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Disposition of docetaxel in the presence of P-glycoprotein inhibition by intravenous administration of R101933

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Abstract

Recently, a study of docetaxel in combination with the new orally administered P-glycoprotein (P-gp) inhibitor R101933 showed that this combination was feasible. However, due to the low oral bioavailability of R101933 and high interpatient variability, no further attempts to increase the level of P-gp inhibition were made. Here, we assessed the feasibility of combining docetaxel with intravenously (i.v.) administered R101933, and determined the disposition of docetaxel with and without the P-gp inhibitor. Patients received i.v. R101933 alone at a dose escalated from 250 to 500 mg on day 1 (cycle 0), docetaxel 100 mg/m^2 as a 1-h infusion on day 8 (cycle 1) and the combination every 3 weeks thereafter (cycle 2 and further cycles). 12 patients were entered into the study, of whom 9 received the combination treatment. Single treatment with i.v. R101933 was associated with minimal toxicity consisting of temporary drowsiness and somnolence. Dose-limiting toxicity consisting of neutropenic fever was seen in cycles 1 and 2 or in further cycles at both dose levels. The plasma pharmacokinetics of docetaxel were not changed by the R101933 regimen at any dose level tested, as indicated by plasma clearance values of 22.5±6.2 l/h/m^2 and 24.2±7.4 l/h/m^2 ($P=0.38$) in cycles 1 and 2, respectively. However, the faecal excretion of unchanged docetaxel decreased significantly after the combination treatment from 2.5±2.1% to less than 1% of the administered dose of docetaxel, most likely due to inhibition of the intestinal P-gp by R101933. Plasma concentrations of R101933 were not different in cycles 0 or 2 and the concentrations achieved in the first 12-h period after i.v. infusion were capable of inhibiting P-gp in an *ex vivo* assay. We conclude that the combination of 100 mg/m^2 i.v. docetaxel and 500 mg i.v. R101933 is feasible, lacks pharmacokinetic interaction in plasma, and shows evidence of P-gp inhibition both in an *ex vivo* assay and *in vivo* as indicated by the inhibition of intestinal P-gp.

Keywords: Multidrug resistance; MDR-reversing agent; Taxanes; Pharmacokinetics

1. Introduction

One of the best described mechanisms of multidrug resistance (MDR), the *in vitro* phenomenon thought to be partly responsible for the failure of cancer treatment with naturally occurring anticancer drugs such as the taxanes, is associated with the overproduction of the transmembrane transport protein P-glycoprotein (P-gp) which acts as an adenosine triphosphate (ATP)-dependent drug efflux pump and thus decreases the intracellular concentrations of these drugs [1,2]. Abundant effort has been put into developing drugs which are able to inhibit P-gp and can be used in combination with anticancer drugs [3,4]. However, it became evident that pharmacokinetic interactions occurred between the P-gp inhibitors and the co-administered anticancer drugs due to the competitive inhibition of cytochrome P-450 enzymes resulting in a significantly increased toxicity associated with the anticancer drugs [5,6]. Recently, we described the results of a phase I and pharmacokinetic study with a new P-gp inhibitor, R101933, administered orally in combination with docetaxel intravenously (i.v.) [7]. In this study, the pharmacokinetic and clinical safety profiles of docetaxel were unchanged indicating a lack of pharmacokinetic interaction between the anticancer drug and the P-gp inhibitor. By means of a functional *in vitro* study, it was shown that the achieved plasma concentrations of R101933 were able to inhibit

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P-gp [8]. However, due to a low oral bioavailability of R101933 and significant interpatient variability in exposure to R101933, no further attempts to increase the level of P-gp inhibition using an oral administration of R101933 were made and a potential pharmacokinetic interaction with docetaxel at higher concentrations of R101933 could not be excluded [7]. Here, we performed a new phase I and pharmacokinetic study with docetaxel in combination with R101933 given i.v. Plasma samples, as well as faeces samples, were collected. Earlier analysis of faecal excretion of docetaxel with and without oral R101933 revealed that the physiological and pharmacokinetic consequences of inhibition of P-gp by effective modulators can not be predicted based on drug monitoring of the plasma alone and collecting faeces samples can be an important element of the pharmacokinetic studies of anticancer drugs given in combination with a P-gp inhibitor [8]. To confirm that the achieved R101933 plasma levels after i.v. administration might be capable of inhibiting P-gp in tumour cells, we developed an *ex vivo* assay as a surrogate measure of P-gp antagonism.

2. Patients and methods

2.1. Eligibility

Patients with a histologically-confirmed diagnosis of a solid tumour 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 $\geqslant 18$ years; Eastern Cooperative Oncology Group performance status <3; life expectancy of at least 3 months; off previous anticancer therapy for at least 3 weeks; no previous treatment with taxanes or high-dose chemotherapy requiring progenitor cell support; adequate bone marrow function absolute neutrophil count (ANC) $>1.5\times10^9$/l, platelet count $>100\times10^9$/l), renal function (serum creatinine $\leqslant 2$ times upper limit of normal), and liver function (bilirubin level normal, aspartate/alanine aminotransferase $\leqslant 2.5$ times the upper limit of normal and alkaline phosphatase $\leqslant 2.5$ times the upper limit of normal); and symptomatic peripheral neuropathy $<$grade 2 (National Cancer Institute (NCI) criteria). Written informed consent was obtained from all of the patients, and the study was approved by the University Hospital Rotterdam Ethics Board.

2.2. 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 tumour measurements. Laboratory studies included a complete blood-cell count analysis, and measurement of white blood cell (WBC) differential, electrolytes (including sodium, potassium, chloride, calcium, magnesium 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 Common Toxicity Criteria (CTC)) were repeated once a week. Complete blood cell counts including WBC differential were performed twice a week. During cycle 0, electrocardiographies were performed at baseline and approximately 1, 2, 6, 24 and 48 h after the start of the infusion with R101933. The electrocardiography was repeated at the end of cycle 2 and further on when clinically indicated. A final assessment was to be made after the patients went off the study. Formal tumour measurements were performed at 6-weeks intervals until documentation of progressive disease. Standard World Health Organization (WHO) response criteria were used.

2.3. Drug administration

First, the patients received R101933 alone (cycle 0) followed by a 72-h wash-out period to allow the assessment of the terminal half-life of R101933. One week later, cycle 1 was initiated with docetaxel alone. Thereafter, the combination was given 3-weekly until progressive disease (PD) or dose-limiting toxicity (DLT) occurred. Docetaxel was administered every 3 weeks as a 1-h infusion and in the combination was started immediately after the end of the i.v. administration of R101933. All patients received pre-medication with dexamethasone orally 8 mg twice daily (b.i.d.), starting 1 day prior to each infusion of docetaxel, and to be taken for a total of 3 days every course. R101933 (Janssen Research Foundation, Beerse, Belgium) was supplied as a 10-mg/ml solution in hydroxypropyl-beta-cyclodextrin, hydrochloric acid, citric acid monohydrate, sodium hydroxide and mannitol. The drug was administered by a syringe infusion pump as a single 1-h infusion. The dose of docetaxel was fixed at 100 mg/m^2 and the dose of R101933 was escalated. The first dose level of R101933 was set at 250 mg, a dose known to be capable of P-gp inhibition and to induce no toxicity in a simultaneous study with paclitaxel [9].

In each cohort, 3 patients were treated unless the DLT of R101933 alone or the DLT of the combination treatment was observed. In that case, the accrual of 3 additional patients was required. The occurrence of a DLT during the first cycle with docetaxel alone led to a replacement of the patient in the same cohort to which the patient was assigned. DLT of R101933 (DLTr)

alone was defined as any non-haematological toxicity >grade 2 before chemotherapy was given. DLT of the combination treatment (DLTc) was defined as grade 3 non-haematological toxicity (with the exception of non-haematological toxicity that was still manageable in an out-patient setting, such as alopecia or nausea/vomiting) or grade 4 neutropenia lasting more than 5 days, grade 4 thrombocytopenia or required delay >2 weeks to a subsequent cycle due to toxicity with a reasonable possibility that the event could be attributed to the combined treatment with R101933 and docetaxel. Febrile neutropenia and neutropenia with severe infection (>grade 2 infection) were also considered as a DLT of the combination treatment. For dose-escalation decisions, only DLTs in cycles 0 and 2 were taken into account. The DLT of R101933 alone was reached when $\geqslant 1$ out of 3 (or $\geqslant 2$ out of 6) patients experienced a DLTr. The DLT of the combination of R101933 with docetaxel was reached when $\geqslant 3$ out of 6 patients experienced a DLTc. The maximum tolerated dose (MTD) was defined as the dose level below the DLT.

2.4. Sample collection and processing

Blood specimens were taken in all patients during cycles 0, 1 and 2. Blood volumes of 5–10 ml were drawn directly into Vacutainer tubes containing lyophilised 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, 4, 9, 24 and 48 h after the start of infusion. For the determination of R101933 concentrations, blood samples were collected immediately before infusion, and at 0.5, 1, 1.5, 2, 3, 5, 10, 24, 48 and 72 h after the start of infusion. 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.

Complete stool collections for docetaxel analysis were obtained in cycles 1 and 2 for the duration of the study (i.e. up to 48 h after the start of docetaxel administration). This interval was chosen based on previous faecal excretion studies with the structural related agent paclitaxel [10]. Faecal specimens were collected in polystyrene containers and stored immediately at −20 °C.

2.5. Drug analysis

Plasma concentrations of docetaxel were determined by solid-phase extraction using Bond Elut nitrile microcolumns (Varian, Harbor City, CA, USA) and a sensitive liquid chromatographic assay with tandem mass spectrometric detection, with a lower limit of quantitation of 1 ng/ml (200 μl-samples) [7]. The analytical method for R101933 and its esterase-mediated metabolite R102207 was based on solid-phase extraction with Bond-Elut Certify microcolumns (Varian) and high-performance liquid chromatography with ultraviolet (UV) absorption measurements at 270 nm, with a lower limit of quantitation of 2 ng/ml (1-ml samples) [7]. Concentrations of docetaxel and its major hydroxylated metabolites M2, M3, and M4 in faeces homogenates were determined by reversed-phase high-performance liquid chromatography with UV detection at 230 nm as described in detail earlier in Refs. [8,11]. This method employs 0.5-ml samples of faeces specimens (homogenised in 0.01 M potassium phosphate buffer (1:3, w/v) and then diluted (1:1, v/v) with human plasma), with a lower limit of quantitation of 2.0 μg/ml.

2.6. Pharmacological data analysis

Plasma concentration–time profiles of docetaxel, R101933 and its metabolite R102207 were pharmacokinetically analysed by compartimental and non-compartimental models using the software package WinNonlin (Pharsight, Mountain View, CA, USA) [7]. The drug disposition half-lives, the area under the plasma concentration–time curve (AUC) extrapolated to infinity, plasma clearance, steady-state volume of distribution and percentage of the absolute docetaxel dose excreted in faeces as parent drug or metabolite were determined using methods and equations as defined elsewhere in Refs. [7,8]. Haematological pharmacodynamics were evaluated by an analysis of the absolute nadir values of blood cell counts or the relative haematological toxicity, i.e. the percentage decrease in blood cell count, which was defined as:

$$\%\ \text{decrease} = \left[\frac{(\text{pretherapy value} - \text{nadir value})}{(\text{pretherapy value})}\right] \times 100\%$$

Within each patient, myelosuppression was described either using continuous variables, consisting of the percentage decrease in WBC, absolute neutrophil count and platelet count or as discrete variables in case of the NCI-CTC myelotoxicity grade.

2.7. Ex vivo *pharmacodynamics*

The expression of high levels of P-gp in the anthracycline-resistant cell line $A2780_{T\text{-}100}$, obtained by transfection of P-gp, allowed us to use an *ex vivo* assay as a surrogate measure of P-gp antagonism in plasma samples of patients treated with R101933. The $A2780_{T\text{-}100}$ cell line was maintained in RPMI 1640 medium without

L-glutamine and phenol red (Gibco, Amsterdam, The Netherlands) supplemented with 10% bovine calf serum, penicillin/streptomycin 50U/50 μg/ml in a 5% CO_2/95% air atmosphere at 37 °C. Cells were grown to 80–90% confluency and treated with trypsin-ethylene diamine tetra acetic acid (EDTA) before subculturing. On the day of the experiment, the cells were centrifuged at room temperature and resuspended in protein-free RPMI1640 medium at a concentration of 80 000 cells/ml. Aliquots (500 μl) of cell suspension were transferred to polypropylene tubes containing 250 μl of incubation medium in the presence of daunorubicin and R101933. Extraction of R101933 was performed from 300-μl plasma samples (patients' samples or spiked calibration samples) using 1500 μl acetonitrile-*n*-butyl chloride (1:4, v/v) in polypropylene microtubes (Eppendorf, Hamburg, Germany) by mixing for 30 s. After centrifugation for 5 min at 23 000*g*, 1300 μl of the upper organic layer was transferred into a glass tube and evaporated to dryness at 50 °C under nitrogen. R101933 was reconstituted in 300 μl RPMI1640 medium by agitation, and the sample was centrifuged again for 1 min at 3000*g*. An aliquot of 250 μl clear supernatant was added to the cell suspension. Next, cells were incubated for 120 min to assure maximal retention of daunorubicin [8]. Preliminary experiments had shown that daunorubicin used at a final concentration of 1 μg/ml provided reasonable fluorescence to distinguish differences between the various anticipated concentrations of R101933, thus making this level suitable for the P-gp inhibition studies. Fluorescence measurements of individual cells were performed with flow cytometry using a Becton-Dickinson fluorescent activated cell sorter (FACS) (San Jose, CA, USA) equipped with an ultraviolet argon laser (excitation at 488 nm, emission at 530/30 and 570/30 nm band-pass filters). Analysis was gated to include single cells on the basis of forward and side light-scatter and was based on the acquisition of data from 7500 cells. Log fluorescence was collected and displayed as single parameter histograms. Geometric mean fluorescence values of duplicate measurements were used for all patients' samples, and fluorescence data obtained from experiments done with the A2780 parental cell line (not expressing P-gp) were used as a positive control. Time–course studies were conducted with samples from the first treatment course in all patients using the fluorescence intensity in the resistant cells relative to a pre-therapy plasma sample (i.e. in the absence of R101933), normalised to a value of 1.0 (arbitrary units). Previous studies have shown that inactivation (with R101933 in $A2780_{T\text{-}100}$ cells) or complete preclusion of P-gp active-efflux function (in A2780 cells) is associated with a normalised fluorescence value of 2.5 on this scale [8]. Therefore, the experimental set-up provides a factor that normalises the degree of inhibition by R101933 in each plasma sample relative to the total absence of P-gp function and allows for normalisation of the results across sample sets both within and between patients. In each patient, the time to maximal inhibition of P-gp function following R101933 administration was determined in addition to the duration above K_i, defined as the concentration required for half-maximum inhibition of P-gp-mediated daunorubicin efflux.

2.8. Statistical considerations

Parameters of all compounds are reported as mean values ± Standard Deviation (S.D.), except where indicated otherwise. The difference in the pharmacokinetic parameters between the docetaxel administration days and between patient cohorts was evaluated statistically using a two-sided parametric matched-pairs Student's *t*-test (after testing for normality) *plus* the 95% confidence intervals. Probability values (two-sided) of less than 0.05 were considered statistically significant. All calculations were done on the Number Cruncher Statistical Systems v5.×software package (J.L. Hintze, East Kaysville, UT, USA, 1992).

3. Results

12 patients were entered into this study. Patient characteristics are listed in Table 1; all patients were eligible. 3 patients were considered not evaluable for toxicity, response and pharmacokinetic analysis. In 1 patient the

Table 1
Patient characteristics

Characteristic	No. of patients
Patients included	12
Sex	
Male	
Female	
Age (years)	
52 (34–66)	Median (range)
Performance score (ECOG)	
0	3
1	8
2	1
Primary tumour	
Urogenital tract	3
Gastrointestinal tract	1
Respiratory tract	4
Breast	3
Sarcoma	1
Prior therapy	
Surgery	7
Radiotherapy	7
Chemotherapy	8
Hormonal therapy	3
None	1

ECOG, Eastern Cooperative Oncology Group.

dose of docetaxel in cycle 2 and further cycles was decreased to 75 mg/m^2 because of disturbed liver function tests after cycle 1. He received seven cycles of the combination treatment without further deterioration of liver function or other severe toxicities related to docetaxel or R101933. 2 patients did not receive the combination treatment due to grade 3 fatigue and neutropenic fever related to chronic sinusitis, respectively, after treatment with docetaxel alone. The *ex vivo* assay was accomplished in all patients as all patients received cycle 0 with R101933 alone according to the protocol.

3.1. Toxicity and haematological effects

A total of 34 cycles docetaxel, including 25 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. At the first dose level of R101933, i.e. 250 mg, 3 patients were treated. Neutropenic fever in 1 patient after administration of docetaxel alone (cycle 1) led to the decision to provide prophylactic antibiotics (ciprofloxacine 500 mg b.i.d., days 4–15). Subsequently, this patient was treated with the combination therapy and experienced no further periods of neutropenic fever, but ceased treatment after cycle 3 due to grade 3 neurotoxicity. Because of grade 4 fatigue and grade 3 myalgia, another heavily pretreated patient at this dose level ended treatment after cycle 5. Since no DLTs were seen in cycles 0 and 2, the dose of R101933 was escalated to 500 mg. At this dose level, all patients experienced fatigue and myalgia from cycle 1 on, but these and other toxicities never exceeded grade 2. At both dose levels, most patients experienced grade 1–2 drowsiness and somnolence after the administration of R101933. As a simultaneously performed study of i.v. R101933 combined with i.v. paclitaxel revealed a MTC of R101933 alone of 500 mg [9], no attempt was made to further increase the dose of R101933 in the present study, despite the fact that formally the DLT had not been reached. All patients without severe toxicity were treated until progressive disease. Partial responses were not seen.

Statistical analysis of paired haematological data, available in all 9 evaluable patients, showed that R101933 co-administration with docetaxel was not associated with a greater percentage decrease in WBC and ANC or lower mean blood cell count nadir (Table 3).

3.2. Docetaxel disposition

Paired plasma concentration–time profiles of docetaxel given alone or in combination with R101933 were available in all 9 patients (Fig. 1). There were no statistically significant differences in any of the studied

Table 2
Main toxicities (worst per cycle) at the two dose levels expressed in number of cycles in which the toxicity occurred

R101933 (mg)	Docetaxel (mg/m^2)	Neutropenia CTC grade[a]					Somnolence CTC grade					Fatigue CTC grade					Myalgia CTC grade				
		uk[b]	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
250	100					10	5	2	3			2	2	5		1	6	2	1	1	
500	100	2	3	2	5	12	21	1	2			15	1	8			11	10	3		

[a] National Cancer Institute (NCI) Common Toxicity Criteria.
[b] uk, unknown.

Table 3
Summary of haematological pharmacodynamics[a]

Parameter	Cycle 1	Cycle 2	Difference	95% C.L.[b]	P^c value
Leucocytes					
Nadir ($\times 10^9$/l)	1.6±1.0 (0.7–3.2)	2.0±1.5 (0.6–4.9)	−0.4±0.7	−2.1, 1.3	0.625
% Decrease WBC	77.5±10.3 (62.5–88.2)	74.5±19.2 (45.2–94.2)	3.0±7.0	−13.3, 19.4	0.675
Neutrophils					
Nadir ($\times 10^9$/l)	0.6±0.7 (0.07–3.7	1.0±1.4 (0.1–3.7)	−0.4±0.6	−2.0, 1.2	0.573
% Decrease ANC	91.2±9.96 (72.5–98.3)	87.8±15.9 (58.4–97.8)	3.4±7.5	−14.9, 21.8	0.664

WBC, white blood cells.
[a] Data were obtained from patients after treatment with a 1-h intravenous (i.v.) infusion of docetaxel at a dose level of 100 mg/m^2 given either alone (cycle 1) or in the presence of i.v. R101933 at a dose level of 250 or 500 mg (cycle 2). The relative haematological 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±Standard Deviation (S.D.), with the observed range shown in parentheses.
[b] C.L., 95% Confidence Limits for the mean difference; ANC, absolute neutrophil count.
[c] Paired Student's *t*-test.

parameters, suggesting that R101933 at the dose levels tested did not influence the plasma pharmacokinetics of docetaxel (Table 4). At the final dose level tested, combining docetaxel at 100 mg/m^2 and R101933 at 500 mg ($n=6$), docetaxel plasma clearance values averaged 22.4±12.6 l/h/m^2 and 23.8±7.9 l/h/m^2 in the absence and presence of R101933, respectively ($P=0.69$). To test whether the pattern of drug excretion altered in the presence of R101933 administered as an i.v. infusion, we also studied the cumulative faecal excretion of docetaxel and its major hydroxylated metabolites over a period of 48 h. In line with previous findings [8], a minor fraction of the administered docetaxel dose (2.5±2.1%) was recovered in the faeces of patients as unchanged parent drug following single agent dosing. In the presence of R101933, faecal excretion of the unchanged drug was markedly reduced to less than 1% of the dose ($P=0.01$, paired Student's *t*-test). In contrast, however, the cumulative faecal excretion of the hydroxylated metabolites increased more than 2-fold ($P=0.01$).

3.3. R101933 plasma levels

No differences were observed in the pharmacokinetics of R101933 given either alone or in the presence of docetaxel (Fig. 2; Table 5). R101933 peak plasma concentrations after the 500 mg i.v. administration ranged from 2580 to 5328 ng/ml. Peak plasma concentrations of its acid metabolite R102207 were approximately 4–5 times higher. The AUC of R101933 ranged from 2.9 to 19.6 μg.h/ml. The AUC of R102207 was approximately 20–25 times higher. Mean plasma clearance values of R101933 given alone were 52.7±19.2 l/h ($n=5$) and 58.5±19.7 l/h ($n=7$) at dose levels of 250 and 500 mg, respectively, suggesting a dose-proportional pharmacokinetic behaviour. No differences were observed in the R101933 plasma clearance given either alone or in the presence of docetaxel (mean values, 53.8±18.4 l/h (cycle 0) versus 57.2±11.6 l/h (cycle 2) ($P=0.47$)), indicating the absence of a pharmacokinetic interaction at these dose levels.

Table 4
Plasma pharmacokinetic parameters of docetaxel in the absence or presence of R101933[a]

Cohort	AUC (ng h/ml)	C_{max} (μg/ml)	$t_{1/2(\alpha)}$ (h)	$t_{1/2(z)}$ (h)	V_{dss} (l/m^2)	MRT (h)
Docetaxel						
100mg/m^2 ($n=3$) without R	4050±490	2700±700	0.3±0.2	20.4±10.0	200±105	8.0±4.1
with R 250 mg	4275±1230	2630±820	0.2±0.02	13.1±1.0	115±55	4.6±1.2
Docetaxel						
100 mg/m^2 ($n=6$) without R	5560±2660	3410±1100	0.3±0.2	14.6±1.5	145±75	6.6±0.8
with R 500 mg	4550±1315	3100±910	0.2±0.02	16.1±4.1	175±105	7.0±2.0

AUC, area under the plasma concentration versus time curve; C_{max}, maximum plasma concentration of drug; $t_{1/2(i)}$, 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.

[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 ±S.D.

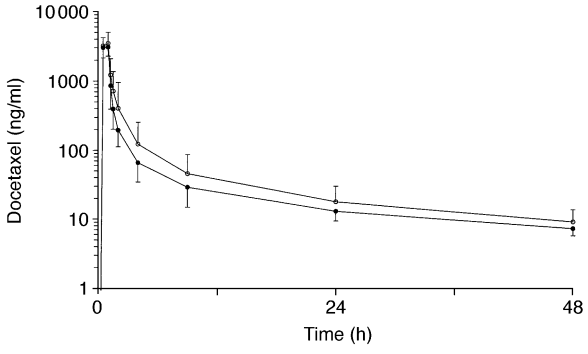

Fig. 1. Plasma concentration–time profiles of docetaxel in patients treated with docetaxel at a dose level of 100 mg/m^2 given either alone (○) or in combination with R101933 given at 500 mg as a 1-h intravenous (i.v.) infusion (●). Data are presented as mean values (○, ●)±Standard Deviation (S.D.) (*error bars*).

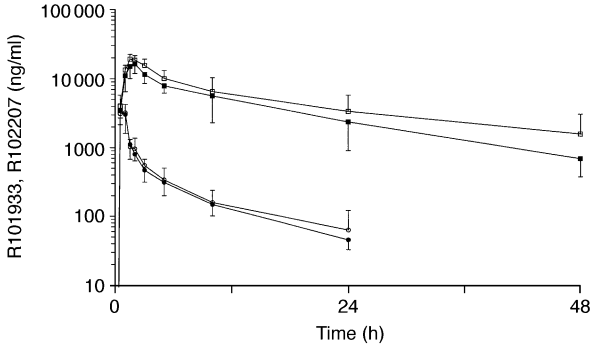

Fig. 2. Plasma concentration–time profiles of R101933 (circles) and its acid metabolite R102207 (squares) in patients treated with R101933 given as a 1-h i.v. infusion at a dose level of 500 mg either given either alone (open symbols) or in combination with docetaxel given at 100 mg/m^2 (closed symbols). Data are presented as mean values (*symbols*)±Standard Deviation (S.D.) (*error bars*).

3.4. P-gp antagonism following R101933

To determine whether the infusion of R101933 would result in systemic concentrations that affected P-gp activity in an *ex vivo* model based on daunorubicin retention in P-gp expressing $A2780_{T\text{-}100}$ cells, plasma samples were obtained before and after the drug infusion in the first treatment course. In this assay, fluorescence values have previously been shown to reflect the degree of inhibition of P-gp-mediated efflux [8]. Control experiments conducted with the A2780 parental cell line provided a maximum fluorescence value of 2.5, whereas the fluorescence measured in the $A2780_{T\text{-}100}$ will depend on the level of R101933 present in the patient's plasma. A total of 107 plasma samples from patients treated with R101933 (5 patients at 250 mg and 7 patients at 500 mg) were analysed for pharmacological activity in the assay. All pre-dose samples showed no measurable activity. Time–course studies with R101933 administered at the two dose levels tested showed that following a single i.v. dose, substantially decreased daunorubicin efflux was found in the plasma samples of all patients collected within 30 min of treatment (Fig. 3). Maximal inhibition of P-gp function was observed in all of the samples collected at times ranging from 30 to 60 min, with fluorescence increase values of 2.4 ± 0.08 and 2.4 ± 0.06 at R101933 dose levels of 250 and 500 mg, respectively, indicating near-complete reversal (i.e. $>97\%$) in the *ex vivo* assay. The duration above K_i, defined as the concentration required for half-maximum

Table 5
Plasma pharmacokinetic parameters of R101933 and its metabolite R102207 in the absence or presence of docetaxel[a]

Parameter	250 mg R101933 ($n=3$)		500 mg R101933 ($n=6$)	
	Without D	With D	Without D	With D
R101933				
C_{max} (μg/ml)	2.2±0.6	2.0±0.3	3.7±1.0	3.7±1.1
AUC (μg h/ml)	5.6±1.0	5.2±0.9	10.0±5.0	8.4±1.6
R102207				
C_{max} (μg/ml)	10.7±6.7	7.5±3.0	20.6±1.8	16.4±4.4
AUC (μg h/ml)	115±65	90±50	310±190	200±80

AUC, area under the plasma concentration versus time curve; C_{max}, maximum plasma concentration of drug; *n*, number of patients evaluated; D, docetaxel.

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

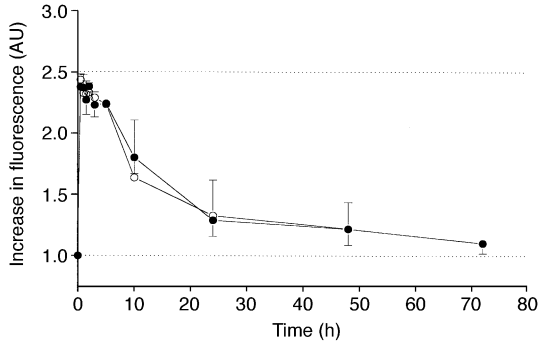

Fig. 3. Time–course of R101933-mediated P-gp antagonism in patient samples obtained following R101933 administration as a 1-h i.v. infusion at dose levels of 250 mg (○) or 500 mg (●). Modulation of daunorubicin uptake in $A2780_{T\text{-}100}$ cells (expressing P-gp) by plasma samples containing R101933 were measured by a change in fluorescence intensity relative to that observed in the A2780 parental cell line in the absence of R101933. Data points are mean values ±Standard Error of the Mean (S.E.M.) of duplicate measurements of plasma samples obtained from 3 patients (250-mg dose) or 7 patients (500-mg dose). The lower dotted line (fluorescence value of 1.0) indicates the base-line fluorescence (i.e. lack of influence on P-gp-mediated efflux), whereas the upper dotted-line (fluorescence value of 2.5) indicates the maximum fluorescence (complete inhibition of P-gp-mediated efflux).

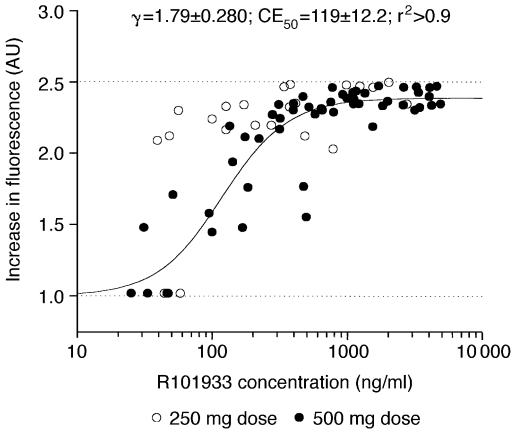

Fig. 4. Modulation of cellular daunorubicin uptake in $A2780_{T\text{-}100}$ cells (expressing P-gp) by R101933 as measured by a change in fluorescence intensity relative to that observed in the A2780 parental cell line in the absence of R101933. The solid line represents a fit of the data based on a Hill function for R101933 concentration-dependent alteration of P-gp inhibition. The lower dotted line (fluorescence value of 1.0) indicates the base-line fluorescence (i.e. lack of influence on P-gp-mediated efflux), whereas the upper dotted line (fluorescence value of 2.5) indicates the maximum fluorescence (complete inhibition of P-gp-mediated efflux).

inhibition of P-gp-mediated daunorubicin efflux, showed considerable interpatient variability (coefficient of variation, >50%). Mean values of the duration above K_i were 12.3 ± 7.2 and 14.1 ± 7.1 h at dose levels of 250 and 500 mg, respectively ($P = 0.709$, unpaired Student's *t*-test). Overall, the data indicate that plasma concentrations achieved in patients within 48 h after single 250 or 500 mg i.v. administration of R101933 inhibit P-gp-mediated drug efflux in tumour cells *ex vivo*. Correlation of P-gp antagonism with R101933 plasma concentrations, as measured by high-performance liquid chromatography, indicated the occurrence of a plateau in efflux antagonism (Fig. 4), in line with previous findings [8].

4. Discussion

MDR is generally regarded to be one of the major stumbling blocks to the efficacy of chemotherapy and since the discovery of the MDR product P-gp, many efforts have been made to convert P-gp-mediated drug resistance into drug sensitivity [6]. However, a plethora of clinical studies involving a wide range of P-gp inhibitors and co-administered anticancer drugs have shown that these combination treatments almost always resulted in increased toxicity of the anticancer drugs solely due to pharmacokinetic interaction between the two agents related to competition on the level of cytochrome-P450 3A isozymes (reviewed in Ref. [5]). Therefore, it has been proposed that the administration of P-gp inhibitors is unlikely to improve the therapeutic index of anticancer drugs such as docetaxel unless such agents lack a pharmacokinetic interaction [5,6]. In a previous study with orally administered R101933 in combination with i.v. docetaxel, we have shown that R101933 did not influence the plasma pharmacokinetic characteristics of docetaxel knowing that the major metabolic route of R101933 is cytochrome-P450-unrelated [7]. Due to the low oral bioavailability of R101933 and significant interpatient variability in exposure to R101933, a further increase of the plasma levels of R101933, in an attempt to achieve higher local concentrations of R101933, can be expected following an i.v. administration. In the present study, this resulted, as expected, in significantly higher plasma levels of R101933 and reduced inter-patient variability. Mean peak concentrations of R101933 after a 500 mg i.v. infusion of R101933 were approximately 3500 ng/ml, compared with only 150 ng/ml after 300 mg of R101933 was given as oral solution [7]. Mean exposure (AUC) to R101933 was approximately 10-fold higher after the i.v. infusion and a relatively lower exposure to the inactive metabolite R102207 was obtained. Furthermore, individual peak concentrations ranged only 2-fold after the i.v. infusion compared with 10-fold after the oral solution, indicating a significantly reduced interpatient variability. Despite the approximately 10-fold higher exposure to R101933 after the i.v. infusion, the plasma pharmacokinetics of docetaxel were still not affected. The toxicity of the combination treatment appeared to be very similar to that reported for docetaxel alone including neutropenic fever, grade 3 neurotoxicity and severe fatigue in combination with myalgia, all being reported in approximately 15, 5, and 6%, of cases treated with docetaxel alone, respectively [12]. Treatment with i.v. R101933 at the dose levels tested was associated with minimal toxicity consisting of temporary drowsiness and somnolence beginning during the infusion and lasting for several hours.

As we have postulated earlier, the physiological and pharmacological consequences of treatment with an effective P-gp inhibitor cannot be predicted based on drug monitoring of the plasma alone [8]. So, we collected faeces up to 48 h after the administration of docetaxel in cycles 1 and 2 to determine an often overlooked and sometimes underestimated parameter of drug disposition, the faecal excretion of the anticancer agent. The excretion of unchanged docetaxel proved to be decreased after co-administration with R101933 indicating a biological activity of the P-gp inhibitor most likely at the level of intestinal P-gp. Inhibition of intestinal P-gp leading to the modification of the enterohepatic cycle was postulated after treatment with orally administered R101933 and now also seems to occur after i.v. administration, indicating sufficient concentrations of R101933 at the apical lumen side of the intestine. The re-absorption of docetaxel from the intestinal lumen did not result in increased plasma levels due to: (i) the relative small fraction of docetaxel that is excreted via the faeces (without R101933 only 2.5%), (ii) a virtually complete first-pass extraction and/or docetaxel metabolism in the liver and intestinal mucosa, where cytochrome P450 3A isozymes are expressed. [13].

In studies like the present one, designed to overcome P-gp-mediated MDR, measurement of the extent of *in vivo* inhibition of the P-gp-mediated drug efflux is deemed essential. To that end, we used an *ex vivo* assay developed as a surrogate measure of P-gp antagonism in the plasma samples of all patients receiving R101933. We deliberately decided to use another approach compared with the two most well-known surrogate assays for determining P-gp antagonism using the known P-gp substrates Tc-99m-sestamibi (sestamibi), a radionuclide imaging agent [14–17], and rhodamine 123 (rhodamine), a lipophilic dye [19,20]. The first method is time-consuming and patient compliance can be a restriction. Moreover, there is no dose–response relationship between sestamibi uptake and the P-gp inhibitor levels [16], and establishment of the sensitivity of sestamibi scintigraphy as an adequate surrogate marker of P-gp inhibition is still needed. In this respect, it is important

to note that a recent pilot study showed no correlation between sestamibi scintigraphy and the expression of P-gp determined in the tumour tissue of patients with high-grade osteosarcoma [18]. Accepting rhodamine-efflux from CD56+ cells as a surrogate assay requires the assumption that P-gp in the CD56+ cells has identical substrate and antagonist specificities as P-gp in cancer cells. Moreover, both methods lack the possibility of interpatient comparison. An essentially different approach of evaluating the extent of P-gp inhibition has been the addition of plasma containing a certain concentration of P-gp inhibitors) from patients treated with this drug to an *in vitro* assay comprised of a known multidrug-resistant cell line and a detectable P-gp substrate [21]. In the present study, we favoured this approach because this method allowed an interpatient and dose level comparison by the use of a cell line known to have a constant level of P-gp expression. Furthermore, this assay allowed the pharmacologically and biologically relevant concentrations of R101933 over a distinguished period of time to be confirmed. Nevertheless, as with the other methods, P-gp expression or the availability of the P-gp inhibitor in the tumour is not provided and the assay has to be performed in protein-free medium because it is expected from *in vitro* studies that protein binding of the inhibitor in the medium can impair the P-gp antagonism activity [21–24]. This binding has raised concern because clinically P-gp inhibitors are exposed to high concentrations of drug-binding proteins and depending on the degree of serum protein binding (for R101933 more than 98%), this is thought to lead to significantly reduced drug availability of the (pharmacologically-active) free drug fraction at the tumour site [24]. However, this view does not justify the generally accepted physiological concept of the extent of distribution of a compound within tissues, including a tumour, involving multiple equilibria, a situation thought not possible outside of the body in the *in vitro* setting as well as in *ex vivo* experiments.

An important finding of the present *ex vivo* study is the observation that a plateau occurred in the inhibition of the P-gp substrate efflux, suggesting that the levels of R101933 achieved in the assay reached a maximum inhibition of P-gp-mediated efflux and complete reversal could not be attained. The plateau phase was reached at *ex vivo* concentrations that corresponded to a plasma concentration of R101933 of approximately 300 ng/ml, a concentration well below that achieved at the maximal-tolerated dose level of i.v. administered R101933. This result is in line with calculations using experimental models with high levels of P-gp expression suggesting that a single P-gp modulator cannot completely inhibit P-gp-mediated efflux [25]. However, the indicated substantial (near maximal) reversal may be sufficient to improve drug efficacy.

In conclusion, we have shown that the combination of i.v. R101933 directly followed by docetaxel infusion is safe, lacking pharmacokinetic interaction in the plasma and that the plasma concentrations of R101933 achieved within 48 h after infusion are capable of inhibiting P-gp in an *ex vivo* assay. These findings justify further development of this combination treatment in which the recommended dose of i.v. R101933 will be 500 mg. A phase II study is possible, for example in patients with metastatic breast cancer failing on treatment with taxanes alone, but as accrual into these kind of studies have proven difficult, we propose adequately powered phase III studies [6].

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