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Mechanism of Enhancement of the Release Rate of Aclarubicin from Poly-β-hydroxybutyric Acid Microspheres by Fatty Acid Esters

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Poly-β-hydroxybutyric acid (PHB) microspheres containing aclarubicin hydrochloride were prepared by a solvent-evaporation process. The release rates of the drug from the microspheres were significantly increased by incorporating ethyl or *n*-butyl esters of fatty acids. In order to clarify the effects of fatty acid esters on drug release, the powder X-ray diffraction patterns of microspheres, contents of fatty acid esters in microspheres and other factors were investigated. Powder X-ray diffraction, scanning electron microscopy and differential scanning calorimetry indicated no significant differences due to incorporation of the esters in the microspheres. The contents of fatty acid esters in the microspheres and the solubility of aclarubicin hydrochloride in the fatty acid esters greatly affected the release rate of the drug from the microspheres.

Keywords——poly-β-hydroxybutyric acid; microsphere; fatty acid ester; aclarubicin

Introduction

Injectable drug delivery systems such as microspheres containing anticancer agents have been developed. These have been employed in intra-arterial chemoembolization for solid tumors. Such selective delivery of anticancer drugs to the target tumor is expected to increase the therapeutic index and to minimize severe side effects.

For long-term release of drugs, biodegradable polymers such as gelatin,[1] albumin,[2,3] polylactic acid,[4,5] polyglycolic acid[6] and polycarbonate[7] have been investigated for use as drug carriers. Now, we have studied poly-β-hydroxybutyric acid (PHB) as a drug carrier. PHB is a new biodegradable polymer and is synthesized by various bacteria.[8] We previously prepared[9] PHB microspheres containing aclarubicin hydrochloride, and showed that the rate of drug release from microspheres was enhanced by the use of fatty acid esters as additives.

The present investigation was undertaken to clarify the role of fatty acid esters in enhancing the rate of drug release from PHB microspheres.

Experimental

Materials——PHB was supplied by ICI Japan Ltd., Tokyo. By using an Ostwald viscometer, its intrinsic viscosity value was determined to be 3.26 dl/g in chloroform at 37°C. The weight-average molecular weight of PHB was then calculated to be 4.4×10^5 according to the relationship between intrinsic viscosity and weight-average molecular weight for PHB reported by Marchessault *et al.*[10] The melting range of PHB was 175—177°C. Aclarubicin hydrochloride (ACR·HCl) was supplied by Sanraku Ocean Co., Tokyo. Ethyl and butyl esters of caproic, caprylic, capric, lauric, myristic, palmitic and stearic acid, all of reagent grade, were purchased from Nakarai Chemicals, Kyoto, except for butyl palmitate, which was purchased from Tokyo Chemical Industry Co., Tokyo, and butyl myristate and butyl stearate, which were gifts from Nikko Chemicals Co., Tokyo. Alkaline-processed gelatin, 200 bloom, was from Nitta Gelatin Co., Osaka. Methylene chloride and hexane of reagent grade were purchased from Wako Pure Chemical Industries Co., Osaka. All chemicals were used without further purification.

Table I. Gas Chromatographic Conditions

	Column oven temp. (°C)		Injection port and detector temp. (°C)	
	Ethyl ester	Butyl ester	Ethyl ester	Butyl ester
Caproate	90	100	120	130
Caprylate	120	120	150	150
Caprate	140	150	170	180
Laurate	160	170	190	200
Myristate	180	190	210	220
Palmitate	210	210	240	240
Stearate	230	230	250	250

Column; 3% Silicone OV-17 on Chromosorb WAW. 2 m. Carrier gas; nitrogen, 50 ml/min.

Preparation of PHB Microspheres——PHB microspheres were prepared by a solvent-evaporation process similar to that reported previously. A weighed amount (7.5 mg) of one of the esters, 6.25 mg of the drug and 30 mg of PHB were dissolved in 1 ml of methylene chloride. The solution was then dispersed in 35 ml of 1% (w/v) gelatin solution under stirring at a rate of 650 rpm by means of a three-bladed propeller. The stirring was maintained for 30 min at room temperature (about 25°C) to evaporate methylene chloride. The microspheres were collected by filtration through a sintered glass disk, washed with distilled water, and dried under reduced pressure at room temperature.

Measurements of Partition Coefficients——The drug sample was dissolved in 0.1 M phosphate buffer solution, pH 6.0 and a 3 ml aliquot of the drug solution was shaken with 3 ml of one of the esters for 20 min at room temperature. After centrifugation, the amounts of the drug left in the aqueous layer were determined spectrophotometrically at 434 nm, and compared with that of the original aqueous solution before partition.

Volatility of Esters——A weighed amount of an ester was dried *in vacuo* and kept at 25°C. After 1, 3, 5 and 7 d, each sample was weighed and its volatility was determined from its weight loss.

Measurements of X-Ray Diffraction——The powder X-ray scattering patterns of the microspheres were measured at room temperature with an X-ray diffractometer, Toshiba ADX-103. The X-ray source was CuK_α, voltage 35 kV, current 15 mA, time constant 2 s. A symmetrical-reflection goniometer was scanned at 2°/min between $2\theta = 3°$ and $2\theta = 70°$.

Microscopic Examination of Microspheres——Surfaces and crosssectional appearances of the microspheres were observed with a scanning electron microscope (MINI-SEM, MSM-102, Akashi Seisakusho, Tokyo) after Au-coating of the microspheres using an ion coater (IB-3, Eiko Engineering Co., Tokyo).

Differential Scanning Calorimetric Measurements——A differential scanning calorimeter (DSC), Rigakudenki TG-DSC, was used. Amounts of samples were about 18 mg, and the heating rate was 10°C/min.

Determination of Ester Contents——The amount of an ester in the microspheres was determined by using a gas chromatograph (GC), Hitachi 063, equipped with a flame ionization detector. The procedure for sample preparation was as follows, and GC conditions are shown in Table I. Weighed amounts of the microspheres were initially dissolved in 0.2 ml of methylene chloride. Then 0.5 ml of hexane was added and the mixture was shaken immediately to extract the ester into the solvent. An aliquot of the solution was injected onto a GC column.

Results and Discussion

Scanning electron photomicrographs of PHB microspheres prepared with three kinds of esters at a level of 25% of PHB or without any ester are shown in Fig. 1. No significant difference in appearance was found between the microspheres with an ester and those without any ester.

There was also no marked degradation or erosion of microspheres after the release experiments, based on observation with the scanning electron microscope.

As PHB is a highly crystalline polymer, the drug release may be expected to be increased if the crystallinity of the PHB matrix is decreased in the presence of an ester. However, differential scanning calorimetric observations indicated that the crystallinity of PHB in the

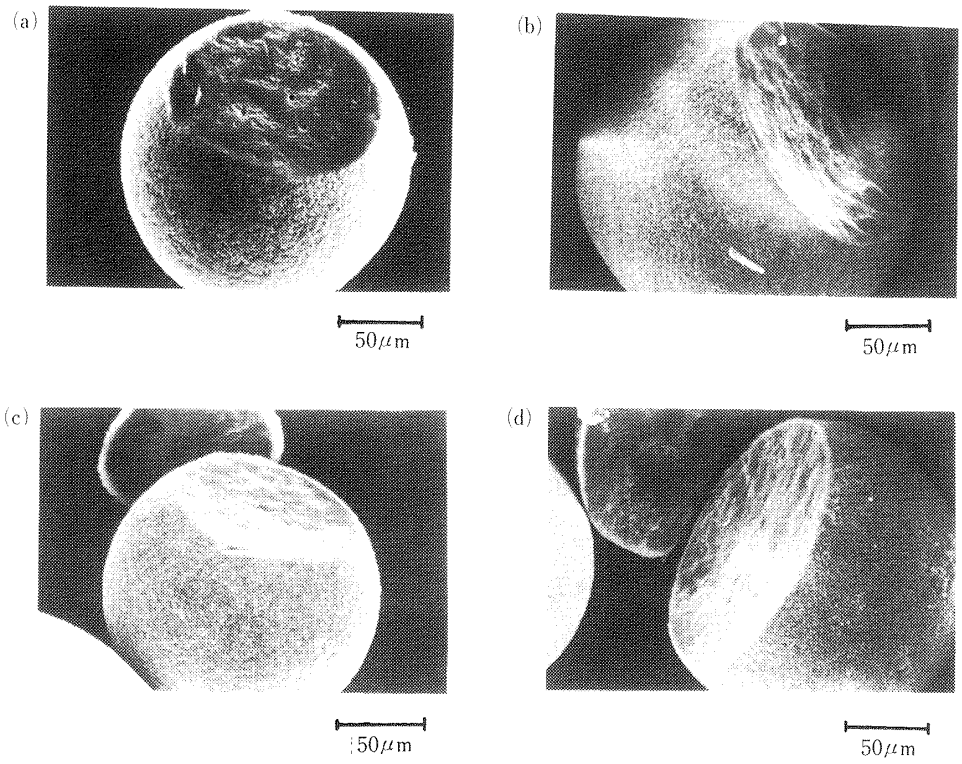

Fig. 1. Scanning Electron Photomicrographs of PHB Microspheres

(a) PHB/drug; (b) PHB/drug/ethyl caproate (C_6); (c) PHB/drug/ethyl laurate (C_{12}); (d) PHB/drug/ethyl stearate (C_{18}). Amounts of the drug and ester added with 16.7% and 25% of PHB, respectively.

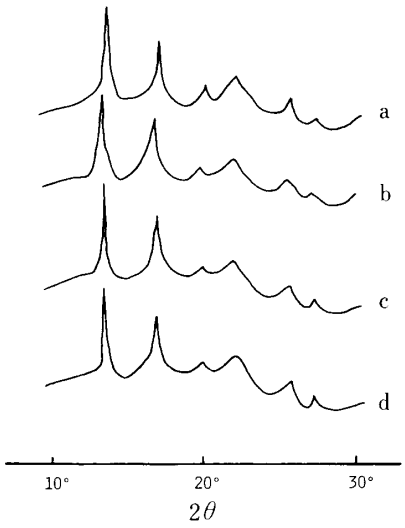

Fig. 2. Powder X-Ray Diffraction Patterns of PHB Microspheres Prepared with Butyl Esters of Fatty Acids (C_6, C_{12}, and C_{18}) at a Level of 25% of PHB

a) PHB only; b) PHB + C_6; c) PHB + C_{12}; d) PHB + C_{18}.

microspheres prepared with addition of ethyl palmitate, which is one of the most effective release enhancers, was not significantly different from that in the microspheres prepared without using an ester (not shown in a figure). It was also found in the study of powder X-ray diffraction patterns of PHB microspheres with butyl esters of fatty acids (C_6, C_{12}, and C_{18}), and without an ester, that the crystallinity of PHB was not changed markedly by addition of release enhancers (esters, Fig. 2).

Since the release rate of the drug from PHB microspheres was very small, diffusivity of the drug in the PHB matrix and/or water channels, if any, is considered to be very small. We

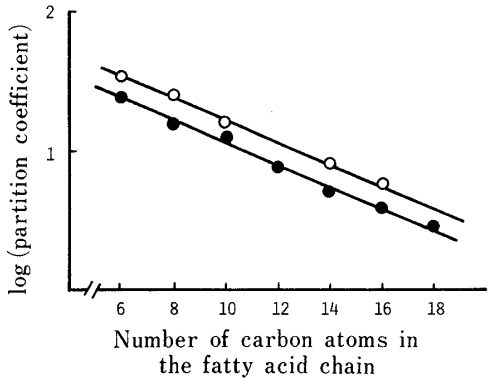

Fig. 3. Dependence of Partition Coefficients of Aclarubicin between Fatty Acid Esters and 0.1 M Phosphate Buffer Solution at pH 6.0 on Fatty Acid Chain Length

○, ethyl esters; ●, butyl esters.

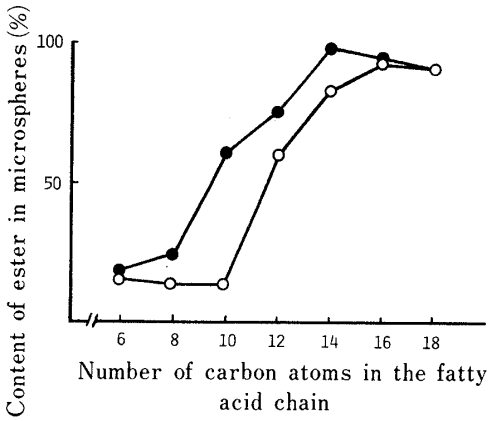

Fig. 4. Dependence of Contents of Fatty Acid Esters in PHB Microspheres on Fatty Acid Chain Length

○, ethyl esters; ●, butyl esters.

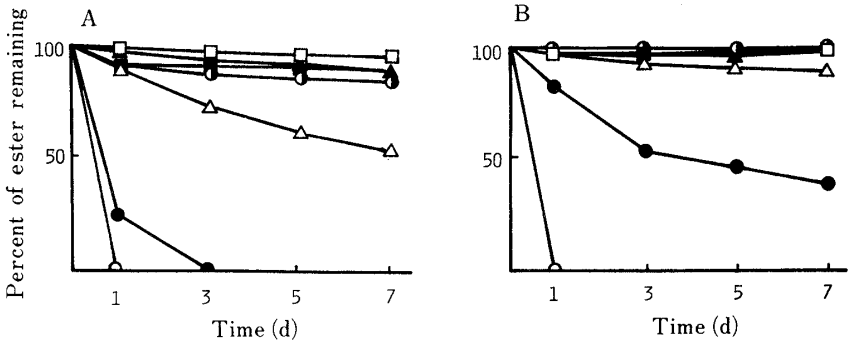

Fig. 5. Dependence of Volatility of Fatty Acid Esters on Fatty Acid Chain Length

○, C_6; ●, C_8; △, C_{10}; ▲, C_{12}; □, C_{14}; ■, C_{16}; ◑, C_{18}. A, ethyl esters; B, butyl esters.

speculate that the additive (ester) forms a kind of channel (channels filled with additives) through which the drug can diffuse out of the microspheres. Figure 3 shows partition coefficients of the drug between fatty acid esters and 0.1 M phosphate buffer solution at pH 6.0. From these results we indirectly obtained the solubility of ACR·HCl in the esters. As shown in Fig. 3, when a fatty acid with a shorter-length alkyl chain was used as the oil phase, the drug was partitioned more in the oily phase.

Figure 4 shows the contents of fatty acid esters as additives in PHB microspheres prepared by incorporating each ester at a level of 25% of PHB. As can be seen in the figure, the longer the carbon chain of the ester, the greater was the amount of the ester incorporated. It is suggested that esters with a shorter acyl chain length have some aqueous solubility, and therefore they dissolved in the dispersion phase during preparation of the microspheres. The release of esters from microspheres during release experiments was also examined and it was observed that percentages of esters which have shorter carbon chains released during release experiments were much greater than those of esters which have longer carbon chains. Namely, 93.1% of ethyl caproate (C_6) and 86.1% of butyl caproate (C_6) were released from microspheres during release experiments. The percentages of esters released also decreased with increase in the carbon number of the fatty acid chain; only 4.4% of ethyl stearate (C_{18}) and 0% of butyl stearate (C_{18}) were released from microspheres.

In addition, the volatility of esters was considered. Weight changes of fatty acid esters

(C_6—C_{18}) under reduced pressure at 25 °C in a desiccator are shown in Fig. 5. Esters with shorter carbon chains would have evaporated during preparation and drying of the microspheres. Due to the resulting low contents of esters with shorter carbon chains in the microspheres, it is expected that their promoting effects on the release rate of the drug were revealed only to a small extent.

Conclusion

No significant difference in crystallinity was found between the PHB matrix which was prepared with fatty acid esters and that without them. Therefore the fact that the drug release is promoted in the presence of the ester in the microspheres cannot be rationalized in terms of a decrease in the crystallinity of the PHB matrix. Another factor has to be considered. As the release rate of the drug from PHB microspheres prepared without an ester was very small, the drug may diffuse out through channels which are formed by the esters. This would explain why the contents of fatty acid esters in the microspheres and the solubility of ACR·HCl in fatty acid esters greatly influence the release rate of ACR·HCl from PHB microspheres.

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References

1) T. Yoshioka, M. Hashida, S. Muranishi and H. Sezaki, *Int. J. Pharmaceut.*, **81**, 131 (1981).
2) P. A. Kramer, *J. Pharm. Sci.*, **63**, 1646 (1974).
3) Y. Morimoto, K. Sugibayashi and Y. Kato, *Chem. Pharm. Bull.*, **29**, 1433 (1981).
4) N. Mason, C. Thies and T. J. Cicero, *J. Pharm. Sci.*, **65**, 847 (1976).
5) K. Juni, J. Ogata, M. Nakano, T. Ichihara, K. Mori and M. Akagi, *Chem. Pharm. Bull.*, **33**, 313 (1985).
6) T. Iwa, M. Ohira, T. Yamada, R. Yamashita and M. Sakatoku, *Igaku No Ayumi*, **135**, 1095 (1985).
7) T. Kojima, M. Nakano, K. Juni, S. Inoue and Y. Yoshida, *Chem. Pharm. Bull.*, **32**, 2795 (1984).
8) D. J. Senior and E. A. Dawes, *Biochem. J.*, **125**, 55 (1971).
9) K. Juni, M. Nakano and M. Kubota, *J. Controlled Release*, **4**, 25 (1986).
10) R. H. Marchessault, K. Okamura and C. J. Su, *Macromolecules*, **3**, 735 (1970).