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Factors affecting the degradation rate of poly(lactide-co-glycolide) microspheres in vivo and in vitro

M.A. Tracy*, K.L. Ward, L. Firouzabadian, Y. Wang, N. Dong, R. Qian, Y. Zhang

Alkermes Inc., 64 Sidney Street, Cambridge, MA 02139, USA

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Abstract

The purpose of this work was to study the degradation of poly(lactide-co-glycolide) (PLG) microspheres in vivo and in vitro. Degradation rate constants were determined by measuring the polymer molecular weight as a function of time by gel-permeation chromatography. The effects of PLG chemistry and the effects of encapsulating the sparingly soluble salt zinc carbonate and the protein recombinant human growth hormone (rhGH) on the degradation rate were assessed. It was found that in vivo degradation was faster than in vitro degradation. In addition, different types of PLGs were found to degrade at different rates depending on the chemistry of the polymer end group and, to a lesser extent, the molecular weight. Finally, zinc carbonate was found to retard the degradation of some PLGs. These degradation studies have proved valuable in the design of sustained release microsphere products.

Keywords: Poly(lactide-co-glycolide); Polymer degradation; Microspheres; Drug delivery; Proteins

1. Introduction

There has been much interest in using microspheres made from biodegradable polymers for the sustained release of protein and peptide drugs. One of the most commonly used polymers is poly(lactide-co-glycolide) (PLG) because of its proven biocompatibility [1, 2]. The properties of the PLG used to encapsulate these drugs play an important role in the rate of microsphere degradation and therefore in achieving desirable release kinetics. Degradation and release may also be affected by the addition of excipients such as sparingly soluble salts to the polymer matrix [3–5]. Recently, we reported that a microsphere formulation has been developed for the continuous release of native recombinant human growth hormone (rhGH) for one month, with desirable release kinetics, from a PLG matrix containing zinc carbonate [6–8]. Here we present data showing the effects on degradation rates of PLG chemistry such as the type of end group and molecular weight, and the effects of encapsulating sparingly soluble salts and a protein.

2. Materials and methods

Polymers: Four types of PLG 50:50 polymers were used in this experiment to investigate the effects of molecular weight and end group on degradation. The polymer end group is determined by the choice of initiator used in the polymerization reaction. Uncapped (or hydrophilic) PLG has free carboxyl groups at the polymer terminus. Capped PLG has ester linkages at the polymer terminus resulting in a more hydrophobic alkyl end. Table 1 summarizes the polymers used in this study and some of their relevant characteristics.

Microsphere preparation: Microspheres containing no additives, zinc carbonate (Spectrum Chemicals Inc., Gardenia, CA) only, and rhGH (Genentech Inc., South San Francisco, CA) with zinc carbonate were prepared using the ProLease® process [9]. Using this process, microspheres were produced by first preparing a PLG/methylene chloride solution and adding materials to be encapsulated such as zinc carbonate and a spray freeze-dried powder containing rhGH. The resulting suspension was atomized using an ultrasonic nozzle into a vessel containing liquid nitrogen over a bed of frozen ethanol, a nonsolvent for PLG. This vessel was placed at a temperature, typically $-80^{\circ}C$, where the liquid nitrogen evaporated and the ethanol melted, allowing extraction of the

* Corresponding author. Tel.: (617) 494-0171 x6519; fax: (617) 252-0915; e-mail: mark_tracy@alkermes.com

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Table 1
PLG 50:50 polymers used in in vitro/in vivo microsphere degradation study

PLG	Source	Intrinsic viscosity (dl/g)	Weight average molecular weight (M_w in Da)	Polydispersity (M_w/M_n)	End group	Average glycolate block length
A	B.P.I. Inc. Birmingham, AL	0.23	9500	1.8	Capped (Ester)	2.9
B	B.I. Chemicals Inc. Montvale, NJ	0.17	8000	1.9	Uncapped (–COOH)	2.8
C	B.I. Chemicals Inc. Montvale, NJ	0.40	21 000	3.0	Uncapped (–COOH)	3.0
D	B.I. Chemicals Inc. Montvale, NJ	0.19	12 700	2.1	Capped (Ester)	3.1

methylene chloride from the droplets over several days. At the end of this period, the solid microspheres were harvested by filtration and dried under vacuum. Residual methylene chloride levels in the microspheres used for this study were found to be $<0.07\%$ (w/w) and the ethanol solvent levels were ~ 1–3% (w/w).

In vivo degradation procedure: 50 mg of microspheres was suspended in 1 ml of a viscous diluent (3% (w/v) low-viscosity sodium carboxymethyl cellulose, 1% (v/v) Tween 20 in 0.9% (w/v) saline), injected into each male Sprague–Dawley rat and excised with the surrounding tissue at each time point. There was one rat for each time point. To extract the polymer from the surrounding tissue, as much excess tissue as possible was first trimmed. Then the remaining microsphere/tissue sample was cut into small portions and added to a 10% acetic acid in chloroform solution. PLG is soluble in both solvents. The acetic acid was added to aid in the separation of the tissue from the polymer. A sufficient amount of the 10% acetic acid in chloroform solution was added to obtain about 1.0 ml solution/10 mg sample. The samples were incubated for 30 min with shaking at room temperature and filtered through a 0.2 μm filter (Acrodisc PTFE, Gelman Sciences, Ann Arbor, MI) to remove the remaining bits of tissue. The filtered solution was frozen using liquid nitrogen and freeze-dried using a lyophilizer (Model 3, Virtis, Inc., Gardner, NY). The dried samples were dissolved in chloroform at a concentration of 10–20 mg ml^{-1} and the polymer molecular weight was measured by gel-permeation chromatography (GPC) (Instrumentation: Waters Corp., Milford, MA, Columns: PLgel 3 μm Mixed-E 300×7.5 mm, Polymer Labs, Amherst, MA for polymers A, B, and D in Table 1, DVB 100000 A, 500×10 mm, Jordi-Gel, Bellingham, MA for polymer C, Standards: Polystyrene Standards, Polymer Labs, Amherst, MA). All samples of the same polymer type were analyzed in a single run. The column run temperature was 35°C. Variability in the determination of the PLG molecular weight by GPC for undegraded samples was about 1% r.s.d. (relative standard deviation). Animal-to-animal variability was estimated to be 3% r.s.d. by injecting seven rats with microspheres made from polymer B (Table 1) and determining the molecular weight of the excised polymer after seven days in vivo.

Time points were taken throughout the degradation period for each sample. For the samples with the 8 kD uncapped polymer, time points were taken weekly for 28 days in vivo and 2–3 times per week for 35 days in vitro. Due to its rapid degradation, quantitation of this polymer's molecular weight was possible only to day 14 in vivo. For the samples containing the 21 kD uncapped polymer, time points were taken twice a week for 28 days in vivo and weekly for 60 days in vitro. For the 12.7 and 9.5 kD capped PLGs without zinc carbonate, time points were taken weekly in vivo for 50–60 days. Time points were taken weekly in vitro for about 60 days for these samples. The 9.5 kD capped PLG with zinc carbonate ($\pm$hGH) was studied out to 90 days in vivo and 60 days in vitro, although quantitation of the molecular weight in vivo was possible only out to 60 days. Microspheres made from the 12.7 kD capped polymer with zinc carbonate ($\pm$hGH) were not produced because its chemistry and molecular weight were similar to the capped 9.5 kD PLG. The time points for the polymer disk studies [3] comparing the 8 kD uncapped and 12.7 kD capped polymers are given in the figures.

In vitro degradation procedure: 50 mg of microspheres was incubated in 5 ml of a 50 mM HEPES, 10 mM KCl, 0.1% sodium azide buffer (pH 7.4) at 37°C. There was one vial for each time point. At each time point, the buffer was decanted from every vial. Fresh buffer was added to all but one vial. The sample in this vial was freeze-dried. When the sample for the last point was dried, each sample was dissolved in chloroform and the molecular weight was determined by GPC. All samples of the same polymer type were analyzed in a single run. Inter-vial variability was estimated to be about 10% r.s.d. by evaluating three samples of microspheres made from polymer B (Table 1), and analyzing each sample's molecular weight by GPC after seven (7) days of incubation.

Data analysis: The molecular weight distribution for each sample was measured as a function of time by GPC.

The weight average molecular weight (M_w) was determined at each time point and was normalized to the initial weight average molecular weight (M_{w_0}). An example plot is given in Fig. 1. PLG degrades by a hydrolytic process [10, 11]. This process may be described by pseudo-first-order kinetics. Thus, the slopes of semilog plots of the normalized weight average molecular weight versus time (in days) give the pseudo-first-order degradation rate constants for the samples. As shown in Fig. 2, these slopes were determined by a linear least-squares fit to the data and are presented in Tables 2–4. The errors in the slopes are the 95% confidence limits determined using JMP software version 3.1.6 (SAS Institute, Inc., Cary, NC). Also, the tables include the approximate time required for the microspheres to fully degrade as determined by a visual examination of the injection site in vivo and of microspheres remaining in each vial in vitro.

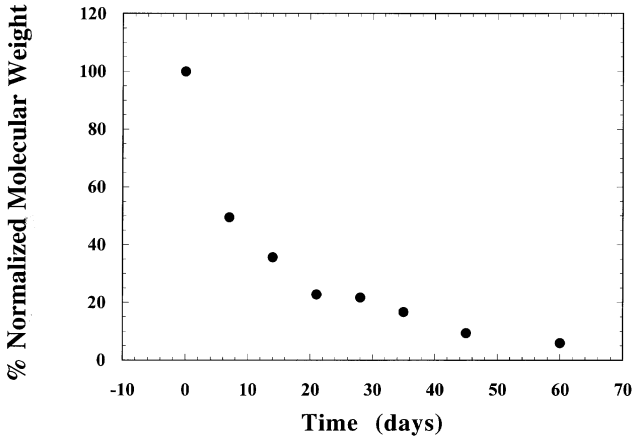

Fig. 1. Example plot of the molecular weight degradation with time for the 21 kD uncapped PLG in vitro. The % normalized molecular weight $= (M_w/M_{w_0}) \times 100$.

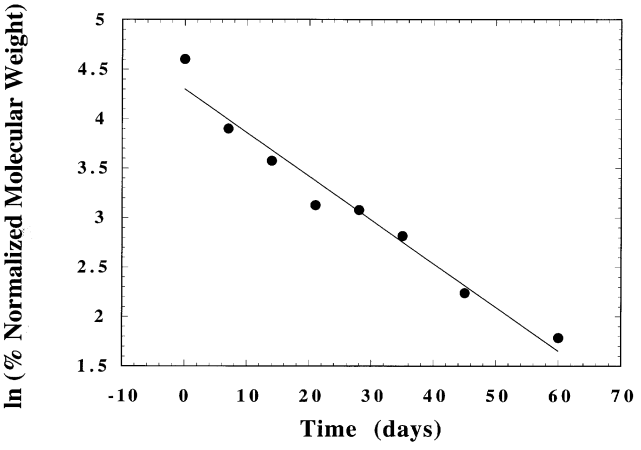

Fig. 2. Example semilog plot of the molecular weight degradation with time for the data in Fig. 1. The line is a linear least-squares fit to the data. The equation is $y = 4.3 - 0.044x$, $R^2 = 0.96$. The degradation rate constant is -0.044 (l/days) as determined from the slope.

3. Results and discussion

In vitro versus in vivo degradation: A comparison of the data in Table 2 shows that the relative rate of degradation of microspheres in vivo is 1.7–2.6 times faster than in vitro. This trend was observed regardless of the polymer end group or molecular weight, or the presence of zinc carbonate and protein (see Tables 3 and 4). Other investigators have observed similar differences between in vivo and in vitro degradation rates for microspheres [12–15]. Experiments described in the literature suggest some possible explanations for this difference [11, 13, 16–18]. For example, Mason et al. [11] reported a degradation rate for poly(D,L-lactide) (PLA) in plasma double that in a buffer or water at 37°C and attributed this to an increase in chain mobility due to lipid components in the plasma which are absorbed by PLA. They also obtained a degradation rate about eight times faster than in aqueous buffer by dissolving the polymer in benzene saturated with water and again attributed the result to enhanced chain mobility. Menei et al. [13] observed faster degradation of PLG microspheres in vivo versus a saline in vitro system and attributed this to lipids or other biological compounds present in vivo acting as plasticizers favoring the uptake of water into the polymer.

In addition, the faster degradation in vivo may be due in part to the foreign body response [16–19]. This response results in the accumulation of cells such as

Table 2
In vivo vs. in vitro degradation results for PLG 50:50 microspheres

Microsphere polymer type	In vivo rate constant ($\times 10^{-2}$) (1/days)#	In vitro rate constant ($\times 10^{-2}$) (1/days)#	Rate constant ratio in vivo/in vitro	Microsphere duration in vivo (days)	Microsphere duration in vitro (days)
Capped, 9.5 kD	-3.3 ± 0.6	-1.8 ± 0.3	1.8	50–60	⩾60*
Capped, 12.7 kD	-4 ± 1	-2.4 ± 0.7	1.7	42–49	⩾60*
Uncapped, 8 kD	-13 ± 5	-5 ± 1	2.6	14–21	~35
Uncapped, 21 kD	-7.9 ± 0.8	-4.4 ± 0.8	1.8	21–28	>60*

* Last time point taken.
# Errors are 95% confidence limits.

Table 3
Effect of zinc carbonate on microsphere degradation in vivo and in vitro

Microsphere polymer type and % zinc carbonate	In vivo rate constant ($\times 10^{-2}$) (1/days)#	Zinc/no zinc rate constant ratio in vivo	Microsphere duration in vivo (days)	In vitro rate constant ($\times 10^{-2}$) (1/days)#	Zinc/no zinc rate constant ratio (in vitro)	Microsphere duration in vitro (days)
Capped, 9.5 kD						
no $ZnCO_3$	-3.3 ± 0.6		50–60	-1.8 ± 0.3		$\gg 60$*
Capped, 9.5 kD						
6% $ZnCO_3$	-1.5 ± 0.8	0.4	~90	-0.7 ± 0.4	0.4	$\gg 60$*
Uncapped, 8 kD						
no $ZnCO_3$	-13 ± 5		14–21	-5 ± 1		~35
Uncapped, 8 kD						
1% $ZnCO_3$	-13 ± 2	1.0	14–21	-5.0 ± 0.5	1.0	~35
6% $ZnCO_3$				-5.6 ± 0.5		
Uncapped, 21 kD						
no $ZnCO_3$	-7.9 ± 0.8		21–28	-4.4 ± 0.8		>60*
Uncapped, 21 kD						
1% $ZnCO_3$	-8 ± 3	1.0	21–28	-4 ± 1	0.9	>60*
6% $ZnCO_3$	-8 ± 2					

* Last time point taken.
# Errors are 95% confidence limits.

Table 4
Effect of protein on the in vivo and in vitro degradation of microspheres containing zinc carbonate

Microsphere polymer type and rhGH content	In vivo rate constant ($\times 10^{-2}$) (1/days)#	rhGH/no rhGH rate constant ratio (in vivo)	Microsphere duration in vivo (days)	In vitro rate constant ($\times 10^{-2}$) (1/days)#	rhGH/no rhGH rate constant ratio (in vitro)	Microsphere duration in vitro (days)
Capped, 9.5 kD						
no rhGH	-1.5 ± 0.8		~90	-0.7 ± 0.4		$\gg 60$*
Capped, 9.5 kD						
w/15% rhGH	-1.4 ± 0.3	0.9	~90	-1.8 ± 0.4**	2.6	$\gg 60$*
Uncapped, 8 kD						
no rhGH	-13 ± 2		14–21	-5.0 ± 0.5		~35
Uncapped, 8 kD						
w/15% rhGH	-14 ± 4	1.1	~21	-5.3 ± 0.5	1.1	~35
Uncapped, 21 kD						
no rhGH	-8 ± 2		21–28	-4 ± 1		>60*
Uncapped, 21 kD						
w/15% rhGH	-9 ± 5	1.1	21–28	-4.9 ± 0.5	1.2	>60*

* Last time point taken.
** Microspheres remained dispersed for 28 days, in contrast to other samples.
# Errors are 95% confidence limits.

macrophages around the foreign body leading to a walling off of the region. Free radicals, acidic products, or enzymes produced by these cells during the foreign body response may accelerate degradation. Local temperature or pH changes due to inflammation may be enhanced by restricted transport of acidic PLG oligomers and other acidic or free radical compounds out of this region.

The degradation rate of PLG devices can also be affected by the size, shape, and porosity of the device [20, 21]. Grizzi et al. [21] showed that PLG device size affected the degradation rate with large size plates and millimeter-sized beads degrading faster than submillimeter films and particles. These authors attributed this to the reduced ability of acidic PLG oligomers to escape from larger or less porous devices. In our study, however, a constant mass of microspheres was tested in vitro and in vivo and the microspheres exhibited a tendency to agglomerate in both environments a short time after hydration.

Effect of polymer end group: Comparing the capped 9.5 and 12.7 kD polymer results in Table 2 to the uncapped 8 kD PLG (similar molecular weight but different end group) shows that the uncapped end group polymer degraded 2–3-fold faster in vitro and 3–4 times faster in vivo than the capped polymer. It was also found that microspheres made from the 21 kD uncapped polymer

degraded faster than those made from the lower molecular weight capped PLG. Smaller rate constants correlated with a longer duration observed in vivo and in vitro.

The uncapped PLG degraded faster than the capped in part because it took up water at a faster rate than the capped polymer [3]. Figure 3 shows the difference in initial water uptake in vitro for disks made from the 12.7 kD capped and 8 kD uncapped PLGs. Also, the uncapped polymer degradation was initially faster because the acidic end groups catalyze hydrolysis of the ester bonds resulting in the production of more acidic groups in an autocatalytic cycle.

Effect of molecular weight: Table 2 also shows that for the 8 and 21 kD uncapped polymers, the molecular weight had a much smaller effect than the end group on the degradation rate constant. The degradation rate for the 8 kD polymer microspheres was no different in vitro. It was 1.6 times faster in vivo than the 21 kD polymer microspheres. Because of its higher initial molecular weight, more time was required to break the latter polymer into soluble oligomers by hydrolysis. Thus, the higher molecular weight material was visible for a longer time at the injection site.

Capped polymer comparison: Table 2 includes the degradation results for capped polymers of similar molecular weight produced by two different manufacturers. The results show that the degradation in vivo and in vitro for these polymers are similar though there is a trend toward a slightly faster degradation for the 12.7 kD PLG. This may be due to a difference in the length of the alkyl ester end group between the two polymers. Carbon-13 NMR was performed on samples of the two capped polymers in order to identify the end groups. The data shows that the 9.5 kD capped PLG has a 6-hydroxyhexyl end group and the 12.7 kD polymer has only an ethyl ester on the end of the chain. These results suggest that the degradation rate of capped PLG's can be modulated by the choice of end group.

Effects of encapsulated salt and protein: The effect of zinc carbonate as an encapsulated excipient in the microspheres is compared in vivo and in vitro in Table 3. Addition of zinc carbonate to the microspheres resulted in about a two-fold decrease in the degradation rate for the capped polymer. On the other hand, zinc carbonate had little, if any, effect on the degradation rate of microspheres made with the uncapped polymers both in vivo and in vitro (Table 3), whether the amount of zinc carbonate was 1% or 6% (w/w). This result was also found for disks made from uncapped and capped polymers of similar molecular weight containing 10% (w/w) zinc carbonate. Figure 4 shows how the presence of zinc carbonate delays the onset of disk mass loss in vitro in the capped PLG, while the salt has no effect on the uncapped polymer. The mass loss was determined gravimetrically by weighing the disks at each time point after drying under vacuum to remove absorbed water.

Zinc carbonate has been found to enhance water uptake but decrease the rate of degradation of PLG films and disks [3–5]. This effect may be due to the neutralization of carboxylic acid groups formed during PLG hydrolysis by the basic salt. In an in vitro experiment where 12.7 kD capped polymer disks were incubated in a pH 7 buffer that was not changed over time, it was found that the buffer pH declined more slowly with zinc carbonate in the disks. There was no effect on the pH for 8 kD uncapped PLG disks containing zinc carbonate. One explanation for this difference may be that there are initially more acid end groups present during the hydration of the uncapped PLG.

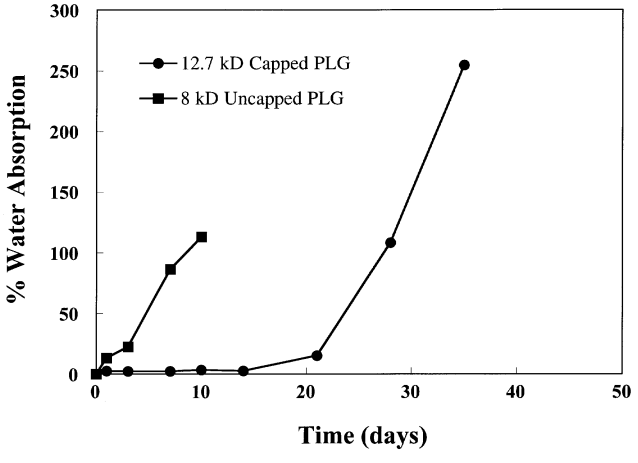

Fig. 3. The time dependence of water uptake into disks made from PLG with different end groups. The squares represent disks of the 8 kD uncapped polymer and the circles the 12.7 kD capped PLG. The disks were prepared as described in [3].

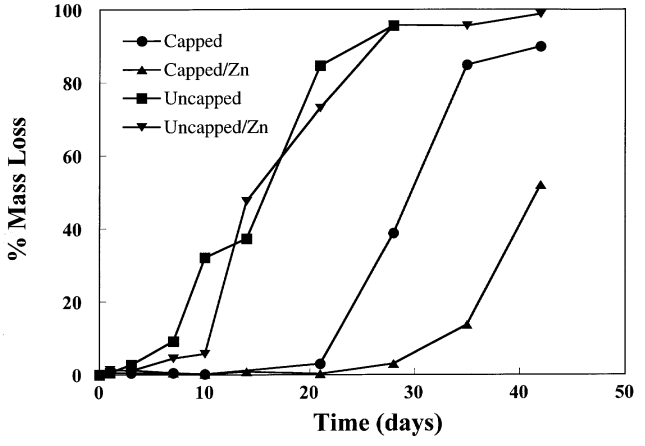

Fig. 4. The time dependence of the mass loss for disks made from 12.7 kD capped PLG (circles) and 8 kD uncapped PLG (squares) end groups. The effect of including 10% (w/w) zinc carbonate in the capped (triangles) and uncapped (upside down triangles) PLG disks is also shown.

Table 4 suggests that the rates of degradation of microspheres containing 15% (w/w) protein are similar to those without protein both in vivo and in vitro, at least for a highly water soluble protein such as rhGH. The higher rate observed in vitro for the capped PLG microspheres with zinc carbonate and rhGH may be due to the fact that these microspheres remained dispersed for greater than 28 days in vitro whereas, for the other samples, the microspheres agglomerated into a single mass after a short period of time. Zinc carbonate may dissolve and be released more rapidly from small, dispersed porous particles than from a larger mass. In fact, the degradation rate for this sample is equal to that of the same polymer without zinc carbonate (Table 2). In contrast, the in vivo degradation rate for these microspheres is virtually identical to the rate seen in the absence of rhGH, perhaps because in both cases the microspheres agglomerated in the subcutaneous space. Therefore, we do not believe the increased in vitro rate is due to the presence of rhGH.

4. Conclusions

PLG microsphere degradation was studied in vivo and in vitro. It was found that degradation was faster in vivo than in vitro in a HEPES buffer at a physiological pH and temperature. Of the polymer chemistry variables tested, the PLG end group had the greatest effect on degradation, with uncapped PLG degrading faster than capped. Though the molecular weight effect was considerably smaller than the end group effect, there was a trend toward faster degradation with the lower molecular weight polymer microspheres. Sparingly soluble salts such as zinc carbonate also retarded degradation in vivo and in vitro for capped but not for uncapped PLGs. The degradation rate was not significantly affected by the protein. These degradation studies have proved valuable in selecting the appropriate PLG polymer to achieve a target release duration in vivo.

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