Pharmacokinetics and Normal Organ Dosimetry Following Intraperitoneal Rhenium-186-Labeled Monoclonal Antibody

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Pharmacokinetics, biodistribution and radiation dose estimates following intraperitoneal administration of a ^{186}Re-labeled murine antibody, NR-LU-10, were assessed in 27 patients with advanced ovarian cancer. **Methods:** Quantitative gamma camera imaging and gamma counting of serum and intraperitoneal fluid radioactivity were used to obtain data for dosimetry estimation. The MIRD intraperitoneal model was used to estimate dose to normal organs from radioactivity within the peritoneal cavity. The absorbed dose to normal peritoneum was estimated in two ways: from the gamma camera activity and peritoneal fluid samples. **Results:** Serum activity peaked at 44 hr and depended on the concentration of radioactivity in the peritoneal fluid. Mean cumulative urinary excretion of ^{186}Re was 50% by 140 hr. Estimates of radiation absorbed dose to normal organs in rad/mCi administered (mean ± s.d.) were whole body 0.7 ± 0.3; marrow 0.4 ± 0.1; liver 1.9 ± 0.9; lungs 1.3 ± 0.7; kidneys 0.2 ± 0.2; intestine 0.2 ± 0.2. Peritoneal surface dose estimates varied depending on the volume of fluid infused and the method of dose determination. Using gamma camera data, the peritoneal dose ranged from 7 to 36 rad/mCi. Using peritoneal fluid sample data, the dose ranged from 2 to 25 rad/mCi. Significant myelosuppression was observed at marrow doses above 100 rad. **Conclusion:** Noninvasive methods of dose estimation for intraperitoneal administration of radioimmunoconjugates provide reasonable estimates when compared with previously described methods.

Key Words: dosimetry; intraperitoneal; rhenium-186; monoclonal antibody

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Treatment of ovarian cancer with abdominal external beam radiation therapy is associated with considerable morbidity (*1,2*). Intraperitoneal administration of radiation with ^{32}P-chromic phosphate is associated with less morbidity, and in patients with Stage I ovarian cancer it may be associated with increased survival (*3*). Phosphorus-32-chromic phosphate, however, is distributed nonspecifically and radiation is delivered to all tissues within 2 mm of the radiocolloid. Surgically confirmed adhesions occur in 7% of patients (*4*). The concentration of radioactivity at the tumor (relative to normal organs) can be increased significantly by intraperitoneal administration of beta-emitting radionuclides conjugated to monoclonal antibodies which are reactive with tumor associated antigens (*5–7*). Clinical trials with intraperitoneal ^{131}I-, ^{90}Y- and ^{186}Re-labeled antibodies have shown encouraging tumor responses in the treatment of patients with minimal ovarian cancer (*6,8–10*).

We previously reported the clinical aspects of a Phase I study with intraperitoneal ^{186}Re-NR-LU-10 antibody (a murine pancarcinoma antibody) in 17 patients with ovarian cancer (*10*). In this paper, we report the pharmacokinetics and dosimetry methods and results following intraperitoneal ^{186}Re-NR-LU-10 from two Phase I trials in greater detail. We estimated absorbed dose to normal organs using noninvasive methods by taking advantage of the 137 keV gamma emission of ^{186}Re.

METHODS

Patients

Twenty-seven patients with Stage III/IV ovarian cancer and recurrence confirmed at laparoscopy, from six institutions, participated in this study. All patients were refractory to cisplatin-based chemotherapy. Detailed eligibility criteria were previously described (*10*). Patients had disease predominantly in the abdomen. An intraperitoneal ^{99m}Tc-sulfur colloid study was performed to confirm distribution of radioactivity throughout the peritoneal cavity (*11*). Massive ascites, as evidenced by bulging flanks on physical examination, were present in five patients.

The study was approved by the Institutional Review Board at each institution and was conducted under an Investigational New Drug Application with the Center for Biologics Evaluation and Research, Food and Drug Administration. All patients granted

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TABLE 1
Absorbed Dose* to Normal Organs Following Intraperitoneal ^{186}Re-NR-LU-10 (rad/mCi)

Patient no.	^{186}Re (mCi)	Peritoneum	Whole body	Marrow†	Liver	Lungs	Kidneys	Small intestine	Large intestine	Spleen
A.1	42	10.3	0.66	0.32	2.09	1.50	0.13	0.25	0.24	0.11
A.2	43	9.4	0.73	0.38	2.35	2.21	0.14	0.21	0.20	—
A.3	96	7.7	0.55	0.28	2.12	1.16	—	0.07	0.07	—
A.4	94	10.5	0.60	0.34	2.63	0.92	0.03	0.09	0.09	—
A.5	98	5.3	0.50	0.35	2.28	1.74	—	0.04	0.04	—
A.6	132	36.0	1.64	0.27	0.45	1.86	0.79	0.77	0.81	0.45
A.7	136	9.4	0.72	0.57	1.62	1.58	0.16	0.22	0.22	—
A.9	123	9.9	0.71	0.52	0.54	1.98	0.17	0.26	0.25	—
A.12	216	6.8	0.42	0.42	3.23	1.00	—	0.07	0.07	0.03
A.13	230	10.2	0.47	0.38	1.60	0.77	—	0.17	0.17	0.08
A.16	236	31.8	1.10	0.46	0.12	1.00	0.27	0.37	0.34	0.12
A.17	255	14.8	0.56	0.40	2.10	1.32	—	0.18	0.17	0.13
B.1	100	18.2	0.46	0.59	1.87	1.43	—	0.17	0.17	0.14
B.2	95	27.0	0.49	0.38	1.57	1.77	0.10	0.11	0.10	0.08
B.3	89	24.3	0.87	0.63	1.40	1.82	—	0.16	0.16	0.22
B.4	91	23.4	0.70	0.87	3.64	1.59	0.30	0.09	0.09	0.29
B.5	107	23.4	0.42	0.40	0.83	3.05	0.11	0.15	0.15	0.09
B.6	116	17.5	0.44	0.40	1.57	0.50	0.12	0.14	0.14	0.11
B.7	137	21.8	0.61	0.31	2.95	0.69	0.08	0.17	0.16	0.06
B.8	133	17.8	0.89	0.79	1.42	0.25	0.31	0.36	0.36	0.29
B.9	175	20.6	0.72	0.25	2.37	1.72	0.32	0.42	0.39	0.31
Mean ± s.d.		17.0 ± 8.8	0.68 ± 0.28	0.44 ± 0.14	1.85 ± 0.91	1.42 ± 0.59	0.22 ± 0.19	0.21 ± 0.16	0.21 ± 0.16	0.17 ± 0.11

*Absorbed dose estimates from gamma camera data to all organs except marrow.
†Marrow absorbed dose estimates based on serum clearance.

informed consent after appropriate explanation of study details and alternatives.

Antibody

NR-LU-10 is a murine IgG2b intact antibody that recognizes a 40 kD glycoprotein antigen expressed on many epithelial cell carcinomas (*12*). The antigen to which NR-LU-10 was developed is a membrane bound antigen which is not shed. The concentration of the antigen in the circulation is extremely low and it is assumed that the concentration on the normal peritoneal surface and in ascites fluid is too low to cause significant binding. In previous studies, ovarian cancer cells from 50 patients demonstrated in vitro reactivity with NR-LU-10 by immunoperoxidase techniques. In this study, tumor cells from the first ten patients were obtained during prestudy surgery or peritoneal lavage and all specimens were reactive with NR-LU-10 (*10*). Subsequently, patients with documented epithelial ovarian cancer were studied without tissue testing.

Antibody Labeling

Labeling of the antibody with ^{186}Re was performed via the preformed chelate approach using the bifunctional chelating agent tetrafluorophenyl mercaptoacetylglycylglycyl-γ-aminobutyrate (TFP MAG_2-GABA), as previously described (*13*). Approximately 40 mg antibody was labeled with increasing amounts of ^{186}Re, to provide doses varying from 25 to 150 mCi/m^2.

Antibody Administration

Groups of two or three patients entered the studies at increasing dose levels (Table 1). In an attempt to increase the maximum tolerated dose (MTD), seventeen patients received a single dose of immunoconjugate (Group A) (*10*) and ten patients received the dose in two equal fractions 7 days apart (Group B). A 7-day interval between administrations was chosen to avoid development of human anti-mouse antibody before the second infusion.

Rhenium-186 NR-LU-10 was infused through an intraperitoneal catheter over 1 hr. Thirteen Group A patients received two liters of normal saline with the radioimmunoconjugate, but Patients A.6, A.14 and A.16 received only one liter because of ascites. Patient A.17 received one liter of saline and all Group B patients received one liter of Ringer's lactate solution.

Quantitation of Activity for Radiation Dose Estimation

Group A patients were imaged immediately, and then daily for 5 of 7 days postinjection. Most Group B patients were imaged less frequently, i.e., immediately and 48 and 140 hr following each infusion. Planar digital images were stored on computer for processing data for quantitative estimation of activity in the source organs. Counts from selected regions of interest (ROIs) were corrected for attenuation and camera sensitivity to derive the percentage of the injected dose in each source organ at each imaging session.

Whole-body activity was expressed as the fraction of administered activity remaining in the whole body at each counting time. This was done with a gamma camera whole-body scan or the patient was counted using a thyroid probe at a distance of 20 feet.

The conjugate-view method was used to quantitate the activity in the chest, abdomen and pelvis (*14,15*). Quantitation of activity in liver and lungs was performed as previously described (*16*). An attenuation correction factor was derived for each patient for liver and lungs from a transmission image prior to ^{186}Re-NR-LU-10 infusion. Because attenuation of the 140-keV ^{99m}Tc, 122-keV ^{57}Co

and 137-keV ^{186}Re photons is similar, either a ^{99m}Tc or a ^{57}Co flood source was used (*16*).

Peritoneal cavity activity was estimated from conjugate-view gamma camera images. The fraction of activity retained was determined as for whole-body activity. Regions of interest (ROIs) were drawn around the entire peritoneal cavity; the bladder and liver activity were excluded. Radioactivity in the kidneys and gastrointestinal tract could not be subtracted in most patients because activity was not sufficiently prominent for ROI definition.

A 250-ml tissue culture flask or a 150-ml saline bag filled with approximately 10 mCi ^{186}Re was counted as a calibration standard after each imaging session to determine the gamma camera detector sensitivity.

Samples of blood, urine and peritoneal fluid were collected for pharmacokinetic analysis up to 140 hr. Stools were collected for 140 hr in three patients to measure cumulative fecal excretion. Samples were counted in a gamma counter and compared with a standard of known activity.

Radiation Absorbed Dose Estimation

The Medical Internal Radiation Dose (MIRD) Committee method for determining absorbed dose was used according to the following formula (*17–20*):

$$\bar{D}(r_k \leftarrow -r_h) = \Sigma \tilde{A}_h \cdot S(r_k \leftarrow -r_h),$$

where $\bar{D}(r_k \leftarrow -r_h)$ is the mean absorbed dose to the target organ (k) from the source regions (h), $\tilde{A}_h$ is the integral cumulated activity from the source regions estimated for each patient and $S(r_k \leftarrow -r_h)$ is the mean dose per unit cumulated activity or S factor.

Time-activity curves for each source organ and the remainder of the body tissues were constructed from the percent of injected dose values and fitted to exponential disappearance curves to estimate initial organ uptakes and disappearance half-times. Whole-body and peritoneal cavity activities were both initially 100% following which exponential disappearance by biological removal and physical decay of activity was observed.

The time-activity curves in the liver and lungs were typically comprised of linear uptake of activity followed by exponential removal. Cumulative activities and residence times for each source organ were estimated from the integral of the area under the time-activity curves.

The absorbed doses to the whole body and organs were estimated using a standard mathematical MIRD anthropomorphic model using the actual weight of each patient in place of the default weight (*21*). Both penetrating and nonpenetrating radiations were considered. Rhenium-186 has a beta particle with a maximum energy of 1.07 MeV and a mean range of 1 mm in tissue. Rhenium-186 S-values were obtained from Monte Carlo calculations on MIRD anthropomorphic mathematical phantoms for photons emitted by ^{186}Re.

Absorbed doses to major organs from activity within the peritoneal cavity from penetrating cross organ gamma irradiation were estimated using the peritoneal dose model of Watson (*22*) scaled for use with MIRDOSE2 software (Internal Dose Information Center, Oak Ridge Institute for Science and Education [ORISE], Oak Ridge, TN). For organs other than the small intestine, the residence time for the peritoneal cavity activity was assigned to the small intestine for MIRDOSE2. Dose estimates for the small intestine were estimated by assigning the peritoneal activity to the large intestine. Dose estimates for the peritoneum were estimated at the peritoneal surface. The initial peritoneal tissue surface dose rate and the total absorbed dose for the peritoneal surface for ^{186}Re were estimated from numerical integration of Berger's scaled absorbed dose distributions for beta-emitting point sources in water (*23,24*). The peritoneal cavity was modeled as a large, unit-density object uniformly filled with a ^{186}Re solution.

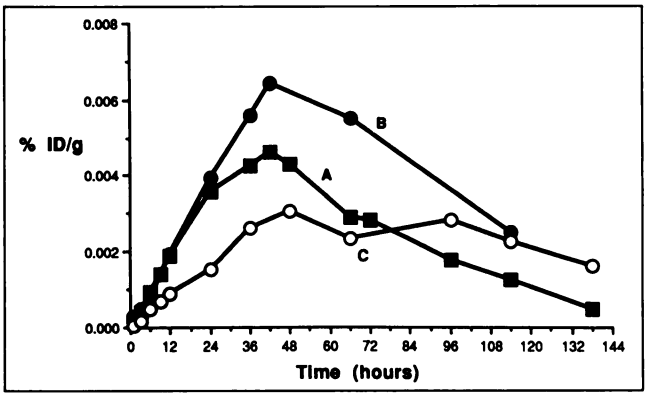


FIGURE 1. Mean serum radioactivity curves following ^{186}Re-NR-LU-10 antibody given intraperitoneally. (A) Two liters of intraperitoneal fluid administered with radioimmunoconjugate. (B) One liter of intraperitoneal fluid. (C) Patients with severe ascites and 1 liter intraperitoneal fluid.

Marrow dose was estimated from the serum time-activity curves, assuming that the primary source of marrow radiation is from circulating ^{186}Re in the blood. The specific activity in the marrow was assumed to be 25% of blood specific activity (*25*).

RESULTS

Pharmacokinetics

The binding of the ^{186}Re label to the antibody, as assessed by FPLC of the serum and peritoneal fluid, exceeded 95% in all cases up to 48 hr. The immunoreactivity of the antibody as assessed by cell binding in both the antibody preparation and the intraperitoneal fluid at 48 to 96 hr remained unchanged from baseline values. These results in serum are similar to those from previous studies in which the ^{186}Re antibody was administered intravenously (*12,13*).

Serum radioactivity (Fig. 1) increased for the first two days, reflecting absorption from the peritoneal cavity. The mean serum activity in patients who received two liters of intraperitoneal fluid with the immunoconjugate was 0.005% ± 0.002% ID/ml, curve A, or an estimated 11.5% of the injected dose in the entire serum at 48 hr (n = 13). Mean maximum serum activity from the patients who received one liter of intraperitoneal fluid was 0.007% ± 0.004% ID/ml (curve B) or 17% in the serum at 42 hr (n = 7). In four patients with massive ascites, peritoneal absorption was significantly lower and peak serum activity ranged from 0.001 to 0.004% ID/ml or 2% to 7% in the serum (curve C). No activity over the background was detected in the serum of the other patient with massive ascites.

Kidneys were the primary route of excretion. Urinary activity consisted of metabolites from ^{186}Re NR-LU-10, primarily the lysine adduct of the ^{186}Re-MAG$_2$GABA che-

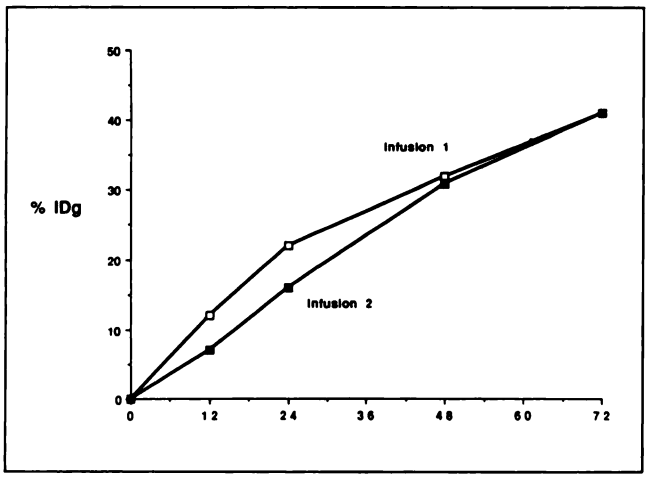


FIGURE 2. Cumulative mean urinary excretion for 72 hr following each administration of ^{186}Re-NR-LU-10 immunoconjugate, 7 days apart, in Group B patients (n = 10).

late (*26*). Mean urinary excretion by 140 hr was 50% ± 11% in patients without severe ascites. Urinary excretion after the first and second infusions in Group B patients was similar (Fig. 2). Cumulative fecal excretion was measured in three patients and ranged from 10% to 14%, respectively by 140 hr.

Sufficient peritoneal fluid samples were obtained to assess peritoneal disappearance in 25 patients as shown in Figure 3. In the 14 patients who received two liters of intraperitoneal fluid, the disappearance half-time was 38 hr (curve A). Curve B shows the activity following one liter intraperitoneal fluid (n = 8), and curve C shows the peritoneal fluid activity in four patients with massive ascites. In the absence of massive ascites, the initial concentration of activity was as anticipated by considering the activity and the volume infused. Thereafter, there was increasing concentration of activity for the first 6–9 hr, due to rapid absorption of the fluid which was infused with the immunoconjugate. With massive ascites present, the concentration was lower due to persistence of a larger fluid volume.

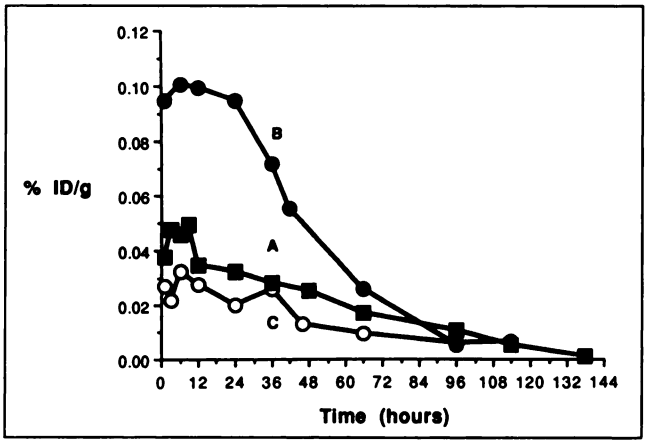


FIGURE 3. Peritoneal fluid aspirate time-activity curves following ^{186}Re-NR-LU-10 antibody. (A) Radioactivity following 2 liters of fluid with radioimmunoconjugate. (B) Following 1 liter of fluid. (C) Patients with severe ascites and 1 liter of fluid.

Biodistribution

Selected images and the decay corrected biodistribution curves of ^{186}Re NR-LU-10 in source organs from a patient without ascites (Patient A.12) are shown in Figures 4A and 4B. Adequate data for quantitation of activity from the images was obtained in 21 patients. The peritoneal cavity disappearance half-times varied over a wide range, from 27 to 312 hr, mean 87 ± 80 hr, as determined by gamma camera counts. In four patients with severe ascites there was very slow disappearance of activity from the peritoneal cavity, with disappearance half-times ranging from 80 to 312 hr. In three of these patients, cardiac blood-pool activity was never visualized. Excluding the patients with severe ascites, the mean peritoneal disappearance half-time was 52 ± 13 hr (n = 17). Patients without obvious ascites did not show prolonged disappearance.

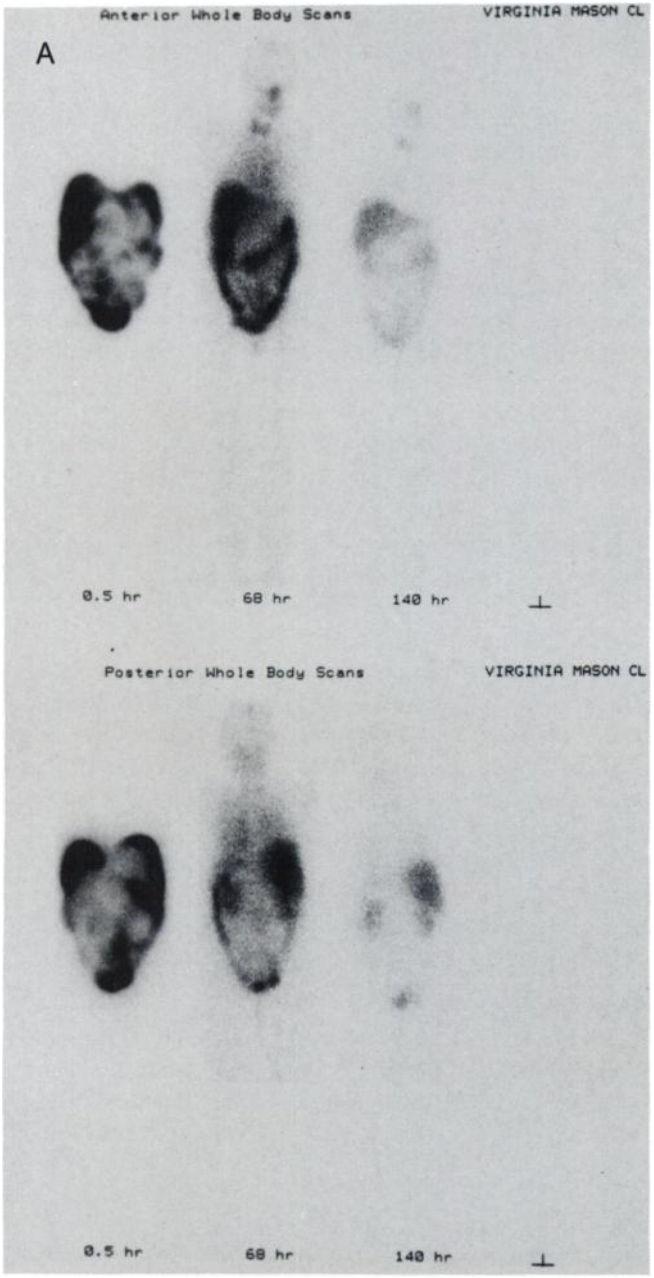
Liver and lung radioactivity increased through the 44-hr images, corresponding with an increase in cardiac blood pool radioactivity. Kidney and thyroid radioactivity were not prominent but were visualized in the majority of patients at 48 or 72 hr. This was due to cross-reactivity of the NR-LU-10 antibody with normal renal collecting duct tissue and thyroid tissue and was not related to release and uptake of free ^{186}Re (*13*). There was insufficient radioactivity for kidney or thyroid to be considered a principal source organ for dosimetry estimates in most patients. Activity in the gut at late time points was due to excretion of immunoconjugate metabolites via the biliary system.

Absorbed Dose Estimates

The absorbed doses (rad/mCi) estimated for 12 patients from Group A and the first dose for nine patients in Group B are presented in Table 1. Results from only the first dose in Group B are provided, data from the second infusion were similar.

The initial peritoneal surface dose rate from fluid in the abdominal space was estimated as 0.18 rad/hr per mCi when two liters of intraperitoneal fluid were administered and 0.36 rad/hr per mCi when one liter was administered with the ^{186}Re-NR-LU-10. The total, infinite-time peritoneal surface dose estimated for patients receiving two liters was 8.8 ± 1.7 rad/mCi and for patients receiving one liter was 20.1 ± 3.6 rad/mCi. Dose rates and peritoneal surface absorbed doses were higher in patients who received only one liter of intraperitoneal fluid with the antibody infusion, because the smaller infusion volume resulted in a higher concentration of activity at the peritoneal tissue surfaces.

Patients A.12 (Fig. 4), A.13 and A.16 showed prominent large intestinal activity at the later time points. When this activity was subtracted from the peritoneal cavity ROI the cumulative activity decreased, and the estimated intestinal wall and peritoneal surface doses were correspondingly lower, peritoneal surface dose decreased by 21%, 40% and 14%. In most of the patients, however, intestinal activity was not prominent enough for a ROI to be defined. Thus,



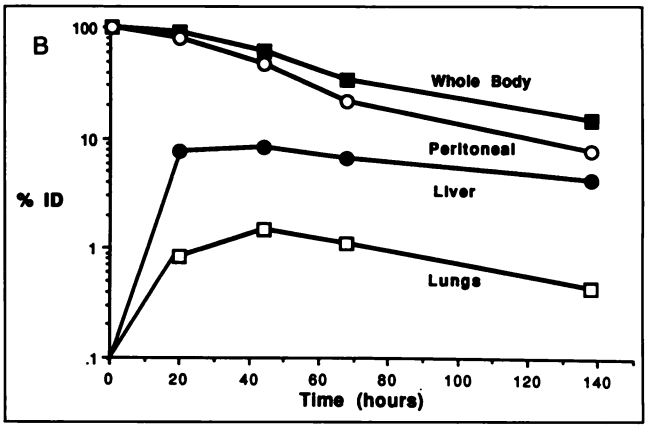


FIGURE 4. (A) Anterior and posterior whole-body images from Patient A.12 who received 216 mCi ^{186}Re-NR-LU-10 antibody. (a) = 0.5 hr. (b) = 68 hr. (c) = 140 hr. (B) Biodistribution curves in normal organs from in vivo quantitation of radioactivity from Patient A.12. The curves show decay-corrected radioactivity.

in most patients, including the gastrointestinal activity as peritoneal activity may result in overestimation of peritoneal surface absorbed doses by 10%–20%.

The daily gamma camera data indicated that there was monoexponential disappearance of radioactivity from the peritoneal cavity. The peritoneal fluid sample counts indicate in curves A and B in Figure 3, however, that there was an initial increase in the concentration of this radioactivity as the saline diluent was absorbed. In patients with massive ascites, even though the ascitic fluid was drained prior to infusion, the concentration of radioactivity in the fluid was lower and there was less of a concentration phase, probably due to continuous formation of ascitic fluid and/or slower absorption of fluid from the peritoneal cavity.

Peritoneal surface radiation absorbed dose was also estimated from radioactivity in the peritoneal fluid samples of nine patients. Table 2 shows a comparison of the dose estimates to the peritoneal surface using the peritoneal disappearance data from both methods for these patients. Note that the dose estimates from the second infusion were similar to those from the first infusion. The gamma camera measured the radioactivity retained in the abdominal region, whereas the peritoneal samples gave actual concentration of activity. Dose estimates from the external camera counts which ranged from 14 to 36 rad/mCi were higher than from the peritoneal sample data. In the five patients without ascites, B.2 to B.6, the dose estimates were very similar and ranged from 10–25 rad/mCi using peritoneal sample data, compared with 14–27 rad/mCi from the external counting. In the four patients with ascites, the dose estimates from the peritoneal sample data were much lower than those estimated from the gamma camera ROI data and ranged from 2 to 7 rad/mCi. This was because the radioactivity concentration remained relatively low in the large volume of ascitic fluid.

Kidney dose estimates in Table 1 are derived from the gamma camera data when the kidney was not a source organ, i.e., in 14 of the 21 patients. In the remaining seven patients, kidney activity was above background activity. In four of these patients dose estimation was possible because there were sufficient data points to derive time activity curves and the dose to the kidney ranged from 0.6 to 6 rad/mCi. The thyroid gland activity was high enough to be considered a source organ in only three patients, and the dose estimates ranged from 0.8 to 2 rad/mCi.

Marrow toxicity was the dose limiting toxicity. When the marrow absorbed dose estimates were above 100 rad, significant toxicity was seen. After a single intraperitoneal administration at the 150 mCi/m^2 dose level, or 230–255 mCi, two patients (A.15 and A.16) experienced Grade III marrow toxicity, i.e., platelet count 25,000 to 50,000/μl or

TABLE 2
Comparison of Absorbed Dose to Peritoneal Surface in rad/mCi of ^{186}Re-NR-LU-10 Using Data from Gamma Camera Measurements and Peritoneal Fluid Aspirate Measurements

Patient no.	Infusion #1		Infusion #2	
	Data from camera	Data from fluid	Data from camera	Data from fluid
B.2	27.0	25.3	22.1	25.1
B.3	24.3	11.7	21.8	10.6
B.4	23.4	21.0	22.9	18.6
B.5	23.4	16.5	19.4	15.6
B.6	17.5	10.1	13.8	not done
B.7*	21.8	2.0	21.4	3.2
B.10*	26.6	2.4	29.7	3.1
A.6*	36.0	7.1	—	—
A.16*	31.8	3.8	—	—

*Patients with severe ascites.

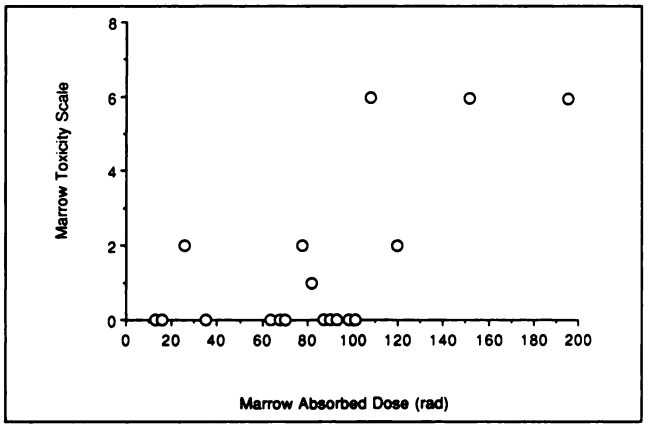


FIGURE 6. Bone marrow suppression (arbitrary units from graded toxicities) compared with total radiation absorbed dose to marrow (rad).

white cell count 1000 to 2000/μl. The total marrow dose estimate in Patient A.16 was 110 rad (Fig. 5), but a total was not available from the other patient. In the second study, two patients, B.4 and B.8, receiving a total of 152 and 196 rad, respectively, from two infusions of 60 mCi/m^2 and 90 mCi/m^2, respectively, given at 1-wk intervals, experienced Grade III marrow toxicity. Figure 6 shows marrow toxicity compared with absorbed dose to marrow using the combined graded toxicity of white cells and platelets as the toxicity scale. In one of the patients with Grade 6 toxicity, dosimetry data were not available.

DISCUSSION

The pharmacokinetics of intraperitoneally administered ^{186}Re-NR-LU-10 is similar to that previously reported for intraperitoneal administration of ^{131}I (*27,28*) and ^{90}Y-labeled (*6,7,9,27*) intact antibodies. The mean peak radioactivity of the ^{186}Re immunoconjugate in the serum occurred at approximately 44 hr and was 12% to 17% of the injected dose depending on the volume infused. For comparison, the mean peak levels were reported as 26% for ^{131}I (*8*) and 23% reported for ^{90}Y-labeled antibodies (*6*). The cumulative urinary excretion of 51% at 140 hr was lower than that following ^{131}I-labeled HFMG1 antibodies, (60% at 110 hr) (*8*). Urinary excretion of ^{90}Y after administration of ^{90}Y-labeled antibody was 11%, probably because of retention of ^{90}Y in the liver, spleen and bone (*27*).

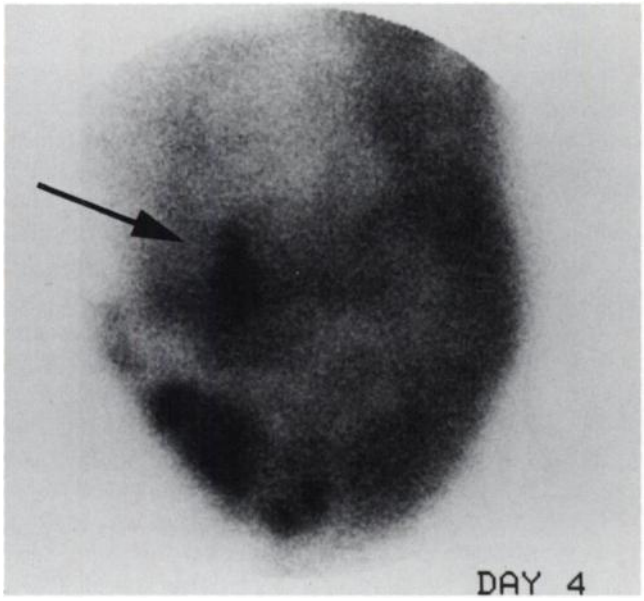


FIGURE 5. Image from Patient A.16 shows localization of radioactivity in the tumor 68 hr after injection of 236 mCi ^{186}Re-NR-LU-10.

We observed low serum radioactivity and prolonged retention of peritoneal activity in patients with severe ascites as have other investigators (*6,8,9*). Prolonged peritoneal disappearance may be attributed to retention of immunoconjugate on tumor tissues such as malignant cells in the ascites fluid (*29*), but the absence of a concentration phase in the peritoneal disappearance curves (Fig. 3) suggests that the delayed clearance is also due to decreased absorption from the peritoneal cavity due to obstruction of lymphatics in the presence of massive ascites (*7*).

By taking advantage of the favorable imaging characteristics of ^{186}Re, we were able to estimate absorbed dose to normal tissues following intraperitoneal NR-LU-10 from data obtained noninvasively by gamma camera imaging, similar to the methodology used in clinical trials with systemically administered radiolabeled antibodies (*16*). One limitation of this method is that by counting radioactivity in the entire abdominal region both organ and metabolite activity, particularly gastrointestinal activity, are included in the peritoneal cavity counts and these cannot always be subtracted. This results in the underestimation of peritoneal clearance, probably by about 10–20%.

To estimate radiation dose accurately to organs bathed in radioactive fluid, it is necessary to know the concentration of the activity. Although disappearance of activity could be estimated with the gamma camera ROI method, the concentration of the activity could not be assessed. The

initial concentration was estimated from the activity and the volume of fluid infused. The dynamics of fluid movement into and out of the peritoneal cavity are complex and cannot be easily measured. Forty to 80% of ascites fluid is believed to leave and enter the peritoneal cavity per hour (*30*). The increasing concentration of activity in the peritoneal aspirate curve for 6 to 9 hr suggested a rapid absorption of the infused fluid and that the concentration of the immunoconjugate at 9 hr was higher than that immediately following injection.

Because of this, we estimated peritoneal surface doses more directly by using the concentration measured in peritoneal fluid samples. The results from both methods were similar in spite of the limitations, except when severe ascites were present. In those patients with massive ascites, the lower dose estimates to the peritoneal surface from the peritoneal fluid data are more realistic because the concentration of activity is considered. Thus, although measuring concentration by peritoneal fluid aspiration does improve the accuracy for estimating peritoneal surface dose, it is somewhat invasive and it is not always possible to obtain adequate peritoneal fluid samples.

The MIRD dosimetry model assumes homogeneous distribution of activity within the peritoneum. The folding of the peritoneum, however, and the inhomogeneous distribution of activity on the images (Fig. 5) suggests that the absorbed dose to both the intestine and peritoneal surface varies. In situations where either the antibody or the radioactivity is deposited in the peritoneal surface, or is complexed to circulating antigen, e.g., CA-125, neither of the methods described above could be used to estimate peritoneal surface dose. Because the antigen to which NR-LU-10 was developed is a membrane-bound antigen, we have assumed that there is no specific localization of the ^{186}Re-NR-LU-10 on the peritoneal surface.

Our dose estimates of 14–27 rad/mCi to the peritoneal surface of patients without severe ascites, even with the limitations of our noninvasive methodology, are in the same range as those determined by more invasive methods, such as biopsy or placement of thermoluminescent dosimeters (TLDs) following ^{131}I- (*27,28*) and ^{90}Y-labeled antibodies (*6,7,9,27*). These procedures were necessary to quantitate activity for dose estimation from ^{32}P-chromic phosphate (*31*) and ^{90}Y-labeled antibodies because of the poor resolution images of the bremsstrahlung radiation resulting from the high-energy beta emissions. Absorbed dose estimates to the peritoneal surface in patients have been obtained from LiF TLDs for ^{131}I- and ^{90}Y-HFMG1 antibodies, infused in 1500 ml, and measured 2.88 ± 0.63 rad/mCi and 21.7 ± 11 rad/mCi, respectively (*8,27*). Hnatowich et al. estimated normal organ and tumor dosimetry from ^{90}Y-OC-125 F(ab')$_2$ antibody by counting biopsy activity (*7*). Using only one data point, additional assumptions were required. It was assumed that uptake was instantaneous and there was no biological clearance from the tissues. As demonstrated in Figure 4B, however, maximal organ activity was not instantaneous but was related to movement of activity from the peritoneal cavity to the circulation, e.g., liver activity highest at 44 hr. Dosimetry from tissue counting also ignores the dose from activity in the surrounding peritoneal fluid, which for small organs and tumors may be significant.

Radiation absorbed dose to the peritoneal surface was measured by TLD in dogs who received ^{32}P chromic phosphate in 400 ml saline (*31*). Dose to the diaphragmatic surface was highest, 8000 rad/mCi, most likely because of the lymphatic drainage of the colloid through the diaphragm. Dose estimates to the peritoneal surface of the liver and spleen were 2000 rad/mCi and to the retroperitoneal nodes were less than 40 rad/mCi.

The greater volume of fluid infused in our studies, two liters compared with one liter, resulted in less absorbed dose to the peritoneal surface. Specific localization of the radiolabeled antibody (Fig. 5) would result in a higher dose to tumor tissue than to the normal peritoneum. Hnatowich et al. demonstrated 3–25 times higher activity in tumor than in normal tissues, and estimated 48 ± 44 rad/mCi from ^{90}Y-labeled Fab$'_2$ fragment of OC-125 antibody (*7*). The effect of the volume of fluid infused on tumor localization of the radioimmunoconjugate is unknown, but increasing the infused fluid volume may possibly increase the tumor-to-normal peritoneal surface dose ratio.

Larson et al. reported the use of the MIRD intraperitoneal dosimetry model in patients with colorectal carcinoma in conjunction with biopsy and gamma camera data to estimate dose to normal organs and tumor. They estimated absorbed doses up to 20 rad/mCi to sites of peritoneal carcinomatosis with ^{131}I-B72.3 (*28*). Tumor dosimetry was not estimated in our studies because tumor volumes were not obtained. However, partial responses at repeat laparoscopy/laparotomy were observed in five patients with tumor masses less than 2 cm. We estimated that eight patients received 4,000 to 6,000 rad to normal peritoneum and this dose was not associated with radiation related toxicity.

Intraperitoneal administration of ^{131}I- and ^{90}Y-labeled antibodies has been limited to total doses of 160 and 20 mCi respectively because of severe myelosuppression (*8,27*). Administration of intravenous EDTA following infusion of ^{90}Y-DTPA antibodies has been successful in reducing marrow toxicity by chelating released ^{90}Y (*6*). With ^{186}Re-NR-LU-10, we were able to administer up to 150 mCi/m^2 as one infusion before significant marrow was observed. Although the numbers of patients in these studies are small, administering the dose in two fractions separated by 7 days resulted in less marrow toxicity than did a single full dose of 150 mCi/m^2. The marrow absorbed dose estimates associated with significant toxicity (100 to 200 rad), although low, are probably realistic, considering that marrow reserve was most likely limited by prior chemotherapy that all these patients had received. These total doses of 150 and 180 mCi/m^2, are 60–90 mCi/m^2 higher than the MTD determined in the trial of ^{186}Re-NR-LU-10 administered intravenously (*13*). Because intraperitoneal administration of

radioimmunoconjugate causes a slow accumulation of activity in serum, and approximately 44 hr of physical decay of the radionuclide had occurred by the time the maximum serum concentration was reached, and the estimated marrow dose per millicurie administered intraperitoneal was approximately 50% of that following intravenous administration.

CONCLUSION

We used the MIRD intraperitoneal model to estimate radiation dose to normal organs following intraperitoneal administration of a ^{186}Re-labeled monoclonal antibody using gamma camera and pharmacokinetic data. Accuracy of absorbed dose estimates to the peritoneal surface is improved when an accurate concentration of radioactivity in the peritoneal fluid is known. Use of gamma camera estimates to determine cumulative activity in the peritoneal cavity requires assumptions relating to the concentration of activity. Measuring peritoneal fluid samples supplied this data, but was not always technically feasible. We found that in patients who did not have severe ascites, the dose estimates to the peritoneal surface were similar using either method. When ascites were present, the peritoneal fluid samples were essential for absorbed dose estimation. Overall, the methods described in this study provide a practical means to estimate radiation absorbed dose from intraperitoneal ^{186}Re-labeled NR-LU-10 monoclonal antibody.

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