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Preparation and Evaluation *in Vitro* and *in Vivo* of Polycarbonate Microspheres Containing Dibucaine

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Polycarbonate microspheres containing dibucaine were prepared and evaluated as injectable sustained release formulations. Poly(ethylene carbonate) and two lots of poly(ethylene propylene carbonate) were used alone or as mixtures to prepare microspheres. The dibucaine contents of these microspheres were about 27 to 30% when they were prepared at a constant drug/polymer ratio of 30/70. The release rate of dibucaine from the microspheres could be varied by changing the composition of polymer matrices. Therefore, various sustained release patterns of dibucaine were obtainable by the employment of polycarbonate microspheres of different polymer compositions.

Three representative preparations of microspheres were examined for local anesthetic effects in guinea pigs. The longest duration of local anesthetic effect was obtained following the implantation of the poly(ethylene propylene carbonate) microspheres with an intermediate release rate of the drug *in vitro*.

Keywords——poly(ethylene carbonate); poly(ethylene propylene carbonate); biodegradable polymer; microsphere; sustained release; dibucaine; local anesthetic effect

In recent years, various drug delivery systems using polymer matrices have been developed. The biodegradability of polymers is advantageous when they are applied parenterally to humans. Biodegradable natural polymers such as gelatin,[1] albumin[2] and starch[3] in the form of micro- or nano-particles have been examined as carrier matrices for drugs in cancer chemotherapy. Synthetic polymers such as polylactic acid,[4] copolymer of lactic acid and glycolic acid[5] and polyalkylcyanoacrylic acid[6] have also been examined as biodegradable carriers of drugs.

In our previous report, poly(ethylene carbonate) and poly(propylene carbonate) microspheres containing local anesthetics were prepared, and the release patterns of the drugs were observed.[7] The biodegradability of poly(ethylene carbonate) was shown in rats[8] and guinea pigs,[9] but poly(propylene carbonate) was not biodegradable in either animal.

Wakiyama *et al.*[10] observed the release patterns of dibucaine from polylactic acid microspheres and the duration of local anesthetic effect of microspheres containing dibucaine in guinea pigs.

In the present study, poly(ethylene carbonate) and two lots of poly(ethylene propylene carbonate) were used. Microspheres containing dibucaine were prepared by using each polymer alone or mixtures of two polymers, and their release patterns of dibucaine *in vitro* and the duration of local anesthetic effect of three representative preparations of microspheres containing dibucaine in guinea pigs were observed.

Experimental

Materials——Dibucaine hydrochloride was purchased from Teikoku Sangyo Co. (Osaka), and it was transformed into its base by treatment with sodium hydroxide solution.

Poly(ethylene carbonate) and two lots of poly(ethylene propylene carbonate) I and II were prepared by the methods reported earlier.[11] The compositions of poly(ethylene propylene carbonate) samples I and II determined by nuclear magnetic resonance (NMR) measurement were $(\text{ethylene oxide})_{0.37}$ $(\text{propylene oxide})_{0.19}$ $(\text{carbon dioxide})_{0.44}$ and $(\text{ethylene oxide})_{0.28}$ $(\text{propylene oxide})_{0.27}$ $(\text{carbon dioxide})_{0.45}$, respectively.

Determination of viscosity was carried out by the method of Kawaguchi *et al.*,[8] and the intrinsic viscosity values of poly(ethylene carbonate) and poly(ethylene propylene carbonate) I and II were 0.37, 0.93 and 0.51 dl/g, respectively, in dioxane at 25 °C.

Alkaline-processed gelatin, 200 bloom, was a gift from Nitta Gelatin Co. (Yao, Osaka). Methylene chloride and chloroform (reagent grade from Wako Pure Chemical Industry Co., Osaka) were used without further purification.

Preparation of Microspheres——Polycarbonate microspheres were prepared by a solvent-evaporation process.[12] The polymer (350 mg) and dibucaine (150 mg) were dissolved in 5 ml of methylene chloride. The organic solution was then added to a round-bottomed flask containing 100 ml of 2% gelatin solution (adjusted to pH 7.5 with 0.1 N NaOH) as a nonsolvent by using a syringe. The stirring rate of the 2% gelatin solution was 850 rpm. Methylene chloride was evaporated off *in vacuo* at room temperature, and the microspheres were collected by centrifugation and filtration. The microspheres were then dried *in vacuo* in a desiccator for at least 24 h and sized through a sieve (100 mesh).

Observation of the Microspheres——The microspheres were observed with a scanning electron microscope (model MSM-102, Akashi Manufacturing Co., Tokyo) to evaluate their shapes and surface structures. The diameters of microspheres were determined by using an optical microscope (model BH-2, Olympus Optical Co., Tokyo).

Dibucaine Contents of the Microspheres——To determine the dibucaine contents of microspheres, a weighed amount of microspheres was dissolved in chloroform, and the concentration of dibucaine in the organic solution was determined spectrophotometrically at 326 nm.

Release Studies——A weighed amount of microspheres (approximately 10 mg) was placed in 25 ml of isotonic citrate–phosphate buffer solution, pH 7.4, preheated to 37 °C in a flask. The flask was set in a shaker bath (model Personal H, Taiyo Scientific Industrial Co., Tokyo) maintained at 37 °C, and shaken horizontally at a rate of 100 cpm. An aliquot of the buffer solution was taken out periodically thereafter and the concentration of dibucaine in the solution was determined spectrophotometrically at 326 nm. Then the amount of dibucaine released from the microspheres was calculated.

Measurement of Local Anesthetic Effect——The examination of local anesthetic effect of polycarbonate microspheres containing dibucaine was carried out by the method of Wakiyama *et al.*[10] Microspheres containing 20 mg of dibucaine (actual weights of preparations A, C, and K were 69, 73, and 75 mg, respectively) were implanted subcutaneously into the dorsum of a guinea pig, and then to mark out the implantation site, the area was encircled in ink. The stimulus was applied to the implantation site with a needle to observe the skin reflex. The stimulus (six times at 3 s intervals) was continued at 5 min intervals for 30 min. The number of stimuli producing no response was noted up to the maximum of thirty-six (100%) as the extent of local anesthetic effect.

In the control experiment, 70 mg of drug-free poly(ethylene carbonate) microspheres was implanted, and the local anesthetic effect was determined in the same way.

Results and Discussion

Release Patterns of Dibucaine from Three Kinds of Polycarbonate Microspheres

The characteristics of polycarbonate microspheres containing dibucaine prepared with a drug/polymer ratio of 30/70 are shown in Table I. Irrespective of the difference of the polymers used, the diameters and drug contents did not differ very much among the three preparations. Figure 1 shows the release patterns of dibucaine from the polycarbonate microspheres of preparations A, B and C. Release of dibucaine from preparation B, which was prepared with poly(ethylene propylene carbonate) I, was very fast, and almost 100% of the drug was released in 8 h. Sustained release of dibucaine was obtained from poly(ethylene propylene carbonate) II and poly(ethylene carbonate) microspheres. Scanning electron photomicrographs of the polycarbonate microspheres are shown in Fig. 2. The cross-sections as well as the surface of drug-free poly(ethylene carbonate) microspheres were very smooth. When dibucaine was contained in the microspheres, the polymer matrix became porous (preparation A). Poly(ethylene propylene carbonate) microspheres containing dibucaine were also porous. In the case of poly(ethylene propylene carbonate) I microspheres (preparation B),

TABLE I. Characteristics of Dibucaine–Polycarbonate Microspheres Prepared by Evaporation *in Vacuo* with Methylene Chloride as a Polymer Solvent and 2% Gelatin (pH 7.5) as a Nonsolvent with a Drug/Polymer Ratio at Preparation of 30/70

Preparation	Polymer	Yield[a] (%)	Diameter (μm)	Drug content (%)
A	Poly(ethylene carbonate)	64.8	57.2 ± 3.0[b]	29.0
B	Poly(ethylene propylene carbonate) I	75.0	60.0 ± 4.2[c]	27.6
C	Poly(ethylene propylene carbonate) II	75.8	55.1 ± 3.0[b]	27.4

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

b) Mean ± Standard Error of the Mean (SEM) ($n = 150$). *c*) Mean ± SEM ($n = 100$).

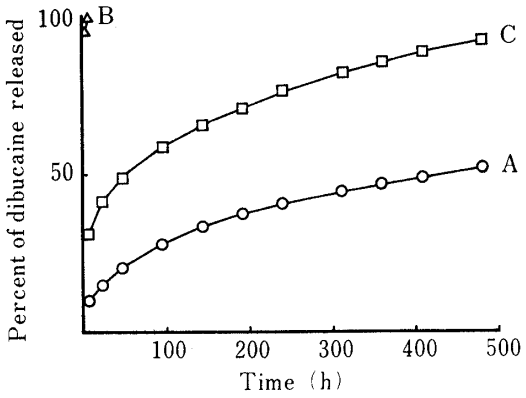


Fig. 1. Release Patterns of Dibucaine from Poly(ethylene carbonate) Microspheres (A, ○), Poly(ethylene propylene carbonate) I Microspheres (B, △), and Poly(ethylene propylene carbonate) II Microspheres (C, □)

Each value represents the mean of two (B) or three (A, C) experiments.

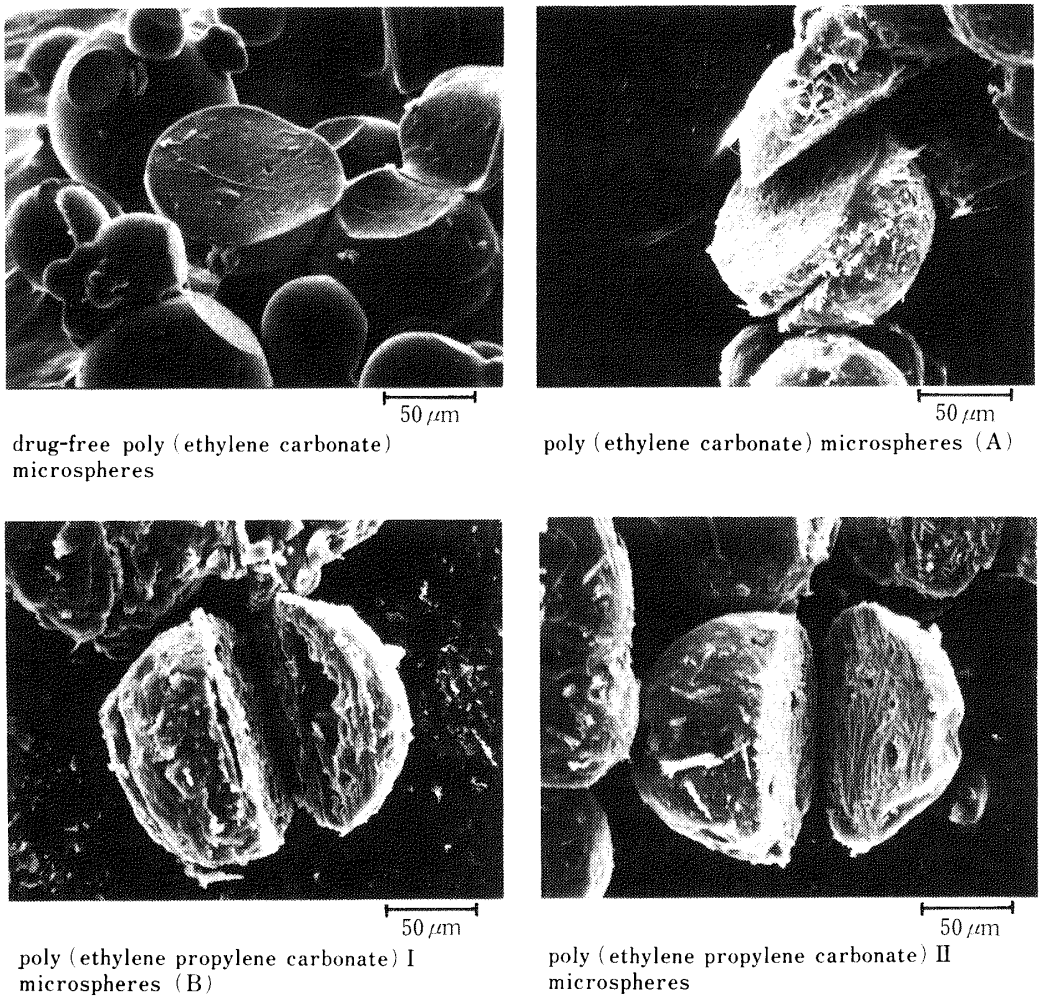
the polymer matrix was especially porous, and many pores were observed on the surface of the microspheres after the release experiment (not shown). This structural effect may explain the rapid release of dibucaine from preparation B. These results may also explain why the release rate of dibucaine was greater from poly(ethylene propylene carbonate) microspheres than from poly(ethylene carbonate) microspheres. Microspheres made of poly(ethylene propylene carbonate) with higher ethylene oxide/propylene oxide ratio seem to give a greater release rate of dibucaine.

Microspheres Prepared from Mixtures of Poly(ethylene carbonate) and Poly(ethylene propylene carbonate) I or II

In order to further modify the release pattern of dibucaine, the effect of mixing two polymers was examined. The characteristics of microspheres prepared from mixtures of poly(ethylene carbonate) and poly(ethylene propylene carbonate) I or II are shown in Table II. Mixing of the polymers did not have a marked influence on the size or drug contents of the microspheres.

Release patterns of dibucaine from microspheres of preparations A, E, and G are shown in Fig. 3. When poly(ethylene propylene carbonate) I was mixed at a level of 10% (preparation D), the release profile (data not shown) did not differ from that of preparation A made of poly(ethylene carbonate) alone. When the mixing level was 20% (preparation E), the initial burst was increased and the release rate of the drug was slightly increased. However, further increase in the mixing level to 40% resulted only in a larger initial burst without any accompanying increase in the release rate.

When poly(ethylene propylene carbonate) II was mixed at a level of 10% (preparation F,



drug-free poly (ethylene carbonate) microspheres

poly (ethylene carbonate) microspheres (A)

poly (ethylene propylene carbonate) I microspheres (B)

poly (ethylene propylene carbonate) II microspheres

Fig. 2. Cross Sections of Polycarbonate Microspheres Containing Dibucaine

TABLE II. Characteristics of Dibucaine–Polycarbonate Microspheres Prepared by Evaporation *in Vacuo* with Methylene Chloride as a Polymer Solvent and 2% Gelatin (pH 7.5) as a Nonsolvent with a Drug/Polymer Ratio at Preparation of 30/70

Preparation	Polymer	Yield[a] (%)	Diameter[b] (μm)	Drug content (%)
	CO_2-EO[c]/(CO_2-EO-PO)I[d]			
D	90/10	75.5	55.5±4.0	28.4
E	80/20	70.9	49.2±3.5	28.6
	CO_2-EO/(CO_2-EO-PO)II[e]			
F	90/10	76.2	60.8±3.0	27.5
G	80/20	73.6	61.1±3.6	30.0

a) Yield (%) = $\frac{\text{total weight of microspheres obtained}}{\text{total weight of polymer and drug used}} \times 100$.
b) Mean ± SEM (n = 100). *c*) CO_2-EO, poly(ethylene carbonate). *d*) (CO_2-EO-PO)I, poly(ethylene propylene carbonate) I. *e*) (CO_2-EO-PO)II, poly(ethylene propylene carbonate) II.

data not shown) or 20% (preparation G), the initial burst was increased, depending on the mixing level, but little increase in the release rate was obtained. Therefore, effective modification of the release pattern of the drug by mixing poly(ethylene carbonate) and

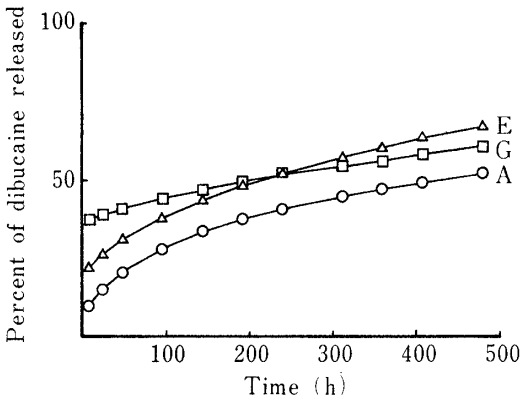


Fig. 3. Release Patterns of Dibucaine from Poly(ethylene carbonate)/Poly(ethylene propylene carbonate) $I_{(80/20)}$ Microspheres (E, △), Poly(ethylene carbonate)/Poly(ethylene propylene carbonate) $II_{(80/20)}$ Microspheres (G, □), and Poly(ethylene carbonate) Microspheres (A, ○)

Each value represents the mean of two (E, G) or three (A) experiments.

TABLE III. Characteristics of Dibucaine–Polycarbonate Microspheres Prepared by Evaporation *in Vacuo* with Methylene Chloride as a Polymer Solvent and 2% Gelatin (pH 7.5) as a Nonsolvent with a Drug/Polymer Ratio at Preparation of 30/70

Preparation	II[a]/I[b] ratio at preparation	Yield[c] (%)	Diameter (μm)	Drug content (%)
H	90/10	77.4	54.6±3.2[d]	27.1
I	80/20	67.2	48.0±3.7[e]	28.3
J	60/40	78.3	45.1±3.3[d]	27.1
K	50/50	78.7	41.8±3.3[e]	26.8

a) II, poly(ethylene propylene carbonate) II. *b*) I, poly(ethylene propylene carbonate) I.

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

d) Mean±SEM ($n=150$). *e*) Mean±SEM ($n=100$).

poly(ethylene propylene carbonate) I or II was unsuccessful.

Microspheres Prepared from Mixtures of Poly(ethylene propylene carbonate) I and II

Table III shows the characteristics of microspheres prepared from mixtures of poly(ethylene propylene carbonate) I and II with various II/I ratios in the preparation. There was little difference in the characteristics of these microspheres (H—K), and they were similar to those of preparations A to G. The surface of preparation K was as rough as that of preparation B, possibly because of the influence of poly(ethylene propylene carbonate) I at a high mixing ratio (50%).

Release patterns of dibucaine from these polycarbonate microspheres are shown in Fig. 4. With increase in the I content in the preparation, the release rate of dibucaine was increased. Therefore, by mixing the two polymers, intermediate release rates could be obtained.

Intensity and Duration of Local Anesthetic Effect of Dibucaine in Microspheres

In order to examine the relationship between the local anesthetic effect and the drug release pattern *in vitro* of microspheres, preparations A, C, and K were chosen as microspheres with small, intermediate, and large *in vitro* release rates of the drug, respectively. Figure 5 shows the intensity and duration of the local anesthetic effect of dibucaine following the implantation of the microspheres. The highest intensity and the longest duration of local anesthetic effect were obtained following the implantation of preparation C. In the case of preparation K, the intensity was comparable to that of preparation C in the initial period for up to 48 h, but it decreased more rapidly than in the case of preparation C, and the anesthetic

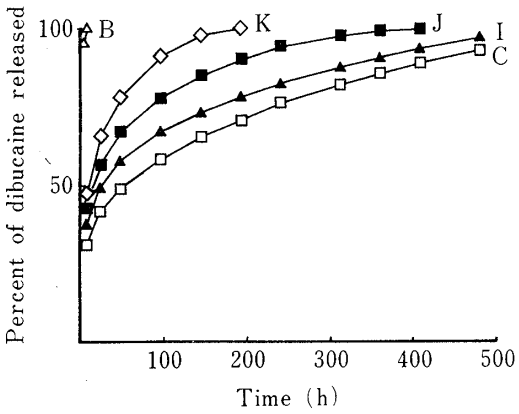


Fig. 4. Release Patterns of Dibucaine from Poly(ethylene propylene carbonate) II/I Microspheres (80/20, I, ▲; 60/40, J, ■; 50/50, K, ◇), Poly(ethylene propylene carbonate) I Microspheres (B, △), and Poly(ethylene propylene carbonate) II Microspheres (C, □)

Each value represents the mean of two (B, I) or three (C, J, K) experiments.

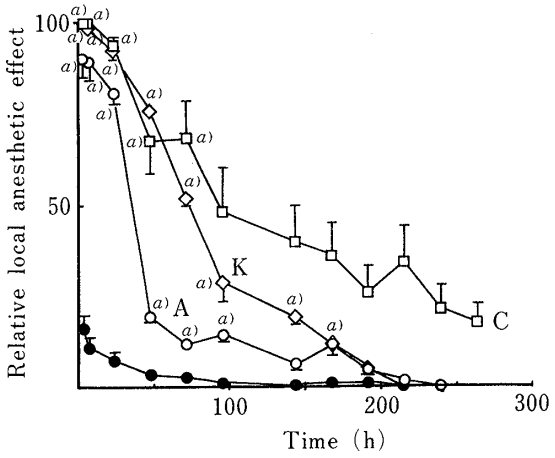


Fig. 5. Relative Local Anesthetic Effects Following Administration of Poly(ethylene carbonate) Microspheres (A, ○; $n=3$), Poly(ethylene propylene carbonate) II Microspheres (C, □; $n=3$), Poly(ethylene propylene carbonate) $I/II_{(50/50)}$ Microspheres (K, ◇; $n=3$), and Drug-Free Poly(ethylene carbonate) Microspheres (●; $n=4$)

a) Statistically significant, $p<0.05$.

effect had almost ceased at about 200 h. In the case of preparation A, the intensity was even lower in the initial period and it decreased more rapidly than in preparations C and K. Lower but nearly constant intensity was maintained thereafter from 48 to 172 h and the anesthetic effect terminated at about 200 h.

Some local anesthetic effect was observed in the case of drug-free poly(ethylene carbonate) microspheres. This might have resulted from physical necrosis produced by the surgical operation.

The relationship between the local anesthetic effect and the *in vitro* drug release pattern of the microspheres can be explained in the following way. In preparation A, only about 50% of the drug was released in 480 h *in vitro*, although the local anesthetic effect terminated at about 200 h. A sufficient local anesthetic effect could be obtained only in the initial period. It seems that the release rate of the drug was not high enough to maintain the local anesthesia.

In the case of preparation K, both the drug release *in vitro* and the local anesthetic effect terminated at about 200 h. The local anesthesia obtained was not as effective as that of preparation C after 48 h. Therefore the release rate of the drug from preparation K was too high.

Among the three preparations examined, preparation C, with an intermediate release rate of the drug *in vitro*, exhibited the most effective local anesthesia in guinea pigs.

General Discussion

The release rates of dibucaine from poly(ethylene propylene carbonate) microspheres were greater than that from poly(ethylene carbonate) microspheres. We have reported previously that the very slow release of a local anesthetic from poly(ethylene carbonate) and poly(propylene carbonate) microspheres after the initial burst might be due partly to the aggregation of the microspheres, resulting in a decrease in the surface area available for the release.[7]

In the present study, only slight aggregation was observed with the poly(ethylene

propylene carbonate) microspheres. The high release rate of dibucaine observed from poly(ethylene propylene carbonate) microspheres may therefore be considered to be a consequence of both the high diffusivity of the drug in the polymer matrix and the low aggregating tendency of the microspheres.

The release rate of dibucaine could be modified by altering the matrix component of microspheres. Mixing poly(ethylene carbonate) and a poly(ethylene propylene carbonate) was not effective in modifying the drug release pattern. On the other hand, mixing poly(ethylene propylene carbonate) I and II resulted in changes in the release rate of the drug depending on the mixing ratio of I and II.

Three preparations of microspheres were evaluated for local anesthetic effect in guinea pigs. The duration of local anesthetic effect of poly(ethylene propylene carbonate) II microspheres, which showed an intermediate release rate of the drug *in vitro*, was longest among the three preparations.

It was evident that effective local anesthesia could be achieved by obtaining a proper rate and duration of drug release from the microspheres. Microspheres which released the drug too slowly or too rapidly failed to provide sufficient intensity or sufficient duration of local anesthesia, respectively.

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