Cancer Chemother Pharmacol (1998) 41: 281–291 © Springer-Verlag 1998

ORIGINAL ARTICLE

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Phase I clinical and pharmacokinetic study of S9788, a new multidrug-resistance reversal agent given alone and in combination with doxorubicin to patients with advanced solid tumors

Received: 13 July 1996 / Accepted: 4 July 1997

Abstract *Purpose*: The objectives of this phase I study were to evaluate the toxic effects and the maximum tolerated dose (MTD) of S9788, a new modifier of multidrug resistance (MDR), when given alone and in combination with doxorubicin to patients with advanced solid tumors; to achieve a potentially active plasma concentration of S9788; and to study the pharmacokinetics of both drugs. *Methods*: A total of 26 patients (median age 58 years) entered the study. S9788 was given alone as a 30-min infusion at day 1 and in combination with a 50-mg/m^2 bolus of doxorubicin at days 8 and 29. Dose levels of S9788 were escalated from 8 to 96 mg/m^2 according to the modified Fibonacci scheme. Plasma samples were taken predose as well as during and up to 48 h after the beginning of infusion for S9788 and doxorubicin quantitation. Fractionated urine samples were also collected for up to 24 h for S9788 determination. *Results*: The dose-limiting side effects of S9788 consisted of bradycardia, sometimes associated with faintness or dizziness. The MTD of S9788 was 96 mg/m^2. No enhancement of doxorubicin toxicity was observed. One partial response (duration 140 days) was observed at 96 mg/m^2 in a patient with multiple lung metastases from a refractory urothelial carcinoma. Pharmacokinetic studies were performed in 24 patients. Since the mean apparent elimination half-life of S9788 was 46 ± 23 h and the last plasma sampling time was 48 h, only model-independent parameters were considered. Plasma levels of S9788 were below the limit of quantitation (4×10^{-3} μM) before each drug administration. S9788 plasma levels of up to 3.7 μM could be obtained with this administration schedule. The urinary elimination of the unchanged drug was negligible, whatever the collection period. In spite of the large inter- and intraindividual variability, plasma pharmacokinetics of S9788 given as a 30-min i.v. infusion were linear up to 96 mg/m^2 and were not modified by doxorubicin administration. Doxorubicin pharmacokinetic parameters did not seem to be influenced by S9788 coadministration. *Conclusion*: The dose-limiting toxicity of S9788 consisted of bradycardia or clinical symptoms suggesting a vasovagal impact such as faintness or dizziness. The MTD of S9788 was 96 mg/m^2. The pharmacokinetic parameters of doxorubicin in this study were close to those usually described and were not influenced by escalation of the S9788 dose. No pharmacokinetic interaction was observed between S9788 and doxorubicin. The clinical tolerability of the combined treatment is in good agreement with the pharmacokinetic findings, since no enhancement of doxorubicin toxicity was observed.

Key words S9788 · Doxorubicin · Pharmacokinetics · Multidrug resistance

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Introduction

Drug resistance to anticancer chemotherapy is considered a major cause of treatment failure. The most extensively studied type of cellular drug resistance is multidrug resistance (MDR). This phenomenon was first described in 1970 by Biedler and Riehm [7] and was subsequently shown to be associated with overexpression of the *mdr1* gene, which encodes the multidrug transporter P-glycoprotein (P-gp) [8, 19, 26]. P-gp is a membrane protein of 170 kDa that acts as an adenosine

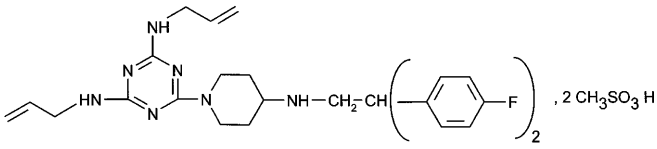

Fig. 1 Structure of S9788

triphosphate (ATP)-dependent drug-efflux pump [25, 27]. MDR leads to decreased cellular drug accumulation due to increased efflux of a broad variety of structurally unrelated natural products and anticancer drugs (epipodophyllotoxins, vinca alkaloids, and anthracyclines) [5, 11, 14].

A number of noncytotoxic pharmacologic agents have been shown to inhibit P-gp in vitro, including cyclosporine A, tamoxifen, quinidine, verapamil, amiodarone, and calmodulin inhibitors [6, 12, 36, 38, 39, 42]. However, their clinical use is limited by the toxicity associated with the doses of these compounds required to achieve plasma concentrations capable of reversing MDR [33]. In this setting the identification of new, less toxic MDR modulators is of primary importance.

S9788, 6-[4-[2,2-Di(4-fluorophenyl)-ethylamino]-1-piperidinyl]-*N*,*N*′-di-2-propenyl-1,3,5-triazine-2,4-diamine, a new triazinoaminopiperidine derivative synthesized by Servier Laboratories (Fig. 1), is capable of reversing MDR, especially that to anthracyclines and vinca alkaloids. It does not belong to any class of compounds known to overcome MDR [16]. S9788 is more potent than verapamil in sensitizing MDR cell lines in vitro, and its optimal in vitro active concentrations range between 0.5 and 5 μM, depending on the cell line, its resistance level, and the cytotoxic agent used [35]. S9788 is highly protein-bound in blood and plasma, especially to lipoproteins [44], but its reversing ability is maintained when cells are incubated in vitro in pure serum medium or when sera of patients treated with S9788 are used to test the reversing potential of the compound [40]. S9788 restores the sensitivity of resistant cells to the cytotoxic substance by P-gp inhibition [20], enhancing drug accumulation as has been described for Adriamycin in MCF7/DOX human breast cancer cells and K562 erythroleukemia cells or C6 rat glioblastoma cells [21, 24]. In vivo studies performed on murine leukemia models (NCI models) showed that S9788 could increase the efficacy of cytotoxic drugs involved in the MDR phenomenon, such as vincristine [13]. The plasma levels that gave MDR reversal in mice ranged between 2 and 6 μM (unpublished data). Preclinical toxicology studies have shown neurologic disorders (ataxia, convulsions) to be the dose-limiting toxicities of S9788. The LD_{10} (lethal dose that kills 10% of animals) in mice after a single i.v. administration was 28 mg/kg.

On the basis of these data we conducted a phase I pharmacokinetic and clinical study to explore the potential for the administration of S9788 alone and in combination with doxorubicin to cancer patients. The objectives of this study were both clinical and pharmacokinetic. The clinical aims were to evaluate the toxic effects and the maximum tolerated dose (MTD) of S9788 given alone and in combination with doxorubicin. The pharmacokinetic aims were, by analyzing plasma levels and pharmacokinetic parameters of S9788, to determine if this administration schedule could achieve S9788 plasma levels between 0.5 and 5 μM, which is the active in vitro concentration range for reversing MDR, to evaluate the dose and time linearity of the S9788 pharmacokinetic parameters at increasing dose levels and on repeated administration, and to study the pharmacokinetic parameters of doxorubicin when combined with S9788.

Patients and methods

Eligibility criteria

Patients included in the present study had a histologically or cytologically confirmed malignant solid tumor for which no potential curative therapy was available. Patients with kidney or colon cancer were preferably chosen due to more frequent intrinsic MDR in these situations. Eligibility criteria included the following: an age of between 18 and 75 years; no chemotherapy or radiotherapy within 4 weeks of entry; in case of prior doxorubicin therapy, a cumulative doxorubicin dose of < 400 mg/m^2; an ECOG/WHO performance status of ≤ 2; evidence of progression within the last 2 months; the possibility of repeated blood sampling; adequate hematologic, kidney, liver, and heart function, defined as a WBC count of $\geq 4{,}000/\mu l$, a platelet count of $\geq 100{,}000/\mu l$, a hemoglobin value of ≥ 10 g/dl, total bilirubin, transaminase, alkaline phosphatase, and creatinine levels of ≤ 1.25 times the upper limit of normal, and a left ventricular ejection fraction (LVEF) of $> 40\%$ as determined by ultrasonography; and, finally, written informed consent. Ineligibility criteria included recent myocardial infarction, arrhythmia, angina pectoris, or cardiac failure; sequelae of prior abdominal radiotherapy; concomitant uncontrolled infection or severe pathology; and pregnancy or lactation. The protocol was approved by the Ethics Committee of Lyon A.

Pretreatment and follow-up studies

Prior to therapy a complete history and physical examination as well as determination of ECOG/WHO performance status were obtained for each patient. The following laboratory tests were obtained at baseline and repeated weekly: complete blood cell count, serum electrolytes, alkaline phosphatase, transaminases, gammaglutamyl transferase, bilirubin, serum creatinine, total protein, serum calcium, inorganic phosphorus, prothrombin time, activated partial thromboplastin time, and urinalysis. A 12-lead EKG as well as blood pressure and heart rate monitoring were performed at baseline and repeated weekly. Evaluation of the LVEF was performed at baseline by cardiac ultrasonography. Patients were followed on a weekly basis for the determination of adverse reactions. Tumor evaluation was assessed before treatment and every 5 or 6 weeks. The toxic effects and tumor responses were classified according to WHO criteria.

Treatment

The *bis*-methane sulfonate salt of S9788 was provided by Servier Laboratories as a 10-mg/ml 5% glucose solution (10-ml vials). The treatment schedule consisted of a 30-min i.v. infusion of S9788 on day 1. After 1 week (day 8), a 30-min i.v. infusion of S9788 was immediately followed by a 50-mg/m^2 doxorubicin i.v. bolus and

the catheter was flushed with saline solution. The co-administration of S9788 and doxorubicin was then repeated every 3 weeks. The starting dose of S9788 represented one-tenth of the LD_{10} in mice, that is, 8 mg/m^2. Doses were escalated to 96 mg/m^2 in successive groups of patients according to a modified Fibonacci scheme. At least three patients were entered at each dose level. However, only one patient was included at each intermediate dose level, i.e., 48 and 80 mg/m^2 (Table 1). In case of grade 3 or 4 hematologic toxicity considered to be dose-limiting (DLT), the number of patients had to be doubled for the corresponding dose level. In case of grade 2–4 non-hematologic toxicity (DLT), the number of patients to be entered at a specific dose level had to be decided between the investigator and the sponsor of the study. The MTD would be the dose at which the DLT appeared in at least two of three patients.

Pharmacokinetics

Whenever possible, pharmacokinetic studies of S9788 and doxorubicin were performed on days 1, 8, and 29.

Sampling

All blood samples for the pharmacokinetic study were drawn from the arm opposite from that receiving the infusion; 5 ml was drawn into heparinized tubes for the S9788 pharmacokinetic study, and 3 ml was collected in ethylenediaminetetraacetic acid (EDTA)-containing tubes for doxorubicin pharmacokinetics determination. After a reference blood sample had been taken, S9788 was infused using a constant-rate infusion pump. On day 1, blood samples were taken predose and at 15 and 30 min (just before the end of infusion) as well as 1, 2, 4, 8, 12, 24 and 48 h after the start of the infusion. On days 8 and 29, to follow the pharmacokinetics of doxorubicin, we added two more sampling times at 40 and 50 min after the start of the S9788 infusion; S9788 and doxorubicin were assayed in all samples. Samples were immediately centrifuged at 2,000 *g* for 5 min. Plasma samples were stored at −20 °C in darkness until analysis. Fractionated urine were collected in glass containers from 0 to 4 h, 4 to 8 h, 8 to 16 h, and 16 to 24 h, and the total volume of each fraction was recorded (urine samples were not pooled). An aliquot from each fraction was then stored at −20 °C for individual analysis.

Sample analysis

S9788 was assayed according to a procedure previously described by Bakes et al. [2], using a solid-phase extraction and high-performance liquid chromatography (HPLC) method with a limit of quantitation of 2 ng/ml. Doxorubicin was assayed according to a procedure using HPLC and fluorimetric detection (excitation 480 nm, emission 592 nm) with a limit of quantitation of 2 ng/ml as previously described by Baurain et al. [4].

S9788 data analysis

Plasma concentrations of S9788 were plotted versus time on a semilogarithmic scale. A model-independent analysis was performed for S9788, the main parameters being C_{max}, t_{max} (maximal observed concentration and its corresponding time); t_{last} (sample time of the last measurable S9788 level); AUC (area under the concentration versus time curve), computed by means of logarithmic trapezoids, for intervals of between 0 and 8 h, 0 and 12 h, 0 and 24 h, and 0 and 48 h; and $t_{1/2}$ (the apparent elimination half-life), computed from the last three points of the curve. The linearity of S9788 pharmacokinetics was assessed using linear regression between AUC_{0-12} and dose on days 1, 8, and 29, respectively. The stability of S9788 pharmacokinetics on repeated administration and the influence of doxorubicin on S9788 pharmacokinetics were studied by comparison of the S9788 AUC_{0-12} between days 8 and 29 and between day 1 and day 8, respectively (Wilcoxon-Mann-Whitney test).

Doxorubicin data analysis

Since some kinetics could be followed only for the first 24 h or less, doxorubicin pharmacokinetic parameters were established not by means of individual evaluation but by using population pharmacokinetics with the parameters determined by Bressolle et al. [9]. The first step was to compute the pharmacokinetic parameters in patients followed for 48 h using individual analysis and then using Bayesian analysis so as to verify the accuracy of the Bayesian calculation in the present study. The efficiency of Bayesian analysis with respect to individual analysis was studied on AUC values, and the AUC predictive performance was analyzed according to the suggestions of Sheiner and Beal [37] by computation of bias and precision between the two sets of AUC values. Thereafter, pharmacokinetic parameters of patients followed for only 24 h or less could be computed using the Bayesian approach. The sampling times retained for the calculation were those closest to the optimal sampling times determined by Bressolle et al. [9] that is, 0.2, 1.5, and 48 or 24 h after the administration of doxorubicin. Bayesian estimation was performed using APIS software [22] on a PC486. The doxorubicin parameters obtained were compared with the literature data (when doxorubicin is given alone) and were compared between day 8 and day 29 for each patient using Student's *t*-test. The influence of increasing doses of S9788 on these parameters was evaluated by the Pearson correlation test.

Results

Between January 1992 and June 1993, 26 patients (12 men and 14 women) were entered in this trial. A majority of the patients had colorectal (14), breast (4), or renal (3) adenocarcinoma, since these tumor types are frequently associated with intrinsic or acquired MDR. In all, 23 of 26 patients had previously been treated with chemotherapy (median number of regimens 2, range 1–5), and 9 of them had received cytotoxic drugs known to induce MDR. The median age was 58 (range 20–72) years, and the median WHO performance status was 1 (range 0–2). Overall, 23 patients had undergone prior surgery, 23 had received prior chemotherapy (median number of regimens 2, range 1–5), 9 had received prior radiotherapy, and 4 had previously been treated by immunotherapy.

A total of 101 courses of S9788, 75 of which were associated with doxorubicin, were given at dose levels ranging from 8 to 96 mg/m^2. The number of patients entered at each dose level and the number of courses given are presented in Table 1. Each patient received 1–7 (median 4) S9788 administrations. One patient treated at the 96-mg/m^2 dose level received only one S9788 infusion since he presented electrocardiographic signs of ventricular and supraventricular hyperexcitability on Holter monitoring during treatment.

Toxicity profile

A total of 26 patients were evaluable for the safety of S9788, 14 of whom experienced some clinical event

Table 1 Demographic and S9788 dosing details of patients

Patient number	Sex	Age (years)	Body weight (kg)	S9788 level (mg/m^2)	S9788 dose (mg/day)	Days of infusion and sampling	Number of cycles	Prior chemother. lines (*n*)	Primary tumor	Response[g]
1[a]	F	65	62	8	13.6	1,8,29	6	1	Colon	SD
2	M	56	66		12.8	1,8,29	3		Colon	
3[c]	M	53	73		14.8	1,8,29	4	1	Kidney	
4[c]	M	51	71	16	28.8	1,8,29	6	1	Colon	
5[a]	M	49	80		32.0	1,8,29	3	1	Colon	
6[a]	M	59	82		32.0	1,8,29	3		Kidney	
7[a]	F	50	70	26	46.8	1,8,29[d]	3	2[h]	Synovial sarcoma	
8	F	67	67		33.8	Not sampled	3	2	Rectum	
9	M	60	93		52.0	1,8,29	5	2	Colon	
10	M	72	89	40	80.0	1,8,29	5	1	Colorectum	
11[a]	F	53	56		66.0	1,8,29	6	4	Colon	SD
12[a]	F	63	48		58.4	1	4	2[h]	Breast	
13[a]	F	60	51	48	69.6	1[d],8,29	3	3[h]	Secondary sarcoma	
14[a]	F	35	45	56	77.3	1[d],8,29	4	5[h]	Breast	
15[a]	M	57	65		96.3	1,8,29	4	2	Rectum	
16[a]	F	20	61		92.0	1,8,29	3	4[h]	Hemangiopericytoma	
17[b]	M	50	62		94.1	1,8,29	3	1	Colorectum	
18	F	58	68		98.5	Not sampled	4	4[h]	Breast	MR
19[b]	F	59	65		94.0	1,8	4	1	Colorectum	
20[a]	M	64	56	72	114.5	1,8,29	3		Kidney	
21[a]	F	55	62		119.5	1,8,29[e]	4	3[h]	Breast	
22	F	47	44		103.0	1,8[f],29[f]	3	1	Colon	
23[a]	M	59	81	80	155.2	1,8,29	4	1	Colon	
24[a]	F	64	43	96	124.8	1,7[d],29[d]	7	2[h]	Renal calix/urothelium	PR
25	M	62	75		180.5	1	1	1	Colon	
26[a]	F	66	78		179.5	1,9,29	3	2[h]	Unknown	

[a] Doxorubicine sampling at day 8 and day 29
[b] Doxorubicine sampling at day 8
[c] Doxorubicine sampling at day 29
[d] Contamination of plasma sample (double lumen catheter)
[e] Irregular infusion
[f] Problem with venous access
[g] *SD* Stable disease, *MR* minor response, *PR* partial response
[h] Prior therapy lines included anthracyclines or vinca alkaloids

Table 2 Tolerance of S9788: clinical events reported in 14/26 patients

Dose level (mg/m^2)	Patient number	Clinical events
26	7.8	Fatigue
40	10	Fatigue
48	13	Palpitations
56	15	Ventricular tachyarrhythmia and convulsions during the 4th course[a]
	16	
	17	Vomiting
	18	Myalgia of thighs
	19	Anorexia, vomiting
		Asymptomatic bradycardia
72	21	Fatigue
80	22	Abdominal pain
96	24	Bradycardia, dizziness, profuse sweating, QTc prolongation
	25	Asymptomatic bradycardia, ventricular hyperexcitability
	26	Faintness, somnolence, profuse sweating

[a] Before the administration of doxorubicin

Table 3 Tolerance of S9788 associated with doxorubicin

S9788 dose level (mg/m^2)	8	16	26	40	48	56	72	80	96	Totals
Patients (*n*)	3	3	3	3	1	6	3	1	2	25
Leukopenia:										
Grade 1–2	1	2	1	1	0	2	2	1	1	11
Grade 3–4	1	0	1	0	1	2	1	0	1	7
Neutropenia:										
Grade 1–2	0	2	0	1	0	0	1	0	0	4
Grade 3–4	1	0	1	0	1	3	1	1	2	10
Nausea-vomiting:										
Grade 1–2	3	1	2	2	1	1	1	1	2	14
Grade 3–4	0	0	0	1	0	3	1	0	0	5
Stomatitis:										
Grade 1–2	2	0	2	2	1	1	0	0	1	9
Grade 3–4	0	0	0	0	0	0	0	0	0	0
Diarrhea:										
Grade 1–2	0	0	1	2	0	1	1	1	0	6
Grade 3–4	0	0	0	0	0	1	0	0	0	1
Alopecia:										
Grade 1–2	2	3	1	1	1	3	1	1	1	14
Grade 3	0	0	1	1	0	1	1	0	1	5

(Table 2), and 25 patients were evaluable for the safety of the combined treatment (Table 3).

Cardiovascular toxicity

The main side effect associated with S9788 administration consisted of bradycardia associated with clinical symptoms suggesting a vasovagal impact such as sweating, faintness, or dizziness. In all, 8 of 26 patients exhibited cardiovascular abnormalities during the infusion of S9788. At the dose level of 56 mg/m^2, two of six evaluable patients presented a S9788-related sinus bradycardia. In one patient, two episodes of asymptomatic bradycardia were found on repeated automatic heart rhythm and blood pressure recordings at the first and the third S9788 infusions. Another patient developed asymptomatic bradycardia at the third S9788 administration. One serious acute side effect occurred in a patient receiving 56 mg/m^2 S9788 for the fourth time. In this case the infusion of S9788 had to be stopped after 15 min (before the administration of doxorubicin) due to convulsions associated with a ventricular tachyarrhythmia. Although this patient had biochemical and electrocardiographic evidence of hypokalemia (3 mmol/l) before the start of the infusion, the added risk of S9788 administration remained unclear.

We therefore decided additionally to monitor all further patients through 24-h electrocardiographic Holter recordings. In all, 9 patients and 18 courses were monitored. At the 72- and 80-mg/m^2 S9788 dose levels (four evaluable patients), no cardiovascular event occurred. However, the three evaluable patients entered at 96 mg/m^2 experienced cardiovascular side effects. One patient (number 24) experienced symptomatic bradycardia after the first S9788 infusion (45 bpm; baseline value 63 bpm), associated with profuse sweating, dizziness, and somnolence. This patient, who presented an objective tumor response, received a further six cycles of the S9788-doxorubicin combination. Sinus bradycardia of varied intensity was noticed during all subsequent courses. Holter monitoring revealed a prolonged QTc interval during cycles 5–7. A second patient (number 25) experienced asymptomatic bradycardia (52 bpm; baseline value 65 bpm) after the first S9788 infusion. This patient also presented electrocardiographic signs of ventricular and supraventricular hyperexcitability on Holter monitoring performed during the treatment. A third patient (number 26) experienced a combination of malaise, sleepiness, and profuse sweating after the second S9788 infusion. In this patient, Holter monitoring revealed the occurrence of second-degree atrioventricular block.

The frequency and increasing severity of these symptoms with increasing dose levels of S9788 led us to consider them as dose-limiting and to define the MTD to be 96 mg/m^2. An echocardiographic follow-up of the left ventricular ejection fraction (LVEF) of ten patients showed no major changes. The median LVEF at inclusion was 65% (range 56–78%), and the median LVEF upon treatment discontinuation between day 50 and day 72 was 60% (range 50–84%).

Hematologic toxicity

The hematologic tolerance of S9788 alone could be explored only between day 1 and day 8. In association with doxorubicin, hematologic tolerance was acceptable. Altogether, 72% of the patients developed leukopenia (grade 3–4 in 28% of patients) and 56% had neutropenia (grade 3–4 in 40% of patients). Anemia was observed in 21 patients (84%), 8 of whom required red blood cell transfusions. No thrombocytopenia was observed. The severity and frequency of myelosuppressive events did not seem to be related to the dose levels of S9788. Aplasia-related fever occurred in only one patient treated at 56 mg/m^2, due to the infection of a sacral fistula.

Other toxicities

The laboratory safety of S9788 alone was excellent. Grade 1 uremia and creatininemia were found in one patient treated at 26 mg/m^2, and spontaneously regressive grade 1 ASAT and alkaline phosphatase levels were found in two patients treated at 96 mg/m^2. Events such as fatigue (four patients), nausea-vomiting (two patients), anorexia (one patient), muscle pain in the thighs (one patient), and abdominal pain (one patient) were observed after the administration of S9788 alone, but were neither redhibitory nor dose-limiting.

Compliance with combined treatment (Table 3) was excellent, since none of the courses had to be delayed

due to drug-related toxicity. Grade 1–3 nausea and vomiting was frequently observed (grade 1–2 in 56% of patients, grade 3 in 20%), regardless of the dose of S9788. Antiemetics had been given not in a preventive manner but according to symptoms (48% of patients). Stomatitis of grade 1 occurred in 36% of patients and was observed at each dose level except at 72 and 80 mg/m^2. Diarrhea was observed in 28% of patients treated at 26 mg/m^2 and above (grade 1 six patients, grade 3 one patient). Grade 1–2 local phlebitis or erythema at the site of injection appeared in 3 of 26 patients (12%). Another patient had uncomplicated moderate extravasation. Grade 1–3 alopecia occurred in 19 of 23 evaluable patients, regardless of the S9788 dose. Unusual events such as headache (three patients), conjunctivitis (two patients), epistaxis (one patient), allergy (urticaria, one patient; urticaria, erythema, and asthma, one patient; pruritus, one patient), or dry mouth (one patient) occurred with the combined treatment. The imputability to either of the compounds was uncertain. The laboratory safety of the combination of S9788 and doxorubicin was satisfactory. An increase in uremia and creatininemia (grade 1) was observed in two patients treated at 16 and 56 mg/m^2, respectively. Of the 25 patients evaluable for toxicity, 17 (68%) had liver metastases. Hepatic enzymes were moderately modified (grade 1–2) in 11 patients. Alkaline phosphatase levels increased in 15 patients (in 5 cases to grade 3–4). Grade 1 hyperbilirubinemia occurred in one patient.

Because of the hypokalemia present in the patient who developed tachyarrhythmia, kalemia was closely followed and analyzed in all patients. At study inclusion, 12 patients had slight hypokalemia, either isolated or associated with other blood chemistry abnormalities. After the first infusion of S9788 alone, the values of 5 of them returned to normal, whereas 7 other patients who had shown normal kalemia at inclusion developed hypokalemia. In three patients, hypokalemia was observed after the combined administration of S9788 and doxorubicin. We have no clear explanation for the frequency of hypokalemia except for the common intake of steroids, diuretics, and laxatives by patients with advanced cancer; the changes in kalemia values observed during the study were not supportive of a drug-induced toxicity.

Antitumor activity

A total of 24 patients were evaluable for antitumor activity. One partial response (140 days) was observed at 96 mg/m^2 in a patient (number 24) with multiple lung metastases from a urothelial carcinoma refractory to MVAC (methotrexate, vinblastine, Adriamycin, cisplatin) chemotherapy. A suggestion of antitumor activity was seen in three patients (numbers 1, 11, and 19) with metastatic colon or rectosigmoid adenocarcinoma (one minor response and two stabilizations).

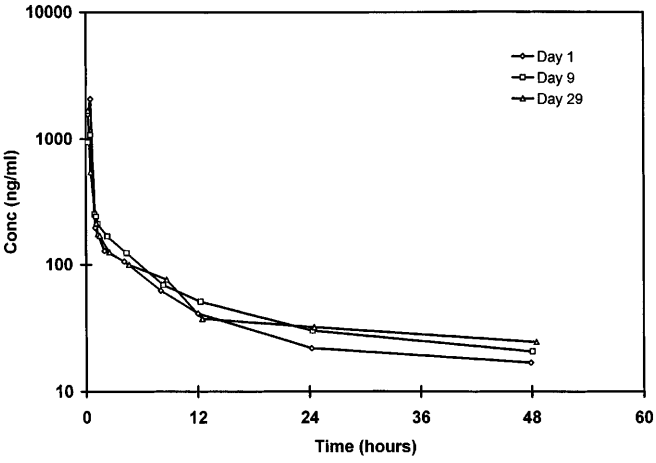

Fig. 2 Plasma decay of S9788 for patient 26, treated at a dose level of 96 mg/m^2, on day 1, day 9, and day 29

Pharmacokinetics

Plasma pharmacokinetics of S9788

Plasma pharmacokinetics of S9788 were followed in 24 patients (67 administrations). Demographic and dosing details for these patients are described in Table 1. Figure 2 shows typical decay curves generated for S9788 in a patient (number 26) treated at a dose level of 96 mg/m^2 on days 1, 9, and 29. In nine cases, pharmacokinetic parameters could not be evaluated (Table 1). Model-independent parameters in the 58 evaluable kinetics are presented in Table 4.

In all cases the plasma concentration before each administration was below the limit of quantitation. C_{max} values higher than 0.5 μM (250 ng/ml) were observed at S9788 dose levels of 26 mg/m^2 and above. The greatest C_{max} value was observed in patient 23 at day 1 (3.74 μM, 1,871 ng/ml) at 80 mg/m^2. Since t_{last}, the time of the last measurable plasma concentration, was in most cases close to $t_{1/2}$ (mean value 46 $\pm$ 23 h), we could not obtain valid parameters by any model or consider AUC from 0 to infinity. The linearity and stability of S9788 pharmacokinetics were evaluated from log-trapezoidal values of AUC_{0-12}. The linearity was considered separately on days 1, 8, and 29. Significant correlations were observed between AUC_{0-12} and doses expressed either in milligrams per square meter of body area or in milligrams for each administration day with an intercept not significantly different from 0 ($P < 0.05$). The stability over time was evaluated by comparison of AUC_{0-12} at days 1, 8, and 29. The Wilcoxon-Mann-Whitney test did not show any significant difference between AUC_{0-12} at day 1 and day 8 ($P = 0.570$) or at day 8 and 29 ($P = 0.359$), showing no influence of doxorubicin on S9788 pharmacokinetics (comparison between day 1 and day 8) or the stability of S9788 pharmacokinetics on repeated administration (day 8 versus day 29). Concerning the urinary elimination, in

Table 4 Individual values for t_{max}, C_{max}, $t_{1/2}$, and log trapezoidal AUC_{0-12} of S9788

Patient N°	S9788 level (mg/m^2)	t_{max} (h)			C_{max} (ng/ml) 1ng/ml = $2 \cdot 10^{-3}$ μM			t_{last} (h)			$t_{1/2}$ (h)			AUC_{0-12} ($ng \cdot ml^{-1} \cdot h$)		
		1	8	29	1	8	29	1	8	29	1	8	29	1	8	29
1	8	0.48	0.25	0.25	121	89	86	4	2	45	4	1	46	–	–	101
2		0.25	0.25	0.25	71	78	201	2	2	4	3	1	3	–	–	–
3		0.48	0.48	0.47	51	61	47	24	8	2	34	10	8	82	–	–
4	16	0.25	0.43	0.47	109	70	143	12	2	2	11	1	1	107	–	–
5		0.25	0.48	0.47	99	116	85	12	4	1	8	5	1	151	–	–
6		0.50	0.50	0.47	117	156	158	4	2	8	4	1	11	–	–	–
7	26	0.25	0.47	–	798	986	–	12	48	47	14	112	32	524	611	–
9		0.47	0.47	0.25	466	459	315	24	48	24	40	91	84	429	449	334
10	40	0.48	0.47	0.48	757	863	893	48	48	44	72	38	77	660	894	729
11		0.48	0.25	0.48	561	181	1411	24	47	48	10	21	69	479	322	876
12		0.48	–	–	129	–	–	24	–	–	29	–	–	220	–	–
13	48	–	–	0.50	–	–	969	–	–	48	–	–	27	–	–	–
14	56	–	0.50	0.25	–	697	370	48	49	24	–	23	13	–	773	464
15		0.48	0.25	0.50	588	646	619	12	12	24	20	9	10	483	543	681
16		0.50	0.47	0.48	1298	1698	588	48	48	25	30	27	14	1104	1173	767
17		0.47	0.25	0.47	585	653	357	12	49	48	5	32	34	408	734	532
19		0.53	0.25	–	1382	828	–	47	48	–	15	67	–	1147	776	–
20		0.25	0.48	0.25	958	1035	273	48	46	42	40	58	37	846	727	383
21	72	0.48	0.25	–	1211	578	–	48	24	–	35	11	–	958	729	–
22		0.25	–	–	620	–	–	45	–	–	46	–	–	592	–	–
23	80	0.47	0.25	0.48	1871	1686	1848	45	46	23	28	20	24	1199	1301	1309
24	96	0.45	–	–	749	–	–	44	–	–	48	–	–	955	–	–
25		0.25	–	–	334	–	–	48	–	–	31	–	–	765	–	–
26		0.48	0.50	0.25	1042	537	842	48	48	48	65	44	62	980	897	853

all cases but one, S9788 remained undetectable in urine samples. In one case (patient 26, day 8) the urinary concentration of parent S9788 was measurable in the first sample (0–4 h) at 11 ng/ml, corresponding to less than 0.1% of the delivered dose.

Plasma pharmacokinetics of doxorubicin

Plasma pharmacokinetics of doxorubicin were assessed in 19 patients (34 kinetics: 15 patients on days 8 and 29, 2 patients on day 8, and 2 patients on day 29). In 20 cases the plasma concentration of doxorubicin was not measured until 48 h. In these cases, Bayesian population pharmacokinetics were used to determine individual pharmacokinetic parameters. The accuracy of the pharmacokinetic parameters was first established from the 14 doxorubicin kinetics, followed for 48 h, using the sampling times closest to the optimal sampling times determined by Bressolle et al. [9]: 0.2, 1.5, and 48 h. AUC values obtained from the Bayesian estimation (AUC_{pop}, median 1.64 mg l^{-1} h, range [1.24, 2.61] mg l^{-1} h) were correlated with AUC values obtained from individual analysis (AUC_{ind}: median 1.71 mg l^{-1} h, range [1.20,

Table 5 Mean pharmacokinetic parameters of Adriamycin, computed by Bayesian estimation

		C_{max} (mg/l)	t_a (h)	t_p (b)	t_g (h)	Cl (l/h)
Day 8	Mean	1.028	0.095	1.68	44.85	53.44
	SD	0.242	0.003	0.37	11.47	15.09
	n	17	17	17	17	17
	Range	0.664–1.756	0.089–.0100	1.30–2.86	30.79–65.91	30.32–85.92
Day 29	Mean	0.981	0.095	1.71	48.88	55.04
	SD	0.146	0.004	0.34	17.28	18.52
	n	17	17	17	17	17
	Range	0.661–1.217	0.088–0.105	1.29–2.34	29.00–93.69	27.22–83.49
Total	Mean	1.005	0.095	1.70	46.86	54.24
	SD	0.198	0.004	0.35	14.59	16.65
	n	34	34	34	34	34

2.90] mg l^{-1} h): $AUC_{pop} = 0.8317\ AUC_{ind} + 0.2358$ ($r = 0.937$). Moreover, bias between the two set of values was nil, with the confidence interval (CI) being [−9.44%, + 2.32%] and the precision 12.97%. Hence, the Bayesian estimation could be used in our patients. Moreover, results obtained with t_{24h} in place of t_{48h} were also validated in the same patients ($r = 0.767$, CI [−11.33%, +11.29%]), but with a lesser precision (25.96%). Consequently, the pharmacokinetic parameters of doxorubicin, presented in Table 5, were computed in each of the 34 courses by means of Bayesian estimation using the following sampling times: 0.2, 1.5, and 48 h – or 24 h when *t*48h was not available. Parameters did not vary between day 8 and day 29 and were independent of the S9788 dose codelivered (as assessed by Student's *t*-test and Pearson correlation test, respectively).

Discussion

Doxorubicin remains a cytotoxic drug of major importance in the chemotherapy of solid tumors. The development of tumor cells exhibiting MDR to doxorubicin and other structurally unrelated cytotoxic agents can be responsible for treatment failure. Major progress in the understanding of the mechanisms of MDR was achieved by the successful cloning of the *mdr*1 gene, which encodes the membrane protein called P-glycoprotein (P-gp) [8, 25, 27]. A wide variety of compounds have been shown to reverse the MDR phenotype in vitro. Concentrations of these agents that are effective in vitro are difficult to achieve in humans without the induction of excessive side effects. Verapamil [32], cyclosporine A [3, 17], quinine [45], and calcium blockers [29] have been used in clinical trials in patients suffering from ovarian, breast, and colorectal cancer, but the results have been disappointing. Therefore, the search for novel and more potent MDR modulators is of major importance. S9788 is a new triazinoaminopiperidine derivative that has demonstrated potent MDR reversal in vitro and in vivo. This compound induces a dose-dependent increase in doxorubicin accumulation within the cells [20, 24, 35].

The aims of the clinical part of this study were to evaluate the safety and the maximum tolerated dose (MTD) of S9788 given alone and in combination with doxorubicin. Clinical follow-up of the included patients showed, on the one hand, the risk of ventricular tachyarrhythmia in the presence of hypokalemia (one patient treated at 56 mg/m^2) and, on the other, the increasing frequency and intensity of vasovagal presyncopal symptoms such as faintness, dizziness, and bradycardia, with increasing doses of S9788. Therefore, the MTD was fixed at 96 mg/m^2, since all three patients enrolled at this dose level presented cardiovascular side effects. An echocardiographic follow-up of the LVEF of the treated patients did not show any evidence of potentiation of the cardiotoxicity of cumulative doses of doxorubicin. The unusually high frequency of hypokalemia observed in the present study (88% of patients) was probably related to the very frequent use of laxatives and diuretics by the patients as well as the concomitant prescription of corticosteroids for this population of patients with well-advanced neoplastic disease. Even though hypokalemia constitutes a major risk for ventricular arrhythmia, continuous electrocardiographic Holter-type recordings did not reveal any case of severe arrhythmia other than that described at 56 mg/m^2.

The dose-limiting cardiovascular toxicity of S9788 has previously been observed in other phase I clinical and pharmacokinetic studies. In the study reported by Awada et al. [1], S9788 was given alone on days 1 and 8 over 30 min by i.v. infusion, and doxorubicin was given alone at 20 mg on day 15. The combination of both drugs was given on day 22 and then repeated weekly. Doses of S9788 were escalated from 8 to 96 mg/m^2 over seven dose levels. At 96 mg/m^2, two patients had an episode of bradycardia, in one case with hypotension and malaise. Using a different administration schedule of S9788 (an i.v. loading dose being given over 30 min before doxorubicin administration, followed by a 2-h continuous i.v. infusion), Tueni et al. [43] observed a dose-related increase in the QTc interval on systematic Holter monitoring as well as other cardiovascular side effects such as atrioventricular block, ventricular arrhythmias, and sinus tachycardia. This cardiac toxicity led to study discontinuation at the 120-mg/m^2 S9788 dose level.

Events known to occur with doxorubicin (mainly cardiotoxicity, leukopenia, and neutropenia) were closely monitored to check whether they were enhanced by the association of the MDR modulator.

Reversible arrhythmias are an extremely uncommon complication of doxorubicin. They can occur at low cumulative doses, usually develop at least 1 day after the last dose, and are often associated with a pericarditis-myocarditis syndrome [10]. Vasovagal symptoms have not been described as a complication of anthracyclines. Therefore, the acute cardiovascular toxicity observed in this study should be considered imputable to S9788.

Anthracycline cardiotoxicity is mostly dose-dependent and is caused by myocyte damage, which progressively manifests with symptoms of congestive heart failure [10, 30]. The follow-up of ten patients for LVEF by means of heart ultrasonography did not show any precipitation of myocardial dysfunction through the adjunction of S9788. However, it could be necessary to monitor further the cardiac function of patients receiving the S9788-anthracycline combination in later phase I studies for better evaluation as to whether S9788 increases the cardiotoxicity of doxorubicin.

Other clinical events that occasionally appeared with S9788 administration (fatigue, nausea-vomiting, anorexia, muscle pain) were not redhibitory or dose-limiting. S9788 did not induce any noteworthy change in renal or hepatic function. In particular, there was no

change in plasma bilirubin levels as might be expected with other MDR modulators such as cyclosporine and derivatives [3, 18]. There was no hematologic change after single-agent administration of S9788. Combining doxorubicin with S9788 did not seem to enhance the usual toxicity of this cytotoxic agent. Although conventional dosage was used (50 mg/m^2 every 3 weeks), compliance with treatment was satisfactory, and none of the courses had to be delayed due to toxicity. Hematologic toxicity did not differ from that usually reported with doxorubicin single-agent chemotherapy. These findings agree with those of Awada et al. [1], who did not observe any increase in the hematologic toxicity of the doxorubicin and S9788 combination as compared with doxorubicin alone. The usual clinical events such as nausea and vomiting, stomatitis, diarrhea, local reaction at the site of injection, or alopecia were not redhibitory and did not increase with the dose level of S9788.

The pharmacokinetic part of the present study was undertaken to describe the plasma pharmacokinetics of S9788 and to see if plasma levels of between 0.5 and 5 μM could be safely achieved in humans since in vitro concentrations known to reverse MDR are within this concentration range (depending on the tumor type and its resistance level). The apparent elimination half-life ($t_{1/2}$) of S9788 depended on the time of the last measurable concentration (t_{last}). For the 29 kinetics followed until 48 h, the $t_{1/2}$ value ranged from 15 to 112 h. Since the mean $t_{1/2}$ value was 46 h and t_{last} was never greater than 48 h (Table 4), the kinetics of S9788 were followed for only one half-life. Consequently, compartmental analysis was not possible, and only model-independent parameters were considered. C_{max} and AUC_{0-12} showed values increasing with doses. In spite of standardized regular infusion of S9788, we observed a large degree of inter- and intraindividual variability for the observed C_{max}. This variability could in part be explained by the variability of t_{max}, since in some cases, plasma samples were taken just after, not just before, the end of infusion.

With regard to the pharmacokinetic parameter AUC, significant correlations were found between AUC_{0-12} at day 1 and dose expressed either in milligrams per square meter or in milligrams ($r = 0.76$, $P < 0.001$); hence, plasma pharmacokinetics could be considered linear up to 96 mg/m^2. Furthermore, the coadministration of doxorubicin did not influence S9788 pharmacokinetics, since no significant difference in AUC_{0-12} was found between days 1 and 8. In the same way, doxorubicin pharmacokinetics were studied to see whether any modification would occur when the anthracycline was combined with S9788, as has been described for reversing agents such as verapamil [28] or cyclosporine [23] and, more recently, for PSC833, a cyclosporine analogue [18]. No significant difference was demonstrated between the doxorubicin pharmacokinetic parameters obtained at day 8 and those found at day 29. The mean values of pharmacokinetic parameters obtained by Bayesian estimation [9], t_a(5.7 $\pm$ 0.3 min), t_b(1.7 $\pm$ 0.3 h), t_g(47 $\pm$ 15 h), and Cl (54 $\pm$ 17 l/h), were close to those found in the literature, with t_a ranging from 3 to 12 min, t_b ranging from 0.5 to 3 h, t_g varying from 13 to 50 h, and Cl ranging from 24 to 75 l/h [31, 41]. These parameters were independent of the S9788 dose up to 96 mg/m^2. Therefore, doxorubicin pharmacokinetic parameters did not seem to be influenced by S9788 coadministration. This is in agreement with the clinical results of the present study since the usual toxicity of doxorubicin did not seem to be enhanced by the addition of S9788. This is also in good agreement with the results obtained by de Valeriola et al. [15], who studied the pharmacokinetic parameters of doxorubicin given as single-agent chemotherapy on day 1 and in combination with S9788 on day 43 and did not observe any pharmacokinetic interaction between S9788 and doxorubicin.

The dose escalation of S9788 up to 96 mg/m^2 led to a C_{max} value of 3.74 μM (patient 23, day 1). C_{max} was always found to be higher than 0.5 μM from the 26-mg/m^2 dose level of S9788. In a previously reported study the mean peak plasma level observed after a 40-mg/m^2 S9788 30-min infusion was 1.2 μM [1]. Taking into account the plasma concentration and the S9788-related side effects observed after a short i.v. infusion, the administration schedule should probably be improved. Preclinical data reported by Perez et al. [34] suggested that a continuous infusion of S9788, starting simultaneously with the administration of the cytotoxic drug and ending 24 h later, might be a more effective schedule for clinical use than a bolus administration. Furthermore, Julia et al. [24] demonstrated the importance of exposure sequence and duration in achieving the maximal reversal effect of S9788 on doxorubicin cytotoxicity against MCF7/DOX cell line. In a comparison of incubation with S9788 before or after treatment with doxorubicin the best reversal factor was obtained with a posttreatment incubation, demonstrating a schedule dependency of S9788 activity.

The partial response we observed in a patient with urothelial papillary carcinoma refractory to MVAC chemotherapy was rather encouraging. Other hints of the antitumor activity of the doxorubicin-S9788 combination have been observed in different phase I studies. Awada et al. [1] reported one minor response in a patient with colorectal cancer. Tueni et al. [43] observed one partial response on peripheral and abdominal lymph nodes in a patient with colorectal cancer whose disease progressed on doxorubicin alone. Considering that the first dose-limiting cardiovascular side effects were observed at a dose of 56 mg/m^2 given over 30 min; that at doses above 24 mg/m^2 infused over 30 min, plasma concentrations with proven activity in vitro would be reached; and, finally, that there were arguments in favor of a prolongatioin of the S9788 infusion, it was suggested that 4.5-h fractionated infusions of S9788 be used: after a loading dose of 48 mg/m^2 given i.v. over 30 min, a maintenance escalating S9788 dose of $\geq$48 mg/m^2 given over 4 h would be infused in combination with either doxorubicin or a vinca alkaloid.

Another alternative was to use a continuous 6-h infusion of S9788 with escalating doses. The initial cumulative dose would not exceed the MTD, 96 mg/m^2, in these phase I studies. The new phase I studies would need close, 24-h, continuous cardiovascular monitoring and a normal or near-normal metabolic (normal kalemia) and cardiovascular status at inclusion.

In conclusion, the dose-limiting toxicity of S9788 given as a 30-min i.v. infusion consisted of bradycardia and/or clinical symptoms suggesting a vasovagal impact such as faintness or dizziness. The MTD of S9788 was 96 mg/m^2, with side effects being observed in all three patients treated at this dose level. No enhancement of doxorubicin toxicity was observed with this combination. S9788 plasma levels of up to 3.7 μM could be obtained with this administration schedule. S9788 plasma pharmacokinetics were linear with doses of up to 96 mg/m^2, and there was no evidence of drug accumulation on repeated S9788 administration at 1- or 2-week intervals. No pharmacokinetic interaction was noted between S9788 and doxorubicin. These results allow further clinical studies to be conducted using conventional dosage of doxorubicin such as that used in this phase I study (50 mg/m^2 every 3 weeks), with the aim being to improve the administration schedule of this new MDR-reversal agent.

Acknowledgements Our colleague and friend Professor Michel Clavel died on 28 February 1993. This paper is dedicated to his memory. This study was supported by Servier Laboratories. We would like to thank all of the clinical staff of the Medical and Pharmacology Unit, Centre Léon Bérard, who participated in this study, particularly Ms. A. Talon, O. Pinero, and M.F. Guicherd.

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