ELSEVIER

International Journal of Pharmaceutics 105 (1994) R1–R3

international journal of pharmaceutics

Rapid Communication

Incorporation in lipospheres of [D-Trp-6]LHRH

Silvia Morel, Maria Rosa Gasco *, Roberta Cavalli

Dipartimento di Scienza e Tecnologia del Farmaco, Università degli studi di Torino, via P. Giuria 9, 10125 Torino, Italy

(Received 23 November 1993; Accepted 20 January 1994)

Abstract

Lipospheres containing a hydrophilic drug, [D-Trp-6]LHRH, were prepared using warm w/o/w microemulsion. Incorporation of the hydrophilic drug, [D-Trp-6]LHRH, was considerable (about 90%). The in vitro release of the peptide from the lipospheres was observed for 8 h.

Key words: [D-Trp-6]LHRH; Microemulsion; Liposphere

Clinical interest in peptides has grown during the last several years. The administration of peptides and of small proteins is a problem, even the parenteral route presenting some difficulty. The most problematic factor is the systemic metabolism of these molecules: indeed, their half-lives are often only a few minutes.

In previous research, w/o microemulsions were used to administer some peptides subcutaneously, in order to achieve prolonged release: insulin was incorporated in a w/o microemulsion and injected into rabbits (Gasco et al., 1992a); the results showed a significant increase of t_{max} and $t_{1/2}$ in comparison with the corresponding data relating to insulin aqueous solution. [D-Trp-6]LHRH was also incorporated in a w/o microemulsion and administered to rats in a single injection; a reduction in plasma testosterone levels was maintained over 3 weeks (Gasco et al., 1990).

Solid lipospheres incorporating different drugs were obtained by microemulsification of molten lipids, as the internal phase, and subsequent dispersion of the water microemulsion in cold water; the drugs incorporated were either rather lipophilic (Gasco et al., 1992b) or became lipophilic by formation of complexes (Gasco et al., 1992c). The vectorization of hydrophilic drugs was problematic, therefore, our interest turned to multiple microemulsions.

Multiple emulsions are complex systems, often called emulsions of emulsions, since the internal phase contains dispersed globules which are miscible with the continuous phase. Two multiple emulsions can exist: o/w/o and w/o/w. Various factors may affect the stability of a multiple emulsion, particularly of a w/o/w emulsion: coalescence of the internal aqueous droplets within the oil phase, coalescence of the oil droplets, and rupture of the oil layer on the surface of the internal droplets (Florence et al., 1982). The most

* Corresponding author.

SSDI 0378-5173(94)00040-C

promising potential application of multiple emulsions in pharmacy is in prolonged delivery systems. Encouraging results have been obtained using multiple emulsions as vehicles to provide sustained release of certain drugs, such as anticancer agents (Omotosho et al., 1989), pilocarpine (Attia et al., 1986), and pentazocine (Mishra et al., 1989).

In order to investigate the vectorization of a hydrophilic drug – the peptide [D-Trp-6]LHRH – in lipospheres, a warm multiple w/o/w microemulsion was prepared with the aim of obtaining lipospheres encorporating the hydrophilic drug. The aqueous internal phase contained the drug, the oil phase consisted of molten lipids and the external phase was an aqueous solution.

The warm w/o/w multiple microemulsion was prepared in two steps: a w/o microemulsion was first prepared by adding, at 70°C, an aqueous solution containing the peptide to a mixture of molten stearic acid, egg lecithin and butyric acid, resulting in a clear system. The w/o microemulsion was then added, at 70°C, to a mixture of water, egg lecithin and butyric acid, again obtaining a clear system.

The composition of the w/o microemulsion was as follows: 55% stearic acid (Merck, Darmstadt, Germany), 14% egg lecithin (Merck), 20% butyric acid (Merck) and 11% aqueous solution of [D-Trp-6]LHRH (Sigma, St. Louis, MO, U.S.A.). The peptide was dissolved in the water phase of the w/o microemulsion, at a concentration of about 30 mg/ml.

The composition of the w/o/w microemulsion was the following: 72% water, 7% egg lecithin, 7% taurodeoxycholate sodium salt (TDC; Sigma), 7% butyric acid and 7% w/o as internal phase. Egg lecithin was purified as described previously (Hanahan et al., 1951).

Solid lipospheres were obtained by dispersing the warm microemulsion in water at 2°C operating in a thermostatic bath, under mechanical stirring. The microemulsion-dispersion medium ratio was 1:20.

The suspension of lipospheres was washed three times with water by an Amicon TCF10A ultrafiltration system (Beverley, MA, U.S.A.), and using Amicon Diaflo YM100 membranes (cut-off 100 000). After washing, the suspension was freeze-dried with a Modulyo freeze-dryer (Edwards, Crawley, U.K.).

Peptide analysis was performed by an HPLC LC-95 detector (Perkin Elmer, CT, U.S.A.). The method proposed by other workers (Sertl et al., 1981) was modified in order to allow the analysis of LHRH-analog samples containing components from the lipospheres. The mobile phase was phosphate buffer pH 3.8/acetonitrile/methanol (40:9:51); the flow rate was 1.0 ml/min. A weighed freeze-dried sample (about 20 mg) of lipospheres, containing [D-Trp-6]LHRH, was dissolved in 1 ml of methanol, then HPLC analysis was performed.

The suspension of the lipospheres containing the peptide in water was placed in a multicavity microdialysis cell designed for separate but simultaneous microdialysis experiments. A Servapor 44155 cellulose membrane (Serva, Heidelberg, Germany) was used.

Liposphere suspensions and peptide solution (500 μg/ml) were tested as donor phase; the medium in both experiments was phosphate buffer 0.025 M, pH 6.5; as acceptor phase the same buffer was used. Liposphere suspension was prepared by dispersing about 60 mg of freeze-dried lipospheres carrying the peptide in 1 ml of buffer solution. Release was observed for 8 h; analysis was by HPLC as above.

As far as we know, multiple microemulsions (warm or cold) have never previously been prepared. Microemulsions might offer some advantages over emulsions in terms of the stability of the system: they are more stable than emulsions and, in the case of our specific application, the multiple microemulsion in any case only had to be stable for a few minutes, the time between the preparation of the clear multiple microemulsion and its dispersion in cold water to prepare the lipospheres. The clear w/o/w system was obtained by adding the previously prepared w/o microemulsion to the aqueous phase, which was a mixture of water, surfactant and cosurfactant. The incorporation in lipospheres of a hydrophilic drug, such as [D-Trp-6]LHRH, which had been dispersed in the w/o microemulsion confirmed the achievement of a warm w/o/w.

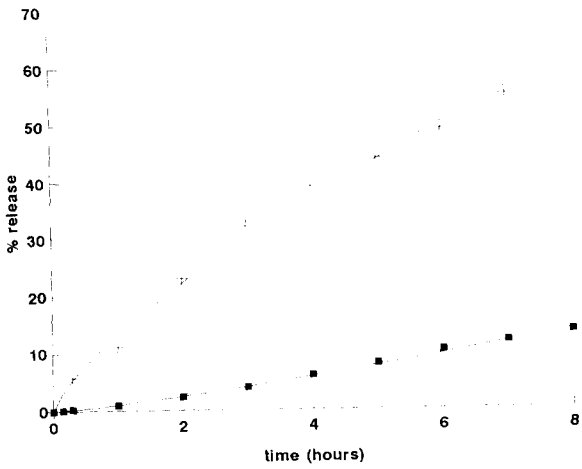

Fig. 1. Percentage release of [D-Trp-6]LHRH from (+) lipospheres and diffusion from (■) solution.

Analysis of peptide in the lipospheres, after the aqueous washings, indicated that the incorporation of the peptide was quite high, about 90%. The average diameter of the lipospheres, dispersed in water, was about 300 nm, determined by a computerized laser light scattering technique at 25°C with a Zetasizer 2C (Malvern, U.K.).

The release of [D-Trp-6]LHRH from freeze-dried lipospheres dispersed in water was observed for 8 h; dialysis cells were used in order to evaluate the difference in [D-Trp-6]LHRH release between aqueous solution and lipospheres (Cavalli et al., 1992). The release of the drug from the lipospheres followed pseudo zero-order kinetics, delivering about 10% of the drug; from the solution, the amount released in 8 h was about 50% (Fig. 1). The possibility of freeze-drying the lipospheres means that the peptide can be maintained in a solid system instead of in a liquid one.

Although further research will be necessary, these preliminary results show that multiple warm microemulsions can be prepared, and that lipospheres carrying hydrophilic drugs can be obtained, realizing a prolonged in vitro release.

1. Acknowledgement

This work was supported by a 40% M.U.R.S.T. grant.

2. References

Attia, M.A. and Habib, F.S., Pilocarpine delivery from multiple emulsions. *STP Pharm.*, 2 (1986) 636–640.

Cavalli, R., Gasco, M.R. and Morel, S., Behaviour of timolol incorporated in lipospheres in the presence of a series of phosphate esters. *STP Pharm. Sci.*, 2 (1992) 514–518.

Florence, A.T. and Whitehill, D., The formulation and stability of multiple emulsions. *Int. J. Pharm.*, 11 (1982) 277–308.

Gasco, M.R., Cavalli, R. and Carlotti, M.E., Timolol in lipospheres. *Pharmazie*, 47 (1992c) 119–122.

Gasco, M.R., Morel, S. and Carpignano, R., Optimization of incorporation of deoxycorticosterone acetate in lipospheres. *Eur. J. Pharm. Biopharm.*, 38 (1992b) 7–10.

Gasco, M.R., Pattarino, F. and Lattanzi, F., Long-acting delivery systems for peptides: reduced plasma testosterone levels in male rats after a single injection. *Int. J. Pharm.*, 62 (1990) 119–123.

Hanahan, D.J., Turner, N.B. and Deyko, M.E., Isolation of egg phosphatidylcholine. *J. Biol. Chem.*, 191 (1951) 623–629.

Mishra, B. and Pandit, J.K., Prolonged release of pentazocine from multiple o/w/o emulsions. *Drug Dev. Ind. Pharm.*, 15 (1989) 1217–1230.

Omotosho, J.A., Whateley, T.L. and Florence, A.T., Release of 5-fluorouracil from intramuscular w/o/w multiple emulsions. *Biopharm. Drug Dispos.*, 10 (1989) 257–268.

Sertl, D.C., Johnson, R.N. and Kho, B.T., An accurate specific HPLC method for the analysis of a decapeptide in a lactose matrix. *J. Liq. Chromatogr.*, 4 (1981) 1135–1156.