Drug Delivery Systems. 2. Camptothecin 20-*O*-Poly(ethylene glycol) Ester Transport Forms

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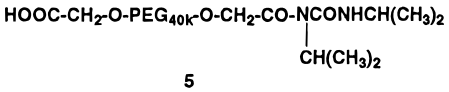
Received January 17, 1996

The potent antitumor natural drug camptothecin (**1**, a topoisomerase I inhibitor) and several of its more water soluble synthetic congeners are currently in various phases of clinical trials.[1] A prodrug that is showing promise in these trials is CPT-11, the salt of a carbamate derivative of 10-hydroxy-7-ethylcamptothecin (SN-38) which is released by metabolic conversion in the liver.[1] Other prodrug approaches which utilize the 20-OH group have also been considered and involve the formation of acid salts of amino acid esters.[2] We sought to solubilize **1** as a nonionic α-alkoxy ester transport form[3] (prodrug) and demonstrate increased circulatory retention as well as continuous therapeutic release of native drug. This was achieved by condensation of **1** with polyethylene glycol (PEG) 40 kDa dicarboxylic acid (**2**) in the presence of diisopropylcarbodiimide, as previously described for paclitaxel.[4] The synthesis resulted in a mixture of camptothecin mono- and disubstituted esters, in the ratio of approximately 2:1 (**3a** + **3b** = **3**) by reaction of the hindered tertiary alcohol at position 20 of ring E. In the case of the monoester **3a**, the opposite end of the polymer consisted of oncolytically inert acyldiisopropylurea arising from rearrangement of the intermediate diisopropylcarbodiimide addition product (Figure 1). All studies carried out utilized the mixture **3** (approximate aqueous solubility = 125 mg/mL), and individual doses were based on percent camptothecin per PEG molecule (determined by a UV assay similar to that reported[4] for PEG taxol, **4**). Additional structural proof of the transport form was provided by controlled hydrolysis of **3** (0.1 M Na_3PO_4, 37 °C, 48 h) which released camptothecin, diacid **2**, and the PEG acid−urea, **5**.



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Examination of the physical properties of **3** provided rates of hydrolysis in water, buffer, and rat and human plasma. These findings are summarized in Table 1 and reveal some startling results. Compared to the similar transport forms of PEG taxol (**4**)[4] and the known prodrug camptothecin 20-glycinate·TFA (**6**),[2a] the hydrolysis of **3** is sufficiently slow at room temperature to allow aqueous formulations to stand for 24 h with less than 10% hydrolysis occurring. Indeed, aqueous solutions (pH 5.6) have been maintained at 4 °C for over 6 months with less than 5% loss of PEG camptothecin (**3**), thus allowing easy formulation and storage of the transport form. Hydrolysis of **3** in phosphate buffer at pH 7.4 and in rat plasma at 37 °C proceeds at a rate approximately 5 times slower than the taxol analog **4** (Table 1).

Table 1. Rates of Hydrolysis of **3**, **4**, **6**, and **7** in Various Media at 37 °C[a]

	$t_{1/2}$ (h)			
compound	H_2O (pH 5.6)	PBS (pH 7.4)	rat plasma	human plasma
camptothecin 20-$PEG_{40\ kDa}$ ester (**3**)	≫72[b]	27	2.0[c]	1.0[c]
taxol-2′-$PEG_{40\ kDa}$ ester (**4**)	>72[b]	5.5	0.4	1.0
camptothecin 20-glycinate·TFA[d] (**6**)	16[e]	3.5	0.5[f]	0.25[f]
camptothecin 20-acetate (**7**)	ND[g]	>>72[h]	ND[g]	ND[g]

[a] All experiments were done in duplicate: Standard deviation of measurements: ± 10%. [b] Studies were done in deionized water only and discontinued after 3 days. [c] These results, more appropriately, represent the half-lives of disappearance of the transport form. [d] TFA = trifluoroacetate. [e] Done in PBS buffer, pH 5.6. [f] Reference 2a. [g] N. D. = Not Determined. [h] DMSO employed as a cosolvent.

In vitro P388 cell toxicity for **3** (IC_{50} = 27 nM) is in the expected range for a prodrug that releases camptothecin, **1** (IC_{50} = 7 nM).[6] *In vivo* studies employing P388-treated mice[6] with transport form **3** (administered ip as an aqueous solution) produced remarkable increased life expectancies (ILS) of almost 200% and a cure rate[7] of 80% at a dose of 16 mg/kg camptothecin equivalents while showing no acute toxicity. Similar *in vivo* survival results were obtained for native **1** (interlipid suspension delivered ip): 70% cured at a total dose of 16 mg/kg, thus demonstrating the equivalency of transport form **3** in this model (Table 2). By comparison, **6** (IC_{50} = 13 nM) also injected ip from aqueous solution (16 mg/kg) gave an ILS of only 64% and demonstrated no cures. This poor showing most likely can be attributed to rapid excretion of water soluble **6** so that the optimum effective dose was not attained and illustrates the advantages of designing drug delivery systems which employ PEG of sufficient molecular weight to guarantee adequate circulating half-lives (i.e. $t_{1/2}$ circulation > $t_{1/2}$ elimination).[4] Pharmacokinetic studies of **3** were done (iv) using CD-1 mice and displayed a blood $t_{1/2\alpha}$ of less than 15 min, but more significantly a $t_{1/2\beta}$ of 3.6 h, with detectable amounts still present after 24 h. Thus, transport form **3** circulates sufficiently to release **1** over a substantial period of time.

Demonstration of equivalent efficacy between **3** and **1** in the murine P388 model prompted the initiation of a pilot xenograft study. Colorectal carcinoma (HT-29) xenografts,[8] 800 mm^3, treated ip five times a week over 4 weeks with an aqueous solution of **3** (2.8 mg/kg/day of camptothecin equivalents), resulted in a striking 60% reduction in the tumor burden without any significant weight loss or lethality occurring. Additionally, we hope to demonstrate the exceptional potency of **3** in ongoing *in vivo* studies which employ this established model.

Two key structural features of camptothecin which are necessary for topoisomerase I inhibition are the lactone (E) ring and the 20-OH group which is α to the lactone.[9] Prior work[10] has shown that certain camptothecin derivatives substituted with lipophilic groups in the A or B ring, such as the 7-ethyl group in the drug CPT-11, preferentially associate with HSA in the lactone form. However, it has not been demonstrated that other chemical modifications, done elsewhere on the pentacyclic framework of **1,** will favor the lactone form of the

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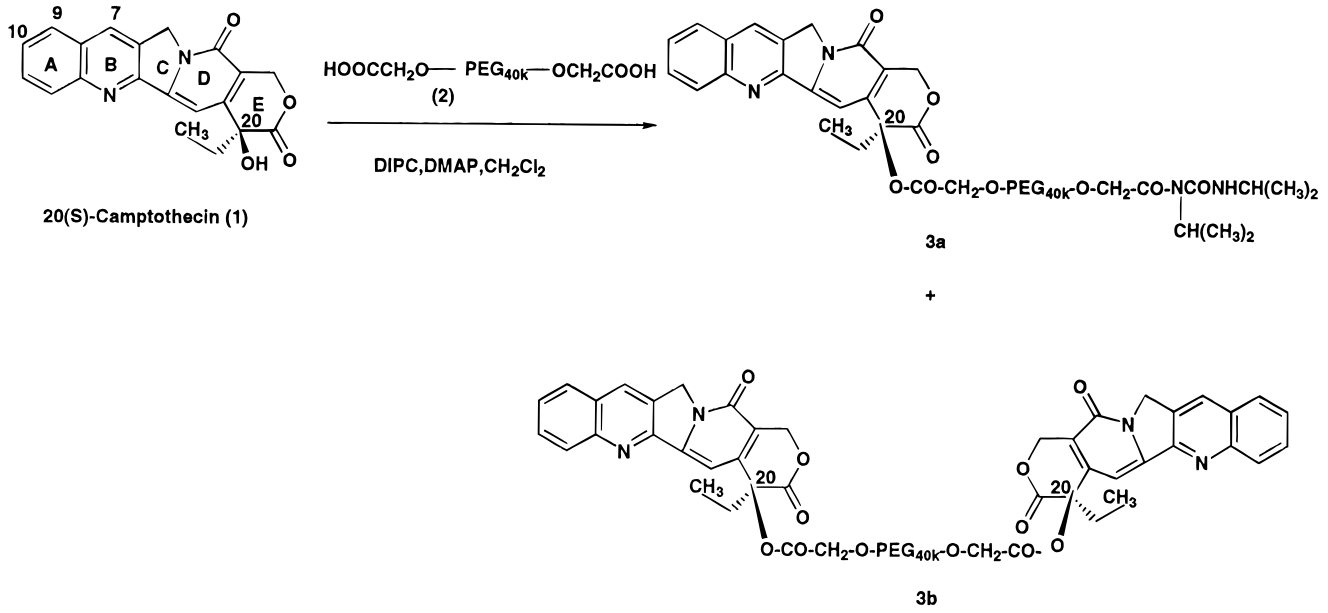

Figure 1.

Table 2. Activity of Camptothecin (**1**) and PEG−Camptothecin (**3**) against P388 Leukemia *in Vivo*

treatment	total dose[a] (mg/kg)	mean time to death (days)	T/C ratio[b]	% ILS[c]	*P* values[d] compared to control	*P* values[d] compared to **1**
control		13.2	0 (0/10)			
compound **1**	16	38.7	2.93 (7/10)	193%	P = 0.001	
compound **3**	16	38.8	2.94(16/20)	194%	P < 0.001	P = 0.47
compound **6**	16	21.7	1.64(0/20)	64%	P < 0.001	P < 0.001

[a] Equivalent dose of camptothecin, mice dosed days 1−5. [b] T/C is mean time to death of treated versus control (survivors at 40 days). [c] ILS is (T/C − 1) × 100. [d] Student *t* test, of two samples assuming equal variances.

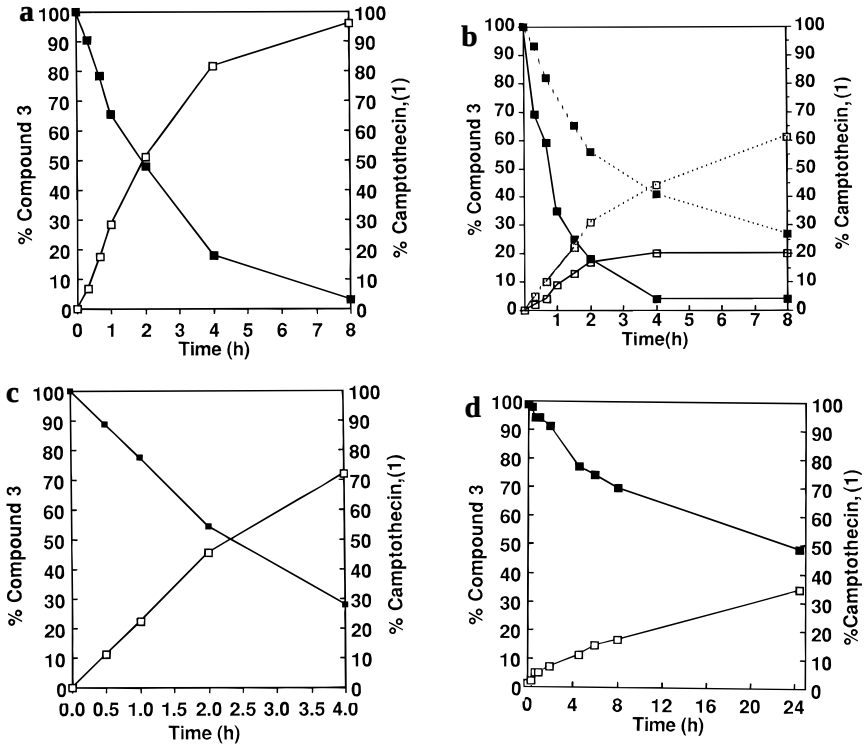

Figure 2. (a) Kinetics of camptothecin (**1**) release from **3** at 37 °C in rat plasma, (□) rate of hydrolysis of **3**; (■) rate of formation of camptothecin (**1**). (b) The solid line represents the kinetics of camptothecin (**1**) release from **3** at 37 °C in human plasma: (■) rate of binding/hydrolysis of **3**; (□) rate of formation of camptothecin (**1**). The dotted line represents the kinetics of camptothecin (**1**) release from **3** at 37 °C in human plasma (diluted 1:4 with PBS): (■) rate of hydrolysis of **3**; (□) rate of formation of camptothecin (**1**). (c) Kinetics of camptothecin (**1**) release from **3** at 37 °C in denatured human plasma (denatured by pretreatment with methanol): (■) rate of hydrolysis of **3**; (□) rate of formation of camptothecin (**1**). (d) Kinetics of camptothecin (**1**) release from **3** at 37 °C in PBS (pH 7.4): (■) rate of hydrolysis of **3**; (□) rate of formation of camptothecin (**1**).

molecule. Indirect evidence that acylation of camptothecin at the 20 position stabilizes the drug in the active lactone form, under physiological conditions, was obtained by the following *in vitro* experiments: Equilibra-

tion of **1** in phosphate buffer (pH 7.4) for 4 h gives a mixture of **1** and its open hydroxy acid salt form[10] which was determined using HPLC analysis. HSA is known to strongly bind to the open acid form of **1**, and a parallel run done in the presence of 50 mg/mL of HSA clearly demonstrated the removal of the open acid form, and a shift of the equilibrium resulting in depletion of the lactone form.[11] An identical experiment performed with **3** shows no change after equilibration in PBS buffer, or in the presence of HSA. Analogous results were also obtained with the known 20-*O*-acetate (**7**)[12] which has been reported[9a] to possess only a fraction of the activity of **1**. These results strongly support the premise that the lactone ring structure of **3** does not engage in hydrolytic ring opening at physiological pH (Table 1). Since both esters **3** and **7** appear to be stable in the lactone form, it follows that the enhanced bioactivity of **3** (an α-alkoxy ester) compared to **7** (an unsubstituted ester) demonstrates that more effective drug delivery, in the case of camptothecin, requires a relatively fast hydrolyzing ester moiety[4] in the transport form in order to free the 20-OH group that is necessary for significant activity.

Lastly, the rate profiles observed for **3** in different species' plasma were completely unexpected and are potentially significant. The disappearance of **3** in rat plasma provided a symmetrical double Y curve and had $t_{1/2}$ = 2 h with 100% recovery of **1** after 8 h. (Figure 2a). However, transport form **3** demonstrated a more rapid interaction with human plasma[13] ($t_{1/2}$ = 0.8 h), and in contrast to the rat, the formation of **1** reached only 20−30% of the expected concentration and was complete after 2 h (Figure 2b). Monitoring was conducted for another 24 h with no further *in vitro* changes detected. The remainder of the PEG camptothecin appears to be tightly bound to a plasma component—possibly through a covalent bond. Two experiments were done which demonstrated that the apparent binding implicates the involvement of protein. Pretreatment of human plasma with methanol, which is a known protein denaturant, should prevent any plasma protein interactions and thus enable **3** to hydrolyze in a predictable manner. Addition of **3** to the denatured plasma provided the normalized graph shown in Figure 2c. In this case a double Y curve was now obtained with $t_{1/2}$ hydrolysis = $t_{1/2}$ formation (2.3 h) and a final recovery of >90% of **1** after 6 h. Additionally, dilution of a human plasma sample 1:4 with PBS buffer followed by the addition of transport form **3** now results in $t_{1/2}$ hydrolysis = 2.5−3 h and a $t_{1/2}$ formation of 5 h with >90% recovery of **1** after 6 h (Figure 2b). From this result it appears that saturation of the specific protein binding site has taken place, followed by aqueous hydrolysis of uncomplexed **3**, presumably esterase catalyzed since $t_{1/2}$ is more rapid than in buffer alone (Figure 2d). The combined evidence indicates that a unique human plasma protein, capable of interacting with water soluble camptothecin in the lactone form, rapidly removes transport form **3** from the aqueous environment, thus preventing, or substantially slowing, its further transformation into **1**. Identification of this protein and its implications in the drug delivery of synthetically constructed water soluble camptothecin congeners is actively being pursued.

The unsymmetrical nature of the plot in Figure 2b offers the intriguing possibility of a timed release of camptothecin from its presumably less toxic plasma bound transport form in humans. We plan to address this hypothesis in future *in vivo* experiments and will report the results at the completion of the study.

Acknowledgment. We wish to thank Dr. Chyi Lee, Dr. Jeffrey McGuire, Dr. Srijata Sarkar, Douglas MacDonald, Mei-mann Hseu, and Hong Hsu for their invaluable assistance on this project.

Supporting Information Available: Experimental section and additional figures (5 pages). Ordering information is given on any current masthead page.

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JM9600555