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Design of triptorelin loaded nanospheres for transdermal iontophoretic administration

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Abstract

Triptorelin is a decapeptide analog of luteinizing hormone releasing hormone, currently used for the treatment of sex-hormones dependents diseases. The aim of this work was to prepare triptorelin-loaded nanospheres useful for transdermal iontophoretic administration. Nanospheres were prepared with the double emulsion/solvent evaporation technique. The effect of three parameters on the encapsulation efficiency has been determined: the role of the pH of the internal and external aqueous phases, the nature of the organic solvent and the effect of three different poly(lactide-co-glycolide) (PLGA) co-polymers. Particle size, zeta potential and release kinetics were also determined. The encapsulation efficiency varied from 4 to 83% reaching the maximum value when both the internal and the external water phases were brought to pH 7 (isoelectric point of the peptide), methylene chloride was used as solvent of the copolymers and PLGA rich in free carboxylic groups was employed. The release profiles obtained with this co-polymer were characterized by the absence of burst effect. This behavior as well as the high encapsulation efficiency was explained by an ionic interaction occurring between the peptide and the co-polymer. This supports the already expressed theory that the release of peptides and proteins from PLGA nanospheres is also governed by the affinity of the encapsulated molecule versus the polymer. The obtained nanoparticles, regarding their size, amount encapsulated and zeta potential, were shown to be suitable for transdermal iontophoretic administration.

Keywords: Multiple emulsion; Iontophoresis; Biodegradable nanospheres; Peptide delivery; Poly(lactide-co-glycolide)

Triptorelin is a decapeptide analog of the luteinizing hormone releasing hormone (LHRH) currently used for the treatment of sex hormone-dependent tumors. In order to optimize the treatments by triptorelin, maintenance of constant plasma levels of drug for prolonged time periods is required (Sandow, 1983). Transdermal delivery is an attractive alternative to injection for trip-

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torelin administration, because it is non-invasive, it avoids degradation by the proteolytic enzymes from the gastrointestinal tract and it provides a controlled and sustained release of the drug.

It has been demonstrated that particles with a diameter up to 10 μm are able to penetrate into the annexes of the skin, i.e. sweat and sebaceous glands and hair follicles (Rolland et al., 1993). The accumulation of triptorelin loaded nanoparticles could create a triptorelin reservoir into the skin. From this reservoir the drug could slowly be released to reach the systemic circulation, generating appropriate plasmatic levels for long time periods. In order to increase the penetration and accumulation of nanoparticles in the skin, iontophoresis — whose main penetration route is the annexial one (Turner and Guy, 1998) — could be applied. Thus, the aim of this work was to prepare triptorelin loaded nanospheres for transdermal iontophoretic administration.

Nanoparticles were prepared by the double emulsion solvent evaporation method, using an ultrasonication probe for the emulsification processes (Blanco and Alonso, 1997). Briefly, 1 mg of triptorelin was dissolved in 100 μl of an aqueous solution (W_1) and emulsified with a 1 ml solution of poly(lactide-co-glycolide) (PLGA) in an organic solvent (50–100–200 mg/ml) by sonicating for 30 s. Then, 2 ml of a 1% PVA solution (W_2) were added, and the resulting $W_1/O/W_2$ emulsion was sonicated for 60 s. The double emulsion was diluted into 15 ml of 0.3% PVA aqueous solution and the solvent was evaporated under vacuum. The obtained nanospheres were separated by ultracentrifugation at $13\,000 \times g$ for 30 min and washed with distilled water.

Different pH of the aqueous phases were tested: pH 5 (maximum of triptorelin stability) and pH 7 (isoelectric point of the peptide) for the internal water phase (W_1), and pH 6 (pH of the PVA 1% solution) and pH 7 for the external one (W_2). The co-polymers tested were Resomer ®RG 756 (PLGA 75/25, intrinsic viscosity in chloroform: 0.8 dl/g), Resomer ®RG 752 (PLGA 75/25, intrinsic viscosity in chloroform: 0.2 dl/g), and Resomer ®RG 503 H (PLGA 50/50, rich in free carboxylic groups, intrinsic viscosity in chloroform: 0.4 dl/g). Ethyl acetate and methylene chloride were used for co-polymers solubilization. Particle size and zeta potential were measured, respectively, by photon correlation spectroscopy (PCS), using an N4 Plus (Coulter), and by microelectrophoresis using a Malvern Zetasizer 4 (Malvern Instruments). For the determination of triptorelin content in nanospheres, a weighed amount of lyophilized nanospheres were dispersed in 0.1 M NaOH containing 1% sodium dodecylsulfate (SDS) and stirred until complete dissolution. The analysis was made by HPLC. The encapsulation results as well as the nanoparticles sizes are illustrated in Table 1.

Using Resomer ®RG 756, ethyl acetate as organic solvent, and a pH 5 buffer for the W_1, nanoparticles with a diameter ranging from 350 to 600 nm were obtained, depending on polymer concentration (formulation A, B, C). The encapsulation efficiency was very low reaching a maximum of 14% when both the internal and the external water phases were brought to pH 7 (isoelectric point of the peptide; formulation E). In these conditions, the substitution of ethyl acetate by methylene chloride induced an increase of the encapsulated efficiency that reached 48% (formulation F). The mean diameter of the obtained nanospheres was about 750 nm with a very high polydispersity index, indicating the presence of particles ranging from 400 to 1200 nm.

The use of a lower molecular weight polymer, Resomer ®RG 752, did not significantly change the encapsulation efficiency and the particle size (formulation G). On the contrary, using Resomer ®RG 503 H, characterized by a high presence of free carboxyl groups, the encapsulation efficiency reached 83% (formulation H).

This may be explained by the existence of an ionic interaction between triptorelin and the copolymer. Such an ionic interaction has indeed been previously demonstrated, using NMR spectroscopy, between the argynil and histidyl residues of a triptorelin analog and the carboxylic acids at the end of PLGA polymers (Okada et al., 1994). It is assumed that a similar interaction occurs between the same residues of triptorelin and the more numerous free carboxylic terminals of RG 503 H, causing an increase in the triptorelin loaded amount. Zeta potential of blank and drug-

Table 1
Preparation conditions, triptorelin encapsulation and average particle size[a]

	Copolymer	PLGA conc. (mg/ml)	pH in W_2	pH in W_1	Organic solvent	Particle size (nm)	μg Tripto loaded/mg PLGA[c]	Encapsulation efficiency (%)
A	Resomer ®RG 756	50	6	5	CH_3COOEt	335 (PI[b] = 0.125)	0.82	3.6
B	Resomer ®RG 756	100	6	5	CH_3COOEt	440 (PI = 0.147)	0.52	4.7
C	Resomer ®RG 756	200	6	5	CH_3COOEt	620 (PI = 0.203)	0.51	8.9
D	Resomer ®RG 756	100	7	5	CH_3COOEt	402 (PI = 0.129)	0.98	9.8
E	Resomer ®RG 756	100	7	7	CH_3COOEt	455 (PI = 0.153)	1.71	14.1
F	Resomer ®RG 756	100	7	7	CH_2Cl_2	743 (PI = 0.412)	5.6	47.9
G	Resomer ®RG 752	100	7	7	CH_2Cl_2	674 (PI = 0.415)	5.7	50.2
H	Resomer ®RG503H	100	7	7	CH_2Cl_2	574 (PI = 0.408)	9.6	83.5

[a] Effect of W_1 and W_2 pH, organic solvent and co-polymer.
[b] PI: polydispersity index.
[c] Theoretical loading: 10 μg triptorelin/mg PLGA.

loaded nanoparticles was measured in order to characterize the particles in view of their iontophoretic administration and also in order to investigate triptorelin localization in the nanospheres. Measurement were performed in a pH 5 buffer (at this pH value triptorelin is positively charged). As shown in Fig. 1, there was no difference between zeta potential of blank and loaded nanospheres, indicating that the drug was not localized at the surface of the particles. Resomer ®RG 756 and Resomer ®RG 752 nanospheres were almost neutral, while Resomer ®RG 503 H nanospheres were negatively charged, due to the presence of free carboxylic groups. In order to investigate iontophoretic transport of nanoparticles, positively charged particles are more suitable. Therefore nanoparticles were magnetically stirred with a solution of cetyltributylammoniumbromide (CTAB) overnight. The zeta potential obtained, measured after ultracentrifugation, was positive: about +15 mV for the formulations F and G (see Fig. 1), indicating an adsorption of the cation onto the surface of the nanospheres.

Release kinetics of formulations F, G and H were studied at 37°C in a pH 5 solution. Fig. 2 illustrates the percentage of triptorelin released with respect to the amount of triptorelin encapsulated. Nanospheres prepared using RG 756 and RG 752 showed a similar release profile, characterized by a burst effect of about 20% followed by a plateau. The burst effect observed with RG 756 and 752 disappears when nanospheres were prepared using RG 503 H, as a consequence of the already mentioned ionic interaction. The release kinetic profile obtained with this polymer was continuous and faster with respect to the 75/25 co-polymers due to the higher hydrophilicity.

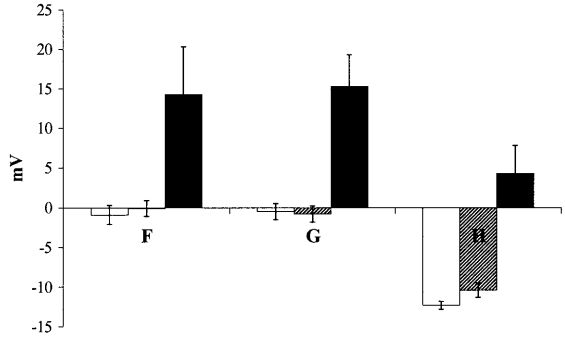

Fig. 1. Zeta potential of formulations F, G and H measured in a pH 5 buffer 0.1 M. Control nanoparticles (white bars), triptorelin loaded nanoparticles (gray bars), CTAB adsorbed onto nanoparticles (black bars).

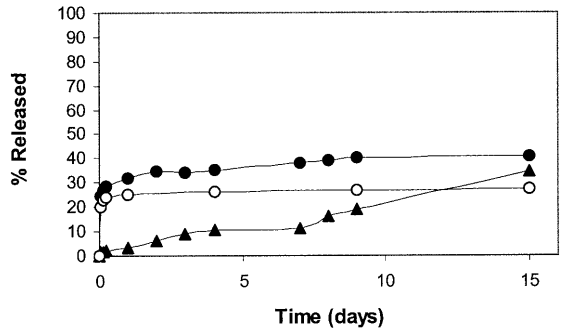

Fig. 2. Release kinetics of triptorelin from different PLGA nanospheres: formulation F — RG 756 (open circles); formulation G — RG 752 (closed circles); formulation H — RG 503 H (closed triangles).

Since the nanoparticles should be designed for iontophoretic administration, being forced to cross the skin by the electrorepulsion, the release kinetics were studied also in presence of current.

The application of current ($d = 0.5$ mA/cm^2; 1 h) did not significantly modify triptorelin release from nanospheres (data not shown). This is particularly important in order to avoid the immediate release of the drug from the 'reservoir' during the current application. We can conclude that it is possible to encapsulate triptorelin into nanospheres and modulate the encapsulation efficiency by adjusting the formulation conditions. In particular, the use of a co-polymer with free terminal carboxylic group allows to obtain a high triptorelin loading and a controlled release characterized by the absence of any burst effect.

This information supports the already expressed theory (Blanco and Alonso, 1997) that the release of peptides and proteins from PLGA nanospheres is also governed by the affinity of the encapsulated molecule versus the polymer.

In conclusion, the obtained nanospheres may be considered as suitable for transdermal iontophoretic administration, due to their size, amount of peptide encapsulated, zeta potential and release kinetic profile.

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