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Thymopentin in solid lipid nanoparticles

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Abstract

The pentapeptide thymopentin was englobed in solid lipid nanoparticles prepared from warm microemulsions following two different methods: from O/W microemulsion by forming the more lipophilic ion-pair with hexadecylphosphate, and from W/O/W microemulsion by dissolving the pentapeptide in the aqueous internal phase. The incorporation of the hydrophilic drug was 5.2% and 1.7% respectively; in both cases, the in vitro release of thymopentin from the solid lipid nanoparticles followed a pseudo-zero-order kinetics.

Keywords: Thymopentin; Warm O/W and W/O/W microemulsions; Solid lipid nanoparticles; Polypeptides

Thymopentin (TP-5) is a synthetic pentapeptide (Arg-Lys-Asp-Val-Tyr) corresponding to the active site of the 49-amino acid human hormone thymopoietin. The half-life is 30 s (Tischio et al., 1979). The efficacy of TP-5, as immunomodulating agent, has been demonstrated in the Sézary syndrome (a cutaneous T-cell lymphoma) after i.v. administration (Bernengo et al., 1992).

Solid lipid nanoparticles (SLN) can be obtained from warm O/W microemulsions whose internal phase consists of low melting components (Gasco and Morel, 1990; Gasco et al., 1992). In a previous paper (Morel et al., 1994) a polypeptide [D-Trp-6]LHRH was englobed in SLN obtained from a warm W/O/W multiple microemulsion by dissolving it in the internal aqueous phase. Lipophilic ion-pairs of drugs, in which dissociation functions are present, were prepared to increase the incorporation (Cavalli et al., 1992, 1993).

The aim of the research reported here was to incorporate a hydrophilic molecule, i.e. TP-5, which has a majority of basic amino acids, in SLN by two different methods: (a) from an O/W microemulsion increasing its lipophilicity by forming an ion-pair with an anionic counter-ion, consequently increasing its partition in the oil phase; (b) from a W/O/W microemulsion, by dissolving it in the internal aqueous phase of a microemulsion.

The warm microemulsion O/W was prepared as follows: a mixture of 74.0% water, 3.9% egg lecithin (Merck, Darmstadt, Germany) purified according to Hanahan et al. (1951); 9.7% taurodeoxycholate sodium salts (TDC) (Sigma, St.

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Louis, MO, USA) and 4.0% butanol (Merck), heated to about 70°C, were added to 5.9% melted stearic acid (Merck), 1.5% sodium hexadecyl phosphate ($NaC_{16}P$) (Brown et al., 1955) and 1.0% TP-5 (Cilag Ag, CH-8201, Schaffhausen). A clear system was obtained.

$NaC_{16}P$ normally used in liposomes (Benita et al., 1984) was used as a counter-ion for the formation of the lipophilic TP-5 ion-pair. TDC was used as a co-surfactant but might also behave as a counter-ion.

The warm microemulsion W/O/W was prepared as follows: a W/O microemulsion was first prepared by adding a mixture of 44.7% molten stearic acid, 26.8% egg lecithin and 21.7% butanol to a 6.8% aqueous solution containing 350 mg/ml of TP-5, at 70°C. Predetermined amounts of W/O microemulsion (14.5%) were added to a mixture of 68.3% water, 3.4% egg lecithin, 5.5% butanol and 8.3% TDC heated to about 70°C. A clear system was obtained. The preparation was completed in the shortest possible time.

The warm microemulsion (O/W or W/O/W), at 70°C, was dispersed in water operating at a temperature of about 2°C, thus producing SLN. The microemulsion/dispersion medium ratio was about 1:10. The dispersion of SLN was washed three times with water by the Amicon TCF10A ultrafiltration system, (Beverley, MA, USA) (membranes Amicon Diaflo YM100), then freeze-dried.

The size and polydispersity of the SLN were measured by photon correlation spectroscopy (PCS) using a Zetasizer 2c (Malvern, Worcestershire, UK). The wave-length of the laser light (He/Ne) was 632.8 nm. Peptide analysis was performed by capillary electrophoresis system, CZE, (Bio-Rad, Hercules, CA, USA) (Wu et al., 1992). A weighed freeze-dried sample of SLN (about 5 mg) was dissolved in 1 ml of methanol and diluted with sodium phosphate buffer (pH 2.5). The washing waters were diluted with sodium phosphate buffer only.

The apparent partition coefficient between stearic acid and water of TP-5 alone was determined by adding a known volume of TP-5 aqueous solution (1.0×10^{-3} M), to a known volume of stearic acid ($d = 0.847$). The system was stirred with a bar magnet at 70°C until equilibrium was reached. After separation of the two phases, the drug concentration was determined in the aqueous phase by CZE. The apparent partition coefficient of TP-5 with $NaC_{16}P$ (molar ratio TP-5/$NaC_{16}P$, 1:5), with TDC (molar ratio TP-5/TDC, 1:5) and with the two salts (molar ratio TP-5/$NaC_{16}P$/TDC, 1:5:5) were then determined.

Release from SLN aqueous suspension (100 mg/ml of freeze-dried SLN) and diffusion from the aqueous solution (5 mg/ml) were observed for about 6 h as previously described (Morel et al., 1994).

The stability of TP-5 in water at 70°C was observed for more than 1.5 h, even though the time required to prepare the microemulsion is about 0.5 h; analysis revealed no degradation.

Freeze-dried SLN analysed by gas chromatography, GC, (Carlo Erba, Milan, Italy) (Aquilano et al., 1993) revealed traces of butanol only in SLN prepared from W/O/W microemulsion.

The apparent partition coefficient of TP-5 alone between stearic acid and water is very low; it increases more than 200 times in the presence of $NaC_{16}P$, in consequence of the formation of the lipophilic ion-pair. The apparent partition coefficient of TP-5 decreases, in the presence of both TDC and $NaC_{16}P$, even if TDC alone has a mild effect on the partition (Table 1). Probably the presence of TDC micelles in the aqueous phase might explain this behaviour.

In the hot O/W microemulsion the molar ratio TDC/$NaC_{16}P$ was about 4:1, but, in this system, a considerable part of the TDC used should be present at the interface as co-surfactant. After washing, an incorporation of 5.2% of TP-5 in SLN was obtained; the average diameter of the

Table 1
Apparent partition coefficients (P) between stearic acid and water of TP-5 alone and in the presence of TDC and $NaC_{16}P$

	Molar ratio	P
TP-5		0.1
TP-5/TDC	1:5	1
TP-5/C_{16}PNa	1:5	28
TP-5/C_{16}PNa/TDC	1:5:5	8

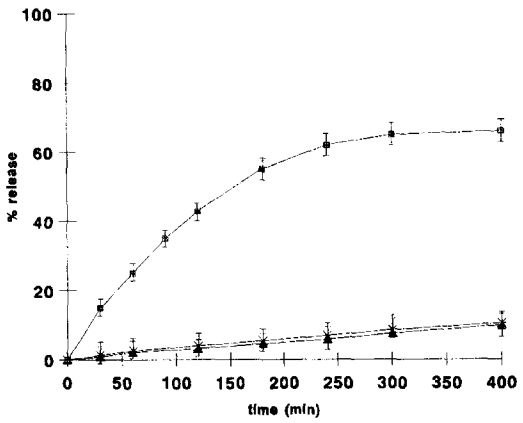

Fig. 1. Percentage release of thymopentin from (▲) SLN obtained by O/W microemulsion, from (X) SLN obtained by W/O/W microemulsion and diffusion from (■) aqueous solution.

SLN was small (about 100 nm, polydispersion 0.29). The recovery of the drug was 47%.

In the second method of preparing SLN, the percentage of incorporation, after washing, was 1.7%, below that obtained by the first method. In fact, in order to obtain a clear multiple W/O/W microemulsion, the internal aqueous phase, in which TP-5 was dissolved, was only 1% of the whole system; thus, despite a high concentration of TP-5 in this phase (350 mg/ml), the concentration in the whole W/O/W microemulsion was 0.35%, while in the case of O/W microemulsion, TP-5 was about 1%; the average diameter of the SLN was 200 nm (polydispersion 0.31). The recovery of the drug was 63%.

The percentage release of the drug from the SLN prepared with the two different methods was practically identical (about 10% in 6 h) while the percentage release from solution was higher (about 65% in 6 h). The release from SLN followed a pseudo-zero-order kinetics in both cases (Fig. 1). Only in vivo tests will be able to show whether, by injecting a dispersion of TP-5 carried by SLN, a longer half-life of the drug can be achieved than by injecting the solution.

In conclusion, the research shows that two different methods may be proposed to incorporate hydrophilic polypeptides, containing a majority of either basic or acidic amino acids, into SLN.

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