Indium-111- and Yttrium-90-Labeled Human Monoclonal Immunoglobulin M Targeting of Human Ovarian Cancer in Mice

Paul E. Borchardt, Syed M. Quadri, Ralph S. Freedman and Huibert M. Vriesendorp
Departments of Experimental Radiation Oncology and Gynecologic Oncology, The University of Texas M.D. Anderson Cancer Center, Houston, Texas

Most patients with ovarian cancer have disease in the peritoneal cavity. Treatment of this region is inadequate because recurrences are frequent. Increased radiation doses to tumor and, hence, greater tumor control may be possible with intraperitoneal (i.p.) administration of radiolabeled human monoclonal immunoglobulin M (IgM), which is reactive with tumor-associated antigens. **Methods:** Biodistribution studies were performed in nude mice bearing i.p. nodules of human ovarian cancer after administration of human monoclonal IgMλ (AC6C3-2B12), labeled with ^{111}In or ^{90}Y. Irrelevant ^{111}In-labeled human IgMλ (CH-1B9) and ^{90}Y-aggregate served as specificity controls. **Results:** Intravenous administration of ^{111}In-labeled AC6C3-2B12 produced low tumor and high liver and spleen uptake. Intraperitoneal administration of AC6C3-2B12 labeled with ^{111}In or ^{90}Y resulted in rapid, high tumor uptake (>45% of injected dose per gram of tumor at 3 hr) that was at least three-fold higher than any normal organ. Biodistribution results were similar for ^{111}In- and ^{90}Y-labeled IgM. Tumor uptake of ^{111}In-labeled AC6C3-2B12 was two-fold greater than that of ^{111}In-labeled CH-1B9. Normal organ uptakes were similar for tumor-reactive and irrelevant IgM. Radioimmunoconjugates were retained in the peritoneal cavity for a prolonged period of time. Yttrium-90 aggregate demonstrated high tumor and bone uptake. **Conclusion:** Higher tumor uptake was observed after i.p. administration of tumor-reactive IgM than after irrelevant IgM. The in vivo behavior of tumor-reactive IgM was similar when it was radiolabeled with either ^{111}In or ^{90}Y. Therefore, ^{111}In-based imaging studies can be used to predict the biodistribution of subsequently administered ^{90}Y-labeled IgM. Further development of radiolabeled AC6C3-2B12 as a diagnostic and therapeutic agent for patients with advanced ovarian carcinoma is warranted.

Key Words: human IgM; intraperitoneal radioimmunotherapy; pharmacokinetics; ovarian carcinoma

J Nucl Med 1998; 39:476–484

Ovarian carcinoma is the fourth most common cause of death from cancer in women, with 14,800 deaths occurring annually in the U.S. (*1*). The 5-yr survival rate ranges from 50% for patients with tumors confined to the ovaries to less than 20% for patients with Stage III and IV disease (*2*). At the time of diagnosis, 75%–85% of all patients have advanced disease (*3*). Current first-line therapy for these patients consists of cytoreductive surgery followed by intravenous (i.v.) platinum-based chemotherapy, which now includes paclitaxel. Response rates range from 60% to 80% (*4,5*); however, the majority of these patients relapse.

The observation that most patients have disease in the peritoneal cavity and that it is a common site of relapse has focused attention on regional treatment approaches. Intraperitoneal (i.p.) chemotherapy, i.p. colloidal ^{32}P and external beam radiotherapy of the entire abdomen have been reported (*6,7*). Tumor response and prolongation of survival is limited to patients with microscopic or very small macroscopic disease. Tissue penetration by i.p. administered chemotherapeutic agents is confined to the outer 1 mm–2 mm of the tumor surface (*8*). Most chemotherapy drugs have short half-lives in the peritoneal cavity, which are directly proportional to their small size (*9*). Intraperitoneal colloidal ^{32}P nonselectively irradiates the surface of the peritoneal cavity. It is also prone to aggregation that produces inhomogeneous dose distributions (*10*). The presence of radiosensitive organs in the abdominal region prevents the delivery of high doses of external beam radiation. The probability of eradicating measurable disease (cancer nodules of ≥5 mm) with low-dose external beam radiation is small.

Intraperitoneally-administered radiolabeled antibodies may provide a more effective regional therapy. Intraperitoneal administration places a high concentration of radiolabeled antibody in close proximity to tumor. The specificity of the antibody combined with its high, local concentration, the absence of an endothelial barrier and a long half-life in the peritoneal cavity could lead to higher doses of radiation to i.p. tumors than was previously possible (*11,12*).

In contrast to earlier studies with i.p. radiolabeled murine immunoglobulin G (IgG), the use of tumor-reactive, radiolabeled human monoclonal immunoglobulin M (IgM) may address three problems that prevent the incorporation of i.p. radioimmunotherapy (RIT) into standard clinical practice: low tumor uptake and retention of the radioimmunoconjugates, myelotoxicity and the development of human antimouse antibodies. Low tumor uptake of radiolabeled IgG results in low tumor response. Dose escalation cannot be used as a means to increase tumor deposition of radioactivity because, with IgG, significant levels of radioactivity reach the blood. This produces myelotoxicity (*13,14*). Bone marrow damage usually results from irradiation of the bone marrow cells by circulation of radioimmunoconjugates in the blood and not by specific targeting of the bone marrow parenchyma by radioimmunoconjugates or incorporation of radioisotopes in the bone matrix (*15*). Human antimouse antibody formation is common among immunocompetent patients given radiolabeled murine monoclonal IgG (*13,16*). The presence of anti-antibodies can induce allergic reactions and may prevent tumor targeting of the administered radiolabeled antibodies. The incidence of anti-antibody formation can be greatly reduced through the use of human antibodies (*17*). Retention of radiolabeled antibody in the peritoneal cavity depends on the presence of tumor antigen (*18*), the size of the immunoglobulin and the size of the host (*19*). Immunoglobulin M is approximately 5 times larger than IgG and has a much higher degree of avidity, due to its 10 antigen-binding sites. Both factors are expected to contribute to a longer half-life in

Received Dec. 6, 1996; revision accepted Apr. 15, 1997.
For correspondence or reprints contact: Syed M. Quadri, PhD, 6701 Rockledge Dr., Room 4144, Center for Scientific Review, National Institutes of Health, Bethesda, MD 20892.

the peritoneal cavity for IgM than for IgG. Moreover, myelotoxicity can be prevented if radiolabeled IgM does not reach the bloodstream in significant amounts.

An outpatient RIT treatment program can be established using two sequential injections, one for diagnosis using a pure gamma-emitting radioisotope and the other for therapy using a pure beta emitter. Previous studies suggest the use of ^{111}In as the diagnostic and ^{90}Y as the therapeutic isotope (*20,21*). Central to this scheme is the similar in vivo behavior of the immunoconjugate when it is radiolabeled with either radioisotope. Imaging can be performed with a low activity of the gamma emitter. If immunoscintigraphy using a gamma camera demonstrates tumor localization and in vivo stability of the radioimmunoconjugate, then dosimetric estimations can be performed to calculate an appropriate activity of the beta-emitting radioisotope to prescribe. Most of the energy emitted by the therapeutic isotope will be absorbed in the vicinity of the tumor, allowing the patients to be treated as outpatients, thereby reducing treatment expense and adding to patient comfort.

This study compares the biodistribution of human monoclonal IgM 2B12 radiolabeled with ^{111}In or ^{90}Y in nude mice bearing peritoneal nodules of a human epithelial ovarian carcinoma cell line (SKOV3 NMP2). The results support further development of i.p. ^{90}Y-labeled IgM for the treatment of peritoneal carcinomatosis in human patients.

MATERIALS AND METHODS

Monoclonal Antibodies

A human monoclonal antibody secreted by the AC6C3 heterohybridoma was used in these experiments (*22,23*). It is of the IgMλ isotype and is reactive with specimens of ovarian, breast and colon carcinomas and certain other malignancies but not normal tissue. At approximately annual intervals, the hybridoma is subjected to recloning, and the secreted antibody is tested for reactivity against an ovarian tumor cell line (SKOV3; American Type Culture Collection, Rockville, MD) by fluorescence-activated cell sorter (FACS) analysis. The experiments presented here were conducted with the antibody secreted by the recloned cell line, AC6C3–2B12, hereafter referred to as 2B12. An isotype-matched human monoclonal IgMλ (CH-1B9) was used as an irrelevant control.

Fluorescence-Activated Cell Sorter Analysis

The reactivity of the immunoglobulins with the cell surface of a human ovarian carcinoma cell line (SKOV3) was demonstrated by FACS. Briefly, cells were cultured in L15 medium containing 10% (v/v) fetal bovine serum (FBS) in the presence of 5% CO_2 at 37°C. Then, cells were detached from the tissue culture flasks with Versene and washed with phosphate-buffered saline (PBS), pH 7.3, containing 2% FBS and 0.02% (w/v) NaN_3. They were then incubated with 100 μl (100 μg) of human IgM for 60 min at 4°C, washed and then incubated in a 1:100 dilution of fluorescein isothiocyanate-conjugated goat antihuman $F(ab')_2$ for 30 min at 4°C. The cells were then washed and resuspended in PBS with 2% FBS and 0.02% NaN_3, followed by fixation in 2.0% (v/v) paraformaldehyde. Cell binding was examined with an EPICS profile analyzer (Coulter, Hialeah, FL).

Preparation of Immunoconjugate

Immunoglobulin M (1×10^{-5} mmol) in 0.05 *M* Hepes buffer, pH 8.6, was reacted with 1.0×10^{-4} mmol of 2-(*p*-isothiocyanato)-benzyl-3-methyl-diethylenetriamine petaacetic acid (ITC-2B3M-DTPA) at 4°C for 12 hr (*24*). The immunoconjugate was purified from unreacted ITC-2B3M-DTPA by filtration in a Centricon-30 (Amicon Corp., Beverly, MA) and washed with 0.1 *M* PBS, pH 7.3. Purity was ascertained by ultraviolet absorption measurements after size-exclusion HPLC using a Bio-Silect SEC 250–5 column (Bio-Rad, Hercules, CA) with 0.2 *M* sodium phosphate buffer, pH 7.2, as the mobile phase. The average number of DTPA molecules per IgM was determined as described previously (*25*).

Radiolabeling of IgM-2B3M-DTPA

The ^{111}In-labeled immunoconjugate was prepared by mixing 100 μl of 0.6 *M* sodium acetate buffer, pH 5.3, with 100 μl of 0.06 *M* sodium citrate buffer, pH 5.5, and 100 μl of immunoconjugate solution (10 mg/ml). Then, 5 μl of $^{111}InCl_3$ solution (1 mCi; New England Nuclear, Boston, MA) was added with mixing and the reaction mixture allowed to incubate for 40 min. This empirically selected incubation time produced a high level of ^{111}In incorporation. The radioimmunoconjugates were challenged with excess chelator by incubating the labeling mixture with 0.01 *M* DTPA, pH 6.5, for 10 min. The radiolabeled IgM was purified on a Sephadex G50 gel column (1.5 × 12 cm) using 0.1 *M* PBS as the elutant. Fractions were collected and assayed with a CRC-15R dose calibrator (Capintec, Ramsey, NJ). The degree of radiometal incorporation and the purity of the radioimmunoconjugate were assessed at each step by instant thin-layer chromatography (ITLC) with saline as the mobile phase and thin-layer chromatography (TLC) using a 1:1 ratio of methanol to 10% (w/v) ammonium acetate in water as the mobile phase. The strips were cut in half and counted in a Cobra II gamma counter (Packard Instrument Co., Meriden, CT). The radioimmunoconjugate remained at the origin, whereas ^{111}In that was poorly bound to the immunoconjugate was stripped off by the excess, free chelate and migrated with the solvent front.

The ^{90}Y-labeled radioimmunoconjugate was prepared by mixing 200 μl of 2.0 *M* sodium acetate, pH 6.0, with 200 μl of immunoconjugate solution (10 mg/ml). To this was added 5 μl of $^{90}YCl_3$ solution (5.0 mCi; Pacific Northwest National Laboratory, Richland, WA). The solution was mixed well and allowed to incubate for 60 min, an empirically selected time that produced high levels of ^{90}Y incorporation. The reaction was quenched with a 100-fold excess of 0.01 *M* DTPA, pH 6.5. After a 10-min incubation, the solution was loaded onto a Sephadex G100 gel column (1.5 × 30 cm), and the radioimmunoconjugate was eluted with 0.1 *M* PBS, pH 7.3. The fractions were collected and assayed for radioactivity using a dose calibrator. Purity was assessed at each step by ITLC with saline as the mobile phase and by TLC using a 1:4 ratio of 30% ammonium hydroxide and ethanol as the mobile phase. The radioimmunoconjugate remained at the origin, whereas ^{90}Y that was poorly bound to the immunoconjugate was stripped off by the excess, free chelate and migrated with the solvent front.

Preparation of Yttrium-90-Aggregate

Five microliters of YCl_3 (7.7 mCi) were mixed with 50 μl of 0.05 *N* NaOH. The solution was vortexed vigorously. One hundred microliters of a 12.5% solution of human serum albumin in PBS, pH 7.3, were added. The mixture was vortexed again and then allowed to incubate for 1 hr. A gelatinous substance formed. An aliquot of the aggregate was resuspended with sterile 0.1 *M* PBS, pH 7.3, for injection into mice. The integrity of the suspension was checked by ITLC with saline as the mobile phase and by TLC using a 1:4 ratio of 30% ammonium hydroxide to ethanol as the mobile phase. The ^{90}Y-aggregate remained at the origin, whereas $^{90}YCl_3$ or low molecular weight ^{90}Y-complex moieties were found at the solvent front.

Tumor Inoculation

A human epithelial ovarian carcinoma cell line, SKOV3-NMP2 (*26*), was obtained from Dr. Kalpana Mujoo (The University of Texas M.D. Anderson Cancer Center). It was grown in minimal

essential medium supplemented with 10% FBS and 5% CO_2 at 37°C. Cells were detached with 0.25% trypsin at 37°C for 2 min. The trypsin was neutralized with medium, and the cells were then centrifuged at 800 × *g*. The cell pellet was resuspended in medium to a density of 12.5 × 10^6 cells per ml. Female athymic nude mice, 6–10 wk old, were injected i.p. with 0.2 ml of cell suspension using a 30-gauge needle. The mice were housed in filter-top cages and provided with sterile food and water. Animal studies were conducted in compliance with the U.S. Department of Agriculture and Animal Welfare Act. Animal protocols were approved by the Animal Care and Use Committee at the M.D. Anderson Cancer Center.

Radioimmunoglobulin and Yttrium-90-Aggregate Administration to Nude Mice

The purified radioimmunoconjugate solutions were sterile-filtered and then diluted to the appropriate activities using sterile 0.1 *M* PBS, pH 7.3. All radioimmunoconjugates were retested for radiochemical purity immediately before administration. All preparations were >95% pure and were used within 2 hr from the completion of column purification. The reagents were administered to mice that had received the tumor inoculate 12 days earlier. Both the i.p. and i.v. treated mice received approximately 200 μl of the radioimmunoconjugate. The ^{111}In-IgM-treated mice received 10–15 μCi of activity, except for the mice treated for the measurement of whole-body retention of radioactivity. They received 55 μCi of activity. The ^{90}Y-IgM-treated mice received 40 μCi of activity. The aggregate was >99% pure and was administered i.p. to the mice. They received approximately 200 μl of the suspension at an activity of 30 μCi. These activity levels were chosen based on the duration of the experiments and the counting efficiency of these radioisotopes with the gamma counter. In general, the number of mice used for each time point ranged from four to six.

Samples of the administered reagents were saved as controls to correct for radioactive decay in the biodistribution experiments.

Biodistribution in Nude Mice

Mice treated i.p. were euthanized at 3, 24, 48 and 96 hr postinjection. Blood was drawn by cardiac puncture and weighed. Normal tissue and tumor nodules were excised, rinsed in PBS, blotted dry, weighed and then counted in a gamma counter along with the blood samples. The iv.-treated mice were euthanized at 24 and 48 hr post-treatment. The organ samples were collected and processed as above. The counting efficiencies were approximately 73.3% (150- to 500-keV window) for ^{111}In and 11.5% (500- to 2000-keV window) for ^{90}Y. The results were corrected for radioactive decay and were expressed as percent injected dose per gram of tissue (%ID/g). Values for s.d. of the mean are given in the figures only. Standards of known radioactivity in 1 ml of PBS were used to convert counts/min into μCi/g. No corrections were made for volume or density of experimental samples.

Whole-Body Autoradiography

Two mice that had received i.p. ^{111}In-labeled 2B12 were killed at 24 hr postinjection. Their extremities were removed, and their bodies were frozen in 4% carboxymethyl cellulose. The frozen blocks were mounted on a cryomicrotome (Hacker Instruments, Fairfield, NJ) and sectioned into 50-μm-thick coronal slices. Photographs were made of the sections, which were then mounted on tape and freeze-dried. The sections were subsequently exposed to X-Omat AR x-irradiation film (Kodak, Rochester, NY) for 48 hr before development of images.

Whole-Body Retention

Five mice that received i.p. ^{111}In-labeled 2B12 were measured daily for whole-body retention of radioactivity in a dose calibrator over a period of 0 hr–165 hr. The measurements were corrected for radioactive decay.

TABLE 1
Quality Control Analysis of Immunoglobulin M Conjugates

Analysis	^{111}In-IgM (2B12)	^{90}Y-IgM (2B12)	^{111}In-IgM (CH-1B9)
Average DTPA/IgM	4	4	4
Purity of immunoconjugate (size-exclusion HPLC)	98%	98%	98%
Purity of radioimmunoconjugate* (ITLC and TLC)	99%	98%	99%
Specific activity (mCi/mg)	1.0	4.1	1.6
Serum stability†			
24 h	94.0%	94.0%	nd
72 h	92.0%	90.5%	nd
Activity injected per animal	10 μCi	40 μCi	15 μCi

*Mean value.
†Protein-bound fraction after incubation at 37°C in human serum; mean values of triplicate tests.
HPLC = high-performance liquid chromatography; nd = not determined.

Statistical Analysis

The differences in the biodistribution values of different experimental groups were compared using the Mann–Whitney rank sum test.

RESULTS

Quality Control Analysis

The purified antibodies demonstrated cell surface reactivity by FACS of 93% for 2B12, compared to 19% for the irrelevant IgM (CH-1B9). Table 1 summarizes the in vitro quality control analysis of the immunoconjugates and radioimmunoconjugates. Size-exclusion high-performance liquid chromatography analysis of the immunoconjugates demonstrated a single ultraviolet absorption peak after derivatization. An average of four 2B3M-DTPA molecules were conjugated per IgM molecule. Moreover, after purification of the radioimmunoconjugates, 98% of the radioactivity was antibody-bound. More than 90% of the radioactivity, using either isotope, remained bound to the immunoconjugate when incubated up to 72 hr at 37°C in human serum. The in vitro immunoreactivity of the radioimmunoconjugates was not analyzed because previously reported tests using a cell-binding assay demonstrated that the immunoreactivity was retained after derivatization and radiolabeling (*27,28*).

Biodistribution of 2B12

Routes of Administration. The biodistribution data comparing the route of administration of ^{111}In-labeled 2B12 with tumor and normal organ uptake are shown in Figure 1. Intraperitoneal administration resulted in a high tumor uptake at 24 hr (39%ID/g) that remained elevated at 48 hr (28%ID/g). Tumor uptake was at least three-fold higher than uptake by any normal organ at both time points ($p < 0.001$ for all organs). Liver and spleen demonstrated the highest normal organ uptake at both time points (12%ID/g and 13%ID/g, respectively, at 24 hr, with a drop to 8%ID/g for both by 48 hr). Blood radioactivity was 3.3%ID/g at 24 hr and decreased to 1.7%ID/g by 48 hr. Uptake of radioactivity for organs outside the peritoneal cavity (heart, lung, muscle and bone) was low (<2%ID/g) at both time points. Intravenous administration resulted in low tumor uptake at 24 hr (0.9%ID/g) and at 48 hr (1.4%ID/g). Liver and spleen showed moderately high uptake at 24 hr (17%ID/g and

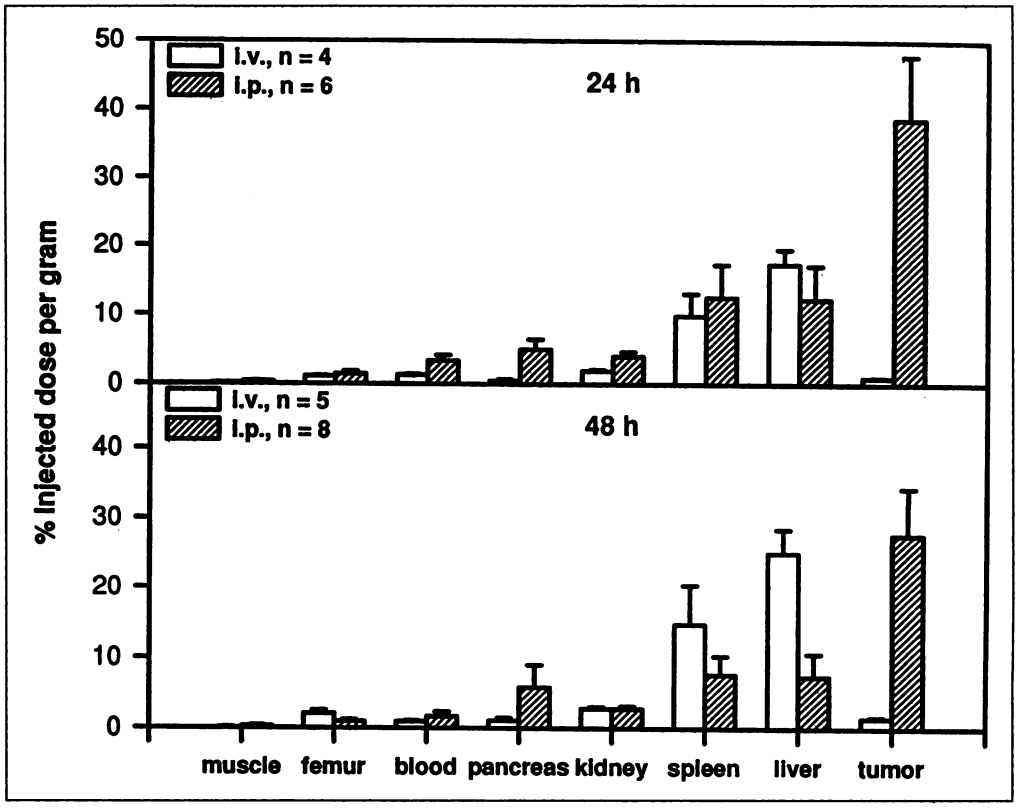

FIGURE 1. Comparison of i.p. and i.v. ^{111}In-labeled 2B12 administered in nude mice bearing SKOV3 NMP2 xenografts (mean ± s.d.).

10%ID/g, respectively), which increased by 48 hr (25%ID/g and 15%ID/g, respectively). All other organs showed low uptake at both time points, with the kidney having the highest uptake at both time points (1.9%ID/g at 24 hr and 2.8%ID/g at 48 hr). Clearance of radioactivity from the blood was nearly complete at 24 hr (1.3%ID/g). Intraperitoneal administration produced significantly higher kidney uptake at 24 hr ($p = 0.005$), whereas i.v. administration produced significantly higher uptake in liver (24 hr, $p = 0.033$; 48 hr, $p < 0.001$), spleen (48 hr, $p = 0.003$) and femur (48 hr, $p < 0.001$).

Comparison of 2B12 Labeled with Indium-111 or Yttrium-90. The biodistribution data comparing the i.p. coinjection of 2B12 labeled with ^{111}In or ^{90}Y are shown in Figure 2. Tumor uptake was high at 3 hr (46%ID/g for ^{111}In and 70%ID/g for ^{90}Y) and remained high for at least 48 hr (28%ID/g for ^{111}In and 30%ID/g for ^{90}Y). At the early time points, tumor uptake was three-fold higher than uptake in the highest normal organs (liver and spleen), but the ratio dropped to two-fold at 48 hr and to about one-fold by 96 hr. Blood activity was the highest at 3 hr (7.2%ID/g for ^{111}In and 8.9%ID/g for ^{90}Y) and decreased rapidly, so that by 24 hr, blood activities were 2.1%ID/g for ^{111}In and 1.5%ID/g for ^{90}Y. Uptake of the radioimmunoconjugate by organs outside the peritoneal cavity was low at all time points, with the highest uptake occurring at 3 hr in the lungs (2.5%ID/g for ^{111}In and 3.8%ID/g for ^{90}Y). Bone uptake was low for both radioimmunoconjugates (1%ID/g for ^{111}In and 2%ID/g for ^{90}Y at all time points). The radioimmunoconjugates showed similar behavior in most organs at most time points. A summary of the statistical analysis comparing the radioimmunoconjugates is shown in Table 2.

Comparison of Tumor-Reactive and Irrelevant IgM. Tumor and normal organ uptake of i.p. ^{111}In-labeled 2B12 and ^{111}In-labeled CH-1B9 (irrelevant) IgM in tumor-bearing mice are compared in Figure 3, along with normal organ uptake of i.p. ^{111}In-labeled 2B12 in nontumor-bearing nude mice. In tumor-bearing mice at 24 hr, tumor uptake of 2B12 (39%ID/g) was 1.8-fold higher than that of CH-1B9 (22%ID/g) ($p = 0.002$). The uptake of radioimmunoconjugate in normal organs was not statistically different at 24 hr, except for kidney, where the irrelevant antibody had an uptake of 5%ID/g, compared to 4%ID/g for 2B12 ($p = 0.015$). At 96 hr, tumor uptake of 2B12 remained two-fold higher than that of the irrelevant antibody ($p = 0.004$). In nude mice without tumor, organ uptake of 2B12 was not statistically different from that in the tumor-bearing mice at both time points, with liver and spleen demonstrating the highest uptake.

Comparison of IgM and Yttrium-90-Aggregate. In Figure 4, the biodistribution of i.p. ^{90}Y-labeled 2B12 is compared with an i.p. suspension of ^{90}Y-aggregate. Immediate, high tumor uptake was demonstrated for both reagents at 3 hr, with the antibody having 71%ID/g and the aggregate having 70%ID/g. Tumor uptake remained high for the antibody through at least 48 hr, whereas tumor uptake for the aggregate remained high until at least 96 hr. The normal organs with the highest uptake for antibody throughout the experiment were liver and spleen. The aggregate demonstrated high levels of uptake initially in the kidney and femur. The kidney levels decreased with time, whereas the femur levels increased. Yttrium-90-labeled 2B12 had significantly higher uptake in liver (3 hr, $p = 0.016$; 48 hr, $p = 0.024$) and spleen (3 hr, $p = 0.004$). Whereas ^{90}Y-aggregate had significantly higher uptake in tumor (96 hr, $p = 0.004$), kidney ($p < 0.016$ for all time points) and femur ($p < 0.008$ for all time points).

Autoradiographs of an Intraperitoneally-Treated Mouse. Autoradiographs of a sectioned mouse treated 24 hr earlier with i.p. ^{111}In-labeled 2B12 is shown in Figure 5, along with corresponding photographs of the sections. The areas of highest activity in the peritoneal cavity corresponded to the periphery of tumor. Other areas of high activity are seen along the margins of the peritoneal cavity, particularly the diaphragm. The liver shows moderate uptake, whereas the gastrointestinal tract shows minimal activity. Outside of the peritoneal cavity,

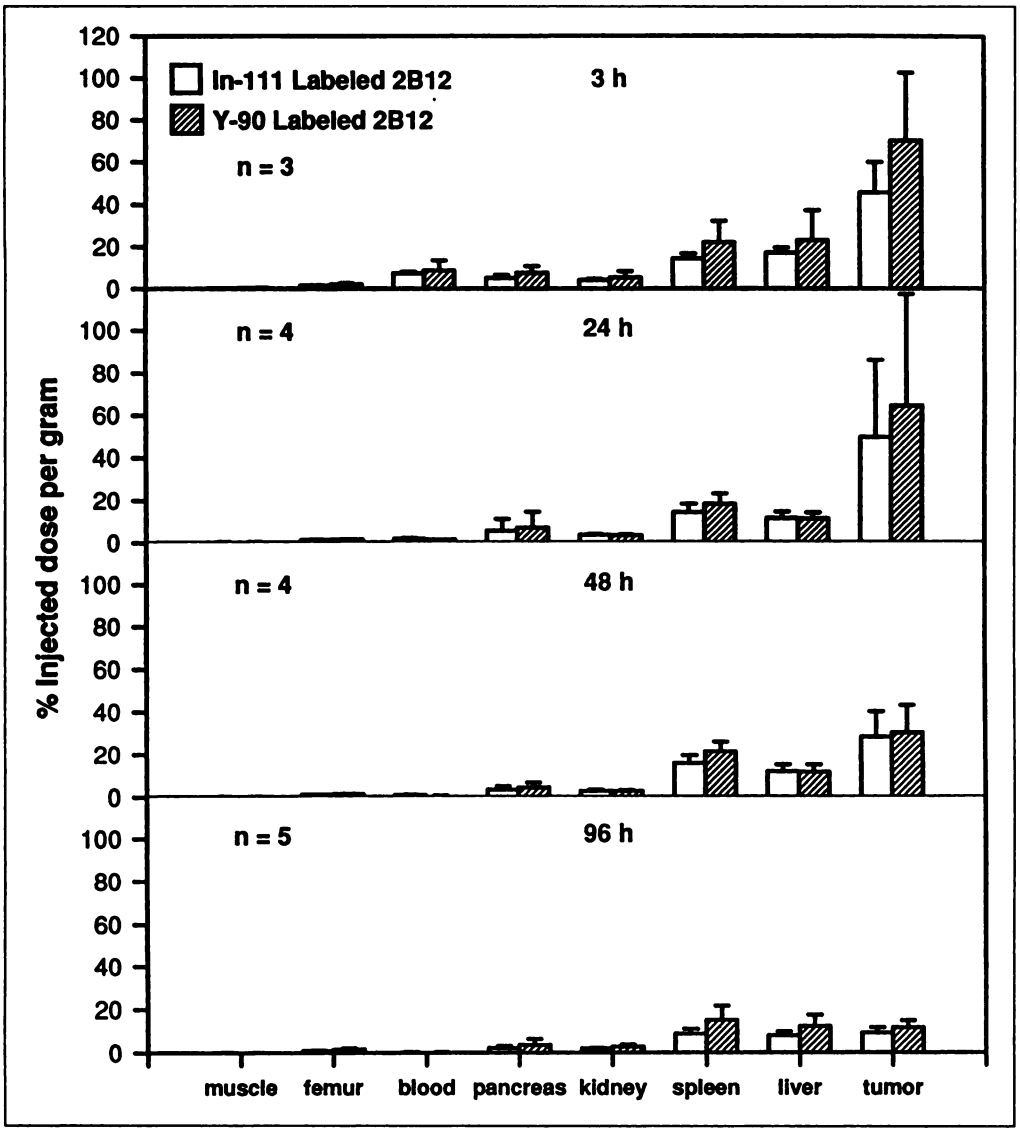

FIGURE 2. Comparison of ^{111}In- and ^{90}Y-labeled human IgM (2B12) coinjected into nude mice bearing i.p. SKOV3 NMP2 xenografts (mean ± s.d.).

activity was limited to the retrosternal lymph nodes. The volume of these nodes was so small that they could not be identified for removal for counting radioactivity in the biodistribution studies.

TABLE 2
Statistical Analysis (p Values) of Indium-111 and Yttrium-90 Coinjection Biodistribution

	Time after injection (hr)		
Organ	24*	48*	96†
Muscle	na	ns	ns
Femur	ns	<0.05‡	<0.05‡
Blood	<0.05§	0.05§	ns
Pancreas	ns	ns	ns
Kidney	ns	ns	ns
Spleen	ns	ns	ns
Liver	ns	ns	ns
Tumor	ns	ns	ns

*n = 4.
†n = 5.
‡^{90}Y > ^{111}In.
§^{111}In > ^{90}Y.
The p values obtained by the Mann–Whitney rank sum test are given. ns = not significant; na = not applicable (n = 3).

Whole-Body Retention of Radioactivity. The whole-body retention of radioactivity in i.p. tumor-bearing mice after i.p. administration of ^{111}In-labeled 2B12 is shown in Figure 6. The radioactivity was measured daily for a week. The graph is biphasic, having a fast metabolic component with a T_α of approximately 14 hr and a slow metabolic component with a T_β of approximately 111 hr.

DISCUSSION

This preclinical study demonstrates rapid and high tumor uptake of radiolabeled human IgM after i.p. administration in nude mice bearing i.p. human ovarian carcinoma nodules. Tumor uptake at the earliest time point, 3 hr, is three-fold higher than it is in the highest normal organs and remains elevated up to at least 48 hr postinjection. This rapid uptake supports the notion that the absence of an endothelial barrier facilitates the binding of antibody to tumor antigen (*11,27*).

The biodistribution of 2B12 is not statistically different when it is radiolabeled with ^{111}In or ^{90}Y, except for higher ^{111}In activity in blood at 24 and 48 hr and higher ^{90}Y activity in bone at 48 and 96 hr. The increased bone radioactivity for ^{90}Y, although statistically significant, is still low at <2%ID/g, indicating that the great majority of ^{90}Y remains effectively chelated with the bifunctional chelator, 2B3M-DTPA. This low level of radioactivity is not anticipated to be a significant source

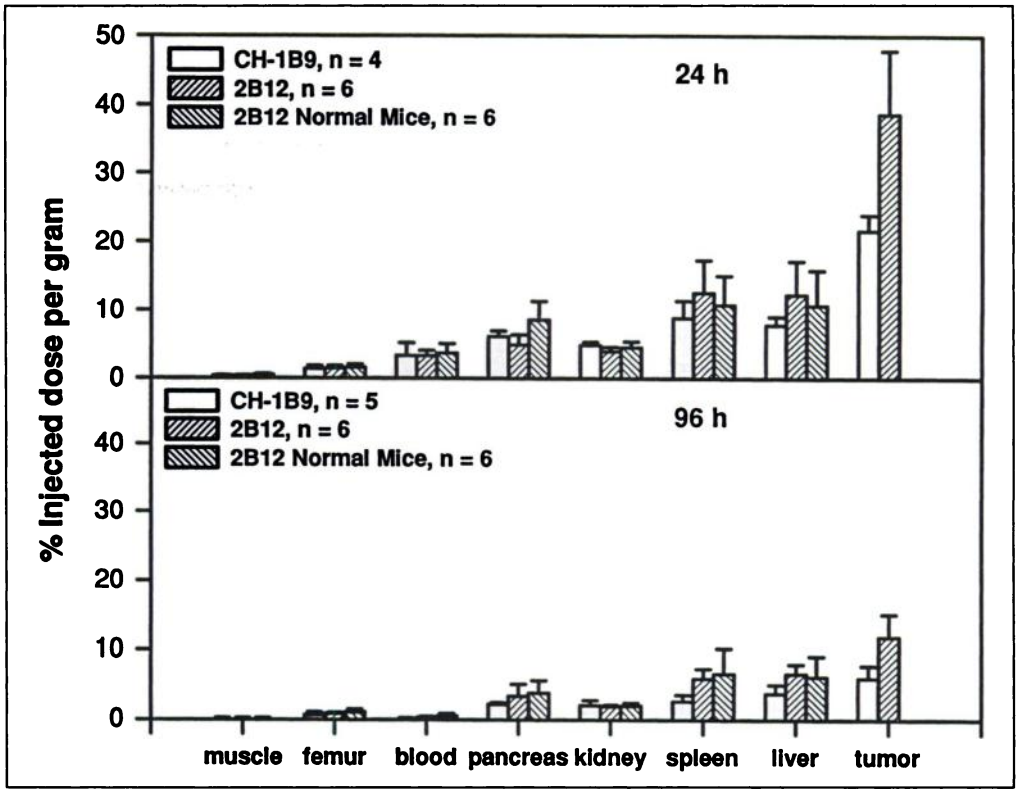

FIGURE 3. Comparison of ^{111}In-labeled tumor-reactive (2B12) and ^{111}In-labeled irrelevant (CH-1B9) IgM administered i.p. into nude mice with or without tumor xenografts (mean ± s.d.).

of bone marrow toxicity when therapeutic activities are used (*15*). The similar biodistributions of the two radioimmunoconjugates suggest that ^{111}In-labeled 2B12 could be used as an i.p. diagnostic agent to demonstrate localization of the radioimmunoconjugate to known tumor sites before treatment with i.p. ^{90}Y-labeled 2B12.

The normal organ uptake of i.p. administered ^{111}In-labeled tumor-reactive IgM (2B12) is not statistically different between tumor-bearing and normal nude mice. This implies that the moderate liver and spleen uptake is not the result of microscopic tumor deposits but may instead be due to nonspecific absorption of IgM onto the peritoneal membrane that covers the organ capsule or the result of cross-reactivity of IgM with normal tissue antigens. Nonspecific absorption onto the peritoneal membrane is the most likely possibility because this tissue shows the highest level of radioactivity in the peritoneal cavity after i.p. administration of ^{111}In-labeled 2B12 in normal beagle dogs (*27*).

Tumor uptake of tumor-reactive (2B12) IgM is twice that of irrelevant IgM. The deposition of radioactivity in normal organs is not statistically different between the radioimmunoconjugates at 24 hr, with the exception of kidney, where uptake was lower for the tumor-reactive IgM. The similarity in liver and spleen uptake between the tumor-reactive and irrelevant IgM supports the hypotheses that IgM is nonspecifically absorbed onto the peritoneal membrane that covers these tissues. By 96 hr, only femur and kidney uptake are similar, with the other organs showing a significant drop in uptake of the irrelevant IgM versus 2B12. This suggests a difference in the rate of metabolism between the two antibodies.

Most of the i.p. administered radiolabeled IgM was retained in the peritoneal cavity, with limited uptake by blood, femur, heart, lungs and muscle. The peritoneal retention appears to be based on size because the irrelevant antibody and 2B12 were retained in the peritoneal cavity to a similar degree. This was also true in another nude mouse model (*28*). Moreover, the peritoneal membrane appears to be an effective barrier to the transport of IgM (*27*). The autoradiographs of the mouse sections demonstrate uptake by the retrosternal lymph nodes, illustrating that some radiolabeled IgM exits the peritoneal cavity through the transdiaphragm lymphatics that drain the peritoneal cavity. In normal beagle dogs, a similar observation was made: 75% of i.p. administered human IgM remained in the peritoneal cavity, whereas the rest was retained by the mediastinal lymph nodes (*27*). In turn, retention of IgM by the lymphatics may be a function of its size, its immunoglobulin class or the presence of tumor deposits. One route for the extraabdominal spread of ovarian carcinoma is through the transdiaphragm lymphatics into the thoracic cavity, which is seen in Stage IV disease. Intraperitoneally-administered IgM may thus allow targeting of involved retrosternal mediastinal lymph nodes.

Initial blood activity in the current study was 8%ID/g–9%ID/g at 3 hr, which is higher than that in a previous nude mouse model (<1%ID/g at 2 hr) (*27,28*). This may be due to differences in expression of tumor antigen, tumor burden or blockage of the lymphatics by tumor. The last two possibilities are supported by these observations. Tumor deposits were routinely visible on the diaphragm at the time of dissection, and the autoradiographs of the mouse sections showed increased activity on the diaphragm. Tumors in this location may block intradiaphragmatic lymph vessels and decrease the transport of immunoglobulins from the peritoneal cavity to the systemic circulation (*29*). When animals received radiolabeled IgM on days 17 and 18 after tumor inoculation instead of on day 12, the blood activity was less than 1% (data not shown). Additionally, normal nude mice had blood activities of 9%ID/g 3 hr after i.p. administration of the radioimmunoconjugate. A previous study demonstrated <1% blood radioactivity of ^{111}In-labeled 2B12 in normal beagle dogs after i.p. administration, suggesting that, in larger recipients, lower blood activities could be expected (*27*).

Intravenously-administered 2B12 was rapidly cleared from the blood so that, by 24 hr postinjection, blood radioactivity was only 1.3%ID/g. Liver and spleen uptake was high and tumor

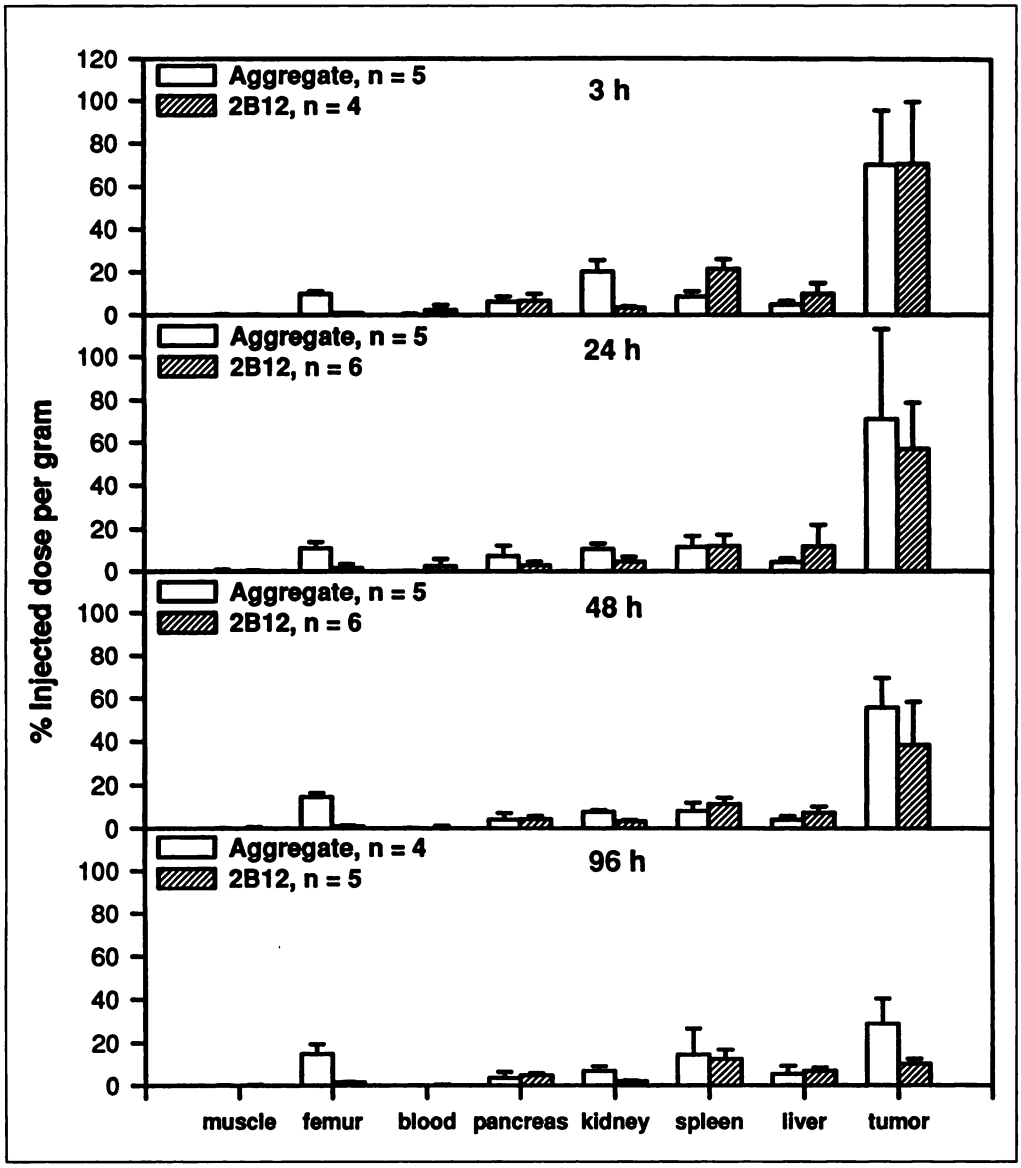

FIGURE 4. Comparison between ^{90}Y-aggregate and ^{90}Y-labeled 2B12 administered i.p. to nude mice bearing i.p. SKOV3 NMP2 xenografts (mean ± s.d.).

uptake was very low (<1%ID/g). Poor tumor uptake is the result of the combined low rate of extravasation for IgM and the competing rapid uptake by the liver and spleen. Immunoglobulin M extravasates into tissue at less than half the rate of IgG (*30*). High liver uptake, but not high spleen uptake, was also noticed for i.v. murine monoclonal IgM (*unpublished observations*). Similar high liver uptake in mice has been reported with both endogenously and exogenously labeled human and murine monoclonal IgM (*31*). In contrast to the radioactivity found on the liver capsule after i.p. administration, liver and spleen uptake is intravascular (endothelial binding of IgM) after i.v. administration.

Intraperitoneal administration of ^{90}Y-aggregate resulted in high, rapid tumor uptake that remained elevated up to 96 hr. The high uptake was surprising and may have been related to phagocytosis of the suspended particles by tumor macrophages. Significant levels of radioactivity left the peritoneal cavity, as evidenced by early bone and kidney activity. Possible explanations are either that small particles of ^{90}Y-aggregate readily escaped from the peritoneal cavity or that some of the i.p. ^{90}Y-aggregate was rapidly hydrolyzed, resulting in free ^{90}Y that quickly entered the circulation. The high kidney activity declined rapidly, suggesting urinary elimination of free ^{90}Y. Femur radioactivity, on the other hand, increased with time, suggesting the slow, continuous leaching of free ^{90}Y from the aggregate in the peritoneal cavity. The risk of developing bone marrow toxicity increases with the length of bone exposure to free ^{90}Y (*15*). In contrast, ^{90}Y that is effectively chelated by a radioimmunoconjugate does not accumulate in bone, and observed myelotoxicity after RIT is usually produced by the circulating radioimmunoconjugate (*15*).

Future radiotoxicology studies to define the dose-limiting organs of i.p. administered ^{90}Y-labeled IgM need to be performed in larger animals. Small animals such as mice cannot be used for toxicity studies because of the small size and proximity of their organs. The long path of the beta particles emitted by ^{90}Y will produce a rather homogeneous dose distribution within their abdomen (*25*). Dogs have been proposed for RIT toxicological studies because their abdominal organs are closer in size to those of humans, and the radiosensitivity of dog bone marrow and liver are similar to those of human bone marrow and liver (*32,33*).

Patients with advanced disease who fail first-line chemotherapy and have disease primarily in the peritoneal cavity may be suitable for novel i.p. treatment strategies. Intraperitoneal administration of murine IgG radiolabeled with ^{90}Y demonstrated efficacy as an adjuvant, but myelotoxicity prevented dose escalation beyond 30 mCi of activity (*34*). The low blood

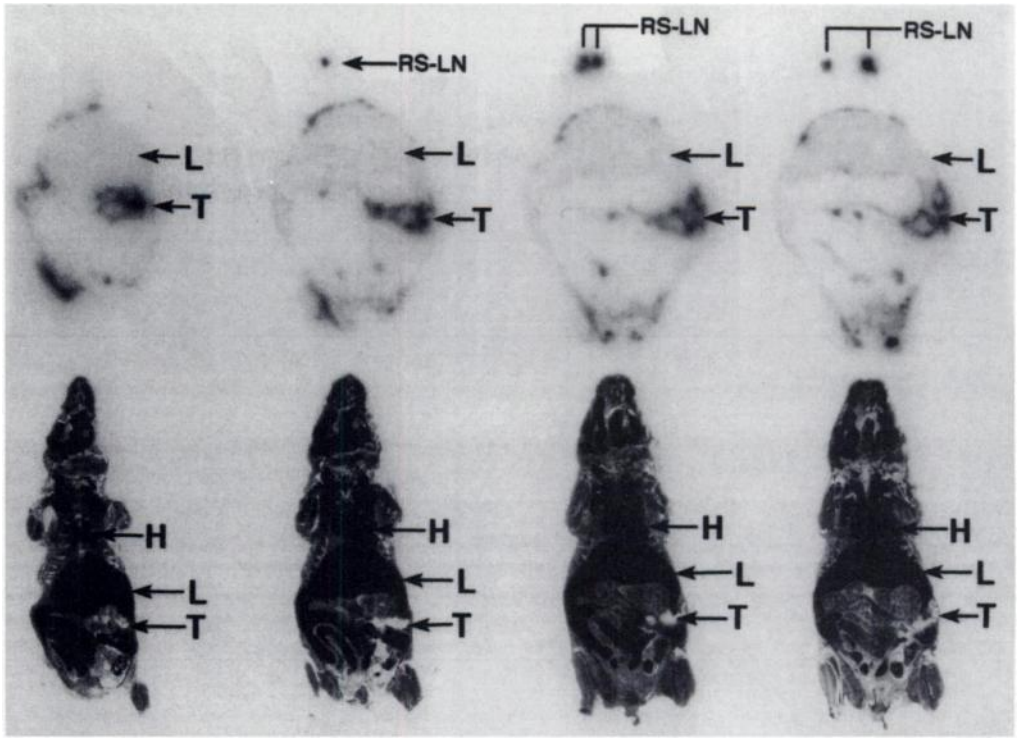

FIGURE 5. Photographs and corresponding autoradiographs of a sectioned tumor-bearing mouse administered ^{111}In-labeled 2B12 i.p. 24 hr earlier. H = heart; L = liver; RS-LN = retrosternal lymph nodes; T = tumor. Radioactivity seen around the lateral pelvis is believed to be due to peritoneal carcinomatosis and not normal tissue uptake. Only low levels of radioactivity were seen in the intestines in the biodistribution studies.

radioactivity and long retention of radiolabeled IgM in the peritoneal cavity demonstrated in this study suggests that higher activities can be administered to patients using radiolabeled IgM without incurring myelotoxicity. This may increase tumor dose and extend treatment response to include macroscopic disease. The localization of radiolabeled IgM to the periphery of tumor nodules, as demonstrated on the autoradiographs of the mouse sections, implies that multiple administrations may be needed to effectively treat macroscopic disease. This would be possible with human IgM because the clinical experience of others has demonstrated a low degree of immunogenicity in human patients (*17*).

CONCLUSION

Biodistribution studies of intraperitoneally-administered radiolabeled IgM show that high levels of radiation can be delivered to tumor with significantly lower doses delivered to normal tissue. Extraperitoneal radioactivity was low, indicating that hematological toxicity may be avoidable with i.p. radiolabeled IgM. Indium-111- and yttrium-90-labeled IgM have similar biodistributions. This may allow the use of ^{111}In-labeled IgM to predict the behavior of ^{90}Y-labeled IgM. An outpatient treatment program may be possible. Further studies are warranted for the clinical development of i.p. radiolabeled human IgM for the treatment of ovarian carcinoma.

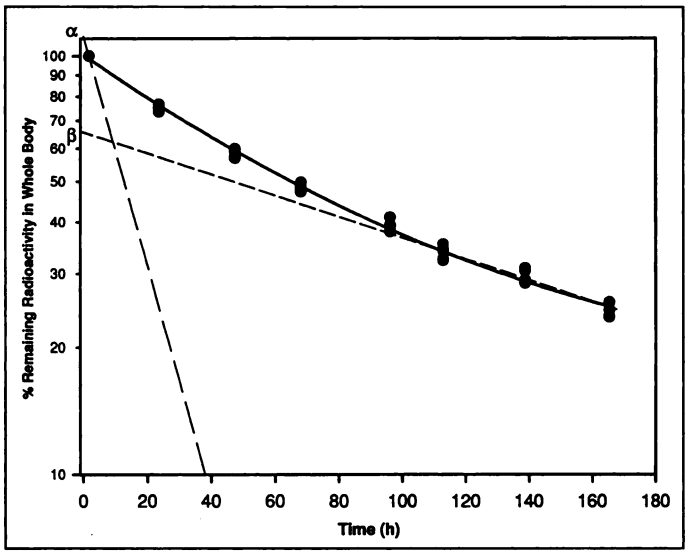

FIGURE 6. Whole-body retention of i.p. administered ^{111}In-labeled 2B12 in nude mice bearing i.p. SKOV3 NMP2 xenografts. Mice were serially measured (n = 5). Data were fitted with second-order regression (r^2 = 0.996). T_α ~ 14 hr; T_β ~ 111 hr.

ACKNOWLEDGMENTS

This project was supported by National Institutes of Health Grants CA 51161 and CA 16672.

REFERENCES

1. Parker SL, Tong T, Bolden S, Wingo PA. Cancer statistics, 1996. *CA Cancer J Clin* 1996;46:5–27.
2. Teneriello MG, Park RC. Early detection of ovarian cancer. *CA Cancer J Clin* 1995;45:71–87.
3. McGuire WP. Primary treatment of epithelial ovarian malignancies. *Cancer* 1993; 71(suppl):1541–1550.
4. McGuire WP, Hoskins WJ, Brady MF, et al. Cyclophosphamide and cisplatin compared with paclitaxel and cisplatin in patients with stage III and stage IV ovarian cancer. *N Engl J Med* 1996;334:1–6.
5. Thigpen JT, Blessing JA, Vance RB, et al. Chemotherapy in ovarian carcinoma: present role and future prospects. *Semin Oncol* 1989;16:58–65.
6. Markman M. Intraperitoneal chemotherapy. *Semin Oncol* 1991;18:248–254.
7. Ozols RF, Rubin SC, Dembo AJ, et al. Epithelial ovarian cancer. In: Hoskins WJ, Perez CA, Young RC, eds. *Principles and practice of gynecologic oncology.* Philadelphia: J.B. Lippincott; 1992:731–781.
8. Los G, Mutsaers PHA, van der Vijgh WJF, et al. Direct diffusion of cis-diamminedichloroplatinum(II) in intraperitoneal rat tumors after intraperitoneal chemotherapy: a comparison with systemic chemotherapy. *Cancer Res* 1989;49:3380–3384.
9. Dedrick RL, Myers CE, Bungay PM, et al. Pharmacokinetic rational for peritoneal drug administration in the treatment of ovarian cancer. *Cancer Treat Rep* 1978;62:1–11.
10. Kaplan WD, Zimmerman RE, Bloomer WD, et al. Therapeutic intraperitoneal ^{32}P: a clinical assessment of the dynamics of distribution. *Radiology* 1981;138:683–688.
11. Thedrez P, Saccavini J-C, Nolibe D, et al. Biodistribution of indium-111-labeled OC 125 monoclonal antibody after intraperitoneal injection in nude mice intraperitoneally grafted with ovarian carcinoma. *Cancer Res* 1989;49:3081–3086.
12. Rowlinson G, Snook D, Busza A, et al. Antibody-guided localization of intraperitoneal tumors following intraperitoneal or intravenous antibody administration. *Cancer Res* 1987;47:6528–6531.
13. Stewart JSW, Hird V, Snook D, et al. Intraperitoneal yttrium-90-labeled monoclonal antibody in ovarian cancer. *J Clin Oncol* 1990;8:1941–1950.
14. Breitz HB, Durham JS, Fisher DR, et al. Pharmacokinetics and normal organ

dosimetry following intraperitoneal rhenium-186-labeled monoclonal antibody. *J Nucl Med* 1995;36:754–761.
15. Vriesendorp HM, Quadri SM, Andersson BS, et al. Hematologic side effects of radiolabeled immunoglobulin therapy. *Exp Hematol* 1996;24:1183–1190.
16. Finkler NJ, Muto MG, Kassis AI, et al. Intraperitoneal radiolabeled OC 125 in patients with advanced ovarian cancer. *Gynecol Oncol* 1989;34:339–344.
17. De Jager R, Abdel-Nabi H, Serafini A, et al. Current status of cancer immunodetection with radiolabeled human monoclonal antibodies. *Semin Nucl Med* 1993;23:165–179.
18. Griffin TW, Collins J, Bokhari F, et al. Intraperitoneal immunoconjugates. *Cancer Res* 1990;50(suppl):1031s–1038s.
19. Horan Hand P, Shrivastav S, Colcher D, et al. Pharmacokinetics of radiolabeled monoclonal antibodies following intraperitoneal and intravenous administration in rodents, monkeys and humans. *Antibody Immunoconj Radiopharm* 1989;2:241–255.
20. Leichner PK, Yang N-C, Frenkel TL, et al. Dosimetry and treatment planning for ^{90}Y-labeled antiferritin in hepatoma. *Int J Radiat Oncol Biol Phys* 1988;14:1033–1042.
21. Vriesendorp HM, Quadri SM, Williams JP. Radioimmunotherapy. In: Armitage JO, Antman K, eds. *High dose cancer therapy*. Philadelphia: Williams and Wilkins; 1992:84–123.
22. Freedman RS, Ioannides CG, Tomasovic B, et al. Development of a cell surface reacting human monoclonal antibody recognizing ovarian and certain other malignancies. *Hybridoma* 1991;10:21–33.
23. Chen P-F, Freedman RS, Chernajovsky Y, et al. Amplification of immunoglobulin transcripts by the non-palindromic adaptor polymerase chain reaction (NPA-PCR). Nucleotide sequence analysis of two human monoclonal antibodies recognizing two cell surface antigens expressed in ovarian, cervix, breast, colon and other carcinomas. *Hum Antibodies Hybridomas* 1994;5:131–142.
24. Quadri SM, Mohammadpour H. Synthesis of 2-*p*-aminobenzyl-3-methyl-and 2-*p*-aminobenzyl-3-benzyl derivatives of diethyleneaminepentaacetic acids (DTPA): carbon backbone modified bifunctional chelating agents. *Bioorg Med Chem Lett* 1992;2:1661–1664.
25. Quadri SM, Vriesendorp HM, Leichner PK, et al. Evaluation of indium-111- and yttrium-90-labeled linker-immunoconjugates in nude mice and dogs. *J Nucl Med* 1993;34:938–945.
26. Mujoo K, Maneval DC, Anderson SC, et al. Adenoviral-mediated p53 tumor suppressor gene therapy of human ovarian carcinoma. *Oncogene* 1996;12:1617–1623.
27. Quadri SM, Malik AB, Tang XZ, et al. Preclinical analysis of intraperitoneal administration of ^{111}In-labeled human tumor reactive monoclonal IgM AC6C3–2B12. *Cancer Res* 1995;55(suppl):5736s–5742s.
28. Quadri SM, Malik AB, Chu HB, et al. Intraperitoneal indium-111 and yttrium-90-labeled human IgM (AC6C3–2B12) in nude mice bearing peritoneal carcinomatosis. *J Nucl Med* 1996;37:1545–1551.
29. Wahl RL, Barrett J, Geatti O, et al. The intraperitoneal delivery of radiolabeled monoclonal antibodies: studies on the regional delivery advantage. *Cancer Immunol Immunother* 1988;26:187–201.
30. Jullien-Vitoux D, Voisin GA. Studies in vascular permeability II. Comparative extravasation of different immunoglobulin classes in normal pig skin. *Eur J Immunol* 1973;3:663–667.
31. Halpern SE, Hagan PL, Chen A, et al. Distribution of radiolabeled human and mouse monoclonal IgM antibodies in murine models. *J Nucl Med* 1988;29:1688–1696.
32. Quadri SM, Shao Y, Blum JE, et al. Preclinical evaluation of intravenously administered ^{111}In- and ^{90}Y-labeled B72.3 immunoconjugate (GYK-DTPA) in beagle dogs. *Nucl Med Biol* 1993;20:559–570.
33. Wang S, Quadri SM, Tang X-Z, et al. Liver toxicity induced by combined external beam irradiation and radioimmunoglobulin therapy. *Radiat Res* 1995;141:292–302.
34. Hird V, Maraveyas A, Snook D, et al. Adjuvant therapy of ovarian cancer with radioactive monoclonal antibody. *Br J Cancer* 1993;68:403–406.

Pharmacokinetic Model of Iodine-131-G250 Antibody in Renal Cell Carcinoma Patients

Angela Loh, George Sgouros, Joseph A. O'Donoghue, Devie Deland, Dennis Puri, Peter Capitelli, John L. Humm, Steven M. Larson, Lloyd J. Old and Chaitanya R. Divgi

Department of Medical Physics and Department of Radiology, Nuclear Medicine Service, Memorial Sloan-Kettering Cancer Center; and Ludwig Institute for Cancer Research, New York, New York

A model that describes the pharmacokinetic distribution of ^{131}I-labeled G250 antibody is developed. **Methods:** Previously collected pharmacokinetic data from a Phase I–II study of ^{131}I-G250 murine antibody against renal cell carcinoma were used to develop a mathematical model describing antibody clearance from serum and the whole body. Survey meter measurements, obtained while the patient was under radiation precautions, and imaging data, obtained at later times, were combined to evaluate whole-body clearance kinetics over an extended period. **Results:** A linear two-compartment model was found to provide good fits to the data. The antibody was injected into Compartment 1, the initial distribution volume (V_d) of the antibody, which included serum. The antibody exchanged with the rest of the body, Compartment 2, and was eventually excreted. Data from 13 of the 16 patients fit the model with unique parameters; the maximum, median and minimum values for model-derived V_d were 6.3, 3.7 and 2.11, respectively. The maximum, median and minimum values for the excretion rate were 8×10^{-2}, 2.4×10^{-2} and 1.3×10^{-2} hr^{-1}, respectively. Parameter sensitivity analysis showed that a change in the transfer rate constant from serum to the rest of the body had the greatest effect on serum cumulative activity and that the rate constant for excretion had the greatest effect on whole-body cumulative activity. **Conclusion:** A linear two-compartment model was adequate in describing the serum and whole-body kinetics of G250 antibody distribution. The median initial distribution volume predicted by the model was consistent with the nominal value of 3.81. A wide variability in fitted parameters was observed among patients, reflecting the differences in individual patient clearance and exchange kinetics of G250 antibody. By selecting median parameter values, such a model may be used to evaluate and design prolonged multiple administration radioimmunotherapy protocols.

Key Words: radioimmunotherapy; modeling; antibody; treatment planning; pharmacokinetics

J Nucl Med 1998; 39:484–489

With the advent of humanized antibodies and the accompanying reduction in immunogenicity, fractionated dosing of antibodies over protracted time periods will be feasible. Because of the increased complexity of such trials, pharmacokinetic modeling will be important in evaluating administration protocols and their potential efficacy and toxicity. Studies of radioimmunotherapy in mice have shown that greater doses can be tolerated, and toxicity reduced, with fractionated administration (*1,2*). Patient studies also have suggested that this is the case but have been limited by the induction of an immune response against murine-derived antibody (*3–5*). Little is known about the potential marrow-sparing effects of prolonged, multiple-administration radioimmunotherapy or how best to design prolonged fractionated treatment protocols. Modeling will allow evaluation of various dosing schedules according to quantifiable criteria, such as predicted clearance rates, residence times and absorbed doses. Information obtained from single-administration protocols may be used to establish mathematical models of radiolabeled antibody distribution and dosimetry. Such models may then be used to simulate prolonged, multiple-

Received Dec. 23, 1996; revision accepted May 6, 1997.
For correspondence or reprints contact: George Sgouros, PhD, Department of Medical Physics, Memorial Sloan-Kettering Cancer Center, New York, NY 10021.