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Original Paper

Experimental Antitumour Activity of S 16020-2 in a Panel of Human Tumours

L. Kraus-Berthier, N. Guilbaud, M. Jan, D. Saint-Dizier, M.H. Rouillon, M.F. Burbridge, A. Pierré and G. Atassi

Institut de Recherches Servier, Division de Cancérologie expérimentale, 14 rue de la République, 92150 Suresnes, France

The antitumour activity of S 16020-2, a new topoisomerase II inhibitor, was evaluated in comparison with doxorubicin against 13 human tumours, including colon (HT-29, Colo320DM), breast (MCF7, MDAMB-231), ovary (SK-OV-3, A2780, NIH:OVCAR-3), non-small cell lung (NCI-H460, A549, Calu-6, NCI-H125) and small-cell lung (NCI-H69, SCLC6) cancers. S 16020-2 was administered weekly intravenous within a dose range of 20–90 mg/kg for 3 weeks. Antitumour responses were obtained in all the tumour types tested except in the two colon cancers. S 16020-2 produced significant growth delays in nine tumour models and induced regressions of all A549 lung tumours. The antitumour activity of S 16020-2 was superior to that of doxorubicin against the NCI-H460, A549, NCI-H69, SCLC6 and NIH:OVCAR-3 xenografts. These results demonstrate the broad spectrum of antitumour activity of S 16020-2 in a large panel of *in vivo* experimental models and confirm its interest as a potential agent in the treatment of malignant disease. © **1997 Elsevier Science Ltd.**

Key words: S 16020-2, human tumours, Pgp, xenografts, topoisomerase II
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INTRODUCTION

TOPOISOMERASE II is a critical intracellular target for a number of antitumour drugs such as doxorubicin [1], ellipticine derivatives [2] and etoposide [3]. Their cytotoxic activity is mainly due to the stabilisation of the covalent DNA-topoisomerase II reaction intermediate, the cleavable complex [4].

S 16020-2 is a new highly cytotoxic olivacine derivative [5, 6] which probably shares the same molecular mechanism since it intercalates into DNA and stabilises the topoisomerase II–DNA complex [7].

In vivo, S 16020-2 has demonstrated a broad range of antitumour activity against a panel of murine tumour models, particularly against the Lewis lung carcinoma and resistant sublines of P388 leukaemia displaying the multidrug resistance phenotype. In these models, S 16020-2 was more active than doxorubicin and elliptinium acetate [8, 9]. The antitumour activity of S 16020-2 was observed after intravenous (i.v) administration, whilst ellipticine derivatives were generally less active by this route [10].

Correspondence to G. Atassi.
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We decided to complete this pharmacological evaluation in accordance with the New Drug Development programme recommended by the NCI and EORTC and based on the disease-oriented strategies for drug screening [11, 12]. For this purpose, S 16020-2 was evaluated in a panel of human solid tumours xenografted into nude mice including various histological types of lung, colon, breast and ovary cancers.

The activity of S 16020-2, administered i.v., was compared to that of doxorubicin, a drug currently included in protocols of chemotherapy and active in the treatment of solid human tumours such as breast [13] and small-cell lung cancers [14]. In the case of the ovarian SK-OV-3 tumour, the activity of S 16020-2 was compared to that of cisplatin, a highly effective drug in the treatment of ovarian cancer [15].

MATERIALS AND METHODS

Drugs

S 16020-2 was synthesised in our institute as described [5]. S 16020-2 and reference compounds, doxorubicin (Adriblastine®, Upjohn/Pharmacia, France) and cisplatin (Cisplatine®, Lilly, France), were diluted in sterile water before administration to animals at 0.1 ml/10 g body weight.

Mice and tumour models

Nude female congenic athymic mice purchased from Iffa Credo (Lyon, France) of Swiss strain homozygous for the nude gene (nu/nu) were used for HT29, Colo320DM, NCI-H460, A549, Calu-6, NCI-H125, SCLC6, NCI-H69, A2780 and SK-OV-3 xenograft models. For the breast tumour xenografts MDA-MB-231, MCF7 and for the ovary carcinoma NIH:OVCAR-3, homozygous inbred BALB/C by-J-nu/nu mice were used. All mice weighed 20–22 g (4–6 weeks old) at the start of experiments.

All cell lines used were provided by the American Type Culture Collection (Rockville, Maryland, U.S.A.) except SCLC6 which was isolated from an untreated patient [16] and was a gift of Dr M. F. Poupon (Institut Curie, Paris). The cells were cultured *in vitro* and 10^7 cells were grafted s.c. into nude mice. Tumours were maintained by serial transplantation of 2–3 mm^3 fragments into the flanks of nude mice and used for chemotherapy after 3–10 *in vivo* passages. For MCF7 xenografts, mice were supplemented with 17β-oestradiol in the form of slow-release pellets (0.72 mg/pellet) purchased from Innovative Research (Rockville, Maryland, U.S.A.). The pellet was placed with the aid of a 14-gauge needle in the interscapular region one day before fragment inoculation.

Evaluation of the therapeutic response

Characteristics of each tumour are listed in Table 1. For each experiment, nude mice were grafted subcutaneously (s.c.) bilaterally and when tumours had reached a mean volume of approximately 50–100 mm^3, mice were distributed among experimental and control groups (day 0). S 16020-2 was administered i.v. within a dose range of 20–90 mg/kg per injection using a weekly schedule for 3 weeks (days 0, 7 and 14) unless otherwise indicated. The optimal dose was considered as that inducing the best response whilst leading to no more than 20% body weight loss (approximately 4 g per mouse). Animal deaths occurring within the treatment period and the following 2 weeks were considered as due to toxicity and these mice were excluded from tumour evaluation.

The volume of each tumour was estimated from two-dimensional measurements performed with a slide caliper following the formula: length (mm) × width^2 (mm^2)/2. The relative tumour volume was expressed as the V_t/V_0 index, where V_t is the tumour volume on a given day of measurement and V_0 is the volume of the same tumour at the start of the treatment. Growth curves expressing the median relative tumour volume in control and treated groups as a function of time were plotted.

For the % *T/C* calculation, the following formula was applied at each day of tumour 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: $SGD = \frac{T_d \text{ treated} - T_d \text{ control}}{T_d \text{ control}}$ where T_d is the time required for the tumour to double in volume.

The antitumour activity was also scored according to Fodstad [17] on the basis of *T/C* and SGD values:

% *T/C*		SGD	Score
≥ 50	and	≤ 1.0	–
≤ 50	or	≥ 1.0	+/–
≤ 50	and	≥ 1.0	+
≤ 40	and	≥ 1.5	++
≤ 25	and	≥ 2.0	+++
≤ 10	and	≥ 3.0	++++

– inactive; +/– marginally active; +, ++, +++, ++++ active to highly active.

The homogeneity in the distribution of the tumour volumes within the different experimental groups was assessed before the beginning of the treatment by a statistical analysis (analysis of variance with one factor). Further statistical analyses based on the Newman–Keuls test were used to compare the treated versus control groups.

RESULTS

The antitumour activity of S 16020-2 compared to reference compounds is presented in Table 2.

Colon tumours

S 16020-2 administered at 60 mg/kg on days 0, 7 and 14 to HT29-bearing mice induced the death of two mice out of nine. Consequently, S 16020-2 was administered at 60 mg/kg only twice (days 0 and 7) to Colo320DM-bearing mice. Tumour growth curves shown in Figure 1(a) (HT29) and (b) (Colo320DM) demonstrate that both S 16020-2 and doxorubicin were inactive in these two human colon xenografts.

Breast tumours

In MCF7 xenograft (Figure 2(a)), S 16020-2 administered at 60 mg/kg led to maximal tumour growth inhibition of 77%

Table 1. *Characteristics of human tumours*

Cell line	Tumour type	Histology	Tumour doubling time (days)
Colo320DM	colon (primary tumour)	adenocarcinoma	6.1
HT29	colon (primary tumour)	adenocarcinoma	5.0
MCF7	breast (pleural effusion)	adenocarcinoma	9.4
MDA-MB-231	breast (pleural effusion)	adenocarcinoma	8.1
NCI-H460	lung (pleural fluid)	large-cell carcinoma	3.6
Calu-6	lung	large-cell carcinoma	5.0
NCI-H125	lung (skin)	adenocarcinoma	5.5
A549	lung	adenocarcinoma	30.5
NCI-H69	lung (bone marrow metastases)	small-cell carcinoma	4.7
SCLC6	lung (pleural fluid)	small-cell carcinoma	2.4
SK-OV-3	ovary (malignant ascites)	adenocarcinoma	0.9
A2780	ovary (malignant ascites)	adenocarcinoma	3.0
NIH:OVCAR-3	ovary (malignant ascites)	adenocarcinoma	8.7

Table 2. Antitumour activity of S 16020-2 against human tumours implanted subcutaneously into nude mice

Tumour	Treatment	Dose range (mg/kg)	Optical dose* (mg/kg)	Number of evaluated tumours	Maximum weight loss (g)	Number of death/ total number of mice (day)	% T/C (day)	SGD	Efficacy
Colon									
HT29	S 16020-2	40–60	60	14	−3.4	2/9 (2)	115 (25)	<0	−
	Doxorubicin	10	10	14	−4.0	1/8 (14)	71 (25)	0.2	−
Colo320DM	S 16020-2	60†	60	8	−2.1	0/8	85 (27)	<0	−
	Doxorubicin	10†	10	8	−1.3	0/8	62 (23)	<0	−
Breast									
MCF7	S 16020-2	60–90	60	10	−2.0	0/5	23 (21)	1.4	+
	Doxorubicin	10	10	8	−7.2	1/5 (24)	9 (21)	>2.0	+++
MDA-MB-231	S 16020-2	20–80	80	10	−3.2	0/6	212 (39)	1.2	+
	Doxorubicin	8–10	8	10	−2.4	0/5	29 (35)	1.3	+
Lung NSCLC									
NCI-H460	S 16020-2	20–80	80	10	−1.2	0/7	19 (22)	1.4	+
	Doxorubicin	5–10	10	10	−1.8	0/7	26 (22)	0.9	+/−
Calu-6	S 16020-2	20–80	80	14	−1.9	0/7	30 (23)	1.2	+
	Doxorubicin	5–10	10	14	−1.8	1/7 (41)	17 (30)	2.0	+++
A549	S16020-2	20–80	80	8	−2.1	1/7 (40)	10 (41)	>1.0	++++
	Doxorubicin	8–10	10	10	−2.2	0/7	43 (41)	0.8	+/−
NCI-H125	S 16020-2	20–80	60	12	−1.1	0/7	66 (27)	0.2	−
	Doxorubicin	5–10	10	11	−1.7	1/6 (29)	40 (27)	0.4	+/−
Lung SCLC									
NCI-H69	S 16020-2	30–90	90	7	−1.4	1/5 (11)	15 (24)	2.0	+++
	Doxorubicin	3–9	9	8	−1.1	0/5	32 (31)	0.4	+/−
SCLC6	S 16020-2	60–90	90	17	−1.2	0/9	31 (23)	1.5	++
	Doxorubicin	10	10	16	0	0/10	46 (30)	<0	+/−
Ovary									
SK-OV-3	S 16020-2	20–80	80	12	−1.2	0/6	13 (12)‡	5.0	++++
	Cisplatin§	5–10	5	10	0	0/5	27 (12)‡	2.0	+++
NIH:OVCAR-3	S 16020-2	20–80	80	13	−3.7	0/8	19 (25)	2.7	+++
	Doxorubicin	8	8	11	−2.1	0/8	46 (25)	0.7	+/−
A2780	S 16020-2	40–80	40	12	−1.0	0/8	31 (21)	0.7	+/−
	Doxorubicin	8	8	11	−2.2	0/8	13 (21)	1.4	+

S 16020-2 and doxorubicin were given i.v. on days 0, 7 and 14. SGD, specific growth delay. *For each drug, the optimal dose was determined as defined in Materials and Methods. †S 16020-2 and doxorubicin were given i.v. on days 0 and 7. ‡*T/C* values were calculated on day 12 when the control group was sacrificed because of the very rapid growth of this tumour. §Cisplatin was used as reference compound.

on day 21 (SGD = 1.4). Doxorubicin given at 10 mg/kg induced maximal growth inhibition of 91% on day 21, but marked body weight loss and one death occurred on day 24. Growth curves of control and S 16020-2 or doxorubicin treated groups were statistically different from day 10 to the end of the experiment ($P \leq 0.05$). S 16020-2 given at 80 mg/kg to MDA-MB-231-bearing mice was active with a maximum growth inhibition of 78% on day 39 and an SGD of 1.2. Doxorubicin administered at 8 mg/kg induced a significant inhibition of tumour growth of 71% on day 35 and an SGD of 1.3. The tumour growth curves of animals treated with 80 mg/kg S 16020-2 or 8 mg/kg doxorubicin (Figure 2(b)) were statistically different from that of control from day 32 ($P \leq 0.05$) to day 46 ($P \leq 0.01$).

Lung tumours

For the non-small cell lung cancers, S 16020-2 was given within a dose range of 20–80 mg/kg and doxorubicin at 5 and 10 mg/kg. Representative growth curves are shown in Figure 3.

S 16020-2 showed a significant dose-dependent antitumour activity against the NCI-H460 tumour (Figure 3(a)), maximal inhibition of tumour growth (81% on day 22) was observed at 80 mg/kg with an SGD of 1.4. Growth curves of control and 80 mg/kg S 16020-2-treated groups were significantly different from days 17 to 24 ($P \leq 0.05$–0.01). Doxorubicin was less active than S 16020-2 in this tumour model.

S 16020-2 administered at 80 mg/kg to mice bearing the Calu-6 tumour induced a maximal inhibition of tumour growth of 70% on day 23 and an SGD of 1.2 (Figure 3(b)). The antitumour efficacy of doxorubicin (10 mg/kg) was superior to that of S 16020-2 with a maximum growth inhibition of 83% on day 30 but one death occurred on day 41. S 16020-2 given at 80 mg/kg induced the regression of A549 tumours in all mice, the tumour growth being inhibited by 90% with respect to control (Figure 3(c)). Growth curves of the S 16020-2-treated group and control were significantly different from days 21 to 55 ($P \leq 0.01$). Against the NCI-H125 tumour, S 16020-2 was found to be active only at 80 mg/kg although high toxicity was observed with the death of 2 mice out of 7 on day 10. Consequently, we cannot consider S 16020-2 as active against this tumour model, the dose of 60 mg/kg being inactive (Figure 3(d)). In A549 and NCI-H125 tumour models, doxorubicin was only marginally active.

S 16020-2 induced a dose-dependent growth inhibition of the two small-cell lung xenografts used (Figures 3(e), (f)). Administered at 90 mg/kg, S 16020-2 induced a significant

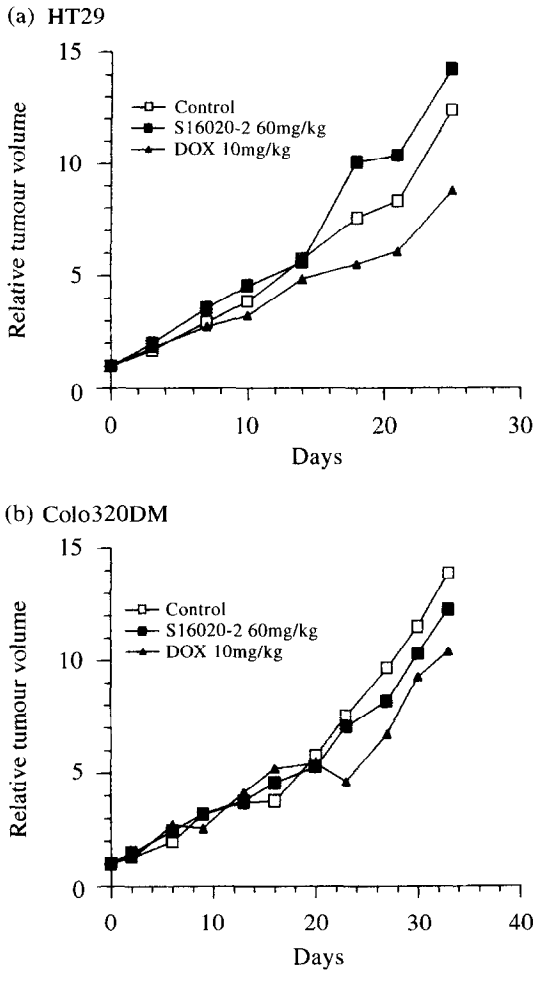

Figure 1. Effect of S 16020-2 on the growth of two colon xenografts. Treatment was started on day 0 when the tumour volume reached 100 mm³. Tumours were measured at the indicated days and the relative tumour volume was expressed as the V_t/V_0 index.

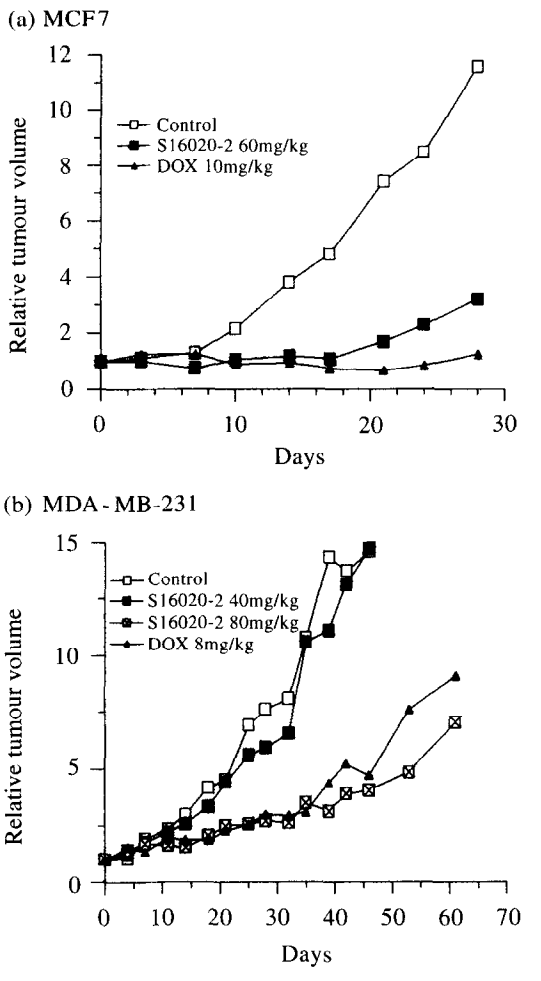

Figure 2. Effect of S 16020-2 on the growth of two breast xenografts. Treatment was started on day 0 when the tumour volume reached 100 mm³. Tumours were measured at the indicated days and the relative tumour volume was expressed as the V_t/V_0 index.

inhibition of NCI-H69 growth from days 10 to 35 ($P \leq 0.05$–0.01) with a maximal inhibition of 85% on day 24 and an SGD of 2.0 but one toxic death was observed during the treatment. In the SCLC6 tumour model, S 16020-2 administered at 90 mg/kg caused a significant inhibition of tumour growth ($P \leq 0.01$ from day 10 to day 31, Figure 3(f)). In these two models, doxorubicin showed only marginal activity.

Ovarian tumours

The doubling time of the SK-OV-3 tumour was very short (0.9 days, Table 1) and tumours became rapidly necrotic so the untreated mice were sacrificed on day 12, 2 days before the last treatment. Consequently, the *T/C* values which could not be calculated beyond day 12 are not representative of the antitumour activity of the tested compounds and the antitumour effects are evaluated on the basis of the SGD values only. As shown in Figure 4(a), S 16020-2 induced a dose-dependent inhibition of tumour growth and the highest inhibition was obtained at 80 mg/kg (SGD = 5.0). Statistical analysis showed that the differences between the tumour growth curves of control and treated groups were significant from day 6 for S 16020-2 at 40 and 80 mg/kg and for cisplatin at 5 mg/kg ($P \leq 0.01$). The antitumour activity of S 16020-2 (80 mg/kg) was significantly superior to that of cisplatin, the growth curves being statistically different from day 12 ($P \leq 0.05$) to day 19 ($P \leq 0.001$). In the NIH:OVCAR-3 tumour model, S 16020-2 administered at 40 and 80 mg/kg induced a dose-dependent inhibition of tumour growth superior to that of doxorubicin (Figure 4(b)). Growth curves of the control and S 16020-2 treated group for the highest dose were statistically different from day 17 to 57 ($P \leq 0.01$). S 16020-2 given at 80 mg/kg to A2780-bearing mice induced significant inhibition of tumour growth which was associated with a dramatic toxicity, since four mice out of eight died after treatment. Hence, only the dose of 40 mg/kg was considered for the evaluation of antitumour activity, the maximum inhibition of tumour growth being 69% on day 21 (Figure 4(c)). In this tumour, doxorubicin showed better activity than S 16020-2, the tumour growth being inhibited by 87% on day 21.

DISCUSSION

The aim of this study was to evaluate the antitumour activity of S 16020-2 in a large panel of human tumours including colon, breast, ovary and lung cancers in which S 16020-2 has shown a potent cytotoxic activity *in vitro* with a mean IC_{50} of approximately 50 nM [6]. The use of human tumour xenografts in the preclinical evaluation of a new drug is now an integral part of the current NCI and

EORTC disease-oriented strategies in which specific characteristics of tumour lines such as hormone dependency or drug resistance can be used to orient future clinical development [11, 12, 18].

The schedule of treatment using i.v. administration on days 0, 7 and 14 was chosen on the basis of previous results obtained in the P388 leukaemia model in which the best antitumour activity was obtained following a repeated sched-

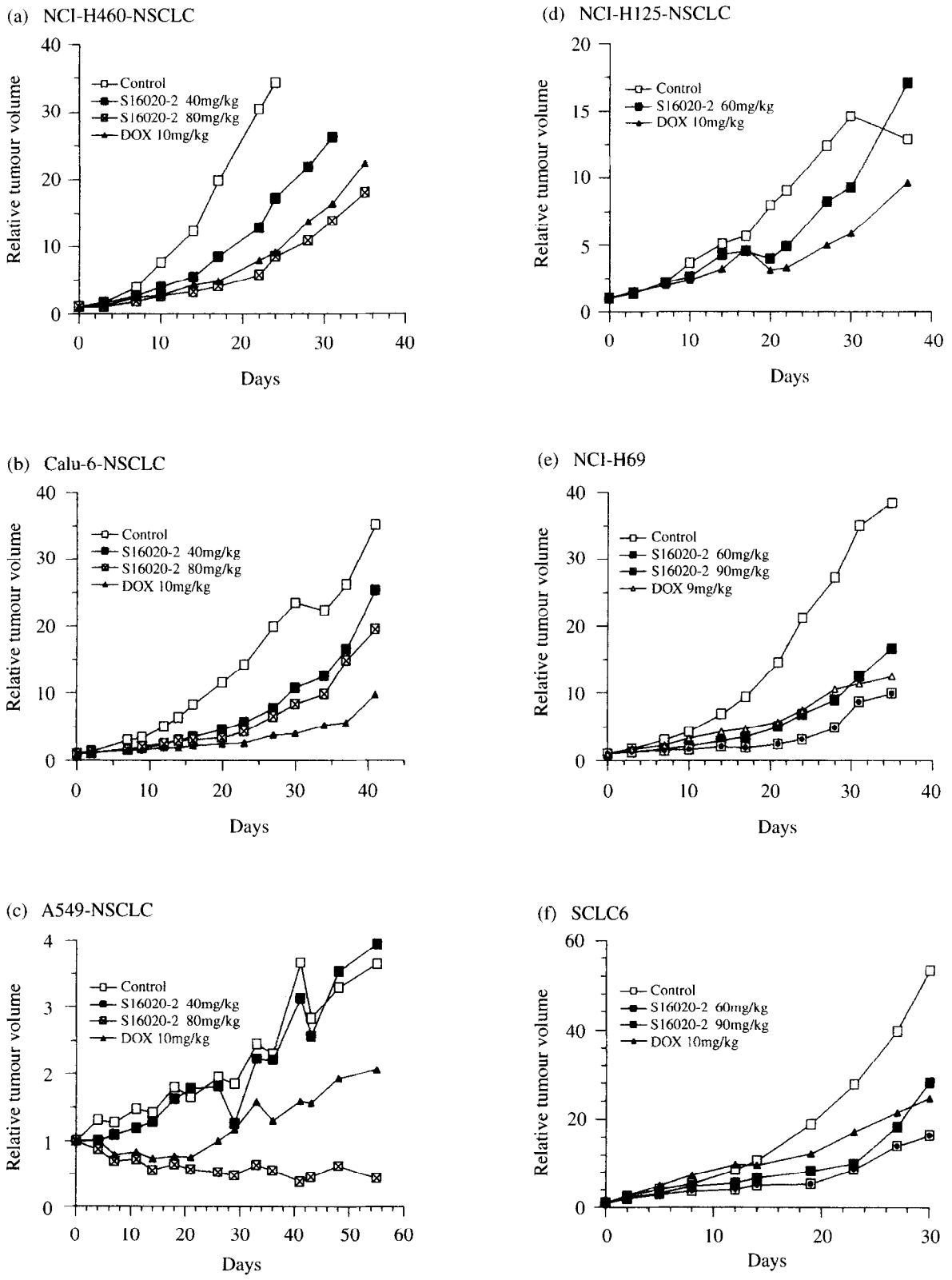

Figure 3. Effect of S 16020-2 on the growth of four non-small cell lung (a–d) and two small-cell lung (e, f) xenografts. Treatment was started on day 0 when the tumour volume reached 100 mm³. Tumours were measured at the indicated days and the relative tumour volume was expressed as the V_t/V_0 index.

ule (days 1, 5, 9) [8]. Because nude mice show higher sensitivity to chemotherapy treatment, the classical weekly schedule was chosen. Significant antitumour responses were obtained in all human tumour xenografts except the two colon cancers Colo320DM and HT29, the latter known to be highly resistant [19]. S 16020-2 was significantly active against the oestrogen (MCF7) and non-oestrogen (MDA-MB-231) dependent breast carcinomas and highly active against two of the three ovarian tumours tested.

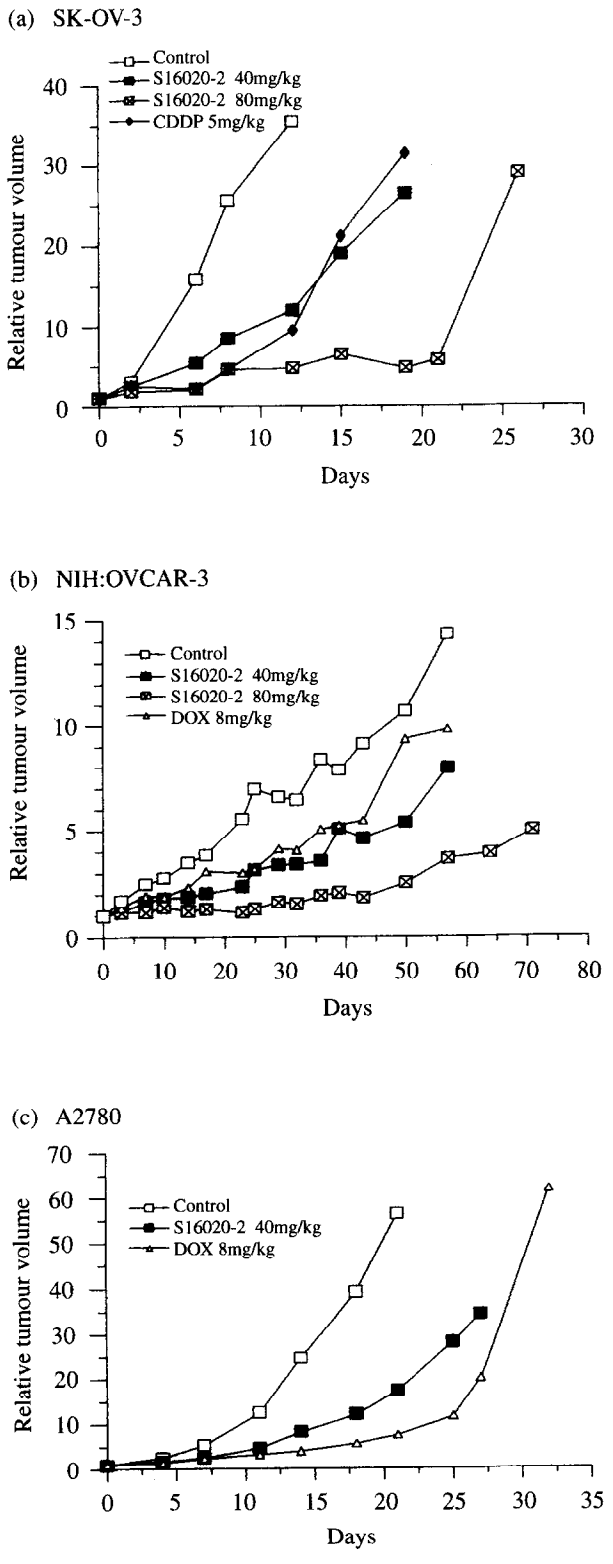

Figure 4. Effect of S 16020-2 on the growth of three ovarian xenografts. Treatment was started on day 0 when the tumour volume reached 100 mm³. Tumours were measured at the indicated days and the relative tumour volume was expressed as the V_t/V_0 index.

In these human tumour models, S 16020-2 and doxorubicin showed a comparable profile of antitumour activity with regard to responses. However, S16020-2 was more active than doxorubicin in five of the 12 tumours, particularly in the lung cancers. At active doses, S 16020-2 induced less severe toxicity than doxorubicin based on animal body weight loss and drug-induced mortality. This is in agreement with the previous observation that S 16020-2 induces a less severe cytotoxic effect than doxorubicin against bone marrow stem cells along with a more rapid return to normal values in mice [8]. Because of its curative effect against the murine Lewis lung carcinoma [8, 9], the antitumour activity of S 16020-2 was evaluated in pulmonary tumours. The non-small cell lung carcinomas, except the NCI-H125 xenograft, responded to S 16020-2. In the A549 model, S 16020-2 induced regressions of the tumours lasting 5–6 weeks after the last treatment. This result is noteworthy, considering the long doubling time of this tumour *in vivo* (30.5 days) and the lower sensitivity of quiescent versus proliferative A549 cells *in vitro* [6], and suggests potential antitumour activity of S 16020-2 even for slowly growing tumours. In the two small-cell lung carcinomas NCI-H69 and SCLC6, S 16020-2 caused a pronounced inhibition of tumour growth. This is of interest particularly in the case of SCLC6, which displays the multidrug resistance phenotype linked to overexpression of the *mdr1* and *GSTπ* genes and which is partially resistant to doxorubicin *in vivo* [20]. S 16020-2 was cytotoxic against a variety of Pgp positive sublines *in vitro* [6] and *in vivo* [8] such as resistant murine leukaemias, including MDR-transfected lines, suggesting a clinical potential of S 16020-2 for tumours displaying a Pgp-mediated multidrug resistance.

Surprisingly, S 16020-2 was inactive *in vivo* against Colo320DM which also displays the multidrug resistance phenotype and was shown to be sensitive *in vitro* to S 16020-2 [6], suggesting in this case, the involvement, *in vivo*, of other types of resistance.

In all these experimental human models, the antitumour effect of S 16020-2 was highest at the maximal tolerated dose for healthy animals, 80 or 90 mg/kg per administration. Similar observations were made with doxorubicin, the active dose (8–10 mg/kg) being the maximal tolerated doses. However, this optimal dose of S 16020-2 induced unexpectedly high mortality in A2780- and in NCI-H125-bearing mice, impeding the administration of doses as high as 80 mg/kg and greater antitumour activity. This mortality might be explained by acute aggressiveness of the disease, which may render the mice more sensitive to chemotherapy.

When compared to doxorubicin which has proven clinical activity, the significant antitumour activity of S 16020-2 against this panel of human tumour xenografts delineates an interesting chemotherapeutic potential for this drug.

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