Does Paclitaxel (Taxol) Given after ^{111}In-Labeled Monoclonal Antibodies Increase Tumor-Cumulated Activity in Epithelial Cancers?

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Abstract

Purpose: Paclitaxel synergized radiolabeled monoclonal antibodies, enhancing therapeutic effect in studies in mice with human xenografts. Paclitaxel was also observed to increase tumor uptake in imaging studies of ^{111}In-DOTA-Gly$_3$Phe-m170 in patients with breast and prostate cancers. Further evaluations of tissue-cumulated activities, therapeutic indices, and pharmacokinetics were done using data for patients with breast and prostate cancer and for mice with human breast cancer xenografts.

Experimental Design: In radioimmunotherapy trials, 12 patients with breast or prostate cancer were given two imaging doses (5 mCi each) of ^{111}In-DOTA-Gly$_3$Phe-m170 1 week apart. Five of these patients were given a single dose of paclitaxel i.v. (75 mg/m^2) 2 days after the second dose of ^{111}In. In a subsequent study, athymic mice with human breast cancer xenografts were given ^{111}In-DOTA-Gly$_3$Phe-ChL6 alone, or in combination with daily paclitaxel i.p. (300 μg) one or more times. Pharmacokinetics were studied for at least 6 days in patients and 5 days in mice. Cumulated activities were determined for tumors and normal tissues.

Results: Tumor-cumulated activity for every patient in the paclitaxel-treated group increased for the second dose of ^{111}In-DOTA-Gly$_3$Phe-m170. The median ratio of cumulated activities in tumors for imaging dose 2 to those for dose 1 was 1.0 (0.8-1.3) in patients that were not given paclitaxel and 1.3 (1.2-1.4) in patients given paclitaxel. Normal tissue-cumulated activities were not different for the two doses. Mice given paclitaxel 1 day after ^{111}In-DOTA-Gly$_3$Phe-ChL6 also showed an increase in tumor-cumulated activity, 22.9 (± 1.3) versus 19.4 (± 3.3) μCi h/g/μCi (P = 0.05). Cumulated activities of normal tissues were similar for all groups of mice.

Conclusions: Paclitaxel given 1 to 2 days after ^{111}In-DOTA-Gly$_3$Phe-monoclonal antibody increased the tumor-cumulated activity in patients and in mice with epithelial cancers and did not alter cumulated activities in normal tissues.

In the development of radioimmunotherapy, investigators have explored various means to increase the therapeutic index including external beam radiation (1–3), fractionation of the radioimmunotherapy dose (4), and combined modality radioimmunotherapy using various drugs to increase uptake or radiosensitivity (5–9). One combined modality radioimmunotherapy method successful in human breast tumors was the addition of paclitaxel to radioimmunotherapy. Paclitaxel is a taxane, a group of commonly structured compounds found in various members of the yew family. Studies have shown that paclitaxel promotes the assembly of microtubules from tubulin dimers and stabilizes microtubules, blocking depolymerization (10). By stabilizing microtubules, paclitaxel interferes with the normal microtubule network dynamics of living cells, especially spindle apparatus formation during mitosis, forming microtubule arrays and multiple asters of microtubules, thereby halting cell division in the G_2-M phase, the most radiosensitive phase (11, 12). Paclitaxel also facilitates apoptosis by inducing tubulin polymerization and bcl-2 phosphorylation (13, 14).

Radioimmunotherapy kills cancer cells by initiating apoptotic mechanisms through low-dose rate radiation damage to DNA (15, 16). This damage is most pronounced in cells that are in the G_2-M phase of the cell growth cycle, when the DNA has condensed into discrete chromosomes. The presence of active bcl-2 makes a cell resistant to apoptosis, and by inducing bcl-2 phosphorylation, paclitaxel may decrease the bcl-2-related apoptosis resistance (17). When a single dose of paclitaxel that was ineffective in mice with breast tumor xenografts by itself was given after radioimmunotherapy, it synergized the radioimmunotherapy to produce a cure rate of 50%, whereas radioimmunotherapy alone produced responses but no cures (18). Further studies of mice with human prostate cancer xenografts showed similar synergy (19). No increased toxicity was observed in mice with human breast or prostate tumors (18, 19).

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Based on these findings, treatment trials were initiated in patients with breast or prostate cancer. When increased tumor uptake was observed in the patients given paclitaxel, the effects of paclitaxel on the pharmacokinetics of ^{111}In-labeled radioimmunoconjugate in athymic mice with human breast cancer xenografts were studied. For the purposes of this report, we have used cumulated activity (area under the activity curve) as a surrogate for radiation dose.

Materials and Methods

Antibodies. For patient studies, m170 (Biomira, Inc., Canada) was used. This is a mouse IgG_1 monoclonal antibody generated using a synthetic asialo-GM1 terminal disaccharide related to the Thomsen-Friedenreich disaccharide. This monoclonal antibody targets MUC1 antigen that is expressed by many adenocarcinomas (20), and binds with high affinity (4×10^8 mol/L) to them. The preparation was human use grade and was >95% monomeric IgG by PAGE.

Because no m170 reactive tumor xenograft exists, chimeric L6 (ChL6; Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, WA) was used for the mouse studies. ChL6 is a chimera consisting of a human IgG_1 constant region and the Fab′ of murine L6. It targets a membrane glycoprotein that is highly expressed on human breast, colon, ovary, and lung carcinomas (21).

Paclitaxel. Paclitaxel (Taxol; Bristol-Myers Squibb, Princeton, NJ), a natural taxane, was furnished as a nonaqueous solution at a concentration of 6 mg/mL, and was diluted further in 0.9% NaCl saline to 1.5 mg/mL just prior to injection. The mice were given 300 μg (200 μL) i.p.

Cells. The HBT 3477 cell line (Bristol-Myers Squibb Pharmaceutical Research Institute) used to produce human breast tumor xenografts was derived from a human breast adenocarcinoma. These cells are aneuploid with a 1.5 DNA index, are negative for both estrogen and progesterone receptors by immunohistochemistry, and 70% of the cells stain with L6 in the type 1 pattern defined by Mattes et al. (22, 23). The genome possesses a mutant p53 gene with a deletion of the region that detects double-stranded DNA breaks. *BCL2* expression is present.

Radiolabeling. Carrier-free ^{111}In (MDS Nordion, Vancouver, Canada) was purchased as chloride in 0.05 mol/L HCl. C.F. Meares and J. Peterson (Department of Chemistry, University of California, Davis, CA) provided the DOTA-peptide linker GGGF (Gly_3Phenylalanine). The method for preparing DOTA-Gly_3Phe-m170 and DOTA-Gly_3Phe-ChL6 has been described previously (24, 25). Both ^{111}In-labeled m170 and ChL6 were prepared, as described (24, 26). ^{111}In was added to DOTA-Gly_3Phe-monoclonal antibodies in 0.1 mol/L ammonium acetate (pH 5.5) and incubated at 37°C for 30 minutes. Nonspecifically bound ^{111}In was scavenged by the addition of EDTA to a final concentration of 10 nmol/L, and the solution was kept at room temperature for 30 minutes; the radioimmunoconjugate was purified from the reaction mixture using Sephadex G25-80 (Pharmacia, Uppsala, Sweden) molecular sieving column centrifugation.

Quality control. Radioimmunoconjugates were examined by molecular sieving high-performance liquid chromatography, cellulose acetate electrophoresis, and immunoreactivity assay (27), as previously described (24, 26). High-performance liquid chromatography and cellulose acetate electrophoresis of radioimmunoconjugates showed that all ^{111}In-DOTA-Gly_3Phe-m170 preparations were >98% monomeric, and all ^{111}In-DOTA-Gly_3Phe-ChL6 preparations were >97% monomeric. ^{111}In-DOTA-Gly_3Phe-m170 immunoreactivity was >70%, and ^{111}In-DOTA-Gly_3Phe-ChL6 immunoreactivity was 100% relative to their unmodified analogues.

Patients. Seven patients (three with prostate cancer and four with breast cancer) participated in the radioimmunotherapy phase of the trials, and another five patients (three with prostate and two with breast cancer) participated in the combined modality radioimmunotherapy phase of the trials. Both patient groups (with and without paclitaxel) were enrolled in phase 1 studies, one for metastatic breast cancer failing at least one chemotherapy regimen and one for hormone refractory metastatic prostate cancer. The entry criteria were identical for those who did and did not receive paclitaxel. All of the patients had advanced stages of disease, and the required Karnofsky score of at least 70%, neutrophil count >1,500/μL, platelet count >100,000/μL, hemoglobin >10.0 mg/dL, normal renal function, liver function tests with normal bilirubin, and aspartate aminotransferase <1.5 times the upper limit of normal, negative human anti-mouse antibody assay result and no anticancer therapy for at least 4 weeks. Every patient had computed tomography and bone scan evaluation. Before treatment, all patients were advised of the investigational nature of the study and signed an informed consent for protocols that was approved by the University of California Davis, Human Subjects and Radiation Use Committees under an Investigation of New Drug authorization from the U.S. Food and Drug Administration.

Antibody infusion. In patients, 5 mg of unlabeled m170 was given before administration of radioimmunoconjugate to reduce nonspecific binding of radiolabeled m170. Both doses of ^{111}In-DOTA-Gly_3Phe-m170 (5 mCi ^{111}In with mean 1.2 mg m170) followed 1 week later by ^{111}In/^{90}Y-DOTA-Gly_3Phe-m170 (5 mCi ^{111}In with mean 8.2 mg m170) were given to all 12 patients. Paclitaxel (75 mg/m^2) was infused over 3 hours, 2 days after the ^{111}In/^{90}Y dose given patients in the combined modality radioimmunotherapy phase. Premedication included dexamethasone at 12, 6, and 0.5 hours before injection as well as diphenhydramine HCl and cimetidine.

Pharmacokinetics and cumulated activity. Because ^{90}Y has no gamma emission, ^{111}In was used as a surrogate for ^{90}Y. A medium energy collimator and an energy window centered at 171 and 245 KeV with 15% photopeak widths were used for image acquisition. Images were acquired on a Siemens Bodyscan dual detector camera interfaced to an ICON-NucliDose computer system in a 128 × 128 pixel matrix for two million counts or 900 seconds, whichever occurred first. The method for calculating pharmacokinetic data obtained from images has been described previously (28, 29). Briefly, transmission images were obtained in the presence and absence of the patient using a rod source containing about 2.5 mCi ^{111}In. A series of planar images with conjugated views of total body, skull, chest, abdomen, pelvis, and additional tumor sites was acquired 1, 2 hours, and 1, 2, 3 and 6 days

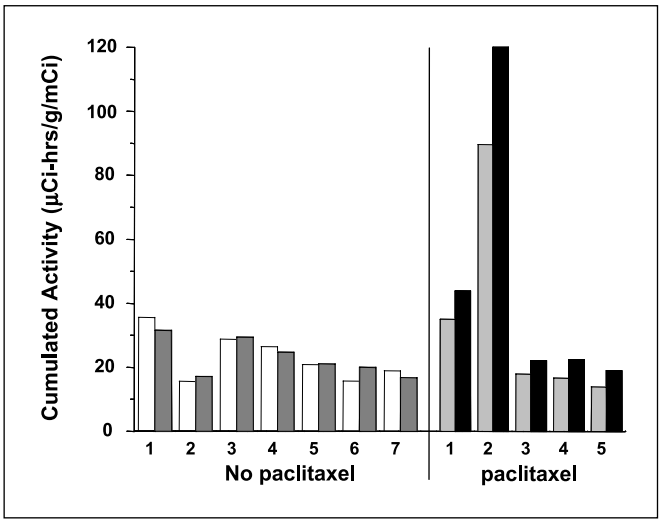

Fig. 1. Patient tumor-cumulated activity. Two groups of patients with breast and prostate cancer were given ^{111}In-DOTA-Gly_3Phe-m170 twice i.v., 1 week apart. One group was given paclitaxel after the second dose. Paclitaxel increased tumor-cumulated activity in every instance ($P = 0.02$). The median ratio of tumor-cumulated activity of the first dose and the second dose was 1.3 (1.2-1.4) for the group given paclitaxel and 1.0 (0.8-1.3) in the group that did not receive paclitaxel. This difference in ratios was significant ($P = 0.01$). No paclitaxel: first dose (▭); second dose (▭); Paclitaxel treatment: first dose (▬); second dose (■).

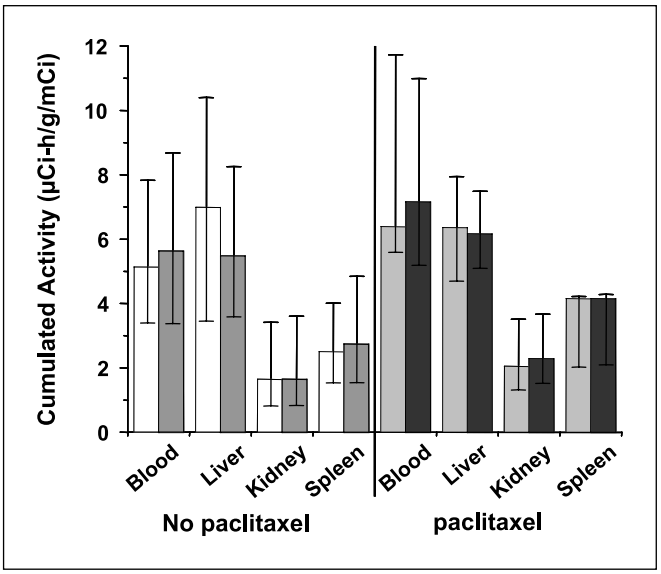

Fig. 2. Patient normal organ-cumulated activity. Two groups of patients with breast and prostate cancer were given ^{111}In-DOTA-Gly$_3$Phe-m170 twice i.v., 1 week apart. One group was given paclitaxel after the second dose. Paclitaxel had no effect on cumulated activity of normal organs; group median value and range shown. No paclitaxel: first dose (□); second dose (□); paclitaxel treatment: first dose (■); second dose (■).

after infusion of ^{111}In-DOTA-Gly$_3$Phe-m170. The patient position was marked and the position of the detector in horizontal and vertical orientation was recorded for each static view to ensure that the detector had a constant position relative to the patient.

Blood samples were collected at 0.5, 1, and 2 hours after infusion of ^{111}In-DOTA-m170 and on each image day, counted in a gamma well counter and % ID calculated by comparing with a standard prepared from the radiopharmaceutical, using the body weight to estimate blood volume.

Tumor sizes were determined as previously described (30). Only tumors with a size of ≥10 g were included. A total of 21 tumors (10 in breast, 2 from combined modality radioimmunotherapy studies, and 11 in prostate cancer, 5 from combined modality radioimmunotherapy, respectively) met the criteria for this study. The regions of interest for the whole body, organs, and tumors were defined manually based on the visual boundary. An identical region of interest, selected on the first dose image with best contrast, was used for the complete image sequences for both the first and second dose studies in each patient. The geometric mean method was employed to quantify activity, as previously described (24).

Cumulated activity in organs and tumors was estimated by fitting pharmacokinetic data to monoexponential functions, except blood. On the few occasions when a monoexponential function was not appropriate, the cumulated activities were determined using the trapezoidal method. The blood clearance of radioactivity was best fitted to a biexponential function. Organs (liver, lungs, kidneys, spleen, marrow, and blood volumes), and 21 tumors, mass >10 g with clearly defined location and edges in images were analyzed. In patients with more than one tumor, the mean tumor-cumulated activity was used so that each patient was equally weighted.

Mouse studies. Female athymic nude mice (Harlan, Frederick, MD), 5 to 8 weeks old, were maintained according to University of California guidelines on a normal diet, ad libitum. HBT 3477 cells were grown in Iscove's medium (Life Technologies BRL, Frederick, MD) in a 5% CO_2 atmosphere at 37°C. When the cells were in log phase, 3×10^6 in 100 μL were implanted s.c. into the lower abdominal wall in bilateral sites. When the xenografts were 50 to 500 mm^3, 15 to 30 μCi ^{111}In-DOTA-Gly$_3$Phe-ChL6 was injected i.v. into a lateral tail vein of each mouse. Some mice were injected i.p. immediately (T0) with 300 μg paclitaxel diluted 1:4 in 0.9% NaCl. Other mice were injected 1 or 2 days after radioimmunoconjugate injection (D1 or D2, respectively), whereas others were given multiple injections; at radioimmunoconjugate injection and 1 day later (T0D1), or at radioimmunoconjugate injection and 1 and 2 days (T0D1D2) later. Following radioimmunoconjugate injection, whole body activity was immediately measured using two opposed, isoresponsive sodium iodide detectors (Canberra Industries, Meriden, CT). To determine whole body clearance, mice were counted serially for 5 days after injection with this detector system. The counts were decay-corrected, and expressed as % ID. Blood clearances were determined by collecting 2 μL blood samples in microcapillary pipettes from the tail veins of mice for 5 days after injection and counting the samples in a gamma well counter. Decay-corrected radioactivity in the blood was expressed as % ID, using a weight-based theoretical blood volume. Pharmacokinetic data for other tissues were obtained by sacrificing mice at 1, 3, and 5 days after injection, removing and weighing tissues, and counting them in a gamma well counter. The concentration in each tissue was expressed as % ID/g. The cumulated activity ($\hat{A}_h$), the sum of all radioactive decays in a tissue (h), during the time interval of interest was obtained from sequential measurements of radioactivity in the tissue. Because of the monoexponential physical decay of short-lived ^{111}In, tissue clearance was assumed to be monoexponential, so that the expected activity concentration at time t could be represented as $A\lambda e^{(-\lambda t)}$, where A represents the activity at time $t = 0$ and λ represents the rate of decrease in activity concentration with time. The cumulated activity was obtained by fitting the data with this monoexponential function using nonlinear regression analysis, SAS procedure NLIN, applied to the activity concentration (SAS OnlineDoc, version 9, February 2000, SAS Institute, Inc., Cary, NC). Based on the monoexponential model, the cumulated activity is A/λ and is estimated using the estimated values for A and λ. The SE of this ratio was estimated from the SE estimates of A and λ. An extension of Fieller's theorem was used to provide an approximation for a SE of the ratio of two random variables based on the SEs of the two variables and their correlation, and assuming that each was approximately normally distributed (31). Cumulated activity concentrations in units of microcuries hour per gram per unit of administered radioactivity (μCi h/g/μCi) were obtained by adjusting cumulated activity for tissue mass and for administered radioactivity.

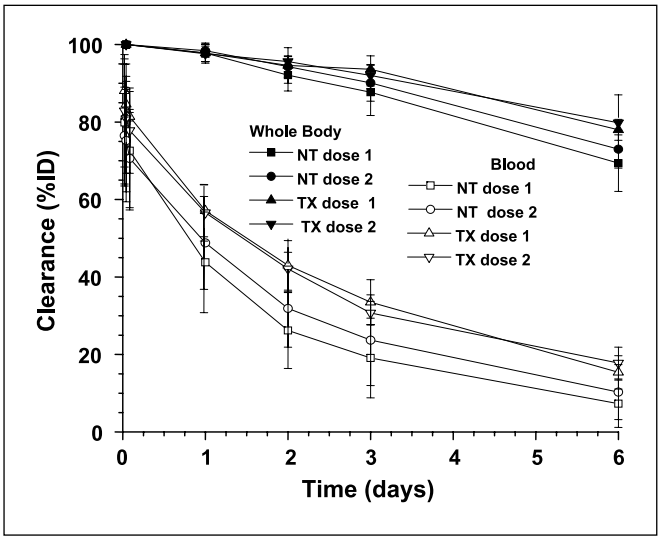

Fig. 3. Patient clearance. Two groups of patients with breast or prostate cancer were given ^{111}In-DOTA-Gly$_3$Phe-m170 twice i.v., 1 week apart. One group was given paclitaxel after the second dose. Clearance of ^{111}In-DOTA-Gly$_3$Phe-m170 was similar for the first and second dose in both untreated and paclitaxel-treated groups. Whole body—no paclitaxel: first dose (■), second dose (●); paclitaxel: first dose (▲), second dose (▼). Blood—no paclitaxel: first dose (□), second dose (○); paclitaxel—first dose (△), second dose (▽).

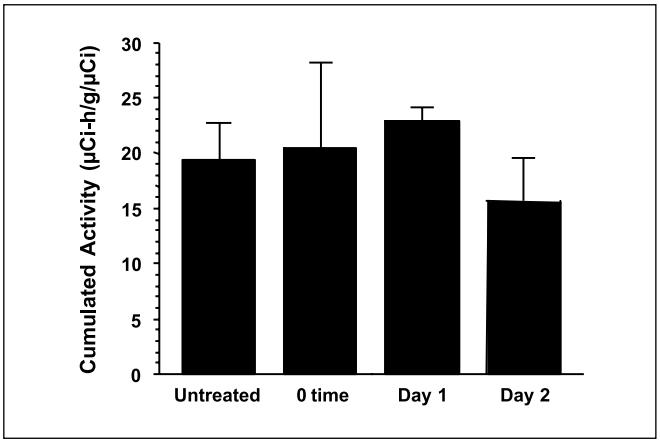

Fig. 4. Mouse tumor-cumulated activity. Groups of mice bearing HBT 3477 xenografts were treated with ^{111}In-DOTA-Gly$_3$Phe-ChL6. Except for one group that served as a control, the other groups were treated with paclitaxel at the same time or 1 or 2 days after ^{111}In-DOTA-Gly$_3$Phe-ChL6. Tumor-cumulated activity increased only when paclitaxel was given just 1 day after radioimmunoconjugate injection (P = 0.05). No paclitaxel treatment, □; paclitaxel given simultaneous with ^{111}In-DOTA-Gly$_3$Phe-ChL6 (T0), □; paclitaxel given 1 day after ^{111}In-DOTA-Gly$_3$Phe-ChL6 (D1), ▩; paclitaxel given 2 days after ^{111}In-DOTA-Gly$_3$Phe-ChL6 (D2), ■.

Mouse study design. Because paclitaxel given to patients 2 days after the second radiopharmaceutical injection was found to increase the ratio of cumulated activity between the first and second dose of ^{111}In-DOTA-Gly$_3$Phe-m170, when compared with data for the patients receiving no paclitaxel, biodistribution studies were conducted in athymic nude mice, comparing mice given paclitaxel to groups of mice not given paclitaxel. Mice were sacrificed 1, 3, and 5 days after radioimmunoconjugate injection in the untreated, T0, D1, and D2 groups. Mice were sacrificed 3 and 5 days after radioimmunoconjugate injection in the groups given multiple paclitaxel treatments, T0D1, and T0D1D2 and cumulated activity was estimated by linear regression of the natural logarithms of organ activities, substituting tissue and organ activity values of mice in the T0 group that were sacrificed 1 day after radioimmunoconjugate injection.

Statistical methods. In patients, the cumulated activities of the first and second dose of radioimmunoconjugate in tissues of each patient were matched and the Wilcoxon signed rank test (32), a method based on ranking for paired samples, was applied to the paired values to test for significance in the differences between the first and second dose-cumulated activities of the tissues of the patients in either untreated or paclitaxel-treated group. The ratio of tumor-cumulated activity of the first dose to the second dose of ^{111}In-DOTA-Gly$_3$Phe-m170 of each patient was calculated and a comparison of the group given paclitaxel and the group that did not receive paclitaxel was done with the Wilcoxon-Mann-Whitney rank sum test (33). To compare the cumulated activity between the various treated groups and untreated groups in mice, the assumption was made that the estimates of the cumulated activity were approximately normally distributed, and a comparison was made by taking the differences in the estimates (untreated and treated) and dividing the difference by the square root of the sum of the squares of the SEs (estimated as described above) for the two cumulated activity estimates. This provides a Z score, which can be compared with normal distribution tables. Results were considered statistically significant if P for a one-sided test was <0.05. No adjustment was made for multiple comparisons.

Results

Patients. Tumor-cumulated activity (μCi h/g/mCi) of the first dose and the second dose of ^{111}In-DOTA-Gly$_3$Phe-m170 dose of each patient are shown in Fig. 1. Tumor-cumulated activity for every patient in the paclitaxel-treated group increased for the second dose of ^{111}In-DOTA-Gly$_3$Phe-m170. The median tumor-cumulated activity in patients given paclitaxel was 17.8 (13.8-89.6) μCi h/g/mCi for the first dose of ^{111}In-DOTA-Gly$_3$Phe-m170 and 22.3 (18.9-124.5) μCi h/g/mCi for the second dose of ^{111}In-DOTA-Gly$_3$Phe-m170, a significant increase for the second dose when tested using the Wilcoxon Signed Rank test (P = 0.02). In the group of patients not given paclitaxel, median tumor-cumulated activity was 20.9 (15.6-35.6) μCi h/g/mCi for the first dose of ^{111}In-DOTA-Gly$_3$Phe-m170 and 21.0 (16.7-31.5) μCi h/g/mCi for the second dose of ^{111}In-DOTA-Gly$_3$Phe-m170, an insignificant difference when tested using the Wilcoxon sign rank test (P = 0.43). The median ratio of first dose tumor-cumulated activity to second dose tumor-cumulated activity was 1.3 (1.2-1.4) in the group given paclitaxel and 1.0 (0.8-1.3) in the group that did not receive paclitaxel, and when the ratios of tumor-cumulated activity of the first dose to the second dose of ^{111}In-DOTA-Gly$_3$Phe-m170 of patients in the group given paclitaxel and the ratios of patients not given paclitaxel were compared using the Wilcoxon-Mann-Whitney rank sum test, the difference between the group given paclitaxel and the group without paclitaxel was significant (P = 0.01). Only tumor-cumulated activity was affected by paclitaxel. No difference was seen for liver, kidney, spleen, or blood-cumulated activities of patients given paclitaxel compared with patients who did not receive paclitaxel (Fig. 2), and neither blood clearance nor whole body clearance were affected (Fig. 3).

Mice. Tumor-cumulated activity (μCi h/g/μCi) of mice given paclitaxel or untreated mice are shown in Fig. 4. Tumor-cumulated activity was 20.5 ± 7.7, 22.9 ± 1.3, and 15.6 ± 4.0 μCi h/μCi/g for mice given paclitaxel at the time of ^{111}In-DOTA-Gly$_3$Phe-ChL6 injection, on days 1 and 2, respectively, and tumor-cumulated activity of the group that was not given paclitaxel was 19.4 ± 3.3 μCi h/g/μCi. The increased tumor-cumulated activity in the mice given paclitaxel on day 1 was

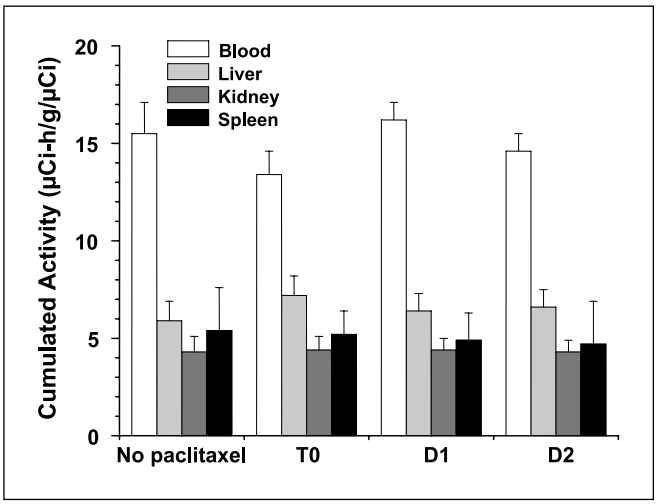

Fig. 5. Mouse normal organ-cumulated activity. Groups of mice bearing HBT 3477 xenografts were treated with ^{111}In-DOTA-Gly$_3$Phe-ChL6. Except for one group that served as a control, the other groups were treated with paclitaxel at the same time or 1 or 2 days after ^{111}In-DOTA-Gly$_3$Phe-ChL6. Although tumor-cumulated activity increased when paclitaxel was given 1 day after radioimmunoconjugate injection, there was no significant change ($P \leq 0.05$) in cumulated activity of normal tissue with paclitaxel treatment. Blood (□), liver (□), kidney (▩), spleen (■).

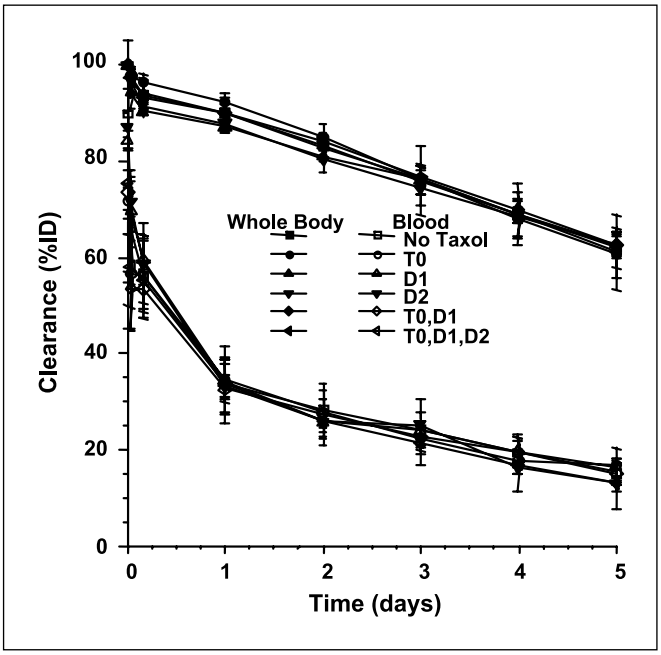

Fig. 6. Mouse clearance. Groups of mice injected i.v. with ^{111}In-DOTA-Gly$_3$Phe-ChL6 were treated with single or multiple i.p. injections of paclitaxel. Whole body and blood clearances were measured. Treatment with paclitaxel at any time or schedule did not affect clearance. Whole body—no paclitaxel treatment (■), paclitaxel treatment at ^{111}In-DOTA-Gly$_3$Phe-ChL6 injection (T0) (●), 1 day after ^{111}In-DOTA-Gly$_3$Phe-ChL6 (D1) (▲), 2 days after ^{111}In-DOTA-Gly$_3$Phe-ChL6 (D2) (▼), at T0 and D1 (♦), at T0 and D1 and D2 (◀). Blood—no paclitaxel treatment (□), paclitaxel treatment at T0 (○), D1 (△), D2 (▽), at T0 and D1 (◇), at T0 and D1 and D2 (◁).

significantly higher than the tumor-cumulated activity in the untreated group (P = 0.05). The ratios of tumor-cumulated activity of mice not given paclitaxel to mice given paclitaxel were 1.1, 1.2, and 0.8 for the mice given paclitaxel at the time of ^{111}In-DOTA-Gly$_3$Phe-ChL6 injection, on days 1 and 2, respectively. Giving successive treatments of paclitaxel to mice did not seem to increase tumor-cumulated activity (17.2 μCi h/g/μCi for mice treated at the time of ^{111}In-DOTA-Gly$_3$Phe-ChL6 injection and on day 1, and 16.6 μCi h/g/μCi for mice treated at the time of ^{111}In-DOTA-Gly$_3$Phe-ChL6 injection and days 1 and 2). Cumulated activity of liver, kidney, spleen, and blood were unaffected by paclitaxel (Fig. 5), nor was blood or whole body clearance affected by paclitaxel (Fig. 6).

Discussion

Paclitaxel has shown synergism with radioimmunotherapy in therapy studies in this and other tumored athymic nude mouse models (19, 34). The cure rate for HBT 3477 tumor xenografts increased from 0% for radioimmunotherapy alone to 50% or 88% when 300 or 600 μg of paclitaxel, respectively, was administered i.p. 48 hours after radioimmunotherapy (18), even though neither dose of paclitaxel alone induced any regression in the tumor xenografts. When 600 μg of paclitaxel was given 24 hours after radioimmunotherapy, the timing that showed the greatest increase in tumor-cumulated activity in the preclinical pharmacokinetic study reported herein, the cure rate was 50%. Neither dose of paclitaxel was associated with additional toxicity in the mice when given 48 hours after radioimmunotherapy. These paclitaxel doses are equivalent to human doses of 42 and 84 mg/m^2, well below paclitaxel doses usually used for treatment of women with metastatic breast cancer (100-250 mg/m^2). The observations in these preclinical studies led to combined modality radioimmunotherapy trials of paclitaxel and radioimmunotherapy in patients using 75 mg/m^2 paclitaxel, a dose similar to the 300 μg dose of paclitaxel shown effective in the mice. Toxicity was limited to moderate myelosuppression, and increased tumor uptake and cumulated activity were observed, as reported here (35). These observations, in turn, led to the tumored athymic nude mouse pharmacokinetic studies that are also reported here.

Paclitaxel is a microtubule stabilizer that interrupts the cell cycle in G_2-M, the most radiosensitive phase of the cell cycle. This effect on cancer cells was originally believed to be the basis for synergy observed with radioimmunotherapy (36, 37). Following the initial preclinical combined modality radioimmunotherapy therapy studies of paclitaxel in tumored athymic mice, evidence began to mount showing that paclitaxel was particularly efficacious against malignancies with p53 mutations, a condition found in many adenocarcinomas (38–40). Recently, paclitaxel has been shown to have an effect on angiogenesis; low concentrations of paclitaxel have antiangiogenic activity, inhibiting endothelial cell motility, chemotaxis, and invasiveness, at levels that do not affect cell proliferation (41). In *in vitro* tube formation assays, paclitaxel inhibited differentiation of human umbilical vein endothelial cells, and in rat mesentery assays, it significantly shortened microvessel sprouts (42, 43). Increased tumor uptake was observed in biodistribution studies of cilengitide, an antiangiogenic agent (44), and preclinical mouse studies of combined modality radioimmunotherapy with cilengitide showed synergy (45), suggesting that the antiangiogenic property of paclitaxel may also increase tumor uptake of radioimmunoconjugate, thereby increasing the cumulated activity. All the paclitaxel properties described above may be responsible individually, or collectively, directly or indirectly, for the enhanced therapeutic efficacy of radioimmunotherapy observed in mice and patients. Increased tumor uptake and cumulated activity may be just one factor leading to the increased tumoricidal effect of combined modality radioimmunotherapy with paclitaxel.

In summary, these studies show that paclitaxel given after radiolabeled monoclonal antibodies increases the cumulated activity in breast cancer in patients and mice when given in the correct sequence and timing.

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