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New biodegradable polymers of L-lactic acid and aromatic hydroxy acids and their applications in drug delivery systems

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Summary

New biodegradable polymers with an aromatic ring in the main chain and with aromatic rings in both the main chain and the pendant group were synthesized by the direct polycondensation of L-lactic acid (LA) and aromatic hydroxy acids such as DL-mandelic (MA), *p*-hydroxybenzoic (HBA), *p*-hydroxyphenylacetic (HPAA), and *p*-hydroxyphenylpropionic (HPPA) acids, using polymer systems composed of LA/HBA, LA/HPAA, LA/HPPA, and LA/MA/HPPA, for example. In an LA copolymer, the degree of degradation in vivo markedly decreases with increasing proportion of aromatic hydroxy acid in the system, in which the degradation behavior resulted in either a linear or a parabolic profile according to the kind of copolymer and its composition. In contrast, an S-type degradation pattern was obtained in a ternary polymer system (e.g. poly(LA/MA/HPPA), 80:10:10 mol%). Estramustine was incorporated into the ternary polymer formulation in the form of small cylinders followed by subcutaneous implantation into male rats. The pharmacological effect of drug released in vivo from the formulation was examined by measuring the changes in weight of the accessory sex organs of male rats, the results demonstrating the maintenance of efficacious pharmacological action over a period of approx. 10 weeks.

Introduction

The use of biodegradable and biocompatible polymers in drug delivery systems has recently been attracting considerable interest, as reported for narcotic agonists (Maa and Heller, 1990), local anesthetics (Wakiyama et al., 1982), steroid hormones (Eenink et al., 1987), physiologically active peptides (Hutchinson and Furr, 1990; Heller et al., 1990; Yamakawa et al., 1990), and anticancer agents (Kawaguchi et al., 1983; Hecquet et al., 1986). For such purposes, the carriers supplied are generally composed of relatively high molecular weight polymers which have been synthesized in the presence of catalysts. The use of catalysts is undesirable, since the catalytic

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residues can affect the polymer (Asano et al., 1990). In order to avoid this drawback, several investigators have recently devoted their efforts towards actively synthesizing low molecular weight polymers via direct polycondensation in the absence of catalysts (Asano et al., 1989; Bodmeier and Chen, 1989; Bodmeier et al., 1989; Yoshikawa et al., 1989; Fukuzaki et al., 1990; Imasaka et al., 1990, 1991a,b, 1992). These materials possess the following characteristics: (i) they are of high purity; (ii) their shape is easily adjustable without the need for organic solvents owing to a low softening point; (iii) their degradation behavior can be readily controlled, whether of the parabolic, linear or S type; and (iv) the time course of degradation can be regulated in a simple manner. We extended our series of investigations to the search for a pure biodegradable polymer having properties such as the undergoing of an ideal pattern of degradation within 2 months, low molecular weight, and the ability to follow degradation behavior of the linear, parabolic, and S types.

In the present article, we describe the use of polymer systems containing an aromatic hydroxy acid in both the main chain and side chain, the systems comprising LA/HBA, LA/HPAA, LA/HPPA and LA/MA/HPPA (LA, L-lactic acid; MA, DL-mandelic acid; HBA, *p*-hydroxybenzoic acid; HPAA, *p*-hydroxyphenylacetic acid; HPPA, *p*-hydroxyphenylpropionic acid), since studies on the polarizability and biodegradability of aromatic hydroxy acids are lacking in the literature.

Estramustine (estradiol-3-[bis(2-chloroethyl) carbamate]) was chosen as a model anticancer agent in order to evaluate the suitability of implantable polymer carriers for application in drug delivery systems (Forsgren et al., 1978; Yamanaka et al., 1981, 1982; Wadsten and Lindberg, 1989).

Materials and Methods

Materials

The low molecular weight polymers (M_w = 2100–4100) consisting of LA/HBA, LA/HPAA, LA/HPPA, and LA/MA/HPPA were synthesized by direct polycondensation without the use of a catalyst, by bubbling nitrogen gas through the solution for 20 h at 200°C, according to a previously reported procedure (Imasaka et al., 1991a). Table 1 lists the analytical data obtained on the resulting polymers, e.g., polymer composition, as determined by ^{1}H-NMR spectroscopy using a Jeol GSX-270 spectrometer and number average (M_n) and weight-average (M_w) molecular weights, as evaluated based on gel permeation chromatography (GPC) using a Waters ALC-244

TABLE 1

Analytical data on poly(LA / MA / HPPA)

No.	Monomer composition (mol%)			^{1}H-NMR Polymer composition (mol%)			GPC		
	LA	MA	HBA/ HPAA/ HPPA/	LA	MA	HBA/ HPAA/ HPPA	M_n	M_w	M_w/M_n
1	50	–	50 HBA	38	–	62 HBA	1600	2700	1.69
2	70	–	30 HBA	66	–	34 HBA	2100	3300	1.57
3	50	–	50 HPAA	44	–	56 HPAA	2100	3000	1.42
4	70	–	30 HPAA	66	–	34 HPAA	2300	3800	1.65
5	30	–	70 HPPA	22	–	78 HPPA	1800	2200	1.67
6	50	–	50 HPPA	42	–	58 HPPA	1800	2800	1.43
7	70	–	30 HPPA	65	–	35 HPPA	2700	3900	1.44
8	80	10	10 HPPA	75	11	14 HPPA	2100	4100	1.92
9	50	40	10 HPPA	51	37	12 HPPA	1300	2200	1.75
10	50	10	40 HPPA	44	10	46 HPPA	1200	2100	1.65

high-performance liquid chromatograph, at 25°C and a flow rate of 1 ml/min through 10^2,10^3 and 10^4 Å Waters ultrastyragel columns, in tetrahydrofuran (Imasaka et al., 1990).

Estramustine (A/B Leo, Helsingborg, Sweden), which has an excellent palliative effect on advanced prostatic cancer, was used as a model chemotherapeutic agent.

Preparation of implantable polymer formulations

A mixture of 80 mg of powdered polymer carrier and 20 mg of powdered drug was first melted at about 100°C to obtain a homogeneous mixture, then allowed to cool to room temperature, and crushed. The crushed mixture was charged into a commercially available poly(tetrafluoroethylene) tubing of 3 mm inner diameter. The piston rods were inserted from both sides of the tube under a pressure of 100 kg/cm^2, according to the so-called melt-pressing technique (Asano et al., 1989), yielding a small cylinder of the polymer formulation (3 mm diameter) which was charged into the tube.

In vivo degradation of pure copolymers

Examination of in vivo degradation was performed by subcutaneous implantation of the small cylinder of polymer formulation (total weight: 100 mg) with and without 20 mg of estramustine in the back of male adult Wistar strain rats weighing 350–400 g (4 samples per rat, 3 rats per group). After killing the rats, the implants were excised at intervals, pooled after being freed of surrounding connective tissue, lyophilized, and weighed to estimate the degree of in vivo degradation (%) using the equation, $100\ (D_0 - D)/D_0$, where D_0 is the weight of the copolyester before degradation and D represents the sample weight after treatment for the desired period of time.

Pharmacological effects of implantable estramustine formulations on male rats

The pharmacological influence of estramustine with and without poly(LA/MA/HPPA, 80:10:10 mol%) (sample 8 in Table 1) formulations was evaluated by measuring the changes in weight of organs such as ventral prostates (VP), dorsolateral prostates (DLP), the right-side seminal vesicle (SV), both adrenal glands (Ad) and the right testis (T). For this purpose, a small cylindrical formulation (carrier, 80 mg; drug, 20 mg) for the controlled release of estramustine (one formulation per rat, 5 rats per group) and pure estramustine without the use of formulations which consisted of a mixture of 0.1 ml dimethyl sulfoxide (DMSO) and 1 mg of drug (single injection per rat, 5 rats per group) were injected subcutaneously into the back of male adult Wistar strain rats weighing 350–400 g. The above-mentioned organs (VP, DLP, SV, Ad, and T) were excised from killed rats at given time intervals, freed from surrounding connective tissue, then pooled, and weighed. Organ weights are expressed in mg per 100 g of body weight at the time of killing. Estramustine remaining in formulations excised from killed rats was determined using a Hitachi L-6000 high-performance liquid chromatograph under the following conditions: column, Inertsil ODS-2 (Gaskuro Kogyo Inc.; inner diameter, 4 mm; length, 150 mm); eluent, 0.1% phosphoric acid/acetonitrile (9:11); flow rate, 1 ml/min; column temperature, 40 °C; absorbance wavelength, 272 nm.

Results and Discussion

Polymer characterization

To investigate the strong dependence of both the polymerizability and biodegradability on the composition of LA/HBA, LA/HPAA, LA/HPPA and LA/MA/HPPA, LA was polycondensed with various aromatic hydroxy acids, i.e., LA/HBA, LA/HPAA, LA/HPPA and LA/MA/HPPA, in the absence of catalysts at 200°C by bubbling nitrogen gas through the solution. The structural formulae of the polymers are shown in Fig. 1. The molar composition of the polymers obtained was determined from the ratio of ^{1}H-NMR peak intensities between the methyl group (3H) of LA and methine group (1H) of MA, or between the methylene group (2H) of HPAA and ethylene group (4H) of HPPA. The results are listed in Table 1. The molecular weights (M_n and M_w) of the polymers and their dispersities (M_w/M_n) are also given in Table 1.

As can clearly be seen from Table 1, the M_n and M_w values are relatively low due to the low degree of polymerizability of MA, HBA, HPAA, and HPPA as compared to the magnitude of the steric hindrance, reactivity and solubility resulting from the presence of aromatic rings in the monomer. The following values were determined: poly(LA/HBA), $M_n < 2100$; poly(LA/HPAA), $M_n < 2300$; poly(LA/HPPA), $M_n < 2700$; poly (LA/MA/HPPA), $M_n < 2100$.

In vivo degradation study

The pattern of in vivo degradation of LA/HBA, LA/HPAA, LA/HPPA and LA/MA/HPPA is depicted in Fig. 2 as a function of implantation time. The degree of in vivo degradation of LA-containing polymers increased with time; a 30 mol% HBA copolymer was found to undergo the largest increase in extent of in vivo degradation (i.e., 100% at the 2nd week after starting implantation) whereas the lowest degree of degradation was observed with poly(LA/HPPA, 30:70 mol%) (approx. 56% after 15 weeks implantation). The results plotted in Fig. 2 clearly demonstrate that the degree of degradation decreases considerably with increase in the proportion of aromatic hydroxy acid content (e.g., HBA, HPAA, and HPPA) in the copolymer system. Copolymers with a relatively small proportion of aromatic hydroxy acid content resulted in a characteristic parabolic degradation vs time plot, however, an increase in the ratio of aromatic hydroxy acids gave rise to an apparently linear pattern of degradation as is evident for the poly(LA/HPPA, 30:70 mol%) system.

In a ternary system comprising LA, MA (with an aromatic ring as pendant group), and HPPA (with an aromatic ring in the main chain), the pattern of in vivo degradation displayed markedly different behavior as compared with that of the above LA/HPPA copolymer, resulting in the appearance of a pseudo S-type pattern for a poly(LA/MA/HPPA, 80:10:10 mol%) system. This is illustrated clearly by the degradation vs time plots shown in Fig. 3 for pure ternary poly(LA/MA/HPPA). The mechanism of in vivo degradation of a pseudo S-type biodegradable ternary polymer is as follows. The small cylindrical specimen that has been implanted swells slowly from its surface. The time period required for complete swelling is approx. 4 weeks. A gradual weight loss of the polymer during the initial 4 weeks is due to erosion of lower molecular weight

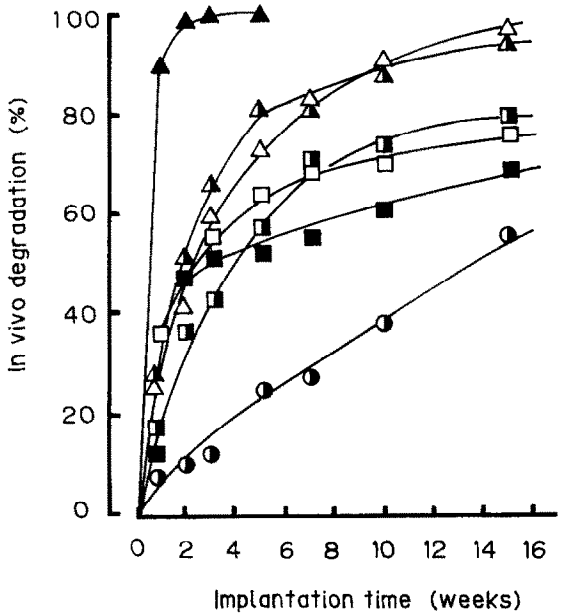

Fig. 2. In vivo degradation profiles of pure copolymers of: (▲) LA/HBA (70:30 mol%) (no. 2 in Table 1); (△) LA/HPAA (70:30 mol%) (no. 4); (▲) LA/HPPA (70:30 mol%) (no. 7); (■) LA/HBA (50:50 mol%) (no. 1); (□) LA/HPAA (50:50 mol%) (no. 3); (◨) LA/HPPA (50:50 mol%) (no. 6); (◑) LA/HPPA (30:70 mol%) (no. 5).

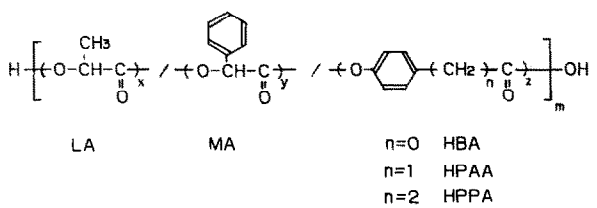

Fig. 1. Structural formulae of polymers consisting of LA/HBA, LA/HPAA, LA/HPPA, and LA/MA/HPPA.

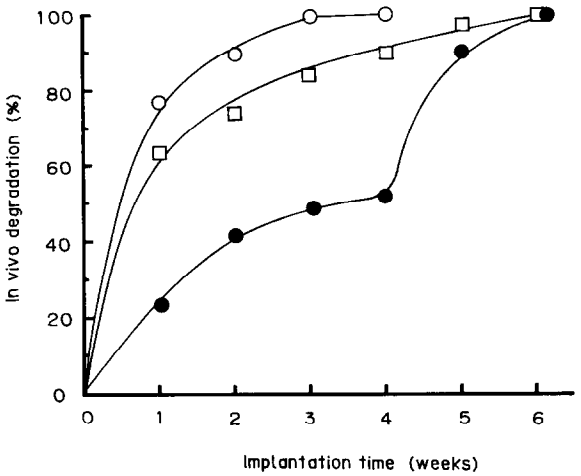

Fig. 3. In vivo degradation of pure ternary polymers of LA/MA/HPPA with compositions of (●) 80:10:10 mol% (no. 8 in Table 1). (○) 50:40:10 mol% (no. 9) and (□) 50:10:40 mol% (no. 10).

polymer. Subsequently, rapid degradation followed by hydrolysis due to the action of enzymes results in the surface area of the device being markedly increased on breaking the device, especially after 4 weeks implantation. This phenomenon was observed only for the poly(LA/MA/HPPA, 80:10:10 mol%) system.

Pharmacological study on rat prostates

A nitrogen mustard derivative of estradiol-17β, estramustine, exerts an excellent palliative effect against advanced prostatic cancer. The pharmacological influence of this drug can be evaluated by measuring the changes in weight of rat prostates. Estramustine (20 mg) was incorporated into a pseudo S-type biodegradable poly(LA/MA/HPPA, 80:10:10 mol%) formulation of M_n = 2100 (80 mg). The formulations were implanted subucutaneously into the back of male rats. The degree of in vivo weight loss of the drug-containing formulation was markedly retarded compared with that of the pure carrier, as demonstrated in Fig. 4, due to the hydrophobicity of the drugs. The resulting total disappearance of the formulation was observed at the 15th week from the initiation of implantation. The cumulative amount of drug released in vivo from the formulation reached almost 50% of the initial

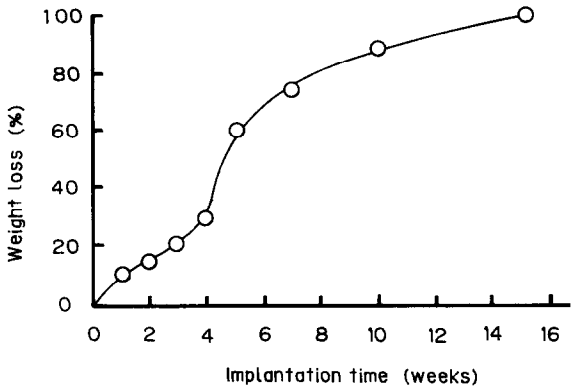

Fig 4. In vivo weight loss profiles of poly(LA/MA/HPPA, 80:10:10 mol%) (no. 8 in Table 1) formulation with estramustine.

drug content after 4 weeks implantation, 85% after 6 weeks and approx. 100% after 10 weeks implantation. For the sake of comparison, the degree of atrophy of organs on administration of pure estramustine without the formulation was monitored by administering single subcutaneous doses of drug, e.g., of 1 mg drug. Pharmacological

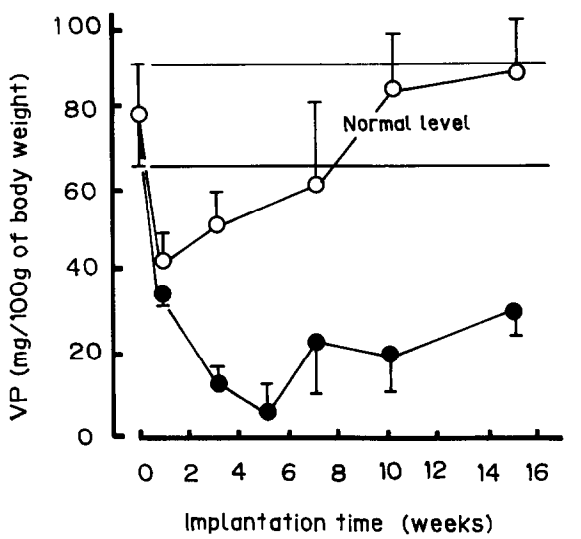

Fig. 5. Changes in weight of VP of male adult rats on administration of estramustine with and without a poly(LA/MA/HPPA, 80:10:10 mol%) (no. 8 in Table 1) formulation. Method of administration: (○) subcutaneous single administration of DMSO solution containing 1 mg of drug; (●) subcutaneous implantation of polymer formulation releasing drug at a controlled rate.

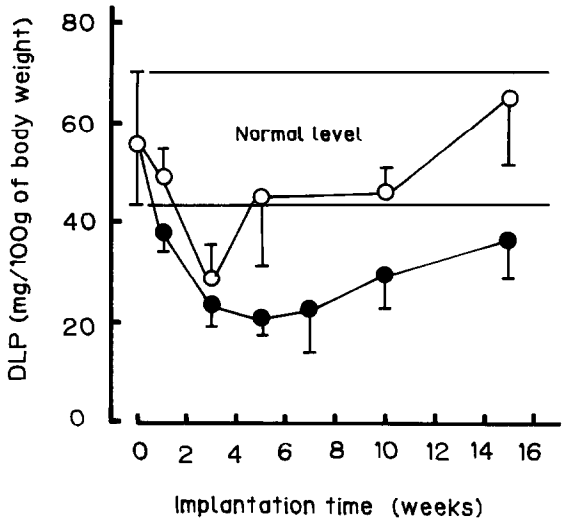

Fig. 6. Changes in weight of DLP of male adult rats on administration of estramustine with and without a poly(LA/MA/HPPA, 80:10:10 mol%) (no. 8 in Table 1) formulation; experimental conditions were the same as those described in Fig. 5.

data on the changes in organ weights (i.e., VT, DLP, SV, Ad and T) are depicted in Figs 5–9. On implantation of the carrier alone (100 mg), the weight of each organ was evaluated, yielding

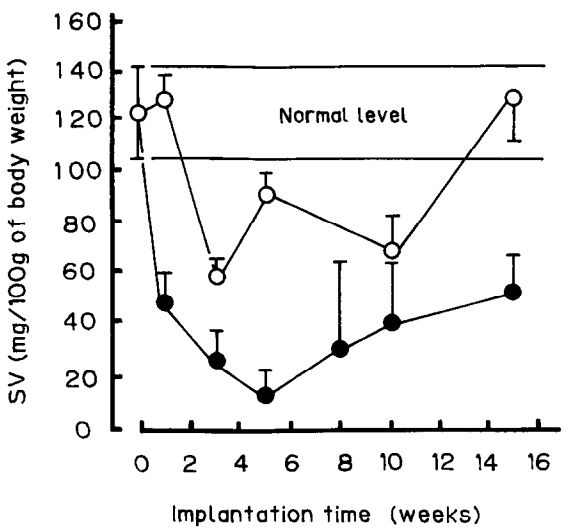

Fig. 7. Changes in weight of SV of male adult rats on administration of estramustine with and without a poly(LA/MA/HPPA, 80:10:10 mol%) (no. 8 in Table 1) formulation; experimental conditions were the same as those described Fig. 5.

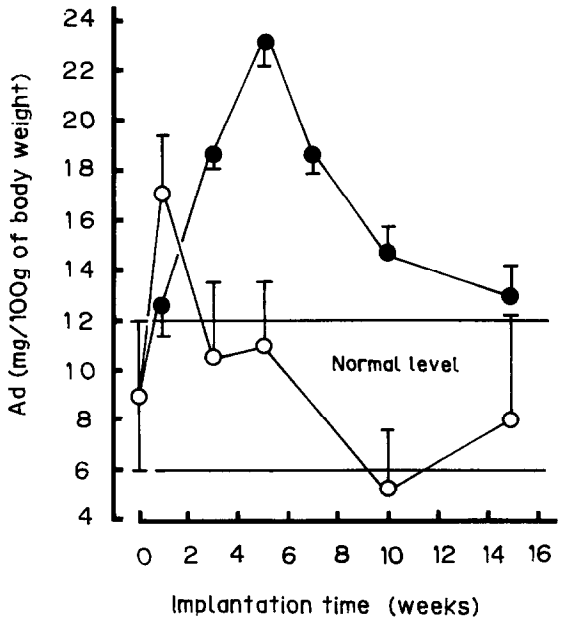

Fig. 8. Changes in weight of Ad of male adult rats on administration of estramustine with and without poly(LA/MA/HPPA, 80:10:10 mol%) (no. 8 in Table 1) formulation; experimental conditions were the same as those described in Fig. 5.

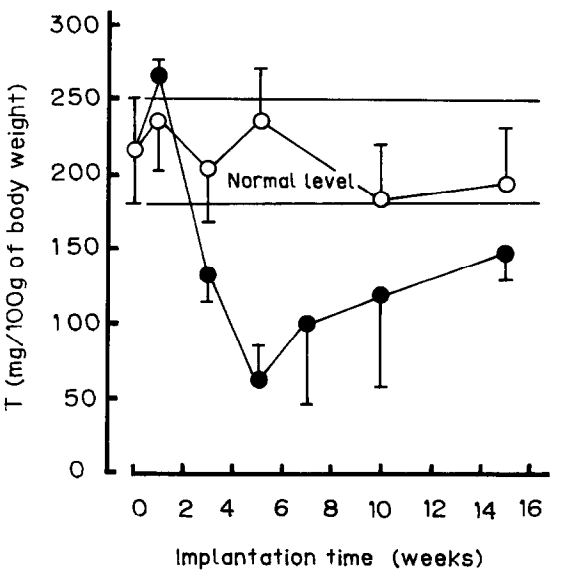

Fig. 9. Changes in weight of T of male adult rats on administration of estramustine with and without poly(LA/MA/HPPA, 80:10:10 mol%) (no. 8 in Table 1) formulation; experimental conditions were the same as those described in Fig. 5.

the following results: 63–90 (VP), 48–70 (DLP), 105–141 (SV), 6–12 (Ad) and 180–250 (T) mg per 100 g body weight ($n = 5$), these values being in close agreement with those of normal rats. For the administration of a single subcutaneous injection of pure estramustine, it was observed that organ weights, e.g., VP, DLP and SV, despite showing a marked fall subsequent to injection, completely recovered within a relatively short period of time. The weight of organ T was maintained at the normal value throughout the experimental period while, in contrast, that of Ad was characterized by an accompanying rapid increase during the initial stages of injection. In the administration of a controlled drug release formulation, a pharmacological effect of estramustine that corresponded to the level in castrates was observed during the first 10 weeks, followed by a gradual recovery to normal levels. This provides evidence in support of the occurrence of controlled release of drug from the formulation over a considerable period of time.

References

Asano, M., Fukuzaki, H., Yoshida, M., Kumakura, M., Mashimo, T., Yuasa, H., Imai, K. and Yamanaka, H., In vivo characteristics of low molecular weight copoly(D,L-lactic acid) formulation with controlled release of LH-RH agonist. *Biomaterials,* 10 (1989) 569–573.

Asano, M., Fukuzaki, H., Yoshida, M., Kumakura, M., Mashimo, T., Yuasa, H., Imai, K. and Yamanaka, H., Application of poly DL-lactic acids of varying molecular weight in drug delivery systems. *Drug Design Delivery*, 5 (1990) 301–320.

Bodmeier, R. and Chen, H., Evaluation of biodegradable poly(lactide) pellets prepared by direct compression. *J. Pharm. Sci.*, 78 (1989) 819–822.

Bodmeier, R., Oh, K.H. and Chen, H., The effect of the addition of low molecular weight poly(DL-lactide) on drug release from biodegradable poly(DL-lactide) drug delivery systems. *Int. J. Pharm.*, 51 (1989) 1–8.

Eenink, M.J.D., Feijen, J., Olijslager, J., Albers, J.H.M., Rieke, J.C. and Greidanus, P.J., Biodegradable hollow fibers for the controlled release of hormones. *J. Controlled Release*, 6 (1987) 225–247.

Forsgren, B., Bjork, P., Carlstrom, K., Gustafsson, J.A., Pousette, A. and Hogberg, B., Purification and distribution of a major protein in the rat prostate that binds estramustine, a nitrogen mustard derivatives of estradiol-17β. *Proc. Natl. Acad. Sci. USA*, 76 (1979) 3149–3156.

Fukuzaki, H., Aiba, Y., Yoshida, M., Asano, M. and Kumakura, M., Synthesis of biodegradable poly(L-lactic acid-co-D,L-mandelic acid) with relatively low molecular weight. *Makromol. Chem.*, 190 (1989) 2407–2415.

Fukuzaki, H., Yoshida, M., Asano, M., Kumakura, M., Mashimo, T., Yuasa, H., Imai, K. and Yamanaka, H., In vivo characteristics of low molecular weight copolymers composed of L-lactic acid and various DL-hydroxyacids as biodegradable carriers for drug delivery systems. *Biomaterials*, 11 (1990) 441–445.

Hecquet, B., Chabot, F., Gonzales, J.C.D., Fournier, C., Hilali, S., Cambier, L., Depadt, G. and Vert, M., In vivo sustained release of cisplatin from biodegradable implants in mice. *Anticancer Res.*, 6 (1986) 1251–1256.

Heller, J., Chang, A.C., Rodd, G. and Grodsky, G.M., Release of insulin from pH-sensitive poly(ortho esters). *J. Controlled Release*, 13 (1990) 295–302.

Hutchinson, F.G. and Furr, B.J.A., Biodegradable polymer systems for the sustained release of polypeptides. *J. Controlled Release*, 13 (1990) 279–294.

Imasaka, K., Nagai, T., Yoshida, M., Fukuzaki, H., Asano, M. and Kumakura, M., Synthesis and in vitro degradations of low-molecular weight copolyesters composed of L-lactic acid and aromatic hydroxy acids. *Makromol. Chem.*, 191 (1990) 2077–2082.

Imasaka, K., Yoshida, M., Fukuzaki, H., Asano, M., Kumakura, M., Mashimo, T., Yamanaka, H. and Nagai, T., A new biodegradable implant consisting of waxy-type poly-(ϵ-caprolactone-co-δ-valerolactone) and estramustine. *Int. J. Pharm.*, 68 (1991a) 87–95.

Imasaka, K., Yoshida, M., Fukuzaki, H., Asano, M., Kumakura, M., Mashimo, T., Yamanaka, H. and Nagai, T., Evaluation of new pasty-type implantable devices consisting of poly(ϵ-caprolactone/δ-valerolactone) and Estracyt or Estramustine. *Chem. Pharm. Bull.*, 39 (1991b) 2096–2099.

Imasaka, K., Nagai, T., Yoshida, M., Fukuzaki, H., Asano, M. and Kumakura, M., Synthesis of erodible ternary polymers respond to external stimuli such as pH, ionic strength and temperature. *Makromol. Chem.*, (1992) in press.

Kawaguchi, T., Nakano, M., Juni, K., Inoue, S. and Yoshida, Y., Plasma levels of tegafur following implantation of polycarbonate pellets containing tegafur or FD-1 into rats. *Chem. Pharm. Bull.*, 31 (1983) 4157–4160.

Maa, Y.F. and Heller, J., Controlled release of naltrexone pamotate from linear poly(ortho esters). *J. Controlled Release*, 14 (1990) 21–28.

Wadsten, T. and Lindberg, N.O., Polymorphism of estramustine, *J. Pharm. Sci.*, 78 (1989) 563–566.

Wakiyama, N., Juni, K. and Nakano, M., Preparation and evaluation in vitro and in vivo of polylactic acid microspheres containing dibucaine. *Chem. Pharm. Bull.*, 30 (1982) 3719–3727.

Yamakawa, I., Kawahara, M., Watanabe, S. and Miyake, Y., Sustained release of insulin by double-layered implant using poly(D,L-lactic acid). *J. Pharm. Sci.*, 79 (1990) 505–509.

Yamanaka, H., Kitamura, K., Imai, K., Yuasa, H., Nakai, K., Matsumura, Y., Uehara, H. and Shida, K., In vivo studies of ^{3}H-estramustine in castrated male rat. *Acta Urol. Jpn.*, 27 (1981) 243–250.

Yamanaka, H., Yuasa, H., Kosaku, N., Bashirelahi, N. and Shida, K., Analysis of estramustine binding protein (EMBP) in rat dorsal prostate by means of high performance liquid chromatography. *Endocrinol. Jpn.*, 29 (1982) 669–674.

Yoshikawa, H., Nakano, Y., Takada, K., Muranishi, S., Wada, R., Tabata, Y., Hyon, S.H. and Ikada, Y., Targeted and Sustained delivery of aclarubicin to lymphatics by lactic acid-oligomer microsphere in rat. *Chem. Pharm. Bull.*, 37 (1989) 802–804.