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Rapid Communication

Immunogenicity studies on diphtheria toxoid loaded biodegradable microspheres

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Summary

Diphtheria toxoid loaded polylactide microspheres were prepared using the solvent evaporation method. The vaccine loaded microspheres were subjected to in vitro antigen release studies and in vivo immune response tests. The immunogenicity of the vaccine after microencapsulation was compared to that against diphtheria toxoid given in the conventional three injection schedule on alum as an adjuvant. The antibody response seen in the two cases is compared. The duration of the immune response from the two schedules is exhibited.

The controlled delivery of macromolecules with complex structures, short half-life and stability is generating a great deal of interest among researchers working in the area of drug delivery (Langer, 1981; Heller et al., 1983; Wise, 1987; Hora et al., 1990). Among the classes of macromolecules most urgently requiring appropriate delivery systems is that of vaccines. This is mainly because most of the vaccines have a multiple injection schedule requiring frequent immunisations. Ideally, it would require the development of a suitable delivery system to deliver three or more doses of the vaccines in a preprogrammed manner, over an extended period of time to generate long-lasting antibody titers. The development of such a single contact point immunisation system would have a great impact on the expanded programme of immunisations of the developing countries, where the population is large and health facilities scarce.

Although this area has generated tremendous interest, progress towards such a development is still in its infancy. Several workers have recently reported the adjuvant effect of biodegradable microparticles for delivering antigens (Gilley, 1988; Eldridge et al., 1990; O'Hogan et al., 1991a). Antibody response generated by microparticles phagocytosed by macrophages has been shown to be better than that obtained with Freund's adjuvant, a proven immune response potentiator in animals (O'Hogan et al., 1991b). Our group has also recently reported (Singh et al., 1991a) the release of diphtheria toxoid (DT) from polylactide microspheres without loss in antigen characteristics. We have additionally reported (Singh et al., 1991b) that a suitable vaccine like LHRH-DT,

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can also be entrapped in a hydrogel matrix wherein the release of the vaccine is governed by the degree of crosslinking of the hydrogel matrix. By varying the crosslinking density, it is possible to control the release rates of the macromolecule.

The present study demonstrates the immunogenicity profile of the microencapsulated vaccine as seen in comparision to the response generated from the conventional three injection schedule of DT on alum.

Polylactide (PLA) of molecular weight 49 000 was procured from Birmingham Polymers Inc., AL, U.S.A. and characterised in the laboratory using gel permeation chromatography (Waters 745B system with refractive index detector and Ultrastyragel columns), intrinsic viscosity measurements and differential scanning calorimetry (Du Pont, DSC 10 TA System). The vaccine, DT was procured from the Serum Institute of India, Pune, India containing 3500 Lf units per ml and a protein concentration of 15 mg per ml. The other chemicals were obtained from Sigma Chemical Co., St. Louis, U.S.A. and were used as received.

The vaccine loaded microspheres were prepared according to our solvent evaporation method as previously reported (Singh et al., 1991a). Briefly, the vaccine was dissolved in 1 ml of phosphate buffer (50 mM, pH 7.4) containing 0.05% gelatin. This aqueous solution was emulsified with 10 ml of 10% solution of PLA in methylene chloride. This w/o emulsion was then added dropwise to 500 ml of 1% polyvinylpyrrolidone solution in distilled water. The w/o/w emulsion was stirred under vacuum for 12 h at room temperature and then filtered, to recover free flowing vaccine loaded biodegradable microspheres. The resulting microspheres were checked for actual loading of vaccine by dissolving the microspheres in methylene chloride and extracting the vaccine in distilled water. The aqueous phase was then estimated for total protein content by following the Lowry procedure for protein assay. The vaccine loaded microsphere batches were subjected to scanning electron microscopic studies on size, surface and batch uniformity. The size of the microspheres ranged from 5 to 85 with maximum particle size in the 40–50 μm range.

The release of the vaccine from such a homogeneously eroding matrix system is a complex phenomenon. Mainly two parameters – diffusion and erosion – contribute to the end release profiles. The diffusional release of large macromolecules occurs initially, as partially surface entrapped protein is released rapidly. This phase is often referred to as the burst phase. The more prominent erosion based release occurs gradually as the polymer matrix erodes homogeneously depending on its molecular weight and monomer ratios. As the degradation of the polymer occurs only by the hydrolytic cleavage of the ester group in the polymer backbone, it was important to see the degradation profile of the polymer. For this the gel permeation chromatographic (GPC) profile of the microspheres suspended in physiological buffer at 37° C for days 0, 10 and 30 was studied. Fig. 1 exhibits the three gel permeation chromatograms clearly indicating a homopolymer (Mol. Wt 49 000) peak at day 0, a loss in molecular weight without mass loss (Mol. Wt 31 000) at day 10 and finally a molecular weight and mass

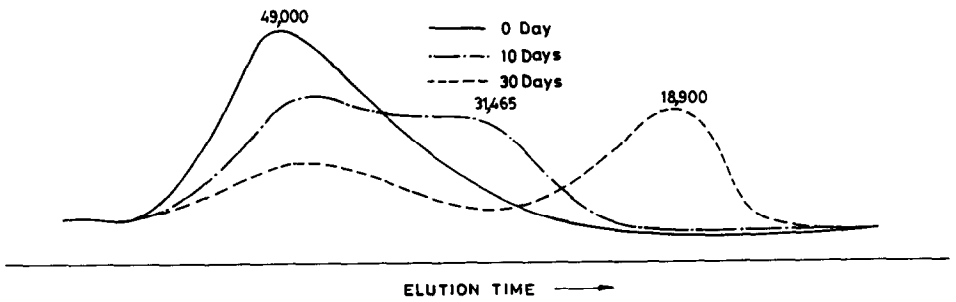

Fig. 1. Degradation profile of polylactic acid microspheres in physiological buffer at day 0, 10 and 30 exhibiting the erosion pattern of the polymer.

loss to Mol. Wt 18900 at day 30. These profiles serve as controlling parameters to select an appropriate polymer to programme accurate vaccine release profiles for optimum immune response in animal models.

The dose of DT being 25 Lf units in humans, microspheres corresponding to three doses (75 Lf Units) were weighed for immunisation in mice. Three groups, each of 10 inbred Balb/C mice, were selected for the immune response studies. The first group was immunised with a single injection of bidegradable microspheres containing 75 Lf units of DT suspended in a vehicle (normal saline with 0.2% methyl cellulose and 0.1% Tween 20). The second group was immunised at day 0, 30 and 60 with individual injections of 25 Lf units of DT adsorbed on alum. The third group was immunised with one injection of dummy microspheres prepared from PLA without any vaccine. All the three groups were bled through the retro orbital plexusus every 15 days until day 75 and every 25 days until 8 months. The serum was separated from each bleed and checked for anti-DT antibodies using enzyme-linked immunosorbent assay (ELISA) developed in the laboratory with standard reagents as in our earlier report (Singh et al., 1991a).

Fig. 2 shows the antibody profiles of the three groups until 8 months in Balb/C mice. The dotted line, indicating the conventional schedule, shows a typical booster response observed after the second and third injections (day 30 and 60) and a gradual fall in anti-DT titers to less than 500 antibody units at the end of the study. On the other hand, the immune response generated by the single injection of biodegradable microspheres of DT, indicated by the continuous line, exhibits a gradual build up of the antibody titers with a boost in response observed at day 45. This boost in the response at day 45 is due to a sudden increase in vaccine release occurring on polymer erosion around day 30–40. The antibody response from the single microsphere injection was stronger than that of the conventional schedule, until the end of the study. This indicates, firstly, that a slow release of DT over an extended period of time does not generate tolerance in the animal model and, secondly, that the erosion pattern of the biodegradable polymer is such that rapid erosion with mass loss occurs around day 30–45.

Further studies are currently underways to show whether a pulsatile release of the vaccine would generate a stronger immune response than

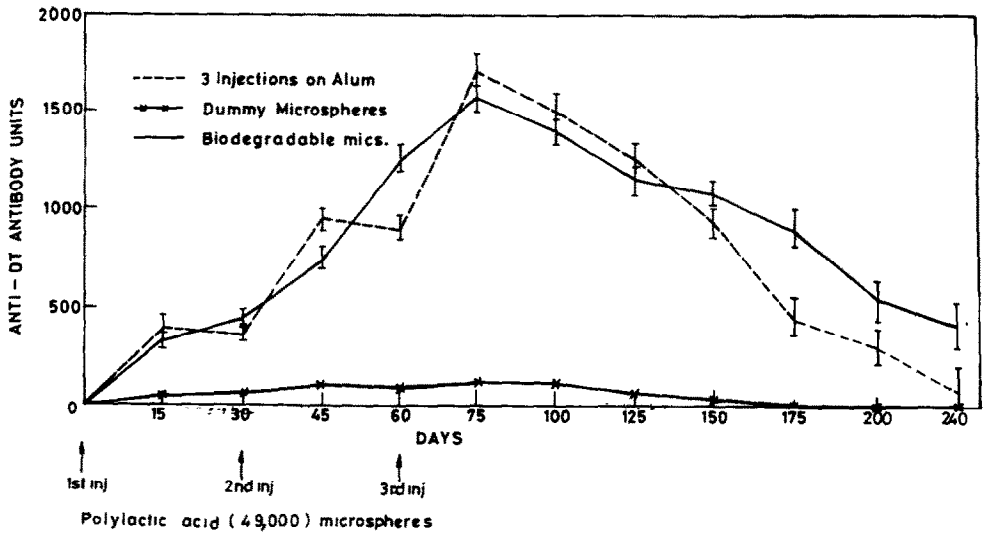

Fig. 2. Immune response profile after immunisation obtained from the three groups of Balb/C mice until 8 months.

that observed here and whether reduction in the size of the microspheres for optimum phagocytic uptake by macrophages would exhibit a stronger and long lasting immune response.

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