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Induction of CYP3A4 by Vinblastine: Role of the Nuclear Receptor NR1I2

Nicola F Smith, PhD[Graduate Fellow],

Clinical Pharmacology Program, Medical Oncology Branch, National Cancer Institute, Bethesda, MD

Sridhar Mani, MD,

Assistant Professor of Medicine and Molecular Pharmacology, Albert Einstein Cancer Center and Department of Medicine, Albert Einstein College of Medicine, Bronx, NY

Erin G Schuetz, PhD[Faculty Member],

Department of Pharmaceutical Sciences, St. Jude Children's Research Hospital, Memphis, TN

Kazuto Yasuda, PhD[Post-doctoral fellow],

Department of Pharmaceutical Sciences, St. Jude Children's Research Hospital

Tristan M Sissung, PhD[Staff Scientist],

Clinical Pharmacology Program, Medical Oncology Branch, National Cancer Institute

Susan E Bates, MD,

Experimental Therapeutics Section, Medical Oncology Branch, Center for Cancer Research, National Cancer Institute

William D Figg, PharmD MBA, and

Head of the Clinical Pharmacology Program, Medical Oncology Branch, National Cancer Institute

Alex Sparreboom, PhD[Associate Faculty Member]

Department of Pharmaceutical Sciences, St. Jude Children's Research Hospital

Abstract

BACKGROUND—Several microtubule targeting agents are capable of inducing CYP3A4 via activation of the pregnane X receptor (PXR; NR1I2).

OBJECTIVE—To evaluate the CYP3A4 induction potential of vinblastine both clinically and in vitro and determine the involvement of the nuclear receptors NR1I2 and the constitutive androstane receptor (NR1I3).

METHODS—Midazolam pharmacokinetics were evaluated in 6 patients who were enrolled in a Phase 1/2 study of infusional vinblastine given in combination with the ABCB1 (P-glycoprotein) antagonist valsopodar (PSC 833) and received the CYP3A4 phenotyping probe midazolam on more than 1 occasion. Genotyping was conducted in *CYP3A4*, *CYP3A5*, and *ABCB1* to rule out potential pharmacogenetic influences. Clinical data were followed-up by Western blotting and reporter assays in HepG2 and NIH3T3 cells treated with vinblastine over a dose range of 150-4800 ng/mL for 48 hours.

RESULTS—In 6 patients with cancer, vinblastine increased the median (95% CI) clearance of the CYP3A4 phenotyping probe midazolam from 21.7 L/h (12.6 to 28.1) to 32.3 L/h (17.3 to 53.9)

Correspondence: Dr. Figg, wdfigg@helix.nih.gov.

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($p = 0.0156$, Wilcoxon signed-rank test). No obvious effect of polymorphisms in *CYP3A4*, *CYP3A5*, and *ABCB1* on midazolam clearance was observed. In vitro, vinblastine induced CYP3A4 protein. Furthermore, cell-based reporter gene assays using transiently transfected HepG2 and NIH3T3 cells indicated that vinblastine (150-4800 ng/mL) weakly activated human and mouse full-length NR1I2, but had no influence on NR1I3.

CONCLUSIONS—Collectively, these findings suggest that vinblastine is able to induce CYP3A4, at least in part, via an NR1I2-dependent mechanism, and thus has the potential to facilitate its own elimination and cause interactions with other CYP3A4 substrates.

Keywords

CYP3A4; induction; NR1I2 (PXR); vinblastine

Vinblastine is a naturally occurring vinca alkaloid that is found in the Madagascar periwinkle plant *Catharanthus roseus* (L.) G. Don (formerly known as *Vinca rosea* L.). This antimicrotubule agent has been used widely in cancer chemotherapy for several decades as a single agent and in combination with other antineoplastic drugs. It is approved for the treatment of testicular cancer, Hodgkin's disease, malignant lymphoma, Kaposi's sarcoma, breast cancer, choriocarcinoma, mycosis fungoides, Letterer-Siwe disease, and malignant histiocytosis.¹

Large intra- and interindividual variability in the pharmacokinetics of vinblastine has been reported.^{2,3} This agent undergoes extensive hepatic metabolism and is mainly excreted in feces. Several studies have demonstrated that members of the cytochrome P450 (CYP) 3A sub-family are involved in the metabolism of vinblastine and other vinca alkaloids.⁴⁻¹⁰ CYP3A4-mediated metabolism of vinblastine was first demonstrated using human liver microsomes. Microsomal metabolism of vinblastine was highly variable and correlated with CYP3A4 protein and mRNA expression.¹⁰ In addition to being a substrate of CYP3A4, vinblastine has been shown to inhibit the metabolism of vindesine¹¹ and dihydropyridine denitronifedipine,⁴ both of which are metabolized by CYP3A4.

Other microtubule-targeting agents, including paclitaxel, discodermolide, and the epothilone B analogue ixabepilone, are capable of inducing CYP3A4 expression in vitro.¹²⁻¹⁴ For each of these agents, induction of CYP3A4 is mediated via activation of the pregnane X receptor (PXR, SXR, NR1I2),^{13,14} a member of the nuclear receptor family of ligand-activated transcription factors that have a central role in regulating the transcription of genes involved in xenobiotic metabolism and transport.¹⁵ The objective of this study was to evaluate whether vinblastine also acts as an inducer of CYP3A4, both in vivo and in vitro, and, if so, to determine the role of NR1I2 in this process.

Methods

PATIENTS

A total of 6 patients (Table 1), who were enrolled in a Phase 1/2 study of infusional vinblastine given in combination with the ABCB1 (P-glycoprotein) antagonist valsopodar (PSC 833)¹⁶ and received the CYP3A4 phenotyping probe midazolam on more than 1 occasion, were included in this study. All patients were white and had a diagnosis of renal cell carcinoma. Inclusion and exclusion criteria have been described previously and all patients had adequate hepatic function (aspartate aminotransferase <2.5 times the upper limit of normal; total bilirubin <1.5 times the upper limit of normal).¹⁶ Based on the knowledge that valsopodar is metabolized by CYP3A^{4,17} the same enzyme that is involved in cyclosporine metabolism, the use of strong inducers of cyclosporine metabolism or CYP3A4

(including nafcillin, rifampin, carbamazepine, phenobarbital, and phenytoin) or the use of inhibitors (including diltiazem, nifedipine, verapamil, fluconazole, clarithromycin, erythromycin, bromocriptine, and danazol) was avoided. The study was approved by the institutional review board and all patients gave informed consent.

DRUG ADMINISTRATION

Valspodar, vinblastine, and midazolam were obtained from commercial sources by the National Institutes of Health Clinical Center Pharmacy Department, Bethesda, MD. Briefly, valspodar was prepared for a 24-hour infusion (50 mg/mL in polyoxyethylated castor oil [Cremophor EL] diluted in 250 mL or 500 mL of 5% dextrose or 0.9% sodium chloride) and was administered on the first 2 days of therapy in cycle 1 followed 4 days later by a 6-day infusion of valspodar. Vials containing 10 mg of lyophilized vinblastine were reconstituted and the drug was then further diluted in 250 mL of sterile 0.9% sodium chloride injection. Vinblastine was given as a 3-day continuous intravenous infusion, which began 24 hours after the initiation of valspodar (1-2.6 mg/m²/day) every 28 days (days 9-11 of cycle 1 and days 2-4 during subsequent cycles). Midazolam was administered as an intravenous bolus injection at a dose of 0.0145 mg/kg on day 1 of cycles 1 and 4 to assess phenotypic CYP3A4 activity.

MIDAZOLAM PHARMACOKINETICS

Blood samples were drawn into heparinized tubes at 0.083, 0.25, 0.5, 1, 2, 3, 4, and 5 hours following midazolam administration. Samples were centrifuged immediately to obtain plasma, which was stored at -80 °C until analysis. Plasma concentrations of midazolam were determined by high-performance liquid chromatography with mass spectrometric detection, as described previously.¹⁸ The lower limit of quantitation of this assay is 1 ng/mL, with values for precision and accuracy of ≤15% and <12% relative error, respectively. Pharmacokinetic parameters were determined by noncompartmental analysis using WinNonlin version 4.0 (Pharsight Corporation, Mountain View, CA). The area under the plasma concentration versus time curve (AUC) was calculated using the linear trapezoidal method from time zero to the time of the final quantifiable concentration. The AUC was then extrapolated to infinity by dividing the last measured concentration by the rate constant of the terminal phase (*k*), which was determined by linear-regression analysis of the final 3 time points of the log-linear concentration-time plot. All reported AUC values were dose normalized. The systemic clearance of midazolam was calculated by dividing the administered dose in milligrams by the observed AUC.

STATISTICAL ANALYSIS

Based on the standard deviation of the changes expected in phenotypic CYP3A4 activity (*s_d*), a power (1-β) of 0.8 (80%), a clinically relevant change in the clearance of midazolam of 30% (δ ; standardized difference, $2\delta/s_d$), and a 2-sided significance level (α) of 0.05 (5%), a patient sample size of 6 was required in a paired, 2-sided analysis. A nonparametric 2-sided Wilcoxon signed-rank test was used to assess the differences in the median AUC and clearance of midazolam between day 1 during cycles 1 and 4. All pharmacokinetic data are reported as median values with 95% confidence interval. This calculation was performed with the software package NCSS version 2004 (Number Cruncher Statistical Systems; J. L. Hintze, Kaysville, UT). The cutoff value for statistical significance was set at $p < 0.05$.

GENOTYPING

DNA was isolated from plasma and individual *CYP3A4*1B* (-392A>G; 5'-regulatory region; dbSNP rs ID number, rs2740574; see <http://www.ncbi.nlm.nih.gov/>), *CYP3A4*17* (15615T>C; F189S; rs4987161), *CYP3A4*18A* (20070T>C; L293P; no rs ID number),

*CYP3A5*3C* (6986A>G; splicing defect; rs776746), *CYP3A5*6* (14690G>A; splicing defect; rs10264272), and *ABCB1 (MDR1)* (3435C>T; I1145I; rs1045652) genotypes were determined as described previously.¹⁹

WESTERN BLOTTING

HepG2 cells, maintained in RPMI 1640 supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin, were treated with vinblastine (150-4800 ng/mL) for 48 hours. CYP3A4 protein expression was determined by Western blot analysis as described previously.¹³ For microsomes preparation, cells were centrifuged at $750 \times g$ for 5 minutes and washed immediately in a storage buffer composed of 50 mM potassium phosphate, 1 mM EDTA, and 20% glycerol (pH 7.4). This was followed by homogenization using a glass-Teflon homogenizer and a buffer containing 1.15 M potassium chloride, 10 mM EDTA, 100 mM potassium phosphate, and 0.2 mM phenylmethylsulfonyl fluoride (pH 7.5). Following differential centrifugation ($12,000 \times g$ for 12 minutes; $100,000 \times g$ for 90 minutes), the pellet was resuspended in storage buffer to a concentration of 4 mg/mL. Fifty micrograms of microsomal protein were loaded per lane and transferred to a nitrocellulose membrane following separation by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Human CYP3A4 + reductase BD Supersomes (BD Biosciences, Bedford, MS) were used as a positive control. The WB-MAB-3A mouse monoclonal antibody and an HRP-conjugated rabbit anti-mouse IgG were purchased from BD Biosciences and diluted as per manufacturer's instructions. Bands on film were optically scanned (Epson Expression 1600, San Diego, CA).

TRANSIENT TRANSFECTION EXPERIMENTS

The reporter plasmids pSG5-hPXR (human NR1I2) and pSG5-mPXR (mouse NR1I2) were provided by Dr. Steven Kliewer (University of Texas Southwestern Medical Center, Dallas, TX).²⁰ The reporter plasmids pDR-hCAR (human NR1I3) and pcDNA3-mCAR (mouse NR1I3) were provided by Drs. David Moore (Baylor College of Medicine, Houston, TX) and Masahiko Negishi (National Institute of Environmental Health Sciences, Research Triangle Park, NC), respectively. The CYP3A4-PXRE-LUC reporter plasmid containing the proximal promoter (-362/+53) and distal XREM (-7836/-7208) was prepared as previously described.²¹ Vinblastine (Sigma-Aldrich, St. Louis, MO) and valsopodar (Novartis Pharmaceutical Corp., East Hanover, NJ; distributed by the Cancer Therapy Evaluation Program, National Cancer Institute, Bethesda, MD) were dissolved in dimethyl sulfoxide (DMSO) and stored at -20 °C. HepG2 and NIH3T3 cells were maintained in minimum Eagle's medium supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin, and 1% L-glutamine. Experiments were performed as described previously.²² Briefly, approximately 3×10^5 cells were seeded into 24-well plates, 24 hours before co-transfection by calcium phosphate precipitation with 0.5 µg of the reporter plasmid and 0.1 µg of the expression plasmid or empty vector plasmid. The SV40-β-galactosidase control vector (0.1 µg) was used as an internal control for transfection efficiency. Twelve hours later, cells were washed and then incubated with drug prepared in medium containing 10% charcoal dextran-treated fetal bovine serum (Hyclone Laboratories, Logan, UT) for 24 hours. Cells were harvested, lysed, and centrifuged at 1500 xg for 4 min, and an aliquot of supernatant was used to determine luciferase (Luciferase Assay System, Promega, Madison, WI) and β-galactosidase (β-Galactosidase Enzyme Assay System, Promega, Madison, WI) activities according to the manufacturers' instructions. Luciferase activity was normalized to β-galactosidase activity. All experiments were performed twice in triplicate. As positive controls, rifampin and pregnenolone-16α-carbonitrile (PCN) were used for human and mouse NR1I2, and CITCO (6-(4-chlorophenyl)imidazo[2,1-b][1,3]thiazole-5-carbaldehyde O-(3,4-dichlorobenzyl)oxime) and TCPOBOP (1,4-bis[2-(3,5-dichloropyridyloxy)]benzene)

for human and mouse NR1I3, respectively. The final concentration of DMSO was $\leq 0.2\%$ in all experiments.

Results

IN VIVO STUDY

The CYP3A4 phenotyping probe midazolam (0.0145 mg/kg) was administered by intravenous bolus to 6 patients with renal cell carcinoma undergoing treatment with vinblastine (72-hour intravenous infusion given every 28 days). None of the patients received any prescription drug other than vinblastine that is known or suspected to alter CYP3A4 activity that could have confounded the results, albeit that valsopodar is a very weak CYP3A4 inhibitor.²³ Midazolam clearance and AUC were determined on day 1 and during cycle 4 (days 92-119; Figure 1). On day 1 of cycle 1, the clearance of midazolam ranged from 16.2 to 28.1 L/h (median 21.7; 95% CI 12.6 to 28.1). All 6 patients exhibited increased midazolam clearance between cycle 1 and cycle 4, indicative of increased CYP3A4 activity following repeat administration of vinblastine (Figure 1). The observed increase in midazolam clearance ranged from 2% to 217%, with a median value of 32.3 L/h (95% CI 17.3 to 53.9; $p = 0.0156$). The corresponding median AUC values decreased from 46.4 ng·h/mL per mg (95% CI 35.6 to 61.7) to 31.0 ng·h/mL per mg (95% CI 18.6 to 57.7; $p = 0.0313$). The half-life of midazolam ranged from 2.19 to 3.52 hours (median 2.54; 95% CI 2.19 to 3.52) on day 1 of cycle 1, and from 2.52 to 4.81 hours (median 3.08; 95% CI 2.52 to 4.81) on day 1 of cycle 4 ($p = 0.0793$). One patient received midazolam on 5 occasions over a period of 1.3 years, at the end of which midazolam clearance was 3.6-fold above the clearance value prior to treatment with vinblastine. The interindividual variability in the extent of CYP3A4 induction by vinblastine was not obviously associated with any of the studied *CYP3A4*, *CYP3A5*, and *ABCB1* genotypes (Table 1).

IN VITRO CYP3A4 PROTEIN EXPRESSION

Expression of CYP3A4 protein was determined following exposure of HepG2 cells to vinblastine for 48 hours at concentrations ranging between 150 and 4800 ng/mL. Vinblastine, at concentrations of 300 ng/mL and above, substantially increased CYP3A4 protein expression compared to that seen in DMSO -treated control cells (Figure 2).

REPORTER GENE ASSAYS

Cell-based luciferase reporter gene assays for the determination of NR1I2 transcriptional activity were performed to establish whether vinblastine is an activating ligand of NR1I2. HepG2 cells were transiently transfected with the expression plasmid for full-length human NR1I2 (pSG5-hPXR) or mouse NR1I2 (pSG5-mPXR) and the respective luciferase reporter plasmids. Treatment with vinblastine resulted in a concentration-dependent increase in transcriptional activity of both human and mouse NR1I2 (Figure 3). The extent of activation by vinblastine (2-fold to 3-fold), however, was relatively weak compared to that of the positive controls rifampin and PCN, respectively, which resulted in a 5-fold to 8-fold increase in reporter activity. Vinblastine had no effect on the transcriptional activity of human and mouse full-length NR1I3 in transiently transfected NIH3T3 cells (Figure 4). Increased activity of the reporter was not observed after treatment with valsopodar, suggesting that this compound is not an activating ligand of NR1I2 or NR1I3 (Figure 3 and Figure 4).

Discussion

In this study, the ability of the antimicrotubule agent vinblastine to induce CYP3A4 was evaluated both clinically and in vitro. Repeated exposure to vinblastine resulted in

significantly increased clearance of the CYP3A4 phenotyping probe midazolam in all patients, indicative of induction of CYP3A4. Midazolam does not interact with ABCB1.²⁴ In addition to vinblastine, patients received the ABCB1 inhibitor valsopodar,¹⁶ which might be considered a drawback of this study. However, since valsopodar is reported to be a potential competitive inhibitor of CYP3A4,¹⁷ it is unlikely that this agent contributed to the observed induction of this drug-metabolizing enzyme; rather, valsopodar would be expected to mask the effect of vinblastine on the clearance of midazolam. Moreover, valsopodar is only a very weak inhibitor of midazolam metabolism through CYP3A4/5 in human liver microsomes, and selective ABCB1 inhibitors do not affect midazolam clearance mechanisms in humans.^{23,25}

To provide mechanistic evidence that induction of CYP3A4 expression by vinblastine is responsible for the increase in midazolam clearance observed clinically, HepG2 cells were exposed to increasing concentrations of vinblastine for 48 hours. Consistent with the clinical observation, vinblastine substantially induced CYP3A4 protein expression in vitro, even at low concentrations. The majority of compounds that induce CYP3A4 do so via activation of the nuclear receptor NR1I2. Furthermore, this has recently been shown to be the mechanism of CYP3A4 induction by other microtubule-targeting agents, including paclitaxel and epothilones,^{13,14} and the mechanism ABCB1 induction through vinblastine itself at a higher concentration (10 μ M, or 8110 ng/mL).²⁶ Therefore, cell-based luciferase reporter gene assays were performed to determine whether vinblastine is an activating ligand of NR1I2 between 150 ng/mL and 4800 ng/mL. The results from these experiments suggest that vinblastine is a weak activator of both human and mouse NR1I2 and that the observed induction of CYP3A4 expression is likely to occur, at least in part, via an NR1I2-dependent mechanism. Of note, activation of another closely related nuclear receptor that can regulate CYP3A4, namely NR1I3,²⁷ by vinblastine was not observed, even at high concentrations. However, activation of another nuclear receptor that can regulate CYP3A4, such as the vitamin D receptor (NR1I1),²⁸⁻³¹ or the glucocorticoid receptor (NR3C1),³² cannot be excluded. Additionally, CYP3A4 induction may occur as a post-translational event as a result of stabilization of mRNA or decreased protein turnover.

The observed induction of CYP3A4 by vinblastine has considerable clinical implications. First, since vinblastine itself is a substrate of this enzyme,¹⁰ autoinduction leading to accelerated metabolism may occur in patients receiving vinblastine over prolonged periods. Second, there is the possibility for a previously unrecognized type of pharmacokinetic interaction between vinblastine and concomitantly administered drugs that are CYP3A4 substrates. Both of these events could result in altered efficacy or toxicity of the drugs involved. Indeed, CYP3A4 induction by vinblastine may provide a plausible explanation for the outcomes of 2 earlier clinical studies involving this drug.^{33,34} For example, it was reported that treatment of patients with testicular cancer using a combination regimen of cisplatin, vinblastine, and bleomycin resulted in a 30% increase in the clearance of antipyrine, which lasted for 6 weeks.³⁴ Although antipyrine is metabolized by several CYP isoenzymes, CYP3A4 is responsible for the formation of a major metabolite, 4-hydroxyantipyrine.³⁵ Additionally, the combination of paclitaxel, vinblastine, and cisplatin was found to be less effective and less toxic than expected in the treatment of advanced transitional cell carcinoma of the urothelium.³³ In this combination, vinblastine was administered as a bolus prior to paclitaxel, which is very extensively metabolized by CYP2C8 and CYP3A4.^{36,37} Thus, induction of the CYP3A4-mediated metabolism of paclitaxel by vinblastine could contribute to the low response rate and the low incidence of peripheral neuropathy reported. However, vinblastine was administered as a bolus injection in the above study, while our data were obtained in patients receiving a continuous infusion, and it remains unclear whether or not the inductive effect on CYP3A4 through vinblastine treatment is schedule dependent in vivo.

Finally, there is substantial evidence for the involvement of CYP3A4 in the metabolism of other vinca alkaloids in addition to vinblastine. Studies with human liver microsomes revealed that CYP3A4 is responsible for the metabolism of vindesine.¹¹ Vincristine and vinorelbine were shown to inhibit vinblastine metabolism,¹⁰ and vincristine also inhibited the metabolism of dihydropyridine denitronifedipine.⁴ The CYP3A4-inducing antiepileptic agents carbamazepine and phenytoin increased the clearance of vincristine in patients with brain tumors.⁷ Furthermore, nifedipine and itraconazole, which are inhibitors of CYP3A4, decreased the clearance⁶ and increased the neurotoxicity⁵ of vincristine, respectively. Since vinblastine acts as an inducer of CYP3A4, in addition to being a substrate and inhibitor of this enzyme, it is possible that similar induction phenotypes are associated with other vinca alkaloids.

In conclusion, this study indicates that vinblastine acts as an inducer of CYP3A4 both clinically and in vitro. Similar to other microtubule-targeted agents known to induce CYP3A4, induction of CYP3A4 by vinblastine appears to occur, at least in part, via an NR1I2-dependent mechanism. This observation has clinical ramifications not only for vinblastine, but also for other vinca alkaloids in clinical use or development.

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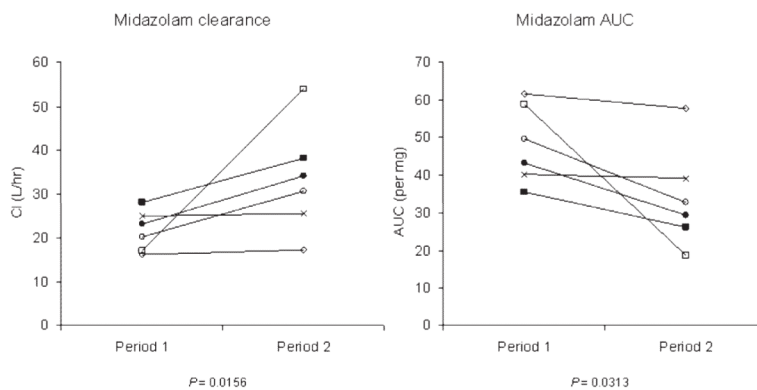


Figure 1. Effect of vinblastine on midazolam clearance (Cl; L/h) and area under the curve (AUC) (ng·h/mL per mg). Data were obtained from 6 patients treated with vinblastine as a 72-hour intravenous infusion every 28 days. Midazolam (0.0145 mg/kg, intravenous bolus) was used as a CYP3A4 phenotyping probe on day 1 of cycle 1 (period 1) and on day 1 of cycle 4 (period 2). A nonparametric 2-sided Wilcoxon signed-rank test was used to assess the differences in medians.

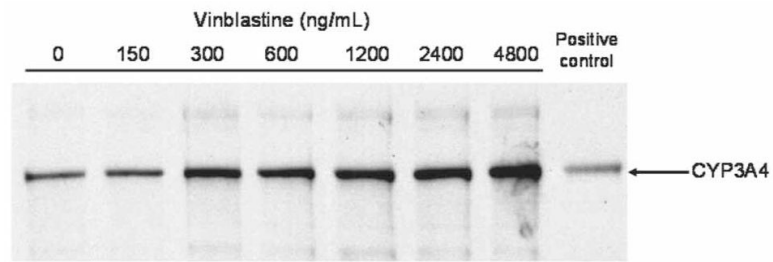


Figure 2. Effect of vinblastine on the expression of CYP3A4 protein in HepG2 cells. Cells were treated with vinblastine for 48 hours as indicated and CYP3A4 protein expression was determined by Western blot analysis. Human CYP3A4 plus reductase BD Supersomes were used as a positive control (far right lane). The range used corresponds to vinblastine concentrations of 0.165 μ M (150 ng/mL) to 5.28 μ M (4800 ng/mL)

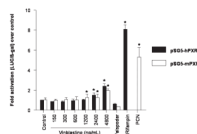


Figure 3.

Effect of vinblastine and valsopodar (PSC 833) on the transcriptional activity of NR1I2 in HepG2 cells co-transfected with the pSG5-hPXR and pSG5-mPXR expression plasmids and CYP3A4-PXRE2-LUC reporter plasmid. Rifampin (10 μ M) and pregnenolone-16 α -carbonitrile (PCN; 50 μ M) were used as positive controls. Experiments were performed twice in triplicate, except for vinblastine 150 ng/mL for pSG5-hPXR (n = 1). Error bars are standard error of the mean. The range used corresponds to vinblastine concentrations of 0.165 μ M (150 ng/mL) to 5.28 μ M (4800 ng/mL).

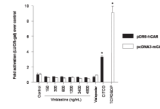


Figure 4.

Effect of vinblastine and valsopodar (PSC 833) on the transcriptional activity of NR1I3 in NIH3T3 cells co-transfected with the pDR6-hCAR and pcDNA3-mCAR expression plasmids and CYP3A4-PXRE2-LUC reporter plasmid. CITCO (6-(4-chlorophenyl)imidazo[2,1-b][1,3]thiazole-5-carbaldehyde *O*-(3,4-dichlorobenzyl)oxime; 100 nM) and TCPOBOP (1,4-*bis*[2-(3,5-dichloropyridyloxy)]benzene; 250 nM) were used as positive controls. Experiments were performed twice in triplicate. Error bars are standard error of the mean. The range used corresponds to vinblastine concentrations of 0.165 μ M (150 ng/mL) to 5.28 μ M (4800 ng/mL).

Table 1

Patient Demographics and Genotypes

Pts. Sex	Age, y	ECOG PS	Midazolam CI	% ↑	CYP3A4*1B	CYP3A4*17	CYP3A4*18A	CYP3A5*3C	CYP3A5*6	ABCB1/3435C>T
Male	55	1	51.7		*1A/*1A	*1A/*1A	*1A/*1A	*3C/*3C	*1A/*1A	CT
Male	60	0	35.9		*1A/*1B	*1A/*1A	*1A/*1A	*1A/*3C	*1A/*1A	TT
Male	58	1	216.7		*1A/*1A	*1A/*1A	*1A/*1A	*3C/*3C	*1A/*1A	CT
Male	40	1	2.5		*1A/*1A	*1A/*1A	*1A/*1A	*3C/*3C	*1A/*1A	CT
Male	50	1	46.9		*1A/*1A	*1A/*1A	*1A/*1A	*3C/*3C	*1A/*1A	CC
Female	66	1	6.9		*1A/*1A	*1A/*1A	*1A/*1A	*3C/*3C	*1A/*1A	CT

CI = clearance; ECOG PS = Eastern Cooperative Oncology Group Performance Status.