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Steady-state plasma concentrations and effects of taxol for a 250 mg/m^2 dose in combination with granulocyte-colony stimulating factor in patients with ovarian cancer

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Abstract. Taxol, a natural product initially isolated from the stem bark of the western yew *Taxus brevifolia*, is undergoing phase II and III evaluation due to its reported activity against a variety of tumors. Previous studies have described correlations between plasma concentrations and toxicity when taxol is given (a) at lower doses, (b) for shorter infusion times, and (c) without granulocyte-colony-stimulating factor. Because the 24-h infusion schedule is most commonly used in current clinical trials, we attempted to correlate steady-state plasma concentrations of taxol achieved with a 24-h continuous i.v. infusion with toxicities and responses. Plasma samples from 48 refractory ovarian cancer patients were obtained 1–2 h prior to the end of the first taxol infusion. Taxol concentrations were measured by high-performance liquid chromatography (HPLC). Interpatient variation of taxol plasma concentrations was small (mean ± SD, 0.85 ± 0.21 μ*M*. Total taxol body clearance was 256 ± 72 ml min^{-1} m^{-2} (mean ± SD). Taxol plasma protein binding was 88.4% ± 1.3% (mean ± SD, n = 9). Grade 3–4 hematologic toxicity, mainly leukopenia, occurred in 92% of the patients. The leukopenia was transient and did not warrant a reduction in the dose of taxol. Grade 3–4 nonhematologic toxicity occurred in 8% of the patients. No severe hypersensitivity reaction or grade 3–4 neurotoxicity was observed. Correlations of plasma concentrations and toxicities were not feasible due to the high frequency of hematologic effects and the low frequency of nonhematologic toxicity. The low degree of interpatient variation in plasma concentrations hindered the development of correlations with response.

Introduction

Taxol (Fig. 1) is a natural product initially isolated from the stem bark of the western yew *Taxus brevifolia* [16]. Taxol binds covalently to the β-subunit of tubulin in vitro [9], shifting the equilibrium between the α- and β-tubulin dimers and the microtubule toward the formation of the latter, inducing polymerization of tubulin. In the presence of taxol, microtubules are resistant to depolymerization by low temperature (4°C) or calcium (4 m*M*) addition [14]. Taxol-treated cells are arrested at the G_2 and M phases of the cell cycle and show characteristic bundles of disorganized microtubules [13]. Taxol is active against L1210, P388 and P1534 cell lines in culture [15]. Taxol also has significant antitumor activity against several human tumor xenografts [10].

Clinically, taxol has demonstrated activity against refractory ovarian cancer, breast cancer, lung cancer, and malignant melanoma [1, 2, 4, 7, 12]. Major toxicities associated with taxol therapy are myelosuppression, particularly of the granulocytic component (dose limiting), neuropathy, mucositis, myalgia, alopecia and cardiac arrhythmias [1, 2, 4, 5, 7, 11, 12]. Leukocyte toxicity can be ameliorated by the addition of granulocyte-colony stimulating factor (G-CSF) to the regimen [12]. Severe hypersensitivity reactions have been reported with taxol infusions [17]. These reactions may be related to the vehicle used for solubilizing taxol (Cremophor EL, a polyethoxylated castor oil). The incidence of these hypersensitivity reactions has been reduced by premedicating the patients with steroids and H_1 and H_2 receptor antagonists and by concomitantly prolonging the duration of the infusion [17].

Earlier studies showed a high degree of interindividual variability in plasma taxol concentrations [1, 3, 6, 18]. Some investigators found correlations between plasma concentrations and toxicity when taxol without G-CSF was given at lower doses or during shorter infusion times [1, 3, 6]. We studied 48 refractory ovarian cancer patients treated at the Clinical Center of the National Cancer Institute (Bethesda, Md.). We report the taxol protein-binding data, steady-state taxol plasma concentrations, total body clearance, area under the plasma concentration versus time curve (AUC), toxicities, and responses observed when this group of patients received 250 mg/m^2 taxol as a 24-h continuous intravenous infusion followed by G-CSF.

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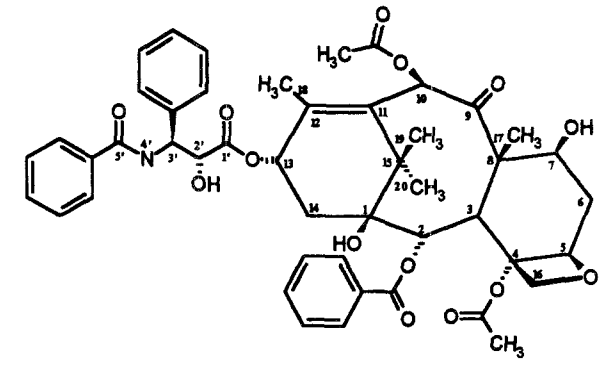

TAXOL

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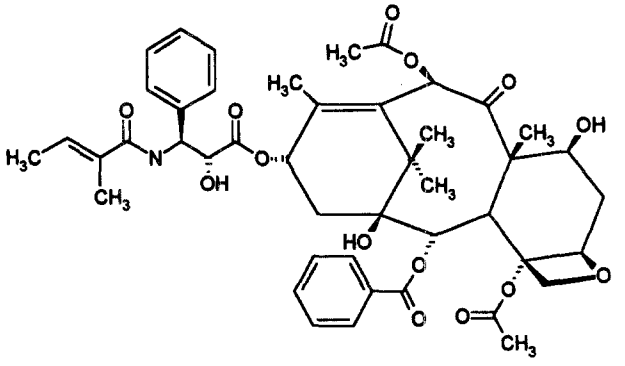

INTERNAL STANDARD

NSC-318735

Fig. 1. Chemical structures of taxol and the internal standard cephalomannine

Patients and methods

Patient eligibility. Only patients with histologic proof of ovarian cancer of epithelial histology, International Federation of Gynecology and Obstetrics (FIGO) stage III or IV, treated with at least one prior cisplatin or carboplatin-based regimen, and objective bidimensionally measurable disease were admitted. Eligibility criteria included an age of ≥ 18 years; an Eastern Cooperative Oncology Group (ECOG) performance status of ≤ 2; no prior chemo- or radiotherapy within 4 weeks of entry to the study; no prior radiation except for intraperitoneal ^{32}P; recovery from all toxicities of prior treatment; and adequate bone marrow, renal, and hepatic function as documented by laboratory parameters. Not eligible were patients with cerebral metastasis; a prior history of myocardial infarction, congestive heart failure, asymptomatic first-degree atrioventricular (AV) block, asymptomatic left anterior hemiblock (LAHB) and right bundle branch block (RBBB), or a cardiac arrhythmia requiring medication; a preexisting peripheral neuropathy of > grade 1; any coexisting malignancy or a history of a malignancy other than ovarian carcinoma (exceptions: local basal cell carcinoma of the skin or carcinoma in situ of the cervix); or an active, uncontrolled infection. All patients gave written informed consent before entering the study.

Each patient provided a medical history and a physical examination was completed prior to taxol administration. Other tests performed before therapy included a complete blood cell count with differential and platelet count, determinations of serum electrolytes, glucose, blood urea nitrogen (BUN), creatinine, bilirubin, alkaline phosphatase, SGOT, SGPT, and lactate dehydrogenase (LDH), urinalysis, an EKG; electromyograph (EMG)/nerve conduction tests; chest Xrays, other radiologic studies to measure disease; and determinations of CA125 levels. Platelet and complete blood cell counts were performed biweekly until two successive determinations after the nadir showed a total granulocyte count of >1500/μl. If toxicity was acceptable and there was no disease progression, the cycle of therapy was repeated every 3 weeks. Patients were evaluated for response after each cycle if the disease was measurable by physical examination, every two cycles if radiologic studies were necessary to evaluate disease response, and every four cycles if peritoneoscopy or laparoscopy was required. Responding patients received two cycles beyond the maximal response. Patients with stable or progressive disease were removed from study after two cycles of therapy. Patients who required evaluation by an invasive procedure were removed from study if they had stable or progressive disease after four cycles of therapy.

Taxol formulation and administration. Taxol was supplied by the Division of Cancer Treatment, National Cancer Institute (Bethesda, Md.) as a concentrated sterile solution of 6 mg/ml in 5-ml ampules (30 mg/ampule) in 50% polyethoxylated castor oil (Cremophor EL; BASF, Ludwigshafen, Germany) and 50% Dehydrated Alcohol, USP. Just prior to administration the taxol infusion was prepared by diluting one-third of the total dose in each of three 1000-ml bottles of 5% Dextrose Injection, USP. In-line filtration of the infusion solution was accomplished by incorporating a hydrophilic filter of pore size 0.22 μ*M* into the i.v. fluid path.

Taxol (250 mg/m^2) was given through a central vein as a 24-h continuous infusion, repeated every 21 days. Patients were premedicated with 20 mg dexamethasone given p.o. or i.v. 14 and 7 h prior to taxol and with 300 mg cimetidine and 25 mg diphenhydramine given i.v. 30 min prior to taxol. Recombinant G-CSF was supplied by the Division of Cancer Treatment, National Cancer Institute (Bethesda, Md.). G-CSF was given s.c. at a dose of 10 μg/kg daily, beginning 24 h after the completion of the taxol infusion and ending after the total granulocyte count had reached ≥ 1500/μl on two successive determinations.

Taxol measurement. Taxol plasma concentrations were measured by reverse-phase high-performance liquid chromatography (HPLC) using cephalomannine (Fig. 1) as the internal standard. Taxol and cephalomannine reference standards were obtained from the Division of Cancer Treatment, National Cancer Institute, (Bethesda, Md.). Cephalomannine was further purified by HPLC. An aliquot of cephalomannine was added to 0.5 ml standard or sample and mixed. Samples were extracted on a C_{18} solid-phase extraction column (1 ml Bond Elut; Varian Sample Preparation Products, Harbor City, Calif.). Each column was conditioned with 1 ml acetonitrile followed by 1 ml water. The sample was applied and the column was washed with 3 ml water. The extract was collected in a 2-ml acetonitrile elution. The eluent was dried in a vacuum concentrator (Savant Instruments, Farmingdale, NY). The extract was reconstituted in 150 μl 45% acetonitrile in water and 70 μl were injected onto the HPLC column.

Extracts were chromatographed isocratically with 45% acetonitrile in water at a flow of 1 ml/min. Separation was accomplished on an ODS Hypersyl C_{18}, 5 μm, 100 × 4.6 mm column (Hewlett-Packard Co., Palo Alto, Calif.) with a C_{18} precolumn insert (Millipore Corp., Milford, Mass.). Precolumn inserts were replaced after every 16 plasma sample injections to maintain optimal resolution. Taxol and cephalomannine were quantitated at 230 nm with peak purity and the identity was confirmed by diode-array detection. After each sample run the column was washed for 2 min with 95% acetonitrile in water and equilibrated for 7 min under the initial conditions. The taxol concentration in plasma samples was quantitated by linear regression analysis of the peak area ratio (taxol/cephalomannine) versus taxol standards prepared in ethylenediaminetetraacetic acid (EDTA)-treated normal human plasma. The typical plasma standard curve was in the range of expected plasma concentrations, between 0.125 and 2.5 μ*M* (0.107–2.13 μg/ml).

Pharmacokinetics. For measurement of steady-state plasma concentrations, blood samples were collected in EDTA-treated tubes prior to and 1 and 2 h before the end of the first taxol infusion. The samples were

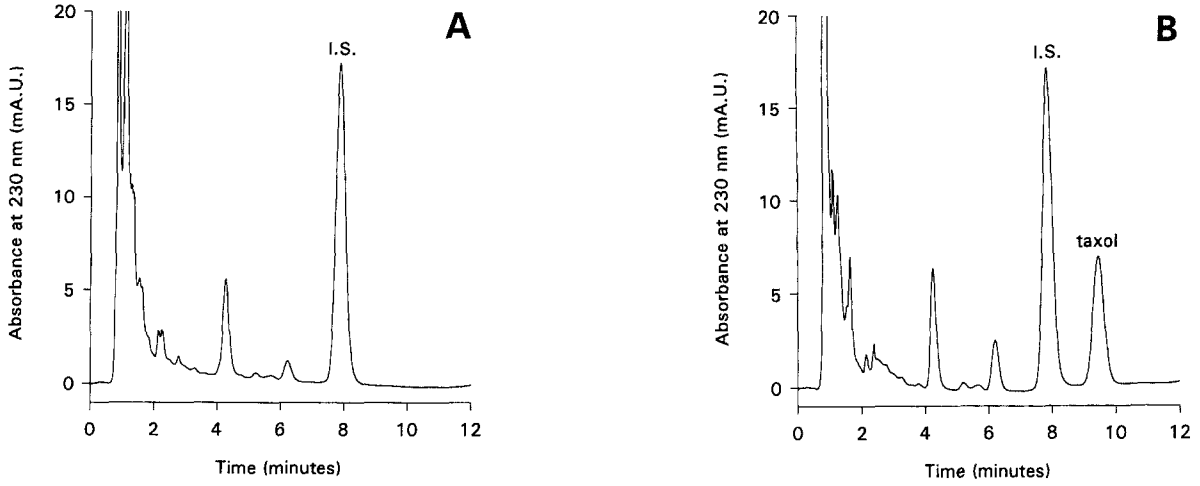

Fig. 2. Chromatograms of plasma sample extracts obtained from a patient **A** before and **B** during taxol infusion ($t = 22$ h). Plasma samples were analyzed as described in Patients and methods. *mA.U.*, milliabsorbance units; *I.S.*, internal standard

centrifuged and the plasma was separated and stored at –20°C until analysis. The reported steady-state plasma value represents the average of the two steady-state samples. Total body clearance was calculated by dividing the infusion rate by the average steady-state taxol concentration. The AUC was calculated by multiplying the average steady-state taxol concentration by the infusion time (24 h).

Taxol protein binding. Preliminary experiments in our laboratory showed extensive taxol binding to filtration devices. Thus, we estimated the plasma protein binding of taxol in vitro by equilibrium dialysis of human plasma against a phosphate-buffered saline solution (PBS; 0.144 *M* sodium chloride + 0.01 *M* potassium phosphate buffer, pH 7.46). Pretreatment plasma samples obtained from 9 ovarian cancer patients were spiked with [^{3}H]-taxol (23 Ci/mmol, 1 mCi/ml; Moravek Biochemicals, Inc., Brea, Calif.) to a concentration of 1 μCi/ml (0.043 μ*M*) and dialyzed for 5.5 h at 37°C through a 12,000–14,000 MWCO Spectra/Por 2 membrane (Spectrum Medical Industries, Inc., Los Angeles, Calif.) against PBS using a Spectrum Equilibrium Dialyzer (Spectrum Medical Industries, Inc., Los Angeles, Calif.). After incubation, a 100-μl aliquot was taken from each cell side and dispensed into a vial containing 10 ml scintillation fluid. The pipette tip was rinsed two times with scintillation fluid to avoid any loss of taxol to the tip. Another aliquot was analyzed by HPLC to confirm the integrity of [^{3}H]-taxol after the equilibrium dialysis. Samples were measured in duplicate.

Clinical effects. All toxicities were graded according to the Common Toxicity Criteria of the Cancer Therapy Evaluation Program of the National Cancer Institute. Disease response was assessed according to the following definitions: clinical complete response, the complete resolution of all evaluable disease, lasting at least 1 month; partial response, a reduction of ≥50% in the sum of the products of the longest perpendicular diameters of all measurable lesions, lasting at least 1 month; and no response, all cases not meeting either of the above definitions.

Results

A total of 49 patients received taxol. One patient's replicate taxol plasma concentrations showed a 6.1-fold difference (10.98 and 1.81 μ*M*). This patient was not included in the analysis. Of the remaining 48 patients, 5 were not formally treated on the protocol and as such were not evaluable for response to therapy.

Taxol analysis

The mean recovery (± SD) from plasma of cephalomannine (2.5 μ*M*) and taxol (1 μ*M*) was 90% ± 7% and 88% ± 5%, respectively ($n = 11$). Figure 2 shows chromatograms from plasma extracts obtained from one patient before and during the taxol infusion. The retention times were 7.9 min for cephalomannine and 9.6 min for taxol. Extracts from blank plasma showed no interfering peaks at the retention times of either the taxol or the internal standard peak (data not shown). No metabolites were detected in plasma.

Detection was linear down to 0.0125 μ*M* (0.0107 μg/ml), with the signal-to-noise ratio being 3 at this concentration (data not shown). The interday coefficients of variation for 1.9, 1.2, and 0.49 μ*M* taxol were 1.6%, 3.4%, and 6.3%, respectively. Samples of normal human plasma were spiked with taxol and stored at –20°C. There was no change in the taxol concentration of these samples measured on five different occasions over an 8-month period (data not shown).

Steady-state taxol plasma concentrations after a 24-h infusion

A total of 48 patients were evaluable for steady-state plasma concentrations. As seen in Fig. 3, the plasma concentrations measured during the first cycle of taxol therapy fit a normal distribution of frequency with a range of 0.40–1.41 μ*M*. The mean steady-state plasma concentration (± SD) was 0.85 ± 0.21 μ*M*. Three patients in this group had only one concentration measured. The total body clearance for taxol was 256 ± 72 ml min^{-1} m^{-2} (mean ± SD, $n = 48$). The AUC was 20.3 ± 5.1 μ*M* h (mean ± SD, $n = 48$).

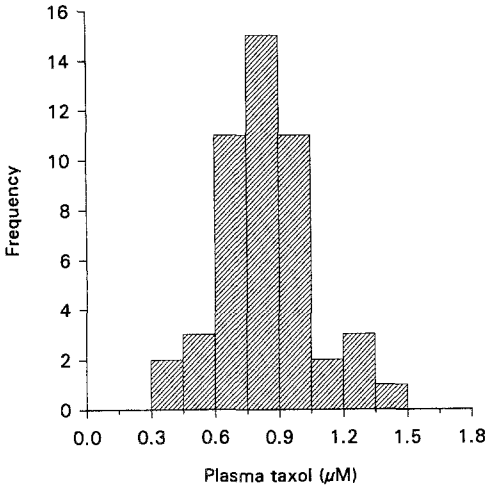

Fig. 3. Frequency distribution of plasma taxol concentrations measured in 48 ovarian cancer patients. Blood samples were collected at 22 and 23 h after the start of the infusion. Samples were centrifuged and the plasma was separated and stored at –20°C until analyzed as described in Patients and methods. The average value of the two steady-state samples was used to construct the histogram

Taxol protein binding

Under our system conditions, equilibrium was reached within 5.5 h. Longer incubations for up to 24 h resulted in increasing degradation of [^{3}H]-taxol. Binding of taxol to pipette tips is a problem when one is working with dilute aqueous solutions. To avoid this problem, the pipette tips were rinsed with scintillation fluid. Following this procedure, the protein binding of taxol was 88.4% ±1.3% (mean ±SD, $n = 9$).

Toxicities

Grade 3–4 hematologic toxicity that included granulocytopenia, lymphocytopenia, thrombocytopenia, or anemia occurred in 44 of 48 evaluable patients (92%). In all, 5 patients (10%) were admitted to the hospital with fever and neutropenia. The leukocyte nadir occurred by the 7th day of the cycle. Grade 3–4 nonhematologic toxicity occurred in only 4 patients (8%) and included mucositis, fever without neutropenia, fatigue, and depression. No grade 3 or 4 neurotoxicity was observed. There was no hypersensitivity reaction to the drug infusion. Alopecia was observed in all patients.

Responses

A total of 43 patients were evaluable for response. The best responses involved 4 patients (9%) who achieved a clinical complete response and 17 patients (40%) who had a partial response. In addition, 6 patients showed clinical improvement that did not meet the criteria for a partial response. In all, 7 patients had stable disease and 9 patients progressed despite therapy. Since these patients form a subset of a larger group evaluated in a phase II trial, additional clinical details will be presented elsewhere. There was no significant relationship between the response rate and the taxol plasma concentration.

Discussion

Previous studies reported wide interpatient variation of taxol plasma concentrations [1, 3, 6, 18] and correlations between plasma concentrations and toxicity when taxol without G-CSF was given at lower doses or during shorter infusion times [1, 3, 6]. The 24-h i.v. infusion schedule is most commonly used in current clinical trials; therefore, we attempted to correlate the steady-state plasma concentrations of taxol with the toxicity and response observed with such a schedule.

For this purpose, we developed a simple, sensitive and specific HPLC method for quantitating taxol in biological fluids. The method uses cephalomannine, a taxane very similar to taxol, as the internal standard. The recovery was high (88%–90%) for both taxol and cephalomannine. The method performed equally well for analyzing taxol in urine samples (results not shown). The assay was very reproducible as demonstrated by the small inter day variation.

Intersubject variation in drug toxicity can be due to differences in the total plasma drug concentration or to differences in the amount of free drug present. In our patients, the steady-state taxol plasma concentrations fell in a narrow range (0.4 – 1.41 μ*M*) with little variation (mean ± SD, 0.85 ± 0.21 μm). There was even less patient-to-patient variation in the amount of taxol bound to plasma proteins (88.4% ± 1.3%, $n = 9$).

The contrast between our findings and previous reports of high interpatient variability [1, 3, 6] could be explained by a variety of factors. Previous reports have been based upon phase I studies, which typically accrue a more variable population. Although we did not find circulating metabolites in our patients' plasma, taxol is metabolized by the liver [8]. Thus, hepatic dysfunction is a credible source of interpatient variation. Analytical methodology is also a potential problem, since taxol is labile, fairly insoluble, and binds extensively to laboratory ware. The last two factors could also account for the difference between our taxol protein binding data and the previously reported [19] higher taxol-bound fraction (97.5%).

Despite the use of G-CSF, grade 3–4 leukopenia was frequently observed (92%). This effect could be due to the poor bone marrow reserve of this group of heavily pretreated refractory ovarian cancer patients. It should be noted that the leukopenia was brief and in most cases did not warrant a reduction in the dose of taxol. Only 8% of patients presented with nonhematologic grade 3–4 toxicity, and no hypersensitivity reaction was observed.

Correlations of steady-state plasma concentrations and clinical effects were not feasible in this study for several reasons:

1. The low degree of interpatient variation in plasma concentrations and taxol-plasma protein binding hindered the development of correlations with effect. Although concentration is generally a more proximate indicator of drug effect than drug dose, the low degree of interpatient varia-

tion indicates that dose itself may be an adequate indicator in relatively homogeneous groups of patients with normal liver function.
2. The low frequency of nonhematologic toxicity precluded correlations.
3. Conversely, the high frequency of hematologic effects was a barrier to discovery of relationships. Since the basic motivation for this study design was to push the taxol dose to the point where intervention with G-CSF was required, nearly every patient had grade 3 or 4 hematologic toxicity.
4. The inability to find a relationship between plasma concentration and antitumor response may indicate that a maximal response could be obtained at lower doses of taxol, but it is equally possible that the range of plasma concentrations was simply too narrow for a definitive statement.

Ongoing trials of taxol doses randomized to 135 versus 170 versus 250 mg/m^2 provide a more suitable opportunity to explore the lower end of the curves of taxol concentration versus antitumor response or hematologic toxicity.

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