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In vivo antitumor activity of S 16020-2, a new olivacine derivative

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Abstract The antitumor activity of S 16020-2, a new olivacine derivative, was investigated in vivo and compared with that of Adriamycin and elliptinium acetate in a panel of murine (P388 leukemia, M5076 sarcoma, Lewis lung carcinoma, and B16 melanoma) and human (NCI-H460 non-small-cell lung and MCF7 breast carcinomas) tumor models. S 16020-2 given i.v. was active against P388 leukemia implanted i.p., s.c., or intracerebrally. The therapeutic effect of an intermittent schedule (administration on days 1, 5, 9) was superior to that of single-dose treatment, allowing the i.v. administration of high total doses of S 16020-2 and resulting in the cure of 60% of mice in the i.p. P388 model. In this model, S 16020-2 was more active than elliptinium acetate and showed a better therapeutic index than Adriamycin: ≥ 8 versus 2. A good therapeutic effect of S 16020-2 was also observed in three P388 leukemia sublines displaying the classic multidrug-resistance phenotype, namely, P388/VCR, P388/VCR-20, and P388/MDRC.04, the latter being totally insensitive to vincristine and Adriamycin. However, S 16020-2 was not active against the P388/ADR leukemia, a model highly resistant to adriamycin in vivo. S 16020-2 was both more active than Adriamycin and curative in the M5076 sarcoma and Lewis lung carcinoma implanted s.c. In the B16 melanoma implanted i.p. or s.c., S 16020-2 was less active than Adriamycin. Against the NCI-H460 human tumor xenograft, S 16020-2 demonstrated activity superior to that of Adriamycin (T/C = 20% versus 43% on day 21). Against the MCF7 breast cancer xenograft, S 16020-2 was active, but less so than Adriamycin (T/C = 23% versus 9% on day 21), whereas elliptinium acetate was marginally active (T/C = 49% on day 24). The hematological toxicity of S 16020-2 given to B6D2F1 mice at pharmacological dose appeared to be less severe than that of Adriamycin, particularly in bone-marrow stem cells. These results demonstrate that S 16020-2 is a highly active antitumor drug in various experimental tumor models and is markedly more efficient than elliptinium acetate. Because of its pharmacological profile, which is globally different from that of Adriamycin, S 16020-2 is considered an interesting candidate for clinical trials.

Key words S 16020-2 · Multidrug resistance · Topoisomerase II · Xenografts

Abbreviations *TI* therapeutic index · *MDR* multidrug resistance · *MST* median survival time · *MTD* maximal tolerated dose · *P-gp* P-glycoprotein · *ADR* Adriamycin · *ELP* elliptinium acetate · *VCR* vincristine · *CFUs* colony-forming units · *LTS* long-term survivors · *SGD* specific growth delay

Introduction

S 16020-2, a new pyridocarbazole derivative, was selected on the basis of its cytotoxicity in vitro and its antitumor activity against P388 leukemia and colon 38 adenocarcinoma in vivo [8]. S 16020-2 has been shown to intercalate into DNA and to stabilize the cleavable complex formed by purified topoisomerase II and DNA [11]. The purpose of the present study was to determine the pharmacological activity of S 16020-2 in vivo against a panel of murine tumors, including P388 leukemia, B16 melanoma, Lewis lung carcinoma, and

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M5076 reticulosarcoma as well as two human tumor xenografts, MCF7 breast adenocarcinoma and NCI-H460 non-small-cell lung carcinoma.

Preliminary in vitro studies have also shown that S 16020-2 retains a high degree of cytotoxic activity against cell lines displaying the multidrug-resistance (MDR) phenotype associated with overexpression of P-glycoprotein (P-gp) [10]. To confirm in vivo these results, we investigated the therapeutic efficacy of S 16020-2 against four MDR P388 leukemia sublines, including three sublines selected for resistance to vincristine (P388/VCR and P388/VCR-20) and Adriamycin (P388/ADR) and a subline transformed with human MDR1 cDNA (P388/VMDRC.04) [16]. Since its structure is related to that of ellipticine derivatives, we compared the antitumor activity of S 16020-2 with that of elliptinium acetate (ELP), which has a modest activity in the treatment of advanced breast cancer [13], and Adriamycin (ADR), an anthracycline currently used for the treatment of malignant hematological diseases as well as for the treatment of solid tumors [7, 17]. These two reference compounds had been shown to interact with topoisomerase II [5, 18]. The preclinical evaluation of S 16020-2 was completed by a comparative study of the hematological toxicity of S 16020-2 and ADR to mice, with particular emphasis being placed on measurement of the kinetics of recovery of hematopoietic tissues after cytotoxic treatment.

Materials and methods

Drugs

S 16020-2, 9-hydroxy-5,6-dimethyl-1-[*N*-[2-(dimethylamino)ethyl]carbamoyl]-6H-pyrido[4,3-*b*]carbazoledichlorhydrate (Fig. 1), was synthesized in our institute as described elsewhere [8]. Reference compounds obtained from various suppliers included ADR (Adriblastine, Upjohn/Pharmacia, France), ELP (Celiptium, Pasteur Vaccins, France), cyclophosphamide (Endoxan, Sarget, France), VCR (Oncovin, Lilly, France), and carmustine (BCNU; Bicnu, Bristol, France). Drugs were first dissolved in sterile water (except for BCNU, which was dissolved in absolute ethanol) for preparation of stock solutions. The stock solutions were diluted in sterile water before administration to animals at 0.1 ml/10 g of body weight.

Mice and tumor models

Female B6D2F1 (C57B1/6 × DBA2) mice were used in murine tumor models. Nude female congenic athymic mice of Swiss and BALB/C strains homozygous for the nude gene (nu/nu) were used for NCI-H460 and MCF7 tumor xenografts, respectively. All mice were purchased from Iffa Credo (Lyon, France). They were aged 4–6 weeks and weighed 20–22 g at the start of the experiments. These were conducted in accordance with the protocols published by the National Cancer Institute (NCI) and European Organization for Research and Treatment of Cancer (EORTC) members [3, 6]. All tumors used for the experiments except P388/VCR-20 and P388/VMDRC.04 leukemias were provided by the Division of Cancer Treatment, Tumor Repository, NCI (Frederick, Md. USA). The resistant leukemia P388/VCR-20 was established in our laboratory by in vitro exposure of P388/VCR cells to 20 n*M* VCR [12], and P388/VMDRC.04 was a gift from Dr. W.N. Hait, Cancer Institute of New Jersey (USA).

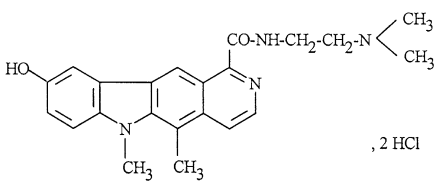

Fig. 1 Chemical structure of S 16020-2

For parental and drug-resistant P388 tumor models, mice were inoculated either i.p. or s.c. with 10^6 leukemic cells or intracerebrally (i.c.) with 5×10^5 leukemic cells. P388/VMDRC.04, a P388 cell line transformed with pHaMDR1/A, a retroviral vector containing cloned human MDR1 cDNA [16], was cultured in vitro and inoculated i.p. (10^6 leukemic cells) into B6D2F1 mice. For the B16 melanoma tumor model, 0.5 ml of a tumor brei, made by disrupting and homogenizing tumor fragments in sterile 0.9% NaCl was inoculated i.p. or s.c. into recipient mice. For M5076 reticulosarcoma models, 10^6 tumor cells were inoculated i.p. or s.c. For the Lewis lung-carcinoma tumor model, fragments of approximately 30 mg were grafted onto B6D2F1 mice. Depending on the tumor model, test compounds were given beginning on day 1, 2, or 3 after tumor implantation. The different treatment schedules and routes of administration are indicated in the legends to the tables.

The two human cell lines MCF7 and NCI-H460 were provided by the American Type Culture Collection (Rockville, Md., USA). MCF7 cells, isolated from a pleural effusion of a breast adenocarcinoma [14], were grafted onto mice previously implanted with slow-release pellets of 17β-estradiol (0.72 mg/pellet) purchased from Innovative Research (Rockville, Md., USA). The pellet was placed with the aid of a 14-gauge needle in the interscapular region at 1 day before fragment inoculation. NCI-H460 cells were isolated from pleural fluid of a non-small-cell lung carcinoma [2]. These tumors were maintained by serial bilateral transplantation of 2-mm^3 fragments into the flanks of female athymic BALB/C-nu/nu mice (for MCF7) and Swiss-nu/nu (for NCI-H460). For experiments with human tumor xenografts, five mice per group were implanted bilaterally and treatments were started when the tumor volumes reached 50 mm^3 (day 0). Mice were treated on days 0,7, and 14 with S 16020-2 (60 mg/kg, i.v.), ADR (10 mg/kg, i.v.), and ELP (3 mg/kg, i.v.)

Evaluation of antitumor activity

Life span

The median survival time (MST) of the treated group (T) was compared with that of the control group (C), and results were expressed as T/C:

$$\text{Median \% T/C (survival)} = \frac{\text{MST of treated group}}{\text{MST of control group}} \times 100.$$

Long-term survivors were registered on day 60 or 90 as indicated. The therapeutic index (TI) of a compound given following a definite schedule of treatment was defined as the ratio of the optimal dose (i.e., the most active nontoxic dose as determined on the basis of a body weight loss lower than 20% and the absence of early death) over the minimal active dose (the dose inducing a T/C value of 125%).

Tumor growth

The volume of each tumor was estimated from two-dimensional tumor measurements performed with a slide caliper following the

formula *length* (*mm*) × *width*^{2} (*mm*^{2}) /2. The median tumor volume of each treated group was compared with that of the control group and the results were expressed as T/C:

$$\text{Median \% T/C (tumor growth)} = \frac{\text{Median tumor volume of treated group}}{\text{Median tumor volume of control group}} \times 100.$$

At the end of each experiment, on day 20 or 90 as indicated, the surviving mice were palpated and the numbers of tumor-free animals were registered.

For human tumor xenografts, treatment started when the tumor reached a volume of 50 mm^3, and the tumor volumes were estimated by the formula *length* (*mm*) × *width*^{2} (*mm*^{2}) /2. The relative tumor volume was expressed as the V_t/V_0 index, where V_t is the tumor volume on a given day of measurement and V_0 is the volume of the same tumor at the start of the treatment. For the % T/C calculation the following formula was applied at each day of tumor measurement:

$$\text{Median \% T/C} = \frac{\text{Median } (V_t/V_0) \text{ treated}}{\text{Median } (V_t/V_0) \text{ control}} \times 100.$$

The specific growth delay (SGD) was calculated as follows:

$$\text{SGD} = \frac{T_d \text{ treated} - T_d \text{ control}}{T_d \text{ control}},$$

where T_d is the time needed for each control and treated tumor to double in volume. The homogeneity in the distribution of the different experimental groups with respect to the tumor volume before the beginning of the treatment was verified by statistical analysis (analysis of variance with one factor).

Hematological toxicity studies

The hematological toxicity of S 16020-2 and ADR was evaluated in B6D2F1 mice after administration of doses found to be optimal in the intermittent schedule for the P388 tumor model (i.e., the non-toxic dose in terms of mortality giving the best antitumor activity). At each indicated time, three mice from each treated (S 16020-2 or ADR) and control group were randomly selected. Peripheral blood samples were collected by retro-orbital bleeding, and femoral bone marrow was flushed by injection of 0.15 *M* NaCl. After red-blood-cell lysis, numeration of nucleated cells was performed with a Zm counter (Coultronics).

Colony-forming unit assay

The toxicity to bone-marrow stem cells of S 16020-2 and ADR given in a single injection at doses corresponding to about half the maximal tolerated dose (MTD) was evaluated in mice following the protocol of Till and McCulloch [15]. B6D2F1 mice were treated with either S 16020-2 (60 mg/kg) or ADR (12.5 mg/kg) by the i.v. route at 1,4,7, and 10 days before grafting of their bone marrow into recipient mice. Their femurs were excised and the bone marrow was flushed by injection of 1 ml of 0.15 *M* NaCl. The cells were counted and adjusted to a cellular density of 10^6 cells/ml; 0.2 ml of the bone marrow suspension was then injected via the tail vein into B6D2F1 mice that had been irradiated by X-rays (9.6 Gy, 115 kV, 13 mA) 24 h before. These animals were killed 7 days later and the numbers of colonies on their spleens were visually scored (colony-forming units, CFUs).

Statistical analysis

For human xenograft studies, data obtained from treated and control groups were analyzed by the Newman-Keuls test. For hematological toxicity studies, leukocyte and bone-marrow-cell numeration data were analyzed by the Newman-Keuls test and CFU-numeration data were analyzed by Student's *t*-test.

Results

P388 leukemia

Against i.p. P388 leukemia, S 16020-2 given i.p., i.v., or p.o. as a single dose on day 1 was found to be highly

Table 1 Antitumor activity of S 16020-2, ELP, and ADR against P388 leukemia models[a]

Tumor model and site of implantation	Experimental group	Schedule and route		Dose range (mg/kg)	Optimal dose (mg/kg)	Median T/C% at optimal dose	LTS at optimal dose[b]
P388 i.p.	S 16020-2	Day 1	i.p.	10–90	60	317	1/8
		Days 1, 5, 9	i.p.	10–30	30	265	0/6
		Day 1	i.v.	10–120	90	190	0/10
		Days 1, 5, 9	i.v.	10–80	60	> 536	6/10
		Day 1	p.o.	100–600	600	217	0/6
	ADR	Day 1	i.v.	2.5–30	20	258	2/10
		Days 1, 5, 9	i.v.	2.5–10	10	> 536	4/10
	ELP	Day 1	i.p.	5	5	171	0/10
		Days 1, 5, 9	i.p.	1–3	3	176	0/6
		Days 1, 5, 9	i.v.	2.5–7.5	7.5	112	0/6
P388 s.c.	S 16020-2	Days 2, 6, 10	i.v.	10–60	60	210	0/10
	ADR	Days 2, 6, 10	i.v.	2.5–10	10	174	0/10
P388 i.c.	S 16020-2	Days 1, 5, 9	i.v.	–	60	195	0/10
	ADR	Days 1, 5, 9	i.v.	–	10	144	1/10

[a] On day 0, 10^6 leukemic cells were inoculated i.p. or s.c. 5×10^5 cells were inoculated i.c. Drugs were given by the indicated schedule and route of administration

[b] Number of long-term survivors on day 60 over the number of mice per group

Table 2 Antitumor activity of S 16020-2 and ADR against i.p. P388 leukemia[a]

Experimental group	Schedule and route	Dose	Median survival time, days (mortality range)	Median T/C%	LTS day 60[b]
S 16020-2	Days 1, 5, 9 i.v.	10	18.4 (18–20)	165	0/10
		20	23.0 (21–25)	206	0/10
		40	30.0 (26–45)	269	2/10
		60	> 60 (33–45)	> 536	6/10
		80	> 60 (10–35)	> 536	6/10
ADR	Days 1, 5, 9 i.v.	2.5	13.3 (12–23)	119	0/10
		5	18.2 (16–27)	163	0/10
		10	37.0 (21–37)	332	4/10
		15	12.2 (12–14)	109	0/10
Control	–	–	11.2 (10–19)	100	0/30

[a] 10^6 leukemic cells were inoculated i.p. on day 0. Drugs were given i.v. on days 1, 5, and 9
[b] Number of long-term survivors on day 60 over the number of mice per group

active. The optimal doses, i.e., the doses inducing the best % T/C value with no sign of side effect, were 60 mg/kg for the i.p. route (T/C = 317%), 90 mg/kg for the i.v. route (T/C = 190%), and 600 mg/kg (T/C = 217%) for the p.o. route (Table 1). The therapeutic effect of S 16020-2 given i.v. was highly increased on an intermittent schedule (days 1, 5, and 9), this resulting, at 60 mg/kg, in the cure of 60% of the treated animals. ADR injected i.v. as a single dose on day 1 was highly active since a T/C value 258% was reached at 20 mg/kg with 20% long-term survivors (LTS). On the intermittent schedule the antitumor activity of ADR was enhanced since, at 10 mg/kg, 40% LTS were registered. On this schedule the T/C values obtained after treatment with increasing doses of S 16020-2 (10–80 mg/kg) and ADR (2.5–15 mg/kg) were used to calculate the corresponding therapeutic indices, ≥ 8 for S 16020-2 and 2 for ADR (Table 2). In the same i.p. P388 leukemia model, ELP proved to be active when given by the i.p. route (T/C = 171–176%) and totally inactive when injected by the i.v. route. In the s.c. implanted P388 leukemia model the survival time of tumor-bearing mice was increased following i.v. administration of S 16020-2 or ADR on days 2, 6, and 10, but no LTS were observed (Table 1). The highest T/C value of 210% was reached for S 16020-2 at the optimal dose of 60 mg/kg versus 174% for ADR at 10 mg/kg.

When the P388 leukemia cells were inoculated i.c., S 16020-2 given i.v. at 60 mg/kg on days 1, 5, and 9 induced a T/C value of 195%, suggesting that the drug partially crossed the blood-brain barrier. In this model, ADR was only moderately active at 10 mg/kg (T/C = 144%).

B16 melanoma

Against the i.p. grafted B16 melanoma, ADR was highly efficient when injected i.p. daily for a total of nine injections (days 1–9), 60% of the animals being cured at the dose of 2 mg/kg, whereas S 16020-2 (5 mg/kg) and ELP (0.6 mg/kg) were moderately active (Table 3). When the i.v. route was used, ADR given at 10 mg/kg following an intermittent schedule (days 2, 6, and 10) was active, although considerably less so than on the daily schedule, and induced a T/C value of 178% with no LTS. When S 16020-2 was injected i.v. at 60 mg/kg a T/C value of 132% was obtained. Against the s.c. implanted melanoma, S 16020-2 given i.v. at 60 mg/kg moderately inhibited tumor growth (T/C = 48%) and increased the survival of treated mice by 62%. ADR totally inhibited tumor growth and increased the survival of treated mice by 112% at 10 mg/kg.

M5076 sarcoma

When M5076 sarcoma cells were implanted i.p., S 16020-2 injected i.p. on days 1, 5, 9, and 13 demonstrated a marked activity at doses ranging from 5 to 20 mg/kg (Table 3). The highest T/C value was > 352% at 20 mg/kg with 60% LTS. ADR showed comparable activity at 2.5 mg/kg with a T/C value of > 352% and 50% LTS. ELP given at 2 mg/kg prolonged the life span of animals with a T/C value of 180% but was not curative. Against s.c. implanted M5076 tumors, S 16020-2 activity was again clearly dependent on the dose. At 60 mg /kg of S 16020-2 given i.v., 90% of treated mice were tumor-free on day 20. In this model, ADR demonstrated moderate activity at 10 mg/kg (T/C = 15%), with only 10% of the mice being tumor-free.

Lewis lung carcinoma

Against s.c. implanted Lewis lung carcinoma, S 16020-2 injected i.v. on days 3, 6, and 9 showed a strong therapeutic effect (Table 4). At doses of 20, 40, and 60 mg/kg, S 16020-2 inhibited tumor growth by 100%. More

Table 3 Antitumor activity of S 16020-2, ADR, and ELP against B16 melanoma and M5076 sarcoma[a]

Tumor model and site of implantation	Experimental group	Schedule and route		Dose range (mg/kg)	Optimal dose (mg/kg)	Median T/C% (survival) at optimal dose	Median T/C% (tumor growth) at optimal dose	LTS at optimal dose[b]
B16 i.p.	S 16020-2	Days 1–9	i.p.	2.5–10	5	139	–	1/10
		Days 2, 6, 10	i.v.	20–60	60	132	–	0/10
	ADR	Days 1–9	i.p.	1–3	2	> 404	–	6/10
		Days 2, 6, 10	i.v.	2.5–10	10	178	–	0/10
	ELP	Days 1–9	i.p.	–	0.6	134	–	1/10
B16 s.c.	S16020-2	Days 2, 6, 10	i.v.	30–60	60	162	48	0/10
	ADR	Days 2, 6, 10	i.v.	5–10	10	212	0	0/10
M5076 i.p.	S 16020-2	Days 1, 5, 9, 13	i.p.	5–40	20	> 352	–	6/10
	ADR	Days 1, 5, 9, 13	i.p.	2.5–10	2.5	> 352	–	5/10
	ELP	Days 1, 5, 9, 13	i.p.	1–2	2	180	–	0/10
M5076 s.c.	S 16020-2	Days 2, 6, 10	i.v.	20–60	60	–	0 (9/10)[c]	–
	ADR	Days 2, 6, 10	i.v.	5–10	10	–	15 (1/10)[c]	–

[a] On day 0, 0.5 ml of B16 tumor brei at 1 g/10 ml was inoculated i.p. or s.c., and 10^6 M5076 cells were inoculated i.p. or s.c. Drugs were given by the indicated schedule and route of administration
[b] Number of long-term survivors on day 90 over the number of mice per group
[c] Number of tumor-free animals over surviving animals scored on day 20

Table 4 Antitumor activity of S 16020-2 and ADR against s.c. Lewis lung carcinoma[a]

Experimental group[b]	Schedule and route	Dose (mg/kg)	Median T/C% on day 20 (tumor growth)	Median survival time, days (mortality range)	Median T/C% (survival)	Tumor-free animals/ LTS on day 90[c]
S 16020-2	Days 3, 6, 9 i.v.	20	1	> 90 (38–49)	> 295	6/6
		40	0	> 90	> 295	10/10
		60	0	> 90 (10–17)	> 295	6/6
ADR	Days 3, 6, 9 i.v.	2.5	63	33 (20–37)	108	0
		5	44	38 (9–45)	125	0
		10	1	39 (14–86)	128	2/2
Cyclophosphamide	Days 3, 6, 9 i.p.	60	0	> 90 (41–44)	> 295	8/8
Control	–	–	100	30.5 (11–47)	100	1/1

[a] Tumor fragments were implanted s.c. on day 0. Drugs were given on days 3, 6, and 9 by the indicated route of administration
[b] 10 mice were used for each treated group and 27 mice were used for the control group
[c] Number of tumor-free animals over surviving animals scored on day 90

importantly, a high number of LTS were scored at the three doses tested. At 40 mg/kg, 100% of the treated mice were considered cured since all 10 mice were alive on day 90 with no detectable tumor. Cyclophosphamide given i.p. at 60 mg/kg totally inhibited tumor growth and achieved a cure rate of 80%. Under the same conditions, ADR was only moderately active at 5 mg/kg and was active but toxic at 10 mg/kg. In another experiment, ELP injected i.v. or i.p. at doses ranging from 2.5 to 20 mg/kg was found to be inactive against Lewis lung carcinoma (T/C = 101% at 5 mg/kg for ELP given i.v.)

P388 resistant leukemias

Against the sensitive P388 leukemia, S 16020-2 injected i.v. on days 1, 5, and 9 increased the time of survival of tumor-bearing mice at doses ranging from 15 to 60 mg/kg and was also curative at a dose of 60 mg/kg with 63% LTS (Table 5).

S 16020-2 was curative against the weakly VCR-resistant P388/VCR subline (63% LTS at 30 mg/kg) and the MDR1-transfected P388/VMDRC.04 subline (25% LTS at 60 mg/kg), which is totally resistant to ADR (T/C = 123% at 5 mg/kg). Against the resistant P388/VCR-20 subline, S 16020-2 retained marked antitumor activity (T/C = 360% at 60 mg/kg) but was not curative at any dose tested. This tumor was clearly more resistant to VCR than was P388/VCR, since VCR injected i.p. at 2 mg/kg only slightly increased the survival time, yielding a T/C value of 155%. The P388/VCR-20 leukemia was also totally resistant to ADR given i.v., since the best T/C value was 117% at

Table 5 Antitumor activity of S 16020-2, VCR, and ADR against sensitive and multidrug-resistant i.p. P388 leukemia models[a]

Tumor model	Experimental group	Schedule and route		Dose range (mg/kg)	Optimal dose (mg/kg)	Median T/C% at optimal dose	LTS at optimal dose
P388	S 16020-2	Days 1, 5, 9	i.v.	15–60	60	> 594	5/8
	VCR	Days 1, 5, 9	i.p.	0.5–2	2	248	1/8
	ADR	Days 1, 5, 9	i.v.	2.5–10	10	281	0/8
	BCNU	Day 1	i.p.	–	30	> 594	7/8
P388/VCR	S 16020-2	Days 1, 5, 9	i.v.	15–60	30	> 488	5/8
	VCR	Days 1, 5, 9	i.p.	0.5–2	2	218	0/8
	ADR	Days 1, 5, 9	i.v.	2.5–10	10	259	1/8
	BCNU	Day 1	i.p.	–	30	278	3/8
P388/VCR-20	S 16020-2	Days 1, 5, 9	i.v.	15–60	60	360	0/8
	VCR	Days 1, 5, 9	i.p.	0.5–2	2	155	0/8
	ADR	Days 1, 5, 9	i.v.	2.5–10	2.5	117	0/8
	BCNU	Day 1	i.p.	–	30	> 652	6/8
P388/VMDRC.04	S 16020-2	Days 1, 5, 9	i.v.	15–60	60	197	2/8
	VCR	Day 1, 5, 9	i.p.	0.5–2	1	115	0/8
	ADR	Days 1, 5, 9	i.v.	2.5–10	5	123	0/8
	BCNU	Day 1	i.p.	–	30	> 382	8/8
P388/ADR	S 16020-2	Days 1, 5, 9	i.v.	20–60	60	102	0/6
	ADR	Days 1, 5, 9	i.v.	2.5–10	2.5	95	0/6
	Cyclophosphamide	Days 1, 5, 9	i.p.	–	60	> 561	3/6

[a]10^6 leukemic cells were inoculated i.p. on day 0. Drugs were given by the indicated schedule and route of administration
[b]Number of long-term survivors on day 60 over the number of mice per group

a dose of 2.5 mg/kg. S 16020-2 and ADR were found to be totally inactive in the P388/ADR leukemia model. As expected, the two non-MDR drugs cyclophosphamide and BCNU were highly active and curative against the MDR leukemias.

Human tumor xenografts

The antitumor activity of S 16020-2 was studied in two human tumor xenografts, a breast adenocarcinoma (MCF7) and a non-small-cell lung carcinoma (NCI-H460). Three i.v. injections of S 16020-2 at 60 mg/kg induced a significant inhibition of MCF7 tumor growth (Fig. 2), with the optimal T/C value being 23% on day 21 and the SGD value being 1.4 (Table 6). Significant reductions in tumor weight were observed between the control and the S 16020-2 treated group ($P < 0.05$ on day 10; $P < 0.01$ on day 28). The growth curve generated for the ADR-treated group (10 mg /kg) showed a similar profile (Fig. 2) with an optimal T/C value of 9% on day 21 and an SGD value superior to 2 (Table 6). However, this activity was associated with the occurrence of one death due to toxicity after the last treatment. In this model, ELP showed marginal activity (T/C = 49% on day 24).

S 16020-2 given at 60 and 90 mg/kg to NCI-H460 xenografted nude mice showed considerable antitumor activity (Fig. 3), producing significant reductions in tumor weight (T/C = 22% and 20%, respectively, on day 21; SGD = 1.2 and 1.3, respectively; see Table 6). Significant reductions in tumor weight were observed between the control and the S 16020-2-treated group ($P < 0.01$ on days 10–21). ADR given at 10 mg/kg showed marginal antitumor activity (T/C = 43% on day 21), and ELP was inactive (Table 6).

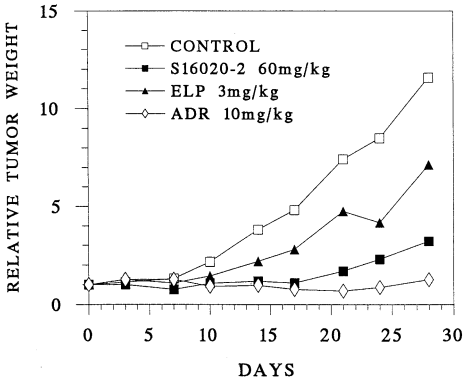

Fig. 2 Effect of S 16020-2 on MCF7 tumor growth. Treatment was started on day 0, i.e., 13 days after tumor implantation bilaterally into 5 mice/group. Mice were treated on days 0, 7, and 14 with S 16020-2 (60 mg/kg, i.v.), ADR (10 mg/kg, i.v.), and ELP (3 mg/kg, i.v.). Tumors were measured on the indicated days. The relative tumor volume was expressed as the V_t/V_0 index, where V_t is the tumor volume on a given day of measurement and V_0 is the volume of the same tumor at the start of the treatment

Table 6 Antitumor activity of S 16020-2 against human tumor models[a]

Tumor cell line	Histological type	Experimental group	Dose (mg/kg)	Deaths total number of mice (day)	Optimal T/C[b] (%)	SGD[c]
MCF7	Breast Adenocarcinoma	S 16020-2	60	0/5	23 (21)	1.4
		ELP	3	0/5	49 (24)	0.4
		ADR	10	1/5 (24)	9 (21)	> 2.0
NCI-H460	Lung carcinoma	S 16020-2	60	1/5 (15)	22 (21)	1.2
			90	0/5	20 (21)	1.3
		ELP	3	0/5	96 (21)	< 0
		ADR	10	0/5	43 (21)	0.8

[a] Mice were treated i.v. on days 0, 7, and 14 with S 16020-2, ELP, and ADR. The optimal %T/C is the lowest median T/C (%) obtained at least 7 days after the final treatment

[b] T/C values were determined twice a week after the end of treatment: T/C – (*median* V_t/V_0 *treated/median* V_t/V_0 *control*) $\times 100$

[c] (*Td treated* − *Td control*) /*Td control*, where *Td* represents the tumor-doubling time

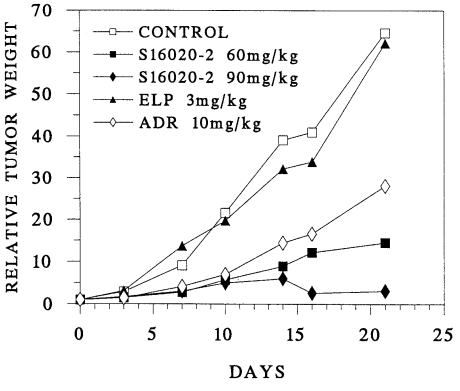

Fig. 3 Effect of S 16020-2 on NCI-H460 tumor growth. Treatment was started on day 0, i.e., 7 days after tumor implantation bilaterally into 5 mice/group. Mice were treated on days 0, 7, and 14 with S 16020-2 (60 mg/kg, 90 mg/h, i.v.), ADR (10 mg/kg, i.v.), and ELP (3 mg/kg, i.v.). Tumors were measured on the indicated days. The relative tumor volume was expressed as the V_t/V_0 index, where V_t is the tumor volume on a given day of measurement and V_0 is the volume of the same tumor at the start of the treatment

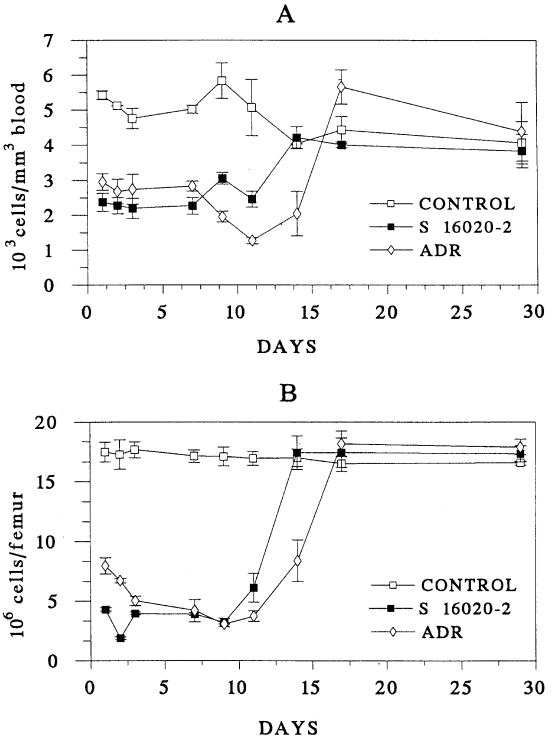

Fig. 4A, B Effect of S 16020-2 on **A** leukocyte counts and **B** bone-marrow cellularity changes. Mice were treated on days 0, 4, and 8 with S 16020-2 (60 mg/kg, i.v.) or ADR (10 mg/kg, i.v.). Each point corresponds to the mean value ± SEM for 3 leukocyte or nucleated marrow-cell counts

Hematological toxicity studies

The hematotoxicity of S 16020-2 injected i.v. at 60 mg/kg on days 0, 4, and 8 was compared with that of ADR given i.v. at 10 mg/kg on the same schedule. Changes in blood leukocyte and bone-marrow cell counts are presented in Fig. 4. After treatment with S 16020-2, a significant decrease in leukocyte (Fig. 4A) and bone marrow counts (Fig. 4B) was observed from day 1 to day 11, with a return to normal values occurring on day 14. After treatment with ADR, the decrease in these two parameters was more pronounced than that induced by S 16020-2, and the return to normal values was observed only on day 17.

The cytotoxicity of S 16020-2 and ADR to CFUs was compared using the protocol of Till and McCulloch at doses corresponding to about half the respective MTD. As can be seen in Fig. 5, the numbers of CFUs were significantly higher in animals treated with S 16020-2 at 60 mg/kg than in animals treated with ADR at 10 mg/kg at 1 day and 4 days after the treatment ($P < 0.001$). Moreover, normal CFU values were obtained at 4 days after treatment with S 16020-2 versus 10 days following ADR administration.

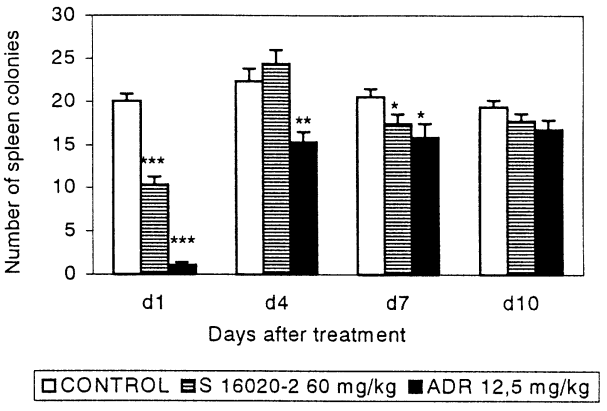

Fig. 5 Cytotoxicity of S 16020-2 for bone-marrow stem cells. The treatment of mice was performed at 1, 4, 7, or 10 days before grafting of their bone marrow cells. Data represent mean values ± SEM for 8 values. Student's *t*-test was used, and significant degrees of difference between control and treated values are represented as follows: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$

Discussion

S 16020-2 is a new, highly cytotoxic olivacine derivative that has been shown to intercalate into DNA and to stabilize the cleavable complex formed by purified topoisomerase II and DNA [11]. In this study, S 16020-2 demonstrated marked antitumor activity against a large panel of murine and human experimental tumor models. Against the i.p. P388 leukemia, S 16020-2 was active when given i.v., i.p., or p.o., suggesting good distribution properties. The bioavailability after oral absorption is probably low as indicated by a high optimal dose in comparison with the i.v. route. The higher potency of S 16020-2 injected i.p. in comparison with i.v. could be explained either by the observation that in the i.p. model, tumor cells are in contact with high drug concentrations or by first-pass metabolism, leading to more active species of the molecule. It is worthwhile to underline the high degree of antitumor activity observed after i.v. administration of S 16020-2, since such activity has not been observed for some ellipticine derivatives [1]. In the P388 leukemia model, S 16020-2 injected i.v. on an intermittent schedule showed higher activity than that observed for a single-treatment schedule and cured a high proportion of tumor-bearing mice. Although ADR was also curative in this model, S 16020-2 presented a better therapeutic index than ADR, ≥ 8 versus 2, respectively. The antitumor activity of S 16020-2 determined in the ascitic P388 leukemia model was also far superior to that of ELP, which was inactive when given by the i.v. route in this model.

The antitumor activity of S 16020-2 against four P388 leukemia sublines displaying the MDR phenotype associated with an overexpression of P-gp was examined. A good therapeutic effect of S 16020-2, superior to that of ADR, was observed in the P388/VCR and P388/VCR-20 leukemias, which were selected for resistance to VCR but are also resistant to ADR. S 16020-2 was also active against the MDR1-transfected P388 leukemia, P388/VMDRC.04, which is totally insensitive to VCR and ADR. It should be noted, however, that stronger antitumor activity for S 16020-2 was observed in the weakly VCR-resistant P388/VCR leukemia than in the other two, more resistant tumors, suggesting that S 16020-2 is partially recognized by P-gp. S 16020-2 was not active against the P388/ADR leukemia, which is highly resistant to ADR in vivo. The P388/ADR subline has been reported to have at least two mechanisms of resistance an overexpression of P-gp and a modification in topoisomerase II activity [4]. It is thus possible that the cross-resistance to S 16020-2 was in this case due to quantitative and/or qualitative modifications of the target enzyme topoisomerse II and/or to differences in the intracellular drug concentration due to P-gp expression. These results are in agreement with those obtained in vitro, which have shown that S 16020-2 is more cytotoxic to purely classic MDR tumor lines than to lines whose resistance has been induced by ADR treatment [10]. They also suggest a clinical potential of S 16020-2 against tumors displaying the MDR phenotype associated with P-gp overexpression.

S 16020-2 injected i.v. on an intermittent schedule was more active than ADR against the s.c. M5076 sarcoma and the s.c. Lewis lung carcinoma and was curative in the latter model. When given i.v. at 30 and 60 mg/kg, S 16020-2 totally inhibited tumor growth of Lewis lung carcinoma-bearing mice and cured 60% and 100% of the mice, respectively. Against the i.p. B16 melanoma, S 16020-2 was moderately active when injected i.p. on days 1–9 and cured 10% of the mice in comparison with ADR, which cured 60%. When injected i.v. on an intermittent schedule, S 16020-2 was moderately active, whereas ADR retained a more pronounced activity, albeit less marked than that observed in the i.p.-i.p. model.

The results obtained with the three solid tumor models of murine origin mentioned above suggest different pharmacological profiles for S 16020-2 and ADR. We thus compared the antitumor activity of S 16020-2 with that of ADR and ELP against a human breast cancer xenograft, MCF7. ADR is one of the most commonly used cytotoxic compounds in the treatment of primary breast cancer [9], and ELP is included in certain protocols for treatment of metastatic breast cancer [13]. S 16020-2 exhibited significant antitumor activity against the MCF7 xenograft, whereas ELP was only marginally active. The marked antitumor activity observed after treatment with ADR (10 mg/kg) was associated with marked toxicity. S 16020-2 was also more active than ADR against a non-small-cell lung carcinoma line (NCI-H460). Considering this result and the curative activity of S 16020-2 against Lewis lung

carcinoma, a more complete evaluation involving a large panel of lung-tumor xenografts is in progress in our laboratory.

Finally, from a toxicological point of view, myelosuppression appeared to be a potential limiting side effect of S 16020-2. However. when given at a pharmacological dose as a single injection, S 16020-2 induced a less severe cytotoxic effect than did ADR against bone-marrow stem cells along with a more rapid return to normal values. The toxicitity of S 16020-2 and ADR to circulating leukocytes and bone marrow cells was also compared using an intermittent schedule. In this case, again, S 16020-2 appeared to be less toxic than ADR since the return to normal values was more rapid for S 16020-2 than for ADR. However, this side effect must be considered as one of the potential limiting toxicities of S 16020-2.

Because of its pharmacological profile, especially its activity against human tumor xenografts and its potency in curing some very aggressive murine tumors, S 16020-2 is considered an interesting candidate for clinical trials.

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