A Dose-finding and Pharmacokinetic Study of Reversal of Multidrug Resistance with SDZ PSC 833 in Combination with Doxorubicin in Patients with Solid Tumors[1]

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ABSTRACT

Forty-two patients with advanced solid tumors were entered into a dose-finding study of the combination of doxorubicin with the cyclosporin analogue SDZ PSC 833 (PSC), given by oral route. Patients received PSC at escalating doses, ranging from 2.5 to 25 mg/kg/day, for 5 days, in doses given every 12 h. Doxorubicin was given by i.v. push on day 3 of PSC administration, 4 h after the morning dose of PSC. Pharmacokinetic analyses of PSC and doxorubicin were performed. A total of 38 patients received a combination of PSC and doxorubicin, and 27 received doxorubicin alone in the first course. The major toxicity of the combination was hematological and was significantly more severe than that with doxorubicin alone; severe myelosuppression was already observed at the first PSC dose level, which required doxorubicin dose reduction from 50 to 35 mg/m^2. At all dose levels of PSC, up to 17.5 mg/kg/day, there were at least two patients with grade 3 or 4 hematological toxicity, which was manageable in less heavily pretreated patients. A further PSC dose escalation was performed to 25 mg/kg/day, together with doxorubicin, at a further reduced dose of 20 mg/m^2. At this dose, central nervous system toxicity became the most relevant side effect. The increase of toxicity in the combined treatment was supported by a significant increase of the area under the plasma concentration-time curve to ∞ of doxorubicin (54%) and a 10-fold increase of the area under the plasma concentration-time curve to ∞ of doxorubicinol. The pharmacological interaction was not dependent on the plasma concentration of PSC. The total body clearance of doxorubicin decreased by 30%. PSC plasma concentrations of >1 μM at the time of doxorubicin administration were, in general, found at a dose of 7.5 mg/kg/day or higher. One patient had a partial response. In conclusion, PSC plasma concentrations that can revert multidrug resistance in experimental models could be achieved in patients who have solid tumors and who are treated with doxorubicin. However, a marked pharmacological interaction was found between doxorubicin and PSC, which led to substantial increase in hematological toxicity and required marked reduction of the doxorubicin dose. Further study of PSC may be warranted, in association with the investigation of P-glycoprotein expression and concentration of drugs in the tumor tissues.

INTRODUCTION

The occurrence of drug resistance is thought to play an important role in chemotherapy failure. Among the many mechanisms described in *in vitro* systems, MDR,[3] *i.e.*, the presence of cross-resistance to a broad number of anticancer drugs that possess different mechanisms of action, is certainly the one that is most relevant to clinical experience. The classical MDR phenotype is usually associated *in vitro* to overexpression of the *MDR1* gene and its gene product, Pgp. Pgp can actively extrude a number of substances from the cytoplasm of the tumor cell, among them, several cytotoxic agents, such as doxorubicin, *Vinca* alkaloids, epipodophyllotoxins, actinomycin D, and taxanes. Alkylating agents and antimetabolites are typically not substrates of Pgp. The reduced intracellular concentration of the cytotoxic drug leads to reduced exposure of the target (*e.g.*, DNA or microtubulin). The expression of Pgp has also often been associated with a more aggressive phenotype in several tumor types, and this is sometimes difficult to dissect from resistance to chemotherapeutic agents (1).

Interestingly, MDR due to overexpression of Pgp can be effectively reversed *in vitro* and *in vivo* by a large number of substances, several of which have been developed for clinical use. The first generation of modulators includes drugs that had been developed for another indication but were incidentally found to have the ability to inhibit Pgp function, *e.g.*, verapamil and CsA. These modulators are relatively weak and toxic at the doses necessary to obtain a plasma concentration that reverts

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[3] The abbreviations used are: MDR, multidrug resistance; Pgp, P-glycoprotein; CsA, cyclosporin A; PSC, SDZ PSC 833; MTD, maximum tolerated dose; CTC, common toxicity criteria; PT, prothrombin time; PTT, partial thromboplastin time; LVEF, left ventricular ejection fraction; HPLC, high-performance liquid chromatography; *t*-BME, *t*-butylmethyl ether; ACN, acetonitrile; AUC, area under the plasma concentration-time curve; MRT, mean residence time.

MDR *in vitro*. The second generation of MDR modulators includes molecules that are less toxic inhibitors of Pgp action, such as dexverapamil. The third generation of Pgp modulators includes the most powerful compounds, such as PSC. The concentration that is able *in vitro* to revert MDR is usually in the micromolar range (>5 μM for verapamil and 2–3 μM for CsA). It was impossible to reach and maintain adequate blood concentrations of several of the early MDR-modifying agents because of unacceptable toxicity; for instance, the cardiac toxicity observed in the first studies with verapamil greatly delayed the broad experimentation of this approach in the clinic. Several dose-finding studies, using many MDR-modifying agents, have been reported in the literature, but only a very limited number of randomized studies have been performed (2).

Dose-finding studies of CsA have been performed in combination with etoposide, doxorubicin, and various combination chemotherapies. When given i.v. by continuous infusion, adequate blood concentrations could be achieved and maintained for several days. CsA is, however, rather toxic and may sometimes lead to severe nephrotoxicity, immunosuppression, and nausea and vomiting. PSC was selected for further development among a number of cyclosporin analogues synthesized by Sandoz laboratories (3). The chemical structure of PSC is (3′-keto-Bmt1)-[val2]-cyclosporin. PSC appeared to be severalfold more potent in reverting MDR than was CsA (4, 5), and it was devoid of immunosuppressive effects and nephrotoxicity. PSC induced an increase both of activity and toxicity of doxorubicin in L1210 leukemia-bearing mice (6). PSC had a relatively wide therapeutic window, with daily oral administration in solid tumor-bearing mice (7). Resistance to most of the MDR drugs, including paclitaxel (8), has been effectively reversed in experimental models. PSC can be given p.o., allowing administration on an outpatient basis.

We performed a dose-finding study of escalating doses of oral PSC, given in combination with doxorubicin in patients with solid tumors. Because pharmacological interaction was shown with CsA and verapamil, careful pharmacokinetic evaluation of both drugs was also performed. The aims of this study were to determine the MTD of PSC p.o. in combination with doxorubicin, to propose a safe dose for Phase II trials with the investigated schedule, and to study the pharmacokinetics of both drugs and document possible antitumor activity.

PATIENTS AND METHODS

Patients. To be eligible for the study, patients had to be between the ages of 18 and 70 years and have microscopically confirmed diagnosis of a solid tumor that was not amenable to curative treatment, a WHO performance status of ≤2, a life expectancy of at least 3 months, a normal bone marrow reserve (WBC count of ≥4,000/μl and platelet count of ≥100,000/μl), normal renal function (serum creatinine of <120 μmol/liter), normal hepatic function (bilirubin of <25 μmol/liter and liver enzymes within 2 times the normal upper limit), and normal clotting (normal PT and PTT). The presence of measurable or evaluable sites of disease was not required in this dose-finding study. Furthermore, patients could not have received chemo-, immuno-, or radiotherapy in the 4 weeks (6 weeks for nitrosoureas, mitomycin C, and extensive radiotherapy) preceding entry into the trial. Prior doxorubicin chemotherapy was allowed, up to a maximum dose of 300 mg/m^2 (600 mg/m^2 for epirubicin). Patients with a history of myocardial infarction within the past 12 months, congestive heart failure, severe arrhythmias, active ischemic heart disease, or uncontrolled hypertension were excluded from the study, as were patients who were concomitantly receiving drugs with possible liver, kidney, or cardiac toxicities. In addition, pregnant or breast-feeding women, patients with bacterial infections, and patients with signs or symptoms of brain or leptomeningeal involvement were not eligible for this study. Written informed consent was required from each participant.

Study Design and Treatment. PSC was provided by Sandoz-Pharma Ltd. (Basel, Switzerland) at 100 mg/ml in a labrafil-based drinking solution. Commercially available doxorubicin, diluted in saline to a concentration of 2 mg/ml, was used and was administered as a 2-min i.v. push. PSC was given p.o. every 12 h for a total of 5 days. Doxorubicin was given by 2-min i.v. push on day 3 of PSC administration, 4 h after the morning administration of PSC. This schedule was suggested by data obtained in healthy volunteers, in whom the peak plasma concentration of PSC appeared to be around 4 h after oral administration.[4] For practical reasons, PSC was administered at around 7:00 a.m., and doxorubicin was administered at 11:00 a.m. In the first cycle, doxorubicin was given alone, and in the following cycles, doxorubicin was given in combination with PSC. In most cases, prophylactic antiemetic medications with metoclopramide or ondansetron were administered.

Although it had been decided that a Fibonacci-like scheme for dose escalation would be followed, several modifications were necessary during the course of the study, based on clinical and pharmacokinetic findings. The initial dose of doxorubicin was 50 mg/m^2, and the initial dose of PSC was 2.5 mg/kg/day. The design was such that the doxorubicin dose would be kept constant, and the PSC dose would progressively increase, with escalation steps of 2.5 mg/kg/day. This design allowed the direct comparison of toxicity and pharmacokinetics in the same patient. The study was aimed at achieving a peak plasma concentration of PSC of 1–2 μM, which is sufficient to revert MDR *in vitro* in a number of model systems (4, 5). Plans were made to increase the PSC dose by steps of 50–100% in the absence of toxicity with a grade of >1. Dose increases of 20–33% were planned in case of higher degrees of toxicities. Because of the results obtained in previous studies of CsA with doxorubicin (9, 10) and of PSC in healthy volunteers, alopecia and bilirubin elevations were not considered in the evaluation of dose escalation.

At least three patients and four courses were evaluated before dose escalation was performed. At any given dose level, at least 1 or 2 weeks were required before additional patients were entered on that dose level. No dose escalation was performed in the same patient. Dose escalation was foreseen until toxicity with a grade of 3 or 4 was observed; at that dose level, three more patients were to be treated at the same dose level. The MTD was established at the dose at which a tolerable,

[4] Sandoz Ltd., unpublished observation.

manageable, and reversible CTC toxicity grade of 3 or 4 was observed in two of six patients. Further dose escalation was foreseen after MTD was reached in less heavily pretreated patients, and after the achievement of the MTD in heavily pretreated patients.

Cycles were repeated if WBC counts of $\geq 4 \times 10^9$/liter and platelet counts of $\geq 100 \times 10^9$/liter were observed, and all signs of toxicity had subsided, including signs of hepatic and renal disturbances. If more than 2 weeks were required for complete recovery, the patient went off the study. In case of tolerable treatment and no tumor progression, treatment was allowed for up to a total of six cycles or to a doxorubicin cumulative dose of 550 mg/m^2 (450 mg/m^2 for patients extensively irradiated on the chest).

Pretreatment Evaluation and Evaluation during Treatment. Prior to entry into the study, each patient was evaluated by physical examination, full blood count, chemistries (electrolytes, serum creatinine and ureum, liver enzymes, serum protein and albumin, glucose, PT, and PTT), electrocardiogram, chest X-ray, and LVEF when prior anthracycline treatment was given. Additional radiological tests were performed to properly evaluate disease extension and to allow response assessment. During treatment, full blood counts were performed at least weekly; chemistries were evaluated once a week, and sites of disease were evaluated before each cycle, whenever possible, or at least every 2 cycles. During PSC administration, multiple assessment of fractionated bilirubin, bile acids, PT, and PTT were also performed. Radionuclide LVEF was assessed at baseline and when indicated, and in patients continuing treatment, radionuclide LVEF was assessed about every three cycles of doxorubicin.

Toxicity was assessed according to CTC criteria, and the response evaluation was performed according to WHO criteria (11). Patients were seen at least weekly for possible signs of toxicity.

Pharmacokinetics. Blood samples for assessment of doxorubicin and metabolites were obtained before doxorubicin administration and at 0, 5, 10, 15, and 30 min and 1, 2, 4, 6, 9, 12, 20, 24, 36, and 48 h after doxorubicin administration. Blood samples were collected in polypropylene heparin-coated Sarstedt Monovette tubes (Nümbrecht, Germany). Samples were cooled on ice and centrifuged for 10 min at 2700 $\times$ *g*; plasma was stored at −20°C until analysis. HPLC analysis was performed as described previously. Detection of metabolites and calculations were also performed as described previously (12).

Sampling for PSC determination was performed at −52, −48, −40, −4, 0, 8, 12, and 20 h, respective to the 0 h time of doxorubicin administration. During the course of the study, some additional sampling times were introduced. PSC plasma concentrations were assessed by HPLC with UV detection (210 nm) after liquid-liquid extraction from blood with *t*-BME-ethylacetate after addition of buffer and a saturated sodium chloride solution. The organic extract was evaporated under a stream of nitrogen and reconstituted in ACN/water before injection into the HPLC system; 200 μl of the sample were injected by the autosampler onto the analytical column (Phenomenex IB Sil Phenyl, 5 μm, 150 $\times$ 3.2 mm) using a solution containing 46.5% ACN, 9% *t*-BME, and 44.5% water (v/v/v) as the mobile phase. The column temperature was 70°C. After the analyte was eluted from the column, the analytical column was washed with a wash phase using a solution containing 80% ACN, 10% *t*-BME, and 10% water (v/v/v) for 2.5 min at a higher flow rate and then reconditioned with the mobile phase prior to the next run. The time between two injection cycles was approximately 28 min. The limit of quantification was set at 50 ng/ml blood for the entire study. Six calibration standards of different PSC concentrations and a blank were freshly prepared before each run and analyzed in duplicate. In addition, three quality control samples with different known concentrations, stored at −20°C and thawed prior to use, were run in parallel.

Table 1 Patient characteristics

Characteristic	Value
Total no. of patients	42
Sex (no. of men/no. of women)	21/21
Median age in yr (range)	52 (30–72)
Median ECOG[a] performance status (range)	1 (0–2)
Tumor type	
Colorectal	11
Renal	6
Non-small cell lung cancer	4
Breast	3
Stomach	3
Head and neck	3
Germ cell tumor	3
Thyroid	2
Soft tissue sarcoma	2
Malignant melanoma	2
Ovary	1
Pancreas	1
Uterus	1
Prior therapy	
Surgery	32
Radiotherapy	14
Chemotherapy	34
MDR drugs[b]	19

[a] ECOG, Eastern Cooperative Oncology Group.
[b] MDR drugs included anthracyclines, *Vinca* alkaloids, epipodophyllotoxins, and paclitaxel.

Statistics. Comparisons of paired samples (*i.e.*, toxicities and pharmacokinetic parameters observed with doxorubicin alone *versus* doxorubicin plus PSC) were assessed by the Wilcoxon test or the Student's *t* test, as specified (13).

RESULTS

Treatment

Forty-two patients were entered in this study between December 1992 and May 1995: 28 patients from the University Hospital Vrije Universiteit (Amsterdam, the Netherlands) and 14 patients from the Leon Bérard Cancer Center (Lyon, France). Patient characteristics are listed in Table 1.

The doses selected for doxorubicin and PSC and the number of patients treated at each dose level are shown in Table 2. As a result of clinical and pharmacological findings, several dose adjustments were made during the execution of the study. Because it was clear from the initial dose of PSC that there was a pharmacological interaction between doxorubicin and PSC, leading to a significant increase of toxicity despite the relatively low doxorubicin dose, the dose of doxorubicin was reduced to

Table 2 Dose escalation scheme: evaluable patients and courses with doxorubicin alone and in combination with PSC

Dose level	PSC dose (mg/kg/day)	Doxorubicin dose (mg/m^2)	Patients (no.)	Courses	
				No PSC	PSC
1	2.5	50	3	4[a]	9
2	5	50	6	6[b]	14
3	5	35	4	5[a]	16
4	7.5	35	6	7[a]	21
5	10	35	6	7[a]	9
6	12.5	35	2	2	4
Subtotal			27	31	73
6b[c]	12.5	35	3		5
7	17.5	35	4		16
8	25	20	4		6
Subtotal			11		27
Total			38	31	100

[a] Four patients received only one cycle of doxorubicin.
[b] In two patients, the doses of doxorubicin were reduced in the second cycle to 30 and 35 mg/m^2.
[c] Starting from dose level 6b, combined treatment was given from cycle 1.

35 mg/m^2 on dose level 3 (Table 2). Furthermore, when the nature and the extent of the pharmacological interaction between PSC and doxorubicin were assessed, to speed up the accrual, the first dosing with doxorubicin alone was dropped, and patients were administered the combination of PSC with doxorubicin from the first cycle of therapy, starting at the PSC dose of 12.5 mg/kg/day (dose level 6b; Table 2). Finally, because the dose-limiting toxicity was myelosuppression due to doxorubicin, further doxorubicin dose reduction was performed, to 20 mg/m^2, to allow additional PSC dose escalation and reach the MTD due to PSC, which was described as central nervous system toxicity (14); the dose of PSC (25 mg/kg/day) was selected on the basis of preliminary results of another study of oral PSC in combination with vinblastine.[5]

Four patients received one course of doxorubicin alone and went off the study before the doxorubicin-PSC combination could be administered (two rapid progressive disease, one treatment delay of >2 weeks, and one refusal). This left 38 patients for whom at least one course of the combination was evaluable. A total of 100 evaluable cycles were given: 31 cycles of doxorubicin alone were given in 27 patients (four patients received only the first cycle of doxorubicin alone), and 73 cycles of doxorubicin in combination with PSC were given (Table 2). A median of two cycles per patient was given (range, 1–12).

Toxicity

From the first PSC step, it was clear that toxicity was higher with the combination of doxorubicin and PSC than it was with doxorubicin alone. In Tables 3 and 4, a comparison of hematological toxicity between cycles 1 (doxorubicin alone) and 2 (doxorubicin with PSC) is shown, by step. In general, at all steps the second cycle, was more myelotoxic than the first cycle. Comparing all cycles 1 with cycles 2, there was a statistical significant difference in leukopenia ($P = 0.0002$; Wilcoxon test), neutropenia ($P = 0.0218$), and thrombocytopenia ($P = 0.0000$). However, significant differences were not detectable on all dose levels (Table 3), basically due to the small number of patients enrolled. The more profound myelosuppression led to an increased chance of severe infection only in the combination regimen (Table 5). Stomatitis was also somewhat more frequent in cycle 2 than it was in cycle 1. Nausea and vomiting were infrequently severe and were slightly more frequent with the combined administration than with doxorubicin alone (data not shown); the use of 5HT3 inhibitors effectively prevented emesis.

By comparing cycle 1 with cycle 2, the MTD was already reached at dose level 1 (Table 4); however, because one patient had already had grade 3 leukopenia with doxorubicin alone, all patients were heavily pretreated, and the hematological toxicity was short-lived and asymptomatic, we decided to further escalate PSC. On level 2, the MTD was clearly reached for heavily pretreated patients (three of six patients with grade 3 or 4 hematological toxicity). At this dose level, severe infection developed in two patients (Table 5). From dose level 3 on, doxorubicin was given at 35 mg/m^2, and less heavily pretreated patients were enrolled into the study. Despite severe leuko-neutropenia in at least two patients per dose level, we decided to proceed to further dose escalation because the length of the hematological toxicity, namely neutropenia, was short and did not lead to complications.

By analyzing all cycles including PSC, there was a tendency to cumulative anemia and thrombocytopenia (data not shown). Anemia of any grade was observed in 83% of patients, and it was severe in 17% of them. Anemia was often already present at study entry, and progressive decrease of hemoglobin levels was frequently observed. Cardiac toxicity was observed in a patient with metastatic renal cell cancer treated on the second dose level; his LVEF fell to 43% after three cycles of doxorubicin 50 mg/m^2, and it decreased further to 38% after treatment suspension due to progression of lung metastases; the patient had received interleukin-2 in the past but no anthracyclines. Another patient with lung cancer had a decrease of LVEF from baseline 61% to 50% after nine cycles at a doxorubicin dose of 35 mg/m^2. Both patients had increasing dyspnea, which

[5] S. Bates, personal communication.

Table 3 Hematological toxicity of doxorubicin alone (cycle 1) and doxorubicin in combination with PSC (cycle 2)

Step (*n*) Cycle	Nadir, cells × 10^9/liter (range)					
	Leukopenia	*P*[a]	Neutropenia	*P*[a]	Thrombocytopenia	*P*[a]
1 (3)						
1	3.2 (1.1–5.6)		NA[b]		256 (206–334)	
2	0.96 (0.7–1.3)		NA[b]		57 (50–66)	
		0.29				0.11
2 (6)[c]						
1	4.08 (1.6–8.1)		2.21 (0.15–4.7)		250 (143–405)	
2	2.45 (0.2–4.8)		0.79 (0.06–2.07)		106 (29–195)	
		0.075		0.14		0.028
3 (4)						
1	3.1 (1.8–4.2)		1.63 (0.07–2.57)		214 (121–289)	
2	2.7 (1.6–4.2)		1.58 (0.62–2.57)		109 (10–188)	
		0.11		0.65		0.14
4 (6)						
1	3.83 (2.3–6.3)		2.07 (0.99–3.86)		168 (71–223)	
2	2.21 (1.2–3.4)		0.76 (0.25–1.92)		83 (15–129)	
		0.028		0.028		0.028
5 (6)						
1	3.5 (1.2–5.5)		1.8 (0.41–3.42)		208 (92–388)	
2	2.4 (1.4–3.4)		1.14 (0.9–1.31)		126 (16–234)	
		0.075		0.22		0.046
6 (2)						
1	5.45 (3.7–7.2)		3.6 (1.5–4.61)		202 (160–243)	
2	4.75 (4.1–5.4)		2.53 (2.05–3.13)		107 (85–128)	
		0.65		0.65		0.18

[a] *P*s were calculated according to the Wilcoxon test for paired samples (13).
[b] NA, not available.
[c] Two patients had doxorubicin dose reduction in the second cycle, one because of persistent leukopenia at recycle day and another because of severe leukopenia after the administration of doxorubicin alone.

Table 4 Myelotoxicity in the first two cycles up to dose level 6 and in cycle 1 in the higher dose levels

		CTC grade														
		Leukopenia					Neutropenia					Thrombocytopenia				
Step (*n*)	Cycle	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
1 (3)	1	1	1		1		NA[a]					3				
	2				1	2	NA[a]							3		
2 (6)[b]	1	2		3	1		2		1	1	1	5	1			
	2	2	1			3	1		2		3	2	1	1	2	
3 (4)[c]	1	1	1	1	1		2				1	3	1			
	2	1	1		2		1	1	1	1		1	2			1
4 (6)	1	3		3			2	2	1	1		5		1		
	2		2	1	3		1			3	2		4	1		1
5 (6)[d]	1	2	2	1	1		2	2		1	1	4	2			
	2		1	3	2				3	2		2	2	1		1
6 (2)	1	1	1				1	1				2				
	2	2					2						2			
6b (3)	1		1		2			1			2	1	1		1	
7 (4)	1	1		1		2	1		1		2	2	1			1
8 (4)	1	3	1				3	1				4				

[a] NA, not available.
[b] Two patients had a dose reduction of doxorubicin in the second cycle, one because of persistent leukopenia at recycle day and another because of severe leukopenia after the administration of doxorubicin alone; at this dose level, five of six patients had neutrophils assessed in cycle 1.
[c] At this dose level, three of four had neutrophil assessment in cycle 1.
[d] At this dose level, neutrophils were assessed in five of six patients in cycle 2.

could partly be accounted by the decrease in ventricular function. Alopecia was total in patients who received more than two courses.

Because the toxicity encountered in PSC dose levels of up to 17.5 mg/kg/day was essentially the toxicity of doxorubicin and because the toxicity of PSC as reported in healthy volunteers and in other studies of MDR modulation was not clearly observed, it was decided to proceed to further dose escalation of

Table 5 Major nonhematological toxicity in the first two cycles up to dose level 6 and in cycle 1 in the higher dose levels

		CTC grade														
		Stomatitis					Infection					Diarrhea				
Step (*n*)	Cycle	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
1 (3)	1	3					3					3				
	2	1		2			3					3				
2 (6)[a]	1	4	1	1			6					4	1		1	
	2	4	1	1			4			1	1	3	2	1		
3 (4)	1	4					4					3	1			
	2	3	1				3			1		4				
4 (6)	1	4	2				5	1				6				
	2	4	1	1			4	2				5	1			
5 (6)	1	5	1				6					4	2			
	2	6					6					4	1	1		
6 (2)	1	2					2					2				
	2	1	1				2					2				
6b (3)	1	2	1				3					2	1			
7 (4)	1	3			1		4					4				
8 (4)	1	2	1	1			4					4				

[a] Two patients had a dose reduction of doxorubicin in the second cycle, one because of persistent leukopenia at recycle day and another because of severe leukopenia after the administration of doxorubicin alone.

PSC up to 25 mg/kg/day, after another dose reduction of doxorubicin to 20 mg/m^2. This PSC dose escalation eventually led to the expected neurological toxicity. Neurotoxicity was observed only in the combined treatment, except in one patient who had dizziness (grade 1) both without and with PSC, treated on dose level 5. On dose level 2, dizziness (grade 1) was observed in one patient, and lethargia (grade 3) developed in another patient when given the combined treatment; on level 3, one patient developed dizziness (grade 2); on dose level 4, grade 1 paresthesia combined with grade 2 somnolence were observed in one patient, and tremor (grade 1) was observed in another patient; on level 5, grade 1 paresthesia was seen in one patient; on level 6, one patient developed severe excitation (grade 3); and on level 7, dizziness (grade 1) was reported in one patient and dizziness (grade 1) together with paresthesia grade 1 in another patient. On dose level 8, all four patients had some peripheral neurotoxicity: one patient had dizziness and grade 2 disarthria (3 days duration during PSC administration), and this patient refused further treatment; the second patient had grade 1 paresthesia of the feet for 3 days; and the third patient had no neurotoxicity after the first cycle, but on the second cycle, she developed grade 2 finger and perioral paresthesia, accompanied with grade 2 ataxia and uncertainty in gait during PSC administration (durations of 4 and 2 days, respectively); the fourth patient, who had preexisting peripheral neurosensory toxicity due to prior cisplatin treatment, developed paresthesias and grade 3 ataxia (walking with great difficulty and only if supported), which abated gradually over several weeks; this patient was later found to have multiple brain metastases, which may have partially contributed to the severity of ataxia.

A mild increase in bilirubin was observed from dose level 2; from dose level 5, severe but transient bilirubin increases were recorded. There was a tendency to have more frequent increases in bilirubin with increasing dose levels of PSC, and this was also accompanied by mild-to-moderate increase of serum bile acids. CTC grade 3 or 4 (1.5–3 times or >3 times the upper normal value, respectively) bilirubin increases were only observed with PSC doses of higher than 10 mg/kg/day. Alterations of bilirubin levels were often accompanied by a transient and slight increase of liver enzymes, which usually resolved within a week after treatment. No signs of renal toxicity were reported.

Antitumor Activity

Of 42 patients entered into this dose-finding study, 36 were evaluable for response assessment: there were 22 with progressive disease, 12 with stable disease, 1 with partial response, and 1 with minor response. The partial response was observed in a patient with non-small cell lung cancer, who was treated on dose level 7 and had progressed after treatment with carboplatin and paclitaxel. The response was assessed in the lung already after two cycles of therapy; the patient refused further treatment after nine cycles of therapy, and progressive disease was observed 2 months after the last chemotherapy administration. Another patient with metastatic renal cancer had a minor response (28% reduction in the product of maximal diameters of mediastinal metastases) that was evident after four cycles of therapy and progressed after seven cycles. This patient was treated on dose level 2.

Pharmacokinetics

PSC Pharmacokinetics. Peak and trough levels of PSC around the time of doxorubicin administration are listed by dose level in Table 6. In Fig. 1, the profiles of the five patients treated on dose level 2 are shown. There was a large variation in PSC levels within and between patients. In general, trough and peak (4-h) PSC levels increased by repetitive dosing, and after the fourth PSC administration, a steady-state level (*i.e.*, constant trough and peak values after repetitive dosing) was reached (Figs. 1 and 2 and data not shown). At the highest PSC dose level (25 mg/kg/day), additional blood samples were assessed in

Table 6 Trough and peak blood levels of PSC (ng/ml) during dose escalation

PSC dose (mg/kg/day)	No. of patients	No. of courses	Mean trough at t = 12 h (range)	Mean peak at t = 4 h (range)
2.5	3	3	125 (117–132)	227 (200–272)
5	5	5	442 (134–915)	790 (498–1548)
7.5	5	8	533 (247–825)	822 (341–1224)
10	3	3	547 (288–698)	554 (395–848)
12.5	5	6	562 (294–889)	1349 (696–2522)
17.5	3	3	1015 (901–1082)	1116 (872–2129)
25	4	4	798 (255–1228)	1613 (578–2822)

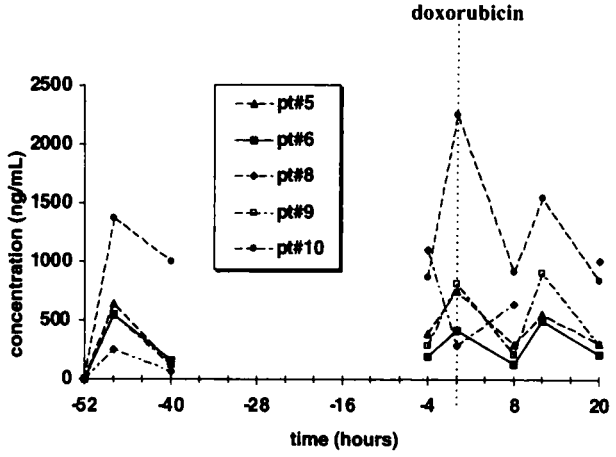

Fig. 1 Concentration-time curves of PSC in five patients treated at dose level 2 during the second cycle of chemotherapy (5 mg/kg/day PSC p.o. and 50 mg/m^2 doxorubicin i.v.). PSC concentration is given in ng/ml, and time is in hours: time 0 corresponds to the administration of the i.v. bolus of doxorubicin. All other times correspond to times of oral PSC administration.

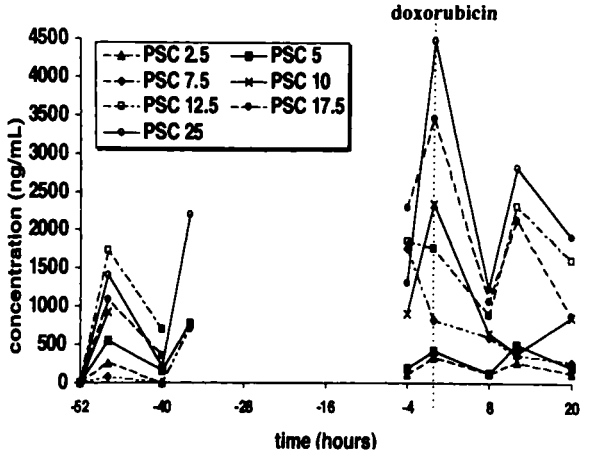

Fig. 2 Representative pharmacokinetic profiles of different PSC dose levels. PSC concentration is given in ng/ml, and time is in hours: time 0 corresponds to administration of the i.v. bolus of doxorubicin. All other times correspond to times of oral PSC administration.

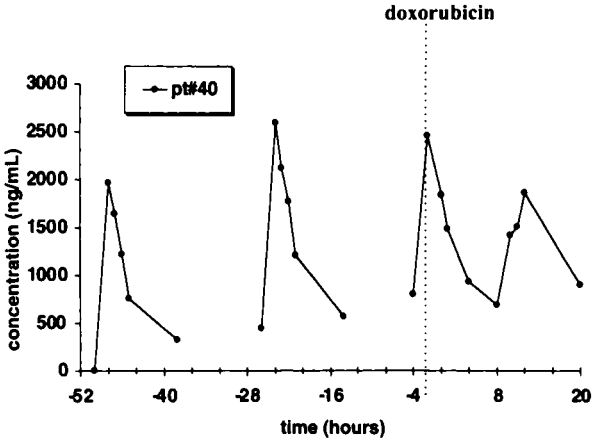

Fig. 3 Representative extended pharmacokinetics curve for PSC in a subject given 25 mg/kg/day. All times correspond to times of oral PSC administration.

four patients, which indicated that the values tended to be peak earlier than 4 h after PSC administration, as was reported in healthy volunteers at the highest PSC doses tested[4] (Fig. 3).

Peak values of PSC of >1000 ng/ml were usually obtained after the fifth dose of PSC at a level of 7.5 mg/kg/day or higher. Interestingly, peak values of PSC after the sixth dose (just after doxorubicin administration) were lower than those after the fifth dosing (before doxorubicin). This occurred reproducibly in the majority of patients, and the difference was statistically significant (n = 31; P = 0.0001, Wilcoxon test) and suggested that the drug interaction between PSC and doxorubicin is mutual. By looking at the highest dose level of PSC, where more extensive sampling was performed, the peak area between +8 and +20 h was 67% of that between −4 and +8 h.

Doxorubicin Pharmacokinetics. An interaction between doxorubicin and PSC was already evident from the first PSC dose level. Comparison of the pharmacokinetics of doxorubicin and its metabolites in the presence and absence of PSC was performed in all patients, up to dose level 6 (Table 7). The AUC of doxorubicin increased by 54% when the two agents were given together. The MRT also significantly increased when PSC was given together with doxorubicin, whereas the plasma clearance decreased markedly. The AUC of the major doxorubicin metabolite doxorubicinol increased to even a greater extent (over 10 times when the AUCs to ∞ were considered, and 2.7 times when AUCs from 0 to 48 h were considered). The plasma levels of the other metabolites were low and not influenced by coadministration of PSC (data not shown). Representative curves of doxorubicin and doxorubicinol are depicted in Fig. 4.

The number of patients used for calculating the pharmacokinetics of doxorubicinol is smaller than that for doxorubicin because patients with increasing doxorubicinol concentrations during the final part of the concentration-time curve were not included. The AUCs (0–48 h) were only calculated for patients for whom both the first course (no PSC) and the second course (with PSC) were sampled and the PSC peak plasma concentration was known at the start of the doxorubicin administration.

Table 7 Pharmacokinetic parameters of doxorubicin and doxorubicinol in absence or presence of PSC

Values are means ± SD. Where appropriate, values were normalized to a doxorubicin dose of 50 mg/m^2 and were calculated with the noncompartment model using TopFit 2.0. *Ps* were calculated by Student's *t* test. According to the three-compartment model, $t_{1/2\alpha}$ and $t_{1/2\beta}$ for doxorubicin were 0.063 and 1.76 h, respectively; these values did not significantly change in the presence of PSC.

Parameter	No PSC[a]	PSC[b]	% change	*P*
Doxorubicin (0 to ∞)				
AUC (nM·h)	3451 ± 823	5326 ± 1706	+54	<0.001
C_{max} (nM)	11763 ± 3892	11328 ± 4430	−3.7	0.88
Plasma clearance (liters/h)	27.1 ± 8.3	18.9 ± 7.8	−30	0.041
MRT (h)	27.7 ± 14.5	41.1 ± 21.3	+48	0.008
V_{ss} (liters)	701 ± 317	690 ± 241	−1.5	0.52
$t_{1/2\gamma}$ (h)	32.9 ± 12.6	38.2 ± 18.2	+16	0.10
Doxorubicin (0–48 h)				
AUC (nM·h)	3215 ± 778	3828 ± 852	+19.0	0.04
Doxorubicinol (0 to ∞)				
AUC (nM·h)	2433 ± 3948	28291 ± 28199	+1063	0.0011
C_{max} (nM)	46 ± 24	92 ± 34	+101	<0.001
t_{max} (h)	1.5 ± 2.3	23.3 ± 11.5	+1453	<0.001
$t_{1/2\gamma}$ (h)	65 ± 95	237 ± 299	+267	0.028
Doxorubicinol (0–48 h)				
AUC (nM·h)	923 ± 374	3454 ± 1429	+274	<0.001

[a] Doxorubicin (0 to ∞), n = 21; doxorubicin (0–48 h), n = 16; doxorubicinol (0 to ∞), n = 20; doxorubicinol (0–48 h), n = 16.
[b] Doxorubicin (0 to ∞), n = 32; doxorubicin (0–48 h), n = 16; doxorubicinol (0 to ∞), n = 19; doxorubicinol (0–48 h); n = 16.

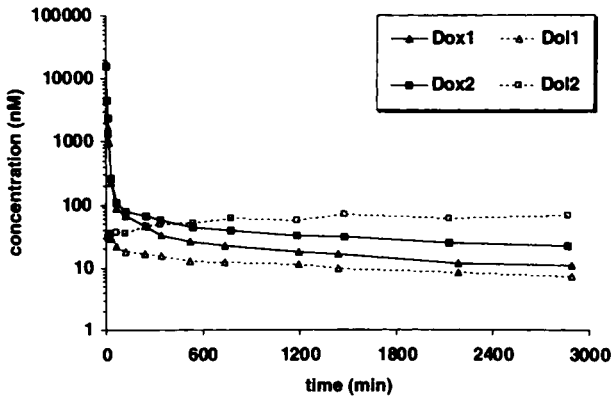

Fig. 4 Representative concentration-time curves of doxorubicin and doxorubicinol when 50 mg/m^2 doxorubicin were administered alone (cycle 1: *Dox1* and *Dol1*, respectively) or in combination with 5 mg/kg/day PSC (cycle 2: *Dox2* and *Dol2*, respectively).

These values were also used to draw Fig. 5. In this figure, the ratios of the AUCs (which also represent the ratios of the total body clearances) of doxorubicin and doxorubicinol up to 48 h, in the presence or absence of PSC coadministration, are shown. This figure indicates that there is an overall increase of the AUC ratio of doxorubicin in the presence of PSC, which is not clearly dependent on the PSC concentration. For doxorubicinol, however, a clear increase in the ratio of the AUCs was observed at PSC concentrations higher than 250 ng/ml (occurring after PSC dose of 1.5 mg/kg but also after 2.5 mg/kg). Beyond that concentration, the ratio remained at a level of about 4. Comparable results were obtained when the AUCs were calculated to infinity (data not shown) or when, instead of the ratios, the differences of AUCs were calculated (data not shown). Similar results were also obtained when, instead of PSC concentration at time 0, the AUCs of PSC (0–20 h or 0–8 h) were used (data not shown).

There was no significant correlation between doxorubicin or doxorubicinol AUCs and any toxicity, including hyperbilirubinemia (data not shown). For myelotoxicity, no correlation was observed, regardless of whether absolute values, toxicity gradings, or percentage of decrease of cell counts were considered.

DISCUSSION

Here, PSC plasma concentrations of >1 μM at the time of doxorubicin administration were, in general, found at a dose of 7.5 mg/kg/day or higher. However, wide variations in PSC levels were observed with our twice-daily administration, and a large interpatient variability was found. Bioavailability of this oral formulation has recently been shown to be only 34%, with a large interpatient variability (3–58%; Ref. 15). More frequent daily administrations may produce a lower but more sustained steady-state PSC level (14). Moreover, better oral formulations are under investigations.

The most frequent toxicity in our study was myelosuppression, in particular, leukopenia and granulocytopenia. Myelosuppression was more frequent in the combination of doxorubicin with PSC than it was with doxorubicin alone, and it rendered dose reduction of doxorubicin necessary. The duration of hematological toxicity was, however, relatively short, and after the dose of doxorubicin was reduced to 35 mg/m^2, severe infectious complications did not occur any longer. For this reason, we proceeded with PSC dose escalation, even in the presence of grade 3 or 4 hematological toxicity, which met the criteria we initially defined as dose limiting.

The increase in hematological toxicity was associated with a remarkable pharmacological interaction between doxorubicin and PSC. This was also shown by preliminary results of another study (16). The increased myelotoxicity observed in our study may be due to the increase of AUC associated with reduced clearance of doxorubicin; however, an alternative explanation may be the presence of Pgp expression on hematopoietic cells, in particular, CD34$^+$ stem cells (17). The fact that thrombocy-

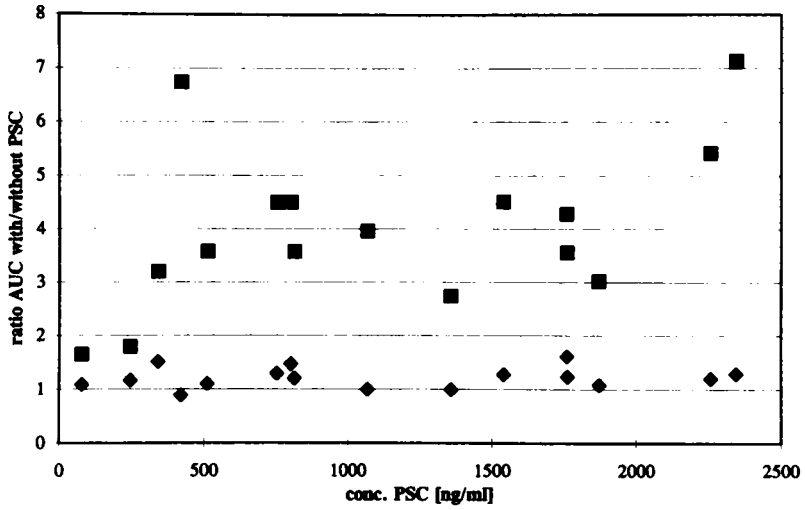

Fig. 5 Ratio of AUCs (0–48 h) of doxorubicin (Dox; ◆) and doxorubicinol (Dol; ■), with and without PSC, plotted against PSC concentration (t = 0).

topenia also increased with the addition of PSC makes the first hypothesis the most convincing one.

In mice and rats given doxorubicin in combination with CsA (18) and in mice given PSC (19), a substantially higher doxorubicin level was found in several tissues, including intestine, liver, kidney, adrenal, and heart, in comparison to the administration of doxorubicin alone. No significant differences in serum concentrations or elimination were observed. This treatment was associated with increased acute and delayed toxicity. In L1210 leukemia-bearing mice, PSC decreased the MTD of doxorubicin about 3 times; despite the much lower dose, doxorubicin exerted significant antitumor activity (6). In mdr1a knockout mice, vinblastine excretion of the drug in liver, kidney, and gut was delayed, and this led to a 3-fold increase in its AUC (20). Furthermore, increased reabsorption of drug from bile, gut lumen, and urine was also observed.

The presence of pharmacological interaction between the reverter and the cytotoxic drug has been seen with several other modulators, *e.g.*, verapamil (21), nifedipine (22), dexverapamil (23), and CsA (10, 24). This effect may vary depending on the cytotoxic drug used (14, 23). The precise nature of this interaction is not fully understood; it may be due to an activity on the excretion phase of the antineoplastic agent, by increasing drug accumulation in Pgp-expressing excretory cells and enhancing reabsorption, as shown for CsA (10, 24). Alternatively, the pharmacological interaction may take place at sites unrelated to Pgp, such as pathways of drug metabolism, like the cytochrome P-450 (25). The major elimination pathway for doxorubicin is through biliary excretion. PSC inhibited doxorubicin biliary clearance in rats, presumably inhibiting Pgp in the hepatocyte canaliculus (26).

Here, a decrease of doxorubicin clearance by 30% was observed in absence of nephrotoxicity. A significant increase of doxorubicin AUC was observed, which was identical to that observed by Barlett *et al.* (10) using CsA. In that study (10), there was no indication that doxorubicin AUC could predict hematological toxicity, but a significant correlation was found with peak bilirubin levels. Because Pgp is expressed in secretory endothelium (*e.g.*, the bile canaliculi of the liver, proximal renal tubules, and lumenal surface of the intestine) and in the vascular endothelial cells lining the blood-tissue barrier in the brain, placenta, and testicle, a physiological role in disposition of natural toxins has been proposed (27). CsA and PSC are extensively metabolized in the liver by cytochrome P450 CYP3A (28). Both CsA and PSC inhibit a bile acid transporter in the canalicular membrane (29), which is likely responsible for the hyperbilirubinemia. Here, hyperbilirubinemia occurred in 37% of patients (30% of cycles) and mainly during PSC administration, and normal bilirubin levels were observed within a week of suspension. This appears to be less frequent than with CsA (10). A tendency of hyperbilirubinemia to increase in frequency and intensity was observed with increasing PSC dose. All these liver function changes were, however, not symptomatic. We could not find a significant correlation between doxorubicin or doxorubicinol AUCs in presence or absence of PSC and any toxicities, including leukopenia, thrombocytopenia, and hyperbilirubinemia.

Here, the increase of AUC of doxorubicinol was much greater than that of doxorubicin. This was also observed with CsA (10). Here, the concentrations of doxorubicinol tended to continue to increase at the end of the period of analysis in several patients; therefore, the AUC (0–48 h) gives a more reliable estimate; an increase of 2.7 times the AUC of doxorubicinol was observed when PSC was given. Doxorubicinol is considerably less cytotoxic than its parent compound, doxorubicin (30), and it has been associated with an increased risk of cardiac damage (31). We observed clear signs of cardiotoxicity in two patients who had never received anthracyclines or chest radiotherapy; both had increased dyspnea, which was associated with a significant decrease of LVEF. Careful monitoring of the cardiac function seems warranted in future studies of PSC or CsA in combination with doxorubicin.

In a Phase I study of continuous i.v. PSC infusion for 5 days, in combination with 2-h daily infusions of etoposide (32), myelosuppression was the most common toxicity, hyperbilirubinemia occurred in 83% of cycles, and the dose-limiting tox-

icity of PSC was severe ataxia. The dose of etoposide was reduced from 100 to 75 mg/m^2 to limit the severity of myelosuppression, which was increased due to the pharmacological interaction with PSC. The AUC of etoposide increased by a mean of 76% in this study, in analogy to a study of the combination of etoposide with CsA (33), and this increase appeared to be present from the initial dose of PSC tested and to plateau at 4 mg/kg/day. Total clearance and V_{ss} decreased by 41%. No change in MRT was observed, and $t_{1/2\alpha}$ and $t_{1/2\beta}$ increased by 34 and 39%, respectively. In our study of oral PSC, in contrast, there was a smaller decrease of plasma clearance by 30% but a substantial increase of MRT by 48%. The increase in $t_{1/2\gamma}$ (noncompartment model) and t_{max} reached a significant level for doxorubicinol only and not for doxorubicin. $t_{1/2\alpha}$ and $t_{1/2\beta}$ of doxorubicin did not change in our study (three-compartment model).

Severe and prolonged ataxia (lasting for weeks) was dose limiting in the study by Boote *et al.* (32), and it was observed in two of nine patients treated at 12 mg/kg/day PSC. Here, mild perioral tingling, paresthesias, and ataxia were the major neurological toxicities due to PSC administration; the duration, however, was relatively short, usually less than a week, except in one patient who had preexistent peripheral sensory neurotoxicity due to cisplatin and who developed severe ataxia, which also lasted several weeks. A computed tomography scan a few weeks later demonstrated multiple brain metastases, which might have contributed, in part, to the severity of ataxia.

Cerebellar ataxia has been described as the dose-limiting toxicity of two other MDR modulators: tamoxifen and dexniguldipine (14). PSC-induced ataxia may be due to the inhibitory effect that PSC has on the endothelial cells in the blood-brain barrier or from the high-dose administration of the drug itself. From mdr1a knock-out mice, it appears that the blood-brain barrier is highly disrupted and much more vulnerable to neurotoxic substances (20). The group in Stanford has performed a study of oral PSC in combination with etoposide or paclitaxel (14). In these studies, the MTD was 5 mg/kg, four times a day for 4 days. A dose of 6 mg/kg four times a day for 4 days produced unacceptable ataxia. Significant pharmacological interaction was observed with both anticancer drugs. The design of these studies, in which PSC was also given alone, clarified that ataxia is a consequence of PSC administration alone and not the result of pharmacodynamic interactions with the chemotherapeutic drugs. Moreover, development of ataxia was closely correlated with serum levels of PSC, with grade 3 ataxia occurring only above serum levels of 2.5 μM. These findings suggest, therefore, that doxorubicin, etoposide, and paclitaxel may not be toxic to the central nervous system (14). PSC has been shown to be able to block Pgp in the blood-brain barrier in rats in a dose-dependent manner and induce neurological toxicity at the higher concentrations (34).

Because the dose of anticancer drug has to be reduced so drastically, future studies should investigate the concentration of the cytotoxic agent at the cancer cell level, also in view of a possible large discrepancy between plasma and tissue levels (18, 19). Furthermore, the determination of Pgp expression and its function should also be investigated in tumor cells from patients. The fact that a reverter may increase the AUC of the anticancer drug may lead to increased drug exposure of the tumor and therefore, by itself, increase antitumor activity and toxicity. These issues should be carefully addressed in future randomized trials of such compounds. Furthermore, concentrations of drug resistance modifiers *in vitro* (usually 5–10% serum) may not be relevant *in vivo*, where high protein binding may necessitate much higher levels.

In conclusion, here, oral PSC could be safely given when the dose of doxorubicin was reduced by 60% and achieved concentrations able to revert MDR *in vitro* with PSC doses above 7.5 mg/kg/day. Studies of PSC are warranted in hematological malignancies and in selected solid tumors (*e.g.*, breast and ovary cancer) where refractoriness to MDR drugs is shown to be associated with increased in Pgp expression. The large intra- and interpatient variations observed with our oral formulation and schedule make the investigation of better absorbed oral formulations or continuous i.v. PSC for the reliable achievement of stable active plasma concentrations desirable.

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