The Orally Administered P-glycoprotein Inhibitor R101933 Does Not Alter the Plasma Pharmacokinetics of Docetaxel

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ABSTRACT

This Phase I study was performed to assess the feasibility of combining docetaxel with the new P-glycoprotein inhibitor R101933 and to determine the dose limiting toxicity of this combination. Fifteen patients received oral R101933 alone at a dose escalated from 200 to 300 mg twice daily (b.i.d.; cycle 0), an escalating i.v. dose of docetaxel (60, 75, and 100 mg/m^2) as a 1-h infusion (cycle 1), and the combination (cycle 2 and further). Dose limiting toxicity consisting of mucositis and neutropenic fever was reached at the combination of docetaxel, 100 mg/m^2, and R101933, 300 mg b.i.d., and the maximum tolerated dose was established at docetaxel, 100 mg/m^2, and R101933, 200 mg b.i.d. Plasma concentrations of R101933 achieved in patients were in the same range as required in preclinical rodent models to overcome paclitaxel resistance. The plasma pharmacokinetics of docetaxel were not influenced by the R101933 regimen at any dose level tested, as indicated by plasma clearance values of 26.5 ± 7.78 liters/h/m^2 and 23.4 ± 4.52 liters/h/m^2 ($P = 0.15$) in cycles 1 and 2, respectively. These findings indicate that the contribution of a P-glycoprotein inhibitor to the activity of anticancer chemotherapy can now be assessed in patients for the first time independent of its effect on drug pharmacokinetics.

INTRODUCTION

Acquired or intrinsic resistance of malignant cells to taxanes and other naturally occurring drugs has been linked to the so-called "classical" mechanism of MDR[2] resulting in decreased intracellular concentrations of these anticancer drugs. This MDR phenotype is characterized by increased levels of P-glycoprotein, a member of the ATP-binding cassette superfamily of transmembrane transport proteins with a molecular weight of M_r 170,000 encoded by the *MDR1* gene and acting as an energy-dependent drug efflux pump with broad substrate specificity (1, 2).

Since the first observation that verapamil could reverse MDR *in vitro*, similar properties have been shown for a wide range of drugs (3). These agents are thought to be competitive substrates for P-glycoprotein and thus can increase the intracellular concentration of a coadministered anticancer agent and consequently restore the antitumoral activity (4). Initially, a number of drugs, marketed for other indications than inhibiting P-glycoprotein, have entered clinical trials (5). However, it became evident that pharmacokinetic interactions occurred between these P-glycoprotein inhibitors and the coadministered anticancer drugs due in part to competitive inhibition of cytochrome P-450 enzymes resulting in significantly increased toxicity of the anticancer drug (6). By rational design, new modulators were developed to specifically inhibit P-glycoprotein and to be more suitable candidates for further clinical evaluations (7, 8). The results of most of these clinical studies have been rather disappointing, and the pharmacokinetic interaction between the cytotoxic and the P-glycoprotein inhibiting agent remains a confounding problem (6, 9).

R101933 (Fig. 1) is a new p.o. administered compound that inhibits P-glycoprotein as demonstrated by various *in vitro* and *in vivo* models (10, 11). The tolerability, cardiovascular and laboratory safety, and the pharmacokinetics were investigated in healthy subjects.[3] Nausea and vomiting were the dose-limiting adverse events and were reported above the 400-mg single oral dose. Drowsiness was also mentioned as a side effect. No clinically relevant changes in laboratory and cardiovascular safety parameters were observed. *In vitro* metabolism studies showed that the major metabolic pathway is not cytochrome P450 3A4-dependent.[3] Plasma levels of R101933 at 200 mg b.i.d. are in the range of concentrations that are active in paclitaxel and Adriamycin-resistant human tumor xenograph rodent models.[3]

Docetaxel is a known substrate of P-glycoprotein and has shown to have a higher affinity for the protein than the related compound paclitaxel (12, 13). It also lacks the problems associated with i.v. use of paclitaxel caused by the presence of the

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[2] The abbreviations used are: MDR, multidrug resistance; AUC, area under the plasma concentration-time curve; b.i.d., twice daily; NCI, National Cancer Institute; DLT, dose-limiting toxicity; DLTc, DLT of chemotherapy; DLTr, DLT of R101933; MTD, maximum tolerated dose; CTC, Common Toxicity Criteria; PD, progressive disease.

[3] Janssen Research Foundation, data on file.

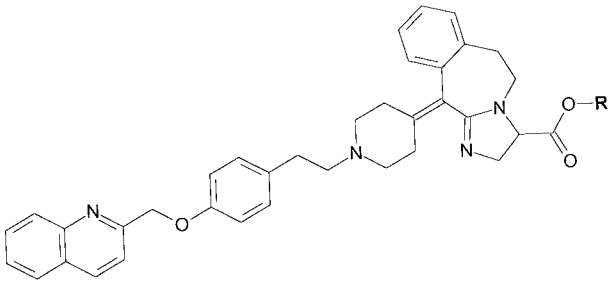

Fig. 1 Structure of R101933 ($R = CH_3$) and its main metabolite R102207 ($R = H$).

formulation vehicle Cremophor EL, which is known to (*a*) alter the pharmacokinetics of the anticancer drug by entrapment in micelles (14) and (*b*) mask the effects, if any, of endogenously expressed P-glycoprotein on the plasma levels of paclitaxel (15). Therefore, the development of agents that could reverse or prevent the development of resistance to docetaxel is of great interest.

The principal objectives of this Phase I and pharmacokinetic study of R101933 and docetaxel were to determine the clinical utility of the combination and to investigate the potential lack of pharmacokinetic interactions.

PATIENTS AND METHODS

Eligibility. Patients with a histologically confirmed diagnosis of a solid tumor for whom docetaxel as monotherapy was a viable therapeutic option or for whom other treatment options were not available were candidates for this study. Additional eligibility criteria were: age $\geq$18 and $\leq$75 years; Eastern Cooperative Oncology Group performance status $<$3; life expectancy of at least 3 months; off previous anticancer therapy for at least 4 weeks; no previous treatment with taxanes or high dose chemotherapy requiring progenitor cell support; adequate bone marrow function (WBC count $>3.5 \times 10^9$/liter, platelet count $>100 \times 10^9$/liter), renal function (serum creatinine $\leq$2 times the upper limit of normal), and liver function (bilirubin level normal, aspartate/alanine aminotransferase $\leq$2 times upper limit of normal, and alkaline phosphatase $\leq$2.5 times upper limit of normal); and symptomatic peripheral neuropathy less than grade 2 (NCI criteria). Written informed consent was obtained from all patients, and the study was approved by the Rotterdam Cancer Institute Ethics Board.

Pretreatment and Follow-up. Pretreatment evaluation consisted of recording the history of the patient, physical examination, laboratory studies, electrocardiography, and chest X-ray. Computer tomographic scans were performed for tumor measurements. Laboratory studies included a complete blood-cell count analysis and measurement of WBC differential, electrolytes (including sodium, potassium, chloride, calcium, and inorganic phosphate), creatinine, urea, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, bilirubin, total plasma proteins, serum albumin, glucose, uric acid, and urinalysis. History, physical examination, and toxicity scoring (according to the NCI-expanded CTC) were repeated once a week. Complete blood cell counts, including WBC differential, were performed twice a week, and the other laboratory tests were done once a week. Electrocardiography was repeated as clinically indicated. A final assessment was to be made after patients went off the study. Formal tumor measurements and chest X-ray were performed at 6-week intervals until documentation of PD. Standard WHO response criteria were used.

Drug Administration. Docetaxel was administered every 3 weeks on day 3 as a 1-h infusion and was started 1 h after intake of R101933. All patients received premedication with dexamethasone, p.o. 8 mg b.i.d., starting 1 day before each infusion of docetaxel for 5 days. R101933 (Janssen Research Foundation, Beerse, Belgium) was supplied as a 10-mg/ml oral solution in 15% hydroxypropyl-β-cyclodextrin. It had to be taken with water at least 1 h after a meal. The drug was administered twice daily from days 1–5. From studies with healthy volunteers, it was known that the terminal half-life of R101933 averaged about 24 h, with peak plasma concentrations attained within 2 h after intake. The MTD after 7-day b.i.d. dosing was 300 mg in healthy volunteers.[3] Seven-day dosing at 200 mg b.i.d. appeared to be safe and well tolerated. Pharmacokinetic data revealed that plasma levels of R101933 at 200 mg b.i.d achieve concentrations that are in the same range as required in *in vivo* models to overcome paclitaxel resistance. Hence, the starting dose for our study was set at this dose level. In view of the terminal disposition half-life of docetaxel, a simultaneous exposure to both R101933 and docetaxel for a 3-day period was considered sufficient. This led to the choice of the 5-day R101933 regimen. In the first stage of the study, the dose of docetaxel was escalated and the dose of R101933 was fixed. In the second stage of the study, the dose of docetaxel was fixed and the dose of R101933 was escalated.

First, the patients received five doses of R101933 alone every 12 h (cycle 0) followed by a 48-h wash-out to allow assessment of the terminal half-life of R101933. One week later, cycle 1 was initiated with docetaxel alone. Thereafter, the combination was given triweekly until PD or DLT occurred.

In each cohort, three patients were treated unless DLTc or DLTr was observed. In that case, the accrual of three additional patients was required. DLTc was defined as grade 3 nonhematological toxicity (with the exception of nonhematological toxicity that was still manageable in an out-patient setting, such as nausea/vomiting) or grade 4 neutropenia lasting $>$8 days or grade 4 thrombocytopenia or required delay $>$2 weeks to a subsequent cycle due to toxicity. Febrile neutropenia and neutropenia with severe infection ($\geq$grade 3 infection) was also considered as DLTc. DLTr was defined as any nonhematological toxicity $>$grade 2 in the first 2 days of treatment before chemotherapy was given. For dose-escalation decisions, only DLTs in cycles 0 and 2 were taken into account. The DLT of the combination of R101933 with docetaxel was reached when greater or equal to three of six patients experienced DLTc. The DLT of R101933 alone was reached when greater or equal to one of three (or greater or equal to two of six) patients experienced DLTr. The MTD was defined as the dose level below DLT.

Sample Collection and Processing. Blood specimens were taken in all patients during the first, second, and third courses of treatment. Blood volumes of 6 ml were drawn di-

rectly into Vacutainer tubes containing lyophilized sodium heparin (Becton Dickinson, Meylan, France) from a peripheral venous access device. In each patient, sufficient plasma was obtained before drug administration to evaluate possible interfering peaks in the chromatographic analysis. Samples for docetaxel analysis were collected immediately before infusion and at 0.5, 1, 1.25, 1.5, 2, 3, 7, 11, 23, and 31 h after the start of infusion. For the determination of R101933 concentrations, blood samples were obtained on day 1 (before the first dosing), day 2 (before the second dosing and 12 h thereafter), and day 3 (before the third dosing and 2, 4, 8, 12, 24, 32, and 48 h thereafter). All blood samples were centrifuged immediately for 10 min at 1000 × *g* to yield plasma, which was stored frozen in polypropylene vials (Eppendorf, Hamburg, Germany) until the time of analysis.

Analytical Methods. A pure reference standard of docetaxel (batch, 14PROC9230; purity, 98.0% by reversed-phase high-performance liquid chromatography) and the clinical docetaxel formulation in polysorbate 80 (Taxotere; 40 mg/ml) were kindly supplied by Rhône-Poulenc Rorer (Vitry-sur-Seine, France) and were used as received. Plasma concentrations of docetaxel were determined by a validated liquid chromatographic/tandem mass spectrometric assay, with a lowest limit of quantitation of 1 ng/ml. Samples (200 μl) were pretreated by solid-phase extraction using endcapped Bond Elut nitrile microcolumns (Varian, Harbor City, CA), based on an earlier procedure described for paclitaxel (16). A stainless steel analytical column (100 × 4.6 mm internal diameter) packed with 3-μm Hypersil BDS C18 material (Alltech, Breda, the Netherlands) was used for chromatographic separation, and gradient elution was performed with a mixture of acetonitrile and 0.02 M ammonium acetate (pH 4.0) at a flow rate of 0.8 ml/min. Paclitaxel (50 μl of 20 μg/ml in acetonitrile) was used as internal standard. Triple quadrupole mass-spectrometric detection was performed with a turboionspray interface used in the positive ion mode with selective monitoring at *m/z* 808.5 (molecular ion docetaxel parent) and *m/z* 854.5 (molecular ion paclitaxel parent) and at *m/z* 527.0 (docetaxel taxane ring fragment) and *m/z* 569.0 (paclitaxel taxane ring fragment) in the first and third quadrupole, respectively. Calibration curves spanning a range of 1–5000 ng/ml were calculated by regression analysis of peak area ratios of docetaxel:internal standard *versus* the spiked drug concentration of the standard.

Blood samples collected and processed to plasma were also analyzed, as appropriate, for R101933 and its esterase-mediated carboxylic acid metabolite R102207 using a validated high-performance liquid chromatography method. This assay used a selective solid-phase extraction with Bond-Elut Certify microcolumns (Varian). The columns were conditioned with 3 ml of ethanol, 3 ml of deionized water, and 1 ml of 1 M aqueous acetic acid. Plasma samples (1 ml) were mixed with three volumes of 1 M acetic acid and 100 μl of the internal standard (R125026; 10 μg/ml in acetonitrile) and then loaded on the extraction columns. Consecutive washing steps with 3 ml of deionized water, 1 ml of 1 M acetic acid, and 3 ml of ethanol were performed before elution in 3 ml of ethanol:ammonia (98:2, v/v). Samples were dried under nitrogen at 65°C and reconstituted in 100 μl of 0.02 M ammonium formate (pH 4.0):acetonitrile:ethanol (50:25:25, v/v/v). The analytes were separated on a 3-μm Hypersil BDS C8 column (100 × 4.6 mm internal diameter; Alltech) using a mobile phase comprising 0.02 M ammonium formate (pH 4.0), acetonitrile, and ethanol delivered with gradient elution at 0.8 ml/min. Detection was performed by UV absorption measurements at 270 nm. The concentrations of R101933 and R102207 were determined from calibration curves constructed in blank human plasma in the range of 2–10,000 ng/ml. The ratio of the log-transformed peak areas of each of the analytes: internal standard were plotted *versus* nominal concentrations for quantitative computations.

Pharmacokinetic Data Analysis. Individual plasma concentration-time profiles of R101933 and its inactive metabolite R102207 were analyzed model independently using a validated macro in the EXCEL software package. The actual times of drug intake and blood sampling were taken into account. Peak plasma concentration (C_{max}) was determined by visual inspection of the data. The AUC within a 12-h dosing interval was calculated by the trapezoidal rule. In all cases, the AUC was extrapolated to infinity by addition of C_{last}/λ, in which C_{last} is the last quantifiable concentration in the curve and λ is the terminal elimination rate constant determined by linear regression analysis of the terminal points of the ln-linear plasma concentration-time curve. The terminal disposition half-life [$t_{1/2(2)}$] was defined as $\ln 2/\lambda$. Individual plasma concentration-time curves of docetaxel were analyzed using the software package WinNonlin (Pharsight, Mountain View, CA) by determining the slopes and intercepts of the plotted curves with multiexponential functions. All curves were fitted using the actual infusion duration and blood sampling times. In all cases, concentration-time profiles of docetaxel were best fitted to a biexponential equation after zero-order input with weighting according to the square of the model predictions of the concentrations. Final values of the iterated parameters of the best-fit function were used to calculate the pharmacokinetic parameters using standard equations (17).

Statistical Considerations. Pharmacokinetic parameters for docetaxel and R101933 are reported as mean values ± SD. Variability in dose-normalized parameters between the various docetaxel dose levels was evaluated by the Kruskal-Wallis statistic followed, if required, by a Dunn's test to determine which group differed. To test pharmacodynamic and pharmacokinetic parameter differences for statistical significance among treatment courses, a two-tailed paired Student's *t* test was performed. Probability values of <0.05 were regarded as statistically significant. All statistical calculations were performed using the Number Cruncher Statistical System version 5.X (Dr. Jerry Hintze, Kaysville, UT; 1992) or using Statgraphics Plus version 2 (Manugistics Inc., Rockville, MA).

RESULTS

Seventeen patients were entered into this study. Patient characteristics are listed in Table 1; all patients were eligible. Two patients were considered not evaluable for toxicity and response. They did not receive the combination therapy, one because of unexpected rapid deterioration of the clinical condition and another because of development of liver enzyme abnormalities due to the malignant disease that would have precluded administration of docetaxel within normal safety limits.

Table 1 Patient characteristics

Characteristic		No.
Patients included		17
Sex		
Male		10
Female		7
Age, yr		
Median	57.5	
Range	42–72	
Performance score (WHO)		
0		8
1		9
Primary tumor		
Urogenital tract		6
Gastrointestinal tract		5
Respiratory tract		2
Melanoma		2
Sarcoma		1
Unknown primary		1
Prior therapy		
Surgery		14
Radiotherapy		7
Chemotherapy		11
None		1

At the dose level for R101933, 300 mg b.i.d., one patient was not evaluable for pharmacokinetics because it was not possible to take blood samples during the second cycle for technical reasons.

Toxicity and Pharmacodynamics. A total of 59 cycles, including 44 cycles of combined docetaxel and R101933, were given. Table 2 lists the number of cycles at each dose level and the main toxicities at each dose level. In the first part of the study, the dose of docetaxel was escalated and the dose of R101933 was fixed at 200 mg b.i.d. Each dose level of docetaxel, 60, 75, and 100 mg/m^2 respectively, consisted of three patients, and no DLT was experienced at this stage. One patient at the dose level of 60 mg/m^2 was hospitalized because of neutropenic fever after cycle 3 and was diagnosed to have pneumonia and sinusitis. Because of this clearly unrelated focus, the patient received two further cycles, which were uneventful. Two patients had grade 2 diarrhea starting 1 week after docetaxel infusion lasting not more than 4 days. Due to infection occurring in the area of the primary head/neck tumor after cycle 0, one patient treated with docetaxel at a dose level of 100 mg/m^2 was given prophylactic antibiotics during both cycles 1 and 2. From all three dose levels of docetaxel studied with R101933, 200 mg b.i.d., it was concluded that the combination was feasible with docetaxel given up to 100 mg/m^2, which is the single agent MTD for docetaxel (12).

In the second part of the study, the dose of docetaxel was fixed at 100 mg/m^2 and the dose of R101933 was escalated to 300 mg b.i.d. Paired analysis of hematological pharmacodynamic parameters indicated that R101933 coadministration had no significant influence on the observed myelotoxicity (Table 3), including the percent decrease in WBC and absolute neutrophil count. DLTs were seen in four of six patients, but in two of these already with single treatment of docetaxel, and thus could not be attributed to the combination (Table 4). DLT consisted of neutropenic fever ($n = 2$), mucositis ($n = 1$), and vomiting ($n = 1$).

DLTr was not reached at any of the investigated dose levels. Nausea/vomiting and drowsiness, known to be side effects of R101933 in healthy subjects,[3] were not seen in our patients after administration of R101933 alone. Fatigue was often mentioned as a side effect after docetaxel but never exceeded grade 2. Nevertheless, for one patient given docetaxel at 100 mg/m^2 and R101933, 200 mg b.i.d., it was a reason to refuse further treatment after cycle 3, although an ongoing partial response was noted. Two patients at docetaxel 100 mg/m^2 and R101933, 300 mg b.i.d., went off the study after cycle 2 because of the observed DLT consisting of mucositis and vomiting, respectively. All other patients went off the study because of PD. Two patients achieved a partial response, and seven had stable disease.

Plasma Pharmacokinetics. For the evaluation of docetaxel pharmacokinetics, only the patients who had sampling and complete kinetic data during both treatment courses were included ($n = 14$ of 15). The results of paired plasma concentration-time profiles of unchanged docetaxel given with and without cotreatment were remarkably similar for all patients studied (Fig. 2). During both treatment courses, disposition phases appeared to be very typical of a biexponential profile, with plasma concentrations of docetaxel decreasing very rapidly immediately after cessation of the infusion, followed by a more prolonged terminal disposition phase of ~11 h, in line with previous observations (18). The mean pharmacokinetic parameters of docetaxel for both treatment courses are summarized as a function of the study cohort in Table 5. The docetaxel total body clearance was normally distributed as judged by the D'Agostino-Pearson omnibus K^2 test, was independent of the administered dose (Kruskal-Wallis, $P = 0.396$), and averaged 26.5 ± 7.78 liters/h/m^2 (mean ± SD) without R101933 and 23.4 ± 4.52 liters/h/m^2 with R101933 (Kruskal-Wallis, $P = 0.608$), which is within the same range as described for this compound previously (18). There were no statistically significant differences in any of the studied docetaxel pharmacokinetic parameters, including the clearance ($P = 0.15$), between the two treatment courses (Table 5), suggesting that R101933 administration did not influence the disposition of the taxane at the dose levels tested. At the final dose level, combining docetaxel at 100 mg/m^2 and R101933, 300 mg b.i.d, statistical analysis indicated that a 1.3-fold change in docetaxel clearance could have been detected with $(1\text{-}\beta) = 0.80$ at the observed SD of the mean difference between cycles ($s_d = 3.13$) and a calculated standardized difference of $2\delta/s_d$ (19).

Similarly, docetaxel did not significantly alter the absorption and elimination routes of R101933 (Table 6). In addition, dose-normalized AUC values for R101933 were similar with or without docetaxel cotreatment. Overall, substantial interpatient variability in R101933 kinetic parameters was apparent, with up to a 10-fold variation in peak plasma levels. Over the total dose range studied, the peak plasma levels of R101933 did not increase with values of 133 ± 74 ng/ml (mean ± SD; $n = 10$) and 136 ± 45 ng/ml ($n = 7$) respectively, suggesting a dose-dependent kinetic behavior of the compound with saturable absorption characteristics. For this reason, no attempt was made to further increase the dose of R101933. In all patients, there was extensive formation of the pharmacologically inactive compound R102207, the principal circulating metabolite of

Table 2 Main toxicities (worst per cycle) at each dose level expressed in number of cycles in which they occurred

Docetaxel mg/m^2	R101933 mg b.i.d.	No. pts/ cycle	Neutropenia CTC grade[a] 0	1	2	3	4	Neutropenic fever	Vomiting CTC grade 0	1	2	3	4	Mucositis CTC grade 0	1	2	3	Fatigue CTC grade 0	1	2	DLTc
60	200	3/11	4		5		2	1	10		1			11				6		5	0
75	200	3/18	1	1	6	3	7		17	1				16	2			10	6	2	0
100	200	3/7			2		5	2	7					5	1	1		2	1	4	0
100	300	6/23	1	1	2	3	16	4	20		1		2	17	3	2	1	12	7	4	4

[a] NCI CTC.

Table 3 Summary of hematological pharmacodynamics[a]

Parameter	Cycle 1	Cycle 2 and further	Mean difference	95% C.L.[b]	P[c]
Leukocytes					
Nadir ($\times 10^9$/liter)	1.51 ± 1.00 (0.43–3.40)	2.23 ± 1.82 (0.20–7.30)	−0.72 ± 0.35	−1.48, 0.038	0.061
% decrease WBC	75.2 ± 18.0 (33.3–96.6)	70.9 ± 17.7 (37.9–96.9)	4.28 ± 4.41	−5.18, 13.7	0.348
Neutrophils					
Nadir ($\times 10^9$/liter)	0.44 ± 0.49 (0.04–1.50)	0.64 ± 0.86 (0.05–2.60)	−0.20 ± 0.17	−0.57, 0.18	0.274
% decrease ANC	90.6 ± 9.48 (75.6–99.3)	88.6 ± 15.7 (44.8–98.7)	2.06 ± 3.89	−6.50, 10.6	0.607

[a] Data were obtained from patients after treatment with a 1-h i.v. infusion of docetaxel at a dose level of 60, 75, or 100 mg/m^2 given either alone (cycle 1) or in the presence of oral R101933 at a dose level of 200 or 300 mg b.i.d. (cycle 2 and further). The relative hematological toxicity (*i.e.*, the percentage decrease in blood cell count) was defined as: % decrease = [(pretherapy value − nadir value)/(pretherapy value)] × 100%. Data are presented as mean values ± SD, with the observed range shown in parenthesis.

[b] C.L., 95% confidence limits for the mean difference; ANC, absolute neutrophil count.

[c] Paired Student's *t* test.

Table 4 Neutropenia [highest CTC grade (NCI common toxicity criteria)] and DLT as per protocol in cycle 1 *versus* cycle 2 and further

Taxotere (mg/m^2)	R101933 (mg b.i.d.)	Patient	Cycle 1 Neutropenia	Cycle 1 DLT	Cycle 2 and further Neutropenia	Cycle 2 and further DLT
60	200	1	2	−	0	−
		2	2	−	4	−
		3	2	−	0	−
75	200	4	4	−	4	−
		5	4	−	4	−
		6	4	−	3	−
100	200	7	4	−	4	−
		8	4	−	4	−
		9	4	−	2	−
100	300	10	4	−	4	+
		11	4	−	4	−
		12	4	+	4	−
		13	0	−	4	+
		14	4	+	4	+
		15	4	−	4	−

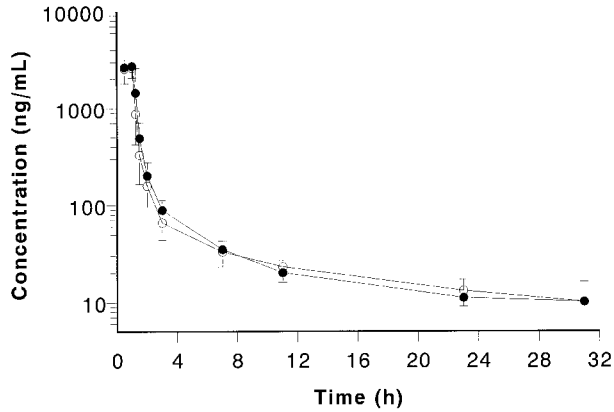

Fig. 2 Plasma concentration *versus* time profiles of docetaxel in patients treated with 100 mg/m^2 of docetaxel alone (○) or in combination with 300 mg b.i.d. oral R101933 (●). Data are presented as mean values (○, ●) ± SD (*error bars*).

R101933, reaching AUC values ~80-fold higher than that of the parent compound. Concentrations of this compound were also not substantially influenced by the administration of docetaxel at any dose level tested (Table 6). Of particular note, plasma levels of R101933 capable of reversal of daunorubicin resistance in A2780 cell cultures with and without P-glycoprotein expression were achieved in all patients (20).

DISCUSSION

In the present study, we observed that the plasma pharmacokinetic characteristics of docetaxel were not substantially influenced by R101933, a new p.o. administered P-glycoprotein inhibitor. The lack of a pharmacokinetic interaction between docetaxel and R101933 is an important finding that makes it possible to study the contribution of an inhibitor of P-glycoprotein to the toxicity and activity of an anticancer drug independ-

Table 5 Plasma pharmacokinetic parameters of docetaxel in the absence or presence of R101933[a]

Cohort	AUC (μg·h/ml)	C_{max} (μg/ml)	$t_{1/2(\alpha)}$[b] (h)	$t_{1/2(z)}$ (h)	V_{dss} (liters/m^2)	MRT (h)
Docetaxel 60 mg/m^2 (n = 3)						
Without R	3.18 ± 0.95	2.06 ± 0.58	0.17 ± 0.02	12.5 ± 3.8	120 ± 43	6.21 ± 2.78
With R, 200 mg	2.88 ± 0.52	1.77 ± 0.27	0.20 ± 0.01	11.3 ± 0.4	119 ± 35	5.54 ± 1.08
Docetaxel 75 mg/m^2 (n = 3)						
Without R	2.83 ± 1.03	1.68 ± 1.01	0.37 ± 0.36	12.0 ± 2.4	253 ± 227	7.49 ± 3.34
With R, 200 mg	3.59 ± 0.30	2.20 ± 0.53	0.32 ± 0.12	12.1 ± 2.5	112 ± 29	5.31 ± 1.22
Docetaxel 100 mg/m^2 (n = 3)						
Without R	3.93 ± 0.67	2.47 ± 0.21	0.15 ± 0.06	11.1 ± 2.3	137 ± 27	5.51 ± 1.92
With R, 200 mg	4.33 ± 1.13	2.95 ± 0.26	0.24 ± 0.06	9.4 ± 4.4	86 ± 41	3.78 ± 2.05
Docetaxel 100 mg/m^2 (n = 5)						
Without R	3.62 ± 0.71	2.54 ± 0.54	0.18 ± 0.03	11.0 ± 2.0	129 ± 32	4.59 ± 0.95
With R, 300 mg	4.04 ± 0.84	2.86 ± 0.55	0.23 ± 0.07	10.9 ± 4.8	104 ± 50	4.27 ± 2.02

[a] Data were obtained from patients after the first (without R101933) and second treatment cycle (with R101933) of a 1-h infusion of docetaxel. The kinetic terms are mean values ± SD.

[b] $t_{1/2(i)}$, half-life of the i-th disposition phase; V_{dss}, volume of distribution at steady state; MRT, mean residence time; n, number of patients evaluated at both treatment courses; R, R101933.

Table 6 Plasma pharmacokinetic parameters of R101933 and its metabolite R102207 in the absence or presence of docetaxel[a]

	200 mg of R101933 (n = 9)[b]		300 mg of R101933 (n = 5)	
Parameter	Without D	With D	Without D	With D
R101933				
C_{max} (ng/ml)	120 ± 66	94.3 ± 26.9	127 ± 50	144 ± 42
$t_{1/2(z)}$ (h)	19.6 ± 7.4	NA	23.0 ± 9.2	NA
AUC (μg·h/ml)	0.55 ± 0.24	0.53 ± 0.15	0.91 ± 0.46	0.86 ± 0.49
R102207				
AUC (μg·h/ml)	50.5 ± 14.2	52.4 ± 26.7	37.9 ± 20.2	45.7 ± 11.0

[a] Data were obtained from patients after cycle 0 (without docetaxel) and after cycle 2 (with docetaxel). The kinetic terms are mean values ± SD.

[b] n, number of patients evaluated; D, docetaxel; NA, not available.

ently of its effects on drug pharmacokinetics. Previous clinical studies have shown that drug interactions between modulator and anticancer drugs occur (even with dose reductions of the anticancer drug), resulting in increased toxicity that can be accounted for by pharmacokinetic interactions alone [reviewed in Ref. (6)]. These clinical investigations indicate that P-glycoprotein inhibitors [verapamil, cyclosporin A, PSC833 (valspodar), and VX-710 (biricodar)] increase the anticancer drug's systemic exposure, thereby potentially (nonselectively) increasing exposure to normal and malignant cells, resulting in increased severity or incidence of toxic adverse effects. Based on these findings, it has been proposed that the administration of P-glycoprotein inhibitors is unlikely to improve the therapeutic index of docetaxel (or any other drug) without dose adjustment unless such agents lack a pharmacokinetic interaction (6, 9).

Our findings show that previously observed drug pharmacokinetic interactions between anticancer drugs and modulators, such as those between the docetaxel analogue paclitaxel and *r*-verapamil (21), cyclosporin A (22), PSC833 (23), or VX-710 (24), are most likely more related to an overlap in specificity of enzymes responsible for metabolism of the compounds than to modulation of P-glycoprotein activity. Although few clinical data are available, several *in vitro* studies have shown that docetaxel is extensively metabolized in humans by the cytochrome P450 3A4 system (25, 26). The main pathway of docetaxel metabolism in humans consists of successive oxidations of the *tert*-butyl propionate group on the C13 side chain, with spontaneous cyclization occurring for the putative aldehyde and acid derivatives. All metabolites thus far characterized have been found to be >100-fold less cytotoxic than docetaxel itself (27, 28). In this context, it is noteworthy that R101933 did not influence the *in vitro* metabolism of docetaxel even at concentrations as high as 1 μg/ml and that the major metabolic route to R102207 is cytochrome-P450-unrelated.[3] Clearly, additional experiments are needed to establish the relevance of this principle in humans and to determine for what drugs it will apply. In addition, when given in combination with docetaxel, biologically relevant R101933 concentrations could be achieved and sustained for several hours, simulating optimal pharmacological conditions required for complete reversal of the MDR phenotype in *in vitro* systems.

Clinically, we observed that single treatment with R101933 given p.o. at the tested dosages was associated with minimal toxicity. The toxicological profile of the combination appeared to be very similar to that reported for docetaxel alone and included neutropenic fever and mucositis as the principal DLTs. Febrile neutropenia requiring hospitalization has been reported in ~15% and severe mucositis in ~10% of cases treated with docetaxel alone (29). In fact, the incidence of neutropenia observed with other inhibitors of P-glycoprotein in studies with anticancer drugs is greater than that observed with the cytotoxic agent alone (24). Fatigue was often mentioned by the patients in this study as a side effect, but never after R101933 alone, and asthenia is also a known side effect of docetaxel.

In conclusion, we have shown that the studied combination

of oral R101933 and i.v. docetaxel is safe and at the achieved dose levels, lacks the significant kinetic interaction with the anticancer drug as observed previously with other modulators. In the case of a Phase II/III study with the combination of R101933 and docetaxel, 100 mg/m^2, and in view of the pharmacokinetic data on R101933 presently presented, the recommended dose of R101933 will be 200 mg b.i.d. p.o.

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