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Modification of the Release Rate of Aclarubicin from Polylactic Acid Microspheres by Using Additives

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Poly(lactic acid) (PLA) microspheres containing aclarubicin were prepared by a solvent-evaporation process and their release patterns were examined *in vitro*. When microspheres were prepared with the drug and PLA, the release rates of the drug were extremely small. When microspheres were prepared with fatty acid esters as additives, the release rates of the drug were significantly increased depending on the amount of the additive incorporated. Two additives, isopropyl myristate and medium-chain triglyceride, exhibited similar effects on the steady-state release rates of the drug from the microspheres. In addition, the effect of alkyl chain length of the additives on the release rate of the drug was examined by using a series of ethyl and butyl esters of fatty acids. A parabolic relationship was obtained between the steady-state release rate of the drug and the alkyl chain length of the additives. Therefore the release rate of the drug could be controlled by proper selection of the additive and by adjusting the amount of the additive used in the preparation of the microspheres.

Keywords—poly(lactic acid); microsphere; aclarubicin; sustained release; fatty acid ester; isopropyl myristate; medium-chain triglyceride; alkyl chain length

In order to deliver anticancer agents selectively to tumor-containing areas, many attempts have been directed towards the development of injectable delivery systems, including microencapsulation of anticancer agents by using insoluble polymers.¹⁾ When selecting polymers for that purpose, biodegradable polymers are preferable to nonbiodegradable polymers. Microspheres prepared with biodegradable polymers such as albumin,²⁻⁴⁾ gelatin,⁵⁾ polyalkylcyanoacrylate,⁶⁾ and poly(lactic acid)⁷⁻⁹⁾ have been examined as carriers mainly for doxorubicin,^{3,8)} mitomycin C,^{4,5)} and 5-fluorouracil.^{2,7)} We have reported on the preparation and release profiles of poly(lactic acid) microspheres containing doxorubicin⁸⁾ and bleomycin.⁹⁾

Aclarubicin is one of the anthracycline anticancer antibiotics, and it has lower cardiotoxicity than other anthracyclines, *i.e.* daunorubicin and doxorubicin.¹⁰⁾ It has been shown that aclarubicin is metabolized more rapidly than other anthracyclines and that repetitive administration or a large dose is necessary to obtain higher antitumor effects.¹¹⁾ Studies on the effect of aclarubicin on cultured cells have also suggested that continuous administration at a low concentration or divided administrations is the most effective for clinical application.¹²⁾ Therefore sustained-release preparations of aclarubicin administered in close proximity to the tumor may provide a superior effect. As far as we know, there has been no report on the sustained release of aclarubicin.

Therefore we have investigated the preparation and release characteristics *in vitro* of poly(lactic acid) (PLA) microspheres containing aclarubicin. When microspheres were prepared with PLA and the drug alone, the release rate of the drug was extremely small. Previously we have reported that the release rates of bleomycin from PLA microspheres were

greatly enhanced by additives such as fatty acid esters.⁹⁾ Therefore in preliminary experiments, various substances were examined as additives which might enhance the release rate of aclarubicin from PLA microspheres. The following substances were examined on the basis of suitable hydrophobicity for incorporation in the microspheres and clinical applicability: lecithin, cholesterol, triethyl citrate and triacetin (often used as plasticizers), olive oil (a vegetable oil), stearyl monoglyceride, medium-chain triglyceride, and a series of alkyl esters of fatty acids. Among the substances examined, fatty acid esters, medium-chain triglyceride and alkyl esters of fatty acids proved to be suitable as additives, *i.e.* they significantly enhanced the release rate of the drug without interfering with the formation of microspheres.

In the present report, the preparation of PLA microspheres containing aclarubicin and the modification of the release profile of the drug by using fatty acid esters as additives are described.

Experimental

Materials—The following materials were supplied by the cited companies; aclarubicin hydrochloride from Sanraku Ocean Co., Tokyo; L-poly(lactic acid) (PLA) with an average molecular weight of 35000 from Mitsui Toatsu Chemicals Co., Ohmuta; gelatin, alkaline processed, 200 bloom, from Nitta Gelatin Co., Osaka; isopropyl myristate (IPM), butyl myristate, and butyl stearate from Nikko Chemicals Co., Tokyo; and medium chain triglyceride (MCT, triglyceride consisting of a 75:25 mixture of caprylic acid and capric acid, ODO®) from Nisshin Seiyu Co., Tokyo. Ethyl caprate, ethyl laurate, ethyl myristate, ethyl palmitate, ethyl stearate, butyl caprate, and butyl laurate, all of reagent grade, were purchased from Nakarai Chemicals Co., Kyoto. Methylene chloride and chloroform of reagent grade were purchased from Wako Junyaku Kogyo Co., Osaka. All chemicals were used without further purification.

Preparation of PLA Microspheres—PLA microspheres were prepared by a solvent-evaporation process as reported previously.¹³⁾ Weighed amounts of the drug powder, an additive, and 75 mg of PLA were dissolved in 1 ml of methylene chloride. When ethyl and butyl esters of fatty acids were used as additives, the loading level of the additives was fixed at 25% of PLA. The solution was then dispersed in 30 ml of 1% (w/v) gelatin solution under stirring at a rate of 500 rpm by means of a magnetic stirrer. The stirring was continued for 30 min at $25 \pm 2^\circ\text{C}$. The microspheres were collected by filtration through a sintered glass disk, washed with distilled water, and dried under reduced pressure at room temperature.

Determination of Drug Contents and Sizes of Microspheres—Weighed amounts of microspheres were dissolved in chloroform and the drug concentration was determined spectrophotometrically at 434 nm. Drug contents of microspheres were then calculated. None of the additives interfered with the determinations.

Microspheres were observed under an optical microscope (BH-2, Olympus Kogaku Co., Tokyo) and their diameters were measured.

Release Studies—Weighed amounts of microspheres were put into a flask containing 20 ml of a saline solution containing 0.01% (w/v) polysorbate 80. The flask was immersed in a shaker bath maintained at $37.0 \pm 0.1^\circ\text{C}$ and shaken horizontally. At predetermined intervals, 5 or 10 ml of the solution was sampled and the same volume of fresh medium was added. The amount of the drug released was calculated by spectrophotometric determination of the sample solution at 258 nm.

Results and Discussion

PLA microspheres containing aclarubicin and various additives were successfully prepared by a process similar to that reported previously.¹³⁾ Table I summarizes the compositions of materials used in the preparation, as well as the sizes and drug contents of microspheres. The diameters of the microspheres did not vary significantly when additives of different species or in different amounts were used. Further, the drug contents of the microspheres did not vary significantly because the weight fraction of the drug in the total materials used in preparation was fixed in every preparation. The diameters and drug contents of the microspheres containing ethyl and butyl esters of fatty acids as additives did not differ significantly from those of the microspheres containing IPM or MCT. If all of the materials used, *i.e.* the drug, the additive, and PLA, were included in the final preparations, the drug content should be 9.1%. In fact, some of the drug was partitioned into the outer gelatin

solution during preparation. It was observed that almost all of the PLA used was incorporated into the microspheres. It may be considered that almost all of the additive used was incorporated into the microspheres because the additives used in this study are practically insoluble in water. In fact, more than 99% of IPM used was incorporated in the final preparations (data not shown). It is considered that 85 to 91% of the drug initially loaded could be incorporated into the microspheres, irrespective of the amount and species of additive used.

As reported previously,⁹⁾ PLA microspheres containing an additive might be regarded as a composite matrix where the additive is dispersed in the PLA matrix. Aclarubicin can dissolve in both the PLA matrix and the oily additive portion because of its lipophilicity. Although diffusion processes of the drug in such a composite matrix can be complex,¹⁴⁾ we have attempted to analyze the release data by using the $Q-t^{1/2}$ relationship reported by Higuchi,¹⁵⁾ where the cumulative amount of drug released (Q) is directly proportional to the square root of time ($t^{1/2}$). In the present study, the fraction of drug released (F), instead of Q ,

TABLE I. Compositions of Materials Used in Preparation, Sizes, and Drug Contents of Microspheres

Additive	Additive/PLA %	Diameter, μm mean \pm SEM ($n=100$)	Drug content, % ^{a)}
None	0	159.8 \pm 5.1	7.7
IPM	10	234.7 \pm 4.8	7.9
	15	236.0 \pm 4.7	7.9
	25	233.0 \pm 4.2	7.9
	50	260.3 \pm 4.9	8.3
	100	263.0 \pm 7.6	8.1
MCT	10	189.3 \pm 6.5	8.2
	15	176.9 \pm 8.1	7.8
	25	220.0 \pm 7.0	8.0
	50	208.8 \pm 8.8	8.1
	100	228.2 \pm 9.0	7.7

The weight ratio, drug/(drug+additive+PLA), was fixed at 9.1%. a) Mean of two separate preparations. SEM, standard error of the mean.

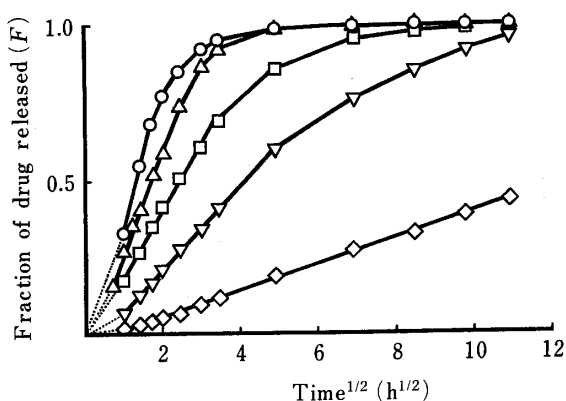


Fig. 1. Release Profiles of Aclarubicin from PLA Microspheres Containing IPM at the Level of 10% (\diamond), 15% (∇), 25% (\square), 50% (\triangle) or 100% (\circ) of PLA

Average of two experiments.

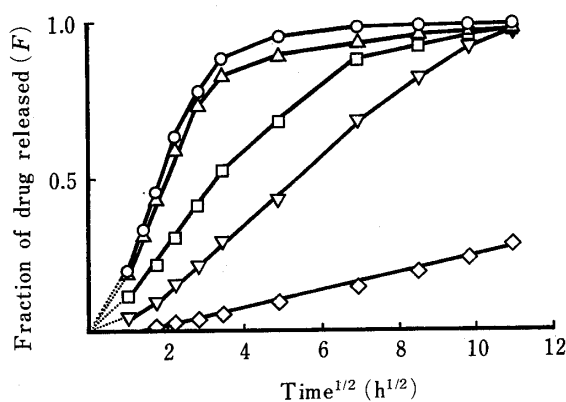


Fig. 2. Release Profiles of Aclarubicin from PLA Microspheres Containing MCT at the Level of 10% (\diamond), 15% (∇), 25% (\square), 50% (\triangle) or 100% (\circ) of PLA

Average of two experiments.

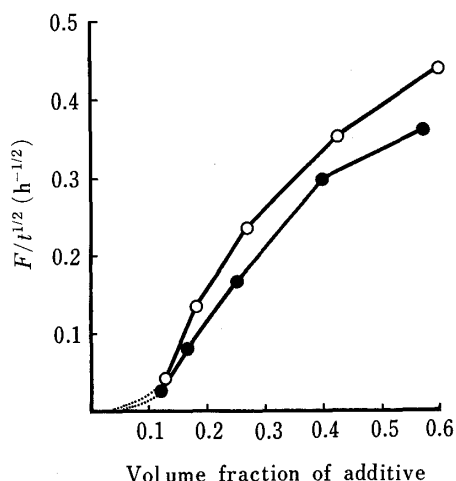


Fig. 3. Steady-State Fractional Release Rates of Aclarubicin as a Function of Volume Fraction of Additive in the PLA Microspheres
Additive: ○, IPM; ●, MCT.

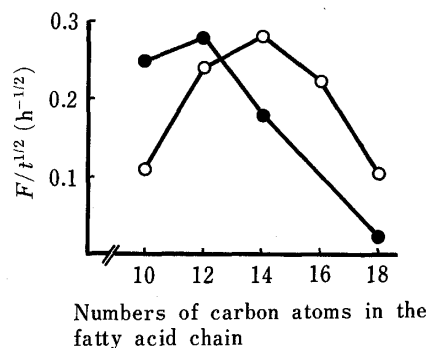


Fig. 4. Relationship between the Steady-State Fractional Release Rates of Aclarubicin and the Alkyl Chain Length of Additives in the PLA Microspheres

○, ethyl esters; ●, butyl esters.
Average of two experiments.

was plotted *versus* $t^{1/2}$.

The effect of the amount of additive used on the release rate of the drug was observed with IPM and MCT as additives. Figures 1 and 2 show the release profiles of aclarubicin from PLA microspheres. When the microspheres were prepared with the drug and PLA, less than 2% of the drug was released in 120 h (data not shown). When IPM or MCT was incorporated into the microspheres, a significant increase in the release rate of the drug was observed depending on the amount of the additive used. In all cases, nearly linear profiles were obtained until the fraction of drug released reached about 0.7. From these results, it is clear that small changes in the additive/PLA ratio between 10 and 25% resulted in large changes in the release rate of the drug. The steady-state fractional release rate of the drug ($F/t^{1/2}$) was estimated from the slope of the linear portion of the $F-t^{1/2}$ profile in each case.

In Fig. 3, the estimated $F/t^{1/2}$ values are plotted against the volume fractions of the additives in the microspheres, which were approximated as the volume of the additive divided by that of PLA plus the additive calculated from the loaded amount of each material in the preparation. As can be seen in the figure, similar dependency of $F/t^{1/2}$ values on the volume fractions of IPM and MCT was noted. Thus, the release rate of the drug from the microspheres can be controlled by changing the amount of IPM or MCT incorporated into the microspheres.

In addition to the effect of difference in the amount of additive, the effect of difference in the alkyl chain length of fatty acid esters as additives on the release rate of the drug was evaluated by using ethyl esters of capric acid (C_{10}), lauric acid (C_{12}), myristic acid (C_{14}), palmitic acid (C_{16}), and stearic acid (C_{18}) and butyl esters of capric acid, lauric acid, myristic acid, and stearic acid. The steady-state fractional release rates of the drug ($F/t^{1/2}$) were estimated from $F-t^{1/2}$ profiles and are plotted against the number of carbon atoms in the fatty acid chain of the esters in Fig. 4.

In the series of ethyl esters, a parabolic relationship between the $F/t^{1/2}$ values and alkyl chain length was obtained. A similar relationship was obtained in the series of butyl esters, with a shift from the case of ethyl esters in the alkyl chain length which gave maximum $F/t^{1/2}$. Although the reason for this relationship is not fully understood, mutual solubility or compatibility of PLA, the drug, and an additive may be dependent on the alkyl chain length

of the additive, and may markedly affect the release rate of the drug from the microspheres.

In summary, PLA microspheres containing aclarubicin were prepared and the release patterns of the drug were determined *in vitro*. Various degrees of sustained release of the drug are obtainable by adjusting the amount and the kind of fatty acid esters with different alkyl chain lengths incorporated into the microspheres as an additive.

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