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In vivo controlled release of a luteinizing hormone-releasing hormone agonist from poly(DL-lactic acid) formulations of varying degradation pattern

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Summary

Biodegradable formulations with a desired lag time were prepared from blends of low molecular weight poly(DL-lactic acid) (low MW-PLA, number-average molecular weight: $\overline{M}_n = 1400$, parabolic-type degradation pattern, 100% in vivo degradation at 5th week of implantation) and high molecular weight poly(DL-lactide) (high MW-PLA, $\overline{M}_n = 11\,500$, S-type degradation pattern with a lag time of 10 weeks). A luteinizing hormone-releasing hormone agonist (LH-RH agonist), des-Gly^{10}-[Leu^6]LH-RH ethylamide monoacetate, was incorporated into the small cylinders of PLA blends. The initial burst of drug release from cylindrical formulation, which was implanted subcutaneously in the back of male rats, could be controlled by adjusting the amount of high MW-PLA in the blend and, as a result, it was found by measuring the pharmacological influence on rat prostate and serum drug levels that the best efficacy and the most constant release over a long period are obtained with a 25/75% low MW-PLA/high MW-PLA blend.

Introduction

Biodegradable and biocompatible polyesters are applicable in a wide range of biomedical fields, e.g., surgical sutures (Chu, 1982; Fredericks et al., 1984), osteoplastic materials (Zimmerman et al., 1987; Chegini et al., 1988; Hay et al., 1988), and carriers for drug delivery systems (Maulding et al., 1986; Bodmeier et al., 1989; Cha and Pitt, 1988; Hecquet et al., 1988; Schakenraad et al., 1988; Tencer et al., 1989). The biodegradability of the polyesters can be controlled by various parameters such as copolyester ratio, molecular weight, molecular weight distribution, crystallinity, kind of polyester, hydrophilicity, and sequence of poly-

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ester (Asano et al., 1984, 1985, 1989a,b,c; Fukuzaki et al., 1989a,b).

The drug release from biodegradable polyesters is often accompanied by initial rapid release, called an initial burst effect, especially in a parabolic-type biodegradable polyester. In contrast, the initial drug release is too slow in an S-type biodegradable polyester (Asano et al., 1990). The object of this investigation is to design a biodegradable polyester formulation with a constant release of drug throughout the desired experimental period. For this purpose, we have prepared small cylinders of biodegradable formulations consisting of blends of low MW-PLA with a typical parabolic-type degradation pattern and high MW-PLA with a typical S-type degradation pattern and the resulting constant release over a long period could be obtained by adjusting the amount of high MW-PLA in the blend. In this study, the confirmation of drug release was also performed by measuring the pharmacological influences on rat prostate and serum drug levels, in which the small cylinders of polymer blends containing LH-RH agonist were implanted subcutaneously in the back of male rats.

Materials and Methods

Materials

DL-Lactic acid (Kanto Chemical Co., Ltd), DL-lactide (C.V. Chemie Combinatie, Amsterdam), and LH-RH agonist (Takeda Chemical Industries, Ltd) (Yoshida et al., 1986) were used.

Methods

Low MW-PLA ($\overline{M}_n = 1400$) was synthesized by direct polycondensation of DL-lactic acid in the absence of catalysts by bubbling nitrogen gas through the reaction vessel at 200 °C for 5 h (Asano et al., 1990). The colorless-transparent products obtained were used without further purification. In a high MW-PLA ($\overline{M}_n = 11\,500$), the synthesis was performed by ring-opening polymerization of DL-lactide (10 g) using stannous octoate (0.003 g) as catalyst and lauryl alcohol (0.2 g) as molecular weight moderator at 180 °C for 2 h in vacuo (Asano et al., 1990), in which it was purified by precipitating the chloroform-soluble product in methanol to remove the impurities.

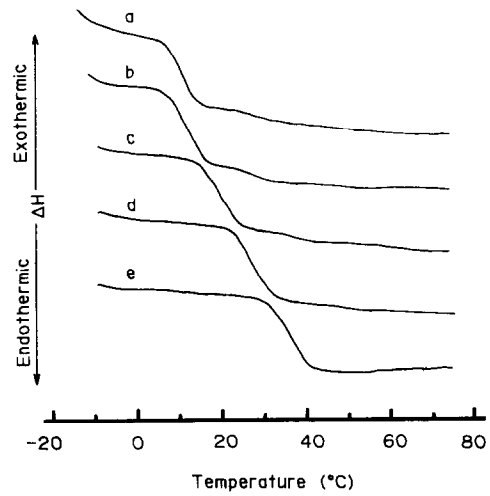

Fig. 1. DSC curves of blends of low and high MW-PLAs which were previously melt-treated at 200 °C; low MW-PLA/high MW-PLA (wt%): (a) 100:0; (b) 75:25; (c) 50:50; (d) 25:75; (e) 0:100.

The blends with the 100:0, 75:25, 50:50, 25:75, and 0:100 wt% low MW-PLA/high MW-PLA compositions were melt-treated at 200 °C for 2 min to obtain a homogeneous mixture, allowed to cool to room temperature, and crushed. The glass transition temperature (T_g) of blended PLAs was determined with a Seiko differential scanning calorimeter (DSC), Model DSC-10, at a heating rate of 5 °C/min. The DSC curves of blends of low and high MW-PLAs are shown in Fig. 1, in which they display only a single T_g, supporting the existence of the monodisperse system.

The molecular weight of PLAs was measured by gel permeation chromatography (GPC). The measurements were performed with a Waters Model ALC-244 high-performance liquid chromatograph at 25 °C at a flow rate of 1 ml/min throughout 10^2, 10^3, 10^4, and 10^5 Å Waters Ultrastyragel columns, in tetrahydrofuran. The values of $\overline{M}_n$ and weight-average molecular weight ($\overline{M}_w$), which were calibrated by the use of standard polystyrene (Asano et al., 1989a), were found to be 1400 and 3800 for a low MW-PLA and 11 500 and 22 400 for a high MW-PLA, respectively.

A schematic diagram for the preparation of small cylinders of blended PLA formulations containing LH-RH agonist is shown in Fig. 2. A mixture of blended PLA (45 mg) with a desired composition and LH-RH agonist (5 mg) was melt-treated in the range of about 70–100 °C to obtain a homogeneous mixture, then allowed to cool to 20 °C, and crushed. The crushed mixture was charged into a commercially available poly(tetrafluoroethylene) tube of 2 mm inner diameter and piston rods were inserted from both sides of the tube under a pressure of 100 kg/cm^2 at 40–60 °C by a so-called melt-pressing technique (Asano et al., 1984), resulting in the formation of a solid formulation in fine cylindrical form (2 mm in diameter, 10 mm long). The formulation tube was sterilized by irradiation at up to 30 kGy at low temperature (dry ice temperature: −78 °C) with γ-rays from a ^{60}Co source. The recovery of LH-RH agonist was determined in order to ascertain its loss (or inactivation) during the shaping treatment and radiation sterilization, according to a radioimmunoassay method (Yamazaki and Okada, 1980) and the results obtained corresponded to recoveries of 90% or above. On the other hand, the degradation and cross-linking of pure copolymer during γ-ray irradiation were checked by measuring the change of molecular weight ($\overline{M}_w$ and $\overline{M}_n$) using GPC equipment and, as a result, it was confirmed that no change in molecular weight of the copolymer occurred under the experimental conditions used in this study.

In the in vivo experiments, the small cylinders of blended PLA formulations with and without drugs were implanted subcutaneously in the back of male adult Wistar strain rats weighing 350–400 g (one formulation per rat, 5 rats per group), in order to determine the in vivo daily dose of LH-RH agonist from the formulation, the serum LH-RH agonist level, and the pharmacological influence on rat prostate induced by the administration of the drug delivery system (Yoshida et al., 1986). For these purposes, at fixed time intervals, the implants were excised from killed rats, freed of surrounding connective tissue, pooled, dried in vacuo, and then weighed. The degree of in vivo

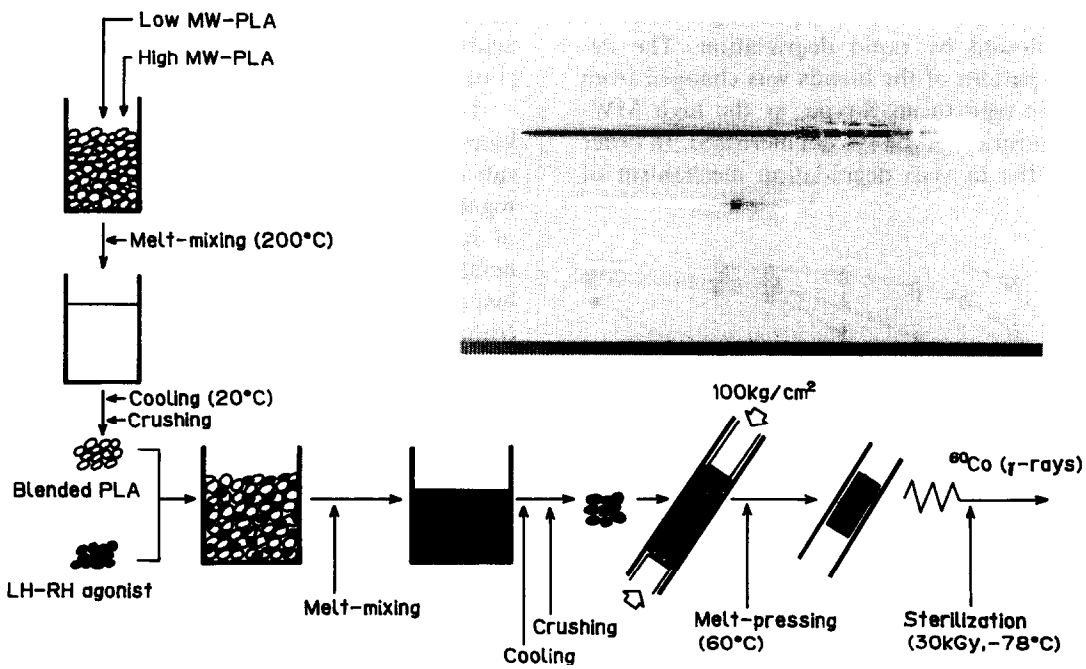

Fig. 2. Schematic diagram illustrating the preparation of small cylinders of blended PLA formulations containing LH-RH agonist by the melt-pressing technique.

degradation of pure PLA was evaluated from the ratio of the weight loss of the carrier after treatment and the initial weight. The daily dose of LH-RH agonist released in vivo from the formulation was estimated from the amount of drug remaining in the formulation collected from killed animals every week, in which the drug concentration was measured by both radioimmunoassay and spectrophotometrical assay at 278 nm with a Hitachi U-3210 spectrophotometer. The pharmacological influence was evaluated from the change in weight of the ventral prostate (VP) as an accessory sex organ. This weight is expressed as mg per 100 g body weight at killing (mg/100 gbw).

Results and Discussion

The in vivo degradation profiles of pure PLA are shown in Fig. 3. In a low MW-PLA with $\overline{M}_n = 1400$, it showed a typical parabolic-type degradation pattern which is characterized by initial rapid degradation, giving 100% degradation after 5 weeks implantation. In contrast, a high MW-PLA with $\overline{M}_n = 11\,500$ had a typical S-type degradation pattern, characterized by an initial lag time of 10 weeks, followed by rapid degradation. The degradation pattern of the blends was changed from a parabolic-type to an S-type, as the high MW-PLA composition in the system increased. In order to clarify the in vivo degradation mechanism of

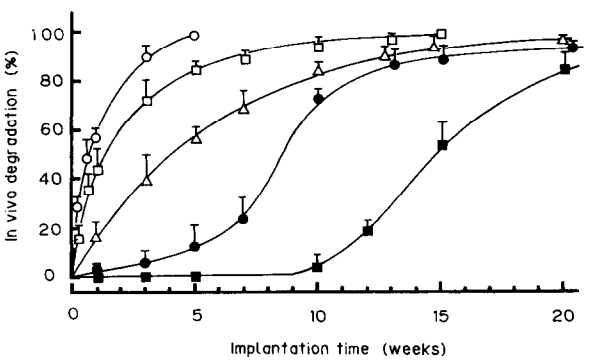

Fig. 3. In vivo degradation profiles of small cylinders of blended PLA formulations without drugs after subcutaneous implantation into the backs of rats; low MW-PLA/high MW-PLA (wt-%): (○) 100:0; (□) 75:25; (△) 50:50; (●) 25:75; (■) 0:100. Data are shown as mean ± SD ($n = 5$).

blends of low and high MW-PLAs, the changes in molecular weight distribution were investigated by GPC, as seen clearly in Fig. 4. The molecular weight distribution of pure low MW-PLA was transformed from a broad peak into a sharp peak with passage of time, due to the preferential degradation of lower molecular weight polymer (Fig. 4a). For a pure high MW-PLA, it showed a different degradation mechanism, in which the peak is gradually shifted to the left with time resulting in the formation of lower molecular weight polymer (Fig. 4e). This effect means that the scission of the high MW-PLA chain occurred preferentially during the induction period (lag time). In a blend, the molecular weight distribution consists generally of two peaks assigned to low and high MW-PLAs, as expected from the degradation involving the additivity of each PLA. In practice, however, the rate of in vivo degradation of the blend is faster than that of each PLA, especially for high MW-PLA (Fig. 3). Possibly this is closely related to the increase in acidity owing to dissolution or hydrolysis of oligomers in the interior of small cylinders, resulting in the acceleration of the scission of the high MW-PLA chain. A similar mechanism has been already found in copoly(D-lactic acid/L-lactic acid) with a typical S-type degradation pattern (Fukuzaki et al., 1989c).

The appearances of small cylinders of PLA blends, implanted subcutaneously in the back of rats over a period of 5 weeks, are shown in Fig. 5, together with results of optical microphotographs of tissues surrounding the implants stained with hematoxylin and eosin. The changes in shape and histopathological findings are markedly distinct from the ratios of low and high MW-PLAs. The histopathological findings of tissue surrounding a pure low MW-PLA cylinder, which gave 100% degradation, showed that the carrier is completely absorbed and replaced by granulation tissue consisting of foaming histiocytes and multinucleated giant cells accompanying the formation of a fibrous capsule around the granulation tissue (Fig. 5a). For a 75%/25% low MW-PLA/high MW-PLA blend with 86% of in vivo degradation, the connective tissue, consisting of fibroblast, collagen fiber, and capillaries, is reticularly invaded by the subcutaneously implanted carrier and many foamy

histiocytes are seen around the implant (Fig. 5b). The fibrous connective tissue is formed around the subcutaneously implanted polymer and a small amount of connective tissue is invaded by the implant for a 50%/50% low MW-PLA/high MW-PLA blend with 58% of in vivo degradation (Fig. 5c). The multinucleated giant cells accompanying the formation of the fibrous capsule are seen for a 25%/75% low MW-PLA/high MW-PLA blend with 9% of in vivo degradation (Fig. 5d). In contrast, for a pure high MW-PLA with no degradation, only a fibrous capsule is formed around the implant (Fig. 5e).

The in vivo daily dose of LH-RH agonist released from small cylinders of blended PLA formulations with different polymer compositions is

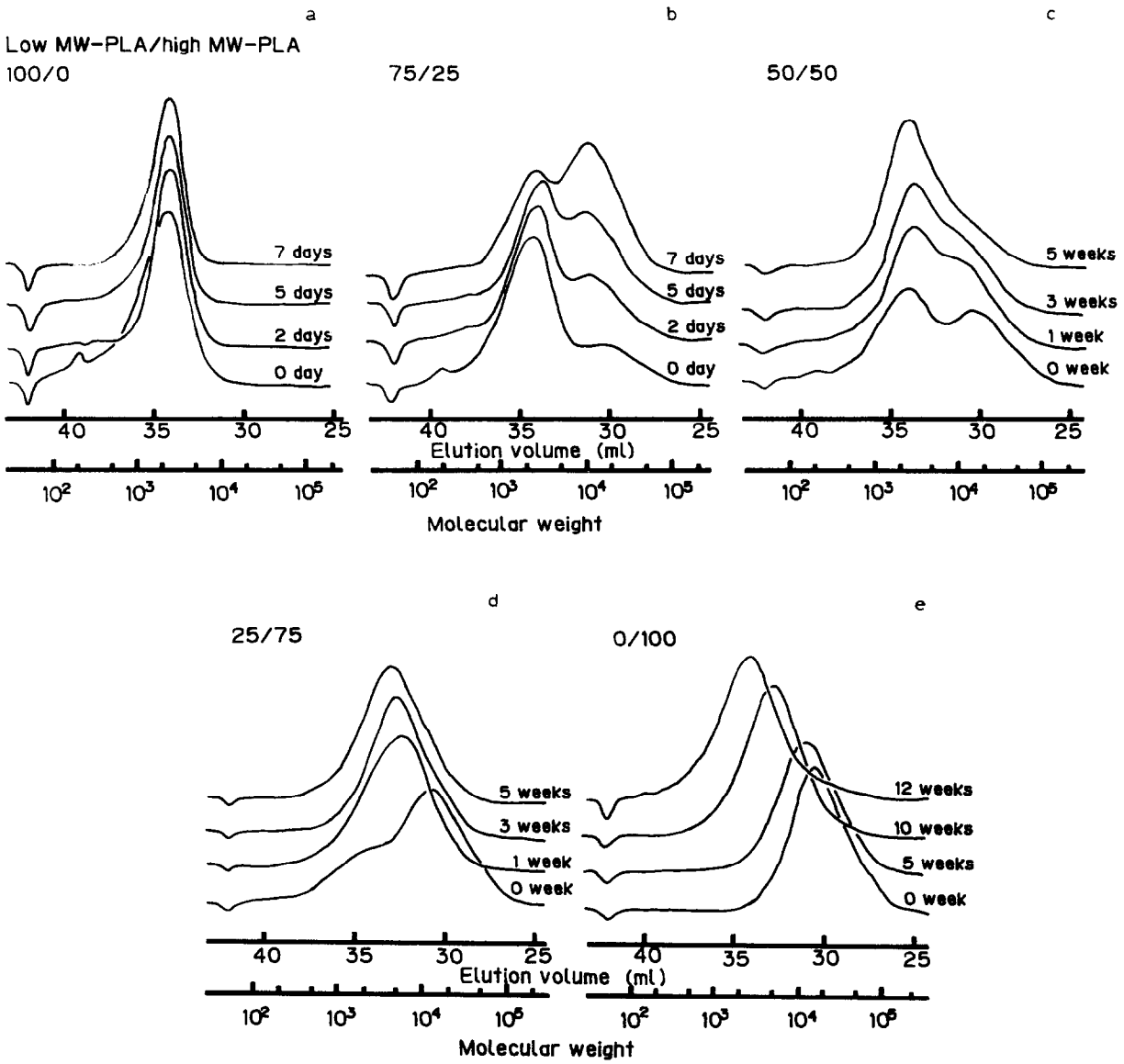

Fig. 4. Changes in molecular weight distribution of blended PLAs as functions of polymer composition and implantation time. The blends (no drug) in small cylinder form were implanted subcutaneously in the backs of rats for the desired period of time and then used for the measurement of molecular weight distribution by GPC. Molecular weights shown refer to that of standard polystyrene.

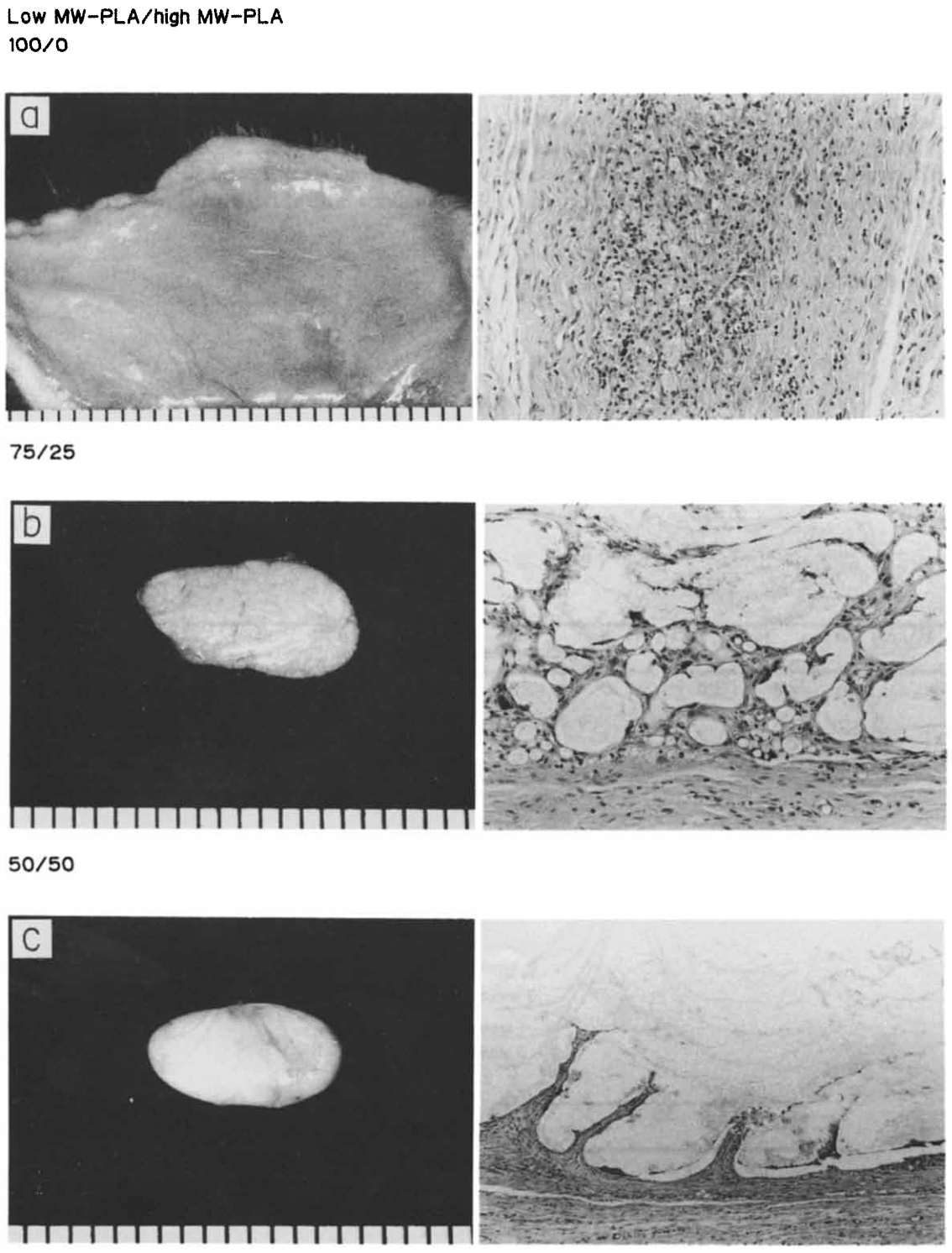

Fig. 5. Appearances of blended PLAs after subcutaneous implantation into the backs of rats for 5 weeks as a function of polymer composition and optical micrographs of tissue surrounding the implants (stained with hematoxylin and eosin). The implants were freed of surrounding connective tissue, pooled, and dried, except for a pure low MW-PLA (only subcutaneous tissue is shown because of 100% degradation within 5 weeks). Degree of in vivo degradation (%): (a) 100; (b) 86; (c) 58; (d) 9; (e) 0 (no weight loss).

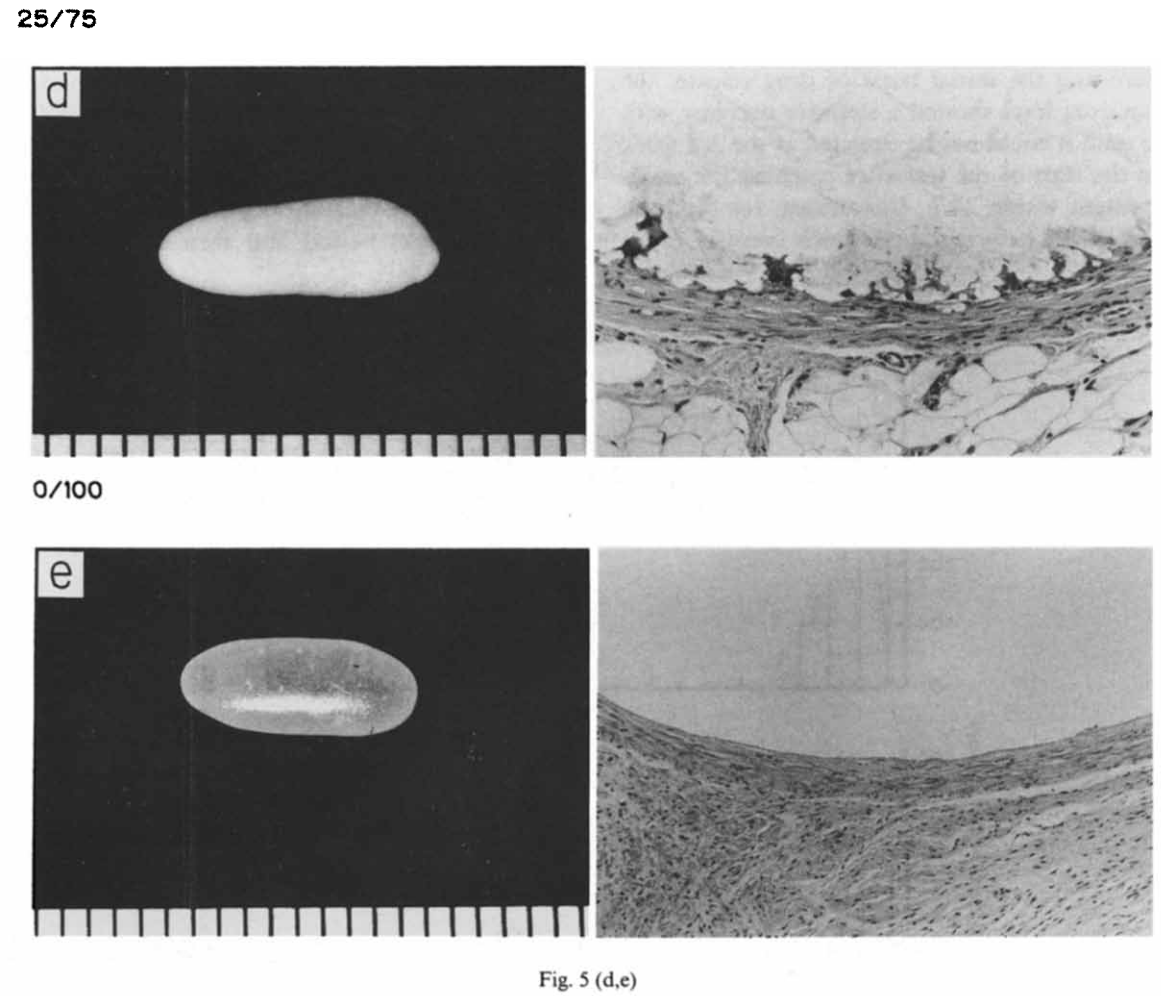

Fig. 5 (d,e)

shown in Fig. 6. The burst effect of drug release within a short time was observed in a cylindrical formulation prepared from pure low MW-PLA of typical parabolic-type degradation pattern (Fig. 6a). In this formulation, no constant release was maintained. Such an initial rapid release of drug should be retarded by incorporating high MW-PLA into the blend. This incorporation clearly led to the initial slow release and the resulting drug release period was prolonged, as the high MW-PLA composition increased (Fig. 6b–d). However, in a pure high MW-PLA formulation of typical S-type degradation pattern, a lag time with no drug release was found in the initial stage, and was followed by a slow release (Fig. 6e). It is obvious from these findings that the drug release at a relatively constant rate can be controlled by adjusting the amount of high MW-PLA and, as a result, the most reasonable release was obtained for a 25% low MW-PLA-containing blend (Fig. 6d), in which its rate was apparently kept constant at 68 ± 32 μg/day during the first 11 week period, followed by a gradual release at approx. 6 μg/day over a period of 10 weeks from the 11th to 20th week.

The serum drug level was determined in order to confirm the controlled drug delivery. This is clearly demonstrated in Fig. 7 which shows the changes in serum LH-RH agonist level in male rats implanted with small cylinders of blended

PLA formulations. In a pure low MW-PLA formulation with both the parabolic-type degradation pattern and the initial burst of drug release, the serum drug level showed a stepwise decrease with time until it could not be detected at the 3rd week from the start of the test after reaching the maximal extent within 24 h. In contrast, for the pure high MW-PLA formulation which consists of an S-type degradation pattern and lag time with no drug release, the low serum level of drug was maintained during the first 10 weeks period, followed by a high level. As a result, the most reasonable serum level of drug was maintained in a 25%/75% low MW-PLA/high MW-PLA blend, remaining constant at 90 ± 30 ng/ml during the first 10 weeks period and then at approx. 0.14

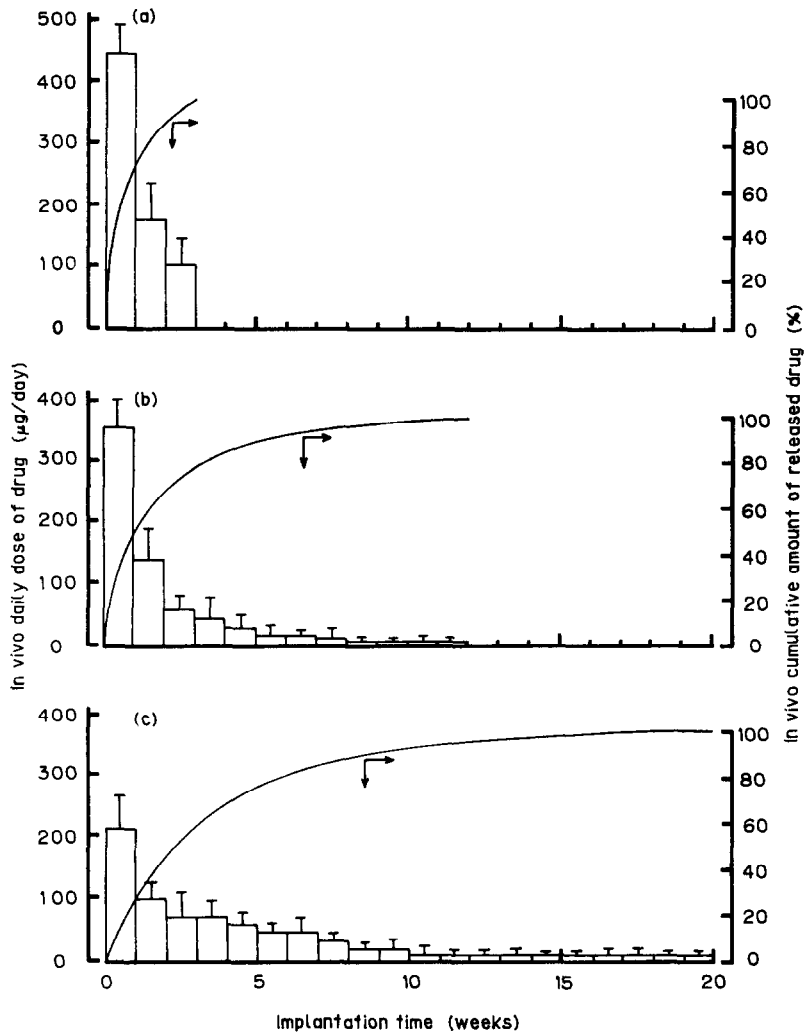

Fig. 6. In vivo daily dose of LH-RH agonist released from small cylinders of blended PLA formulations with different polymer compositions. The formulations were implanted subcutaneously in the backs of rats at the start of the experiment, and then excised from killed animals every week. The amount of drug released in vivo was estimated from the amount of drug remaining in the formulation; low MW-PLA/high MW-PLA (wt%): (a) 100:0; (b) 75:25; (c) 50:50; (d) 25:75; (e) 0:100. Data are shown as mean $\pm$ SD ($n = 5$).

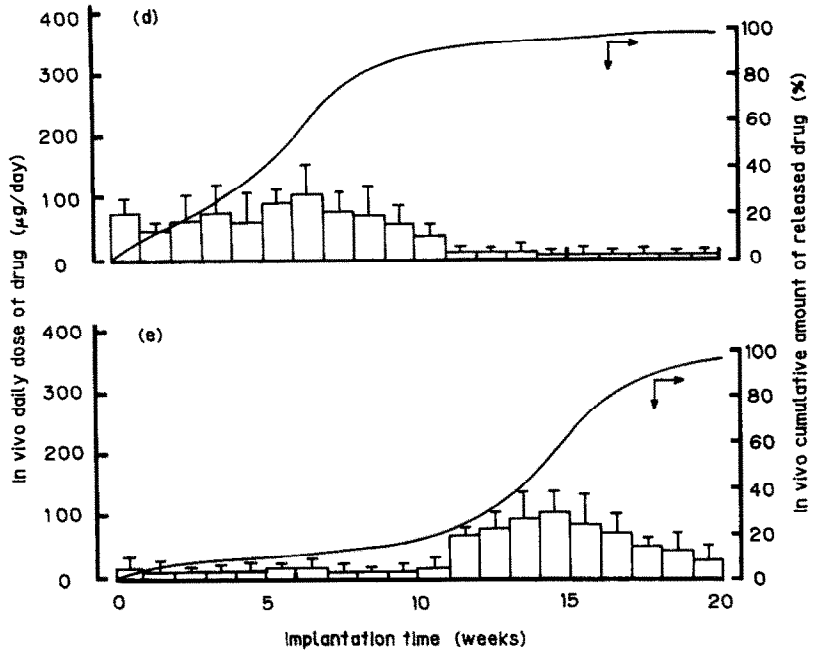

Fig. 6 (continued).

ng/ml for a period of 9 weeks from the 11th to the 20th week. These effects agreed very closely with the in vivo controlled drug delivery.

The pharmacological influence on rat prostate induced by the action of LH-RH agonist released from small cylinders of blended PLA formulations

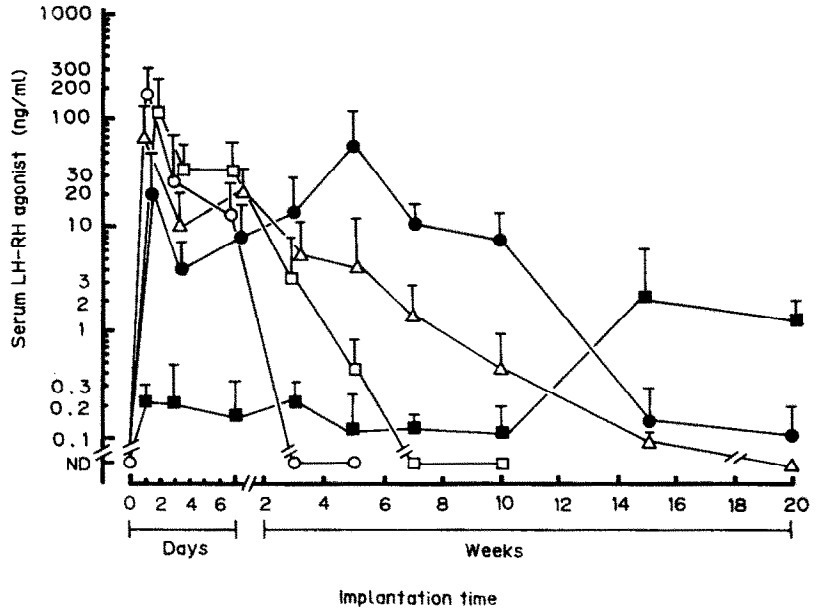

Fig. 7. Changes in serum LH-RH agonist levels in male rats implanted with small cylinders of blended PLA formulations as a function of polymer composition; low MW-PLA/high MW-PLA (wt%): (○) 100 : 0; (□) 75 : 25; (△) 50 : 50; (●); 25 : 75; (■) 0 : 100. Data are shown as mean ± SD ($n = 5$).

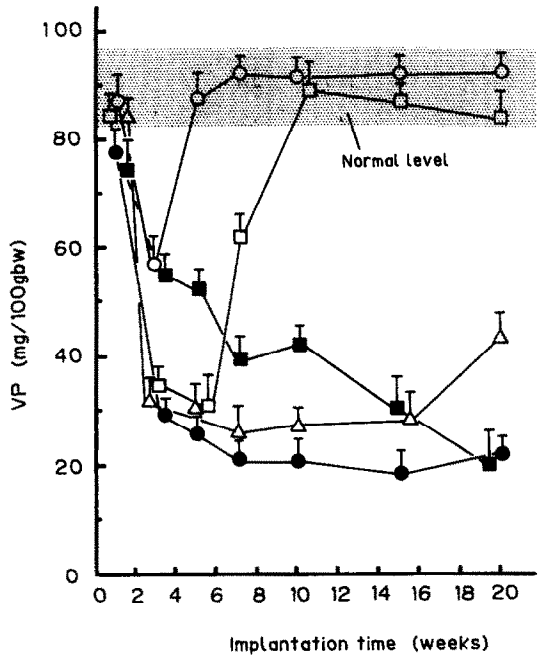

Fig. 8. Changes in weight of ventral prostate (VP) following subcutaneous implantation into male rats with small cylinders of blended PLA formulations as a function of polymer composition. Symbols refer to polymer composition given in Fig. 7. Data are shown as mean ± SD ($n = 5$).

was examined by changing in weight of VP, as seen in Fig. 8. In general, the weights of VP decreased until the castrate weight was reached by controlled drug release administration, followed by a rebounding effect, and finally recovered to reach normal organ weight. Of these formulations, the strongest pharmacological influence was observed with a 25%/75% low MW-PLA/high MW-PLA blend, in which the rate of change in the VP weight corresponded to that seen following castration.

In conclusion, the S-type biodegradable formulation with the desired lag time was successfully prepared from blends of low MW-PLA ($\overline{M}_n$ = 1400, parabolic-type degradation pattern, 100% degradation at 5th week of implantation) and high MW-PLA ($\overline{M}_n$ = 11 500, S-type degradation pattern with a lag time of 10 weeks). The drug release was dependent on the amount of high MW-PLA used. By measuring the pharmacological influence on rat prostate and serum drug levels, it was proved that the best efficacy and the most constant release over a long period are observed with a 25%/75% low MW-PLA/high MW-PLA blend.

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