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Factors influencing the profiles of TRH release from copoly(d,l-lactic/glycolic acid) microspheres

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Summary

Copoly(d,l-lactic/glycolic acid) (PLGA) microspheres containing thyrotropin releasing hormone (TRH) were prepared using an in-water drying method through a (w/o)/w emulsion. Various factors which affect TRH release from microspheres were examined both in vitro and in vivo. The drug release was affected by the drug loading level, the molecular weight of PLGA, and the lactic acid/glycolic acid (LA/GA) ratio of PLGA. The effect of the drug loading level on the initial burst differed widely with changes in the molecular weight of PLGA. The initial burst increased with decreasing molecular weight of PLGA. After the initial burst, the release rate was dominated mainly by the degradation of PLGA; the lower the molecular weight of PLGA, the faster the release rate. The release rate was also affected by the LA/GA ratio of the PLGA. The microspheres prepared with a higher GA content of PLGA exhibited a faster release rate. In the in vivo study in rats, the microspheres also released the peptide sustainedly over a long period after s.c. injection.

Introduction

Thyrotropin-releasing hormone (TRH) has a number of central nervous effects independent of its endocrine function and has been reported to have possibilities in the treatment of various CNS dysfunctions, such as spinocerebellum degeneration or unconsciousness (Sobue et al., 1980). However, long-term treatment with daily injections is often required in these diseases. To overcome this inconvenience, we have developed sustained release microspheres of TRH prepared with a biodegradable and biocompatible polymer, copoly(d,l-lactic/glycolic acid) (PLGA), by an in-water drying method through a (w/o)/w emulsion. Our previous studies (Heya et al., 1991) revealed that microspheres prepared by the in-water drying method only at the appropriate concentrations of TRH in PLGA achieved a fairly small initial burst in spite of the high water solubility of the drug. This phenomenon was explained by the rigid microsphere structure formed as a result of the ionic interaction between TRH and PLGA.

From the standpoint of reliable therapeutic effects with less side effects, it would be desirable to decrease the initial burst to achieve a constant

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release rate, and to control the duration of release. Various factors have been studied in an effort to control the release of lipophilic drugs from biodegradable microspheres (Leong et al., 1985; Suzuki and Price, 1985; Chang et al., 1986). On the other hand, there are few reports dealing with controlled release of water-soluble drugs except for LH-RH analogues (Sanders et al., 1986; Ogawa et al., 1988b). The present study was undertaken to clarify the factors influencing the release profiles of a water-soluble compound, TRH, from PLGA microspheres.

Materials and Methods

Materials

TRH synthesized in the Pharmaceutical Production Research Laboratories of Takeda Chemical Industries, Ltd (Osaka), was used. Copoly-(d,l-lactic/glycolic acid) (PLGA), poly(d,l-lactic acid) (PLA) were supplied by Wako Pure Chemical Industries, Ltd (Osaka).

Preparation of PLGA microspheres

PLGA microspheres were prepared using an in-water drying method as reported previously (Heya et al., 1991). A weighed amount of TRH (0.05–0.5 g) was dissolved in 0.29–0.5 ml water, and 4.5–5.0 g PLGA was dissolved in 4.6–5.82 ml dichloromethane. These solutions were vigorously homogenized with a Polytron (Kinematica GmbH, Luzern, Switzerland) for a few minutes to make a w/o emulsion. The emulsion, after being cooled to 15–20°C, was poured through a nozzle of about 1.5 mm diameter into 1000 ml of 0.25% polyvinyl alcohol aqueous solution under stirring with an Autohomomixer (Tokshu Kika Kogyo Co., Osaka), and the resulting mixture was stirred for a few minutes to make a w/o/w emulsion. To evaporate the dichloromethane, the w/o/w emulsion was further stirred gently with a propeller mixer for 3 h. After removing particles larger than 74 μm by sieving, the resulting microspheres were collected by centrifuging at 1500 rpm for 10 min, rinsed with water three times and then lyophilized into a powder.

The shape and surface characteristics of the dried microspheres were examined with a scanning electron microscope (model JSM T-300, JEOL Co., Ltd, Tokyo).

Determination of the TRH content in microspheres

Microspheres were dissolved in 10 ml of dichloromethane and 20 ml of 1/30 M phosphate buffer, pH 6.0, and TRH in the buffer layer was assayed using high-performance liquid chromatography (HPLC, Shimadzu LC-5A) with an ultraviolet (UV) detector as follows: column, Zolbax ODS (250 mm in length, 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.

In vitro release studies

The in vitro release of TRH from microspheres was determined as described in the previous paper (Heya et al., 1991). Microspheres (50 mg) were suspended in 10 ml of 1/30 M phosphate buffer, pH 7.0, containing 0.02% Tween 80 (Kao Atlas, Tokyo) at 37°C. The amount of residual TRH in the microspheres was determined using the analytical method described above after filtering the microspheres with a 1.2 μm Millipore® filter.

In vivo release studies

The in vivo release profiles were evaluated by a method similar to that of Okada et al. (1988). The microspheres were injected s.c. into rats after being dispersed in the vehicle containing 1% sodium carboxymethylcellulose and 0.5% Tween 80. The periodically excised microspheres, surrounded by thin connective tissue, were homogenized with the Polytron in 10 ml of 1/30 M phosphate-buffered saline (PBS) containing 0.02% Tween 80, pH 6.0, and were shaken with an additional 10 ml of the PBS solution and 10 ml of dichloromethane. After the homogenate was centrifuged, the drug content in the aqueous layer was analyzed using HPLC.

Determination of the average molecular weight

The molecular weight of the polymer was determined by a gel permeation chromatography procedure (GPC) under the following conditions

using a polystyrene reference standard: column, Shimpack HSG 40s, HSG 30s, HSG 20s and two columns of HSG 15s; mobile phase, THF; flow rate, 1 ml/min; column temperature, 50°C; polystyrene standard mol. wt. 92600, 50000, 19000, 9000, 4000, 2100, 800 (purchased from Dupont, U.S.A.); detection, refractive index (Shodex RI SE-31, Showa Denko KK, Japan). The polymer was dissolved in 0.5 ml of tetrahydrofuran (THF), and 50 μl of the solution was injected into the GPC equipment. The average molecular weight was calculated with reference to the polystyrene standard by microcomputer software developed by Shimadzu. The molecular weight was expressed as the weight average molecular weight.

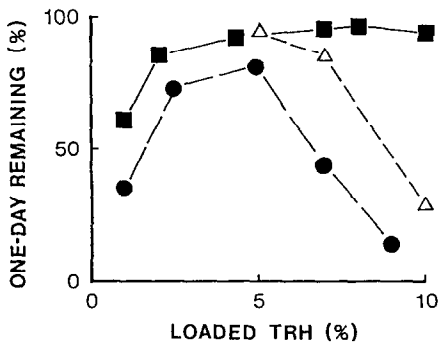

Fig. 1. Effect of molecular weight of PLGA(75/25) on initial release of TRH from PLGA microspheres with different levels of drug loading. (●) Molecular weight 6000; (△) molecular weight 8000; (■) molecular weight 11000.

Results and Discussion

Effect of molecular weight on initial release

In the previous study (Heya et al., 1991), we found that the profile of TRH release from PLGA microspheres is roughly divided into two stages: an initial burst phase followed by an erosion mediated phase. Accordingly, effects of various factors were interpreted differently with respect to the phase.

Firstly, the effects of the molecular weight of PLGA (molar ratio of lactide and glycolide of 75/25; referred to as PLGA(75/25)) on the initial burst were investigated with different levels of drug loading. Our previous report demonstrated that the profiles of TRH release from PLGA microspheres varied widely with the amount of peptide loaded. In Fig. 1, the percentage remaining at day 1 in the in vitro release test is plotted vs drug loading using PLGA of different molecular weights. The molecular weight is expressed as the weight-average molecular weight. At a low level of drug loading (1% TRH), a large initial burst was observed at all molecular weights of PLGA, indicating that a certain amount of the peptide is necessary for the formation of the rigid structure of the microspheres independent of the molecular weight of PLGA.

The microspheres prepared with PLGA of low molecular weight exhibited a larger initial burst with an increase in drug loading, although they retained their spherical shape with a relatively smooth surface according to scanning electron microscope observation (Fig. 2). The hydrophilicity of the microsphere increases with decrease in the molecular weight of PLGA as a result of the relative increase in the hydrophilic end region. Drug overloading also increases the hydrophilic region. The large initial burst with the decrease in molecular weight of PLGA and increase in drug loading can be attributed to the increase in the hydrophilic region producing aqueous channels. Consequently, the optimum TRH loading range for preparing microspheres with a small initial burst varies with the molecular weight of PLGA.

As shown in Table 1, the preparation of microspheres with a small initial burst was hindered by the addition of acid to the inner water phase in the preparation. This indicates that ionic interaction between TRH and PLGA is necessary to prepare microspheres with a small initial burst regardless of the molecular weight of PLGA.

Effect of molecular weight on release profiles over a long period

The effect of the molecular weight of PLGA(75/25) on drug release in the second phase was investigated. The level of drug loading (4.8%) was chosen to minimize the effect of the initial burst for any molecular weight of PLGA. As shown in Fig. 3, constant release was obtained over a period of 2–4 weeks. However, using microspheres prepared with PLGA 5000, constant

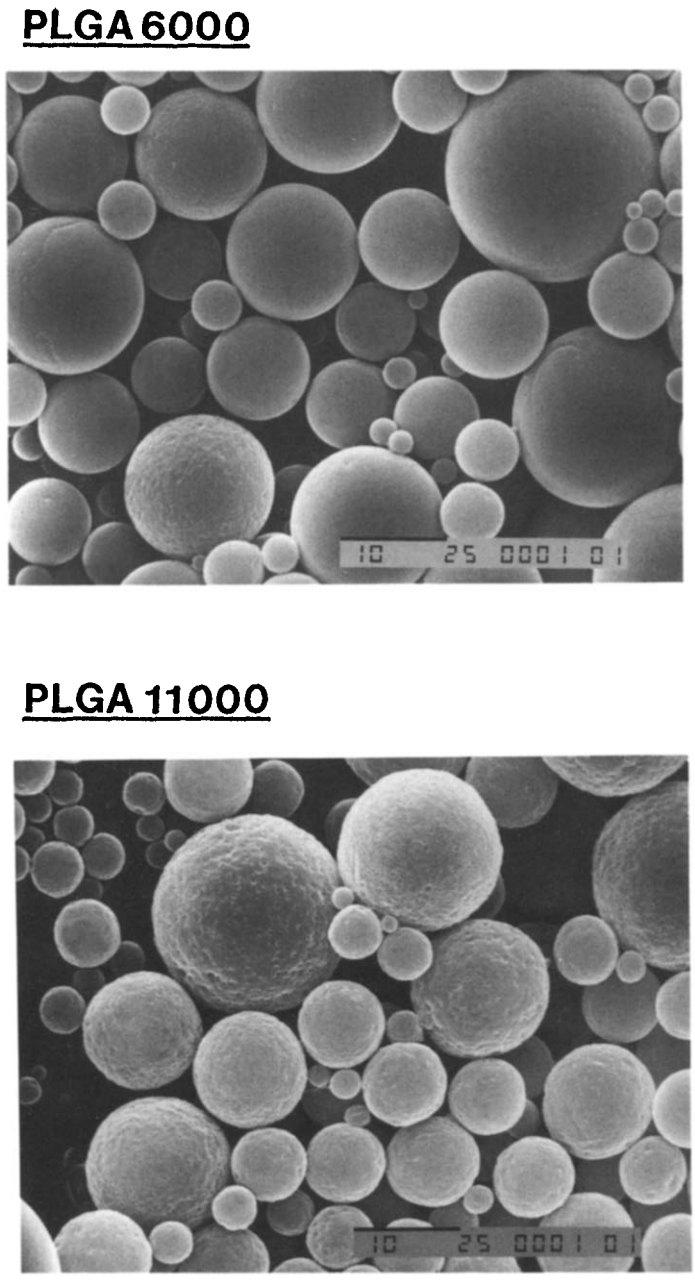

Fig. 2. Scanning electron micrographs of TRH microspheres consisting of PLGA 6000 (upper) and PLGA 11 000 (lower) at a loading amount of 7%.

TABLE 1

Effect of addition of tartaric acid on the entrapment ratio and initial release of TRH in PLGA microspheres prepared with different molecular weight of PLGA

Molecular weight	Entrapment ratio (%)	Remaining (%, 1 day)
Prepared without tartaric acid		
6000	77.8	65.6
8000	90.0	90.8
11000	104.8	84.5
Prepared with tartaric acid [a]		
6000	62.1	0
8000	72.7	0
11000	64.7	0

[a] Equimolar with TRH.

release could not be achieved, probably due to excessive hydrophilicity of the polymer.

The microspheres prepared with a smaller molecular weight provided a faster release rate. The difference in release rate can be attributed mainly to the difference in the biodegradation rate. It was found that the rate of TRH release following the initial release was governed primarily by the molecular weight of PLGA. This is similar to the case of LH-RH analogues (Sanders et al., 1986; Ogawa et al., 1988b).

Relationship between PLGA degradation and drug release

Table 2 shows the in vitro drug release from TRH microspheres and the changes in the polymer using two polymers with different initial PLGA(75/25) molecular weights. Changes in the polymer were identified by determining the molecular weight of the polymer and the weight changes. There were no significant differences in the relative decrease in molecular weight of PLGA between the two polymers. However, weight loss in the polymer with the lower molecular weight of PLGA was faster than that in those with higher molecular weight, presumably due to the greater amount of soluble fractions as a result of hydrolysis and to a higher degradation rate. The drug release rate correlated with the erosion of the polymer: the faster the erosion rate, the faster the release rate. The drug release from the polymer was much more rapid than the polymer weight loss. This suggests that the drug is able to diffuse through the aqueous channels in a polymer matrix especially during the advanced stage of biodegradation before the polymer becomes completely soluble. This diffusion is much more prominent in microspheres prepared with a low molecular weight of PLGA probably due to the higher hydrophilicity and the lower wall intensity of the polymer.

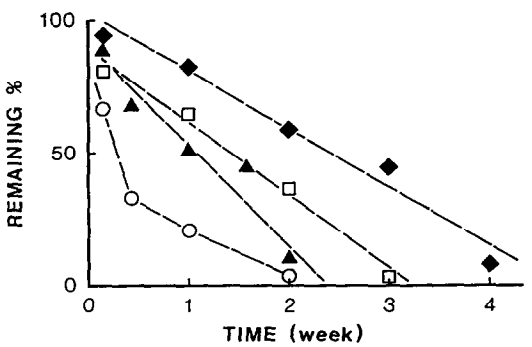

Fig. 3. Effect of molecular weight of PLGA(75/25) on release profiles of TRH from microspheres. (○) Molecular weight 5000; (▲) molecular weight 6000; (□) molecular weight 8000; (◆) molecular weight 11000.

TABLE 2

Relationship between PLGA erosion and TRH release from microspheres in vitro

Time (week)	TRH Remaining [a] (%)	PLGA MW [b]	PLGA Remaining [c] (%)
PLGA(75/25) 6000			
Initial	100	6400	100
1	57.3	5600 (87.5) [d]	75
2	17.3	4300 (67.2)	55
3	0	2800 (43.8)	37
PLGA(75/25) 11000			
Initial	100	10500	100
1	78.1	7800 (74.3)	82
2	64.3	6500 (61.9)	66
3	15.4	4500 (42.9)	56

[a] TRH remaining in microspheres in the in vitro release test.
[b] Weight-average molecular weight.
[c] Weight changes.
[d] % of initial molecular weight.

Release profiles of microspheres with different PLGA copolymer ratios

The TRH release rate was also affected by the

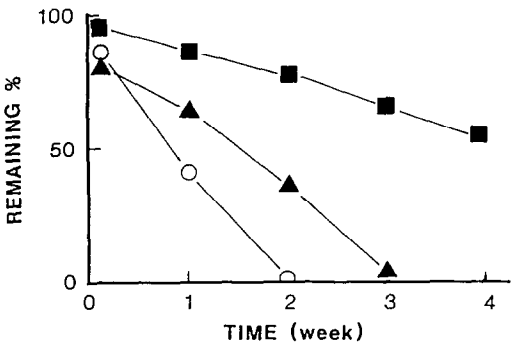

Fig. 4. Release profiles of TRH from PLGA microspheres with different copolymer ratios of PLGA. (○) LA/GA = 50/50, molecular weight 8000; (▲) LA/GA = 75/25, molecular weight 8000; (■) LA/GA = 100/0, molecular weight 11000.

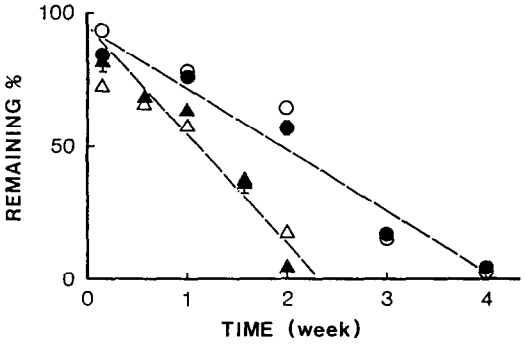

Fig. 5. In vitro and in vivo release profiles of TRH from PLGA(75/25) microspheres. PLGA 6000: (△) in vitro, (▲) in vivo; PLGA 11000: (○) in vitro, (●) in vivo.

LA/GA ratio of the PLGA used to prepare the microspheres. Fig. 3 shows the TRH release profiles with different PLGA copolymer ratios and drug loading of 4.8%. Sustained release was observed with regard to all copolymer ratios of PLGA. In comparison with the release rates of the microspheres with the same molecular weight of PLGA with different LA/GA ratio (Figs 3 and 4), the rate of TRH release from the microspheres prepared with the copolymer ratio of 50/50 was faster than that of 75/25; furthermore, the rate of TRH release with the copolymer ratio of 100/0 was slower than that of 75/25. These results indicate that the rate of TRH release increases with increase in GA content in PLGA. This is probably due to the difference in degradation rate of the polymer: the higher the GA content, the faster the degradation rate (Ogawa et al., 1988).

With the microspheres prepared with different copolymer ratios, 100% release within 1 day was observed with the addition of tartaric acid to the inner phase in preparation (data not shown). These results suggest that the ionic interaction between TRH and PLGA is an important factor in the entrapment of water-soluble peptides regardless of the PLGA/copolymer ratio.

In vivo release profiles

Fig. 5 shows the in vitro and in vivo release profiles of PLGA (75/25) microspheres. In vivo release was estimated by determining the remaining TRH at the injection site as described in Materials and Methods. Microspheres also exhibited sustained release in vivo, and the duration of release correlated with that in vitro, indicating that PLGA degradation is mediated mainly by hydrolysis not by enzymatic degradation. These results are similar to those observed with leuprolide (Ogawa et al., 1988b; Okada et al., 1988). Although TRH is rapidly metabolized in the body, the microsphere protects it from enzymatic degradation and releases the drug over a long period.

It was found that sustained release was reconfirmed in vivo, suggesting the possible application of these TRH microspheres as more convenient and reliable TRH therapy for brain diseases.

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