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RESEARCH PAPER

In Vivo Characteristics of Injectable Poly(DL-Lactic Acid) Microspheres for Long-Acting Drug Delivery

Daishiro Kobayashi,[1] Satoru Tsubuku,[2] Hidetoshi Yamanaka,[1] Masaharu Asano,[3] Masaharu Miyajima,[2] and Masaru Yoshida[3,*]

[1]*Department of Urology, Gunma University School of Medicine, Maebashi, Gunma 371, Japan*
[2]*Department of Pharmaceutical Research, Central Research Laboratories, Zeria Pharmaceutical Co., Ltd., 2512-1 Oshikiri, Kohnan-machi, Ohsato-gun, Saitama 360-01, Japan*
[3]*Department of Material Development, Takasaki Radiation Chemistry Research Establishment, Japan Atomic Energy Research Institute, 1233 Watanuki-machi, Takasaki, Gunma 370-12, Japan*

ABSTRACT

Poly(DL-lactic acid) (PLA) microspheres containing testosterone (T) were prepared by the solvent evaporation process to evaluate their physical properties such as size distribution, shape, drug content, in vivo controlled drug release, pharmacological influences on the prostate gland in castrated rats, and histopathological findings of tissues surrounding the implants. The in vivo release of T from PLA microspheres containing 30 mg of drug obtained with chloroform was continued over a 6-week period. This effect is attributed to high dispersibility of T in the device when obtained with chloroform. Both serum drug levels and prostate gland weight recovery suggested the effects of a long-acting drug delivery system. The histopathological findings showed that the devices used were completely degraded 10 weeks after injection.

* To whom correspondence should be addressed.

INTRODUCTION

It is well-known that testosterone (T) replacement therapy improves aspects of sexual function in men, e.g., libido, erection, ejaculation, and frequency of sexual intercourse (1). T therapy has thus far been administered by intramuscular injection once every 2 weeks, during which time serum drug levels became greater than the normal physiological range in the initial stage of administration and lower than normal physiological levels at the end of the dosing period before the next injection. To achieve more constant levels, Meikle et al. developed the permeation-enhanced T transdermal system and reported that applying the T transdermal system for 24-hr at the physiological drug level showed the normal circadian patterns noted in healthy men (2). We performed T replacement therapy for male hypogonadism using a long-acting implantable testicular prosthesis which consists of a nonbiodegradable, biocompatible, and hydrophilic copolymer gel of 2-hydroxyethyl methacrylate and polyethylene glycol dimethacrylate at an average serum drug level of more than 200 ng/dl during a maximum period of 48 months (3–5). In contrast to the above nonbiodegradable materials, we also developed a new method to synthesize biodegradable polymer materials with a relatively low molecular weight by means of direct polycondensation without the use of any catalyst, e.g., poly(L-, D-, and DL-lactic acids), poly(glycolic acid), poly(γ-butyrolactone), and poly(ϵ-caprolactone) (6–11). We studied the application of these biodegradable polymers in drug delivery systems (12,13).

In the present study, we used poly(DL-lactic acid) (PLA) with a relatively low molecular weight obtained by direct polycondensation. Microspheres containing T were prepared by the solvent evaporation process to evaluate their physical characteristics with regard to the in vivo release of drugs, serum drug level, pharmacological influences on the prostate gland in castrated rats, and histopathological findings of tissues surrounding the implants.

MATERIALS AND METHODS

Materials

T, 90% aqueous solution of DL-lactic acid (LA), poly(vinyl alcohol) (PVA) (degree of polymerization, 500; degree of saponification, 86.5–89.0 mol%) were purchased from Sigma Chemical Co., St. Louis, Mo; Wako Pure Chemical Industries, Ltd. (Tokyo, Japan); and Kanto Chemical Co., Inc. (Tokyo, Japan), respectively.

Synthesis of PLA

PLA was synthesized by directly polycondensing a 90% aqueous solution of LA at 200°C for 20 hr without the use of a catalyst. Nitrogen gas was bubbled into the solution at a rate of 200 ml/min during the reaction, according to a previously reported procedure (14).

The molecular weight of the PLAs obtained was measured with a Waters ALC-244 (Waters Associates, Milford, MA) gel permeation chromatograph at 25°C and a flow rate of 1 ml/min using 10^2, 10^3, and 10^4 Å Waters ultrastyra gel columns in tetrahydrofuran. The number-average molecular weight (M_n), weight-average molecular weight (M_w), and molecular weight distribution (M_w/M_n) were found to be 5100, 11730, and 2.3., respectively; these values were calibrated by the use of standard poly(styrene) (15).

Preparation of Drug-Loaded PLA Microspheres

A schematic diagram illustrating the preparation of PLA microspheres containing T as a drug by the solvent evaporation process is shown in Fig. 1. A homogeneous solution consisting of 1 g of T, 2 g of PLA, and 10 ml of solvents such as dichloromethane and chloroform was added dropwise to 200 ml of a 1% aqueous solution of PVA. The mixed solution was stirred at 400 rpm at 15°C until the solvent was evaporated. The drug-loaded PLA microspheres were collected by filtration, followed by a lyophilization step.

The average particle diameter of the drug-loaded PLA microspheres was measured with a Coulter TA-II

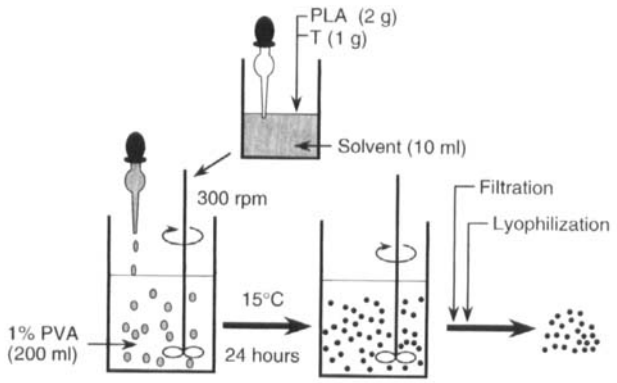

Figure 1. Schematic diagram illustrating the preparation of drug-loaded PLA microspheres by the solvent evaporation process.

counter (Coulter K.K., Tokyo, Japan), and the shape was observed with a Jeol JXA-733 (Jeol Ltd., Tokyo, Japan) scanning electron microscope. The miscibility of T and PLA in the microsphere was evaluated with a Seiko DSC-10 (Seiko Instrument Inc., Tokyo, Japan) differential scanning calorimeter (DSC) at a heating rate of 5°C/min.

Animal Experiments

The size distribution of drug-loaded PLA microspheres is shown in Fig. 2. The microspheres obtained with dichloromethane showed a wide distribution of sizes of 53–212 μm, in contrast to a relatively narrow distribution of 75–150 μm for those obtained with chloroform. In this study, the devices with particle sizes of 75–150 μm were employed for animal experiments. The amount of T loaded in the device was spectrophotometrically assayed at 249 nm after the drug was completely extracted with ethanol at 37°C. The results obtained were 10 mg of T in 36 mg in the device obtained with dichloromethane (loading 28%) and 10 mg of T in 26 mg in the device with chloroform (loading 38%).

PLA microspheres containing 10 or 30 mg of T previously suspended in 1.5 ml of a mixture consisting of 50 ml of distilled water, 2 g of D-mannitol, 0.4 g of sodium carboxymethylcellulose, and 0.04 ml of Tween 80 were injected subcutaneously in the back of castrated male Wistar strain rats weighing approximately 300 g. This injection was performed 3 weeks after castration to allow sufficient atrophy of the prostate gland.

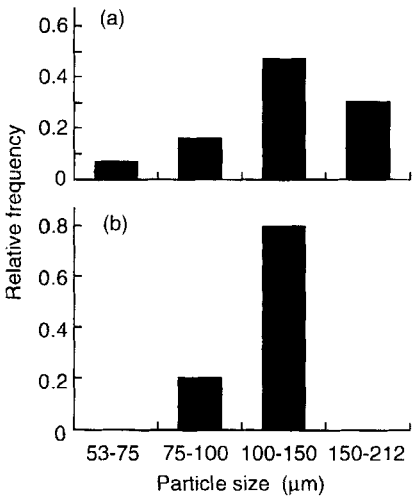

Figure 2. Size distribution of drug-loaded PLA microspheres obtained by the solvent evaporation process using (a) dichloromethane and (b) chloroform.

To evaluate the amount of T released in vivo from PLA microspheres, the implants were excised from rats (five rats per group). In these animals, the implants became a big mass under the back skin immediately after injection, as shown in Fig. 5. This was due to the low glass transition temperature (T_g) of the PLAs used. The excised device was immersed into ethanol at 37°C to extract T, and the drug concentration in the medium was assayed spectrophotometrically at 249 nm. The amount of drug released in vivo was thus estimated from the amount of drug remaining in the device.

The serum T concentration was measured by radioimmunoassay according to the method of Makino (16).

The efficacy of T released in vivo from PLA microspheres was evaluated by the changes in weight of ventral prostates (VP), dorsolateral prostates (DLP), and right-side seminal vesicles (SV), which were excised separately from sacrificed rats, pooled after being freed from surrounding connective tissues, and then weighed (17). These weights are expressed as milligrams per 100 g of gross rat body weight at sacrifice (mg/100 gram body weight [gbw]).

Microscopic Observations

The tissues surrounding the drug-loaded PLA microspheres and VP were preserved in 10% buffered formalin. For the purpose of optical microscopy, they were embedded in paraffin, sectioned at approximately 4 μm, and stained with hematoxylin and eosin.

RESULTS AND DISCUSSION

Physical Properties of Drug-Loaded PLA Microspheres

The drug-loaded PLA microspheres were prepared by the solvent evaporation process using such solvents as dichloromethane (boiling point 39.95°C) and chloroform (boiling point 61.15°C). The amount of T loaded in microspheres is influenced by the kind of solvent; 28% T was found with dichloromethane and 38% with chloroform. To clarify the cause of this difference, the surface structure of microspheres was observed microscopically. Results are shown in Fig. 3. For dichloromethane, a number of small drug crystals were deposited on the surface of the device, but no such crystals were evident inside the device. In contrast to this, drug-loaded PLA microspheres obtained with chloroform showed a highly homogeneous and molecular dispersion of T not only outside but also inside the device. Figure 4 clearly shows the DSC curves

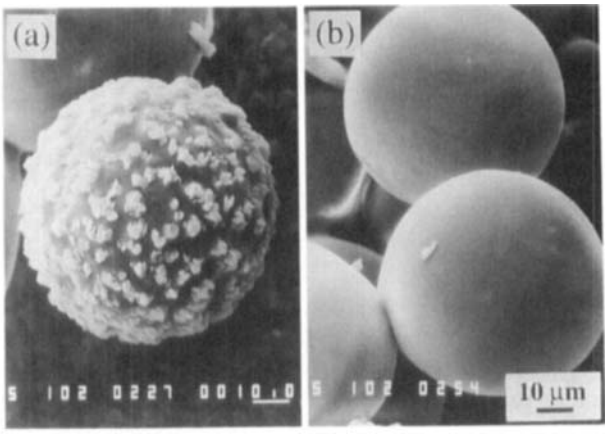

Figure 3. Microscopic view of drug-loaded PLA microspheres obtained with (a) dichloromethane and (b) chloroform.

of bulk T, bulk PLA, T-loaded PLA microspheres obtained with dichloromethane, and T-loaded PLA microspheres obtained with chloroform. The DSC pattern of bulk T showed a melting point (mp) of 155°C, in contrast to a T_g of 43°C for bulk PLA. The peaks, corresponding to T_g of bulk PLA and mp of bulk T shifted slightly to the left, existed with T-loaded PLA microsphere obtained with dichloromethane, indicating that it is a poor solvent because of heterogeneous deposition of T from the device. In this case, a peak appeared at a temperature of 65°, though the cause is not clear at present. Conversely, these peaks were not present in T-loaded PLA microspheres obtained with chloroform. This finding suggests that drug molecules homogeneously distribute in the device obtained with chloroform.

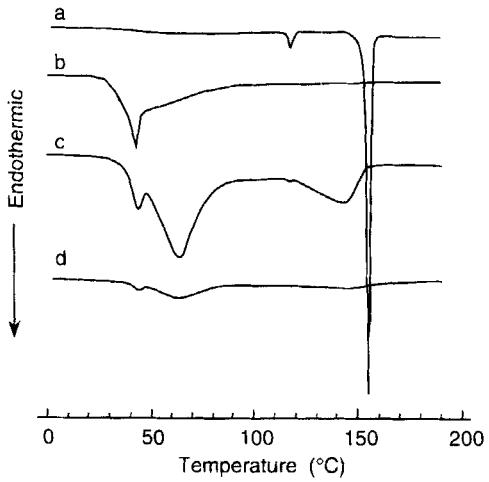

Figure 4. DSC curves of (a) bulk T, (b) bulk PLA, (c) drug-loaded PLA microspheres obtained with dichloromethane, and (d) drug-loaded PLA microspheres obtained with chloroform.

In Vivo Characteristics of Drug-Loaded PLA Microspheres

PLA microspheres obtained with chloroform and containing 30 mg of T with diameters of 75–150 μm were injected subcutaneously in the back of castrated rats. It was confirmed that drug-loaded PLA microspheres aggregated under the back skin of rats within 1 hr of implantation to form a flexible mass. The appearance of such a mass on the second week after implantation is shown in Fig. 5. Owing to in vivo degradation, the size of implants became smaller with the passage of time until they completely disappeared 10 weeks after implantation.

Histopathological findings in rat tissues surrounding the implants were investigated with regard to in vivo degradation and the results are shown in Fig. 6. This implant degraded approximately 15% in the first week of implantation and, at this time histopathological findings showed that the injected particles had become a tablet-like mass under the back skin, and also that this implant had a thin fibrous capsule which contained fibroblasts and histiocytes [Fig. 6(a)]. After 4 weeks of implantation (approximately 85% degradation), we observed a small amount of granular tissues in the implant which contained fibroblasts, histiocytes, foreign body giant cells, and blood capillaries [Fig. 6(b)]. The implant had been almost completely replaced by granular tissue after 8 weeks of implantation as the degree of degradation reached 95% or

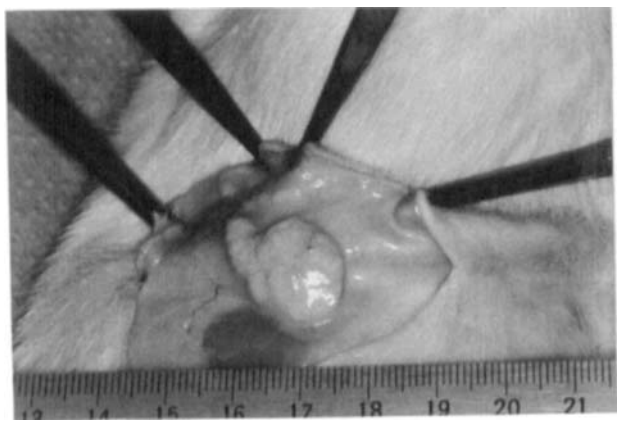

Figure 5. The injection site of implant insertion. Drug-loaded PLA microspheres with diameters of 75–150 μm were injected subcutaneously in the back of castrated rats for 2 weeks.

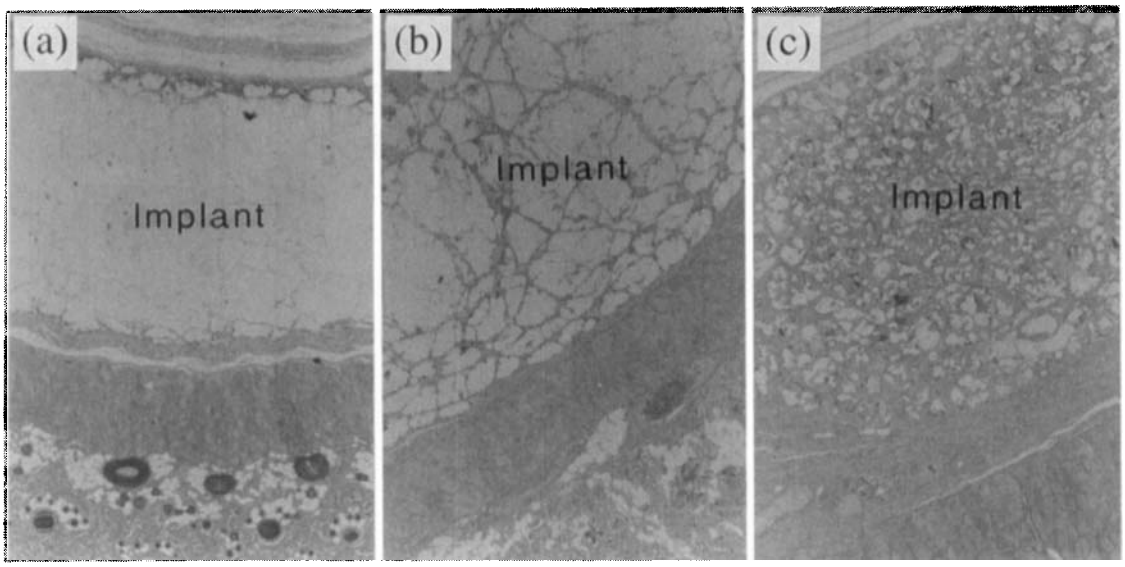

Figure 6. Optical photomicrographs of tissues surrounding the implants obtained with chloroform and implanted for periods of (a) 1, (b) 4, and (c) 8 weeks. Original magnification 40×.

more. It was confirmed that the histopathological findings of tissues surrounding the drug-loaded PLA microspheres obtained with dichloromethane were the same as those obtained with chloroform.

The daily dose of T released in vivo from the biodegradable PLA microspheres containing 10 and 30 mg of drug is shown in Fig. 7. The rate of drug release showed a stepwise decrease with the passage of time, but its release from the device obtained with dichloromethane is much faster than that obtained with chloroform, e.g., T was released completely from the device containing 30 mg of drug obtained with dichloromethane during the first 4 weeks, in contrast to 6 weeks for the device obtained with chloroform. This is due to the difference in dispersion of T in PLA.

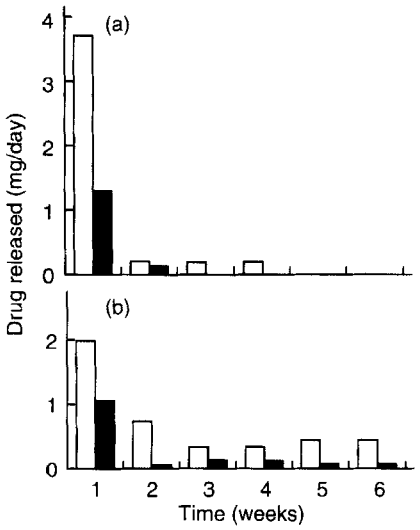

Figure 7. In vivo daily dose of T released from PLA microspheres and containing (■) 10 and (□) 30 mg of drug obtained with (a) dichloromethane and (b) chloroform.

The serum T level was determined in order to study the period of controlled drug release from biodegradable PLA microspheres as shown in Fig. 8. The results obtained suggest the amount of drug released in vivo on a daily basis.

Pharmacological Influences in Castrated Rats with Drug-Loaded PLA Microspheres

The pharmacological influence in castrated rats of drug-loaded PLA microspheres obtained by evaporating dichloromethane and chloroform was studied by determining the changes in weight of accessory sex organs such as VP, DLP, and right-side SV. The results for dichloromethane and chloroform are shown in Figs. 9 and 10, respectively. VP, DLP, and SV were atrophied by castration, achieving minimal weights after 3 weeks of castration. However, these weights should return to those of normal rats if T is administered with drug-loaded PLA microspheres.

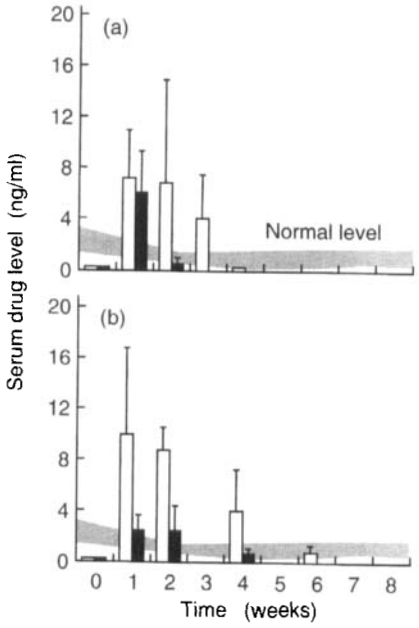

Figure 8. Changes in serum T level of castrated rats injected with PLA microspheres containing (■) 10 and (□) 30 mg of drug and obtained with (a) dichloromethane and (b) chloroform.

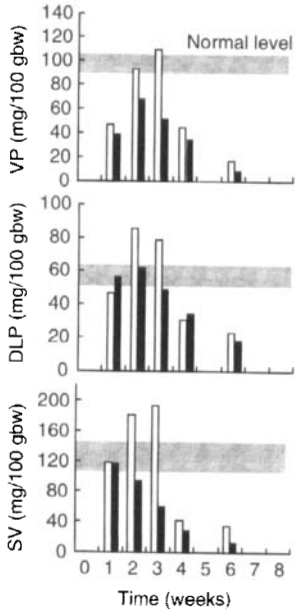

Figure 9. Changes in weight of prostate glands in castrated rats injected with PLA microspheres containing (■) 10 and (□) 30 mg of drug and obtained with dichloromethane.

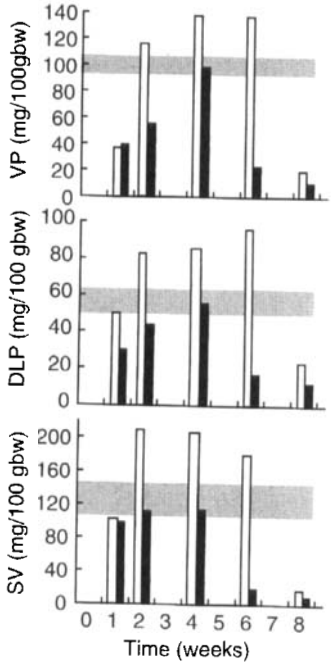

Figure 10. Changes in weight of prostate glands in castrated rats injected with PLA microspheres containing (■) 10 and (□) 30 mg of drug and obtained with chloroform.

In the case of drug-loaded PLA microspheres obtained with dichloromethane (Fig. 9), the weight of rat prostate glands was maintained at or above normal levels during the first 3 weeks while 30 mg of T was administered. After the first 3 weeks, levels showed a marked decrease with time because of no drug release. In contrast, weight recovery to normal levels continued for 6 weeks when PLA microspheres containing 30 mg of T obtained with chloroform were used, as is clearly shown in Fig. 10. This also supports the results on controlled drug release and serum drug level.

The optical photomicrographs of VP in castrated rats injected with and without drug-loaded PLA microspheres obtained with chloroform are shown in Fig. 11. In one castrated rat [Fig. 11(a)], VP showed a marked diffuse-glandular atrophy accompanied by a disappearance of papillary tissue enfolding the epithelium lining owing to relatively increased stromal volume. When PLA microspheres containing 30 mg of T were implanted subcutaneously in the back of castrated rats for a period of 4 weeks [Fig. 11(b)], normal VP was satisfactorily recovered because of long-acting controlled drug release.

In conclusion, we successfully incorporated T into PLA microspheres degraded in vivo after 10 weeks by the solvent evaporation process. Drug-loaded PLA mi-

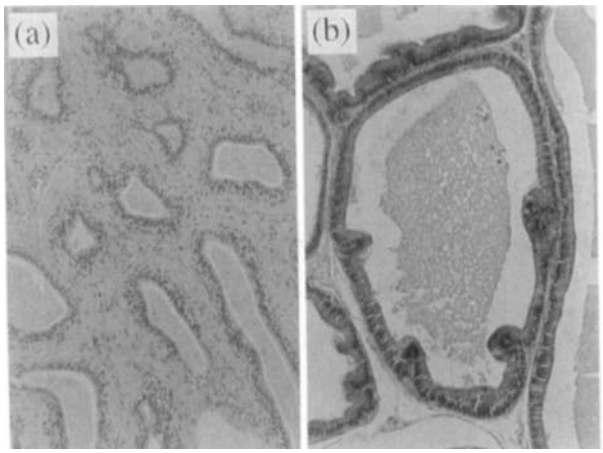

Figure 11. Light photomicrographs of VP in castrated rats injected (a) without and (b) with PLA microspheres obtained with chloroform. The device containing 30 mg of T was injected for a period of 4 weeks. Original magnification 40×.

crospheres which provide a long-acting controlled drug release are applicable to a wide range of clinical medicines.

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