Efficacy of the Novel Camptothecin Gimatecan against Orthotopic and Metastatic Human Tumor Xenograft Models

Michelandrea De Cesare,[1] Graziella Pratesi,[1] Silvia Veneroni,[1] Raffaella Bergottini,[2] and Franco Zunino[1]

[1]Istituto Nazionale Tumori, Milan; and [2]Dipartimento Patologia Animale, Università degli Studi, Milan, Italy

ABSTRACT

Purpose: **Gimatecan, a novel oral lipophilic camptothecin characterized by favorable features at molecular/cellular level and by a promising profile of preclinical activity, is currently in clinical phase I/II. The aim of the study was to additionally investigate the therapeutic potential of the drug in human tumor xenografts growing in different organs as models representative of tumor growth in the clinical setting.**

Experimental Design: **The models include two orthotopic central nervous system tumors, two melanomas growing intracranially, and an ovarian carcinoma growing i.p. In addition, gimatecan was tested against experimental lung metastases of two tumor types (lung and ovarian carcinomas). Gimatecan was delivered by oral gavage according to various schedules (daily or intermittent). The time (in days) mice required to show evident signs of disease was used as end point for drug efficacy.**

Results: **Gimatecan was highly effective in delaying disease manifestations in all tumor systems investigated. In the intracranially growing tumors, a significant time increase (*versus* control mice) was achieved by the drug administered according to all of the schedules. In addition, almost all treated mice were alive and tumor-free at the end of the experiment in the metastatic models and in the ascitic ovarian tumor. The daily prolonged treatment schedule was the best one.**

Conclusions: **In all tumor systems investigated, including orthotopic tumor growth models and lung metastases, the oral administration of gimatecan showed a therapeutic benefit in terms of survival increase. The good oral availability allowed a prolonged daily treatment regimen, which seems the most promising to exploit the therapeutic potential of the drug.**

INTRODUCTION

Camptothecins represent an important class of antitumor drugs (1). Two synthetic, water-soluble camptothecins, topotecan and irinotecan, represent an important achievement of clinical cancer chemotherapy (2). Reasons for clinical drawbacks of camptothecin have been identified, thus allowing a rational design of novel agents and a better exploitation of their therapeutic potential. Many synthetic analogues have been studied in the past years or are currently under study (3). More recently, new lipophilic analogues have been selected for clinical studies (4), which were designed and screened to overcome the known drawbacks of the "first generation camptothecin" (5). Lipophilicity may provide some advantages in terms of increased drug-target interaction, stability of the closed lactone form, and oral bioavailability.

In a series of camptothecins substituted in position 7 with lipophilic chains, gimatecan (ST1481) was selected for clinical development by oral route and is currently in phase I/II studies (6). Preclinical studies indicated a very promising pharmacological profile of the compound. At the cellular level, gimatecan has a strong cytotoxic potency, which is likely related to multiple factors. They include a potent inhibition of the target topoisomerase I and a persistent stabilization of the cleavable complex (DNA-enzyme-drug; ref. 7). In addition, it rapidly accumulates in tumor cells where it remains for long time (much longer than topotecan; ref. 8) and exhibits a peculiar localization in the lysosomal compartment where it might be stored in the active lactone form (9). Such properties are reflected in a strong antitumor activity in a large panel of human tumor xenografts of various tumor types where oral gimatecan was very potent and equally or more effective than the established camptothecin topotecan against s.c. implanted tumors and liver metastases (8, 10). Moreover, in contrast to other camptothecins, gimatecan showed antitumor effect even in tumors expressing multidrug resistance mechanisms mediated by transport systems such as P-gp170, BCRP, and MRP (6, 8, 11).

The aim of the study was to additionally investigate the pharmacological profile of gimatecan in orthotopic and metastatic tumor models. Because of the high lipophilicity of the molecule, the ability of overcoming P-gp170–mediated resistance, and the rapid distribution in brain tissues (8), the compound was tested against four human tumor xenografts intracranially (i.c.) growing and representative of central nervous system tumors (glioblastoma) and brain-metastasizing tumors (melanoma). Moreover, gimatecan was tested against experimental lung metastases achieved by i.v. injection of human tumor cells of different tumor types (lung and ovarian carcinomas). A human ovary carcinoma was grown i.p. in mouse, thus achieving an orthotopic ascitic tumor model, which reproduces

Received 5/25/04; revised 7/8/04; accepted 7/20/04.
Grant support: Associazione Italiana Ricerca sul Cancro, Milan, by the Ministero della Salute, Rome, by the Consiglio Nazionale delle Ricerche-Ministero Istruzione Universita Ricerca, Rome, Italy.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Requests for reprints: Graziella Pratesi, Istituto Nazionale Tumori, via Venezian 1, 20133 Milan, Italy. Phone: 39-02-23902626; Fax: 39-02-23902692; E-mail: graziella.pratesi@istitutotumori.mi.it.

the tumor spread of ovarian carcinoma patients. In addition to the systemic administration of gimatecan by oral route, which was used in the previous studies, the intratumor drug delivery in s.c. growing tumors was also studied. The overall results show antitumor efficacy of gimatecan in all of the preclinical systems investigated, thus providing additional support to the pharmacological potential of the compound.

MATERIALS AND METHODS

Animals and Anesthesia. All experiments were carried out using female athymic Swiss nude mice, 8–10 weeks-old (Charles River, Calco, Italy). Mice were maintained in laminar flow rooms, keeping temperature and humidity constant. Mice had free access to food and water. Experiments were approved by the Ethics Committee for Animal Experimentation of the Istituto Nazionale Tumori of Milan, according to the institutional guidelines. For animal anesthesia, a solution of ketamine (Ketavet 50, Farmaceutici Gellin, Aprilia, Italy), xylazine (Ronpum; Bayer AG, Leverkusen, Germany) and saline (20: 2.5: 77.5) was prepared and delivered i.p. in a volume of 10 mL/kg of body weight.

Drugs. Gimatecan (ST1481, 7-t-butoxyiminomethylcamptothecin, Sigma-Tau, Pomezia, Italy) was dissolved in DMSO and stored at −20°C until use. At treatment days, the drug was thawed and suspended in sterile, distilled water (DMSO 10% final concentration). The drug was delivered p.o. in a volume of 10 mL/kg or intratumorally in a volume of 5 mL/kg body weight. All control mice were treated in parallel with the drug solvent (DMSO 10% in water).

Intracranial Tumor Models and Antitumor Activity. The human tumor cell lines used in the study were derived from one melanoma patient (LP and LM, from the primary tumor and a lymph node metastasis, respectively; ref. 12) and two glioblastoma patients (U87-MG and SW1783; both from the American Type Culture Collection, Manassas, VA). For experiments, tumor cells were implanted i.c. at various concentration levels, depending on tumor cell malignancy (see tables). Exponentially growing cells from *in vitro* culture were collected, washed, and injected (0.01 mL/mouse, in saline) into anesthetized mice, stereotactically into the right frontal lobe to a depth of 5 mm, with an exmire microsyringe MS-N100 (PBI International, Milan, Italy) with a N50B gauge needle tip. Tumor growth in brain induces weight loss with lethal effects on mice. Mice were monitored daily. For ethical reasons, mice with a body weight loss ~25 to 30% were sacrificed because natural death was expected in 1 to 2 days. The day of sacrifice was considered as day of death.

Gimatecan was delivered by oral gavage, according to different schedules and doses (see tables), starting 3 days after cell inoculum. Drug activity was assessed as T/C%, *i.e.,* the ratio of median survival time in treated over control mice (T/C) × 100. For statistical analysis, the days of death (actually, sacrifice) of treated mice were compared with those of control mice by Student's *t* test. Deaths occurring in treated mice before the death of the first control mouse were ascribed to drug toxicity.

At sacrifice, the brains of control mice were removed, fixed in 10% phosphate-buffered formalin and embedded in paraffin in an orientated way, with the encephalon-cap above. Serial longitudinal 4-μm thick sections were cut and processed for conventional histologic procedures by using H&E staining. The samples, representative of the general brain morphology, were analyzed under an optical microscope.

Experimental Lung Metastases. The IGROV-1 ovarian (13) and the NCI-H460 lung carcinoma (14) tumor lines were maintained by serial i.p. passages in healthy mice. Tumor grows as ascites and small solid masses (15). For experimental lung metastases, mice were inoculated via the tail vein with IGROV-1 (5×10^5) or NCI-H460 (20×10^5) ascitic cells suspended in saline (0.4 mL/mouse). Experimental groups consisted of 8 to 10 animals. Three days after cell injection, treatment started by delivering gimatecan p.o. every day for 5 days a week (qd×5/week) for ≥2 weeks. Mice developing experimental metastases undergo cachexia and die because of lung invasion by multiple tumor foci. For ethical reasons, in our study, animals were not allowed to die. Thus, they were inspected daily and weighed twice a week. When abrupt body weight loss or respiratory distress or any other sign of suffering were evident, mice were sacrificed and carefully autopsied. Lungs were removed and weighed. The day of sacrifice was considered as day of death. Control mice had a median survival time of 31 (IGROV-1) or 39.5 (NCI-H460) days. Approximately 3 months after median survival time of controls (see tables), all mice still alive were sacrificed, autopsied, and their lungs were removed and weighed. Lethal toxicity was assessed as for the i.c. growing tumors.

Intraperitoneally Growing Tumor. IGROV-1 ascitic cells were collected and prepared as described for the experimental lung metastases, and 2.5×10^6 cells/mouse (in 0.2 mL of saline) were i.p. injected. Mice develop hemorrhagic and diffuse carcinomatosis and eventually die by 25 to 30 days (15). Mice were inspected daily and weighed three times a week. For ethical reasons, the day of ascites onset (increase in body weight of 15 to 20%) was considered as the experimental end point instead of the survival time. Animals were sacrificed 1 to 2 days later. Experimental groups consisted of seven mice.

The compound was delivered orally, at a dose of 0.25 mg/kg, qd×4 to 5/week for 5 weeks, starting the day after cell injection. Lethal toxicity was assessed as for i.c. growing tumors.

Subcutaneous Tumor Models and Intratumoral Drug Treatment. The A2780 ovarian (16) and the NCI-H460 lung carcinoma lines were maintained by s.c. passages of tumor fragments (~2× 2 × 6 mm) in athymic nude mice. For experiments, four to five mice per group were inoculated s.c. with one tumor fragment in the right flank. Tumors were implanted on day 0, and tumor growth was followed by biweekly measurements of tumor diameters with a Vernier caliper. Tumor volume was calculated according to the formula: tumor volume (mm^3) = $d^2 \times D/2$, where d and D are the shortest and the longest diameter, respectively. When tumors reached a large size (tumor volume = 500 to 600 mm^3), a single treatment of gimatecan was delivered intratumorally, with a 26 gauge-needle, in a volume of 5 mL/kg. Tumor growth was followed.

Statistical Analysis. Curves reporting the percentage of survivors and of disease-free mice over time were estimated by the Kaplan-Meier product limit method and compared with the log-rank test. Student's *t* test was used to determine the statis-

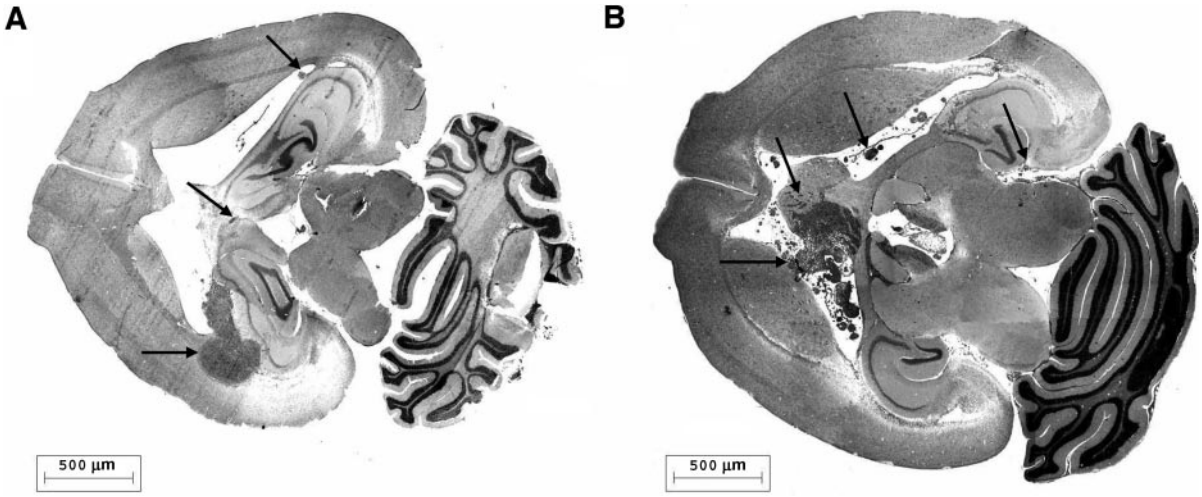

Fig. 1 Brain sections of mice i.c. implanted with U87-MG glioma (*A*) and LM melanoma (*B*) cells. *Arrows:* tumor nodules. H&E. *Bar:* 500 μm.

tical significance of the difference between treated and control mice in tumor volumes for s.c. implanted mice and in survival times for i.c. implanted mice. Analyses were done with GraphPad Prism, version 4.0 (GraphPad Software, Inc., San Diego, CA). All tests were two-sided.

RESULTS

Efficacy of Gimatecan on Brain Tumor Models. Histologic sections of brains of tumor-bearing mice are presented in Fig. 1. SW1783 and U87-MG glioblastomas grew as a large mass and few small nodules. The U87-MG, which is shown in Fig. 1*A*, expanded the choroids plexus of the lateral ventricle, invaded the external capsule, and compressed the cerebral cortex. On the other hand, the LM and LP melanoma cells injected into mice brains produced small scattered tumoral lesions predominantly within the lumen of the lateral ventricles, infiltrating the fimbria hippocampus and multifocally expanding the meninges (LM is reported in Fig. 1*B*).

Table 1 reports the results of the antitumor effect of gimatecan against two human glioblastomas orthotopically implanted in mouse brain tissue. U87-MG glioblastoma-bearing mice were treated with gimatecan every fourth or eighth day for three to four times. All tolerated regimens significantly ($P < 0.01$) increased mice survival time over control mice without an apparent dose dependence. The dose of 4 mg/kg every eighth day was toxic in two of five mice. Against the SW1783 glioblastoma, various treatment regimens were investigated, and gimatecan was effective and tolerated by all of them. The best efficacy was achieved by the daily administration of gimatecan, 0.25 mg/kg, for 4 weeks (T/C% 195). The daily dose of 0.5 mg/kg was toxic in two of eight mice.

Table 2 reports the results of the antitumor effects of gimatecan against human melanoma xenografts i.c. growing in nude mice. Mice that received injections of LM melanoma cells were treated with the drug according to various schedules. When gimatecan was delivered every fourth or every seventh day, all tested doses were well tolerated and very active, inducing a significant increase of median survival time compared with controls ($P < 0.0001$). A similar level of antitumor efficacy was observed even after treatment with the drug according to a daily

Table 1 Effects of oral gimatecan in athymic nude mice bearing i.c. implanted human glioblastomas

Tumor (no. of cells)	Days of treatment	Dose (mg/kg)		T/C* %	P <*	Disease free/ total number†	Toxicity‡
		Single	Total				
U87-MG (2×10^5)	3, 7, 11, 15	1.5	6	161	0.01	0/5	0/5
		2	8	147	0.01	0/5	0/5
	3, 11, 18	3	9	172	0.005	0/5	0/5
		4	12	172	n.s.	0/5	2/5 (7,24)
SW1783 (2.5×10^5)	3, 7, 11, 15	2	8	148	0.0001	0/9	0/9
	3, 11, 18	3	9	130	n.s.	1/9	1/9 (10)
	3→7, 10→14, 17→21, 24→28	0.25	5	195	0.0001	0/8	0/8
		0.5	10	148	n.s.	0/8	2/8 (10,11)

* Median survival time in treated (T) over control (C) mice × 100. Median survival time in control mice was 31 and 22 to 23 days (two experiments) for U87-MG and SW1783, respectively. *P* values were calculated *versus* control mice by Student's *t* test.

† At 80 days (end of experiment).

‡Number of toxic deaths/total number of mice; in parentheses the day of death.

Abbreviation: n.s., not significant.

Table 2 Effects of oral gimatecan in athymic nude mice bearing i.c. implanted human melanomas.

Tumor (no. of cells)	Days of treatment	Dose (mg/kg)		T/C* %	P <*	Disease free/ total number†	Toxicity‡
		Single	Total				
LM (0.1×10^5)	3, 7, 10, 14, 17, 21, 24, 28	1	8	163	0.0001	0/8	0/8
	3, 7, 11, 15	2	8	161	0.0001	0/8	0/8
	3, 10, 17, 24	2	8	159	0.0001	0/8	0/8
		3	12	167	0.0001	0/7	0/7
	3→7, 10→14, 17→21, 24→28	0.25	5	165	0.001	0/8	0/8
	3→7, 10→14, 17→21	0.5	7.5	159	n.s.	0/8	1/8 (15)
LP (2.5×10^5)	3, 7, 11, 15	2	8	150	0.05	0/9	1/9 (10)
	3, 11, 19	3	9	147	n.s.	1/9	2/9 (10,10)

* Median survival time in treated (T) over control (C) mice × 100. Median survival time in control mice was 23 to 24.5 (two experiments) and 36 days for LM and LP, respectively. P values were calculated *versus* control mice by Student's t test.

† At 80 days (end of experiment).

‡ Number of toxic deaths/total number of mice.

Abbreviation: n.s., not significant.

schedule (d×5 days/week) for several weeks. Again, according to such protracted treatment schedule, the dose level of 0.5 mg/kg was somewhat toxic, causing the death of one mouse at early time (day 15). Possibly for such reason, no statistical significance was reached *versus* controls survival time ($P >$ 0.05). No dose- or schedule-related differences in the antitumor effects of gimatecan against the LM melanoma were evident. Against the LP melanoma gimatecan, 2 mg/kg, administered every fourth day was well tolerated and significantly effective in increasing mice life span. The dose of 3 mg/kg, every eighth day was partially toxic, but achieved one tumor-free mouse, at the end of experiment, 80 days after tumor cell injection.

A comparable efficacy of gimatecan was achieved against the two tumor lines. Survival curves of controls and mice treated with the daily administration of gimatecan, 0.25 mg/kg, are shown in Fig. 2.

Efficacy of Gimatecan on Experimental Lung Metastases. The effects of the oral gimatecan were investigated in experimental lung metastases induced by IGROV-1 or NCI-H460 human tumor cells (Table 3). After i.v. injection of IGROV-1 ascitic cells, all control mice developed evident signs of disease such as dispnea and marked body weight loss. Mice presenting such manifestations were sacrificed (median time, 31 days), and their lungs were widely invaded by metastatic lesions (range in lung weight, 0.63 to 1.87 g). In the mice receiving gimatecan (0.25 mg/kg/d×5 days/week for 5 weeks), the disease onset and the metastases development were dramatically affected. In fact, no mouse presented manifestations of disease at a very advanced time (132 days after cell injection), when the experiment was closed and mice were sacrificed. At necropsy, five of nine mice presented macroscopically healthy lungs and only four mice showed metastatic nodules (range in lung weight, 0.17 to 0.81 g). When a higher (0.4 mg/kg) gimatecan dose level was administered by the daily schedule, treatment was toxic after short time and was discontinued (two of eight death for toxicity after 2 weeks). At sacrifice, half the group presented lung metastases. The weights of metastatic lung in the treated mice were much smaller than in the control mice, due to the few, countable, tumor nodules and only two treated mice presented invaded lungs (range in lung weight, 0.19 to 0.80 g).

Mice i.v. injected with NCI-H460 lung carcinoma cells (Table 3) presented evidence of disease later than the IGROV-1-bearing mice (median time: 39.5 days). When treated with daily administration of gimatecan, mice showed no evidence of

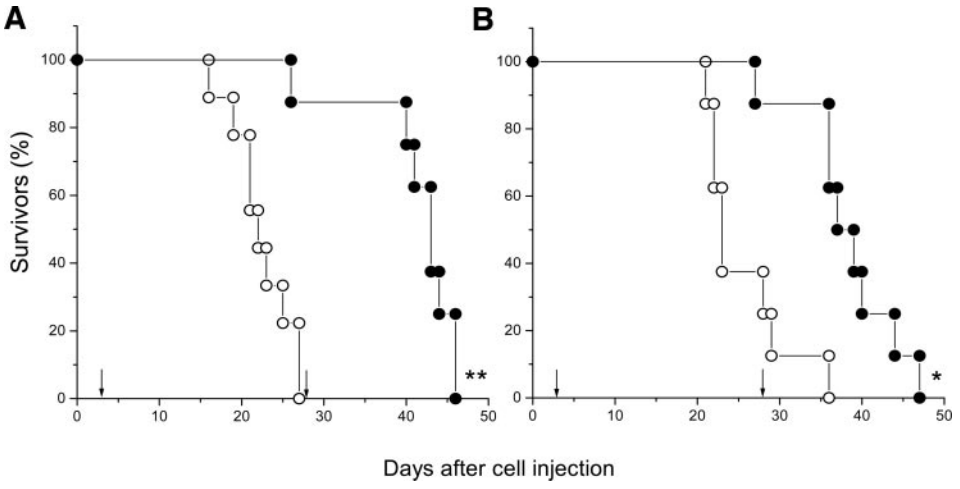

Fig. 2 Kaplan-Meier plot of the percentage of survivors over time among mice that received i.c. injection of SW1787 astrocytoma cells (*A*) or LM melanoma cells (*B*): vehicle-treated mice (○); gimatecan-treated mice, 0.25 mg/kg (●). Treatments were delivered orally by gavage, daily for 5 days/week for 4 weeks (*arrows* indicate the first and the last day of treatment). Experimental groups consisted of eight to nine mice. $*P <$ 0.001 and $**P < 0.0002$ by two-sided log-rank χ^2 test *versus* vehicle-treated mice.

Table 3 Effects of oral gimatecan on experimental lung metastases

Tumor (no. of cells)*	Dose (mg/kg) Single	Total	Toxicity†	Survivors/total (day)‡	Mice with lung metastases/total§	Lung weight (g)§ (mean ± SD)
IGROV-1 (5×10^5)				0/10 (132)	10/10	1.30 ± 0.35
	0.25¶	6.25	0/9	9/9 (132)	4/9	0.29 ± 0.19
	0.4‖	4	2/8	6/8 (132)	3/6	0.32 ± 0.22
NCI-H460 (20×10^5)				1/9 (120)	8/9	0.93 ± 0.31
	0.25¶	6.25	0/9	9/9 (120)	6/9	0.19 ± 0.05
	0.5**	7.5	1/9	8/9 (120)	3/8	0.27 ± 0.22

NOTE. The drug was delivered daily, 5 days a week, for several weeks starting 3 days after i.v. cell injection.

* Tumor cells were injected i.v. Median day of death in control mice was 31 and 39.5 for IGROV-1 and NCI-H460, respectively.

† Number of toxic deaths/total number of mice.

‡ Mice alive without signs of disease/total number of mice. In parentheses, the day of sacrifice of the mice (end of experiment).

§ Lungs were removed and weighed at sacrifice. Mice with lung metastases/total number of mice. In lung weight, metastases-free lungs are included.

¶ Delivered for 5 weeks.

‖ Delivered for 2 weeks.

** Delivered daily, every other week × 3.

disease for at least 4 months (120 days), *i.e.,* at the end of the experiment. At necropsy, mice were or metastasis-free (8 of 17) or presented only small metastatic burden in lungs. The high dose tested (0.5 mg/kg, delivered d×5 days/week, every other week for three times) was more effective in terms of number of metastasis-free mice (more than half the group) but was partially toxic, thus indicating the low dose of gimatecan, which could be delivered safely for many weeks, as the optimal treatment regimen.

Fig. 3 shows survival curves of control and treated mice of the two experimental systems.

Efficacy of Gimatecan against i.p. Growing Ovarian Tumor. After i.p. injection of IGROV-1 ascitic cells, all control mice developed ascites with increase of abdomen volume and body weight in a median time of 11 days. The oral gimatecan exhibited an impressive activity against this model, which is relatively refractory to conventional agents. Indeed, in mice receiving oral gimatecan (0.25 mg/kg/d×4 to 5 days/week for 5weeks), the ascites onset was dramatically delayed, and only three mice developed ascites at a very advanced time (88 days, end of experiment), with a T/C > 800% (Fig. 3*C*). The protracted treatment with the low dose was well tolerated.

Drug Activity and Toxicity of Gimatecan after Intratumoral Injection. The results achieved delivering gimatecan directly into very large-size tumors (~500 mm^3) are reported in Fig. 4. The antitumor efficacy shown by the drug against the A2780 ovarian tumor was impressive: a single treatment with 3 mg/kg of the drug induced a marked regression of the large tumors, and drug efficacy was evident up to 7 days after the treatment, when tumors were inhibited of 97% compared with control ($P < 0.001$, by Student's *t* test). In the NCI-H460 lung tumor model, the same dose was again very effective, inducing

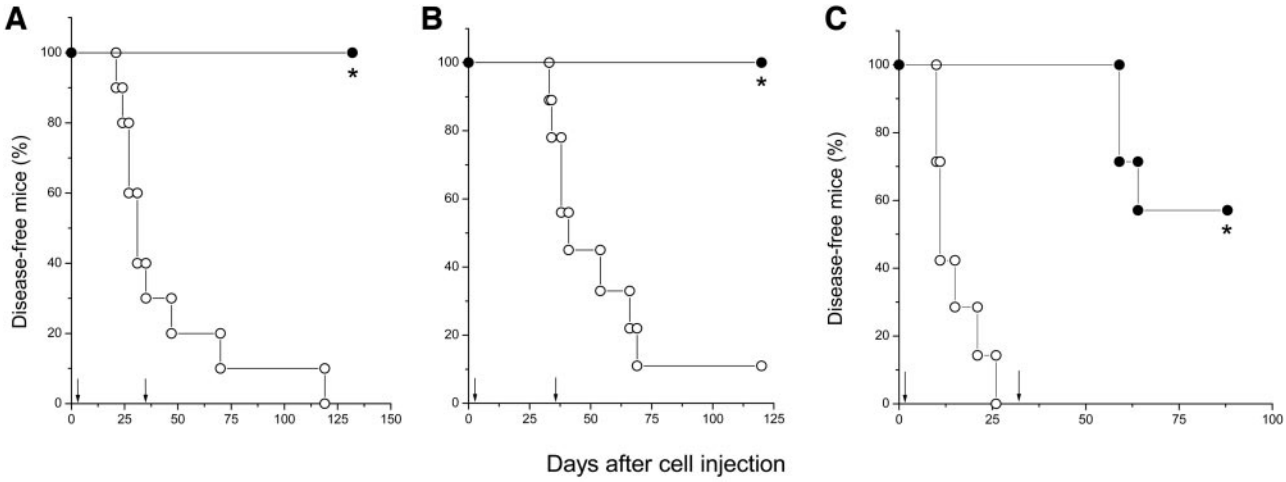

Fig. 3 Kaplan-Meier plot of the percentage of disease-free mice over time among mice that received i.v. injection of IGROV-1 ovarian carcinoma cells (*A*) or NCI-H460 lung carcinoma cells (*B*) or i.p. injection of IGROV-1 ovarian carcinoma cells (*C*): vehicle-treated mice (○); gimatecan-treated mice, 0.25 mg/kg (●). Treatments were delivered orally by gavage, daily for 5 days/week for 5 weeks (*arrows* indicate the first and the last day of treatment). Experimental groups consisted of 8 to 10 mice in *A*, 9 mice in *B*, and 7 mice in *C*. $*P < 0.0001$ by two-sided log-rank χ^2 test *versus* vehicle-treated mice.

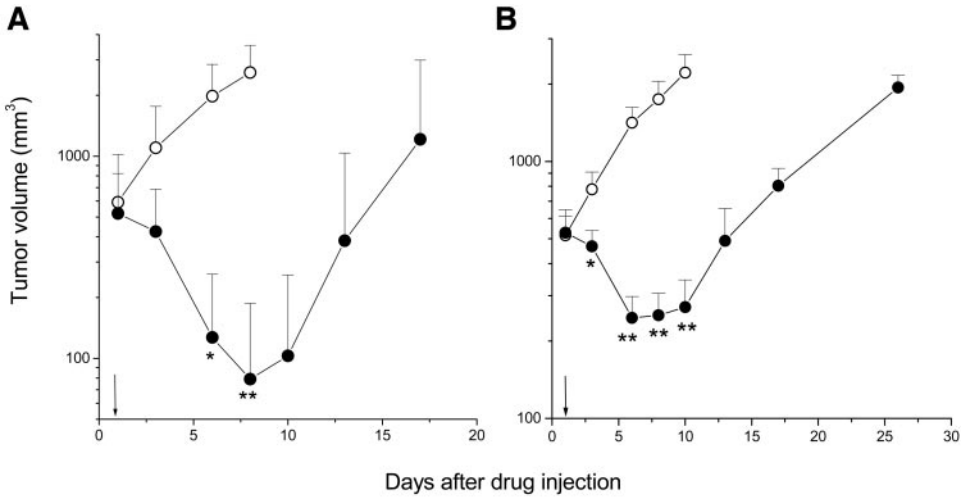

Fig. 4 Effects on tumor growth of gimatecan against the A2780 ovarian carcinoma (*A*) and the NCI-H460 lung carcinoma (*B*) growing s.c.: vehicle-treated mice (○); gimatecan-treated mice, 3 mg/kg (●). A single treatment was delivered intratumor (*arrow*). Points report the mean values of four to five mice. *Bars:* SD. $*P < 0.01$; $**P < 0.001$ by Student's *t* test *versus* vehicle-treated tumors.

a tumor growth inhibition of 88% *versus* control mice ($P < 0.0001$, by Student's *t* test). Tumor volumes in treated and control mice statistically differed at all time points investigated. Surprisingly, the toxicity of the drug was dramatically enhanced by the intratumoral route of administration, and additional treatments were not delivered. In contrast, by oral route, the dose level of 3 mg/kg could be administered safely for three to four times (Tables 1 and 2).

DISCUSSION

The results presented in the paper indicate that the novel camptothecin, gimatecan, is effective in inhibiting the growth of brain- and lung-growing tumors. The drug was delivered p.o. by various treatment regimens, and all of them were active. The best antitumor efficacy was achieved by the daily protracted low-dose (0.25 mg/kg) administration. In addition to achieving the highest increase in mice life span with the lowest total dose used, gimatecan was well tolerated for a long period of treatment, *i.e.,* at least 5 weeks (maximum time investigated). This observation indicated that the therapeutic index could be improved with this schedule. It is likely that the tolerability of low-dose schedule is closely related to the dose level and therefore to the plasma level achieved during the protracted treatment. Indeed, 0.25 mg/kg could be safely administered for many weeks without cumulative toxicity, whereas 0.4 to 0.5 mg/kg daily injections induced lethal toxicity after few weeks of treatment. The long half life of gimatecan in mouse (8), as well as in man (17, 18), may underlie such behavior. Phase I studies clearly indicated higher drug plasma levels on day 5 than on day 1 after daily drug treatment (17), thus indicating drug accumulation as well as increasing plasma levels after each successive weekly dose (18). The finding that the daily schedule was the optimal one for gimatecan was expected on the basis of mechanism of camptothecin cytotoxicity (19) and antiangiogenic effect (20). In fact, cytotoxic drugs delivered by low-dose/frequent treatment (the so called metronomic chemotherapy) have been reported to affect tumor angiogenesis more than by the classical high-dose/intermittent treatment schedules (21). Such a pattern of response has been demonstrated for gimatecan in a human tumor xenograft (20).

Many schedules and doses of treatment were investigated in the different tumor models. The lack of a clear dose dependence in the antitumor effects likely reflects the increased toxicity of the high-dose levels. Indeed, relatively high-dose levels used in a daily protracted schedule may affect the median survival time of treated animals as a consequence of early toxic deaths or of reduction of treatment duration (due to reduced tolerability). Both events are expected to affect the therapeutic benefit of doses above the maximum tolerated ones.

The growth of orthotopically implanted human central nervous system tumor xenografts was evident in the brain parenchyma. In tumor-bearing mice, the systemic administration of gimatecan by oral route was able to significantly increase mice survival time, although all mice eventually died for tumor progression. The efficacy of established camptothecins, topotecan and irinotecan, against central nervous system tumor xenografts either s.c. or i.c. growing was previously reported (22, 23), and both drugs appear to have activity against malignant glioma patients (24, 25). Against s.c. growing brain tumor xenografts, gimatecan was more effective than topotecan (10), thus making the novel camptothecin a candidate for clinical studies on such tumor type. A phase I/II clinical study with oral gimatecan in glioma patients is ongoing, and radiographic response and disease stabilization have been already observed (26).

Central nervous system is a common site of metastatic spread in melanoma patients and established cytotoxic drugs produce <20% of response rate and no survival benefit (27). In our study, cells from a primary melanoma (LP) and from a lymph node metastasis (LM) of the same patient were implanted in mice brain, and gimatecan significantly increase mice survival in both experimental systems. The LM tumor was as sensitive as the LP tumor to the drug treatment, which is in contrast to what was observed with the same two tumor xenografts s.c. implanted, where complete response (in 100% of mice) was observed only in the LP-bearing mice (10). Thus,

tumor sensitivity is influenced by the site of growth. The fact that organ microenvironment can influence tumor response to chemotherapy by regulating the expression of drug resistance systems (such as mdr-1) or angiogenesis-related factors (vascular endothelial growth factor, basic fibroblast growth factor, and interleukin 8) has been reported previously (28, 29).

The antitumor effect of gimatecan in brain-growing tumors is related to its ability to cross the blood-brain barrier. Gimatecan is not a substrate for transport systems (6), which are expressed not only in the blood-brain barrier but also in brain tissues (30). The ability of gimatecan to overcome the blood-brain barrier was reflected in the rapid distribution in brain tissue, which has been documented in mouse (8). Histologic analysis showed tumor growth in different regions of the brain, but a comparable response was achieved by gimatecan in the four tumors investigated, thus indicating its ability to diffuse in all brain areas. An additional factor contributing to antitumor efficacy may be related to the ability of gimatecan of inhibiting growth factors expression such as basic fibroblast growth factor (20). Indeed, some tumor types, including melanoma and glioma, exhibit a paracrine or autocrine stimulation mediated by fibroblast growth factor, which is a proangiogenic factor and likely plays a role in tumor development (31).

Again, the remarkable effect against experimental lung metastases achieved by gimatecan in two different human tumor systems, a lung and an ovarian carcinoma, could reflect the favorable pharmacokinetic behavior and the rapid distribution in lung tissues (8), as well as inhibitory effects of the drug on angiogenesis. Indeed, preliminary studies with gimatecan against a s.c. growing NCI-H460 tumor indicated a 30 to 40% microvessel density inhibition in the primary tumors together with a significant inhibition of spontaneous lung metastases in treated *versus* control mice (not shown). Interestingly, the preliminary results of an ongoing phase I study of gimatecan document partial responses in two lung carcinoma patients (32).

Finally, the impressive antitumor activity of gimatecan against an ascitic ovarian carcinoma supports the potential interest of the drug for the treatment of such tumors. Gimatecan is a promising candidate for clinical studies, even considering that in a previous preclinical study, it was significantly more effective than topotecan against ovarian carcinoma and cisplatin-resistant tumor xenografts s.c. growing (10). Topotecan is now approved for use in relapsed ovarian cancer patients, and results indicate the antitumor effect to be schedule dependent, with the prolonged infusion of $\geq$5 days achieving improved response rates (33). Thus, the oral route, which has been investigated in our study for gimatecan, is the most suitable for prolonged daily administrations.

The lack of histolesive potential of camptothecins allows a locoregional or intratumor delivery with expected advantage in terms of active drug concentration at tumor site (34). The results of intratumoral administration of gimatecan indicated its ability to strongly reduce tumor burden of bulky tumors. However, the therapeutic relevance of the finding remains to be defined because only one treatment with a suboptimal dose was allowed for an unexpected drug toxicity, possibly related to a massive tumor destruction (35). Other regimens of the drug might be investigated by such a route, but the role of intralesional therapy in clinical practice is very limited, and a clinical study with intratumoral topotecan in an ovarian carcinoma patient achieved disappointing results (36).

In conclusion, in all tumor systems investigated, including orthotopic tumor growth models and lung metastases, gimatecan demonstrated a therapeutic benefit in terms of survival increase. A prolonged low-dose treatment regimen, easily allowed by the good oral availability of the drug, seems the most promising to exploit the therapeutic potential of the drug.

ACKNOWLEDGMENTS

We thank Nives Carenini and Loredana Cleris for technical help and Laura Zanesi for editorial assistance.

REFERENCES

1. Giovanella BC, Stehlin JS, Wall ME, et al. DNA topoisomerase I-targeted chemotherapy of human colon cancer in xenografts. Science (Wash. DC) 1989;246:1046–8.

2. Takimoto CH, Wright J, Arbuck SG. Clinical applications of the camptothecins. Biochim Biophys Acta 1998;1400:107–19.

3. Zunino F, Dallavalle S, Laccabue D, Beretta G, Merlini L, Pratesi G. Current status and perspective in the development of camptothecins. Curr Pharm Des 2002;8:2505–20.

4. Zunino F, Pratesi G. Camptothecins in clinical development. Expert Opin Investig Drugs 2004;13:269–84.

5. Burke TG, Mi Z. The structural basis of camptothecin interactions with human serum albumin: impact on drug stability. J Med Chem 1994;37:40–6.

6. Pratesi G, Beretta GL, Zunino F. Gimatecan, a novel camptothecin with a promising preclinical profile. Anticancer Drugs 2004;15:545–52.

7. Dallavalle S, Ferrari A, Biasotti B, et al. Novel 7-oxyiminomethyl derivatives of camptothecin with potential in vitro and in vivo antitumor activity. J Med Chem 2001;44:3264–74.

8. De Cesare M, Pratesi G, Perego P, et al. Potent antitumor activity and improved pharmacological profile of ST1481, a novel 7-substituted camptothecin. Cancer Res 2001;61:7189–95.

9. Croce AC, Bottiroli G, Supino R, Favini E, Zuco V, Zunino F. Subcellular localization of the camptothecin analogues, topotecan and gimatecan. Biochem Pharmacol 2004;67:1035–45.

10. Pratesi G, De Cesare M, Carenini N, et al. Pattern of antitumor activity of a novel camptothecin, ST1481, in a large panel of human tumor xenografts. Clin Cancer Res 2002;8:3904–9.

11. Perego P, De Cesare M, De Isabella P, et al. A novel 7-modified camptothecin analog overcomes breast cancer resistance protein-associated resistance in a mitoxantrone-selected colon carcinoma cell line. Cancer Res 2001;61:6034–7.

12. Leonetti C, Biroccio A, Candiloro A, et al. Increase of cisplatin sensitivity by c-myc antisense oligodeoxynucleotides in a human metastatic melanoma inherently resistant to cisplatin. Clin Cancer Res 1999;5:2588–95.

13. Bènard J, Da Silva J, De Blois M-C, et al. Characterization of a human ovarian adenocarcinoma line, IGROV-1, in tissue culture and in nude mice. Cancer Res 1985;45:4970–9.

14. Brower M, Carney DN, Oie HK, Gazdar AF, Minna JD. Growth of cell lines and clinical specimens of human non-small cell lung cancer in a serum-free defined medium. Cancer Res 1986;46:798–806.

15. Pratesi G, Manzotti C, Tortoreto M, Audisio RA, Zunino F. Differential efficacy of flavone acetic acid against liver versus lung metastases in a human tumour xenograft. Br J Cancer 1991;63:71–4.

16. Behrens BC, Hamilton TC, Masuda H, et al. Characterization of a cis-diamminedichloroplatinum(II)-resistant human ovarian cancer cell line and its use in evaluation of platinum analogues. Cancer Res 1987;47:414–8.

17. Zucchetti M, Pace S, Frapolli R, et al. Clinical pharmacokinetic profile of gimatecan (ST1481), a new oral camptothecin derivative. Proc Am Soc Clin Oncol 2002;21:476.

18. Zhu AX, Ready NE, Clark JW, et al. Phase I trial of gimatecan given orally once a week for 3 of 4 weeks in patients with advanced solid tumors. Proc Am Soc Clin Oncol 2003;22:139.

19. Liu LF. DNA topoisomerase poisons as antitumor drugs. Annu Rev Biochem 1989;58:351–74.

20. Petrangolini G, Pratesi G, De Cesare M, et al. Antiangiogenic effects of the novel camptothecin ST1481 (Gimatecan) in human tumor xenografts. Mol Cancer Res 2003;1:863–70.

21. Schirner M. Antiangiogenic chemotherapeutic agents. Cancer Metastasis Rev 2000;19:67–73.

22. Friedman HS, Houghton PJ, Schold SC, Keir S, Bigner DD. Activity of 9-dimethylaminomethyl-10-hydroxycamptothecin against pediatric and adult central nervous system tumor xenografts. Cancer Chemother Pharmacol 1994;34:171–4.

23. Hare CB, Elion GB, Houghton PJ, et al. Therapeutic efficacy of the topoisomerase I inhibitor 7-ethyl-10-(4-[1-piperidino]-1-piperidino)-carbonyloxy-camptothecin against pediatric and adult central nervous system tumor xenografts. Cancer Chemother Pharmacol 1997;39:187–91.

24. Friedman HS, Kerby T, Fields S, et al. Topotecan treatment of adults with primary malignant glioma. The Brain Tumor Center at Duke. Cancer (Phila.) 1999;85:1160–5.

25. Turner CD, Gururangan S, Eastwood J, et al. Phase II study of irinotecan (CPT-11) in children with high-risk malignant brain tumors: the Duke experience. Neuro-oncol 2002;4:102–8.

26. Alderson L, Supko J, Maestri X, et al. Phase I/II trial of gimatecan in patients with recurrent malignant glioma. Proc Am Soc Clin Oncol 2003;22:416.

27. Douglas JG, Margolin K. The treatment of brain metastases from malignant melanoma. Semin Oncol 2002;29:518–24.

28. Fidler IJ. Critical determinants of metastasis. Cancer Biol 2002;12:89–96.

29. Laccabue D, Tortoreto M, Veneroni S, et al. A novel taxane active against an orthotopically growing human glioma xenograft. Cancer (Phila.) 2001;92:3085–92.

30. Lee G, Dallas S, Hong M, Bendayan R. Drug transporters in the central nervous system: brain barriers and brain parenchyma considerations. Pharmacol Rev 2001;53:569–96.

31. Powers CJ, McLeskey SW, Wellstein A. Fibroblast growth factors, their receptors and signalling. Endocr Relat Cancer 2000;7:165–97.

32. Gianni L, Hess D, Baselga J, et al. A phase I study of the oral camptothecin gimatecan with a design of concerted dose escalation in three schedules of different dosing-duration. Proc Am Soc Clin Oncol 2003;22:554.

33. Hochster H, Wadler S, Runowicz C, et al. Activity and pharmacodynamics of 21-day topotecan infusion in patients with ovarian cancer previously treated with platinum-based chemotherapy. New York Gynecologic Oncology Group. J Clin Oncol 1999;17:2553–61.

34. Verschraegen CF, Jaeckle K, Giovanella B, Knight V, Gilbert BE. Alternative administration of camptothecin analogues. Ann NY Acad Sci 2000;922:237–46.

35. Chasty RC, Liu-Yin JA. Acute tumour lysis syndrome. Br J Hosp Med 1993;49:488–92.

36. Nicoletto MO, Padrini R, Palumbo M, et al. Intralesional topotecan in advanced ovarian cancer: a clinical report, based on a preclinical study. Oncol Rep 2002;9:1351–4.