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Report

The effect of different doses of cyclosporin A on the systemic exposure of orally administered paclitaxel

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The objective of this study was to define the minimally effective dose of cyclosporin A (CsA) that would result in a maximal increase of the systemic exposure to oral paclitaxel. Six evaluable patients participated in this randomized cross-over study in which they received at two occasions two doses of 90 mg/m^2 oral paclitaxel 7 h apart in combination with 10 or 5 mg/kg CsA. Dose reduction of CsA from 10 to 5 mg/kg resulted in a statistically significant decrease in the area under the plasma concentration–time curve (AUC) and time above the threshold concentrations of 0.1 μM ($T>0.1$ μM) of oral paclitaxel. The mean ($\pm$SD) AUC and $T>0.1$ μM values of oral paclitaxel with CsA 10 mg/kg were 4.29$\pm$0.88 μM·h and 12.0$\pm$2.1 h, respectively. With CsA 5 mg/kg these values were 2.75$\pm$0.63 μM·h and 7.0$\pm$2.1 h, respectively ($p=0.028$ for both parameters). In conclusion, dose reduction of CsA from 10 to 5 mg/kg resulted in a significant decrease in the AUC and $T>0.1$ μM values of oral paclitaxel. Because CsA 10 mg/kg resulted in similar paclitaxel AUC and $T>0.1$ μM values compared to CsA 15 mg/kg (data which we have published previously), the minimally effective dose of CsA is determined at 10 mg/kg. [© 2001 Lippincott Williams & Wilkins.]

Key words: cyclosporin A, oral administration, paclitaxel.

Introduction

Paclitaxel is an important anticancer agent widely applied for the treatment of breast, ovarian and lung cancer, and AIDS-related Kaposi's sarcoma.[1,2] The drug is currently administered i.v. at different dosages and time schedules, and optimization of the clinical application is still pursued. Recently we reported on the feasibility of oral administration of paclitaxel.[3–7] Oral administration is investigated because it is convenient to patients, it reduces administration costs and facilitates the use of more chronic treatment regimens. The latter is important as there are strong indications in both preclinical[8–12] and clinical studies[13–16] that the pharmacological activity of paclitaxel is related to duration of exposure to the drug.

Oral administration of paclitaxel appeared feasible with co-administration of cyclosporin A (CsA),[6,7] an efficacious inhibitor of the gastrointestinal drug efflux pump P-glycoprotein (P-gp), which was shown in our preclinical studies to cause the low oral bioavailability of paclitaxel.[3] In our clinical proof of principle study, co-administration of 15 mg/kg CsA resulted in an approximately 7-fold increase in the systemic exposure to 60 mg/m^2 orally administered paclitaxel.[6,7] These first promising results with low paclitaxel dosages encouraged us to investigate dose increment of CsA and dose escalation of paclitaxel in order to further increase the systemic exposure. A dose increment of CsA from 15 to 30 mg/kg did not result in an additional increase in the systemic exposure of paclitaxel and hence CsA 15 mg/kg was used in further studies.[17] Dose escalation of paclitaxel from 60 up to 300 mg/m^2 resulted in a significant increase in systemic exposure of paclitaxel; however, these increases were moderate and not proportional with the increases in dose, indicating limited absorption for orally administered paclitaxel.[17] In order to further increase the systemic exposure we consecutively

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investigated dose escalation of a twice-daily dose regimen of oral paclitaxel. Compared to single-dose administration, hyperfractionated administration resulted in consistently higher values of the systemic exposure of paclitaxel. As observed for single-dose paclitaxel, twice-daily dose administration also revealed limited absorption of the drug. Based on these observations the oral paclitaxel dose level of 2×90 mg/m^2 in combination with CsA 15 mg/kg was recommended for further studies.[18]

In this study we investigated dose reduction of CsA from 10 to 5 mg/kg co-administered to 2×90 mg/m^2 oral paclitaxel, in order to define the minimally effective dose of CsA that would result in a maximal increase in the systemic exposure to paclitaxel. CsA is an immunosuppressive drug widely used in transplantation to prevent rejection of the transplanted organ.[19] It felt important that the immunosuppressive effect is minimized. In addition, a dose-related nephrotoxicity of CsA should be considered.[20] It was our aim to determine the minimally effective dose of CsA resulting in a maximal increase in systemic exposure to paclitaxel.

Patients and methods

Patient population

Patients with a histologic 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 function (white blood cells $> 3.0 \times 10^9$/l; platelets $> 100 \times 10^9$/l), liver function (serum bilirubin $\leqslant 25$ μmol/l; serum albumin $\geqslant 25$ g/l), renal function (serum creatinine $\leqslant 160$ μmol/l or clearance $\geqslant 50$ ml/min) and a WHO performance status $\leqslant 2$. Patients were not eligible if they suffered from uncontrolled infectious disease, neurologic disease, bowel obstruction or symptomatic brain metastases. Other exclusion criteria were concomitant use of known P-gp inhibitors and chronic use of H2 receptor antagonists or proton pump inhibitors. The study protocol was approved by the Medical Ethics Committee of the Institute and all patients had to give written informed consent.

Study design

The study had a randomized cross-over trial design. Patients received, at one occasion, two doses of 90 mg/m^2 paclitaxel orally 7 h apart in combination with 10 or 5 mg/kg CsA. Three weeks later, these patients received the two doses of 90 mg/m^2 oral paclitaxel in combination with the alternate CsA dose. If it was considered to be in their best interest, patients continued on a 3-weekly schedule of i.v. paclitaxel 175 mg/m^2 administered as a 3-h infusion.

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, FL) was used for both i.v. and oral administration of paclitaxel. CsA (Neoral®; Novartis, Basel, Switzerland) was administered 30 min prior to each oral paclitaxel dose. The first oral paclitaxel dose was administered at least 2 h after a standard light breakfast (two crackers and a cup of tea) and patients fasted until 2 h following administration. For the second oral paclitaxel dose, patients refrained from food and drinks for 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, patients received oral granisetron 1 mg (Kytril®) 2 h prior to the first CsA dose and 1 h prior to the second CsA dose. Prior to i.v. administration of paclitaxel patients received standard i.v. premedication to prevent hypersensitivity reactions, i.e. 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. Oral paclitaxel doses were given without this premedication regimen as previous studies of oral paclitaxel have shown that the co-solvent Cremophor EL, suspected of causing the hypersensitivity reactions,[21] was not absorbed following oral intake of the drug.[6,7,17,18]

Patient evaluation

Pre-treatment evaluation included a complete medical history and complete physical examination. Before each course, an interim history including concomitant medications taken, toxicities and performance status were registered, and a physical examination was performed. Hematology was checked twice weekly after course 1, 2 and 3, 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).[22] Tumor measurements were performed every other cycle, but initially after the first two i.v. courses. Responses were evaluated according to the WHO criteria.[23]

Sample collection and analysis

After oral paclitaxel administration 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).[24] For plasma paclitaxel and metabolite concentrations, the remainder of the blood samples was centrifuged and plasma samples were stored at −20°C until analysis. Paclitaxel and metabolite concentrations were determined using a validated high-performance liquid chromatography (HPLC) assay.[25] In addition to measuring CsA and paclitaxel levels after oral drug administration, Cremophor EL concentrations were measured in one patient during both oral courses. The plasma samples obtained for paclitaxel analysis were used for analysis of Cremophor EL. Plasma Cremophor EL concentrations were measured at six time points up to 13 h after the first oral dose of paclitaxel using a validated HPLC assay[26] with minor modifications as described elsewhere.[27] 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.[28]

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.[29] Blood samples were collected in heparinized tubes, centrifuged and plasma samples were stored at −20°C until analysis. Paclitaxel concentrations were determined using a validated HPLC assay.[25]

Pharmacokinetic analysis

Non-compartmental pharmacokinetic methods were applied to process the results.[30] For orally administered paclitaxel, the maximal drug concentration (C_{max}) and time to maximal drug concentration (T_{max}) 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 (AUC_t) and extrapolated to infinity using the terminal rate constant k. The terminal half-life ($t_{1/2}$) was calculated as $\ln 2/k$. The time above the previously defined threshold concentrations of 0.05 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.[29] The percentage of the administered dose recovered in the urine (U_{excr}) was calculated as the amount excreted in urine divided by the actual administered dose times 100%. Statistical analysis of the data was performed using the non-parametric Wilcoxon matched-pairs signed-rank test. The *a priori* level of significance was $p = 0.05$.

Results

Patients and treatment

A total of eight patients (five males and three females) was enrolled in the study. At study entry, the median age of the patients was 47 years (range 36–69) and the median WHO performance status was 0 (range 0–1). Primary tumor types included non-small cell lung cancer (2), breast (1), stomach (2), cervix (1) and uterus (1) tumors, and adenocarcinoma of unknown primary site (1). All patients, except one, had received prior surgical therapy, radiotherapy and/or chemotherapy.

The oral combination of paclitaxel and CsA was in general well tolerated. Hematological toxicities after oral intake of paclitaxel plus CsA (in total 16 courses) consisted of anemia (12), leukocytopenia (4) and granulocytopenia (3), which were generally mild (CTC grade 1–2), except for two patients who experienced leukocytopenia grade 3. No pronounced differences could be observed in hematological toxicities between the two CsA dose levels. Non-hematological toxicities after oral administration of paclitaxel plus CsA were nausea (7), vomiting (2), diarrhea (5), mucositis (1), arthralgia/myalgia (5), alopecia (7) and fatigue (2). Non-hematological toxicities did not exceed grade 1 in severity, except for one patient who experienced alopecia grade 2. Again, no pronounced differences between the two CsA levels were observed. However, the limited number of observations does not allow a definite comparison of toxicities. After the first course of i.v. paclitaxel (in total eight courses) the pattern of hematological and non-hematological toxicities was as expected for i.v. paclitaxel, and consisted of anemia (3), leukocytopenia (4), granulocytopenia (4), arthralgia/myalgia (7), alopecia (3), neurotoxicity (2) and mucositis (1).

In this study two partial responses were observed, one in a patient with a cervix tumor after six courses

of paclitaxel (two oral and four i.v.) and one in a patient with adenocarcinoma of unknown primary site after five courses of paclitaxel (two oral and three i.v.).

Pharmacokinetics

A total of six patients was evaluable for pharmacokinetic analysis. One patient was considered not evaluable because of vomiting within 1 h after intake of oral paclitaxel. For another patient, plasma samples of one oral course were lost due to power failure of the freezer.

The mean (±SD) values of the plasma pharmacokinetic parameters of orally administered paclitaxel in combination with 5 and 10 mg/kg CsA are given in Table 1. In addition, the data of 2×90 mg/m^2 oral paclitaxel in combination with 15 mg/kg CsA, data from our previously performed dose-escalation study of twice daily dosing of oral paclitaxel are presented in Table 1.[18] Dose reduction of CsA from 10 to 5 mg/kg resulted in a significant decrease in the AUC, $T > 0.1$ μM and $T > 0.05$ μM values of orally administered paclitaxel ($p = 0.028$ for all three parameters). Compared to CsA 15 mg/kg (in a different cohort of patients),[18] co-administration of 10 mg/kg CsA resulted in comparable paclitaxel AUC, $T > 0.1$ μM and $T > 0.05$ μM values. Figure 1 shows the plasma paclitaxel concentration–time curves of a patient receiving oral paclitaxel 2×90 mg/m^2 in combination with 5 and 10 mg/kg CsA on two occasions. Plasma Cremophor EL levels were lower than the lower limit of quantitation of the assay (below 0.01% v/v) at all investigated time-points.

Table 2 presents the AUC$_t$ values of the paclitaxel metabolites 6α-hydroxypaclitaxel, 3′*p*-hydroxypaclitaxel and 6α,3′*p*-dihydroxypaclitaxel after oral administration of paclitaxel in combination with CsA 5, 10 and 15 mg/kg. The latter data are again derived from our previously performed dose-escalation study of twice-daily dosing of oral paclitaxel.[18] In this study of CsA 5 and 10 mg/kg, metabolite data of two patients could not be determined due to interference of (unknown) compounds in the analytical assay. AUC$_t$ values have been calculated because extrapolation of the AUC could not be performed properly due to erratic profiles and the limited time that these metabolites could be detected. Higher doses of CsA appeared to result in a relative decrease in the formation of the 3′*p*-hydroxypaclitaxel metabolite. The AUC$_t$ ratio for the metabolites 6α-hydroxypaclitaxel and 3′*p*-hydroxypaclitaxel was 1.0 (0.49/0.48) after CsA 5 mg/kg, 4.0 (2.63/0.66) after CsA 10 mg/kg and 6.9 (6.19/0.90) after CsA 15 mg/kg.

CsA whole blood pharmacokinetic parameters of 5, 10 and 15 mg/kg co-administered to 2×90 mg/m$_2$ oral paclitaxel are shown in Table 3. As done for paclitaxel, we added in this table CsA data from our previous study of oral paclitaxel in combination with 15 mg/kg CsA.[18] A dose increase of CsA from 5 to 10 mg/kg resulted in an approximately 2-fold increase in C_{max1}, C_{max2} and AUC values of CsA, whereas T_{max1}, T_{max2} and $t_{1/2}$ revealed rather constant values. Administration of CsA 15 mg/kg revealed further increases in C_{max} and AUC values of CsA in proportion with the further increase in dose.

Urinary excretion after orally administered paclitaxel in combination with CsA was minimal, as was shown in our previous clinical studies of oral paclitaxel.[17,18] Different CsA doses did not seem to result in pronounced differences in urinary excretion of the drug. Paclitaxel was excreted as unchanged drug for 2.0±1.2% after CsA 5 mg/kg and for 2.8±0.9% after CsA 10 mg/kg. After CsA 15 mg/kg paclitaxel was excreted in the urine for 2.2±1.0%.[18]

Table 1. Pharmacokinetic parameters of paclitaxel after oral administration at a dose of 2×90 mg/m^2 in combination with CsA 5, 10 and 15 mg/kg

Paclitaxel data	CsA 5 mg/kg (n=6)	CsA 10 mg/kg (n=6)	CsA 15 mg/kg (n=3)
AUC (μM·h)	2.75±0.63[a]	4.29±0.88	4.57±2.43
$T > 0.1$ μM (h)	7.0±2.1[a]	12.0±2.1	12.1±8.8
$T > 0.05$ μM (h)	14.5±4.9[a]	23.7±4.4	21.8±10.3
$t_{1/2}$ (h)	10.5±5.6	13.6±7.5	11.4±2.2
C_{max1} (μM)	0.16±0.05	0.21±0.05	0.23±0.16
C_{max2} (μM)	0.28±0.08	0.35±0.11	0.32±0.16
T_{max1} (h)	2.5±1.4	2.3±1.0	3.4±0.6
T_{max2} (h)	2.7±0.9	2.5±0.5	3.2±1.0

The data of CsA 5 and 10 mg/kg were obtained in the same group of patients at two occasions which were randomized. The data of CsA 15 mg/kg are retrieved from a previously performed study[18] [data listed as mean±SD].
[a] $p < 0.05$ compared to CsA 10 mg/kg (randomized cross-over trial design).

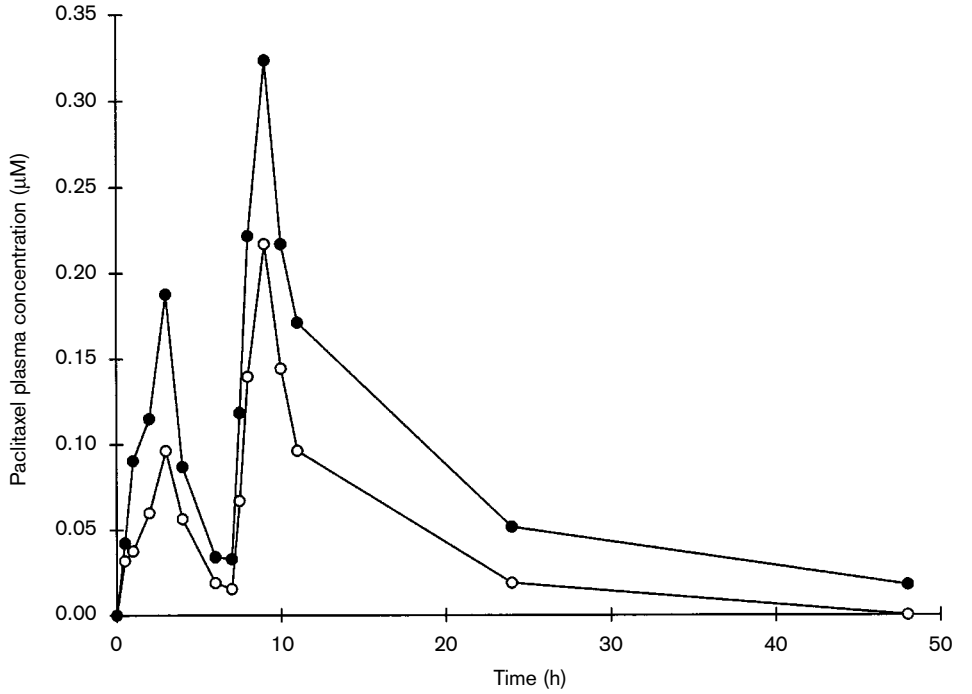

Figure 1. Paclitaxel plasma concentration-time curves obtained in one patient dosed with oral paclitaxel 2×90 mg/m^2 in combination with 5 (○) and 10 (●) mg/kg CsA.

Table 2. AUC_t values of the paclitaxel metabolites 6α-hydroxypaclitaxel (6α-HP), 3′*p*-hydroxypaclitaxel (3′*p*-HP) and 6α,3′*p*-dihydroxypaclitaxel (6α,3′*p*-DHP) after oral paclitaxel administration at a dose of 2×90 mg/m^2 in combination with CsA 5, 10 and 15 mg/kg

Metabolite data	CsA 5 mg/kg ($n=4$)	CsA 10 mg/kg ($n=4$)	CsA 15 mg/kg ($n=3$)
AUC_t 6α-HP (μM·h)	0.49 ± 0.24	2.63 ± 0.87	6.19 ± 6.79
AUC_t 3'p-HP (μM·h)	0.48 ± 0.51	0.66 ± 0.21	0.90 ± 0.88
AUC_t 6α,3′*p*-DHP (μM·h)	0.35 ± 0.62	1.73 ± 1.06	4.66 ± 6.75

The data of CsA 5 and 10 mg/kg were obtained in the same group of patients at two occasions which were randomized. The data of CsA 15 mg/kg are retrieved from a previously performed study[18] [data listed as mean ± SD].

Table 3. Pharmacokinetic parameters of CsA administered at doses of 2×5, 2×10 and 2×15 mg/kg combined with 2×90 mg/m^2 oral paclitaxel

	CsA 5 mg/kg ($n=6$)	CsA 10 mg/kg ($n=6$)	CsA 15 mg/kg ($n=3$)
AUC (mg/l·h)	16.64 ± 6.00	37.09 ± 6.77	62.75 ± 23.53
$t_{1/2}$ (h)	10.3 ± 2.4	10.5 ± 1.1	12.4 ± 2.4
C_{max1} (mg/l)	1.43 ± 0.93	2.44 ± 0.78	3.01 ± 0.69
C_{max2} (mg/l)	1.26 ± 0.52	2.37 ± 0.73	3.65 ± 1.42
T_{max1} (h)	1.9 ± 1.1	1.1 ± 0.2	2.1 ± 1.5
T_{max2} (h)	2.8 ± 0.8	2.7 ± 1.4	3.5 ± 2.2

The data of CsA 5 and 10 mg/kg were obtained in the same group of patients at two occasions which were randomized. The data of CsA 15 mg/kg are retrieved from a previously performed study.[18] [data listed as mean ± (SD)].

The pharmacokinetic data of i.v. paclitaxel (175 mg/m^2 as a 3 h infusion) were in good agreement with earlier observations.[13,16] The mean plasma AUC and $T>0.1$ μM values were 15.40 ± 1.79 μM·h and 16.3 ± 0.7 h, respectively ($n=4$). For two patients i.v. paclitaxel pharmacokinetics could not be determined because infusion duration or blood sampling were not within the range of the limited sampling model.[29]

Discussion

In this randomized cross-over trial we investigated dose reduction of CsA from 10 to 5 mg/kg co-administered to oral paclitaxel 2×90 mg/m^2 in order to define the minimally effective dose of CsA that would still result in a maximal increase of the systemic exposure to paclitaxel. A dose reduction of CsA from 10 to 5 mg/kg resulted in a significant decrease in the AUC, $T>0.1$ μM and $T>0.05$ μM values of orally administered paclitaxel at a dose of 2×90 mg/m^2 in the same group of patients. After CsA 10 mg/kg these parameters were approximately 1.6- to 1.7-fold higher than after CsA 5 mg/kg. Compared to CsA 15 mg/kg,[18] co-administration of 10 mg/kg CsA revealed similar paclitaxel AUC, $T>0.1$ μM and $T>0.05$ μM values. Parameters differed in a range of 0.9- to 1.1-fold between the CsA doses of 10 and 15 mg/kg. Previously we investigated the effect of dose increment and dose scheduling of CsA on the systemic exposure of orally administered paclitaxel 60 mg/m^2 in a single-dose regimen.[17] Increasing the CsA dose from 15 to 30 mg/kg to achieve higher levels of the inhibitor did not result in an increase in the systemic exposure of paclitaxel.[17] In addition, administration of two doses of 15 mg/kg CsA, 10 min prior to and 2 h after the oral intake of paclitaxel, to achieve more sustained levels of the inhibitor, also did not result in a further increase in the systemic exposure to paclitaxel.[17] Thus, combining the results from dose increment and dose scheduling of CsA with those of dose reduction of CsA, P-gp inhibition by CsA appears to be maximal at CsA 10 mg/kg, which is therefore recommended for further studies of orally administered paclitaxel.

In our proof of principle study of oral paclitaxel with and without CsA[6,7] we suggested that the increase in systemic exposure to orally administered paclitaxel by CsA was most likely caused by inhibition of intestinal P-gp by CsA. In addition, we hypothesized that inhibition of paclitaxel metabolism by CsA may have contributed as we observed altered paclitaxel metabolism following CsA co-administration.[7] Paclitaxel is metabolized by the cytochrome P450 (CYP) isoenzymes 2C8 and 3A4, resulting in the metabolites 6α-hydroxypaclitaxel and 3′*p*-hydroxypaclitaxel, respectively (Figure 2).[31,32] Both metabolites are substantially less active than the parent compound.[32] CsA itself is also metabolized by CYP 3A4.[33] In our proof of principle study of oral paclitaxel[7] 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. In this study, we were able to compare metabolite levels after three different doses of CsA. Higher doses of CsA resulted in a pronounced increase in the AUC_t ratio of the metabolites 6α-hydroxypaclitaxel and 3′*p*-hydroxypaclitaxel, indicating a relative decrease in the formation of the 3′*p*-hydroxypaclitaxel metabolite. These data also suggest inhibition of the CYP 3A4-mediated metabolic pathway of paclitaxel by CsA. Interpretation of the metabolite data should, however, be done with caution because of the small number of patients enrolled at each CsA dose level and the very large interpatient variability in the metabolite data of paclitaxel. Furthermore, it is important to note that inhibition of the CYP 3A4-mediated pathway will not necessarily result in prolonged exposure of the active parent compound because drug not handled by CYP 3A4 may escape through the CYP 2C8 pathway, which is, in general, the predominant metabolic pathway of paclitaxel.

As plasma levels of Cremophor EL, the co-solvent suspected of causing the hypersensitivity reactions related to paclitaxel administration,[21] were undetectable in our previous studies of oral paclitaxel,[6,7,17,18] patients in this study, and previous studies of oral paclitaxel,[17,18] received oral paclitaxel without premedication. We have confirmed these previously established data by measuring Cremophor EL levels in one patient at both oral courses, which were at all investigated time points lower than the lower limit of quantitation. No hypersensitivity reactions were observed and, evidently, paclitaxel (Paxene®) can be administered orally without the premedication regi-

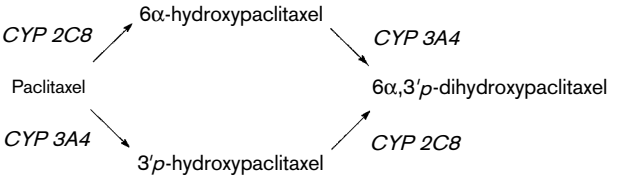

Figure 2. Major metabolic pathways of paclitaxel.

men. Furthermore, absence of systemic Cremophor EL is important, because the co-solvent is responsible for the non-linear pharmacokinetic behavior of i.v. paclitaxel.[14,34] 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[27] implies that after oral paclitaxel 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.[27] Therefore, pharmacokinetics of i.v. paclitaxel and orally administered paclitaxel, with and without systemic Cremophor EL, are substantially different and comparison of the pharmacokinetic parameters should be done with caution.

In conclusion, dose reduction of CsA from 10 to 5 mg/kg resulted in a significant decrease in the AUC, $T>0.1$ μM and $T>0.05$ μM values of orally administered paclitaxel. However, the 5 mg/kg dose still provided paclitaxel levels that were greater than after paclitaxel alone.[6] CsA 10 mg/kg resulted in paclitaxel AUC, $T>0.1$ μM and $T>0.05$ μM values comparable to CsA 15 mg/kg, which was previously shown to reveal maximal inhibition of P-gp. Thus, CsA 10 mg/kg is determined as the minimally effective dose of CsA with a maximal increase in the systemic exposure to paclitaxel and is recommended for further studies of orally administered paclitaxel.

References

1. Rowinsky EK, Donehower RC. Paclitaxel (Taxol). *N Engl J Med* 1995; **332**: 1004–14.
2. Huizing MT, Sewberath Misser VH, Pieters RC, *et al*. Taxanes: a new class of antitumor agents. *Cancer Invest* 1995; **13**: 381–404.
3. Sparreboom A, Van Asperen J, Mayer U, *et al*. Limited oral bioavailability and active epithelial secretion of paclitaxel caused by P-glycoprotein in the intestine. *Proc Natl Acad Sci USA* 1997; **94**: 2031–5.
4. Van Asperen J, Van Tellingen O, Sparreboom A, *et al*. Enhanced oral bioavailability of paclitaxel in mice co-treated with the P-glycoprotein blocker SDZ PSC 833. *Br J Cancer* 1997; **76**: 1181–3.
5. Van Asperen J, Van Tellingen O, Van der Valk MA, Rozenhart M, Beijnen JH. Enhanced oral absorption and decreased elimination of paclitaxel in mice co-treated with cyclosporin A. *Clin Cancer Res* 1998; **4**: 2293–7.
6. Meerum Terwogt JM, Beijnen JH, ten Bokkel Huinink WW, Rosing H, Schellens JHM. Co-administration of cyclosporin enables oral therapy with paclitaxel. *Lancet* 1998; **352**: 285.
7. Meerum Terwogt JM, Malingré MM, Beijnen JH, *et al*. Co-administration of cyclosporin A enables oral therapy with paclitaxel. *Clin Cancer Res* 1999; **5**: 3379–84.
8. Kelland LR, Abel G. Comparative *in vitro* cytotoxicity of Taxol and Taxotere against cisplatin-sensitive and -resistant human ovarian carcinoma cell lines. *Cancer Chemother Pharmacol* 1992; **30**: 444–50.
9. Liebmann JE, Cook JA, Lipschultz C, Teague D, Fisher J, Mitchell JB. Cytotoxic studies of paclitaxel (Taxol) in human tumour cell lines. *Br J Cancer* 1993; **68**: 1104–9.
10. Lopes NM, Adams EG, Pitts TW, Bhuyan BK. Cell kill kinetics and cell cycle effects of Taxol on human and hamster ovarian cell lines. *Cancer Chemother Pharmacol* 1993; **32**: 235–42.
11. Baguley BC, Marshall ES, Whittaker JR, *et al*. Resistance mechanisms determining the *in vitro* sensitivity to paclitaxel of tumour cells cultured from patients with ovarian cancer. *Eur J Cancer* 1995; **31**: 230–7.
12. Raymond E, Hanauske A, Faivre S, *et al*. Effects of prolonged versus short-term exposure paclitaxel (Taxol) on human tumor colony-forming units. *Anti-Cancer Drugs* 1997; **8**: 379–85.
13. Huizing MT, Keung AC, Rosing H, *et al*. Pharmacokinetics of paclitaxel and metabolites in a randomized comparative study in platinum-pretreated ovarian cancer patients. *J Clin Oncol* 1993; **11**: 2117–35.
14. Gianni L, Kearns CM, Giani A, *et al*. Nonlinear pharmacokinetics and metabolism of paclitaxel and its pharmacokinetic/pharmacodynamic relationships in humans. *J Clin Oncol* 1995; **13**: 180–90.
15. Ohtsu T, Yasutsuna S, Tamura T, *et al*. Clinical pharmacokinetics and pharmacodynamics of paclitaxel: a 3-hour infusion versus a 24-hour infusion. *Clin Cancer Res* 1995; **1**: 599–606.
16. Huizing MT, Giaccone G, Van Warmerdam LJC, *et al*. Pharmacokinetics of paclitaxel and carboplatin in a dose-escalating and sequencing study in patients with non-small cell lung cancer. *J Clin Oncol* 1997; **15**: 317–29.
17. Malingré MM, Meerum Terwogt JM, Beijnen JH, *et al*. Phase I and pharmacokinetic study of oral paclitaxel. *J Clin Oncol* 2000; **18**: 2468–75.
18. Malingré MM, Beijnen JH, Rosing H, *et al*. A phase I and pharmacokinetic study of bi-daily dosing of oral paclitaxel in combination with cyclosporin A. *Cancer Chemother Pharmacol*, in press
19. Kahan BD. Cyclosporine. *N Engl J Med* 1989; **321**: 1725–38.
20. Myers BD, Ross J, Newton L, Luetscher J, Perlroth M. Cyclosporine-associated chronic nephropathy. *N Engl J Med* 1984; **311**: 699–705.
21. Dye D, Watkins J. Suspected anaphylactic reaction to Cremophor EL. *Br Med J* 1980; **280**: 1353.
22. *Guidelines for reporting of adverse drug reactions*. Bethesda, MD: National Cancer Institute, Division of Cancer Treatment 1988.
23. *WHO handbook for reporting results of cancer treatment*. Geneva: WHO 1979.

24. Chan GL, Weinstein SS, Lefor WW, Spoto E, Kahana L, Shires DL. Relative performance of specific and non-specific fluorescence immunoassay for cyclosporin in transplant patients. *Ther Drug Monit* 1992; **14**: 42–7.
25. Huizing MT, Sparreboom A, Rosing H, Van Tellingen O, Pinedo HM, Beijnen JH. Quantification of paclitaxel metabolites in human plasma by high-performance liquid chromatography. *J Chromatogr B* 1995; **674**: 261–8.
26. Sparreboom A, Van Tellingen O, Huizing MT, Nooijen WJ, Beijnen JH. Determination of polyoxyethyleneglycerol triricinoleate 35 (Cremophor EL) in plasma by pre-column derivatization and reversed-phase high-performance liquid chromatography. *J Chromatogr B* 1996; **681**: 355–62.
27. Van Tellingen O, Huizing MT, Nannan Panday VR, Schellens JHM, Nooijen WJ, Beijnen JH. Cremophor EL causes (pseudo)-non-linear pharmacokinetics of paclitaxel in patients. *Br J Cancer* 1999; **81**: 330–5.
28. Huizing MT, Rosing H, Koopman FJ, Keung ACF, Pinedo HM, Beijnen JH. High-performance liquid chromatographic procedures for the quantitative determination of paclitaxel (Taxol) in human urine. *J Chromatogr B* 1995; **664**: 373–82.
29. Huizing MT, Van Warmerdam LJC, Rosing H, *et al*. Limited sampling strategies for investigating paclitaxel pharmacokinetics in patients receiving 175 mg/m^2 as a 3-hour infusion. *Clin Drug Invest* 1995; **9**: 344–53.
30. Gibaldi M, Perrier D. Noncompartmental analysis based on statistical moment theory. In: Swarbrick J, ed. *Pharmacokinetics*. New York, NY: Marcel Dekker 1982: 409–17.
31. Cresteil T, Monsarrat B, Alvinerie P, Treluyer JM, Vieira I, Wright M. Taxol metabolism by human liver microsomes: identification of cytochrome P450 isozymes involved in its biotransformation. *Cancer Res* 1994; **54**: 386–92.
32. Monsarrat B, Mariel E, Cros S, *et al*. Taxol metabolism. Isolation and identification of three major metabolites of taxol in rat bile. *Drug Metab Disp* 1990; **18**: 895–901.
33. Shet MS, Fisher CW, Holmans PL, Estabrook RW. Human cytochrome P450 3A4: enzymatic properties of a purified recombinant fusion protein containing NADPH-P450 reductase. *Proc Natl Acad Sci USA* 1993; **90**: 11748–52.
34. Sparreboom A, Van Tellingen O, Nooijen WJ, Beijnen JH. Nonlinear pharmacokinetics of paclitaxel in mice results from the pharmaceutical vehicle Cremophor EL. *Cancer Res* 1996; **56**: 2112–5.
35. Sparreboom A, Van Tellingen O, Nooijen WJ, Beijnen JH. Tissue distribution, metabolism and excretion of paclitaxel in mice. *Anti-Cancer Drugs* 1996; **7**: 78–86.

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