DURATION OF THE LOCAL ANESTHETIC EFFECT OF TETRACAINE HYDROCHLORIDE SOLUTIONS AND TETRACAINE IN MICROSPHERES

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The local anesthetic effect of tetracaine in polylactic acid microspheres was compared with that of tetracaine hydrochloride solutions. As a first step, the local anesthetic effect of 0.1 ml tetracaine hydrochloride solutions was examined at four concentrations *in vivo*. Then, the local anesthetic effect of 0.2 ml tetracaine hydrochloride solutions was compared with that of 0.1 ml tetracaine hydrochloride solutions. The more the amount of tetracaine, the stronger and longer was the local anesthetic effect. The local anesthetic effect of tetracaine in microspheres was much longer and lasted over 100 h.

Keywords—tetracaine; microsphere; local anesthetic effect; sustained release; sustained action; polylactic acid; biodegradable polymer

Cancer pain is most distressing to terminal cancer patients. In these patients, relief from pain should be the first treatment.^{1,2)} For palliative pain treatment, three approaches; administration of analgesics, neurosurgery, and nerve blocks, have been applied.²⁾ However, none of them has achieved the relief of pain. In addition to terminal cancer, trigeminal neuralgia also produces a severe pain and has no remedy except nerve blocks. Recently, clinical aspects of local anesthetics have been studied.^{3–6)} In the present study, the local anesthetic effect of tetracaine in microspheres has been compared with that of tetracaine hydrochloride solutions in order to examine a possible use in nerve blocks. The choice of tetracaine is based on its potency.³⁾ Polylactic acid was used as a release controlling matrix because of its biodegradability.⁷⁾

MATERIALS AND METHODS

Materials—DL-Polylactic acid (PLA) was synthesized from DL-lactic acid⁸⁾ purchased from Wako Pure Chemical Ind. (Osaka). Its average molecular weight was determined viscometrically to be 9100. Tetracaine hydrochloride was

purchased from Hoei Yakuhin Kogyo Co. (Osaka). Its base was obtained by alkalization of the salt solution. Male guinea pigs of 350–500 g body weight were used.

Preparation of Microspheres—Microspheres were prepared by a solvent-evaporation process similar to that of Beck *et al.*⁹⁾ PLA and tetracaine were dissolved in methylene chloride, a polymer solvent. The solution was then added dropwise into a round bottom flask containing 100 ml of 1% gelatin solution in water, a non-solvent for the polymer. The stirring rate was kept at 800 rpm. Reduced pressure was applied to the suspension to evaporate off methylene chloride, then the microspheres were collected by filtration. The collected microspheres were dried at room temperature under a vacuum, and were sized through a standard sieve. Preparation conditions and characteristics of microspheres are shown in Table I.

Measurement of Tetracaine Activity—Guinea pig skin techniques were used for the measurement of tetracaine activity.¹⁰⁾ A tetracaine hydrochloride solution (0.1 or 0.2 ml) in the injectable isotonic saline solution was injected sub-

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TABLE I. *Preparation Conditions and Characteristics of Microspheres*

Preparation	Tetracaine/PLA ratio at preparation	Non-solvent	Yield, %	Diameter, μm	Drug content, %
A	30/70	1% gelatin	51	54.6 ± 2.3	18.9
B	20/80	0.5% tetracaine · HCl in 1% gelatin	66	56.8 ± 3.1	16.3

FIG. 1. *Local Anesthetic Effects following Administration of 0.1 ml of 0.1% (○), 0.05% (△), 0.02% (□), and 0.01% (◇) Tetracaine HCl Solution and Saline (×)*
Each value represents the mean $\pm$ S.E. of 4 experiments.

FIG. 2. *Local Anesthetic Effects following Administration of 0.2 ml of 0.1% (●), 0.1 ml of 0.1% (○), 0.2 ml of 0.05% (▲), 0.1 ml of 0.05% (△), 0.1 ml of 0.02% (□), 0.2 ml of 0.01% (◆), and 0.1 ml of 0.01% (◇) Tetracaine HCl Solution*
Each value represents the mean $\pm$ S.E. of 4 experiments.

cutaneously to the dorsum and the injected area was encircled in ink. The examination of anesthesia commenced 5 min later by stimulating repeatedly every 3 s, stopping as soon as the reflex reappeared, *i.e.* at the slightest quiver of the skin. The number of stimulus producing no response was noted by checking the activity up to the max-

imum of six. Scores represent the extent of local anesthetic effect. The exploration was continued for 30 min every 5 min. The activity of tetracaine hydrochloride solution was compared with respect to tetracaine concentrations and amounts (concentration multiplied by volume) of tetracaine. The activity of tetracaine in microspheres

FIG. 3. Similar to Fig. 1 but Scores are added up for Each 30 min Period

Each value represents the mean $\pm$ S.E. of 4 experiments.

FIG. 5. Release Rate of Tetracaine from 10 mg Microspheres A

FIG. 4. Local Anesthetic Effects following Administration of 15 mg of 79 mg Microspheres A ($\circ$) and Drug Free Microspheres (Δ)

Each value represents the mean $\pm$ S.E. of 3 experiments.

FIG. 6. Local Anesthetic Effects following Administration of 15 mg of 92 mg Microspheres B ($\circ$) and Drug Free Microspheres (Δ)

Each value represents the mean $\pm$ S.E. of 3 experiments.

was measured after dorsal subcutaneous implantation of microspheres containing 15 mg of tetracaine. To mark out the implanted site, the area was encircled in ink. The examination of anesthesia was carried out similarly to tetracaine hydrochloride solution. Scores were added up for each 30 min period to be maximum of thirty six (maximum score of six times 6 measurements). Further, 5% tetracaine hydrochloride solution containing the same amount (15 mg) of tetracaine was injected subcutaneously to the back of the guinea pig to examine its toxicity.

RESULTS AND DISCUSSION

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

Intensity and duration of the local anesthetic effect following injection of 0.1 ml of 0.1, 0.05, 0.02, and 0.01% tetracaine hydrochloride solutions are shown in Fig. 1. There were significant differences between 0.1, 0.05, and 0.02% tetracaine hydrochloride solutions and saline (control). These 0.1, 0.05, and 0.02% tetracaine hydrochloride solutions had markedly increased local anesthetic effect compared with saline (control) till 30 min after injection. The difference in local anesthetic effect is statistically significant ($p < 0.05$) when tested using the Student's t-test, and error of the second kind is 0% except 0.1 ml of 0.05% tetracaine hydrochloride solution at 30 min after injection. However, there was no significant difference (5% level) between 0.01% tetracaine hydrochloride solution and saline when tested using the Student's t-test. It is evident that 0.1 ml of 0.01% tetracaine hydrochloride solution (10 μ g of tetracaine hydrochloride) exhibits little local anesthetic effect. Moreover, with the increase in tetracaine concentration, the intensity and the duration of the local anesthetic effect tended to increase. Effects of volume of the drug solution on intensity and duration of the local anesthetic effect of tetracaine hydrochloride solutions are shown in Fig. 2. Although the difference is not statistically significant, the local anesthetic effect of 0.2 ml tetracaine hydrochloride solutions tended to be stronger and longer than that of 0.1

ml tetracaine hydrochloride solutions, and in addition 0.2 ml tetracaine hydrochloride solutions produced broader anesthetized area than 0.1 ml tetracaine hydrochloride solutions. Consequently, the local anesthetic effect is related to the amount of tetracaine hydrochloride solutions and about 20 μ g of tetracaine hydrochloride was required to produce the local anesthetic effect in guinea pigs.

Intensity and Duration of the Local Anesthetic Effect of Tetracaine Hydrochloride, Tetracaine in Microspheres, and Drug-free Microspheres

Intensity and duration of the local anesthetic effect of tetracaine hydrochloride solution and tetracaine in microspheres are shown in Fig. 3, and Figs. 4 and 6, respectively. Fig. 3 shows the sum of effects of six 5-min intervals for two 30-min periods, the first 30 min shown in Fig. 1 and third 30-min period (from 60 to 90 min). Even in 0.1% tetracaine hydrochloride solution, the local anesthetic effect disappeared 1 h after the injection. However, tetracaine in microspheres A and B maintained the local anesthetic effect for a longer period of time. The difference in local anesthetic effect compared with drug-free microspheres for Microspheres A is statistically

FIG. 7. Release Rate of Tetracaine from 10 mg Microspheres B

significant ($p < 0.05$) when tested using the Student's t-test and error of the second kind is 0% till 80 h after implantation. That for Microspheres B is statistically significant ($p < 0.05$) till 120 h after implantation and error of the second kind is 0% till 96 h and 19% at 120 h after implantation. Moreover, when 5% tetracaine hydrochloride solution containing about 15 mg tetracaine was injected to a guinea pig, the guinea pig died of the tetracaine overdose. When drug-free microspheres were implanted subcutaneously to guinea pigs, some local anesthetic effect was observed. In this case, a cutaneous necrosis might have been produced by the surgical operation, so that some numbers of stimuli producing no response were observed.

Release rates of tetracaine from 10 mg Microspheres A and B are shown in Fig. 5 and Fig. 7, respectively. When the profiles of local anesthetic effect (Figs. 4 and 6) are compared with these figures, the release rate profiles tended to be reflected on profiles of the local anesthetic effect. Thus, it is likely that the release *in vitro* is reflected in the release *in vivo*. Therefore, we might be able to predict duration of the local anesthetic effect by the release studies *in vitro*.

General Discussion

Poorly soluble salts of lidocaine were prepared to increase duration of local anesthetic effect by slow dissolution.¹¹⁾ In the present approach, sustained release through a polymer matrix was examined. A plan was made to prepare microspheres containing bupivacaine, which has been used extensively in pain clinic, and to examine release characteristics of the drug from the microspheres. Although more animal studies are required before clinical use, local anesthetics in microspheres may be applicable in control of pain in pain clinic since administration of local anesthetics have been used as one of the methods in control of pain in pain clinic for the treatment

of cancer pains and trigeminal neuralgia. Preparation of microspheres exhibiting more sustained release characteristics than those described may be required to control pain for more extended periods of time.

REFERENCES

- 1) T. Matsumae, A. Watanabe and A. Murakami: Our management of pains suffering from terminal malignant tumor — especially with nerveblocks, *Iryo.*, **35**, 69–71 (1981).
- 2) J. Gybels, H. Adriaensen and P. Cosyns: Treatment of pain in patients with advanced cancer, *Europ. J. Cancer*, **12**, 341–351 (1976).
- 3) L. E. Mather and M. J. Cousins: Local anesthetics and their current clinical use, *Drugs.*, **18**, 185–205 (1979).
- 4) G. T. Tucker and L. E. Mather: Clinical pharmacokinetics of local anaesthetics, *Clinical Pharmacokinetics.*, **4**, 241–278 (1979).
- 5) F. Magora, L. Stern, E. Zylber-Kaltz, D. Olshwang, Y. Donchin and A. Magora: Prolonged effect of bupivacaine hydrochloride after cuff release in *i.v.* regional anesthesia, *Br. J. Anaesth.*, **52**, 1131–1135 (1980).
- 6) H. Rudolph and J. D. Bonin: Local anesthetics: Injection route alters relative toxicity of bupivacaine, *Anesth. Analg.*, **59**, 925–928 (1980).
- 7) R. K. Kulkarni, E. G. Moore, A. F. Hegyeli and F. Leonard: Biodegradable poly(lactic acid) polymers, *J. Biomed. Mater. Res.*, **5**, 169–181 (1971).
- 8) A. Schindler, K. Jeffcoat, G. L. Kimmel, C. G. Pitt, M. E. Wall, and R. Zweidinger, in "Contemporary Topics in Polymer Science," Vol. 2, ed. by E. M. Pearce and J. R. Schaefgen, Plenum Pub. Corp., New York, 1977, pp. 251–289.
- 9) L. R. Beck, D. R. Cowsar, R. J. Cosgrove, C. T. Riddle, S. L. Lowry and T. Epperly: A new long-acting injectable microcapsule system for the administration of progesterone, *Fertil. Steril.*, **31**, 545–551 (1979).
- 10) A. Quevauviller in Local Anesthetics, 2nd, ed., R. H. Jong, ed., Experimental methods for comparing local anesthetic activity, C. C. Thomas, Publisher, Springfield, Illinois, 1977, pp. 291–318.
- 11) N. I. Nakano, N. Kawahara, T. Amiya, Y. Gotoh, and K. Furukawa: 3-Hydroxy-2-naphthoates of lidocaine, mepivacaine, and bupivacaine and their dissolution characteristics, *Chem. Pharm. Bull.*, **26**, 936–941 (1978).