

Oral Paclitaxel and Concurrent Cyclosporin A: Targeting Clinically Relevant Systemic Exposure to Paclitaxel¹

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ABSTRACT

Oral paclitaxel is not inherently bioavailable because of the overexpression of P-glycoprotein by intestinal cells and the significant first-pass extraction by cytochrome P450-dependent processes. This study sought to simulate the toxicological and pharmacological profile of a clinically relevant schedule of paclitaxel administered on clinically relevant i.v. dosing schedules in patients with advanced solid malignancies using oral paclitaxel administered with cyclosporin A, an inhibitor of both P-glycoprotein and P450 CYP3A.

Nine patients were treated with a single course of oral paclitaxel in its parenteral formulation at a paclitaxel dose level of 180, 360, or 540 mg. Cyclosporin A was administered at a dose of 5 mg/kg p.o. 1 h before and concurrently with oral paclitaxel. Blood sampling was performed to evaluate the pharmacokinetics of paclitaxel, 6- α -hydroxypaclitaxel, 3- ρ -hydroxypaclitaxel, and cyclosporin A. The pharmacokinetic behavior of paclitaxel was characterized using both compartmental and noncompartmental methods. Model-estimated parameters were used to simulate paclitaxel concentrations after once daily and twice daily oral administration of paclitaxel and cyclosporin A.

Aside from an unpleasant taste, the oral regimen was well tolerated, and there were no grade 3 or 4 drug-related toxicities. The systemic exposure to paclitaxel, as assessed by maximum plasma concentration (C_{max}) and area under the plasma concentration *versus* time curve (AUC) values, did not increase as the dose of paclitaxel was increased from 180 to 540 mg, and there was substantial interindividual varia-

bility (4–6-fold) at each dose level. Mean paclitaxel C_{max} values approached plasma concentrations achieved with clinically relevant parenteral dose schedules, averaging 268 ± 164 ng/ml. AUC values averaged 3306 ± 1977 ng·h/ml, which was significantly lower than AUC values achieved with clinically relevant i.v. paclitaxel dose schedules. However, computer simulations using pharmacokinetic parameters derived from the present study demonstrated that pharmacodynamically relevant steady-state plasma paclitaxel concentrations of at least $0.06 \mu\text{M}$ would be achieved after protracted once daily and twice daily dosing with oral paclitaxel and cyclosporin A. Paclitaxel metabolites were detectable in three patients, and the 6- α -hydroxypaclitaxel:paclitaxel and 3- ρ -hydroxypaclitaxel:paclitaxel AUC ratios averaged 0.63 and 0.86, respectively; these values were substantially higher than values reported in patients treated with i.v. paclitaxel.

Oral paclitaxel was bioavailable in humans when administered in combination with oral cyclosporin A 5 mg/kg 1 h before and concurrently with paclitaxel treatment, and plasma paclitaxel concentrations achieved with this schedule were biologically relevant and approached concentrations attained with clinically relevant parenteral dose schedules. However, treatment of patients with oral paclitaxel using a single oral dose administration schedule failed to achieve sufficiently high systemic drug exposure and pharmacodynamic effects. In contrast, computer simulations demonstrated that clinically relevant pharmacodynamic effects are likely to be achieved with multiple once daily and twice daily oral paclitaxel-cyclosporin A dosing schedules.

INTRODUCTION

The prototypic taxane paclitaxel, which disrupts tubulin dynamics, has demonstrated impressive clinical activity against many common types of solid malignancies, including ovarian, breast, and non-small cell lung carcinomas, and its use in the management of both early and advanced malignant diseases is increasing (1–5). Because paclitaxel is not bioavailable when administered p.o. (6, 7), the agent is administered exclusively by the i.v. route, most commonly over 3 h (8), which is cumbersome and limits the use of intermittent and frequent dosing schedules that can achieve prolonged systemic exposure to this cell cycle phase-specific agent. Moreover, paclitaxel is formulated for systemic administration in a mixture of ethanol and polyoxyethylated castor oil that appears to be primarily responsible for drug-related hypersensitivity reactions (9). Because paclitaxel can induce severe hypersensitivity reactions, premedication with corticosteroids and both H₁- and H₂-histamine antagonists is indicated, and long administration schedules are commonly used (3, 9). The polyoxyethylated castor oil emulsi-

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fier is also a substrate for Pgp,³ which plays a critical role in the biliary excretion of paclitaxel, doxorubicin, and other xenobiotics (9). In fact, the principal mechanism proposed to account for the increased incidence of cardiomyopathy in patients treated with brief infusions of paclitaxel and doxorubicin in combination over that achieved with identical cumulative doses of doxorubicin as a single agent is a decrement in the Pgp-mediated biliary clearance of doxorubicin secondary to high plasma concentrations of polyoxyethylated castor oil (9–11). In addition, the polyoxyethylated castor oil vehicle appears to be, in part, responsible for the nonlinear pharmacokinetic behavior of paclitaxel (12).

Many of the aforementioned limitations of paclitaxel could be reduced or eliminated if oral administration was feasible; however, the systemic bioavailability in humans after oral paclitaxel administration is less than 6% (7). The explanations proposed to account for the low oral bioavailability of paclitaxel are multifactorial. The most likely explanation is that enterocytes constitutively overexpress the ATP-dependent plasma membrane transporter Pgp, which actively extrudes many types of xenobiotics, particularly structurally bulky natural product toxins such as the taxanes, *Vinca* alkaloids, anthracyclines, and epipodophyllotoxins (13–16). Although Pgp was initially discovered as a drug pump in multidrug-resistant malignant cells (13–18), it is constitutively overexpressed in many types of normal tissues (14–15). For example, Pgp is localized to the apical surface of the columnar cells in the jejunum and colon, suggesting that Pgp prevents the absorption of some p.o. administered drugs (14, 15, 19, 20). Further support that Pgp is principally responsible for the low bioavailability of oral paclitaxel is derived from studies in *mdr1a* (–/–) knockout mice lacking intestinal Pgp, in which the bioavailability of 10 mg/kg of paclitaxel increased from 11% in wild-type mice to 35% in *mdr1a* (–/–) knockout mice (21).

Another explanation that may account for the low bioavailability of oral paclitaxel is first-pass extraction by cytochrome P450-dependent metabolic processes, particularly P450 isoenzymes CYP2C8 and CYP3A4, which metabolize paclitaxel to 6 α -hydroxypaclitaxel and 3 ρ -hydroxypaclitaxel, respectively (22–26). In humans, 6 α -hydroxypaclitaxel is the principal metabolite of paclitaxel (22, 23, 26), although 3 ρ -hydroxypaclitaxel may predominate in patients with hepatic dysfunction and after treatment with agents that induce P450-dependent metabolism (27, 28). Both CYP2C8 and CYP3A4 are primarily found in the hepatocytes (29, 30); however, CYP3A4 is also found in the villous columnar epithelial cells of the jejunum (30). Paclitaxel administered by the oral route may undergo cytochrome P450-mediated first-pass metabolism in both enterocytes and hepatocytes, thereby significantly limiting the systemic bioavailability of an p.o. administered dose.

The dual expression of Pgp and CYP3A4 in mature enterocytes and their overlapping substrate specificities suggest that the functions of these two protein complexes are complementary

(31, 32). The transporter, by actively expelling drug from enterocytes into the intestinal lumen, may prevent sufficient quantities of paclitaxel from reaching the portal circulation (32). In this manner, paclitaxel may be reabsorbed into the enterocyte, where it is repeatedly subjected to metabolism by CYP3A4 (32). Regardless of the mechanisms underlying the potential interactions between Pgp and CYP3A4 function in the enterocyte, Pgp inhibition combined with CYP3A4 inhibition may provide a clinical strategy to improve the oral bioavailability of paclitaxel.

Cyclosporin A, which inhibits the functions of both Pgp and CYP3A (33), has been shown to render oral paclitaxel bioavailable *in vivo* (34–36). In mice, the bioavailability of oral paclitaxel has been shown to increase from 9.3% without cyclosporin A to 67% with the concurrent administration of 10 mg/kg of oral cyclosporin A (34). The concurrent oral administration of 10 mg/kg of paclitaxel and 50 mg/kg of the nonimmunosuppressive cyclosporin A analogue SDZ PSC-833 has also been demonstrated to augment the bioavailability of oral paclitaxel (35). Furthermore, the bioavailability of oral paclitaxel was reported to be 40% in rats treated with 5 mg/kg of oral cyclosporin A 1 h prior to and concurrently with 9 mg/kg of oral paclitaxel, and biologically relevant plasma paclitaxel concentrations (>0.07 μ M) were maintained for 12 h posttreatment (1, 36).

Because oral administration of paclitaxel would obviate the need for i.v. access and increase patient convenience and the feasibility of administering paclitaxel on a multiple dosing schedule, this study was performed to replicate a single dosing i.v. schedule with the administration of oral paclitaxel 1 h after and concurrently with 5 mg/kg of oral cyclosporin A in patients with advanced solid malignancies. The principal objectives of the study were as follows: (a) to characterize the pharmacological behavior of paclitaxel, paclitaxel metabolites, and cyclosporin A in plasma after treatment with this oral regimen; and (b) to determine the principal toxicities of this regimen.

PATIENTS AND METHODS

Patient Selection. Patients with histologically confirmed malignancies refractory to standard therapy, or for whom no effective therapy existed, were candidates for this study. Patients with metastatic non-small cell lung cancer were eligible at any time during their treatment course. Patients with metastatic breast cancer were required to have previously received a doxorubicin-containing regimen. Other eligibility criteria included the following: (a) age >18 years; (b) Eastern Cooperative Oncology Group performance status of ≤ 2 (ambulatory and capable of self care); (c) life expectancy of at least 12 weeks; (d) no chemotherapy in the previous 4 weeks (6 weeks for nitrosoureas or mitomycin C); (e) no investigational study participation in the previous 30 days; (f) adequate hematopoietic (absolute neutrophil count ≥ 1500 μ l and platelet count $\geq 100,000$ μ l), hepatic (total serum bilirubin ≤ 2.0 mg/dl and serum transaminases ≤ 3 times the upper limit of normal in the absence of liver metastases and ≤ 5 times upper limit of normal in the presence of liver metastases), and renal (serum creatinine concentration ≤ 2.0 mg/dl) functions; (g) no gastrointestinal disease that could interfere with the absorption of oral drug treatment; (h) no known active central nervous system metastases (*i.e.*, no

³ The abbreviations used are: Pgp, P-glycoprotein; AUC, area under the plasma concentration *versus* time curve; LC/MS/MS, liquid chromatography with tandem mass spectrometry.

progressive lesions or edema) or organic brain syndrome; (i) no moderate or severe peripheral neuropathy; (j) no known intolerance or hypersensitivity to the components of the vehicles used to formulate oral paclitaxel and cyclosporin (polyoxyethylated castor oil, ethanol, and olive oil) or to the agents themselves; and (k) no active systemic infection or other coexisting medical problem of sufficient severity to prevent full compliance with the study. In addition, females of childbearing potential were required to have had a negative serum pregnancy test prior to study entry. Written informed consent was obtained according to federal and institutional guidelines.

Drug Dosage and Escalation. During the first course of therapy, 5 mg/kg of cyclosporin A was administered p.o. to all patients 1 h before oral paclitaxel administration and repeated at the time of oral paclitaxel administration. The rationale for this schedule was based on a preclinical study, in which rats receiving 5 mg/kg of oral cyclosporin A 1 h before and concurrently with 9 mg/kg (approximately 54 mg/m²) of oral paclitaxel achieved plasma paclitaxel concentrations in the range of those known to inhibit microtubulin depolymerization *in vitro* (0.05–0.1 μM; Refs. 1 and 36). Based on rodent studies in which the bioavailability of oral paclitaxel with cyclosporin A was 40–67% (34, 36), the oral bioavailability of paclitaxel combined with cyclosporin A was estimated (for the purpose of determining the starting doses) to be approximately 50% in humans. For the current clinical study, the average body surface area was assumed to be 1.8 m². It was planned to enroll at least three patients at each of the following dose levels in order to evaluate the paclitaxel exposure that could be achieved after oral administration: 180, 360, and 540 mg.

Toxicities were graded according to the National Cancer Institute common toxicity criteria (37). Dose-limiting toxicity was defined as either grade 4 hematological toxicity (including absolute neutrophil count <500 μl, platelet count <25,000 μl, and hemoglobin <6.5 g/dl) or any nonhematological toxicity of at least grade 3 severity. If more than one dose-limiting toxicity occurred at any of the aforementioned dose levels, at least three additional patients were to be treated at the next lower dose level. If a maximum tolerated dose level was not defined in the aforementioned dosing range, and if pharmacokinetic parameters demonstrated a dose response, further oral paclitaxel dose escalation would be considered.

Patients who completed the first course of oral paclitaxel were eligible to receive i.v. paclitaxel (Paxene, Baker Norton Pharmaceuticals Inc., Miami, FL) at doses of 175–250 mg/m² every 3 weeks beginning 2 weeks after oral paclitaxel administration.

Drug Administration. Cyclosporin A was obtained from Sandoz Pharmaceuticals Corp. (East Hanover, NJ) as the commercially available Neoral oral solution (100 mg/ml) and stored at 22°C until dispensing. A glass syringe was used to dispense 5 mg/kg of cyclosporin A into a glass containing 50 ml of water. The solution was then stirred gently and administered within 10 min of preparation to patients who had fasted overnight. The first dose of 5 mg/kg of cyclosporin A was administered p.o. 1 h prior to treatment with oral paclitaxel, and the second dose was administered concurrently with oral paclitaxel.

Paclitaxel for oral and i.v. administration (Paxene) was supplied by Baker Norton Pharmaceuticals, Inc., as a concen-

trated sterile solution in 5-ml vials containing 30 mg/ml. Each ml of sterile solution contained 527 mg polyoxyethylated castor oil (Cremophor EL) and 49.7% (w/v) absolute alcohol. Vials were stored at room temperature. For each oral dose of paclitaxel, contents from the appropriate number of vials were dissolved in 5% dextrose in water in a glass. Doses of paclitaxel of 180 mg were dissolved in 120 ml of 5% dextrose solution in water, whereas doses of 360 and 540 mg were dissolved in 180 and 240 ml of 5% dextrose solution in water, respectively. The solution was stirred gently for 1 min and consumed within 4 h of preparation. Premedications for prophylaxis of hypersensitivity reactions were not administered prior to oral paclitaxel. In addition, antiemetic agents were not routinely administered.

Pretreatment and Follow-up Studies. Prior to administration of oral paclitaxel, a history and physical examination were performed, and complete blood count, differential WBC count, routine chemistry and electrolyte profiles, urinalysis, electrocardiogram, chest radiograph, and appropriate tumor markers were obtained. Each weekly evaluation on days 8, 15, and 22 consisted of an interval history with an assessment of toxicity, physical examination, complete blood count, routine chemistry and electrolyte profiles, and urinalysis. On day 15, patients with an absolute neutrophil count of at least 1000 μl were permitted to be treated with i.v. paclitaxel. After treatment with i.v. paclitaxel, the complete blood count was determined each week. An interval history with an assessment of toxicity, physical examination, complete blood count, routine chemistry and electrolyte profiles, urinalysis, and electrocardiogram was performed every 3 weeks, prior to the administration of i.v. paclitaxel.

Appropriate radiological studies for documentation of measurable disease were performed prior to enrollment, after the first course of i.v. paclitaxel, and then as felt to be appropriate for the individual subject. A complete response was scored if there was disappearance of all known disease on two measurements, separated by a minimum of 4 weeks. A partial response required at least a 50% reduction in the sum of the products of the bidimensional measurements, separated by at least 4 weeks. Progressive disease was defined as an increase in the sum of the bidimensional measurements of all known disease by at least 25% or the appearance of new lesions.

Blood Sampling and Assay. To study the pharmacokinetics of oral paclitaxel and its metabolites, 6-α-hydroxypaclitaxel and 3-ρ-hydroxypaclitaxel, blood was sampled immediately before treatment with oral paclitaxel and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 24, and 48 h after ingestion of oral paclitaxel. The samples were collected in heparinized tubes. The tubes were gently inverted several times and then placed on ice prior to centrifugation. The plasma was separated by centrifugation at 2500 × g for 10 min within 1 h of collection. After centrifugation, the plasma was transferred to a polypropylene storage tube and stored at –20°C until analyzed.

Plasma samples were analyzed for paclitaxel using LC/MS/MS. Before quantification, the samples were thawed on ice, and a 500-μl aliquot was transferred to an 8-ml polypropylene tube. One hundred μl of calibration standard solution were added to each sample, followed by 100 μl of internal standard (D₅-paclitaxel), 100 μl of 50:50 (v/v) mixture of ethanol and 0.01 M ammonium acetate with acetic acid (pH 5), and 1 ml of PBS.

The samples were applied to an Isolute C18 solid phase extraction cartridge (International Sorbent Technology Ltd., Hengoed, Wales), preconditioned by washing with methanol (2 ml) and water (2 ml). Paclitaxel was eluted with acetonitrile, and the eluates were evaporated to dryness with nitrogen at 40°C. Extracts were reconstituted in a 40:10:50 (v/v) mixture of acetonitrile, methanol, and 0.01 M ammonium acetate with acetic acid (pH 5). The resulting mixtures were transferred to autosampler vials and analyzed by LC/MS/MS.

LC/MS/MS was performed on a PE-Sciex (Concord, Ontario, Canada) Model API III^{Plus} mass analyzer interfaced via an ion spray atmospheric pressure ionization inlet to a Shimadzu (Kyoto, Japan) liquid chromatograph consisting of 2 LC-10AD high-performance liquid chromatography pumps with a mixing tee, a SCL-10A controller, and a SIL-10A autoinjector. Chromatography was performed using a Betasil C-18 column (100 × 2.1 mm; internal diameter, 5 μm) from Keystone Scientific (State College, PA), and 30-μl aliquots of the extract were injected for analysis. The mobile phase consisted of 5 mM ammonium formate, acetonitrile, and water with 5 mM ammonium formate in a ratio of 40:54:6 (v/v) at a flow rate of 0.3 ml/min. The Turbo Ionspray temperature setting was 400°C. The nebulizing gas pressure and auxiliary flow were set at 80 p.s.i. and 8 l/min, respectively. Positive ionization was effected using ammonium adducts as precursors. The following reactions were monitored: m/z 871.4 → 509.0 and m/z 876.4 → 514.0 for paclitaxel and D₅-paclitaxel, respectively. The orifice potential and electron multiplier settings were +38 V and -4.5 kV, respectively. The dwell time was 800 ms. Peak area ratios of the analyte with respect to the internal standard were computed using PE-Sciex MacQuan software. The calibration curves were constructed using least squares regression with a weighting factor of 1/ x . The lower limit of quantification for paclitaxel was 1 ng/ml, and the range of linear response was 1–500 ng/ml.

Plasma samples were analyzed for paclitaxel metabolites (6α-hydroxypaclitaxel and 3β-hydroxypaclitaxel) using high-performance liquid chromatography with solid-phase extraction as the sample pretreatment procedure and UV detection, as described previously (38–40).

To determine the concentration of cyclosporin A in whole blood, blood was sampled just prior to the first dose of cyclosporin A and 0.5, 1, 2, 3, 4, 6, 8, 10, and 24 h after the second dose of cyclosporin A. These samples were collected into EDTA tubes and stored at -20°C. Whole blood cyclosporin A levels were measured by RIA using the Cyclo-Trac SP-Whole Blood kit (Ingstar, Stillwater, MN) with a lower limit of quantification of 25 ng/ml (41).

Pharmacokinetic Analysis. Individual paclitaxel, paclitaxel metabolite, and cyclosporin concentrations were analyzed using model-independent methods (42). C_{\max} and T_{\max} were determined by inspection of the concentration *versus* time curves. The terminal rate constant, k , was calculated as the negative of the slope of the log-linear terminal portion of the concentration *versus* time curve using linear regression. The terminal half-life, $t_{1/2}$, was calculated as $0.693/k$. The AUC from time 0 to the time of the final quantifiable sample, $AUC(tf)$, was calculated using the linear trapezoidal method and was extrapolated to infinity according to the following equation:

$$AUC_{\infty} = AUC(tf) + C(tf)/k \quad (A)$$

where $C(tf)$ was the estimated concentration at time tf . Systemic clearance (Cl_s/F) was calculated by dividing the dose by AUC_{∞} . The apparent volume of distribution (V_{area}/F) was calculated by dividing Cl_s/F by k . Relative systemic exposure of 6α-hydroxypaclitaxel and 3β-hydroxypaclitaxel to that of paclitaxel was calculated as the AUC ratio of 6α-hydroxypaclitaxel to paclitaxel and 3β-hydroxypaclitaxel to paclitaxel, respectively.

In addition, paclitaxel plasma concentration data were analyzed using model-dependent methods. This exercise was undertaken to characterize oral paclitaxel concentrations to simulate paclitaxel systemic exposure with other dosing schedules. Individual data were fit with either a linear one- or two-compartment model using maximum likelihood estimation (ADAPT II release 4, Biomedical Simulations Resource, Los Angeles, CA; Ref. 43). Model discrimination (*i.e.*, zero- *versus* first-order absorption and one- *versus* two-compartment model) was guided by inspection of the weighted sum of squares and the coefficient of variation of the fitted pharmacokinetic parameters and by the Akaike information criterion (44). Nonlinear models were not explored for the following reasons: (a) the nonlinear pharmacokinetic behavior is primarily manifested with the achievement of sufficiently high plasma concentrations, as with paclitaxel doses of at least 175 mg/m² administered over 3 h or with larger doses administered over longer durations (45); and (b) the majority of the maximum concentration values achieved after administration of oral paclitaxel were substantially lower than those achieved after administration of paclitaxel on the aforementioned dose schedules (38, 46, 47). Drug input was modeled as either zero- or first-order absorption. Estimated parameters included T_{\max} (for zero-order absorption input), K_a (for first-order absorption input), V_c , and K_e . Bioavailability (F) was modeled as 1.0 because the unique F for each patient was unknown. However, given the recent data from Meerum-Terwogt *et al.* (7) demonstrating that the F for oral paclitaxel in combination with cyclosporin is <100%, the reported values for volume of distribution (V_c/F) and clearance (Cl/F) are apparent values relative to the value of F for each patient. The duration of time above a plasma concentration of 0.05 μmol/liter was determined from concentration *versus* time profiles generated for each patient by using the model-estimated parameters and the simulation module in ADAPT II (43). Simulated paclitaxel plasma concentrations after once daily and twice daily oral administration of paclitaxel were generated using mean and individual model-estimated parameters and the simulation module in ADAPT II (43).

Paclitaxel and cyclosporin A pharmacokinetic parameters were summarized using descriptive statistics. Statistical analysis was performed using the JMP version 3.1.6.2 statistical program (SAS Institute, Cary, NC).

RESULTS

General. Nine patients, whose characteristics are detailed in Table 1, each received 1 course of oral paclitaxel and cyclosporin A. Three patients were enrolled at each of the 180-, 360-, and 540-mg dose levels, and all patients completed the toxicity and pharmacokinetic evaluations. After treatment with

Table 1 Patient characteristics

	No. of patients
Total no. of patients	9
Sex (male:female)	4:5
Median age, yr (range)	56 (43–73)
ECOG ^a performance status	
0	3
1	4
2	2
Previous treatment	
Chemotherapy only	6
Chemotherapy and radiation therapy	1
Radiation therapy and biological therapy	1
None	1
Tumor type	
Non-small cell lung cancer	3
Breast	1
Cervix	1
Kidney	1
Prostate	1
Stomach	1
Vagina	1

^a ECOG, Eastern Cooperative Oncology Group.

oral paclitaxel and cyclosporin A, the nine patients received a median of 4 courses of i.v. paclitaxel (range, 1–13) at doses ranging from 175 to 250 mg/m² administered over 3 h at 3-week intervals.

Paclitaxel Pharmacokinetic Studies. Paclitaxel plasma concentration data were analyzed by both noncompartmental and compartmental methods. Representative plasma paclitaxel concentration *versus* time profiles after oral administration are shown in Fig. 1. Paclitaxel absorption was best described by a zero-order process, and paclitaxel plasma disposition was best described by a linear one-compartment model. In several patients, it appeared that a first disposition phase was missed by this model. However, because of the absence of sampling after 24 h posttreatment and the lack of data to adequately describe a second disposition phase, a two-compartment model could not be fit to the plasma concentration.

Although paclitaxel absorption parameters (T_{max} and C_{max}) were similar between dose levels, there was considerable interpatient variability within each dose level. The pharmacokinetic parameters that were determined using noncompartmental methods are outlined in Table 2. The mean (\pm SD) values for T_{max} and C_{max} across all dose levels were 3.7 ± 1.8 h (range, 1.0–6.0 h) and 268 ± 164 ng/ml (range, 28–492 ng/ml), respectively. In addition, AUC values did not vary with dose, averaging 3306 ± 1977 ng·h/ml (range, 851–6577 ng·h/ml).

Paclitaxel pharmacokinetic parameters that were determined using model-dependent methods are outlined in Table 3. The mean V_c/F and $t_{1/2}$ values ranged from 1285 to 3250 liters and from 9.0 to 11.4 h, respectively. The mean values for apparent oral clearance, which ranged from 41 to 140 l/h/m², were similar ($\pm 16\%$) to those derived using noncompartmental methods.

To further define the systemic exposure to paclitaxel after oral administration, the time that plasma concentrations remained above $0.05 \mu\text{M}$ was determined, and both mean and individual values achieved at all dose levels overlapped. Plasma

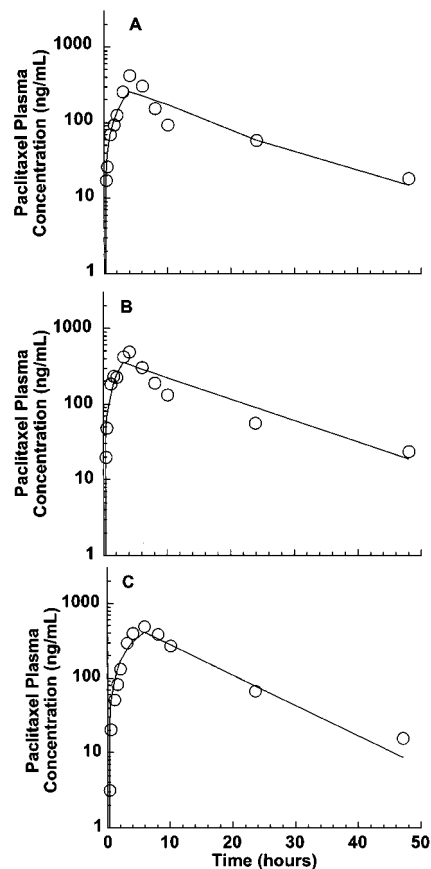


Fig. 1 Representative paclitaxel plasma concentrations after administration of 180 (A), 360 (B), and 540 (C) mg of oral paclitaxel in combination with 5 mg/kg oral cyclosporin 1 h before and concurrently with oral paclitaxel. Lines, best-fit curves from the model-estimated parameters.

concentrations were maintained above $0.05 \mu\text{M}$ for an average time of 22 ± 11 h (range, 9.4–35 h). Simulations were performed to determine whether paclitaxel steady-state plasma concentrations ($0.06 \mu\text{M}$) achieved with protracted i.v. infusion schedules at clinically relevant doses (140 mg/m^2 administered over 96 h) could be achieved with once daily dosing of oral paclitaxel in combination with oral cyclosporin A (48). As shown in Fig. 2A, the simulations demonstrated that steady-state plasma concentrations of at least $0.06 \mu\text{M}$ could be achieved in four of nine patients. Simulations of twice daily dosing of oral paclitaxel indicated that minimum steady-state plasma concentrations of $0.06 \mu\text{M}$ could be achieved in seven of nine patients; however, concentrations exceeding $0.07 \mu\text{M}$, a threshold level associated with the occurrence of neutropenia and mucositis when maintained for 96 h, could be achieved in six of nine patients (48).

Paclitaxel Metabolite Studies. In most patients, metabolite concentrations in plasma were below the lower limit of quantitation at the majority of sampling times; however, metabolite concentrations were measurable at most time points in a single patient at each dose level, permitting assessment of ex-

Table 2 Paclitaxel pharmacokinetic parameters derived using noncompartmental methods^a

Dose level (mg)	T_{\max} (h)	C_{\max} (ng/ml)	V_{area}/F (liters)	Cl_r/F (l/h/m ²)	$t_{1/2}$ (h)	AUC _∞ (ng·h/ml)
180	4.1 ± 0.2	261 ± 164	2188 ± 1987	39 ± 25	18.1 ± 2.1	3238 ± 1810
360	3.7 ± 2.5	280 ± 205	5916 ± 7592	126 ± 141	16.2 ± 7.7	3072 ± 2350
540	3.3 ± 2.5	263 ± 195	5933 ± 6279	111 ± 56	18.1 ± 14.6	3609 ± 2571
Mean values	3.7 ± 1.8	268 ± 164	4679 ± 5361	92 ± 87	17.5 ± 8.4	3306 ± 1977

^a Mean values ± SD.Table 3 Paclitaxel pharmacokinetic parameters derived using compartmental methods^a

Dose level (mg)	V_c/F (liters)	K_e (h ⁻¹)	Cl_r/F (liters/h/m ²)	$t_{1/2}$ (h)
180	1285 ± 955	0.062 ± 0.010	41 ± 29	11.4 ± 2.0
360	2938 ± 2536	0.075 ± 0.008	140 ± 152	9.3 ± 1.1
540	3250 ± 2529	0.082 ± 0.024	129 ± 63	9.0 ± 3.1

^a Mean values ± SD.

posure. In these three patients, the mean 6- α -hydroxypaclitaxel:paclitaxel AUC ratio was 0.63 (range, 0.14–0.98), and the mean 3- ρ -hydroxypaclitaxel:paclitaxel AUC ratio was 0.86 (range, 0.09–2.4).

Cyclosporin A Pharmacokinetic Studies. The mean (\pm SD) total dose of cyclosporin A administered to each patient was 822 \pm 240 mg. The mean (\pm SD) cyclosporin A C_{\max} was 1598 \pm 456 ng/ml, and the mean (\pm SD) cyclosporin A AUC_∞ was 13162 \pm 6716 ng·h/ml. No clear relationship between cyclosporin A exposure and paclitaxel exposure was observed.

Toxicity. Nausea and vomiting were the most common toxicities after treatment with oral paclitaxel and cyclosporin A. The entire dose of paclitaxel was assumed to be in the emesis of the first patient treated at the 180-mg dose level who experienced vomiting within 2 min of paclitaxel administration. This patient was successfully retreated with oral paclitaxel and cyclosporin A 24 h later, after premedication with ondansetron and lorazepam. Of the remaining eight patients, three were premedicated with ondansetron at the discretion of the treating physicians. Grade 1–2 nausea and/or vomiting within 8 days of dosing were experienced by eight patients across all dose levels, and two patients reported that the paclitaxel solution had an unpleasant taste. Other toxicities, which were attributable to treatment with oral paclitaxel and cyclosporin, were less frequent and were grade 1–2 in severity, as outlined in Table 4. Of note, grade 2 thrombocytopenia was observed at the 180-mg dose level in a 51-year-old female with a squamous cell carcinoma of the vagina who had previously been treated with 6 courses of doxorubicin and cisplatin and 2 courses of a 5-fluorouracil-based regimen. In addition, grade 2 neutropenia and grade 2 alopecia were observed at the 540-mg dose level in a 61-year-old male with leiomyosarcoma of the stomach who had received extensive prior treatment with myelosuppressive chemotherapy.

The severity and frequencies of the toxicities after treatment with i.v. paclitaxel, including nausea, vomiting, myalgias, arthralgias, sensory neuropathy, mucositis, alopecia, and myelosuppression, were similar to those that have been reported previously on comparable dose schedules.

DISCUSSION

Paclitaxel, which is an antimicrotubule agent that disrupts tubulin dynamics and has impressive activity against many common malignancies, has poor bioavailability after oral administration (6, 7). However, studies in rodents have revealed that cyclosporin A and the cyclosporin analogue SDZ PSC-833 can increase the systemic bioavailability of paclitaxel administered p.o. (34–36). In addition, a recent study in humans has demonstrated that the oral bioavailability of paclitaxel increases from 5.9 to 47.4% with the concurrent administration of cyclosporin A (7). Several mechanisms to account for this phenomenon have been proposed. Perhaps the most plausible mechanism is that cyclosporin A, by interfering with Pgp, may prevent the active extrusion of paclitaxel from enterocytes (7, 21, 34, 35). An alternate or supplemental mechanism is that cyclosporin A may inhibit cytochrome P450 CYP3A4-mediated first-pass metabolism of paclitaxel in both enterocytes and hepatocytes (7, 34, 35). This study provides evidence that the administration of cyclosporin A, 5 mg/kg p.o. 1 h before and concurrently with oral paclitaxel, renders paclitaxel bioavailable, but when administered intermittently, this regimen does not replicate the pharmacological and toxicological profiles of clinically efficacious i.v. schedules.

Systemic paclitaxel exposure (C_{\max} , AUC) did not increase as the absolute oral dose of paclitaxel was increased from 180 to 540 mg. The constant systemic exposure despite a 3-fold increase in paclitaxel dose could not be explained by changes in clearance or volume of distribution, suggesting that the absorption of oral paclitaxel is a saturable process. These results may be, in part, attributable to interactions between paclitaxel and the polyoxyethylated castor oil component of its formulation vehicle (49). Hypothetically, trapping of paclitaxel in polyoxyethylated castor oil micelles in the intestinal lumen may substantially reduce the availability of free paclitaxel for absorption (50). The micelles may be subsequently degraded by intestinal lipases, precipitating paclitaxel in the intestinal lumen because of the poor aqueous solubility of paclitaxel. In addition to formulation issues, an important consideration is whether the inherently large inter-

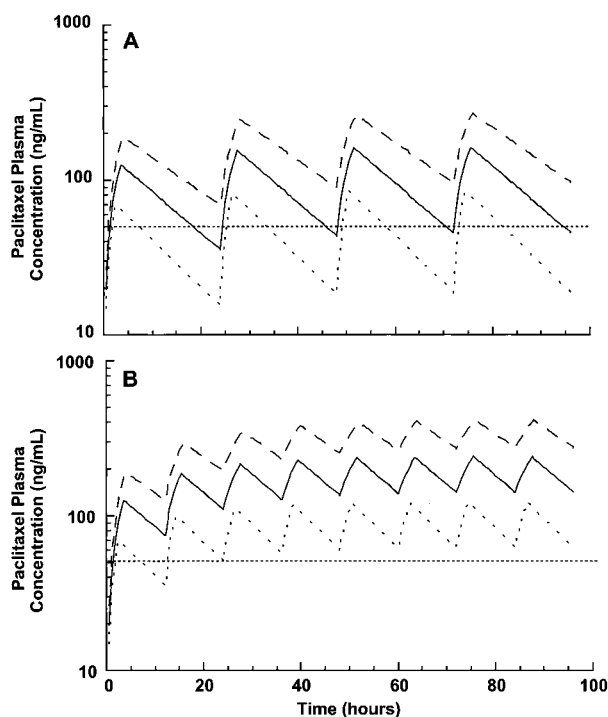


Fig. 2 Simulated paclitaxel plasma concentrations after administration of 180 mg of oral paclitaxel daily (A) and twice daily (B) in combination with 5 mg/kg oral cyclosporin 1 h before and concurrently with oral paclitaxel. Solid line, predicted curve using the mean model-estimated parameters at the 180-mg dose level. Dashed and dotted lines, predicted curves using the model-estimated parameters from two patients with the lowest (40 liters/h) and highest (167 liters/h) paclitaxel clearance values, respectively, at the 180-mg dose level. Horizontal line, 0.06 μM .

individual variability in parameters reflecting both drug absorption and disposition, as well as the small numbers of patients treated at each dose level, may confound the analysis of saturability. Indeed, although mean C_{max} and AUC values were similar at all dose levels, interpatient variability within each dose level was large. Another potentially confounding factor is the large interindividual variability in constitutive Pgp content in enterocytes. Pgp content in enterocytes in small intestine biopsy samples have been shown to vary by approximately 8-fold (51), and the range of paclitaxel doses in the present study may have been too small to appreciate true differences in systemic exposure after oral administration. However, the results of the present study have been corroborated by a larger study involving 53 patients (7, 49). In that study, paclitaxel AUC values were demonstrated to increase as oral doses of paclitaxel were increased from 60 to 180 mg/m^2 ; however, the values did not increase further as the dose of paclitaxel was increased above 180 mg/m^2 (49).

Paclitaxel was metabolized to a much greater extent after oral administration with cyclosporin A than in single-agent studies in which paclitaxel was administered i.v. without cyclosporin A. The mean 6- α -hydroxypaclitaxel:paclitaxel AUC ratio in this study was 0.63, whereas ratios of 0.09 and 0.11 were previously reported after parenteral drug administration (28, 46). Likewise, the mean 3- ρ -hydroxypaclitaxel:paclitaxel AUC ratio in the present study was 0.86,

Table 4 Toxicities

Toxicity	Most severe grade of toxicity experienced by each patient				
	0	1	2	3	4
Neutropenia	7	2	1	0	0
Thrombocytopenia	8	1	1	0	0
Mucositis	6	3	0	0	0
Myalgia	8	1	0	0	0
Arthralgia	8	1	0	0	0
Alopecia	8	2	1	0	0

whereas a ratio of 0.02 has been reported with i.v. administration (28). The elevated metabolite:paclitaxel AUC ratios obtained in the present study were consistent with the results of another study of oral paclitaxel and cyclosporin in which the 6- α -hydroxypaclitaxel:paclitaxel and 3- ρ -hydroxypaclitaxel:paclitaxel AUC ratios were 0.87 and 0.16, respectively (7). The most plausible explanation for the elevated metabolite:paclitaxel AUC ratios is a substantial "first-pass" with oral drug administration attributable to both biotransformation in the intestinal mucosa and hepatic metabolism. It is also possible that cyclosporin A, by inhibiting Pgp-dependent transport processes in the biliary canaliculi that excrete xenobiotic substrates into the bile, increases the availability of parent compound for hepatic metabolism. However, the results of studies in mice deficient in hepatic Pgp, with mutations in both *mdr 1a* and *mdr 1b*, indicate that Pgp is not essential for normal biliary excretion of paclitaxel (21).

The systemic disposition of paclitaxel after oral administration fits a linear one-compartment model. The overall mean (\pm SD) $t_{1/2}$ value determined using compartmental methods (9.9 ± 2.2 h) was within the range of mean terminal $t_{1/2}$ values (6.5–18.75 h) reported with paclitaxel administered as a 3-h i.v. infusion (38, 46, 47). However, apparent oral clearance values ranged from 21 to 315 $\text{liters}/\text{h}/\text{m}^2$, which were much higher than clearance values that have been previously reported with paclitaxel administered as a 3-h IV infusion (range, 6.7–18 $\text{liters}/\text{h}/\text{m}^2$; Refs. 38, 46, and 47). The increased clearance of oral paclitaxel in the present study likely results from both incomplete absorption and substantial first-pass metabolism.

AUC values of cyclosporin were similar to those previously reported after the administration of Neoral oral solution. In the present study, a total dose of Neoral averaging 822 ± 240 mg resulted in cyclosporin A AUC values averaging 13162 ± 6716 $\text{ng}\cdot\text{h}/\text{ml}$, which were similar to AUC values achieved after the administration of 800 mg of Neoral to healthy volunteers (mean \pm SD, 12364 ± 3145 $\text{ng}\cdot\text{h}/\text{ml}$; Ref. 52). Interestingly, cyclosporin A C_{max} values (mean \pm SD, 1.3 ± 0.4 μM) were less than the concentrations (2–4 μM) required for reversal of multidrug resistance *in vitro* (53).

The regimen of oral paclitaxel-cyclosporin A was well tolerated. Premedication to prevent serious hypersensitivity reactions was not administered from the outset because polyoxyethylated castor oil has not been detected in plasma after oral paclitaxel administration to humans (7). However, minuscule quantities of polyoxyethylated castor oil that are below the

lower limit of assay sensitivity, albeit capable of inducing histamine release and hypersensitivity phenomena, may still be observed after oral drug administration. Although hypersensitivity reactions did not occur in the present study, these results are not absolute assurance that hypersensitivity reactions will not occur after treatment with oral paclitaxel formulated in polyoxyethylated castor oil. The paclitaxel solution had an unpleasant taste, which resulted in regurgitation of the entire dose within a few minutes of drug administration in one individual. The most common adverse effects related to drug were mild to moderate (grade 1–2) nausea and/or vomiting, which were successfully prevented and readily managed with 5-hydroxytryptamine 3 receptor antagonists. One patient each treated at the 180- and 540-mg dose levels experienced grade 2 hematological toxicity. These patients were not among those with the highest paclitaxel systemic exposure, but both individuals had received extensive prior treatment with myelosuppressive therapies. In addition, patients did not experience any clinical immunosuppressive effects attributable to the administration of cyclosporin A.

Paclitaxel plasma concentrations achieved with the oral paclitaxel-cyclosporin A regimen were substantially higher than those capable of disrupting tubulin dynamics, which occurs at subnanomolar to low nanomolar concentrations *in vitro* (1). In addition, paclitaxel C_{\max} values averaged 268 ± 164 ng/ml (0.31 ± 0.19 μM), which is in the range of C_{\max} and C_{ss} values achieved with several paclitaxel dose schedules of potential therapeutic relevance. To illustrate this point, the range of paclitaxel C_{\max} values in the present study exceeded mean paclitaxel C_{ss} values that are readily achieved with 140 mg/m² paclitaxel as a 96-h i.v. infusion (0.060 μM) and approached C_{\max} values achieved with 175 mg/m² paclitaxel as a 24-h i.v. infusion (0.4–0.5 μM ; Refs. 38, 46, and 48).

Although clinically relevant paclitaxel plasma concentrations were achieved, other pharmacokinetic indices of drug exposure were inadequate to achieve a therapeutic effect. Paclitaxel AUC values (range, 1.0–7.7 $\mu\text{M}\cdot\text{h}$) achieved with the oral paclitaxel-cyclosporin A regimen were substantially lower than AUC values achieved with 175 mg/m² paclitaxel as a 3-h i.v. infusion (16.8 and 18.5 $\mu\text{M}\cdot\text{h}$; Refs. 38 and 46). Furthermore, paclitaxel concentrations exceeded 0.05 μM for an average of 22 h (range, 5.2–35 h) after treatment with the oral paclitaxel-cyclosporin A regimen; these concentrations fall somewhat short of the C_{ss} values (mean, 0.06 μM) that are maintained for most of the 96-h treatment period when paclitaxel is administered at its maximum tolerated dose (140 mg/m²) as a 96-h i.v. infusion (48). Thus, a single treatment with oral paclitaxel-cyclosporin A on an intermittent schedule (*i.e.*, every 3 weeks) does not achieve clinically relevant pharmacological end points.

The lack of significant myelosuppression with the oral paclitaxel-cyclosporin A regimen and the failure to approach a therapeutic range with respect to other pharmacokinetic indices of drug exposure led to the exploration of alternate schedules using computer simulations with pharmacokinetic parameters derived from this study. The plasma paclitaxel concentrations generated with single and twice daily oral dosing indicate that such treatment schedules can readily

achieve and maintain plasma paclitaxel concentrations of at least 0.060 μM for time periods similar to those achieved with parenteral dosing schedules associated with clinical efficacy. Alternatively, daily administration of oral paclitaxel and cyclosporin A may alter the pharmacokinetic behavior of paclitaxel, a conjecture that remains to be explored in further studies.

The results of this study indicate that cyclosporin A renders paclitaxel bioavailable after oral administration. The paclitaxel C_{\max} values achieved approach those achieved with several parenteral administration schedules that are known to be efficacious (38, 46). However, the lack of toxicity after a single treatment with the oral paclitaxel-cyclosporin A regimen, as well as the fact that most pharmacokinetic indices of drug exposure fall short of those achieved with clinically efficacious dose schedules, indicates that intermittent treatment with single doses of the paclitaxel-cyclosporin A regimen using conventional dosing intervals (*e.g.*, every 3 weeks) will not achieve relevant pharmacodynamic effects. In contrast, clinically relevant pharmacodynamic effects are likely to be achieved with the administration of the oral paclitaxel-cyclosporin A on repetitive daily or twice daily dosing schedules as demonstrated by the simulations using pharmacokinetic data generated in the present study. Although simulating a 96-h paclitaxel infusion with oral paclitaxel-cyclosporin A appears to be feasible, more protracted administration of therapeutic doses of cyclosporin A may result in immunosuppression, a complication that could be circumvented by the use of nonimmunosuppressive cyclosporin analogues. The validity of the predictions regarding multiple dosing regimens with oral paclitaxel-cyclosporin A will require validation before disease-directed evaluations can be performed.

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