Irinophore C: A Liposome Formulation of Irinotecan with Substantially Improved Therapeutic Efficacy against a Panel of Human Xenograft Tumors

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Abstract

Purpose: To assess the pharmacokinetics, tumor drug accumulation, and therapeutic activity of Irinophore C, a novel liposomal formulation of irinotecan (CPT-11).

Experimental Design: The plasma lactone/carboxy levels of CPT-11 and SN-38 were determined in mice after a single i.v. dose of irinotecan (Camptosar), or Irinophore C, and the plasma t1/2, plasma area under the curve, plasma Cmax, and plasma clearance were calculated. Further, plasma and tumor drug levels were also measured in tumor-bearing mice following Irinophore C treatment. The efficacy of Irinophore C was compared with that of Camptosar in five s.c. human tumor xenografts using single-dose treatment (LS 180), a total of three doses administered at 4-day intervals (H460), or a total of three doses administered at 7-day intervals (Capan-1, PC-3, and HT-29).

Results: Compared with Camptosar, Irinophore C mediated an 8-fold increase in t1/2, a 100-fold increase in Cmax, a 1,000-fold increase in area under the curve, and a 1,000-fold decrease in clearance for the active lactone form of CPT-11. Further, the plasma and tumor SN-38 lactone levels were consistent for at least 48 h post-Irinophore C injection. Camptosar treatment (40 mg/kg) mediated a delay in the time required for tumors to increase to four times their pretreatment size compared with controls (T-Cs). T-Cs ranged from 2 days (LS 180 model) to 87 days (Capan-1 model). Irinophore C (40 mg/kg) engendered T-Cs ranging from 14 days (LS 180 model) to 87 days (Capan-1 model).

Conclusion: Irinophore C improved CPT-11/SN-38 pharmacokinetics, promoted tumor drug accumulation, and increased therapeutic efficacy in a panel of five distinct human tumor xenografts.

Camptothecins mediate their anticancer activity by stabilizing the cleavable complex formed between topoisomerase I and DNA during the S phase of the cell cycle (1); however, this mechanism is dependent on the α-hydroxy-ω-lactone ring of camptothecins (2), which is problematic because physiologic conditions promote the reversible hydrolysis of the lactone ring to yield the inactive carboxylate form (3). Recently, research has focused on strategies to stabilize the lactone ring structure, including the use of drug carrier technologies such as hydrogels (4), polymer conjugates (5), microspheres (6), and lipid-based systems (7–12).

The water-soluble camptothecin derivative irinotecan (CPT-11) is an attractive candidate for formulation in a nanocarrier because it has proven clinical activity against colorectal (13) and small-cell lung cancers (14) and may be active in other cancer indications (15). CPT-11 has a complicated pharmacologic profile and is extensively metabolized in vivo to yield a number of derivatives including the potent metabolite SN-38 (16). As with the parent drug, the cytotoxic activity of SN-38 is also dependent on the maintenance of the lactone ring (16).

The physicochemical characteristics of CPT-11 make it amenable to efficient encapsulation in pharmaceutically viable liposome systems. It is already established that CPT-11 can be actively loaded into liposomes via a transmembrane pH gradient (11). The weakly basic drug is added to the outside of liposomes suspended in buffer at pH 7.4, and at this pH a substantial proportion of the drug exists in the neutral form, which can easily permeate the liposomal membrane. On contact with the acidic environment of the aqueous core, CPT-11 is ionized and consequently trapped inside the liposome (17–19). A further benefit of this technology is that the encapsulated CPT-11 exists predominately as the active lactone, which improves therapeutic efficacy (10, 11, 20, 21).
For many anticancer drugs, particularly those that are cell cycle specific, improved drug retention is associated with increases in drug exposure at sites of disease (15, 16). We have recently described a formulation technology that involves the use of entrapped copper ions and a transmembrane pH gradient (acidic inside), which seems to result in significantly improved CPT-11 retention in the liposomes following systemic administration (20). Drugs such as CPT-11 that have protonizable amine groups have been shown to be better retained by liposomes comprising acidic interior buffers (17); further, we have shown that CPT-11 can form a transition metal complex with copper (22). Consequently, following systemic administration, a liposome formulation with a stable internal acidic environment with copper ions mediated substantial improvements in CPT-11 retention, which translated to improved therapeutic activity in a model of colorectal cancer, when compared with carrier systems using pH gradients or copper ion gradients alone. We have named this liposome formulation Irinophore C.

The series of studies described herein investigated the influence of Irinophore C on the pharmacokinetics and tumor accumulation of both the lactone and carboxylate forms of CPT-11 and SN-38. The results will show that Irinophore C mediated a 1,000-fold increase in the plasma CPT-11 lactone and SN-38 lactone area under the curve (AUC) compared with Camptosar and mediated a 1,000-fold increase in the plasma CPT-11 lactone and SN-38. The results will show that Irinophore C accumulation of both the lactone and carboxylate forms. For many anticancer drugs, particularly those that are cell cycle specific, improved drug retention is associated with increases in drug exposure at sites of disease (15, 16). We have recently described a formulation technology that involves the use of entrapped copper ions and a transmembrane pH gradient (acidic inside), which seems to result in significantly improved CPT-11 retention in the liposomes following systemic administration (20). Drugs such as CPT-11 that have protonizable amine groups have been shown to be better retained by liposomes comprising acidic interior buffers (17); further, we have shown that CPT-11 can form a transition metal complex with copper (22). Consequently, following systemic administration, a liposome formulation with a stable internal acidic environment with copper ions mediated substantial improvements in CPT-11 retention, which translated to improved therapeutic activity in a model of colorectal cancer, when compared with carrier systems using pH gradients or copper ion gradients alone. We have named this liposome formulation Irinophore C.

The series of studies described herein investigated the influence of Irinophore C on the pharmacokinetics and tumor accumulation of both the lactone and carboxylate forms of CPT-11 and SN-38. The results will show that Irinophore C mediated a 1,000-fold increase in the plasma CPT-11 lactone area under the curve (AUC) compared with Camptosar and maintained continuous plasma levels of SN-38 lactone for at least 24 h after i.v. administration. Further, CPT-11 lactone and SN-38 lactone accumulated in tumors following Irinophore C treatment. Collectively, the pharmacokinetic improvements and tumor accumulation resulted in substantial gains in therapeutic efficacy in five different xenograft models of human cancer.

### Materials and Methods

**Liposome preparation.** 1,2-Distearoyl-sn-glycero-phosphocholine and cholesterol (DSPC/Chol; 55:45 mol%; Avanti Polar Lipids) large unilamellar vesicles were prepared as previously described (23). Briefly, lipids were dissolved in chloroform at the required molar ratio, labeled with the nonexchangeable, nonmetabolizable lipid marker 1′H-CHE (Perkin-Elmer Life Sciences), and dried to a thin film under a stream of nitrogen gas. Subsequently, the lipid was placed in a high vacuum for 2–3 h to remove any residual solvent. The lipid films were hydrated at 65°C by mixing with 300 mmol/L copper sulfate solution before five cycles of freeze-and-thaw (5 min each, freezing in liquid nitrogen and thawing at 65°C). The multilamellar vesicle suspensions were then extruded 10 times through stacked polycarbonate filters of 0.08- and 0.1-μm pore sizes at 65°C (extruder, Northern Lipids). The resultant large unilamellar vesicles typically possessed mean vesicular diameters in the range of 110 ± 30 nm as determined using Phase Analysis Light Scattering methods (ZetaPALS, Brookhaven Instruments Corp.). The large unilamellar vesicle external buffer was exchanged using Sephadex G-50 size exclusion chromatography with SHE buffer pH 7.5 (300 mmol/L sucrose, 20 mmol/L HEPES, 15 mmol/L EDTA).

**Accumulation of irinotecan into preformed DSPC/Chol liposomes.** The divalent metal ionophore A23187 [calcium ion; Sigma; 1 mg/mL (1.9 mmol/L solution in 100% ethanol)] was preincubated with liposomes (0.5 μg/mg lipid) at 60°C for 30 min. Subsequently, irinotecan hydrochloride trihydrate (Camptosar, Pharmacia; BC Cancer Agency Pharmacy) was added to liposomes (~ 50 mmol/L) at 50°C at a drug-to-lipid ratio of 0.2:1 (mol/mol). Drug uptake was determined after 60-min incubation by separating encapsulated drug from free drug using a Sephadex G-50 column equilibrated with PBS buffer. The excluded fractions, containing the liposomes, were analyzed to determine drug-to-lipid ratios. Lipid concentrations were measured by liquid scintillation counting (Packard 1900TR Liquid Scintillation Analyzer). Irinotecan concentration was determined by measuring absorbance at 370 nm. Briefly, a portion of the sample collected from the column was adjusted to a final volume of 100 μL with PBS. Subsequently, 900 μL of Triton X-100 1% was added and the samples were heated in a water bath at >90°C until the cloud point of the detergent was observed. The samples were then cooled to room temperature and the absorbance was determined against a freshly prepared irinotecan standard curve (Hewlett Packard UV-Vis spectrophotometer, model 8453). For efficacy studies, Irinophore C was concentrated using Amicon Ultra-15 centrifugal Filter Tubes (3,000 × g for 30 min; Millipore) to achieve the desired dose (mg/kg) administered in a volume of 200 μL. The concentrations of lipid and irinotecan present in the final samples were confirmed as described above.

**Pharmacokinetic studies.** Female BALB/c mice (Taconic; 20–25 g; four per time point) were injected i.v. with a single dose of Camptosar (50 mg/kg irinotecan) or Irinophore C (50 mg/kg irinotecan; 225 mg/kg lipid) and the plasma concentrations of liposomal lipid (1H scintillation counting as described above to measure associated 3H-CHE) and irinotecan were determined over time (11). In addition, female RAG-2M mice (n = 4) with established s.c. human non–small-cell lung cancer cell line (NSCLC) H460 tumors (see "Efficacy studies") were given a single i.v. dose of Irinophore C (20 mg/kg irinotecan; 180 mg/kg lipid) and the plasma levels of drug and lipid were determined as above. High-performance liquid chromatography separation of irinotecan (CPT-11) and SN-38 lactone and carboxylate forms was done using a 250 × 4.6 mm Symmetryshield RP18 5-μm column and Symmetryshield RP18 guard column (Waters). Gradient elution was used with mobile phase A composed of 75 mmol/L ammonium acetate and 7.5 mmol/L tetrabutylammonium bromide adjusted to pH 6.4 with glacial acetic acid (Fisher Scientific) and mobile phase B was acetonitrile. Gradient profile was as follows: time, 0 min: 78% A:22% B; time, 10 min: 64% A:36% B; time, 16 min: 78% A:22% B; time, 20 min: 78% A:22% B. A 10-μL sample was injected onto the column (column temperature, 40°C) and eluted at a flow rate of 1 mL/min. CPT-11 and SN-38 forms were detected using a Waters 2475 multi-wavelength fluorescence detector (Waters) set with time program events of λex = 370 nm; λem = 425 nm between times 0 and 12.5 min for CPT-11 forms, and λex = 370 nm; λem = 535 nm between times 12.5 and 20 min for SN-38 forms. Before injection, all samples were maintained at 4°C to reduce conversion between lactone and carboxylate forms. Standard curves of CPT-11 and SN-38 lactone form were prepared by serial dilutions in a 2:1:1 sodium acetate (100 mmol/L)/methanol/acetonitrile pH 4.0 buffer. For the carboxylate form of CPT-11 and SN-38, serial dilutions were prepared in a 2:1:1 sodium borate (100 mmol/L)/methanol/acetonitrile pH 9.0 buffer. The limit of quantitation for CPT-11 and SN-38 lactone and carboxylate forms was 10 ng/mL. Irinotecan pharmacokinetic parameters were calculated using the noncompartmental analysis function of WinNonLin software version 5.0.1 (Pharsight).

**Cell culture.** All cell lines were purchased from the American Type Culture Collection and were cultured in the appropriate base media (StemCell) with fetal bovine serum (FBS; Cansera) for up to 10 passages. After 10 passages, new cells were expanded from a frozen stock stored in liquid nitrogen. The human NSCLC H460 was cultured in RPMI 1640 supplemented with 2 mmol/L l-glutamine, 1.5 g/L sodium bicarbonate, 4.5 g/L glucose, 10 mmol/L HEPES, 1.0 mmol/L sodium pyruvate, and 10% FBS. The human colorectal adenocarcinoma cell line LS 180 was cultured in Eagle's MEM with 2 mmol/L l-glutamine and Earle's balanced salt solution, 1.5 g/L sodium bicarbonate, 0.1 mmol/L nonessential amino acids, 1.0 mmol/L sodium pyruvate, and 10% FBS. The human colorectal adenocarcinoma cell line HT-29 was cultured in modified McCoy's 5A medium with

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1.5 mmol/L L-glutamine and 2.2 g/L sodium bicarbonate and 10% FBS. The prostate adenocarcinoma cell line PC-3 was cultured in Ham’s F12K medium supplemented with 2 mmol/L L-glutamine, 1.5 g/L sodium bicarbonate, and 10% FBS. Capan-1 pancreatic adenocarcinoma cells were cultured in Iscove’s modified Dulbecco’s medium with 4 mmol/L L-glutamine, 1.5 g/L sodium bicarbonate, and 20% FBS.

**Tumor drug accumulation studies.** RAG2-M mice (129S6/SvEvTac-Rag2tm1Flw, Taconic, 20-25 g, three per group) were inoculated s.c. into the center of the lower back with 2 x 10^6 H460 cells (50 μL). Tumor growth was monitored every Monday, Wednesday, and Friday with calipers and the measured dimensions (in millimeters) were converted to tumor weight (in milligrams) using the following equation: length x width x height / 2. The equilibrium between the lactone and carboxylate forms; which has been reported to be 100- to 1,000-fold more potent than CPT-11 (24). In addition, SN-38 also exists in the lactone and carboxylate forms. The pharmacokinetic parameters calculated for Irinophore C-CPT-11 carboxylate form supported this belief. For example, the plasma half-life of CPT-11 in the presence of carboxylesterase to yield SN-38, which has been reported to be 100- to 1,000-fold more potent in vitro than CPT-11 (24). In addition, SN-38 also exists in the lactone and carboxylate forms; therefore, the relative plasma levels of the two forms were quantified following Camptosar and Irinophore C i.v. administration. SN-38 lactone and carboxylate forms were both detectable in the plasma 5 min after injection of Camptosar (Fig. 1C). Equilibrium was achieved by the 30-min time point, and this decreased to 100 μg/mL after 24 h. These levels represent a 10- to 100-fold increase over the maximum recorded concentration achieved following injection of an identical dose of Camptosar. The carrier-mediated increase in CPT-11 levels is highlighted by the pharmacokinetic parameters shown in Table 1 and is best emphasized by the 1,000-fold increase in plasma CPT-11 lactone AUC levels afforded by Irinophore C.

It is assumed that the majority of CPT-11 detected in the plasma following Irinophore C administration is associated with the drug carrier. The pharmacokinetic parameters calculated for Irinophore C-CPT-11 carboxylate form supported this belief. For example, the plasma half-life of CPT-11 in the presence of carboxylesterase to yield SN-38, which has been reported to be 100- to 1,000-fold more potent in vitro than CPT-11 (24). In addition, SN-38 also exists in the lactone and carboxylate forms; therefore, the relative plasma levels of the two forms were quantified following Camptosar and Irinophore C i.v. administration. SN-38 lactone and carboxylate forms were both detectable in the plasma 5 min after injection of Camptosar (Fig. 1C). Equilibrium was achieved by the 30-min time point, and the plasma elimination profiles of the two forms were comparable for the remainder of the experiment, with the SN-38 lactone/SN-38 carboxylate ratio consistent at 2:1 (w/w). In
contrast, only SN-38 lactone was detectable in the plasma following Irinophore C administration (Fig. 1D). An SN-38 lactone plasma concentration of 2 \( \mu \text{g/mL} \) was recorded 1 h postinjection. This level decreased to 1 \( \mu \text{g/mL} \) after 4 h and remained constant for the duration of the experiment achieving continuous plasma SN-38 levels over 24 h, which were greater than those observed 10 min after Camptosar administration.
The plasma elimination profiles for both forms of CPT-11 (Fig. 1E) and SN-38 (Fig. 1F) were also quantified following a single dose of Irinophore C 20 mg/kg injected i.v. via the tail vein of RAG-2M mice bearing established s.c. human NSCLC H460 xenografts. The duration of this study was extended to 72 h postinjection and the relative levels of CPT-11 lactone and carboxy for the first 24 h are comparable to that seen in non-tumor-bearing BALB/c mice treated with a higher dose of 50 mg/kg Irinophore C (compare Fig. 1E-B). At 48 h postinjection, the CPT-11 lactone levels had decreased by 2 orders of magnitude (Fig. 1E); in contrast, the concentration of CPT-11 carboxy had remained reasonably constant. Resultantly, the ratio of CPT-11 lactone/CPT-11 carboxy changed from ~1,000:1 over the first 24 h to 10:1 by 72 h. The pharmacokinetic parameters associated with Irinophore C administration to tumor-bearing mice are shown in Table 1 and are comparable to those for BALB/c mice, with a plasma half-life for CPT-11 lactone of 6 h in both models.

The plasma SN-38 levels in the H460 xenograft model following Irinophore C injection (20 mg/kg) are illustrated in Fig. 1F. Consistent with the plasma profile in BALB/c mice (Fig. 1D), SN-38 lactone levels remained steady over 24 h, albeit at concentrations approximately half of that seen with a higher dose of Irinophore C (50 mg/kg), before decreasing by 2 orders of magnitude by 72 h; further, there were no detectable levels of SN-38 carboxy (Fig. 1F). Although SN-38 is known to be highly protein bound (>90%) and the lactone form has a higher affinity thereby forcing equilibrium for unbound plasma SN-38 toward the lactone form (16), it is unclear why SN-38 carboxy was not detected after Irinophore C administration (limit of detection, 10 ng/mL; ref. 25). It may be that SN-38 can be generated when the CPT-11 is still associated with the liposome, thereby facilitating the maintenance of the lactone form (26).

The concentration of CPT-11 and SN-38 in H460 NSCLC tumors following treatment with Irinophore C. Enhanced permeability and retention is a putative mechanism by which nanoparticle drug carrier systems can exert their antitumor effects (27). Particles in the nanometer size range that are normally too large to extravasate from blood vessels are able to passively accumulate in tumor tissue via leaky capillaries associated with the disease. Figure 2 shows the concentrations of CPT-11 and SN-38 present in human NSCLC H460 xenografts grown on the back of RAG-2M mice following a single i.v. dose of Irinophore C (20 mg/kg irinotecan). The drug concentrations have been corrected to account for the blood volume associated with the tumor (28) and, therefore, represent the drug present in the tissue.

The concentration CPT-11 lactone (black columns) was the greatest of the species detected over the duration of the experiment, peaking at ~12 μg/g of tumor tissue after 24 h before decreasing to ~5 μg/g of tumor tissue at 48 h. CPT-11 carboxy levels followed the same trend as CPT-11 lactone, albeit at markedly lower levels with a peak of 1 μg/g of tumor tissue detected after 24 h. Because the tumor levels of SN-38 lactone have been corrected for circulating plasma levels, the concentrations shown in Fig. 2 represent local concentrations of SN-38 (29). SN-38 lactone was detectable 1 h postinjection, but levels increased to ~1 μg/g of tumor tissue after 4 h and remained constant for the duration of the experiment, mirroring what was observed in the plasma (see Fig. 1F). SN-38 carboxy was not detected using the experimental methods described in this article.

### Table 1. The calculated CPT-11 pharmacokinetic parameters following a single i.v. dose of Camptosar or Irinophore C

<table>
<thead>
<tr>
<th>Treatment</th>
<th>( t_{1/2} ) (h)</th>
<th>( C_{max} ) (μg/mL)</th>
<th>AUC (h μg/mL)</th>
<th>Cl (mL/h/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lactone</td>
<td>Carboxy</td>
<td>Lactone</td>
<td>Carboxy</td>
</tr>
<tr>
<td>Camptosar</td>
<td>0.8</td>
<td>1.0</td>
<td>9.9</td>
<td>5.1</td>
</tr>
<tr>
<td>Irinophore C</td>
<td>6.2</td>
<td>9.0</td>
<td>1,071.3</td>
<td>3.9</td>
</tr>
<tr>
<td>Irinophore C ⁶</td>
<td>6.1</td>
<td>8.5</td>
<td>654.0</td>
<td>1.5</td>
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</table>

Abbreviation: Cl, clearance.

*Pharmacokinetic parameters were estimated using noncompartmental analysis (WinNonLin software version 5.0.1).

⁰BALB/c mice were given a single i.v. bolus dose equivalent to 50 mg/kg irinotecan (CPT-11).

⁶Human NSCLC H460 tumor-bearing RAG-2M mice were given a single i.v. bolus dose equivalent to 20 mg/kg irinotecan (CPT-11).
Fig. 3. The therapeutic effectiveness of Camptosar and Irinophore C measured against a panel of human tumor xenografts. RAG2-M mice bearing established s.c. human tumor xenografts were treated with saline (▲), Camptosar 40 mg/kg (●), Camptosar 60 mg/kg (▼, H460 only), Irinophore C 20 mg/kg (○), Irinophore C 30 mg/kg (□), Irinophore C 40 mg/kg (◇), and Irinophore C 60 mg/kg (◇, H460 only). Tumor growth is represented by the median relative TGI and is expressed as a function of time. The number of doses and dosing schedule for each tumor model, as described in Materials and Methods, are indicated by the arrows. Each data point represents \( n = 8 \), except for Capan-1 where * indicates that this data point and subsequent data points for this treatment represent \( n = 7 \) because there was one durable cure. A, H460 NSCLC; B, LS 180 colorectal adenocarcinoma; C, HT-29 colorectal adenocarcinoma; D, PC-3 prostate carcinoma; E, Capan-1 pancreatic carcinoma.
The therapeutic efficacy of Camptosar and Irinophore C against a panel of five human tumor xenografts. Following i.v. administration of Irinophore C, there was a substantial change in both CPT-11 and SN-38 pharmacokinetics, represented by significant increases in AUC for the lactone forms of these drugs, and the pharmacokinetics suggested that SN-38 levels were maintained over extended time periods; further, CPT-11 lactone and SN-38 lactone were shown to be present in the tumor. The next series of studies aimed to determine whether the altered pharmacokinetic profile of Irinophore C was associated with improvements in therapeutic activity. Camptosar and Irinophore C were used to treat RAG2-M mice bearing established s.c. human tumor xenografts. The results from five different tumor models are summarized in Fig. 3, where tumor growth is expressed as the median relative TGI as a function of time (days).

A dose of Camptosar (40 or 60 mg/kg) or Irinophore C (20, 30, 40, or 60 mg/kg) was administered every 4 days for a total of three doses to treat mice with human H460 NSCLC xenografts (Fig. 3A). Camptosar did not result in a significant delay in TGI when compared with the saline-treated controls. Significant improvements (relative to controls and the Camptosar treated animals) were seen following treatment with Irinophore C. This is emphasized by the T-C, which represents the median difference in days for treated tumors (T) compared with control tumors (C) to reach a TGI equal to 4 (Table 2). The calculated T-C for the highest Camptosar dose tested (60 mg/kg) was 8 days, significantly lower than the lowest dose of Irinophore C (20 mg/kg), which mediated a T-C of 15 days. The mean %BWL was recorded and used as a marker for toxicity (Table 2). The %BWL associated with Irinophore C 20 mg/kg was 8.4%, which was lower than all treatment groups. There was a trend toward greater %BWL at the highest doses, particularly was 8.4%, which was lower than all treatment groups. There was no significant improvements in therapeutic activity (Fig. 3A; Table 2). For this reason, further efficacy experiments focused on doses of Irinophore C induced tumor regression whereas Camptosar treated animals. Resultantly, Irinophore C mediated a 10-fold increase in T-C (32 days for Irinophore C 40 mg/kg compared with 3 days for Camptosar 40 mg/kg; Table 2). Interestingly, there was no significant difference in T-C recorded for the three doses of Irinophore C (Table 2), and this was supported by similar tumor growth curves (Fig. 3C). Analysis of the associated %BWL indicated that administering the treatments at 7-day intervals resulted in similar %BWL observed for single-dose treatment (Table 2; cf. LS 180) and did not result in a cumulative dosing effect that was apparent for H460 (Table 2).

The results for the treatment of RAG2-M mice with established PC-3 prostate carcinoma xenografts followed a pattern similar to that observed for the HT-29 model (i.e., all doses of Irinophore C induced tumor regression whereas Camptosar only delayed tumor growth; Fig. 3D). Further, there were no notable differences in the therapeutic activity of the three Irinophore C doses (Table 2; Fig. 3D), and the %BWL associated with the different treatments were comparable and

<table>
<thead>
<tr>
<th>Treatment</th>
<th>H460</th>
<th>LS 180</th>
<th>HT-29</th>
<th>PC-3</th>
<th>Capan-1</th>
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<tr>
<td></td>
<td>T-C</td>
<td>%BWL</td>
<td>T-C</td>
<td>%BWL</td>
<td>T-C</td>
</tr>
<tr>
<td>Irinophore C (20 mg/kg)</td>
<td>15*</td>
<td>8.4 (3.8)</td>
<td>12 †</td>
<td>5.0 (2.2)</td>
<td>30 †</td>
</tr>
<tr>
<td>Irinophore C (30 mg/kg)</td>
<td>19 †</td>
<td>14.7 (4.3)</td>
<td>15 †</td>
<td>4.8 (6.5)</td>
<td>24 †</td>
</tr>
<tr>
<td>Irinophore C (40 mg/kg)</td>
<td>23 †</td>
<td>15.0 (1.9)</td>
<td>14 †</td>
<td>5.4 (2.8)</td>
<td>32 †</td>
</tr>
<tr>
<td>Camptosar (40 mg/kg)</td>
<td>5</td>
<td>12.1 (3.4)</td>
<td>2</td>
<td>4.9 (2.9)</td>
<td>3</td>
</tr>
<tr>
<td>Irinophore C (60 mg/kg)</td>
<td>24‡</td>
<td>18.5 (3.6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Camptosar (60 mg/kg)</td>
<td>8</td>
<td>13.9 (3.9)</td>
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</tbody>
</table>

NOTE: Treatment schedules are described in Materials and Methods section. T-C represents the median difference in days for treated tumors (T) compared with control tumors (C) to reach a TGI = 4. %BWL represents the maximum mean body weight loss associated with the indicated treatment. The SD is included in parentheses.

*Significantly different from Camptosar 40 mg/kg and Camptosar 60 mg/kg (P < 0.005).
†Significantly different from Camptosar 40 mg/kg (P < 0.005).
‡Significantly different from Irinophore C 20 mg/kg (P < 0.05).
§Significantly different from Irinophore C 20 mg/kg (P = 0.001) and Irinophore C 30 mg/kg (P < 0.05).
similar to that recorded for the single-dose treatment of LS 180 (Table 2).

The final model tested was a Capan-1 pancreatic xenograft and the results are shown in Fig. 3E. This was the only model within the panel of human tumor xenografts where there was a marked difference in therapeutic efficacy associated with the dose of Irinophore C. Clearly, all doses of Irinophore C were substantially more active than Camptosar 40 mg/kg (Fig. 3E). Moreover, the highest dose of Irinophore C (40 mg/kg) was able to cure one mouse and also mediated a T-C (87 days) that was significantly greater than those recorded for the 20 and 30 mg/kg doses, as well as being 15-fold higher than the T-C for the equivalent dose of Camptosar (6 days; Table 2). Consistent with the previous models using the dosing schedule of three doses administered every 7 days, the %BWL were comparable to those observed following a single dose (Table 2).

Discussion

Liposomes represent the preeminent nanoscale drug delivery technology for the i.v. administration of cytotoxic drugs (30), with three anthracycline preparations currently licensed for clinical use (31–33) and many other liposomal formulations of antineoplastic drugs in preclinical or clinical trials (12, 34–37). The beneficial effects of liposomes have been attributed to decreased toxicity, increased drug stability, and improved biodistribution of the encapsulated drugs (38).

The clinical effect of CPT-11 on overall patient survival and quality of life has been significant (39) despite the physiologic instability of the active lactone forms of the drug and its potent metabolite, SN-38 (3). Consequently, CPT-11 is an attractive candidate for formulation with lipid nanocarriers. Burke and Gao (7) first showed that lipid-based carrier formulations could stabilize the lactone ring of topotecan; subsequently, the stabilization of CPT-11 lactone, following i.v. administration, by liposome encapsulation has been associated with improved therapeutic activity (10, 11, 20). Irinophore C is a novel liposome formulation of CPT-11 in which drug retention following systemic administration was greatly improved by entrapping the drug in an acidic copper ion environment (40).

Following a single i.v. bolus injection, Irinophore C mediated a substantial increase in the CPT-11 lactone plasma half-life, maximum plasma concentration \(C_{\text{max}}\), and plasma AUC and a substantial decrease in plasma clearance, when compared with an equivalent dose of Camptosar (Table 1). Likewise, Irinophore C altered the pharmacokinetic profile for CPT-11 carboxylate (Table 1; Fig. 1A and B). Presumably, this indicates that the majority of both forms of CPT-11 detected in the plasma are associated with the carrier system, which would explain why the high CPT-11 \(C_{\text{max}}\) levels following i.v. administration of Irinophore C did not precipitate a “cholinergic syndrome.” This toxicity is attributed to CPT-11–mediated acetylcholinesterase inhibition (41) and limits the maximum single i.v. dose of Camptosar that RAG2-M mice can tolerate to 80 mg/kg. In contrast, Irinophore C is well tolerated at this dose in RAG2-M mice and has been administered to the plasma carboxylesterase–deficient murine strain aES1/J (42) at single doses up to 350 mg/kg with no immediate or long-term toxicities. Drummond et al. (10) reported similar results for a liposomal irinotecan formulation in which multivalent anionic trapping agents were used to increase retention of the drug.

Protracted dosing schedules would be expected to be beneficial for S-phase active drugs like CPT-11 (15, 16). Clinical studies have shown that the maximum CPT-11 dose intensity achievable with long-term continuous infusion is actually two to three times lower than that observed with short-term infusions; however, the SN-38 plasma AUC levels were comparable (15, 16). This implies that higher CPT-11 doses may saturate the hepatic carboxylesterases that are predominately responsible for the generation of SN-38.

Further, there is evidence to suggest that optimal anticancer activity is dependent on the maintenance of a threshold level of exposure to SN-38 (43). Furman et al. (44) reported that a prolonged CPT-11 dosing schedule optimized in xenograft models to maintain a minimum threshold plasma concentration of SN-38 could be mimicked in children with a comparable relationship between SN-38 levels and response. The long half-life of CPT-11 release from the carrier (44.4 h; ref. 40) could prevent saturation of the carboxylesterase enzymes in the liver, enabling Irinophore C to mediate a constant plasma SN-38 lactone level over the duration of the experiment (Fig. 1D and F). Indeed, the inherent accumulation of liposomes in the liver might act as a drug depot where the macrophage disruption of the carrier could slowly free CPT-11 for enzymatic conversion (45). If the lowest dose of Irinophore C (20 mg/kg) still maintained the plasma SN-38 levels above the critical threshold (Fig. 1F), this could explain the lack of dose-response effect observed for the majority of the xenograft models tested (Fig. 3).

The mechanism of tumor growth delay, or shrinkage, observed in the five xenograft models following Irinophore C treatment remains to be determined. Camptothecins have been reported to have antiangiogenic effects (46), and it may be that maintenance of a threshold plasma level of SN-38 by Irinophore C could mimic metronomic dosing, in which the anticancer action is primarily through disruption of the tumor vasculature. However, despite SN-38 being reported as up to 1,000 times more potent than CPT-11 in vitro (24), it is difficult to estimate the contribution of each compound to the overall cytotoxic effect in vitro (47). There have been in vivo survival studies using HT-29 cells (48) and H460 cells (25) that have attributed the observed cytotoxicity solely to CPT-11. Analysis of H460 tumor levels of CPT-11 (Fig. 2) showed that the lactone form predominates, suggesting either that CPT-11 is still encapsulated within the liposome or, alternatively, that the lower pH of the tumor microenvironment is limiting the conversion of bioavailable CPT-11 lactone to the carboxy form (49). SN-38 lactone levels within the tumor, after accounting for the presence of the active metabolite in the plasma, could be due to CPT-11 lactone that leaked from regionally localized liposomes, or it could be from the plasma compartment. The latter could be a result of drug release from liposomes within the plasma compartment or following localization in other

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*Unpublished observations.*
tissues such as the liver. However, it is also reasonable to speculate that tumor accumulation of liposomes via the enhanced permeability and retention effect and subsequent leakage of CPT-11 could also contribute to the anticancer effect of Irinophore C. We are currently investigating whether the activity of Irinophore C is mediated by a dual-action mechanism encompassing antivascular and direct tumor cell cytotoxic actions. Further, ongoing studies in our laboratory are also assessing the therapeutic efficacy of Irinophore C against a systemic melanoma model grown in aE512J mice. These mice are deficient in plasma carboxylesterase and are believed to offer a better model of SN-38 metabolism in humans. It is hoped that these experiments will provide a more mechanistic basis for the improved therapeutic effects of Irinophore C.

Obviously, further clinical development of Irinophore C will also require a better understanding of the toxicity of this new formulation. The principal dose-limiting toxicity of irinotecan is delayed diarrhea, which is attributed to the high intestinal concentrations of SN-38 excreted directly via the biliary canal, or as the result of β-glucuronidase cleavage of SN-38G to SN-38 by the gut flora (50). Ongoing studies in our laboratory are investigating the effect of CPT-11 encapsulation on the principal toxicities associated with irinotecan therapy.

In conclusion, Irinophore C is a novel liposomal formulation of irinotecan, which substantially increases the plasma levels of CPT-11 lactone and mediates a prolonged plasma and tumor exposure to SN-38 lactone. This favorable pharmacokinetic profile translated into superior therapeutic activity compared with Camptosar when tested against a panel of five human cancer xenografts. These results would support the further development of Irinophore C for future clinical trials.

References


Irinophore C: A Liposome Formulation of Irinotecan with Substantially Improved Therapeutic Efficacy against a Panel of Human Xenograft Tumors

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