Patient Biodistribution of Intraperitoneally Administered Yttrium-90-Labeled Antibody

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Although ^{90}Y is one of the best radionuclides for radioimmunotherapeutic applications, the lack of gamma rays in its decay complicates the estimation of radiation dose since its biodistribution cannot be accurately determined by external imaging. A limited clinical trial has been conducted with tracer doses (1 mCi) of ^{90}Y in five patients who then received second-look surgery such that tissue samples were obtained for accurate radioactivity quantitation by in vitro counting. The anti-ovarian antibody OC-125 as the F(ab')$_2$ fragment was coupled with diethylenetriaminepentaacetic acid, radiolabeled with ^{90}Y and administered intraperitoneally to patients with suspected or documented ovarian cancer. Size exclusion and ion exchange high performance liquid chromatography analysis of patient ascitic fluid and serum samples showed no evidence of radiolabel instability although a high molecular weight species (presumably immune complex) was observed in three patients. Total urinary excretion of radioactivity prior to surgery averaged 7% of the administered radioactivity while at surgery the mean organ accumulation was 8% of the administered radioactivity in serum, 10% in liver, 7% in bone marrow, and 19% in bone with large patient to patient variation. The mean tumor/normal tissue radioactivity ratio varied between 3 and 25. On the assumption that the above radioactivity levels were achieved immediately following administration, that the radioactivity remained in situ until decayed and that the dimensions of tumor were sufficient to completely attenuate the emissions of ^{90}Y, the dose to tumor for a 1-mCi administration would be ~50 rad with normal tissues receiving ~8 rad.

The clinical experiences of this laboratory in the use of the anti-ovarian monoclonal antibody OC-125 conjugated with diethylenetriaminepentaacetic acid (DTPA) and radiolabeled with indium-111 (^{111}In) have been encouraging (1,2). As a result, therapy trials are now under consideration in which the OC-125 antibody will be radiolabeled with yttrium-90 (^{90}Y), a pure beta-ray emitting radionuclide. In this trial, the preferred route of administration may be intraperitoneal since ovarian cancer is generally localized within the peritoneum and since administration by this route may restrict the accumulation of activity in normal organs such as liver.

Yttrium-90 is considered to be one of a small number of radionuclides suitable for the treatment of cancer by radioimmunotherapy (3,4). Among the advantages of

this radionuclide is the fact that it is available conveniently and inexpensively from in-house strontium-90-yttrium-90 (^{90}Sr-^{90}Y) radionuclide generators (5) and that it forms stable chelates with DTPA conjugated antibodies (6,7).

The pure beta-ray decay of ^{90}Y is also an advantage in the delivery of radiation therapy, however, it makes difficult the estimation of radiation dose. The calculation of dose requires knowledge of the time-dependent accumulation of radioactivity in tumor and normal tissues. For pure beta-ray emitters such as ^{90}Y, the accumulation of radioactivity in tissues cannot be determined accurately by external imaging. Furthermore, it is unlikely that the biodistribution of ^{111}In-labeled antibodies may be used to estimate the biodistribution of the same DTPA-coupled antibodies labeled with ^{90}Y since these two radiolabels most probably behave differently in vivo (6). It is also unlikely that the biodistribution of ^{111}In-labeled antibodies in animals may be used to predict the behavior of this agent in patients. A partial solution to the determination of the biodis-

tribution of ^{90}Y is to consider patients scheduled for exploratory surgery so that tissue samples may be obtained by biopsy. A limited clinical trial was therefore conducted in which a tracer dose (1 mCi) of ^{90}Y-labeled OC-125 fragments was administered to each of five patients who were to receive second-look surgery for ovarian cancer. Several serum and urine samples were obtained prior to surgery and, during surgery, small samples of liver, bone and bone marrow were obtained along with samples of tumor (if any) and normal peritoneal tissues. The radioactivity content of these samples was then determined and the results used to estimate the biodistribution of the label at the time of surgery. The biodistribution was used in the calculation of radiation absorbed doses.

MATERIALS AND METHODS

The anti-ovarian tumor antibody OC-125 was developed by Bast et al. by immunizing BALB/c mice with human epithelial cells obtained originally from a patient with serous papillary cystadenocarcinoma (8). The antibody was prepared for this study as previously described (1) by intraperitoneal administration of hybridoma cells into pristane-primed BALB/c mice. The OC-125 IgG1 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 0.1M citrate, pH 4.2. Progress of the digestion was monitored by size exclusion high performance liquid chromatography (HPLC) analysis and the digestion terminated by neutralization after ~18 hr 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 6B 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 FDA.

Antibody coupling with DTPA, purification and radiolabeling of the fragments was performed as described previously (9). The five samples of DTPA-coupled antibody used in this study were from two lots of coupled fragments; each was prepared by coupling 20–30 mg of fragments using the cyclic anhydride of DTPA (10) such that the average number of attached DTPA groups per fragment molecule was ~1. Following purification from free, unconjugated DTPA, the preparations were divided into sterile vials such that each vial contained 1.0 mg of fragments in 200–800 μl of saline. The vials were then sealed and immediately frozen in liquid nitrogen for storage. Vials from the same lots were used both in this investigation and in our continuing study of the ^{111}In-labeled OC-125 antibody for detection of ovarian cancer.

A chromogenic limulus amoebocyte lysate (LAL) 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 selected at random from each lot.

The immunoreactivity of the DTPA-coupled fragments was determined using a competitive binding assay as previously described (9). Serial dilutions of a coupled (but unlabeled) antibody fragment preparation were diluted in 0.1M citrate buffer containing 50% calf serum, pH 6.0, at antibody concentrations between 0.2 mg/ml and 10^{-6} mg/ml. OC-125 IgG antibody, radioiodinated with ^{125}I using Bolton-Hunter reagent (Dupont Company, No. Billerica, MA) to a specific activity of 10 mCi/mg, was then diluted to 100 ng/ml and an equal volume added to each serial dilution such that the final concentration of iodinated antibody in these solutions was 50 ng/ml. Polyethylene beads (Precision Plastic Ball Co., Chicago, IL) which were previously coated with OC-125 IgG and then CA-125 antigen (recognized by OC-125 antibody), were incubated with 0.2-ml aliquots of each serial dilution in duplicate for 16 hr at room temperature. The beads were then washed three times with distilled water and counted in a gamma-well counter. Uncoupled OC-125 F(ab')$_2$ was also assayed as a positive control.

The ^{90}Y used in this investigation was obtained from a ^{90}Sr-^{90}Y radionuclide generator. The construction of the generator, its elution and the conversion of the [^{90}Y]EDTA to the acetate complex have been previously described (5).

Prior to protein labeling, each ^{90}Y eluant intended for patient use was analyzed by paper chromatography as a check against catastrophic generator failure resulting in excessive ^{90}Sr breakthrough (5). On Whatman No. 1 paper and with saline eluant, ^{90}Sr migrates with the solvent front while ^{90}Y in the acetate form remains near the origin. After patient administration, the more involved and accurate method for ^{90}Sr determination of Doering et al. (11) was performed on each eluant (5). To an aliquot of the acetate eluant, containing ~1 mCi of ^{90}Y activity, 2 mg of strontium and 10 mg of yttrium carrier were added. The solution was then added to the top of a 1 cm diameter by 16 cm long anion exchange column containing AG 1 × 4 (50–100 mesh) (BioRad Laboratories, Richmond, CA) in the hydroxide form. The column was then eluted with 10 ml of water into a 10 ml volumetric flask. The flask was filled up to the mark, the contents mixed by agitation, and exactly 1 ml removed for evaporation to dryness on a planchette. The planchette was then counted at fixed geometry on an end-window GM counter with and without a 220 mg/cm^2 aluminum absorber. The mica window on the GM counter is of a thickness which does not appreciably absorb the beta rays of either ^{90}Sr or ^{90}Y whereas the aluminum attenuates all the soft beta rays from ^{90}Sr and about 50% of the harder beta-rays from ^{90}Y. A standard containing 0.01 μCi of ^{90}Sr-^{90}Y (DuPont Company), also mounted on a planchette, was counted in the same manner and at the same geometry.

Before each patient study, a vial of DTPA-coupled OC-125 F(ab')$_2$ fragments was allowed to warm to room temperature and 1.0 mCi of ^{90}Y in ~50 μl of sterile 0.5M acetate buffer, pH 6.0 was added. The contents of the vial was then left undisturbed for 30 min to 1 hr to maximize chelation and was then drawn into a 3-ml syringe along with 2.2 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 using a single 7.5 × 300 mm TSK 250 column (BioRad Laboratories) and 0.1M phosphate pH 7.0 eluant. After labeling the fragments were diluted and infused without delay in order to minimize the possibility of radiolytic degradation of the protein.

All patients signed Informed Consent and were studied with the approval of the FDA (IND BB 1863) and the appropriate institution review committees. A total of five patients were each administered ~1 mCi of ^{90}Y-labeled OC-125 fragments, along with ~150 ml of saline, by slow (10 ml/min) intraperitoneal infusion via a Tenckhoff catheter. The proper location and patency of the catheter was established prior to the infusion by the injection of a small quantity (100 μCi) of [^{99m}Tc]pertechnetate followed by imaging on a gamma camera.

In each case, a pre-injection serum sample was obtained as well as frequent serum samples and a complete urine collection during the period between antibody infusion and surgery. The pre-injection serum sample was analyzed for CA-125 by radioimmunoassay (Centocor, Inc., Malvern, PA). During surgery, small samples of normal peritoneal tissues such as bowel, omentum, muscle, paracolic gutters, subcutaneous fat, parietal peritoneum, abdomen wall, abdominal scar tissues, cervix, bladder flap, and sigmoid were removed along with tumor tissue when present. In addition, liver, bone, bone marrow and bone marrow aspirate were also removed by needle biopsy. In each case, approximately half of each tissue was reserved for histopathological examination while the remainder was immediately weighed and counted.

The beta-ray activity of each tissue, serum and urine sample was counted first in a NaI(Tl) well counter. The serum and urine samples were counted against a standard of the injectate contained in a volume equal to the sample and each tissue was counted while covered in water as described previously (6). In most cases, the samples were counted several times to ensure that the activity was decaying with the half life of ^{90}Y and therefore free of contamination with ^{99m}Tc used to evaluate the location and patency of the catheter. After well counting, most tissues were re-counted with a liquid scintillation detector. Each tissue was reweighed and, with the exception of bone, dissolved in 1 ml or protosol (DuPont Company) and the solution added to 20 ml of Biofluor (DuPont Company). The resulting solutions were generally colorless and clear. Bone samples were first digested in 5 ml of conc. HCl for 1 hr at 60°C. To each sample was then added 0.5 ml of Protosol, 0.1 ml of 30% hydrogen peroxide solution and the sample was again placed in an oven at 60°C for an additional hour. After cooling, the contents were added to 20 ml of Biofluor. Standards were prepared by subjecting aliquots of the injectate to the identical procedure. Once again, most samples were clear and colorless and were usually counted several times and always with several standards of the injectate so that the results could be expressed in percentage of the injected dose.

One or more serum samples for each patient and one sample of ascites were analyzed by size exclusion HPLC using a single 7.5 × 300 mm TSK 400 column (BioRad) and 0.5M phosphate pH 7.0 buffer eluant. Fractions (0.2 ml) were collected and an aliquot of each was transferred to Biofluor, with or without Protosol, for counting against an aliquot of

the injectate. In addition, a sample of labeled antibody, incubated in ascitic fluid, was also analyzed by cation exchange HPLC using a gradient flow (1 ml/min) from 0.02M sodium acetate, pH 5.0 to 0.5M sodium sulfate, 0.02M tris, pH 8.0 in 30 min. on a 7.5 × 75 mm SP 5PW column (Waters, Milford, MA).As before, fractions were collected for liquid scintillation counting.

Dosimetric calculations were based on the assumption that the ^{90}Y activity was uniformly distributed throughout each organ and tissue and remained constant at the level determined at surgery during the time interval from administration until complete decay. The MIRD formalism (12) was then used to calculate the radiation dose to tissues resulting from ^{90}Y within the tissues. The radiation dose is a product of the absorbed dose per cumulated activity which was determined from the product of the measured activity per unit mass in the tissue samples, the mass of the organ (12) and the mean life of [90]Y (92 hr). Because ^{90}Y is a pure beta-ray emitter, it was not normally necessary to consider any additional dose resulting from the accumulation of activity in surrounding tissues. One exception is bone marrow which will be irradiated by radioactivity deposition in bone; the extent of dose contribution depends on whether cortical or trabecular bone is under consideration. The MIRD formalism was used in this case to estimate dose to marrow from ^{90}Y uniformly distributed in both bone marrow and in bone. The other exception is the dose to organs in the peritoneum resulting from irradiation by radioactivity in the peritoneal fluid or on the surfaces of the peritoneum. Irradiation will be restricted to the first centimeter of tissue thickness due to the finite range of beta particles and tissue doses will depend on whether the ^{90}Y is in the fluid or on the surface of the tissues since in the former case some attenuation will occur in the fluid itself. A radiation dose distribution may be estimated by summing both over the beta-ray energy (range) spectrum and depth in tissue.

RESULTS

The results of all tests to which the fragments were subjected, both before and after coupling and labeling, demonstrated that the injectate was safe for human use. Two lots of DTPA-coupled OC-125 fragments were used in this investigation. The first (used in Patients 1–3) contained an average of 1.0 DTPA groups per fragment while for the second this value was 0.7. The percentage dimeric species (generated during conjugation with DTPA) was 9% and 6%, respectively, and when radiolabeled with ^{111}In, ~2% of the activity was present as radiolabeled DTPA in both cases. The immunoreactivity assay, performed over seven orders of magnitude in fragment concentration, showed unimpaired ability of the coupled antibody from both lots to compete for CA-125 with respect to the native, unmodified antibody.

The elution efficiency of the [90]Sr-^{90}Y radionuclide generator, expressed in percentage of the theoretical yield, averaged 95 ± 5 (s.d., N = 5). Paper chromatographic evaluation of the ^{90}Y activity, after conversion

to the acetate, failed to show any signs of ^{90}Sr breakthrough. Using column chromatography, the mean ^{90}Sr breakthrough, expressed as percentage of the ^{90}Y activity at the end of elution, was 0.003 ± 0.004 (s.d., N = 5). Therefore a 1-mCi administration of ^{90}Y-labeled OC-125 contained ~0.04 μCi of ^{90}Sr. At this level, the ^{90}Sr likely to be present in patient samples would be undetectable in this investigation.

Following radiolabeling, the radiochemical purity, defined as the percentage of activity present as labeled antibody, averaged 95 ± 1 (s.d., N = 5) by HPLC analysis. Since the preparations of coupled fragments used in this investigation contained ~2% free DTPA, only ~3% of the injected ^{90}Y was in an unknown chemical form. The absence of radiocolloids and radiolabeled aggregates in the labeled fragment preparations was demonstrated by the absence of radioactivity voiding in the HPLC analysis and by recoveries in this analysis averaging 98 ± 3%.

Most patient samples were counted both in a NaI(Tl) well counter and in a liquid scintillation counter. The agreement between both determinations was consistent throughout this study and the ratio of values (in % ID/g) obtained by both these methods averaged 0.70 ± 0.26 (s.d., N = 53). The results obtained by well counting were used to calculate activity levels presented below. However, liver, bone marrow, and bone tissues for Patient 1 were counted at background levels in the well and, in these three cases, the activity levels obtained by liquid scintillation counting have been multiplied by 0.7 to maintain consistency. Likewise, bone uptake for Patient 2 was determined only by liquid scintillation counting and this value has been similarly corrected.

Table 1 lists separately for each patient the pre-injection CA-125 serum levels, the time between anti-

body infusion and surgery and the percentage of the administered radioactivity (corrected for decay) which appeared in urine throughout the study. Also in the table are listed the serum, liver, bone marrow, and bone activity at surgery, both in percentage of injected radioactivity per gram and per organ (corrected for decay). The latter were determined by multiplying the percentage of the injected radioactivity per gram by the number of grams using the patients body weight and the assumption that serum, liver, bone marrow and bone constitute 4% (13), 2.6%, 4.3%, and 7.1% (12) of whole-body weight, respectively.

Urinary excretion of radioactivity was at a fairly constant rate for each patient although a slight maximum was usually apparent at 4–12 hr postadministration. Serum levels tended to be low initially and to rise steadily throughout. The exceptions were Patient 4 where serum levels went through a maximum at ~10 hr postadministration and Patient 5 where maximum values were reached almost immediately. Only Patient 1 presented with ascites; 2.5 l of fluid, drained at surgery, were subsequently shown to contain tumor cells. Determination of radioactivity levels in ascites, obtained by sampling via the catheter, showed a dramatic drop in the first 18 hr, due most likely to deposition of radioactivity within peritoneal surfaces, and fairly constant activity levels thereafter. At surgery, ~80% of the administered radioactivity was contained in the ascitic fluid with ~10% of this in a filterable form, possibly on cells.

The radioactivity accumulated in tumor and surrounding normal tissues at the time of surgery is presented in Table 2. Histologic examination of tissues obtained at surgery from Patients 4 and 5 revealed no evidence of tumor. In the case of Patient 3, this exam-

TABLE 1
Pre-injection Serum CA-125 Levels, Total Urinary Radioactivity Excretion, and Radioactivity Levels in Normal Tissues at Surgery*

Patient	Pre-injection CA-125 serum levels (U/ml)	Time to surgery (hr)	Urine excretion (% ID)	Serum activity (% ID/g) (% ID/total volume)	Liver activity (% ID/g) (% ID/organ)	Bone marrow activity (% ID/g) (% ID/organ)	Bone activity (% ID/g) (% ID/organ)
1	>500	47	1	0.00028 1	0.0028 5	0.0020 6	0.0007 4
2	70	44	5	0.0047 12	0.0085 14	0.0007 2	0.011 49
3	13	43	7	0.0029 8	0.0062 10	0.0017 5	0.0045 21
4	12	24	12	0.0046 14	0.0052 10	0.0041 13	0.0035 18
5	19	16	6	0.0019 5	NA† NA	0.0038 10	0.0004 2

* Results tabulated for each of five patients receiving ^{90}Y-labeled OC-125 and expressed in percentages of injected radioactivity per gram and per organ.
† NA = Not Available

TABLE 2

Radioactivity Levels at Surgery in a Number (N) of Tumor and Normal Tissues and Their Ratios[*]

Patient	Tumor activity (s.d.)	Normal tissues (s.d.)	Tumor/normal tissue ratio
1	0.050 (0.035, N = 6)	0.002 (0.001, N = 6)	25
2	0.020 (0.004, N = 6)	0.003 (0.004, N = 10)	7
3	0.009 (0.005, N = 10)	0.003 (0.002, N = 5)	3
4	NT†	0.004 (0.004, N = 13)	—
5	NT†	0.002 (0.001, N = 13)	—

[*] Results tabulated for each of the five patients receiving ^{90}Y-labeled OC-125 and expressed in percentage of injected radioactivity per gram of tissue.

† NT = no tumor.

ination revealed tumor involvement in most of the tissue samples removed at surgery (N = 10); however, the degree of involvement was variable with many tissues showing a low percentage and others a high percentage of tumor cells. In the following analysis, all tissues found to contain tumor cells whether classified as +4 (predominately tumor cells) or +1 (few tumor cells) were included under the category of tumor tissue. Values in the table for the accumulation in normal tissue were determined by including in this category all tissues obtained at surgery which were shown to be normal, including liver, bone marrow, and bone.

As part of this investigation, serum and ascitic samples were analyzed by HPLC to help establish the chemical forms of activity present in these fluids. Figure 1 presents a radiochromatogram obtained by TSK 400 size exclusion HPLC analysis of the serum obtained at 12 hr postinfusion of Patient 5. Superimposed on the image is the radiochromatogram obtained under identical conditions for the injectate. In this case, serum radioactivity coelutes with the labeled fragments and is probably present in this chemical form. No evidence of immunocomplexes or other species are apparent. This is in contrast to the results obtained in an identical analysis of serum obtained at 24 hr postinfusion from Patients 3 and 4 in which a predominant second peak at earlier retention times (i.e., higher molecular weight) is apparent. A high molecular weight peak was not observed in the analysis of the 28-hr serum from Patient 2. The same analysis, performed on ascitic fluid obtained at 1 hr postinfusion of Patient 1, showed essentially all the radioactivity to be present in a high molecular weight form with only a small fraction coeluting with the fragments.

A sample of ascites from Patient 1 was stored at 4°C and used several weeks later for incubation with labeled OC-125 at a concentration of 10 μg/ml for 26 hr at 37°C. Cation exchange HPLC analysis performed as previously described (1) with liquid scintillation counting of fractions showed a number of radioactive peaks, most of which were present following an identical analysis of the labeled fragments themselves. Whereas only a single peak is apparent by size occlusion HPLC of labeled OC-125, this preparation separates into several peaks by cation exchange HPLC (1). Significantly, a radioactive peak in the analysis of the incubate corresponding to the presence of ^{90}Y-labeled transferrin was not observed.

Table 3 presents radiation dose estimates for 1 mCi

FIGURE 1
Radiochromatogram obtained by size exclusion HPLC analysis of a patient serum sample obtained 12 hr after infusion of ^{90}Y-labeled OC-125 fragments. Radiochromatogram of the injectate is superimposed for comparison.

o——o SERUM, 12 HOURS
▲——▲ INJECTATE

COUNTS (ARBITRARY SCALE)

RETENTION TIME
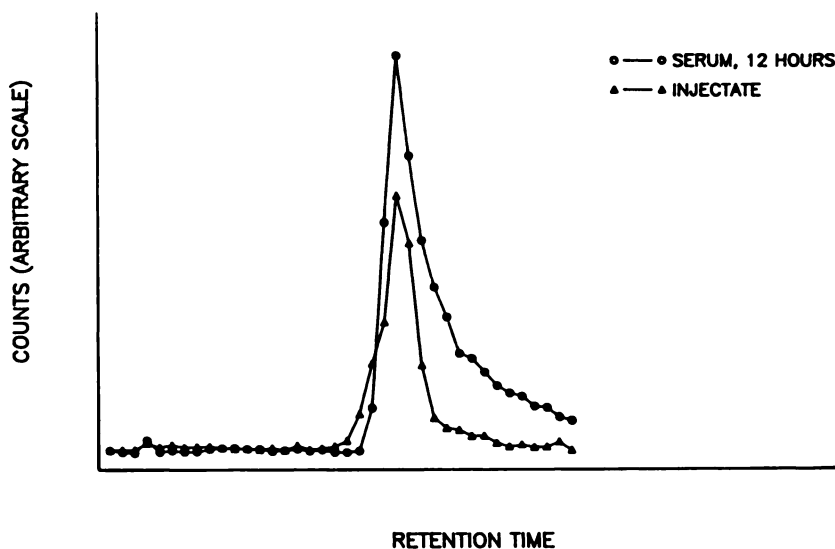
TABLE 3
Mean Radiation Dose Estimates for 1 mCi of ^{90}Y-Labeled
OC-125 Administered i.p. to Patients*

	Dose (rad)†
Serum	5.3 ± 3.4
Liver	8.8 ± 3.7
Bone marrow	8.2 ± 2.8‡
Bone	7.1 ± 6.1‡
Tumor	48 ± 44

* Values based on the mean radioactivity accumulation in serum and tissue at surgery and on the assumptions described in the text.

† Excluding dose due to radioactivity in peritoneum.

‡ Assuming bone activity uniformly distributed throughout bone mass.

of administered ^{90}Y-labeled OC-125 based on the mean levels of accumulated activity and on the assumptions described in the previous section. Dose estimates have been presented without consideration of contributions to tissue resulting from radioactivity in peritoneal fluid. Since the contribution to the radiation dose received by bone marrow from bone varies with the thickness and the type of bone, values have been presented in the table for the assumption that bone activity was uniformly distributed and therefore that 20% of the activity was in trabecular bone and 80% in cortical bone (12). If bone activity was deposited entirely in trabecular bone, the average dose to bone marrow would be 16 rad/mCi administered.

An estimate of the dose rate to peritoneal tissues from activity deposited on their surface may be made by summing both over the beta-ray energy (range) spectrum of ^{90}Y and depth in tissue and shows that for 1 μCi/cm^2 of activity deposited on the surface of tissue, the dose rate averaged over the first 1 cm thickness is 1 rad/hr or 92 rad. Under the assumption that 1 mCi of ^{90}Y is deposited over a surface area in the peritoneum of 30,000 cm^2 (14), radiation dose to the first centimeter of tissues would be 3 rad while if the same activity is contained in a volume of fluid sufficient to irradiate the same surface area, the radiation dose will be <3 rad and will depend on the volume. For organs the size of liver, this additional dose only to the surface represents a negligible dose to the entire organ. Radiation dose to tumor and to thin-walled organs such as the gut due to this source may be more significant.

DISCUSSION

Using ^{90}Y obtained from an in-house ^{90}Sr-^{90}Y radionuclide generator, no difficulties were experienced in achieving the desired specific activity of ^{90}Y-labeled OC-125 (1 mCi/mg) without the need for post-labeling purification. Thus labeling efficiencies and HPLC re-

coveries were both consistently high. Both are considered to be important determinants of radiochemical purity (4). Moreover, the generator breakthrough of ^{90}Sr provided only a negligible increase in patient radiation dose, estimated at ~0.7 rad to bone assuming the entire 0.04 μCi of ^{90}Sr to be deposited in this organ.

No evidence for label instability was observed in this investigation. Size exclusion HPLC analysis of serum samples occasionally demonstrated the presence of a high molecular weight peak, however, this is likely due to immune complex formation rather than label instability. These analyses failed to show evidence of low molecular weight radioactivity peaks which would have indicated label instability. These results are in agreement with that of our previous investigation which has shown that when chelated to DTPA-coupled antibodies, ^{90}Y is stable in serum (6). Furthermore, cation exchange HPLC analysis of antibody incubation in ascitic fluid showed no evidence of transcomplexation of ^{90}Y to transferrin.

The observation that at 1 hr postinfusion in Patient 1, radioactivity in ascites was present entirely in a high molecular weight form is not surprising considering the high circulating CA-125 level in Patient 1 (Table 1). More surprising is the apparent absence of immune complex in the serum of Patients 2 and 5 and its presence in Patients 3 and 4, despite similar circulating CA-125 levels in these four patients. These observations are consistent with earlier observations regarding ^{111}In-labeled OC-125 in which no correlation was observed between circulating CA-125 and immune complex levels (1).

An important observation of this study is the variable retention of radioactivity within the peritoneum following i.p. administration. Although not directly determined, the degree of diffusion may be related to the level of radioactivity in circulation and to the radioactivity accounted for at the time of surgery (Table 1). By both considerations, Patient 1 retained a significant fraction of the administered dose; not only were serum levels much lower than that of other patients in this study but only ~20% of the injected activity appeared in urine, serum, liver, bone marrow, and bone at surgery. That this patient also presented with ascites may indicate blockage of the peritoneum and may offer an explanation for the low levels of diffusion. However, Patient 5 showed similarly poor diffusion without evidence of ascites. In this case, circulating activity was also low at surgery and only ~35% of the dose is accounted for, assuming liver activity (which was not determined in this case) to be equal to the average value of the remaining four patients. The highest serum levels were observed in the case of Patients 2, 3, and 4 while the activity accounted for in these Patients was 80%, 50%, and 70%, respectively. It is therefore likely that diffusion in these cases was pronounced and, in the case

of Patient 4, occurred rapidly judging by the high levels of circulating activity present in serum from this patient obtained at the end of infusion.

Accumulation of ^{90}Y activity in bone varied considerably from patient to patient. Some accumulation of activity in this organ is expected since ^{90}Y resulting in an overestimate of bone activity is a bone seeker in its uncomplexed form (4) and may be released from the antibody at sites of catabolism in a chemical form which accumulates in bone. Nevertheless, the high levels of bone accumulation in Patient 2 was unexpected (Table 1). It is possible that the bone biopsy sample in this case was contaminated with ^{90}Y since 80% of the administered activity is accounted for, without including activity most likely to be still present in the peritoneum. Likewise it is possible that some of the contribution to bone activity in Patients 3 and 4 is due to contamination with blood which, in these patients, contained significant activity.

The accumulation of radioactivity in tumor varied from patient to patient as shown in Table 2. Activity accumulation in normal tissues was fairly consistent throughout at ~0.003% ID/g while accumulation in tumor was 3 to 25 times this value. These values are consistent with those obtained in one patient following i.v. administration of ^{111}In-labeled OC-125 (1).

The radiation dose estimates presented in Table 3 are based on the mean tissue values from Table 1. Of concern is bone marrow where, under the assumption of uniform deposition of activity in bone, the dose to marrow may reach 8 rad/mCi. Bone marrow is thus likely to be the dose-limiting organ. The radiation dose to tumor of ~50 rad/mCi is marginal considering the need to limit exposure to bone marrow especially since a tumor dose of 1,500 rad may be required for therapy (15). However, tumor dose estimates depend on tumor geometry and are prone to large uncertainties; just as the radiation dose will decrease in small tumors, it will increase due to radiation received from activity within the peritoneum. The assumption that the activity appears in each tissue immediately upon injection leads to the highest possible radiation dose. The doses reported will be less by ~10% for each 10 hr delay, following injection, in the appearance of activity in the tissue. The doses will also be reduced if there is a biologic removal of the activity from the tissues. To date there is not enough pharmacokinetic data for this labeled antibody to predict more realistic doses.

In conclusion, the results of this investigation indicate that under the conditions of this study, therapeutic doses of ^{90}Y-labeled OC-125 fragments may be used to treat ovarian cancer, although it will be necessary to proceed with caution. The use of intraperitoneal rather than i.v. administration may be important to this application since it probably offers a means of reducing radiation exposures to bone marrow, the critical organ,

without reducing exposure to tumor within the peritoneum. It is possible that preinjection of a tracer dose of ^{90}Y-labeled antibody may be desirable to estimate the degree and rate of peritoneal diffusion prior to the administration of therapeutic doses. The success of the treatment will then depend on the extent and rate of radioactivity uptake in tumor, tumor size, and its sensitivity to radiation.

ACKNOWLEDGMENTS

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