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ORIGINAL ARTICLE

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Differences in the induction of cytochrome P450 3A4 by taxane anticancer drugs, docetaxel and paclitaxel, assessed employing primary human hepatocytes

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Abstract *Purpose*: The induction of cytochrome P450 (CYP) 3A4 by drugs and other xenobiotics is a common cause of serious drug interactions. The aim of this study was to comparatively examine the effects of paclitaxel and docetaxel, two structurally related taxane anticancer agents, on the activity and expression of hepatic CYP3A4. *Methods*: Employing primary cultures of human hepatocytes from multiple donors, we investigated the differences in the magnitude of CYP3A4 induction and relative accumulation of paclitaxel and docetaxel. The CYP3A4 activity of intact hepatocytes was measured as the rate of testosterone 6β-hydroxylation. The CYP3A4-specific immunoreactive protein and mRNA levels were measured employing Western blot and Northern blot analysis, respectively. Furthermore, employing cell-based reporter gene assay in CV-1 cells, we evaluated the capacity of paclitaxel and docetaxel to activate human pregnane X receptor (hPXR), an orphan nuclear receptor that plays a key role in the transcriptional regulation of CYP3A4. *Results*: In concurrence with previous reports, we observed that paclitaxel potently induced *CYP3A4* activity and expression in hepatocytes treated for 48–96 h. However, docetaxel did not increase the activity or the CYP3A4 immunoreactive protein levels for treatment periods up to 96 h. A marginal increase in the CYP3A4 mRNA levels was observed in cells treated with higher levels (5 and 10 μM) of docetaxel. Furthermore, while paclitaxel effectively activated hPXR (the half-maximal effective concentration, EC_{50}, being about 5.2 μM), docetaxel weakly activated hPXR, and moreover the activation occurred only at high concentrations relative to paclitaxel. A comparison of the cellular concentrations of paclitaxel and docetaxel, in the cell culture models employed for evaluating CYP3A4 induction and hPXR activation, revealed that the intracellular paclitaxel levels were three-fold higher than that of docetaxel. Thus, it appears that both pharmacokinetic (drug concentration) and pharmacodynamic differences (hPXR activation) may account for the observed differences in CYP3A induction by paclitaxel and docetaxel. *Conclusion*: Our studies suggest that docetaxel has markedly reduced propensity to cause drug interactions that may entail hepatic CYP3A4 induction.

Keywords CYP3A4 · hPXR · Paclitaxel · Docetaxel · Enzyme induction · Hepatocytes · Cotransfection assay

Abbreviations CYP: Cytochrome P450 · HMM: Hepatocyte maintenance medium · HPLC: High pressure liquid chromatography · hPXR: human pregnane X receptor · MTT: 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide · XREM: Xenobiotic response element module · DAS: Drug accumulation studies

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Introduction

Taxanes are a family of structurally related compounds that share a core ring structure referred to as baccatin III. Two members of this family, paclitaxel and docetaxel, are important anticancer drugs extensively used in the treatment of various malignancies including ovarian cancer, breast cancer and lung cancer [7]. These agents share a unique mechanism of action acting as promoters of microtubule polymerization, which leads to a variety of effects within dividing cells, including cell cycle arrest in the G_2/M phase, apoptosis, and acute cytotoxicity [14]. Given their high potency, unique mechanism of action, partially non-overlapping toxicities and relative

lack of cross-resistance with other agents, taxanes are widely used in combination chemotherapy regimens. Extensive investigations are underway to pharmacokinetically optimize chemotherapy regimens containing taxanes with the goal of enhancing the antitumor efficacy of the combinations while reducing the toxicity and adverse drug–drug interactions. Furthermore, significant efforts are also being made in designing second-generation taxanes with improved efficacy and toxicity profiles [33]. Attempts to elucidate the effects of taxanes on the activity of drug-metabolizing enzymes are an integral part of these efforts.

Cytochrome P450 (CYP) enzymes constitute a superfamily of hemoproteins that catalyze mainly monooxygenation reactions of a diverse array of substrates. These enzymes play a vital role in the detoxification and systemic clearance of xenobiotics. Of more than 55 human CYP isozymes presently known, CYP3A4 is considered the drug-metabolizing enzyme of principal importance [24]. It is the most abundant CYP enzyme present in the liver and intestine and it participates in the metabolism of over 60% of all marketed drugs that are eliminated by enzyme-catalyzed processes [45]. As such, modulation of the CYP3A4 activity is known to be a major cause of drug–drug interactions. Recent studies have significantly advanced our understanding of the processes that regulate the expression of CYP3A4. Accordingly, an orphan nuclear receptor termed the human pregnane X receptor (hPXR), also known as the steroid xenobiotic receptor (SXR), has been shown to serve as a key regulator of the *CYP3A4* gene [1, 2, 19, 23]. Human PXR is activated by a variety of known *CYP3A4* inducers, notably rifampicin, phenobarbital, and clotrimazole [1, 2, 17, 23]. Upon ligand binding, hPXR binds its cognate response elements in the 5′-flanking region of the *CYP3A4* gene as a heterodimer with the 9-*cis*-retinoid X receptor (RXRα), resulting in the transcriptional activation of *CYP3A4*and other target genes [15].

Pharmacokinetic studies have shown that the systemic elimination of paclitaxel predominantly involves hepatic metabolism by the CYP isozymes, CYP2C8 and CYP3A4 [6, 12], and docetaxel metabolism is largely catalyzed by CYP3A4 [28]. Since a number of anticancer agents used in combination with taxanes are also substrates for CYP3A4 [18, 34], a significant potential for adverse drug–drug interactions exists. Kostrubsky et al. [20] first reported that paclitaxel markedly increases the activity and expression of CYP3A4 enzyme in primary cultures of human hepatocytes. In previous studies, we have noted that docetaxel induces the rat CYP3A enzyme [31]. Recently, Synold et al. have reported that paclitaxel induces CYP3A4 in human hepatocytes and activates hPXR, whereas docetaxel is unable to do so when employed at a concentration of 10 μM[41].

In the present study, we conducted a detailed comparative evaluation of the effects of paclitaxel and docetaxel on the activity and expression of CYP3A4 in human hepatocytes. The emphasis was to elucidate the differences in the CYP3A4-inductive capacity of the two drugs employing a range of drug concentrations and drug treatment periods. We also examined the differences in the total cellular accumulation of these compounds in hepatocytes. Finally, employing cell-based reporter assays we examined the ability of the two drugs to activate hPXR.

Materials and methods

Chemicals and reagents

Paclitaxel, rifampicin, testosterone and 6β-hydroxytestosterone were obtained from Sigma Chemical Company (St. Louis, Mo.). Docetaxel was a gift from Aventis Pharmaceutical Company (Bridgewater, N.J.). Hepatocyte maintenance medium (HMM; Modified Williams' E culture medium) and medium supplements, dexamethasone, gentamicin and insulin, were obtained from BioWhittaker (San Diego, Calif.). Monoclonal antibody for human CYP3A4 was obtained from Gentest Corporation (Woburn, Mass.). The 780-bp CYP3A4 cDNA probe and the horseradish peroxidase-conjugated anti-mouse secondary antibody were obtained from Oxford Biomedical Research (Oxford, Mich.). ECL chemiluminescence detection system was obtained from Amersham Pharmacia Biotech (Piscataway, N.J.).

Hepatocyte culture and drug treatment

Human hepatocytes, isolated from lobes of liver from several separate donors, were provided by Dr Stephen Strom, Department of Pathology, University of Pittsburgh, Pa., under the auspices of the liver tissue procurement and distribution system (LTPADS) and with the approval of institutional review board (IRB). Table 1 summarizes the medical history of the donors and the medications they received prior to organ donation. The protocols for the use of human hepatocytes were also approved by the University of Cincinnati IRB. Hepatocytes were maintained in HMM supplemented with dexamethasone (0.1 μM), insulin (0.1 μM) and gentamicin (0.05%). Hepatocytes from each donor were plated in several formats (all collagen-coated for optimal plating efficiency and maintenance) based on the experimental requirements to facilitate parallel processing. For determining the CYP3A4 activity and immunoreactive levels, hepatocytes were plated in six-well plates (1×10^6 cells/well), for measuring mRNA levels, cells were plated in T-25-cm^2 flasks, and for assessing cell viability using the 3-(4,5-dimethylthiazol-2yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay, hepatocytes in 24-well format (1.25×10^5) were employed. Hepatocytes were treated, 48 h after isolation, with HMM containing paclitaxel (1–10 μM), docetaxel (1–10 μM), rifampicin (10 μM) or solvent used for dissolving drugs (DMSO, 0.1%) for periods of 48–96 h.

Table 1 Human liver donor information. Human hepatocytes were used for studying taxane-mediated CYP3A4 induction following 48, 72 and 96 h of treatment. Hepatocytes were also used for drug accumulation studies (*DAS*) in which total cellular concentrations of paclitaxel and docetaxel were compared

Batch	Age (years)	Sex	Race	Cause of death	Drugs used in emergency room/operating room	Incubation period/Used for
HH788	7	M	African American	Vehicle accident	Dopamine	48 h
HH804	24	F	Caucasian	Vehicle accident	Neosynephrine, erythromycin	48 h
HH873	53	M	Caucasian	Intracranial bleeding	Dopamine	96 h
HH877	63	M	Caucasian	Subarachnoid hemorrhage	Dopamine	48 h
HH894	40	M	Caucasian	Intracranial bleeding	Dopamine	72 h
HH899	23	F	Caucasian	Vehicle accident	Dopamine pentobarbital, neosynephrine, insulin	72 h
HH921	6	F	Caucasian	Vehicle accident	Dopamine, epinephrine	72 h
HH954	62	M	Caucasian	Intracranial bleeding	–	48, 72, 96 h
HH961	34	F	Caucasian	Vehicle accident	Dopamine, insulin, methyl prednisolone; regular Prozac, amitriptyline	72 h/DAS
HH965	17	M	Caucasian	Anoxia	–	72 h/DAS
HH969	46	M	Caucasian	Pulmonary hypertension	Spironolactone	DAS

Hepatocytes were then processed for determining CYP3A4 activity and expression as described below. Drug-containing medium was replaced every 24 h. At the end of drug treatment, cell viability was assessed employing the MTT assay as described previously [3]. At the concentration employed, DMSO did not alter cell viability or CYP3A4 activity or expression [8, 20].

CV-1 Cell culture

CV-1 cells (African green monkey kidney fibroblasts) obtained from ATCC (Manassas, Va.) were plated (2×10^6 cells/plate) in 10-cm dishes and maintained in Dulbecco's modified Earl's medium (DMEM) containing 10% fetal bovine serum, supplemented with L-glutamine, non-essential amino acids, sodium pyruvate, penicillin and streptomycin.

Measurement of CYP3A4 activity

The conversion of testosterone to 6β-hydroxytestosterone, a reaction catalyzed by CYP3A4, was employed as a marker for enzyme activity in intact hepatocytes [44]. 6β-Hydroxytestosterone levels were measured employing a reverse-phase high-pressure liquid chromatography (HPLC) method as described in our previous studies [8].

Immunodetection of CYP3A4 protein

Western blot analysis of S9 protein fractions (3 μg) prepared from hepatocytes was performed exactly as described previously [8]. A primary monoclonal anti-CYP3A4 antibody (Gentest, Woburn, Mass.), which cross-reacts with CYP3A5, was used, followed by horseradish peroxidase-conjugated anti-mouse secondary antibody. The protein bands were visualized using enhanced chemiluminescence detection (Amersham, Piscataway, N.J.) and quantitated employing a NucleoVision image analyzer (Nucleotech, San Mateo, Calif.).

Northern blot analysis of CYP3A4 mRNA

Northern blot analysis of total RNA isolated from hepatocytes was performed as described previously [8]. Equal loading per lane was verified by ethidium bromide staining of 18S and 28S rRNA, which was visualized and photographed under UV illumination. Isolated CYP3A4 cDNA probes were labeled with [α^{32}P]-dCTP (NEN, Boston, Mass.) using the random primer method. Hybridization procedures were conducted as described by Church and Gilbert (1984) [4]. The membranes were exposed to X-ray film and the bands developed were quantitated employing a NucleoVision image analyzer.

Total cellular taxane concentration in human hepatocytes and CV-1 cells

Primary cultures of human hepatocytes plated in 75-cm^2 flasks were incubated with medium containing paclitaxel (10 μ*M*) or docetaxel (10 μ*M*). Flasks were incubated with drug-containing medium for 0.5, 1, 2, 4, 8, 18 and 24 h. At the indicated time-points cells were washed thrice with cold phosphate-buffered saline (PBS), pH 7.4, scraped and collected in 2 ml PBS. Cells were homogenized and an aliquot (100 μl) was collected for protein assay by the Lowry method. Paclitaxel and docetaxel in the samples were extracted employing a solid-phase extraction procedure followed by HPLC analysis [16]. Briefly, cephalomannine (2 μg), used as an internal standard, was added to the cell homogenates and to the calibration standards, where a known amount of the respective drug was added to solvent-treated hepatocytes. Samples and calibration standards were extracted on a C_{18} solid-phase extraction column (6 ml Bond-Elut,

500 mg cartridge; Varian, Walnut Creek, Calif.). Each column was conditioned with 3 ml water followed by 3 ml acetonitrile and a final wash with 3 ml water. The sample was loaded on to the column and washed with 5 ml water. The drug bound to the column was extracted by eluting the cartridge twice with 4 ml acetonitrile. The eluent (acetonitrile) was dried under a stream of nitrogen and reconstituted in 200 µl mobile phase. Extracts were chromatographed isocratically with a mobile system that comprised a 34:9:57 ratio of acetonitrile/methanol/water, employing a flow of 1 ml/min. Separation was accomplished on a symmetry C_{18} column (particle size 5 µm; dimensions 3.9×150 mm). Docetaxel, paclitaxel and cephalomannine were detected at 230 nm employing a UV detector (Waters 410) with elution times of 26, 31 and 35 min, respectively. Recoveries of paclitaxel, docetaxel and cephalomannine were determined using solid-phase extraction. The recoveries of paclitaxel, docetaxel and cephalomannine in the concentration range 0.5–10 µ*M* were 89.9%, 87.6% and 86.9%, respectively. The interday and intraday variability in the HPLC analysis was less than 5% and the detection limit was about 100 pmol for paclitaxel and docetaxel.

For determining paclitaxel and docetaxel concentration in CV-1 cells were plated at 2×10^6 cells/plate in 10-cm dishes and maintained in DMEM. Cell monolayers were incubated with medium containing paclitaxel (10 µ*M*) or docetaxel (10 µ*M*) for 24 h. The intracellular drug content was determined as described above.

Cotransfection studies

CV-1 cells transiently transfected with luciferase reporter gene construct p3A4-362 (7836/7208ins) were used to examine the activation of hPXR by paclitaxel and docetaxel exactly as described by Jones et al. [17]. Transfection mixes contained 8 ng *CYP3A4* reporter gene [CYP3A4 (+53 to −13,000)–LUC] construct [11], 5 ng PXR expression vector pSG5-ΔATG-hPXR, 8 ng pβ-actin-SPAP (an expression vector containing the secreted placental alkaline phosphatase cDNA under control of the β-actin promoter), and 52 ng pBluescript (Stratagene, La Jolla, Calif.). The proximal promoter region of the *CYP3A4*gene contains an everted repeat with a six-nucleotide spacer (ER-6) of the $AG^G/_TTCA$ hexad. This element binds the hPXR–RXRα heterodimer with high affinity. Heterologous reporter gene constructs containing multimerized copies of this element are activated in a hPXR-dependent manner [1, 2, 23]. However, maximal activation of the *CYP3A4* promoter requires additional sequences located about 8 kb upstream of the transcription initiation site in the xenobiotic responsive enhancer module (XREM) region of the *CYP3A4* gene [11, 15]. CV-1 cells transfected with *CYP3A4* reporter gene construct were then treated with paclitaxel, docetaxel and rifampicin, a well-known activator of hPXR, for 24 h. The concentration range of each compound employed was 1 n*M* to 10 µ*M*. An aliquot of medium was withdrawn for SPAP assay and the cells lysed before luciferase determination. Luciferase activities were normalized to SPAP expression. Dose–response curves were generated and analyzed employing a non-linear data analysis algorithm using WinNonlin Standard, version 1.5 (Pharsight Corporation, Mountain View, Calif.). In additional samples, at the end of drug treatment, cell viability was also determined by the MTT assay performed exactly as described previously [3].

Statistical and data analysis

The differences in the formation of 6β-hydroxytestosterone, CYP3A4 protein and mRNA levels and cell viability assessed using the MTT assay in control vs the treated groups were analyzed by one-factor ANOVA. This was followed by the post-hoc Tukey-Kramer test to compare the mean of corresponding measures to that of the control group at a significance level of $\alpha = 0.05$.

Results

We compared the extent to which docetaxel and paclitaxel induced CYP3A4 in human hepatocytes following 48, 72 and 96-h treatments. The effects of clinically relevant concentrations of paclitaxel (1–10 µ*M*) and docetaxel (1–10 µ*M*) on the activity and expression of CYP3A4 in primary cultures of human hepatocytes were examined [5, 39]. Rifampicin (10 µ*M*) was used as positive control for the induction of CYP3A4 in hepatocytes. Results from the MTT assay indicated that the viability of hepatocytes treated for 48, 72 and 96 h with taxanes (1–10 µ*M*) and rifampicin (10 µ*M*) was comparable to that of the vehicle-treated controls. Information on the liver donors and the purpose for which each hepatocyte batch was used are indicated in Table 1.

Effect of taxanes on the activity and expression of CYP3A4 in human hepatocytes

72-h treatment

We employed a total of six batches of human hepatocytes (Table 1) for studying the effect of a 72-h exposure to taxanes on CYP3A4 expression and activity. The fold increase in the CYP3A4 activity, immunoreactive protein levels and mRNA in drug-treated hepatocytes compared to untreated controls following a 72-h exposure are shown in Table 2. CYP3A4-specific immunoreactive protein and mRNA levels are shown in Fig. 1a and b, respectively. Paclitaxel (1–10 µ*M*) significantly ($P < 0.05$) increased the CYP3A4 activity and protein levels (Western blots; Fig. 1a) by 3- to 10-fold, with the maximal induction occurring at a concentration of 10 µ*M*. Surprisingly, even after 72 h exposure docetaxel

did not increase the CYP3A4 activity or protein levels in any of the human hepatocyte batches employed in this study. Rifampicin increased CYP3A4 activity and immunoreactive protein levels from 3- to 20-fold, and it was the most potent inducer of CYP3A4 activity in all batches of human hepatocytes. On an equimolar basis, paclitaxel (10 μ*M*) was approximately 53% as effective as rifampicin (10 μ*M*) in increasing CYP3A4 activity and protein levels. Northern blots showing CYP3A4 mRNA induction in human hepatocytes in response to drug treatments for 72 h are shown in Fig. 1b. As summarized in Table 2, rifampicin was the most potent inducer of CYP3A4 mRNA, followed by paclitaxel, which caused a 6- to 20-fold increase. Maximal induction response with paclitaxel was observed at a concentration of 10 μ*M*. Although docetaxel treatment did not result in increased CYP3A4 activity or protein levels, we did observe an increase in the CYP3A4 mRNA. While the CYP3A4 mRNA increase by docetaxel (2- to 7-fold) was considerably lower than that observed with paclitaxel (6- to 21-fold), it was statistically significant ($P < 0.05$).

The magnitude of CYP3A4 induction response to paclitaxel and rifampicin here is in agreement with previously published reports [20, 21]. In general, we observed a good correlation between the paclitaxel-mediated fold increase in immunoreactive content and the fold increase in CYP3A4 activity ($R^2 = 0.97$, $P < 0.001$; Fig. 2a). Paclitaxel-mediated fold induction of CYP3A4-specific mRNA levels and the fold increase in immunoreactive protein content were also well correlated ($R^2 = 0.94$, $P < 0.006$; Fig. 2b).

48-h treatment

We also evaluated the changes in CYP3A4 activity and expression in human hepatocytes treated with taxanes for 48 h. Paclitaxel (5–10 μ*M*) caused a 1.7- to 2.4-fold ($P < 0.05$) and rifampicin (10 μ*M*) caused a 6-fold increase in CYP3A4-mediated testosterone 6β-hydroxylation. However, docetaxel (0.5–20 μ*M*) did not increase CYP3A4 activity compared to untreated controls at any of the concentrations employed. As revealed by Western blot analysis (Fig. 3a), treatment of hepatocytes with paclitaxel (10 μ*M*) for 48 h increased CYP3A4 protein levels by 12.5-fold, but docetaxel (0.5–20 μ*M*) treatment did not increase CYP3A4 protein levels in any of the batches employed. Surprisingly, docetaxel (10 μ*M*) weakly induced (up to 4-fold) CYP3A4 mRNA levels (Fig. 3b) compared to paclitaxel (1–10 μ*M*) which resulted in a 6- to 10-fold increase.

96-h treatment

The pattern of CYP3A4 induction observed following a 96-h drug treatment of hepatocytes with taxanes was similar to that observed following the 72-h exposure. Rifampicin was the most potent inducer of CYP3A4 activity followed by paclitaxel. No increase in CYP3A4 activity was observed in hepatocytes treated with docetaxel (5–10 μ*M*). Paclitaxel also induced CYP3A4 protein levels and mRNA levels (data not shown). While docetaxel (1–10 μ*M*) did increase CYP3A4 mRNA levels up to four-fold, it did not result in corresponding increases in CYP3A4 protein levels. Our studies in human hepatocytes reveal interesting results pertaining to docetaxel-mediated CYP3A4 induction. Overall, following 48–96 h exposure we observed that the capacity to enhance CYP3A4 expression and activity for the drugs employed followed the order rifampicin > paclitaxel, with docetaxel exhibiting no increase in the activity but marginal increase in the CYP3A4 mRNA levels.

Taxane-mediated activation of human PXR

The activation of hPXR by taxanes was examined employing CV-1 cells that were transiently transfected with a reporter gene construct harboring hPXR-responsive regions of the *CYP3A4* gene. In parallel experiments, we compared hPXR activation profiles of paclitaxel, docetaxel, and rifampicin (Fig. 4) in the

Table 2 Fold increase (with respect to untreated control) in the CYP3A4 activity, immunoreactive protein and mRNA levels in human hepatocytes following treatment with various drugs for 72 h (means ± SEM, $n = 4$). Various batches of hepatocytes ($n = 4$) were treated with the drugs at the indicated concentrations for 72 h. Cells were then exposed to testosterone-containing medium (250 μ*M*) for 30 min. The 6β-hydroxytestosterone levels (as marker for CYP3A4 activity) in the medium supernatant were determined by HPLC/UV analysis. The immunoreactive CYP3A4 levels in protein fractions prepared from harvested hepatocytes were determined by Western blot analysis employing a monoclonal CYP3A4 antibody. The CYP3A4 mRNA levels were determined by Northern blot analysis of total RNA using a radiolabeled 780 bp cDNA probe. The CYP3A4 mRNA levels were normalized to the 18S rRNA levels in respective groups

	Rifampicin[a]	Paclitaxel			Docetaxel		
	10 μ*M*	1 μ*M*	5 μ*M*	10 μ*M*	1 μ*M*	5 μ*M*	10 μ*M*
CYP3A4 activity[b]	14.6 ± 2**	4.3 ± 0.4**	7.8 ± 0.9*	7.5 ± 0.9**	0.7 ± 0.1	0.4 ± 0.1	0.6 ± 0.1
CYP3A4 protein levels	15.8 ± 4*	4.4 ± 1.2*	7.2 ± 2.7*	8.8 ± 1.4**	1.7 ± 0.2	1.5 ± 0.3	1.6 ± 0.5
CYP3A4 mRNA levels	21.6 ± 5*	6.6 ± 3.1*	14.9 ± 5.6*	21 ± 6.4*	2.1 ± 0.8*	4.9 ± 0.8*	7.1 ± 1**

*$P < 0.05$, **$P < 0.01$, compared to vehicle treated controls
[a]Positive control for CYP3A4 induction
[b]Determined by measuring the testosterone 6β-hydroxylation, means ± SEM from four batches of hepatocytes

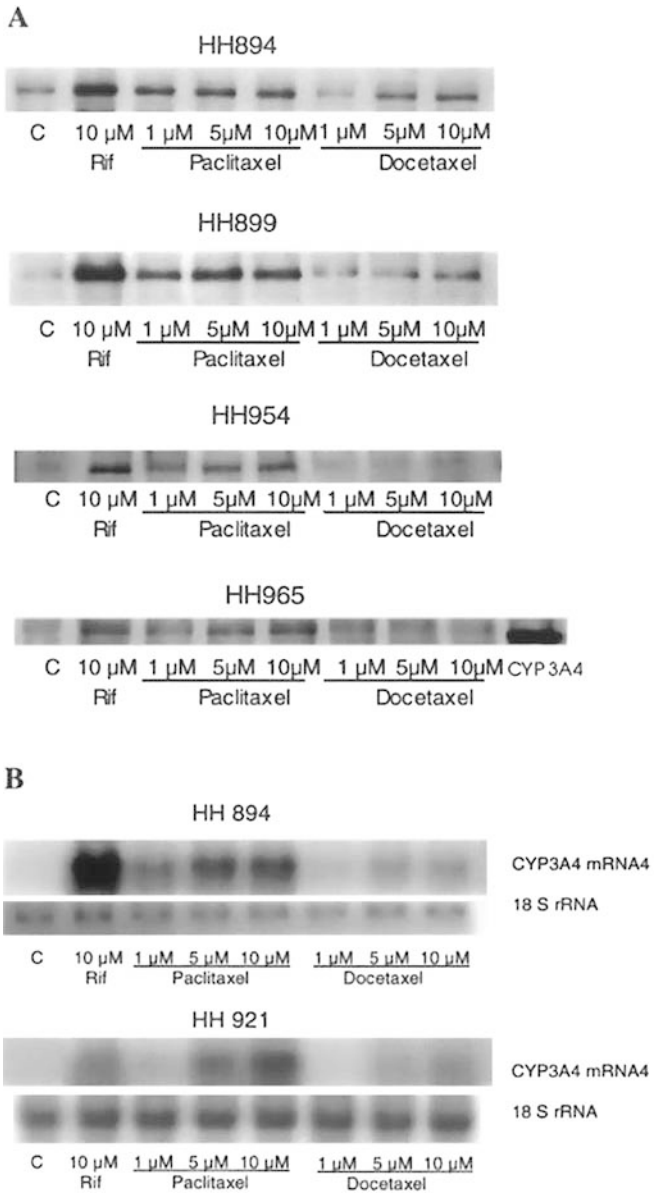

Fig. 1a, b Western blot (**a**) and Northern blot (**b**) showing CYP3A4 immunoreactive protein and mRNA levels, respectively, in human hepatocytes treated for 72 h with docetaxel (1–10 μ*M*), paclitaxel (10 μ*M*) and rifampicin (10 μ*M*). **a** The S9 protein fractions of the drug-treated and untreated control hepatocytes were resolved electrophoretically. The immunoreactive CYP3A4 protein was probed with a monoclonal anti-human CYP3A4 primary antibody and visualized using a horseradish peroxidase-linked secondary antibody followed by enhanced chemilumescence reaction. **b** Northern blots showing CYP3A4 mRNA expression (normalized to 18S rRNA) in hepatocytes treated for 72 h with paclitaxel (1–10 μ*M*), docetaxel (1–10 μ*M*) and rifampicin (*Rif*, 10 μ*M*). Total RNA was isolated from the hepatocytes, fractionated on denaturing agarose gel, and transferred on to a nitrocellulose membrane. The CYP3A4 mRNA was probed using CYP3A4 cDNA randomly labeled with [^{32}P]CTP and the autoradiogram was developed using Kodak X-OMAT X-ray film

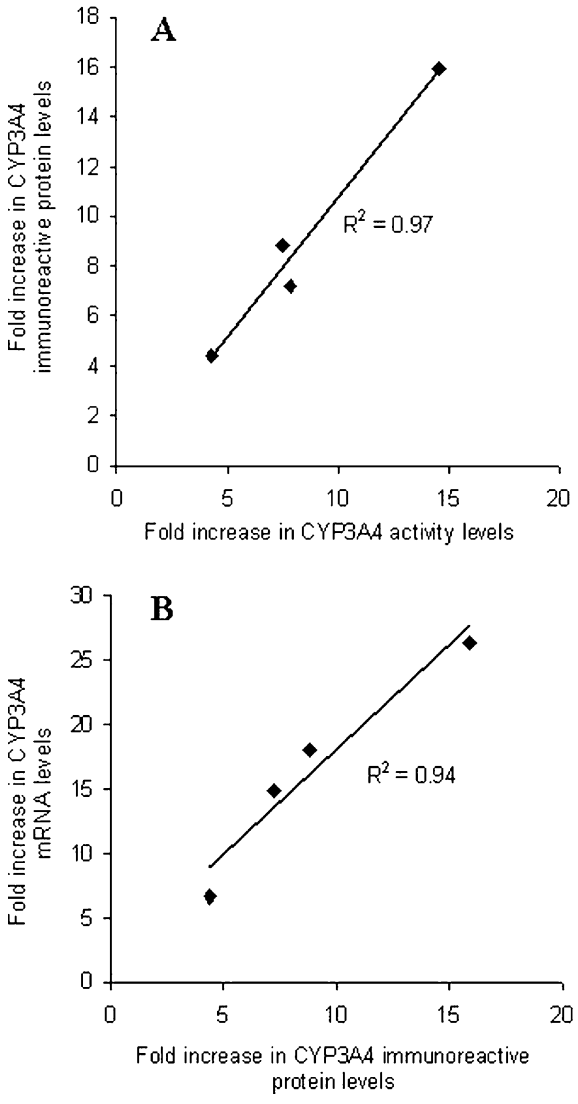

Fig. 2 a Correlation between fold increase (with respect to untreated controls) in CYP3A4 activity (means, $n=4$) and immunoreactive CYP3A4 levels (means, $n=4$) in human hepatocytes treated for 72 h with paclitaxel (1–10 μ*M*) and rifampicin (10 μ*M*). **b** Correlation between fold increase (with respect to untreated controls) in immunoreactive CYP3A4 levels (means, $n=4$) and CYP3A4-specific mRNA levels (means, $n=4$) in human hepatocytes treated for 72 h with paclitaxel (1–10 μ*M*) and rifampicin (10 μ*M*)

transfected cells. The dose–response for each compound was fitted to a sigmoidal E_{max} model employing WinNonlin Standard, version 1.5 (Pharsight Corporation) and the maximal effect (E_{max}) and half-maximal effective concentration (EC_{50}) were determined from this model. Paclitaxel and rifampicin activated the hPXR with EC_{50}values of about 5.2 and 0.5 μ*M*, respectively. This EC_{50} value for rifampicin is in close agreement with earlier reports [1, 2, 17, 23]. Notably, the highest concentration of paclitaxel (10 μ*M*) induced reporter gene expression to a greater extent than rifampicin (by about 1.5-fold). Relative to rifampicin and paclitaxel, docetaxel was a weak hPXR activator. With the concentrations range employed, we were unable to acceptably determine E_{max}. MTT assays indicated that paclitaxel, docetaxel and rifampicin at the employed concentrations

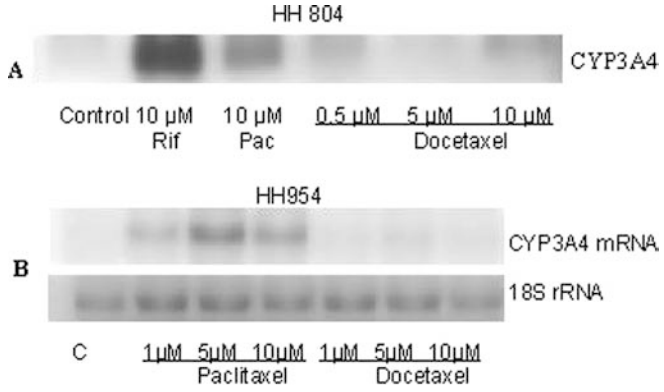

Fig. 3 **a** Western blot showing CYP3A4 immunoreactive protein levels in human hepatocytes treated for 48 h with docetaxel (0.5–10 μ*M*), paclitaxel (*Pac*, 1–10 μ*M*) and rifampicin (*Rif*, 10 μ*M*) compared to untreated control (*Control*). **b** Northern blots showing increases in CYP3A4 mRNA levels in human hepatocytes treated for 48 h with docetaxel (1–10 μ*M*), paclitaxel (1–10 μ*M*) and rifampicin (10 μ*M*) compared to untreated control (*C*)

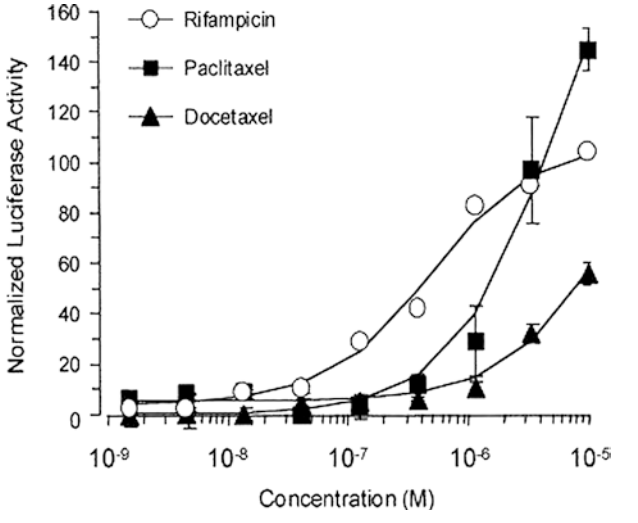

Fig. 4 Dose–response profiles of human PXR activation by docetaxel (*triangles*), paclitaxel (*squares*) and rifampicin (*circles*). CV-1 cells were transiently transfected with the pSG5-hPXR ΔATG and the XREM–CYP3A4–luciferase reporter plasmid and treated with various drugs (1 n*M* to 10 μ*M*). Cell extracts were subsequently assayed for luciferase activity and normalized to *β*-actin serum placental alkaline phosphatase activity to take account of transfection efficiency (means ± SEM, $n = 3$)

(1–10 μ*M*) were not cytotoxic to CV-1 cells over a 24-h exposure period. It is noteworthy that previous reports have indicated that paclitaxel is non-cytotoxic to CV-1 cells under experimental conditions similar to those used here [26].

Total cellular content of docetaxel and paclitaxel in human hepatocytes and CV-1 cells

To test whether the observed contrast in the propensity of paclitaxel and docetaxel for CYP3A4 induction or hPXR activation may have been due to differences in the intracellular drug levels, we assessed the total cellular concentration in human hepatocytes and CV-1 cells.

Total cellular concentrations of docetaxel (10 μ*M*) and paclitaxel (10 μ*M*) over different times of incubation in three different batches of hepatocytes are shown in Fig. 5a. Treatment with paclitaxel at 10 μ*M* led to a rapid increase in the intracellular drug levels (peak concentration 36 nmol/mg protein) in the hepatocytes. This was followed by a gradual decrease in the paclitaxel levels over the course of the study (24 h). In contrast, the intracellular docetaxel peak levels in hepatocytes treated with docetaxel (10 μ*M*) occurred 1 h following the drug administration and, moreover, it was considerably lower than that observed with paclitaxel (10.6 nmol/mg protein). The total exposure, measured as the area under the concentration vs time profile curve, of paclitaxel (AUC 411 nmol × min/mg protein) in human hepatocytes was significantly ($P = 0.03$) higher than that of docetaxel (AUC 142 nmol × min/mg protein).

Since the hPXR activation assays employed the CV-1 cells, to assess whether any differences in the "availability" of the drug may be a contributing factor to the observed contrast in CYP3A4 induction and hPXR activation, we also evaluated the total cellular concentration of docetaxel and paclitaxel in CV-1 cells. Cells were treated with paclitaxel (10 μ*M*) or docetaxel (10 μ*M*) for 24 h. Figure 5b shows the concentrations of paclitaxel and docetaxel achieved in CV-1 cells following a 24-h exposure period. The total cellular concentration of paclitaxel in CV-1 cells was 15.6 nmol/mg, but that of docetaxel was significantly lower (3.1 nmol/mg), a difference of about five-fold.

Previous studies suggest that PXR may also regulate drug transporters [40, 41]. Considering that P-glycoprotein (P-gp) is expressed in hepatocytes, albeit weakly [9], potential complicating factors in our drug accumulation studies were (a) that paclitaxel and docetaxel are substrates for P-gp [30, 43], and (b) that paclitaxel is known to induce MDR1 in human hepatocytes [41]. However, we believe that these factors are not likely to have had an impact on our intracellular drug accumulation studies because our studies were performed over a 24-h period with most of the drug efflux occurring probably within the first 8 h. In addition, as suggested previously, induction of MDR1 in hepatocytes usually requires exposure periods exceeding 48 h [36, 41].

Discussion

In this study we observed that two structurally related taxane anticancer drugs, docetaxel and paclitaxel, markedly differ in their capacity to increase the activity and expression of the principal drug-metabolizing enzyme, CYP3A4, in primary human hepatocytes. Whereas paclitaxel potently enhanced the testosterone 6*β*-hydroxylation activity and CYP3A4-specific protein and mRNA levels, docetaxel did not increase the enzymatic activity or the protein content. We did observe a

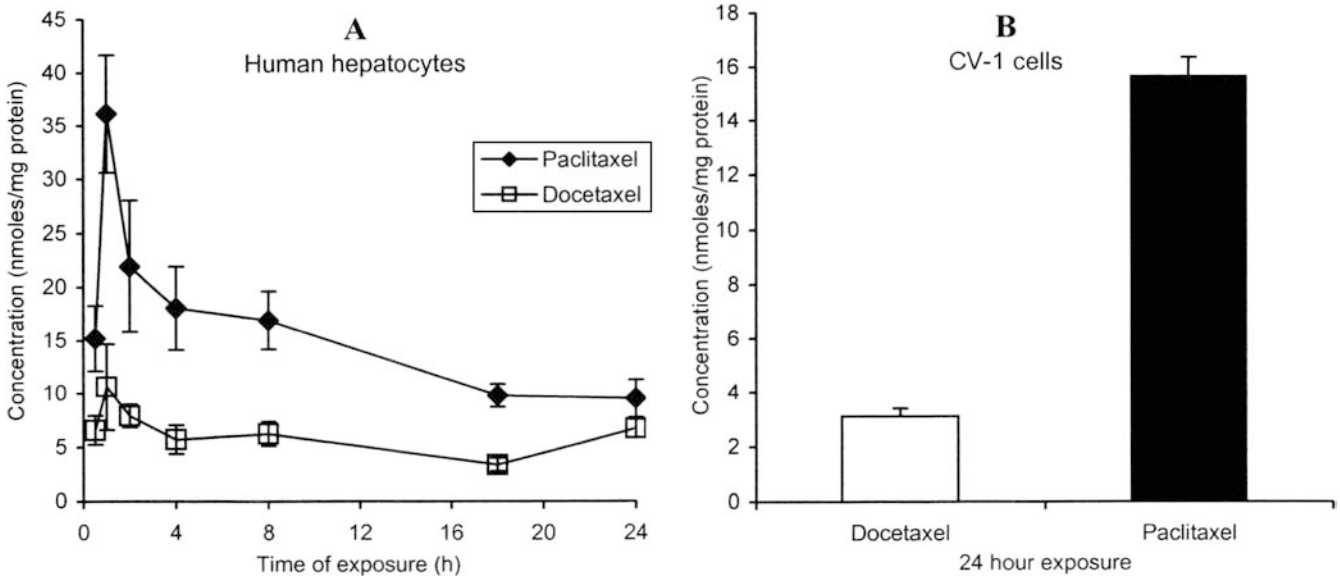

Fig. 5a, b Total cellular concentration of paclitaxel and docetaxel in human hepatocytes and CV-1 cells. **a** Total cellular drug levels vs time profile of paclitaxel (10 μ*M*) and docetaxel (10 μ*M*) in primary cultures of hepatocytes. Hepatocytes from three different liver donors were incubated with paclitaxel (10 μ*M*, *filled diamonds*) or docetaxel (10 μ*M*, *open squares*) in HMM for 30 min, and 1, 2, 4, 8, 18 and 24 h. **b** Total cellular concentration of docetaxel (*open box*) and paclitaxel (*filled box*) in CV-1 cells following a 24-h exposure to docetaxel (10 μ*M*) and paclitaxel (10 μ*M*). The cell monolayers following drug exposure were washed with cold PBS following which the cells were harvested and homogenized. Paclitaxel and docetaxel in the homogenates were analyzed by solid-phase extraction followed by HPLC/UV analysis. The drug levels in hepatocytes or CV-1 cells was normalized to the amount of protein in the sample and plotted against time of incubation (means ± SEM, $n = 3$)

statistically significant, albeit relatively weak, increase in the CYP3A4-specific mRNA levels in cells treated with higher concentrations of docetaxel. Concurrently, in CV-1 cells transfected with the CYP3A4 reporter gene and a vector expressing hPXR, paclitaxel was observed to be a considerably more effective hPXR activator than docetaxel. We also observed that the total cellular concentration of docetaxel was considerably lower than that of paclitaxel in both hepatocytes and CV-1 cells.

As indicated above, Synold et al. have recently shown that docetaxel does not induce CYP3A4 or other hPXR (also known as SXR) target genes in human hepatocytes [41]. Employing a batch of human hepatocytes, the investigators compared paclitaxel and docetaxel at a concentration of 10 μ*M*and a treatment period of 48 h. For measuring the CYP3A4 activity, they employed 3′-*p*-hydroxylation of paclitaxel as a marker. Although, our studies confirm the previous results, there are several important differences in the methodology employed and between our observations and the previous findings. Firstly, we employed human hepatocytes from a number of donors and employed a range of clinically relevant drug levels (1–10 μ*M*) and drug treatment periods (48–96 h). In most of our studies the 72 h treatment period was used, since this period facilitates more distinct differentiation between CYP3A4 inducers and non-inducers [21, 25]. Secondly, we employed testosterone 6*β*-hydroxylase activity as a marker, the most widely used CYP3A4 substrate [24, 32]. Thirdly, while Synold et al. employed the yeast two-hybrid assay comprising of the Gal4 reporter system [41], we used the CV-1 cells transfected with the CYP3A4–luciferase reporter gene that harbors the potent enhancer module 8 kb distal to the transcription initiation point, and confers maximal activation [11]. Our studies confirm the initial findings by these authors that paclitaxel is markedly more effective than docetaxel in activating hPXR and inducing CYP3A4, and as such, is more likely to induce drug clearance. In addition, we observed a distinct, albeit small, increase in the CYP3A4-specific mRNA levels in all batches of hepatocytes tested. Further, we also observed that docetaxel causes hPXR activation, which again was considerably lower than that caused by paclitaxel, but much higher than that reported by the previous investigators.

As described above, we observed a good correlation between CYP3A4 immunoreactive protein levels and testosterone 6*β*-hydroxylation activity and between CYP3A4-specific mRNA and immunoreactive protein levels, excluding the docetaxel data. Clearly, the increased transcription resulting from docetaxel treatment did not translate into increased CYP3A4 protein levels. The reasons underlying these observations are not clear at this stage. This may entail post-transcriptional changes by docetaxel in the expression of CYP3A4. Another observation that needs further investigation is that, compared to untreated controls, we consistently noticed somewhat lower, albeit statistically insignificant, testosterone 6*β*-hydroxylation activity in cells treated with docetaxel. In our experiments, prior to measuring enzymatic activity, the hepatocytes were washed and incubated for 30 min to 1 h in drug-free medium with the intention of facilitating cellular drug elimination. However, the possibility cannot be ruled out that small

quantities of drug remaining associated with the cells may have inhibited testosterone hydroxylation.

An important issue not addressed in our study is whether paclitaxel induces CYP3A in extrahepatic tissues. For instance, CYP3A4 is also expressed in intestinal tissues, whereas most other tissues such as the lung, kidney and polymorphonuclear leukocytes express CYP3A5, which exhibits polymorphic expression and is not induced by most prototype inducers [22]. While most compounds that induce hepatic CYP3A4 also exhibit induction of intestinal enzyme, there are some exceptions [29]. The role of PXR in regulation of intestinal CYP3A4 is unclear since other receptors such as the vitamin D receptor (VDR) appear to have a significant influence on CYP3A4 expression in intestinal cell lines [42]. There is considerable impetus to develop orally active taxanes including orally bioavailable forms of paclitaxel. When the oral route of administration is employed, potential induction of intestinal metabolism and its influence on compounds such as imatinib and gefitinib will need to be carefully evaluated. On a related note, Synold et al. have also shown that paclitaxel induces MDR1 in hepatocytes and intestinal cell lines [41]. This may contribute to increased biliary clearance and/or reduced drug uptake.

Studies were performed to compare the total cellular concentration of paclitaxel and docetaxel in the model systems employed for assessing CYP3A4 induction (i.e., hepatocytes) or hPXR activation (i.e., CV-1 cells). Most notably, our studies indicate that docetaxel intracellular levels in hepatocytes and CV-1 cells were much lower than those of paclitaxel, resulting in markedly lower overall drug exposure. The reason for the difference in taxane concentration in CV-1 cells is unclear and counter-intuitive, since it is known that CV-1 cells overexpress P-gp [26] and paclitaxel is a better substrate for P-gp than docetaxel [30, 43]. However, the net cellular drug content depends on the total influx, retention and efflux, and since we did not measure efflux, further investigation of this issue is warranted. The observed differences in hPXR activation by docetaxel and paclitaxel may have been due to differences in their binding affinities to the ligand-binding domain of the hPXR [41]. Our observations suggest that the difference may also have been partly due to reduced intracellular drug availability.

The striking difference in CYP3A4 induction response between docetaxel and paclitaxel, two taxane anticancer drugs with nearly identical cytotoxicity profiles, has important clinical implications. It is well known that the classic *CYP3A4* inducers, such as rifampicin and phenobarbital, markedly alter the systemic clearance and consequently the efficacy and/or toxicity of numerous drugs [10, 18]. These drug–drug interactions are of particular concern in cancer chemotherapy since most antineoplastic agents have extremely narrow therapeutic indices, and relatively minor changes in plasma levels may have considerable influence on the drug pharmacodynamics. Both paclitaxel and docetaxel are routinely administered together with anticancer drugs including etoposide, and doxorubicin and palliative agents such as, granisetron and ondansetron that are CYP3A4 substrates [18, 27, 37]. Our finding that *CYP3A4* induction and hPXR activation occurs at clinically relevant paclitaxel concentrations (1–10 μM) but not with docetaxel, suggests that the potential for drug–drug interaction is higher for paclitaxel than for docetaxel. However, the potential clinical impact of induction by paclitaxel may depend on the frequency and dose of administration, which will influence the net hepatocyte exposure (concentration and duration) to the drug.

Currently, paclitaxel is used in cycles of 3 weeks and comedication such as etoposide is administered within 1–2 h of paclitaxel administration. Under this scenario, the potential for increased enzymatic clearance of co-administered drugs may be limited. However, if alternative paclitaxel dosing schemes, such as twice weekly [13] or weekly [38], are used and when the coadministered drug is also dosed more frequently, the likelihood of enzyme induction-related interaction increases. Indeed, there is evidence in the literature that suggests that the drug–drug interactions associated with paclitaxel may be attributed to its ability to induce *CYP3A4*[35]. Accordingly, it has been reported that the plasma clearance of CYP3A4 substrates such as the novel antiangiogenic agent, SU5416, is considerably increased with prior treatment with paclitaxel [35].

To summarize our observations, employing multiple batches of human hepatocytes, we demonstrated that, whereas paclitaxel treatment markedly induced CYP3A4 activity and expression, docetaxel did not increase the CYP3A4 activity. However, docetaxel did enhance the transcriptional activation of hPXR and the CYP3A4 mRNA levels in human hepatocytes, although compared to those of paclitaxel these effects were fairly weak. Our studies and those published previously [41] suggest that compared to paclitaxel, docetaxel is a weak hPXR activator. This may be related to their intrinsic binding affinities to hPXR ligand binding sites. It appears that reduced intracellular availability of docetaxel compared to paclitaxel may also contribute to the weaker hPXR activation by docetaxel. Finally, our studies provide evidence in support of the contention that compared to paclitaxel, docetaxel has markedly lower potential to cause drug–drug interactions through enzyme induction.

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