International Journal of Pharmaceutics, 76 (1991) R5–R8

ADONIS 037851739100358N

IJP 10037

Rapid communication

Controlled release of LHRH-DT from bioerodible hydrogel microspheres

Manmohan Singh [1], Ramesh Rathi [2], Amarjit Singh [3], Jorge Heller [4], G.P. Talwar [1] and Jindrich Kopecek [2]

[1] *National Institute of Immunology, New Delhi 110067 (India),* [2] *Center for Controlled Chemical Delivery, Salt Lake City, UT (U.S.A.),* [3] *Pharmax Corp. Ltd, New Delhi 110020 (India) and* [4] *SRI International, Menlo Park, CA 94025 (U.S.A.)*

(Received 7 August 1991)
(Accepted 24 August 1991)

Key words: Bioerodible hydrogel; Unsaturated polyester; LHRH-DT vaccine; Degree of crosslinking; ELISA, in vitro

Summary

Bioerodible hydrogels prepared from water-soluble unsaturated polyesters, crosslinked through the double bonds, can immobilise a vaccine preparation such as LHRH-DT. The release of the entrapped macromolecule takes place by hydrolytic cleavage of the ester linkage in the polymer backbone. The in vitro rate of hydrolysis and vaccine release can be controlled by synthesising polyesters with varying proportions of esters activated by electron-withdrawing substituents placed vicinally to the ester group and by varying the crosslink density of the hydrogel. The in vitro release of the vaccine from three different crosslinking densities and two different pendant unsaturated polymers is studied.

The recent interest in the field of macromolecular delivery has led to studies in the area of vaccine delivery (Langer, 1981; Wise, 1987; Gilley, 1988; Eldridge et al., 1990 and Hora, 1990). Bioerodible hydrogels have been studied for the controlled release of water-soluble macromolecules (Heller, 1983, 1985; Heller et al., 1983). The release of bovine serum albumin as a model macromolecule has been reported. We have reported earlier that a vaccine consisting of a denatured protein preparation such as diphtheria toxoid, can be encapsulated using biodegradable poly (DL-lactide) polymer without any significant loss in immunogenicity of the vaccine (Singh et al., 1991).

The vaccine selected for the present study was LHRH-DT. In this vaccine, the synthetic decapeptide, leutinising hormone-releasing hormone (LHRH), is conjugated to an immunogenic carrier-diphtheria toxoid (DT). Immunization with this vaccine generates antibodies to the hapten (LHRH) as well as to the carrier (DT). The circulating anti-LHRH antibodies bio-neutralise the native LHRH, thereby showing regression of

Correspondence: M. Singh, National Institute of Immunology, Shaheed Jeet Singh Marg, New Delhi 110067, India.

prostrate carcinoma. The same vaccine can also serve as an anti-fertility vaccine by depleting the circulating LHRH so very necessary for spermatogenesis in males and ovulation in females.

Two water-soluble polyesters, one with backbone unsaturation and the other with pendant unsaturation, were synthesised. The synthesis of the polyester with backbone unsaturation was carried out using 50.19 g of PEG-600, 9.34 g of fumaric acid and 12.2 mg of hydroquinone, heated at 190 °C for 7.5 h in a 100 ml three-necked flask equipped with a thermometer, distillation condenser and a nitrogen inlet. The product was a greenish-yellow liquid at room temperature. Two polyesters with pendant unsaturation were synthesised. One was synthesised using 61 g of PEG-600, 13 g of itaconic acid, 6.5 g of ketoglutaric acid, 83 mg of hydroquinone, and 830 μl of a 0.5% solution of *p*-toluenesulphonic acid in 95% ethanol, heated at 150 °C for 22 h under vacuum. The second polymer was synthesised similarly, but without ketoglutaric acid

For the preparation of vaccine-loaded microspheres from backbone unsaturated polyester, 2 g of the polymer was dissolved in 5 ml of 0.1 M phosphate buffer containing 50 μg of LHRH-DT. For a formulation with 20 wt% crosslinking, 400 mg of *N*-vinylpyrrolidine was added along with 0.1 ml of 0.1% ferrous ammonium sulphate solution, 1 ml of 1 *M* Tris buffer of pH 8.6 and 1 ml of 1.12% aqueous solution of *N,N,N',N'*-tetramethylethylenediamine. Oxygen was removed from the solution by bubbling nitrogen through it for 20 min. The polymerisation was then initiated by adding 1 ml of a nitrogen-purged 0.5% aqueous solution of ammonium persulphate. The resulting solution was then added dropwise to 100 ml of rapidly stirred *n*-hexane containing 4% Arlacel C (sorbitan sesquioleate) at 37 °C. The temperature was maintained at 37 °C for 30 min. The microspheres so formed were partially dehydrated by addition of 100 ml of absolute alcohol. Hexane was decanted off and the solution filtered. The microspheres were dried overnight in a desiccator. Very fine, free-flowing microspheres were obtained. Similar products with the same vaccine loading, but with 40 and 60 wt% crosslinking were also prepared.

The bioerodible hydrogel microspheres from polyester with pendant unsaturation were prepared by free radical coupling. 5 g of the polymer was dissolved in 5 ml of 0.1 M PBS containing 50 μg of the vaccine. To this solution, 1 ml of 0.1% ferrous ammonium sulphate, 1 ml of Tris buffer and 1 ml of a 1.12% aqueous solution of *N,N,N',N'*-tetramethylethylenediamine was added.The final solution was purged with nitrogen for 20 min. The polymerisation was then initiated by adding 5 ml of a nitrogen-purged 25% aqueous solution of ammonium persulphate. This solution was added dropwise to 100 ml of stirred *n*-hexane containing 4% Arlacel C at 37 °C. After 30 min the microspheres were dehydrated with 100 ml of absolute ethanol. The microspheres were filtered and dried overnight.

All batches were subjected to SEM studies for surface morphology and size distribution. The prepared microspheres were in the size range of 20–60 μm. The total vaccine loading was determined by digesting 10 mg of the vaccine-loaded microspheres in 5% SDS solution in 0.1 N NaOH for 12 h. The in vitro antigen release was quantitatively estimated by an enzyme-linked immunosorbent assay (ELISA) developed in the laboratory. The ELISA was also used to estimate total vaccine loading. All batches were subjected to in vitro release studies in a shaking water bath at 37 °C. 100 mg of the product was dispersed in 1 ml of PBS buffer pH 7.6 and the antigen release monitored every week for 12 weeks.

The in vitro release of LHRH-DT from bioerodible hydrogel microspheres prepared from backbone unsaturated polyester with 20, 40 and 60 wt% crosslinking was estimated (Fig. 1). The release from 20% crosslinked hydrogel is complete in 9 weeks in comparison to 12 weeks from 60% crosslinked hydrogel. The difference in the amount of vaccine released from 20 and 60% crosslinking is not as large as was expected. The difference in the release of BSA as reported by Heller et al. from 20 and 60% crosslinking is more significant than observed here. This could be due to the different hydrodynamic structure that the vaccine possesses in comparison to BSA although both have nearly the same molecular weight. Thus, a higher degree of crosslinking can

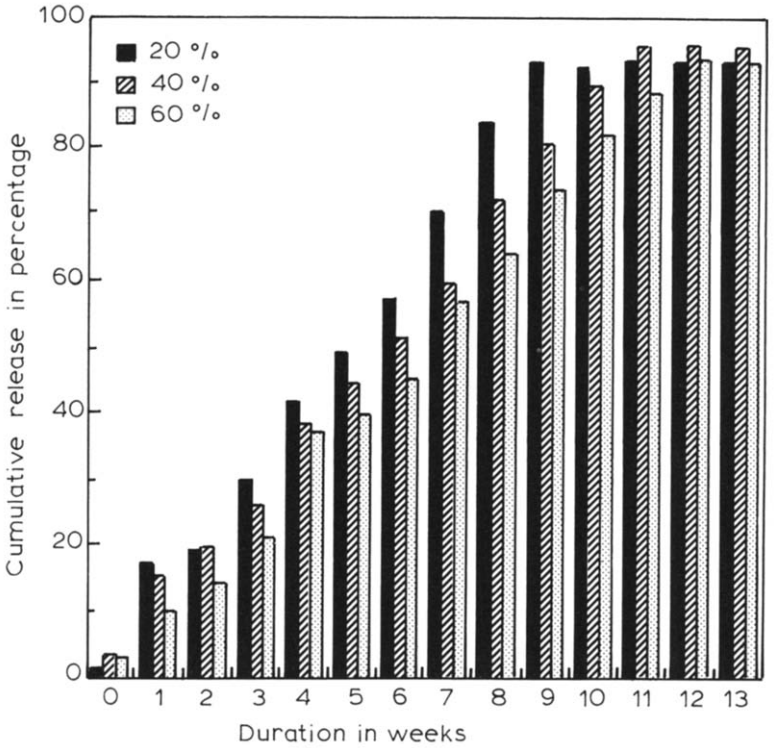

Fig. 1. Percent cumulative vaccine release from bioerodible hydrogel microspheres prepared from backbone unsaturated polymer at 20, 40 and 60 wt% crosslinking density.

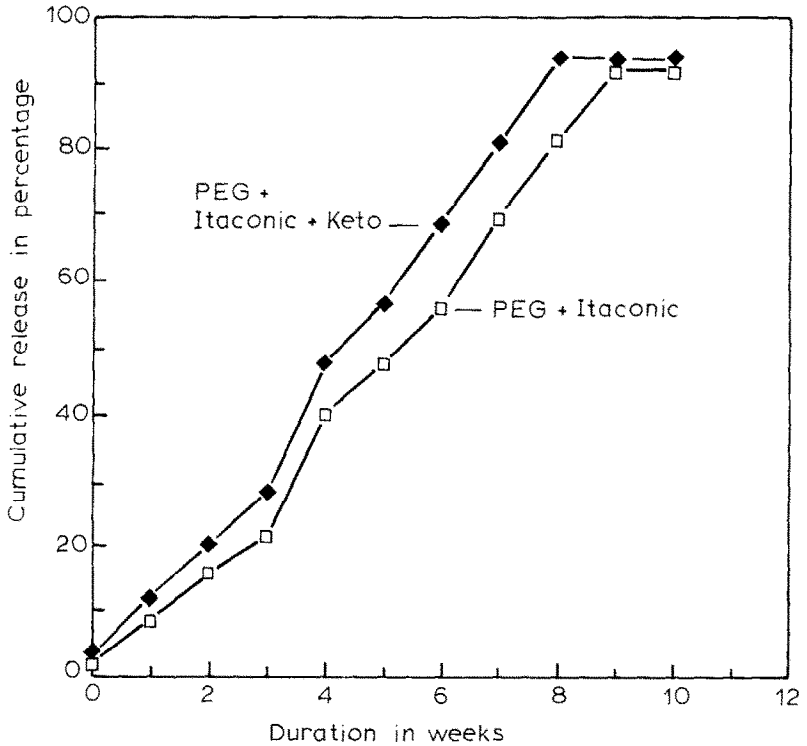

Fig. 2. Percent cumulative vaccine release from bioerodible hydrogel microspheres prepared from two pendant unsaturated polymers.

more tightly entrap the macromolecule and retard the rate of release which occurs only through hydrolytic cleavage of the polymer and subsequent loosening of the crosslinked network. Therefore, by varying the degree of crosslinking, one could achieve a desired rate of release over a prolonged period of time.

The in vitro release of the vaccine from bioerodible hydrogel microspheres prepared from two pendant unsaturated polymers was determined (Fig. 2). Total cumulative release occurs in 8 weeks from the polyester with ketoglutaric acid and itaconic acid, whereas it has a duration of 10 weeks from the polyester without ketoglutaric acid. Therefore, the addition of a dicarboxylic acid nucleus having an electron-withdrawing group placed vicinally to a carbonyl group of an acid, aids in the hydrolysis of the polymer, thereby giving rise a rapid release of the vaccine in our case. An increase in the ratio of ketoglutaric acid to itaconic acid in the polymer would show a more rapid release of the vaccine.

In both cases, the vaccine is incorporated into the polymer to form bioerodible masses in which the vaccine is entrapped within the hydrogel network without any chemical bonding to the network structure. The release of the vaccine from the microspheres occurs only when the polymer chain is cleaved and the network gradually swells. Further studies on the immunogenicity, biocompatibility and toxicity of the hydrogel microspheres need to be undertaken.

Acknowledgement

The work was carried out at the Center for Controlled Chemical Delivery, Salt Lake City, U.S.A., as part of CONRAD fellowship (CF-91-003) awarded to M.S.

References

Eldridge, H.J., Hammad, J.C., Meulbroek, J.A., Stass, J.K., Gilley, R.M. and Tice, T.R., Controlled vaccine release in the gut associated lymphokine tissue – I Orally administered biodegradable microspheres target the peyer's patches. *J. Control. Rel.*, 11 (1990) 205–214.

Gilley, R.M., Developments of secretory and systemic immunity following oral administration of microencapsulated antigens. *Proc. Int. Symp. Rel. Bioact. Mater.*, 15 (1988) 123–124.

Heller, J., Use of polymers in controlled drug release. In Szychei, M. (ed.), *Biocompatible polymers, metals and composites*, Technomic, 1983, 551–584.

Heller, J., Water soluble polyesters, *U.S. Patent No.* 4,502,976 (1985).

Heller, J., Heiwing, R.F., Baker, R.W. and Tuttle, M.E., Controlled release of water-soluble macromolecules from bioerodible hydrogels. *Biomaterials*, 4 (1983) 262–266.

Hora, M.S., Controlled release of interleukin-2 from biodegradable microspheres. *Biotechnology*, 8 (1990) 755–758.

Langer, R., Polymers for the sustained release of macromolecules: Their use in a single step method of immunization. *Methods Enzymol*, 73 (1981) 56–75.

Singh, M., Singh, A. and Talwar, G.P., Controlled delivery of diphtheria toxoid using biodegradable Poly(D,L-lactide) microcapsules. *Pharm. Res.*, 8 (1991) in press.

Wise, D.L., Opportunities and challenges in the design of implantable biodegradable polymeric systems for the delivery of anti-microbial agents and vaccines. *Adv. Drug. Del. Rev.*, 2 (1987) 19–39.