Pharmacokinetics in patients of an anti-carcinoembryonic antigen antibody radiolabeled with indium-111 using a novel diethylenetriamine pentaacetic acid chelator

Donald J. Hnatowich
University of Massachusetts Medical School

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Pharmacokinetics in Patients of an Anti-Carcinoembryonic Antigen Antibody Radiolabeled with Indium-111 Using a Novel Diethylenetriamine Pentaacetic Acid Chelator


ABSTRACT

The pharmacokinetics of the C110 anti-carcinoembryonic antigen antibody radiolabeled with 111In via a novel benzylisothiocyanate derivative of diethylenetriamine pentaacetic acid have been determined in 12 patients. The chelator was attached to the protein via a thiourea bond and in such a way that all 5 carboxymethyl arms were presumably able to participate in chelation. Patients with known or suspected colorectal carcinoma received between 5 and 20 mg of the IgG antibody labeled with 5 mCi of 111In. Individual organ radioactivity levels were quantitated, and serum and urine samples were analyzed, principally by size exclusion high-performance liquid chromatography (HPLC). Total urinary excretion averaged 0.18% of the injected dose/h with large patient to patient variation. At early times postadministration (<8 h) the predominant radiolabeled species in urine was free diethylenetriamine pentaacetic acid most probably administered as a small radioccontaminant in the injectate. Thereafter, radioactivity in urine was primarily present as a low molecular weight catabolic product. Analysis of serum by size exclusion HPLC occasionally showed 3 radioactivity peaks, 2 of which are due to circulating immune complexes and labeled antibody. The third peak is of low molecular weight and is due to one or more products of antibody catabolism. Transchelation of 111In to circulating transferrin was observed but at modest levels. Quantitation of organ radioactivity showed that 18 ± 4 (SD)% of the injected dose was in the liver at 1 day postadministration and 1.4 ± 1.1 and 1.2 ± 0.9% was in the spleen and in both kidneys, respectively, at this time. The mean-life for clearance of total injected radioactivity was fitted to a single exponential and was found to be 34 h (SD, 14 h; N = 13) and that for antibody alone, assessed by size exclusion HPLC analysis of serum samples, was calculated to be 22 h (SD, 8 h; N = 10). Neither of these values nor organ radioactivity levels were affected by antibody-loading dose.

INTRODUCTION

A derivative of the bifunctional chelator DTPA has recently been synthesized in which a p-isothiocyanatobenzyl moiety is attached to a methylene carbon atom on one of the carboxymethyl arms (1). This chelator was intended to be covalently conjugated to antibodies as a means of radiolabeling these proteins with 111In (2). At present, the method most often used for this purpose attaches DTPA through either its mixed (3) or cyclic anhydrides (4). There are at least 2 major differences in this new approach to labeling antibodies: (a) whereas both anhydrides conjugate DTPA via amide bonds, the new chelator is conjugated via a thiourea bond, and (b) when attached via its anhydrides, DTPA is conjugated through one of its carboxymethyl arms which may not then be available to participate in chelation of indium. By contrast, conjugation of the new chelator preserves all 5 carboxymethyl arms. It has been suggested that all 5 arms are necessary for the stable chelation of indium (5, 6).

The object of this investigation was to evaluate the pharmacokinetics in patients of one antibody conjugated with the new chelator and radiolabeled with 111In. The antibody selected for this study was the anti-CEA C110 developed by Sumerson et al. (2). For this investigation, the intact IgG antibody was conjugated with an average of 6 chelators/protein molecule and radiolabeled with 111In. 111In, 5 mCi, and the antibody, between 5 and 20 mg, were administered to 12 patients with documented or suspected colorectal carcinoma. In this report we describe the pharmacokinetic behavior of the label; a detailed description of the imaging results obtained in these patients will appear elsewhere.

MATERIALS AND METHODS

The C110-DTPA antibody-chelator conjugate, prepared and characterized as previously described (2), was supplied by Abbott Laboratories as a sterile, pyrogen-free solution in 0.05 M citrate buffer, pH 6, at a concentration of 5 mg/ml.

The conjugated antibody was radiolabeled with carrier-free 111In (NEN Dupont, Billerica, MA) by adding 5 mCi of this activity to the desired weight of antibody. The labeled antibody preparation was assayed by size exclusion HPLC using a single 7.5 x 300-mm TSK 250 column (BioRad Laboratories, Richmond, CA) or a single 10 x 300-mm Superose 12 column (Pharmacia, Piscataway, NJ) and 0.05 M phosphate buffer, pH 7.0, eluant. The output of an in-line radioactivity detector was digitized and stored in a multichannel analyzer to facilitate the calculation of peak areas (7). Recovery was determined by counting the effluent against a standard of the injectate.

Patients enrolled in this investigation gave informed consent and were studied with the approval of the Food and Drug Administration (IND BB 2732) and the appropriate institutional review committees. Each patient received 5 mCi and either 5, 10, or 20 mg of the labeled antibody in 180 ml of normal saline for injection by slow infusion (10 ml/min) into a peripheral vein. One predose administration and several postadministration blood samples were collected over 3–4 days in red-top Vacutainers so that serum would be available after clotting. A complete urine collection over the same period was also obtained. Regular whole body and spot images were obtained for patients 1–9 on an Ohio-Nuclear LFOV gamma camera and for subsequent patients on a Siemens Body Scan. Attenuation correction was achieved as previously described (8).

Several blood samples were collected in heparinized tubes so that the percentage of radioactivity bound to formed elements could be determined.

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2 To whom requests for reprints should be addressed, at the Department of Nuclear Medicine, University of Massachusetts Medical Center, 55 Lake Avenue North, Worcester, MA 01655.

3 The abbreviations used are: DTPA, diethylenetriamine pentaacetic acid; CEA, carcinoembryonic antigen; HPLC, high performance liquid chromatography; ID, injected dose; BSA, bovine serum albumin; PBS, phosphate-buffered saline; HAMA, human anti-mouse antibody.

For most patients, 3–5 serum samples were analyzed by size exclusion HPLC using a single 7.5 x 300-mm TSK 400 column (BioRad) or a single 10 x 300-mm Superoxide 12 column (Pharmacia) with 0.05 M phosphate buffer, pH 7.0, eluant. Serum samples were analyzed semiautomatically using a WISP injector (Waters Associates, Milford, MA) and an automatic fraction collector. Fractions were then counted in an automatic well gamma counter. In addition to the analysis of serum samples by size exclusion, sera from the first 4 patients were also analyzed by cation exchange HPLC using a single 7.5 x 75-mm SP 5PW column (Waters) with gradient elution from 0.02 M sodium acetate, pH 5.0, to 0.5 M sodium sulfate, 0.02 M Tris hydrochloride, pH 8.0, in 30 min. Urine samples from the first 5 patients were analyzed both by open column Sephadex G25 chromatography and by size exclusion HPLC using a single 7.5 x 300-mm I-60 column (Waters) and 0.05 M phosphate buffer, pH 7.0. Urine samples from subsequent patients were analyzed by size exclusion HPLC using the Superoxide 12 column and by anion exchange HPLC using a 7.5 x 75-mm DEAE column (Waters) with gradient elution from 0.02 M Tris buffer, pH 8.5, to 0.02 M Tris buffer, 0.5 M sodium chloride, pH 7.0, in 30 min.

Regardless of the method of analysis for both serum and urine samples, the in-line radiation detectors were too insensitive to record radioactivity levels so that fractions (0.2–0.8 ml) were collected for counting in a well gamma counter.

Radioactivity levels in all serum and urine samples were also determined by counting an aliquot of each sample in a well gamma counter. In a limited number of analyses, the percentage of serum radioactivity present as labeled antibody, immune complex, and occasionally as catabolic product(s) could be determined. In these cases, the SAAM29 computer program (9) was used to estimate serum clearance curves for each species separately. Because of the larger number of reliable measurements of antibody concentration, this analysis was most successful when applied to this species.

The determination of the rate and extent of transcomplexation of 111In from antibody in circulation to transferrin was achieved by goat anti-human transferrin affinity chromatography as described previously (10). The percentage of serum activity binding to the column is proportional to the concentration of 111In-labeled transferrin in the sample.

Serum samples obtained at between 14 and 210 days after antibody administration were also analyzed for HAMA. To each of several test tubes, 1 polyethylene bead (Precision Plastic Ball Co., Chicago, IL) previously coated with a mouse antibody (19–20; Centocor, Malvern, PA) was added along with 300 μl of bicarbonate buffer, pH 9.6, containing 1% human serum albumin. The beads were incubated for 1 h at 37°C and were then washed 2 times with PBS containing 1% human serum albumin and 0.05% Tween 20. A sample of a patient’s serum with a known titer of anti-mouse antibody was used as a standard. Three hundred μl of the control sera (1:5 serial dilutions in PBS) and the sera under investigation (diluted 1:4 in PBS) were added to separate test tubes containing the washed beads. The test tubes containing the beads were agitated on a laboratory rocker at room temperature for 1.5 h. The beads were then washed with PBS/Tween 20, and the tracer, 111In-DTPA-OC-125 F(ab’)2, at 50 ng/ml, was added. The beads were incubated for 16 h at room temperature and were then washed as before and counted in a well gamma counter.

RESULTS

The results of all tests to which the antibody was subjected, both before and after coupling and labeling, demonstrated that the injectate was safe for human use. Thus, the preparation was shown to be sterile, pyrogenic, and free of murine retroviruses, Mycoplasma, and potential toxic substances (Quality Biologics, Camden, NJ). Two preparations of conjugated C110 antibodies were used in this investigation: patients 1–9 received antibody from the first lot, while the remaining patients received antibody from the second. The average number of chelator groups per antibody molecule was 6 for both lots. Despite this relatively high degree of conjugation, the immunoreactivity of the antibody was unaffected (2).

Following radiolabeling, analysis by size exclusion HPLC showed that 96% (SD, 4%; N = 13) of the label was bound to antibody. The recovery during these analyses averaged 93% (SD, 4%; N = 13).

Twelve patients were enrolled in this study, each with documented colorectal carcinoma. The median age was 54 years, with a range of 36–71 years. Six patients received 5 mg, 5 received 10 mg, and 2 received 20 mg. The first patient received 5 mg of the labeled antibody preparation and 15 months later received a second administration of 10 mg. Table 1 identifies patients by number and lists the preinjection circulating CEA levels, antibody dose administered, the percentage of administered dose in the liver at 1 day, and the percentage of radioactivity in serum present as immune complex at approximately 1 day postadministration.

Organ Quantitation. The time-dependent radioactivity levels in liver are plotted in Fig. 1 with each patient identified by number. In most cases, liver accumulation of label is consistent with that observed in this laboratory for 2 other antibodies labeled with 111In (8, 10). Maximum values are reached immediately, with blood pool activity contributing significantly to the earliest measurement. Thereafter, high values persist throughout each investigation (not shown in Fig. 1) as a value for patient 3 of 16% ID in the liver at 142 h. Excluding patients 4 and 1 (second study of patient 1), the mean liver radioactivity level at 1 day postadministration is 18% (4% SD, N = 9).

Mean values for spleen uptake of radiolabel at approximately 1 day postadministration was 1.2% (SD, 1.1%; N = 13) and for both kidneys was 1.4% (SD, 0.9%; N = 13). As in the liver, spleen and kidney radioactivity levels were generally unchanged throughout each investigation.

Urinary Analysis. Release of radioactivity to urine was slow but steady in all patients, averaging 0.18% ID/h (SD, 0.08%; N = 13), a value which is less than that observed by us for 2 other antibodies (8, 10). Fig. 2 presents urinary radioactivity in percentage of injected dose (corrected for decay) present in each collection plotted at the middle of the collection period. Presented in this manner, it is clear that release of radioactivity into urine goes through a pronounced minimum at about 8 h postadministration.

Chromatography by G25 open column and I-60 HPLC showed that radioactivity in urine elutes predominantly in one peak. Size exclusion HPLC analysis using the Superoxide 12

Table 1. Antibody dose, preadministration circulating CEA antigen levels, percentage of serum activity present as immune complex, and percentage of administered activity in liver, at approximately 1 day postadministration

<table>
<thead>
<tr>
<th>Patient</th>
<th>Dose (mg)</th>
<th>Preadministration CEA levels (ng/ml)</th>
<th>Immune complex at 20–29 h (%)</th>
<th>Liver activity at 19–29 h (%) ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>24</td>
<td>9</td>
<td>18</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>11</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>145</td>
<td>26</td>
<td>20</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>2546</td>
<td>63</td>
<td>70</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>20</td>
<td>0</td>
<td>*</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>56</td>
<td>6</td>
<td>*</td>
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<tr>
<td>11</td>
<td>5</td>
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<td>34</td>
<td>26</td>
</tr>
<tr>
<td>12</td>
<td>5</td>
<td>0.6</td>
<td>7</td>
<td>21</td>
</tr>
</tbody>
</table>

*Not available.

1*, second administration to patient 1.
column showed a slight difference in retention time of this prominent peak between early and late urine collections. The resolution was improved by anion exchange DEAE HPLC analysis, and radiochromatograms obtained of a mixture of urine collected from one patient at 0-4 and 24-48 h showed 2 distinct peaks. The first was exclusively present in an identical analysis of the 0- to 4-h urine collection, while the second was alone in the 24- to 48-h urine collection. This behavior is similar to that observed by us previously in clinical trials with the 19-9 and OC-125 antibodies radiolabeled with 111In via the cyclic anhydride of DTPA. At early times, radioactivity in urine was primarily due to radiolabeled free DTPA clearing to urine following its introduction into serum as a small radiocontaminant of the injectate. At later times, however, this chelate was no longer observed in urine. Instead, the predominant species in late urines, while also of low molecular weight, no longer coeluted with labeled DTPA and was a product of antibody catabolism (8, 10).

Accumulations of 111In activity in the gastrointestinal tract was occasionally seen in late images.

Serum Analysis. Figs. 3 and 4 show the individual serum levels of total radioactivity for each patient identified by number. As seen best in Fig. 3, in almost every case a transient increase in serum radioactivity occurred at 15 min to 2 h postadministration. Since the initial time point in these figures is 15-30 min after the start of antibody infusion, the maximum is unlikely to be due simply to buildup of serum activity during a slow infusion. Furthermore, serum samples were removed from the arm contralateral to that receiving the infusate; thus this behavior cannot be attributed simply to contamination.

Size exclusion HPLC was performed on a total of 67 serum samples from the 12 patients. Serum samples from the first 10 patients were analyzed on a TSK 250 column (BioRad), while serum from later patients was analyzed on a Superose 12 column (Pharmacia). The radiochromatograms most often show a prominent peak corresponding to that of labeled antibody. However, species with both shorter and longer retention times were occasionally observed. Fig. 5 shows the most pronounced example of a radiochromatogram in which all 3 species are apparent. The radiochromatogram was obtained by analysis of the 24-h serum from patient 4. In this case the first peak (shortest retention time) predominates and is due to immune complexes resulting from high circulating CEA levels (Table 1). The second peak coelutes with the labeled antibody and is therefore at least largely due to radiolabeled antibody still present in serum. The third peak (longest retention time) coelutes in this analysis with radiolabeled DTPA. However, radiolabeled DTPA, administered as a small radiocontaminant in the injectate, may be expected to clear rapidly into urine from serum by glomerular filtration. Since this peak was observed only in sera collected after about 20 h postadministration, it is likely that the species responsible is not labeled DTPA but is a catabolic product with a similar retention time by size exclusion HPLC.

The analysis of each serum sample by size exclusion HPLC and under the assumption that each peak was due to a single radiolabeled species resulted in estimates for the fraction of radioactivity in serum present in each of 3 species. An attempt
preinjection CEA levels, organ uptake, immune complex, and catabolic species formation. Possibly because of the limited dose range (5–20 mg) and the limited number of patients at each dose, no correlation was apparent for most parameters. The sole exception was the relationship between preinjection CEA levels and the rate of formation of immune complex. The rate of formation was determined by the method of Beck and Rescigno (11) and was found to be directly proportional to CEA level.

The extent of transcomplexation of the label from antibody to transferrin was first investigated by cation exchange HPLC. In a previous study of the OC-125 antibody, this chromatographic system was used to identify the presence of circulating $^{111}$In-labeled transferrin and to estimate its concentration (10). In this study an identical analysis of serum from a patient who received 5 mg of antibody showed no evidence of labeled transferrin. Furthermore, a similar negative result was obtained using immunoprecipitation and electrophoresis with autoradiography. Consequently, in subsequent studies, an anti-human transferrin affinity column was used (10). In this way, the mean transcomplexation rate was found to be 3.3%/day (SD, 2.8%/day; N = 7) in patients receiving 10–20 mg of antibody. This value is lower than that observed earlier by us (8, 10) of about 9%/day for other antibodies labeled with $^{111}$In after conjugation with DTPA. In our previous studies, only 1 mg of antibody was administered to each patient and the antibody was conjugated with only an average of 1 chelator/molecule. In the present study of the C110 antibody, up to 20 mg was infused and an average of 6 chelators were attached. Thus the possibility was

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**Fig. 3.** Radioactivity in serum presented as a percentage of administered $^{111}$In (corrected for decay)/ml compared with time after administration. Patients identified by number. Only early time points (0–7 h) are presented.

A search for correlation was performed among antibody dose,
considered that the transchelation rate may depend on chelator concentration. Accordingly, in vitro 37°C serum incubations were performed at two different concentrations of labeled C110 antibody. The results are shown in Table 3. When the chelator concentration in serum was adjusted to be roughly equal to that of our previous patient studies (5 ng/ml), the transchelation rate was found to be approximately 5%/day. However, when the concentration was increased a factor of 25, the rate dropped to approximately 2%/day. The 5%/day value for transchelation obtained by in vitro incubation may be compared to the 9%/day value observed by us previously. The fact that the former rate is lower indicates that the chelator used in this study forms a chelate which is more stable to transchelation of 111In to transferrin.

With the exception of the second administration to patient 1, all preinjection sera showed no evidence of HAMA activity (i.e., HAMA levels of <30 ng/ml). Postadministration HAMA levels were determined once for each patient (with the exception of patients 9 and 11) on sera collected between 14 and 205 days. Patients 2, 4, 5, 7, 10, and 12 were negative for HAMA (i.e., titers of <80 ng/ml). The remaining patients showed HAMA titers of 140–8250 ng/ml and are therefore all considered positive. These results are in general agreement with a more complete analysis of anti-isotype and anti-idiotypic HAMA titers.

FIG. 1*.

Formed Element Binding. The binding of radioactivity to formed elements in blood was determined at several time points in 5 patients and in in vitro incubations in which the labeled antibody was added to normal blood at a concentration of 1 µg/ml. Analysis was by centrifugation and counting of the packed cells and platelets after multiple rinses with cold saline. At approximately 2 days postinjection 4.7% (SD, 4.3%; N = 5) of radioactivity in blood was bound to formed elements. Using differential centrifugation (12), we determined that almost all this radioactivity was on RBCs.

Patient Imaging. The image presented in Fig. 6 is typical of those obtained in this investigation. Shown is a posterior whole body image obtained 22 h postadministration of 20 mg of labeled antibody (patient 8). Activity is present in kidneys and liver and, to a lesser extent, in bone marrow. An intense focal lesion appears in the pelvis that is suspected of being due to metastatic colorectal cancer from a primary removed surgically 3 months previously. A detailed description of the imaging results obtained in these patients will appear elsewhere.4

DISCUSSION

This laboratory has conducted 2 previous pharmacokinetic investigations of antibodies in patients with the 19–9 (8) and the OC-125 antibodies (10) radiolabeled with 111In. A comparison of these results with that obtained in the present study is inexact even though all 3 antibodies are of the IgG1 isotype because the 19–9 and OC-125 antibodies were administered as the F(ab')2 fragment, while the C110 was used as the intact IgG. Furthermore, only 1 mg of 19–9 and OC-125 antibody was administered, whereas between 5 and 20 mg of C110 was used. A final significant difference is the chelator used. Whereas the 19–9 and OC-125 antibodies were both conjugated with DTPA using the cyclic anhydride, in the present study a derivative of DTPA was attached. Nevertheless, some similarities are apparent.

The level of radioactivity in the liver was similar in all 3 studies (18, 20, and 12% ID for the C110, 19–9, and OC-125, respectively, at 24 h). However, a higher liver level may be expected for the intact C110 antibody over that of the F(ab')2 fragments since Fc receptors in the liver are thought to contribute to the accumulation of IgG antibodies in this organ (13). Furthermore, it may have been anticipated that liver levels in this investigation would have been low (and possibly decreasing) judging from the favorable results obtained in mice with antibodies radiolabeled with 111In via an isothiocyanatobenzyl DTPA chelator (5). It has been suggested that the decreased liver levels observed in animals may be due to dissociation of

the thiourea bond with the release from the liver of the intact chelate (14). If so, it is likely that the extent of this phenomena may be limited in patients such that liver levels are not greatly affected (15).

Particularly high liver radioactivity levels were observed in patients 4 and 1° (Table 1). The exceptionally high liver levels observed in the case of patient 4 are most likely due to antigen-antibody immune complex formation resulting from a high circulating CEA level with clearance into the liver. Size exclusion HPLC radiochromatograms show that a high molecular weight species formed in serum immediately upon antibody administration and persisted as the predominant species throughout this investigation. It is interesting that spleen and kidney levels were unremarkable in this case (0.6 and 0.4%, respectively, at 24 h). The explanation for the high liver levels obtained in the second administration to patient 1 may also be

the elevated CEA levels, although, in this case, the high liver levels (and the rapidly clearing serum radioactivity) may be due to a HAMA response resulting from the first injection of the C110 antibody to this patient 15 months previously. A serum sample from this patient obtained 7 months after the first antibody administration showed mildly elevated HAMA levels by two assays. The size exclusion HPLC radiochromatograms show that a high molecular weight peak appeared in serum within 1 h of the second antibody administration and became the predominant species at 20 h. These 2 cases illustrate the profound effect that immune complex formation can have on biodistribution.

The analysis of serum and urine samples in this investigation showed patterns which had been previously observed in the case of the 19–9 and OC-125 antibodies. A catabolic radiolabeled species with a molecular weight similar to that of labeled free DTPA appears in urine after approximately 8 h. It is likely that this species is generated in the liver since patient 4 exhibited levels among the highest urine levels and also had the highest level of radioactivity in liver (Fig. 1).

An important feature of this investigation was the determination of the number and concentrations of radiolabeled species in a large number of patient sera. As a result, it was possible to determine the half-life for clearance and the volume of distribution for the radiolabeled antibody itself rather than for the mixture of radiolabeled species which makes up the total serum radioactivity. Since these analyses showed that radiolabeled antibody was often the predominant species, the concentration of this species was determined with more certainty than that of the immune complex or catabolite especially in late samples. Because of physical decay and biological clearance, the counting rates of fractions obtained by HPLC analysis of these late samples were often close to the limit of detection. As such only values for the radiolabeled antibody are presented in Table 2. It is apparent from the table that, under the assumption of single compartment clearance, the terminal clearance half-time varied over a considerable range from patient to patient.

The volumes of distribution at steady state are not reported because of uncertainties in these values even in the case of radiolabeled antibodies. The error inherent in estimating the area under the clearance curve out to steady state became unmanageable due to low radioactivity levels in late serum samples. The initial volume of distribution for the antibody are more useful, although also subject to uncertainty, in this case because the antibody was administered by slow infusion, and thus the initial collection times are not well defined.

Based on the results presented in Table 2, the terminal clearance half-time for the antibody is 22 h. When the identical analysis is performed on the total serum radioactivity, this value becomes 34 h. The significant difference (P < 0.02) in these values may reflect the contribution to total radioactivity clearance of other species especially immune complexes. The longer clearance half-life found for total radioactivity over that of antibody itself reflects the fact that immune complexes and catabolites were accumulating in blood during the observation period. As expected, the plasma volumes calculated by height and weight and the initial volumes of distribution are in good agreement, especially for total radioactivity.

One surprising result of this investigation is the lack of influence of antibody dose on pharmacokinetics. Other investigators have reported decreasing liver accumulation and decreased serum clearance with increasing antibody dose. How-
ever these previous studies involved higher antibody doses and covered a larger range (16, 17).

The image presented in Fig. 6 illustrates some of the pharmacokinetic behavior of the C110 antibody; activity levels in blood pool are low at 22 h postadministration, and activity has accumulated in normal organs such as kidneys, liver, bone marrow, and spleen. Nevertheless, the image also shows striking accumulation of label in one suspected lesion in the pelvis. This accumulation demonstrates that the C110 antibody possesses interesting properties for imaging CEA-expressing tumors and that the method of labeling used in this investigation provides a useful alternative to standard methods of radiolabeling.

REFERENCES