molecular pharmaceutics

articles

Antitumor Activity of β-Cyclodextrin Polymer−Camptothecin Conjugates

Jianjun Cheng,[†] Kay T. Khin,[†] and Mark E. Davis*[,‡]

Insert Therapeutics, Inc., 2585 Nina Street, Pasadena, California 91107, and Chemical Engineering, California Institute of Technology, Pasadena, California 91125

Received February 23, 2004

Abstract: Antitumor activity of linear, β-cyclodextrin polymer (CDP)−camptothecin (CPT) conjugates (HGGG6, LGGG10, HG6, and HGGG10) is investigated in nude mice bearing human LS174T colon carcinoma tumors. These conjugates differ in polymer molecular mass [97 kDa (H) or 35 kDa (L)], CDP−CPT linker structure [glycine (G) or triglycine (GGG)], and CPT loading [ca. 6 wt % (6) or 10 wt % (10)]. Maximum tolerable doses (MTDs) of the three conjugates, LGGG10, HG6, and HGGG10, are determined to be 36, 9, and 9 mg of CPT/kg, respectively, while the MTD of the CDP alone exceeds 240 mg/kg (highest value investigated). The three CDP−CPT conjugates with high polymer molecular masses (HGGG6, HG6, and HGGG10) demonstrate antitumor activity at their MTDs superior to that of CPT at the same amount and to that of irinotecan at its optimal dose. They also show tumor growth inhibition that is superior to that of the conjugate containing the low-molecular mass polymer (LGGG10) at the same dose of CPT. No significant effects of CPT weight loading or linker structure on tumor growth delay are observed. However, conjugates containing G appear to be less toxic than these with GGG. These antitumor studies demonstrate that the CDP-based conjugates of CPT exhibit tumor growth inhibition superior to that of CPT or irinotecan at the conditions employed in this study. The striking observation is that a short course of treatment with the polymer conjugates gives long-term control of tumor growth that does not occur with either CPT or irinotecan. Intracellular CDPs are demonstrated by analyzing cells that were cultured in the presence of rhodamine-labeled CDP (HRhod) containing medium using both confocal microscopy and flow cytometry. The long-term therapeutic efficacy of CDP−CPT conjugates observed in mice may in part be due to the sustained release of CPT from these conjugates in the acidic, intracellular compartments since these conjugates are shown to have significantly slower release rates at acidic pH than at physiological pH.

Keywords: Camptothecin; cyclodextrin-based polymer; polymer−drug conjugates; antitumor activity; sustained release; drug delivery

Introduction

20(*S*)-Camptothecin (CPT) was first isolated from the Chinese tree *Camptotheca acuminata* in the 1960s[1] and has shown a broad range of anticancer activity in animal models.[2,3] CPT has low aqueous solubility and can be highly toxic in some forms.[4] The structure of CPT is illustrated in Figure 1. It is known that CPT has a pH-dependent

* To whom correspondence should be addressed: Chemical Engineering, California Institute of Technology, Pasadena, CA 91125. Phone: (626) 395-4251. Fax: (626) 568-8743. E-mail: mdavis@cheme.caltech.edu.

[†] Insert Therapeutics, Inc.

[‡] California Institute of Technology.

(1) Wall, M. E.; Wani, M. C.; Cook, C. E.; Palmer, K. H.; McPhail, A. T.; Sim, G. A. *J. Am. Chem. Soc.* **1966**, *88*, 3888−3890.

(2) Muggia, F. M.; Dimery, I.; Arbuck, S. G. *Ann. N.Y. Acad. Sci.* **1996**, *803*, 213−223.

(3) Rothenberg, M. L. *Ann. Oncol.* **1997**, *9*, 837−855.

10.1021/mp049966y CCC: $27.50
Published on Web 04/03/2004

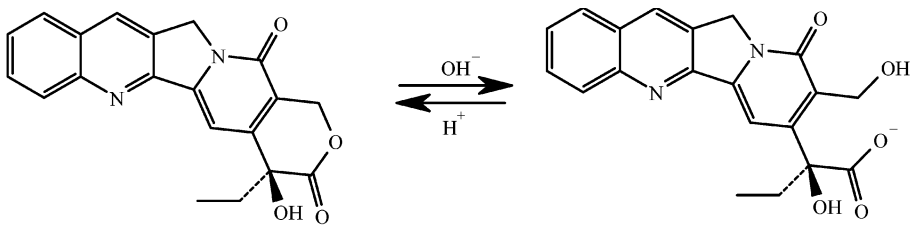

Figure 1. CPT structure and pH-dependent equilibrium of the lactone and carboxylate forms. The lactone form is favored at acidic pH.

equilibrium between its lactone form and carboxylate form in aqueous solution. The lactone form is essential for antitumor activity, while the carboxylate form is inactive and favored at physiological pH.[5,6] Serum albumin preferentially binds the carboxylate form of CPT and forces the distribution of CPT to further disfavor the lactone form.[4−6] These features of CPT can result in rapid lactone ring opening and a loss of antitumor activity. In attempts to circumvent these problems, a number of CPT analogues have been synthesized in an effort to achieve better aqueous solubility and higher lactone stability.[7] Two of these CPT analogues, topotecan and irinotecan, are currently approved for treatment of certain cancers in humans.[8,9]

An alternative approach to overcoming the pharmaceutical and pharmacokinetic shortcomings of CPT is to covalently attach the active lactone form to water soluble polymers via the 20-OH position. CPT conjugates with polyethylene glycol (PEG),[10−12] poly-*N*-(2-hydroxypropyl)methacrylamide (HPMA copolymer),[13,14] and poly-L-glutamic acid (PG)[15−17] have been reported. In addition to improving the solubility and stability of CPT, the polymer drug conjugation approach can also enhance the accumulation of the drug in tumors by taking advantage of the ability of macromolecules to penetrate and be trapped in tumor tissue (due to the abnormally leaky vasculature of tumors) through the so-called enhanced permeability and retention (EPR) effect.[18]

Interest in small molecule drug delivery using biocompatible materials has continued to grow in the past two decades.[19−24] Recently, β-cyclodextrin (CD)-based, linear polycations were synthesized by polymerizing a difunctionalized CD monomer with other difunctionalized comonomers through condensation reactions.[25,26] These polymers were used as nonviral vectors for systemic gene delivery.[26,27] We prepared CD-based linear polyanions and formed CPT conjugates (Figure 2) with these new polymers. Complete details of the polymer and conjugate syntheses can be found elsewhere.[28] The aqueous solubility of CPT is increased by more than 3 orders of magnitude when it is conjugated to this hydrophilic polymer, and the conjugated CPT remains in the lactone form and releases CPT (confirmed by HPLC and mass spectrometry).[28] Here, a series of studies were performed to evaluate the antitumor effects of the CDP−CPT conjugates that involved investigating the polymer molecular mass (MW), the structure at the linker between the polymer and the CPT, and the CPT weight percent loading. The efficacy study was conducted in athymic

(4) Hertzberg, R. P.; Caranfa, M. J.; Hecht, S. M. *Biochemistry* **1989**, *28*, 4629−4638.
(5) Mi, Z.; Burke, T. G. *Biochemistry* **1994**, *33*, 12540−12545.
(6) Mi, Z.; Burke, T. G. *Biochemistry* **1994**, *33*, 10325−10336.
(7) Kehrer, D. F. S.; Soepenberg, O.; Loos, W. J.; Verweij, J.; Sparreboom, A. *Anti-Cancer Drugs* **2001**, *12*, 89−105.
(8) ten Bokkel Huinink, W. W.; Gore, M.; Carmichael, J. *J. Clin. Oncol.* **1997**, *15*, 2183−2193.
(9) Van Cutsem, M.; Cunningham, D.; ten Bokkel Huinink, W. W. *Eur. J. Cancer* **1999**, *35*, 54−59.
(10) Greenwald, R. B.; Pendri, A.; Conover, C. D.; Gilbert, C. W.; Yang, R.; Xia, J. *J. Med. Chem.* **1996**, *39*, 1938−1940.
(11) Rowinsky, E. K.; Rizzo, J.; Ochoa, L.; Takimoto, C. H.; Forouzesh, B.; Schwartz, G.; Hammond, L. A.; Patnaik, A.; Kwiatek, J.; Goetz, A.; Denis, L.; McGuire, J.; Tolcher, A. W. *J. Clin. Oncol.* **2003**, *21*, 148−157.
(12) Conover, C. D.; Greenwald, R. B.; Pendri, A.; Shum, K. L. *Anti-Cancer Drug Des.* **1999**, *14*, 499−506.
(13) Caiolfa, V. R.; Zamai, M.; Fiorino, A.; Frigerio, E.; Pellizzoni, C.; d'Argy, R.; Ghiglieri, A.; Castelli, M. G.; Farao, M.; Pesenti, E.; Gogli, M.; Angelucci, F.; Suarato, A. *J. Controlled Release* **2000**, *65*, 105−119.
(14) Zamai, M.; vandeVen, M.; Farao, M.; Gratton, E.; Ghiglieri, A.; Castelli, M. G.; Fontana, E.; d'Argy, R.; Fiorino, A.; Pesenti, E.; Suarato, A.; Caiolfa, V. R. *Mol. Cancer Ther.* **2003**, *2*, 29−40.
(15) Singer, J. W.; Bhatt, R.; Tulinsky, J.; Buhler, K. R.; Heasley, E.; Klein, P.; Vries, P. D. *J. Controlled Release* **2001**, *74*, 243−247.
(16) Bhatt, R.; Vries, P. D.; Tulinsky, J.; Bellamy, G.; Baker, B.; Singer, J. W.; Klein, P. *J. Med. Chem.* **2003**, *46*, 190−193.
(17) Zou, Y.; Wu, Q.; Tansey, W.; Chow, D.; Hung, M.; Charnsangavej, C.; Wallace, S.; Li, C. *Int. J. Oncol.* **2001**, *18*, 331−336.
(18) Matsumura, Y.; Maeda, H. *Cancer Res.* **1986**, *6*, 6387−6392.
(19) Duncan, R.; Gac-Breton, S.; Keane, R.; Musila, R.; Sat, Y. N.; Satchi, R.; Searle, F. *J. Controlled Release* **2001**, *74*, 135−146.
(20) Duncan, R. *Anti-Cancer Drugs* **1992**, *3*, 175−210.
(21) Duncan, R.; Dimitrijevic, S.; Evagorou, E. *S.T.P. Pharma Sci.* **1996**, *6*, 237−263.
(22) Duncan, R.; Kopecek, J. *Adv. Polym. Sci.* **1984**, *57*, 51−101.
(23) Duncan, R.; Spreafico, F. *Clin. Pharmacokinet.* **1994**, *27*, 290−306.
(24) Putnam, D.; Kopecek, J. *Adv. Polym. Sci.* **1995**, *122*, 55−123.
(25) Gonzalez, H.; Hwang, S. J.; Davis, M. E. *Bioconjugate Chem.* **1999**, *10*, 1068−1074.
(26) Hwang, S. J.; Bellocq, N. C.; Davis, M. E. *Bioconjugate Chem.* **2001**, *12*, 280−290.
(27) Pun, S. H.; Davis, M. E. *Bioconjugate Chem.* **2002**, *13*, 630−639.
(28) Cheng, J.; Khin, K. T.; Jensen, G. S.; Liu, A.; Davis, M. E. *Bioconjugate Chem.* **2003**, *14*, 1007−1017.

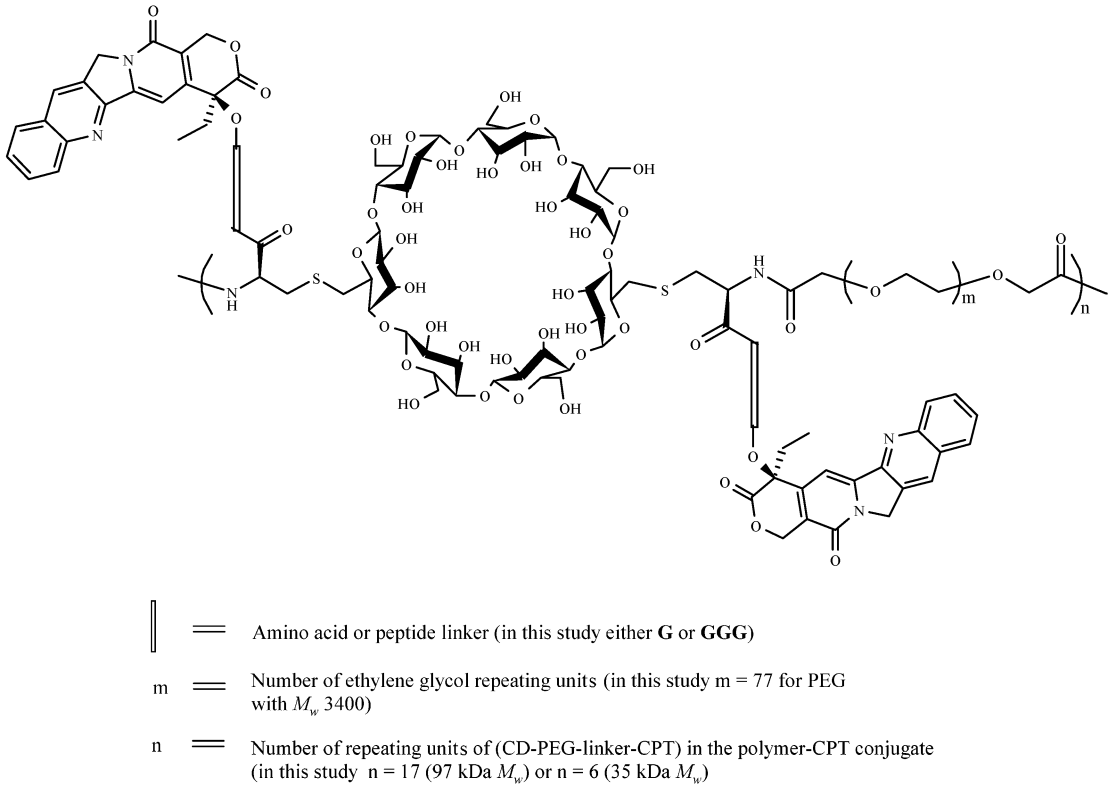

Figure 2. Schematic illustration of β-cyclodextrin polymer−camptothecin conjugates.

Table 1. Properties of Polymer−CPT Conjugates[a]

conjugate[b]	M_w of the parent polymer ($\times 10^{-3}$)	M_w/M_n[c]	linker	CPT (wt %)
HGGG6	97	1.7	triglycine	6.1
LGGG10	35	1.6	triglycine	10.2
HG6	97	1.7	glycine	6.8
HGGG10	97	1.7	triglycine	9.6

[a] Four CDP−CPT conjugates that differ in their polymer molecular mass, linker structure, and weight percent loading of CPT are summarized. A combination of letters and numbers were used to denote the conjugates. [b] Abbreviations: H, high-M_w polymer (97 kDa); L, low-M_w polymer (35 kDa); GGG, triglycine linker; G, glycine linker; 6, drug loading around 6 wt %; 10, drug loading around 10 wt %. [c] Polymer polydispersity as measured by light scattering techniques.[26]

nude mice bearing LS174T human colon carcinoma tumors that are moderately sensitive to treatments with topoisomerase I inhibitors.

Experimental Section

CDP−CPT Conjugates. The complete details for the synthesis of the linear, β-cyclodextrin polymers (CDPs) and their CPT conjugates with a glycine or a triglycine linker have been reported elsewhere.[28] Four conjugates with a variable polymer MW (97 or 35 kDa), linker (triglycine or glycine), and drug loading (ca. 6 or 10 wt %) were prepared, and some of their properties are described in Table 1. A combination of letters and numbers were used to denote the conjugates in this study. For example, HGGG6 represents a conjugate with a high MW (H, M_w = 97 kDa) and a triglycine linker (GGG) at ca. 6 wt % CPT loading (6). The other three conjugates were denoted accordingly (Table 1).

Release of CPT from HGGG6 and HG6. The complete details of the experimental protocols used for the study of release of CPT from CDP−CPT conjugates in PBS, human plasma, and solutions at various pHs are reported elsewhere using HPLC and mass spectrometer analyses.[28] CPT is released from CDP−CPT conjugates.

CDP−Lissamine Rhodamine Conjuagte. Lissamine rhodamine B ethylenediamine (2 mg, 3.3 mmol) and high-molecular mass CDP (M_w = 97 kDa, 37.8 mg) were dried under vacuum for 4 h. Dimethyl sulfoxide (DMSO, 3.7 mL) was added to the mixture, followed by 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDC, 2.6 mg, 13.6 mmol) and *N*-hydroxysuccinimide (NHS, 1.6 mg, 13.6 mmol). The mixture was stirred for 24 h and dialyzed against water using a MWCO of 25 000 for 72 h in the dark. The polymer−dye conjugate (HRhod) was obtained in 80% yield (32 mg) after lyophilization. The weight percent of rhodamine on the conjugate was determined by measuring the absorbance from rhodamine at 560 nm.

Cell Culture and Uptake Analysis Using Confocal Laser Scanning Microscopy. PC-3 (human prostate carcinoma) cells were purchased from American Type Culture Collection (Manassas, VA). Media and supplements were purchased from Gibco BRL (Gaithersburg, MD). PC-3 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS) at 37 °C in a humidified incubator containing 5% CO_2. Media containing HRhod and LysoTracker Green DND-26

(Molecular Probes) were prepared by diluting stock solutions into DMEM. Exponentially growing PC-3 cells were dissociated with trypsin (0.25%), plated on six-well Lab-Tek coverslips (Nunc Inc.), and cultured for 24 h. The culture medium was discarded, and the cells were washed once with DMEM. The cell monolayers were incubated at 37 °C with 10 mM HRhod and DMEM for 24 h. The culture medium was discarded, and the cells were rinsed six times with PBS and incubated with 1 mM LysoTracker Green DND-26 and DMEM for 24 h at 37 °C. The culture medium was discarded, and the cells were rinsed six times with PBS and fixed in paraformaldehyde [4% (w/v) in PBS] for 10 min at room temperature. The cells were washed three times with PBS and mounted onto glass microscope slides using mounting medium for fluorescence. The distribution of the fluorescence was analyzed at the California Institute of Technology Biological Imaging Center using a Zeiss 410 laser scanning confocal microscope set up from an inverted Axiophot microscope. Excitation wavelengths of 488 and 568 nm were used for LysoTracker Green DND-26 and rhodamine, respectively. LysoTracker Green DND-26 was detected with a 515−540 nm band-pass filter, and rhodamine was detected with a 580−620 nm band-pass filter. Images were analyzed with Adobe Photoshop version 6.0.

Uptake Analysis by Fluorescence-Activated Cell Sorting (FACS). PC-3 cells were cultured as described above. Exponentially growing PC-3 cells were dissociated with trypsin (0.25%), plated on a six-well plate at a density of 1×10^6 cells per well, and cultured for 24 h. The culture medium was discarded, and the cells were washed once with DMEM. The cell monolayers were incubated at 37 °C with 5 mM HRhod in DMEM (2 mL) for 24 h. The culture medium was discarded. The cells were rinsed three times with PBS, dissociated with trypsin (0.25%), and centrifuged at 1000*g* for 10 min. The cells were subsequently washed with Hank's balanced saline solution (Invitrogen) three times and analyzed on a FACScalibur instrument (Becton Dickinson, Franklin Lakes, NJ) with a 488 nm excitation line.

In Vivo Study. All of the animal studies reported here were performed by Piedmont Research Center (Morrisville, NC).

Determination of the Maximum Tolerable Dose (MTD) for CDP−CPT Conjugates. The MTD was determined using female Charles River nude mice (15−16 weeks old) for all drug conjugates except HGGG6 (identical to HGGG10 except for a lower drug loading). A 5% (w/v) dextrose solution (D5W) of the polymer−CPT conjugates was freshly prepared before each injection. Doses for the treatment groups ranged from 2.25 to 54 mg of CPT/kg (Table 3). The doses were administered intravenously (iv) by tail vein injection on days 1, 5, and 9. The dosing volume was determined based upon a ratio of 200 μL for a 20 g mouse and was scaled appropriately according to the actual body weight (BW) of the mice. Three to five mice were used in each treatment group. The BWs of the mice were followed daily for the first 5 days and then twice a week thereafter. The MTD is defined as the largest administered dose that resulted in a decrease of mean group BW of less than 20% or the largest administered dose that does not result in death of any animal in that group.

Table 2. Half-Life ($t_{1/2}$, in hours) of the Release of CPT from HG6 and HGGG6[a]

conjugate	PBS[b]	human plasma[c]	pH 4.5 buffer[d]
HG6	59	1.7	>300
HGGG6	32	1.6	>300

[a] $t_{1/2}$ is defined as the time required for the release of half of the total conjugated CPT. [b] pH 7.4 PBS 1× buffer. [c] Reconstituted human plasma. [d] Sodium phthalate (0.1 M, pH 4.5).

Table 3. Treatment Response for the MTD Study[a]

agent	mg/kg[b]	maximum %BW loss (day)[c]	N_{TR}/N[d]
D5W	−	−2.5, day 3	0/6
CDP	240	−2.0, day 3	0/6
CDP	160	−3.5, day 13	0/6
CDP	80	−2.3, day 3	0/6
LGGG10	54	−20.6, day 3	3/3
LGGG10	36	−9.3, day 13	0/3
LGGG10	18	0	0/3
LGGG10	9	0	0/5
LGGG10	4.5	−0.8, day 13	0/5
HG6	54	−28.5, day 3	3/3
HG6	36	−23.9, day 3	3/3
HG6	18	−22.1, day 3	3/3
HG6	9	−6.1, day 9	0/5
HG6	4.5	−4.4, day 5	0/5
HG6	2.25	−2.9, day 9	0/5
HGGG10	54	−	3/3
HGGG10	36	−34, day 5	3/3
HGGG10	18	−16, day 3	1/3
HGGG10	9	−3.3, day 9	0/5
HGGG10	4.5	−2.5, day 9	0/5

[a] Nude mice ($n = 3-6$) were treated iv by tail vein injection with D5W ($n = 6$), CDP ($M_w = 97$ kDa, $n = 6$), and three conjugates (LGGG10, HG6, and HGGG10, $n = 3-5$) on days 1, 5, and 9 with selected doses. The body weights of the mice were followed daily for the first 5 days and then twice a week. [b] Milligrams of CDP per kilogram for the CDP polymer and milligrams of CPT per kilogram for the three conjugates that were tested. [c] Maximum body weight (BW) loss observed post injection. [d] Number of treatment-related deaths (N_{TR}) and the number of mice treated (N).

In Vivo Toxicity and Blood Chemistry Analysis after Treatment with the Parent Polymer. The toxicity of the parent polymer and its effects on blood chemistry were evaluated in female Charles River nude mice (13−14 weeks old). The CDP that was used for the synthesis of the high-MW conjugates (HGGG6, HG6, and HGGG10; see Table 1) was employed in this study. Each of the six mice in the four treatment groups was treated with a DSW solution of CDP at a dose of 240, 160, and 80 mg/kg or D5W alone by iv tail vein injection on days 1, 5, and 9, respectively (same dosing schedule that was used for the CPT-conjugated versions) (Table 3). The dosing volume was determined based upon a ratio of 200 μL for a 20 g mouse and was scaled appropriately according to the actual BW of the mice. The BWs of the mice were followed daily for the first 5 days and then twice a week thereafter. Blood samples (150−

Table 4. Dosing Protocol for the Efficacy Study[a]

group	agent	dose (mg of CPT/kg)[b]	route[c]	schedule[d]
1	D5W	−	iv	Q4D × 3
2	CPT	9	ip	Q4D × 2[e]
3	irinotecan	100[f]	ip	Qwk × 3
4	HGGG6	9	iv	Q4D × 3
5	HGGG6	4.5	iv	Q4D × 3
6	LGGG10	36	iv	Q4D × 3
7	LGGG10	18	iv	Q4D × 3
8	LGGG10	9	iv	Q4D × 3
9	HG6	9	iv	Q4D × 3
10	HG6	4.5	iv	Q4D × 3
11	HGGG10	9	iv	Q4D × 3
12	HGGG10	4.5	iv	Q4D × 3

[a] D5W, CPT, irinotecan, and four CDP−CPT conjugates using selected dosing schedules were administered to nude mice bearing LS174T colon carcinoma tumors. Seven mice were used in each group. [b] Doses are equivalents of CPT except for group 3. [c] ip is intraperitoneal and iv intravenous. [d] Administration schedules are abbreviated as follows: Q4D × 3, three injections with 4 day intervals; Qwk × 3, three injections with a 1 week interval. The first dose was administered on day 1 for all groups. [e] The scheduled third dose was not given due to the emerging toxicity. [f] Dose of 100 mg of irinotecan/kg.

200 μL) were collected from each mouse by retro-orbital bleeding under isoflourane on day 12. Samples from three mice in each group were used for complete blood count (CBC) analyses, while blood samples from the remaining three mice in each group were processed for blood chemistry analyses. The study was stopped at day 23, and no substantial change in BW was observed during the time period. All mice were euthanized by cardiac puncture under CO_2, and blood from each mouse was collected for CBC and blood chemistry analysis in the same manner as on day 12.

Antitumor Efficacy Study. The antitumor efficacy study was performed using female Charles River nude mice (15−16 weeks old). A fragment (1 mm^3) of human LS174T colon carcinoma tissue was implanted subcutaneously (sc) into the right flank of each test mouse approximately 14−18 days before dosing. The tumor volume was determined by measuring the tumor in two dimensions with calipers and calculated using the formula tumor volume = (length × $width^2$)/2. Tumor volume was converted to tumor weight assuming 1 mm^3 is equal to 1 mg of tumor in weight. Treatment was initialized when the mean tumor size reached approximately 60−100 mg (day 1). The animals were sorted into 12 groups. Each group consisted of seven mice with tumor sizes ranging from 62.5 to 144.0 mg with group mean tumor sizes of 88.6−90.7 mg.

Mice in each group were treated according to the protocol given in Table 4. Group 1 mice were treated with D5W and used as the control. CPT and irinotecan were administered by ip injection. CPT is very insoluble in aqueous solution and is acutely lethal when given to mice iv at 9 mg/kg due to embolization induced by the particulate matter in the drug suspension. Thus, CPT was suspended in a vehicle of 0.5% methylcellulose and 0.1% Tween 80 and administered ip in an attempt to maximize its efficacy. Mice were given CPT at 9 mg/kg once daily on days 1 and 5 (Q4D × 2). A scheduled third dose was not given on day 9 because of emerging toxicity. Mice were treated ip with irinotecan in a D5W solution at 100 mg/kg once a week on days 1, 8, and 15 (Qwk × 3). This administration schedule was suggested by the Piedmont Research Center on the basis of their extensive in vivo experience with irinotecan and this tumor model. It is also their experience that very similar antitumor efficacy is observed when irinotecan is administered at the optimal dose by either an ip or iv route in various tumor models. The conjugate solutions were freshly prepared in D5W prior to each treatment. All conjugate treatments (groups 4−12, Table 4) were administered intravenously by tail vein injection every 4 days, for a total of three doses (days 1, 5, and 9) at 9 and 4.5 mg of CPT/kg except for LGGG10, which was tested at 36, 18, and 9 mg of CPT/kg. Tumor sizes were measured twice a week for the duration of the experiment.

Each animal was euthanized when the tumor weight reached the predetermined end point size (1500 mg). The time to end point (TTE) for each mouse was calculated from the equation TTE = [log(end point − b)]/m, where b and m are the intercept and slope, respectively, of the line obtained by linear regression of a log-transformed tumor growth data set comprised of the first observation that exceeded the study end point volume and the three consecutive observations that immediately preceded the attainment of the end point volume. Animals that did not reach the end point were assigned a TTE value equal to the last day of the study (114 days). Animals classified as treatment-related deaths (TR) were assigned a TTE value equal to the day of death. Animals classified as non-treatment-related death (NTR) were excluded from TTE calculations. Tumor growth delay (TGD), defined as the increase in the median time to end point (TTE) in a treatment group compared to the control group, was one parameter investigated to evaluate treatment efficacy. TGD is calculated as the difference between the median TTE for a treatment group and the median TTE of the control group (TGD = $T - C$) and is expressed in days, and as a percentage of the median TTE of the control group; %TGD = $(T - C)/C$, where T is equal to the median TTE for a treatment groups and C is equal to the median TTE for the control (group 1).

Toxicity. Animals were weighed daily on days 1−5, and then twice weekly thereafter. Mice were examined frequently for overt signs of any adverse, drug-related side effects. Acceptable toxicity for cancer drugs in mice is defined by the NCI as a group mean body weight loss of less than 20% during the study, and not more than one toxic death among seven treated animals.

Statistical and Graphical Analyses. Logrank tests were employed to analyze differences in the median TTE between treatment groups. The logrank test was used to analyze the data for all animals except the NTR deaths. Two-tailed statistical analyses were conducted at $P = 0.05$. Results were deemed significant at $0.01 \leq P \leq 0.05$ and highly significant at $P < 0.01$. Median tumor growth curves prepared for each

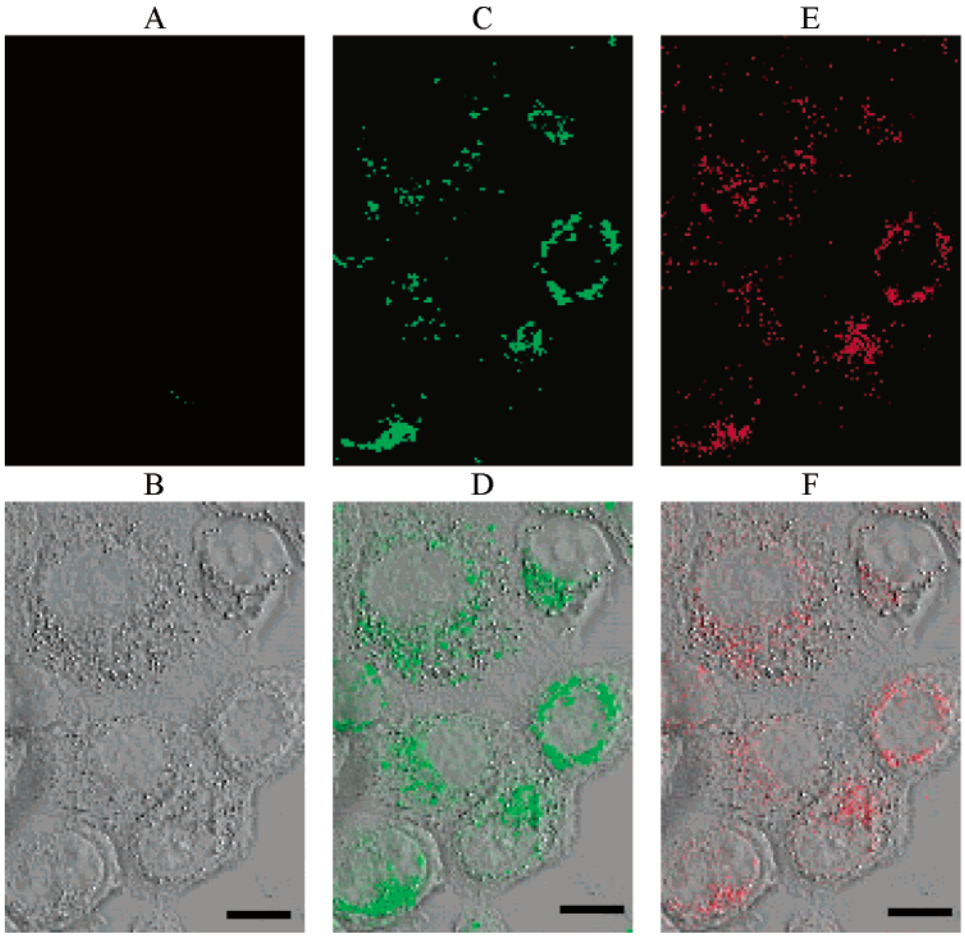

Figure 3. Uptake of the fluorescently labeled polymer (HRhod) and LysoTracker Green DND-26 by laser scanning confocal microscopy. (A) Cells treated with CDP for 24 h and analyzed under condition 1 (control). CDP-treated cells under condition 2 give an identical image (image not shown). (B) Cells were treated with HRhod and LysoTracker Green DND-26 and analyzed under condition 3. (C) Same as panel B except that cells were analyzed under condition 1. (D) Overlay of panels C and B. (E) Same as panel B except that cells were analyzed under condition 2. (F) Overlay of panels E and B. Condition 1: excitation wavelength of 488 nm and emission band-pass filter of 515−540 nm. Condition 2: excitation wavelength of 568 nm and emission band-pass filter of 580−620 nm. Condition 3: bright field. Bars are 5 μm long.

group depicted the median tumor size as a function of time. When an animal exited the study due to tumor size or TR death, the final tumor size recorded for the animal was included with the data used to calculate the median size at subsequent time points. Kaplan−Meier plots were constructed to show the percentage of animals remaining in the study versus time.

Results

Cellular Uptake of CDP. Lissamine rhodamine B ethylenediamine was conjugated to high-molecular-mass CDP (M_w = 97 kDa) using the conventional EDC/NHS coupling method. The weight percent rhodamine on the conjugate HRhod was determined to be 5% by measuring the absorbance of the conjugated rhodamine at 560 nm as compared to the standard curve for this dye. Cells treated with HRhod exhibit punctated staining patterns (Figure 3E,F). HRhod is likely internalized into cells via endocytosis since the staining patterns are very similar to those for cells treated with LysoTracker Green DND-26 (Figure 3C,D), a commercial, organic dye known to be internalized into acidic intracellular compartments, such as endosomes and lysosomes.[29] Uptake of HRhod into cells was also observed by flow cytometry analysis (Figure 4).

Release of CPT from Conjugates. The kinetics of releasing CPT from HG6 and HGGG6 were measured at 37 °C in various solutions (Table 2). In PBS (pH 7.4), the half-lives ($t_{1/2}$) for releasing CPT from HG6 and HGGG6 were 59 and 32 h, respectively. The half-lives decreased dramatically to 1.7 and 1.6 h, respectively, in 100% human plasma. In a buffer solution mimicking the pH of lysosomes (pH 4.5),[30] less than 25% of total conjugated CPT was released from both HG6 and HGGG6 for times up to 300 h (Table 2).

(29) Haugland, R. *Handbook of Fluorescent Probes and Research Products*, 9th ed.; Molecular Probes: Eugene, OR, 2002.
(30) Mach, L.; Mort, J. S.; Glossl, J. *J. Biol. Chem.* **1994**, *269*, 13030−13035.

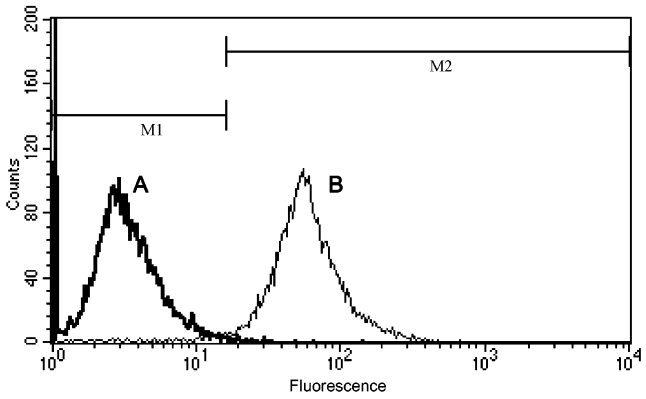

Figure 4. Uptake of fluorescently labeled CDP by flow cytometry. CDP and HRhod were administered to cells using various means. Twenty-four hours after being treated, cells were collected, washed, and analyzed by flow cytometry: (A) CDP-treated cells (mean fluorescence in arbitrary units of 3.48) and (B) HRhod-treated cells (mean fluorescence in arbitrary units of 63.5).

MTD of CDP−CPT Conjugates. The MTDs of LGGG10, HG6, and HGGG10 were determined to be 36, 9, and 9 mg of CPT/kg, respectively, from the data listed in Table 3. On the basis of the structural similarities between HGGG6 and HGGG10, it is expected that the MTDs for these two groups are similar. Therefore, the MTD of HGGG6 (not tested) was assumed to be 9 mg of CPT/kg.

Determination of MTDs. The BWs and survival of the polymer-treated mice were followed for 23 days (Table 3). The CDP is comprised of β-CD and PEG, each of which has low cytotoxicity and low immunogenicity. Like the individual components that make up the polymer, the CDP also exhibited very low toxicity as measured by the BW loss data (Table 3). There was no significant difference in BW weight loss between any of the CDP groups and the control throughout the study. Although the immunogenicity of the CD polymer shown here has not been investigated, other CD-based polymers do not elicit an immune response in rabbits.[25] All six mice survived in each treatment group. CDP was well tolerated by mice at the maximum dose that was administered (240 mg/kg).

Blood was drawn on days 12 and 23 for CBC and blood chemistry analyses. The median red blood cell count (RBC) and platelet count (PLT) and the mean value of creatinine (CRT), blood urea nitrogen (BUN), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) from each of the study groups are listed in Table 5. No significant differences were observed for the CBC and blood chemistry data between the CDP-treated groups and the D5W group, and no time-dependent effects were observed over 23 days for all the treated groups.

For the three CDP−CPT conjugates tested for toxicity (Table 3), HG6 and HGGG10 produced a similar MTD of 9 mg of CPT/kg. LGGG10 was ∼4-fold less toxic, with a dose of 36 mg of CPT/kg giving moderate weight loss but no deaths.

Antitumor Efficacy Study. The efficacy study was performed in accordance with the protocol illustrated in Table 4. Twelve groups of seven athymic nude mice bearing 60−100 mm^3 LS174T colon carcinoma tumors were treated. The experimental plan was to euthanize mice at the point when the tumor grew to 1500 mg or at day 60, the time expected for tumors to reach the end point in this model with irinotecan at the dose schedule that was used. Because of the protracted antitumor effect seen in animals treated with the conjugates at their MTD, the study was extended to 114 days. Results for the study that include median TTE values, median tumor burden on day 114, treatment response, and deaths are summarized in Table 6.

One NTR death on day 72 was observed in the control animals treated with D5W. Tumors in the other six control mice grew to the end point size of 1500 mg, yielding a median TTE of 34.9 days (Table 6).

Two treatment-related deaths were reported on day 9 for CPT at 9 mg/kg. Thus, CPT must be considered to be toxic at this dose in this experiment. The median TTE for this group was 51.4 days, corresponding to a 16.5 day $T - C$ and a 47% TGD, relative to untreated control mice (not significant). No animal in group 2 survived until day 114.

Members of group 3 received irinotecan ip at 100 mg/kg (Qwk × 3). The median TTE for group 3 was 68.7 days, corresponding to a highly significant 33.8 day $T - C$ and a 97% TGD, relative to control mice ($P < 0.01$). Three animals survived until day 114 with a median tumor burden of 1152 mg. No regressions were recorded.

Members of groups 4 and 5 received HGGG6 iv Q4D × 3 at 9 and 4.5 mg of CPT/kg, respectively. One treatment-related death was observed on day 16 in group 4, and one NTR death was recorded on day 37 in group 5. The median TTE for group 4 was 114 days, the maximum possible value in this study. This TTE value corresponds to a significant 79.1 day $T - C$ and a 227% TGD, relative to control ($P < 0.01$). Tumors in five mice from group 4 did not reach the end point of 1500 mg. These five mice had a median tumor burden of 256 mg on day 114. Comparison between the survival of mice in groups 1−4 is illustrated in the Kaplan−Meier plot shown in Figure 5. The median TTE for group 5 was 65.6 days and corresponds to a significant 30.7 day $T - C$ and a 88% TGD, relative to control ($P < 0.01$).

Groups 6−8 were treated with LGGG10 iv Q4D × 3 at 36, 18, and 9 mg of CPT/kg, respectively. Although no death was observed in the MTD study using this conjugate in non-tumor-bearing mice at 36 mg of CPT/kg (Table 3), four treatment-related deaths were recorded in group 6 when tumor-bearing mice were given this dose, two on day 16 and one each on days 75 and 100. These results indicate that 36 mg of CPT/kg is probably greater than the MTD of LGGG10. As shown Table 3, no significant body weight loss was recorded in the MTD study when the mice were treated with 18 mg of CPT/kg, indicating that this dose is less than the MTD. Therefore, the MTD of LGGG10 lies somewhere between 18 and 36 mg of CPT/kg. The median

Table 5. Selected CBC and Blood Chemistry Data[a]

group	D5W	240 mg/kg[b]	160 mg/kg[b]	80 mg/kg[b]
RBC (M/μL),[c] day 12	8.95	9.23	8.93	10.10
RBC (M/μL), day 23	9.69	9.15	9.49	10.20
PLT (K/μL),[d] day 12	1239	1347	1504	1247
PLT (K/μL), day 23	1696	1138	926	1372
CRT (mg/mL),[e] day 12	0.33 $\pm$ 0.12	0.33 $\pm$ 0.12	0.23 $\pm$ 0.06	0.30 $\pm$ 0.1
CRT (mg/mL), day 23	0.23 $\pm$ 0.06	0.37 $\pm$ 0.06	0.20 $\pm$ 0	0.33 $\pm$ 0.15
BUN (mg/mL),[f] day 12	21 $\pm$ 6.43	22 $\pm$ 2	17 $\pm$ 3.06	21 $\pm$ 3.06
BUN (mg/mL), day 23	25 $\pm$ 2.08	24 $\pm$ 3	23 $\pm$ 2.89	28 $\pm$ 4.04
ALT (units/L),[g] day 12	105 $\pm$ 33	50 $\pm$ 15	114 $\pm$ 59	54 $\pm$ 15
ALT (units/L), day 23	46 $\pm$ 26	44 $\pm$ 25	47 $\pm$ 22	44 $\pm$ 10
AST (units/L),[h] day 12	156 $\pm$ 20	135 $\pm$ 25	139 $\pm$ 52	119 $\pm$ 44
AST (units/L), day 23	108 $\pm$ 44	136 $\pm$ 58	117 $\pm$ 10	129 $\pm$ 38

[a] Four treatment groups of six mice each were treated with a D5W solution of CDP at a dose of 240, 160, or 80 mg/kg or D5W alone (iv, tail vein injection, Q4D $\times$ 3, $n = 6$). Blood samples (150−200 μL) were collected from each mouse by retro-orbital bleeding under isoflourane on day 12. Blood from each mice was withdrawn for complete blood count (CBC) and blood chemistry analyses on days 12 and 23. [b] Milligrams of CDP per kilogram (Q4d $\times$ 3), iv tail vein injection. [c] Red blood cell count (RBC) in millions per microliter. [d] Platelet (PLT) count in thousands per microliter. [e] Creatinine (CRT). [f] Blood urea nitrogen (BUN). [g] Alanine aminotransferase (ALT). [h] Aspartate aminotransferase (AST).

Table 6. Antitumor Efficacy Study in Nude Mice Bearing Subcutaneously Implanted LS174T Colon Carcinoma Tumors[a]

group	median TTE[b]	$T - C$[c]	%TGD[d]	median tumor burden (mg) (N_s[e])	N_{TR}[f]/N_{NTR}[g]/N_{EU}[h]	P vs D5W[i]	P vs CPT[j]
1	34.9	–	–	– (0)	0/1/6	–	–
2	51.4	16.5	47	– (0)	2/0/5	0.2128	–
3	68.7	33.8	97	1152 (3)	0/0/4	0.0002	0.0253
4	114.0	79.1	227	256 (5)	1/0/1	0.0040	0.0115
5	65.6	30.7	88	566 (2)	0/1/4	0.0046	0.1369
6	100.0	65.1	187	666 (3)	4/0/0	0.0272	0.0289
7	75.6	40.7	117	221 (3)	0/0/4	0.0018	0.0601
8	63.2	28.3	81	700 (1)	1/0/5	0.0006	0.1064
9	114.0	79.1	227	394 (4)	0/0/3	0.0002	0.0028
10	74.2	39.3	113	668 (2)	1/1/3	0.0016	0.0673
11	114.0	79.1	227	500 (5)	1/0/1	0.0040	0.0050
12	78.0	43.1	123	1010 (2)	0/0/6	0.0006	0.0392

[a] Each mouse was euthanized when the tumor size reached the end point (1500 mg) starting from ca. 90 mg or at day 114. [b] TTE is the time (days) to the end point (1500 mg). [c] $T - C$ is the difference between TTE (days) of the treated group vs the control group. [d] %TGD = $(T - C)/C$. [e] Number of mice surviving. [f] N_{TR} is the number of treatment-related deaths. [g] N_{NTR} is the number of non-treatment-related deaths. [h] N_{EU} is the number of mice euthanized after the end point had been reached. [i] P value vs the D5W treatment group (group 1). [j] P value vs the CPT treatment group (group 2).

TTE for group 7 (18 mg of CPT/kg) was 75.6 days. This TTE value corresponds to a significant 40.7 day $T - C$ and a 117% TGD, relative to control mice ($P < 0.01$). Three mice in this group had a median tumor burden of 221 mg on day 114. One late TR death was recorded on day 103 in group 8 (9 mg of CPT/kg). The median TTE for group 8 was 63.2 days. This TTE value corresponds to a significant 28.3 day $T - C$ and a 81% TGD, relative to untreated control mice ($P < 0.01$). The remaining mouse in this group had a tumor burden of 700 mg on day 114.

Groups 9 and 10 were treated with HG6 iv Q4D $\times$ 3 at 9 and 4.5 mg of CPT/kg, respectively. One TR and one NTR deaths were recorded in group 10 on days 47 and 84, respectively. No regressions were recorded in either group. The median TTE for group 9 was the maximum, 114 days. This TTE value corresponds to a significant 79.1 day $T - C$ and a 227% TGD, relative to untreated control mice ($P < 0.01$). Four mice in group 9 had a median tumor burden of 394 mg on day 114. The survival of mice in group 9 is illustrated in the Kaplan−Meier plot shown in Figure 5. The median TTE for group 10 was 74.2 days. This TTE value corresponds to a significant 39.3 day $T - C$ and a 113% TGD, relative to control mice ($P < 0.01$). The remaining two mice in group 10 had a median tumor burden of 668 mg on day 114.

Groups 11 and 12 were treated with HGGG10 iv Q4D $\times$ 3 at 9 and 4.5 mg of CPT/kg, respectively. One treatment-related death was recorded on day 16 in group 11. The median TTEs for groups 11 and 12 were 114 and 78 days, respectively. The TTE value for group 11 corresponds to a significant 79.1 day $T - C$ and a 227% TGD, relative to control mice ($P < 0.01$). Tumors in five mice in group 11 did not reach the end point; these five mice had a median tumor burden of 500 mg on day 114. The TTE value of group 12 corresponds to a significant 43.1 day $T - C$ and a 123% TGD, relative to control mice ($P < 0.01$). The remaining two mice in this group had a median tumor burden of 1010 mg on day 114.

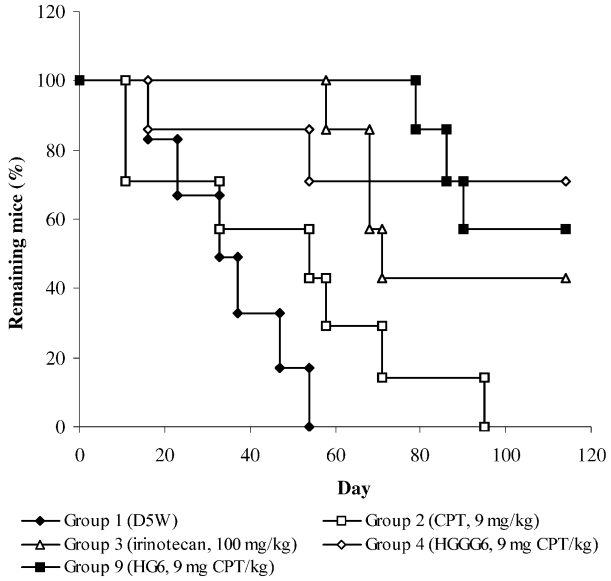

Figure 5. Kaplan−Meier plot for groups 1−4 and 9. Loss of mice was due to either treatment-related death or euthanasia after the end point (1500 mg) had been reached.

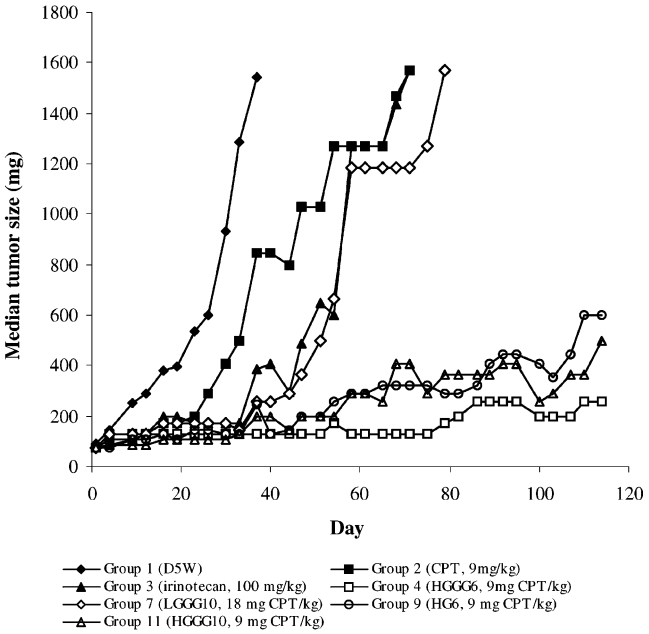

Figure 6. Antitumor efficacy using D5W, CPT, irinotecan, and CDP−CPT conjugates in nude mice bearing subcutaneously implanted LS174T tumors.

The tumor growth curves as a function of time for the D5W, CPT, irinotecan, LGGG10 at the highest nontoxic dose that was tested, and the other three conjugates with the high-MW polymer (HGGG6, HG6, and HGGG10) at their MTDs are shown in Figure 6. The three high-MW conjugates administered at their MTDs displayed more prolonged tumor growth inhibition than D5W, CPT, and irinotecan. The median tumor growth curves for HGGG6, HG6, and HGGG10 that are illustrated in Figure 7 show that there is a distinct dose response for both of these polymers when they are administered at their MTD and at half of their MTD.

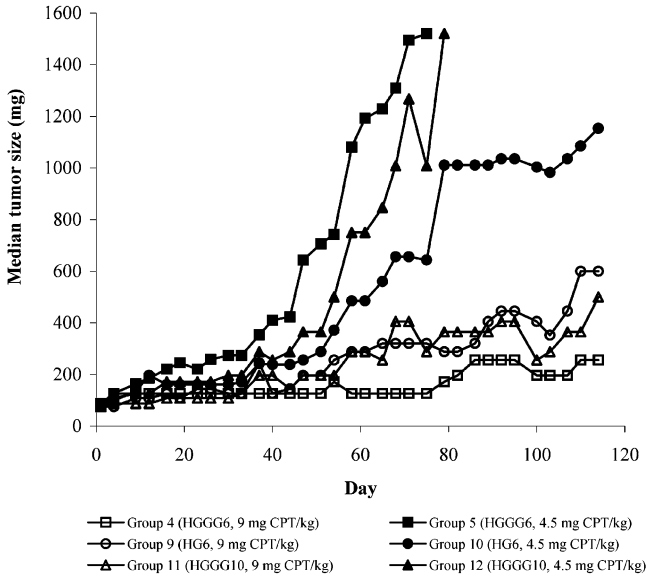

Figure 7. Dose responses using CDP−CPT conjugates. In each case, $n = 7$ and iv tail vein injection at Q4D × 3 was used.

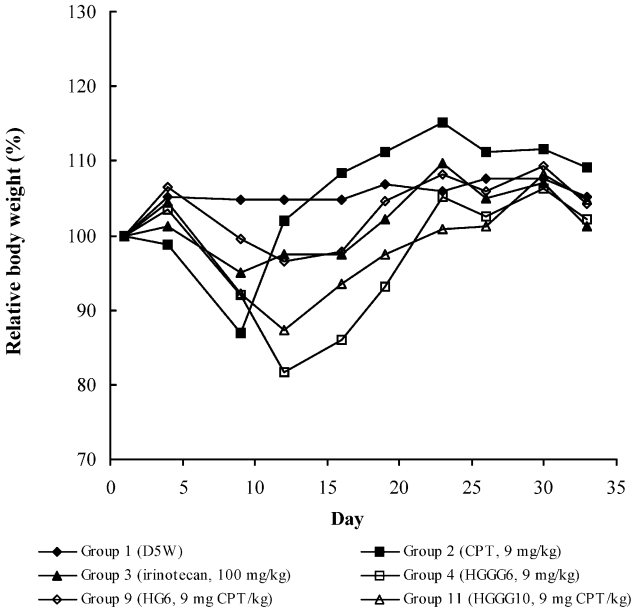

Figure 8. Relative mean body weight change for D5W, CPT, irinotecan, and CDP−CPT conjugates with the high-MW polymer at their MTDs.

Mean Body Weight Loss of Mice. Mean body weight (MBW) losses as a function of time are plotted for D5W, CPT, irinotecan, and the three conjugates containing the high-MW polymer at their MTDs (Figure 8). Maximum MBW losses observed in group 2 (CPT) and the two conjugates with the triglycine linker administered at their MTDs (groups 4 and 11) were 13.1, 18.3, and 12.6%, respectively. The maximum MBW loss of HG6 (3.4%), the only conjugate with a glycine linker, was similar to the maximum MBW loss recorded for irinotecan (5.0%). Negligible (<5%) maximum group mean body weight losses were recorded in all the other treatment groups and in the D5W group.

Discussion

The purpose of this study was to evaluate the influence of polymer size, drug loading, and linker structure on the antitumor efficacy and toxicity of the β-cyclodextrin-based polymer−CPT conjugates by observing tumor size reduction and toxicity obtained in an LS174T colon carcinoma xenograft mouse model. It has been reported that PG−CPT conjugates with a triglycine linker exhibited a stronger antitumor effect than that with a glycine linker in HT29 and NCI-H460 mice models.[16,31] Additionally, numerous polymers have inhibited renal clearance when their MWs exceed 40−50 kDa.[32] As summarized in Table 1, HGGG6 and HGGG10 conjugates are high-molecular mass polymers and utilize the same linker structure, but differ only in CPT loading (6.13 and 9.63%, respectively). LGGG10 and HGGG10 have similar drug loading (10.15 and 9.63%, respectively) and the same linker structure, but differ in MW. HGGG6 and HG6 have the same polymer MW and have similar CPT loadings (6.13 and 6.75%, respectively), but differ in that HGGG6 has a triglycine linker while HG6 has a glycine linker.

The lactone form of CPT is extremely insoluble in water. Conjugation of CPT to hydrophilic polymers can substantially increase its solubility in aqueous solution, and this enhanced solubility can be achieved by linking the 20-OH group (to stop lactone ring hydrolysis) with a pendent side chain of the polymer through an amino acid/peptide spacer.[33] Drug release rates can be controlled by adjusting the size and nature of the amino acid linker.[12] Several polymers have been investigated for delivery of CPT (PEG, HPMA copolymer, and PG)[10−17] or CPT analogues (dextran),[34−36] and a variety of preclinical studies with these polymers have shown stabilized and sustained release of these drug molecules.[12,14] It has been demonstrated that antitumor efficacy is affected by the MW of the polymeric delivery system. Higher-MW polymer prodrugs (>50 kDa) with sizes in excess of the renal clearance threshold generally lead to better efficacy. This behavior is presumably due to the prolonged circulation of these prodrugs in the blood stream, allowing for greater tumor accumulation (EPR effect).[15]

The difference in MTD between LGGG10 (18−36 mg of CPT/kg) and HGGG10 (9 mg of CPT/kg) shows that polymer molecular mass has a large effect on the maximum tolerable dose, and this behavior is presumably due to the faster renal clearance of the low-MW CDP−drug conjugate (LGGG10). Urine samples collected 30 min after treatment from selected mice reveal larger quantities of CPT when they are treated with L rather than H polymers.

As shown in Figure 6, the three conjugates with the high-MW polymer administered at their MTD show significantly greater antitumor activity than D5W and CPT, and more prolonged tumor growth inhibition than irinotecan. HGGG6 and HGGG10 administered at 9 mg of CPT/kg (groups 4 and 11, respectively) produced the greatest tumor growth delay based on TGD and the numbers of tumors that did not reach the end point size of 1500 mg within 114 days. For mice treated with HGGG6 at 9 mg of CPT/kg (group 4), the median tumor volume does not increase until day 80. The 36 mg of CPT/kg dose of LGGG10 likely exceeds the MTD since four TR deaths were observed in mice that received LGGG10, and maximum mean weight loss of this group exceeded 20%. LGGG10 given at a smaller dose (18 mg of CPT/kg, group 7), which is likely below the MTD in this experiment, exhibited tumor inhibition similar to that of irinotecan (Figure 6).

At the same CPT equivalent dose, the higher-MW polymer demonstrated much more protracted antitumor effects than the lower-MW polymer. This is likely due to a slower renal clearance in the high-MW group resulting in more CPT reaching the tumor with the higher-MW polymer. Initial biodistribution studies are consistent with this hypothesis, and full biodistribution studies will be completed and reported soon. HGGG6, HG6, and HGGG10 administered at large and small doses demonstrated clear dose responses (Figure 7).

Although conjugates with GGG and G linkers have similar antitumor efficacy as shown in Figure 7, mice treated with these conjugates show different MBW losses. These data indicate conjugates with different linker chemistries have different in vivo toxicities (Figure 8). When treated with 9 mg of CPT/kg, mice treated with the conjugates containing a GGG linker (both HGGG6 and HGGG10) show MBW losses greater than 10% as compared to less than 5% MBW losses for mice treated with the conjugate with a G linker (HG6). We are currently investigating a possible mechanism for these trends in toxicity.

There was no significant difference in median TTE between the toxic 9 mg/kg (Q4D $\times$ 2) CPT treatment group and the vehicle control. Treatment with irinotecan and all CDP−CPT conjugate groups resulted in highly significant antitumor efficacy as measured by TTE as compared to vehicle control. Additionally, treatment with all the large dose groups of the high-MW polymer−CPT conjugates resulted in significantly greater TGD than with CPT (Table 6).

The striking finding from this in vivo evaluation of the antitumor effects of the high-MW CDP−CPT polymers is that a very brief course of treatment (three doses over 9 days) results in a very protracted control of tumor growth. At the MTD dose levels, there were no complete regressions, but there was static tumor growth out to 114 days, with some individual tumors growing no more than 2−3-fold from their starting size.

(31) Bhatt, R.; de Vries, P.; Klein, J. P.; Lewis, R. A.; Singer, J. W.; Tulinsky, J. PCT WO 0170275, 2001.
(32) Jorgensen, K. E.; Moller, J. V. *Am. J. Physiol.* **1979**, *236*, F103−F111.
(33) Zhao, H.; Lee, C.; Sai, P.; Choe, Y.; Boro, M.; Pentri, A.; Guan, S.; Greenwald, R. B. *J. Org. Chem.* **2000**, *65*, 4601−4606.
(34) Inoue, K.; Kumazawa, E.; Kuga, H.; Susaki, H.; Masubuchi, N.; Kajimura, T. *Adv. Exp. Med. Biol.* **2003**, *519*, 145−153.
(35) Harada, M.; Murata, J.; Sakamura, Y.; Sakakibara, H.; Okuno, S.; Suzuki, T. *J. Controlled Release* **2001**, *71*, 71−86.
(36) Harada, M.; Sakakibara, H.; Yano, T.; Suzuki, T.; Okuno, S. *J. Controlled Release* **2000**, *69*, 399−412.

Upon analyzing tumor tissues of mice in group 9 at the end point (day 114), we detected CPT at concentrations around 0.03−0.2 ng/mg of tumor tissue and found that more than 95% of detected CPT is in the conjugated form (data not shown). The protracted tumor growth inhibition may in part be due to slow release of CPT over time in the tumor tissues. As shown in Table 2, the CPT hydrolysis rates from both HG6 and HGGG6 decrease substantially at acidic pH. Therefore, a possible explanation for the protracted retention of CDP−CPT conjugates in tumor is that CDP−CPT conjugates are internalized to tumor cells through the endocytotic pathway and are contained in intracellular compartments such as lysosomes that have a low pH (3.5−5).[37] We confirmed the uptake of rhodamine-labeled CDP (HRhod) into PC-3 cells (Figures 3C and 4). The punctated staining patterns of PC-3 cells treated with HRhod are very similar to those of cells treated with LysoTracker Green DND-26, a commercial, organic dye used for staining acidic, intracellular compartments.[29] Since HRhod and CDP−CPT conjugates have very similar weight percent loading and since both rhodamine and CPT have similar hydrophobic properties, the CDP−CPT conjugate is likely endocytosed into cells and contained in acidic compartments in a manner similar to that of HRhod. Substantially reduced rates of release of CPT from CDP−CPT polymer conjugates contained within intracellular acidic compartments likely contribute to the long-term therapeutic efficacy of these conjugates.

In summary, a linear, cyclodextrin-based polymeric drug delivery system (CDP) was prepared and used for the study of antitumor efficacy in mice when conjugated with camptothecin (CPT). CDPs were synthesized at two different molecular masses (one above and one below the size threshold for the renal clearance). CPT was conjugated to these two polymers via either a glycine or a triglycine linker. The effects of polymer molecular mass (MW), drug loading, and linkage chemistry on toxicity and tumor growth inhibition were investigated. All four types of CDP−CPT conjugates demonstrated excellent antitumor activity against human LS174T carcinoma tumors in nude mice. The high-MW conjugates produced greater antitumor activity than CPT at the same dose and irinotecan given at a dose approximately 1 order of magnitude higher than those of the conjugates. The conjugates of the high-MW polymer (97 kDa) displayed greater tumor growth inhibition than those using low-MW polymer (35 kDa) at the same dose of conjugated CPT. Optimizing the dosing frequency of the conjugates with the high-MW polymer may lead to reduced toxicity and even better antitumor efficacy, since the effects of the dosing schedule on antitumor efficacy have been demonstrated with HPMA−CPT conjugates.[14] These initial antitumor efficacy and toxicity studies demonstrate that cyclodextrin-based polymers can be used for anticancer drug delivery to improve solubility and enhance antitumor activity compared to the unconjugated compound.

Acknowledgment. We thank Dr. Shih-Fong Chen, Anna Avrutskaya, Walter Kruger, Beth Hollister, and Alan Meshaw at the Piedmont Research Center; our colleagues Drs. Nathalie Bellocq, Leonard Borrmann, Christopher Dartt, John Guastella, and Suzie Hwang Pun and Mr. Gregory Jensen at Insert Therapeutics for their helpful discussions; and Dr. Randall Johnson for his critical suggestions and comments throughout this study. We especially thank Swaroop Mishra (California Institute of Technology) for his help with the confocal microscopy.

MP049966Y

(37) Duncan, R. *Nat. Rev. Drug Discovery* **2003**, *2*, 347−360.