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ANTI-TUMOR ACTIVITY OF CPT-11 IN EXPERIMENTAL HUMAN OVARIAN CANCER AND HUMAN SOFT-TISSUE SARCOMA

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CPT-11, a semi-synthetic derivative of camptothecin, was investigated for its activity in panels of 15 human ovarian-cancer lines and 10 human soft-tissue sarcoma lines grown s.c. in nude mice. Various factors were analyzed that may be of influence on the extent of tumor-growth inhibition induced by CPT-11. At equitoxic doses, CPT-11 was more effective in the daily ×5 schedule than the weekly ×2 schedule, although a 2-fold higher dose was administered in the weekly ×2 schedule. Since i.p. and i.v. injections were similarly effective, the selected treatment schedule was 20 mg/kg i.p. daily ×5, starting when tumors measured approximately 150 mm³. Growth inhibition of ≥75% was obtained in 8/15 human ovarian-cancer lines and in 6/10 human soft-tissue sarcoma lines. A weak correlation was found between topoisomerase-I mRNA in xenograft tissues and sensitivity to CPT-11. Relative topoisomerase-I expression was highest in MRI-H-207 and WLS-160 xenografts, in which CPT-11 was able to induce cures of all tumors. The high efficacy in the 2 panels of human tumor lines suggests over-prediction of its potential clinical activity in these tumor types. The difference in efficacy of CPT-11 between species may be related to the metabolism of the drug, since CPT-11 is converted more efficiently into SN-38 in mice. In addition, mice may be less sensitive to SN-38-induced side-effects. On the basis of the preclinical data, frequent administration of lower doses of CPT-11 should be considered in order to increase response rates in the clinic. *Int. J. Cancer* 73:891–896, 1997.

CPT-11 (irinotecan or 7-ethyl-10[4-(1-piperidino)-1-piperidino]-carbonyloxy camptothecin) is a water-soluble derivative of camptothecin, an inhibitor of the nuclear enzyme topoisomerase I. CPT-11 is a drug with limited activity, since to exert its action it needs to be converted into SN-38 (7-ethyl-10-hydroxy-camptothecin) *in vivo* by a carboxylesterase (Dancey and Eisenhauer, 1996). The metabolite SN-38 is more potent *in vitro* when compared with CPT-11. In 5 unselected human colon-cancer cell lines, SN-38 was found to be 130- to 570-fold more active than the parent compound (Jansen *et al.,* 1997). The anti-tumor activity of camptothecins both *in vitro* and *in vivo* is significantly greater for the lactone form than the carboxylate form. The closed lactone ring is important both for passive diffusion into the cell and for inhibition of the activity of topoisomerase I. Thus, factors of influence on the lactone-carboxylate equilibrium of camptothecins, such as the pH, will determine their biological activity (Dancey and Eisenhauer, 1996). At pH 7 or above, the lactone ring of the drug hydrolyses into the less active carboxylate form.

CPT-11, when given by i.p., i.v. or oral route, has shown substantial activity in a broad spectrum of mouse tumors (Kunimoto *et al.,* 1987). Impressive activity of the compound administered i.v. was measured in human tumor xenografts, such as colon-cancer and childhood-rhabdomyosarcoma xenografts (Houghton *et al.,* 1995). Growth inhibition was also obtained in pediatric and adult central-nervous-system xenografts when the drug was administered i.p. (Hare *et al.,* 1997). Clinical evaluation of CPT-11 has confirmed the therapeutic potential in a number of malignancies, particularly colorectal cancer, non-small-cell lung cancer and cervical cancer (Dancey and Eisenhauer, 1996).

Camptothecins are particularly toxic to cells in the S-phase of the cell cycle, although the cellular levels of topoisomerase I appear to be relatively constant during the phases of the cell cycle (Dancey and Eisenhauer, 1996). Therefore, increased activity may be expected by altering the treatment regimen. Houghton *et al.* (1995) have suggested that more potent topoisomerase-I inhibition may be achieved by using a low-dose protracted schedule, as demonstrated in human tumor xenografts. In other experimental tumor models, Bissery *et al.* (1996) have demonstrated that CPT-11 was not markedly schedule-dependent with respect to toxicity, as has been confirmed in humans.

We have established a panel of 15 human ovarian-cancer lines and a panel of 10 human soft-tissue-sarcoma lines grown as (s.c.) tumors in nude mice. The retention of the histological and antigenic characteristics of the tumor tissue of origin has been described (Boven *et al.,* 1989; Molthoff *et al.,* 1991). In the present experiments, CPT-11 was investigated for its activity in these human tumor xenografts, and various factors of possible influence on efficacy were analyzed. In the experiments, we first compared the growth inhibition induced by CPT-11 when given in a weekly ×2 or a daily ×5 schedule and when given i.p. or i.v. Thereafter, the best effective schedule of CPT-11 was studied in the 2 panels of human tumor xenografts. Topoisomerase-I mRNA expression was measured in the xenograft tissues, to determine whether a possible relation was present between gene content and the growth inhibition induced by CPT-11.

MATERIAL AND METHODS

Animals

Female nude mice (Hsd: athymic nude-*nu*) were purchased from Harlan CPB (Zeist, The Netherlands) at the age of 6 weeks. The animals were maintained in cages with paper filter covers, in controlled atmospheric conditions. Cages, covers, bedding, food and water were changed and sterilized weekly. Animals were handled in a sterile manner in a laminar down-flow hood.

Tumor lines

Human ovarian-cancer xenografts (Table I) and soft-tissue sarcoma xenografts (Table II) had been established earlier from fresh tumor tissue of patients or from *in vitro* cell lines. Ovarian-cancer xenografts (6) were generated from patients during second-look laparotomy after treatment, and 5 soft-tissue sarcoma xenografts were grown from progressive or recurrent disease during or after treatment. None of the patients had been given CPT-11. Other xenografts were established from untreated patients, except for 3 patients whose pre-treatment was unknown. Tumors were grown s.c. in both flanks. For transplantation, fragments with a diameter of 2 to 3 mm were implanted through a small skin incision which was closed with a metal clamp. The panels represent a variety of histological sub-types occurring in ovarian-cancer patients or soft-tissue sarcoma patients. In serial passages, the tumor lines retained their histological sub-type and a consistent growth rate.

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TABLE I – HUMAN OVARIAN-CANCER LINES

Tumor line	Histology	T_d[1]
Ov.Ri(C)	moderately differentiated serous	11.0
FKo	moderately differentiated serous	12.0
Ov.Gl	poorly differentiated serous	10.0
OVCAR-3[2]	poorly differentiated serous	8.0
Ov.Sh	poorly differentiated serous	15.0
Ov.Pe	moderately differentiated serous	8.0
Ov.He	moderately differentiated serous	9.0
Ov.Gr	moderately differentiated serous	15.0
FMa	poorly differentiated serous	5.5
FCo	clear cell carcinoma	6.5
Ov.Vg	clear cell carcinoma	8.0
Ov.Me	carcinosarcoma	6.0
MRI-H-207	undifferentiated	3.5
A2780[2]	undifferentiated	3.5
H134[2]	undifferentiated	11.0

[1]T_d, tumor volume doubling time (days).–[2]Established from cell line.

TABLE II – HUMAN SOFT-TISSUE-SARCOMA LINES

Tumor line	Histology	T_d[1]
S.Ba	liposarcoma	10.0
S.Lt	synovial sarcoma	11.0
S.Hh	leiomyosarcoma	19.5
S.La(C)	malignant fibrous histiocytoma	12.5
S.Hu	leiomyosarcoma	14.5
S.Ho	malignant fibrous histiocytoma	5.0
S.To	synovial sarcoma	14.5
S.Sin	neurofibrosarcoma	6.0
WLS-160[2]	liposarcoma	7.0
S.Zu	malignant fibrous histiocytoma	5.0

[1]T_d, tumor volume doubling time (days).–[2]Established from cell line.

Treatment and evaluation

CPT-11 as a solution of 20 mg/ml was kindly provided by Rhône-Poulenc Rorer (Vitry-sur-Seine, France) and was further diluted in NaCl 0.9%. Tumors were measured weekly or twice a week in 3 dimensions with a Vernier caliper by the same observer. The volume was calculated by the equation length × width × thickness × 0.5, and expressed in mm^3. At the start of treatment, groups of 6 tumor-bearing mice were formed to provide a mean tumor volume of approximately 150 mm^3 in each group (designated as day 0). Treatment experiments included the comparison of a weekly ×2 and daily ×5 schedule, as well as i.v. and i.p. administrations of CPT-11.

Doses of CPT-11 for the weekly ×2 and daily ×5 schedule were administered according to the maximum tolerated dose (MTD) for tumor-bearing nude mice. At this MTD, the mice should have a weight loss of approximately 10% of the initial weight within the first 2 weeks after treatment (Boven *et al.*, 1992; Langdon *et al.*, 1994). Recovery of the weight loss should be completed on day 14; consequently, mice were weighed on weekdays for 2 weeks and thereafter weekly. The MTD was assessed in groups of 3 non-tumor-bearing nude mice per dose level. Thereafter, doses were adjusted in tumor-bearing animals if required.

For the evaluation of treatment, we used relative tumor volumes which were calculated with the formula V_T/V_0, where V_T is the volume on any given day and V_0 is the volume at the start of the treatment. The ratio of the mean relative volume of treated tumors over that of control tumors multiplied by 100% (T/C%) was assessed on each day of measurement. From the lowest T/C% obtained within 5 weeks after the last injection, the growth inhibition (100%-T/C%) was calculated to express drug efficacy. The drug was considered to be active if the growth inhibition obtained in a given tumor line was ≥50%, very active if ≥75%, and inactive if growth inhibition was <50% (Boven *et al.*, 1992; Langdon *et al.*, 1994). Complete remission was defined as the total disappearance of tumor after treatment for a period of at least one month.

Topoisomerase-I expression

Total RNA was isolated from frozen xenograft tissue samples with RNAzol B (Campro, Veenendaal, The Netherlands. [α-^{32}P]-labeled RNA complementary to the topoisomerase-I-cDNA 703-bp sequence (nucleotides 835-1538), inserted into pGEM3, was transcribed from *Fok*I-linearized DNA using T7 polymerase. RNase protection was carried out as described by Giaccone *et al.* (1995). In all experiments, a probe for γ-actin was included to control for RNA loading. The hybridized probe was visualized after gel electrophoresis through a denaturing 6% acrylamide gel. For autoradiography, the gel was exposed at −70°C to a Kodak BIOMAX MR film. The amount of topoisomerase-I mRNA relative to the amount of γ-actin was calculated by densitometric scanning of autoradiograms. Topoisomerase-I expression was determined at least twice in 3 separate xenografts of a tumor line.

Statistics

Anti-tumor effects were evaluated by Student's *t*-test. Linear-regression analysis was used to determine the sensitivity of the tumor lines to CPT-11 in relation to topoisomerase-I mRNA expression.

RESULTS

Maximum tolerated doses

The MTD of CPT-11 for the weekly ×2 and daily ×5 injections was first determined in non-tumor-bearing nude mice (Table III). For the weekly ×2 schedule, a dose range of 100 mg/kg to 225 mg/kg was used. The injections were given i.p. instead of i.v., since i.v. injections of higher doses caused acute deaths. The 150 mg/kg i.p. dose was chosen as the MTD for the weekly ×2 schedule. For the daily ×5 schedule, a dose range of 10 mg/kg i.v. to 30 mg/kg i.v. was used. The 25 mg/kg i.v. dose was selected as the MTD.

The MTD of CPT-11 was further defined by studying the selected schedule in tumor-bearing animals. The weight loss for the weekly ×2 schedule was determined in S.Ho-bearing mice. At the dose of 150 mg/kg i.p., however, the weight loss (±SD) was 15.2% (±3.9%), and toxic deaths were observed in 2/6 mice. For the next injections, the dose was reduced to 100 mg/kg i.p., which was tolerated well in the weekly ×2 schedule. Initially, 25 mg/kg i.v. was chosen for the daily ×5 schedule, but in view of the excessive weight loss of 18% ± 9.6% in S.Ho-bearing mice this dose had to be reduced to 20 mg/kg i.v.

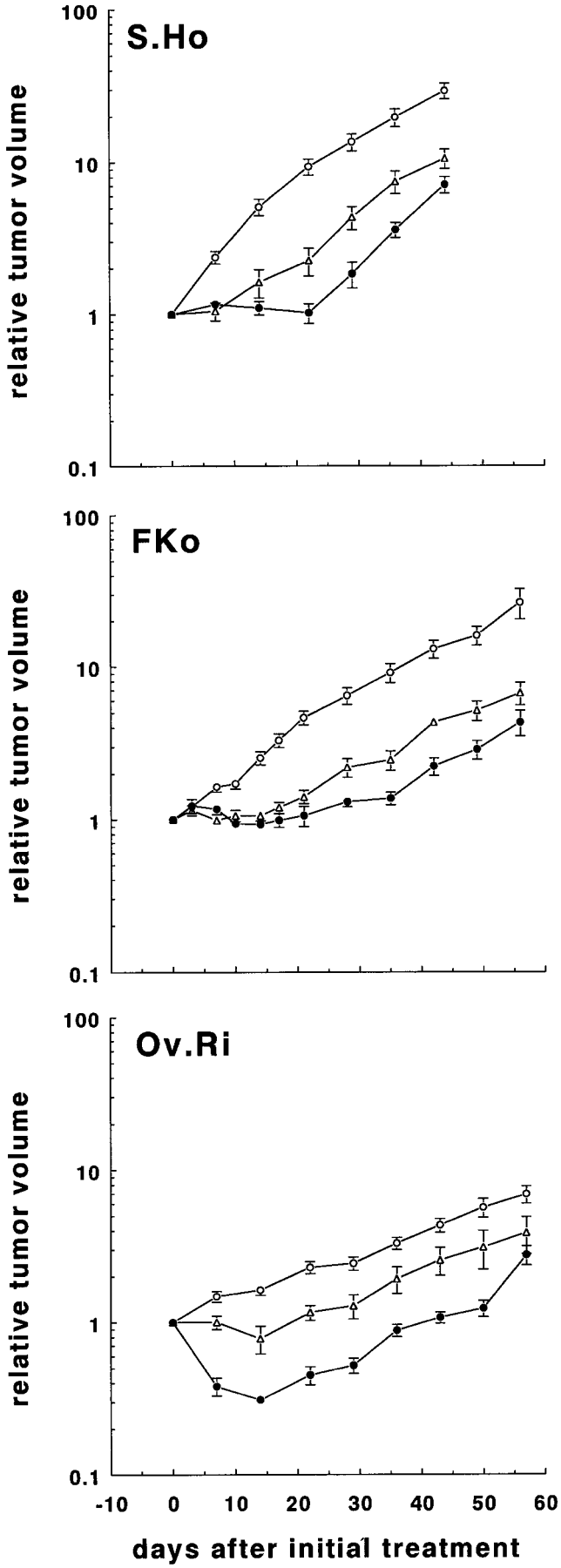
Schedule dependency

In mice bearing S.Ho, F.Ko and Ov.Ri(C) tumors, the daily ×5 and weekly ×2 schedules were compared for the efficacy at the MTD. Both schedules produced remarkable growth inhibition (Fig. 1). The weekly ×2 schedule was less effective than the daily ×5 schedule. The maximum growth inhibition in S.Ho tumors was 76% for the weekly schedule and 89% for the daily schedule ($p < 0.05$). The values in FKo tumors were 73% and 85% ($p < 0.02$) and in Ov.Ri(C) tumors 47% and 79% ($p < 0.01$) respectively. The degree of weight loss varied between 2.6 and 11.8% for the daily ×5 schedule and between 11.9% and 15.2% for the weekly ×2 schedule.

TABLE III – MAXIMUM-TOLERATED-DOSE STUDIES OF CPT-11 IN NUDE MICE

Dose (mg/kg)	Route	Days	Maximum weight loss[1]	Weight, day 14[1]	Deaths
100	i.p.	0, 7	13.4 (±1.8)	93.1 (±0.5)	0
125	i.p.	0, 7	5.1 (±1.6)	98.2 (±2.0)	0
150	i.p.	0, 7	14.3 (±10.3)	99.7 (±2.1)	0
175	i.p.	0, 7	4.3 (±2.5)	99.8 (±0.3)	0
200	i.p.	0, 7	18.6 (±1.1)	85.1 (±9.7)	0
225	i.p.	0, 7	13.6 (±2.2)	90.4 (±3.4)	0
10	i.v.	daily ×5	5.5 (±0.6)	100.0 (±2.9)	0
20	i.v.	daily ×5	14.1 (±8.2)	93.8 (±6.5)	1
30	i.v.	daily ×5	20.1 (±4.4)	96.2 (±3.6)	0

[1]% of weight on day 0 (±SD).



Administration route

The route of administration of CPT-11 was determined in S.La(C)- and S.Ho-bearing mice for the daily ×5 schedule. Figure 2 shows that the i.p. injections were as effective as the i.v. injections. The S.La(C) tumors showed a growth inhibition of 92% regardless of the route of administration, while a growth inhibition of 92% was calculated in S.Ho tumors. Both routes were equitoxic on the basis of weight loss. For further injections of CPT-11, the i.p. route was selected for the daily ×5 schedule.

Ovarian-cancer xenografts

The daily ×5 schedule of CPT-11 20 mg/kg i.p. was studied in 15 human ovarian-cancer xenografts (Table IV). CPT-11 caused a reversible weight loss between 1.0 and 14.3%, and there were no toxic deaths. In 14/15 tumor lines the drug was effective, resulting in a growth inhibition of ≥50%. Extensive growth inhibition of ≥75% CPT-11 was obtained in 8/15 tumors. lines. Complete remissions of tumors were observed in Ov.Gl, OVCAR-3, Ov.Vg and Ov.Me xenografts, while cures of all tumors were observed in mice bearing MRI-H-207 xenografts.

The expected 84-bp transcript size for topoisomerase-I mRNA was detected in all 15 ovarian-cancer lines. Among the tissues, little variation was detected in the extent of topoisomerase-I mRNA, although the growth inhibition induced by CPT-11 differed between 38% and 100%. A weak relation appeared to be present between topoisomerase-I expression and sensitivity to CPT-11, but the r value of 0.45 was not significant. MRI-H-207 tumors showed highest topoisomerase-I expression.

Soft-tissue-sarcoma xenografts

The 10 human soft-tissue-sarcoma lines were investigated for their sensitivity to CPT-11 20 mg/kg i.p. administered daily ×5 (Table V). CPT-11 caused a reversible weight loss between 5.0 and 12.6%, and there were no toxic deaths. In 6/10 soft-tissue-sarcoma lines, growth inhibition of ≥75% was obtained. Complete remissions were observed in S.La(C) xenografts, while all WLS-160 tumors could be cured by CPT-11.

Topoisomerase-I mRNA was detectable in all soft-tissue-sarcoma lines. Small variations were seen among the tissues, in contrast to the wider range of sensitivity to CPT-11 in these xenografts. The relation between topoisomerase-I expression and sensitivity to CPT-11 was weak ($r = 0.60$), but not significant. WLS-160 xenografts showed highest topoisomerase-I expression. Simultaneously with the soft-tissue-sarcoma-xenograft tissues, MRI-H-207 xenografts were analyzed for topoisomerase-I expression. The relative expression was 1.08 ± 0.18, which means that values in ovarian-cancer-xenograft tissues were slightly higher than in tissues from soft-tissue sarcoma.

DISCUSSION

In general, comparison of the response rate generated by drugs in cancer patients and that obtained in xenografts grown s.c. in nude mice reveals good correlation for the anti-tumor profile of a particular drug. In the past, we have confirmed the reliability of the human tumor xenograft model for preclinical drug testing of both standard and investigational drugs (Boven *et al.*, 1992; Langdon *et al.*, 1994). For the topoisomerase-I inhibitor CPT-11, we demonstrate here that extensive growth inhibition of ≥75% was obtained in 8/15 (53%) human ovarian-cancer xenografts and in 6/10 (60%) human soft-tissue-sarcoma xenografts. This high efficacy in the 2 tumor types selected has not been observed in the clinic. In a clinical phase-II trial of CPT-11 administered at 100 mg/m^2 i.v. every week, Takeuchi *et al.* (1991) observed 3 responses in 14

FIGURE 1 – Growth curves of S.Ho, FKo and Ov.Ri xenografts in nude mice. The mean relative volume (±SEM) of untreated tumors (○) and that of tumors treated with CPT-11 20 mg/kg i.p. or i.v. daily ×5 (●) or CPT-11 100 mg/kg i.p. weekly ×2 (△) are drawn.

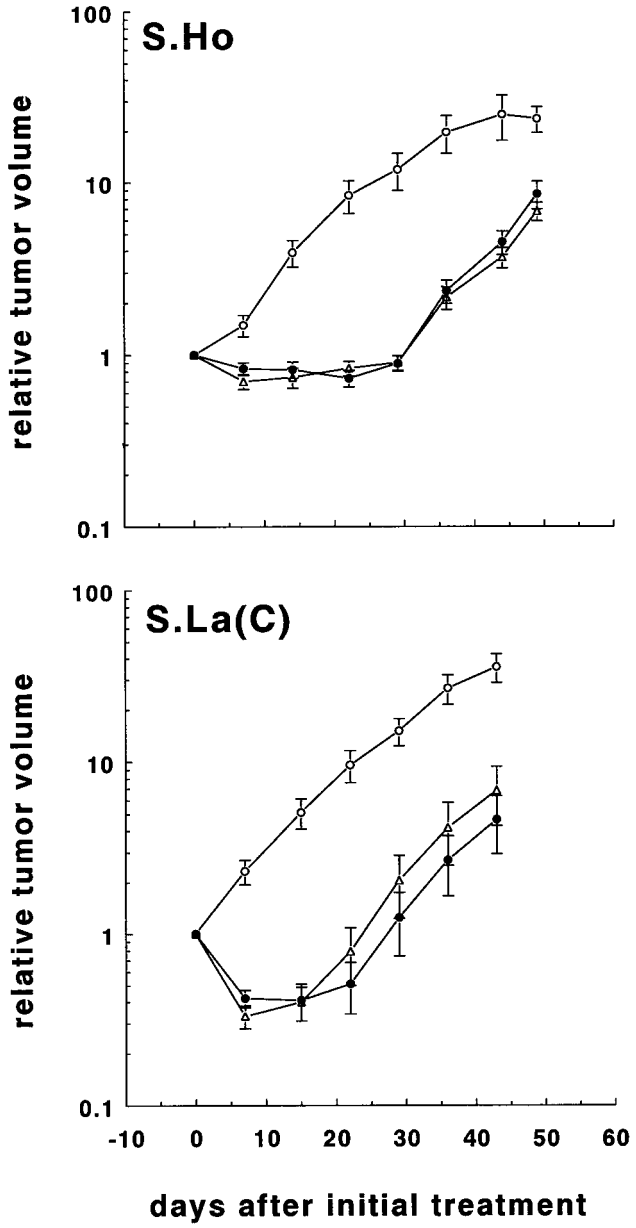


FIGURE 2 – Growth curves of S.Ho and S.La(C) xenografts in nude mice. The mean relative volume (±SEM) of untreated tumors (○) and that of tumors treated with CPT-11 daily ×5 are drawn. S.Ho xenografts were treated with 25 mg/kg CPT-11 given i.v. (△) or i.p. (●). S.La(C) xenografts were treated with 20 mg/kg CPT-11 given i.v. (△) or i.p. (●).

TABLE IV – CPT-11[1] IN A PANEL OF HUMAN OVARIAN-CANCER LINES: TOXICITY, EFFICACY AND TOPOISOMERASE-I EXPRESSION

Tumor line	Maximum weight loss (mean ± SD)	GI%[2]	CR[3]	Relative Topo-I expression (mean ± SD)
Ov.Ri(C)	11.8 ± 3.7	79 (++)	0/11	1.65 ± 0.00
FKo	2.6 ± 4.3	85 (++)	0/11	1.32 ± 0.03
Ov.Gl	7.7 ± 3.5	100 (++)	9/10	1.44 ± 0.17
OVCAR-3	6.9 ± 4.2	98 (++)	2/11	1.30 ± 0.01
Ov.Sh	9.4 ± 5.1	64 (+)	0/11	0.92 ± 0.08
Ov.Pe	1.0 ± 2.0	64 (+)	0/12	1.40 ± 0.26
Ov.He	14.3 ± 11.0	51 (+)	0/9	1.11 ± 0.13
Ov.Gr	5.2 ± 3.0	54 (+)	0/12	1.51 ± 0.34
FMa	1.7 ± 3.7	73 (+)	0/12	1.30 ± 0.31
FCo	8.7 ± 1.6	73 (+)	0/12	1.00 ± 0.00
Ov.Vg	4.5 ± 2.3	85 (++)	1/11	1.70 ± 0.57
Ov.Me	10.1 ± 6.4	99 (++)	4/11	1.49 ± 0.24
MRI-H-207	6.9 ± 6.4	100 (++)	12/12	1.71 ± 0.27
A2780	11.1 ± 2.8	81 (++)	0/11	1.52 ± 0.36
H134	9.1 ± 4.6	38 (−)	0/12	1.27 ± 0.24

[1]CPT-11 20 mg/kg i.p. daily ×5.–[2]Chemosensitivity expressed as percentage of growth inhibition: ≥75% (++); <75% to ≥50% (+); <50% (−).–[3]CR, number of complete remissions per total number of tumors.

TABLE V – CPT-11[1] IN A PANEL OF HUMAN SOFT-TISSUE-SARCOMA LINES: TOXICITY, EFFICACY AND TOPOISOMERASE-I EXPRESSION

Tumor line	Maximum weight loss (mean ± SD)	GI%[2]	CR[3]	Relative Topo-I expression (mean ± SD)
S.Ba	12.6 ± 7.0	65 (+)	0/12	0.32 ± 0.15
S.Lt	9.0 ± 4.4	80 (++)	0/10	0.51 ± 0.12
S.Hh	8.1 ± 3.8	41 (−)	0/14	0.37 ± 0.01
S.La(C)	8.8 ± 1.9	92 (++)	4/12	0.56 ± 0.43
S.Hu	9.2 ± 10.0	68 (+)	0/8	0.40 ± 0.08
S.Ho	5.0 ± 2.1	89 (++)	0/12	0.48 ± 0.12
S.To	10.0 ± 5.8	73 (+)	0/12	0.62 ± 0.40
S.Sin	6.7 ± 5.5	94 (++)	0/11	0.45 ± 0.27
WLS-160	10.0 ± 4.5	100 (++)	9/9	1.26 ± 0.65
S.Zu	7.0 ± 4.2	83 (++)	0/12	0.71 ± 0.38

[1]CPT-11 20 mg/kg i.p. daily ×5.–[2]Chemosensitivity expressed as percentage of growth inhibition: ≥75% (++); <75% to ≥50% (+); <50% (−).–[3]CR, number of complete remissions per total number of tumors.

ovarian-cancer patients (21.4%), whereas we observed a response rate of 53% in the panel of human ovarian-cancer xenografts. A phase-II trial of topotecan, given at 1.5 mg/m^2 i.v. daily ×5 every 3 weeks, has been carried out in 16 soft-tissue-sarcoma patients. A modest response rate for topotecan (13%) was detected; 2 responses were noted among the 16 patients (Eisenhauer *et al.*, 1994). In our panel of human-soft-tissue-sarcoma xenografts, however, a response rate of 60% was observed for CPT-11. A number of reasons may account for the discrepancy.

CPT-11 was more effective in the human tumor xenografts in the daily ×5 schedule than in the equitoxic weekly ×2 schedule, although the cumulative drug dose was 50% of the weekly ×2-schedule dose. Inhibitors of topoisomerase I appear to be highly S-phase-specific, since the replicating DNA is more susceptible to irreversible damage in the presence of the drug (Dancey and Eisenhauer, 1996). A relatively brief exposure to a topoisomerase-I inhibitor is lethal to only a small proportion of cells, particularly among tumor-cell populations that have long cell-cycle times or low growth fractions. Houghton *et al.* (1995) have also demonstrated in 3 panels of human tumor lines (colon cancer, childhood rhabdomyosarcoma and pediatric brain tumors) that protracted low-dose exposure to CPT-11 or to topotecan was more effective than bolus injections of higher doses. For evaluation of CPT-11 in European phase-II trials, the single i.v. infusion every 3 weeks was recommended at a starting dose of 350 mg/m^2 (Armand *et al.*, 1996). The preclinical data suggest, however, that frequent administration of lower doses of CPT-11 may be more effective than higher doses given at longer intervals.

The route of administration may play an important role in the efficacy of a drug, but presumably not in the case of CPT-11, which was equally effective given by i.p. or by i.v. injections in the daily ×5 schedule. Consequently, for further administration we used the i.p. route. Kawato *et al.* (1991) have shown that, for the ×3/every 4 days schedule in human tumor xenografts, oral administration of CPT-11 was as effective as the i.v. route. To achieve the same extent of growth inhibition, however, the oral dose had to be 2- to 4-fold the i.v. dose. Similar data have been reported by Bissery *et al.* (1996) in murine tumor models. These observations suggest that the schedule of CPT-11, rather than the route of administration, appears to be important for efficacy.

The influence of the schedule on the efficacy of CPT-11 may not be the only factor to explain the discrepancy between the experimental data and the clinic. Other factors should therefore be taken into account, such as the binding of camptothecins to human serum albumin (HSA). In the case of the parent compound camptothecin, HSA was found to bind the carboxylate form more tightly than serum albumins of other species (Mi and Burke, 1994). HSA exhibited a marked 200-fold-binding preference for the carboxylate relative to the lactone. As a result, the lactone-carboxylate equilibrium shifts to the right and the drug is extensively converted into its biologically inactive form. In contrast, for the camptothecin analogs CPT-11, SN-38 and topotecan, binding to HSA was shown preferentially for the lactone form, which in fact indicates a gain in stability in the presence of HSA (Burke and Mi, 1994).

The explanation for the difference in efficacy of CPT-11 in human tumor xenografts and in cancer patients may be found in differences of drug activation, detoxification and, perhaps, elimination between species. CPT-11 is converted to SN-38 more efficiently in mice. As an example, Abigerges *et al.* (1995) have reported that a dose of CPT-11 350 mg/m^2 in patients (dose recommended for phase-II trials) resulted in SN-38 mean C_{max} of 56 ng/ml, while the area under the curve (AUC) of SN-38 was 1.3% of that of CPT-11. Bissery *et al.* (1996) have measured, at a dose of CPT-11 52.5 mg/kg i.v. in mice (MTD), that SN-38 mean C_{max} was 1.6 µg/ml, while the AUC of SN-38 was 54.3% of that of CPT-11. Mean C_{max} and mean AUC values for CPT-11 in patients and mice were comparable. The low efficiency in conversion of CPT-11 into SN-38 in patients does not seem to be related to carboxylesterase activity, since the proportion of SN-38 formed was similar at a CPT-11 dose range of 100 mg/m^2 to 750 mg/m^2, and varied between 1.3% and 5.8% (Abigerges *et al.,* 1995). A likely explanation may be a difference in the metabolic pathway of CPT-11. Lokiec *et al.* (1996) have reported the occurrence of at least 16 metabolites of CPT-11 in the bile of a patient. In between the peaks of CPT-11, SN-38 and SN-38 glucuronide, oxidized and decarboxylated forms could be identified. We hypothesize that in mice the enzymatic pathway is more favorable to the formation of SN-38.

The dose-limiting toxicity of CPT-11 in patients is diarrhea, the severity of which appears to be related to the AUC of SN-38 (Gupta *et al.,* 1994). SN-38 glucuronidation by the liver is necessary for detoxification. Gupta *et al.* (1994) have described a ''biliary index'' as the product of the area ratio of SN-38 to SN-38 glucuronide and the AUC of total CPT-11. A high ''biliary index'' was most likely to occur in a patient with severe diarrhea. In patients and in rats, SN-38 glucuronide may be de-conjugated by intestinal microflora producing SN-38 for enterohepatic circulation (Narita *et al.,* 1993; Takasuna *et al.,* 1996). Treatment-related diarrhea was not observed in our experiments in nude mice, even at the highest doses used to determine the MTD.

Topoisomerase I has been identified as the intracellular target of camptothecins. Niwa *et al.* (1994) have reported that the sensitivity of cells to camptothecins was directly related to topoisomerase-I mRNA content. Of interest, in the tumor lines MRI-H-207 and WLS-160 that could be cured by CPT-11, the highest topoisomerase-I expression was measured. However, we found only a weak relation between topoisomerase-I expression and sensitivity to CPT-11 in the panel of ovarian-cancer xenografts and in that of soft-tissue-sarcoma xenografts. In an earlier study in 5 unselected human colon-cancer cell lines *in vitro,* we found no relation between the extent of topoisomerase I and the anti-proliferative effects of carboxylesterase-activated CPT-11 or SN-38 (Jansen *et al.,* 1997). A stronger relation could be found by measuring the topoisomerase-I activity. Goldwasser *et al.* (1995) have demonstrated a positive relation between drug-stabilized topoisomerase-I-cleavable complexes and camptothecin sensitivity in a panel of 7 unselected human-colon-cancer cell lines. These assays, however, cannot easily be transferred to the clinic, since topoisomerase-I activity can best be measured in fresh tumor tissue, and since the alkaline-elution method works best when cellular DNA is labeled.

In conclusion, CPT-11 exhibits potent anti-tumor activity in human-ovarian-cancer and human-soft-tissue-sarcoma xenografts. The results appear to over-predict its clinical efficacy in these tumor types. This observation may be caused by the more effective daily schedule. In addition, mice appear to tolerate SN-38 better than humans. Further efforts should be put into the design of clinical trials with protracted low doses of CPT-11, which may cause less diarrhea in patients.

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