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Synthesis and Antitumor Activity of Phthalimide-Based Polymers Containing Camptothecin

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Abstract: The objective of this study was to develop a polymeric drug delivery system for camptothecin (CPT), capable of improving its therapeutic index and reducing its side effects. A monomeric conjugate, 3,6-*endo*-methylene-1,2,3,6-tetrahydrophthalimidoethanoylcamptothecin (ETECPT) between CPT and 3,6-*endo*-methylene-1,2,3,6-tetrahydrophthalimidoethanoic acid was synthesized. Its homo- and copolymer with acrylic acid (AA) were prepared by photopolymerization using 2,2-dimethoxy-2-phenylacetophenone (DMP) as a photoinitiator. The monomer and its polymers were characterized by IR, ^{1}H- and ^{13}C-NMR spectra. The ETECPT content in poly(ETECPT-*co*-AA) obtained by elemental analysis was 82 wt%. The number-average molecular weights of the polymers determined by gel permeation chromatography were as follows: M_n = 11,400 for poly(ETECPT), M_n = 17,900 for poly(ETECPT-*co*-AA). The IC_{50} values of ETECPT and its polymers against cancer cells were much larger than that of CPT. Our results from the *in vivo* antitumor activity indicated that all polymers show high antitumor activity than CPT at a dose of 100 mg/kg.

Keywords: 3,6-*endo*-methylene-1,2,3,6-tetrahydrophthalimidoethanoylcamptothecin, camptothecin, photopolymerization, *in vitro* cytotoxicity, *in vivo* antitumor activity.

Introduction

Efficient use of potent drugs with strong side effects requires their selective delivery at the site of action at a controlled rate.[1-3] Among these methods, a particularly promising one relies on conjugation of the parent drug to polymers to enhance both its tissue specificity and effective concentration at tumor sites. In contrast to low molecular weight anticancer drugs, polymer-anticancer drug conjugates can prolong the antitumor activity of the parent drug by releasing it at a controlled rate at the targeted site.[4]

The potential of the above strategy is illustrated by the numerous studies dealing with conjugation of polymers with anticancer drugs such as 5-fluorouracil (5-FU),[5,6] 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 documentled side effects. More specifically, CPT 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. CPT injected intramuscularly induced complete remission in mice inoculated with a variety of human cancer xenograft lines. However, antitumor and toxic effects were found to vary remarkably with schedule and route of administration. Despite its promising antitumor activity in animal models,

This article is dedicated to professor Won-Jai Cho on the occasion of his retirement.

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clinical development of the drug was halted for unpredictable toxicities such as myelosuppression, vomiting, and diarrhea.

The limitations of CPT can be overcome 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-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]

To improve the biological profile of CPT, we describe herein the synthesis and antitumor activity of polymers containing CPT based on the tetrahydrophthalimide template. The monomer unit was built by linking CPT to tetrahydrophthalic acid with glycine as a spacer. Its homopolymer and copolymer with acrylic acid (AA) were prepared by photopolymerization. The obtained monomer and its polymers were identified by Infrared (IR), ^{1}H Nuclear Magnetic Resonance (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.

Experimental

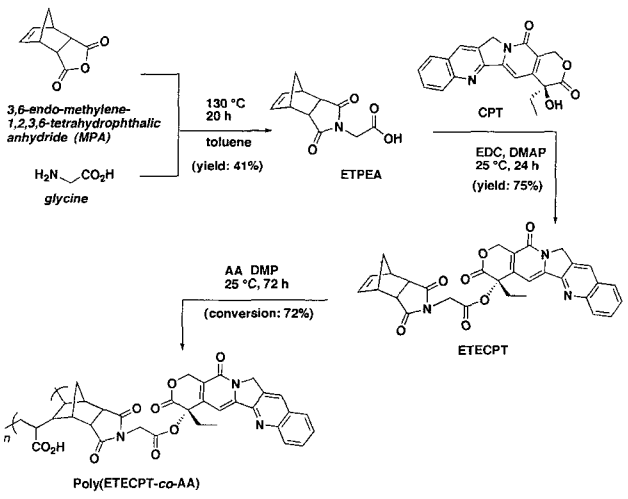
Materials. Glycine (Aldrich, USA), 3,6-*endo*-methylene-1,2,3,6-tetrahydrophthalic anhydride (MPA, Aldrich), triethylamine (TEA, Aldrich), camptothecin (CPT, Aldrich), dimethylaminopyridine (DMAP, Aldrich), 1-[3-(dimethylaminopropyl)]-3-ethylcarbodiimide hydrochloride (EDC, Aldrich), and 2,2-dimethoxy-2-phenylacetophenone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

Studies for 50% inhibitory concentration (IC_{50}) using FM3A, P388, U937, and AC2F cell lines were conducted. For the *in vivo* antitumor activity, 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 ± 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 Kosin University and were performed according to the NIH Guide for The Care and Use of Laboratory Animals.

Instruments. ^{1}H and ^{13}C NMR spectra were measured on a FT-300 MHz Varian Gemini 2000 spectrophotometer. The chemical shifts are reported in parts per million (ppm) relative to internal solvent (2.50 ppm in DMSO-d_6). IR spectra were recorded on a Perkin-Elmer 397 spectrometer using KBr pellets. Elemental analyses were performed on a Carlo Erba model 180 elemental analyzer. 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).

Synthesis of Monomer. The ETECPT monomer and poly (ETECPT-*co*-AA) were synthesized according to Scheme I.

3,6-*endo*-Methylene-1,2,3,6-tetrahydrophthalimidoethanoic acid (ETPEA). Water was distilled azeotropically with a Dean-Stark apparatus from a mixture of MPA(15.0 g, 91.4 mmol), glycine (6.9 g, 92 mmol), and TEA (1.2 mL) in toluene (150 mL). After cooling to room temperature, the reaction mixture was then concentrated under reduced pressure to near dryness. The residue was triturated with 0.1 N HCl (50 mL) and dissolved in saturated aqueous $NaHCO_3$ (60 mL). The aqueous solution was washed with ethyl acetate (45 mL), acidified to pH 2 by adding conc. HCl and extracted twice with dichloromethane (50× 2 mL). The dichloromethane layers were dried over anhydrous $MgSO_4$ and evaporated to obtain pure ETPEA in 41% yield. ^{1}H NMR (DMSO-d_6): δ(ppm) = 1.54(s, 2H, $CHCH_2CH$ of MPA), 3.25 (m, 2H, -*CH*CH=CH*CH*- of MPA), 3.42 (s, 2H, -*CH*CON-COC*H*- of MPA), 3.88 (s, 2H, -NCH_2COOH), 6.01 (s, 2H,



Scheme I

-C*H*=C*H*- of MPA).

3,6-*endo*-Methylene-1,2,3,6-tetrahydrophthalimidoethanoylcamptothecin (ETECPT). A mixture of ETEHA (221 mg, 1.0 mmol), CPT (348 mg, 1.0 mmol), DMAP(134 mg, 1.1 mmol), and EDC (210 mg, 1.1 mol) in dry dichloromethane (5 mL) was stirred for 18 h at room temperature followed by dilution with dichloromethane (100 mL). This mixture was washed with 0.1 N HCl (30× 2 mL), saturated aqueous sodium bicarbonate (30× 2 mL), and water (30× 2 mL). The organic layer was dried over anhydrous $MgSO_4$ and filtered, followed by removal of the solvent by evaporation 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 white solid (413 mg, 75%). ^{1}H NMR (DMSO-d_6): δ(ppm) = 0.90 (t, 3H, J = 7.3 Hz, H-18), 1.54 (s, 2H, H-c), 2.11-2.17 (m, 2H, H-19), 3.23-3.26 (m, 2H, H-b), 3.41-3.46 (m, 2H, H-d), 4.20 (d, 1H, J = 18.0 Hz, H-e), 4.37 (d, 1H, J = 18.0 Hz, H-e), 5.26 (d, 1H, J = 17.0 Hz, H-5), 5.31 (d, 1H, J = 17.0 Hz, H-5), 5.49 (s, 2H, H-17), 5.98-6.00 (m, 1H, H-a), 6.06-6.08 (m, 1H, H-a), 7.11 (s, 1H, H-14), 7.73 (t, 1H, J = 7.5 Hz, H-10), 7.89 (t, 1H, J = 7.5 Hz, H-11), 8.13 (d, 1H, J = 8.0 Hz, H-9), 8.19 (d, 1H, J = 8.0 Hz, H-12), 8.69 (s, 1H, H-7). ^{13}C NMR (DMSO-d_6): δ(ppm) = 7.5, 30.4, 38.8, 44.26, 44.29, 45.46, 45.50, 50.2, 51.8, 66.3, 76.8, 94.9, 118.9, 127.7, 128.0, 128.5, 129.0, 129.8, 130.4, 131.5, 134.30, 134.34, 144.6, 146.1, 147.9, 152.3, 156.4, 165.9, 166.6, 176.29, 176.30.

Synthesis of Poly(ETECPT). A solution of ETECPT (0.1 g) and DMP (0.004 g) as an initiator in THF (30 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 72 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 homo-polymer was dried under reduced pressure to a constant weight. The conversion was 88%.

Syntheses of Poly(ETECPT-*co*-AA) A solution of ETECPT (0.1 g) and AA (0.021 g) with DMP (0.008 g) as an initiator in dry THF (30 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(ETECPT-*co*-AA) was the same as that described for the homopolymerization of ETECPT except for the monomer pairs. The copolymerization conversion of ETECPT with AA was 72%.

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 ETECPT moiety in the copolymer were calculated from C, H and N data obtained by elemental analysis.

Biological Activity Tests

***In Vitro* Cytotoxicity.** The cytotoxicity of ETECPT and its polymers against three cancer cell lines *in vitro* was performed with the MTT assay according to the Mosmann's method.[26] The MTT assay is based on the reduction of the soluble 3-(4,5-dimethyl-2-thiazolyl)-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 (2×10^4/mL) were plated in 96-well culture plates and incubated at 37 °C in a 5% CO_2 incubator. After 12 h, the test sample (2 μL) 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. The results were expressed as the IC_{50}, which is the concentration of the drugs inducing a 50% inhibition of cell growth of treated cells when compared to the growth of control cells. Each experiment was performed at least 3 times. There was a good reproducibility between replicate wells with standard errors below ± 10%.

***In Vivo* Antitumor Activity.** Antitumor activity of ETECPT and its polymers was performed on mice with sarcoma 180 cell. 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 solutions were given i.p. to mice once a day for 4 consecutive days starting from 24 h after the cell injection. 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 Statistical Package for Social Science software program.

Results and Discussion

Direct conjugation of CPT to ETPEA through the 20-hydroxyl group was accomplished using EDC as a coupling reagent with DMAP as a organic base. Chemical structure and purity of ETECPT were proven using IR, ^{1}H-NMR, and ^{13}C-NMR spectroscopic techniques. As shown in Figure 1, the ^{1}H-NMR spectrum of ETECPT showed two multiplets at 5.98-6.00 and 6.06-6.08 ppm, which were assigned to vinyl group protons. The peaks at 0.90 and 2.11-2.17 ppm were assigned to ethyl group protons of CPT moiety in ETECPT. The IR spectrum of ETECPT showed two peaks at 1756 and 1706 cm^{-1} which were assigned to carbonyl group and a peak at 1669 cm^{-1} which are characteristic peak for vinyl group. As shown in Figure 1 the peaks of vinyl protons in ETECPT appeared at 5.98-6.00 and 6.06-6.08 ppm, and ethyl proton of CPT moiety in ETECPT appeared at 0.90 and 2.11-2.17 ppm.

Poly(ETECPT) was synthesized via radical polymerization through the carbon-carbon double bond on the norbornene moiety. The disappearance of vinyl group peak at 1669 cm^{-1} which appeared in ETECPT monomer confirmed a complete conversion of ETECPT to poly(ETECPT). The peaks for the vinyl protons of monomeric ETECPT at 5.98-6.00 and 6.06-6.08 ppm were not observed. The FTIR spectrum of poly(ETECPT-*co*-AA) indicated absorption at 3500-2800 cm^{-1} (COOH stretching of AA moiety) and 1710 cm^{-1} (C=O stretching). The absorption peaks caused by protons of ETECPT moiety in poly(ETECPT-*co*-AA) were assigned to the same as those of poly(ETECPT). The peaks assigned to the olefinic proton of ETECPT and AA moiety disappeared. ETECPT and its polymers were soluble in acetone, ethanol, methanol, DMF, DMSO and THF and were insoluble in diethyl ether, *n*-hexane and water. The average molecular weights and polydispersity indices of the polymers are listed in Table I. The number average molecular weights (M_n) and the polydispersity index of poly(ETECPT) were 11,400 and was 1.57, respectively.

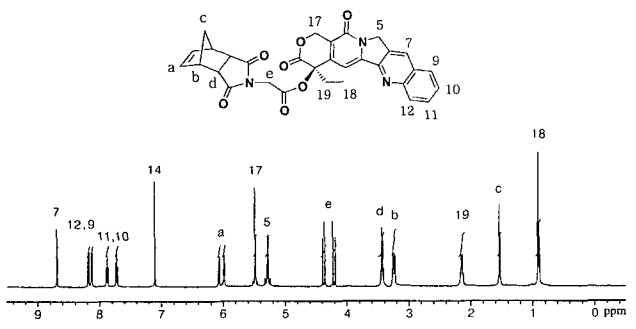


Figure 1. The ^{1}H NMR spectrum of ETECPT.

The elemental analysis value of poly(ETECPT-*co*-AA) is as follows: C, 57.52; H, 5.93; N, 5.52. The ETECPT composition in poly(ETECPT-*co*-AA) was calculated from N content and was 82 wt%. The *in vitro* cytotoxicity of ETECPT and its polymers against three cancer cell lines and a normal cell line are shown in Table II. As shown in Table II, the values of 50% cytotoxicity (IC_{50}) of the conjugates were in the range of 1.0-3.6 ng/mL against cancer cell lines. The cytotoxicity of ETECPT against U937 cell was comparable to its homopolymer and higher than that of its copolymer. The cytotoxicity of ETECPT and its polymers against P388 cell increased in the following order: ETECPT = poly (ETECPT) > poly(ETECPT-*co*-AA). In normal cell line (AC2F), the cytotoxicities of CPT-conjugates were much lower that of free CPT.

We evaluated the antitumor activity of monomer and its polymers against intraperitoneally inoculated sarcoma 180 cell in Balb/C mice. The survival data of mice treated with ETECPT and its polymers are shown in Table III together with that of CPT for comparison. Mortality was recorded and mean survival time was calculated for each compound. The activity of drug was expressed as a survival effect (*T*/*C*), where *T* is the mean survival time of mice treated with drug and *C* is the survival time of mice in a control group. Entry 4 low dosage of CPT (10 mg/kg) led to good antitumor activity (612% increase as compared to the control group) and resulted in 100% survival of mice beyond the experimental period of 93 days. However, increase of drug dosage to 100 mg/kg (Table III, entry 3) led to a sharp decrease of

Table I. Average Molecular Weights and Polydispersity of the Polymers

Polymers	M_n	M_w	M_w/M_n
Poly(ETECPT)	11,400	17,900	1.57
Poly(ETECPT-*co*-AA)	17,800	25,200	1.41

Molecular weights were determined by GPC in DMF.

Table II. *In Vitro* Cytotoxicity of ETECPT 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
ETECPT	1.6±0.7	3.2±0.5	1.3±0.1	1.5±0.4
Poly(ETECPT)	1.0±0.1	3.6±0.4	1.3±0.2	10±0.9
Poly (ETECPT-*co*-AA)	15±1.6	16±0.9	12±2.5	10±0.9

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

Table III. *In vivo* Antitumor Activity of ETECPT 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	ETECPT	100	60	93.0 ± 0.0	612	10/10*
6		10	6.0	70.5 ± 17.0	464	6/10*
7	Poly(ETECPT)[d]	100	60	90.3 ± 8.1	594	9/10*
8		10	6.0	25.3 ± 5.9	166	6/10*
9	Poly(ETECPT-*co*-AA)[e]	100	49	93.0 ± 0.0	612	10/10*
10		10	4.9	51.5 ± 12.7	339	4/10*

* $P < 0.0001$.
[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 × 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(ETECPT), the drug composition is 60 wt%.
[e]For poly(ETECPT-*co*-AA), the ETECPT composition is 82 wt% which means that the drug composition is 49 wt%.

the mean survival time (33% as compared to the control group) and loss of all animals within the first 5 days. This is attributed to the inherent toxicity of CPT. At low and high drug dosages (10 and 100 mg/kg) we observed a significant increase (464% and 612% respectively) of the mean survival time versus that of the control group. In both cases, such treatment led to a significant prolongation of the life of mice beyond the experimental period. In addition, comparison between the activities obtained for CPT and ETECPT at high dosage (entries 3 and 5 respectively) indicates that even at a high concentration ETECPT displays a low toxicity which could be attributed to a slow release of the active drug from the monomer. Evaluation of poly(ETECPT) at two different dosages is presented in Table III, entries 7 and 8. High drug dosage (100 mg/kg) led to an increase of the mean survival time by 594% versus that of the control group. At this dosage 9 out of the 10 mice survived the experimental period of 93 days, indicating that the polymer has a low toxicity level and is well tolerated by the animals. However, at low drug dosage (10 mg/kg) the mean survival time was only slightly increased (166%) and only 6 mice survived the experimental period. This may be attributed due to the low concentration of CPT in the polymer. As indicated in Table III, entries 9 and 10 treatment of mice with poly(ETECPT-*co*-AA) resulted in a substantial increase of the mean survival time at high drug dosage (100 mg/kg). At this concentration we observed an increase in mean survival time by 612% leading to a 100% survival of treated mice beyond the experiment period of 93 days. However, at low drug dosage (10 mg/kg) the mean survival time was 339% and only 4 mice survived beyond the 93 days. These results demonstrate that this polymer has a very low toxicity even at high dosages and can thus overcome the inherent toxicity associated with high doses of CPT.

The lack of toxicity may be the result of the slow and sustained release of free CPT from its conjugate. Intraperitoneal administration of the CPT-conjugates to sarcoma 180 tumor-bearing mice resulted in a significant improvement in the life span of the treated animals and induced no acute toxicity. These results demonstrate that the binding of CPT to phthalimide polymer has a very low toxicity even at high dosage, and can therefore overcome the inherent toxicity associated with high doses of CPT.

Conclusions

In the present study we have synthesized 3,6-*endo*-methylene-1,2,3,6-tetrahydrophthalimidoethanoylcamptothecin (ETECPT) from camptothecin (CPT) and 3,6-*endo*-methylene-1,2,3,6-tetra-hydrophthalimidoethanoic acid (ETPEA). Its homopolymer and copolymer with acrylic acid (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 1.6. The content of ETHCPT in poly(ETECPT-*co*-AA) was found to be 82 mol%. The range of IC_{50} values obtained from the *in vitro* test for ETECPT, poly(ETECPT), and poly(ETECPT-*co*-AA) were from 1.0 to 16 μg/mL against cancer cell lines. In a normal cell, the cytotoxicity of monomer was stronger than those of its homopolymer and its copolymer. Our data obtained from *in vivo* test indicate that the synthesized monomer and it polymers exhibit higher antitumor activities and lower toxicities than CPT. These results provide a basis for delivery systems of CPT for therapeutic use.

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References

(1) G. Khang, J. H. Lee, J. W. Lee, J. C. Cho, and H. B. Lee, *Korea Polym. J.*, **8**, 80 (2000).
(2) G. Khang, H.-S. Choi, S. C. Yoon, J. C. Cho, and H. B. Lee, *Korea Polym. J.*, **8**, 253 (2000).
(3) G. Khang, S.-A Seo, H. S. Choi, J. M. Rhee, and H. B. Lee, *Macromol. Res.*, **10**, 246 (2002).
(4) K. E. Uhrich, S. M. Cannizzaro, R. Langer, and K. M. Shakesheff, *Chem. Rev.*, **99**, 3181 (1999).
(5) W. M. Choi, I. D. Jung, N. J. Lee, S. H. Kim, C. S. Ha, and W. J. Cho, *Polym. Adv. Technol.*, **8**, 701 (1997).
(6) Z. Liu and S. Rimmer, *J. Control. Release*, **81**, 91 (2002).
(7) A. H. Thomson, P. A. Vasey, L. S. Murry, J. Cassidy, D. Fraier, E. Frigerio, and C. Twelves, *Br. J. Cancer*, **81**, 99 (1999).
(8) P. A. Vasey, S. B. Kaye, R. Morrison, C. Twelve, P. Wilson, R. Duncan, A. H. Thomson, L. S. Murray, T. E. Hilditch, T. Murray, S. Burtles, D. Fraier, E. Frigerio, and J. Cassidy, *Clin. Cancer Res.*, **5**, 83 (1999).
(9) A. Pendri, C. D. Conover, and R. B. Greenwald, *Anti-Cancer Drug Design*, **13**, 387 (1998).
(10) J. M. M. Terwogt, W. W. B. Huinink, J. H. M. Schellens, M. Schot, I. A. M. Mandjes, M. G. Zurlo, M. Rocchetti, H. Rosing, F. J. Koopman, and J. H. Beijnen, *Anti-Cancer Drugs*, **12**, 315 (2001).
(11) H. Maeda, *Med. Res. Revs.*, **6**, 181 (1991).
(12) R. B. Greenwald, C. D. Conover, A. Pendri, Y. H. Choe, A. Martinez, D. Wu, S. Guan, Z. Yao, and K. L. Shum, *J. Control. Release*, **61**, 281 (1999).
(13) R. B. Greenwald, A. Pendri, C. D. Conover, C. Lee, Y. H. Choe, C. Gilbert, A. Martinez, J. Xia, D. Wu, and M. Hsue, *Bioorg. Med. Chem.*, **6**, 551 (1998).
(14) D. Fraier, E. Frigerio, G. Brianceschi, M. Casati, A. Benecchi, and C. James, *J. Pharm. Biom. Anal.*, **22**, 505 (2000).
(15) C. D. Conover, R. B. Greenwald, A. Pendri, C. W. Gilbert, and K. L. Shum, *Cancer Chemother. Pharmacol.*, **42**, 407 (1998).
(16) V. R. Caiolfa, M. Zamai, A. Fiorino, E. Frigerio, C. Pellizzoni, R. d'Argy, A. Ghiglieri, M. G. Castelli, M. Farao, E. Pesenti, M. Gigli, F. Angelucci, and A. Suarato, *J. Control. Release*, **65**, 105 (2000).
(17) B. Giovanella, H. Hinz, A. Kozielski, J. Stehlin, R. Silber, and M. Potmesil, *Cancer Res.*, **51**, 3052 (1991).
(18) Y. H. Hsiang, L. F. Liu, M. E. Wall, M. C. Wani, A. W. Nicholas, G. Manikumar, S. Kirschenbaum, R. Silber, and M. Potmesil, *Cancer Res.*, **49**, 4385 (1989).
(19) R. P. Hertzberg, M. J. Caranfa, and S. M. Hecht, *Biochemistry*, **28**, 4629 (1989).
(20) P. Pantazis, *Clin. Cancer Res.*, **1**, 1235 (1995).
(21) J. G. Park, W. M. Choi, N. J. Lee, C. S. Ha, and W. J. Cho, *J. Polym. Sci., Polym. Chem. Ed.*, **36**, 1625 (1998).
(22) W. M. Choi, I. D. Chung, N. J. Lee, Y. W. Lee, C. S. Ha, and W. J. Cho, *J. Polym. Sci., Polym. Chem. Ed.*, **36**, 2177 (1998).
(23) E. Y. Jung, I. D. Chung, N. J. Lee, J. S. Park, C. S. Ha, and W. J. Cho, *J. Polym. Sci., Polym. Chem. Ed.*, **38**, 1247 (2000).
(24) N. J. Lee, K. H. Kim, H. Y. Rhew, W. M. Choi, I. D. Chung, and W. J. Cho, *Polym. Int.*, **49**, 1702 (2000).
(25) N. J. Lee, J. C. Koo, S. S. Ju, S. B. Moon, W. J. Cho, I. C. Jeong, S. J. Lee, M. Y. Cho, and E. A. Theodorakis, *Polym. Int.*, **51**, 569 (2002).
(26) T. Mosmann, *J. Immunol. Methods*, **65**, 55 (1983).