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Synthesis and biological activity of medium molecular weight polymers of camptothecin

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Abstract

A new monomer, 3,6-endo-methylene-1,2,3,6-tetrahydrophthalimidohexanoylcamptothecin (ETHCPT) was synthesized from 3,6-endo-methylene-1,2,3,6-tetrahydrophthalimidohexanoic acid. Its homopolymer and copolymer with acrylic acid (AA) were synthesized and spectroscopically characterized. The ETHCPT content in poly(ETHCPT-*co*-AA) obtained by elemental analysis was 37 wt.%. The number-average molecular weights of the polymers determined by gel permeation chromatography were as follows: $M_n = 9700$ for poly(ETHCPT), $M_n = 25\,500$ for poly(ETHCPT-*co*-AA). The IC_{50} value of ETHCPT and its polymers against cancer cells was much larger than that of CPT. The *in vivo* antitumor activity of all polymers in Balb/C mice bearing the sarcoma 180 tumor cell line was greater than that of CPT at a dose of 100 mg/kg.

Keywords: 3,6-Endo-methylene-1,2,3,6-tetrahydrophthalimidohaxanoylcamptothecin; Camptothecin; Poly(ETHCPT-*co*-AA); Photopolymerization; *In vitro* cytotoxicity; *In vivo* antitumor activity

1. Introduction

Despite their many clinical benefits, anticancer drugs often cause significant side effects due to their overall cytotoxicity and their lack of tissue specificity. In principle, these issues can be addressed by conjugating the parent drugs to polymers [1–3], thereby enhancing both their tissue specificity and effective concentration at tumor sites. In fact, in contrast to low molecular weight anticancer drugs, polymer-based therapeutics have been found to accumulate more in tumor tissues due to an enhanced permeability and retention effect at these sites [4,5]. Moreover, they can prolong the antitumor activity of the parent drug by releasing it at a controlled rate at the targeted site [6].

The value of the above strategy is illustrated by the numerous studies dealing with conjugation of polymers with drugs such as doxorubicin [7,8], taxol [9,10], neocarzinostatin [11], podophyllotoxin [12] and camptothecin (CPT) [13–16]. Among these compounds the CPT-conjugates are particularly appealing since the parent molecule (CPT) has a well understood mode of action, a well established clinical potential and well documented side effects. More specifically, CPT

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experiments *in vivo* have also shown that CPT displays a significant antitumor activity in nude mice bearing human lung, ovarian, breast, pancreas and stomach cancers [17]. It has also been demonstrated that CPT is an antimitotic drug acting at the S-phase (DNA synthesis phase) of the cell cycle and as such it has low toxicity against normal resting cells [18]. The mechanism of action of this drug involves stabilization of the topoisomerase I-induced DNA strand breaks, thereby preventing subsequent strand relegation and leading ultimately to apoptotic cell death [19]. Consequently, prolonged inhibition of topoisomerase I was postulated to be an important factor for the therapeutic activity of CPT. However, in biological media, CPT was found to undergo an unfavorable chemical modification, during which the active δ-lactone form present at pH 4–5 is converted to an inactive open ring structure. This low stability together with the poor aqueous solubility of CPT have led to unpredictable toxic effects and have slowed the clinical development of this drug.

The limitations of CPT can be addressed by attaching it to a polymeric support that could act as a transport form for this drug and enhance its biodistribution while keeping intact its therapeutic profile. Both water soluble and insoluble CPT polymers have been reported and have interesting pharmacological properties. While the water soluble conjugates promise to solve the poor solubility of the natural product [14], the water insoluble polymers exhibit a superior antitumor activity against *in vitro* human cancers and *in vivo* animal xenografts [20].

Inspired by the clinical potential and current limitations of the CPT-based therapeutics, we sought to construct and study phthalimide-based polymers of CPT. Such polymers have been found in our laboratories to be efficient carriers of 5-fluorouracil (5-FU), retaining the antitumor efficacy while decreasing the toxicity of the parent molecule. Moreover, tetrahydrophthalic acid-based polymers showed a strong antitumor activity against cancer cell lines [21–24], while they displayed very low toxicity against normal cells [25].

In this report we describe the synthesis of new antitumor active polymers of CPT based on the tetrahydrophthalimide template and present results of their antitumor activity evaluation in comparison with CPT as the reference drug. The monomer was built by linking CPT to tetrahydrophthalic anhydride using 6-aminohexanoic acid. Its homopolymer and copolymer with acrylic acid (AA) were prepared by photopolymerization. The obtained monomer and its polymers were identified by ^{1}H NMR and ^{13}C NMR spectroscopy, and elemental analysis. The average molecular weights of the polymers were measured by gel permeation chromatography (GPC). The *in vitro* cytotoxicities were evaluated with mouse mammary carcinoma (FM3A), mouse leukemia (P388), and human histiocytic lymphoma (U937) as cancer cell lines and mouse liver cells (AC2F) as a normal cell line. The *in vivo* antitumor activities of the synthesized polymers against mice bearing the sarcoma 180 tumor cell line were evaluated. Our data indicate that the synthesized polymers exhibit higher antitumor activities than monomer (ETHCPT) and CPT. In addition, they display a low cytotoxicity against normal cell lines, suggesting that they can be used for further pharmacological and clinical studies.

2. Experimental

2.1. Materials

6-Aminohaxanoic acid (Aldrich, USA), CPT (Aldrich, USA), 3,6-*endo*-methylene-1,2,3,6-tetrahydrophthalic anhydride (MPA, Aldrich, USA), 2,2-dimethoxy-2-phenylacetophenone (DMP, Aldrich, USA), dimethylaminopyridine (DMAP, Aldrich, USA), 1-[3-(dimethylaminopropyl)]-3-ethylcarbodiimide hydrochloride (EDC, Aldrich, USA), and triethylamine (TEA, Aldrich, USA) were used without further purification. Acrylic acid (AA, Junsei Chem.) was distilled under vacuum (7 mm Hg, 45 °C).

For the *in vitro* tests, FM3A, P388, and U937 were used as cancer cell lines and AC2F was used as a normal cell line. For the *in vivo* test, Balb/C mice and sarcoma 180 cell line were purchased from the Center of Genetic Engineering, Korea Institute of Science and Technology. Mice were maintained under specific pathogen-free conditions of the experimental animal house at 22 ± 1 °C and $55 \pm 5\%$ humidity. They were fed a Purina chow diet and water ad libitum during the experiment. Animal experiments were approved by the Ethical Committee for Animal Experimentation of our University and were performed according to the NIH Guide for The Care and Use of Laboratory Animals.

2.2. Instruments

IR spectra were recorded on a Perkin–Elmer 397 spectrometer using KBr pellets. ^{1}H and ^{13}C NMR spectra were measured on a FT-300 MHz Varian Gemini 2000 spectrophotometer. DMSO-d_6 was used as the solvent. Elemental analyses were carried out with an elemental analyzer (Carlo Erba model EA 180). Number- and weight-average molecular weights (M_n and M_w) and polydispersity (M_n/M_w) were estimated by gel permeation chromatography (GPC; Waters 410 Differential Refractometer).

2.3. Synthesis of monomer

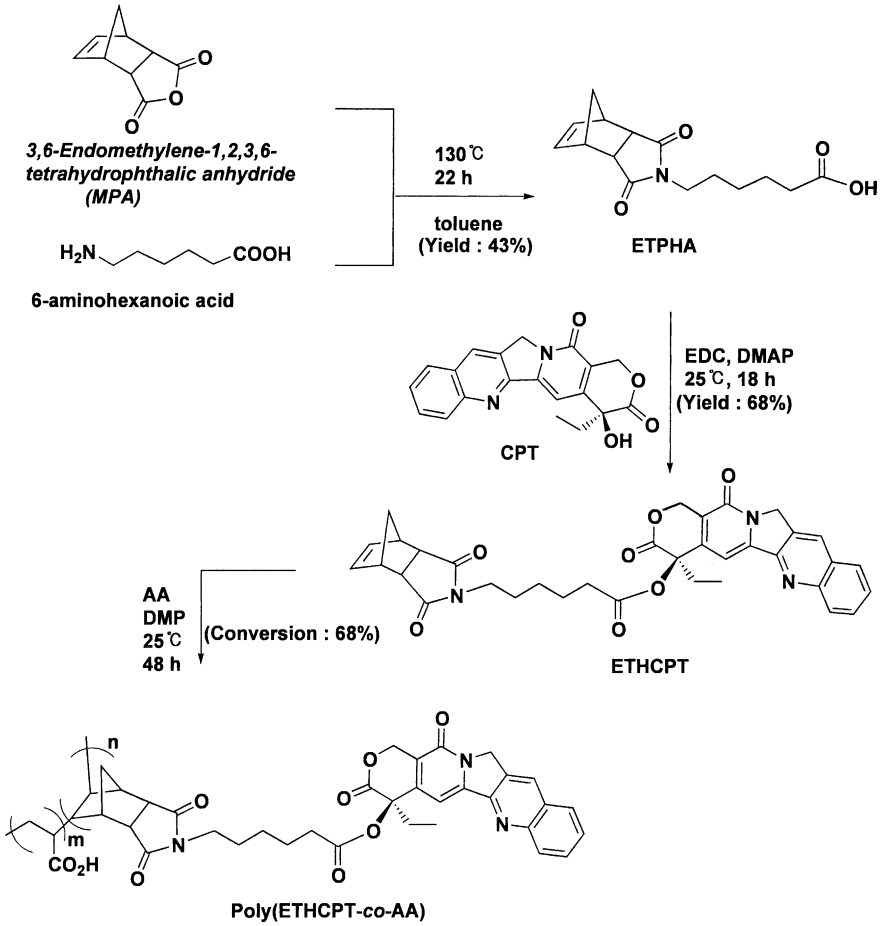
Scheme 1 gives monomer and polymer synthesis.

2.4. 3,6-Endo-methylene-1,2,3,6-tetrahydrophthalimido-hexanoic acid

MPA(4.93 g, 30.0 mmol), 6-aminohexanoic acid (3.98 g, 30.4 mmol), and TEA (1 ml) were refluxed in toluene (100 ml) for 22 h with azotropic removal of water via a Dean–Stark apparatus. After evaporation of the solvent, the residue was triturated with 0.1 N HCl (30 ml), and dissolved in saturated aqueous $NaHCO_3$ (10 ml). The aqueous solution was washed with ethyl acetate (20 ml), acidified to pH 2 by adding conc. HCl and extracted twice with chloroform (2×30 ml). The chloroform layers were dried over anhydrous $MgSO_4$ and evaporated to obtain pure ETPHA in 43% yield. ^{1}H NMR (DMSO-d_6): δ(ppm) = 11.9 (s, 1H, COO*H*), 6.01 (s, 2H, –C*H*=C*H*– of MPA), 3.27 (s, 2H, –NCH_2CH$_2$–), 3.26 (s, 2H, –C*H*CONCOCH– of MPA), 3.14 (m, 2H, –C*H*CH = CHC*H*– of MPA), 2.13 (t, 2H, –CH$_2$CH_2COOH), 1.52 (m, 2H, CH_2CHCHCON of MPA) 1.40 (m, 2H, NCH$_2$CH_2CH$_2$CH$_2$CH$_2$COOH), 1.28 (m, 2H, NCH$_2$CH$_2$CH$_2$CH_2CH$_2$COOH), 1.14 (m, 2H, NCH$_2$CH$_2$CH$_2$CH_2CH$_2$COOH).

2.5. 3,6-Endo-methylene-1,2,3,6-tetrahydrophthalimido-hexanoylcamptothecin

A mixture of ETPHA (277 mg, 1.0 mmol), CPT (348 mg, 1.0 mmol), DMAP (134 mg, 1.1 mmol), and EDC (210 mg, 1.1 mol) in anhydrous dichloromethane (5 ml) was stirred at room temperature. After 18 h stirring, the reaction mixture was diluted with dichloromethane (150 ml) and washed with 0.1 N HCl (30 ml), saturated aqueous $NaHCO_3$ (30 ml), and water (30 ml). The organic layer was dried over anhydrous $MgSO_4$ and evaporated under reduced pressure to give a pale yellow solid. The product was purified by flash silica gel column chromatography using CH_2Cl_2–MeOH (97:3, v/v) as eluent to yield as a yellow solid (413 mg, 68%). ^{1}H NMR



Scheme 1.

(DMSO-d_6): δ(ppm) = 8.69 (s, 1H, H-7), 8.14 (d, 1H, $J = 7.0$ Hz, H-12), 8.12 (d, 1H, $J = 7.0$ Hz, H-9), 7.86 (t, 1H, $J = 7.5$ Hz, H-10), 7.71 (t, 1H, $J = 7.5$ Hz, H-11), 7.04 (s, 1H, H-14), 5.95–5.98 (m, 2H, HC=CH of MPA), 5.51 (d, 1H, $J = 17.0$ Hz, H-17), 5.47 (d, 1H, $J = 17.0$ Hz, H-17), 5.30 (d, 1H, $J = 17.0$ Hz, H-5), 5.27 (d, 1H, $J = 17.0$ Hz, H-5), 3.41–3.46 (m, 4H, NCH_2CH_2 and CHCONCOCH– of MPA), 3.23–3.26 (m, 2H, C*H*CHCONCOCHC*H* of MPA), 2.10 (t, 2H, CH_2CH_2COO), 1.45–1.56 (m, 4H, H-19 and $CH_2CHCHCON$ of MPA), 1.17–1.36 (m, 6H, NCH_2-$CH_2CH_2CH_2CH_2COOCPT$), 0.91 (t, 3H, H-18). ^{13}C NMR (DMSO-d_6): δ(ppm) = 177.35, 177.33, 172.0, 167.3, 156.6, 152.3, 147.9, 146.0, 145.4, 134.23 (a), 134.20 (a), 131.6, 130.4, 129.8, 128.9, 128.5, 128.0, 127.7, 119.0, 94.7, 75.6, 66.3, 51.7, 50.2, 45.1 (d), 44.2 (b), 37.2, 32.9, 30.3, 26.8, 25.4, 23.8, 7.5, HRMS calcd for $C_{35}H_{34}N_3O_7$ M^++1, 608.2398, found 608.2389.

2.6. Synthesis of poly(ETHCPT)

A solution of ETHCPT (0.88 g, 1.3 mmol) and DMP (0.02 g, 0.065 mmol) as an initiator in dry acetone (10 ml) was introduced into a dry Pyrex polymerization tube. The tube was flushed twice with N_2 gas, sealed and placed in a photochemical chamber where it was irradiated at 313 nm (115 V, 60 Hz power supply) at 25 °C for 48 h. After polymerization, the tube was opened and the viscous liquid obtained was slowly precipitated into a large excess of *n*-hexane (300 ml). The precipitated polymer was collected by filtration and washed several times with acetone. The obtained homopolymer was dried under reduced pressure to a constant weight. The conversion was 72%.

2.7. Syntheses of poly(ETHCPT-co-AA)

A solution of ETHCPT (0.88 g, 1.3 mmol) and AA (0.1 ml, 1.3 mmol) with DMP (0.04 g, 0.13 mmol) as an initiator in dry acetone (10 ml) was introduced into a dry Pyrex polymerization tube. The tube was sealed after flushing twice with bubbling purified N_2 gas. The preparation procedure for poly(ETHCPT-*co*-AA) was the same as that described for the homopolymerization of ETHCPT except for the monomer pairs. The copolymerization conversion of ETHCPT with AA was 68%.

2.8. Measurements of average molecular weight and compositions

To compare the average molecular weights of the synthesized polymers, we determined the apparent molecular weights by GPC using a microstyragel column and low polydispersity polystyrene as a standard at 40 °C. Dimethylformamide was used as an eluent. The contents of ETHCPT moiety in the copolymers were calculated from C, H and N data obtained by elemental analysis.

2.9. Biological activity tests

2.9.1. In vitro cytotoxicity

The cytotoxicities of ETHCPT and its polymers against three cancer cell lines *in vitro* were determined by the MTT assay according to the Mosmann's method [26]. The MTT assay is based on the reduction of the soluble 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) into a blue-purple formazan product, mainly by mitochondrial reductase activity inside living cells. The cells used in cytotoxicity assay were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum. Cells suspended in the medium were plated in 96-well culture plates and incubated at 37 °C in a 5% CO_2 incubator. After 12 h, the test sample was added to the cells (2×10^4) in 96-well plates and cultured at 37 °C for 3 days. The cultured cells were mixed with 20 μl of MTT solution and incubated for 4 h at 37 °C. The supernatant was carefully removed from each well and 100 μl of DMSO was added to each well to dissolve the formazan crystals which were formed by the cellular reduction of MTT. After mixing with a mechanical plate mixer, the absorbance of each well was measured by a microplate reader using a test wavelength of 570 nm. All experiments were carried out in triplicate. Cytotoxic activities were expressed as IC_{50} values, the drug concentrations required for 50% inhibition of cell growth. There was a good reproducibility between replicate wells with standard errors below ±10%.

2.9.2. In vivo antitumor activity

Antitumor effects of ETHCPT and its polymers were evaluated *in vivo* after treatment of mice with Sarcoma 180. Sarcoma 180 cells were kept as an ascitic tumor in balb/C mice with weekly transplants. The cells (1×10^6) were injected intraperitoneally (i.p.) into Balb/C mice (6 weeks old, 25 g). Because the test samples were insoluble in phosphate-buffered solution (PBS), we dissolved those in PBS under alkali condition and then neutralized the solutions. The solution was given i.p. to mice every 24 h after the first cell injection during 4 days. The different doses tested were 10 and 100 mg/kg. Groups of ten animals were used. For comparison, antitumor activity of CPT was also tested by the same method. The control group was divided into two groups: one subgroup was treated with sarcoma 180 cells together with neat saline by replacing the sample solution; the other subgroup was treated with sarcoma 180 cells alone. Observation was carried out for 93 days. The evaluated parameter of activity was increase in life-span (ILS), calculated from

average survival times of treated and control mice (T/C). The differences between control and treated groups were assessed by Mann–Whitney test. Statistical significance was defined as $P < 0.0001$ to reject a null hypothesis. Statistical analysis was conducted using SPSS 10.0 for Windows.

3. Results and discussion

3.1. Identification of monomer and its polymers

The structures of the synthesized monomer and polymers were confirmed by IR, ^{1}H NMR and ^{13}C NMR spectroscopies. The FTIR characteristic absorption peaks for ETHCPT appeared at 1747 and 1694 cm^{-1} (C=O stretching), and 1666 cm^{-1} (C=C stretching). ^{1}H NMR spectrum of ETHCPT is shown in Fig. 1. The peaks of vinyl protons in ETHCPT appeared at 5.9 ppm and ethyl proton of CPT moiety in ETHCPT indicated at 1.45–1.56 ppm and at 0.91 ppm, respectively.

Poly(ETHCPT) was synthesized via radical polymerization through the carbon–carbon double bond on the norbornene moiety. The FTIR spectrum of poly(ETHCPT) showed the characteristic absorption peaks at 1750 and 1695 cm^{-1} with disappearance of vinyl absorptions at 1666 cm^{-1} which appeared in ETHCPT monomer. The characteristic peaks for the methine protons of the polymer backbone of poly(ETHCPT) was observed at 0.7 ppm in the ^{1}H NMR spectrum. The peak for the vinyl protons of monomeric ETHCPT at 5.95–5.98 ppm was not observed. The FTIR spectrum of poly(ETHCPT-*co*-AA) indicated absorption at 3400–2800 cm^{-1} (COOH stretching of AA moiety) and 1750–1700 cm^{-1} (C=O stretching). The absorption peaks caused by protons of ETHCPT moiety in poly(ETHCPT-*co*-AA) were assigned to the same as those of poly(ETHCPT). In ^{1}H NMR spectrum, the proton peaks for the carboxylic acid, methine, and methylene protons of the AA moiety in poly(ETHCPT-*co*-AA) were observed at 12.0, 1.0, and 1.2 ppm, respectively. The characteristic peaks for the methine proton on the ETHCPT moiety were observed at 0.9 ppm. The peak assigned to the olefinic proton of ETHCPT and AA moiety at 5.9 and 7.1 ppm disappeared, respectively.

3.2. Solubility of ETHCPT and its polymers

The solubility of ETHCPT and its polymers are shown in Table 1. ETHCPT was soluble in acetone, 2-butanone, DMSO, DMF, EtOH, MeOH and THF. Its polymers were insoluble or poorly soluble in acetic acid, diethyl ether, *n*-hexane and water.

3.3. Average molecular weights and compositions of polymers

The average molecular weights and polydispersity indices of the polymers are listed in Table 2. The number average molecular weights (M_n) of poly(ETHCPT) we synthesized using a previously reported method [21–25] was 9700 and molecular weight distribution (M_w/M_n) was 1.38.

The elemental analysis value of poly(ETHCPT-*co*-AA) is as follows: C, 43.34; H, 8.28; N, 2.56. The

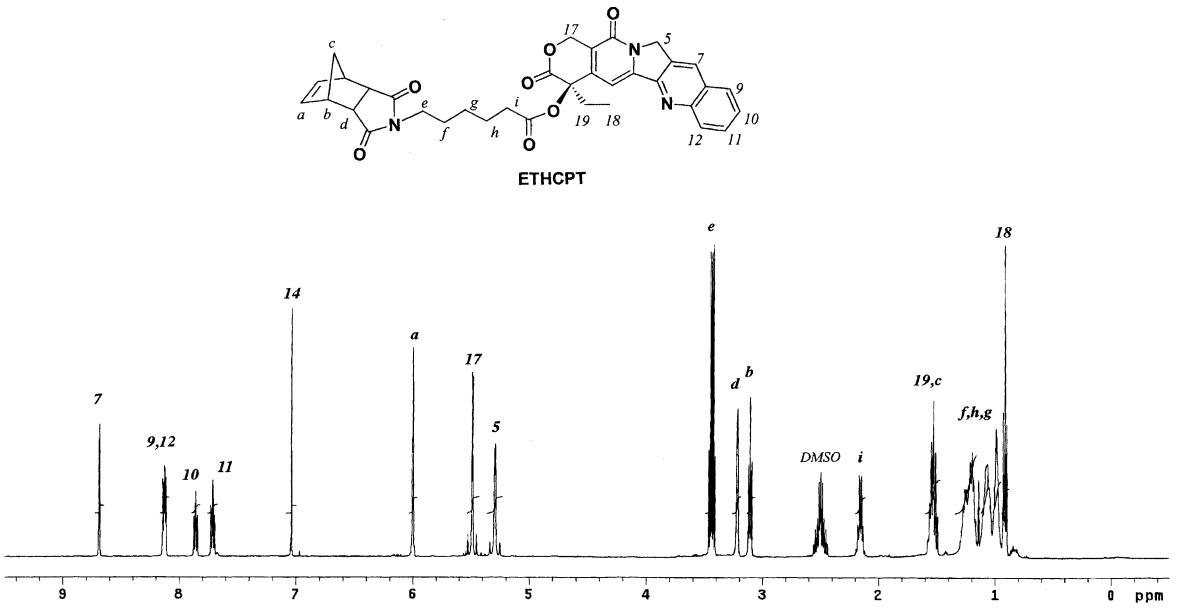


Fig. 1. The ^{1}H NMR spectrum of ETHCPT.

Table 1
Solubility of ETHCPT and its polymers

Solvent	Sample		
	ETHCPT	Poly-(ETHCPT)	Poly(ETHCPT-*co*-AA)
Water	IS	IS	IS
PBS	IS	IS	IS
Acetic acid	IS	IS	IS
MeOH	S	S	S
EtOH	S	S	S
DMSO	S	S	S
DMF	S	S	S
Acetone	S	S	S
2-Butanone	S	S	S
THF	S	S	S
Ethyl ether	IS	IS	IS
n-Hexane	IS	IS	IS

S : soluble, IS : insoluble

Table 2
Average molecular weights and polydispersity of the polymers

Polymers	M_n	M_w	M_w/M_n
Poly(ETHCPT)	9700	13 400	1.38
Poly(ETHCPT-*co*-AA)	25 500	37 900	1.49

Molecular weights were determined by GPC in DMF.

ETHCPT composition in poly(ETHCPT-*co*-AA) calculated from N content was 37 wt.%.

3.3.1. In vitro cytotoxicity of ETHCPT and its polymers

The *in vitro* cytotoxicity of ETHCPT and its polymers were evaluated against three cancer cell lines and one normal cell line. As shown in Table 3, the values of 50% cytotoxicity (IC_{50}) for ETHCPT and its polymers were in the range of 10–65 μg/ml against cancer cell lines. The cytotoxicity of monomer and its polymers were lower than that of free CPT, because the drugs attached on monomer and its polymers are not active and they take certain period to release the active parent drug. The cytotoxity of ETHCPT against FM3A cell was higher than those of poly(ETHCPT) and poly(ETHCPT-*co*-AA), but its cytotoxity against P388 cell was lower than those of poly(ETHCPT) and poly(ETHCPT-*co*-AA). The cytotoxicity of ETHCPT and its polymers against U937 cell increased in the following order: poly-(ETHCPT) > ETHCPT > poly(ETHCPT-*co*-AA). In normal cell line (AC2F), the cytotoxicities of ETHCPT and its polymers were much lower than that of free CPT.

3.3.2. In vivo antitumor activity of ETHCPT and its polymers

The survival data of mice treated with ETHCPT and its polymers are shown in Table 4 together with that of CPT for comparison. Mortality was recorded and mean survival time was calculated for each compound. The activity of polymers was expressed as a survival effect (T/C), where T is the mean survival time of mice treated with sample and C is the survival time of mice in a control group.

As shown in Table 4, entries 4 low dosage of CPT (10 mg/kg) led to good antitumor activity (625% increase as compared to the control group). However, increase of drug dosage to 100 mg/kg led to a sharp decrease of the mean survival time (33% as compared to the control group). This is attributed to the inherent toxicity of CPT.

The anticancer activity of the monomer (ETHCPT) at different dosages is shown in entries 5 and 6. At a low and high drug dosages (10 and 100 mg/kg) we observed a significant increase (above 612%) of the mean survival time versus that of the control group. This suggests that at a high concentration the monomer displays a low toxicity and at a low dosage it led to a statistically significant prolongation of the life of mice beyond the experimental period (93 days).

Evaluation of poly(ETHCPT) at different dosages is presented in Table 4, entries 7 and 8. Independently of

Table 3
In vitro cytotoxicity of ETHCPT and its polymers against cell lines

Samples	IC_{50} (ng/mL) for cell lines[a]			
	Cancer cells			Normal cells
	FM3A[b]	P388[c]	U937[d]	AC2F[e]
CPT	0.05 ± 0.004	0.22 ± 0.01	0.21 ± 0.02	0.04 ± 0.002
ETHCPT	10 ± 0.8	45 ± 2.6	50 ± 4.7	30 ± 1.1
Poly(ETHCPT)	14 ± 0.3	23 ± 1.2	10 ± 0.3	40 ± 2.3
Poly(ETHCPT-*co*-AA)	64 ± 2.7	10 ± 0.3	65 ± 3.7	110 ± 9.5

[a] The 50% growth inhibition.
[b] Mouse mammary carcinoma cell
[c] Mouse leukemia cell.
[d] Human histiocytic lymphoma cell.
[e] Mouse liver cell.

Table 4
In vivo antitumor activity of ETHCPT and its polymers

Entries	Samples	Dose (mg/kg)	mg of CPT (equivs/kg)	Survival time (days)[a]	T/C (%)[b]	S/E[c]
1	Control	–		14.7 ± 2.3	100	0/10
2		Saline		15.7 ± 0.5	100	0/10
3	CPT	100	100	5.0 ± 0.0	33	0/10*
4		10	10	93.0 ± 0.0	612	10/10*
5	ETHCPT	100	57	93.0 ± 0.0	612	10/10*
6		10	5.7	93.0 ± 0.0	612	10/10*
7	Poly(ETHCPT)[d]	100	57	93.0 ± 0.0	612	10/10*
8		10	5.7	91.4 ± 4.8	601	9/10*
9	Poly(ETHCPT-*co*-AA)[e]	100	21	88.5 ± 6.8	582	7/10*
10		10	2.1	90.0 ± 6.0	592	8/10*

[a] Mean survival time of animals dying within the experimental period of 93 days.
[b] T/C (%) represents the ratio of the survival time of the mice treated with a sample (T) to the control (C) mice $\times$ 100.
[c] S/E denotes the ratio of the number of survival mice (S) to that of experimental mice (E) after the experimental period of 93 days.
[d] For poly(ETHCPT), the drug composition is 57 wt.%.
[e] For poly(ETHCPT-*co*-AA), the ETHCPT composition is 37 wt.% which means that the drug composition is 21 wt.%.
* $P < 0.0001$.

the dose administered, we recorded a notable increase of mean survival time (≈600%) versus that of the control group. Administration of this polymer resulted in 100% and 90% survival of mice beyond the experiment period of 93 days at 10 and 100 mg/kg, respectively.

As indicated in entries 9 and 10, treatment of mice with poly(ETHCPT-*co*-AA) resulted in a substantial increase of about 500% of the mean survival time as compared to the control group. Administration of this polymer at high dosage (100 mg/kg) resulted in 70% survival of mice beyond the experiment period of 93 days. Similar results were recorded at a low polymer dosage (10 mg/kg) resulting in 590% survival. These results demonstrate that this polymer has a very low toxicity even at high dosage, and can therefore overcome the inherent toxicity associated with high doses of CPT.

4. Conclusions

A new monomer, 3,6-*endo*-methylene-1,2,3,6-tetrahydrophthalimidohexanoylcamptothecin (ETHCPT), was synthesized from CPT and 3,6-*endo*-methylene-1,2,3,6-tetrahydrophthalimidohexanoic acid (ETPHA). Its homopolymer and copolymer with AA were prepared by photopolymerization and were identified by ^{1}H NMR and ^{13}C NMR spectroscopies. The polydispersity indices of all synthesized polymers ranged from 1.3 to 1.49. The content of ETHCPT in poly(ETHCPT-*co*-AA) was 37 wt.%.

IC_{50} values obtained from the *in vitro* test for ETHCPT, poly(ETHCPT), and poly(ETHCPT-*co*-AA) were 10–65 ng/ml against cancer cell lines, which were much lower than those of CPT. In a normal cell line, the cytotoxicities of ETHCPT and its polymers were much lower than that of CPT. From estimation in mice bearing sarcoma 180 tumor cell line, the *in vivo* antitumor activities of ETHCPT and its polymers were greater than that of CPT at a dose of 100 mg/kg.

Acknowledgement

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References

[1] Sinko P, Kohn J. Polymeric drug delivery systems. In: Nokali ME, Piatt DM, Charpantier, editors. Polymeric delivery systems. Properties and applications. ACS Symposium Series 520, Washington DC; 1993. p. 18.
[2] Uhrich KE, Larrier DR, Laurencin CT, Langer R. J Polym Sci, Part A: Polym Chem 1996;34:1261.
[3] Uhrich KE, Ibim SEM, Larrier DR, Langer R, Laurencin CT. Biomaterials 1998;19:2045.
[4] Matsumura Y, Maeda H. Cancer Res 1986;46:6387.
[5] Maeda H, Seymour LW, Miyamota Y. Bioconjugate Chem 1992;3:351.
[6] Uhrich KE, Cannizzaro SM, Langer R, Shakesheff KM. Chem Rev 1999;99:3181.
[7] Thomson AH, Vasey PA, Murry LS, Cassidy J, Fraier D, Frigerio E, et al. Br J Cancer 1999;81:99.
[8] Vasey PA, Kaye SB, Morrison R, Twelve C, Wilson P, Duncan R, et al. Clin Cancer Res 1999;5:83.
[9] Pendri A, Conover CD, Greenwald RB. Anti-Cancer Drug Des 1998;13:387.
[10] Terwogt JMM, Huinink WWB, Schellens JHM, Schot M, Mandjes IAM, Zurlo MG, et al. Anti-Cancer Drugs 2001;12:315.
[11] Maeda H. Med Res Rev 1991;6:181.

[12] Greenwald RB, Conover CD, Pendri A, Choe YH, Martinez A, Wu D, et al. J Control Release 1999;61:281.
[13] Greenwald RB, Pendri A, Conover CD, Lee C, Choe YH, Gilbert C, et al. Bioorg Med Chem 1998;6:551.
[14] Fraier D, Frigerio E, Brianceschi G, Casati M, Benecchi A, James C. J Pharm Biom Anal 2000;22:505.
[15] Conover CD, Greenwald RB, Pendri A, Gilbert CW, Shum KL. Cancer Chemother Pharmacol 1998;42:407.
[16] Caiolfa VR, Zamai M, Fiorino A, Frigerio E, Pellizzoni C, d'Argy R, et al. J Control Release 2000;65:105.
[17] Giovanella B, Hinz H, Kozielski A, Stehlin J, Silber R, Potmesil M. Cancer Res 1991;51:3052.
[18] Hsiang YH, Liu LF, Wall ME, Wani MC, Nicholas AW, Manikumar G, et al. Cancer Res 1989;49:4385.
[19] Hertzberg RP, Caranfa MJ, Hecht SM. Biochemistry 1989;28:4629.
[20] Pantazis P. Clin Cancer Res 1995;1:1235.
[21] Park JG, Choi WM, Lee NJ, Ha CS, Cho WJ. J Polym Sci, Polym Chem 1998;36:1625.
[22] Choi WM, Chung ID, Lee NJ, Lee YW, Ha CS, Cho WJ. J Polym Sci, Polym Chem 1998;36:2177.
[23] Jung EY, Chung ID, Lee NJ, Park JS, Ha CS, Cho WJ. J Polym Sci, Polym Chem 2000;38:1247.
[24] Lee NJ, Kim KH, Rhew HY, Choi WM, Chung ID, Cho WJ. Polym Int 2000;49:1702.
[25] Lee NJ, Koo JC, Moon SB, Cho WJ, Jeong IC, Lee SJ, Cho MY, Theodorakis EA. Polym Int 2002;51:569.
[26] Mosmann T. J Immunol Methods 1983;65:55.