Poly(ethylene glycol) Anticancer Drug Delivery Systems

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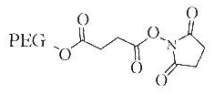
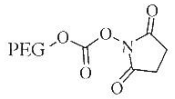
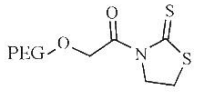
The advent of PEG chemistry has been attributed to Abuchowski, Davis et al (1) in 1977 with the findings that the alteration of immunological properties of bovine serum albumin (BSA) had been achieved by the covalent attachment of poly(ethylene) glycol, more conveniently referred to as PEG. These researchers soon demonstrated that conjugating PEG (PEGylation) to *E. Coli* produced PEG-L-asparaginase, an anticancer enzyme (2) that was now essentially non-immunogenic. This compound was further developed clinically and is currently sold under the trade name Oncaspar®.

Without doubt the most important feature of PEG modification is the greatly extended half-life ($t_{½}$) of the protein conjugates which results in a greatly increased plasma presence. This can be attributed, in part, to the increase in molecular weight of the conjugate beyond the limits of renal filtration; reduced proteolysis of the PEG conjugated enzyme also appears to be a factor. Usually, the specific activity of the protein declines after modification, but this is more than compensated for by the greater area under the pharmacokinetic (PK) curve, and increased water solubility is an additional benefit. Most PEG conjugates (of both high and low molecular weight) have been utilized by first activating PEG at the OH termini of either diol or mono methoxy PEG.

Examples of activated mPEGs are provided in Table 1. A more recent modification is provided by branched chain PEG which provides an umbrella like covering (U-PEG, PEG 2) (3,4) and has demonstrated utility in protein conjugates.

Permanently Bonded PEG-Drugs. The paucity of information in the literature relating to PEG-low molecular weight (lmw) organic molecules and drugs prior to the early 1990's is striking and is in contrast to the voluminous work on PEGylated proteins. Several *in vivo* biological studies of the few PEG-drugs synthesized did not reflect the initial *in vitro* results observed (5,6). Biologically active protein conjugates were prepared using well characterized and easily prepared activated PEG linkers of molecular weight (mw) 2,000-5,000 (7,8). These lmw PEG linkers were also used to couple drugs containing several different types of moieties (9). In the early 1990's we initiated a drug delivery research program that also utilized lmw PEG, bonded in a permanent fashion, in the hope that anticancer drugs that were insoluble could be more easily formulated. The most striking example demonstrating the enhanced solubility of PEG modification was provided by modifying paclitaxel at the 7-position with lmw PEG (5,000) with formation of a permanent carbamate bond(10). *In vitro*, these water-soluble taxane derivatives (solubility >600mg/mL) demonstrated activity in the micromolar range; substantially less than the native drug which exhibited activity at nanomolar concentrations. Subsequently, permanently bonded PEG-7-carbamate-paclitaxel, (IC_{50}, 8.2 μM) was examined at a level of 10 μmol/mouse and was also found to be non-toxic (11). From this and related results obtained with other permanently bonded PEG-anticancer drugs, it was concluded that a PEG prodrug approach to anticancer drug delivery would provide an attractive route for enhancement of therapeutic index (12).

Table 1. PEG Linkers

Name	Structure	Properties
SS-PEG$^{\alpha}$		-Good reactivity -Maintain Enzyme activity -Distal ester hydrolysis at physiological pH
SC-PEG$^{\beta}$		-Good reactivity & selectivity -Forms stable carbamate bonds
T-PEG$^{\chi}$		-Excellent selectivity -Forms stable amide

α Abuchowski A, et al. *Cancer Biochem Biophys* 1984;7:175.
β Zalipsky S, et al. *Biotechnol Appl Biochem* 1992;*15*:100.
χ Greenwald R B, et al. *Bioconjugate Chem* 1996;7:638.

Non-Permanently Bonded PEG-Drugs: PEG Prodrugs.

A. Low Molecular Weight (lmw < 20,000) PEG Prodrugs.

Prodrug design comprises an area of drug research that is concerned with the optimization of drug delivery. A

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prodrug is a biologically inactive derivative of a parent drug molecule that usually requires an enzymatic transformation within the body in order to release the active drug, and has improved delivery properties over the parent molecule (25-28). PEG prodrugs of highly insoluble anticancer agents should be especially advantageous since the solubility of the prodrug will exceed that of the original drug, thus increasing the possibility of more effective drug delivery. Accordingly, we prepared prodrugs based on ester formation. Esters with PEG as an electron-withdrawing substituent (alkoxy) in the α-position proved to be especially effective linking groups in the design of prodrugs since they aid in the rapid hydrolysis of the ester carbonyl bond, and are thus able to release alcohols (paclitaxel, 2° alcohol in the 2-position required for activity) in a continuous and effective manner. These highly water soluble 2'-PEG 5,000 esters of paclitaxel were synthesized by us in the early 1990's (13) and shown to function as prodrugs, *i.e.*, breakdown occurred in a predictable fashion *in vitro*: the half-life ($t_{1/2}$) in PBS buffer at pH 7.4 was 5.5 h, while in rat plasma a more rapid breakdown was observed, with a $t_{1/2}$ of about 1 h. Cell tissue culture employing P338/0 and L1210 murine leukemia cells with **3a** gave IC_{50} values which were comparable to Taxane formulations. It was therefore surprising that no acute toxicity was exhibited in mice when treated i.p. **3a** at a dose of 5.25 μmol since Taxane formulations (Cremophor® EL formulated paclitaxel) at this dose was profoundly toxic(14). A lack of *in vivo* activity was also observed for **3a** when tested i.p. in a P388/0 murine leukemia model (Table 2). This example clearly illustrates the necessity for *in vivo* testing to verify *in vitro* cytotoxicity results.

Table 2. *In Vivo* Activity of Paclitaxel and PAEG Paclitaxel Prodrugs Against P388 Leukemia

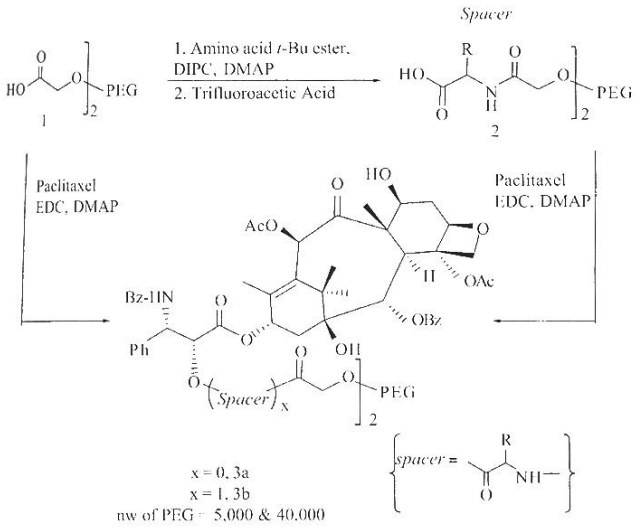
Group	Total Dose (mmol/mouse)	Mean Time to Death (days)	% ILS
Control		12.5 ± 0.8	
Paclitaxel	1.75	18.7 ± 1.3	50%
	5.25	6.7 ± 1.4	-46%
PEG 5,000 Paclitaxel	1.75	14.1 ± 2.3	13%
	5.25	15..7 ± 2.1	26%
PEG 40,000 Paclitaxel	1.75	19.0 ± 1.1	52%
PEG 40,000 Glycine Paclitaxel	1.75	21.8 ± 1.0	74%

B. High Molecular Weight (hmw >20,000) PEG Prodrugs. In 1994 a detailed study conducted by Yamaoka et al (15) measured the distribution and tissue uptake of PEG of different molecular weights after *i.v.* administration to mice. Yamaoka reported that the renal clearance of PEG decreased with an increase in molecular weight, with the most dramatic change occurring at 30,000. The $t_{1/2}$ of PEG circulating in blood also showed a concomitant and dramatic increase. For example, the $t_{1/2}$ for PEG went from approximately 18 min to 16.5 h as the molecular weight increased from 6,000 to 50,000. It has long been recognized that for dendritic (branched) polymer drug conjugates, the biodistribution of the polymer alone will determine the fate of the conjugate. Similar reports (16) detailing the effect of molecular weight of HPMA copolymers on body distribution and rate of excretion identified a molecular weight threshold limiting glomerular filtration of 45,000; below this limit the $t_{1/2}$ of the polymer was quite short, e.g., $t_{1/2}$ for a 12,000 mw copolymer was reported to be only 3 min.

Traditional prodrugs are generally designed to be cleaved efficiently and rapidly ($t_{1/2}$ <20 min) by enzymatically mediated processes resulting in an accelerated rate of conversion of the inert form to the biologically active parent (17). Thus, the PK of the parent drug is only minimally affected by prodrug modification. However, in addition to this approach, an alternative solution to the problem of prodrug efficacy would be to extend the circulating lifetime of the water-soluble modification. By increasing the circulating life of the prodrug in plasma relative to its rate of hydrolysis, equivalent or greater potency should result with a gradual controlled release of the drug as long as therapeutic levels can be reached and maintained without causing toxicity. One way to accomplish this objective is to prevent rapid renal excretion of the hydrophilic form of the drug by increasing the molecular weight of the solubilizing agent, as was demonstrated for HPMA-doxorubicin (16,18). Accordingly, the first application of hmw PEG to prodrugs was the synthesis of a PEG 40,000 ester of paclitaxel using PEG diacid. After it was established that acute toxicity resulted from high doses of **3a** (14) (PEG 40,000), the efficacy of the hmw PEG prodrug was re-examined. In a P388/0 mouse leukemic model, the hmw paclitaxel prodrug was found to be essentially equivalent to a Taxane formulation.

Hmw PEG paclitaxel prodrug strategies were extended to tripartate (19,20) prodrugs, which require the use of heterobifunctional spacer groups. Of the various spacers tried, amino acids appear to be the most useful, reducing toxicity while increasing efficacy for most of the anticancer drugs tried (21-25) (Figure 1). By first preparing the hmw PEG conjugated amino acid, PEG glycine (**2**), condensation with the 2'-OH of paclitaxel resulted directly in a relatively stable PEG amide derivative of paclitaxel-2'-glycinate (**3b**) (21) which had a useful solubility of ~ 125 mg/mL, or 5 mg paclitaxel equivalent / mL. The relative *in vivo* equivalency of paclitaxel and the conjugated forms was assessed by

Figure 1. PEG Prodrugs of Paclitaxel.



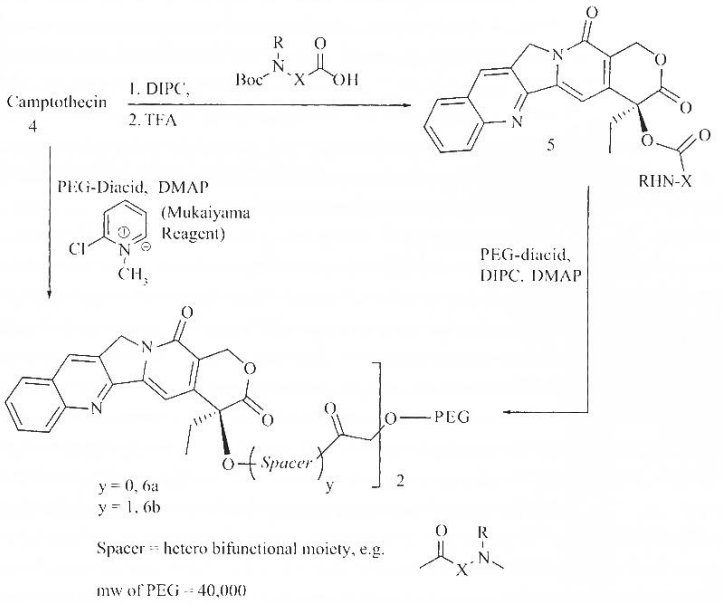
monitoring survival in a P388/0 murine leukemia model (Table 2). The mean time to death for animals treated with unconjugated paclitaxel at a total dose of 75 mg/kg was 17.5 days, resulting in an increased life span (ILS) of 33% with no cures. Similarly, the mean time to death for animals treated with an equivalent dose of PEG-paclitaxel was 19 days (ILS = 45%). In contrast, PEG-gly-paclitaxel (**3b**) treated animals had a mean time to death of 21.8 days (ILS = 65%), which was significantly ($P < 0.02$) longer than the paclitaxel group. At a total dose of 100 mg/kg, the paclitaxel group's ILS was reduced to 4% due to acute toxicity, while the same active dose of PEG-gly-paclitaxel increased the ILS to 82% with one cure. The PEG-paclitaxel lmw prodrug showed only half the activity of native paclitaxel. Thus, this screen suggests a greater therapeutic index can be achieved with hmw (40,000) PEG-gly-paclitaxel. In addition, treatments with this prodrug against HT-29 (colon), A549 (lung) and SKOV3 (ovarian) solid tumor bearing mice demonstrated significant antitumor activity.

The hmw PEG paclitaxel prodrug conjugates offer improved therapeutic efficacy that likely arises from two outstanding features. First, due to the hmw of the polymer, this transport form (*i.e.* hmw PEG prodrug) has a longer circulatory $t_{1/2}$ relative to the native drug. Secondly, and perhaps most significantly, the hmw polymer-drug conjugate is expected to selectively accumulate in the tumor. This accumulation, known as the enhanced permeability and retention (EPR) effect, or passive tumor targeting, has been postulated by Maeda et al to occur for hmw polymer conjugate therapeutics (26-28). In addition, the reduced toxicity exhibited by these compounds probably results from a more controlled ester bond hydrolysis compared with the non-attenuated release of unconjugated paclitaxel. The two properties, passive targeting and continuous release from a depot of the polymeric prodrug, provide what has been termed double targeting (29), and produce enhanced efficacy.

The insoluble anticancer drug camptothecin (CPT) illustrates the general utility of the hmw PEG prodrug approach. CPT has the unique structural elements of a lactone ring and a 3° alcohol (20-OH), both of which are requirements for activity (topoisomerase I inhibition) (30-32). The biggest drawback to the use of this potent drug is that camptothecin is virtually insoluble in water. Thus, ester prodrug strategies based on the 20-OH group have been developed as the means to solving this problem. However, another element must be added to the promoiety in order to effect solubilization (33,34). Until recently that was often an amino group, and a salt of the prodrug was made in order to effect solubilization. Using a PEG prodrug delivery strategy, we reported that CPT can be solubilized as a non-ionic α-alkoxyester conjugated to PEG carboxylic acid with a molecular weight of 40,000 (35). CPT's solubility as the 20-camptothecin PEG 40,000 ester prodrug form (**6a**) was approximately 2 mg/mL in water, and is dramatically greater than that of CPT (0.0025 mg/mL, water). PEG-CPT has been shown to hydrolyze *in vivo* and gradually release native CPT (36). Fortuitously, it was found that modifying CPT at the 20 position as a PEG ester additionally stabilizes the active lactone ring (essential for activity) under physiological conditions (35). We believe that stabilization by acylation of the 20-OH group of CPT is due to a low degree of intramolecular H-bonding (37). However, while this simple α-alkoxy ester was efficacious (36), introduction of various spacer groups between the PEG solubilizing portion of the molecule and the CPT alcohol again led to significant differences in biological activity, as was the case for paclitaxel (22) (Figure 2, **6a** and **6b**). Thus, by comparing the rates of breakdown of the PEG ester prodrugs in rat plasma with *in vivo* results, some predictive rules could be winnowed out of the study (38,39).

By careful selection of different spacer groups, the rates of hydrolysis can be adjusted, leading to significant differences in biological activity (Table 3). The circulatory retention for three derivatives illustrates this point (Table 4). Using ^{3}H-CPT and PEG-gly-^{3}H-CPT, a study was performed using HT-29 (colorectal) tumor bearing mice. The results of the study clearly demonstrated the EPR effect, with a tumor accumulation of the PEG prodrug about 30-fold greater than the native species (40).

Figure 2. PEG Prodrugs of Camptothecin.



Ref. R. B. Greenwald, et al. *J. Med. Chem.* 1996, *39*, 1938-4
Bioorg. & Med. Chem. 1998, 6, 551-562.

Table 3. *In Vitro* an *in vivo* Results of PEG-camptothecin (CPT) Derivates

Entry	Compound (CPT-)	IC_{50} (nM) P388/0	$t_{1/2}$ (h)		P388/0 (16 mgkKg)	
			PBS (pH 7.4)	Rat Plasma	% ILS	Survival on Day 40
1	Camptothecin (CPT)	7	-	-	-	0/10
2	-O-CO-CH_2-CO-PEG	15	27	2.0	192	9/10
3	-O-CO-CH_2-O-CH_2-CO-H-PEG	16	5.5	0.8	34	4/10
4	-O-CO-CH_2-O-CH_2-CO-N(CH_3)-PEG	21	27	3	143	6/10
5	-O-CO-CH_2-O-CO-N(CH_3)-PEG	18	28	5	80	0/10
6	-O-CO-CH_2-NH-CO-CH_2-O-PEG	12	40	6	169	8/10
7	-O-CO-CH_2-N(CH_3)-CO-CH_2-O-PEG	15	97	10	48	0/10
8	-O-CO-CH_2-NH-PEG	24	12	3	135	0/10
9	-O-CO-CH_2-N(CH_3)-PEG	42	102	49	65	0/10

After great consideration, PEG-ala-CPT (Prothecan®) was ultimately chosen for a Phase I clinical trial because of its relatively extended $t_{1/2}$, lower toxicity in mice, and efficacy compared to other PEG-CPT derivatives. Human PK studies demonstrated a dose dependent area under the curve (AUC), with extended levels of CPT present even after 70 h (Figure 3). Thus far, single doses of 7,000 mg/m^2 have been reached in MTD studies, with neutropenia and leukopenia being the major toxicities encountered. Out of fourteen patients treated, five exhibit stable disease states, with one showing a partial response.

Table 4. Circulatory Retention of PEG-Camptothecin in Mice.

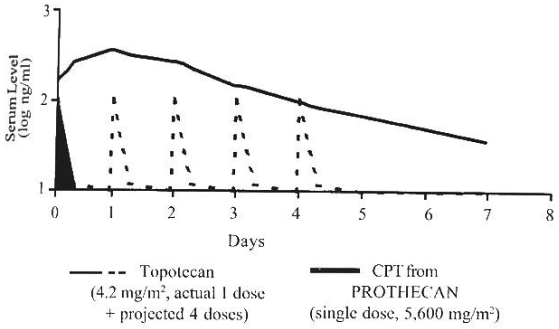
Compound	$t_{1/2a}$ (Distribution)	$t_{1/2b}$ (Elimination)	Mean Reisdence Time (AUMC/AUC)
PEG-CPT	~ 4 min	3.4 h	4.9 h
PEG-Gly-CPT	~ 5 min	5.3 h	7.5 h
PEG-Ala-CPT	~ 8 min	11.3 h	15.9 h

Ref. Conover CD, et al. Cancer Chemother Pharmacol 1998;42:407-414
Conover CD, et al. Anticancer Drug Des 1999; 14:499-506

PEG Amino Prodrug Methods. *A. Benzyl Elimination (BE)*. Until recently there have been very few published methods available for the practical synthesis of PEG amino prodrugs. While most amine drugs can be solubilized as acid salts, their rate of renal excretion is high. When converted to neutral small prodrug species, the ability to form salts is lost, and solubility may again become problematic. This is not the case for PEG-drug conjugates, where PEG confers water solubility on insoluble small organic compounds without the need for forming salts. PEG conjugated specifiers (19) or "triggers" (41) were synthesized as part of a double prodrug strategy that relied, first, on enzymatic separation of PEG, followed by the classical and rapid 1,4- or 1,6-benzyl elimination reaction releasing the amine (drug) latentiated in the form of a carbamate (42). This release technology has been developed extensively and is generally referred to as the double prodrug approach (43) since, in essence, a pro-prodrug has been made. In such systems, the hydrolytic sequence involves a first step, which usually is an enzymatic cleavage, followed by a second, faster step that is a molecular decomposition. One of the first applications of the 1,4- or 1,6-elimination (or BE) concept was in designing model tripartate prodrugs and involved the latentiation of an aromatic NH_2 by forming an carbamate with lysine (19).

Further refinements that enabled us to easily modify the rates of hydrolysis of 1,6-elimination prodrugs were accomplished by the introduction of steric hindrance through the use of *ortho* substituents on the aromatic component of the prodrug (Figure 4). This modification led to a longer plasma $t_{1/2}$ for the final tripartate form. The "*ortho*" effect also had the beneficial effect of directing nucleophilic attack almost exclusively to the activated

Figure 3. PROTHECAN Clinical Pharmacokinetics.

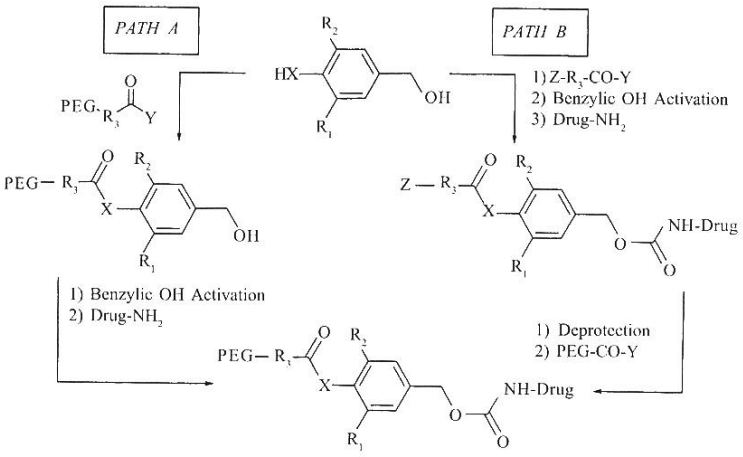


Ref. L. Ohoa, et al., *Proc. ASCO,* 19, 770 (2000).
E. K. Rowinsky, et al., *J. Clin. Oncol.* 14, 1224 (1996).

benzyl 6-position of the heterobifunctional intermediates (44). This technology has extended the usefulness of the PEG prodrug strategy to amino-containing anticancer compounds; it was also felt that the methodology should be applicable to other amino-containing drugs of diverse activities.

The efficacy of PEG-DNR conjugates prepared using the BE methodology was tested within a solid M109 tumor model, and their relative activities varied according to route of administration and their rate of *in vitro* dissociation (Table 5). Those compounds with a $t_{1/2}$ rat plasma dissociation of 2-4 h were the most effective in inhibiting solid tumor growth without causing toxicity, and displayed a lower %T/C than an equivalent dose of DNR. The reason behind this phenomena probably lies in the biodistribution of the PEG-drug conjugates, especially with respect to their rates of drug elimination versus tumor uptake.

Figure 4. Synthesis of PEG-Spacer-BE Prodrugs.



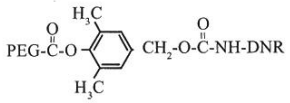
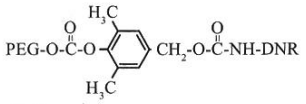
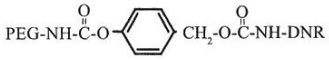
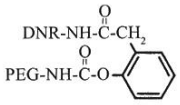
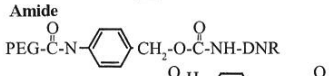
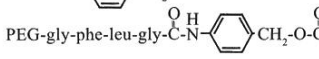
$R_1 = R_2 = H, OCH_3, CH_3$, X = O or NH, R_3 = Spacer Moiety, Y = activating group, Z = Protecting group, Activation = NHS, PNP, etc., Drug = Daunorubicin, Doxorubicin, etc.

B. Trimethyl Lock (TML) Lactonization. In our continuing efforts to extend the limits of the PEG prodrug strategy, it was apparent that the use of lactonization reactions (45-47) could be incorporated into the strategy, and would provide a practical alternative to the BE system. Our laboratory at Enzon has extended and refined existing PEG technology to embrace the concept of the TML tripartate system (48). In order to utilize the TML system for polymer conjugated prodrugs, it was necessary to first establish various methodologies which allowed the efficient synthesis of different acyl functionalities (triggers), such as esters, carbonates, and carbamates conjugated to the phenolic hydroxyl group (Figure 5). The acylating agents were by necessity bifunctional and offered a site for easy PEGylation. Thus, introduction of PEG into the TML system as part of the specifier or trigger resulted in a neutral and highly water soluble tripartate polymeric prodrug capable of passive tumor targeting. The PEG prodrugs were designed to attain predictable rates of hydrolysis by changing the nature of the trigger/linker bond, by adding steric hindrance on the aromatic ring of the linker, and by the use of spacer groups (Figure 5). This approach resulted in a versatile methodology for easily altering the final design of the prodrug: it enabled a "mix and match" of spacers, triggers, and linkers that could be designed to produce variation of half-lives, and ultimately provided optimal plasma concentrations for delivery of different types of drugs (Table 6). Among the 7 compounds examined in Table 6, rat plasma hydrolysis data showed only one derivative with a $t_{1/2}$ between 2 and 17 h. While further combinations of triggers and linkers should produce more intermediate $t_{1/2}$ values, it is evident that adjusting $t_{1/2}$ of TML linkers is not as simplistic as was the case for the BE system.

The TML–ala derivative ($t_{1/2}$ = 2 h) and a BE-carbamate derivative ($t_{1/2}$ = 4 h) were both chosen as the best representative examples for comparative evaluation using chemotherapeutic activity against a small panel of human tumor xenografts as the measure. Both prodrugs were quite similar in their ability to significantly inhibit the growth of SKOV3 tumors (Table 7), and were more effective than native daunorubicin. By contrast, PEG conjugation did not appear to enhance the activity of DNR against tumor lines which were insensitive to DNR (MX-1; mammary and PC-3; prostate) (48).

The safety of both systems was demonstrated by synthesizing the simple

Table 5. *In vitro* and *In Vivo* Results of PEG BE-Daunorubicin Prodrugs

Compound	$t_{1/2}$ (h)		IC_{50} (nM) P388/0	% T/C	
	PBS	Rat Plasma		M109 i.p.	M109 i.v.
Daunorubicin (DNR)			3	44.8	117.0
Ester					
			55	90.3	67.9
Carbonate					
	>48	1.9	179	90.3	74.4
Carbonate					
	>48	2.9	15	84.1	64.6
	>48	>24	415	75.3	129.0
	>48	3.0	35	91.3	82.2
Amide					
	>48	>24	457	122.7	NA
	>24	13	160	87.6	82.6

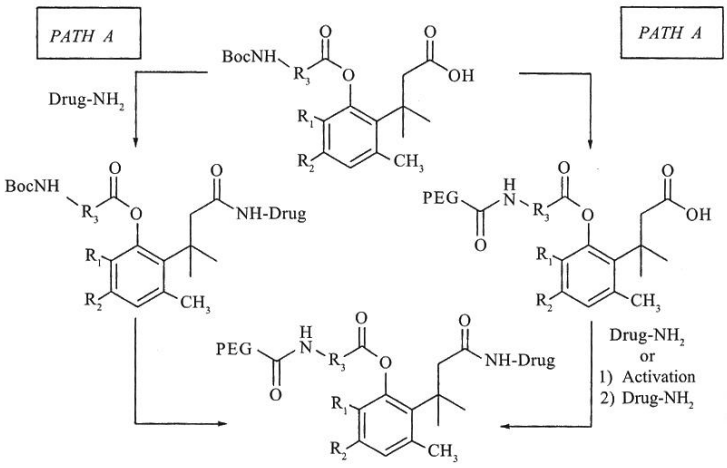
amine containing prodrugs shown in Table 8. These compounds were tested *in vitro* and were shown to be inactive. Also, no toxicity was observed when mice were treated at three times the normal dose of the PEG-DNR conjugates. This strongly implies that the breakdown products associated with both the BE and TML series are innocuous.

C. Further Applications of PEG Amino Prodrugs: Releasable PEG (rPEG) Protein Conjugation. Using lysozyme as a representative protein substrate that loses its activity when PEGylation takes place on the ε-amino group of lysine residues, various amounts of a novel releasable lmw PEG (5,000) linker (rPEG) were conjugated to the protein (Figure 6). rPEG-lysozyme conjugates were relatively stable in pH 7.4 buffer for over 24 h. However, regeneration of native protein from the rPEG conjugates occurred in a predictable manner during incubation in high pH buffer or rat plasma as demonstrated by enzymatic activity and structural characterization (49). The rates of regeneration were also correlated with PEG number: native lysozyme was released more rapidly from the monosubstituted conjugate than from the disubstituted conjugate, suggesting possible steric hindrance to the approach of cleaving enzymes. Recovery of normal activity and structure for the regenerated native lysozyme was shown by a variety of assays. This demonstration of rPEG, in and of itself, will no doubt be useful in anticancer drug delivery. rPEG has the potential to be used in practical ways by extension of the system to other proteins such as cytokines by providing a depot of protein drug in the rPEG form. Thus, spiking, which can cause severe side effects when large amounts of native protein drug are delivered in a short period of time, can be eliminated using the rPEG linker.

Polymer Therapeutics: Why PEG?

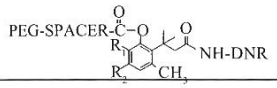
PEG is essentially non-toxic, is easily manufactured with low polydispersity, is relatively inexpensive for large-scale processes, is easily activated for

Figure 5. Synthesis of PEG-Spacer-TML Prodrugs.



$R_1 = R_2 = H$ or CH_3, R_3 = Spacer Moiety,
Drug - Daunorubicin, Doxorubicin, Ara-C,
Melphalan, Anilinonitrogen Mustard.

Table 6. *In Vitro* and *In Vivo* Results of PEG TML/DNR Prodrugs.

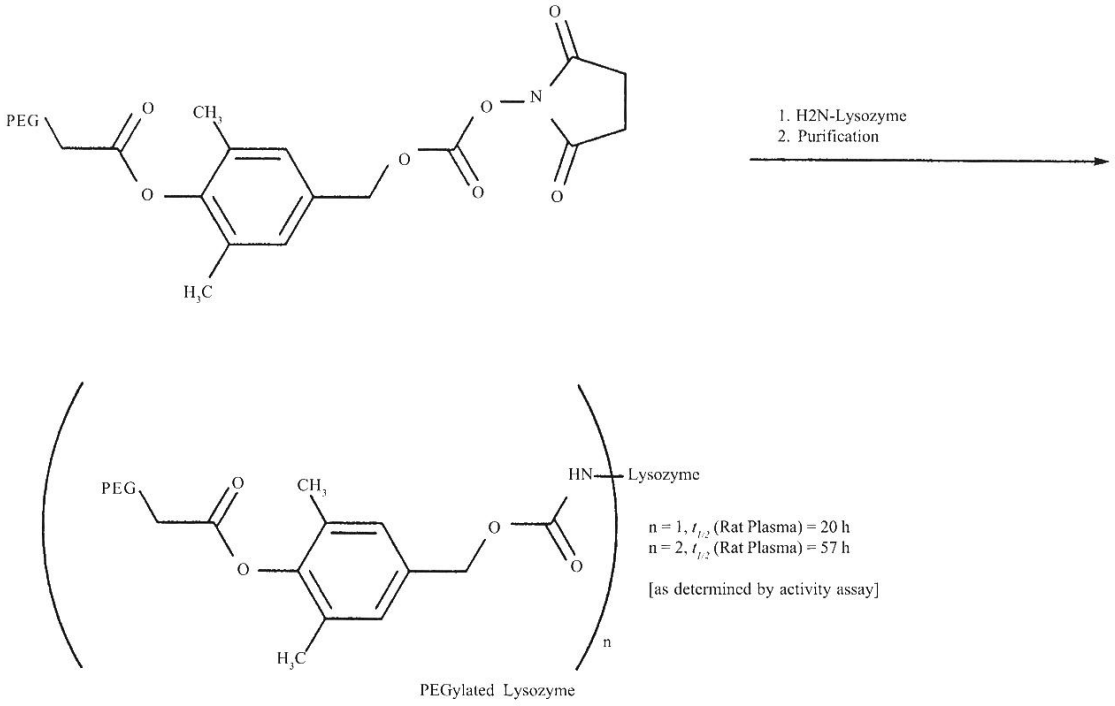


Compound			$t_{1/2}$ (h)		IC_{50} (nM) P388/0	%T/C M109 i.v.
R_1	R_2	Spacer	PBS	Rat Plasma		
Daunorubicin (DNR)			-	-	3	117.0
Esters						
H	CH_3	Alanine	>24	1.9	43	92.5
H	CH_3	Proline	>24	17	301	122.6
H	CH_3	ß-Alanine	>24	0.2	203	63.7
CH_3	H	Alanine	>24	21	389	72.5
CH_3	H	ß-Alanine	>24	8	411	31.6
Carbonate						
H	CH_3	NH(CH2CH2O)2	>24	1.1	142	118.4
Carbamate						
H	CH_3	$NH(CH_2CH_2O)_2CH_2CH_2NH$	>24	>24	203	93.8

conjugation, and PEG conjugated proteins have already been approved for human use.

PEG chemistry has experienced a resurgence in the last five years that in large part may be attributed to the use of hmw PEG conjugates. The successful application of α-interferon PEGylation has now centered attention on the use of fewer strands of hmw PEG with proteins. The successful application of the PEG prodrug (40,000) concept to anticancer agents and the initiation of a clinical trial of PEG-camptothecin by Enzon may be viewed as the beginning of a drug delivery methodology which can be extended to many other classes of compounds: cytokines, blood factors, peptides, antifungals, antibiotics, and immunosuppressive agents, to mention a few.

Figure 6. PEGylation of Lysozyme with PEG-BE Linker.



Ref. S. Lee, et al., submitted for publication.

Table 7. Efficacy of PEG/BE & TML/DNR Against s.c. Human Ovarian Tumors (SKOV3) in Nude Mice

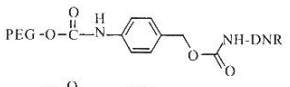
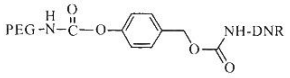
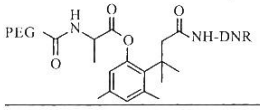
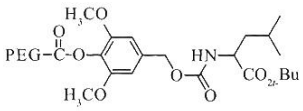
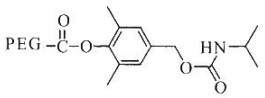
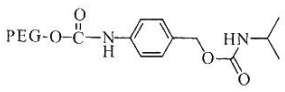
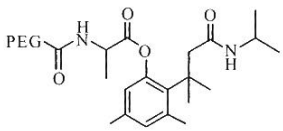
Compound	Tumor Vol. by week 6 (Mean, mm ^{3})	% from basal by week 6 (Mean)	T/C (%) by week 5
Control	**1557.8**	**3091.4**	--
Daunorubicin HCl	**1242.4**	**3008.1**	**35.2**
	777.1	1224.8	51.7
	512.9	699.1	7.6
	50.0	78.5	4.5

Table 8. *Safety* of PEG BE & TML Linkers.

Compound	IC_{50} P388/0 (5mg/mL)	Dose (mg/kg)	% Toxity (Death)
	No Inhibition	830	0
	No Inhibition	830	0
	No Inhibition	830	0
	No Inhibition	830	0

However, as the technology inevitably becomes validated by more successes, the drug delivery community will in time, no doubt, adapt PEG strategies for new applications that are constrained only by one's imagination.

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