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Dependence of Pharmacokinetics and Biodistribution on Polymer Architecture: Effect of Cyclic versus Linear Polymers

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Long circulation times of water-soluble polymers are essential for the successful delivery of drugs to solid tumors. The circulation time of such a polymer depends upon molecular weight (MW) and polymer architecture.[1−4] This is because physiological barriers in the kidneys have a nanoporous structure that retards the permeation of soluble polymers but allows the passage and elimination from the body of low-MW substances.[5,6] Linear polymers traverse a nanopore by the end-on motion of the polymer chain, and since only one polymer segment needs to enter the pore for a linear polymer to traverse it, linear polymers cross nanopores more easily than star polymers.[1,5] Cyclic polymers lack chain ends, so two chain segments would need to enter the pore for the cyclic polymer to transit. Therefore, we predicted that cyclic polymers would behave differently in vivo than linear polymers of the same MW.

The linear polymer precursor and cyclic copolymer of α-cholo-ε-caprolactone (ClCL) and ε-caprolactone (ε-CL) were synthesized following published procedures.[7−9] Briefly, the cyclic random copolymer of ε-CL and ClCL were polymerized using the cyclic tin-based catalyst 2,2-dibutyl-2-stanna-1,3-dioxepane. ClCL was introduced as a copolymer to allow further modification of the polymer chain. To create the cyclic polymers, α-(1-acryloxyethyl)-ε-caprolactone was added to the polymerization medium to enable cyclization via intramolecular photocrosslinking (Table S1 in the Supporting Information).

A comparison of the linear polymer precursor and the cyclic polymer by gel permeation chromatography (GPC) (Figures S2−S4) showed a shift of the elution time toward a smaller MW for the cyclic polymer, indicating the formation of intramolecular cyclization. The number-average MWs of the linear polymer precursor and cyclic polymer measured by GPC were 11 900 Da (PDI = 1.46) and 9300 Da (PDI = 1.38), respectively (Figure S2). The larger MWs of the linear polymers are expected because these polymers have larger hydrodynamic volumes than cyclic polymers of the same MW.[4,10]

An alkyne phenol moiety was quantitatively introduced on the azide-containing polyester [P(N_3CL-co-CL)] backbone using very mild conditions to avoid hydrolytic degradation. The polyester backbone was further modified by cycloaddition of an alkyne, ω-methoxyl-PEG, to increase water solubility (Figures S5 and S6). The grafting density of PEG chains on the linear and cyclic polyesters was stoichiometrically controlled using "click chemistry".[7] The MWs of the linear and cyclic polymers was tuned from less than to greater than the elimination thresholds in the kidney by the addition of 1.1, 2, or 5 kDa PEG chains.[11]

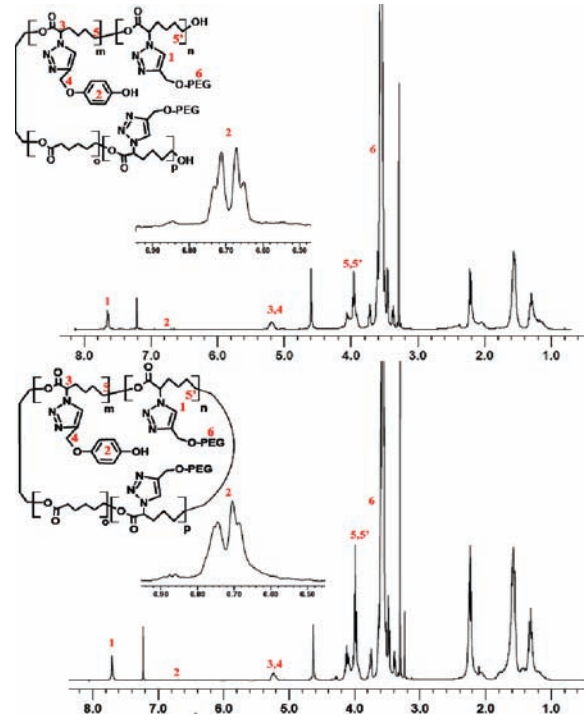

Figure 1. NMR spectra (^{1}H, 400 MHz) of (A) linear and (B) cyclic phenols containing PEG(2 kDa)-*g*-PCL(9 kDa).

The grafting of PEG was confirmed by a characteristic chemical shift of the $-CH_2O-$ resonance at 3.6 ppm and the triazole proton at 7.7 ppm in the ^{1}H NMR spectrum (Figure 1). Calculation of the integral intensities of the chemical shifts at 3.6 ppm ($-CH_2CH_2O-$) and 4 ppm ($-CH_2CH_2CH_2O-$) for PEG and ε-caprolactone, respectively, indicated that ∼21 1.1 and 2 kDa PEG chains and 16 5 kDa PEG chains were grafted onto poly(N_3CL-co-CL) (72 units). The number-average MWs of the grafted polymers were ∼32, 50, and 90 kDa for conjugation of 1.1, 2, and 5 kDa PEG, respectively (Table 1). We adjusted the amount of propargyl phenol in the reactions to give on average one phenol moiety per polyester chain.

We determined the dependence of the blood circulation time and biodistribution as a function of the polymer architecture and MW using polymers radiolabeled on the phenol group with ^{125}I via the chloramine T method (Figure S13).[5] The polymer backbone degraded over the course of 10 days in phosphate buffered saline at 37 °C (Figures S9 and S11). The degradation rate was faster in human plasma (Figures S10 and S11), but degradation did not interfere with the determination of the pharmacokinetic elimination rates (Figure S15).

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Table 1. Polymer Characterization and Pharmacokinetic Data

polymer	M_n (kDa)	PDI	half-life (h) $t_{1/2,\alpha}$	half-life (h) $t_{1/2,\beta}$	$AUC_{0\rightarrow\infty}$ (%ID h g^{-1})
cyclic 90	93	1.33	1.00 ± 0.30	20.9 ± 4.4	587.1 ± 13.6
cyclic 50	49	1.14	0.09 ± 0.08	13.6 ± 2.7	343.3 ± 8.8
cyclic 32	33	1.21	0.27 ± 0.02	2.7 ± 1.3	11.7 ± 1.4
linear 90	90	1.25	0.51 ± 0.04	14.7 ± 1.5	218.6 ± 1.0
linear 50	52	1.21	0.26 ± 0.03	4.4 ± 1.3	22.7 ± 1.0
linear 32	32	1.21	0.17 ± 0.02	3.0 ± 0.5	22.8 ± 0.3

Figure 2 shows a plot of polymer concentration in the blood as a function of time. Elimination half-lives ($t_{1/2,\beta}$) were calculated using the residuals method in a two-compartment model (Table 1). Cyclic polymers with MWs greater than the renal filtration threshold (50 and 90 kDa) had longer plasma circulation times than linear polymers of similar mass.[11] This is because linear polymers can reptate through the nanopores in the glomeruli by an "end-on" motion more easily than cyclic polymers. Interestingly, the effect of polymer architecture was more profound for the 50 kDa polymer (the R_h determined from GPC was ~4.4 nm) than the 90 kDa polymer. Similar behavior has been reported for elongated polymers and more densely structured dendrimers.[5,12] This behavior was manifested by a lower amount of cyclic polymer in the urine for the 50 and 90 kDa polymers but not for the 32 kDa polymer. Radioactivity in the urine was found for both polymer and lower-MW fragments (Figure S14). The 32 kDa polymers showed very rapid elimination. The 32 kDa cyclic polymer was eliminated faster than the linear 32 kDa polymer because the MW of both were below the nominal MW cutoff (30−40 kDa) of the renal filtration of linear PEG[11] but the cyclic polymer is a more compact molecule.

The accumulation of radioactivity in different organs at 24 h is displayed in Figure 3. First, as observed with other polymers, a larger polymer MW leads to retention of a greater percentage of polymer in the body (Figures S15 and S16). The 32 kDa cyclic polymer had low accumulations in all organs because of its short circulation time and was secreted in urine 13% more than the 32 kDa linear polymer. The linear 32 kDa, although rapidly eliminated, had a higher kidney accumulation than did any of the other polymers. The reason for this is not apparent. When the MW was increased to 50 kDa, the cyclic polymer was secreted in urine 12% less than the linear polymer. As a result, the 50 kDa cyclic polymer accumulated in organs more than the 50 kDa linear polymer. This tissue accumulation advantage of the cyclic polymer was also observed in the 90 kDa polymers. The comb polymers described herein exhibit blood retention values at 24 h that are greater than those of linear PEG[11] and less than those of dendritic bow-tie polymers.[1] The longer $t_{1/2,\beta}$ of the cyclic polymer compared

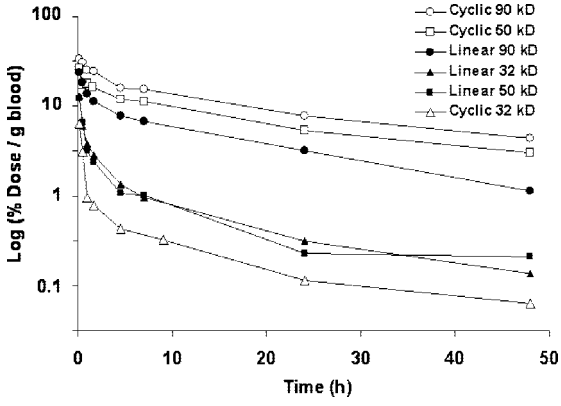

Figure 2. Linear and cyclic polymer concentration in the blood as a function of time.

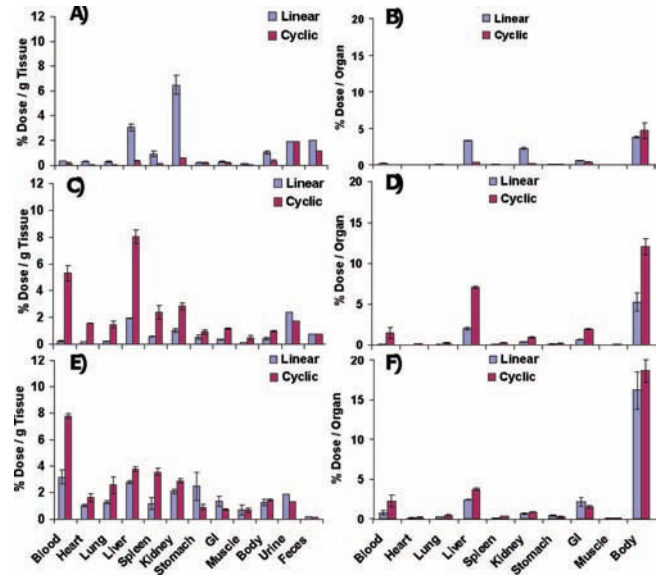

Figure 3. Biodistribution of linear and cyclic polymers in mice at 24 h: (A, B) 32 kDa; (C, D) 50 kDa; (E, F) 90 kDa. Data are plotted as % injected dose per gram of tissue vs time (left panels; urine and feces were calculated by dividing % injected dose by animal weight) and % injected dose per organ vs time (right panels). Error bars have been omitted from the feces and urine values because the respective excrements for each time point were not collected individually but were pooled together. The % recoveries of linear and cyclic polymers with MWs of 32, 50, and 90 kDa were 80.2, 68.3, 72.8, 76.9, 69.8, and 61.4, respectively.

with the linear polymer of the same MW may provide a window of opportunity for cyclic polymers as drug carriers or imaging agents: in the cyclized state, the polymer would circulate, releasing the drug; when the chain was broken, the polymer would be more rapidly eliminated. This is not a particular advantage for degradable polymer backbones. It could be an advantage for cyclic nondegradable backbones, such as in the methacrylate polymers widely used as drug carriers.[4,13]

In conclusion, we have demonstrated that polymer topology (cyclic vs linear) can play an important role in blood circulation time. This finding adds to the range of macromolecules whose architectures influence their behavior in circulation and may be useful for the creation of drug carriers with improved drug delivery attributes

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Supporting Information Available: Table S1 and Figures S1−S16. This material is available free of charge via the Internet at http://pubs.acs.org.

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