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ORIGINAL ARTICLE

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P450 induction alters paclitaxel pharmacokinetics and tissue distribution with multiple dosing

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Abstract *Purpose*: Paclitaxel (Taxol) is an effective agent against a broad range of human cancers. Studies on the metabolism and disposition of paclitaxel have shown that it is primarily eliminated via hepatic metabolism by P450 enzymes (2C8 and 3A4) to essentially inactive metabolites, and that biliary and gut transport by P-glycoprotein (PGP) as well as urinary elimination of the parent compound play relatively minor roles. Recent studies in vitro have shown that paclitaxel treatment increases the level of CYP2C8 and CYP3A4 in human hepatocytes as well as PGP in colon tumor cells. The data suggest that previous paclitaxel exposure may influence metabolism and elimination of subsequent doses. Further, since weekly paclitaxel dose schedules are becoming more common as opposed to the original every 21-day dosing, the likelihood of enzyme induction from previous doses impacting that from subsequent doses is increased. *Methods*: To study the potential for such sequence-dependent alterations in paclitaxel pharmacokinetics, we carried out pharmacokinetic studies in mouse plasma and tissues following day 1 and days 1 and 5 dosing at 20 mg/kg. Paclitaxel concentrations were determined by a sensitive LC/MS/MS assay out to 16 h post-dosing in plasma, liver, kidney, gut and heart. The effect of paclitaxel treatment on hepatic expression of PGP and P450 isoforms (CYP2C and CYP3A) was determined to elucidate the mechanism by which paclitaxel disposition is altered by previous drug exposure. *Results*: Pharmacokinetic analysis of the data showed that plasma and tissue AUC values after treatment on day 5 following a dose on day 1 were between 50% and 74% of those determined following a single dose on day 1. The terminal elimination half-life was not different. Activity and protein levels for CYP2C in liver were elevated at 24 and 96 h after paclitaxel dosing. Cremophor EL, a carrier solvent for paclitaxel, also caused elevated CYP2C activity. Neither CYP3A nor PGP levels in liver were altered by paclitaxel or Cremophor EL treatment at the 24-h and 96-h time points. The levels of 6α-OH-paclitaxel in feces were increased on day 5 as opposed to day 1 while paclitaxel levels in feces were unchanged. *Conclusions*: The results of our studies showed that paclitaxel pharmacokinetics are altered by previous paclitaxel exposure up to 96 h earlier.

Keywords Paclitaxel · Cytochrome P450 · Pharmacokinetics · P-glycoprotein · Mice

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Introduction

Paclitaxel (PTX) is a member of a class of antitumor agents known as the taxanes. Taxanes act by interacting with tubulin microtubes [21] thus preventing normal mitosis, and show cell cycle specificity for cytotoxicity, being much more toxic to mitotic cells than interphase cells [15]. The taxanes have shown considerable activity in the treatment of breast, ovarian and non-small-cell lung cancer [12], and are currently being investigated in combination therapy for a number of cancers with other chemotherapeutic agents.

Following intravenous administration of PTX, the drug is extensively distributed to most tissues, but is excluded from the central nervous system and testes [4, 6, 13]. The major route of elimination is hepatic metabolism by CYP2C8 and CYP3A4, the human cytochrome P450s responsible for the biotransformation of PTX, and subsequent biliary elimination [9, 20].

Studies have also shown that transport of PTX mediated by P-glycoprotein (PGP) plays a role in the elimination kinetics as well as limiting oral bioavailability [25, 30]. Therefore, alterations in P450 and/or PGP expression could be expected to alter PTX pharmacokinetics.

A complicating factor in PTX pharmacokinetics is that it is administered as a pharmaceutical preparation containing 50% Cremophor EL and 50% dehydrated ethanol. Cremophor EL is known to alter the pharmacokinetics of PTX in both mice [26] and humans [31]. The mechanisms by which Cremophor EL affects pharmacokinetics are thought to involve alterations in plasma drug partitioning due to micelle-like structures carrying the PTX in the plasma [10, 27], hemodynamic changes [3] and alteration in PGP function [23].

PTX has been shown to activate the steroid and xenobiotic receptor (SXR) to regulate expression of CYP3A4 and PGP in human cells [29]. The potential to modulate its own metabolism and elimination suggests that the pharmacokinetics of PTX may differ across courses of treatment and contribute to interpatient variability. Further, clinical success of weekly PTX dosing [7] may increase the potential for altered metabolism due to decreased interdose time compared to traditional protocols. The studies presented here address the potential for course-dependent changes in PTX pharmacokinetics using an in vivo model by determining changes in pharmacokinetics and PTX-dependent changes in P450 and PGP expression in mouse liver.

Materials and methods

Chemicals and reagents

PTX was purchased from the University of Colorado Hospital Pharmacy. Docetaxel was purchased from LKT laboratories (St. Paul, Minn.). Cremophor EL was purchased from Aldrich Chemical Co. (Milwaukee, Wis.). Bovine serum albumin, erythromycin, formaldehyde, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, glutathione (reduced), glycine, HEPES, Ipegal CA-630, magnesium chloride, $NADP^+$, phenylmethylsulfonyl fluoride, sodium chloride, sodium dodecyl sulfate, Tris and Tween 20 were purchased from Sigma Chemical Co. (St. Louis, Mo.). Acetonitrile, ammonium acetate and methanol were purchased from Fisher Scientific (Pittsburgh, Pa.). All other reagents were of analytical grade.

Study design and animal treatments

Female, Balb/c mice (8–10 weeks old) were purchased from Charles River Laboratories (Wilmington, Mass.) and allowed to acclimatize for 7 days. Animals were housed three per cage in polycarbonate cages and kept on a 12-h light/dark cycle. Food and water were given ad libitum. Following acclimation, mice were randomly divided into two groups for the pharmacokinetic studies and three groups for the PGP and P450 induction studies and were treated via intravenous injection (tail vein) as shown in Fig. 1. All studies were conducted in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals, and animals were housed in a facility accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC).

Following treatment, animals were killed by cardiac stick exsanguination under isoflurane anesthesia and tissue and blood samples were collected at 15 and 30 min, and 1, 2, 8, 12 and 16 h following the PTX injection for pharmacokinetic studies. Plasma, heart, liver, kidney and intestine were removed, frozen in liquid nitrogen, and stored at −80°C prior to extraction and analysis. Animals killed at the 8-, 12- and 16-h time points were housed in metabolic cages and feces collected for analysis of drug content. For studies measuring the induction of PGP, CYP3A4 and CYP2C8 following Cremophor EL or PTX treatment, liver tissue was collected at 24 and 96 h after PTX treatment and frozen in liquid nitrogen and stored at −80°C prior to preparation of membrane-enriched fractions and microsome isolation.

Analysis of paclitaxel and 6α-OH-paclitaxel

Tissue homogenate (100 µl; 100 mg/ml in water) or 100 µl plasma was spiked with 25 pmol docetaxel (25 µl of 1 µ*M*; internal standard), mixed and added to 750 µl of 10 m*M* ammonium acetate in acetonitrile to

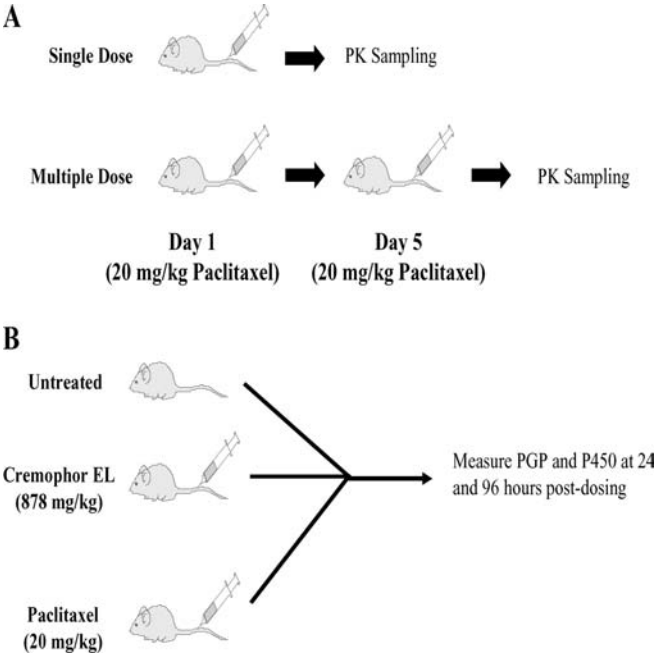

Fig. 1 Study design schematics for the pharmacokinetic study (**a**) and studies determining changes in PGP and P450 expression (**b**)

precipitate proteins. Following centrifugation at 10,000 RCF for 10 min, the supernatant was collected, the volume adjusted to 1 ml with mobile phase and 20 µl injected onto the LC/MS/MS system. Analyses were performed with a PE Sciex API-3000 with a turbo ionspray source. The LC system consisted of a 150 mm C_{18} column (2 mm i.d.) with an isocratic mobile phase consisting of 65% acetonitrile and 35% 10 m*M* ammonium acetate. The flow rate was 100 µl/min. The instrument was operated in the selected reaction monitoring (SRM) mode (positive ion), monitoring the ion transitions from m/z 808 → 226 for docetaxel (internal standard), m/z 854 → 286 for PTX and m/z 872 → 286 for 6α-OH-PTX. The SRM peaks eluted at 7.6 min for 6α-OH-PTX, 9.6 min for docetaxel and 10.5 min for PTX. Chromatographic resolution of the SRM peaks was necessitated by an ammonium adduct formed with PTX giving rise to an m/z of 872 that mimicked the 6α-OH-PTX parent–daughter ions.

Immunoblotting for CYP2C, CYP3A and P-glycoprotein

For immunoblot analysis of PGP in liver samples, membrane-enriched fractions from liver homogenates were used. Liver samples were homogenized in ten times tissue volume of 10 m*M* Tris (pH 8.6), 140 m*M* NaCl, 1.5 m*M* $MgCl_2$, 1% Ipegal CA-630 and 2 m*M* PMSF. Homogenates were then centrifuged at 31,000 RCF for 20 min at 4°C and the resulting supernatant collected. Liver membrane-enriched fractions (100 µg) were separated on SDS-PAGE gel (6%) and transferred to PVDF membranes. Membranes were blocked for 2 h at room temperature in 2% non-fat dried milk (NFDM) in TBST (10 m*M* Tris, pH 7.5, 100 m*M* NaCl, 0.1% v/v Tween 20) and then incubated overnight at 4°C in a 1:500 dilution of rabbit polyclonal anti-PGP antibody (cat. no. PC03; Oncogene Research Products, Boston, Mass.) in TBST with 2% NFDM. Following the overnight incubation with primary antibody, membranes were washed three times for 15 min each with TBST. The secondary antibody (1:3000 dilution in TBST with 2% NFDM), goat anti-rabbit IgG HRP conjugate (cat. no. sc-2030; Santa Cruz Biotechnology, Santa Cruz, Calif.), was then added followed by incubation at room temperature for 2 h. Following incubation with the secondary antibody, the blots were washed three times for 15 min in TBST and developed using an ECL-plus detection kit (Amersham Biosciences, Piscataway, N.J.) and visualized and quantitated using a STORM-gel and blot imaging system (Molecular Dynamics, Sunnyvale, Calif.) equipped with ImageQuant software (Molecular Dynamics).

For immunoblot analysis of CYP3A and CYP2C in mouse liver, microsomal fractions were isolated using a standard differential centrifugation procedure and total protein concentration determined. Microsomal protein samples (5 µg) were separated on 10% SDS-PAGE gels and transferred to PVDF membranes. The membranes were blocked overnight at 4°C in TBST with 5% NFDM. A rabbit polyclonal antibody to CYP3A (cat. no. AB1253) was purchased from Chemicon International (Temecula, Calif.) and a rabbit polyclonal antibody to human CYP2C8 was purchased from Serotec (Oxford, UK). Human CYP2C8 Supersomes were purchased from Gentest Corporation (Woburn, Mass.) and used as a positive control for CYP2C blots. Incubation with the primary antibody dilutions for CYP3A (1:500) and CYP2C (1:5000) were carried out for 2 h at room temperature in TBST with 5% NFDM. Following primary antibody incubation, membranes were washed three times for 15 min and then incubated with secondary antibody and developed as described above for PGP blots.

Determination of CYP2C8 and CYP3A4 enzymatic activity

CYP2C activity was measured in mouse microsomes using the P450-Glo CYP2C8 assay (Promega Corporation, Madison, Wis.) according to the manufacturer's protocol (Promega Technical Bulletin no. 325). A standard curve using human CYP2C8 Supersomes was used to show linearity in the assay at the enzyme levels used and to quantify activity in mouse microsomes in units relative to the human Supersome activity. To verify the relationship between the immunoreactive protein from immunoblot studies and the measured activity, the ability of the antibody used for immunoblot analysis to inhibit the measured CYP2C activity in mouse microsomes was verified (data not shown). The human CYP2C8 antibody used for immunoblotting has previously been shown to be a neutralizing antibody and a potent inhibitor of CYP2C8 activity (Serotec product data sheet). CYP2C activity in mouse microsomes is expressed as picomoles human CYP2C8 equivalents per milligram microsomal protein.

CYP3A activity was determined spectrophotometrically by measuring the formation of formaldehyde as previously described [19, 33]. Briefly, microsome isolations were diluted to 60 µg total protein in 100 µl water. To the microsome sample was added, 4.8 µl 250 m*M* erythromycin, 85.2 µl water and 60 µl of assay buffer (250 n *M* HEPES/KOH, pH 7.4, 12 m*M* reduced glutathione and 150 m*M* $MgCl_2$) and the sample was incubated at 37°C for 3 min. The reaction was started with the addition of 30 µl of a NADPH-regenerating system (100 m*M* glucose-6-phosphate, 5 m*M* $NADP^+$, and 25 U/ml glucose-6-phosphate dehydrogenase) and was incubated for 10 min at 37°C. The reaction was stopped with the addition of 60 µl 40% (w/v) trichloroacetic acid and 300 µl of NASH reagent (5 g ammonium acetate, 100 µl acetyl acetone in 6 ml 3% w/v acetic acid) was immediately added and the solution incubated at 65°C for 10 min. The samples were allowed

to cool and the absorbance measured at 412 nm. A standard curve of formaldehyde was used to quantify the amount of formaldehyde formed in the reaction.

Total protein determination

Total protein in samples was determined using the BCA protein assay reagent (Pierce Biochemicals, Rockford, Ill.) with bovine serum albumin as a standard.

Pharmacokinetic modeling and data analysis

Pharmacokinetic parameters were calculated by compartmental and non-compartmental analysis using WinNonlin Professional (version 4.0.1; Pharsight Corporation, Mountain View, Calif.). Treatment groups were compared at defined time points by one-way ANOVA using the Tukey post-test for pairwise comparison. Statistical analyses were carried out using Sigma Stat version 2.03 (SPSS, Chicago, Ill.).

Results

The plasma pharmacokinetics of PTX measured on day 1 and on day 5 following days 1 and 5 dosing were determined and pharmacokinetic calculations carried out using from the two-compartment model shown in Table 1. Plasma pharmacokinetic data showed that the model-derived C_{max} was increased on day 1 (79.2 ± 5.8 μM) as opposed to day 5 (32.6 ± 6.8 μM), and these initial higher concentrations led to an increase in drug exposure as measured by AUC on day 1 (125.6 ± 3.8 μM·h) when compared to day 5 (86.2 ± 5.5 μM·h). Neither the distribution ($t_{1/2}\alpha$) nor the elimination ($t_{1/2}\beta$) half-lives was dramatically altered on day 5 as compared to day 1. In comparing the measured plasma levels at each individual time point on day 1 to those on day 5, the only individual time point that was significantly different ($P < 0.05$) was the 30-min point where day-1 levels were 51.5 ± 15.2 μM and day-5 levels 25.2 ± 15.6 μM as determined in the three animals at each point.

Tissue levels of PTX were measured in gut, heart, kidney and liver concurrent with plasma level determinations and calculated tissue pharmacokinetic data shown in Table 2. Significant reductions ($P < 0.05$) in tissue PTX levels were seen in gut, heart and liver at 12 h after dosing on day 5 when compared to day 1 and at 8 h in heart and kidney tissue. No significant changes were measured prior to 8 h after dosing. Consistently, however, tissue levels were decreased on day 5 in comparison to day 1, with a few exceptions, and this translated to tissue AUC values on day 5 being decreased between 25% and 40% in comparison to day 1. C_{max} levels were decreased on day 5 as compared to day 1 in all tissues except gut. Terminal half-life ($t_{1/2}\lambda$) values were not changed between day 1 and day 5.

The effect of Cremophor EL and PTX treatment on levels of mouse CYP2C and CYP3A enzyme activities in liver microsomes was determined 24 and 96 h after dosing and the results are shown in Fig. 2. Both Cremophor EL and PTX treatment caused a significant increase in CYP2C activity. At 24 h, levels in the control, Cremophor EL, and PTX treatment groups were 2.6 ± 1.2, 10.0 ± 5.3, and 13.7 ± 8.2 pmol human CYP2C8 equivalents per milligram microsomal protein, respectively. At 96 h, only the level in the PTX group (5.6 ± 1.5 pmol) was significantly elevated as compared to that in the control group (2.4 ± 2.7 pmol). Immunoblot analysis of CYP2C protein in microsomes showed similar results to those determined from activity measurements, and representative blots are shown in Fig. 3. Densitometry of CYP2C bands showed significant elevation of CYP2C protein in both the Cremophor EL and PTX treatment groups at 24 h after treatment, but no significant differences at 96 h. When normalized to control densitometric values (1.00 ± 0.11), at 24 and 96 h levels in the Cremophor EL treatment group were 1.62 ± 0.28 and 1.34 ± 0.37 and in the PTX treatment group were 1.85 ± 0.22 and 1.52 ± 0.28, respectively. CYP3A activity levels were not significantly altered by either Cremophor EL or PTX treatment (Fig. 2b), and immunoblot analysis showed no change in CYP3A protein levels (data not shown).

The effects of Cremophor EL or PTX on PGP levels in membrane-enriched liver fractions were measured by immunoblotting at 24 and 96 h after treatment. Immunoblots were quantitated by densitometry of mdr1a and

Table 1 Plasma pharmacokinetics of PTX following a 20 mg/kg i.v. dose on days 1 and 5. Pharmacokinetic parameters were calculated using a two-compartment model. Values are the calculated pharmacokinetic parameter ± the standard error of the estimate using model-dependent calculations

Parameter	Day 1	Day 5
C_{max} (μM)	79.2 ± 5.8	32.6 ± 6.8
AUC (μM h)	125.6 ± 3.8	86.2 ± 5.5
$t_{1/2}\alpha$ (h)	0.60 ± 0.07	0.66 ± 0.21
$t_{1/2}\beta$ (h)	3.13 ± 0.18	3.52 ± 0.46

Table 2 Tissue pharmacokinetics of paclitaxel following a 20 mg/kg i.v. dose on days 1 and 5. Tissue pharmacokinetics were calculated using non-compartmental analysis

Tissue	Day	C_{max} (nmol/g)	$AUC_{0 \rightarrow \infty}$ (nmol/g h)	$t_{1/2}\lambda$ (h)
Gut	1	29.4	151	2.8
	5	31.3	117	2.3
Heart	1	32.7	81	3.2
	5	24.6	57	2.7
Kidney	1	44.3	127	1.9
	5	32.1	95	2.0
Liver	1	100.2	415	2.1
	5	76.9	251	2.7

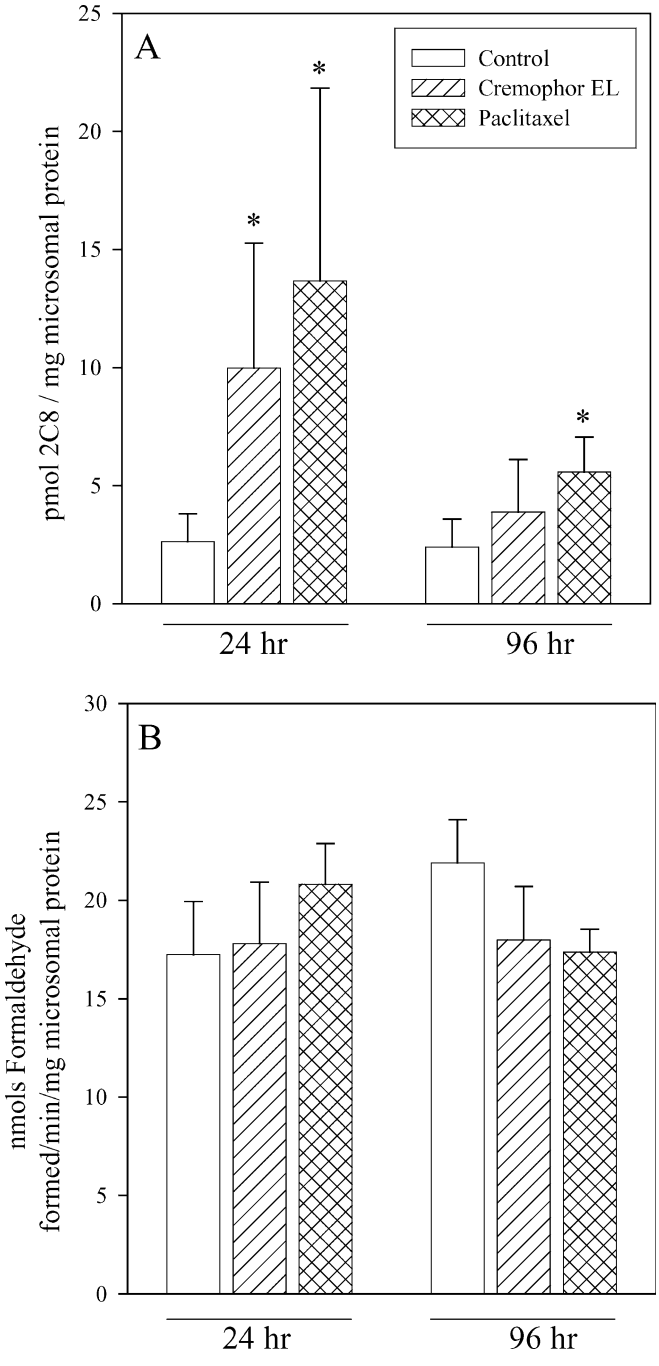

Fig. 2 Cytochrome P450 activity in control, Cremophor EL and PTX treated mouse liver microsomes as shown in Fig. 1b. CYP2C (**a**) and CYP3A (**b**) activities were determined as described in Materials and methods. Values are the means ± SD of six determinations for each treatment group and time point. *$P < 0.05$ vs control group

the results are shown in Fig. 4. No significant changes were measured in mdr1a (band of about 160 kDa) or mdr1b (band of about 180 kDa, data not shown) [5] levels in either the Cremophor EL or PTX treatment groups. When normalized to the density of the control band (100.0 ± 8.4), mdr1a protein levels at 24 and 96 h in the Cremophor EL treatment group were 72.5 ± 8.5 and 106.4 ± 10.9 and in the PTX treatment group were 90.9 ± 9.0 and 86.8 ± 16.9, respectively.

Fecal elimination of PTX and 6α-OH-PTX were determined by measuring accumulation in the feces at 8, 12 and 16 h after dosing. The results in Table 3 show that PTX elimination in the feces was not different between day 1 and day 5 dosing. 6α-OH-PTX accumulation in the feces was increased on day 5 compared to day 1. The amounts of the 6α-OH-PTX in the feces were 5.9, 60.4 and 164.7 nmol at 8, 12 and 16 h after dosing on day 1, and amounts at the same time points on day 5 were 9.1, 106.4 and 206.8 nmol. These results are consistent with elevations in metabolizing capability via CYP2C as shown in Fig. 2a and no change in transport activity (PGP) of the parent compound (Fig. 4).

Discussion

PTX has considerable activity in the treatment of breast, ovarian and non-small-cell lung cancer [12] and is actively being investigated for use in combination therapy for other cancers with many other agents. Generally, PTX has been given at a dose between 100 and 200 mg/m^2 every 21 days. However, recent studies have shown a response to weekly dosing in patients who were refractory to PTX on a standard 3-week schedule [7]. There is some evidence that PTX may be effective when dosed using metronomic schedules, which involve frequent low doses targeting the cytotoxic drug to proliferating endothelial cells within the tumor as opposed to neoplastic cells themselves. The taxanes (PTX and docetaxel) have been shown to fulfill the criteria spelled out by Miller et al. [16] to be considered as antiangiogenic. These criteria include inhibiting endothelial cell proliferation at concentrations lower than those required for tumor cells [2, 28], interfering with endothelial cell functions at doses that are not toxic to the cells [2, 11], and altering tumor architecture such that therapeutic benefit for drug delivery and decreased angiogenic signaling by the tumor can be achieved [8]. Further, both taxanes have shown antiangiogenic activity in vivo [2,

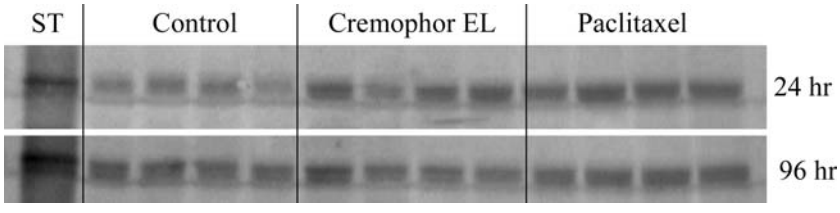

Fig. 3 Immunoblots of CYP2C protein in mouse liver microsomes 24 and 96 h after treatment with Cremophor EL or PTX as shown in Fig. 1b. *ST* human CYP2C8 Supersomes (2 pg)

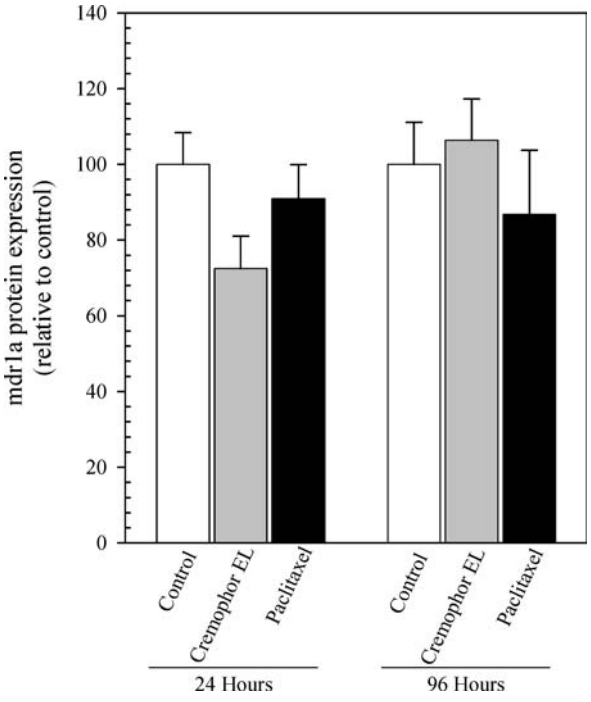

Fig. 4 mdr1a protein levels in mouse liver membrane-enriched fractions at 24 and 96 h after treatment with Cremophor EL or PTX. Values are the means ± SD of six determinations from each treatment group and time point. The values from the treated samples were normalized to those from the control samples run on the same gel

14, 28]. The antiangiogenic activity of PTX makes it an ideal candidate for study in metronomic schedules.

A potential issue in more frequent dosing of PTX is that metabolism and elimination may be induced with more constant drug exposure. The time-dependent alteration in PTX pharmacokinetics could necessitate time-dependent changes in dosing. PTX is metabolized by the cytochrome P450 system to hydroxylated metabolites. The primary metabolites formed in humans

Table 3 Fecal elimination of paclitaxel and 6α-OH-paclitaxel following a 20 mg/kg i.v. dose on days 1 and 5. Total drug and metabolite amounts were determined in pooled total feces samples from three animals housed in metabolic cages for the indicated times after dosing. Calculation of the percent of dose was based on a dose of 20 mg/kg to a 20-g mouse being equal to 469 nmol PTX per mouse, with three mice totaling 1407 nmol PTX

	PTX		6α-OH-PTX	
	nmol	Percent of dose	nmol	Percent of dose
8 h after dosing				
Day 1 dose	67.1	4.8	5.9	0.4
Day 5 dose	71.4	5.1	9.1	0.7
12 h after dosing				
Day 1 dose	210.1	14.9	60.4	4.3
Day 5 dose	215.5	15.3	106.4	7.6
16 h after dosing				
Day 1 dose	470.3	33.4	164.7	11.7
Day 5 dose	492.1	35.0	206.8	14.7

are the 6α-OH-PTX by CYP2C8 [20] and the 3-OH-PTX by CYP3A4 [9] and the 6α-3-dihydroxypaclitaxel metabolite formed by the action of both P450 isoforms. The predominant metabolite is 6α-OH-PTX [32]. PTX is also excreted unchanged in the feces through biliary transport from the liver and direct transepithelial transport from the intestine [32]. Small fractions of drug are also excreted in the urine [32]. Studies have shown correlations between drug elimination and P450 activity in humans for PTX [17, 24] and induction of P450 enzymes has shown alteration in PTX pharmacokinetics in animal models [1]. PGP has also been shown to play a role in the elimination of PTX and for the low oral bioavailability [22, 25, 30].

Induction of CYP2C8, CYP3A4 and PGP has been shown in human hepatocytes and CYP3A4 and PGP in human LS1080 colon cancer cells, and this induction has been shown to proceed through the SXR with the displacement of transcriptional corepressors being the putative mechanism of gene activation by PTX [29]. Interestingly docetaxel was not capable of activating either PGP or P450 through the SXR in these studies. The concentrations of PTX shown to activate human SXR reporter gene expression and murine pregnane X receptor (PXR) is in the micromolar range, which is well within the concentration range for PTX that we determined for liver in these studies. The murine PXR has been shown to be important in the induction of CYP3A by PTX in mice and these studies have shown large inductions in CYP3A mRNA, protein and enzymatic activity [18]. However, in the study, animals were treated with intraperitoneal doses of PTX at 40 mg/kg for four consecutive days prior to measurements 24 h after the last treatment. The difference in dosing and route of delivery may help explain the lack of CYP3A activation shown in our studies.

In the studies presented here, we showed that PTX treatment on day 1 altered PTX pharmacokinetics following drug administration on day 5. Further, measurement of primary PTX metabolizing and elimination pathway induction by treatment on day 1 suggested that CYP2C activation is the major determinant of pharmacokinetic changes in a mouse model. It has been shown that PTX can alter the expression of CYP3A4, CYP2C8 and PGP in human cells and that the induction proceeds coordinately through the SXR [29]. Coordinate regulation has not been shown in mice, although CYP3A activation in mice by PTX has been shown to be through the PXR. The potential for differential coordinate regulation of these genes in mice and humans may explain why in our studies we see CYP2C activation in the absence of CYP3A and PGP activation. Multiple activation pathways and dose- and time-dependent factors could also be factors.

An important focus for preclinical studies is the dose–response relationships in animal models and how they relate to clinical use. Therefore, studying time and dose-dependent changes in drug pharmacokinetics in relevant animal models will allow refinement of animal dosing

and reflection back to the clinical situation. This is becoming increasingly more important when studying metronomic schedules in that maintenance of plasma levels at the endothelial cell proliferation inhibition threshold may be the relevant pharmacokinetic endpoint relating to therapeutic benefit. In frequent, lower dose protocols, the more constant exposure to PTX may lead to pharmacokinetically relevant changes in drug metabolism and elimination such that modification in dosing may be beneficial.

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References

1. Anderson CG, Wang J, Kumar GN, McMillan JM, Walle UK, Walle T (1995) Dexamethasone induction of taxol metabolism in the rat. Drug Metab Dispos 23:1286
2. Belotti D, Vergani V, Drudis T, Borsotti P, Pitelli MR, Viale G, Giavazzi R, Taraboletti G (1996) The microtubule-affecting drug paclitaxel has antiangiogenic activity. Clin Cancer Res 2:1843
3. Bowers VD, Locker S, Ames S, Jennings W, Corry RJ (1991) The hemodynamic effects of Cremophor-EL. Transplantation 51:847
4. Clarke SJ, Rivory LP (1999) Clinical pharmacokinetics of docetaxel. Clin Pharmacokinet 36:99
5. Devault A, Gros P (1990) Two members of the mouse *mdr* gene family confer multidrug resistance with overlapping but distinct drug specificities. Mol Cell Biol 10:1652
6. Eiseman JL, Eddington ND, Leslie J, MacAuley C, Sentz DL, Zuhowski M, Kujawa JM, Young D, Egorin MJ (1994) Plasma pharmacokinetics and tissue distribution of paclitaxel in CD_{2F1} mice. Cancer Chemother Pharmacol 34:465
7. Fennelly D, Aghajanian C, Shapiro F, O'Flaherty C, McKenzie M, O'Connor C, Tong W, Norton L, Spriggs D (1997) Phase I and pharmacologic study of paclitaxel administered weekly in patients with relapsed ovarian cancer. J Clin Oncol 15:187
8. Griffon-Etienne G, Boucher Y, Brekken C, Suit HD, Jain RK (1999) Taxane induced apoptosis decompresses blood vessels and lowers interstitial fluid pressure in solid tumors: clinical complications. Cancer Res 59:3776
9. Harris JW, Rahman A, Kim BR, Guengerich FP, Collins JM (1994) Metabolism of taxol by human hepatic microsomes and liver slices: participation of cytochrome P450 3A4 and an unknown P450 enzyme. Cancer Res 54:4026
10. Henningsson A, Karlsson MO, Vigano L, Gianni L, Verweij J, Sparreboom A (2001) Mechanism-based pharmacokinetic model for paclitaxel. J Clin Oncol 19:4065
11. Hotchkiss KA, Ashton AW, Sparano J, Schwartz EL (2000) Inhibition of endothelial cell function by docetaxel (Taxotere). Proc Am Assoc Cancer Res 41:647
12. Huizing MT, Misser VH, Pieters RC, ten Bokkel Huinink WW, Veenhof CH, Vermorken JB, Pinedo HM, Beijnen JH (1995) Taxanes: a new class of antitumor agents. Cancer Invest 13:381
13. Innocenti F, Danesi R, Di Paolo A, Agen C, Nardini D, Bocci G, Del Tacca M (1995) Plasma and tissue disposition of paclitaxel (taxol) after intraperitoneal administration in mice. Drug Metab Dispos 23:713
14. Lau DH, Xue L, Young LJ, Burke PA, Cheung AT (1999) Paclitaxel (Taxol): an inhibitor of angiogenesis in a highly vascularized transgenic breast cancer. Cancer Biother Radiopharm 14:31
15. 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
16. Miller KD, Sweeney CJ, Sledge GW Jr (2001) Redefining the target: chemotherapeutics as antiangiogenics. J Clin Oncol 19:1195
17. Monsarrat B, Chatelut E, Royer I, Alvinerie P, Dubois J, Dezeuse A, Roche H, Cros S, Wright M, Canal P (1998) Modification of paclitaxel metabolism in a cancer patient by induction of P450 3A4. Drug Metab Dispos 26:229
18. Nallani SC, Goodwin B, Maglich JM, Buckley DJ, Buckley AR, Desai PB (2003) Induction of cytochrome P450 3A by paclitaxel in mice: pivotal role of the nuclear xenobiotic receptor, pregnane x receptor. Drug Metab Dispos 31:681
19. Nash T (1953) The colorimetric estimation of formaldehyde by means of the Hantzsch reaction. Biochem J 55:416
20. Rahman A, Korzekwa KR, Grogan G, Gonzalez FJ, Harris JW (1994) Selective biotransportation of taxol to 6α-hydroxytaxol by human cytochrome P450 2C8. Cancer Res 54:5543
21. Schiff PB, Fant J, Horwitz SB (1979) Promotion of microtube assembly in vitro by taxol. Nature 277:665
22. Schinkel AH, Mayer U, Wagenaar E, Mol CAAM, van Deemter L, Smit JJM, van der Valk MA, Voordouw AC, Spits H, van Tellingen O, Zilmans JMJM, Fibbe WE, Borst P (1997) Normal viability and altered pharmacokinetics in mice lacking mdr1-type (drug-transporting) P-glycoproteins. Proc Natl Acad Sci U S A 94:4028
23. Schuurhuis GJ, Broxterman HJ, Pinedo HM, van Heijningen TH, van Kalken CK, Vermorken JB, Spoelstra EC, Lankelma J (1990) The polyoxyethylene castor oil Cremophor EL modifies multidrug resistance. Br J Cancer 62:591
24. Sonnichsen DS, Liu Q, Schuetz EG, Schuetz JD, Pappo A, Relling MV (1995) Variability in human cytochrome P450 paclitaxel metabolism. J Pharmacol Exp Ther 275:566
25. Sparreboom A, van Asperen J, Mayer U, Schinkel AH, Smit JW, Meijer DK, Borst P, Nooijen WJ, Beijnen JH, Tellingen O (1997) Limited oral bioavailability and active epithelial excretion of paclitaxel (Taxol) caused by P-glycoprotein in the intestine. Proc Natl Acad Sci U S A 94:2031
26. 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
27. Sparreboom A, van Zuylen L, Brouwer E, Loos WJ, de Bruijn P, Gelderblom H, Pillay M, Nooter K, Stoter G, Verweij J (1999) Cremophor EL-mediated alteration of paclitaxel distribution in human blood: clinical pharmacokinetic implications. Cancer Res 59:1454
28. Sweeney CJ, Miller KD, Sissons SE, Nozaki S, Heilman DK, Shen J, Sledge GW Jr (2001) The antiangiogenic property of docetaxel is synergistic with a recombinant humanized monoclonal antibody against vascular endothelial growth factor or 2-methoxyestradiol but antagonized by endothelial growth factors. Cancer Res 61:3369
29. Synold TW, Dussault I, Forman BM (2001) The orphan nuclear receptor SXR coordinately regulates drug metabolism and efflux. Nat Med 7:584
30. van Asperen J, van Tellingen O, Sparreboom A, Schinkel AH, Borst P, Nooijen WJ, Beijnen JH (1997) Enhanced oral bioavailability of paclitaxel in mice treated with the P-glycoprotein blocker SDZ PSC 833. Br J Cancer 76:1181
31. 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
32. Walle T, Walle UK, Kumar GN, Bhalla KN (1995) Taxol metabolism and disposition in cancer patients. Drug Metab Dispos 23:506
33. Werringloer J (1978) Assay of formaldehyde generated during microsomal oxidation reactions. Methods Enzymol 52:297