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Preparation and Evaluation *in Vitro* and *in Vivo* of Polylactic Acid Microspheres containing Dibucaine

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DL-Polylactic acid microspheres containing dibucaine were prepared, and release patterns of dibucaine from the microspheres as well as the local anesthetic effects of the drug in the microspheres were examined. The influences of nonsolvents and dibucaine concentration at preparation on the characteristics and dibucaine contents of the microspheres were investigated. Higher pH in nonsolvents and higher dibucaine concentration at preparation resulted in increased dibucaine contents in the microspheres. The release patterns of dibucaine from microspheres varied significantly among microspheres with different dibucaine contents, and the release mechanisms of dibucaine from microspheres were greatly influenced by disintegration of the microspheres.

The local anesthetic effects of 0.1 ml aliquots of dibucaine hydrochloride solutions were examined at four concentrations *in vivo*. Then, the local anesthetic effects of dibucaine in microspheres were compared with those of the above solutions in relation to the release rate of dibucaine from microspheres *in vitro*. The more the amount of dibucaine, the stronger was the local anesthetic effect. The local anesthetic effect of dibucaine in microspheres lasted much longer (300 h) than that of dibucaine hydrochloride solutions. The profile of local anesthetic effect of dibucaine in microspheres *in vivo* was different from the release profile *in vitro*.

Keywords——DL-polylactic acid; biodegradable polymer; microsphere; dibucaine; local anesthetic effect; sustained release; release pattern

Biodegradable polymers such as polylactic acid[1–3] can be advantageously used in injectable sustained release formulations. They have been evaluated as tools in fertility control,[4] antimalarial chemotherapy,[5,6] and anticancer chemotherapy.[7]

Although dibucaine is one of the most potent local anesthetics, it tends to exhibit very strong side-effects when it enters the blood.[8] Therefore, sustained release formulations of dibucaine for topical use can be expected to increase the duration of local anesthetic effect and to decrease the incidence of side-effects.

Recently, clinical pharmacological aspects of local anesthetics have been reviewed.[8,9] Long-acting local anesthetic formulations are desired for the relief of severe pain associated with terminal cancer and trigeminal neuralgia, although various nerve blocks are carried out in pain clinics. For that reason, efforts to prepare long-acting local anesthetic formulations have been made.[10,11] Even when bupivacaine, etidocaine (a new long-acting local anesthetic agent) or dibucaine in liposomes were used, the local anesthetic action lasted for only a few hours.[9,10]

We have been examining polylactic acid (PLA) systems to achieve sustained release of local anesthetics and a long-term local anesthetic effect. In our previous publications, we reported the use of PLA microspheres as a means to achieve sustained release of local anesthetics[12] and we found that the local anesthetic effect of tetracaine in microspheres was statistically superior ($p<0.05$) to the control until 120 h after implantation.[13] Dibucaine from PLA microspheres was proved to give better sustained release than butamben and tetracaine from the microspheres.[12]

In the present study, the preparation and release characteristics *in vitro* of PLA microspheres containing dibucaine were investigated. Moreover, the local anesthetic effect of dibucaine in microspheres was compared with that of dibucaine hydrochloride solutions in order to

examine its possible use in nerve blocks for the control of intractable pain caused by cancer and trigeminal neuralgia.

Experimental

Materials——Dibucaine hydrochloride was purchased from Teikoku Kagaku Sangyo Co. (Osaka) and transformed into its base by treatment with sodium hydroxide solution. PLA was prepared from DL-lactic acid[2] purchased from Wako Junyaku Kogyo Co. (Osaka). The mean molecular weights of two lots of PLA, PLA's 1 and 2, were determined viscometrically to be 9100 and 17000, respectively, by using an Ostwalt viscometer with benzene as a polymer solvent at 30°C.[14]

Gelatins, acid and alkaline process, 200 bloom, were gifts from Nitta Gelatin Co. (Yao, Osaka). Methylene chloride of reagent grade from Wako Junyaku Kogyo Co. was used without further purification.

Preparation of PLA Microspheres——PLA microspheres were prepared by a solvent-evaporation process.[12] Weighed amounts (500 mg) of PLA and dibucaine were dissolved in methylene chloride. The solution was added dropwise into a round-bottomed flask containing 100 ml of 1 or 2% gelatin solution. The stirring rate was kept at 800 rpm. Reduced pressure was applied to the suspension to evaporate off the methylene chloride, and then microspheres were centrifuged and collected by filtration. The collected microspheres were dried at room temperature under a vacuum and were sized through a standard sieve.

Observation of PLA Microspheres by Scanning Electron Microscopy——The dried microspheres were observed with a scanning electron microscope (model MSM-102, Akashi Manufacturing Co., Tokyo) to examine their shapes and surface characteristics. In an *in vivo* study, PLA microspheres were kept in subcutaneous tissue in guinea pigs, and taken out 72 and 120 h after implantation.

Release Studies——Microspheres (10 mg) were placed inside a 50 ml flask containing 25 ml of an isotonic citrate-phosphate buffer solution, pH 7.4. The flask was placed in a shaker bath (model R-100, Taiyo Scientific Industrial Co., Tokyo) maintained at 37°C and was shaken horizontally at a rate of 90 cpm. Release patterns were obtained by measuring the concentration of dibucaine released from the microspheres spectrophotometrically at 326 nm.

Partition Studies——Dibucaine (5 mg) was dissolved in 0.5 ml of methylene chloride and this solution was added to 4 ml of one of four kinds of nonsolvents (aqueous solutions). The solutions were equilibrated for 2 h by reciprocal shaking (model YS-8D, Yayoi Co., Tokyo) at a rate of 240 cpm. Partition coefficients of dibucaine between each nonsolvent and methylene chloride were obtained by measuring the concentration of dibucaine remaining in the nonsolvent spectrophotometrically at 326 nm.

Measurement of Dibucaine Activity——Guinea pig skin techniques[15] were used for measurement of the local anesthetic activity of dibucaine. A 0.1 ml portion of dibucaine hydrochloride solution in the injectable isotonic saline solution was injected subcutaneously into the dorsum and the injected area was encircled in ink. The examination of anesthesia commenced 5 min later by applying stimulation repeatedly every 3 s, stopping as soon as the reflex reappeared, *i.e.* at the slightest quiver of the skin. The number of stimuli producing no response was noted up to the maximum of six (100%). Scores represent the extent of local anesthetic effect. The stimulus was continued at 5 min intervals for 30 min. The activity of dibucaine hydrochloride solution was compared at various dibucaine concentrations.

The activity of dibucaine in microspheres was measured after dorsal subcutaneous implantation of microspheres containing 20 mg of dibucaine. To mark out the implantation site, the area was encircled in ink. The examination of anesthesia was carried out in the same way as with dibucaine hydrochloride solution. Scores were added up for each 30 min period, the maximum (100%) being thirty-six (maximum score of six in 6 measurements).

Results and Discussion

Physical Characteristics and Drug Contents in Microspheres

Table I shows the physical characteristics and dibucaine contents of PLA 2 microspheres prepared using 2% alkaline processed gelatin solution as a nonsolvent. Since PLA 2 tended to aggregate easily in 1% gelatin solution, the PLA 2 microspheres obtained were mixtures of spherical and nonspherical forms when 1% alkaline processed gelatin solution was used as a nonsolvent. Therefore, 2% alkaline processed gelatin solution was used, and in this case, the PLA 2 microspheres obtained were all spherical, possibly because the higher viscosity of 2% alkaline processed gelatin solution favored suspension of PLA-methylene chloride droplets. With increase in the dibucaine content at preparation, the dibucaine content in the resultant microspheres was increased. The average diameter of the microspheres was about 50 μm as measured from photomicrographs.

Table I. Characteristics and Dibucaine Contents of Microspheres prepared by Evaporation *in Vacuo* employing Methylene Chloride as a Polymer Solvent and 2% Gelatin as a Nonsolvent

Preparation	Drug/polymer ratio at prep.	Yield, %[a)]	Diameter, μm	Drug content, %
A	20/80	60	57.1±11.7	13.4
B	30/70	70	46.5± 6.8	20.3
C	35/45	74	58.9± 9.8	29.9
D	40/60	70	53.2± 6.8	35.9
E	45/55	62	65.1± 5.2	39.8
F	50/50	67	62.2± 8.3	44.7

a) Yield (%) = $\frac{\text{total weight of microspheres obtained}}{\text{total weight of drug and polymer used}} \times 100$.

Table II. Effects of Nonsolvents on the Characteristics and Dibucaine Contents of Microspheres prepared by Evaporation *in Vacuo* employing Methylene Chloride as a Polymer Solvent with a Drug/Polymer Ratio of 30/70 at Preparation

Preparation	Nonsolvent	Yield, %	Diameter, μm	Drug content, %	Shape
G	1% acid pr. gelatin	56	44.4±2.1	9.2	80% sphere[a)]
H	1% alkaline pr. gelatin	57	56.4±2.6	24.4	Sphere
I	0.27 M Na_2HPO_4 in 1% alkaline pr. gelatin	50	71.7±2.5	29.0	Sphere

a) Calculated from duplicate microscopic observations of 200 randomly selected particles.

Table III. Dependence of Partition Coefficient of Dibucaine on Nonsolvents

Nonsolvent	pH	Partition coeff. (Nonsolvent/CH_2Cl_2)
1% acid pr. gelatin	3.8	2.5×10^{-2}
1% alkaline pr. gelatin	7.5	2.2×10^{-3}
0.27 M Na_2HPO_4 in 1% alkaline pr. gelatin	8.6	5.3×10^{-4}

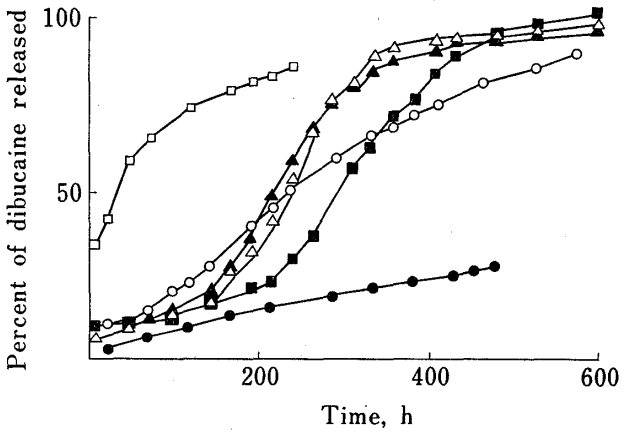

Fig. 1. Release Patterns of Dibucaine from PLA Microspheres containing 13.4% (A, ●), 20.3% (B, ○), 29.9% (C, ▲), 35.9% (D, △), 39.8% (E, ■), and 44.7% (F, □) Dibucaine

Each value represents the mean of 3 experiments.

Table II shows the effects of nonsolvents on the dibucaine contents in PLA 1 microspheres. It was found that the dibucaine contents in microspheres were in the order of Preparation I> H>G. Physicochemical properties of three kinds of nonsolvents are summarized in Table III. It was found that the higher the pH value of the nonsolvent, the smaller was the partition coefficient (nonsolvent/methylene chloride) of dibucaine. Therefore, the dibucaine contents in the

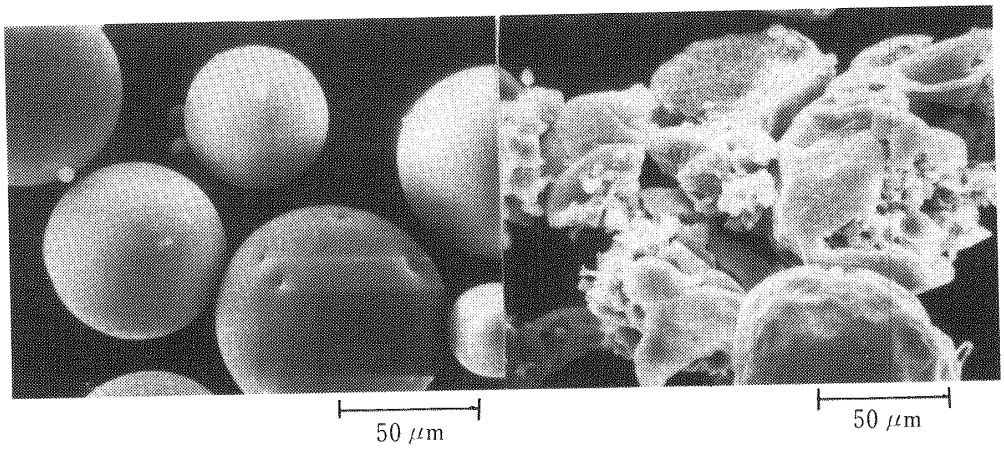

Fig. 2. Scanning Electron Photomicrographs of PLA Microspheres containing 13.4% Dibucaine (Preparation A) before Release (Left) and after Release for 480 h (Right)

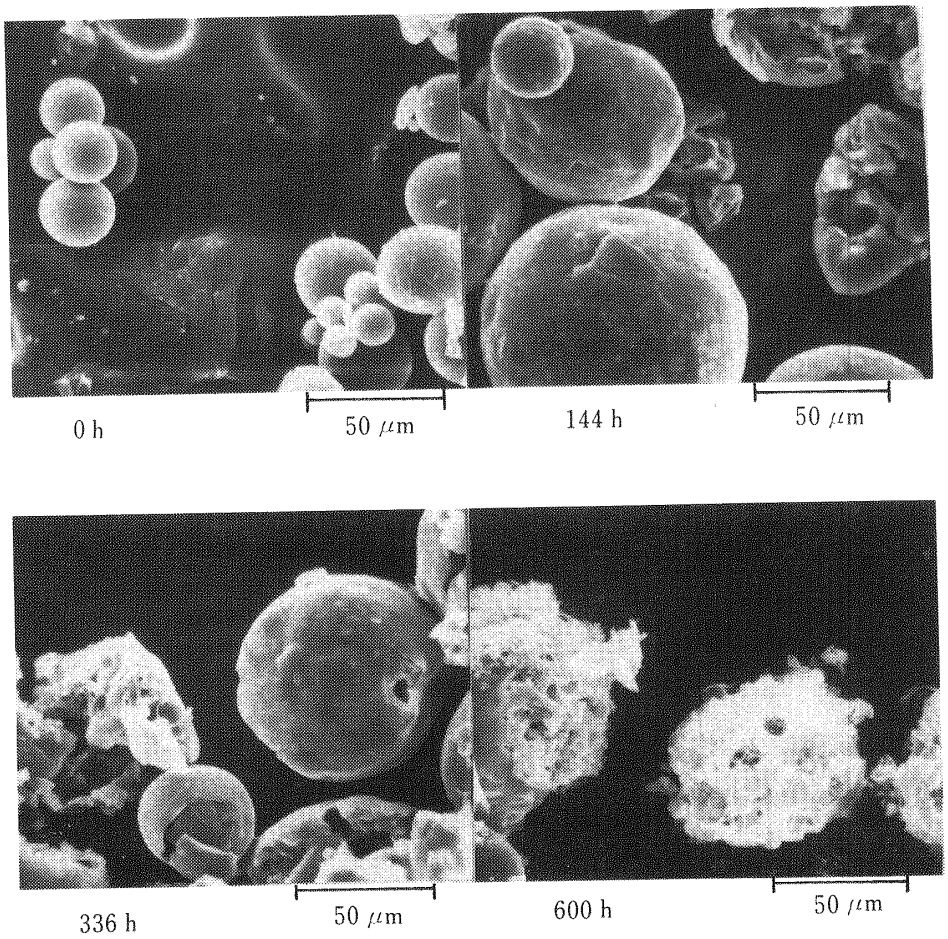

Fig. 3. Scanning Electron Photomicrographs of PLA Microspheres containing 20.3% Dibucaine (Preparation B) before Release (0 h) and after Release for 144, 336, and 600 h

microspheres increased with increase in the pH value of nonsolvents, because dibucaine (pK_a= 1.6 and 8.3) is almost wholly in an ionized form in 1% acid processed gelatin solution at pH 3.8, predominantly in an ionized form in 1% alkaline processed gelatin solution at pH 7.5, and predominantly in an unionized form in 0.27 M sodium phosphate (dibasic) in 1% alkaline processed galatin solution at pH 8.6.

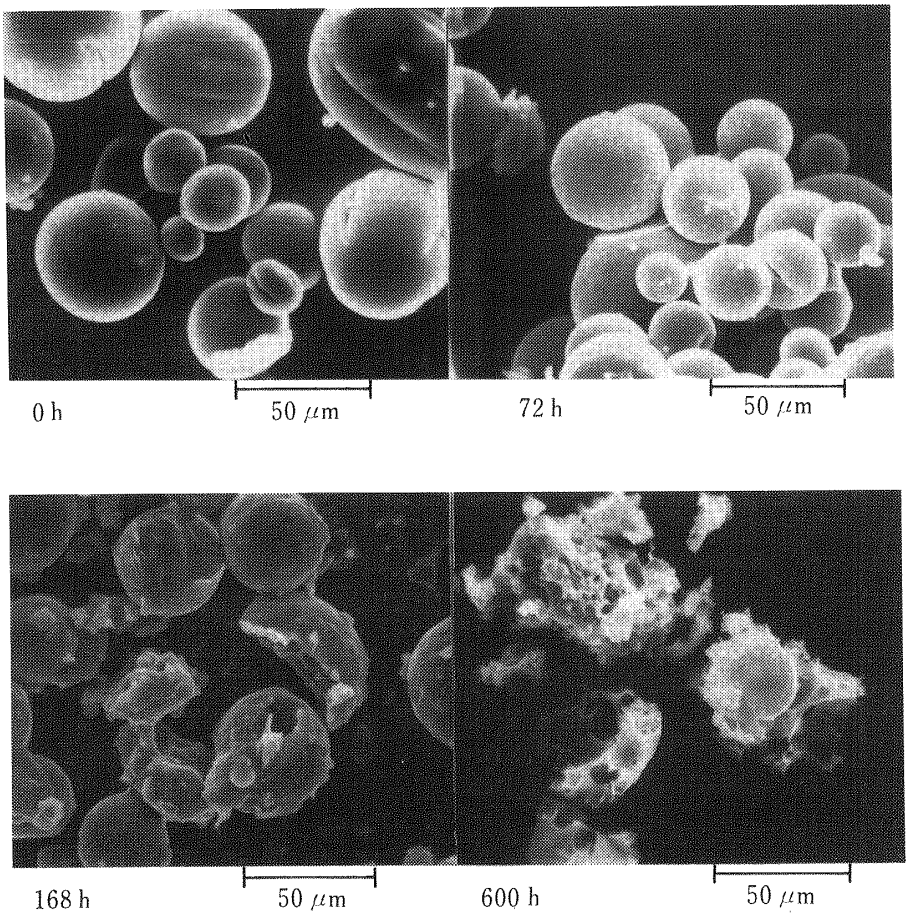

Fig. 4. Scanning Electron Photomicrographs of PLA Microspheres containing 35.9% Dibucaine (Preparation D) before Release (0 h) and after Release for 72, 168, and 600 h

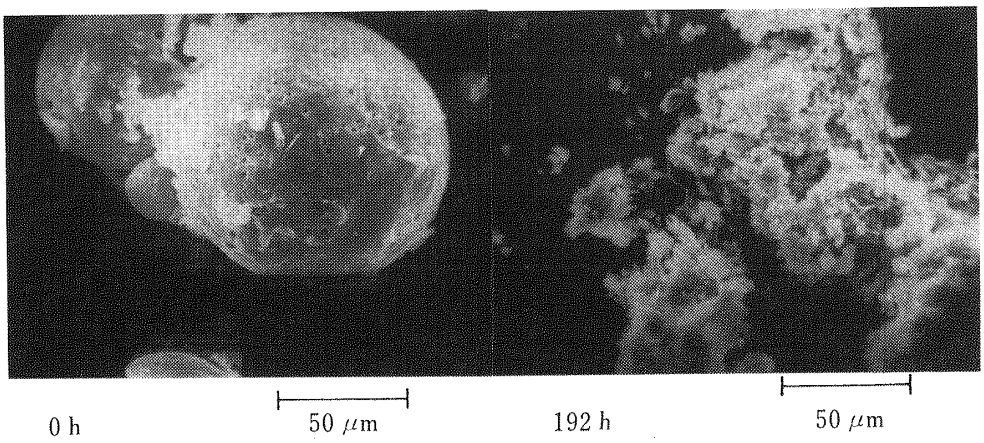

Fig. 5. Scanning Electron Photomicrographs of PLA Microspheres containing 44.7% Dibucaine (Preparation F) before Release (Left) and after Release for 192 h (Right)

Release Patterns

Figure 1 shows the release patterns of dibucaine from PLA 2 microspheres prepared by using 2% alkaline processed gelatin solution as a nonsolvent (Preparations A, B, C, D, E, and F). The release rate of dibucaine from Preparation A was very slow, whereas that from Preparation F was very rapid. Although the release pattern of Preparation A was nearly zero-order, those of Preparations B, C, D, and E were sigmoid. Scanning electron microscopic observations of Preparations A, B, D, and F showed changes in the surfaces and shapes of microspheres with time (Figs. 2—5). In Preparation A, microspheres before release showed round and smooth surfaces, but they were disintegrated after release for 480 h (Fig. 2). In Preparation B, microspheres before release showed round and smooth surfaces, but they became mixtures of porous and disintegrated forms after release for 144 and 336 h, and were then totally disintegrated (about 100% released) after release for 600 h (Fig. 3). Moreover, in Preparation D, microspheres before release and after release for 72 h showed round and smooth surfaces, but they were disintegrated at 168 and 600 h (about 100% released) (Fig. 4). In Preparation F, microspheres before release were porous and they were disintegrated after release for 192 h (Fig. 5).

It seems likely that the release mechanism of dibucaine from microspheres involves both disintegration of microspheres and diffusion through the PLA matrix, and that increase in dibucaine content leads to a faster disintegration rate. From the scanning electron photomicrographs, it may be considered that the sigmoid-type rise in the release rate of Preparation D was a result of disintegration of the microspheres. Nearly zero-order release observed in Preparation A may be attributable to the high density of PLA and a slower disintegration rate of the matrix due to the fact that this preparation had the smallest content of the drug.

Intensity and Duration of the Local Anesthetic Effect of Dibucaine Hydrochloride Solutions

Figure 6 shows the intensity and duration of the local anesthetic effect following injection of 0.1 ml of 0.1, 0.05, 0.02, and 0.01% dibucaine hydrochloride solution and saline (control). During the first 30 min period, there were significant differences ($p<0.05$) between the effects of 0.1, 0.05, and 0.02% dibucaine hydrochloride solutions and saline, but there was a significant difference (5% level) between the effects of 0.01% dibucaine hydrochloride solution and saline only at 5 and 15 min according to Student's t-test. In the third 30 min period (from 60 to 90 min), there were no significant differences (5% level) between the effects of 0.1, 0.05, 0.02, and 0.01% dibucaine hydrochloride solutions and saline. The local anesthetic effect is related to the amount of tetracaine hydrochloride rather than the concentration of tetracaine

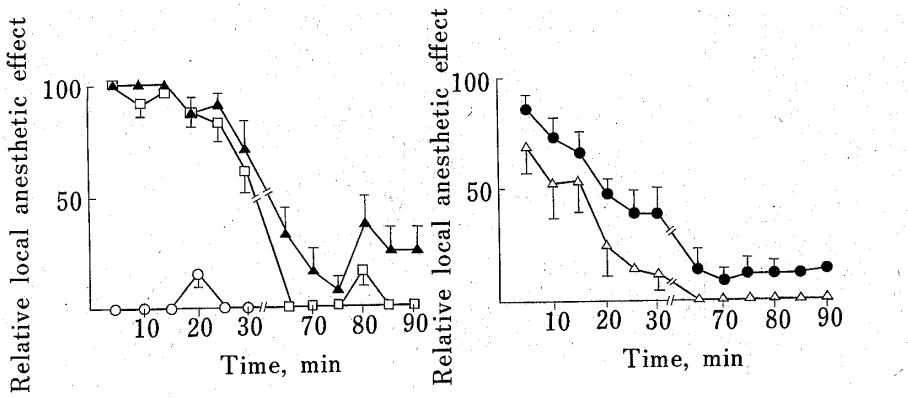

Fig. 6. Relative Local Anesthetic Effects following Administration of 0.1 ml of 0.1% (▲), 0.05% (□), 0.02% (●), and 0.01% (△) Dibucaine HCl Solutions and Saline (○)

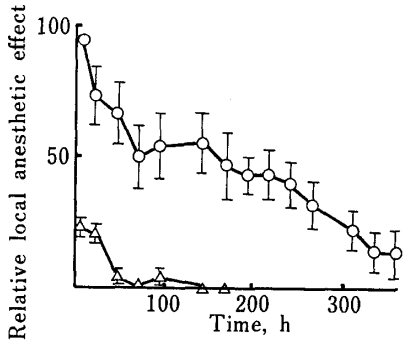

Fig. 7. Relative Local Anesthetic Effects following Administration of 20 mg of Dibucaine in 100 mg of Microspheres (Preparation B, ○) and Drug-free Microspheres (△)

Each value represents the mean±S.E. of 6 experiments.

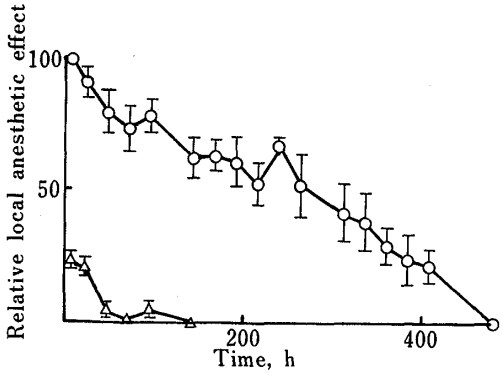

Fig. 8. Relative Local Anesthetic Effects following Administration of 20 mg of Dibucaine in 56 mg of Microspheres (Preparation D, ○) and Drug-free Microspheres (△)

Each value represents the mean±S.E. of 6 experiments.

hydrochloride solutions.[13] About 20 μg of dibucaine hydrochloride was required to produce local anesthetic effect in guinea pigs.

Intensity and Duration of the Local Anesthetic Effect of Dibucaine in Microspheres and Drug-free Microspheres

Figure 7 and 8 show the intensity and duration of the local anesthetic effect of dibucaine in microspheres. Even when 0.1% dibucaine hydrochloride solution was administered, the local anesthetic effect disappeared 1 h after injection (Fig. 6). However, dibucaine in microspheres (Preparations B and D) exhibited local anesthetic effect for a much longer period than dibucaine solution or drug-free microspheres. Preparation B exhibited a statistically significant ($p<0.05$) effect according to Student's t-test until 240 h except at 168 h after implantation. The effect of Preparation D was statistically significant ($p<0.05$) until 360 h except at 336 h after implantation. On the other hand, the effect of tetracaine in microspheres was statistically significant ($p<0.05$) until about 100 h after implantation.[13] It is evident that the release of dibucaine from the microspheres *in vivo* was more sustained than that of tetracaine. When drug free microspheres were implanted subcutaneously into guinea pigs, some local anesthetic effect was observed until 96 h after implantation (Figs. 7 and 8). In this case, cutaneous necrosis might have been produced by the surgical operation, this could account for the observed effect.

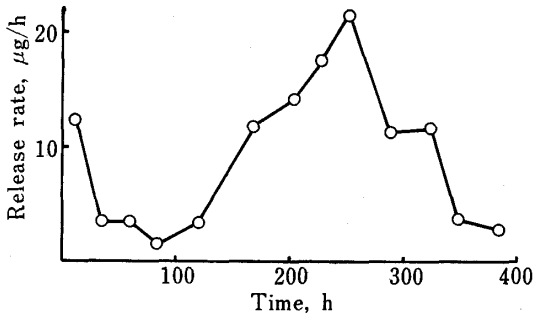

Fig. 9. Release Rate of Dibucaine from 10 mg of Microspheres containing 35.9% Dibucaine (Preparation D)

Each value represents the mean of 3 experiments.

Figure 9 shows the release rate of dibucaine from 10 mg microspheres (Preparation D). When the profile of local anesthetic effect (Fig. 8) is compared with this result, it can be seen that the *in vitro* release rate decreases in the initial period, then it increases after about 80 h, reaches a peak after about 250 h, and finally decreases again, whereas the profile of local anesthetic effect gradually decreases with time. Therefore, the release pattern of dibucaine from micro-

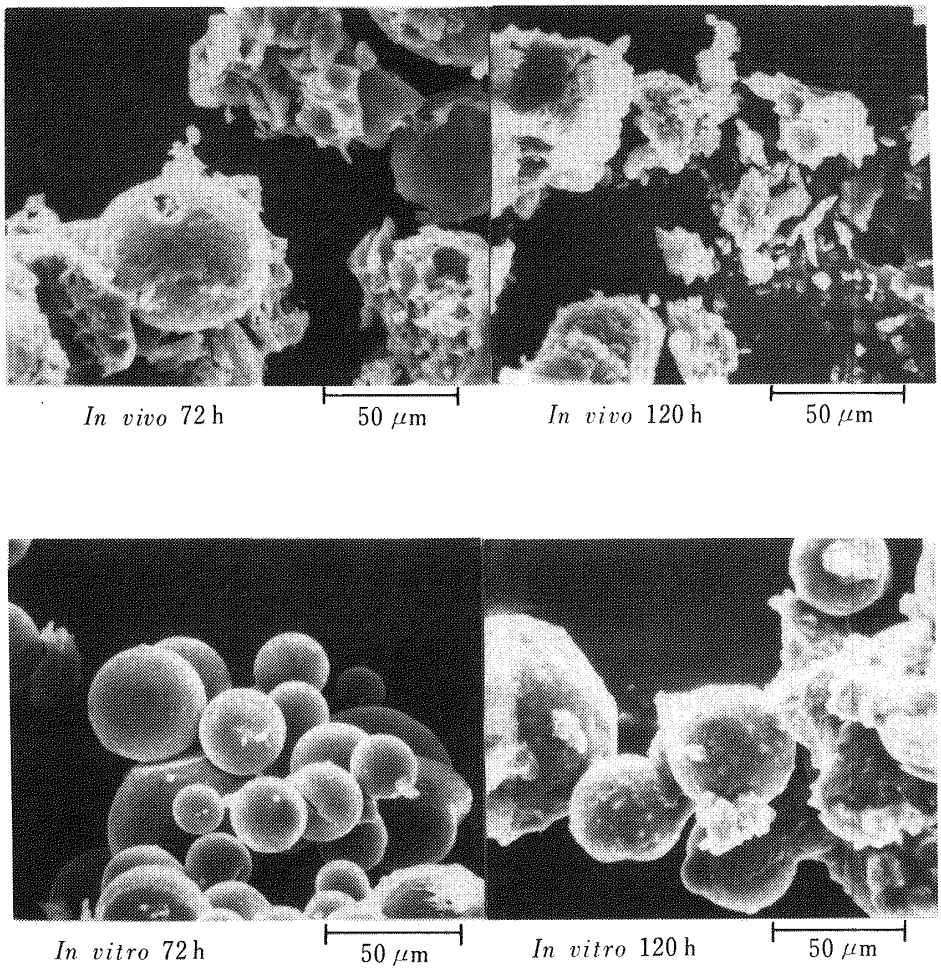

Fig. 10. Scanning Electron Photomicrographs of PLA Microspheres containing 35.9% Dibucaine (Preparation D) after Implantation (Above) and *in Vitro* Release (Below) for 72 and 120 h

spheres *in vivo* might be different from that *in vitro*. Figure 10 shows scanning electron photomicrographs illustrating changes in the surface characteristics of Preparation D *in vivo* and *in vitro* with time (72 and 120 h). It can be seen that the microspheres were disintegrated at 72 and 120 h after implantation (*in vivo*) but they were smooth at 72 h and only slightly disintegrated after release for 120 h (*in vitro*). The disintegration rate of microspheres *in vivo* was faster than that *in vitro* and the release rate of dibucaine from Preparation D *in vivo* was also faster than that *in vitro*. PLA is a biodegradable polymer, and the half-life of degradation of 100% PLA was reported to be 6.6 months, whereas that of 50 : 50 lactic and glycolic acid copolymer was only 2 weeks.[16] We examined the degradation rate of PLA containing tetracaine in 0.01 N NaOH in comparison with that of drug-free PLA. The degradation rate of PLA containing tetracaine was much faster than that of drug-free PLA. With increase in the tetracaine content, the degradation rate increased (unpublished data). Therefore, it seems likely that the disintegration and biodegradation rates of microspheres containing as much as 35.9% dibucaine (Preparation D) were rapid.

General Discussion

The present study revealed that the release rate of dibucaine from PLA microspheres was slow and was greatly influenced by the disintegration of the microspheres. The release rate of dibucaine by diffusion was very slow (Fig. 1) and the solubility of dibucaine in water permeated into microspheres should be high because dibucaine is in an ionic form in water.

Release of dibucaine from PLA microspheres was sustained. Since dibucaine has a strong local anesthetic action,[8] a long-term local anethetic effect may be obtained with dibucaine in microspheres.

Although more animal studies are required before clinical use, dibucaine in microspheres may be applicable to the control of pain in pain clinics, since administration of local anesthetics has been used as one of the methods for control of pain due to cancer and trigeminal neuralgia. The microspheres can be injected as a suspension.

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