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Effects of counteranion of TRH and loading amount on control of TRH release from copoly(dl-lactic/glycolic acid) microspheres prepared by an in-water drying method

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Summary

The preparation of injectable sustained release microspheres of TRH was investigated. The drug was encapsulated in copoly(dl-lactic/glycolic acid) (PLGA) using an in-water drying method through a w/o/w emulsion. The predominant factors influencing the entrapment ratio of TRH in the microspheres and the release rate of TRH from the microspheres were the loading amount and the dissociation state of the drug. Microspheres of TRH encapsulated as a free base at an adequate concentration provided a small initial burst followed by a zero-order prolonged release regardless of the high water solubility of the drug. The addition of strong acids to the inner aqueous phase interfered with the encapsulation efficacy and caused a large initial burst. The ionic interaction between the basic functional group of TRH and the carboxylic acid end group of PLGA during the preparation is necessary to produce a rigid matrix of the microspheres. This method is applicable to the microencapsulation of other water-soluble basic peptides in PLGA.

Introduction

Thyrotropin releasing hormone (TRH; 5-Oxo-prolyl-L-histidyl-L-prolinamide; 5-Oxo-Pro-His-Pro-NH_2) is widely distributed in the brain, and several investigators have paid attention to its profiles of CNS-arousing activity and clinical utility (Nagawa, 1980). Long term treatment with daily injections was often required for clinical use of TRH analogues as CNS arousing drugs. Therefore we attempted to develop injectable microspheres prepared with a biodegradable polymer, copoly(d,l-lactic/glycolic acid) (PLGA), to produce a prolonged controlled injectable dosage form of TRH.

The successful preparation of controlled release dosage forms of several lipophilic drugs had already been reported (Tice and Cowsar, 1984;

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Preliminary reports of this work appeared as abstracts in 1988 (Heya et al., 1988).

Wakiyama et al., 1981; Wise, 1984). However, as for water soluble compounds, there are very few reports except for LH-RH analogues (Ogawa et al., 1989; Okada et al., 1989; Redding et al., 1984). To avoid a large initial burst of water soluble compounds from microspheres was assumed to be difficult. Concerning the LH-RH analogues, the burst could be reduced by steric hindrance factors or by using proper additives (Ogawa et al., 1988; Sanders et al., 1986).

In this study, the preparation of microspheres of a water soluble compound, TRH, which represents a small burst followed by a zero-order release of the drug was investigated.

Materials and Methods

Materials

TRH (Lot T-2) synthesized in the Pharmaceutical Production Research Laboratories of Takeda Chemical Industries, Ltd (Osaka, Japan) was used. PLGA samples with an average molecular weight of 14000 (molar ratio of lactide and glycolide of 75 : 25), were supplied by Wako Pure Chemical Industries (Osaka, Japan). These compounds were synthesized without catalysts, and the average molecular weight was determined by gel-permeation chromatography (GPC) by the supplier.

Preparation of PLGA microspheres

PLGA microspheres were prepared by an in-water drying method similar to that of Ogawa et al. (1988). 50 mg to 1 g of TRH was dissolved in 0.625 ml of water and 5 g of PLGA was dissolved in 6.25 ml of dichloromethane. The PLGA solution and TRH solution were vigorously homogenized with Polytron (Kinematica GmbH, Luzern, Switzerland) for a few minutes to make a w/o emulsion. The emulsion was poured through a nozzle into 1.25 l of an aqueous 0.5% polyvinylalcohol solution under constant stirring with an Autohomomixer (Tokushu Kika Kogyo Co., Osaka, Japan) to make a w/o/w emulsion. To evaporate the dichloromethane, the w/o/w emulsion was stirred gently with a propeller mixer for 3 h. After removing the particles larger than 125 μm by sieving, the resulting microspheres were collected by centrifuging at 1500 rpm for 10 min and rinsed with water three times and then lyophilized.

The viscosity of the w/o emulsion in preparations was measured using a Viscometer EHD (Tokyo Keiki, Tokyo, Japan). The shape and surface of the microspheres were observed with a scanning electron microscope (model JSM T-300, Jeol-Technics Co., Ltd, Tokyo, Japan). The size of the microspheres was determined by a Coulter TA-II.

Determination of the TRH content in microspheres

TRH was analyzed by a high-performance liquid chromatography procedure (HPLC, Shimadzu LC-5A, Kyoto, Japan) with an ultraviolet detector (UV) under the following conditions: column, Zorbax ODS, 250 mm in length and 4.6 mm i.d.; column temperature, room temperature; mobile phase, a mixture of 20 ml of acetonitrile and 300 ml of 1/30 M phosphate buffer, pH 6.7; flow rate, 0.8 ml/min; wavelength, 215 nm. To determine the content of TRH, microspheres were dissolved in a mixture of 10 ml dichloromethane and 20 ml of 1/30 M phosphate buffer, pH 6.0. TRH in the water layer was assayed by HPLC.

In vitro release study

50 mg of TRH microspheres was dispersed in 10 ml of 1/30 M phosphate buffer, pH 7.0, and kept stirred with an RT-50 rotator (Taiyo Scientific Industrial Co., Tokyo, Japan) at 37°C. Since TRH decomposes in the medium, the residual TRH in the microspheres was periodically determined by the analytical method mentioned above after collecting the microspheres using a Millipore® filter (pore size: 1.2 μm).

Results and Discussion

Microspheres of low drug loading

It is generally difficult to avoid a large initial burst of a highly water-soluble drug from PLGA microspheres. The release rate of the drug from PLGA microspheres is assumed to be slow when the amount of the drug contained in the microspheres is small. A higher content results in rapid release due to the formation of aqueous channels

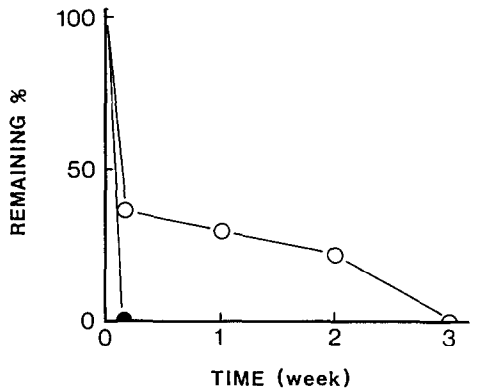

Fig. 1. Release profiles of TRH from PLGA microspheres containing TRH (○) and TRH tartrate (●) at a loading amount of 1.0%.

(Siegel and Langer, 1984). Therefore, microspheres with a loading amount of 1% of TRH were investigated first. Fig. 1 shows the release profiles of TRH from PLGA microspheres prepared with TRH or TRH tartrate. Both of the microspheres provided a large initial burst. When TRH tartrate was incorporated into the microspheres, in particular, 100% burst was observed after 1 day in the in vitro release test.

In order to control better the release rate, the influence of the concentration of PLGA in the oil phase was examined. The concentration of PLGA in the oil phase was an important factor for the preparation of the microspheres (Ogawa et al., 1988). When the concentration of the PLGA in the oil phase was elevated from 33.4 to 50.1%, the 1-day release of TRH decreased from 73.0 to 67.0%. However, the extent of improvement was not enough to achieve a constant release rate.

The large burst is attributable to the drug localized near the surface of the microspheres. The complete removal of the drug from the surface region was considered to be a useful method to reduce the large burst. In order to wash out the drug in the surface region, the evaporation process of dichloromethane was extended. Although the evaporation time was extended to 24 h in the preparation of TRH tartrate microspheres, 100% burst was still observed after 1 day.

TABLE 1

Effect of loading amount of TRH on the entrapment ratio and initial release of TRH in PLGA microspheres

Loaded TRH (%)	Entrapment ratio (%)	Remaining (%, 1 day)
1.0	82.1	34.3
2.4	88.6	85.4
4.8	104.8	84.5
7.0	95.9	91.2
9.1	105.9	74.0
16.7	47.5	61.2

Effect of drug loading

Table 1 shows the entrapment ratio of TRH in the microspheres and 1-day release of the drug from microspheres prepared with various loading amounts of TRH free base. The 1-day remaining percent in the in vitro release test increased with increase in loading amount up to 7.0% and then decreased with further increase in drug loading. The entrapment ratio was on the whole correlated with the 1-day remaining percent. The increase in drug loaded generally leads to a partial destruction of the w/o/w emulsion, which leads to a decreased entrapment ratio, and to a large initial burst through increased aqueous channels. However, the increase in loaded TRH reduced the burst and elevated the entrapment ratio. A satisfactorily small burst and high entrapment ratio were unexpectedly observed at loading amounts from 2.4 to 9.1%. On the other hand, microspheres prepared with TRH tartrate released all of the incorporated drug after 1 day at any loading amount of the drug (Table 2). These results suggest that some amounts of TRH free base are necessary to construct rigid walls of the micro-

TABLE 2

Effect of loading amount of TRH tartrate on the entrapment ratio and initial release of TRH in PLGA microspheres

Loaded TRH (%)	Entrapment ratio (%)	Remaining (%, 1 day)
1.0	82.8	0
2.4	72.7	0
4.8	64.7	0
9.1	60.0	0

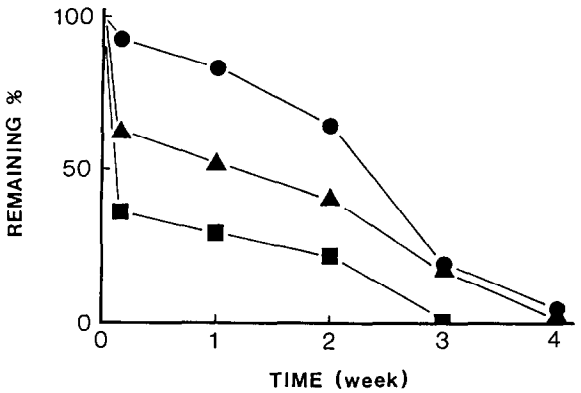

Fig. 2. Effect of the loading amount of TRH on release profiles of TRH from PLGA microspheres. (■) 1.0%; (●) 7.0%; (▲) 16.7%.

spheres to achieve a small initial burst and that tartaric acid inhibits this construction.

The in vitro release profiles of the microspheres with different loading amounts of the drug over 4 weeks are shown in Fig. 2. The microspheres loaded with 1.0 and 16.7% of TRH produced a large initial burst and then exhibited a prolonged release for 3–4 weeks. The microspheres loaded with 7.0% of TRH exhibited a fairly small initial burst followed by a constant release rate over 4 weeks. Thus, it is suggested that the use of appropriate loading amounts of TRH free base makes it possible to obtain microspheres that provide a constant release for a long period after a small initial burst.

Effect of acid addition

In order to examine the mechanism that caused the large initial burst in microspheres of TRH tartrate, various amounts of tartaric acid were added to the water phase of w/o emulsions containing TRH free base. As shown in Table 3, a slight reduction of the entrapment ratio and a large increase in burst were observed with the addition of tartaric acid. This suggests that the pH in the water phase plays a significant role. The 1-day remaining percent after the release test can be a more sensitive indicator than the entrapment ratio to evaluate the structure of the microspheres. This is probably because the microspheres are placed at higher temperature for a longer duration (37 °C, 24 h) in the in vitro release test than in the in-water drying process (room temperature, 3 h). Furthermore, aqueous channels will be formed by the freeze drying in preparation to cause the burst.

TABLE 3

Effect of the addition of tartaric acid on the entrapment ratio and initial release of TRH in PLGA microspheres

Added tartaric acid [a]	Entrapment ratio (%)	Remaining (%, 1 day)
0	98.5	84.2
0.2	87.7	40.1
0.5	82.3	1.7

[a] Molar ratio to TRH.

The effects of the addition of other acids were also examined. Table 4 lists the entrapment ratio of TRH in the microspheres and 1-day remaining percent after the release tests. When strong acids such as hydrochloric, tartaric and citric acids were added, almost all of the drug was released after 1 day. On the other hand, the addition of weak acids like acetic or butyric acid caused only a slight increase in the burst. TRH is a basic compound with pK_a 6.9, and PLGA has carboxylic acid residues in the terminal region. It seems that rigid structures of the microspheres are based on an ionic interaction between the cationic residue of TRH and the anionic residue of PLGA. The addition of a strong acid makes the terminal groups of the carboxylic acid of PLGA undissociated, resulting in interference with the interaction.

TABLE 4

Effect of addition of acids on the entrapment ratio and initial release of TRH in PLGA microspheres

Acid (pK_{a_1})	Entrapment ratio (%)	Remaining (%, 1 day)
None	98.5	84.2
Hydrochloric acid	85.7	1.2
Tartaric acid (2.9)	93.2	0
Citric acid (2.9)	91.8	0
Acetic acid (4.6)	90.4	66.8
Butyric acid (4.6)	100.4	77.2
Sodium citrate	36.9	40.3

Loaded amount of TRH was 2%.

Makino et al. (1988) reported that the dissociation constant of the terminal groups of carboxylic acid of PLGA is 4.0. If it is assumed that the dissociation constant of this PLGA is also 4.0, weak acids are scarcely likely to inhibit the ionic interaction.

When a TRH derivative with a carboxylic acid residue (2-hydroxy-4-carboxybutyryl-L-histidyl-L-prolinamide) was incorporated into PLGA microspheres, all of the drug was released after 1 day. The experimental results also suggest that the ionic interaction between the drug and the polymer is of importance. The microspheres containing sodium citrate exhibited a small initial burst compared with those containing citric acid, suggesting that the ionic interaction between TRH and PLGA is increased by elevation of the pH. However, the addition of sodium citrate gave rise to a low entrapment ratio and still a large burst probably due to the high osmotic pressure in the inner water phase.

The viscosity of the w/o emulsion

Table 5 shows the viscosity of w/o emulsions containing different amounts of TRH with or without tartaric acid. The viscosity of the w/o emulsion increased with increasing drug loading. The increment of viscosity of the emulsion was much larger than could be expected from the increase in volume of the inner water phase, indicating an interaction between the drug and the polymer. The viscosity of the emulsion decreased with addition of tartaric acid which should interfere the ionic interaction of TRH in competition with PLGA. These results indicate that the increase in viscosity was caused by the ionic interaction between TRH and PLGA and likely resulted in making the microsphere matrix becoming rigid, thereby eliminating the large initial burst.

TABLE 5

Viscosity of the w/o emulsion consisted of water containing TRH and dichloromethane containing PLGA

Loading TRH (%)	Addition of acid	Viscosity (cp)	Temperature (°C)
1.0	none	200	18
4.8	none	2800	18
9.1	none	11800	18
2.0	none	800	15
2.0	tartaric acid	500	15

Scanning electron micrography

Fig. 3 shows typical scanning electron micrographs of the microspheres prepared with TRH or TRH tartrate at a loading amount of 4.8% in PLGA. The microspheres prepared with TRH tartrate had a rough porous surface. The porosity was found to be well correlated with the large initial burst. On the other hand, the microspheres with a smooth surface which showed a small initial burst could be obtained by encapsulating TRH as the free base. The morphological changes in microspheres after 1 day in the release test were closely correlated with the initial burst: the larger the initial burst, the larger was the deformation of the microspheres. As reported by Sato et al. (1988), surface morphology is important for the release characteristics of the microspheres. The effect of ionic interaction was reconfirmed by this microscopic observation.

The particle size of the microspheres prepared with TRH free base was about 30 μm. The particle size distribution complied with the logarithmic normal distribution. The particle size of the microspheres prepared with TRH tartrate was much larger than those prepared with TRH free base. During the evaporation process, water might penetrate into the inner water phase from the outer water phase along the osmotic pressure gradient through the porous polymer matrix as a result of the weak interaction. The penetration of water causes swelling of the microspheres, resulting in large porous particles. It is presumed that the differences in release characteristics between these particles are ascribed to the differences in the interaction between the drug and the polymer.

In conclusion, effective incorporation of a basic drug in the PLGA microspheres is highly dependent on the loading amount of the drug and on the dissociation state of the drug and the polymer. A rigid structure of polymer matrix can be ob-

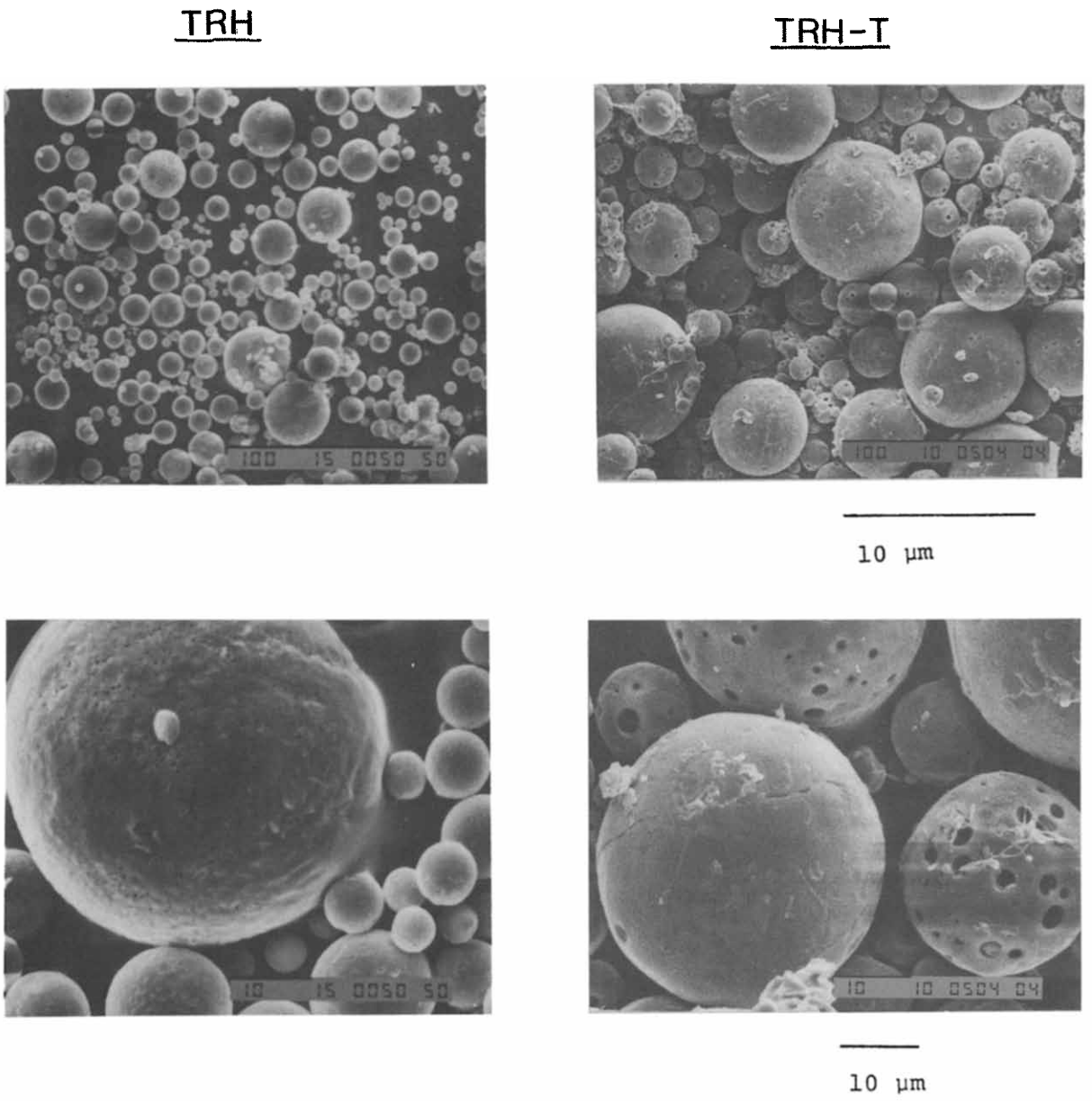

Fig. 3. Scanning electron micrographs of PLGA microspheres of TRH (left) and TRH tartrate (right) at the loading amount of 4.8% as TRH.

tained as a result of the interaction between a basic drug and PLGA. The rigidity makes controlled release of a water-soluble drug for long periods possible.

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References

Heya, T., Tanigawara, Y., Okada, H., Ogawa, Y. and Toguchi, H., Controlled release injectable microspheres of TRH (1): Preparation and release profiles of microspheres (Abstract). 108th Annual Meeting of the Pharmaceutical Society of Japan, 1988, p. 560.

Makino, K., Ohshima, H. and Kondo, T., Transfer of protons from bulk solution to the surface of poly(l-lactide) microcapsules. *J. Microencapsulation*, 3 (1986) 195–202.

Nagawa, Y., Pharmacology on the central nervous system (CNS) Effects of thyrotropin-releasing hormone (TRH). *J. Takeda Res. Labs.*, 39 (1980) 151–191.

Ogawa, Y., Yamamoto, M., Okada, H., Yashiki, T. and Shimamoto, T., A new technique to efficiently entrap leuprolide acetate into microcapsules of polylactic acid or copoly(lactic/glycolic) acid. *Chem. Pharm. Bull.*, 36 (1988) 1095–1103.

Ogawa, Y., Okada, H., Heya, T. and Shimamoto, T., Controlled release of LHRH agonist, leuprolide acetate, from microcapsules: Serum drug level profiles and pharmacological effects in animals. *J. Pharm. Pharmacol.*, 41 (1989) 439–444.

Okada, H., Heya, T., Igari, Y., Ogawa, Y. and Shimamoto, T., One-month release injectable microspheres of leuprolide acetate inhibit steroidogenesis and genital organ growth in rats. *Int. J. Pharm.*, 54 (1989) 231–239.

Redding, T.W., Schally, A.V., Tice, T.R. and Meyers, W.E., Long-acting delivery systems for peptides: inhibition of rat prostate tumors by controlled release of (D-TRP^6)-luteinizing hormone-releasing hormone from injectable microcapsules. *Proc. Natl. Acad. Sci. USA*, 81 (1984) 5845–5848.

Sanders, L.M., Kell, B.A., MacRae, G.I. and Whitehead., G.W., Prolonged controlled-release of nafarelin, a luteinizing hormone-releasing hormone analogue from biodegradable polymeric implants: Influence of composition and molecular weight of polymer. *J. Pharm. Sci.*, 75 (1986) 356–360.

Sato, T., Kanke, M., Schroeder, H.G. and Deluca, P., Porous biodegradable microspheres for controlled drug delivery. I. Assessment of processing conditions and solvent removal techniques. *Pharm. Res.*, 5 (1988) 21–30.

Siegel, R.A. and Langer, R., Controlled release of polypeptides and other macromolecules. *Pharm. Res.*, 1 (1984) 2–9.

Tice, T.R. and Cowsar, D.R., Biodegradable controlled-release parenteral systems. *Pharm. Technol.*, 8 (1984) 26–36.

Wakiyama, N., Juni, K. and Nakano, N., Preparation and evaluation in vitro of polylactic acid microspheres containing local anesthetics. *Chem. Pharm. Bull.*, 29 (1981) 3363–3368.

Wise, D.L., Biopolymer system design for sustained release of biological active agents. In Wise D.L. (Ed.), *Biopolymeric Controlled Release Systems*, Vol. 1, CRC Press, FL, 1984, pp. 3–28.