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Short report

Controlled release of poly-D,L-lactic acid containing bleomycin

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By use of four types of *in vivo* degradable polylactic acid (PLA), i.e. PLA with an average molecular weight of 1500 (1500DL), 2200 (2200DL), 2800 (2800DL) and 3500 (3500DL), preparations of bleomycin (BLM)-containing solid forms (polymers) were tested. The *in vitro* release of BLM from the polymers was also examined in an immersion system. By the melt-pressing technique, five types of BLM (2.5 mg) containing solid forms, i.e. 1500DL polymer, 2200DL polymer, 2800DL polymer, 3500DL polymer and 1500DL + 3500DL (a mixture of 1500DL and 3500DL) polymer were prepared. In all five types of polymers, cumulative BLM release was controlled to less than 5% by the third day and no initial burst of the release was observed. BLM release from the polymer continued for 3 weeks at the shortest and 6 weeks at the longest. Various polymers containing BLM could be useful for the site of drug administration or anti-cancer release pattern.

Key words: Bleomycin, degradable poly-D,L-lactic acid, polymer, solid form PLA.

Introduction

To improve the therapeutic effect and suppress severe side effects in cancer chemotherapy,[1,2] various drug delivery systems for anti-cancer agents have been developed: a particulate form made by mixing with activated carbon was used in some cases[3,4] and a lipiodol-suspension,[5,6] microcapsules[7,8] or microspheres[9–11] were employed in others.

For the treatment of cancer patients in which complete surgical removal of the tumor or metastatic lymph nodes is difficult, implantation of a solid form anti-cancer drug in the body is expected to be a very useful therapy. However, various problems in solidification, such as the safety of the base material, slow and steady release of drug from the base material, and stability of the solid form against bursting, have to be solved before clinical application.

Using poly-D,L-lactic acid (PLA),[12] which is hydrolyzable enzymatically or non-enzymatically *in vivo*, and therefore is a highly biocompatible material, as a solidifying base, we prepared a solid form PLA containing bleomycin (BLM) (hereinafter, polymer) in the present study. The kinetics of the release of BLM from the polymer *in vitro* was also examined.

Materials and methods

Materials

PLAs with an average molecular weight of 1500 (1500DL), 2200 (2200DL), 2800 (2800DL) and 3500 (3500DL) were supplied by Taki Chemical (Hyogo, Japan). BLM was obtained from Nippon Kayaku (Tokyo, Japan).

Preparation of polymers

A fine cylindrical PLA formulation that contains BLM was made according to the melt-pressing technique.[7] A homogeneous mixture of 47.5 mg PLA powder and 2.5 mg BLM powder was prepared by mixing and melting at 50°C. The molten mixture was cooled to 20°C and solidified. The solid was crushed and the crushed particles were put into a 1 ml cylinder (polyethylene) with an inner diameter of 4.5 mm. The cylinder was compressed with a piston from one end at 100 kg cm^2 in a 50°C water bath and a thin cylindrical-shape polymer was obtained. By using different PLAs, different types of polymers were prepared. This preparation was carried out under semi-aseptic conditions.

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Observation of polymers

The surface structure of the polymers was examined with a scanning electron microscope (Model JXA-840A, JEOL Technics, Tokyo, Japan).

Quantitative determination of BLM

The BLM concentration in solution was measured at 293 nm with a spectrophotometer (Model 150-20, Hitachi Seisakusho, Tokyo, Japan).

Standard BLM solutions of various concentrations (0.049, 0.098, 0.195, 0.39, 0.78, 1.56, 3.13, 6.26, 12.5 and 31.3 μg/ml) were made and used in the preparation of a calibration curve for the photometry of BLM.

Release test

Each polymer was immersed in 10 ml of saline in a glass tube and the tube was incubated at 37°C in an incubator. After 1 and 3 days, and 1, 2, 3, 4, 5, 6 and 7 weeks, the BLM concentration in the immersing saline in each tube was measured photometrically. At each observation point, the saline in a tube was measured five times and the mean amount of BLM release was calculated.

Results

Properties of the polymers

Each polymer was a cylinder of diameter 4.5 mm, length 2.0 mm and weight 50 mg. As is shown in Figure 1, the polymer (right) is opaque in comparison with the BLM-free PLA (left), indicating an even distribution of BLM in the polymer.

An electron micrograph of a section of the mixture is presented in Figure 2. Since no impurities are observed, the mixture appears to be homogeneous. According to the electron micrograph of a section of the polymer in Figure 3, BLM particles are enclosed in PLA.

Calibration curve

Table 1 shows the relationship between the BLM concentration and optical density. By plotting each value on a graph with absorbance on the ordinate and logarithm of BLM concentration on the abscissa,

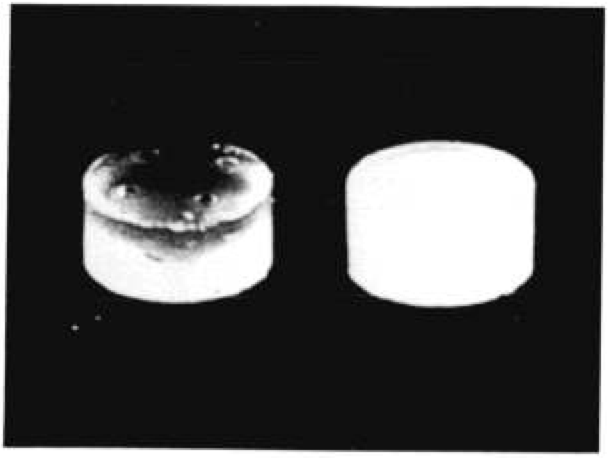

Figure 1. Photographs of PLA formulation (diameter 2 mm, weight 50 mg). Left: BLM-free PLA formulation. Right: polymer (containing 2.5 mg of BLM).

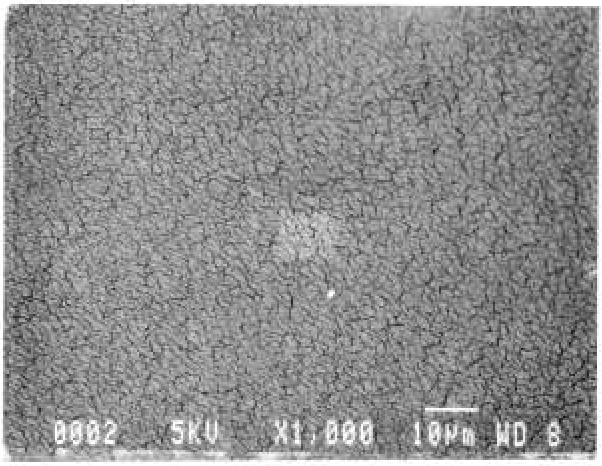

Figure 2. Scanning electron photomicrograph of BLM-free PLA formulation.

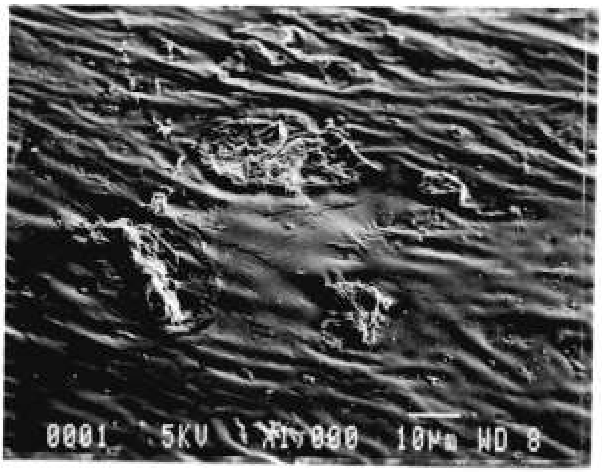

Figure 3. Scanning electron photomicrograph of polymer.

Table 1. Relationship between the BLM concentration and optical density

	BLM concentration (μg/ml in saline)								
	31.3	12.5	6.25	3.13	1.56	0.78	0.39	0.195	0.098
Absorbance at 293 nm	0.186 ± 0.0014	0.075 ± 0.0014	0.037 ± 0.0014	0.021 ± 0.0014	0.009 ± 0.0014	0.007 ± 0.0014	0.006 ± 0.0014	0.006 ± 0.0014	0.006 ± 0.0014

Data are presented as mean ± SD (n=20).

a calibration curve, $Y = 0.912X - 2.13$, was obtained. This formula indicated a high linearity between the concentration and absorbance ($p < 0.001$).

Changes in shape of cylindrical polymers during immersion

The 1500DL polymer was divided into two pieces on the first day; one piece consisted of about 9/10 of the initial amount and stayed as a cylinder at the bottom of the tube, and the other piece spread and floated as a thin film on the surface. On the seventh day, the cylinder at the bottom turned into a jelly-like sphere and became smaller as seen in the left photograph in Figure 4. In the fourth week, almost all of the polymer dissolved and none of the original shape was detectable (right photograph, Figure 4). In the case of the 1500DL + 3500DL polymer, no change in shape was observed on the first day, although some air-bubbles were attached to it. However, on the seventh day, it separated into two pieces, and one piece spread and floated as a thin film. Later, the polymer shrunk and was mostly dissolved by the fourth week.

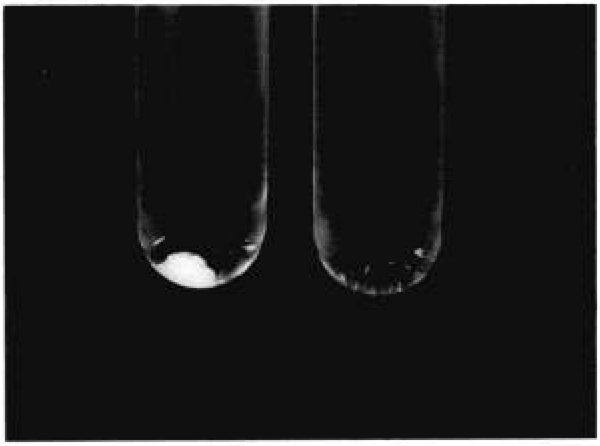

Figure 4. *In vitro* changes in shape of 1500DL polymer. Left photo: day 7. Right: week 4.

BLM release pattern

Table 2 shows the time-course changes of cumulative BLM release from polymers. Total BLM release from various polymers by the third day was in a range of 29.38–97.55 μg, and this amount was less than 4% of BLM in each polymer (2.5 mg). After the

Table 2. Changes of cumulative BLM release from various polymers

	Cumulative BLM amount (μg)							
	1 day	3 days	7 days	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks
1500DL	48.87 ± 15.73	97.55 ± 32.88	621.59 ± 239.70	2347.83 ± 131.76	2574.57 ± 15.73			
2200DL	22.72 ± 4.96	49.61 ± 11.94	80.43 ± 10.29	355.16 ± 46.63	977.38 ± 96.60	1651.01 ± 173.06	2330.51 ± 93.49	2585.21 ± 19.03
2800DL	49.26 ± 11.70	64.49 ± 12.47	77.01 ± 15.22	97.59 ± 21.87	701.15 ± 318.90	2651.94 ± 77.10		
3500DL	35.72 ± 0.96	47.21 ± 5.74	55.64 ± 5.94	98.69 ± 17.59	203.74 ± 37.90	1350.85 ± 109.10	2152.45 ± 55.69	2556.50 ± 7.93
1500DL + 3500DL	11.81 ± 3.59	29.38 ± 5.84	89.63 ± 59.26	562.08 ± 160.89	1423.92 ± 259.98	2063.20 ± 188.93	2505.51 ± 68.46	

Data are presented as mean ± SD (n = 5).

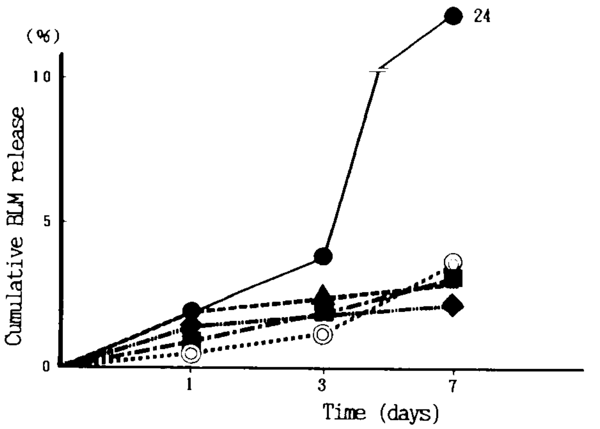

Figure 5. Release patterns of BLM from polymers within 7 days: 1500DL (●), 2200DL (▲), 2800DL (■), 3500DL (◆) and 1500DL+3500DL (◎).

third day, BLM release was different among the polymers. The total release of BLM reached 100% in the third week with the 1500DL polymer, in the sixth week with the 2200DL polymer, in the fourth week with the 2800DL polymer, in the sixth week with the 3500DL polymer and in the fifth week with the 1500DL+3500DL polymer. Figure 5 shows the BLM release pattern of various polymers within seven days. Figure 5 was prepared by calculation of the cumulative release (%) for each time point assuming that the overall release was 100%. In the 1500DL polymer, less than 5% was released by the third day and 24.0% by the seventh day. However, for the other polymers, less than 5% release was seen by the seventh day. Figure 6 shows the BLM release pattern of various polymers within 7 weeks. In the 1500DL polymer, the release rapidly increased to 91.2% in the second week and reached 100% in the third week. In the 2200DL polymer, after a slow release of less than 5% by the second week, 26.4% release was observed in the third week and the release increased rapidly to 100% in the fourth week. In the 2800DL polymer, less than 5% release was seen by the seventh day, 13.7% in the second week, 37.8% in the third week, 63.9% in the fourth week, 90.1% in the fifth week and 100% in the sixth week. In the 3500DL polymer, less than 5% release was observed by the second week, 8.0% release in the third week, 52.8% in the fourth week, 84.2% in the fifth week and 100% in the sixth week. In the 1500DL + 3500DL polymer, less than 5% release was found by the seventh day, and the release increased to 22.4% in the second week, 56.8% in the third week and 82.3% in the fourth week. In the fifth week, it reached 100%.

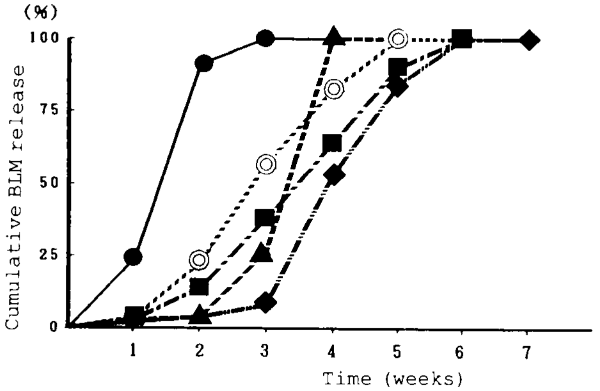

Figure 6. Release patterns of BLM from polymers within 7 weeks: 1500DL (●), 2200DL (▲), 2800DL (■), 3500DL (◆) and 1500DL+3500DL (◎).

Discussion

Similarly to Juni *et al.*,[9] we confirmed the high linearity between the BLM concentration and absorbance by measurement of the UV absorption of BLM in saline at 293 nm and preparation of a calibration curve.

A few reports on the controlled release of anticancer drugs using PLA have been published. Ike *et al.*[10] prepared PLA microspheres and PLA beads containing cisplatin and reported that about 60% of the cisplatin in the microspheres was released in 24 h, whereas the cisplatin in the beads was released in 30–40 days. However, from the beads, about 40% was released by the third day, then the release became a plateau and, from about the 20th day, all the remaining was released in about 5 days. Therefore, the release did not fit the zero-order kinetics. No preparations which showed a long-term steady release of drugs were found by Juni *et al.*[9] or Mori *et al.*[11]

The four types of PLA used for the solid preparation in this study were amorphous solids of DL compounds, and their average molecular weights were 1500, 2200, 2800 and 3500. Due to a low molecular weight, these PLAs were degraded enzymatically and non-enzymatically *in vivo* and excreted.[7] Therefore, their compatibility with the body should be high. According to the study of the *in vivo* degradation by Fukuzaki *et al.*,[7] the 1500DL preparation degraded showing a parabola curve in 5–6 weeks, and the preparations of 2200DL, 2800DL and 3500DL degraded in 10–15 weeks showing a sigmoid curve.

In the present release test *in vitro*, the release of the drug was maintained at less than 5% by the third day in all five types of polymer and no burst of the release was observed. In particular, in the 3500DL polymer, the release was suppressed below 10% until the third week, indicating a good delaying effect for a long period. A 100% release of BLM was observed with the 1500DL polymer in the third week, the 2200DL polymer in the fourth week, the 1500DL + 3500DL polymer in the fifth week, the 2800DL and 3500DL polymers in the sixth week. As to the release pattern, the 1500DL polymer released about 90% of BLM from the first to the second week showing a parabolic curve, whereas the 2200DL, 2800DL, 3500DL and 1500DL+3500DL polymers showed sigmoid curves. The release patterns from these polymers are almost the same as the degradation pattern obtained by Fukuzaki *et al.*[7] These observations suggest that a long-lasting release of BLM can be expected *in vivo* as well as *in vitro*. The enclosed BLM in the polymer was also indicated to be released in accordance with the degradation of the polymer.

Implantation of a solid form anti-cancer drug in the body of patients with carcinoma appears to be an effective therapy. In such a case, the solidifying material should have low toxicity to the body and provide long-lasting release without any initial burst. The polymers prepared in this study are thought to be highly compatible with the body because they were made of an *in vivo* degradable PLA base material and no burst in the release was observed. Therefore, they could be useful drug delivery forms for cancer chemotherapy. A more suitable polymer for chemotherapy with an improved release pattern is expected to be produced by further examination of the mixing ratio of PLA and BLM, and the combination of PLA with different molecular weights.

References

1. Gregoriadis G. Targeting of drugs. *Nature* 1977; **265**: 407–10.
2. Sezaki H. Trends in drug delivery. *Yakugaku Zasshi* 1984; **104**: 1211–22 (in Japanese).
3. Hagiwara A, Ahn T, Ueda T, *et al.* Anticancer agents adsorbed by activated carbon particles. A new form of dosage enhancing efficacy on lymph nodal metastases. *Anticancer Res* 1985; **6**: 1005–8.
4. Shimada M, Natsugoe S, Kumanohoso T, *et al.* Local chemotherapy of esophageal cancer with bleomycin adsorbed to activated carbon particles. In Nabeya K, Hanaoka T, Nogami H, eds. *Recent advances in diseases of the esophagus.* Tokyo: Springer-Verlag 1993: 905–10.
5. Konno T, Maeda H, Iwaki K, *et al.* Effect of arterial administration of high molecular weight anticancer agent SMANCS with lipid lymphographic agent on hepatoma, A preliminary report. *Eur J Cancer Clin Oncol* 1983; **19**: 1053–65.
6. Inoue H, Miyazono N, Kanetuki I, *et al.* Anticancer and side effect of arterial subsegmental chemoembolization using cisplatinum/pirarubicin lipiodol suspension (CTL) for hepatocellular cacinoma. *Jpn J Cancer Chemother* 1993; **20**: 1973–6 (in Japanese).
7. Kato T, Nemoto R. Microencapsulation of mitomycin C for intra-arterial infusion chemotherapy. *Proc Japan Acad* 1978; **54**: 413–17.
8. Ogawa Y, Yamamoto M, Okada H, *et al.* A new technique to efficiently entrap leuprolide acetate into microcapsules of polylactic acid or copoly(lactic/glycolic) acid. *Chem Pharm Bull* 1988; **36**: 1095–103.
9. Juni K, Ogata J, Nakano M, *et al.* Preparation and evaluation *in vitro* and *in vivo* of polylactic acid microspheres containing doxorubicin. *Chem Pharm Bull* 1985; **33**: 313–18.
10. Ike O, Wada R, Kusanoi Y, *et al.* *Cis*-diamminedichloroplatinum delivery system using poly(lactic acid). *Drug Delivery System* 1990; **5**: 29–32 (in Japanese).
11. Spenlehauer G, Vert M, Benoit J-P, *et al.* Biodegradable cisplatin microspheres prepared by the solvent evaporation method: morphology and release characteristics. *J Controlled Rel* 1988; **7**: 217–29.
12. Fukuzaki H, Yoshida M, Asano M, *et al.* Synthesis of copoly(D,L-lactic acid) with relatively low molecular weight and *in vitro* degradation. *Eur Polym J* 1989; **25**: 1019–26.

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