[Chem. Pharm. Bull. 36(7)2576—2581(1988)]

In Vivo Release Profiles of Leuprolide Acetate from Microcapsules Prepared with Polylactic Acids or Copoly(Lactic/Glycolic) Acids and *in Vivo* Degradation of These Polymers

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(Received January 13, 1988)

To screen a suitable polymer for preparing a controlled-release dosage form effective for one month, square plates of polylactic acid (PLA) with average molecular weights of 6000 to 50000 and copoly(lactic/glycolic) acid (PLGA) with average molecular weights of 6000 to 13500 and a copolymer ratio of 90/10 to 55/45 were implanted in rats subcutaneously, and the weight losses were determined over 100 d. Typically the pattern of weight loss was biphasic with a lag time followed by a period when the weight fell exponentially. The lag times and the half lives of the degradation period increased with increase in the molecular weight or with decrease in glycolide content. Release of leuprolide acetate from PLA microcapsules at the injection site in rats (*in vivo* release) tended to be comparable to the bioerosion rate of the polymer used as the wall substance, and the *in vivo* release of the drug from PLGA microcapsules tended to be comparable to the biodegradation rate of the polymer. The *in vivo* release profile of the drug from microcapsules prepared with PLGA of average molecular weight 14000 and copolymer ratio 75/25 was ideal for one month's controlled release. The *in vivo* release profile from microcapsules injected subcutaneously in rats was comparable to the *in vitro* release.

Keywords——*in vivo* release; biodegradation; injectable controlled-release formulation; leuprolide acetate; polylactic acid; copoly(lactic/glycolic) acid; one month release; *in vitro–in vivo* release comparison

Polylactic acid (PLA) and copoly(lactic/glycolic) acid (PLGA) are biocompatible, biodegradable materials that are widely used in many controlled-release systems for pharmaceutical agents such as narcotic antagonists, local anesthetics, and steroid hormones.[1] Recently, some applications of PLGA as a carrier for peptide hormones, hydrophilic compounds which have low oral activity and require chronic daily injection, have increased interest in controlled-release technology.[2] It has been reported that the release of a hydrophilic drug from a bioerodible polymer matrix such as PLGA occurs in parallel with degradation of the polymer[3] and that the degradation rate of PLGA depends on the ratio of lactic and glycolic acid.[4] Long-term daily injection of leuprolide actate, a highly potent LHRH analogue, is obviously effective for the treatment of prostatic cancer.[5] We wished to prepare a controlled-release dosage form that would release leuprolide acetate at a constant rate for one month, and such that the polymer used for the wall substance would be completely eliminated from the injection site within two months. In the previous report we showed that the *in vitro* release rate of leuprolide acetate from PLA or PLGA microcapsules depended on the molecular weight of the polymer and the ratio of lactic and glycolic acid.[6] The most suitable polymer was a PLGA with an average molecular weight of 14000 and a molar ratio of lactic to glycolic acid of 75/25.

The purpose of the present study was to find a suitable polymer among PLAs and

PLGAs for a dosage form for controlled release of leuprolide acetate for one month by suitably controlled the *in vivo* degradation rate of the polymer and release rate of the drug. In addition, the *in vivo* and *in vitro* release patterns of the drug from microcapsules were compared.

Experimental

Materials——Leuprolide acetate (Lot M548-131) synthesized in the Chemical Development Laboratories of Takeda Chemical Ind., Ltd. (Osaka) was used. PLA with an average molecular weight of more than 20000 was synthesized by the method described in the previous paper,[7] the ring-opening polymerization of DL-lactide using a zinc catalyst. PLGA was also synthesized by the method described in the previous paper,[7] *i.e.*, a polycondensation process of lactic and glycolic acid using an acid ion-exchange resin catalyst; PLA with an average molecular weight of less than 20000 was synthesized by the same method. PLA-6000, PLA-12000, PLA-22500, PLGA(90/10)-21000, PLGA-(75/25)-14000, and PLGA(77/23)-12000 were purchased from Wako Pure Chemical Ind. (Osaka); purified gelatin from Nitta Gelatin Co. (Osaka); and Gosenol EG-40 (polyvinyl alcohol) from Nihon Synthetic Chemical Ind., Ltd. (Osaka). Other chemicals were of reagent grade.

Preparation of Microcapsules and Plates——PLA and PLGA microcapsules were prepared by an in-water drying method as described previously.[6] About 500 mg of leuprolide acetate and 80 mg of gelatin were dissolved in 1 ml of distilled water to make the inner water phase, and the solution was gradually poured under stirring into a solution of about 4000 mg of PLA or PLGA in 5.5 ml of methylene chloride (oil phase), to make a w/o emulsion. The emulsion obtained was cooled and poured into 400 ml of a 0.1% solution of polyvinyl alcohol in water to make a (w/o)/w emulsion. The oil phase was hardened to obtain microcapsules by evaporation of the methylene chloride. The microcapsules obtained were fairly spherical and a large number of micropores was observed on the surface, as shown in the previous paper.[7] The mean diameter of the microcapsules was about 20 μm and the range of particle size was 5 to 100 μm. The content of the drug in the microcapsules was about 10%.

PLA and PLGA plates were prepared by a hot-press method. These plates were cut into square plates of dimensions $10 \times 10 \times 1$ mm and the four corners were rounded with a file.

Determination of Leuprolide Acetate, Measurement of Average Molecular Weight and Copolymer Ratio of Polymer, and *in Vitro* Release Procedures——The methods used for determination of leuprolide acetate and the average molecular weight of the polymers and the *in vitro* release test were the same as described in the previous paper.[6] Leuprolide acetate was assayed by the high performance liquid chromatography (HPLC) procedure and the average molecular weight of the polymer was measured by gel permeation chromatography. Copolymers were dissolved in $CDCl_3$ and the integrated signal ratio between the methyl moiety of lactic acid and methylene moiety of glycolic acid was obtained from the nuclear magnetic resonance (NMR) spectrum. The copolymer ratio was calculated from the above ratio. The *in vitro* release pattern was determined by the rotating bottle method by using medium consisting of 1/30 M phosphate buffer, pH 7.0, containing 0.05% Tween-80.

***In Vivo* Erosion of PLA and PLGA Plates**——Plates were sterilized by immersion in a solution of benzalkonium chloride (Osvan, Takeda Chemical Ind.), and were implanted subcutaneously into the nuchal regions of 6-week-old SD male rats (one plate per rat). Animals were sacrificed at various times and the plate was removed. After drying under reduced pressure for 12 h, the plates were weighed and the average molecular weights of polymer taken from the inner and outer portions of the plates were measured.

***In Vivo* Release Study of Leuprolide Acetate from Microcapsules**——Microcapsules containing 0.9 mg of leuprolide acetate (about 9 mg as microcapsules) dispersed in 0.3 ml of the diluent (an aqueous solution containing 1% sodium carboxymethyl cellulose, 0.2% Tween-80, 0.14% methyl *p*-hydroxybenzoate, 0.014% propyl *p*-hydroxybenzoate, and 5% sorbitol) were injected subcutaneously into the nuchal region of 6-week-old SD rats. Animals were killed by aortic exsanguination at various times after administration of microcapsules and the injection site was excised and microcapsules remaining at the site were recovered. Leuprolide acetate in microcapsules remaining at the site of injection was quantified by HPLC. The polymer was extracted from the microcapsules with methylene chloride and the average molecular weight was measured by gel permeation chromatography.

Results and Discussion

Synthesis of Polylactic Acid and Copoly(Lactic/Glycolic) Acid

The average molecular weight and copolymer ratio of PLA and PLGA synthesized by the two methods are shown in Table I. In the ring-opening method, the reaction occurred instantaneously and PLA of various molecular weights could be obtained by regulation of the temperature; for example PLAs with average molecular weights of 20000 and 73000 were

TABLE I. Average Molecular Weight and Copolymer Ratio of PLA and PLGA Synthesized by Different Methods

Lot	Method of synthesis	Copolymer ratio (latic acid/glycolic acid)	Average molecular weight
08109-4	RO	100/0	50000
08212-1	RO	100/0	73000
08212-3	RO	100/0	63000
08303-1	RES	100/0	6800
08303-3	RES	78/22	10000
08303-4	RES	*ca.* 25/75	(10000)
08303-5	RES	54/46	12000
08304-5	RES	100/0	15000
08304-7	RES	55/45	20000
08305-8	RES	89/11	19000
a)	PC	100/0	6000
a)	PC	100/0	12000
a)	PC	100/0	22500
a)	PC	90/10	21000
a)	PC	75/25	14000
a)	PC	77/23	12000

RO, ring-opening method; RES, resin-catalyzing method; PC, polycondensation method without catalyst. *a)* Polymer synthesized by Wako Pure Chemical Ind.

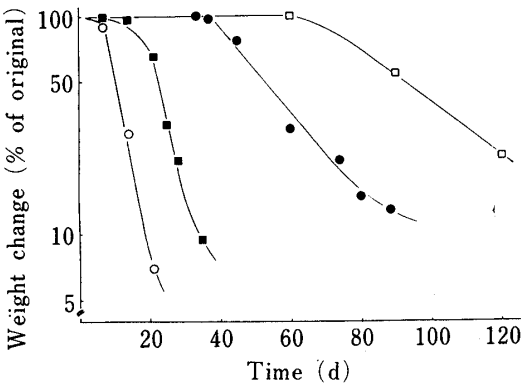

Fig. 1. Weight Changes of PLA and PLGA Implanted Subcutaneously in Rats

Square plates (10 × 10 × 1 mm) of each polymer were implanted in the nuchal area. Each point represents the mean of the results for three rats. □, PLA-42000; ●, PLA-22700; ■, PLGA(89/11)-13600; ○, PLGA(78/22)-8400.

obtained at 135 to 140 and 160°C, respectively. However, the Zn catalyst had to be removed from the polymer, and removal of the catalyst was difficult.

The polycondensation procedure, involving an ion exchange resin, could be used to synthesize copoly(lactic/glycolic) acid with any copolymer ratio, and the catalyst could be removed easily. However, polymers with an average molecular weight of more than 20000 could not be synthesized.

Bioerosion and Biodegradation of PLA and PLGA at Subcutaneous Implantation Site in Rats

Figure 1 shows semi-log plots of typical weight changes in PLA and PLGA after implantation of the polymer plates in three rats. The weight changes of each polymer had two phases: in the first phase the weight did not change (lag time), and in the second phase the weight changed remarkably (erosion phase). The half life of the erosion phase were calculated by applying a first-order degradation equation which was obtained from simulation of experimental points in the erosion phase and the lag time was obtained as the time when the plate weight was 100 in the equation. Table II shows the mean values of the lag times and the half lives with the standard errors. Average molecular weights in Table II are shown as those

TABLE II. Biodegradation of PLA and PLGA Implanted Subcutaneously in Rats

Lot	Copolymer ratio	Molecular[a] weight	Lag time (d)	Half-life[b] (d)
08109-4	100/0	42000	53.8 (2.9)	37.0 (2.7)
08212-3	100/0	24700	42.1 (2.9)	17.2 (1.5)
08212-1	100/0	22700	31.4 (1.6)	19.8 (1.0)
08304-5	100/0	14100	22.6 (0.2)	9.4 (0.9)
08303-1	100/0	5400	7.7 (0.9)	7.2 (0.5)
08305-8	89/11	13600	14.5 (0.3)	6.0 (0.4)
08303-3	78/22	8400	6.8 (0.3)	3.6 (0.2)
08304-7	55/45	13200	7.2 (0.1)	1.8 (0.1)
08303-5	54/46	4000	4.2 (0.1)	2.1 (0.1)
08303-4	*ca.* 25/75	5200	1.6 (0.3)	2.6 (0.3)

Square plates (10 × 10 × 1 mm) of each polymer were implanted in the nuchal area of rats. *a*) Before implantation. *b*) Estimated by simulating the weight change on the basis of a first-order degradation, mean (S.E.), $n=3$.

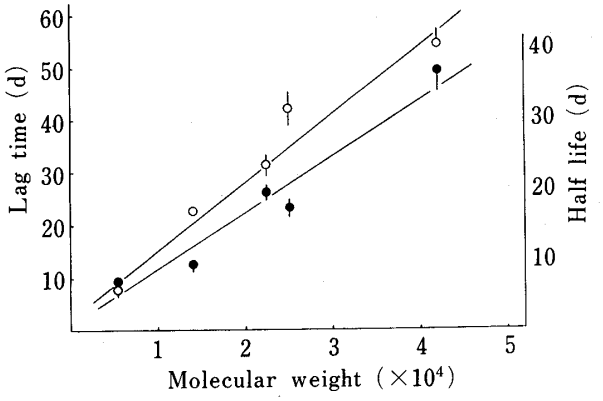

Fig. 2. Half Life and Lag Time of PLAs of Various Molecular Weights Implanted Subcutaneously in Rats

Each point represents the mean of the results for three rats with standard error. ●, half life; ○, lag time.

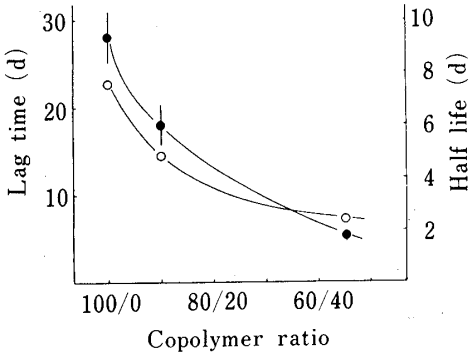

Fig. 3. Half Life and Lag Time of PLGAs with Various Copolymer Ratios Implanted in Rats

Each point represents the mean of the results for three rats with the standard error. ●, half life; ○, lag time.

just before implantation, because the molecular weights decreased during the hot-press process.

For PLA, both the lag time and the half life increased with an increase in molecular weight. The relationships between molecular weight and the lag time, and half life were linear, as shown in Fig. 2. As a wall substance, a polymer which is completely eliminated from the injection site within two months was required. A polymer which has a lag time of about 10 d and a half life of about 5 d, *i.e.* average molecular weight of 5000 to 6000, seemed most suitable for a dosage form for controlled-release of the drug for one month. For PLGA, both the lag time and the half life decreased with an increase in the content of glycolic acid. Figure 3 shows the correlation between bioerosion and the copolymer ratio of PLGA with an average molecular weight of about 13000. The gradient was steeper at a low content of glycolic acid than that at a high content and this profile is similar to that obtained by Miller *et al.*,[4] who calculated the degradation of the polymer from radioactivity of ^{14}C. The organic solvent solubility of PLGA whose copolymer ratio is less than 50/50 decreases with an increase in glycolic acid content.[3] PLGA (25/75)-5200 (Lot 08303-4) synthesized did not dissolve in any

of the solvents used, as described previously,[3] and therefore, its molecular weight could not be determined accurately; its molecular weight is shown in parenthesis in Table I. It was difficult to prepare microcapsules of PLGA (25/75)-5200 by an in-water drying method, since the polymer did not dissolve in any organic solvent. PLGA with a copolymer ratio of 70/30 to 80/20 and an average molecular weight of about 13000 is suitable as the wall substance of a controlled-release dosage form for one month, because the PLGA has a lag time of about 10 d and a half life of about 4 d.

The average molecular weights of the outer and inner portions of plates of PLGA (77/23)-12000 were 4400 and 4100 on day 7, respectively, and were 2300 and 1700 on day 12. There was no significant difference between the molecular weight of PLGA from the outer and inner portions at either time. On day 7, the plate had swollen to form an ovoid due to rehydration by biological fluid. From these results, it is concluded that biological fluid rapidly moves into the PLGA matrix and the hydrolysis of the polymer occurs simultaneously throughout the plate.

In Vivo Release of Leuprolide Acetate from Microcapsules of Various Polymers in Rats

The time courses of the release of leuprolide acetate from microcapsules injected into rats are shown in Fig. 4. Leuprolide acetate was scarcely released from microcapsules prepared with PLA-22500, PLA-12000, and PLGA (90/10)-21000 over the 4-week test period. Release of the drug was slightly greater from microcapsules prepared with PLA-6000, which was bioeroded more rapidly than the other three polymers. However, PLA-6000 was not suitable for a dosage form for controlled-release for one month. The *in vivo* release profile of leuprolide acetate from microcapsules prepared with PLA-6000 showed a linear decrease after a short lag time. This indicates that release of leuprolide acetate from the PLA-6000 microcapsules is a process dependent only on bioerosion (weight loss by dissolution) of the polymer. The profile for the release of leuprolide acetate from PLGA (75/25)-14000 microcapsules was approximately zero-order without a lag time over the 4-week test period. This indicates that release of the drug from the PLGA (75/25)-14000 microcapsules is not a process dependent only on bioerosion. On day 28 there were almost no microcapsules left at the injection site. From these results, it was concluded that the PLGA (75/25)-14000 microcapsules represent a suitable dosage form to meet the stated purpose of this study.

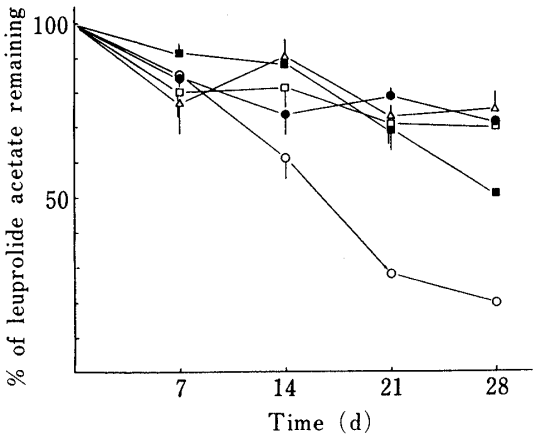

Fig. 4. Leuprolide Acetate Remaining after Subcutaneous Injection of PLA and PLGA Microcapsules to Rats

Each point represents the mean of the results for five rats with standard error. ●, PLA-22500; △, PLA-12000; ■, PLA-6000; □, PLGA(90/10)-21000; ○, PLGA(75/25)-14000.

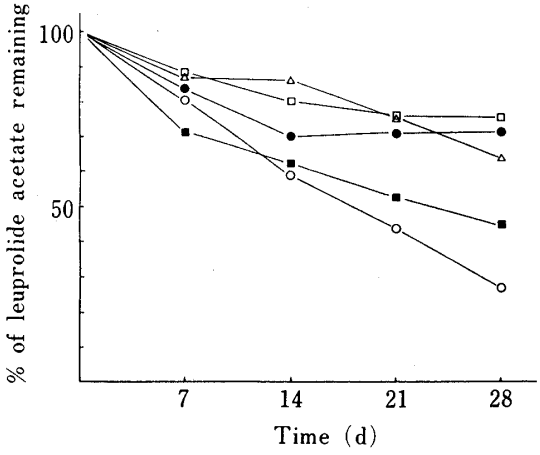

Fig. 5. Leuprolide Acetate after Immersion of PLA and PLGA Microcapsules into pH 7.0 Phosphate Buffer Solution at 37 °C

●, PLA-22500; △, PLA-12000; ■, PLA-6000; □, PLGA(90/10)-21000; ○, PLGA(75/25)-14000.

TABLE III. Comparison of *in Vitro* and *in Vivo* Release of Leuprolide Acetate and Degradation of PLGA(73/23)-12000

Time (d)	*In vitro*		*In vivo*	
	Leuprolide acetate remaining (%)	Average molecular weight of polymer	Leuprolide acetate remaining (%)	Average molecular weight of polymer
0	100	12000	100	12000
7	70	10500	79	10500
14	57	9400	60	8800
21	37	5600	37	5000
28	21	5000	30	2800
35	0	2600	20	2200

Comparison of *in Vivo* with *in Vitro* Release

Figure 5 shows the *in vitro* release of leuprolide acetate from microcapsules prepared with various polymers. The *in vitro* release patterns were similar to those *in vivo*. Release of the drug from microcapsules prepared with PLA-22500, PLA-12000, and PLGA (90/10)-21000 was slow; release from PLA-6000 microcapsules was slightly faster. Release from PLGA (75/25)-14000 approximately followed zero-order kinetics over 4 weeks.

The time courses of release of leuprolide acetate and the change in the average molecular weight of the polymer were determined in *in vitro* and *in vivo* release tests using microcapsules prepared with PLGA (77/23)-12000. As shown in Table III, the time courses of the release of the drug and the change in the molecular weight in the *in vivo* test were comparable to those in the *in vitro* test. The results show that the polymer is hydrolyzed by a non-enzymatic process and that the release of leuprolide acetate approximately correlates with degradation (the change in the molecular weight) of the polymer in the microcapsules when PLGA with the copolymer ratio of 75/25 is used as the wall substance.

Acknowledgements The authors are indebted to Dr. Hatanaka for supplying leuprolide acetate. We would like to thank Drs. K. Morita and Y. Sugino for their encouragement. We wish to thank Dr. P. Hardman for her linguistic advice.

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