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Pharmacokinetics of ^{111}In-labeled OC-125 Antibody in Cancer Patients Compared with the 19-9 Antibody[1]

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ABSTRACT

We recently reported on the pharmacokinetics in 14 cancer patients of the 19-9 antibody radiolabeled with ^{111}In. We have now repeated this investigation in 18 cancer patients using the OC-125 antibody, in part to compare the *in vivo* behavior of two murine monoclonal antibodies of the same subclass administered as the F(ab')$_2$ fragments, by the same route and at the same dose. As in the earlier investigation, 1 mg of fragments was infused i.v., and organ quantitation was obtained for up to 72 h along with frequent blood and urine samples for chromatographic evaluation. Analysis of urine showed that activity clearance by this route amounted to 0.29%/h and consisted of labeled DTPA only in early samples and metabolic products thereafter. Analysis of serum samples often showed the presence of a high-molecular-weight species appearing within 24 h. This species is probably due to antibody binding to circulating antigen, although the percentage of circulating activity present as this species did not correlate well with circulating antigen levels. As before, organ accumulation was greatest in the liver, although levels were significantly reduced (12% compared to 20% of administered dose at 24 h, $P < 0.01$). Plasma clearance was also significantly different: whereas the label in the case of the OC-125 antibody showed one-compartment clearance kinetics and remained in the plasma compartment, in the 19-9 case the label diffused to a second, unidentified compartment.

INTRODUCTION

Reports on clinical investigations using radiolabeled antibodies for the diagnosis of cancer now appear with increasing frequency (1). The majority of these studies have concentrated primarily on imaging and, as such, are important to the evaluation of the efficacy of radioimmunoscintigraphy. Also important, however, is the determination of pharmacokinetic parameters which may explain features within the images. Few clinical studies have investigated pharmacokinetics by measuring blood activity clearance curves and the rate of urinary excretion of activity, by quantitation of activity levels in normal tissues and in tumor and especially by the analysis of serum and urine samples to determine the chemical forms of the radioactivity present (2–5).

This laboratory recently reported on the results of a pharmacokinetic investigation in which the anti-colorectal tumor antibody 19-9 was administered, labeled with indium-111 (^{111}In), to 14 cancer patients (6). The 19-9 antibody is an IgG1 and was used in that study as the F(ab')$_2$ fragment after coupling via the cyclic anhydride of DTPA.[3] Each patient was studied following slow i.v. administration of 1 mg of fragments radiolabeled with 1 to 2 mCi of ^{111}In. Imaging was then performed at regular intervals, and radioactivity levels in liver, spleen, and kidneys were determined. Affinity and gel filtration chromatography was performed on serum and urine samples.

One important observation of that study was the slow whole-body clearance of radioactivity. The major route of excretion was found to be to urine (0.26% injected dose per hour) which by itself largely accounted for the observed 160-h whole-body clearance half-time. At 24 h postadministration, 30% of whole-body radioactivity was in liver, spleen, and kidneys, and 20% was in blood. Since focal areas of radioactivity accumulation in addition to the above organs were not apparent in the images, the remaining 50% whole-body radioactivity was uniformly distributed.

In the interest of determining these pharmacokinetic parameters for a similar antibody, we have extended this investigation to include the anti-ovarian tumor antibody OC-125. This antibody was developed by Bast *et al.* by immunizing BALB/c mice with human epithelial cells obtained originally from a patient with serous papillary cystadenocarcinoma (7). Like 19-9, the OC-125 antibody is an IgG1 and was used in this study as the F(ab')$_2$ fragment after labeling in the identical manner with ^{111}In. Furthermore, the OC-125 antibody recognizes CA-125, a high-molecular-weight ($M_r > 200{,}000$) glycoprotein (8), which, like that recognized by the 19-9 antibody, is released to the circulation (9).

The OC-125 antibody was administered to a total of 18 patients. As in the 19-9 study, each patient received 1 mg of fragments, containing 1 to 2 mCi of ^{111}In, by slow i.v. infusion. In addition to the imaging of all patients (10), accumulation in liver, spleen, and kidneys was quantitated in 16 patients by the planar technique used in the 19-9 study. Serum and urine samples were analyzed by open column and HPLC.

MATERIALS AND METHODS

The OC-125 antibody was produced by i.p. injection of hybridoma cells into pristane-primed BALB/c mice. The OC-125 IgG was then purified from filtered ascitic fluid by Protein A-Sepharose (Pharmacia, Piscataway, NJ) chromatography. The F(ab')$_2$ fragments were produced by digestion of the IgG antibody with 1% (w/w) pepsin in 37°C 0.1 M citrate, pH 4.2. Progress of the digestion was monitored by size exclusion HPLC analysis, and the digestion was terminated by neutralization after about 18 h when the IgG peak disappeared. The F(ab')$_2$ fragments were purified by passing the digest through the Protein A-Sepharose column to remove any undigested IgG or intact Fc antibody followed by passage through a Superose 6 filtration column (Pharmacia) to remove other protein fragments. The resulting OC-125 F(ab')$_2$ preparations were routinely >95% pure by size exclusion HPLC analysis. Prior to release for human use, each preparation was shown to be sterile, apyrogenic, and free of murine retroviruses, *Mycoplasma*, and potential toxic substances (BioSafe Systems, Inc., New Hyde Park, NY) as required by the Food and Drug Administration.

Antibody coupling with DTPA, purification, and radiolabeling of the fragments were performed as described previously (6). Three lots of coupled fragments were used throughout this study; each was prepared by coupling 20 to 30 mg of fragments using the cyclic anhydride of DTPA (11) such that the average number of attached DTPA groups

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[3] The abbreviations used are: DTPA, diethylenetriaminepentaacetic acid; HPLC, high-performance liquid chromatography; Cl, serum clearance; MRT, mean serum residence time; V_c, volume of the central compartment; V_{dss}, volume of distribution at steady state.

per fragment molecule was approximately one. Following purification from free, unconjugated DTPA, the fragments were divided into sterile vials such that each vial contained 1.0 mg of fragments contained in 200 to 800 μl of 0.05 M bicarbonate buffer, pH 8.4. The vials were then sealed and immediately frozen in liquid nitrogen for storage.

The immunoreactivity of the DTPA-coupled fragments was determined using a competitive binding assay. Samples of a coupled (but unlabeled) antibody fragment preparation were diluted in 0.1 M citrate buffer containing 50% calf serum, pH 6.0, at antibody concentrations between 200 μg/ml and 0.001 μg/ml. OC-125 IgG antibody, radioiodinated with ^{125}I using Bolton-Hunter reagent (DuPont NEN Medical Products, N. Billerica, MA) to a specific activity of 10 mCi/mg, was then diluted to 100 ng/ml, and an equal volume was added to each serial dilution such that the final concentration of iodinated antibody in each was 50 ng/ml. Polyethylene beads (Precision Plastic Ball Co., Chicago, IL), which were previously coated with OC-125 IgG and then CA-125, were incubated with 0.2-ml aliquots of each serial dilution in duplicate for 16 h at room temperature. The beads were then washed 3 times with distilled water and counted in a gamma-well counter. Uncoupled OC-125 F(ab')$_2$ was also assayed as a positive control.

A chromogenic Limulus Amebocyte Lysate pyrogen test (QCL-1000; Whittaker M. A. Bioproducts, Walkersville, MD), general safety test (21CFR610.11), and sterility tests (21CFR610.12) were performed on one vial of each lot selected at random.

Before each patient study, a vial was allowed to warm to room temperature, and 1 to 2 mCi of research-grade ^{111}In (DuPont NEN Medical Products), which had previously been made 0.25 M in sterile sodium acetate at pH 5.0, was added with a sterile plastic pipet tip. The contents of the vial were then diluted with about 0.2 ml of saline and drawn into a 3-ml syringe along with an additional 2.0 ml of sterile saline. Several aliquots of the preparation were removed for radioactivity standards and for the analysis of radiochemical purity. The latter was determined by size exclusion HPLC usually using a single 7.5- x 300-mm TSK 250 column (Bio-Rad Laboratories, Richmond, CA) and 0.1 M phosphate, pH 7.0 eluant.

Eighteen patients with documented malignancies were studied. Median age was 54 with a range of 36 to 71. Ten patients received chemotherapy (Adriamycin, cisplatin, and occasionally Cytoxan) prior to this study. All patients signed informed consent and were studied with the approval of the Food and Drug Administration (IND BB 1863) and the appropriate institutional review committees. Patients 8 and 9 were readministered the labeled antibody after 10 and 4 mo, respectively.

Each patient received by slow i.v. infusion (30 to 45 min) approximately 150 ml of saline containing 1.0 mg of fragments radiolabeled with 1 to 2 mCi of ^{111}In. One preadministration and several postadministration blood samples were usually obtained along with a complete urine collection throughout the study (usually 72 h). In most cases, quantitation of radioactivity in liver, spleen, and kidneys was determined at several times postadministration by planar imaging on a Picker International DynaScan SPECT camera equipped with a medium-energy collimator as described previously (6).

Several blood samples were collected in heparinized tubes, and the percentage of radioactivity bound to formed elements was determined by counting in a gamma-well counter after repeat rinsing and centrifugation. Most blood samples were collected in red-top vacutainers and were allowed to clot to provide serum for analysis.

In addition to counting in a well counter along with a standard of the injectate, all serum samples were analyzed for CA-125 levels by radioimmunoassay (Centocor, Inc., Malvern, PA) after decay of ^{111}In. Most of the serum samples obtained in this study were also analyzed for affinity using the CA-125-coated beads described above, to determine the relative percentage of radioactivity in serum which is antibody bound. Since binding of labeled antibody, even in the absence of serum, is not quantitative in this assay, the trends in binding, rather than absolute binding, among serum samples collected at different times were examined.

In a search for radiolabeled species in serum of high molecular weight (possibly signifying antigen-antibody or human anti-mouse response), serum samples were also analyzed by gel filtration chromatography. Initially this was accomplished by open column chromatography using a 1- x 170-cm Sephadex G200 column (flow rate, 5 ml/h) and saline eluant. More recently size exclusion HPLC was used for this purpose using a single 7.5- x 300-mm TSK 400 column (Bio-Rad) and 0.1 M phosphate buffer, pH 7, eluant (12). Fractions were collected and counted in a well counter along with a standard of the injectate. Cation exchange HPLC was also performed on serum samples to measure the percentage of radioactivity bound to transferrin. A single 7.5- x 75-mm SP 5PW cation exchange column (Waters Associates, Milford, MA) was used 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. Samples of urine were analyzed by gel filtration chromatography and, occasionally, by anion exchange chromatography to help identify the radiolabeled species in urine. Initially, a 1- x 150-cm column of Sephadex G50 (flow rate, 40 ml/h) was used with saline eluants, while more recently a single 7.5- x 300-mm I-60 size exclusion HPLC column (Waters) was used with 0.1 M phosphate, pH 7, eluant. A single 7.5- x 75-mm DEAE-anion exchange HPLC column (Waters) was also used with gradient elution from 20 mM tris buffer, pH 8.5, to 20 mM tris buffer, 0.5 M sodium chloride, pH 7.0, in 30 min. As in the case of serum, urines were analyzed by collecting fractions for counting in a well counter.

The serum clearance curves obtained by counting aliquots of each serum sample were analyzed by the AUTOAN program (13) to obtain initial estimates of pharmacokinetic parameters and to determine whether the data were best fit by a one- or two-compartment model. The data were then fitted to the model using NONLIN84 (Statistical Consultants, Lexington, KY) weighted by the square of the reciprocal of serum concentration. The model parameters were used to calculate the intercompartmental rate constants by conventional methods (14). Noncompartmental parameters, Cl, MRT, V_c, and V_{dss}, were calculated using the area under the clearance curve and the area under the first moment curve (13). The identical analysis was performed on serum clearance curves obtained in the 19-9 study.

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. Three lots of DTPA-coupled OC-125 fragments were used in this investigation. The first (used in Patients 1 to 7) contained an average of 1.2 DTPA groups per fragment, while for the second (used in Patients 8 to 14) and third (used in Patients 15 to 18), this value was 0.8 and 0.9, respectively. The percentage of dimeric species (generated during conjugation with DTPA) was 20, 5, and 6% for the three lots, respectively. These dimeric species were not removed in part because no decrease in immunoreactivity was observed for preparations of DTPA-conjugated fragments of OC-125 with up to 23% dimer by the above competitive binding assay.[4] Fig. 1 presents the results of the competitive binding assay performed on the first lot of DTPA-conjugated antibody which demonstrate that the immunoreactivity of this product was not detectably altered from that of the antibody before conjugation.

Following radiolabeling, radiochemical purity, defined as the fraction of activity present as labeled antibody, averaged 98 ± 7% (SD, n = 18) by HPLC analysis. The absence of radiocolloids and radiolabeled aggregates in these labeled fragment preparations was demonstrated by the absence of radioactivity voiding in the HPLC analysis, by recoveries in this analysis averaging 92 ± 7% (SD, n = 18) and by the absence of detectable radioactivity removed by centrifugation at 110,000 × g for 2 h.

Analysis of Serum Samples. The analysis of whole blood showed less than 3% of the radioactivity on formed elements

[4] Unpublished observations.

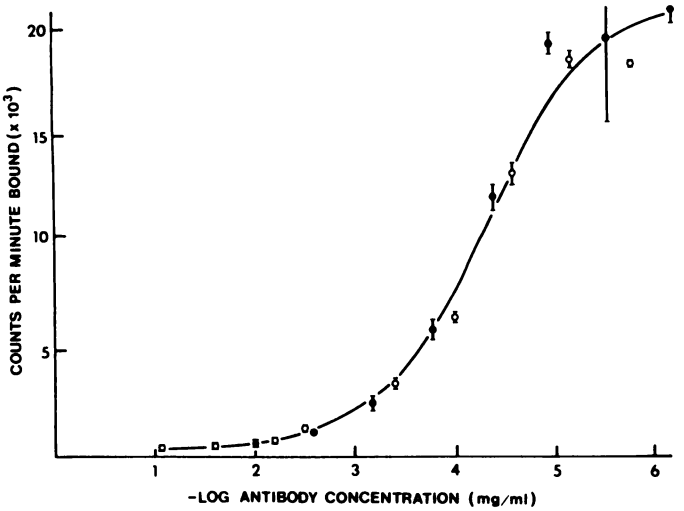

Fig. 1. Results of competitive binding assay in which uncoupled (○) and DTPA-coupled (●) F(ab')$_2$ fragments of OC-125 were each placed in competition with radioiodinated OC-125 IgG for antigen bound to beads (see text). *Points*, mean counts per minute on beads compared with the negative log of unlabeled antibody concentrations; *bars*, range of repeat measurements (n = 2).

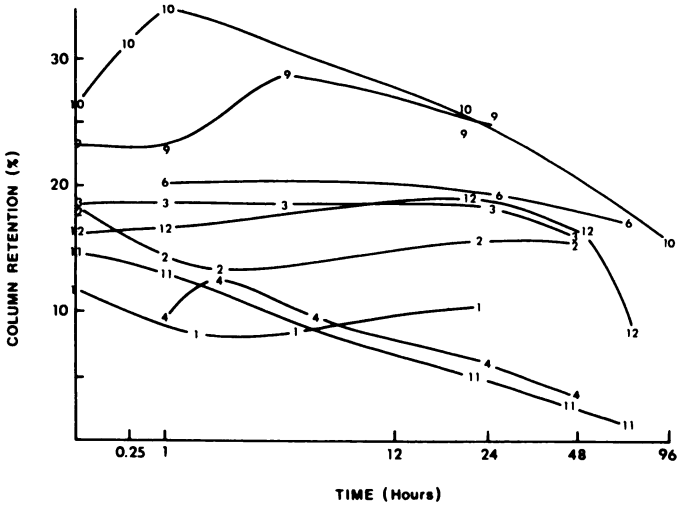

Fig. 2. Percentage of radioactivity in serum which binds to anti-OC-125 affinity beads compared with time following administration of labeled antibody (semilog scale). Patients identified by *number*. *Points*, mean of repeat measurements (n = 2 to 3).

Table 1 *Preadministration circulating CA-125 antigen levels, percentage of serum activity present in a high-molecular-weight species at 19 to 24 h post-antibody administration, percentage of administered activity in liver at 24 h postadministration, plasma clearance, and volume of distribution at steady state obtained in patients administered ^{111}In-labeled OC-125 (F(ab')$_2$ (a partial list)*

Patient	Preadministration CA-125 (units/ml)[a]	Serum high-molecular-wt species (%)	Liver activity at 24 h (% of injected dose)	Cl (liters/h)	V_{dss}
1	NA[b]	24	NA	0.0838	2.2
2	9	7	16.5	0.0639	3.0
3	40	16	10.7	0.0247	1.7
4	423	43	NA	0.0388	1.3
6	0	31	11.2	0.0379	2.1
7	22	15	9.2	0.0240	1.2
9	20	9	11.2	0.0711	2.7
10	39	61	14.4	0.0495	2.9
11	141	39	17.5	0.0968	2.9
12	44	6	12.3	0.0897	2.3
9[c]	31	0	9.1	NA	NA
17	>500	0	10.5	0.0493	2.6
18	9	0	12.1	0.0393	1.7
8[c]	20	22	16.2	0.1130	3.4

[a] Each unit equals 0.015 ng of antigen.
[b] NA, not available.
[c] Repeat study.

at all time points. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis with autoradiography of labeled OC-125 and 19-9 antibody incubated at 37°C in serum for up to 48 h showed only a few percentages of Fab' fragments present in both cases at all time points.

The analysis of preadministration serum samples for CA-125 provided the values presented in Table 1. Analysis of postadministration serum samples showed that most CA-125 levels were below detectable limits. The exceptions are Patient 11, in whom the concentration immediately dipped below the preadministration level and was not reestablished for more than 24 h, and Patient 14, in whom all samples showed the same value of 200 to 400 units/ml. The behavior of circulating antigen in only these two patients approximates the behavior observed consistently in the 19-9 investigation.

The percentage of radioactivity in serum which binds to the CA-125 derivatized beads is presented in Fig. 2 against time postadministration. In each study, an aliquot of the injectate, diluted in serum from a normal volunteer to approximately the same labeled antibody concentration as in the initial patient serum, was also analyzed. Binding of the control serum was approximately that of the initial patient serum in all cases.

In the 19-9 investigation, a rapid decrease was observed in the percentage of serum radioactivity which bound to the affinity column, followed by an equally rapid increase such that initial values were reestablished in about 1 h postadministration. This trend was not as dramatic in the present study as both the decline and rise were less pronounced in general and occurred more slowly, taking about 20 h. As in the 19-9 case, decrease in binding is most likely due to the formation of antigen-antibody complex which cannot bind to the affinity chromatographic medium whereas the subsequent increase in binding results from the clearance of these species from circulation.

After about 24 h postadministration, a second decline is evident in the figure. A similar decline, estimated at 9% per day, was observed in the 19-9 study and was attributed to transcomplexation of the ^{111}In from antibody to circulating transferrin. Estimates of the rate of ^{111}In transcomplexation in serum for a number of proteins conjugated with DTPA vary from 2 to 10% per day (15). The results presented in the figure may be used to estimate that, in the case of the OC-125 antibody, this decline averaged 16 ± 11% per day. This value is, therefore, in overall agreement with previous studies and suggests that the rate of transcomplexation is independent of the labeled protein.

A more accurate measurement of the rate of transcomplexation was provided by analysis of serum samples on a cation exchange HPLC column. Several radiochromatograms obtained on this column are presented in Fig. 3. Analysis in this way of the native, unconjugated antibody fragment with UV detection showed several peaks, most likely resulting from the presence of several species differing in the degree of glycosylation. These multiple peaks are also apparent in the radiochromatogram of the injectate shown in the figure. These peaks persist in serum however, an additional peak, not present in the injectate and due to ^{111}In-transferrin, is also apparent in serum. The rate with which this peak increases provides a value of 6% per day for the rate of transcomplexation.

The percentage of serum radioactivity present as a high-molecular-weight species (*i.e.*, eluting substantially before the radiolabeled fragment on both open column G200 and TSK 400 HPLC) at 19 to 24 h postadministration is also listed in

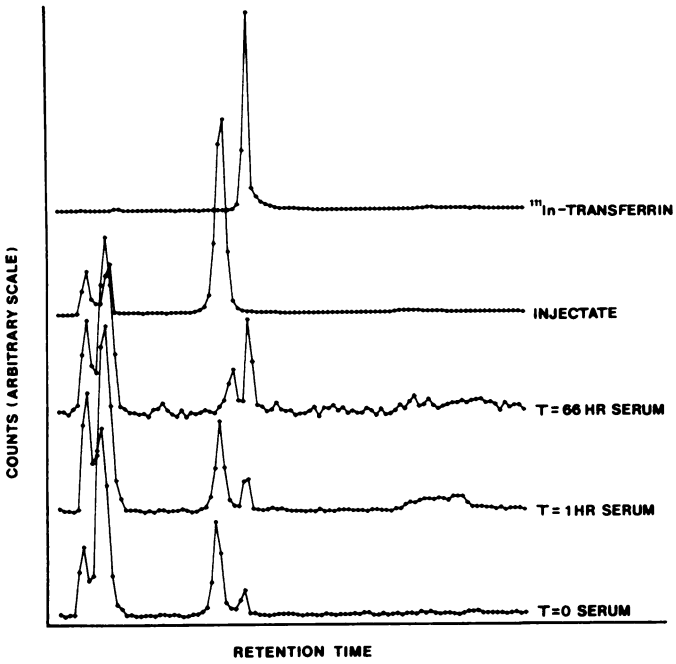

Fig. 3. Several radiochromatograms obtained by the analysis of the indicated samples on an SP 5PW cation exchange HPLC column. Analysis of three serum samples shows the presence of one peak, not present in the injectate, which coelutes with ^{111}In-labeled transferrin. The percentage of serum radioactivity present as labeled transferrin is seen to increase with time postadministration.

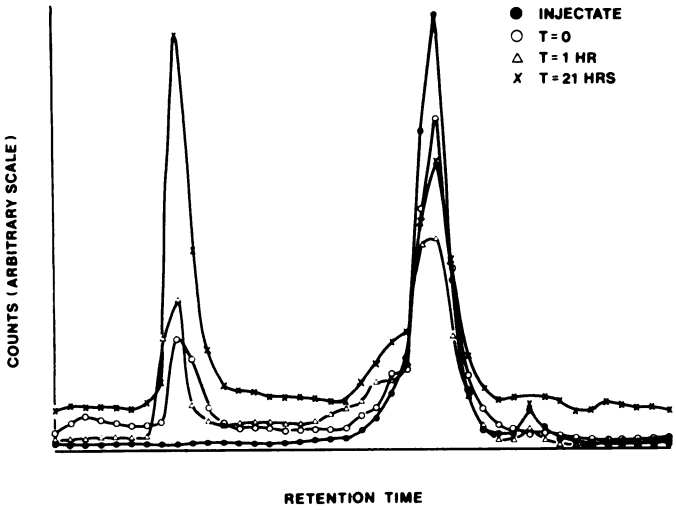

Fig. 4. Radiochromatograms obtained by size exclusion HPLC analysis of radiolabeled OC-125 fragments and of three sera from one patient administered the labeled fragments. With increasing time postadministration, the percentage of radioactivity present in serum as labeled fragments is found to decrease relative to radioactivity present in a high-molecular-weight form.

Table 1. The speed of HPLC permitted the analysis of several serum samples from each patient. As shown in Fig. 4, the percentage of activity in a high-molecular-weight form was thus found to increase in one patient from about 17% initially (*i.e.*, approximately 45 min after the start of antibody infusion) to 39% at 21 h postadministration. A similar slow accumulation of radioactivity in a high-molecular-weight species has been reported for this antibody and attributed to antigen-antibody complex formation (16).

Analysis of the serum clearance curves provided the mean values for Cl, MRT, V_c, and V_{dss} listed in Table 2. Also in the table are the values obtained by identical analysis of the 19-9 clearance curves (6) not previously reported. Statistically significant ($P < 0.05$) differences in the pharmacokinetic measurements were seen in Cl, V_{dss}, and MRT by a pooled t test (MINITAB Statistical Package, University Park, PA). The volume of the central compartment is statistically identical for both antibodies at about 2.4 liters, approximately the plasma volume of the patients. However, the volume of distribution at steady state is significantly larger for the 19-9 antibody, suggesting that the label in this case only diffuses from plasma. Serum clearance of radioactivity calculated from the observed mean pharmacokinetic parameters assuming administration of the same radioactive dose for both antibodies is illustrated in Fig. 5.

Analysis of Urines. As in the case of the 19-9 study, radioactivity released to urine following administration of the OC-125 antibody was slow but steady in all patients, averaging 0.29% of the injected dose per hour. This value is essentially identical with the 0.26% per hour value observed in the 19-9 investigation. Furthermore, G50 chromatography and I-60 HPLC analysis of urine showed identical radioactivity profiles to those observed in the 19-9 study. Two peaks were observed with the peak at longer retention time decreasing in intensity relative to the peak at the void volume from early to late urine collections. The urine samples were also analyzed by DEAE-anion exchange chromatography. Fig. 6 shows typical radiochromatograms obtained in this manner in the analysis of three urine samples from one patient. Activity in the earliest urine collection coelutes with ^{111}In-DTPA and is most probably present largely as labeled free DTPA (since a small amount of this species is in the injectate), whereas in later collections activity is clearly present in another chemical form(s) as shown by the shift in retention time.

Organ Quantitation. Liver radioactivity levels are shown graphically in Fig. 7 in the percentage of the administered activity, corrected for decay, compared with time postadministration. Trends apparent in the figure are similar to those observed in the 19-9 study in that liver levels are high initially, possibly reflecting blood pool in this organ, but do not decrease significantly throughout the study. As in the 19-9 study, a large variation in liver accumulation is apparent among patients receiving the OC-125 antibody. Values for spleen and kidneys remained essentially constant throughout each study, although at much lower levels than those of liver.

A noteworthy difference with the 19-9 investigation is the lower radioactivity levels in liver, spleen, and kidneys. A comparison of these values obtained in both studies is presented in Table 3.

In a search for correlations between liver activity accumula-

Table 2 *Analysis of serum clearance curves obtained in the study of the 19-9 and the OC-125 antibodies*

Values for Cl, MRT, half-times of serum clearance, V_c, and V_{dss} are listed along with the significance of the difference in these values between the two antibodies.

Antibody	Cl (liters/h)	MRT (h)	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)	V_c	V_{dss}
19-9 ($n = 7$)	0.13 ± 0.05[a]	28 ± 10	5.1 ± 3.8	27 ± 6.6	2.5 ± 1.0	3.5 ± 1.5
OC-125 ($n = 13$)	0.056 ± 0.025	44 ± 14		21 ± 8.6	2.3 ± 0.7	2.2 ± 0.6
Significance	$P < 0.005$	$P < 0.05$		NS[b]	NS	$P < 0.05$

[a] Mean ± SD.
[b] NS, not significant.

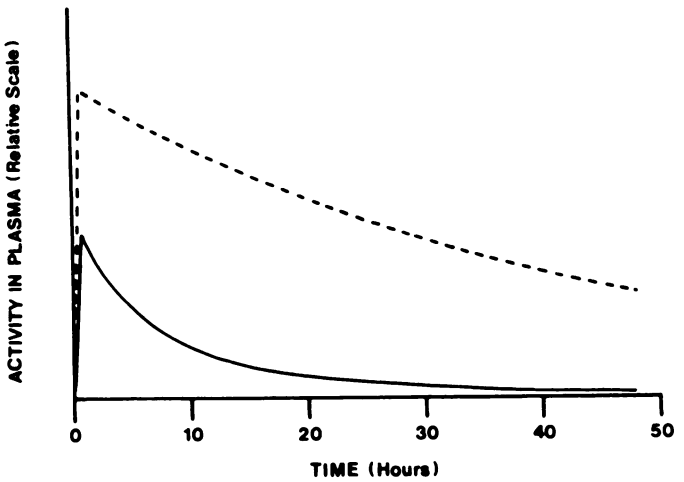

Fig. 5. Serum radioactivity clearance curves for both the 19-9 and OC-125 antibodies calculated from pharmacokinetic parameters derived from clearance measurements. *Curves* are calculated for the same administered radioactivity; nevertheless the 19-9 radioactivity levels are lower as label diffuses from the plasma space.

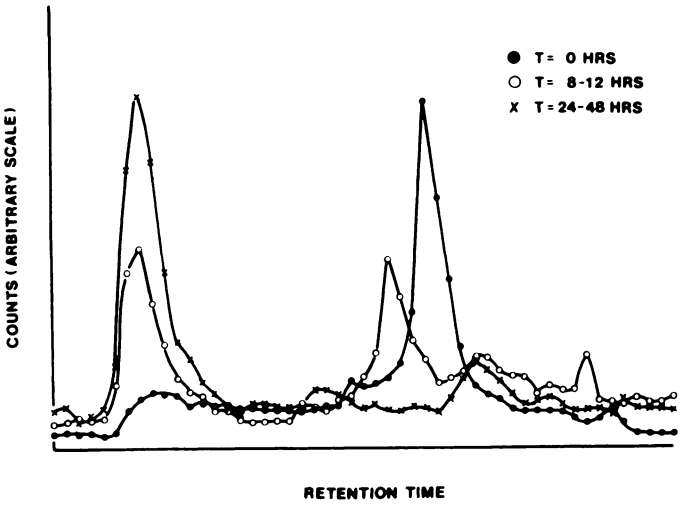

Fig. 6. Radiochromatograms obtained by anion exchange HPLC analysis of three urine samples obtained from one patient. With increasing time postadministration the percentage of radioactivity in urine which coelutes with labeled free DTPA decreases, while radioactivity at the void volume increases.

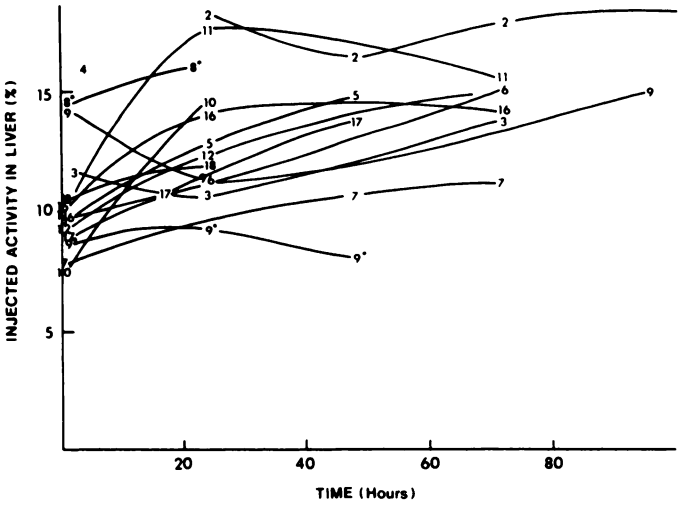

Fig. 7. Radioactivity in liver presented as percentage of administered ^{111}In (corrected for decay) compared with time after administration of labeled antibody. Patients identified by *number*. One *curve* has been extended to a data point off scale. Radioactivity levels obtained following a second administration of labeled antibody are indicated by an *asterisk*.

Table 3 *Results of quantitation of liver, spleen, and kidneys at 24 h postadministration obtained in the study of the 19-9 and OC-125 antibodies along with the significance of the difference in these values between the two antibodies*

Antibody	Liver	Spleen	Kidneys
19-9 (n = 10)	20 ± 8[a]	1.7 ± 1.1	7.3 ± 5.3
OC-125 (n = 13)	12 ± 3	1.1 ± 0.6	3.6 ± 2.0
Significance	$P < 0.01$	NS[b]	NS

[a] Mean ± SD of the percentage of injected dose.
[b] NS, not significant.

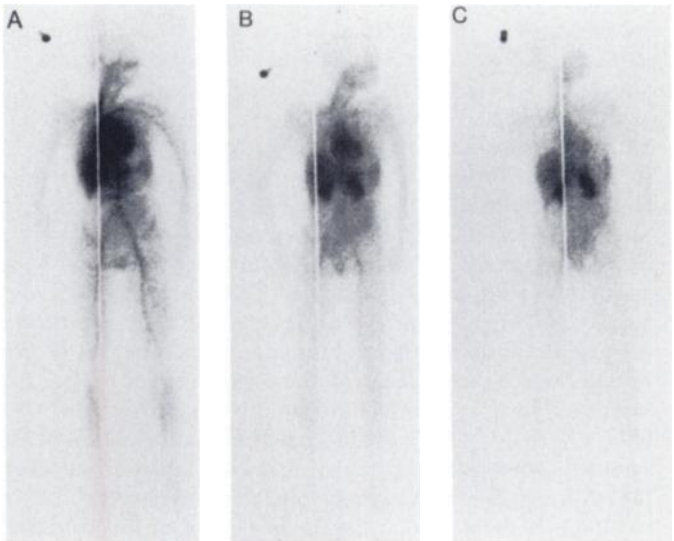

Fig. 8. Series of whole-body posterior images obtained at 1 (*A*), 24 (*B*), and 48 (*C*) h postadministration with ^{111}In-labeled OC-125 fragments. An aliquot of the injectate was placed near the shoulder during each acquisition.

tion at 24 h postadministration and the results of other measurements, a linear regression analysis for two components was conducted (MINITAB) to assess the relationship among liver radioactivity at 24 h, preinjection CA-125 serum levels, radioactivity in serum at 19 to 24 h postadministration in a high-molecular-weight form, Cl, and V_{dss}. The analysis was performed on the mean values of these measurements obtained in the OC-125 and 19-9 study, but in the latter case without the inclusion of high-molecular-weight serum values. Significance ($P < 0.05$) was found in the case of OC-125 antibody for liver accumulation and Cl. Significance was not found in the 19-9 case.

Tissue Counting. Among the patients administered the OC-125 antibody, three underwent surgery before radioactivity in tissues decreased to background levels. In one patient a peritoneal nodule was removed at 3 days postadministration and found to contain a metastatic poorly differentiated adenocarcinoma with features consistent with an ovarian primary. Radioactivity in this tissue was 2 times background at 0.0083% of the injected dose per gram. Only normal tissues were obtained from the second patient at 4 days postadministration. Mean radioactivity in these tissues was 0.002% injected dose per gram. Finally, a supraclavicular lymph node containing recurrent adenocarcinoma was removed from a patient at 18 days postadministration which contained 0.031% of the injected dose per gram. Although normal tissue was not removed, it may be estimated from the previous two patients that, in this case, the tumor/normal tissue ratio exceeded 15.

Imaging. Fig. 8 illustrates the biodistribution of ^{111}In following administration of the labeled OC-125 antibody. Three

whole-body images obtained at 1, 24, and 48 h postadministration are shown of the same patient. These images are typical of those obtained in patients thought to be disease free, and they show early and persistent high activity levels in liver, spleen, and kidneys. Blood activity is still evident even at 48 h, especially in the blood pool of the heart. Very little activity is apparent in bone marrow or in gut.

DISCUSSION

Since an object of this investigation was to compare the *in vivo* pharmacokinetic properties of two antitumor antibodies, the antibodies were selected to be as similar as possible. Thus, both the OC-125 and 19-9 are of the IgG1 subclass and are directed against shed antigen. Both were used in these investigations as the DTPA-coupled $F(ab')_2$ fragments radiolabeled with ^{111}In in the identical fashion. Finally, both were administered by i.v. infusion at a loading dose of 1 mg of protein and 1 to 2 mCi of radioactivity.

Several noteworthy similarities are apparent in the results of these studies. Despite a greater tendency toward dimer protein formation during DTPA conjugation, the immunoreactivity of the OC-125 antibody, like the 19-9 antibody, was unaffected by the conjugation, as determined by competitive binding. Size exclusion HPLC and ultracentrifugation also showed no evidence of radiocolloids or other labeled aggregates. Furthermore, an estimate of the rate of *in vivo* transcomplexation of ^{111}In from antibody in serum to transferrin (6%/day) is consistent with that (9%/day) obtained for the 19-9 antibody.

Since the antigen CA-125 is shed into the circulation, the presence of circulating activity in a high-molecular-weight form signifying antigen-antibody complex formation was expected and, as in the 19-9 case, was most likely observed (Fig. 4). The extent to which these species formed at 19 to 24 h postadministration was, however, variable and does not correlate with circulating CA-125 levels. Also in agreement with the 19-9 study, liver activity levels reached maximum values soon after antibody infusion and did not decline thereafter.

As in the 19-9 case, the only major route of whole-body elimination of radioactivity was via the kidneys, and essentially identical elimination rates into urine (0.29 compared to 0.26%/h) were observed. In both cases, elimination was rapid within the first 4 to 8 h, due most likely to glomerular filtration of the small amount of ^{111}In-labeled DTPA in the injectate. This suggestion is supported by chromatographic analysis of urine samples which shows the presence of this species only in early samples. However, elimination by this route continues thereafter at a steady rate, and chromatographic evaluation of later samples shows the presence of species distinct from labeled DTPA which are most probably metabolic products.

Although these similarities in *in vivo* behavior of the two antibodies are important, it may be the differences which are most significant. From the point of view of radioactivity accumulation in liver, often considered to be problematic in the use of ^{111}In-labeled antibodies, the OC-125 antibody showed significantly decreased liver levels at 24 h postadministration with respect to those observed with 19-9 (12% compared to 20%). A similar decrease was observed for spleen and kidneys as well (Table 3). The reason for these decreased levels is not apparent but may be related to differences also observed in plasma clearance. When administered as the labeled OC-125 antibody, ^{111}In is cleared monoexponentially with V_{dss} equal to the V_c which, in turn, is roughly equal to the plasma volume. Apparently this labeled antibody does not diffuse appreciably from the plasma space. In the case of the 19-9 antibody, by contrast, ^{111}In followed a biexponential clearance curve with an apparent volume of distribution at steady state significantly larger than the initial volume of distribution. In this case, the label is diffusing from plasma and appears to distribute within a compartment with a volume of approximately 1.4 liters. This compartment has not been identified; nevertheless, it must be uniformly distributed throughout the whole body; otherwise radioactivity in the compartment (approximately 50% of the injected dose at 24 h) would appear in the whole-body images as one or more areas of increased activity. This radioactivity is not bound to formed elements in blood, and it is unlikely that the compartment represents either total body water, extracellular, or intrinsic fluid since these volumes are reported to be approximately 40 to 45, 13 to 16, and 10 to 13 liters for a 70-kg subject (17). This analysis is, however, based only on radioactivity levels in serum and is therefore complicated by the presence in serum of at least two chemically distinct forms of ^{111}In: radiolabeled antibody and a radiolabeled high-molecular-weight species, most likely antigen-antibody complex. If the latter clears more slowly from serum compared to labeled antibody, the result will be a decrease in the apparent clearance rate. Since the volume of distribution has been calculated using the area under the clearance curve, a decreased clearance can have the effect of lowering the apparent volume of distribution. In the absence of information on the separate rate of clearance for each labeled species, a volume of distribution for the antibody itself cannot be calculated and, thus, an anatomical location for the second compartment cannot be identified.

In summary, the properties of the ^{111}In radiolabel itself were found to be independent of whether attached to the 19-9 or OC-125 antibodies. Although certain similarities were observed in the *in vivo* behavior of the antibodies, the differences in pharmacokinetics for two similar antibodies administered in identical fashion were surprising. Decreased liver levels observed for the OC-125 antibody suggest that further decreases in these levels may result from the use of other antibodies with even more favorable pharmacokinetics. It is possible that the selection of new antibodies for tumor localization should be made not only on the basis of affinity and specificity but on pharmacokinetic properties as well.

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