International Journal of Pharmaceutics, 68 (1991) 87–95

ADONIS 037851739100078

IJP 02278

A new biodegradable implant consisting of waxy-type poly(ϵ-caprolactone-co-δ-valerolactone) and estramustine

Kazumichi Imasaka [1], Masaru Yoshida [2], Hironobu Fukuzaki [2], Masaharu Asano [2], Minoru Kumakura [2], Tohoru Mashimo [3], Hidetoshi Yamanaka [3] and Tsuneji Nagai [1]

[1] *Department of Pharmaceutics, Hoshi University, Ebara 2-4-41, Shinagawa-ku, Tokyo 142 (Japan),* [2] *Department of Development, Takasaki Radiation Chemistry Research Establishment, Japan Atomic Energy Research Institute, Watanuki-machi 1233, Takasaki, Gunma 370-12 (Japan) and* [3] *Department of Urology, School of Medicine, Gunma University, Maebashi, Gunma 371 (Japan)*

(Received 9 April 1990)
(Modified version received 24 August 1990)
(Accepted 27 August 1990)

Key words: Implantable device; Estramustine; Biodegradable polyester; Waxy-type polymer; Drug delivery system; Rat prostate

Summary

Biodegradable waxy-type copolyesters were prepared by direct copolycondensation of ϵ-caprolactone (CL) and δ-valerolactone (VL) in the absence of catalysts, to apply as implantable matrices for drug delivery systems. The copolyesters are much more subject to erosion than their homopolyesters, in which the degradation is further accelerated by the action of lipase-type enzyme. Estramustine was incorporated into a small cylinder of waxy-type poly(CL-co-VL) with 92 mol% CL unit device by the so-called melt-pressing technique. The in vivo capability of this device was evaluated by implanting into the back of male rats. The drug release, although accompanying a burst phenomenon in the initial stage, was kept constant throughout an experimental period of 19 weeks from the 1st to 20th week. In this case, the release pattern was parallel to the degradation pattern, in support of the release being the rate-limiting step in the degradation of the polymer. The results showed about 75% of the initial drug content was still present in the device even after 20 weeks implantation. This finding means that the biodegradable poly(CL-co-VL) wax is very useful as an implantable matrix for a drug delivery system which controls the release over a relatively long period of time.

Introduction

In chronic diseases, the pharmacological effects of drugs are most frequently apparent during daily administration, and, in order to maintain efficacy, drug should be administered over a long period of time, in which pulsative administration, for example oral and injectable types accompanying a transient toxicity problem, is generally employed. To resolve this problem, implantable devices with sustained release of drugs have been developed as a tool by many researchers. Implantable devices are characterized by relief from side-effects and decrease in number of administrations, producing durable and moderate pharmacological effects.

Correspondence: M. Yoshida, Department of Development, Takasaki Radiation Chemistry Research Establishment, Japan Atomic Energy Research Institute, Watanuki-machi 1233, Takasaki, Gunma 370-12, Japan.

Several researchers have chosen non-biodegradable matrices as implantable devices, e.g. vinyl polymer (Yoshida et al., 1985, 1989), and silicone (Oda et al., 1985; Yanagiya, 1986; Imasaka et al., 1989a,b). However, such matrices must be removed from the living body after the complete release of the drug. This disadvantage has been modified for the preparation of biodegradable matrices involving the ester (-COO-) and amide (-CONH-) bonds in polymer chains (Asano et al., 1982, 1989b,c; Kawaguchi et al., 1983). This is because the bonds are easily degraded by the action of enzymes. The biodegradable polymers are further subdivided into two groups according to whether they are of high or low molecular weight. The high molecular weight polymers can be synthesized only in the presence of catalysts, which are unsuitable regarding biocompatibility because of impurities remaining in the polymer as terminal groups (Hecquet et al., 1986; Schakenraad et al., 1988). In contrast, low molecular weight polymers have been actively prepared by means of polycondensation of α-hydroxy acids and lactones without the use of catalysts, resulting in good biocompatibility. This implantable matrix is characterized by the ease with which control can be exercised over biodegradation, pattern (parabolic, linear and S types), and shaping treatment between the matrix and drug without use of organic solvents by the so-called melt-pressing technique (Asano et al., 1989a, 1990a,b; Fukuzaki et al., 1989a). Recently, we have synthesized low molecular weight copolyesters with different morphologies such as pasty and waxy states, by direct copolycondensation of ϵ-caprolactone (CL) and δ-valerolactone (VL) in the absence of catalysts (Imasaka et al., 1990). In this paper, we report on the in vivo capability of a new class of biodegradable copolyesters which was evaluated by subcutaneous implantation in the back of male rats, especially for waxy-type copolyesters as implantable matrices. Estramustine, estradiol-3-[bis(2-chloroethyl)carbamate], which is one of the main metabolites of estramustine phosphate (Estramustine®), used in the treatment of prostate cancer, was incorporated into the polymeric wax (Forsgren et al., 1978; Yamanaka et al., 1981, 1982; Wadsten et al., 1989).

Materials and Methods

Materials

Low molecular weight poly(CL-co-VL) was synthesized by direct copolycondensation without catalysts by bubbling nitrogen gas through the monomer solution at 200 °C, according to the procedures reported previously (Imasaka et al., 1990). Analytical data such as polymer composition determined by ^{1}H-NMR spectroscopy, number-average molecular weight ($\overline{M}_n$) and weight-average molecular weight ($\overline{M}_w$) determined by gel permeation chromatography (GPC), and melting point (m.p.) measured by differential scanning calorimetry (DSC) are listed in Table 1. It should be pointed out here that the molar ratio in the copolyester obtained is markedly distinct from those in the initial monomer, e.g. the CL contents in the copolyesters are 92, 78, 71, 53, and 19 mol% for initial CL contents in the monomers of 85, 70, 50, 30 and 15 mol%, respectively, leading to the preferential incorporation of CL units into the copolyester (Imasaka et al., 1990).

TABLE 1

Analytical data of poly(CL-co-VL)

No.	^{1}H-NMR		GPC		DSC	Appearance
	Polymer composition (mol%)		$\overline{M}_n$	$\overline{M}_w$	m.p. (°C)	
	CL	VL				
1	100	0	5800	14800	61	waxy
2	92	8	5700	14200	49	waxy
3	78	22	4700	13600	29	waxy
4	71	29	4500	14500	20	pasty
5	53	47	4600	13400	17	pasty
6	19	81	4200	13800	32	waxy
7	0	100	4100	14100	56	waxy

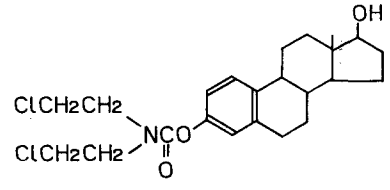

Fig. 1. Structural formula of estramustine.

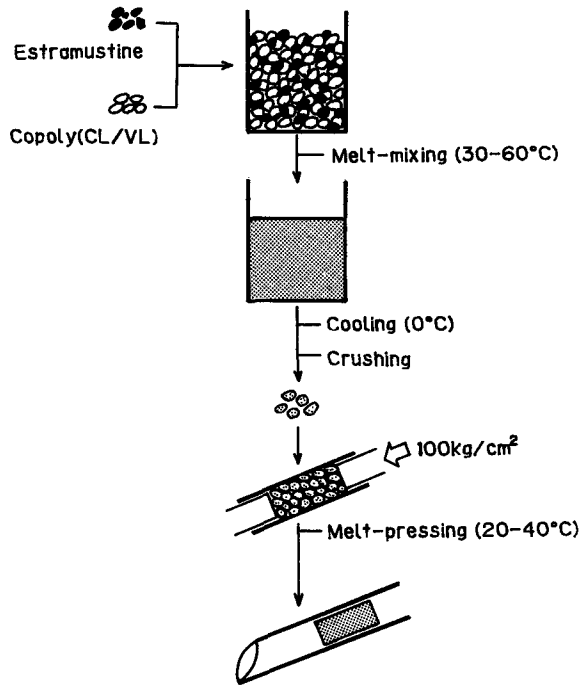

Fig. 2. Schematic diagram for the preparation of small cylinders of implantable devices consisting of poly(CL-co-VL) and estramustine by the melt-pressing technique.

Estramustine (structural formula as shown in Fig. 1: estradiol-3-[bis(2-chloroethyl)carbamate]; A/B Leo, Helsingborg, Sweden), which has an excellent palliative effect on advanced prostatic cancer, was used as a chemotherapeutic agent.

Preparation of implantable poly(CL-co-VL) devices

A schematic diagram for the preparation of small cylinders of poly(CL-co-VL) devices containing estramustine is shown in Fig. 2. A mixture of 80 mg of polymer and 20 mg of powdered drug was first melted at 30–60 °C to obtain a homogeneous mixture, then allowed to cool to 0 °C, and crushed. The crushed mixture was charged into a Teflon® tube of 3 mm inner diameter. The piston rods were inserted from both sides of the tube under a pressure of 100 kg/cm^2 at 20–40 °C, according to the melt-pressing technique (Asano et al., 1984), obtaining waxy-type and pasty-type cylinders of copolyester devices charged into the tube, of which a pasty-type cylinder was stored at 5 °C up to immediately before use.

In vitro-in vivo degradation of pure poly(CL-co-VL)

The degree of in vitro degradation of pure copolyester without drugs shaped into the small cylindrical specimens was evaluated by measuring the weight loss of the specimen which was treated in 30 ml of M/15 phosphate buffer solution (pH 7.2) with and without 0.01% w/v of lipases from hog pancreas (Sigma). The degradation was carried out at 37 °C on standing (one specimen per vial, 5 vials per group). The media were replaced by fresh media every 2 days. At intervals, the specimens were collected from the vial, rinsed with distilled water, lyophilized, and weighed to estimate the degree of in vitro degradation (%) from the following equation $[100(D_0-D)/D_0]$, where D_0 and D are the weights of copolyester before degradation and after treatment for the desired period of time, respectively. For comparison, in vivo degradation was also investigated, in which the small cylinders of pure copolyesters were implanted subcutaneously in the back of male adult Wistar strain rats weighing 350–400 g (2 specimens per rat, 3 rats per group). At certain time intervals, the implants were excised from killed rats, pooled after being freed of surrounding connective tissue, lyophilized, and weighed to estimate the degree of in vivo degradation analogously with the in vitro case.

In vivo release of estramustine from implantable waxy-type poly(CL-co-VL) devices

The cumulative amount of estramustine released in vivo from small cylinders of waxy-type poly(CL-co-VL) with 92 mol% CL unit (sample 2 in Table 1) devices was estimated from the amount of drug remaining in the device which was excised from killed rats at appropriate time intervals. In this case, the remaining drug was extracted with ethanol and, after centrifugation, its concentration in the supernatant solution was assayed at 269 nm with a Hitachi U-3210 spectrophotometer.

Pharmacological influence of implantable estramustine-containing devices in male rat

The pharmacological influence of estramustine with and without waxy-type poly(CL-co-VL) with 92 mol% CL unit (sample 2 in Table 1) devices was estimated in male rats by measuring the

change in weight of sex organs such as ventral prostates (VP) and right-side seminal vesicle (SV). For this purpose, the estramustine-containing device (one device per rat, 5 rats per group) and pure drug without the use of a device which consists of mixtures of 0.1 ml of dimethylsulfoxide (DMSO) and 1 or 5 mg of pure drugs (single injection per rat, 5 rats per group) were administered subcutaneously in the back of male adult Wistar strain rats weighing 350–400 g. At the required time intervals, the animals were killed. Each organ was freed from surrounding connective tissue, then pooled, and weighed (Asano et al., 1990a). The organ weights are expressed in mg per 100 g of body weight at the time of killing.

Results and Discussion

Degradation study

The in vitro degradation profiles of pure poly(CL-co-VL) treated over a period of 20 weeks at maximum in the buffer solutions with and without lipase are shown in Fig. 3. No poly(CL) was degraded non-enzymatically and enzymatically throughout the experimental period. A poly(VL) showed non-enzymatic degradation of approx. 15% after 20 weeks treatment, however, degradation was not accelerated by the action of enzymes. Such a low rate of degradation is due to the high crystallinity of waxy-type homopolymers (Fukuzaki et al., 1989b). However, both non-enzymatic erosion and enzymatic degradation showed a marked increase for the copolymer, especially for the action of enzyme. Maximum degradation was observed for a poly(CL-co-VL) with 53 mol% CL unit (sample 5, Table 1) in which the degree of degradation rose to over 55% at the 4th week from the start of the test. The cause of this is closely related to the difference in crystallinity and melting point of poly(CL-co-VL) with 53 mol% CL unit (sample 5, Table 1) which is pasty. This pasty-type copolyester is characterized by low crystallinity, low T_g and undergoing fusion at room temperature (Imasaka et al., 1990). For comparison, the in vivo degradation of poly(CL-co-VL), which led to different morphologies depending on the composition, was examined by subcutaneous implantation in the back of rats. This is demonstrated in Fig. 4 which shows the changes in degree of in vivo degradation of the copolyesters with the passage of implantation time as a function of polymer composition. The relationship between in vivo degradation and composition showed a similar tendency to that in vitro, giving a maximal degree of degradation for a pasty-type poly(CL-co-VL) with 53 mol% CL unit (sample 5, Table 1). The changes in shape of small cylindrical copolyesters with such typical morphologies as the waxy and pasty types, implanted into the backs of rats over a period of 7 weeks, are shown in Fig. 5, in which the degree of in vivo degradation was approx. 12% for a waxy-type poly(CL-co-VL) with 92 mol% CL unit (sam-

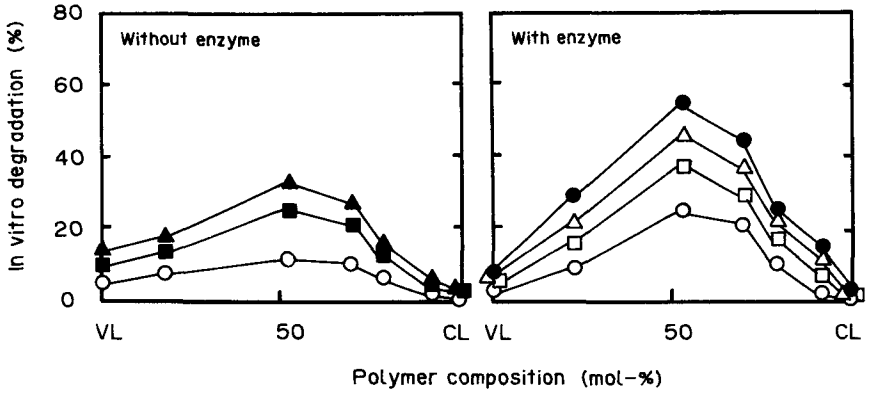

Fig. 3. In vitro degradation profiles of small cylinders of pure poly(CL-co-VL) with and without lipase; degradation time (weeks): (○) 1: (□) 2; (△) 3; (●) 4: (■) 10; (▲) 20.

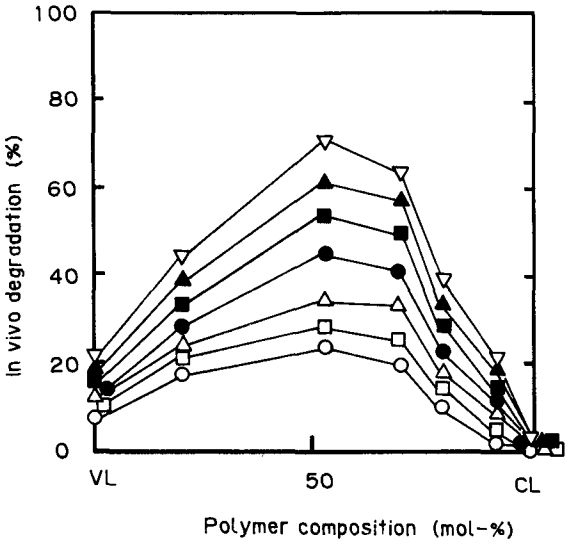

Fig. 4. In vivo degradation profile of small cylinders of pure poly(CL-co-VL); implantation time (weeks): (○) 1; (□) 3; (△) 5; (●) 7; (■) 10; (▲) 15; (▽) 20.

ple 2, Table 1) and 42% for a pasty-time poly(CL-co-VL) with 71 mol% CL unit (sample 4, Table 1). The appearance of the pasty-type copolyester showed a marked change during in vivo degradation, whereas no change was observed for the waxy-type copolyester. This means that the in vivo degradation mechanism differs remarkably as concerns the morphology of the polymer, being divided roughly into two types. Thus, one is the pasty type which is characterized by rapid degradation with time accompanying a deformation in shape, the other being the waxy type displaying moderate degradation with time and keeping its initial shape. In this case, the degradation proceeds mainly from the surface of the polymer during the rate-determining step. In conclusion, the present results suggested that the biodegradation of poly(CL-co-VL) is strongly influenced by parameters such as crystallinity and morphology according to the composition, leading to faster degradation of the pasty-type copolymer rather than its wax.

Drug release study from waxy-type poly(CL-co-VL)

A waxy-type poly(CL-co-VL) with 92 mol% CL unit (sample 2, Table 1) was chosen as an implantable matrix for drug delivery device because of its

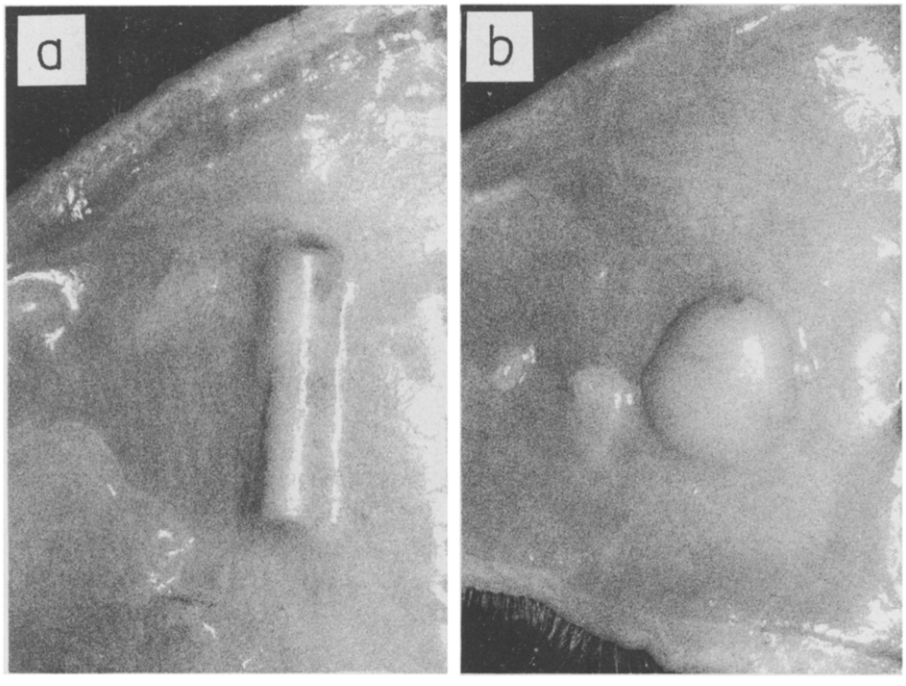

Fig. 5. Appearance of small cylinders of pure poly(CL-co-VL) with different morphologies implanted subcutaneously in the back of rats for 7 weeks; morphology of copolyester: (a) waxy-type poly(CL-co-VL) with 92 mol% CL unit, (b) pasty-type poly(CL-co-VL) 71 mol% CL unit.

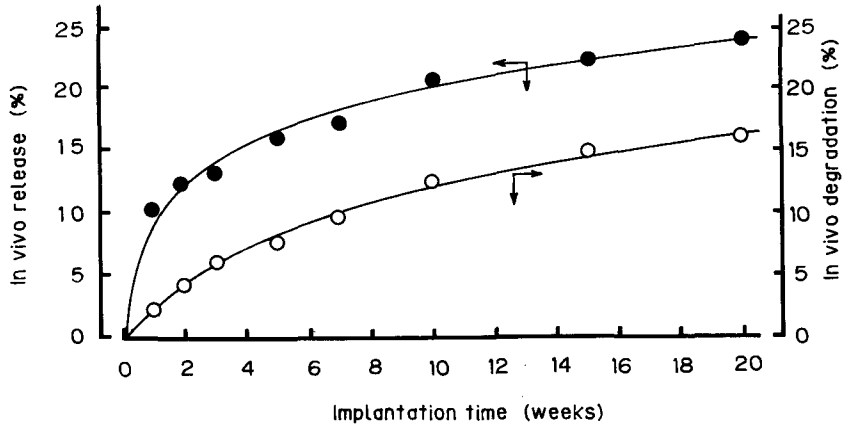

Fig. 6. Relationship between the in vivo cumulative amount of estramustine release from poly(CL-co-VL) with 92 mol% CL unit device and the in vivo degradation of its copolyester as a function of implantation time.

mild degradation in vivo over a very long period of time. Estramustine was incorporated into the waxy-type copolyester device in a small cylinder form by the melt-pressing technique. An important characteristic of this technique is that the mixing and shaping treatments between the matrix

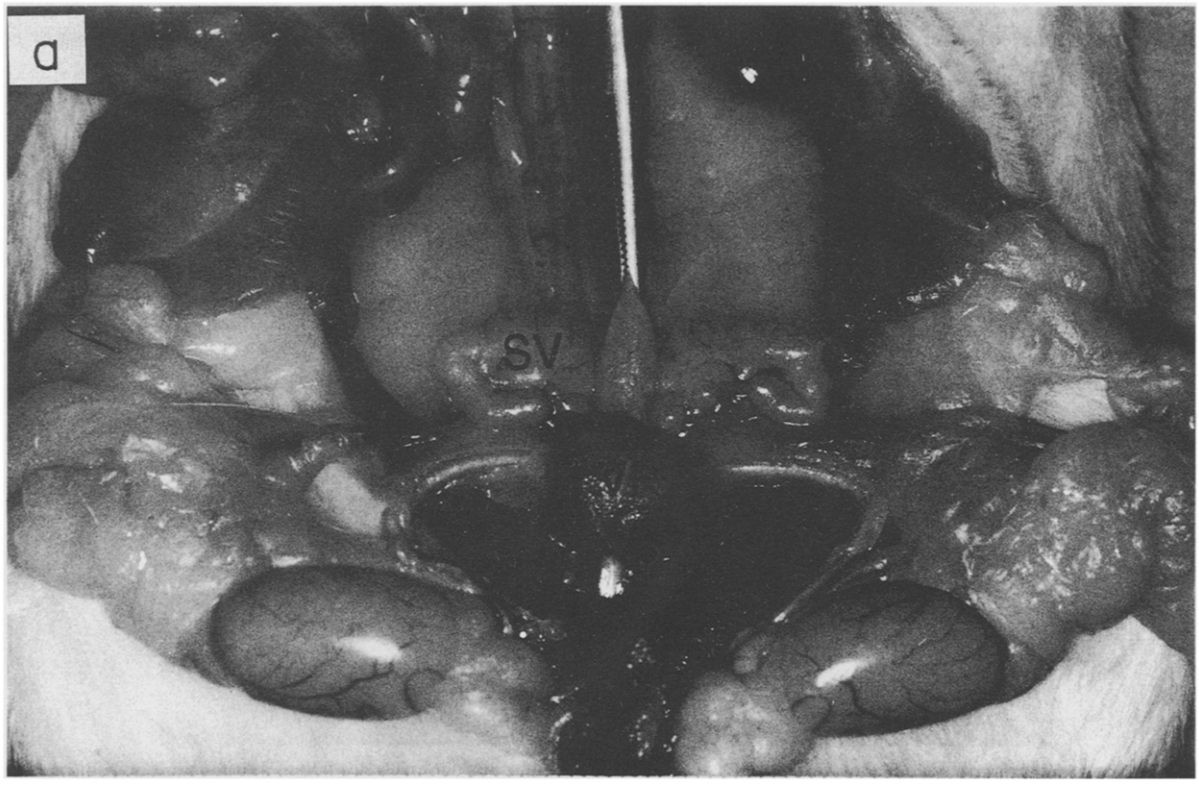

Fig. 7. Atrophy of VP and SV in male rats by waxy-type device administration consisting of poly(CL-co-VL) with 92 mol% CL unit and estramustine; implantation time: (a) before implantation (control); (b) after 10 weeks implantation.

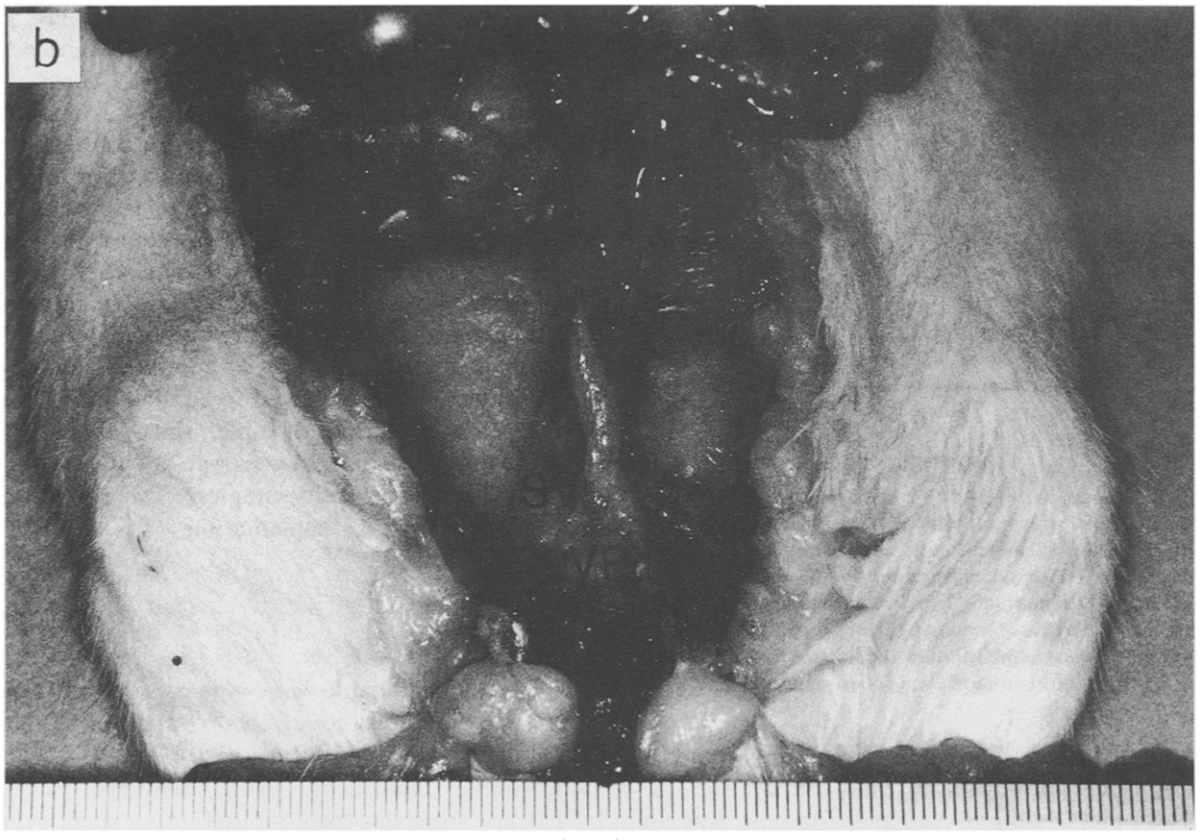

Fig. 7 (b).

and drug can be performed at relatively low temperature without the use of organic solvents, yielding homogeneous mixture. A resulting satisfactory recovery of drugs of more than 98% was demonstrated throughout the experimental procedure.

The cumulative amount of estramustine released in vivo from waxy-type copolyester devices is shown in Fig. 6. The release pattern led to a steady release with time after the initial rapid leakage corresponding to the burst phenomenon, e.g. the amount of released drug was found to be approx. 2.0 mg during the first week (burst effect of drug release), and 2.8 mg (21 μg/day) for 19 weeks from the 1st to the 20th week (steady state of drug release). Except for the initial burst of drug release, the release profile is roughly compatible with the in vivo degradation pattern as seen clearly in Fig. 6. This means that the rate of release of estramustine depends much more strongly on the rate of degradation of waxy-type copolyester than on the rate of diffusion of drug from the device. This aspect remains the most important subject to be resolved in future work for the initial burst of drug release because the waxy-type implantable copolyesters are very useful as a biodegradable device for drug delivery systems.

Pharmacological study on rat prostate

The pharmacological influence of estramustine released in vivo from waxy-type poly(CL-co-VL) with 92 mol% CL unit (sample 2, Table 1) devices was evaluated by measuring the change in weight of accessory sex organs such as VP and SV as shown clearly in Fig. 7. Such organs were atrophied by the administration of the drug delivery device until attainment of the castration level, followed by recovery to the normal level after the complete

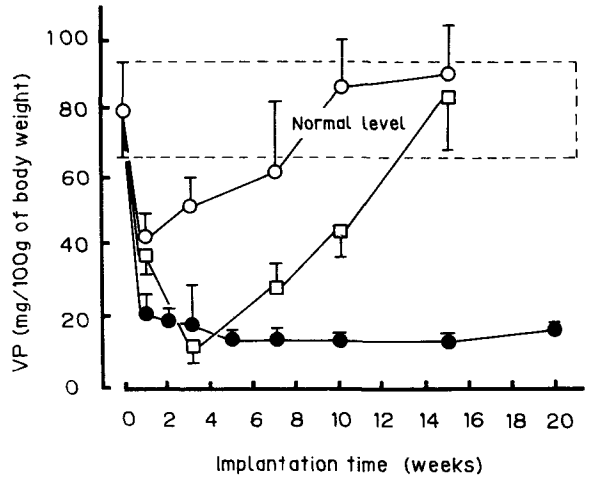

Fig. 8. Changes in weight of VP of male rats by estramustine administration with and without waxy-type poly(CL-co-VL) with 92 mol% CL unit devices; administration method: (○) subcutaneous single administration (DMSO solution containing 1 mg of estramustine); (□) subcutaneous single administration (DMSO solution containing 5 mg of estramustine); (●) waxy-type device administration copolyester device with controlled release of 20 mg of estramustine.

release of drug. For comparison, the extent of atrophy of sex organs by pure estramustine administration without polymer devices was examined analogously with that of the drug delivery device and the results are shown in Fig. 8 for VP and Fig. 9 for SV. In a single injection of pure

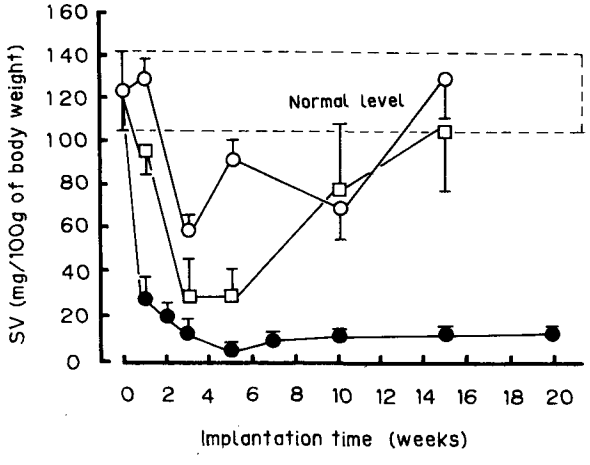

Fig. 9. Changes in weight of SV of male rats by estramustine administration with and without waxy-type poly(CL-co-VL) with 92 mol% CL unit devices. Symbols refer to administration method given in Fig. 8.

estramustine (1 and 5 mg), the organ weight, although showing a striking decrease after injection, completely recovered within 15 weeks. Such administration may be accompanied by a transient side-effect during the initial stage. In contrast, the most reasonable efficacy was obtained for an implantable device with controlled release of estramustine and, as a result, it was found that the pharmacological effect of estramustine corresponding to the castration level is maintained throughout an experimental period of 20 weeks by giving continuously a dose of approx. 21 μg/day. This effect can be expected to be maintained over much longer period of time, since about 76% initial drug remains in a way-type poly(CL-co-VL) with 92 mol% CL unit (sample 2, Table 1) device even after 20 weeks implantation.

Conclusion

The biodegradable waxy-type copolyesters were prepared by direct copolycondensation of CL and VL in the absence of catalysis, to use as an implantable matrix for drug delivery systems. Estramustine was incorporated into a small cylinder of waxy-type poly(CL-co-VL) with 92 mol% unit (sample 2, Table 1) device by the melt-pressing technique, giving a homogeneous mixture. The drug release, although accompanying a burst phenomenon in the initial stage, was strongly dependent on the biodegradability of the copolyester and was the rate-limiting step in the degradation of waxy-type copolyester. The in vivo data demonstrated that the biodegradable polymer wax is very useful as an implantable matrix for drug delivery systems, which control the release over relatively long periods 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 (1989a) 569–573.

Asano, M. Fukuzaki, H., Yoshida, M., Kumakura, M., Mashimo, T., Yuasa, H., Imai, K. and Yamanaka, H.,

Application of polyDL-lactic acids of varying molecular weight in drug delivery systems. *Drug Design Delivery*, 5 (1990a) 301–320.

Asano, M., Fukuzaki, H., Yoshida, M., Kumakura, M., Mashimo, T., Yuasa, H., Imai, K., Yamanaka, H. and Suzuki, K., In vivo characteristics of low molecular weight copoly(L-lactic acid/glycolic acid) formulations with controlled release of luteinizing hormone-releasing hormone agonist. *J. Controlled Release*, 9 (1989b) 111–122.

Asano, M., Yoshida, M., Fukuzaki, H., Kumakura, M., Mashimo, T., Yuasa, H., Imai, K. and Yamanaka, H., Effect of crystallinity on the in vivo degradation of poly(β-propiolactone) prepared by radiation-induced solid polymerization in the organic solvent system at low temperature. *Eur. Polym. J.*, 26 (1990b) 29–33.

Asano, M., Yoshida, M., Fukuzaki, H., Kumakura, M., Oya, M., Mashimo, T., Yuasa, H., Imai, K. and Yamanaka, H., Preparation and in vivo characteristics of biodegradable copoly(DL-alanine-ethyl L-aspartate) formulations with various structures of drug dispersion. *Yakuzaigaku*, 49 (1989c) 116–126.

Asano, M., Yoshida, M. and Kaetsu, I., Controlled slow release of anticancer drugs from device of thermally denatured proteins. *Kobunshi Ronbunshu*, 39 (1982) 327–332.

Asano, M., Yoshida, M., Kaetsu, I., Yamanaka, H., Nakai, K., Yuasa, H., Shida, K. and Oya, M., Fusibility of poly(N-carboxy α-amino acid anhydride) materials treated under pressure-heat conditions and in vitro-in vivo degradation of hot-pressed materials. *J. Macromol. Sci. Chem.*, A21 (1984) 783–791.

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 derivative of estradiol-17β. *Proc. Natl. Acad. Sci. USA*, 76 (1979) 3149–3156.

Fukuzaki, H., Yoshida, M., Asano, M., and Kumakura, M., Synthesis of copoly(D,L-lactic acid) with relatively low molecular weight and in vitro degradation. *Eur. Polym. J.*, 25 (1989a) 1019–1026.

Fukuzaki, H., Yoshida, M., Asano, M., Kumakura, M., Mashimo, T., Yuasa, H., Imai, K., Yamanaka, H., Kawaharada, U. and Suzuki, K., A new biodegradable pasty-type copolymer of L-lactic acid and δ-valerolactone with relatively low molecular weight for application in drug delivery systems. *J. Controlled Release*, 10 (1989b) 292–303.

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.

Imasaka, K., Nagai, T., Yoshida, M., Fukuzaki, H., Asano, M. and Kumakura, M., Direct copolycondensation of ϵ-caprolactone with δ-valerolactone in the absence of catalysis. *Eur. Polym. J.*, 26 (1990) 831–836.

Imasaka, K., Ueda, H., Azuma, T., Kawaguchi, T. and Nagai, T., Application of new silicone gel to sustained release dosage form of antitumor drug. *Drug Design Delivery*, 4 (1989a) 237–246.

Imasaka, K., Ueda, H., Azuma, T. and Nagai, T., Application of PHYCON 6600® sustained release of antitumor drug (carmofur). *Drug Design Delivery*, 5 (1989b) 159–165.

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.

Oda, Y., Kamijyo, Y., Kang, Y., Okamura, T., Tokuriki, Y., Handa, H., Mori, K. and Hashi, K., Evaluation of the sustained release of anticancer agents from silicone pellets. *J. Jap. Soc. Cancer Ther.*, 20 (1985) 2236–2242.

Schakenraad, J.M., Oosterbaan, J.A., Nieuwenhuis, P., Molenaar, I., Olijslager, J., Potman, W., Eenink, M.J.D. and Feijen, J., Biodegradable follow fibres for the controlled release of drug. *Biomaterials*, 9 (1988) 116–120.

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

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. Jap.*, 3 (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. Jap.*, 29 (1982) 669–674.

Yanagiya, K., Controlled release of Adriamycin from silicone elastomer and its antitumor effects on Yoshida sarcoma. *Kokubyou Gakkai Zassi*, 53 (1986) 386–405.

Yoshida, M., Asano, M., Kaetsu, I., Mashimo, T., Imai, K., Yuasa, H. and Yamanaka, H., Pharmacological response in male rats with controlled release formulations of luteinizing hormone-releasing hormone agonist. *Polym. J.*, 18 (1986) 287–296.

Yoshida, M., Asano, M., Morita, Y., Kaetsu, I., Imai, K., Mashimo, T., Yuasa, H., Yamanaka, H., Kawaharada, U. and Suzuki, K., In vivo release of cisplatin from a needle-type copolymer formulation implanted in rat kidney. *Biomaterials*, 10 (1989) 16–22.