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Preclinical report

Preclinical activity of an i.v. formulation of rubitecan in IDD-P™ against human solid tumor xenografts

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An i.v. formulation of rubitecan (9-nitrocamptothecin) was evaluated in five human solid tumor xenograft models. Rubitecan in IDD-P™, a particulate suspension of the insoluble analog, produced significant tumor growth delay in athymic nude mice bearing A375 melanoma, and MX-1 breast, SKMES non-small-cell lung, Panc-1 pancreatic and HT29 colon carcinomas. The activity of i.v. rubitecan was similar or somewhat superior to those of i.p. regimens with the reference drugs, irinotecan and topotecan. Tumor sensitivity to rubitecan in IDD-P was MX-1 > A375 > SKMES > Panc-1 > HT29. Some complete regression responses were seen with MX-1, A375 and SKMES tumors treated with 2.5 mg/kg on a schedule of two 5-day dosing cycles separated by 2 drug-free days. In nude mice, the MTD of rubitecan in IDD-P lies between 2 and 2.5 mg/kg on this schedule; antitumor efficacy was achieved with doses between 2.5 and 1.25 mg/kg. Dosing with 6.6 mg/kg rubitecan in IDD-P on intermittent schedules (4- or 7-day intervals) was tolerated, but less efficacious, when tested in the A375 model. The good responses obtained with rubitecan in IDD-P suggest it could be used clinically in circumstances where an i.v. formulation offers advantages to oral or aerosol formulations. [© 2002 Lippincott Williams & Wilkins.]

Key words: Carcinoma, intravenous, melanoma, rubitecan, xenograft.

Introduction

Camptothecin [20(*S*)-camptothecin (CPT)], the parent compound of rubitecan [20(*RS*)-9-nitroCPT; Orathecin], was identified as an antitumor agent in 1966 by Wall *et al.*[1] After its salt form demonstrated clinical toxicity with little antitumor effect, CPT was abandoned until interest was revived by the discovery of its mechanism of action.[2] CPT interacts specifically with the enzyme topoisomerase I (Topo I), which relieves torsional strain in DNA by inducing reversible single-strand breaks. CPT binds to the DNA–Topo I complex and prevents religation of these single-strand breaks. Cytotoxicity may be incurred when replication enzymes, interacting with the CPT–DNA–Topo I complex, produce double-strand DNA damage.[3] Mammalian cells cannot efficiently repair such double-strand breaks.

This unique mechanism of action prompted the development of CPT derivatives, and two water-soluble compounds, irinotecan and topotecan, have been approved for medical use in the US. The excellent response of human tumor xenografts to CPT and 20(*RS*)-9-aminoCPT (9-AC)[4] stimulated further interest in some of the insoluble CPTs. Both 9-AC and rubitecan had been partially synthesized and evaluated by Wani *et al.*[5] The finding that a small amount (approximately 6%) of rubitecan converts to 9-AC in humans[6] has suggested the possibility that rubitecan is a prodrug for 9-AC. Rubitecan is, however, a highly potent and direct inhibitor of the DNA-religating activity of purified Topo 1.[7] Studies on a tumor cell subline, selected for resistance to rubitecan, confirm Topo I as the specific cellular target. The subline expressed a mutant Topo I bearing a single amino acid residue substitution, while its Topo I catalytic activity was similar to that of the parental cells.[8] Partial cross-resistance to the parent compound, CPT, was observed. These results parallel those obtained with a cloned Topo I cDNA, in which a single point mutation conferred resistance to CPT.[9] Thus, rubitecan possesses intrinsic Topo I inhibiting activity, which does not depend on

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conversion to 9-AC. Rubitecan, rather than 9-AC, was chosen for further development because it has equivalent antitumor efficacy, but maintains a higher percentage of the active lactone form in human plasma.[10,11]

In athymic mice, oral administration of rubitecan resulted in 100% growth inhibition of 30 different human tumor xenografts (lung, colorectal, breast, pancreatic, ovarian, prostate, stomach, melanoma and leukemia).[12] In contrast, i.v. administration has yielded poor and variable responses, and there are no detailed reports of its i.v. antitumor efficacy. Based on these findings, oral rubitecan is now undergoing clinical trials for the treatment of pancreatic cancer and other chemotherapy-resistant solid and hematologic tumors. An aerosol formulation has also entered clinical trials.

Concurrent with these development efforts is the exploration of an i.v. formulation of rubitecan that might, under certain conditions, provide therapeutic advantages over the oral formulation. The candidate vehicles considered for i.v. administration contain IDD-P™ (Insoluble Drug Delivery MicroParticle), which provides a particulate suspension of rubitecan, and IDD-D™ (Insoluble Drug Delivery MicroDroplet), which provides a droplet suspension. In the studies reported here, the IDD-P and IDD-D formulations were both tested for *in vivo* activity against A375 human melanomas in athymic mice. Based on its higher activity in the initial test, the IDD-P formulation was selected for further evaluation in human breast, lung, pancreatic and colon tumor models.

Materials and methods

Materials

Rubitecan in IDD-P (2 mg/ml; mean particle size, 1.29–1. 3 μm) and IDD-P vehicle (0.07–0.06 μm), and rubitecan in IDD-D (0.2 mg/ml; 0.10–0.2 μm) and IDD-D vehicle (0.15 μm) were prepared for these studies by RTP Pharma (now known as SkyePharma Canada, Nuns Island, Quebec, Canada). Rubitecan in IDD-P and its vehicle were diluted 1:3 with water to render them isotonic. Further dilutions were made with the isotonic vehicle. IDD-D was used undiluted and at 1:2 dilution with 5% dextrose in water, pH ~4.8 (D_5W) for the 2 and 1 mg/kg doses, respectively. After dilution, rubitecan suspensions were stored at 2–8°C. For its oral formulation, rubitecan was dissolved in DMA (*N*,*N*-dimethylacetamide; Sigma, St Louis, MO) and diluted with D_5W to yield a final DMA concentration of 3%.

Irinotecan (Camptosar; Pharmacia & Upjohn, Kalamazoo, MI) and dacarbazine (DTIC-Dome; Bayer, West Haven, CT) were diluted with D_5W. Topotecan (Hycamtin; SmithKlein Beecham, Philadelphia, PA) was reconstituted with water. Fresh solutions were prepared each day of dosing.

Toxicity evaluation

The maximum tolerated dose (MTD) for rubitecan in IDD-P and IDD-D was determined in female athymic nude mice (*nu/nu*, Harlan) as a dose that produced less than 20% group mean body-weight loss and no deaths among five animals. Body weight was monitored twice per week during the 28-day studies. Mice were treated i.v. (0.2 ml/20 g body weight) with rubitecan in IDD-D or with rubitecan in IDD-P on a 5/2/5 (5 days on, 2 days off and 5 days on) or qd × 5 (five consecutive daily doses) schedule. The studies were conducted under an AAALAC-accredited program.

Human xenograft models

The human cell lines, HT29 colon, MX-1 breast, Panc-1 pancreatic and SKMES non-small-cell lung carcinomas, and A375 melanoma were obtained from ATCC (Rockville, MD). Each nude mouse received a 1-mm^3 tumor fragment as an s.c. implant in the left flank. Tumor growth was monitored twice weekly by caliper measurements. Tumor volume was estimated using the formula: volume (mm^3) = $(w^2 \times l)/2$, in which w = width and l = length in mm of the tumor, and converted to tumor weight in mg, assuming a tumor density of 1 mg/mm^3. When the tumors exceeded 60 mg, mice were sorted to give nearly identical group mean tumor sizes, within the 71–105 mg range, and treatment was started. This was day 1 of each study. All treatments were body-weight adjusted at 0.2 ml/20 g.

Antitumor efficacy

An animal was euthanized when its tumor reached a designated endpoint size. Based on the tumor growth rates, the following endpoints were selected: 1.0 g, HT29; 1.2 g, Panc-1; 1.5 g, MX-1 and SKMES; and 2.0 g, A375. The mean time (in days) for tumors

in a treatment group to reach the endpoint size was based on the calculated time for the tumor in each mouse to reach the endpoint weight, which is given by the formula: time to endpoint (calculated)=time to endpoint (observed) – {[Wt_2 – endpoint weight]/[(Wt_2 – Wt_1/D_2 – D_1)]}, where D_2 is the day the tumor weight exceeded the endpoint, D_1 is the day of the last caliper measurement before the weight exceeded the endpoint, and Wt_2 and Wt_1 are the tumor weights on D_2 and D_1, respectively.

Tumor growth delay (TGD) is defined as the percentage increase in the mean time to endpoint for mice in a treatment group compared to that of the control group. Therapeutic responses in animals alive at the end of the studies (after approximately 2 months) were classified as complete regression (CR), partial regression (PR) and stable disease/progressive disease (SD/PD). CR indicates that the tumor was not detected and PR refers to a tumor size lower than on day 1. SD/PD indicates a stable or progressively increasing tumor size near the end of the study.

Statistics

The unpaired *t*-test was used to determine the statistical significance of any difference in the mean time to endpoint values of a treatment group and the control group. The *t*-test was performed with Welch's correction when the variances differed significantly. Log-rank tests were used to compare the overall survival of two groups of mice. Survival data consist of the times of treatment-related death(s), the times at which individual mice reached the endpoint and (if the endpoint was not reached) the time to the end of the study. Thus, log-rank analysis includes long-term survivors and treatment-related deaths, which are excluded from data analyzed by the *t*-test, but does not distinguish among the treatment responses (CR, PR and SD/PD) of the survivors. Analyses for statistical significance were conducted at $p=0.05$ (two-tailed), using Prism (GraphPad) version 3.

Results

Safety of i.v. rubitecan formulated in IDD-P and IDD-D in nude mice

Preliminary studies were conducted to determine the safety of the IDD-P and IDD-D vehicles, and the MTD of i.v. rubitecan in these vehicles. IDD-P required 1:3 dilution with water to render it isotonic, whereas IDD-D is an isotonic vehicle. Administration of 1:3 and 1:6 diluted IDD-P, and of undiluted and 1:2 diluted IDD-D, produced no toxicity in nude mice (data not shown). A second study determined the MTD of rubitecan in each of these vehicles. Rubitecan in IDD-P, a particulate suspension, and rubitecan in IDD-D, a droplet suspension, produced no toxicity on a 5/2/5 schedule at 2 and 1 mg/kg doses (data not shown). Rubitecan in IDD-P was also tolerated on a qd × 5 schedule at 6 and 4 mg/kg doses, with acceptable maximum body weight losses of around 17% (data not shown). Higher doses of rubitecan in IDD-D were not feasible.

Activity of rubitecan in IDD-P and IDD-D against A375 melanoma xenografts: effects of i.v. dosage, i.v. schedule and the route of administration

The rubitecan suspensions in IDD-P and IDD-D were initially evaluated for i.v. activity against A375 melanoma xenografts on a 5/2/5 schedule. The treatment regimens and their results are summarized in Table 1. Based on the preliminary toxicity screen, the IDD-P formulation was tested at 3 and 1.5 mg/kg doses. At 3 mg/kg, rubitecan in IDD-P exceeded its MTD. At 1.5 mg/kg (15 mg total dose/kg), rubitecan in IDD-P produced a significant TGD of 67%, relative to the vehicle-treated mice and three 62-day survivors (two CR and one SD/PD).

At its maximum feasible dosage, 2 mg/kg (20 mg total dose/kg), rubitecan in IDD-D yielded a significant TGD of 56% relative to the vehicle-treated mice and two survivors (one CR and one PR). Figure 1 shows that 2 mg/kg doses of rubitecan in IDD-D were less therapeutic than 1.5 mg/kg doses of the particulate IDD-P formulation. At 1 mg/kg doses, the rubitecan in IDD-D droplet formulation was ineffective (Table 1).

The i.v. rubitecan regimens were compared to several reference treatments: oral therapies with rubitecan in 3% DMA at two dosing levels, and i.p. therapies with irinotecan, topotecan and dacarbazine (Table 1). The two oral rubitecan regimens (4 mg/kg and 2 mg/kg, q3d × 4, total doses of 16 and 8 mg/kg, respectively) produced TGD values of 50–57% and seven long-term survivors. Each produced four CR responses. The soluble CPTs, irinotecan (100 mg/kg, qwk × 3) and topotecan (10 mg/kg, q4d × 4), gave significant TGD values of 54–56% relative to untreated mice and each produced a single CR response. Dacarbazine (150 mg/kg, qd × 5), a first-line drug for clinical treatment of melanoma, was the least effective reference agent. Log-rank analysis

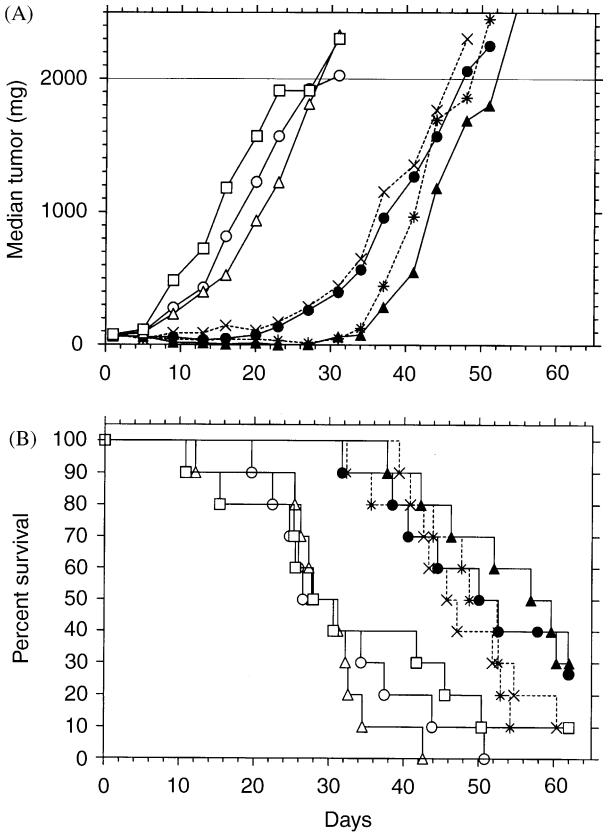

Figure 1. Responses of A375 human melanoma xenografts to therapy with i.v. formulations of rubitecan in IDD-P and IDD-D. (A) Median tumor growth curves and (B) Kaplan–Meier survival curves of mice that received: no treatment (□), IDD-P vehicle i.v. on a 5/2/5 schedule (△), IDD-D vehicle i.v. on a 5/2/5 schedule (○), rubitecan in the IDD-P vehicle i.v. on a 5/2/5 schedule at 1.5 mg/kg (▲), rubitecan in the IDD-D vehicle i.v. on a 5/2/5 schedule at 2 mg/kg (●), irinotecan i.p. on a qwk × 3 schedule at 100 mg/kg (×) and topotecan i.p. on a q4d × 4 schedule at 10 mg/kg (*).

(Table 1) of overall survival in treated versus untreated mice indicates that the oral rubitecan therapies produced highly significant increases, the 1.5 mg/kg i.v. doses of rubitecan in IDD-P produced a significant increase, and the irinotecan, topotecan and dacarbazine therapies did not yield significant increases.

Based on its superiority over the droplet i.v. formulation in the first evaluation, the particulate i.v. formulation was selected for further study. Groups of nine A375 melanoma-bearing mice were used to determine how dosing schedules influence the antitumor activity of i.v. rubitecan in IDD-P. A dosing regimen of 2.5 mg/kg on a 5/2/5 schedule was compared to a single 6.6 mg/kg dose (the highest dose that is feasible in this vehicle) and to 6.6 mg/kg doses administered on two intermittent schedules (Table 2). The 5/2/5 schedule produced a TGD of 97.1% and three survivors (one CR and two SD/PD). The single 6.6 mg/kg dose had no therapeutic effect. Four 6.6 mg/kg doses at 4-day (q4d × 4) or weekly (qwk × 4) intervals were less effective than the lower doses on the 12-day 5/2/5 schedule, even though the total dosage was slightly higher on the intermittent schedules (26.5 versus 25 mg/kg). The intermittent regimens produced fewer survivors and TGD values of 59–63%. These results suggest that rubitecan concentrations dropped to ineffectual levels between the doses of the 13- and 22-day intermittent regimens.

The same study determined how the route of administration influences the activity of rubitecan in IDD-P. The same formulation was administered to groups of mice by the s.c. and p.o. routes at 2.5 mg/kg on a 5/2/5 schedule (Table 2). The oral regimen was well tolerated and yielded eight survivors (three CR and five SD/PD). In contrast, the s.c. regimen produced severe toxicity that resulted in the death or euthanasia of all animals.

Activity of i.v. rubitecan in IDD-P against human breast, lung, colon and pancreatic tumor xenografts

Rubitecan in IDD-P was evaluated in several human xenograft models in order to determine the sensitivity of different tumor types to the i.v. therapy. Rubitecan was administered at 2.5, 1.75 and 1.25 mg/kg on the 5/2/5 schedule. Irinotecan and topotecan were given on the same regimens used in the A375 study.

The MX-1 human breast carcinoma provided a moderately fast-growing hormone-independent target that is very sensitive to therapy with cytotoxic agents. All treatments, except the lowest dose of rubitecan in IDD-P, produced 100% 60-day survival, whereas the mean time to 1.5 g tumor size in untreated mice was 24.8 days (Table 3). At 2.5 and 1.75 mg/kg doses, rubitecan in IDD-P therapy appeared somewhat superior to the two reference treatments, based on the higher numbers of CR responses and the profound suppression of tumor growth in all animals (Figure 2). At 1.25 mg/kg, i.v. rubitecan produced a TGD of 98.4% and four survivors, including two CR responses.

SKMES non-small cell lung carcinoma xenografts were responsive to all the CPTs, which produced highly significant TGD values of 79–121% (Table 4). All dosages of rubitecan in IDD-P suppressed tumor

Table 1. Activity of i.v. rubitecan formulated in IDD-P and IDD-D in A375 melanoma growth-bearing nude mice[a]

Treatment regimen				Time to 2 g (days)		TGD (%)[b]	*t*-test[c]	Day 67 survivors			Log-rank test[c]	Maximum % BW loss	Death
Agent	mg/kg	Route	Schedule	Mean ± SEM	(*n*)			CR	PR	SD/PD			
No treatment	NA[d]	NA	NA	30.3 ± 4.4	(9)			0	0	1		–	
IDD-P vehicle	NA	i.v.	5/2/5[f]	29.2 ± 2.5	(10)	–	NS	0	0	0	NS	–	
IDD-D vehicle	NA	i.v.	5/2/5	31.6 ± 3.2	(10)	4.3	NS	0	0	0	NS	0.40	
D_5 W–3% DMA vehicle	NA	p.o.	q3d × 4	26.0 ± 2.3	(8)	–	NS	1	0	0	NS	–	1[e]
Rubitecan in IDD-P	3	i.v.	5/2/5	52.0 ± 0.6	(2)	71.6	NS	1	3	1	NS	13.1	3
Rubitecan in IDD-P	1.5	i.v.	5/2/5	50.7 ± 3.4	(7)	67.3	**	2	0	1	*	2.2	
Rubitecan in IDD-D	2	i.v.	5/2/5	47.2 ± 3.6	(8)	55.8	*	1	1	0	NS	–	
Rubitecan in IDD-D	1	i.v.	5/2/5	32.6 ± 2.3	(10)	7.6	NS	0	0	0	NS	–	
Rubitecan in D_5 W–3% DMA	4	p.o.	q3d × 4	45.3 ± 7.0	(3)	49.5	NS	4	0	3	**	–	
Rubitecan in D_5 W–3% DMA	2	p.o.	q3d × 4	47.6 ± 3.4	(3)	57.1	NS	4	1	2	***	–	
Irinotecan	100	i.p.	qwk × 3	47.3 ± 2.3	(9)	56.1	**	1	0	0	NS	–	
Topotecan	10	i.p.	q4d × 4	46.7 ± 2.6	(9)	54.1	**	1	0	0	NS	–	
Dacarbazine	150	i.p.	qd × 5	37.6 ± 4.0	(8)	24.1	NS	1	0	0	NS	5.2	1

[a] Treatments were initiated on day 1 of a 67-day study. Treatment group = 10 mice.
[b] TGD values calculated with respect to untreated mice.
[c] *p* values: NS, > 0.05, *, < 0.05; **, < 0.01; ***, < 0.001.
[d] Not applicable.
[e] Non-treatment-related death.
[f] Dosing schedule of 5 days on, 2 days off and 5 days on.

Table 2. Effect of different routes of administration and schedules on the activity of i.v. rubitecan formulated in IDD-P in A375 melanoma growth-bearing nude mice[a]

Treatment regimen				Time to 2 g (days)		TGD (%)[b]	*t*-test[c]	Day 61 survivors			Log-rank test[c]	Maximum % BW loss	Death
Agent	mg/kg	Route	Schedule	Mean ± SEM	(*n*)			CR	PR	SD/PD			
No treatment	NA[d]	NA	NA	24.3 ± 2.7	(9)			0	0	0		–	
IDD-P vehicle	NA	i.v.	5/2/5[e]	22.9 ± 2.2	(7)	–	NS	0	0	2	NS	–	
Rubitecan in IDD-P	2.5	i.v.	5/2/5	47.9 ± 3.4	(6)	97.1	***	1	0	2	***	–	
Rubitecan in IDD-P	2.5	s.c.	5/2/5			–	NA	0	0	0	NA	23.4	9
Rubitecan in IDD-P	2.5	p.o.	5/2/5	45.9	(1)	88.9	NA	3	0	5	***	–	
Rubitecan in IDD-P	6.6	i.v.	qd × 1	26.5 ± 3.2	(8)	9.1	NS	0	1	0	NS	–	
Rubitecan in IDD-P	6.6	i.v.	q4d × 4	38.7 ± 4.1	(7)	59.3	**	0	0	1	***	–	1
Rubitecan in IDD-P	6.6	i.v.	qwk × 4	39.6 ± 4.1	(9)	63.0	**	0	0	0			

[a]Treatments were initiated on day 1 of a 61-day study. Treatment group = 9 mice.
[b]TGD values calculated with respect to untreated mice.
[c] *p* values: NS, > 0.05; **, < 0.01; ***, < 0.001.
[d]Not applicable.
[e]Dosing schedule of 5 days on, 2 days off and 5 days on.

Table 3. Activity of i.v. rubitecan formulated in IDD-P against MX-1 human breast carcinoma in nude mice[a]

Treatment regimen				Time to 1.5 g (days)		TGD (%)[b]	*t*-test[c]	Day 60 survivors			Log-rank test[c]	Maximum % BW loss	Death
Agent	mg/kg	Route	Schedule	Mean ± SEM	(*n*)			CR	PR	SD/PD			
No treatment	NA[d]	NA	NA	24.8 ± 1.2	(10)			0	0	0		–	–
IDD-P vehicle	NA	i.v.	5/2/5[e]	24.4 ± 1.3	(10)	–	NS	0	0	0	NS	–	–
Rubitecan in IDD-P	2.5	i.v.	5/2/5	NA		NA	NA	5	1	4	***	–	–
Rubitecan in IDD-P	1.75	i.v.	5/2/5	NA		NA	NA	6	1	3	***	–	–
Rubitecan in IDD-P	1.25	i.v.	5/2/5	49.2 ± 2.6	(6)	98.4	***	2	1	1	***	–	–
Irinotecan	100	i.p.	qwk × 3	NA		NA	NA	3	2	5	***	–	–
Topotecan	10	i.p.	q4d × 4	NA		NA	NA	2	1	7	***	–	–

[a]Treatments were initiated on day 1 of a 60-day study. Treatment group = 10 mice.
[b]TGD values calculated with respect to untreated mice.
[c] *p* values: NS, > 0.05; ***, < 0.001.
[d]Not applicable.
[e]Dosing schedule of 5 days on, 2 days off and 5 days on.

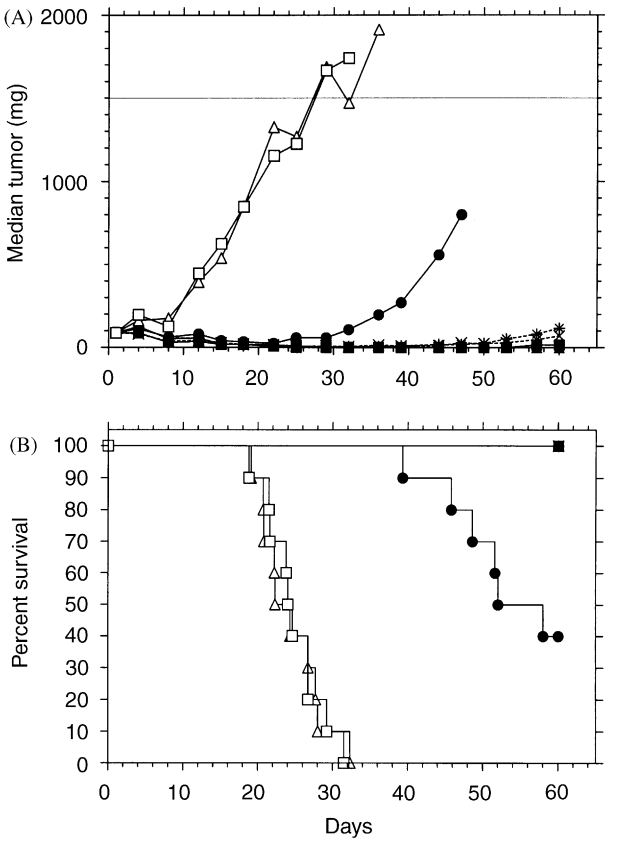

Figure 2. Effects of i.v. rubitecan in IDD-P on tumor growth and survival in nude mice bearing MX-1 human breast carcinomas. (A) Median tumor growth curves and (B) Kaplan–Meier survival curves of mice that received: no treatment (□), IDD-P vehicle i.v. on a 5/2/5 schedule (○), rubitecan in IDD-P i.v. on a 5/2/5 schedule at 2.5 (■),1.75 (▲) and (●) 1.25 mg/kg, irinotecan i.p. on a qwk × 3 schedule at 100 mg/kg (×), and topotecan i.p. on a q4d × 4 schedule at 10 mg/kg (*).

growth (Figure 3A) and caused highly significant increases in overall survival (Figure 3B). The strongest antitumor effect occurred with 1.25 mg/kg doses, which yielded three CR responses. Irinotecan and topotecan produced two survivors (one CR and one SD/PD) and one survivor (CR), respectively.

In the first study using the human Panc-1 pancreatic carcinoma model (Table 5 and Figure 4), therapy with 2.5 mg/kg rubitecan in IDD-P suppressed tumor growth and yielded a highly significant TGD of 68.2%. This response compared favorably with the TGD values of 37.2 and 44.8% that were obtained with irinotecan and topotecan, respectively. In a second study (Table 5), the same dose produced a TGD value of 102.5%, but caused two treatment-related deaths among 10 animals. In both Panc-1 experiments the lower i.v. rubitecan doses produced

Table 4. Activity of i.v. rubitecan formulated in IDD-P against SKMES human non-small cell lung carcinoma in nude mice[a]

Treatment regimen				Time to 1.5 g (days)		TGD (%)[b]	*t*-test[c]	Day 61 survivors			Log-rank test[c]	Maximum % BW loss	Death
Agent	mg/kg	Route	Schedule	Mean ± SEM	(*n*)			CR	PR	SD/PD			
No treatment	NA[d]	NA	NA	16.4 ± 1.1	(10)			0	0	0		–	–
IDD-P vehicle	NA	i.v.	5/2/5[e]	19.5 ± 1.3	(10)	18.9	NS	0	0	0	*	–	–
Rubitecan in IDD-P	2.5	i.v.	5/2/5	33.1 ± 1.7	(10)	101.8	***	0	0	0	***	–	–
Rubitecan in IDD-P	1.75	i.v.	5/2/5	35.1 ± 2.4	(10)	114.0	***	0	0	0	***	–	–
Rubitecan in IDD-P	1.25	i.v.	5/2/5	29.3 ± 1.7	(7)	78.7	***	3	0	0	***	–	–
Irinotecan	100	i.p.	qwk × 3	32.7 ± 1.3	(8)	99.4	***	1	0	1	***	–	–
Topotecan	10	i.p.	q4d × 4	36.2 ± 2.1	(9)	120.7	***	1	0	0	***	–	–

[a]Treatments were initiated on day 1 of a 61-day study. Treatment group = 10 mice.
[b]TGD values calculated with respect to untreated mice.
[c]*p* values: NS, > 0.05; *, < 0.05; ***, < 0.001.
[d]Not applicable.
[e]Dosing schedule of 5 days on, 2 days off and 5 days on.

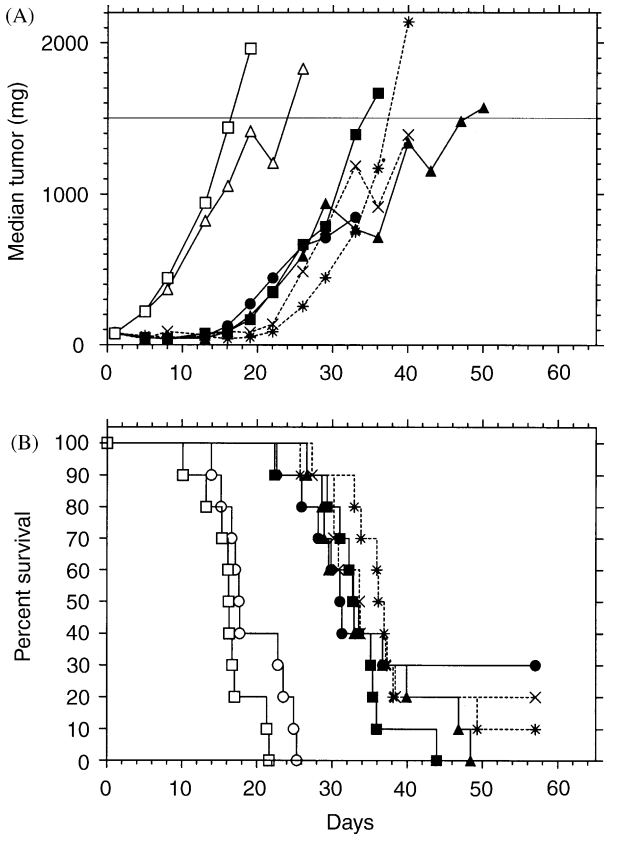

Figure 3. Activity of i.v. rubitecan in IDD-P against SKMES lung carcinoma xenografts. (A) Median tumor growth curves and (B) Kaplan–Meier survival curves of mice that received: no treatment (□), IDD-P vehicle i.v. on a 5/2/5 schedule (○), rubitecan in IDD-P i.v. on a 5/2/5 schedule at 2.5 (■),1.75 (▲) and 1.25 (●) mg/kg, irinotecan i.p. on a qwk × 3 schedule at 100 mg/kg (×), and topotecan i.p. on a q4d × 4 schedule at 10 mg/kg (*).

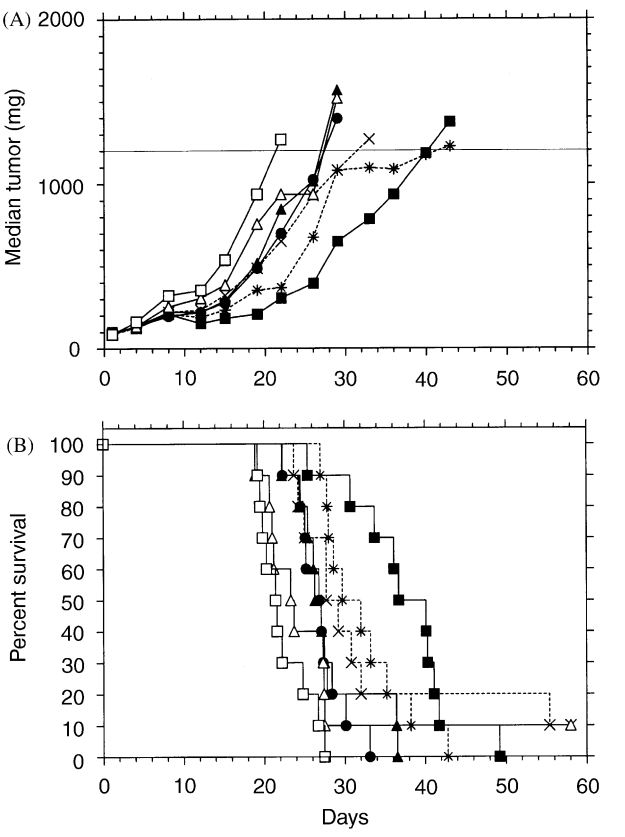

Figure 4. Activity of i.v. rubitecan in IDD-P against Panc-1 lung carcinoma xenografts. (A) Median tumor growth curves and (B) Kaplan–Meier survival curves of mice that received: no treatment (□), IDD-P vehicle i.v. on a 5/2/5 schedule (○), rubitecan in IDD-P i.v. on a 5/2/5 schedule at 2.5 (■),1.75 (▲) and 1.25 (●) mg/kg, irinotecan i.p. on a qwk × 3 schedule at 100 mg/kg (×), and topotecan i.p. on a q4d × 4 schedule at 10 mg/kg (*).

smaller, but statistically significant TGD values. The PR and SD/PD responses in both Panc-1 studies probably represent somewhat poor tumor takes, since they occurred with similar low frequencies in the control, vehicle-treated and drug-treated groups.

Although HT29 colon carcinomas were weakly responsive to the CPTs, all therapies caused significant increases in overall survival, based on log-rank analysis (Table 6). The i.p. reference treatments produced significant TGD values of 33–40%, while the rubitecan in IDD-P therapies yielded somewhat lower but significant TGD values of 27–28%. There was one treatment-related death among nine animals that received 2.5 mg/kg i.v. rubitecan. The two lower i.v. rubitecan doses caused no toxicity, and produced two and one survivors, respectively, each of which represented an SD/PD response. All tumors in other treatment groups reached the 1-g endpoint.

Discussion

Although oral and aerosol formulations of rubitecan are currently undergoing clinical trials, no i.v. formulation of rubitecan, or other lipophilic CPTs, is available for clinical testing. The low therapeutic index of i.v. sodium CPT in clinical trials, as well as the low and variable activity of i.v. CPT analogs in prior preclinical tests, discouraged the development of i.v. CPT therapy. Therefore, two novel formulations of rubitecan were prepared and evaluated for i.v. activity against human tumor xenografts. Rubitecan in IDD-P, a particulate suspension, with a greater capacity and larger particle size than a droplet suspension of rubitecan in IDD-D, proved more efficacious in the initial study with mice bearing A375 melanomas. In subsequent studies, i.v. rubitecan in IDD-P exhibited significant antitumor activity in

Table 5. Activity of i.v. rubitecan formulated in IDD-P against Panc-1 human pancreatic carcinoma in nude mice[a]

Treatment regimen				Time to 1.2 g (days)		TGD (%)[b]	*t*-test[c]	Day 58 survivors			Log-rank test[c]	Maximum % BW loss	Death
Agent	mg/kg	Route	Schedule	Mean ± SEM	(*n*)			CR	PR	SD/PD			
First study													
no treatment	NA[d]	NA	NA	22.3 ± 1.0	(10)			0	0	0		–	–
IDD-P vehicle	NA	i.v.	5/2/5[e]	23.4 ± 1.1	(9)	4.9	NS	0	0	1	NS	4.9	–
rubitecan in IDD-P	2.5	i.v.	5/2/5	37.5 ± 2.1	(10)	68.2	***	0	0	0	***	–	–
rubitecan in IDD-P	1.75	i.v.	5/2/5	28.0 ± 1.5	(10)	25.6	**	0	0	0	**	–	–
rubitecan in IDD-P	1.25	i.v.	5/2/5	27.0 ± 1.0	(10)	21.1	**	0	0	0	**	–	–
irinotecan	100	i.p.	qwk × 3	30.6 ± 3.2	(9)	37.2	*	0	0	1	***	–	–
topotecan	10	i.p.	q4d × 4	32.3 ± 1.6	(10)	44.8	***	0	0	0	***	–	–
Second study													
no treatment	NA	NA	NA	23.5 ± 1.2	(9)			0	1	0		–	–
IDD-P vehicle	NA	i.v.	5/2/5[e]	31.5 ± 3.2	(8)	41.3	*	0	1	1	NS	–	–
rubitecan in IDD-P	1.25	i.v.	5/2/5	47.6 ± 2.3	(6)	113.5	***	0	1	1	NS	10.5	2
rubitecan in IDD-P	2.5	i.v.	5/2/5	39.6 ± 2.6	(9)	77.6	***	0	0	1	*	–	–

[a]Treatments were initiated on day 1 of a 58-day study. Treatment group = 10 mice.
[b]TGD values calculated with respect to untreated mice.
[c]*p* values: NS, > 0.05; *, < 0.05; **, < 0.01; ***, < 0.001.
[d]Not applicable.
[e]Dosing schedule of 5 days on, 2 days off and 5 days on.

Table 6. Activity of i.v. rubitecan formulated in IDD-P against HT-29 human non-small cell lung carcinoma in nude mice[a]

Treatment regimen				Time to 1 g (days)		TGD (%)[b]	*t*-test[c]	Day 64 survivors			Log-rank test[c]	Maximum % BW loss	Death
Agent	mg/kg	Route	Schedule	Mean ± SEM	(*n*)			CR	PR	SD/PD			
No treatment	NA[d]	NA	NA	28.0 ± 1.9	(8)			0	0	0		–	–
IDD-P vehicle	NA	i.v.	5/2/5[e]	31.6 ± 1.4	(8)	12.9	NS	0	0	0	NS	–	–
Rubitecan in IDD-P	2.5	i.v.	5/2/5	36.1 ± 2.1	(8)	28.9	*	0	0	0	**	5.7	1
Rubitecan in IDD-P	1.75	i.v.	5/2/5	35.6 ± 1.2	(8)	27.1	**	0	0	1	**	–	–
Rubitecan in IDD-P	1.25	i.v.	5/2/5	36.0 ± 2.7	(7)	28.6	*	0	0	2	*	–	–
Irinotecan	100	i.p.	Qwk × 3	39.1 ± 2.1	(9)	39.6	**	0	0	0	***	–	–
Topotecan	10	i.p.	Q4d × 4	37.2 ± 1.9	(9)	32.9	**	0	0	0	**	–	–

[a]Treatments were initiated on day 1 of a 64-day study. Treatment group = 8–9 mice.
[b]TGD values calculated with respect to untreated mice.
[c]*p* values: NS, > 0.05; *, < 0.05; **, < 0.01; ***, < 0.001.
[d]Not applicable.
[e]Dosing schedule of 5 days on, 2 days off and 5 days on.

human breast, lung, pancreatic and colon tumor xenograft models.

Intravenous rubitecan was well tolerated at doses of 6.6 mg/kg on intermittent schedules (q4d × 4 and qwk × 4), but these regimens were less active than doses ranging from 2.5 to 1.25 mg/kg on a 5/2/5 schedule. This indicates that the IDD-P formulation requires frequent dosing for antitumor efficacy. The limited 12-day dosing schedule used in the present studies demonstrates that i.v. rubitecan has significant activity against solid tumors. Longer dosing regimens might produce even stronger therapeutic effects. In earlier preclinical studies with rubitecan and other CPTs, three or more cycles of 5-day administration, separated by 2 drug-free days, increased the numbers of regression responses.[13,14] In the present studies, three toxic deaths were observed among a total of 68 nude mice treated with 2.5 mg/kg i.v. rubitecan in seven separate studies. Therefore, on the 5/2/5 schedule, doses slightly below 2.5 mg/kg may be safer in mouse xenograft models. The 1.75 and 1.25 mg/kg i.v. doses were well tolerated, and body-weight losses were negligible or absent. Rubitecan in IDD-P was also well tolerated orally, but was not suitable for s.c. administration in mice (Table 2). When 2.5 mg/kg was administered s.c. on a 5/2/5 schedule, all animals died or required euthanasia before dosing was completed.

The rank order of sensitivity of tumors to i.v. rubitecan in IDD-P was: MX-1 breast A375 ≫ melanoma ≅ SKMES squamous cell lung > Panc-1 pancreatic > HT29 colon. The responses in these five tumor models also exhibited different dose-dependence patterns. Both the 2.5 and the 1.75 mg/kg doses of rubitecan in IDD-P caused regression of MX-1 carcinomas and suppressed regrowth until after day 50 (Figure 2). The onset of SKMES tumor growth was suppressed by all doses of i.v. rubitecan while therapy was in progress (Figure 3). In the slower-growing HT-29 tumors, the three i.v. regimens failed to induce transient regression, but caused similar decreases in the slopes of the median tumor growth curves (Figure 5). The Panc-1 tumors exhibited a steeper dose–response relationship. The 2.5 mg/kg regimen was the only one to transiently suppressed tumor growth and it produced substantially higher TGD values than the two lower doses (Figure 4). The relative activities of i.v. rubitecan and the reference therapies also varied among the different tumor types. Rubitecan in IDD-P was somewhat more active against Panc-1, MX-1 and A375 tumors, whereas SKMES and HT29 carcinomas responded similarly to rubitecan and the reference treatments.

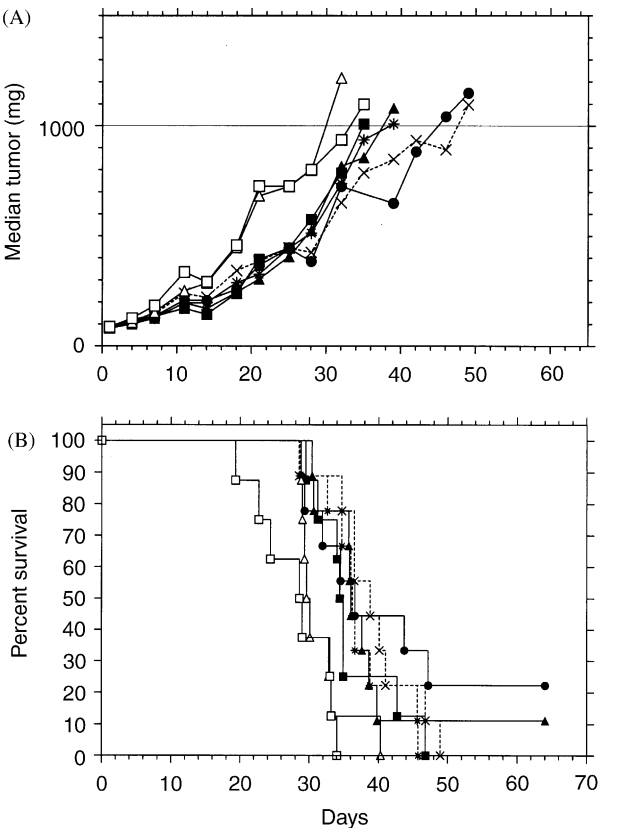

Figure 5. Activity of i.v. rubitecan in IDD-P against HT-29 lung carcinoma xenografts. (A) Median tumor growth curves and (B) Kaplan–Meier survival curves of mice that received: no treatment (□), IDD-P vehicle i.v. on a 5/2/5 schedule (○), rubitecan in IDD-P i.v. on a 5/2/5 schedule at 2.5 (■), 1.75 (▲) and 1.25 (●) mg/kg, irinotecan i.p. on a qwk × 3 schedule at 100 mg/kg (×), and topotecan i.p. on a q4d × 4 schedule at 10 mg/kg (*).

In mice, more than 50% of plasma rubitecan is in the active lactone form and oral rubitecan is highly effective against human tumor xenografts.[12] Accordingly, oral rubitecan in IDD-P was equi-efficacious with oral rubitecan in 3% DMA and produced a stronger therapeutic response in the A375 melanoma model than the i.v. rubitecan in IDD-P regimen. (Tables 1 and 2). It is hoped, however, that i.v. administration will provide advantages in humans, where the lactone represents less than 15% of the plasma drug concentration[10] and therapeutic levels are more difficult to maintain. With i.v. rubitecan, it may be feasible to increase the area under the plasma concentration curve and thus increase therapeutic efficacy. An i.v. formulation might also alter the pharmacokinetics and influence rubitecan's spectrum of activity. Furthermore, i.v. rubitecan could be used to prime patients for oral dosing or to treat patients who are too ill for oral medication.

In conclusion, an i.v. formulation of rubitecan in IDD-P was tested for activity in five human solid tumor xenograft models. The responses to i.v. rubitecan were similar and sometimes superior to those produced by i.p. reference treatments with the soluble CPTs, irinotecan and topotecan. These positive results provide the impetus for continued development of an i.v. rubitecan formulation that might reduce gastrointestinal tract toxicity and allow better clinical management of drug plasma levels.

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