Pharmacokinetics of an Indium-111-Labeled Monoclonal Antibody in Cancer Patients

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We have evaluated the pharmacokinetics in patients of a monoclonal antibody (19-9) $F(ab')_2$ fragment coupled with DTPA and labeled with ^{111}In. In addition to imaging and organ uptake determinations, serum and urine samples were analyzed to help determine the in vivo behavior of the label. Using a competitive binding assay, the immunoreactivity of the coupled fragment was found to be indistinguishable from that of the unmodified fragment. The absence of radiocolloids in the injectate was confirmed as was the in vivo stability of the attached DTPA groups. By a variety of techniques, we show that the only significant source of label instability was transcomplexation to circulating transferrin. About 9% per day of label exposed to transferrin (about 1–2% of the injected dose) dissociated with slight bone marrow accumulation. Following i.v. administration, serum activity levels fell rapidly ($T_{1/2\alpha}$ 2 hr, $T_{1/2\beta}$ 19 hr). Whole-body clearance of the label was slow ($T_{1/2}$ 160 hr) and may be attributed entirely to urinary excretion (0.26% of the injected dose per hour). Organ accumulation was greatest in the liver and persisted after rapidly attaining high values (20% of the injected dose). A total of 14 cancer patients were studied, nine with identifiable sites of metastatic disease from colorectal [8], pancreatic [2], ovarian [3], or small cell lung [1] primaries. Eight of the 12 sites of documented tumor were visualized by external imaging (67%) most distinctly at 48–72 hr postadministration.

J Nucl Med 26:849–858, 1985

Recent interest has focused on the application of monoclonal antibodies directed against tumor-associated antigens as in vivo diagnostic (*1–6*) and therapeutic reagents (*7*). These applications require knowledge of the pharmacokinetics and biodistribution of antibodies after administration to patients. Only through the use of antibodies stably radiolabeled with gamma-ray emitting radionuclides can these properties be determined in patients. Although radioisotopes of iodine have been employed for labeling antibodies, these labels are not stable and significant deiodination may occur rapidly in vivo (*8*).

We have recently described a method of labeling antibodies and other proteins (*9,10*) using the bifunctional chelate approach (*11*) in which the strong chelator diethylenetriaminepentaacetic acid (DTPA) is covalently attached to the antibody and used to bind metallic radionuclides such as indium-111 (^{111}In).

We have now conducted a clinical trial in 14 patients using the $F(ab')_2$ fragment of a murine monoclonal antibody (19-9) labeled with ^{111}In. The primary purpose of this study was to evaluate the stability and the in vivo distribution of the label in humans. Information on the biodistribution is necessary in planning diagnostic and therapeutic applications of monoclonal antibodies directed against tumor-associated antigens.

MATERIALS AND METHODS

Antibody labeling and testing

The 19-9 antibody used in this investigation was originally developed by Koprowski et al. (*12*) and is directed against an oligosaccharide (designated CA 19-9) found to be elevated in sera from patients with

Received Nov. 11, 1984; revision accepted May 7, 1985.

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carcinomas of the upper gastrointestinal tract and large bowel (*13*). Imaging studies in patients with radioiodinated intact 19-9 and its $F(ab')_2$ fragment showed significant accumulation of activity in the majority of colorectal cancer sites studied (*4*).

The 19-9 antibody was produced by intraperitoneal injection of hybridoma cells into pristane-primed BALB/c mice and purified from the ascitic fluid by chromatography on Protein-A-Sepharose CL-4B. The $F(ab')_2$ fragments were produced by adding 1.0% (w/w) pepsin to the IgG in 0.1*M* citrate buffer at pH 4.0 and incubating 16–18 hr at 37°C. Analysis by high performance liquid chromatography (HPLC) of the digest showed no remaining intact antibody. After purification by Protein-A-Sepharose CL-4B chromatography, the $F(ab')_2$ fragments were routinely found to be 95% pure by HPLC analysis. Prior to release for human use, the antibody* was independently shown to be apyrogenic by rabbit tests, to be sterile, and to be free of murine viruses, ecotropic viruses, and reverse transcriptases.

The coupling, purification, and labeling of the antibody fragments were performed under sterile conditions in a laminar flow hood equipped with an uv lamp. About 30 mg of the antibody was coupled with DTPA using the cyclic anhydride (*10*) so that the average number of attached DTPA groups per molecule was less than one. The preparation was purified from free DTPA on a 1 cm × 13 cm column of sterile Sephadex G50. Following purification, protein concentration was determined by uv absorbance ($E_{0.1\%}$ = 1.4 at 280 nm) and the preparation made 1% with respect to sterile HSA. The protein solution was then divided into 15 sterile 3-ml V vials so that each vial contained 1.0 mg of antibody in about 70 μl. The vials were then sealed and immediately frozen for storage.

The immunoreactivity of the DTPA coupled 19-9 $F(ab')_2$ was determined in a competitive binding assay against radioiodinated 19-9. Uncoupled $F(ab')_2$ fragments and fragments which had been coupled with an average of 1.6 DTPA groups per molecule were prepared in 0.05*M* citrate buffer, pH 4.0 containing 1% BSA and 0.1% thimerosal. Each antibody was then successively diluted in this buffer a total of 12 times so that antibody solutions at 12 concentrations between 0.1 mg/ml and 2.5×10^{-8} mg/ml were available. The 19-9 IgG antibody was radioiodinated with iodine-125 (^{125}I) using Bolton-Hunter reagent† to a specific activity of 12 μCi/μg. The radioiodinated protein was diluted in the same buffer and an equal volume added to each of the 24 antibody solutions. The final concentration of the iodinated antibody in these solutions was 0.03 μg/ml. Polyethylene beads‡ which were first derivatized and coupled with 19-9 IgG (*14*) and then exposed to the multivalent antigen CA 19-9 were incubated with each of the protein solutions in duplicate for 16 hr at 22°C. Each bead was then washed three times with distilled water and counted in a well counter.

Before each patient study, a vial was allowed to warm up to room temperature, and research grade ^{111}In§, which had been previously made 0.25*M* in sterile acetate ion at pH 5.0 was added with a sterile plastic pipette tip. About 2.0 mCi of activity was usually added in about 40 μl. The content of the vial was then diluted with 0.2 ml of sterile saline and drawn into a 3 ml syringe along with an additional 2.0 ml of saline. A 0.2-ml aliquot was then transferred to serve as an activity standard and as a determination of radiochemical purity. The latter was determined by HPLC analysis using a single 7.8 × 300 mm I-125 gel exclusion column¶ and an in-line radioactivity detector connected both to a chart recorder and to a multichannel analyzer operating in multiscale mode (*15*).

Prior to clinical trials, several vials were selected at random and reconstituted. In vitro pyrogen** tests were performed and the results were confirmed with a rabbit pyrogen‡‡ test performed commercially. Sterility and safety testing were performed according to 21CFR 610.11 and 610.12.

Patient characteristics

Fourteen patients were studied, eight females and six males. Median age was 54, with a range of 29–70. Ten patients were receiving chemotherapy at the time of antibody administration; four of these received chemotherapeutic regimens containing cis-platinum. Histologic diagnosis included colorectal carcinoma [8], pancreatic carcinoma [2], ovarian carcinoma [3], and small cell lung cancer [1]. Nine patients had identifiable sites of metastatic disease by conventional staging methods, while five patients were considered disease free by conventional methods. All patients underwent a pre-study evaluation consisting of a complete history and physical examination, chest x-ray, complete blood count, differential and platelet count, SMA 12, urinalysis, and serum creatinine. All patients signed Informed Consent and were studied with the approval of the FDA (IND BB1863) and the appropriate institutional review committees.

Antibody administration and imaging

Each patient was administered 1.0 mg of fragments containing 1–2 mCi of ^{111}In either by rapid injection or slow infusion (over 45–60 min) into a peripheral vein. The patients were then observed continuously for the first 2 hr. Following administration, blood samples, urine samples, and feces were obtained. Blood studies were repeated to measure changes from pre-injection values.

Whole-body posterior images were obtained on an Ohio-Nuclear large-field-of-view camera equipped with a medium-energy collimator. Whole-body activity

was estimated without absorption correction using a program to convert the output of the scanning camera into a digitized whole-body computer image (*16*) and by imaging with an aliquot of the injectate in the field of view.

Quantitation of organ activities with attenuation correction was obtained by planar imaging on a Picker International DynaScan SPECT camera equipped with a medium-energy collimator. Computer-acquired anterior and posterior images were obtained and were combined using an empirically determined relation of arithmetic and geometric means which yield a uniform sensitivity with depth for a given thickness of absorber for point sources of ^{111}In. Using a ^{57}Co flood source, a posterior transmission image and an image without the patient were also obtained. The ratio of these two images was used in a quadratic relation to estimate regional patient thickness. This effective thickness was used in a second quadratic relation which relates patient thickness to camera sensitivity to yield regional activity in microcuries (*17*). The quadratic coefficients used in these two equations had been previously determined using an organ scanning phantom in which organ activity was obtained to 10% accuracy (*18*).

Sample analyses

Immediately upon clotting in vacutainers, blood samples were spun down and the serum recovered. An aliquot of each serum and urine sample was counted in a well counter against a standard of the injectate. Serum and urine samples were analyzed by gel filtration chromatography using both open-column and HPLC to help establish the chemical forms of ^{111}In in these samples. A 1 cm × 173 cm column of Sephadex G200 (flow rate 5 ml/hr) and a 1 cm × 150 cm column of Sephadex G 25 (flow rate 40 ml/hr) were both used at room temperature with 0.1*M* phosphate buffer pH 7.3 eluant. For the rapid analysis of multiple samples, HPLC was employed using a single 7.8 mm × 300 mm 300 SW column (molecular weight separation range 10,000 − 500,000 daltons for globular proteins) or a single 7.8 × 300 mm I-125 column (molecular weight separation range 2,000 − 80,000 daltons for globular proteins) again with 0.1*M* phosphate buffer pH 7.0 eluants. In the case of serum samples, and occasionally urine samples as well, the in-line radioactivity detector was too insensitive to detect eluant radioactivity so that the eluant was collected in fractions for counting in a well counter.

Serum samples were also analyzed by affinity chromatography using an anti-19-9 affinity column and an anti-human transferrin affinity column. The former was used to determine the fraction of activity in serum which was antibody-bound and the latter to determine the fraction of activity in serum which was bound to transferrin.

The anti-19-9 affinity column was prepared by covalently attaching 19-9 IgG to cyanogen bromide-activated Sepharose CL-4B and dividing the resin among several 1.0 cm × 4.0 cm columns. The resin was rinsed with a minimum of 4 ml of 0.05*M* sodium citrate at pH 4.0 containing 1% BSA and 0.001% thimerosal. Antigen for the column was conditioned cell medium harvested from confluent SW 1116 cells derived from a human colorectal adenocarcinoma (*19*). After adding the multivalent antigen, the columns were washed with 5 ml of the citrate buffer. The sample to be analyzed was then added and the column washed with 5 ml of the buffer. Finally, the column was regenerated by washing with 4 ml of 3*M* sodium thiocyanate followed by the citrate buffer. The effluent was counted in a well counter to determine the fraction of added activity which was retained. The anti-transferrin column was prepared by conjugating 4.0 mg of goat anti-human transferrin antibody[§§] with 1 g of cyanogen bromide activated Sepharose CL-4B using standard methodology. Several 0.5 cm × 1.0 cm columns were prepared and washed with 0.2*M* PBS. A sample for analysis was added to a column and the column rinsed with 5 ml of PBS. The fraction of activity which eluted was determined by counting the eluant in a well counter against a standard. The columns were regenerated between uses by washing with 5 ml of 3*M* sodium thiocyanate and re-equilibrated with PBS.

Samples were analyzed as soon as possible after collection. When necessary, the samples were stored at refrigerator temperatures. To ensure that significant changes were not occurring during storage, the earliest sample was always re-analyzed at the end of a study.

In vitro serum incubations were performed by adding labeled antibody to a concentration of 1 μg/ml in serum (a concentration similar to that circulating in vivo) to fresh serum either from patients or from healthy volunteers. Incubations were performed at 37°C and the pH of the serum was monitored to ensure that it remained near neutrality throughout each study.

The levels of circulating CA 19-9 antigen were determined by radioimmunoassay (*14*) on serum samples stored until ^{111}In had decayed beyond detection limits.

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. Radiochemical purity of the injectates averaged 95% ± 3% as determined by HPLC analysis. Radioactivity recovery during HPLC analysis averaged 97 ± 3% showing the absence of colloidal ^{111}In activity (which is retained by the HPLC column). Following the labeling procedure outlined above, nonspecific binding of ^{111}In to uncoupled 19-9 antibody fragments was undetecta-

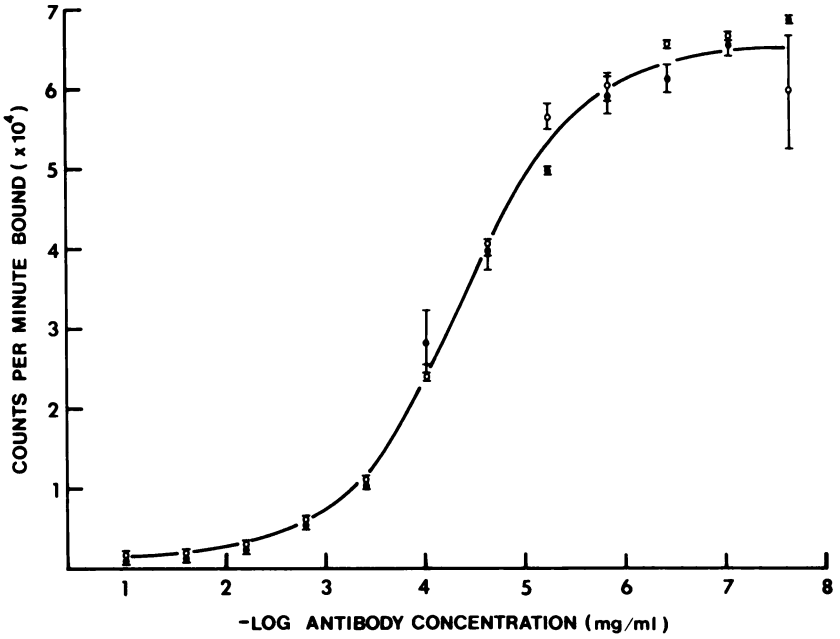

FIGURE 1
Results of competitive binding assay in which uncoupled $F(ab')_2$ fragments of 19-9 (open circles) and coupled (closed circles) were each separately placed in competition with radioiodinated 19-9 IgG for antigen bound to beads (see text), expressed as cpm on beads compared with negative log of unlabeled antibody concentration. Error bars show range of repeat measurements (N = 2)

ble by HPLC analysis even in the absence of free DTPA. HPLC analysis showed that about 4% of the protein was present in dimeric forms generated during the coupling with DTPA. These species were not removed, in part because they possess immunoreactivity (unpublished observations). The results of the competitive binding assay (Fig. 1) show that coupled and uncoupled $F(ab')_2$ fragments of 19-9 compete identically within experimental error at all concentrations studied.

Each injection, whether infused or rapid, was tolerated without visible effect. No acute toxicity (hypotension, tachycardia, skin rash, bronchospasm) was seen in any patient. No significant change in white cell and platelet count, hemoglobin levels, renal function, or liver function tests were seen by repeat blood tests performed up to 2 wk following antibody infusion. Counting of formed elements separated by centrifugation from blood showed no appreciable binding of radioactivity at any time.

Antigen-antibody complex formation and transcomplexation

Figure 2 shows the levels of circulating CA 19-9 in patients following administration of the antibody. Antigen levels varied considerably from patient to patient and ranged from essential zero to more than 200 units/ml. The majority of patients in this study had circulating antigen levels below 40 units/ml, a level which is considered to be normal (*13*). Moreover, in most patients, the antigen level dropped rapidly following antibody administration so that a minimum was reached in 30 min to 1 hr. Subsequently, the antigen levels slowly increased until initial levels were again attained about 4 days later.

The results of 19-9 affinity chromatography are presented in Fig. 3. In each case there was a rapid decrease in the percentage of activity in serum which binds to the column followed by an equally rapid increase so that a maximum was reached at about 1 hr. Thereafter, column retention decreased slowly at about 5–9%/day.

Figures 2 and 3 show the effects of circulating antigen and the formation of antigen-antibody complex.

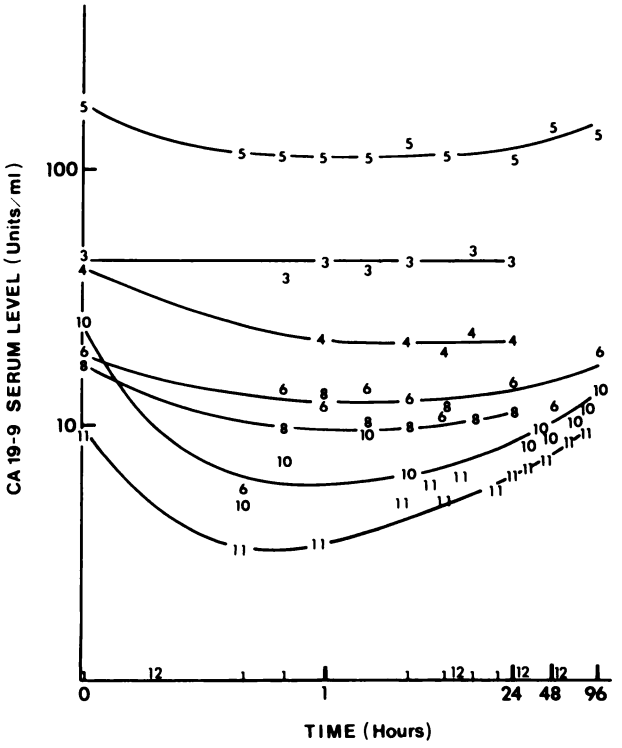

FIGURE 2
Level of circulating antigen (CA 19-9) in patients compared with time following administration of ^{111}In-labeled 19-9 antibody (log-log scale). Patients identified by number

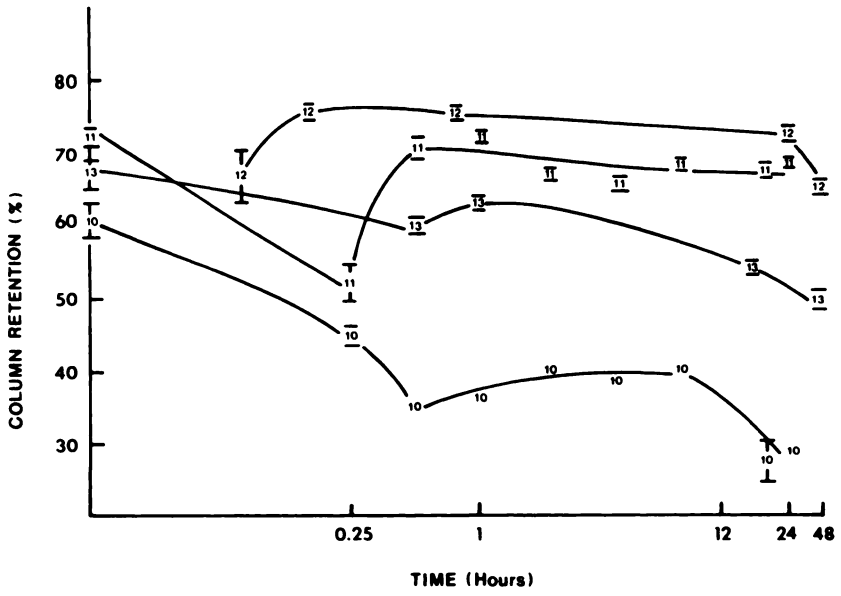

FIGURE 3
Percentage of activity in serum which binds to 19-9 affinity column vs. time following administration of labeled antibody (semi-log scale). Patients identified by number. Error bars indicate range of repeat measurements

The presence of antigen-antibody complex is also apparent in Fig. 4 which presents radiochromatograms obtained by G200 chromatography. Trace A is a radiochromatogram obtained by analysis of a serum sample collected at 20 hr post administration. The majority of activity is labeled antibody but a high molecular weight peak appears in the void volume in fractions 22-27, due probably to antigen-antibody complex. Trace B is the elution profile of [^{111}In]DTPA-transferrin; comparison with trace A shows that only 9–10% of the activity in serum at 20 hr was bound to transferrin as evidenced by the slight shoulder at fractions 40–43 on the antibody peak.

The degree of transcomplexation of ^{111}In from antibody to transferrin may also be estimated from the results presented in Fig. 5. The percentage of activity binding to the anti-human transferrin affinity column was determined for serum samples obtained from one patient. However, since the degree of transcomplexation will be underestimated if ^{111}In-transferrin clears from serum, in vitro serum incubation studies were also performed. Labeled antibody was incubated in sera from five normal volunteers and samples were removed periodically for analysis in duplicate. As shown in the figure, the results of the in vivo and in vitro analysis are in good agreement with both showing about 8–9% transcomplexation per day.

Figure 6 presents typical radiochromatograms obtained by Sephadex G25 gel filtration chromatography of urine samples. The chromatograms show a high and low molecular weight species varying in relative intensity from patient-to-patient. Trace A, obtained by the

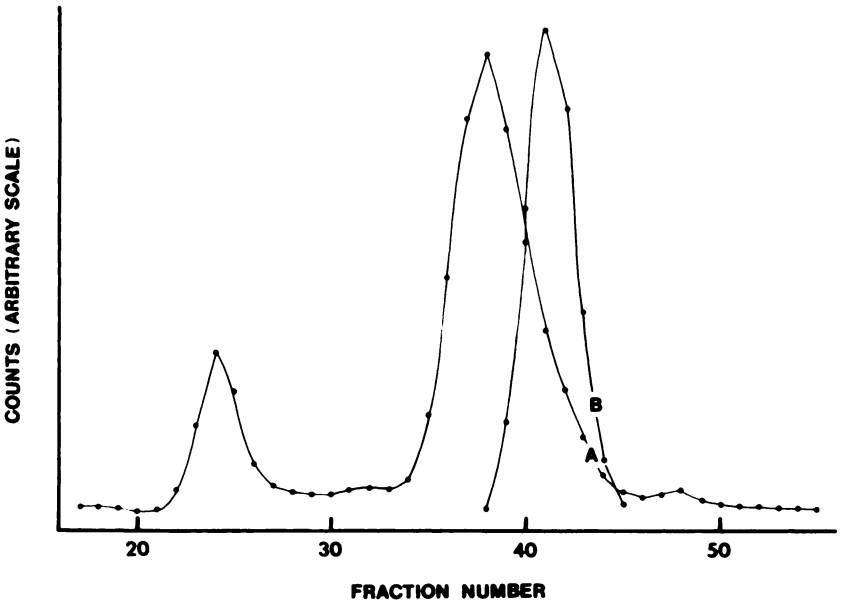

FIGURE 4
Radiochromatograms obtained by G200 Sephadex chromatography. Trace A: Radiochromatogram of serum obtained 20-hr postadministration. Trace B: Radiochromatogram of ^{111}In-labeled transferrin

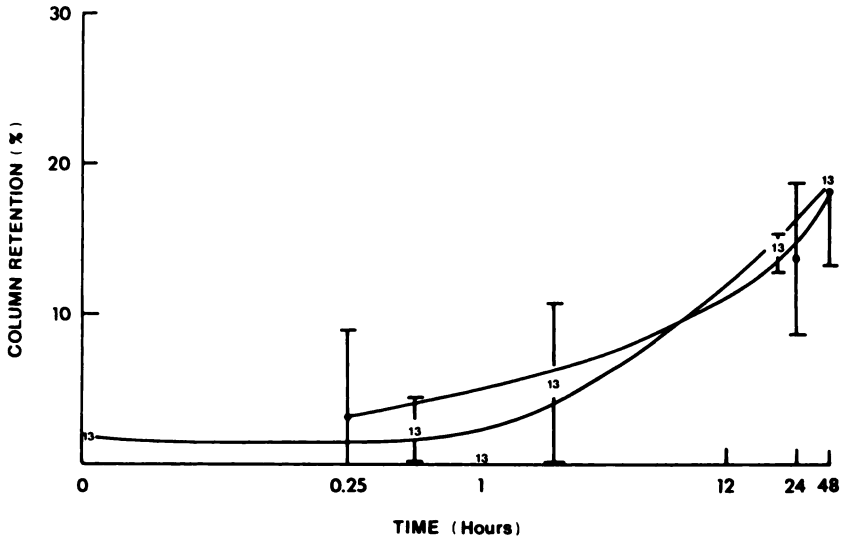

FIGURE 5
Percentage of activity in serum which binds to anti-human transferrin affinity column compared with time following administration of labeled antibody to Patient 13 or time of incubation of labeled antibody in five sera from normal volunteers (semi-log scale). Error bars indicate range of repeat measurements

analysis of a urine sample collected during the first 4 hr postadministration, shows that the majority of activity eluted in fractions 37–40. Since labeled-free DTPA also elutes in these fractions, the majority of activity appearing early in urine is probably labeled-free DTPA. This radiocontaminant is occasionally present at low levels in the injectate. However, as shown by traces B and C (urine samples collected at 12 and 44 hr post injection, respectively), there is a progressive decrease in activity eluting in these fractions and an increase in activity appearing in fractions 33–36. At the same time, there is a progressive increase in activity present as a higher molecular weight species appearing in fractions 22–25. Analysis of these samples by HPLC using the 300 SW column showed that this later species does not co-elute with labeled antibody but exhibits a somewhat lower molecular weight.

Free [^{111}In]DTPA, either administered along with the labeled antibody as a radiocontaminant or produced in vivo by dissociation of the covalent bond attaching the DTPA group, will clear serum by glomerular filtration and appear rapidly in urine. However, it is clear from Fig. 6 that after several hours, activity appearing in urine is not exclusively or even largely present in this chemical form.

Biodistribution studies

The biological clearance of activity in patients was determined relative to a standard by whole-body counting as previously described. By calculating whole-body

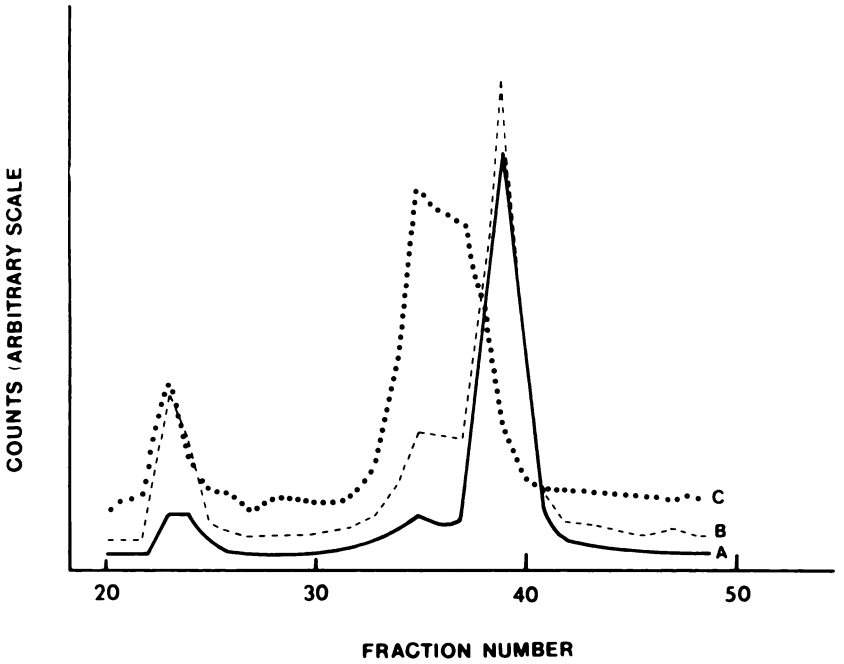

FIGURE 6
Radiochromatograms obtained by G25 Sephadex chromatography of urine samples. Trace A: Radiochromatogram of urine sample obtained 4 hr after administration of labeled antibody. Trace B: Radiochromatogram of urine collected at 12-hr post administration. Trace C: Radiochromatogram of urine collected at 44 hr postadministration

clearance half time from the loss of activity (after decay correction) between each pair of data points, a mean value of 160 hr (range 120–270 hr, N=10) was obtained.

The serum activity levels were determined by counting an aliquot of each serum sample in a gamma-ray well counter. The serum clearance curve was analyzed using a multiexponential least squares program (*20*) and found to be best represented by a two component curve. The first component has a half-life ($T_{1/2\alpha}$) of 1.9 hr and a time-zero value of 61% of the administered activity in total serum while the second component has a half-life ($T_{1/2\beta}$) of 19.3 hr and a time-zero value of 38%.

Activity in urine, when represented as percent of injected dose per 4 hr urine collection, regardless of urine volume, decreased slowly although in the case of four patients a maximum occurred at 4–12 hr. The mean excretion rate was 0.26% of the administered activity per hour (corrected for decay).

Activity appearing in spleen and kidneys were also quantitated and both show a tendency towards maximum accumulation at about 20-hr postadministration followed by a slow clearance. The mean value at maximum accumulation is about 2% of the administered activity (corrected for decay) for spleen and about 5% for each kidney.

The comparable results obtained in liver is presented in Fig. 7. The time course of activity accumulation in liver appears to be different from that observed for spleen and kidneys in that near maximum levels are usually reached almost immediately and thereafter decrease only slowly or remain steady. Patients 10 and 14 are clearly anomalous in that a maximum occurred and at a much higher level than that normally observed. The mean value in liver was 20 ± 8% of the administered activity at 24 hr postadministration.

A fairly complete stool collection was obtained in only one patient. About 1.1% of the injected dose appeared in feces between 18–66 hr postadministration.

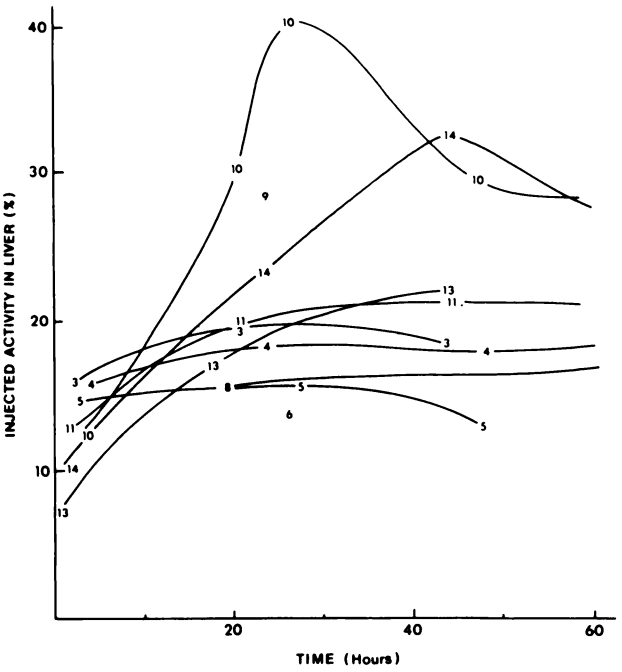

FIGURE 7
Activity in liver presented as percentage of administered activity (corrected for decay) vs. time after administration of labeled antibody. Patients identified by number. Most curves have been extended to data points off scale

Patient images

Figure 8 presents a series of whole-body posterior images obtained at 1, 24, 48, and 72 hr postadministration. The figure confirms the results described above in that the major sites of accumulation were liver and kidneys and that this accumulation was rapid and persistent. In the initial image, some activity appears in bladder and is due to [^{111}In]DTPA present as a radiocontaminant in the injectate. Also in the initial image, activity is prominent in blood pool which decreased such that at 48-hr blood vessels are barely discernable. At 48 hr some activity can be seen in the bowel. The amount of activity present in bone marrow increased slightly; however, the degree of accumulation was minimal.

Figure 9 represents a patient with both diffuse and focal metastases from a colon cancer which involved the mesentery. Imaging was performed at 48 hr and shows diffused increased abdominal uptake (arrow) with two regions of more focal uptake corresponding with the large tumor masses identified by CT (right panel).

Figure 10 presents an anterior image obtained at 72 hr postadministration. Both dark arrows point to sites of abnormal localization of activity (which progressively increased with time) at the biopsy site of a colorectal recurrence.

DISCUSSION

An object of this investigation was to confirm in patients our earlier observations that, when administered chelated to a DTPA-coupled antibody, ^{111}In remains attached to the protein in vivo. A phenomenon which would likely lead to in vivo instability is the nonspecific attachment of ^{111}In to the protein other than at the DTPA sites. The results of this investigation are consistent with our previous studies in showing no evidence for this nonspecific binding. When the activity is added as the acetate complex, labeling occurs only at the DTPA sites as shown by hydrolyzed control studies (*10*) and by attempts to label uncoupled antibodies in the absence of free DTPA. HPLC analyses of these samples consistently show no detectable radioactivity co-eluting with the protein. Furthermore, it is unlikely

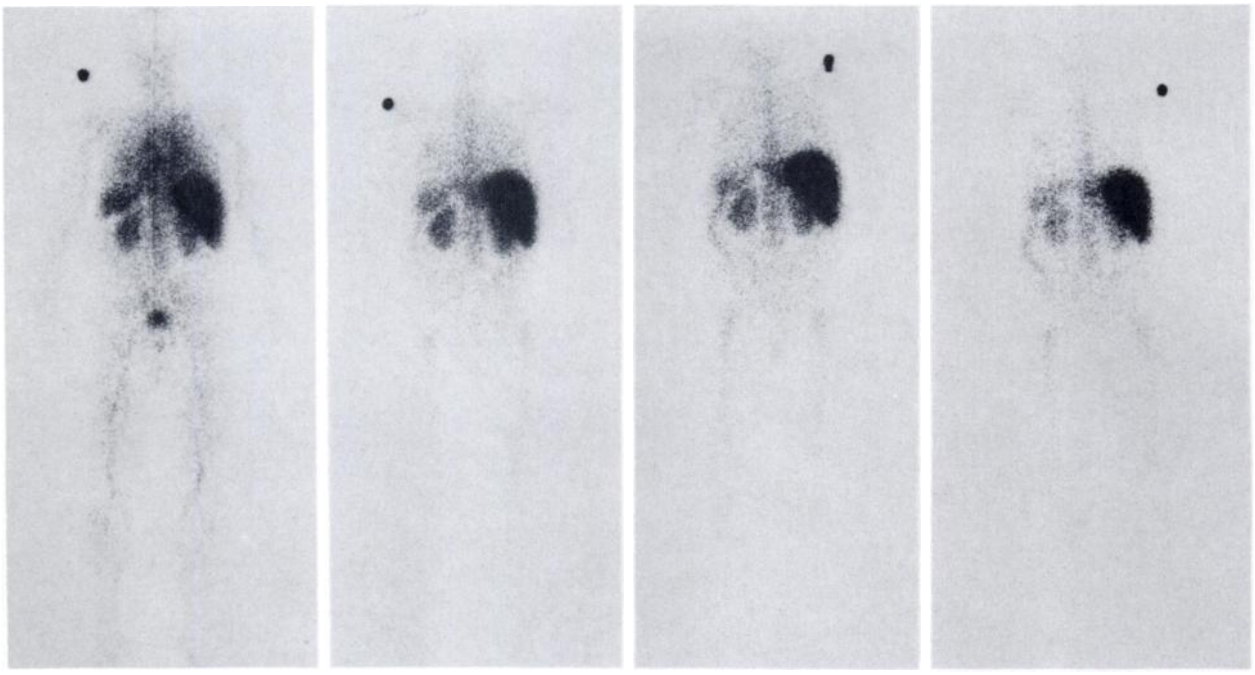

FIGURE 8
Series of whole-body posterior images obtained at 1, 24, 48, and 72 hr postadministration with labeled antibody (earliest image on the left). Aliquot of injectate was placed near shoulder during acquisition of each image

that nonspecific binding has occurred which dissociates during HPLC analysis since under these conditions we normally observe only low recovery of activity following HPLC analysis due to trapping of loosely bound activity on the HPLC column. As described above, recoveries were consistently high.

Another possible instability would result from the dissociation of the covalent bond attaching DTPA to the protein leading to the release of free DTPA still labeled with ^{111}In. Labeled free DTPA would rapidly appear in urine after clearance through the kidneys by glomerular filtration. However, as shown in Fig. 6, except for early time points, labeled DTPA is not present in urine.

The most likely form of instability is the extraction of ^{111}In from the DTPA chelate. The stability constant for the indium chelate of free DTPA [and coupled DTPA (*10*)] is extremely high so that dissociation is usually unlikely. However, this is not the case in the presence of unsaturated transferrin since the stability constant of the indium chelate with this metal-binding protein exceeds that of DTPA (*21*). Since transferrin is likely to be present in serum at a high concentration relative to that of DTPA, transcomplexation of ^{111}In from antibody to transferrin in serum is favored (*21*). Fortunately, the rate of transcomplexation is only about 9%/day of ^{111}In-labeled antibody exposed to transferrin as is apparent from Figs. 3–5. This value is consistent with our previous measurements with a different labeled antibody of 6%/day in mice (*10*) and 10%/day in serum incubations (*22*). This degree of transcomplexation probably has only minimal effect on the images obtained with ^{111}In-labeled antibody. Under the assumption that only labeled antibody in serum is exposed to transferrin, the degree of transcomplexation was only about 1–2% of the administered activity since serum levels decreased rapidly.

One important observation of this investigation is that ^{111}In clears slowly in patients administered labeled 19-9 F(ab')$_2$ fragments. The major route of excretion is into urine since the observed rate of this clearance (0.26% of administered activity per hour) by itself largely accounts for the observed 160-hr whole-body clearance half time and because only insignificant activity was observed in bowel and feces. At 24 hr postadministration, activity accumulation in liver, spleen, and kidneys accounts for about 30% of the injected dose, circulating serum contains a further 20%, leaving about 50% of the activity remaining. This remaining activity is uniformly distributed, probably throughout the interstitial spaces. The slow clearance may increase the exposure of tumor to antibody thereby improving tumor accumulation of activity.

The uptake of radioiodine in liver in the case of radiolabeled antibodies is reported to be lower than that observed in this work (*3*). If, as is likely, both radioiodinated and ^{111}In-labeled antibody behave essentially identically in vivo, then differences in activity accumulation in liver and other organs may be the result of catabolism occurring at these sites so that only radioio-

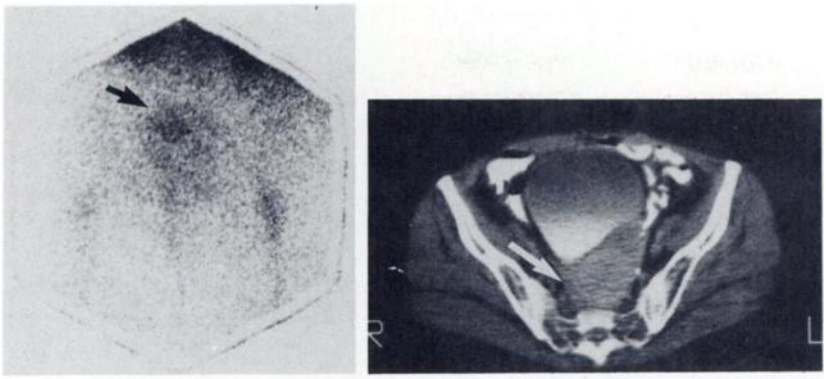

FIGURE 9
Images obtained at 48-hr postadministration of labeled antibody in patient with both diffuse and focal metastases from colon cancer involving mesentery (dark arrow). Corresponding CT image is presented on right and shows area of involvement corresponding to that seen in radioactivity scan (white arrow)

dine is re-released into circulation (probably as I^-). This is consistent with the observation that radioiodinated proteins are usually stable toward in vitro dehalogenation in serum (Krohn KA, personal communication) but yet considerable dehalogenation occurs in vivo with up to 50% of the administered activity appearing in urine in 24-hr postadministration (*8*).

Investigators comparing radioiodinated and ^{111}In-labeled antibodies in animal tumor models consistently report greater accumulation of ^{111}In in tumor (*9,23*). Presumably catabolism with the release only of radioiodine must be occurring in tumor as well as liver to explain these results. If elevated ^{111}In levels in tumor occur in humans as well, the resulting tumor/liver ratios may be similar for radioiodine and ^{111}In.

On the basis of images obtained in this investigation, eight out of 12 sites of documented tumor were identified. The earliest any lesion was apparent was 24 hr and all lesions became more clearly defined at 48 to 72 hr. No significant uptake occurred in tumor in four patients with large liver metastasis from colon primaries and the lesions appeared as cold defects.

The reasons for the large accumulation of activity in liver and its retention were not established in this study. Large accumulation of ^{111}In activity in liver has been previously noted in animal studies and has been shown, using ^{75}Se-labeled antibody, to be related to antibody localization in this organ (*24*). In this study, accumulation may be related to antigen-antibody complex formation. However no obvious correlation is apparent between hepatic uptake and circulating antigen levels or the decrease in circulating antigen levels following antibody administration (Fig. 1). Likewise, no obvious correlation exists between chemotherapy regimen, type of cancer, or presence of liver metastasis. For example, Patient 10 showed the highest liver levels (Fig. 7) yet was not receiving chemotherapy at the time of the study and has not been diagnosed as having liver metastasis.

A more likely explanation is that under certain conditions circulating antibody itself is cleared by the liver. The murine origin of the 19-9 antibody used in this study may contribute to this clearance. In addition, it is recognized that certain glycoproteins are rapidly cleared by liver nonparenchymal cells by a receptor-mediated process (*25*). If this process was occurring and was easily saturated, then rapid and persistent liver uptake would be expected.

The liver accumulation observed in this work for one antibody may have implications in the use of antibodies in therapy. Since specific localization of antibody in tumor is essential for adequate therapy to tumor tissue with acceptable collateral damage to normal tissue,

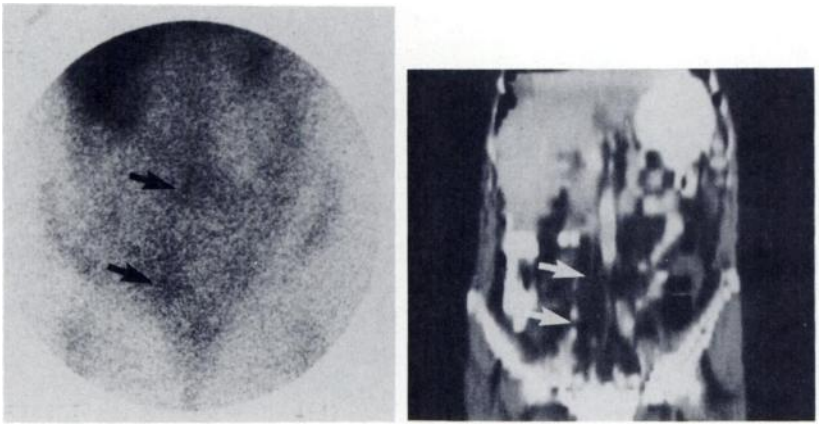

FIGURE 10
Image obtained at 72-hr postadministration of labeled antibody in patient with recurrent colorectal tumor. Both dark arrows point to sites of abnormal activity accumulation corresponding to tumor masses (white arrows) seen in saggital CT scan on right

extensive localization of antibody in liver may restrict therapeutic applications.

FOOTNOTES

* Biosafe Systems, Inc., New Hyde Park, NY.
† DuPont NEN Medical Products, No. Billerica, MA.
‡ Precision Plastic Ball Co., Chicago, IL.
§ Medi-Physics Inc., Richmond, CA.
¶ Waters Associates, Milford, MA.
** QCL-1000, Whittaker M.A. Bioproducts, Walkersville, MD.
‡‡ Findley Research, Inc., Assonet, MA.
§§ U.S. Biochemical Corp., Cleveland, OH.

ACKNOWLEDGMENTS

The authors thank L. E. Braverman for his help and support, J. Roche and P. McNabb for obtaining the images and M. King and R. Schwinger for their assistance in image quantitation. The authors are grateful to Medi-Physics for supplying the ^{111}In used in this study and above all, are indebted to the patients who cheerfully submitted to the rigors of this study and who did so often despite considerable discomfort. This work was supported in part by NIH grant CA 33029 and DOE contract ACO2-83ER60175.

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