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ORIGINAL ARTICLE

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Comparison of the pharmacological profile of an olivacine derivative and a potential prodrug

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Abstract *Background:* The new olivacine derivative S 16020-2 (NSC-659687) has entered clinical trials on the basis of a marked antitumor activity in experimental models. Amongst the analogues which were synthesized to improve both therapeutic index and antitumor activity, the most active ones were those esterified on the 9-OH group such as S 30972-1, the glutaric acid monoester derivative. *Purpose:* To compare the pharmacological profile of S 30972-1 and S 16020-2 in vitro and in vivo and to investigate whether S 30972-1 could act as a prodrug of S 16020-2. *Methods:* The two compounds were compared in vitro in terms of their activity in inhibiting cellular proliferation and perturbing the cell cycle and in vivo in terms of their antitumor activity in murine transplantable tumors and human orthotopic models. The plasma concentrations of S 16020-2 and S 30972-1 were determined in mice, in a comparative pharmacokinetic study after i.v. administration, using an HPLC assay. *Results:* Although tumor cell proliferation and accumulation of cells in the G_2 phase of the cell cycle were similarly affected by the two compounds after a continuous exposure (IC_{50} values of 30–50 n*M*), S 30972-1 was about tenfold less potent than S 16020-2 after short exposures. In vivo, S 30972-1 induced more long-term survivors than S 16020-2 among mice with Lewis lung carcinoma and sensitive or multidrug resistant P388 leukemias. The growth of Colon 38 carcinoma was slightly more inhibited by S 30972-1 than S 16020-2. In the more relevant human orthotopic models, using the optimal doses of each drug, 160 mg/kg S 30972-1 was significantly more active than 80 mg/kg S 16020-2 in the NCI-H460 lung carcinoma. The two compounds were significantly active in A549 lung carcinoma, moderately active in the NIH:OVCAR-3 ovary carcinoma and inactive in the NCI-H125 lung and DU145 prostate carcinomas. Pharmacokinetic study demonstrated that S 30972-1 is a prodrug of S 16020-2: the conversion was rapid and complete within 1 h of the administration of S 30972-1. *Conclusions:* The in vivo profile of these two compounds appeared very similar, although S 30972-1 exhibited globally a wider therapeutic index. The rapid conversion of S 30972-1 to S 16020-2 shows that S 30972-1 acts mainly as a prodrug of S 16020-2. This should be taken into account before considering S 30972-1 as a valuable back-up of S 16020-2.

Keywords Cytotoxic drugs · S 16020-2 · S 30972-1 · Topoisomerase II inhibitor · Orthotopic models

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Introduction

A number of anticancer drugs are known to poison the nuclear enzyme DNA topoisomerase II [29]. S 16020-2 (NSC-659687) is a 6*H*-pyrido(4,3-*b*)carbazole derivative characterized by a basic *N*-dimethylaminethylaminocarbonyl side chain grafted onto an olivacine chromophore [12]. S 16020-2 binds to DNA through intercalation and stimulates DNA cleavage catalyzed by purified DNA topoisomerase II [19, 28], which confers on S 16020-2 potent cytotoxic properties, even against tumor cells expressing high levels of P-glycoprotein [15, 16, 20, 26]. In vivo, S 16020-2 administered i.v. has demonstrated a

broad range of antitumor activity against a panel of murine transplantable tumors [6]. Further investigations using human tumors such as breast, lung and ovary carcinomas xenografted subcutaneously into nude mice have confirmed the antitumor properties of this compound [7, 17]. On the basis of its marked antitumor activity, favorable pharmacokinetic parameters and acceptable toxicity in different animal species, S 16020-2 has been subjected to phase I clinical trials [1, 2, 4, 5].

To increase the antitumor activity and to improve the therapeutic index of S 16020-2, more than 100 derivatives have been synthesized and evaluated in vitro in a panel of tumor cell lines and in vivo against P388 leukemia and B16 melanoma. Amongst them, the most active compounds are those resulting from esterification of the 9-OH group with various aliphatic diacids, as exemplified by S 30972-1 (Fig. 1) [8]. The main characteristic of these derivatives is their antitumor efficiency, which is at least equal to that of S 16020-2, and their low toxicity, since their optimal doses (bolus i.v. injection) are in the range 160–320 mg/kg. Consequently, their therapeutic index in these two screening models has been significantly improved [8]. Due to the presence of an ester bearing a charged COO^- group which could hinder uptake by cells, we hypothesized that S 30972-1 could act as a prodrug of S 16020-2.

The aim of this work was to further characterize the cytotoxic and antitumor properties of S 30972-1 in comparison with that of the parent molecule, S 16020-2, and to determine whether S 30972-1 could be converted into S 16020-2 in vivo in mice. In order to complete the pharmacological evaluation of S 30972-1, its antitumor activity was investigated in an extended panel of murine tumors. However, the relevance and predictability of murine tumor models have recently been questioned and their replacement by more sophisticated orthotopic human tumor xenografts has been proposed [10, 13, 14]. We have recently described the characterization of such orthotopic models, which exhibit metastatic potential and chemosensitivity patterns close to those observed in the clinic [3, 18], making them more suitable than classical murine models for the in-depth comparison of two analogues. Consequently, S 30972-1 and S 16020-2 were evaluated in a set of aggressive models of human lung (NCI-H460, NCI-H125, A549), ovary (NIH:OV-CAR-3) and prostate (DU145) cancers. To better mimic the clinical setting, the treatment was initiated when the disease was established and the two drugs were administered i.v. following an intermittent schedule, previously shown to be optimal for this class of compounds [6, 7, 17].

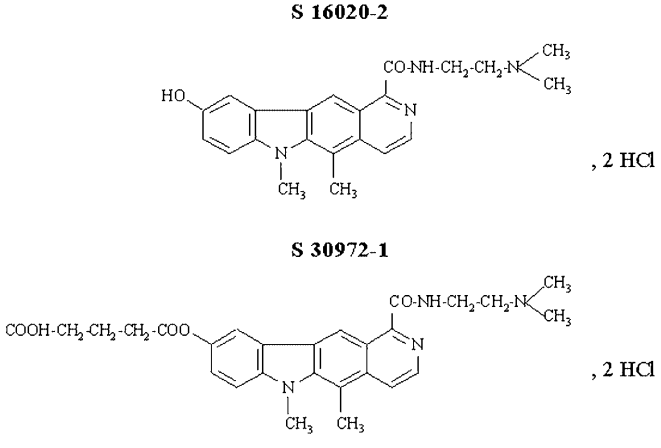

Fig. 1. Chemical structures of S 16020-2 and S 30972-1

Materials and methods

Drugs

S 30972-1 and S 16020-2 (Fig. 1) were synthesized in our institute as described elsewhere [8, 12]. Both compounds were solubilized at 10^{-2} *M* in dimethyl sulfoxide, aliquoted and stored at –20°C for the in vitro experiments. Reference compounds included doxorubicin (Adriblastine; Pharmacia & Upjohn, St Quentin-en-Yvelines, France), cyclophosphamide (Endoxan; Sarget, Merignac, France), vinorelbine (Navelbine; Pierre Fabre Oncology, Boulogne, France), 5-fluorouracil (Fluoro-uracile; Roche, Neuilly sur Seine, France), vincristine (VCR, Oncovin; Lilly, St Cloud, France) and paclitaxel (Sigma, France). For the in vivo experiments, compounds were solubilized and diluted in sterile water except paclitaxel which was prepared in ethanol and polyoxyethylated castor oil as previously described [3]. Compounds were prepared just before administration to animals at 0.1 ml/10 g body weight. The optimal dose of compound was defined as the most active nontoxic dose, i.e. body weight loss less than 20% and absence of early death.

Cell lines

All murine tumors were provided by the Division of Cancer Treatment, Tumor Repository, NCI (Frederick, Md.). The resistant leukemia P388/VCR-20 was established in our laboratory by in vitro exposure of P388/VCR cells to 20 n*M* VCR [26]. Human tumor cell lines were obtained from the American Type Culture Collection (Rockville, Md.). Cells were maintained in RPMI-1640 medium supplemented with 10% decomplemented fetal calf serum (FCS), 2 m*M* L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, and 10 m*M* Hepes, pH 7.4. Cells were grown at 37°C in an atmosphere comprising 5% CO_2/95% air. All media and supplements were from Life Technologies (Cergy-Pontoise, France) except FCS which was purchased from Sigma Chemical Company (St. Louis, Mo.).

Standard proliferation assay

The MTT assay has been previously described [20, 24]. Briefly, cells were incubated for four doubling times (continuous exposure) with the compounds. For kinetic experiments, A549 cells were incubated with the drugs for 1 to 48 h, then washed and incubated in drug-free medium for an overall period of 96 h. The IC_{50} (concentration reducing by 50% the optical density at 540 nm) was calculated by a linear regression performed on the linear zone of the dose-response curve. All the measurements were performed in triplicate.

Cell cycle analysis

L1210 cells in exponential phase of growth were exposed to the drugs for 21 h, then washed, fixed with 70% ethanol and incubated for 30 min in phosphate-buffered saline containing 100 µg/ml RNAse and 50 µg/ml propidium iodide (Sigma). For each sample, 10^4 cells were analyzed on an Epics XL/MCL flow cytometer (Beckman Coulter, France).

Mice and tumor models

Female B6D2F1 (C57Bl/6×DBA2) mice were used as murine models. Nude female congenic athymic BALB/C mice homozygous for the nude gene (nu/nu) were used in the human models except in the prostate adenocarcinoma for which male SCID mice (CB-17/Icr) homozygous for the scid gene (scid/scid) were used. All mice were purchased from Iffa Credo (Lyon, France). They weighed 20–22 g (6–8 weeks of age) at the start of the experiments. Mice received proper care and maintenance in accordance with institutional guidelines.

Murine tumor models were used as previously described [6]. For parental and drug-resistant P388 leukemias, mice were inoculated either i.p. or i.v. with 10^6 leukemic cells. For B16 melanoma, 0.5 ml of a tumor brei was inoculated i.p. into recipient mice. For Lewis lung carcinoma or Colon 38 adenocarcinoma, fragments of approximately 50 mg were grafted s.c. into B6D2F1 mice.

Human pulmonary tumor cell lines were cultured and grafted into immunodeficient mice as previously described [18]. Briefly, 10^6 A549, NCI-H460 or NCI-H125 cells in a volume of 100 µl were implanted through the chest wall into the left pleural space of anesthetized BALB/C nude mice. The NIH:OVCAR-3 ovarian tumor was adapted in vivo, maintained by serial passages and 10^7 cells were injected i.p. into BALB/C nude mice [3]. For the prostatic tumor model, 5×10^6 DU145 cells were transplanted i.p. into male SCID mice.

Evaluation of antitumor activity

Tumor growth. Colon 38 tumors were measured twice a week and tumor volumes (V_t) were calculated using the following formula: length (mm)×width^2 (mm^2)/2. The relative tumor volume (RTV) was expressed as the V_t/V_0 index, where V_t is the tumor volume on the day of measurement and V_0 is the volume of the same tumor at the start of the treatment. The results are expressed as median T/C where T/C (%) = median RTV of treated animals/median RTV of control animals ×100.

Survival. All experiments were approved by an internal ethical committee and in accordance with the guidelines approved by the UKCCCR for the welfare of animals in experimental neoplasia [31]. For each group, the results are expressed as median survival time (MST) in days and as percentage of median T/C where T/C = MST of treated group/MST of control group ×100. Cured animals were defined as long-term survivors (LTS) which, when killed at the end of the experiment, showed no tumor on macroscopic examination.

Statistical analysis

A comparison of the survival curves between each treated and control group was performed using a log-rank test, which took censored values into account. If the log-rank test showed a significant difference ($P \leq 0.05$), each treated group was compared with the control group using a log-rank test followed by a Holm's adjustment to control the overall risk at 5% [11].

Tumor weights in treated mice were compared with those of control animals using Student's *t*-test. If several doses of compound were tested, a one-way ANOVA was carried out, followed, in cases of significance of the overall analysis, by a Newman-Keuls test for pairwise comparisons. All significance thresholds were fixed at 5% [30].

Comparative pharmacokinetics in mice

Two parallel groups of male Swiss mice (30 animals per group) were given a single i.v. dose (as a 1-min infusion) of either S 30972-1 (39 mg base/kg or 80 µmol/kg) or S 16020-2 (30 mg base/kg or 80 µmol/kg) formulated in 5% glucose. A single terminal blood sample was collected from each animal at 1, 10 and 30 min, and at 1, 2, 4, 6, 8, 16 and 24 h after the start of infusion (three animals per time-point). Following S 30972-1 administration, samples were analyzed for both S 30972-1 and S 16020-2. Plasma was prepared by immediate centrifugation and the addition of 1% hydrochloric acid for compound stabilization. Samples were diluted fourfold and 50 µl directly injected onto an inline extraction column (Biotrap C18, 500 MS C18 20×4 mm), with backflushing after 3 min to a Waters Xterra RP18 (100×4.6 mm, 3.5 µm) column. The flow rate on injection was 0.3 ml/min, increasing to 1 ml/min over 3 min and maintained at 1 ml/min for the remainder. Gradient separation was achieved with mobile phase A comprising 0.05% trifluoroacetic acid (TFA) in water and mobile phase B comprising 0.05% TFA in acetonitrile. The total run time was 13.5 min. Compounds were detected by MS-MS (Quattro LC, Z-spray). The assay working range was 2 to 300 ng/ml for each of the compounds.

Results

Inhibition of cellular proliferation and perturbation of cell cycle

The antiproliferative activities of S 16020-2 and S 30972-1 were investigated in a panel of murine and human tumor cells continuously exposed to the drugs. As shown in Fig. 2, S 30972-1 was as potent as S 16020-2 except in KB-3-1 cells in which S 16020-2 was 2.3-fold more potent than S 30972-1. The mean IC_{50} values in this panel of cell lines were 30 and 46 n*M* for S 16020-2 and S 30972-1, respectively. S 16020-2 has previously been shown not to be recognized by P-glycoprotein which is often overexpressed in multidrug-resistant cell lines. To investigate the effect of P-glycoprotein overexpression on the antiproliferative properties of S 30972-1, the two compounds were tested in the resistant P388/VCR-20 cell line, a classical multidrug-resistant line [26]. As shown in Fig. 2, P388/VCR-20 cells were as sensitive as the parental P388 cells to the two compounds.

S 16020-2 has been shown by flow cytometry to induce a significant accumulation of cells in the G_2+M phases of the cell cycle [20]. L1210 cells treated with 100 n*M* S 16020-2 or S 30972-1 accumulated in the G_2+M phases (Fig. 3A). The percentages of G_2+M phase cells were measured after they had been exposed to a wide range of concentrations from 1 to 250 n*M*. As

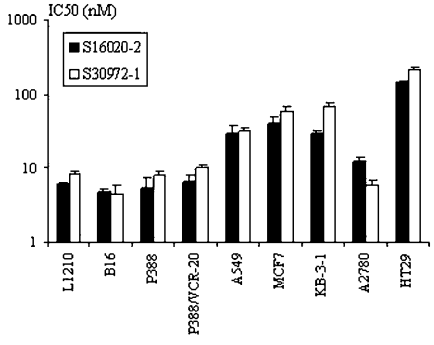

Fig. 2. Inhibition of cell proliferation (continuous exposure). Cells were incubated for four doubling times with various concentrations of S 16020-2 or S 30972-1, and cell viability was determined by the MTT assay. Results are expressed as $IC_{50} \pm$ SEM obtained from at least three independent experiments

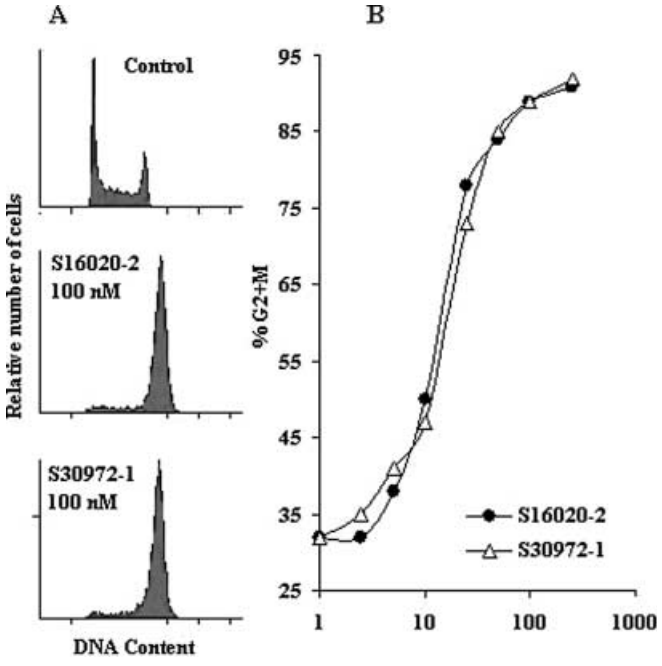

Fig. 3A, B. Effect on the cell cycle of L1210 cells. **A** Typical flow cytometric histograms of cells treated for 21 h with 100 n*M* S 16020-2 or S 30972-1. **B** G_2+M arrest of L1210 cells treated with increasing concentrations of S 16020-2 (*closed circles*), or S 30972-1 (*open triangles*)

shown in Fig. 3B, S 16020-2 and S 30972-1 induced a similar dose-dependent accumulation of cells in the G_2+M phases. These results demonstrate that, after a continuous exposure, S 16020-2 and S 30972-1 are equipotent and suggest that they share the same mechanism of action at the cellular level.

The inhibition of cellular proliferation and cell cycle perturbation after short exposures were then investigated. The A549 human lung cell line was chosen on the basis of its similar sensitivity to both compounds after a continuous exposure. Briefly exposed cells (< 10 h) were significantly less sensitive to S 30972-1 than to S 16020-2 (Fig. 4), the ratio of their IC_{50} values being 12 after 1 h of contact. This ratio then decreased as the time of exposure increased, to reach a value of 1 after 24 h (Fig. 4).

Antitumor activity in experimental models

Five murine and five human tumor models were used to compare the in vivo antitumor activities of S 30972-1 and S 16020-2. In all the following experiments, S 16020-2 and S 30972-1 were administered i.v. and compared with the best reference drug in each model except for P388 and P388/VCR, for which VCR was used to illustrate the resistance of P388/VCR-20. For each compound the optimal dose was defined as the dose inducing the highest antitumor activity without early toxic death and excessive weight loss (not greater than 20%).

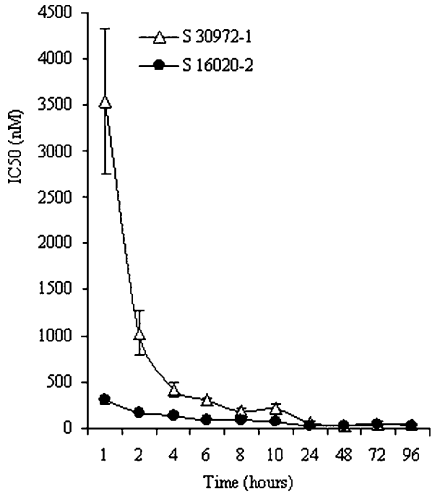

Fig. 4. Inhibition of cell proliferation (shorts exposures). A549 cells were incubated with S 16020-2 (*closed circles*), or S 30972-1 (*open triangles*) for 1 to 48 h, then washed and incubated in drug-free medium for an overall period of 96 h. The results are expressed as $IC_{50} \pm SEM$ obtained from at least three independent experiments

Murine tumor models

The antitumor activity of the compounds in P388 leukemia models is illustrated in Fig. 5. Against i.p. P388 leukemia, S 30972-1 administered i.v. on day 1 was highly active from 40 mg/kg (T/C 160%) to 320 mg/kg, inducing LTS at 160 and 320 mg/kg (Fig. 5A). In this schedule, 160 mg/kg S 16020-2 was lethal and the highest antitumor activity was observed at the optimal dose of 80 mg/kg, with a T/C of 214% and no LTS. Administered following an intermittent schedule (i.e. days 1, 5 and 9), S 30972-1 induced 67% LTS at 80 and 160 mg/kg, while S 16020-2 was curative only at 80 mg/kg (Fig. 5B) and toxic at 160 mg/kg. Taken together, these results show that S 30972-1 was better tolerated

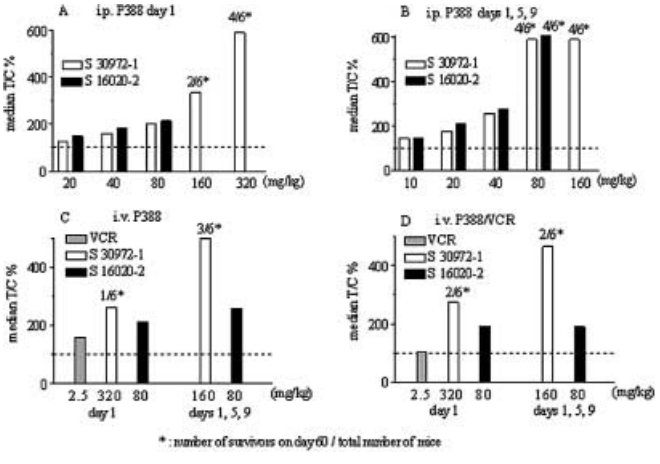

Fig. 5A–D. Antitumor activity of S 30972-1 against sensitive (**A**, **B**, **C**) and resistant (**D**) P388 leukemia. On day 0, 10^6 leukemic cells were inoculated i.p. (**A**, **B**) or i.v. (**C**, **D**) into B6D2F1 mice. S 30972-1 and S 16020-2 were given i.v. by the indicated schedules

and more active than S 16020-2 against i.p. P388 leukemia.

To better model a hematological disease, the sensitive P388 leukemia and the resistant P388/VCR-20 subline exhibiting the classical multidrug resistance phenotype were implanted by the i.v. route. S 30972-1 administered either in a single dose or in an intermittent schedule was as efficient against the sensitive (Fig. 5C) as the resistant (Fig. 5D) leukemia inducing LTS in the two models. The best results were obtained after three administrations of 160 mg/kg S 30972-1 which induced 50% and 33% LTS in the P388 and P388/VCR-20 models, respectively. S 16020-2 was significantly less active than S 30972-1 in these two models since no LTS were registered.

Against the i.p.-grafted B16 melanoma, S 30972-1 and S 16020-2 were moderately active at their optimal doses, increasing the life-span of treated mice with maximum T/C values of 160% and 141%, respectively (Table 1). Doxorubicin was more active than the two compounds, with a T/C value of 220%.

In the s.c. Lewis lung carcinoma model, 160 mg/kg S 30972-1 was toxic, the optimal dose being 80 mg/kg. At this dose, S 30972-1 was curative, the seven mice of the group being tumor-free at the end of the experiment. Under the same experimental conditions, S 16020-2 and cyclophosphamide were slightly less active than S 30972-1 since they induced three and four tumor-free survivors, respectively (Table 1).

In the model of established Colon 38 carcinoma, S 30972-1 administered at 160 mg/kg and S 16020-2 at 80 mg/kg were highly active, inhibiting tumor growth 7 days after the last treatment by 98% and 93%, respectively (Table 1) while 5-FU demonstrated less activity. Tumor growth curves for animals treated with S 30972-1 (160 mg/kg) and S 16020-2 (80 mg/kg) were significantly different from those of the control group from day 21 to day 45 ($P \leq 0.001$) and the inhibition of tumor growth induced by S 30972-1 was dose-dependent (Fig. 6).

Human orthotopic tumor models

Three human non-small-cell lung carcinomas (NCI-H460, A549 and NCI-H125) were implanted into the pleural cavity of nude mice to obtain metastatic models of lung cancer [18]. Vinorelbine, used as a reference compound, was administered i.v. at its optimal dose, 10 mg/kg. Treatments began when the disease was well established, 7 days (NCI-H460) or 14 days (A549 and NCI-H125) after tumor cell implantation. Treatment of mice bearing the NCI-H460 tumor with 160 mg/kg S 30972-1 induced an increase in life-span of 106% versus 65% for 80 mg/kg S 16020-2 (Table 2, Fig. 7). Moreover the difference in the survival curves of animals treated at 80 mg/kg S 16020-2 versus 160 mg/kg S 30972-1 was statistically significant ($P = 0.001$). In this model, vinorelbine was less active than S 30972-1 inducing a T/C of 179%. Against the A549 tumor, 160 mg/kg S 30972-1 and 80 mg/kg S 16020-2 showed a strong therapeutic effect, doubling the life-span of treated mice (Table 2, Fig. 7).

In this model, the higher optimal dose of S 30972-1 did not translate into a better antitumor effect. Vinorelbine was less active than the two compounds. Against the NCI-H125 squamous cell carcinoma, S 30972-1 and S 16020-2 were inactive, in contrast to vinorelbine which was highly active, increasing the life-span of treated mice with a T/C of 250%. The activity of S 30972-1 was also evaluated in an orthotopic model of human ovarian adenocarcinoma. The i.p. injection of NIH:OVCAR3 cells resulted in a progressive peritoneal carcinomatosis and treatments started 7 days after tumor cell injection, corresponding to an established disease as shown by histological analysis [3]. Paclitaxel was administered i.v. at 40 mg/kg once every 10 days for three cycles and induced 100% LTS (Table 2). S 30972-1 administered at 160 mg/kg and S 16020-2 at 80 mg/kg, each induced a significant but moderate increase in life-span (T/C 135% and 149%, respectively), without however inducing LTS.

Table 1. Antitumor activity of S 30972-1 and S 16020-2 administered i.v. against murine models

Tumor model	Compound	Schedule	Dose range (mg/kg)	Optimal dose (mg/kg)[a]	Median T/C (%)	Tumor-free animals[b]
B16 melanoma	S 30972-2	Days 2, 6, 10	10–160	160	160[c]	0
	S 16020-2	–	10–80	80	141[c]	0
	Doxorubicin	–	8	8	220[c]	0
Lewis lung carcinoma	S 30972-1	Days 3, 7, 11	20–160	80	> 316[c] 0[d]	7/7
	S 16020-2	–	20–80	80	> 316[c] 0[d]	3/5
	Cyclophosphamide	–	90	90	> 316[c] 0[d]	4/4
Colon 38 carcinoma	S 30972-1	Days 10, 17, 24	20–160	160	2[d]	2/3
	S 16020-2	–	20–160	80	7[d]	1/4
	5-Fluorouracil	–	80	80	22[d]	0/5

[a]Dose inducing the highest antitumor effect without any toxic death or weight loss greater than 20%
[b]Number of tumor-free animals over surviving animals scored on day 90 for B16 and Lewis lung carcinoma models, and on day 60 for Colon 38 carcinoma
[c]Value of the median T/C (survival time)
[d]Value of the median T/C (tumor growth) measured on day 21 for Lewis lung carcinoma and on day 31 for Colon 38 carcinoma

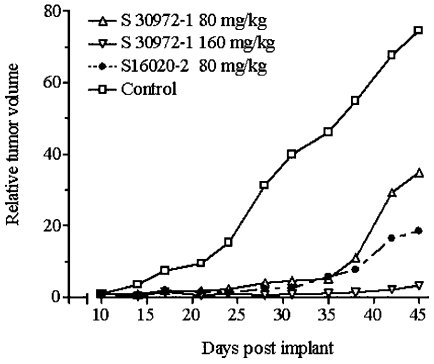

Fig. 6. Antitumor activity of S 30972-1 against murine C38 adenocarcinoma. S 30972-1 and S 16020-2 were administered i.v. at the indicated doses on days 10, 17 and 24 after s.c. grafting of tumor fragments into B6D2F1 mice. The relative tumor volume is expressed as the V_t/V_0 index, where V_t is the tumor volume on a given day of measurement and V_0 is the volume of the same tumor at the start of the treatment

We also evaluated the two compounds against the prostatic carcinoma DU145. The i.p. transplantation of DU145 cells into SCID mice resulted in progressive disease which invaded the peritoneum, the pelvic organs such as seminal vesicle and prostate, and the diaphragm, and led to the death of all the animals within 6 to 9 weeks. Due to the high sensitivity of SCID mice to DNA-interacting drugs, the optimal dose of S 30972-1 and S 16020-2 was 40 mg/kg, a dose which was devoid of antitumor activity.

Comparative pharmacokinetics in mice

Significant levels of S 16020-2 were observed after administration of S 30972-1 (Fig. 8B). Apart from the 1-min time-point (end of infusion), at which mean plasma concentrations of S 16020-2 reached 49.7 μg/ml after S 30972-1 infusion compared to 6.04 μg/ml after administration of S 16020-2 itself, the plasma concentration-time profiles were virtually superimposable from the 10-min time-point onwards. S 30972-1 was no longer measurable 2 h after administration, showing that its conversion to S 16020-2 was rapid and complete (Fig. 8A).

Discussion

S 30972-1, the glutaric ester of S 16020-2, was initially selected for further evaluation on the basis of its wide therapeutic index in P388 leukemia and B16 melanoma. The aim of this work was to compare, in vitro and in vivo, the pharmacological profile of the two compounds. One key issue was whether S 30972-1 is active per se, or acts as a prodrug of S 16020-2, generated by the cleavage of the ester bond by non-specific esterases. This is a very important question for the clinical improvement potentially offered by S 30972-1, considering the dose-limiting toxicity encountered in clinical trials with S 16020-2 (see below).

Table 2. Antitumor activity of S 30972-1 and S 16020-2 administered i.v. against human orthotopic models (*MST* median survival time, *NS* not significant)

Tumor model	Histological type	Treatment	Dose (mg/kg)	Schedule	MST (range of mortality) days	T/C (%)	*P* value[a]
NCI-H460	Large-cell lung carcinoma	S 30972-1	80	Days 7, 14	38.0 (31–41)	163	0.001
		S 30972-1	160	–	48.0 (41–57)	206	0.001
		S 16020-2	80	–	38.3 (35–40)	165	0.001
		Vinorelbine	10	–	41.8 (35–80)	179	0.001
		Control	–	–	23.3 (20–29)	100	–
A 549	Non-small cell lung carcinoma	S 30972-1	80	Days 14, 21, 28	70.0 (51–93)	175	0.001
		S 30972-1	160	–	85.0 (72–150)	213	0.001
		S 16020-2	80	–	79.0 (71–87)	198	0.001
		Vinorelbine	10	–	69.0 (64–156)	172	0.001
		Control	–	–	40.0 (27–46)	100	–
NCI-H125	Squamous cell carcinoma	S 30972-1	80	Days 14, 21, 28	54.0 (28–75)	108	NS
		S 30972-1	160	–	57.0 (28–91)	114	NS
		S 16020-2	80	–	58.0 (50–72)	116	NS
		Vinorelbine	10	–	125.0 (77–170)	250	0.001
		Control	–	–	50 (41–63)	100	–
NIH:OVCAR-3	Ovarian adenocarcinoma	S 30972-1	80	Days 7, 17, 27	70.5 (59–76)	123	0.05
		S 30972-1	160	–	77.5 (73–99)	135	0.01
		S 16020-2	80	–	85.5 (70–133)	149	0.01
		paclitaxel	40	–	–	>336[b]	0.001
		Control	–	–	57.5 (42–68)	100	–
DU 145	Prostatic carcinoma	S 30972-1	40	Days 14, 24, 35	55.0 (51–60)	117	NS
		S 30972-1	80	–	45.0 (22–64)	96	NS
		S 16020-2	40	–	55.0 (52–72)	117	NS
		Control	–	–	47.0 (43–62)	100	–

[a]Survival curve of each treated group was compared with that of the control group as indicated in Material and methods
[b]100% of the animals were considered as cured at the end of the experiment on day 180 since the macroscopic examination showed no evidence of tumor

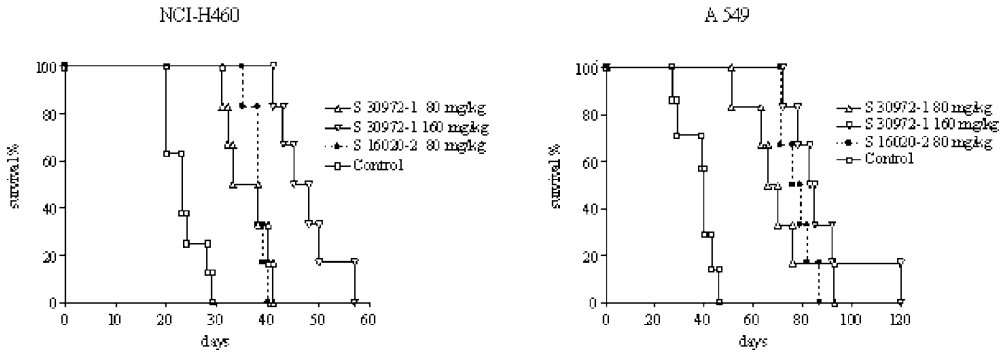

Fig. 7. Antitumor activity of S 30972-1 against the NCI-H460 and A549 human non-small-cell lung carcinomas. S 30972-1 and S 16020-2 were administered i.v. at the indicated doses on days 7 and 14 (NCI-H460) and on days 14, 21 and 28 (A549) after the implantation of 10^6 tumor cells into the pleural space of Balb/c nude mice, respectively

S 16020-2 is a potent poison of DNA topoisomerase II [19]. Its glutaric ester, S 30972-1, is markedly less potent as shown by the 20-fold reduction in potency found in a sensitive assay of cleavable complex formation using ^{32}P-labeled DNA [22]. These assays were performed in purified systems, at a physiological pH and for very short times of incubation (< 1 h), experimental conditions under which S 30972-1 proved to be chemically stable, i.e. not degraded into S 16020-2. Hence, it can be concluded that the efficiency of S 30972-1 in interacting with DNA and DNA-topoisomerase II in the ternary complex is markedly lower than that of S 16020-2. This result is in agreement with the known importance in the olivacine and ellipticine series of the free 9-hydroxy group, which has been shown to increase the affinity for DNA, the stabilization of the DNA-topoisomerase II cleavable complex and consequently cytotoxicity [21, 25].

The two molecules, when added to cells in culture for long exposure times, were equipotent in inhibiting the proliferation of various murine and human cell lines. Under the same conditions, S 30972-1 and S 16020-2 induced a similar accumulation of cells in the G_2 phase of the cell cycle, which is a consequence of the poisoning of topoisomerase II. In contrast, cells briefly exposed to the compounds were less affected by S 30972-1 than by S 16020-2. It can be assumed that, due to its charged side chain, S 30972-1 is less easily taken up by cells and interacts with topoisomerase II less efficiently than S 16020-2. Consequently, relatively long exposure times are necessary to quantitatively release S 16020-2 from S 30972-1 by the esterases. Taken together, these in vitro findings suggest that S 30972-1 acts mainly as a prodrug of S 16020-2. When given i.v. to mice, S 30972-1 plasma concentrations were only measurable for 1 h. This rapid disappearance kinetic was associated with immediate formation of S 16020-2, as reflected by plasma concentrations reaching a maximum at the end of the S 30972-1 infusion. This provides positive evidence that S 30972-1 is a prodrug of S 16020-2. Concentration-time profiles of S 16020-2 were similar after i.v. administration of either compound, demonstrating that the conversion of S 30972-1 into S 16020-2 was complete and rapid.

The pharmacological profile of the two compounds was investigated in a panel of experimental models, including classical murine transplantable tumors and orthotopic models of human solid tumors [3, 18]. Impressive results were obtained against the i.p. P388 leukemia, S 30972-1 inducing significantly more LTS than S 16020-2. This outstanding antitumor activity, which was maintained in an i.v.-grafted P388 subline displaying the multidrug-resistant phenotype, is due to the very high dosage of S 30972-1 which can be administered without signs of major toxicity. Against the B16 melanoma and Colon 38 adenocarcinoma, the optimal dose of S 30972-1 was twice that of S 16020-2, which translated into a moderate increase in the antitumor response. In the Lewis lung carcinoma model, S 30972-1 cured 100% of the animals against 43% cured by S 16020-2.

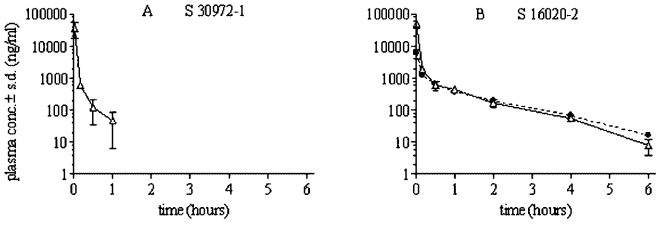

Fig. 8. **A** Mean ± SD plasma concentration-time profiles of S 30972-1 after i.v. infusion of 39 mg/kg S 30972-1 (*open triangles*). **B** Mean ± SD plasma concentration-time profiles of S 16020-2 in mice after i.v. infusion of 39 mg/kg S 30972-1 (*open triangles*) or 30 mg/kg S 16020-1 (*closed circles*). For clarity, the graphs are limited to the first 6 h

The organ-specific environment is an important factor for the growth and progression of tumors in vivo [23]. To closely mimic the human pathology, orthotopic models of human lung, ovary and prostate cancers were used. These models can be considered more appropriate when compared with subcutaneous xenografts because they exhibit several important characteristics of metastatic disease and chemosensitivity patterns close to those observed in the clinic [3, 14, 18]. Consequently, they are more adapted than the murine models to discriminate two structurally related clinical candidates. As previously observed, in murine models, the optimal dose of S 30972-1 was twice that of S 16020-2. This was associated with a moderate increase in antitumor activity and consequently with a widening of the therapeutic index against the NCI-H460 and A549 lung carcinomas. In the NIH:OVCAR-3 model, S 30972-1 did not present a clear advantage over S 16020-2. The two orthotopic models which are resistant to S 16020-2, the squamous-

cell lung carcinoma NCI-H125 and the prostatic carcinoma DU145, were also resistant to S 30972-1, even though the optimal dose of the latter was twofold higher. Interestingly, NCI-H460 and A549 cells harbor the K-ras mutation [9, 16, 27], which previously has been shown to be an important determinant for the sensitivity of the NCI cell line panel to S 16020-2 [15]. In contrast, the resistant NCI-H125 and DU145 express the wild-type ras allele [9, 27].

Taken together, these results demonstrate that the in vivo profiles of the two compounds are similar. These results also indicate that S 30972-1 would at best double the therapeutic index of S 16020-2, and moderately increase its antitumor activity.

These results are somewhat different from those of Malonne et al. [22], who found that S 30972-1 is much more effective in vivo than S 16020-2. In fact, a comparison of their results with ours is difficult because they used the i.p. route of administration and a repeated schedule, which could have underestimated the antitumor activity of S 16020-2. The i.v. route of administration was chosen for two reasons: (1) this is the route used in the clinic and (2) S 16020-2 had been previously shown to be toxic when administered by the i.p. route, especially following a repeated schedule. For example, with the days 1, 5 and 9 schedule, the optimal dose of S 16020-2 was 30 mg/kg i.p. versus 80 mg/kg i.v. (not shown). In addition, they used only murine tumors, which are not representative of the clinical pattern of the different tumor types [14]. Moreover, this study shows that murine models appeared globally more sensitive to S 16020-2 and S 30972-1 than did human solid tumors.

From a clinical point of view, what could be the advantage of S 30972-1 over S 16020-2? Phase I studies of S 16020-2 have shown that the drug is better tolerated when administered following an every-3-week schedule, the maximum tolerated dose being 100 mg/m^2 [1, 2, 4, 5]. Despite the observation of partial responses and symptom improvement during phase I, and subsequent phase II, the main limiting side effects of this compound are a severe cutaneous erythematous rash and acne which preclude dose escalation. This cutaneous toxicity has not been observed in any of the animal species studied so far and its mechanism is currently under study. Although hematological toxicity was predicted in animal toxicological studies, its absence in patients suggested that fully active doses were not attained. No significant renal or hepatic toxicities were observed. Clearly, to be considered as a valuable back-up to S 16020-2, S 30972-1 must be devoid of cutaneous toxicity. The fact that S 30972-1 rapidly generates S 16020-2 is perhaps not encouraging in this respect.

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