Enhanced therapeutic efficacy of a novel liposome-based formulation of SN-38 against human tumor models in SCID mice

Sabrina Lei[a], Pei-Yu Chien[a], Saifuddin Sheikh[a], Allen Zhang[a], Shahid Ali[a] and Imran Ahmad[a]

SN-38 is an active metabolite of CPT-11. The poor solubility of SN-38 in any pharmaceutically acceptable solvent and pH-dependent activity has limited its clinical use. Our objective was to evaluate an easy-to-use liposome-based formulation of SN-38 (LE-SN38) and compare the antitumor activity with its pro-drug CPT-11 against cancer cell lines and human xenograft tumor models. The cytotoxicity of LE-SN38 and CPT-11 was determined in four human cancer cell lines using the sulforhodamine B assay. The therapeutic efficacy was tested against human colon (HT-29) and breast (MX-1) xenograft tumor models in SCID mice. LE-SN38 with greater than 95% drug entrapment was found to be highly cytotoxic against four different cell lines with GI_{50} values of less than 0.1 μM. In the HT-29 tumor model, LE-SN38 (q × d5) at 2, 4 or 8 mg/kg resulted in 33, 81 and 91% tumor growth inhibition, respectively, compared to the drug-free liposome group. In contrast, similar dose levels of CPT-11 treatment led to only 2, 36 and 46% growth inhibition. For the MX-1 model, LE-SN38 (q × d5) regressed tumor growth by 44 and 88% at 4 and 8 mg/kg dose, respectively, whereas no regression was observed in the CPT-11-treated group. We conclude that LE-SN38 is a novel liposome-based formulation with enhanced therapeutic efficacy against human tumor models. *Anti-Cancer Drugs* 15:773–778 © 2004 Lippincott Williams & Wilkins.

Anti-Cancer Drugs 2004, **15**:773–778

Keywords: CPT-11, cytotoxicity, human tumor xenografts, liposome-based formulation, SN-38

[a]Research and Development, NeoPharm Inc., Waukegan, IL, USA.

The first two authors contributed equally to this work.

Correspondence to I. Ahmad, Research and Development, NeoPharm Inc., 1850 Lakeside Drive, Waukegan, IL 60085, USA.
Tel: +1 847 887-0800; fax: +1 847 887-9281;
e-mail: Imran@neophrm.com

Received 14 October 2003 **Revised form accepted** 9 June 2004

Introduction

SN-38 (7-ethyl-10-hydroxy-camptothecin) is an active metabolite of CPT-11 (irinotecan or 7-ethyl-10[4-(1-piperidino)-1-piperidino]-carbonyloxy camptothecin) that had been approved by the FDA for colon cancer patients who failed to respond to 5-fluorouracil and developed liver metastasis. CPT-11, a semisynthetic derivative of camptothecin (CPT), is also shown to be effective for the treatment of non-small cell lung, breast and ovarian cancers [1–4]. CPT is a DNA topoisomerase (Topo) I inhibitor [5,6]. Topo I acts as a 'DNA swivel' and relieves torsional strain in DNA by inducing reversible single-strand breaks which are required for DNA transcription and replication. The formation of a CPT–DNA–Topo I complex causes a collision between moving replication forks and CPT-stabilized cleavable DNA–Topo I complexes, and thereby generates irreversible double-strand breaks [6,7]. CPT-11 itself has a very limited activity and functions by converting to SN-38 in the presence of carboxylesterases, which are mainly present in the liver and blood serum [8]. Less than 10% of CPT-11 is converted into SN-38 in humans [9]. Even at this minimal conversion rate, CPT-11 has been an effective anticancer drug in the market. It is likely that direct administration of SN-38 may produce better therapeutic efficacy in patients. Unfortunately, SN-38 is virtually insoluble in all pharmaceutically acceptable solvents.

Liposomes as drug carriers have been studied since the 1970s, and have been proven to alter drug distribution patterns, prolong circulation time, improve efficacy and reduce toxicity [10–12]. In the present study we have evaluated a well-characterized liposome-based formulation of SN-38 with high drug entrapment, and compared its activity with CPT-11 against human cancer cell lines and xenograft tumor models.

Materials and methods

Materials

Trichloroacetic acid (TCA), sulforhodamine B (SRB) and dextrose were purchased from Sigma (St Louis, MO). CPT-11 (Camptosar; irinotecan hydrochloride injection, 20 mg/ml) was purchased from Pharmacia & Upjohn (Kalamazoo, MI). Dioleoylphosphocholine, cardiolipin and cholesterol were purchased from Avanti Polar Lipids (Alabaster, AL).

Preparation of LE-SN38

The liposome-based formulation of SN-38 was prepared using the modified thin-film hydration method. In brief, dioleoylphosphocholine, cardiolipin (chain length 14), cholesterol (molar ratio of 50:40:10) and α-tocopherol were dissolved in organic solvent. The solvent was removed under reduced pressure to form a thin dry film. The lipid film was hydrated with SN-38 in 10–20% sucrose solution, and liposome dispersion was extruded through two stacks of 0.2- and 0.1-μm polycarbonate filters (Whatman, Clifton, NJ) under nitrogen atmosphere. The resulting liposome-based SN-38 formulation was then sterilized by passing through 0.2-μm filters and lyophilized. The lyophilized LE-SN38 was reconstituted with lactate buffer at pH 2.0 and further dilutions were made with 0.9% NaCl. The drug:lipid ratio in LE-SN38 is 1:18 [13]. The entrapment efficiency of SN-38 in liposomes was above 95% and the average particle size was 150 ± 20 nm (mean ± SD). The diameter of the liposome was measured by a Nicomp 380 submicron particle sizer (Particle Sizing Systems, Santa Barbara, CA). The *in vitro* release study also showed that SN-38 remained with the formulation for up to 120 h in PBS, pH 7.2. The detailed description of LE-SN38 formulation development and characterization has been described elsewhere [13,14].

Cell culture

HT-29 (human colon cancer), A549 (human lung cancer), MX-1 (human breast cancer) and OVCAR-3 (human ovarian cancer) cell lines were obtained from the National Cancer Institute (Fredrick, MD). These cells were maintained in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum (FBS), penicillin 100 U/ml and streptomycin 100 μg/ml, and incubated at 37°C in a 5% CO_2 incubator. All culture media were purchased from Invitrogen (Carlsbad, CA).

Cytotoxicity assay

Cytotoxicity/growth inhibition was determined by SRB assay [15]. Different cancer cells (25 000–50 000 cells/well) with 30–50% confluency were plated on 96-well microtiter plates and placed in a CO_2 incubator at 37°C for 24 h. After incubation, all control and test articles were diluted in 100 μl control medium (RPMI 1640 medium containing 5% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin) and incubated for another 48 h. Drug-free liposomes and 5% dextrose were also diluted in parallel, and used as controls for LE-SN38 and CPT-11, respectively. In a separate T_0 plate, each cell type was seeded at the same time as other plates, but cells were fixed before any drug treatment. After the treatment, the cells were fixed with 10% TCA at 4°C for 1 h. TCA was washed off with tap water and the plates were air-dried. After the plates were dried, SRB (0.4% in 1% acetic acid) was added to the well to stain the cells for 30 min at room temperature. Following staining, the plates were washed with 1% acetic acid to remove unbound dye and air-dried. The bound dye was solubilized with 10 mM Tris base for 5 min on a gyratory shaker and the optical density (OD) was measured at 492 nm using a plate spectrophotometer (MultiScan; ThermoElectron, Milford, MA). The percent net growth of cells in each well was calculated using the following formula: (final OD − mean T_0 OD)/(mean control OD − mean T_0 OD) × 100. Mean T_0 OD was the average of the final ODs per cell type on the T_0 plate. Mean control OD was the average of the final ODs from the control wells of each plate. The net growth of each dilution of LE-SN38, CPT-11, drug-free liposomes or 5% dextrose was the mean of six wells with the same drug concentration. The growth inhibition curves were plotted with the average net growth and the drug concentration was expressed in a logarithmic scale. GI_{50} was calculated as the concentration of the drug that gives 50% growth inhibition.

Tumor models

Female C.B-17 SCID mice, 4–6 weeks old, were purchased from Harlan Sprague-Dawley (Indianapolis, IN). Mice were housed in a facility maintained as a specific pathogen-free environment. Mice (two to five per cage) were kept in microisolation cages with individual ventilation (Techniplast, Milan, Italy) in an environmentally monitored, well-ventilated room maintained at a temperature of 18–26°C with a relative humidity of up to 70%. Fluorescent light provided the illumination for approximately 12 h/day. Mice were able to access *ad libitum* autoclaved Teklad Global 18% Protein Rodent Diet (Harlan Teklad, Madison, WI) and autoclaved water during the acclimation and study periods. Aseptic animal handling techniques were applied.

HT-29 and MX-1 cells at 80–90% confluent were harvested by trypsin–EDTA and centrifuged (300 *g*) at 4°C for 5 min. The cell pellet was resuspended in Hank's balanced salt solution (HBSS). Mice were implanted with 2.5×10^6 HT-29 cells or 4×10^6 MX-1 cells. A volume of 0.1 ml per mouse was injected s.c. to the right lateral side of the body. The treatment initiation date was designated as day 1 when the tumors were well established (100–150 mm^3). The tumors and body weight were measured 1–2 times per week and the mice were sacrificed after 19–21 days from the initial treatment.

Data analysis

The mice were monitored daily and the tumor size was calculated using the following formula [10]: $a \times (b/2)^2 \times \pi$, where $\pi = 3.14$, and a and b are the tumor length and width, respectively.

The percent of initial tumor volume was calculated according to the following equation: $V_x/V_1 \times 100$, where V_x represents the tumor volume in a mouse on any given day and V_1 represents the tumor volume of the mouse on Day 1.

To evaluate the effect of the drug on the tumors, the percent inhibition was calculated as: $(V_B - V_T)/V_B \times 100$, where V_T represents the mean tumor volume in the treatment group on any given day and V_B represents the mean tumor volume in the control group on the same day.

The percent regression was employed to evaluate drug efficacy when the regression was observed. The percent regression was calculated as: $(V_1 - V_x)/V_1 \times 100$, where V_1 is the tumor volume of a mouse on day 1 and V_x is the tumor volume of the mouse on any given day.

All data were expressed as mean ± SEM. The comparison between groups was conducted with Student's *t*-test (two-tailed, unpaired). $p < 0.05$ was considered to be statistically significant.

Results

In vitro cytotoxicity of LE-SN38

The growth inhibition/cytotoxicity of LE-SN38 and CPT-11 was evaluated against four human cancer cell lines (HT-29, MX-1, A549 and OVCAR-3). The growth inhibition curves of each cell line are shown in Figure 1 and the mean GI_{50} values are summarized in Table 1. The results showed that potency of CPT-11 against colon, breast, lung and ovarian cancers was lower compared to LE-SN38. The ratio of LE-SN38 to CPT-11 indicated that LE-SN38 was approximately 300- to 1700-fold more cytotoxic than CPT-11 in all four human cancer cell lines (Table 1).

In vivo efficacy of LE-SN38: HT-29 human colon cancer xenograft model

Single-dose treatment

HT-29 tumor-bearing mice were treated with drug-free liposome, LE-SN38 or CPT-11 on day 1 via the tail vein injection. At 10, 20 and 40 mg/kg of LE-SN38, the tumor growth inhibition on day 10 was 51, 79 and 90%, respectively, compared to the drug-free liposome group. Only 6, 12 and 34% inhibition was observed in CPT-11 treated animals at the same dose level (Fig. 2). The difference in the tumor growth inhibition of 20 or 40 mg/kg LE-SN38 compared to drug-free liposome or CPT-11 was statistically significant ($p < 0.05$). Even though greater body weight loss (25%) was observed in the group of mice treated with 40 mg/kg LE-SN38 (Table 2), all the animals recovered the weight loss by day 13. No significant loss of body weight was observed in other treatment groups (data not shown).

Multiple-dose treatment

HT-29 tumor-bearing mice were treated with 2, 4 and 8 mg/kg/day of LE-SN38 or CPT-11 i.v. via the tail vein for 5 consecutive days (days 1–5). Drug-free liposome at volume of 10 ml/kg was included as a control. At 2, 4 and 8 mg/kg of LE-SN38, a tumor growth inhibition of 33, 81 and 91% was observed on day 11, respectively, compared to the drug-free liposome group (Fig. 3). HT-29 tumor was even regressed by 39% after being treated with 8 mg/kg of LE-SN38. In contrast, the tumor growth inhibition due to CPT-11 treatment at 2, 4 and 8 mg/kg was only 2, 36 and 46% compared to the drug-free liposome group. The difference in the tumor growth inhibition of 4 or 8 mg/kg LE-SN38 compared to drug-free liposome or CPT-11 was statistically significant ($p < 0.05$). No major body weight loss was observed at all doses of LE-SN38 (Table 2). When saline was injected, no inhibition of tumor was observed (data not shown).

In vivo efficacy of LE-SN38: MX-1 human breast cancer xenograft model

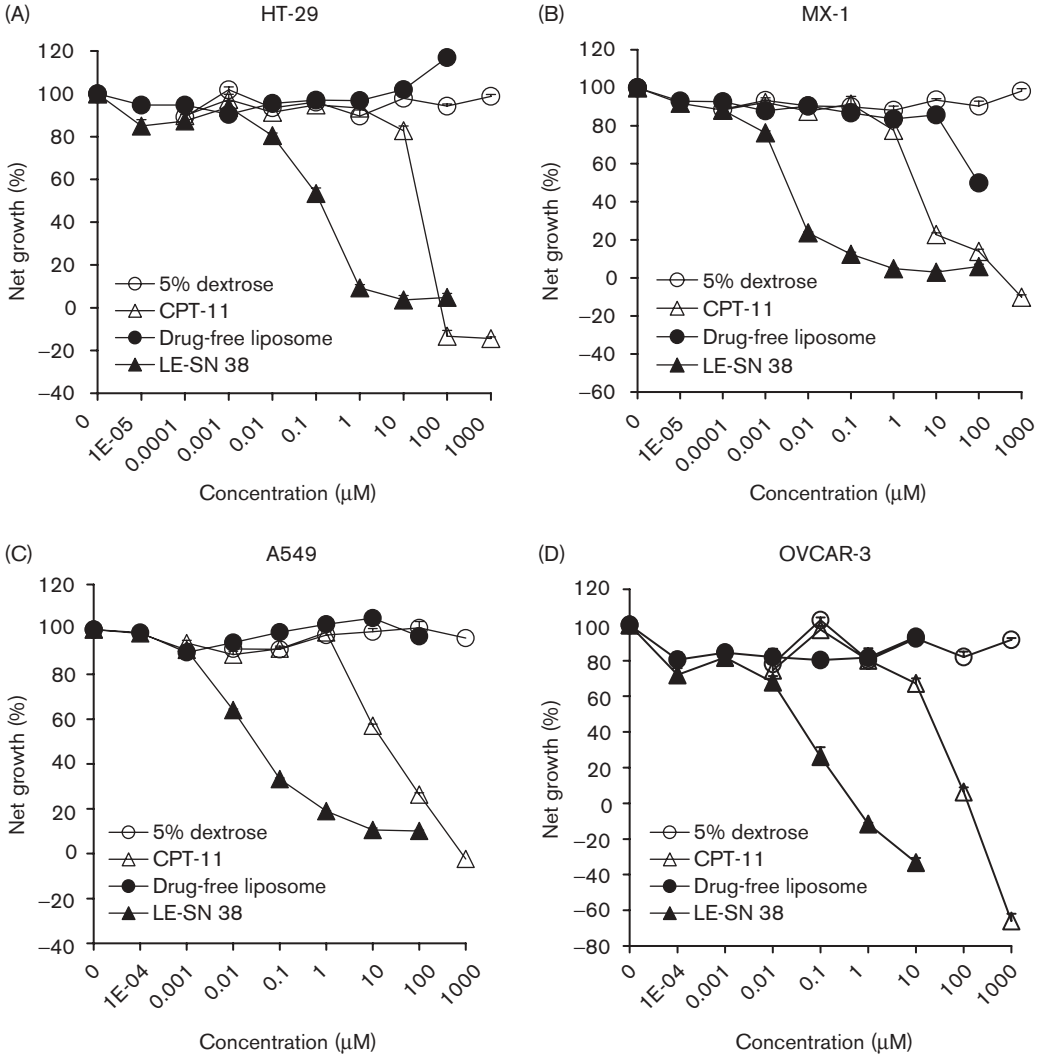
Multiple-dose treatment

MX-1 tumor-bearing mice received drug-free liposome, 4 or 8 mg/kg/day of LE-SN38 or 8 mg/kg CPT-11 for 5 consecutive days. At 4 and 8 mg/kg LE-SN38 on day 14, the tumor was regressed by 44 and 88%, respectively, compared to the tumor size before initiation of treatment. No tumor regression was observed in the animals treated with 8 mg/kg of CPT-11 and only 26% of tumor growth inhibition was achieved compared to drug-free liposome (Fig. 4). No significant body weight loss was observed in the mice treated with LE-SN38 or CPT-11 (data not shown).

Discussion

CPT-11 has been used for the treatment of colon cancer, non-small cell lung cancer and ovarian cancer [1,2,4,16]. However, side-effects, such as myelosupression and gastrointestinal disorders, have been linked to the administration of CPT-11 [17]. To improve the therapeutic potential and reduce the toxicity of CPT-11, a liposomal CPT-11 was formulated to show an enhanced antitumor effect of CPT-11 [18]. Nevertheless, this liposomal formulation resulted in decrease effectiveness of CPT-11 due to reduced liposome uptake in the reticulo-endothelial system (RES) [18]. CPT-11 itself has a very weak anticancer activity and functions by converting into the active metabolite, SN-38, in the presence of carboxylesterases [19]. Two isozymes of human carboxylesterases (hCE-1 and hCE-2) have been characterized to be primarily present in liver and serum [8,20], and can also be found in other tissues, including tumors [20]. Different isozymes may play variable roles in different species or tumor type. For instance, no cytotoxicity was seen in SQ20B cells after incubation of CPT-11 with hCE-1, compared to 60% reduction in cell survival with hCE-2 [21], suggesting that hCE-2 may play

Fig. 1



Cytotoxicity of CPT-11 and LE-SN38 on human cancer cell lines. Growth inhibition was determined by the SRB assay after 48 h of drug exposure. The test range of CPT-11 or LE-SN38 was between 10^{-5} and 1000 μM. Dextrose (5%) or drug-free liposomes were diluted in parallel and used as controls. (A) HT-29 cells, (B) MX-1 cells, (C) A549 cells and (D) OVCAR-3 cells.

Table 1 GI_{50} and potency ratio of CPT-11 and LE-SN38 in human cancer cell lines

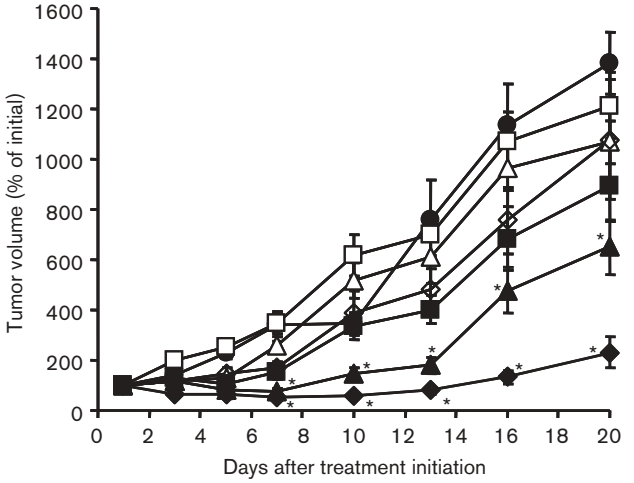
Cell lines	LE-SN38		CPT-11		Potency ratio (LE-SN38 versus CPT-11)
	GI_{50} (μM)	*N*	GI_{50} (μM)	*N*	
HT-29	0.0656	3	19.1	2	291
MX-1	0.00409	3	6.99	3	1709
A549	0.0172	3	14.0	3	814
OVCAR-3	0.0857	3	27.7	2	323

The GI_{50} values of four cancer cell lines in the presence of CPT-11 and LE-SN38 were determined by the SRB assay. After the growth inhibition curves were plotted for each cell type, the GI_{50} was determined as the concentration that gives 50% growth inhibition and the mean GI_{50} values are shown. The potency ratio was calculated as the ratio of GI_{50} obtained from LE-SN38 and CPT-11 treatment. *N*=number of experiments performed

a more important role in CPT-11 conversion in cancer patients.

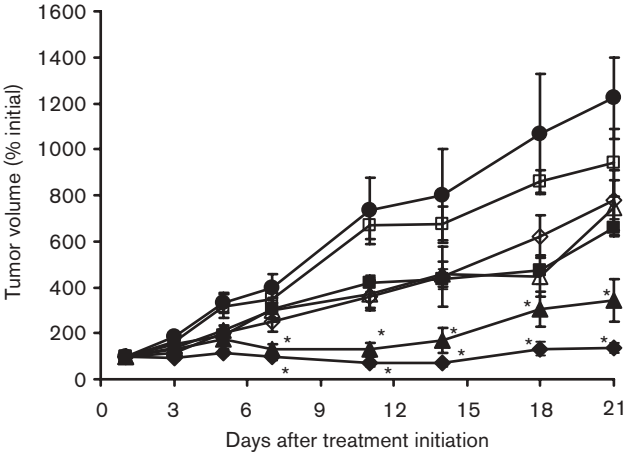
The availability of carboxylesterase also varies from patient to patient and differs among tumor types. For example, only 70% of human non-small cell lung cancers express human carboxylesterase [22] and there has been a wide variability of carboxylesterase activity in colon cancer patients [20]. Different esterases are also involved in the conversion of CPT-11 to SN-38. In mouse plasma, esterases are present at high levels and convert CPT-11 much better than carboxylesterases observed in human plasma [23]. Therefore, it is often difficult to predict and

Fig. 2



Effect of LE-SN38 against human colon cancer (HT-29) model: single treatment. The SCID mice were transplanted with 2.5×10^6 HT-29 cells in 0.1 ml HBSS and treated after the tumor size was established (100–150 mm^3). LE-SN38 was administered at doses of 10 (filled squares), 20 (filled triangles) or 40 (filled diamonds) mg/kg or CPT-11 at doses of 10 (open squares), 20 (open triangles) or 40 (open diamonds) mg/kg. Drug-free liposome 10 ml/kg (filled circles) was injected as a control. Animals were sacrificed 19 days after treatment initiation. Data were expressed as mean ± SEM ($N=5$ per group). An asterisk indicates statistically significant differences ($p<0.05$) comparing LE-SN38 with CPT-11 groups.

Fig. 3



Effect of LE-SN38 against human colon cancer (HT-29) model: multiple-dose treatment. The SCID mice were transplanted with 2.5×10^6 HT-29 cells in 0.1 ml HBSS and treated after the tumor size was established (100–150 mm^3). LE-SN38 was administered for 5 consecutive days at doses of 2 (filled squares), 4 (filled triangles) or 8 (filled diamonds) mg/kg or CPT-11 at doses of 2 (open squares), 4 (open triangles) or 8 (open diamonds) mg/kg. Drug-free liposome 10 ml/kg (filled circles) was injected as a control. Animals were sacrificed 21 days after the first injection. Data were expressed as mean ± SEM ($N=5$ per group). An asterisk indicates statistically significant differences ($p<0.05$) comparing LE-SN38 with CPT-11 groups.

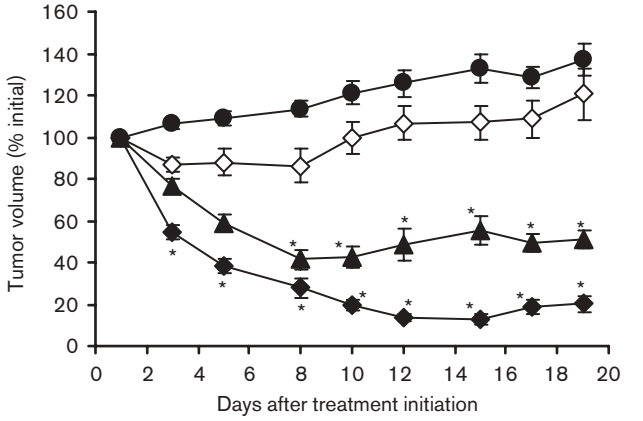
Table 2 Comparison of single- versus multiple-dose LE-SN38 on tumor growth and mouse body weight loss in human colon cancer HT-29 xenograft model

No. of injections	Dose (mg/kg/day)	Total accumulation (mg/kg)	Maximal tumor growth inhibition (%)	Maximal body weight loss (%)
1	10	10	56	2.8
1	20	20	80	11.2
1	40	40	89	25
5	2	10	49	4.8
5	4	20	81	4.9
5	8	40	91	6.2

evaluate the preclinical and clinical outcome of CPT-11. To overcome the problems associated with direct administration of pro-drug CPT-11, we have successfully developed a well-characterized liposome-based formulation of highly insoluble SN-38, and tested its potency against cancer cell lines and human xenograft tumor models.

In vitro results demonstrated that LE-SN38 induced cytotoxicity in lung (A549), colon (HT-29), breast (MX-1) and ovarian (OVCAR-3) cancer cell lines. These results are similar to those obtained from SN-38

Fig. 4



Effect of LE-SN38 against human breast cancer (MX-1) model: multiple-dose treatment. The SCID mice were transplanted with 2.5×10^6 HT-29 cells in 0.1 ml HBSS and treated after the tumor size was established (100–150 mm^3). LE-SN38 was administered for 5 consecutive days at doses of 4 (filled triangles) or 8 (filled diamonds) mg/kg or CPT-11 at a dose of 8 (open diamond) mg/kg. Drug-free liposome 10 ml/kg (filled circles) was injected as a control. Animals were sacrificed 19 days after the first injection. Data were expressed as mean ± SEM ($N=5$ per group). An asterisk indicates statistically significant differences ($p<0.05$) comparing LE-SN38 with CPT-11 groups.

dissolved in DMSO [24,25], suggesting that liposome entrapment did not affect the activity of SN-38. LE-SN38 was found to be significantly more potent than CPT-11 in cancer cells, and the results were comparable to earlier reports obtained by using cytotoxic and Topo I assays [26,27].

The potent anticancer activity of LE-SN38 was further supported by *in vivo* studies using human tumor models. LE-SN38 at 8 mg/kg/day (q × d5) was found to significantly inhibit tumor growth (91%) in human colon model and to regress the tumor growth by 88% in human breast tumor model. This dose is comparable with maximum tolerance dose (MTD) observed in CD2F1 mice (7.5 mg/kg for female and 5 mg/kg for male, q × d5) (unpublished observations). The MTD doses also indicated no histopathological lesions in the small intestine, liver and lungs after 20 days of injection (unpublished observations). Statistical analysis also showed that the efficacy of LE-SN38 was much greater than CPT-11 in both models ($p < 0.05$). This is also be supported by prolong exposure of SN-38, where SN-38 can still be detected 24 h after single injection [14].

In conclusion, both *in vitro* and *in vivo* studies described here indicated that LE-SN38 is a potent anticancer drug that possesses great advantages compared to the currently marketed drug, CPT-11. It is likely that direct administration of SN-38 in a well-characterized liposome-based formulation may produce better therapeutic efficacy in patients. Phase I clinical trials with LE-SN38 are currently in progress.

References

1 Shimada Y, Yoshino M, Wakui A, *et al.* Phase II study of CPT-11, a new camptothecin derivative, in metastatic colorectal cancer. *J Clin Oncol* 1993; **11**:909–913.

2 Cardenal F, Domine M, Massuti B, *et al.* Three-week schedule of irinotecan and cisplatin in advanced non-small cell lung cancer: a multicenter phase II study. *Lung Cancer* 2003; **39**:201–207.

3 Taguchi T, Tominaga T, Ogawa M, Ishida T, Morimoto K, Ogawa N. A late phase II study of CPT-11 (irinotecan) in advanced breast cancer. *Gan To Kagaku Ryoho* 1994; **21**:1017–1024.

4 Bodurka DC, Levenback C, Wolf JK, *et al.* Phase II trial of irinotecan in patients with metastatic epithelial ovarian cancer or peritoneal cancer. *J Clin Oncol* 2003; **21**:291–297.

5 Hsiang YH, Hertzberg R, Hecht S, Lui LF. Camptothecin induces protein-linked DNA breaks via mammalian DNA topoisomerase I. *J Biol Chem* 1985; **260**:14873–14878.

6 Hsiang YH, Lihou MG, Lui LF. Arrest of DNA replication by drug-stabilized topoisomerase I–DNA cleavable complexes as a mechanism of cell killing by Camptothecin. *Cancer Res* 1989; **49**:5077–5082.

7 Holm C, Covey JM, Kerrigan D, Pommier Y. Differential requirement of DNA replication for the cytotoxicity of DNA–topoisomerase I and II inhibitors in Chinese hamster DC3F cells. *Cancer Res* 1989; **49**:6365–6368.

8 Slatter JG, Su P, Sams JP, Schaaf LJ, Wienkers LC. Bioactivation of the anticancer agent CPT-11 to SN-38 by human hepatic microsomal carboxylesterases and the *in vitro* assessment of potential drug interactions. *Drug Metab Disp* 1997; **25**:1157–1164.

9 Slatter JG, Schaaf LJ, Sams JP, *et al.* Pharmacokinetics, metabolism, and excretion of irinotecan (CPT-11) following I.V. infusion of [^{14}C]CPT-11 in cancer patients. *Drug Metab Disp* 2000; **28**:423–433.

10 Gokhale PC, Pei J, Zhang C, Ahmad I, Rahman A, Kasid U. Improved safety, pharmacokinetics and therapeutic efficacy profiles of a novel liposomal formulation of mitoxantrone. *Anticancer Res* 2001; **21**:3313–3321.

11 Gabizon A, Papahadjopoulos D. Liposome formulations with prolonged circulation time in blood and enhanced uptake by tumors. *Proc Natl Acad Sci USA* 1988; **85**:6949–6953.

12 Waterhouse DN, Tardi PG, Mayer LD, Bally MB. A comparison of liposomal formulations of doxorubicin with drug administered in free form: changing toxicity profiles. *Drug Saf* 2001; **24**:903–920.

13 Zhang JA, Xuan T, Parmar M, Ma L, Ugwu S, Ali S, Ahmad I. Development and characterization of a novel liposome-based formulation of SN-38. *Int J Pharm* 2004; **270**:93–107.

14 Khan S, Ahmad A, Guo W, Wang Y-F, Abu-Qare A, Ahmad I. A simple and sensitive LC/MS/MS assay for 7-ethyl-10-hydroxycamptothecin (SN-38) in mouse plasma and tissues:application to pharmacokinetic study of liposome entrapped SN-38 (LE-SN38). *J Pharmac Biomed Anal* 2004; in press.

15 Skehan P, Storeng R, Scudiero D, *et al.* New colorimetric cytotoxicity assay for anticancer-drug screening. *J Natl Cancer Inst* 1990; **82**: 1107–1112.

16 Dancey J, Eisenhauer EA. Current perspectives on camptothecins in cancer treatment. *Br J Cancer* 1996; **74**:327–328.

17 Cersosimo RJ. Irinotecan: a new antineoplastic agent for the management of colorectal cancer. *Ann Pharmacother* 1998; **32**:1324–1333.

18 Sadzuka Y. Effective prodrug liposome and conversion to active metabolite. *Curr Drug Metab* 2000; **1**:31–48.

19 Takayanagi I, Koike K, Tagawa M, Mitsuhashi E. Some pharmacological properties of a new antitumor drug, CPT-11, in isolated muscle preparations. *Gen Pharmacol* 1989; **20**:763–776.

20 Guichard S, Terret C, Hennebelle I, *et al.* CPT-11 converting carboxylesterase and topoisomerase I activities in tumor and normal colon and liver tissues. *Br J Cancer* 1999; **80**:364–370.

21 Humerickhouse R, Lohrbach K, Li L, Bosron WF, Dolan ME. Characterization of CPT-11 hydrolysis by human liver carboxylesterase isoforms hCE-1 and hCE-2. *Cancer Res* 2000; **60**:1189–1192.

22 Ohtsuka K, Kameyama M, Kanetoshi A, Fujimoto T, Takaoka K, Shida AY. Intracellular conversion of irinotecan to its active form, SN-38, by native carboxylesterase in human non-small cell lung cancer. *Lung Cancer* 2003; **41**:187–188.

23 Mathijssen RH, van Alphen RJ, Verweij J, *et al.* Clinical pharmacokinetics and metabolism of irinotecan (CPT-11). *Clin Cancer Res* 2001; **7**: 2182–2194.

24 Pavillard V, Agostini C, Richard S, Charasson V, Montaudon D, Robert J. Determinants of the cytotoxicity of irinotecan in two human colorectal tumor cell lines. *Cancer Chemother Pharmacol* 2002; **49**:329–335.

25 Van Hattum AH, Pinedo HM, Schluper HM, Hausheer FH, Boven E. New highly lipophilic camptothecin BNP1350 is an effective drug in experimental human cancer. *Int J Cancer* 2000; **88**:260–266.

26 Kawato Y, Aonuma M, Hirota Y, Kuga H, Sato K. Intracellular roles of SN-38, a metabolite of the camptothecin derivative CPT-11, in the antitumor effect of CPT-11. *Cancer Res* 1991; **51**:4187–4191.

27 Jansen WJ, Zwart B, Hulscher ST, Giaccone G, Pinedo HM, Boven E. CPT-11 in human colon-cancer cell lines and xenografts: characterization of cellular sensitivity determinants. *Int J Cancer* 1997; **70**:335–340.