Cancer Chemother Pharmacol (2001) 47: 347–354
DOI 10.1007/s002800000226

ORIGINAL ARTICLE

**Mirte M. Malingré · Jos H. Beijnen · Hilde Rosing
Franciska J. Koopman · Olaf van Tellingen
Ken Duchin · Wim W. Ten Bokkel Huinink
Martha Swart · Jan Lieverst · Jan H. M. Schellens**

A phase I and pharmacokinetic study of bi-daily dosing of oral paclitaxel in combination with cyclosporin A

Received: 17 July 2000 / Accepted: 31 October 2000 / Published online: 16 February 2001

Abstract *Purpose*: To investigate dose escalation of bi-daily (b.i.d.) oral paclitaxel in combination with cyclosporin A in order to improve and prolong the systemic exposure to paclitaxel and to explore the maximum tolerated dose and dose limiting toxicity (DLT) of this combination. *Patients and methods*: A total of 15 patients received during course 1 two doses of oral paclitaxel (2×60, 2×90, 2×120, or 2×160 mg/m^2) 7 h apart in combination with 15 mg/kg of cyclosporin A, co-administered to enhance the absorption of paclitaxel. During subsequent courses, patients received 3-weekly intravenous paclitaxel at a dose of 175 mg/m^2 as a 3-h infusion. *Results*: Toxicities observed following b.i.d. dosing of oral paclitaxel were generally mild and included toxicities common to paclitaxel administration and mild gastrointestinal toxicities such as nausea, vomiting, and diarrhea, which occurred more often at the higher dose levels. Dose escalation of b.i.d. oral paclitaxel from 2×60 to 2×160 mg/m^2 did not result in a significant increase in the area under the plasma concentration-time curve (AUC) of paclitaxel. The AUC after doses of 2×60, 90, 120, and 160 mg/m^2 were 3.77 ± 2.70, 4.57 ± 2.43, 3.62 ± 1.58, and 8.58 ± 7.87 μM.h, respectively. The AUC achieved after intravenous administration of paclitaxel 175 mg/m^2 was 17.95 ± 3.94 μM.h. *Conclusion*: Dose increment of paclitaxel did not result in a significant additional increase in the AUC values of the drug. Dose escalation of the b.i.d. dosing regimen was therefore not continued up to DLT. As b.i.d. dosing appeared to result in higher AUC values compared with single-dose administration (data which we have published previously), we recommend b.i.d. dosing of oral paclitaxel for future studies. Although pharmacokinetic data are difficult to interpret, due to the limited number of patients at each dose level and the large interpatient variability, we recommend the dose level of 2×90 mg/m^2 for further investigation, as this dose level showed the highest systemic exposure to paclitaxel combined with good safety.

Key words Oral paclitaxel · Cyclosporin A · Pharmacokinetics

M. M. Malingré (✉) · J. H. Beijnen · O. van Tellingen
W. W. T. Bokkel Huinink · M. Swart · J. Lieverst
J. H. M. Schellens
Department of Medical Oncology, The Netherlands Cancer Institute/Antoni van Leeuwenhoek Hospital,
Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands
E-mail: apmmg@slz.nl
Tel.: +31-20-5124657; Fax: +31-20-5124753

M. M. Malingré · J. H. Beijnen · H. Rosing · F. J. Koopman
Department of Pharmacy and Pharmacology,
The Netherlands Cancer Institute/Slotervaart Hospital,
Louwesweg 6, 1066 EC Amsterdam, The Netherlands

J. H. Beijnen · J. H. M. Schellens
Division of Drug Toxicology, Faculty of Pharmacy,
Utrecht University, Sorbonnelaan 16,
3584 CA Utrecht, The Netherlands

K. Duchin
Baker Norton Pharmaceuticals, 4400 Biscayne Boulevard,
Miami, Florida, USA

Introduction

Paclitaxel is an important anticancer agent widely applied for the treatment of breast, ovarian, and lung cancer and AIDS-related Kaposi's sarcoma [13, 31]. The cellular target for paclitaxel has been identified as the tubulin/microtubule system that plays a significant role in mitosis, intracellular transport, cell motility, and maintenance of cell shape. Paclitaxel promotes the assembly of stable microtubules and inhibits their depolymerization, resulting in the arrest of cells in the G_2-M phase of the cell cycle [11, 23, 32].

Paclitaxel is currently administered intravenously (i.v.) at different dosages and time schedules and optimization of the clinical application is under investigation. Preclinical data from a variety of human cancer cell lines reveal that the cytotoxicity of paclitaxel is schedule dependent. In studies in which investigators evaluated both concentration and exposure duration, prolongation

of drug exposure seemed more important for the activity of paclitaxel than an increase in concentration [1, 19, 20, 21, 30]. Furthermore, paclitaxel, like some other drugs to which resistance is conferred by the multidrug resistance (mdr) phenotype, was more effective in vitro when applied to mdr cells for a longer duration [43]. In clinical studies, prolongation of the infusion duration was associated with an increase in severity of bone marrow suppression. Myelosuppression appeared to be related to the duration of plasma paclitaxel concentrations above either a threshold concentration of 0.05 μM [7, 29] or 0.1 μM [12]. This increased toxicity with longer infusion duration suggests that tumor cell cytotoxicity may also increase as exposure duration increases. Huizing et al. [17] found, in the presence of carboplatin, a positive correlation between the duration of paclitaxel exposure above 0.1 μM and the median survival time in non-small-cell lung cancer patients, indicating that prolongation of paclitaxel exposure may improve the response rate and overall survival.

Oral administration of paclitaxel is investigated because the oral route of administration is more practical and convenient to patients and may enable chronic continuous dosing of paclitaxel. However, paclitaxel shows very low oral bioavailability, which has limited treatment of the drug by the oral route. Preclinical studies in mice have shown that the low oral bioavailability is due to efficient transport of the drug by the multidrug efflux pump P-glycoprotein (P-gp) abundantly present in the gastrointestinal tract [37]. Efficient oral uptake of paclitaxel has recently been made possible in mice [39] and men [25, 26] by co-administration of oral cyclosporin A (CsA), an inhibitor of P-gp and cytochrome P-450 (CYP) 3A4-mediated drug metabolism. In men, co-administration of CsA resulted in a significant increase of at least sevenfold in the systemic exposure of paclitaxel, and plasma concentrations increased from negligible to therapeutic levels [25, 26]. The first promising clinical results at low paclitaxel dosages in a proof of principle study [25, 26] and those obtained in a phase I dose-escalating study using a once daily dosing schedule [22] encouraged us to explore a twice-daily dosing schedule in an attempt to further increase and prolong the systemic exposure to orally administered paclitaxel.

Patients and methods

Patient population

Patients with histological proof of cancer for whom no standard therapy of proven benefit existed were eligible for the study. Previous radiotherapy or chemotherapy other than taxoid therapy was allowed, provided that the last treatment was at least 4 weeks prior to study entry and any resulting toxicities were resolved. Patients had to have acceptable bone marrow (white blood cells $>3.0 \times 10^9$/l, platelets $>100\times10^9$/l), liver function (serum bilirubin ≤ 25 μmol/l, serum albumin ≥ 25 g/l), renal function (serum creatinine ≤ 160 μmol/l or clearance ≥ 50 ml/min), and a World Health Organization (WHO) performance status ≤ 2. Patients were not eligible if they suffered from uncontrolled infectious disease, neurological disease, bowel obstruction, or symptomatic brain metastases. Other exclusion criteria were concomitant use of known P-gp inhibitors and chronic use of H_2 receptor antagonists or proton pump inhibitors. The study protocol was approved by the medical ethics committee of The Netherlands Cancer Institute, and all patients had to give written informed consent.

Study design

Patients received oral paclitaxel at doses of 2×60, 2×90, 2×120, or 2×160 mg/m^2 during course 1 and i.v. paclitaxel administered as a 3-h infusion at a dose of 175 mg/m^2 during course 2. If it was considered to be in their best interest, patients continued on a 3-weekly schedule of i.v. paclitaxel. Per dose level of oral paclitaxel, 3 eligible patients were entered; 3 additional patients (total of 6) were treated at a dose level if 1 of the first 3 patients exhibited dose-limiting toxicity (DLT). DLTs were defined as grade 4 granulocytopenia of a duration of >5 days, grade 4 thrombocytopenia of any duration, or any grade 3/4 non-hematological toxicity except untreated nausea and vomiting. The maximum tolerated dose (MTD) was defined as the highest dose level producing DLTs in <2 of 6 patients. The i.v. formulation of paclitaxel (Paxene, paclitaxel 6 mg/ml, dissolved in Cremophor EL and ethanol 1:1 w/v, Baker Norton Pharmaceuticals, Miami, Fla., USA) was used for both i.v. and oral administration of paclitaxel. Oral paclitaxel was administered in two doses 7 h apart, and 30 min prior to each paclitaxel dose patients received 15 mg/kg CsA (Neoral, Novartis, Basel, Switzerland). The first oral paclitaxel dose was administered after an overnight fast and patients remained fasted until 2 h following administration. For the second oral dose patients were refused food and drinks 1.5 h prior to paclitaxel administration and up to 1 h after administration. To prevent nausea and vomiting following administration of CsA and oral paclitaxel, 1 patient at the dose level 2×90 mg/m^2 and all patients at dose levels 2×120 and 2×160 mg/m^2 received 1 mg oral granisetron (Kytril) 1 h prior to CsA administration. In addition, 1 patient at dose level 2×160 mg/m^2 received a light breakfast at least 2 h prior to the first oral paclitaxel dose. Prior to i.v. administration of paclitaxel, patients received standard i.v. premedication to prevent hypersensitivity reactions, consisting of dexamethasone 20 mg orally 12 and 6 h prior to, and clemastine 2 mg i.v. and cimetidine 300 mg i.v. 30 min prior to paclitaxel administration. Premedication was not administered prior to oral administration of paclitaxel.

Patient evaluation

Pretreatment evaluation included a complete medical history and complete physical examination. Before each course, an interim history, including concomitant medications taken, toxicities, and performance status, was recorded and a physical examination was performed. Hematology was checked twice weekly after course 1 and 2 and weekly after subsequent courses. Blood chemistries, including liver and renal function, serum electrolytes, total protein, and albumin and glucose levels, were checked weekly. All toxicities observed were graded according to the National Cancer Institute Common Toxicity Criteria (NCI CTC) [28]. Tumor measurements were performed every other cycle, but initially after the first two i.v. courses. Responses were evaluated according to the WHO criteria [42].

Sample collection and analysis

After oral drug administration of paclitaxel and CsA, blood samples and urine were collected for pharmacokinetic analysis. Blood samples were obtained in heparinized tubes, pre dose, 30 min and 1, 2, 3, 4, 6, 7, 7.5, 8, 9, 10, 11, 13, 24, and 48 h after ingestion of the two oral doses. For CsA whole-blood concentrations, an aliquot of the blood sample was stored at 4 °C and analyzed within 1 week using a specific fluorescence polarization immunoassay (FPIA, Abbott TDx-FLx, Amstelveen, The Netherlands) [3]. For paclitaxel plasma concentrations, the remainder of the blood

samples was centrifuged and plasma samples were stored at –20 °C until analysis. Paclitaxel plasma concentrations were determined using a validated high-performance liquid chromatography (HPLC) assay [15]. In addition to measuring CsA and paclitaxel levels after oral drug administration, ethanol and Cremophor EL concentrations were measured. The plasma samples obtained for paclitaxel analysis were used for analysis of ethanol and Cremophor EL. Plasma ethanol levels were measured for all patients at the dose levels of 2×90, 120, and 160 mg/m^2 at 30 min and 1 h following each oral dose of paclitaxel and analyzed by gas chromatography. Plasma concentrations of Cremophor EL were measured for 2 patients (dose level 2×160 mg/m^2) at six time points up to 13 h after the first oral dose of paclitaxel using a validated HPLC assay [34] with minor modifications as described elsewhere [40]. Urine was collected in 24-h aliquots for 48 h. Urine samples were stabilized with a mixture of 5% Cremophor EL/ethanol 1:1 v/v and stored at –20 °C until analysis. Paclitaxel concentrations in urine were determined using a validated HPLC assay [16].

During i.v. administration of paclitaxel blood samples for paclitaxel analysis were obtained according to a previously established limited sampling model using two concentration-time points at 1 and 8 h after the end of paclitaxel infusion [14]. Blood samples were collected in heparinized tubes, centrifuged, and plasma samples were stored at –20 °C until analysis. Paclitaxel plasma concentrations were determined using a validated HPLC assay [15].

Pharmacokinetic analysis

Non-compartmental pharmacokinetic methods were applied to process the results [8]. For orally administered paclitaxel, the maximal drug concentration (Cmax) and time to maximal drug concentration (Tmax) were obtained directly from the experimental data. The area under the plasma paclitaxel concentration-time curve (AUC) was estimated by the trapezoidal rule up to the last measured concentration-time point (AUCt) and extrapolated to infinity using the terminal rate constant k. The terminal half-life ($t_{1/2}$) was calculated as ln2/k. The time above the previously defined threshold concentrations of 0.05 μM and 0.1 μM ($T > 0.05$ μM, $T > 0.1$ μM) was determined using linear interpolation. For i.v. administered paclitaxel the parameters AUC and $T > 0.1$ μM were determined using our previously established limited sampling model [14]. The percentage of the administered dose recovered in the urine (U_{excr}) was calculated as the amount excreted in the urine divided by the actual administered dose times 100%. Statistical analysis of the data was performed using the non-parametric Jonckheere-Terpstra test [10] and the Mann-Whitney U-test. The a priori level of significance was $P = 0.05$.

Results

Patients and treatment

A total of 15 patients (3 males and 12 females) was enrolled in the study. At study entry, the median age of the patients was 57 years (range 34–75 years) and the median WHO performance status was 0 (range 0–1). Primary tumor types included breast (3), ovarian (3), non-small-cell lung cancer (3), adenocarcinomas of unknown primary site (3), colon (2), and pancreas (1) tumors. All patients, except 2, had received prior surgical therapy, radiotherapy, and/or chemotherapy.

Toxicities observed following b.i.d. dosing of oral paclitaxel and after the first i.v. course of paclitaxel are presented in Tables 1 and 2. After oral intake of paclitaxel, hematological toxicities observed included anemia, which was often pre-existing, and leukocytopenia/granulocytopenia. The main non-hematological toxicities were alopecia, arthralgia/myalgia, fatigue, neurotoxicity, mucositis, diarrhea, nausea, and vomiting. Other incidental toxicities were gastric pain (1 patient), skin reactions (1 patient), flushes (2 patients), and mild and reversible hypotension (1 patient) (not listed in Table 2). Toxicities observed were generally mild (grade 1–2); 1 patient experienced granulocytopenia grade 3 (dose level 2×160 mg/m^2). Toxicities clearly related to CsA administration were nausea and vomiting, which were observed in 3 patients. These toxicities arose prior to paclitaxel intake. During the first course of i.v. paclitaxel, a similar profile of hematological and non-hematological toxicities was observed as after oral intake of the drug. Toxicities observed were generally mild; 1 patient developed leukocytopenia grade 3 and another patient experienced granulocytopenia grade 4. In this study 1 partial response, which was documented after the third course (1 oral and 2 i.v.), was observed in a patient with ovarian cancer (dose level 2×120 mg/m^2).

Table 1 Hematological toxicities observed following bi-daily (b.i.d.) dosing of oral paclitaxel and after the first i.v. course of paclitaxel

	Oral paclitaxel 2×60 mg/m^2	Oral paclitaxel 2×90 mg/m^2	Oral paclitaxel 2×120 mg/m^2	Oral paclitaxel 2×160 mg/m^2	Oral paclitaxel all dose levels	i.v. paclitaxel 175 mg/m^2 (3-h infusion)
No. of patients	4	3	3	5	15	11
Anemia						
Grade 1	1	0	1	3	5	5
Grade 2	2	2	1	0	5	1
Grade 3	0	0	0	0	0	1
Leukocytopenia						
Grade 1	0	0	0	0	0	2
Grade 2	0	1	0	2	3	1
Grade 3	0	0	0	0	0	1
Granulocytopenia						
Grade 1	0	0	0	0	0	2
Grade 2	0	1	0	1	2	2
Grade 3	0	0	0	1	1	0
Grade 4	0	0	0	0	0	1

Pharmacokinetics

Three patients were considered not evaluable for pharmacokinetic analysis. In 1 patient the oral course was interrupted due to respiratory problems (not drug-related) and 2 other patients vomited within 2 h of intake of oral paclitaxel. Therefore, 12 patients, 3 at each dose level, were considered eligible for pharmacokinetic analysis.

Pharmacokinetic parameters of b.i.d. dosing of oral paclitaxel are presented in Table 3. Dose increment of oral paclitaxel from 2×60 to 2×160 mg/m^2 did not result in a significant increase in the AUC of paclitaxel nor in a significant increase in time above the threshold concentrations of 0.05 μM and 0.1 μM (Jonckheere-Terpstra test). An individual plasma concentration-time curve of b.i.d. dosing of 2×90 mg/m^2 oral paclitaxel is depicted in Fig. 1.

CsA whole-blood pharmacokinetic parameters are shown in Table 4. Cremophor EL plasma levels after oral administration of paclitaxel were measured in 2 patients (dose level 2×160 mg/m^2) and were at all investigated time points lower than the limit of quantitation of the assay ($< 0.01\%$ v/v). Maximal blood ethanol concentrations were reached within 1 h of oral intake of either dose of paclitaxel. Paclitaxel doses of 2×90, 2×120, and 2×160 mg/m^2 (corresponding to 2×7.5, 2×10, and 2×13.3 ml/m^2 ethanol) resulted in mean maximal ethanol concentrations of 0.07 ± 0.05, 0.21 ± 0.04, and $0.29 \pm 0.12‰$, respectively after the first oral dose. After the second oral dose mean maximal ethanol concentrations were comparable to those after the first dose.

The pharmacokinetic data of i.v. paclitaxel (175 mg/m^2 as a 3-h infusion) were in good agreement with earlier observations [12, 17]. The mean plasma AUC and $T > 0.1$ μM values were 17.95 ± 3.94 μM.h and 17.1 ± 6.7 h, respectively ($n = 11$).

In Table 5 a comparison is made between the pharmacokinetic data of b.i.d. dosing of oral paclitaxel (2×60, 2×90, and 2×120 mg/m^2) and those of single-dose administration of oral paclitaxel (120, 180, and 250 mg/m^2) [22]. At all dose levels fractionated administration of oral paclitaxel resulted in consistently higher values of AUC and $T > 0.1$ μM. Differences were, however, not statistically significant (Mann-Whitney U-test).

Table 2 Non-hematological toxicities observed following b.i.d. dosing of oral paclitaxel and after the first i.v. course of paclitaxel

	Oral paclitaxel 2×60 mg/m^2	Oral paclitaxel 2×90 mg/m^2	Oral paclitaxel 2×120 mg/m^2	Oral paclitaxel 2×160 mg/m^2	Oral paclitaxel all dose levels	i.v. paclitaxel 175 mg/m^2 (3-h infusion)
No. of patients	4	3	3	5	15	11
Alopecia						
Grade 1	2	1	2	0	5	0
Grade 2	0	1	1	2	4	3
Arthralgia/myalgia						
Grade 1	1	1	2	2	6	3
Grade 2	1	0	0	0	1	2
Fatigue						
Grade 1	2	0	1	1	4	3
Grade 2	0	1	1	0	2	1
Neurotoxicity						
Grade 1	1	1	0	2	4	3
Mucositis						
Grade 1	2	1	1	0	4	4
Diarrhea						
Grade 1	0	1	1	3	5	1
Nausea						
Grade 1	0	1	1	1	3	1
Vomiting						
Grade 1	2	1	0	1	4	1

Table 3 Pharmacokinetic parameters of b.i.d. dosing of oral paclitaxel [data listed as mean ± (SD)] (*CsA* cyclosporin A, U_{excr} percentage of administered dose recovered in urine)

Paclitaxel dose (mg/m^2)	CsA dose (mg/kg)	No. of patients	Tmax (h)	Cmax (μM)	$AUC_{0\rightarrow\infty}$ (μM.h)	$T > 0.1$ μM (h)	$T > 0.05$ μM (h)	U_{excr} (% dose)
2×60	2×15	3	3.4 (0.6) 3.4 (0.6)	0.21 (0.10) 0.21 (0.08)	3.77 (2.70)	11.4 (10.9)	26.9 (11.1)	1.7 (1.1)
2×90	2×15	3	3.4 (0.6) 3.2 (1.0)	0.23 (0.16) 0.32 (0.16)	4.57 (2.43)	12.1 (8.8)	21.8 (10.3)	2.2 (1.0)
2×120	2×15	3	3.0 (1.0) 0.9 (1.5)	0.20 (0.09) 0.25 (0.17)	3.62 (1.58)	8.7 (7.7)	17.1 (7.9)	1.2 (0.7)
2×160	2×15	3	3.5 (2.5) 2.3 (1.6)	0.44 (0.37) 0.49 (0.41)	8.58 (7.87)	19.1 (18.6)	28.6 (23.1)	1.0 (1.0)

Discussion

Dose escalation of b.i.d. dosing of oral paclitaxel plus CsA was performed starting at 2×60 mg/m^2 up to 2×160 mg/m^2. Pharmacokinetic analysis revealed that dose increment of oral paclitaxel from 2×60 mg/m^2 to 2×90 mg/m^2 or higher doses did not result in a significant additional increase in the systemic exposure to paclitaxel nor in the time above the threshold concentrations of 0.05 and 0.1 μM. Apparently, the absorption of orally administered paclitaxel is limited. Saturation of absorption after oral paclitaxel administration was also observed in the dose escalation study of single-dose oral paclitaxel [22]. It was then hypothesized that limited dissolution, due to release of paclitaxel from its pharmaceutical formulation and precipitation as a result of its poor aqueous solubility, could cause the apparent saturation in absorption of orally administered paclitaxel. At the highest dose level of 2×160 mg/m^2, Cmax and AUC values appear to be higher than those at the lower paclitaxel dose levels. However, at this dose level the extremely high Cmax (0.80 and 0.90 μM) and AUC (16.79 μM.h) values of 1 patient contribute largely to the high mean values. It remains unclear why this patient absorbed oral paclitaxel this well. Saturation of drug absorption has also been observed for other anticancer agents, including methotrexate, etoposide, and leucovorin [9, 24, 38]. This has led to hyperfractionated approaches, whereby the drug has been administered multiple times a day rather than as one large daily dose, to achieve a greater overall daily systemic exposure. Comparison of the pharmacokinetic data of b.i.d. dosing of oral paclitaxel with those of single-dose administration of the drug [22] showed that fractionated administration of oral paclitaxel resulted in consistently higher values of systemic exposure (AUC) to paclitaxel and the duration of systemic exposure ($T > 0.1$ μM and $T > 0.05$ μM) to the drug. However, due to the large interpatient variability and the small number of patients enrolled at each dose level, these differences were not statistically significant. Nevertheless, we suggest that for oral paclitaxel, administration of a multiple-dose regimen is a realistic option to further increase and prolong the systemic exposure to paclitaxel.

An important pharmacokinetic parameter of paclitaxel is the time period of exposure above a certain threshold concentration. Earlier data indicate a strong positive relationship between duration of the paclitaxel plasma concentration above 0.05 or 0.1 μM and pharmacological activity [7, 12, 17, 29]. The feasibility of oral paclitaxel administration may enable the development of more chronic treatment schedules with the aim of

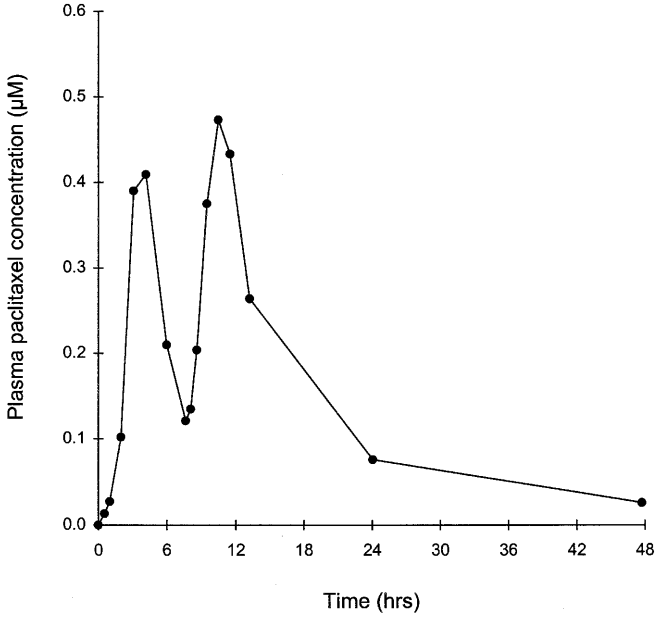

Fig. 1 Typical individual paclitaxel plasma concentration-time curve of bi-daily dosing of 2×90 mg/m^2 oral paclitaxel

Table 5 Pharmacokinetic parameters of b.i.d. dosing of oral paclitaxel compared with single-dose administration of the drug [18] [data listed as mean ± (SD)]

Paclitaxel dose (mg/m^2)	AUC (μM.h)	$T > 0.1$ μM (h)	$T > 0.05$ μM (h)
2×60	3.77 (2.70)*	11.4 (10.9)*	26.9 (11.1)*
120	2.55 (2.29)	7.9 (8.0)	13.0 (12.7)
2×90	4.57 (2.43)**	12.1 (8.8)**	21.8 (10.3)**
180	3.33 (2.39)	7.9 (6.7)	14.6 (12.3)
2×120	3.62 (1.58)***	8.7 (7.7)***	17.1 (7.9)***
250	3.27 (2.94)	7.0 (9.3)	13.6 (11.1)

* Not statistically significant compared with 120 mg/m^2
** Not statistically significant compared with 180 mg/m^2
*** Not statistically significant compared with 250 mg/m^2

Table 4 Pharmacokinetic parameters of b.i.d. dosing of CsA [data listed as mean ± (SD)]

Paclitaxel dose (mg/m^2)	CsA dose (mg/kg)	No. of patients	Tmax (h)	Cmax (mg/l)	$AUC_{0\rightarrow\infty}$ (mg.h/l)
2×60	2×15	3	2.1 (1.3) 5.2 (2.0)	2.75 (0.34) 2.81 (1.10)	55.43 (29.73)
2×90	2×15	3	2.1 (1.5) 3.5 (2.2)	3.01 (0.69) 3.65 (1.42)	62.75 (23.53)
2×120	2×15	3	1.4 (0.4) 2.6 (1.9)	2.60 (0.62) 2.22 (0.87)	42.40 (12.49)
2×160	2×15	3	1.1 (0.0) 0.7 (2.5)	2.98 (1.58) 2.90 (2.25)	50.37 (37.03)

achieving sustained plasma concentrations above these pharmacologically relevant threshold levels. However, it is unclear whether for orally administered paclitaxel the threshold concentrations of 0.05 and 0.1 μM are relevant and should be pursued. The plasma Cremophor EL concentrations are a key factor in this discussion. After oral administration of paclitaxel, plasma Cremophor EL plasma levels were undetectable, which was also seen in our previous studies of orally administered paclitaxel [22, 25, 26]. Thus, after oral administration of the paclitaxel i.v. formulation (Paxene) the co-solvent Cremophor EL is not absorbed. This is important, first because systemic exposure to Cremophor EL can induce severe hypersensitivity reactions requiring extensive premedication [5, 41]. In the current study patients did not receive premedication prior to oral administration of paclitaxel. Potential hypersensitivity reactions observed following orally administered paclitaxel were very mild (grade 1) and consisted of flushes (2 patients), skin reactions (1 patient), and mild and reversible hypotension (1 patient), which did not require additional measures. Evidently, paclitaxel (Paxene) can be administered orally without premedication. Furthermore, Cremophor EL is responsible for the non-linear pharmacokinetic behavior of i.v. paclitaxel [7, 36]. It entraps paclitaxel in the plasma compartment, which results in a more than proportional increase in plasma paclitaxel levels with increasing doses. However, studies in mice show that these higher total drug levels in plasma do not result in higher drug levels in tissues [35]. This pseudo non-linearity of i.v. paclitaxel [40] has two important implications for the pharmacology of oral paclitaxel. First, oral bioavailability of paclitaxel, calculated by comparing plasma AUC values after oral and i.v. administration, will be underestimated as the affinity of paclitaxel for the plasma compartment is increased after i.v. administration due to the presence of systemic Cremophor EL. Secondly, the pseudo non-linearity of i.v. paclitaxel implies that after oral administration, when Cremophor EL is not present, plasma levels of paclitaxel represent a higher fraction of free drug, which will result in enhancement of the availability of paclitaxel for the (tumor) tissues [40]. Consequently, the optimal value of the threshold level may be lower for orally administered paclitaxel than i.v. paclitaxel; this needs further confirmation. Thus, pharmacokinetics of i.v. paclitaxel and oral paclitaxel, with and without Cremophor EL in the systemic circulation, respectively, are substantially different and therefore comparison of pharmacokinetic parameters of i.v. and oral paclitaxel should be performed with caution.

In this study we have used CsA to increase the systemic exposure to oral paclitaxel. CsA is an efficacious inhibitor of P-gp and was one of the first agents applied to modulate P-gp [6]. In addition to CsA, more-potent inhibitors of P-gp have been developed, such as the CsA analogue SDZ PSC 833 [2] or the acridone carboxamide derivative GF120918 [18]. Importantly, these newly developed modulators of P-gp have no known immunosuppressive activity such as CsA, and may therefore be better candidates for clinical use, especially for repeated administration. CsA is, however, commercially available and an advantage in its use to increase the systemic exposure to oral paclitaxel is its potential to inhibit metabolism of paclitaxel. Metabolism of paclitaxel is catalyzed by two cytochrome P-450 (CYP) isoenzymes; CYP 2C8 catalyzes the degradation to the 6α-hydroxypaclitaxel metabolite and CYP 3A4 results in formation of the 3′p-hydroxypaclitaxel metabolite [4, 27]. Both metabolites are substantially less active than the parent drug [27]. CsA itself is also metabolized by CYP 3A4 [33]. In our previous study of single-dose oral paclitaxel in combination with CsA [26] we found that after oral paclitaxel administration in combination with CsA the relative contribution of formation of the metabolite 3′p-hydroxypaclitaxel was substantially lower than after i.v. administration of the drug, indicating inhibition of CYP 3A4-mediated paclitaxel metabolism by CsA.

Because pharmacokinetic analysis revealed limited absorption of orally administered paclitaxel, we did not continue dose escalation of b.i.d. dosing oral paclitaxel up to DLT. Toxicities observed following oral paclitaxel administration in combination with CsA were mild (CTC grade 1–2) at all investigated dose levels. At the dose level 2×160 mg/m^2 diarrhea occurred more often (3 of 5 patients) than at other dose levels, and at this dose level 1 patient continued to experience acute nausea and vomiting despite granisetron administration. Therefore, we considered the lower dose levels more suitable for future studies.

In conclusion, dose escalation of b.i.d. dosing of oral paclitaxel was not continued up to DLT, as the pharmacokinetic data revealed no significant additional increase in the systemic exposure to paclitaxel with increment of the administered dose. Because fractionated administration of oral paclitaxel resulted in consistently higher values of the paclitaxel pharmacokinetic parameters, we will continue with additional clinical studies focused on multiple dose regimens of oral paclitaxel. Although pharmacokinetic data are difficult to interpret, due to the limited number of patients at each dose level and the large interpatient variability, we recommend the dose level of 2×90 mg/m^2 for further investigation, as this dose level showed the highest systemic exposure to paclitaxel combined with good safety.

Acknowledgements We thank Harm van Tinteren, MSc., for assistance with the statistical analysis of the data. We also express our gratitude to the medical and nursing staff of the Antoni van Leeuwenhoek Hospital for the care and support of the patients in this study.

References

1. Baguley BC, Marshall ES, Whittaker JR, Dotchin MC, Nixon J, McCrystal MR, Finlay GJ, Matthews JH, Holdaway KM, Van Zijl P (1995) Resistance mechanisms determining the

in vitro sensitivity to paclitaxel of tumour cells cultured from patients with ovarian cancer. Eur J Cancer 31:230
2. Boesch D, Gaveriaux C, Jachez B, Pourtier-Manzanedo A, Bollinger P, Loor F (1991) In vivo circumvention of P-glycoprotein-mediated multidrug resistance of tumor cells with SDZ PSC 833. Cancer Res 51:4226
3. Chan GL, Weinstein SS, Lefor WW, Spoto E, Kahana L, Shires DL (1992) Relative performance of specific and nonspecific fluorescence immunoassay for cyclosporin in transplant patients. Ther Drug Monit 14:42
4. Cresteil T, Monsarrat B, Alvinerie P, Treluyer JM, Vieira I, Wright M (1994) Taxol metabolism by human liver microsomes: identification of cytochrome P450 isozymes involved in its biotransformation. Cancer Res 54:386
5. Dye D, Watkins J (1980) Suspected anaphylactic reaction to Cremophor EL. BMJ 280:1353
6. Ford JM, Hait WN (1990) Pharmacology of drugs that alter multidrug resistance in cancer. Pharmacol Rev 42:155
7. Gianni L, Kearns CM, Giani A, Capri G, Viganó L, Locatelli A, Bonadonna G, Egorin MJ (1995) Nonlinear pharmacokinetics and metabolism of paclitaxel and its pharmacokinetic/pharmacodynamic relationships in humans. J Clin Oncol 13:180
8. Gibaldi M, Perrier D (1982) Noncompartmental analysis based on statistical moment theory. In: Swarbrick J (ed) Pharmacokinetics. Dekker, New York, p 409
9. Hande KR, Krozely MG, Greco FA, Hainsworth JD, Johnson DH (1993) Bioavailability of low dose oral etoposide. J Clin Oncol 11:374
10. Hollander M, Wolfe DA (1973) Nonparametric statistical methods. Wiley, New York
11. Horwitz SB (1994) Taxol (paclitaxel): mechanisms of action. Ann Oncol 5 [Suppl 6]:S3
12. Huizing MT, Keung AC, Rosing H, Van der Kuij V, Ten Bokkel Huinink WW, Mandjes I, Dubbelman AC, Pinedo HM, Beijnen JH (1993) Pharmacokinetics of paclitaxel and metabolites in a randomized comparative study in platinum-pretreated ovarian cancer patients. J Clin Oncol 11:2117
13. Huizing MT, Sewberath Misser VH, Pieters RC, Ten Bokkel Huinink WW, Veenhof CHN, Vermorken JB, Pinedo HM, Beijnen JH (1995) Taxanes: a new class of antitumor agents. Cancer Invest 13:381
14. Huizing MT, Van Warmerdam LJC, Rosing H, Ten Bokkel Huinink WW, Stewart MB, Pinedo HM, Beijnen JH (1995) Limited sampling strategies for investigating paclitaxel pharmacokinetics in patients receiving 175 mg/m^2 as a 3-hour infusion. Clin Drug Invest 9:344
15. Huizing MT, Sparreboom A, Rosing H, Van Tellingen O, Pinedo HM, Beijnen JH (1995) Quantification of paclitaxel metabolites in human plasma by high-performance liquid chromatography. J Chromatogr B 674:261
16. Huizing MT, Rosing H, Koopman FJ, Keung ACF, Pinedo HM, Beijnen JH (1995) High-performance liquid chromatographic procedures for the quantitative determination of paclitaxel (Taxol) in human urine. J Chromatogr B 664:373
17. Huizing MT, Giaccone G, Van Warmerdam LJC, Rosing H, Bakker PJM, Vermorken JB, Postmus PE, Van Zandwijk N, Koolen MGJ, Ten Bokkel Huinink WW, Van der Vijgh WJF, Bierhorst FJ, Lai A, Dalesio O, Pinedo HM, Veenhof CHN, Beijnen JH (1997) Pharmacokinetics of paclitaxel and carboplatin in a dose-escalating and sequencing study in patients with non-small cell lung cancer. J Clin Oncol 15:317
18. Hyafil F, Vergely C, Du Vignaud P, Grand-Perret T (1993) In vitro and in vivo reversal of multidrug resistance by GF120918, an acridonecarboxamide derivative. Cancer Res 53:4595
19. Kelland LR, Abel G (1992) Comparative in vitro cytotoxicity of Taxol and Taxotere against cisplatin-sensitive and -resistant human ovarian carcinoma cell lines. Cancer Chemother Pharmacol 30:444
20. Liebmann JE, Cook JA, Lipschultz C, Teague D, Fisher J, Mitchell JB (1993) Cytotoxic studies of paclitaxel (Taxol) in human tumour cell lines. Br J Cancer 68:1104
21. Lopes NM, Adams EG, Pitts TW, Bhuyan BK (1993) Cell kill kinetics and cell cycle effects of Taxol on human and hamster ovarian cell lines. Cancer Chemother Pharmacol 32:235
22. Malingré MM, Meerum Terwogt JM, Beijnen JH, Rosing H, Koopman FJ, Van Tellingen O, Ten Bokkel Huinink WW, Swart M, Lieverst J, Schellens JHM (2000) Phase I and pharmacokinetic study of oral paclitaxel. J Clin Oncol 18:2468
23. Manfredi JJ, Parness J, Horwitz SB (1982) Taxol binds to cellular microtubules. J Cell Biol 94:688
24. McGuire BW, Sia LL, Leese PT, Gutierrez ML, Stokstad ELR (1988) Pharmacokinetics of leucovorin calcium after intravenous, intramuscular, and oral administration. Clin Pharm 7:52
25. Meerum Terwogt JM, Beijnen JH, Ten Bokkel Huinink WW, Rosing H, Schellens JHM (1998) Co-administration of cyclosporin enables oral therapy with paclitaxel. Lancet 352:285
26. Meerum Terwogt JM, Malingré MM, Beijnen JH, Ten Bokkel Huinink WW, Rosing H, Koopman FJ, Van Tellingen O, Swart M, Schellens JHM (1999) Co-administration of cyclosporin A enables oral therapy with paclitaxel. Clin Cancer Res 5:3379
27. Monsarrat B, Mariel E, Cros S, Gares M, Guenard D, Gueritte-Voegelein F, Wright M (1990) Taxol metabolism. Isolation and identification of three major metabolites of taxol in rat bile. Drug Metab Dispos 18:895
28. National Cancer Institute, Division of Cancer Treatment (1988) Guidelines for reporting of adverse drug reactions. National Cancer Institute, Division of Cancer Treatment, Bethesda, Maryland
29. Ohtsu T, Yasutsuna S, Tamura T, Miyata Y, Nakanomyo H, Nishiwaki Y, Saijo N (1995) Clinical pharmacokinetics and pharmacodynamis of paclitaxel: a 3-hour infusion versus a 24-hour infusion. Clin Cancer Res 1:599
30. Raymond E, Hanauske A, Faivre S, Izbicka E, Clark G, Rowinsky EK, Von Hoff DD (1997) Effects of prolonged versus short-term exposure paclitaxel (Taxol) on human tumor colony-forming units. Anticancer Drugs 8:379
31. Rowinsky EK, Donehower RC (1995) Paclitaxel (Taxol). N Engl J Med 332:1004
32. Schiff PB, Fant J, Horwitz SB (1979) Promotion of microtubule assembly in vitro by taxol. Nature 277:665
33. Shet MS, Fisher CW, Holmans PL, Estabrook RW (1993) Human cytochrome P450 3A4: enzymatic properties of a purified recombinant fusion protein containing NADPH-P450 reductase. Proc Natl Acad Sci USA 90:11748
34. Sparreboom A, Van Tellingen O, Huizing MT, Nooijen WJ, Beijnen JH (1996) Determination of polyoxyethyleneglycerol triricinoleate 35 (Cremophor EL) in plasma by pre-column derivatization and reversed-phase high-performance liquid chromatography. J Chromatogr B 681:355
35. Sparreboom A, Van Tellingen O, Nooijen WJ, Beijnen JH (1996) Tissue distribution, metabolism and excretion of paclitaxel in mice. Anticancer Drugs 7:78
36. Sparreboom A, Van Tellingen O, Nooijen WJ, Beijnen JH (1996) Nonlinear pharmacokinetics of paclitaxel in mice results from the pharmaceutical vehicle Cremophor EL. Cancer Res 56:2112
37. Sparreboom A, Van Asperen J, Mayer U, Schinkel AH, Smit JW, Meijer DKF, Borst P, Nooijen WJ, Beijnen JH, Van Tellingen O (1997) Limited oral bioavailability and active epithelial secretion of paclitaxel caused by P-glycoprotein in the intestine. Proc Natl Acad Sci USA 94:2031
38. Teresi ME, Crom WR, Choi KE, Mirro J, Evans WE (1987) Methotrexate bioavailability after oral and intramuscular administration in children. J Pediatr 110:788
39. Van Asperen J, Van Tellingen O, Van der Valk MA, Rozenhart M, Beijnen JH (1998) Enhanced oral absorption and decreased elimination of paclitaxel in mice cotreated with cyclosporin A. Clin Cancer Res 4:2293

40. Van Tellingen O, Huizing MT, Nannan Panday VR, Schellens JHM, Nooijen WJ, Beijnen JH (1999) Cremophor EL causes (pseudo)-non-linear pharmacokinetics of paclitaxel in patients. Br J Cancer 81:330
41. Weiss RB, Donehower RC, Wiernik PH, Ohnuma T, Gralla FJ, Trump DL, Baker JR, Van Echo DA, Von Hoff DD, Leyland-Jones B (1990) Hypersensitivity reactions from Taxol. J Clin Oncol 8:1263
42. World Health Organisation, Geneva, Switzerland (1979) WHO Handbook for reporting results of cancer treatment
43. Zhan Z, Scala S, Monks A, Hose C, Bates S, Fojo T (1997) Resistance to paclitaxel mediated by P-glycoprotein can be modulated by changes in the schedule of administration. Cancer Chemother Pharmacol 40:245