Development of Two Radioimmunoassays to Detect Paclitaxel in Sera and in Cerebrospinal, Ascitic, and Pleural Fluids

Kevin P. O'Boyle, M.D. Yuexian Wang, M.D. Edward L. Schwartz, Ph.D. Donna Lee Regl, M.S. Avi Einzig, M.D. Janice P. Dutcher, M.D. Peter H. Wiernik, M.D. Susan Band Horwitz, Ph.D.

Departments of Oncology and Molecular Pharmacology, Montefiore Medical Center/Albert Einstein Cancer Center, Bronx, New York.

Presented in part, in poster form, at the 1994 annual meeting of the American Association for Cancer Research, and published in abstract form in *Proc AACR* 1994;35:241.

Supported in part by a Cancer Center Core Grant (NIH CA 13330-22).

K.O.B. was a recipient of an American Cancer Society Career Development Award and an American Society of Clinical Oncology Young Investigator Award.

The authors are indebted to Xioping Hu for help with the statistical analysis.

Address for reprints: Kevin O'Boyle, M.D., Department of Oncology, Montefiore Medical Center, 111 East 210th Street, Bronx, NY 10467.

Received July 23, 1996; revision received November 7, 1996; accepted November 7, 1996.

© 1997 American Cancer Society

BACKGROUND. Paclitaxel is an antimitotic agent isolated from the Pacific yew tree. It has demonstrated antitumor activity in several cancers and is the first of a new class of antineoplastic agents containing a taxane ring system. Its levels in serum and urine have been measured previously by high performance liquid chromatog-raphy (HPLC). In this study, the authors developed two competitive radioimmuno-assay methods to determine whether they could reliably be used to measure levels of paclitaxel in sera and in cerebrospinal, ascitic, and pleural fluids.

METHODS. A monoclonal antibody prepared against paclitaxel was employed in an immunoradiometric assay (IRMA), in which ¹²⁵I-labeled antibody was used, and in a more conventional tritiated radioimmunoassay (RIA),in which ³H-paclitaxel was used.

RESULTS. Both radioimmunoassays detected levels of paclitaxel in sera that were comparable to those observed with HPLC. However, the IRMA was the most sensitive. Only the IRMA was able to detect low levels of paclitaxel in cerebrospinal fluid after paclitaxel infusion and in sera 3 weeks after infusion. Both the IRMA and RIA methods were able to detect paclitaxel in ascitic and pleural fluids.

CONCLUSIONS. Monitoring paclitaxel levels reliably in sera and other bodily fluids is possible with these radioimmunoassays and may be of value in predicting and preventing toxicity and optimizing paclitaxel treatments. *Cancer* **1997;79:1022–30.** © *1997 American Cancer Society.*

KEYWORDS: paclitaxel, radioimmunoassay, monoclonal antibody, cerebrospinal fluid, carcinoma patients.

Paclitaxel has demonstrated antitumor activity in human ovarian, breast, lung, and head and neck carcinomas as well as leukemia, lymphoma, melanoma, and other malignancies¹⁻⁴. The drug, originally isolated from the bark of the tree *Taxus brevifolia*, is a potent inhibitor of cell replication and has antimitotic activity that results in the blockage of cells in the late G₂ and M phases of the cell cycle. Paclixatel is unusual in its ability to bind to and stabilize microtubules and to induce abnormal stable bundles of microtubules in cells.⁵⁻⁷ Recently, paclitaxel has also been found to induce tyrosine phosphorylation of microtubule-associated protein kinase and expression of tumor necrosis factor- α in macrophages.^{8,9}

The extreme hydrophobicity and aqueous insolubility of paclitaxel has resulted in a drug formulation with Cremophor EL, a mixture of polyoxyethylated castor oil and ethanol for use in patients. Slow infusions over 24 hours were designed to reduce the occurrence of anaphylactic reactions that were seen in early studies with the drug and may have been due to Cremophor EL.^{10,11} Additionally, it was found that pretreatment with dexamethasone and diphenhydramine hyrochloride were important factors in reducing the chance of hypersensitivity reactions. As a result of pretreatment, 3-hour infusions of paclitaxel that can be given on an outpatient basis have been studied.^{12,13} At the same time, other investigators are examining longer (96-hour) infusions of paclitaxel to determine if they may be more effective.¹⁴

Phase I studies revealed neutropenia to be doselimiting.^{15–18} Recombinant granulocyte-colony stimulating factor (G-CSF) was introduced so that the concentration of paclitaxel could be increased.^{19–21} At higher doses allowed by the inclusion of G-CSF, the dose-limiting toxicity was peripheral neuropathy, characterized by a glove-and-stocking distribution of sensory loss affecting proprioception. Other toxicities included nausea, vomiting, myalgia, mucositis, totalbody alopecia, diarrhea, and phlebitis.

The distribution of paclitaxel in normal body fluids, except in sera, is unknown, and a method of detecting low levels of paclitaxel would be valuable. Paclitaxel was not detected in cerebrospinal fluid (CSF) of leukemic patients by high performance liquid chromatography (HPLC) with a lower limit of sensitivity of 50 nmol/L.¹⁷. Tissue distribution studies in rodents using tritiated paclitaxel, a more sensitive technique, revealed negligible penetration of paclitaxel into CSF and the brain.²² No significant evidence of central nervous system toxicity due to paclitaxel has yet been demonstrated.²

Pharmacokinetic studies of 24-hour infusions of paclitaxel in cancer patients have been performed using reverse-phase HPLC.^{2-4,10-19} The lower limit of sensitivity of this method was approximately 50 nM, and research has focused on alternative procedures for the detection of paclitaxel. Recently, immunologic assays have been developed to detect paclitaxel. Enzyme immunoassays for the quantification of paclitaxel, using polyclonal antisera, have been reported.²³⁻²⁵

The development of a quick and sensitive radioimmunoassay to study the pharmacokinetics of paclitaxel would be an asset in clinical trials now exploring the combination of the drug with growth factors and other chemotherapeutic agents. A monoclonal antibody against paclitaxel has been examined to determine its clinical usefulness in two radioimmunoassays that were used to detect paclitaxel in the sera and the cerebrospinal, ascitic, and pleural fluids of patients with cancer who had been treated with the drug. The accuracy, sensitivity, and reliability of these assays was compared with results obtained by HPLC.

MATERIALS AND METHODS

Reagents

A murine immunoglobulin(Ig)G2a monoclonal antibody (MoAb) prepared against paclitaxel in mice that were immunized with a paclitaxel–keyhole limpet hemocyanin (KLH) complex will be described elsewhere. Ascitic fluid, from mice with severe combined immunodeficency injected with hybridoma cells producing this MoAb, was purified using a protein G column (Pierce, Rockford, IL). Paclitaxel (Taxol) was obtained from the Drug Synthesis and Chemistry Branch of the National Cancer Institute.Tritiated paclitaxel (³Htaxol, specific activity 11.1 Ci/mmol, 0.05 mCi/0.1 mL) was purchased from Moravek Biochem. Inc., Brea, CA. Sera and cerebrospinal, ascitic, and pleural fluids were obtained from patients treated with 24-hour intravenous infusions of paclitaxel at Montefiore Medical Center after informed consent and Internal Review Board consent was obtained.

Preparation of Paclitaxel–Bovine Serum Albumin Plates

A paclitaxel–bovine serum albumin (BSA) complex was dissolved in PBS at a concentration of 10 μ g/mL; 100 μ L of this solution was added to each well of 96-well, specially coated breakapart ELISA plates from Costar, Inc., and incubated for 2 hours at room temperature. Excess paclitaxel-BSA was then removed, and the plates were blocked at 4 °C with 1% BSA and 0.05% fetal calf serum (FCS) and stored at 4 °C until use.

Competitive Immunoradiometric Method

The antipaclitaxel MoAb was radiolabeled with iodine-125 (¹²⁵I) by the iodogen (Pierce) method.^{26 125}I-labeled MoAb was serially diluted and added to 96-well plates coated with paclitaxel-BSA for 1 hour at room temperature, to select the best concentration of antibody to obtain approximately 5000 counts per minute (cpm) bound per well after washing with phosphate buffered saline (PBS). This dilution (in human serum) of antibody (50 μ L /well) was added to each well, and a competitive inhibition assay was established.27,28 Free paclitaxel, initially dissolved in dimethylsulfoxide, was serially diluted in 100% ethanol. Each concentration was further diluted 100-fold in blocking buffer (1% BSA and 0.5% FCS). Fifty μ L was added to each well of a 96-well plate and preincubated with labeled MoAb for 1 hour at 37 °C. The mixture was transferred to the plates coated with paclitaxel-BSA, binding occurred for 1 hour at room temperature, and the plates were washed with PBS. The plates were then broken apart and the individual wells counted in a gamma counter. A standard curve was plotted based on the amount of labeled MoAb bound to the paclitaxel-BSA plates after inhibition with free paclitaxel Free baccatin, a biologically inactive analogue of paclitaxel that was used as a control, was diluted and added in a similar manner as paclitaxel. A standard curve also was obtained by

serial dilution of paclitaxel in ethanol and then dilution (1:100) in normal human sera instead of blocking buffer. Standard curves employing human sera were used to calculate all paclitaxel levels in sera as well as in ascitic and pleural fluids and CSF. Sera and fluids containing high concentrations of paclitaxel were diluted into human sera, and 50 μ L of this was preincubated with 50 μ L of MoAb as described above. CSF, containing very low levels of paclitaxel, was not diluted in human serum, but 50 μ L was added directly to MoAb in 50 μ L of human sera for the preincubation step. Each data point was performed in triplicate with a standard deviation calculated.

Competitive Tritiated Radioimmunoassay

³H-paclitaxel was diluted in radioimmunoassay (RIA) buffer (PBS + 0.1% Tween 20 + 0.1% gelatin + 0.1% NaN₃) to 20,000 cpm per 100 μ L and combined with 100 μ L(1 μ g) of antipaclitaxel MoAb, which bound approximately 50% of the counts. A standard curve for this competitive assay was generated by adding sera (100 μ L) containing different known amounts of unlabeled paclitaxel to each tube and incubating for 2 hours at room temperature. Charcoal in 2.5% dextran was added (100 μ L) to precipitate the ³H-paclitaxel not bound by MoAb. After 30 minutes, the tubes were centrifuged in an eppendorf centrifuge, and the radioactivity in the supernatant was determined in a scintillation counter. A value of 100% bound in the standard curve was achieved when there was no inhibition of MoAb binding to ³H-paclitaxel (i.e., approximately 10,000 cpm in the supernatant). Standard plots were made of the percentages of bound versus unlabeled paclitaxel, and values from patient sera were calculated from standard curves. Each data point was performed in triplicate with a standard deviation calculated.

High Pressure Liquid Chromatography

Paclitaxel concentration in sera was determined by a modification of previously described procedures.^{1,11} To prepare samples for high pressure liquid chromatography (HPLC) analysis, 0.5 mL of sera was combined with 1 mL acetonitrile, then centrifuged (for 10 minutes at 10,000 RPM). The supernatant was combined with 1.5 mL water and applied to a C18 Bond Elut solid phase extraction column (J. T. Baker, Phillipsburg, NJ). The column was washed with 2 mL of acetonitrile:water (3:7), and the paclitaxel was eluted with 1 mL of acetonitrile:water (7:3). The sample was evaporated to dryness under reduced pressure and then resuspended in 0.2 mL of the mobile phase (65% acetonitrile:water containing 0.5% phosphoric acid). Extracted samples were analyzed on an HP1090 HPLC,

using an Absorbosphere 5 micron C18 column (4.6 x 250 mm) (Alltech, Deerfield, IL), and run at a flow rate of 1.5 mL/minute, with detection at 227 nm. Standard curves were constructed from human plasma samples infused with known amounts of paclitaxel, and the paclitaxel concentrations in unknown samples were calculated by comparing peak heights to the standard curve.

Statistical Methods

Differences among mean paclitaxel levels measured by RIA, immunoradiometric assay (IRMA), and HPLC were tested using analysis of variance. The 95% simultaneous confidence intervals for differences in mean paclitaxel levels among the three assays are given using Bonferroni criteria. The intraclass correlation coefficient of reliability was calculated within each of the assays and among the three assays. Reliability was defined as the variance of true patient-to-patient paclitaxel levels divided by the variance of observed paclitaxel levels with measurement errors.²⁹ Coefficients of variation for each assay were calculated by dividing standard deviations of results by the mean values for different concentrations of paclitaxel.

RESULTS

Competitive Immunoradiometric Assay

Paclitaxel completely inhibited the binding of radiolabeled MoAb to paclitaxel-BSA at a concentration equal to or greater than 1 μ M. Fifty percent inhibition was achieved at a paclitaxel concentration of 0.7 nM (Fig. 1A). The MoAb had much greater specificity for paclitaxel then for baccatin, a biologically inactive paclitaxel analogue that lacks the side chain at C-13. Complete inhibition with baccatin was seen at 25 μ M and 50% inhibition at 0.1 μ M. The usefulness of the IRMA as a clinical tool for detecting paclitaxel levels in the sera of patients is demonstrated in Figure 1B. Normal serum was infused with free paclitaxel after initial serial dilutions of the drug in ethanol were made. This curve shows the same S-shaped pattern as free paclitaxel diluted first in ethanol and subsequently diluted into blocking buffer, and revealed an even greater sensitivity in detecting drug levels. Complete inhibition of MoAb binding is seen again at a 1 μ M concentration of free paclitaxel and 50% inhibition is seen at 0.08 nM, which makes this assay 10 times more sensitive than for free paclitaxel diluted in blocking buffer. This suggests that this assay can detect paclitaxel levels in patients' sera down to 0.10 nM. The sensitivity of this assay for measuring paclitaxel levels in patients' sera was maximized by selecting an amount of radiolabeled MoAb (approximately 5000 cpm/well), the binding of which to paclitaxel-BSA could be easily inhibited. For

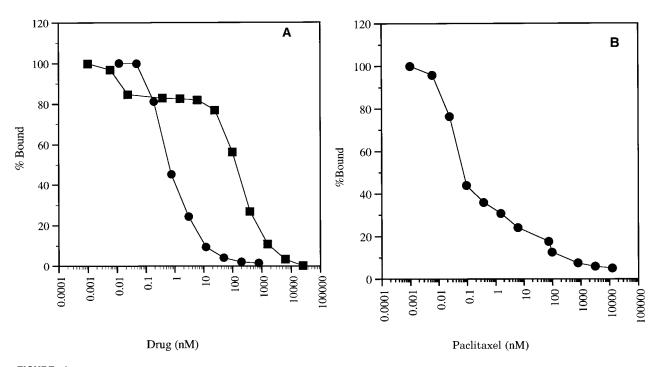


FIGURE 1. (A) Competitive immunoradiometric assays (IRMAs), with serial dilutions of free paclitaxel (λ) and baccatin (ν) diluted in blocking buffer, are shown. The points are means of triplicate results expressed as percentages of ¹²⁵I-labeled monoclonal antibody (MoAb) bound to plates coated with paclitaxel–bovine serum albumin relative to maximum MoAb bound in the absence of free paclitaxel. (B) Competitive IRMA, with serial dilutions of paclitaxel diluted in normal human serum, is shown.

analysis of paclitaxel in clinical samples, sera from patients were diluted in normal human serum to obtain inhibition values falling between 40% and 60%. Samples were run in triplicate, and paclitaxel levels with standard deviations were calculated from the standard curve that was repeated with each assay (Table 1). The interassay coefficient of variation for the IRMA was 19.0% at 1.0 μ M, 10.2% at 0.1 μ M, and 17.0% at 0.01 μ M. The lower limit of paclitaxel detection using the IRMA was 0.001 μ M.

Competitive Tritiated Radioimmunoassay

Standard tubes, with no unlabeled paclitaxel added, yielded approximately 10,000 cpm per supernatant, indicating no inhibition of ³H-paclitaxel binding by MoAb. When excessive unlabeled paclitaxel was added to the tubes, few counts were found in the supernatant. A typical standard curve for this competitive RIA is illustrated in Figure 2. The interassay coefficient of variation for the RIA was 18.2% at 1.0 μ M and 27.8% at 0.1 μ M. The requirement for MoAb in this assay was significantly greater than for the IRMA, and, as a result, this assay was not as sensitive as the IRMA. The lack of sensitivity is attributable to the fact that a proportionally greater amount of free paclitaxel is needed to inhibit the increased amount of MoAb used in this

assay. Therefore, this assay, unlike the IRMA, was not able to detect paclitaxel levels in CSF or serum from patients who had been treated with the drug 3 weeks previously. The lower limit of detection of the tritiated RIA was 0.05 μ M, the same as for HPLC.

High Performance Liquid Chromatography

The results of the HPLC tests confirmed the validity of both the IRMA and RIA radioimmunoassays (Table 1). The interassay coefficient of variation for HPLC was 14.9% at 0.1 μ M and 4.6% at 1 μ M, but this assay had a lower sensitivity for paclitaxel (0.05 μ M) than the IRMA. The HPLC method was not able to detect paclitaxel in CSF or pre-retreatment levels in sera.

Detection of Serum Paclitaxel Levels Using IRMA, RIA, and HPLC

Data were obtained on the pharmacokinetics of paclitaxel infusion for 8 patients receiving different concentrations of the drug. Data points were obtained at 0, 2, 4, 8, 12, and 24 hours. IRMA and RIA data were compared with HPLC for four patients to demonstrate the validity of the assays. Patients had been treated with paclitaxel 3 weeks earlier, and in 6 of 8 patients low levels of paclitaxel could be detected at preretreatment (Table 2). Figure 3 presents the pharma-

TABLE 1
Comparison of the Levels of Paclitaxel in Sera during 24-Hour Drug Infusions by Three Different Methods

Patient no.	Cancer	Paclitaxel (mg/m ²)	Time (hrs)	RIA (μM)	SD	IRMA (µM)	SD	HPLC (µM)	
1.	Ovarian ca	135	4	0.74	0.240	0.44	0.077	0.55	
	ovanan ca	Ovariari ca 1	100	8	0.74	0.190	0.49	0.070	0.83
			12	0.88	0.220	0.70	0.119	0.70	
				24	0.90	0.073	0.62	0.054	0.75
2.	Ovarian ca	135	2	0.15	0.040	0.25	0.045	0.30	
			100	4	0.16	0.060	0.26	0.020	0.34
				8	0.32	0.020	0.34	0.029	0.44
			24	0.54	0.060	0.44	0.051	0.43	
3.	Melanoma	300	2	0.61	0.070	0.38	0.045	0.54	
		000	4	0.71	0.065	0.80	0.136	0.72	
			8	1.43	0.251	1.00	0.108	1.19	
			12	1.80	0.187	1.44	0.368	1.71	
			24	2.06	0.500	2.16	0.689	2.20	
1.	Lung ca	250	2	0.33	0.016	0.30	0.033	0.53	
	Lung Ca	230	4	0.66	0.010	0.49	0.111	0.82	
			8	0.68	0.098	0.58	0.152	1.28	
			12	0.89	0.196	1.16	0.320	1.20	
			24	1.02	0.232	0.82	0.198	1.61	
5.	Ovarian ca	135	24	0.27	0.114	0.39	0.064	N.D.	
J .	Ovaliali ca		0.29	0.009	0.35	0.004	N.D.		
		4 8		0.23	0.003	0.44	0.113		
			12	0.32	0.027	0.40	0.113		
			24	0.32	0.001	0.62	0.152		
6.	Ovarian ca	135	24	0.31	0.009	0.02	0.032		
D.	Ovariali ca	155	4	0.19	0.039	0.26	0.032		
			8	0.19	0.000	0.20	0.086		
			o 12	0.19	0.002	0.32	0.000		
			24			0.48			
-	0	105		0.24 0.37	0.076		0.018		
7.	Ovarian ca	135	2		0.033	0.24	0.043		
			4	0.27	0.012	0.35	0.022		
			8	0.30	0.020	0.34	0.350		
		12	0.31	0.004	0.70	0.211			
2	Lung	250	24	0.33	0.012	0.36	0.045		
3.	Lung ca	250	4	0.84	0.160	0.58	0.049		
			8	0.87	0.090	0.60	0.052		
			12	1.14	0.150	0.64	0.056		
			24	1.33	0.130	0.83	0.099		

RIA: radioimmunoassay; SD: standard deviation; IRMA: immunoradiometric assay; HPLC: high performance liquid chromatography; ca: carcinoma; N.D.: not done.

cokinetic curves for two patients treated with different doses of paclitaxel. Detection was achieved by all three methods. In one patient paclitaxel levels were monitored after infusion using the IRMA (Fig. 4). The paclitaxel levels reached a maximum at the end of the 24-hour infusion, which is similar to results published in Phase I studies.^{1,9}

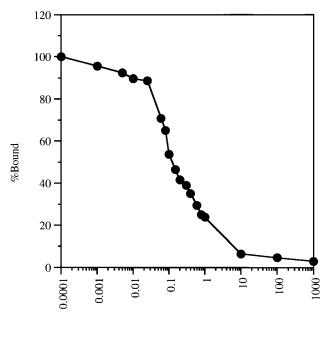
Detection of Paclitaxel Levels in Human Cerebrospinal, Ascitic, and Pleural Fluids with IRMA

Very low paclitaxel levels were detected in CSF in a patient with acute myelogenous leukemia treated with 315 mg/ m^2 of paclitaxel (Fig. 5). CSF was obtained from an Om-

maya reservoir on the day the 24-hour paclitaxel infusion was finished (Day 1), and Day 2, Day 3, and Day 4 after infusion. These low levels were about 0.1% of the expected serum levels for that dose of paclitaxel. The interesting finding was that the paclitaxel levels increased in the postinfusion CSF up to a maximum on Day 3 and then declined, which is consistent with a diffusion phenomena into and out of the CSF. Paclitaxel levels were also detected in malignant ascitic and pleural fluids with this method (Table 2).

Statistical Analysis

No significant differences in mean paclitaxel levels among the three assays (RIA, IRMA, and HPLC) were



Paclitaxel (µM)

FIGURE 2. Standard curve of competitive tritiated RIA is shown. The points are mean values of triplicate results expressed as percentages of maximal binding of ³H-paclitaxel to monoclonal antibody as the concentration of unlabeled paclitaxel is increased.

found (P > 0.5). The simultaneous 95% confidence intervals for differences in mean paclitaxel levels were -0.21 versus 0.09 for RIA versus HPLC, -0.14 versus 0.16) for IRMA versus HPLC, and -0.10 versus 0.20 for RIA versus IRMA. The reliability for RIA was 86.4%, and for IRMA, 85.7%. The reliability among RIA, IRMA, and HPLC was 72.5%. The reliability for RIA and IRMA individually were excellent (Reliability [R] > 0.75). The reliability among RIA, IRMA, and HPLC was good (0.4 < R < 0.75).

DISCUSSION

In this report, the development of two new competitive RIAs, using a MoAb to detect paclitaxel, are described. One RIA is based on a traditional approach using tritiated pacliaxel. The second is a newer, more sensitive IRMA, similar to a competitive ELISA, employing ¹²⁵I-labeled MoAb. Both methods proved reliable when compared to HPLC. These assays can be used to determine accurately and quickly paclitaxel levels in sera and other bodily fluids, including CSF, ascitic fluid, and pleural fluid, which may facilitate the design of experimental clinical protocols and be helpful in predicting toxicity. The latter may allow more judicious use of paclitaxel and enable investigators to give higher doses to some patients. TABLE 2

Paclitaxel Levels in Sera (Before and After Infusion) and in Cerebrospinal, Ascitic, and Pleural Fluids

Pre-retreatment paclitaxel levels detected using IRMA in patients previously treated			
Patient no.	IRMA (µM paclitaxel)	SD	
1.	0.0026	0.0024	
2.	0.0039	0.0010	
3.	0.0068	0.0004	
5.	0.0098	0.0014	
6.	0.0074	0.0009	
7.	0.0105	0.0024	

Paclitaxel levels in sera detected by IRMA from a patient with lung carcinoma treated with 350 mg/m² paclitaxel

Day	IRMA (μM paclitaxel)	SD
	0.004	0.0070
0 (Prior to infusion)	0.034	0.0070
1 (End of infusion)	2.250	0.2690
6	0.087	0.0070
8	0.044	0.0070
12	0.039	0.0090

Detection of paclitaxel in cerebrospinal fluid using IRMA from a patient with acute myelogenous leukemia treated with 315 mg/m² paclitaxel

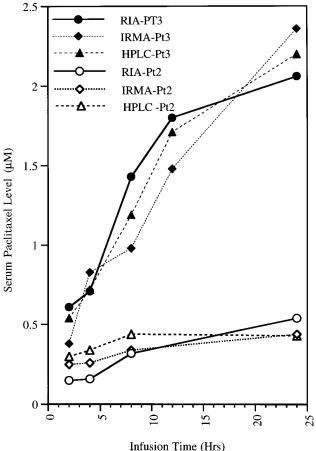
Day	IRMA (µM paclitaxel)	SD
1 (End of infusion)	0.0012	0.0003
2	0.0016	0.0004
3	0.0022	0.0004
4	0.0011	0.0002

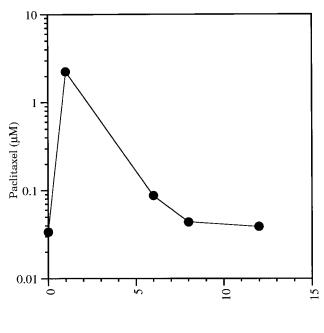
Detection of paclitaxel in ascitic fluid using IRMA from a patient with ovarian carcinoma treated with 135 mg/m² paclitaxel

Time (hrs)	IRMA (µM paclitaxel)	SD	
	4 1		
Prior to infusion	0.000		
7	0.112	0.0100	
22	0.260	0.320	
6 (Serum level)	0.260	0.0320	

Detection of paclitaxel in pleural fluid using IRMA from a patient with lung carcinoma treated with 250 $\rm mg/m^2$ paclitaxel

Time (hrs)	IRMA (µM paclitaxel)	SD
22 (During		
infusion)	0.629	0.067





Time (Days Post Infusion)

FIGURE 3. Paclitaxel levels in the sera of two patients are shown (Patient 2, with 135 mg/m², and Patient 3, with 300 mg/m²), as determined by 3 different assays: immunoradiometric assay (IRMA), tritiated radioimmuno-assay (RIA), and high performance liquid chromatography (HPLC). Values represent means of triplicate results obtained at different time points during

24-hour infusions.

The competitive IRMA method employed large amounts of paclitaxel-BSA but required only small amounts of ¹²⁵I-labeled MoAb. The tritiated paclitaxel RIA was fast and very accurate but consumed larger amounts of MoAb and was not as sensitive as the IRMA. Both RIA techniques were found to be less labor intensive than the HPLC method. The IMRA was more sensitive than both the tritiated RIA and HPLC.

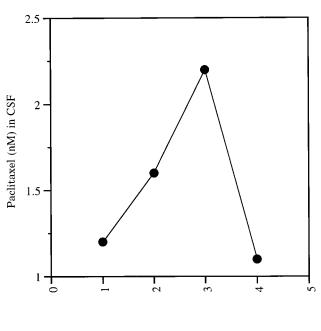
Using the IRMA, it was possible to detect paclitaxel in the CSF of one patient with leukemia and meningeal involvement (Table 1), the malignant ascites of a patient with ovarian carcinoma, and the pleural fluid of a patient with nonsmall cell lung carcinoma. The sensitivity of the IRMA was maximized by selecting the minimal amount of ¹²⁵I-labeled MoAb necessary to give accurate results but which would be the easiest to inhibit. The sensitivity of the assay was determined

FIGURE 4. Time course of preinfusion and postinfusion paclitaxel levels is shown, as determined by immunoradiometric assay in a patient with lung carcinoma treated with 350 mg/m² of paclitaxel. Values represent triplicate results calculated from a standard curve run simultaneously.

from the inhibition curve, which demonstrated that this assay could detect down to 1 nanomolar. HPLC has been reported to be sensitive only to about 50 nanomolar, therefore making the IRMA, with a lower detection limit of 1 nanomolar, significantly more sensitive than HPLC. The other advantage of this assay is the speed with which it can be done (about 3 hours) for large numbers of samples (several patients' pharmacokinetic curves can be plotted per day).

This is the first report of the detection of paclitaxel in human CSF. This was possible because of the sensitivity of the IRMA. The low levels of pacliataxel detected in CSF (approximately 1/1000 of serum levels) is consistent with the clinical observation that paclitaxel, even at high doses, does not cause central nervous system toxicity. The finding that pretreatment levels of paclitaxel could be detected from previous cycles of drug administration suggests that the terminal halflife of paclitaxel is long. Detection of paclitaxel in ascitic and pleural fluids may be associated with blood in the malignant fluids, but it may also represent diffusion of paclitaxel into these fluids.

The ability to determine the pharmacokinetic curves quickly for each patient's treatment could yield important information about response, toxicity, and paclitaxel resistance. As higher doses of paclitaxel are



Time (Days)

FIGURE 5. Time course of paclitaxel levels in cerebrospinal fluid is shown, as determined by immunoradiometric assay from a patient with acute myelogenous leukemia who received 315 mg/m² of paclitaxel. Cerebrospinal fluid collections from the Ommaya reservoir began on Day 1 at the end of the 24-hour intravenous infusion. Values represent triplicate results calculated from a standard curve run simultaneously.

used in combination with growth factors and other antineoplastic agents, it will be important to continue to quantify the pharmacokinetics of paclitaxel. There is considerable interest in attempting to reverse multidrug resistance by the use of compounds, such as verapamil and cyclosporine.³⁰ The ability to monitor serum levels of paclitaxel accurately during clinical trials with reversing agents could be instrumental in avoiding paclitaxel toxicity and ascertaining the proper dose of both the drug and the reversing agent.

In summary, we have developed two reliable radioimmunoassays that will be useful in the detection of paclitaxel in sera and other bodily fluids. The more sensitive IRMA, using radiolabeled antipaclitaxel antibody, may be particularly helpful for quantifying low levels of paclitaxel, such as those found in CSF.

REFERENCES

- 1. Wiernik PH, Schwartz EL, Einzig A, Strauman JJ, Lipton RB, Dutcher JP. Phase I trial of taxol given as a 24-hour infusion every 21 days: responses observed in metastatic melanoma. *J Clin Oncol* 1987;5:1232–9.
- Rowinsky EK, Cazenave LA, Donehower RC. Taxol: a novel investigational antimicrotubule agent. J Natl Cancer Inst 1990;82:1247–59.

- 3. Rowinsky EK, Onetto N, Canetta RM, Arbuck S. Taxol: the first of the taxanes, an important new class of anti-tumor agents. *Semin Oncol* 1992;19:646–62.
- Einzig A, Wiernik PH, Sasloff J, Runowicz CD, Goldberg GL. Phase II trial and long-term follow-up of patients treated with taxol for advanced ovarian adenocarcinoma. *J Clin Oncol* 1992;10:1748–53.
- 5. Schiff PB, Fant J, Horwitz SB. Promotion of microtubule assembly in vitro by taxol. *Nature* 1979;277:665–7.
- Schiff PB, Horwitz SB. Taxol stabilizes microtubules in mouse fibroblast cells. *Proc Natl Acad Sci U S A* 1980; 77:1561–5.
- 7. Horwitz SB. Mechanisms of action of taxol. *Trends Pharma-col Sci* 1992;13:134–6.
- Ding A, Sanchez E, Nathan CF. Taxol shares the ability of bacterial lipopolysaccharide to induce tyrosine phosphorylation of microtubule-associated protein kinase. *J Immunol* 1993;151:5596–6002.
- Burkhart C, Berman JW, Swindell CS, Horwitz SB. Relationship between the structure of taxol and other taxanes on induction of tumor necrosis factor-α gene expression and cytotoxicity. *Cancer Res* 1994;54:5779–82.
- Weiss RB, Donehower RC, Wiernik PH, Ohnuma T, Gralla RJ, Trump DL, et al. Hypersensitivity reactions from taxol. *J Clin Oncol* 1990;8:1263–8.
- Wiernik PH, Schwartz EL, Strauman JJ, Dutcher JP, Lipton RB, Paietta E. Phase I clinical and pharmacokinetic study of taxol. *Cancer Res* 1987;47:2486–93.
- Schiller JH, Storer B, Tutsch K, Arzoomanian R, Alberti D, Feierabend C., et al. Phase I trial of 3-hour infusion of paclitaxel with or without granulocyte colony-stimulating factor in patients with advanced cancer. *J Clin Oncol* 1994;12:241– 8.
- Younes A, Sarris A, Melnyk A, Romaguera J, McLaughlin P, Swan F, et al. Three-hour paclitaxel infusion in patients with refractory and relapsed non-Hodgkin's lymphoma. *J Clin Oncol* 1995;13:583–7.
- Wilson WH, Berg SL, Bryant G, Wittes RE, Bates S, Fojo A, et al. Paclitaxel in doxorubicin or mitoxantrone refractory breast cancer: a Phase I/II trial of 96-hour infusion. *J Clin Oncol* 1994;12:1621–9.
- Kris MG, O'Connell JP, Gralla RJ, Wertheim MS, Parente RM, Schiff PB, et al. Phase I trial of taxol given as a 3-hour infusion every 21 days. *Cancer Treat Rep* 1986;70:605–7.
- Grem JL, Tutsch KD, Simon KJ, Alberti DB, Willson KV, Tormey DC, et al. Phase I study of taxol administered as a short i.v. infusion daily for 5 days. *Cancer Treat Rep* 1987;71:1179– 84.
- Rowinsky EK, Burke PJ, Karp JE, Tucker RW, Ettinger DS, Donehower RC. Phase I and pharmacodynamic study of taxol in refractory acute leukemias. *Cancer Res* 1989; 49:4640–7.
- Brown T, Havlin K, Weiss G, Cagnola J, Koeller J, Kuhn J, et al. A Phase I trial of taxol given by 6-hour intravenous infusion. *J Clin Oncol* 1991;9:1261–7.
- Sarosy G, Kohn E, Stoner D, Rothenberg M, Jacob J, Adamo DO, et al. Phase I study of taxol and granulocyte colonystimulating factor in patients with refractory ovarian cancer. *J Clin Oncol* 1992;10:1165–70.
- Reichman BS, Seidman AD, Crown JPA, Heelan R, Hakes TB, Lebwohl DE, et al. Paclitaxel and recombinant human granulocyte colony-stimulating factor as initial chemotherapy for metastatic breast cancer. *J Clin Oncol* 1993;11:1943– 51.

- Seidman A, Norton L, Reichman BS, Crown JPA, Yao TJ, Heelan R, et al. Preliminary experience with paclitaxel (TAXOL) plus recombinant human granulocyte colony-stimulating factor in the treatment of breast cancer. *Semin Oncol* 1993;20(S3): 40–5.
- 22. Klecker RW, Jamis-Dow CA, Egorin MJ, Erkmen K, Parker RJ, Collins JM. Distribution and metabolism of ³H-taxol in the rat. *Proc Am Assoc Cancer Res* 1993;34:380.
- 23. Jaziri M, Diallo BM, Vanhaelen MH, Vanhaelen-Fastre RJ, Zhiri, A, Becu AG, et al. Enzyme-linked immunosorbent assay for the detection of taxol and semi-quantitative determination of taxane diterpinoids related to taxol in Taxus sp. and tissue cultures. J Pharm Belg 1991; 46: 93–9.
- 24. Grothaus PG, Raybould TJG, Bignami GS, Lazo CB, Byrnes J. An enzyme immunoassay for the determination of taxol and taxanes in Taxus sp. tissues and human plasma. *J Immunol Methods* 1993;158:5–15.
- 25. Leu J-G, Chen B-X, Schiff PB, Erlanger BF. Characterization of polyclonal and monoclonal anti-taxol antibodies and

measurement of taxol in serum. *Cancer Res* 1993;53:1388-91.

- Fraker PJ, Speck JC. Protein and cell membrane iodinations with a sparingly soluble chloroamide, 1,3,4,6-tetrachloro-3a,6a-diphenylglycoluril. *Biochem Biophys Res Comm* 1978; 80:849–57.
- 27. Hunter WM, Budd PS. Immunoradiometric versus radioimmunoassay: a comparison using alpha-foetoprotein as the model analyte. *J Immunol Methods* 1981;45:255–73.
- Hunter WM, Bennie JG, Brock DJH, Van Heyningen V. Monoclonal antibodies for use in an immunoradiometric assay for alpha-foetoprotein. *J Immunol Methods* 1982; 50:133–44.
- 29. Fleiss JL. The design and analysis of clinical experiments. 1st edition. New York: John Wiley & Sons, Inc., 1986.
- Jachez B, Nordmann R, Loor F. Restoration of taxol sensitivity of multidrug-resistant cells by the cyclosporine SDZ PSC 833 and the cyclopeptolide SDZ 280-446. J Natl Cancer Inst 1993;85:478–82.