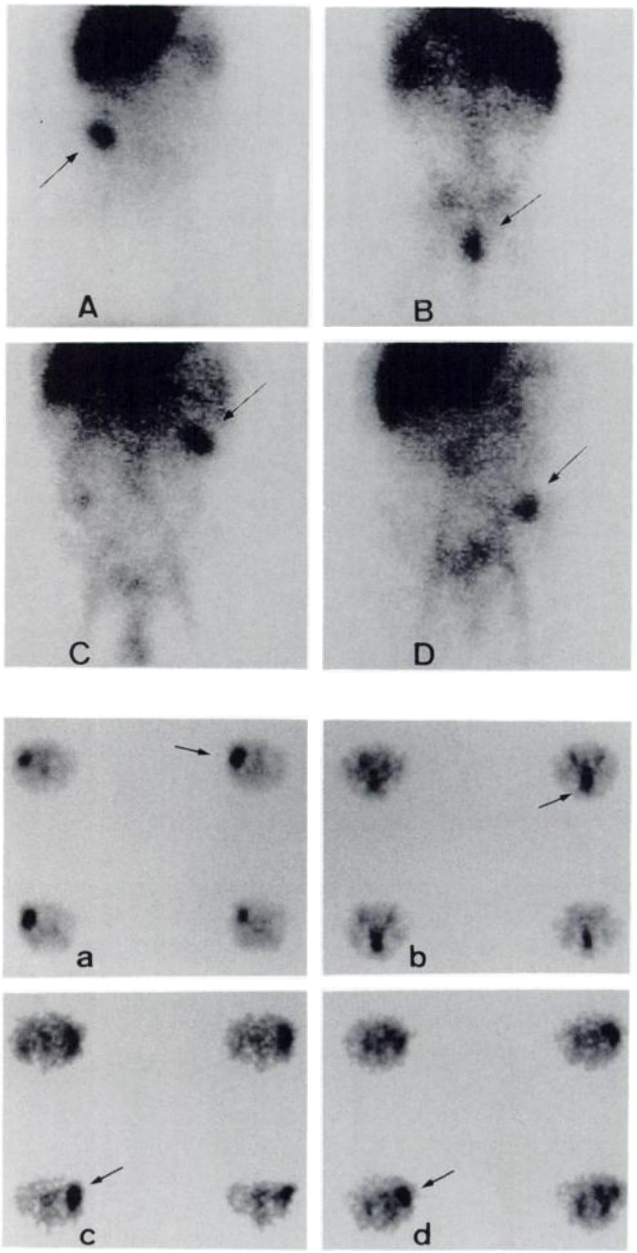

FIGURE 2. Planar and transaxial SPECT slices of four patients. One planar image (A–D) and a set of 4 slices (a–d) are displayed for each patient. The patients depicted in A/a, B/b, C/c and D/d are numbered 4, 9, 13 and 14 in Table 1, respectively. Planar scintigraphies include the whole abdomen in either anterior (A, C, D) or posterior view (B). The transaxial slices (2 pixels) are displayed from top to bottom, with a 2-pixel space between the slices which are shown. The slices were selected to pass through the upper, center and lower part of each tumor. Arrows point to the tumors.

(Patient 2) was a tumor of the sigmoid colon with much fibrosis. We cannot explain why, in spite of high ^{67}Ga recovery in the surgical specimens relative to normal tissue, it was not seen by scintigraphy. A possible explanation is that the tissue samples were not representative of the whole tumor. The other false-negative primary tumor (Patient 6) was associated with a very high serum CEA

concentration, 1600 ng/ml, which resulted in low recoveries of both ^{67}Ga and ^{125}I in the tumor specimens (0.0104% and 0.0015% ID/g for ^{67}Ga and ^{125}I, respectively). Interestingly, enlarged thoracic and abdominal lymph nodes could be seen on the images of this patient five days after the injection. Numerous microscopic metastatic tumor deposits were present in other nodes removed during surgery in the same case.

In the patients who received free deferroxamine from the time of the antibody injection, five of five primary tumors were visualized by both planar scintigraphy and SPECT, taking into account the patient with two primary tumors (Table 1). Figures 2C and 2D (planar) and Figures 2c and 2d (SPECT) show the images from two of these patients. A tumor of the left flexure (Patient 13) is shown 40 hours after the injection in Figures 2C and 2c, with low activity in the large bowel, blood pool and genitalia. Figures 2D and 2d show a tumor of the descending colon obtained one day after the injection in Patient 14. It is best seen on the transaxial sections because the activity in blood is still high.

Specimens from the tumor and normal tissue (blood, normal mucosa, fat, skin) were obtained in all patients; in addition, a liver biopsy was obtained during the operation in eight cases. The mean %ID/g recovered in all tumors three to nine days after injection was 0.019% for ^{67}Ga and 0.005% for ^{125}I, a statistically significant difference ($p < 0.001$, Student's paired t-test). Figures 3 through 5 compare the recoveries of ^{67}Ga and ^{125}I in three subgroups of patients who had surgery at different times after the injection: 3 days, 5–6 days or 7–9 days. The results appear more favorable for both radionuclides in the two patients who underwent surgery on Day 3 (Fig. 3). This is in good agreement with the comparison of the ^{67}Ga images obtained early (1–2 days) or late (5 days) after the antibody injection. For the six patients who had surgery on Days 5 and 6, the ^{67}Ga %ID/g is definitely higher than ^{125}I in the tumor, but this advantage is at the cost of a higher liver uptake of ^{67}Ga (Fig. 4). In the five patients who had surgery later, significant ^{67}Ga concentrations remain in the tumor and liver, while the ^{125}I in the tumor is definitely lower (Fig. 5).

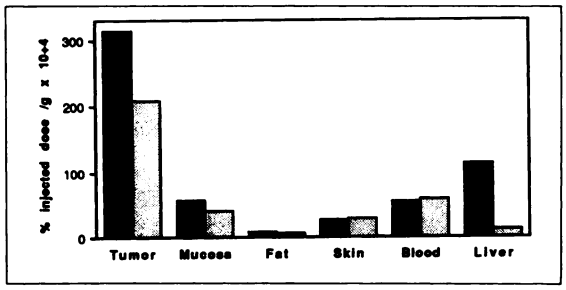

FIGURE 3. Comparison of the dose recovery of ^{67}Ga and ^{125}I in tumor and normal tissue. Results from 2 patients operated 3 days after the Mab injection. The numbers on the y-axis are %ID/g multiplied by 10^4 (i.e., 10^{-6} ID/g). Black columns represent ^{67}Ga and gray columns ^{125}I.

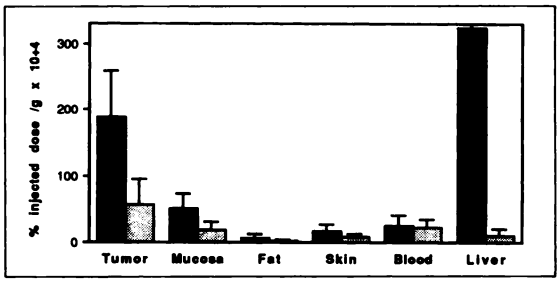

FIGURE 4. Comparison of the dose recovery of ^{67}Ga and ^{125}I in tumor and normal tissue. Results from 6 patients who had surgery 5 to 6 days after the Mab injection. Same scale as in Figure 3. Black columns represent ^{67}Ga and gray columns ^{125}I.

The individual ^{67}Ga and ^{125}I recovery values in the surgical specimens from all patients are shown in Table 2. Clearly, the high specific radioactivity of the ^{67}Ga-labeled antibody did not alter its biodistribution. The recovery values of ^{67}Ga and ^{125}I are indistinguishable in blood, and in 58 out of 60 samples of tumor, normal mucosa, skin, subcutaneous fat and liver, the percentage ^{67}Ga injected dose recovery per gram is higher than the corresponding ^{125}I recovery value. Table 2 also shows the statistical analysis of the difference between the two radionuclides, performed by the Student's paired t-test on the 13 samples of tumor and normal tissues and the eight samples of liver.

The ^{67}Ga tumor-to-normal-tissue ratios were higher than that of ^{125}I in 43 out of 52 samples of blood, normal mucosa, subcutaneous fat and skin. The mean tumor-to-normal-tissue radioactivity ratios for the specimens of all patients are shown in Figure 6. The mean ^{67}Ga ratios are higher than five for the samples of blood, skin and fat. The difference between the tumor-to-mucosa ratios of ^{67}Ga and ^{125}I is small when compared to the difference between the tumor-to-normal-tissue ratios for blood, skin and fat.

DISCUSSION

Twelve of 14 primary tumors in this study were clearly visualized by using the ^{67}Ga-labeled anti-CEA Mab. We had one false-positive result in a patient who had an ill-defined, nontumoral lesion of the cecum, and four false-negatives consisting of two missed primary tumors and two liver metastases. The four false-negative results were in patients not receiving deferroxamine infusion.

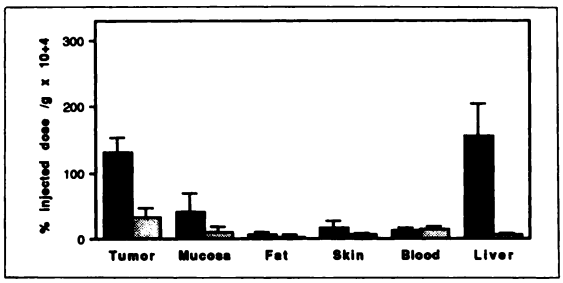

FIGURE 5. Comparison of the dose recovery of ^{67}Ga and ^{125}I in tumor and normal tissue. Results from 5 patients who had surgery 7 to 9 days after the Mab injection. Same scale and symbols as in Fig. 3.

TABLE 2
Recoveries of the Injected Doses in Tumors and Normal Tissues

Patient no.	Tumor		Mucosa		Fat		Skin		Blood		Liver	
	^{67}Ga	^{125}I	^{67}Ga	^{125}I	^{67}Ga	^{125}I	^{67}Ga	^{125}I	^{67}Ga	^{125}I	^{67}Ga	^{125}I
2	157*	28†	54	11	12	7.1	1.8	1.1	18	20	n.a.	
3	128	39	23	7.6	4.4	1.2	12	5.3	11	14	n.a.	
4	227	47	52	12	1.5	1.0	9.2	3.0	4.2	7.9	n.a.	
5	92	20	25	12	2.5	1.6	12	5.5	48	44	458	25
6	104	15	21	4.9	2.0	1.2	17	2.5	13	13	203	7.9
7	245	81	70	15	7.4	2.5	19	10	15	12	334	4.8
8	261	56	72	27	8.2	3.6	28	13	37	28	269	5.1
9	195	120	62	37	15	4.6	27	14	23	24	n.a.	
10	152	51	16	4.2	3.1	1.4	29	9.1	9.3	10	160	4.6
11	119	28	84	25	9.5	2.7	19	8.4	12	15	103	6.9
12	108	17	18	5.3	3.1	1.2	8.1	2.9	14	16	236	7.3
13	306	208	42	27	8.6	4.7	34	22	51	54	113	12
14	321	210	73	56	6.6	7.2	19	35	57	62	n.a.	
p value‡	<0.001		<0.001		0.002		0.007		0.576		0.001	

* ^{67}Ga, % ID/g × 10^4 (i.e., 10^{-6} ID/g).
† ^{125}I, % ID/g × 10^4.
‡ Paired t-test, ^{67}Ga compared to ^{125}I.
n.a. = not available.

The comparison of the dose recovery in tumor and normal tissues of ^{67}Ga with that of ^{125}I-labeled antibody allows us to draw some general conclusions from a relatively low number of patients studied. The mean percentage of injected dose of ^{67}Ga in resected tumors is significantly higher (almost four times) than that of ^{125}I. It is interesting that this higher tumor localization is obtained despite the fact that the specific radioactivity of the ^{67}Ga-labeled antibody was higher than that of the ^{125}I-labeled antibody, since antibodies labeled to higher specific radioactivity usually have a less favorable biodistribution (e.g., *10–12*). Also, we noted that the mean ^{67}Ga recovery in all tumors (0.019 %ID/g) is high, i.e. comparable to the highest results published for antibodies labeled with other radionuclides (*13–18*), despite the fact that tumor radioactivity was measured relatively late after the antibody injection (average six days).

The high affinity of deferroxamine for gallium and the stability of the oxime link between aminooxyacetyldeferroxamine and the oxidized carbohydrate of the antibody probably contributed to the improved localization of ^{67}Ga- as compared to ^{125}I-labeled antibody in tumors. Nevertheless, our results with ^{67}Ga-labeled antibody may have been influenced by the delabeling of antibody by tissue enzymes, by general antibody catabolism, or by the biodistribution of the free radionuclide.

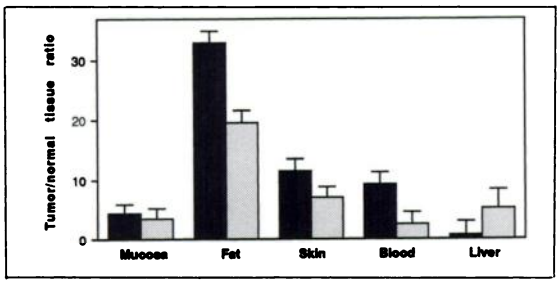

FIGURE 6. Tumor-to-normal-tissue ratios. The means and standard deviations were calculated from the logarithm of the individual tumor-to-normal-tissue ratios. Black columns represent ^{67}Ga and gray columns ^{125}I. The vertical bars are the standard deviations.

Antibodies can be delabeled by tissue enzymes even if the antibody label is chemically quite stable in vitro (*19–21*). In spite of the lack of firm evidence to support this hypothesis in our case, we cannot exclude the possibility that the better localization of ^{67}Ga in the tumors depended upon the susceptibility of the iodinated antibody to dehalogenases. There is stronger experimental evidence that the particular metabolism of the free radionuclides can influence the radioactivity distribution in immunoscintigraphy. Comparisons between radioiodinated and ^{111}In-DTPA-labeled antibody in both animal and clinical studies have shown that, following the delabeling of the antibody intra- or extracellularly, ^{111}In remained at the tumor site while free iodine was partially washed out (*20–21*). It is likely that our results depended in part on a similar difference between the metabolisms of free ^{67}Ga and free ^{125}I. However, the good imaging of tumors with ^{67}Ga-labeled antibody in patients receiving deferroxamine argue against an important fraction of accessible free ^{67}Ga in the tumors, since one would expect this fraction to be washed out.

As in the tumors, the recovery of ^{67}Ga and ^{125}I also differed in normal tissue. In spite of a higher percentage injected dose recovery of ^{67}Ga than of ^{125}I in all normal tissue specimens tested, with the exception of blood, the tumor-to-normal-tissue ratios of ^{67}Ga were higher than those for ^{125}I in 43 of 52 samples of blood, normal mucosa,

subcutaneous and skin. The situation was different for the liver, where the tumor-to-normal-tissue ratio for ^{67}Ga was lower than that for ^{125}I. This prevented positive visualization of liver metastases; the only liver metastases demonstrated were photopenic lesions. The high level of ^{67}Ga in liver was a constant finding, including the patient without tumor, and it was not dependent of the formation of immune complexes: high molecular weight material compatible with circulating immune complexes was detectable in the blood of only four patients.

Although the causes of the organ differences in the localization of ^{67}Ga after the injection of ^{67}Ga-labeled antibody remain conjectural, ^{67}Ga accumulation in liver is clearly similar to the localization of ^{111}In in that organ, which has been recognized by all authors using that radionuclide for antibody labeling (*20–25*). In an experimental system where anti-CEA antibodies biosynthetically labeled with ^{75}Se-selenomethionine were used, we also observed an accumulation of the radionuclide in the liver (*26–27*). We found evidence that the radionuclide in the liver was not the result of a simple delabeling of the antibody, but a consequence of the release of the radionuclide during antibody catabolism. We could obtain a significant decrease of the ^{75}Se deposited in the liver and other normal tissue by increasing the excretion of the free radionuclide, through the administration of cold selenium (*26*). Since this excretion of ^{75}Se was associated with significant increases of the tumor-to-normal-tissue ratios, it demonstrated that the free ^{75}Se was preferentially located in normal tissue, in particular the liver, kidneys and gastrointestinal tract. Preliminary results (unpublished) in tumor-bearing mice injected with ^{67}Ga-labeled antibody also showed a significant improvement of the tumor-to-normal-tissue ratios with deferroxamine infusions.

The administration of deferroxamine to increase the excretion of ^{67}Ga after the injection of ^{67}Ga-labeled antibody in patients is based on the hypothesis that the ^{67}Ga-labeled antibody might analogously be catabolized and that the free nuclide could be preferentially located or more easily mobilized in normal tissue. If this is correct, deferroxamine infusion could be a safe and convenient approach to improve the tumor-to-normal-tissue ratios of ^{67}Ga by increasing the excretion of the free ^{67}Ga. The approximately three-fold increase of the ^{67}Ga excretion rate in the urine which we have obtained from patients receiving deferroxamine is consistent with our hypothesis. However, it is obvious that, due to the quite small number of scintigraphic studies available in patients receiving deferroxamine infusions, no conclusion can be drawn yet concerning the expected improvement of tumor detection with deferroxamine infusion.

In conclusion, high specific radioactivity anti-CEA antibody labeled with ^{67}Ga using aminooxyacetyldeferroxamine attached through oxidized carbohydrate seems to be a good agent for imaging primary colorectal carcinomas. This radioimmunoconjugate appears to be particularly advantageous in the pelvic region. By comparing the percentage of the injected dose of ^{67}Ga per gram in surgical specimens to that of the iodinated antibody, we demonstrated higher levels of ^{67}Ga-labeled antibody as well as higher tumor-to-normal-tissue ratios, except for the liver. While the accumulation of ^{67}Ga in the liver is obviously a disadvantage (and we envisage further steps with a view to reducing or eliminating it), the ^{67}Ga-complex described above seems to offer promising advantages over ^{125}I-antibody labeled by the iodogen method.

ACKNOWLEDGMENTS

We thank Professors A. Rohner and Y. Kapanci for their encouragement. We are grateful to Mr. M. Velasquez for the imaging procedures; to the nuclear medicine laboratory team for processing the surgical specimens; to Ms. N. Lavanchy for analysis of serum samples; and to the nurses of the clinic of Digestive Surgery who cared for the study patients. We thank Mr. Ho Ing for endotoxin assays and Dr. G. Ducel for the bacteriological controls. This work was supported by the Swiss National Foundation for Scientific Research (J.E.R., K.R., R.E.O.) and by the Ligue Suisse contre le Cancer and the Zentralschweizerische Krebsliga Luzern (I.F., R.E.O.).

REFERENCES

1. Haskell, CM, Buchegger F, Schreyer M, Carrel S, Mach JP. Monoclonal antibodies to carcinoembryonic antigen: ionic strength as a factor in the selection of antibodies for immunoscintigraphy. *Cancer Res* 1983;43:3857–3864.
2. Pochon S, Buchegger F, Pélegrin A, et al. A novel derivative of the chelon desferrioxamine for site-specific conjugation to antibodies. *Int J Cancer* 1989;43:1188–1194.
3. Ward MC, Roberts KR, Westwood JH, Coombes RCC, McCready VR. The effect of chelating agents on the distribution of monoclonal antibodies in mice. *J Nucl Med* 1986;27:1746–1750.
4. Rodwell JD, Alvarez VL, Lee C, et al. Site-specific covalent modification of monoclonal antibodies: in vitro and in vivo evaluations. *Proc Natl Acad Sci USA* 1986;83:2632–2636.
5. Cohen J, McConnell JS. Observations on the measurement and evaluation endotoxemia by a quantitative Limulus lysate microassay. *J Infect Dis* 1984;150:916–924.
6. Markwell MAK, Fox CF. Surface-specific iodination of membrane proteins of viruses and eucaryotic cells using 1,3,4,6-tetrachloro-3a,6a-diphenylglycoluril. *Biochemistry* 1978;17:4807–4817.
7. Buchegger F, Mach JP, Leonnard P, Carrel S. Selective tumor localization of radiolabeled anti-human melanoma monoclonal antibody fragment demonstrated in the nude mouse model. *Cancer* 1986;58:655–662.
8. Sephton RG, de Abrew S. Effects of desferroxamine on Ga-67 distributions in tumor-bearing mice. *Proc Soc Exp Biol Med* 1979;161:402–406.
9. Oster ZH, Som P, Sacker DF, Atkins HL. The effects of deferoxamine mesylate on gallium-67 distribution in normal and abscess-bearing animals: concise communication. *J Nucl Med* 1980;21:421–425.
10. Meares CF, Goodwin DA. Linking radiometals to proteins with bifunctional chelating agents. *J Protein Chem* 1984;3:215–228.
11. Matzku S, Kirchgessner H, Dippold WG, Brüggen J. Immunoreactivity of monoclonal anti-melanoma antibodies in relation to the amount of radioactive iodine substituted to the antibody molecule. *Eur J Nucl Med* 1985;11:260–264.
12. Saccavini JC, Bohy J, Bruneau J. Radiolabeling of monoclonal antibodies. *Nucl Med Biol* 1986;13:191–194.
13. Mach JP, Carrel S, Forni M, Ritschard J, Donath A, Alberto P. Tumor localization of radiolabeled antibodies against carcinoembryonic antigen in patients with carcinoma. *N Engl J Med* 1980;303:5–10.
14. Miraldi FD, Nelson AD, Kraly C, et al. Diagnostic imaging of human neuroblastoma with radiolabeled antibody. *Radiology* 1986;161:413–418.

15. Carrasquillo AC, Bunn PA, Keenan AM, et al. Radioimmunodetection of cutaneous T-cell lymphoma with In-111-labeled T101 monoclonal antibody. *N Engl J Med* 1986;315:673–680.
16. Colcher D, Esteban JM, Carrasquillo JA, et al. Quantitative analyses of selective radiolabeled monoclonal antibody localization in metastatic lesions of colorectal cancer patients. *Cancer Res* 1987;47:1185–1189.
17. Eary JF, Press OW, Badger CC, et al. Imaging and treatment of B-cell lymphoma. *J Nucl Med* 1990;31:1257–1268.
18. Granowska M, Mather SJ, Britton KE. Diagnostic evaluation of In-111 and Tc-99m radiolabelled monoclonal antibodies in ovarian and colorectal cancer: correlations with surgery. *Nucl Med Biol* 1991;18:413–424.
19. Halpern SE. The advantages and limits of indium-111 labeling of antibodies; experimental studies and clinical applications. *Nucl Med Biol* 1986;13:195–201.
20. Khaw BA, Cooney J, Edgington T, Strauss W. Differences in experimental tumor localization of dual-labeled monoclonal antibody. *J Nucl Med* 1986;27:1293–1299.
21. Perkins AC, Pimm MV. Difference in tumour and normal tissue concentrations of iodine- and indium-labelled monoclonal antibody. *Eur J Nucl Med* 1985;11:295–304.
22. Halpern SE, Dillman RO, Witztum KF, et al. Radioimmunodetection of melanoma utilizing In-111 96.5 monoclonal antibody: a preliminary report. *Radiology* 1985;155:493–499.
23. Taylor A, Milton W, Eyre H, et al. Radioimmunodetection of human melanoma with indium-111-labeled monoclonal antibody. *J Nucl Med* 1988;29:329–337.
24. Carrasquillo JA, Abrams PG, Schroff RW, et al. Effect of antibody dose on the imaging and biodistribution of Indium-111 9.2.27 anti-melanoma monoclonal antibody. *J Nucl Med* 1988;29:39–47.
25. Halpern SE, Hagan PL, Chen A, et al. Distribution of radiolabeled human and mouse monoclonal IgM antibodies in murine models. *J Nucl Med* 1988;29:1688–1696.
26. Ryser JE, Carrel S, Buchegger F, Donath A. Immunoscintigraphy with biosynthetically-labeled ^{75}Se-antibodies. I: biodistribution of the ^{75}Se-MABs and of the free radionuclide. *Nucl Med Biol* 1990;17:487–494.
27. Ryser JE, Carrel S, Buchegger F, Donath A. Immunoscintigraphy with biosynthetically-labeled ^{75}Se-antibodies. 2: comparison with antibodies labeled by radioiodination. *Nucl Med Biol* 1990;17:495–497.

CORRECTION

Due to a production error, in the August 1992 issue of the *Journal*, Figures 1 and 2 in the article "Reversible Thallium-201 Perfusion Defects of the Septal and Inferoapical Segments in a Patient with Incomplete Right-to-Left Bundle Branch Block and Normal Coronary Angiogram" by Shih et al. were labeled incorrectly. The corrected figures are shown below.

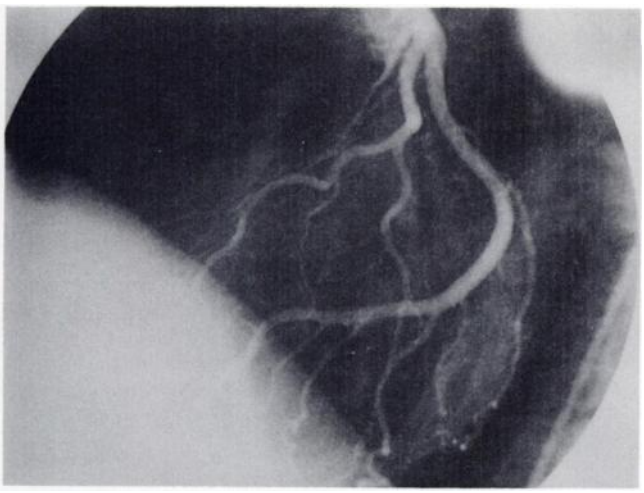

FIGURE 1. Left anterior oblique projection of coronary angiogram shows normal appearance of septal, left anterior descending, diagonal and circumflex arteries.

FIGURE 2. Thallium-201-chloride myocardial images on left anterior oblique views show decrease perfusion in the septal and inferoapical walls during stress (S) and redistribution of these areas at rest (R) consistent with an ischemic pattern.