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POTENT THERAPEUTIC ACTIVITY OF IRINOTECAN (CPT-11) AND ITS SCHEDULE DEPENDENCY IN MEDULLOBLASTOMA XENOGRAFTS IN NUDE MICE

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The anti-tumor activity of irinotecan (CPT-11), a DNA-topoisomerase I inhibitor, was evaluated in 5 advanced stage subcutaneous medulloblastoma xenografts in nude mice, using different schedules of administration. With a 5-day schedule, the highest i.v. dose tested (40 mg kg^{-1} day^{-1}) induced complete regressions in all xenografts but 1, and delays in tumor growth always exceeded 30 days. Two xenografts, IGRM11 and IGRM33, were highly sensitive, and animals survived tumor-free beyond 120 days after treatment. CPT-11 clearly retained its anti-tumor activity at a lower dosage (27 mg kg^{-1} day^{-1}). CPT-11 was significantly more active than cyclophosphamide, thiotepa and etoposide against the 3 xenografts evaluated. To study the schedule dependency of its anti-tumor activity, CPT-11 was given i.v. at the same total doses over the same period (33 days) using either a protracted or a sequential schedule in IGRM34-bearing mice. With a dose of 10 mg kg^{-1} day^{-1} given on days 0–4, days 7–11, days 21–25 and days 28–32 (total dose, 200 mg kg^{-1}), 3 of 6 animals were tumor free on day 378. The same total dose given with a sequential schedule, *i.e.,* 20 mg kg^{-1} day^{-1} on days 0–4 and days 28–32, failed to induce complete regression. The plasma pharmacokinetics of CPT-11 and SN-38 were studied in IGRM34-bearing animals after a single i.v. dose of 10 and 40 mg kg^{-1}. The plasma clearance rate of CPT-11 was dose dependent. The ratio between the SN-38 and CPT-11 area under the curve in plasma was 0.4–0.65, *i.e.,* significantly higher than that observed in humans at the maximum tolerated dose (0.01–0.05). Conversely, this ratio was 10-fold lower in tumor than in plasma. Clinical development of irinotecan is warranted in pediatric malignancies. *Int. J. Cancer* 73:156–163, 1997.

Medulloblastomas are frequent malignant brain tumors of the posterior fossa occurring in the first decade of life. These cancers are both chemo- and radiosensitive. The current clinical protocols yield a 40–70% cure rate at 5 years. New active drugs are needed to improve survival and the quality of life of children with medulloblastoma, in particular of 2 subsets of patients, namely, children with metastases or incompletely resected tumor, and children younger than 3–6 years of age, to avoid the deleterious effects of radiation therapy administered to developing brain. We have developed and characterized a panel of medulloblastoma xenografts in athymic mice to identify potentially active new drugs for the treatment of children with medulloblastoma (Vassal *et al.,* 1996*b*).

Irinotecan [CPT-11 or 7-ethyl-10-(4-[1-piperidino]-1-piperidino)-carbonyloxy camptothecin] is a semi-synthetic water-soluble analogue of camptothecin. Irinotecan belongs to a new class of anti-cancer drugs, the DNA-topoisomerase I inhibitors. These drugs stabilize the covalent topoisomerase I-DNA cleavable complex. Irinotecan has shown a wide spectrum of preclinical anti-tumor activity against murine tumors and human xenografts (Lavelle *et al.,* 1996), including several pediatric tumors (Houghton *et al.,* 1993; Komuro *et al.,* 1994). Phase II studies in adults have exhibited promising response rates in several cancers (Rothenberg, 1996). We have previously reported the therapeutic activity of irinotecan against peripheral primitive neuro-ectodermal tumor (PNET) and neuroblastoma xenografts (Vassal *et al.,* 1996*a*). Here, we report the preclinical *in vivo* evaluation of irinotecan against 5 medulloblastoma xenografts, using different administration schedules.

Irinotecan is converted *in vivo* into an active metabolite, SN-38, by carboxylesterase. SN-38 is 1,000-fold more potent for the stabilization of the cleavable complexes than CPT-11. In rodents, intense carboxylesterase activity in plasma is responsible for the conversion of irinotecan into SN-38 in plasma (Kaneda *et al.,* 1990). In humans, CPT-11 activation does not occur in plasma. As metabolic differences between species could undermine the predictibility of *in vivo* preclinical studies in terms of potential activity in humans, we decided to study the plasma and tumor pharmacokinetics of CPT-11 and SN-38 in tumor-bearing mice to check whether the preclinical anti-tumor activity of CPT-11 was observed at clinically achievable levels of systemic exposure of CPT-11 and SN-38.

MATERIAL AND METHODS

Drugs

Irinotecan (Campto) was kindly provided by Rhône-Poulenc Rorer-Bellon (Neuilly-sur-Seine, France). Cyclophosphamide was purchased from Asta-Medica (Mérignac, France), thiotepa and cisplatin from Rhône-Poulenc Rorer-Bellon and etoposide from Sandoz (Rueil-Malmaison, France). Drugs were dissolved in a 0.9% sodium chloride solution immediately before injection on each day of treatment. Drugs were administered as a 0.2 ml volume of the appropriate solution per mouse. In the control groups, mice received 0.2 ml of 0.9% sodium chloride according to the same schedule as the treated animals. Camptothecin was purchased from Sigma (Saint-Quentin Fallavier, France) and used as an internal standard for the drug assay. SN-38, provided by Rhône-Poulenc Rorer-Bellon, was used for standard curve calibration.

Animals

Female SPF-Swiss nude mice were bred in the Animal Experimentation Unit of the Institut Gustave-Roussy. Animals were

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housed in sterile isolators and fed with irradiated nutriments (UAR, Villemoisson/Orge, France) and filtered water *ad libitum.* Experiments were carried out under the conditions established by the European Community (directive 86/609/CEE).

Xenografts

The panel comprised 5 medulloblastoma xenografts derived from primary tumors of the posterior fossa by subcutaneous transplantation of small fragments in previously irradiated athymic mice (Vassal *et al.,* 1996*b*). IGRM11, IGRM13 and IGRM21 were derived from metastatic medulloblastomas and IGRM33 from a recurrent medulloblastoma after conventional chemotherapy and radiotherapy in a 7-year-old boy. These xenografts exhibited the histological features of undifferentiated medulloblastoma. IGRM34 was derived from a metastatic PNET of the posterior fossa in a 1.6-year-old boy and exhibited the histological features of a PNET with a rhabdoid phenotype. The human origin of these xenografts was confirmed by the presence of abundant human lactate dehydrogenase (LDH) isoenzymes and by cytogenetic studies. All xenografts were maintained *in vivo* by sequential passaging from an s.c. implant, with an engraftment success rate exceeding 75%. Xenograft stability was verified at each passage by analysis of doubling time and by histological analysis every 3–6 passages. Therapeutic experiments were started after the 4th passage *in vivo* for all the xenografts.

Experimental design

Drug activity was evaluated against advanced stage tumors, as previously described (Vassal *et al.,* 1996*a*). For each experiment, 3 × 3 mm tumor fragments were xenotransplanted s.c. (unilaterally or bilaterally) in 30–50 athymic mice aged 6–8 weeks. Animals bearing bilateral tumors were used to evaluate cyclophosphamide, thiotepa, etoposide and prolonged treatments with CPT-11. On day 0 of the treatment, mice bearing a 100–300 mm^3 subcutaneous tumor were pooled and randomly assigned to 2–4 groups of 5–10 mice (1 control group and 1–3 groups treated at different dose levels). Animals with tumors outside the desired volume range were excluded. Two perpendicular tumor diameters were measured 3 times a week with a caliper by the same investigator. The volume of each tumor was calculated according to the following equation: $V(mm^3) = d^2(mm^2) \times D\ (mm)/2$, where d and D are the smallest and largest perpendicular tumor diameters, respectively. Each group of mice was treated according to the average weight of the group. Animal body weights were recorded 3 times a week, and mortality was checked daily. Body weight loss (BWL) was reported as the maximum treatment-related weight loss. The experiments lasted until tumor volumes reached 1,500–2,000 mm^3. The experiment was stopped after 120 days when there were tumor-free survivors.

Treatment

CPT-11 was administered i.v. in a caudal vein at a daily dose ranging from 6.75 to 40 mg kg^{-1}. In the experiments evaluating a short course of treatment, 27 and 40 mg kg^{-1} doses were given daily over 5 consecutive days (1 cycle). A total dose of 200 mg kg^{-1} was previously shown to be the maximum tolerated dose (MTD) of CPT-11 in animals of the same strain bearing neuroblastoma xenografts (Vassal *et al.,* 1996*a*). The schedule-dependent antitumor activity of CPT-11 was studied in IGRM34-bearing mice. The first experiment was designed to deliver the same total doses as those used in the short-schedule experiments, *i.e.,* 135 and 200 mg kg^{-1}, over a prolonged period (33 days) using a protracted i.v. schedule. CPT-11 (6.75 and 10 mg kg^{-1} day^{-1}) was given in 4 5-day cycles (days 0–4, days 7–11, days 21–25 and days 28–32). A 1 week rest was required in the middle of the treatment period to resolve local toxicity at the site of the i.v. injection. The second experiment was designed to administer the same total doses but with a sequential i.v. schedule. CPT-11 (13.5, 20 and 40 mg kg^{-1} day^{-1}) was given in 2 5-day cycles (days 0–4 and days 28–32). Thus, during these 2 prolonged schedules, the same total doses (135 and 200 mg kg^{-1}) were administered over the same period (33 days) using either a protracted or a sequential schedule. The dose intensity was defined as the total dose divided by the total duration of treatment and was expressed in mg kg^{-1}/week.

Cyclophosphamide (200–500 mg kg^{-1}) and thiotepa (24 and 36 mg kg^{-1}) were administered as a single i.p. injection. Etoposide was administered i.v. at a daily dose of 17 and 20 mg kg^{-1} over 5 consecutive days.

Evaluation of anti-tumor activity

The activity of each drug tested was evaluated according to 3 criteria: 1) the number of complete and partial tumor regressions; 2) the tumor growth delay (TGD); and 3) the number of tumor-free survivors (TFS). Complete regression (CR) was defined as tumor regression beyond the palpable limit (15 mm^3) and partial regression (PR) as tumor regression greater than 50% of the initial tumor volume. CR and PR had to be observed for at least 2 consecutive tumor measurements to be retained. TGD was defined as the difference in the median time to reach a tumor volume 5-fold the initial tumor volume between the treated and the control group (Vassal *et al.,* 1996*a*). Tumor-free survivors were defined as animals free of palpable tumor at the end of the experiment (at least 120 days).

Pharmacokinetic study

Animals bearing a unilateral IGRM34 tumor of 100–300 mm^3 were randomly assigned to receive an i.v. dose of either 10 or 40 mg kg^{-1}. Three animals were sacrificed by CO_2 inhalation before and after the injection at each of the following time points: 5, 15, 30 min and 1, 1.5, 2, 4, 6 and 8 hr. A total blood sample (1 ml) was immediately collected by cardiac puncture in a heparinized vial. Plasma was separated by centrifugation and frozen at −80°C until analysis. Each tumor was removed, sampled and frozen at −80°C. Prior to analysis, tumor samples (50–100 mg) were homogenized in Tris HCl, pH 8.1, with a Potter homogenizer (Heidolph, Germany) on ice and centrifuged at 4°C (11,000 *g* for 5 min).

Total CPT-11 and SN-38, *i.e.,* the addition of the lactone and carboxylate forms of either compounds, were assayed by HPLC as previously described (Barilero *et al.,* 1992). Briefly, prior to extraction, 100 µl of plasma or tumor homogenate were acidified in 0.01 N HCl and spiked with camptothecin (final concentration, 1 µg/ml). Solid-phase extraction was carried out on columns (Bond Elut cartridges, 100 mg, 40 µm; J.T. Baker, Phillipsburg, NY). After evaporation to dryness, each sample was dissolved in 250 µl of the mobile phase (acetonitrile-0.1 M KH_2PO_4 buffer containing 3 mM heptane-sulphonate acid, adjusted to pH 4 with 1 M HCl). HPLC analysis was performed on a Waters workstation (Milford, MA). Separation of CPT-11, SN-38 and the internal standard was achieved using a reversed phase Nucleosil C18 column (S.F.C.C., Neuilly-Plaisance, France). The fluorescence detector wavelengths were set at 380 nm (excitation) and 515 nm (emission). The detection limit of CPT-11 and SN-38 was 1 ng/ml. Standard curves in mouse plasma and tumor homogenate were linear from 0 to 10,000 ng/ml for CPT-11 and from 0 to 1,000 ng/ml for SN38. The assay was highly reproducible, with coefficients of variation of less than 6%.

Model independent analysis of CPT-11 and SN-38 concentration *vs.* time curves in plasma and tumor was performed using APIS software (MIIPS, Marseille, France). Elimination half-lives were calculated according to the following equation: $t_{1/2\beta} = 0.693/\beta$, where β is the slope of the log-linear regression analysis of the second phase of the curve. The area under the curve ($AUC_{0-\infty}$) was calculated with the log-linear trapezoidal rules from 0 to 8 hr and extrapolated to infinity according to the slope of the elimination part of the curve. The total clearance rate of CPT-11 was calculated according to the following equation: $CI = dose/AUC_{0-\infty}$.

TABLE I – ANTI-TUMOR ACTIVITY OF i.v. CPT-11 GIVEN AS A 5-DAY TREATMENT SCHEDULE AGAINST 4 s.c. MEDULLOBLASTOMA XENOGRAFTS[1]

Xenograft	DT (days)	Dose (mg kg^{-1} day^{-1})	Schedule	Total dose (mg kg^{-1})	Number	BWL (max. %)	Toxic death	CR	PR	TGD (days)	Tumor-free survivors
IGRM11	10.8	27	Daily × 5	135	7	0	0	7	—	>93	7/7 on day 120
		40	Daily × 5	200	7	0	1[2]	7	—	>93	5/7 on day 120
IGRM13	11	27	Daily × 5	135	8	0	0	0	0	28	0/8
		40	Daily × 5	200	8	0	0	0	0	37	0/8
IGRM21	9.0	40	Daily × 5	200	7	12	0	2	2	36	0/7
IGRM33	9.9	27	Daily × 5	135	8	0	0	7	1	105	2/8 on day 153
		40	Daily × 5	200	8	0.5	0	7	1	>125	3/8 on day 153

[1]DT, median doubling time measured in each control group during the exponential phase of tumor growth curve; number, number of animals/group; BWL, maximum body weight loss; CR, complete regression; PR, partial regression; TGD, tumor growth delay.–[2]Animal died on day 45.

TABLE II – SCHEDULE-DEPENDENT ANTI-TUMOR ACTIVITY OF i.v. CPT-11 AGAINST s.c. IGRM34 XENOGRAFT[1]

Treatment course	DT (days)	Dose (mg kg^{-1} day^{-1})	Schedule	Duration (days)	Total dose (mg kg^{-1})	Number	BWL (max. %)	Toxic death	CR	PR	TGD (days)	Tumor-free survivors
Short	3.5	27	Daily × 5	5	135	8	0	0	3	5	41	0/8
		40	Daily × 5	5	200	8	1.4	0	2	6	38	0/8
Protracted	4.2	6.75	(Daily × 5) × 4	33	135	6	0	2[2]	6	—	125	0/6
		10	(Daily × 5) × 4	33	200	6	0	0	6	—	>120	3/6 on day 378
Sequential	3.1	13.5	(Daily × 5) × 2	33	135	5	0	0	0	0	49	0/5
		20	(Daily × 5) × 2	33	200	5	4	0	0	5	57	0/5
		40	(Daily × 5) × 2	33	400	5	5	0	0	1	50	0/5

[1]DT, median doubling time measured in each control group during the exponential phase of tumor growth curve; number, number of animals/group; BWL, maximum body weight loss; CR, complete regression; PR, partial regression; TGD, tumor growth delay.–[2]Died free of tumor on days 121 and 211.

RESULTS

Anti-tumor activity of CPT-11

At the MTD (40 mg kg^{-1} day^{-1}) with a short schedule (5 days), CPT-11 induced complete tumor regressions of all xenografts except IGRM13 (Tables I, II). IGRM11 proved to be the most sensitive xenograft, with 100% complete regressions and 71–100% tumor-free survivors on day 120 at the 2 doses tested (Fig. 1*a*). IGRM33 was also very sensitive since 40 mg kg^{-1} day^{-1} over 5 days allowed 3 of 8 mice to survive tumor-free on day 153 with a tumor growth delay exceeding 125 days (Fig. 1*b*). At the same dose level, complete regressions (28 and 25%) were achieved in IGRM21 and IGRM34 with tumor growth delays of 36 and 38 days, respectively (Fig. 1*c*,*d*). No complete or partial regression was observed in IGRM13, but irinotecan induced a significant tumor growth delay of 36 days (Fig. 1*e*). The anti-tumor activity of CPT-11 was clearly retained at a lower dosage (27 mg kg^{-1} day^{-1}) in the 4 xenografts evaluated (Tables I, II).

Schedule-dependent anti-tumor activity of CPT-11

To evaluate the effect of the schedule on CPT-11 anti-tumor activity in IGRM34 tumor-bearing mice, 2 experiments were carried out in which the same dose intensity was used in both the protracted and the sequential schedule (Table II). At 42.4 mg kg^{-1} week^{-1} (200 mg kg^{-1} over 33 days), the protracted schedule was devoid of toxicity and proved to be the most active against IGRM34, with 100% complete regressions and 3 of 6 animals remaining free of tumor on day 378 (Fig. 2*a*,*b*). The same dose intensity with the sequential administration was far less active (no complete regression, tumor growth delay of 57 days) (Fig. 2*c*,*d*). Anti-tumor activity did not increase when a higher dose intensity was used in the sequential schedule (84.8 mg kg^{-1} week^{-1}, *i.e.*, 400 mg kg^{-1} over 33 days). A clear difference was also observed between the protracted and sequential administrations at a lower dose-intensity level (28.6 mg kg^{-1} week^{-1}, *i.e.*, 135 mg kg^{-1} over 33 days) (Table II).

Anti-tumor activity of other anticancer drugs

Three anticancer drugs commonly used in the treatment of medulloblastoma in children were also evaluated against 3 xenografts of the panel, at several dose levels including the MTD (Table III). IGRM11 was highly sensitive to alkylating agents. Cyclophosphamide induced 50% of complete regressions, and 1 of 6 mice survived tumor free at the dose of 300 mg kg^{-1}, with a clear dose-effect relationship in terms of toxicity and activity at a higher dose level. Thiotepa allowed 50% of animals to survive tumor free, while etoposide induced only a significant tumor growth delay of 30 days. Overall, CPT-11 can be considered as being the most active drug against IGRM11 since 100% of mice survived tumor free at the lowest dose tested. Activity of the 3 anticancer drugs was poor (cyclophosphamide) or absent (thiotepa and etoposide) against the IGRM33 xenograft derived from a recurrent medulloblastoma after chemo- and radiotherapy. This markedly contrasts with the potent activity of the highest dose of CPT-11 tested (87% complete regressions with 3/8 tumor-free survivors). Finally, IGRM34 was refractory to thiotepa and etoposide. Cyclophosphamide and a 5-day cycle of CPT-11 displayed anti-tumor activity of the same order of magnitude (tumor growth delays of 41 and 38 days, respectively).

Pharmacokinetics

Mean CPT-11 plasma levels decayed biphasically after the 10 and 40 mg kg^{-1} dose, with elimination half-lives of 2.0 and 3.0 hr, respectively (Fig. 3*a*). Plasma pharmacokinetics of CPT-11 were dose dependent since the clearance rate decreased from 7.26 L h^{-1} kg^{-1} at 10 mg kg^{-1} to 4.1 L hr^{-1} kg^{-1} at 40 mg kg^{-1} (Table IV). Mean SN-38 plasma levels decayed biphasically, with a clear rebound being observed at 90 min after the highest dose of CPT-11 (Fig. 3*a*). Systemic exposure to SN-38 appeared to increase linearly as the dose increased, from 902 ng hr ml^{-1} at 10 mg kg^{-1} to 3,780 ng hr ml^{-1} at 40 mg kg^{-1}, *i.e.*, a 4.2-fold increase (Table IV). The concentration-*vs.*-time curves of CPT-11 and SN-38 tumor levels are shown in Figure 3*b*. The elimination half-life of CPT-11 was 2.4–2.5 hr in tumor (Table IV). SN-38 was detectable in tumor up to 8 and 6 hr after doses of 10 and 40 mg kg^{-1}, respectively. The ratio between the SN-38 and CPT-11 AUC in tumour (0.02–0.04) was more than 10-fold lower than in plasma (0.4–0.65). The tumor AUC for CPT-11 increased from 1,075 ng hr ml^{-1} at 10 mg kg^{-1} to 2,640 ng hr ml^{-1} at 40 mg kg^{-1}. No difference was observed in the AUC of SN-38 between the 2 dose levels. This suggests that

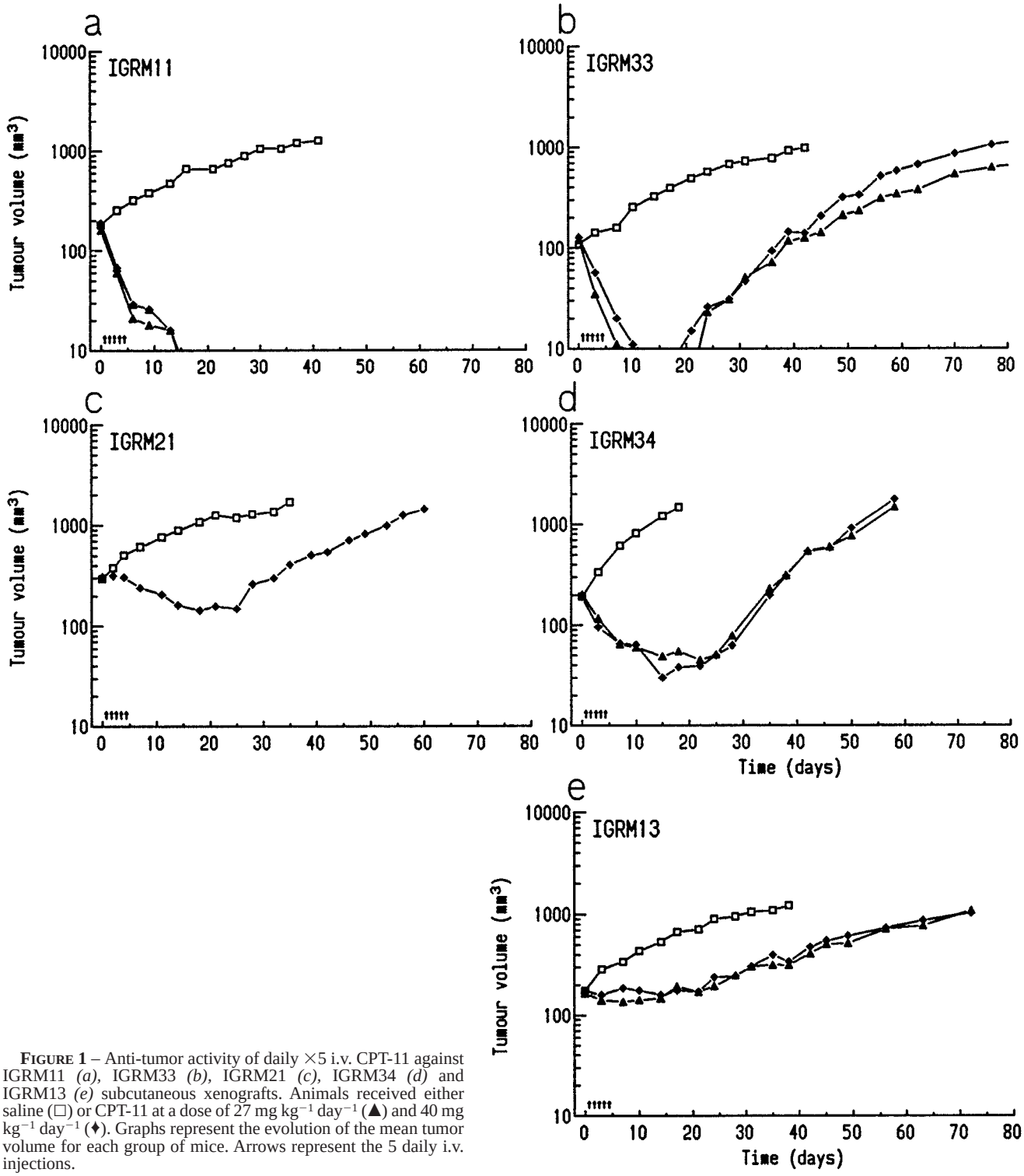

FIGURE 1 – Anti-tumor activity of daily ×5 i.v. CPT-11 against IGRM11 *(a)*, IGRM33 *(b)*, IGRM21 *(c)*, IGRM34 *(d)* and IGRM13 *(e)* subcutaneous xenografts. Animals received either saline (□) or CPT-11 at a dose of 27 mg kg^{-1} day^{-1} (▲) and 40 mg kg^{-1} day^{-1} (♦). Graphs represent the evolution of the mean tumor volume for each group of mice. Arrows represent the 5 daily i.v. injections.

intra-tumor distribution and/or metabolism is saturated when the dose increases.

DISCUSSION

Irinotecan is a DNA-topoisomerase I inhibitor that has shown a wide spectrum of anti-tumor activity in preclinical studies (Lavelle *et al.*, 1996) and promising clinical results in adult cancers such as colorectal and lung cancers, squamous cell carcinoma of the uterine cervix, and hematological malignancies (Rothenberg, 1996). Several preclinical studies in pediatric tumor xenografts have suggested that irinotecan might be active in childhood malignancies. Significant anti-tumor activity has already been demonstrated in rhabdomyosarcoma (Houghton *et al.*, 1993), neuroblastoma (Ko-

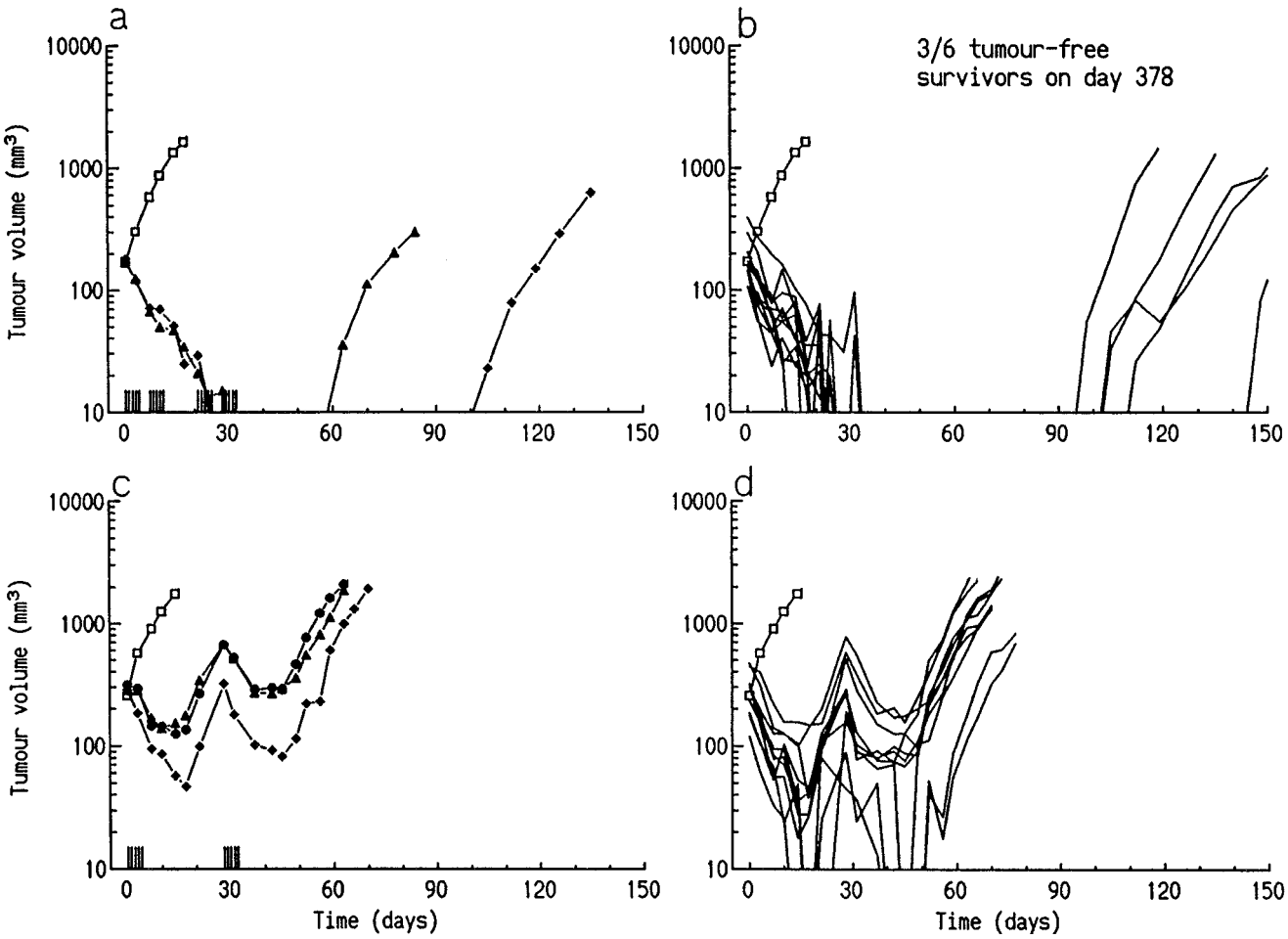

FIGURE 2 – Anti-tumor activity of i.v. CPT-11 against IGRM34 xenograft, using a protracted, *i.e.*, days 0–4, days 7–11, days 21–25 and days 28–32 *(a,b)*, or a sequential, *i.e.*, days 0–4 and days 28–32 *(c,d)*, schedule. Animals received either saline (□) or CPT-11 at a total dose of 135 mg kg^{-1} (▲), 200 mg kg^{-1} (♦) or 400 mg kg^{-1} (●) over 33 days. Left-hand graphs *(a,c)* represent the evolution of the mean tumor volume for each group of mice. Right-hand graphs *(b,d)* represent the evolution of each individual tumor volume (—) at the total dose of 200 mg kg^{-1} day^{-1}. Spikes from the X-axis represent the daily i.v. injections.

muro *et al.*, 1994; Vassal *et al.*, 1996*a;* Thompson *et al.*, 1997) and pediatric brain tumor xenografts (Houghton *et al.*, 1995). In the present study, we have shown that irinotecan, given over 5 consecutive days, is active against a panel of 5 subcutaneous medulloblastoma xenografts. Two of these 5 xenografts were highly sensitive, with animals surviving tumor free beyond 120 days after the start of treatment. In addition, the therapeutic index was wide in nude mice: no major toxicity was observed at the dose levels tested, and irinotecan clearly retained its anti-tumor activity at the lowest dose level.

The anti-tumor activity of irinotecan compares favorably with those of cyclophosphamide, thiotepa and etoposide. Cyclophosphamide and thiotepa are 2 alkylating agents commonly used in the treatment of children with medulloblastoma, either in conventional or high-dose chemotherapy regimens (Mograbi *et al.*, 1995; Kalifa *et al.*, 1992). Etoposide has been evaluated mainly in combination with platinum compounds such as carboplatin and has proved very active (Gentet *et al.*, 1994). However, single-agent etoposide failed to demonstrate significant anti-tumor activity in children with medulloblastoma (Boor *et al.*, 1994). Irinotecan was more active against the 3 xenografts evaluated than these 3 drugs at the doses tested. Of particular interest is the IGRM33 xenograft, derived from a recurrent medulloblastoma after conventional treatment including chemotherapy and radiotherapy. This xenograft was resistant or weakly sensitive to the 3 common anticancer drugs. Nevertheless, irinotecan proved to be highly effective against this refractory tumor since animals survived tumor free at the 2 dose levels tested. The absence of cross-resistance between irinotecan and other anticancer drugs has already been demonstrated (Tsuruo *et al.*, 1988). Houghton *et al.* (1993) showed that irinotecan was active against rhabdomyosarcoma xenografts selected for their resistance to vincristine, melphalan and topotecan.

The cytotoxicity of topoisomerase I inhibitors appears to be mainly directed toward dividing cells. Camptothecin and its derivatives stabilize the cleavable complex between topoisomerase I and single-strand DNA, and this is reversible upon drug removal. During the S-phase, the collision between an advancing replication fork and the camptothecin-trapped cleavable complex results in an arrest in fork progression and irreversible DNA damage characterized by double-strand breaks (Hsiang *et al.*, 1989). As with other S-phase-dependent anticancer drugs, the extent of camptothecin-induced cytotoxicity is likely related to the time of exposure to the drug rather than to its concentration. If such is the case, the duration of exposure should be an important aspect in attempts to define the optimal schedules of administration of camptothecins in humans. This question has already been addressed. For example, the *in vivo* toxicity of topotecan, another camptothecin derivative, has clearly been demonstrated to be schedule dependent in preclinical models. Several schedules of administration, from bolus to 21-day continuous infusion, have been evaluated in humans (Herben *et al.*, 1996).

TABLE III – ACTIVITY OF 3 ANTICANCER DRUGS AGAINST s.c. MEDULLOBLASTOMA XENOGRAFTS[1]

Xenograft	Drug	Dose (mg kg^{-1} day^{-1})	Schedule/ route	Total dose (mg kg^{-1})	Number	BWL (max. %)	Toxic death	CR	PR	TGD (days)	Tumor-free survivors
IGRM11	Thiotepa	24	Single/i.p.	24	6	0	0	4	1	>107	2/6 on day 153
		30	Single/i.p.	30	6	5	0	4	2	>107	4/6 on day 153
	Cyclophosphamide	300	Single/i.p.	300	6	5	1[2]	3	3	68	1/6 on day 117
		400	Single/i.p.	400	8	10	3[3]	8	—	>100	5/6 on day 117
	Etoposide	20	Daily × 5/i.v.	100	7	7	0	0	2	30	0/7
IGRM33	Thiotepa	24	Single/i.p.	24	7	10	0	0	1	—	0/7
		30	Single/i.p.	30	7	22	5	0	1	—	0/7
	Cyclophosphamide	400	Single/i.p.	400	7	3	0	0	1	18	0/7
		500	Single/i.p.	500	7	2	0	0	3	24	0/7
	Etoposide	17	Daily × 5/i.v.	85	9	2	0	0	0	5	0/9
IGRM34	Thiotepa	24	Single/i.p.	24	7	1	0	0	0	2	0/7
		30	Single/i.p.	30	7	4	0	0	0	6	0/7
	Cyclophosphamide	200	Single/i.p.	200	8	0	0	0	1	22	0/8
		300	Single/i.p.	300	8	3	0	0	7	41	0/8
		400	Single/i.p.	400	8	10	7	2	6	NA	NA
	Etoposide	17	Daily × 5/i.v.	85	7	5	0	0	0	4	0/7
		20	Daily × 5/i.v.	100	7	15	0	0	0	4	0/7

[1]DT, median doubling time measured in each control group during the exponential phase of tumor growth curve; number, number of animals/group; BWL, maximum body weight loss; CR, complete regression; PR, partial regression; TGD, tumor growth delay.–[2]Animal died on day 56.–[3]Animal died on days 56–64; NA, not available.

Since topotecan exhibits a short half-life in humans (3 hr), the duration of administration is critical in efforts to sustain effective plasma drug levels.

The schedule dependency of irinotecan is less clearly established. In preclinical studies, Bissery *et al.* (1996) showed that irinotecan was not markedly schedule dependent using 2 rapidly growing murine tumors. In these experiments, irinotecan was given with different schedules (once or twice daily, intermittent, infusion) for the same short course of treatment (4 days). No change was observed in the MTD or in the anti-tumor effects when the number of injections was increased. Houghton *et al.* (1993, 1995) showed that prolonged administration of irinotecan over 54 days was more effective than a short treatment over 12 days against several pediatric tumor xenografts. In these studies, irinotecan was administered 5 days a week for 2 consecutive weeks, and this was considered as a cycle of chemotherapy. In the first study, irinotecan, given at the MTD (300 mg kg^{-1}) in 3 consecutive cycles, was shown to be significantly more active than 400 mg kg^{-1} given in 1 cycle against xenografts of a juvenile colon cancer and a rhabdomyosarcoma (Houghton *et al.,* 1993). Prolonged treatment with 3 cycles of irinotecan was highly active against a large panel of xenografts: 6 of 7 colon cancers, 6 of 6 rhabdomyosarcomas, 2 of 2 medulloblastomas and 1 glioblastoma (Houghton *et al.,* 1995). These results clearly show that, for equivalent dosages of irinotecan, prolonged administration is more effective than intensive treatment in shorter schedules in human tumor xenografts.

To analyze further the schedule dependency of irinotecan *in vivo* anti-tumor activity, we compared its activity at the same dose intensity levels, *i.e.,* same total doses over the same period of treatment, using either sequential treatment or protracted administration. Sequential administration (1 5-day cycle every 28 days) resembles the schedules commonly used in chemotherapy protocols in humans. The continuous schedule, *i.e.,* 5 days a week for 5 consecutive weeks, was not possible in mice because of local toxicity at the injection site. One week of rest was needed during the protracted treatment schedule. Our data show that the anti-tumor activity of irinotecan, when given as a prolonged treatment, is clearly schedule dependent in one PNET xenograft. Conversely, an increase in the number of injections does not increase the activity of irinotecan in a short course of treatment (Bissery *et al.,* 1996). Different schedules have been explored in phase I studies in humans (once every 3 weeks, 3 times a day every 3 weeks, weekly for 3 consecutive weeks), but CPT-11 was not found to be markedly schedule dependent (Armand, 1996; Bissery *et al.,* 1996). However, our data, along with those of Houghton *et al.* (1995), suggest

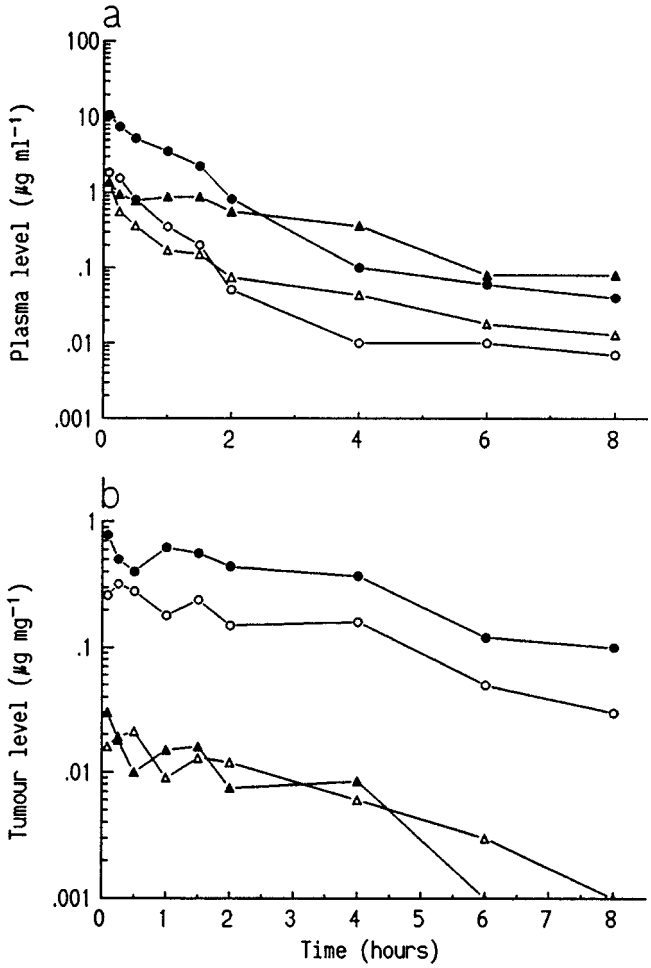

FIGURE 3 – Pharmacokinetics of CPT-11 (circles) and SN-38 (triangles) in the plasma *(a)* and tumor *(b)* of IGRM34 xenograft-bearing nude mice. CPT-11 was administered as an i.v. bolus at a dose of 10 mg kg^{-1} day^{-1} (open symbols) or 40 mg kg^{-1} day^{-1} (solid symbols).

TABLE IV – PLASMA AND TUMOR PHARMACOKINETICS OF CPT-11 AND SN-38 IN IGRM34-BEARING MICE[1]

	CPT-11 pharmacokinetics		SN-38 pharmacokinetics	
Dose of i.v. CPT-11 (mg/kg)	10	40	10	40
Plasma				
C_{max} (ng/ml)				
Mean	1,850	10,680	1,370	1,380
Range	1,420–2,330	9,470–12,730	1,240–1,500	1,230–1,520
$t_{1/2}\beta$ (hr)	2.0	3.0	1.85	2.1
$AUC_{0-\infty}$ (ng hr ml^{-1})	1,378	9,744	902	3,780
CI (L hr^{-1} kg^{-1})	7.26	4.1	—	—
Tumor				
C_{max} (ng/mg)				
Mean	320	780	18	21
Range	270–350	450–1,100	10–26	15–30
$t_{1/2}\beta$ (hr)	2.4	2.5	—	—
$AUC_{0-\infty}$ (ng hr mg^{-1})	1,075	2,640	52[2]	57[2]

[1]C_{max}, maximum concentration; $t_{1/2}\beta$, elimination half-life; $AUC_{0-\infty}$ area under the curve from 0 to infinity; Cl, total plasma clearance rate.–[2]AUC from 0 to 8 hr. Results of intratumor pharmacokinetics are expressed in ng/mg of tumor.

that prolonged administration in a continuous i.v. infusion or orally probably deserves further investigation in humans.

The third aim of our study was to analyze the pharmacokinetics and metabolism of irinotecan in tumor-bearing mice. Irinotecan is indeed mainly active when converted into its active metabolite SN-38 by carboxylesterase. Other metabolic pathways, such as SN-38 glucuronidation, have also been identified. The ways in which irinotecan is metabolized may play a key role in the nature and extent of toxicity in humans. Pharmacodynamic relationships have been shown to exist between irinotecan and SN-38 plasma levels or systemic exposure and the main toxicities, *i.e.,* hematologic toxicity and diarrhea (Chabot *et al.,* 1995). Moreover, the rate of SN-38 glucuronidation may be involved in the occurrence of diarrhea (Gupta *et al.,* 1994). Major differences between species have been identified for several anti-cancer drugs that are highly metabolized *in vivo.* This may markedly interfere with the predictibility of the results of preclinical anti-tumor activity in experimental systems. In tumor-bearing nude mice, we showed that irinotecan clearance was dose-dependent, as previously shown in BALB/c mice (Kaneda *et al.,* 1990). The ratio between SN-38 and CPT-11 AUC in plasma was 0.65 and 0.4 at the 10 and 40 mg kg^{-1} doses, respectively, and differed significantly from the metabolic ratios observed in humans (0.01–0.05) (Chabot *et al.,* 1995). Assuming that there is no time-dependent alteration in the disposition of irinotecan in nude mice, the AUC of CPT-11 was expected to be 48.7 μg hr ml^{-1} at the highest daily dose of irinotecan tested (40 mg kg^{-1} $day^{-1} \times 5$). In humans, the mean AUCs of CPT-11 at the MTDs ranged from 28 to 47 μg hr ml^{-1} in phase I studies (Rowinsky *et al.,* 1994; Chabot *et al.,* 1995). By contrast, a more than 10-fold difference was observed in the SN-38 AUC between nude mice (18,900 ng hr ml^{-1}) and humans (410–941 ng hr ml^{-1}). Thus, the preclinical anti-tumor activity of CPT-11 in xenograft-bearing nude mice after a short administration schedule was observed for the CPT-11 systemic exposure levels in plasma that are commonly reached at the MTD in humans, and for the SN-38 systemic exposure levels that are more than 10-fold higher than those observed in humans. The consequences of these interspecies variations cannot be presently analyzed. It remains to be determined whether the anti-tumor activity of CPT-11 is correlated with plasma systemic exposure to CPT-11 and/or SN-38 in humans. The AUC may not be the most appropriate pharmacokinetic parameter for such studies. Clinical data showed that the activity of etoposide, a schedule-dependent topoisomerase II inhibitor, was better correlated with the number of hours at plasma levels above 1 μg/ml rather than with the AUC (Slevin *et al.,* 1989).

Study of tumor pharmacokinetics of CPT-11 and SN-38 after i.v. administration of a single dose of CPT-11 revealed 2 major findings: the ratio between the SN-38 and CPT-11 AUC in tumor was 10-fold lower (0.02–0.04) than in plasma; the dose increase from 10 to 40 mg kg^{-1} was associated with an increase in the CPT-11 AUC, but not with an increase in the SN-38 AUC. The complex mechanisms responsible for these differences are not readily open to analysis since several processes may be involved, such as tumor distribution of CPT-11 and SN-38 from plasma, intra-tumor conversion of CPT-11 into SN-38, *in situ* glucuronidation of SN-38 and other metabolic pathways. Kawato *et al.* (1991) have shown that CPT-11 is converted into SN-38 by human tumor xenografts, but that the sensitivity of these tumors to CPT-11 *in vivo* was independent of their ability to produce SN-38. The absence of increase in the SN-38 AUC in tumor following the dose increase may be related to the absence of clear dose-dependent anti-tumor activity observed during the 5-day treatment schedule in the 4 xenografts evaluated.

In conclusion, irinotecan was found to be highly active against medulloblastoma xenografts. Given the preclinical data generated in pediatric tumor xenografts, irinotecan deserves clinical evaluation in pediatric malignancies. A phase I study in children is ongoing in France, using a once-every-3-week schedule (Vassal *et al.,* 1997). Future clinical development will need to address the tolerance and anti-tumor activity of CPT-11 following prolonged administrations. In addition, further metabolic and pharmacodynamic studies will be required during the phase II studies in children to elucidate the consequences of the inter-species differences so far identified.

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