International Journal of Pharmaceutics, 93 (1993) R1–R5

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IJP 10062

Rapid Communication

Biodegradable delivery system for single step immunization with tetanus toxoid

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(Received 19 March 1993)
(Accepted 1 April 1993)

Key words: Biodegradable polymer; Poly(DL-lactide-co-glycolide); Tetanus toxoid; Vaccine; Controlled delivery; Neonatal tetanus

Summary

Tetanus toxoid (TT) loaded biodegradable microspheres were prepared from poly(DL-lactide-co-glycolide) (monomer ratio 65:35) of Mol. Wt 75000. The method of preparation was emulsification-solvent evaporation. In vitro vaccine release data are discussed. An in vivo immune response study was performed on rats. Results of immunization with the conventional schedule of TT and encapsulated TT until 5 months after immunization are presented, which were found to be comparable.

With the success of biotechnology and recombinant technology, protein/peptides are being looked upon as future therapeutic agents. New vaccines consisting of only a few amino acids, in place of the complete protein chain, are also being examined for immunization with positive results. The short biological half-life and oral instability are an inherent problem with most of the biologically active proteins and hence they offer a greater challenge in their development as a controlled delivery system.

Most of the vaccines have a multi-injection schedule, comprising two or more injections. The oral instability, short biological half-life and repeated injection schedule make these vaccines an ideal candidate for controlled delivery research. Although there are a few reports of oral delivery of vaccines (Gilligan et al., 1991; O'Hagan et al., 1992; Santiago et al., 1992), little success has been attained in this field. However, controlled parenteral delivery of vaccines using biodegradable microspheres has shown promising results. Reports of encapsulating antigens like BSA (Heller et al., 1983), OVA (O'Hagan et al., 1991a,b), TT (Alonso et al., 1992; Hazrati et al., 1992), staphylococcal enterotoxin B toxoid (Eldridge et al., 1991), hCG (Stevens et al., 1992), influenza virus protein M1 (Santiago et al., 1992), malarial antigen (Bathrust et al., 1992), etc., have recently been published, showing the increased interest in this area. Our group has also recently reported the controlled delivery of diptheria toxoid and LHRH-DT conjugate (Singh et al., 1991a,b, 1992) using these polymers.

The present work has been undertaken to develop a single injection formulation of tetanus toxoid (TT) which can replace the conventional

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multidose (usually two) schedule. Pregnant women are immunized with two injections of 5 lf each, adsorbed on alum, at an interval of 30 days for prevention of newborn from neonatal tetanus (NT), and protection of the mother. NT is a major cause of infant mortality in underdeveloped and developing countries, where limited physician and clinical services are available and a large number of deliveries take place at home or in remote rural areas under unhygienic conditions. Such a formulation will hold great utility for public health and mass immunization programmes.

For the above purpose biodegradable microspheres were prepared from poly(DL-lactide-co-glycolide) (65:35) (mol. Wt 75000). It was obtained from Birmingham Polymers Inc., U.S.A., and was characterized in the laboratory using gel permeation chromatography (Waters 745 B system with refractive index detector and Ultrastyragel columns), intrinsic viscosity measurements and DSC (Du Pont, DSC, 10 TA System). The vaccine TT, free as well as alum adsorbed, was obtained from the Serum Institute of India, Pune, India. The strength of the vaccine was 4000 lf/ml and the protein concentration of 15 μg/ml. Other chemicals such as polyvinyl alcohol (PVA) gelatin and dichloromethane (DCM) were obtained from Sigma Chemical Co., U.S.A., and were used as received.

The method of preparation was a slight modification of that reported earlier (Singh et al., 1991b). Briefly 10 ml of the 10% (w/w) polymer in DCM was emulsified with 1 ml of aqueous phase containing 0.1% gelatin as stabilizer and the required amount of vaccine in PBS (pH 7.4, 50 mM) to give a w/o primary emulsion (PE). This PE was then added, in the form of fine droplets, to 100 ml of 1% PVA solution in DW using a no. 23 gauge needle. The fine droplets of PE thus formed were kept suspended in 1% PVA solution by continuous stirring with a magnetic stirrer for 8 h under mild vacuum. After the evaporation of DCM during this period, vaccine loaded microspheres were filtered, washed twice with distilled water and dried under vacuum dessication to yield free flowing microspheres.

Scanning electron microscopy of the microspheres demonstrated them to be spherical with smooth topography. The size of the microspheres ranged from 5 to 70 μm with maximum concentration ranging from 20 to 40 μm.

The microspheres were checked for actual loading of vaccine by dissolving a known weight in DCM and extracting the vaccine in PBS-Tween (0.1%). The aqueous phase was then assayed by Lowry's method for total protein.

Vaccine release from the bulk eroding polymer like polyesters is a complex phenomenon which is a combined contribution of two different factors, diffusion and erosion (Shah et al., 1992). Diffusional release mainly contributes to the initial release which involves the surface protein that has been partially or incompletely entrapped. They are released rapidly giving rise to initial burst release. The subsequent release is controlled mainly by the erosion of the polymer. The erosion is due to the hydrolytic cleavage of the ester bond in the polymer chain backbone. It is polymer molecular weight and monomer ratio dependent (Wang et al., 1990). The degradation of polymer occurs in three stages. The initial stage of degradation involves a random chain scisson process. Here, the molecular weight of the polymer decreases significantly but there is no appreciable weight loss and no soluble monomeric products are formed. In the middle phase, a decrease in molecular weight is accompanied by rapid loss of mass and soluble oligomeric and monomeric products are formed. In the final stage, soluble monomeric products are formed from the soluble oligomeric fragments. The final stage is that of complete polymer solubilization.

Encapsulated drug release is mainly polymer degradation dependent. Thus, in the initial phase, it is very slow due only to chain scission but not solubilization. Then slightly faster release occurs due to small soluble oligomeric fragments being formed. Finally, when the polymer is in the third phase of complete solubilization, the maximum release of drug occurs.

For in vitro studies, a known weight of microspheres was taken in 2 ml vials along with 1 ml of PBS-Tween (0.1%). Vials were kept in a shaking water bath maintained at 37°C. Three vials were

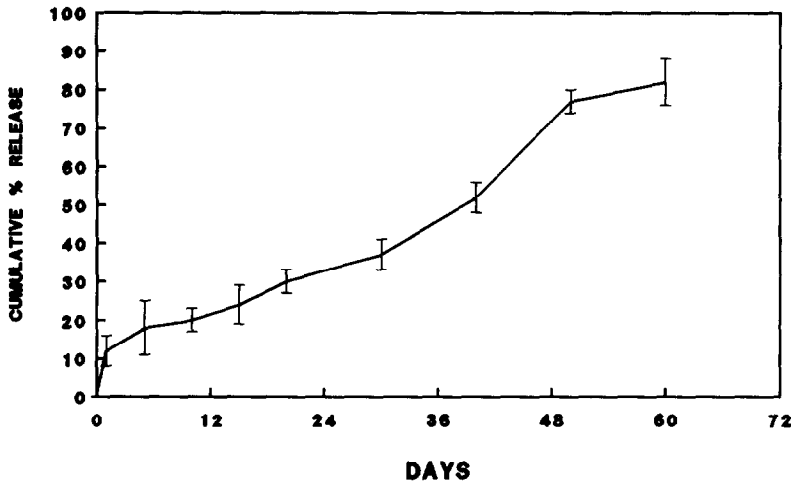

Fig. 1. Percent cumulative vaccine release from TT loaded biodegradable PDLLGA (65 : 35) microspheres.

removed at each specified time point and the dissolution fluid analysed for the presence of TT, using a sensitive enzyme-linked immunosorbent assay (ELISA) developed in the laboratory. The in vitro release profile of the microspheres as shown in Fig. 1 represents a phenomenon similar to polymer degradation discussed above. The initial release of 12% of the loaded vaccine within the first 2 days is due to the burst release. The subsequent period of slow vaccine release up to day 30, releasing only a further 25% of the loaded vaccine, is a period of chain scisson. Only small pores are formed in the microsphere matrix which gives rise to the slow release. The period of rapid release after day 30, releasing the remaining 60% vaccine from day 30 to 60, is the time when

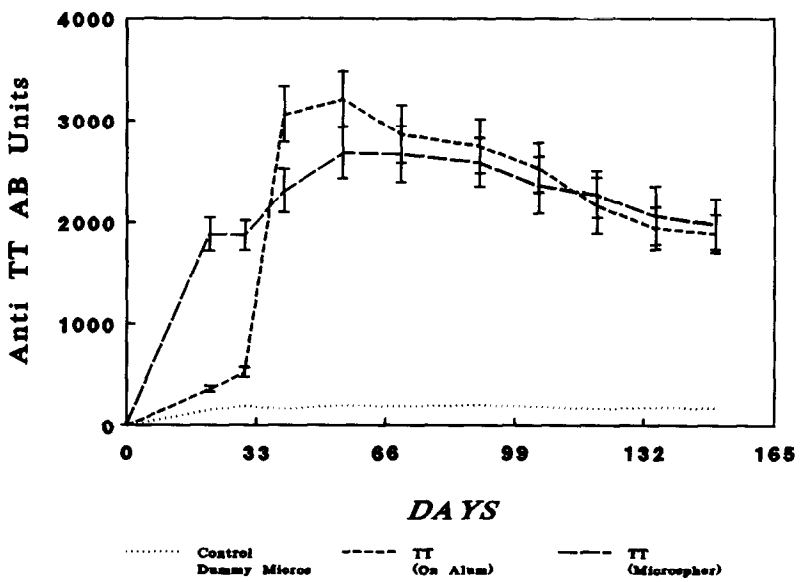

Fig. 2. Comparative anti-TT antibody titres from single injection of TT loaded biodegradable PDLLGA (65 : 35) microspheres and two injections of alum adsorbed TT.

polymer solubilization starts to release the entrapped vaccine.

The unit dose of alum adsorbed TT in conventional immunization is 5 lf. The immunization schedule for prevention of NT involves two injections of 5 lf each given at an interval of 30 days. For in vivo studies, three groups of outbred Wistar rats (6–8 weeks old), each containing six rats were immunized as follows: group A, dummy microspheres; group B, vaccine loaded microspheres; and group C, alum adsorbed TT from market. Group B was given one injection of microspheres containing 10 lf of TT. Group C was given two injections of 5 lf TT each at an interval of 30 days.

Rats were bled through the retro orbital plexus at specific time intervals and anti-TT-antibody titres estimated using a sensitive ELISA developed in our laboratory. The results of in vivo experiments until day 150 are shown in Fig. 2. The control group (group A) shows no antibody (Ab) formation in response to dummy microsphere injection, showing non-immunogenicity of the polymer. The titres in group C show a typical booster response after day 30 but the encapsulated TT (group B) displays a gradual build up of pl68 in Ab titres from day 0 onwards. Group C shows a low Ab titre (around 300 units) until day 30. In contrast, group B exhibits a titre of roughly 1800 units on day 30. This is because the sustained release of TT from the microspheres provides a small amount of TT continuously to be presented to the immune system for better stimulation. As a result, the immune cells show continuous proliferation producing the circulating Ab from the very beginning. Whilst in the case of the conventional schedule the injection of 5 lf TT causes initial proliferation of the immune cells, as the source of antigen (Ag) is limited it is depleted rapidly. After their depletion, most of the proliferating immune cells, in response to TT challenge, wither off leaving only few with strong Ag receptor affinity. In the absence of Ag, proliferation stops, resulting in the absence of Ab production. On giving the second injection, rapid proliferation coupled with fast Ab titre build up is seen, as depicted in Fig. 2.

The study shows that slow release of TT for extended periods does not generate tolerance in the animal model and both the systems, conventional as well as encapsulated, show comparable Ab titres. Work is continuing in order to determine the time when titres fall back to their initial level. It will be interesting to note whether the anti-TT titres in the microsphere immunized group are longer lasting than the group receiving two injections of TT on alum. Efforts are also being directed to determine whether a combination of more than two types of polymer microspheres give a better immune profile.

Acknowledgements

This work was funded by the Department of Biotechnology, Government of India. The authors are grateful to Mr Upinder Kak and Mr Amit Misra for their help in in vivo experiments.

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