Microsphere technology as a carrier for delivering macromolecular drugs

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ABSTRACT

Smooth, highly spherical, crosslinked chitosan microspheres in the size range of 100-300µm were prepared by emulsion cross linking procedure. A simple pre formulation study was carried out to get discrete microspheres without aggregation by optimizing the concentration and volume of cross linking agent, stirrer speed, volume of emulsifying agent and cross-linking time. The tetanus toxoid (TT) was incorporated on chitosan microspheres by adsorption method. The microspheres showed good adsorption of TT. The extent of TT loading and release from microspheres had a remarkable dependence on the viscosity grade and concentration of polymer used for preparing the microspheres. The microspheres prepared with lower viscosity grade the release of protein antigen was onset and could release 47% of antigenic TT in 100 days. The microspheres prepared with higher viscosity grade the release was slow and could release 49% of TT in 100 days and could prove to be an alternative drug delivery technology in controlling the protein antigen to avoid the subsequent booster dose.

Key words: Immunization, Biodegradable, lysozyme, Chitosan, TT, Limes Flocculation (Lf)

INTRODUCTION

In controlled release technology, biodegradable polymeric carriers offer potential advantages for prolonged release of macromolecular drugs. [1-3] Drugs in this class include polypeptides, hormones, polysaccharides, antigens, antibodies and other biologically active agents. Prolong release technology offers reduction in number of doses required for complete immunization. The number of doses required for a vaccine to be effective against an infectious disease is a critical factor in achieving the appropriate level of immunity. The conversion of multiple dose vaccines to a single dose vaccine containing priming and successive booster doses will have a dramatic impact on the immune response. [4-6] In developing world, dropout rate from the subsequent doses of vaccines like the combined diphtheria - pertussis-tetanus (DPT) are high though still in its inception stages, sustained vaccine release is one area where there has been a considerable amount of research activity done, because of the need to immunize large populations particularly in the developing world.

Here we have tried with a natural and cheaper poly saccharide chitosan, which have many biomedical applications. [7-10] The susceptibility of chitosan to lysozyme makes it biodegradable in vivo. Chitosan is a deacetylate derivative of chitin, a biopolymer second in abundance to cellulose. Chitin is reported to degrade completely when implanted intramuscularly in a considerable amount of research activity done, because of the need to immunize large populations particularly in the developing world.

4) MATERIALS AND METHODS

4.1) Materials

Chitosan having a viscosity (77cps and 150 cps) was obtained from Central Institute of Fisheries and Technology, Cochin, India and used without further purification. Plain TT (800 Lf/ml), Tetanus Anti TT (20 Lf/ml) from Pasteur Institute of India, Coonoor, Nilgiri Dist. India. Glutaraldehyde (50% biological grade) from Sigma, Chemical Company, St Louis, USA. Sodium Chloride, Span 80 and Tween 80 from Himedia Lab Ltd., Mumbai, India. Acetic Acid from Ranbaxy Lab Ltd., SAS Nagar, India, Tolueene, Acetone and Hydrochloric Acid from Merck Ltd., Worli, Mumbai, India.

4.2) Methods

Chitosan microspheres crosslinked with Glutaraldehyde were prepared as water in oil (W/O) emulsion. A simple pre formulation studies were carried out by optimizing various parameters to formulate stable microsphere without aggregation. The preformulation was carried out by choosing the parameters like polymer concentration, volume of emulsifying agent, stirrer speed, concentration and volume of cross linking agent and cross linking time. The optimized procedure for preparing stable microsphere was formulated by taking 4% of 1% chitosan gel dissolved in 1ml of 0.01N HCL. This aqueous solution was added to 50ml of toluene containing 10% volume of span 80. The solution was stirred well at 2000 RPM for 1 hour to get a W/O type emulsion. To this emulsion 10ml of glutaraldehyde saturated toluene (equal proportion of 50% glutaraldehyde and toluene stirred and kept for stabilization overnight and upper layer is utilized) was added in drop wise and stirred for four hours. The product so obtained were centrifuged at 3000 RPM for 10 minutes and washed thrice with tolune and acetone respectively. The microspheres was then dried at room temperature and collected as dry free flowing powder stored in a desicator until use. Four different batches of microspheres were prepared by utilizing two different viscosity range and concentration of chitosan polymer (77 cps and 150 cps), (1% and 2%). A mixed batch was prepared by mixing the above four batches of microspheres were included in the study. The scanning electron microscopic (SEM) examination of microsphere was done by using a Hitachi (Model S-2400 Japan) instrument. The average diameter of chitosan microspheres were taken from self-scaled SEM photograph. The TT was incorporated by adsorption method by swelling the microspheres in sterile water for 24 hours and later separated by centrifugation and dried at 37°C. The diluted TT 10ml volume containing 105Lf/ml was added in a conical flask containing the swelled microspheres and kept in an incubator at 37°C for 24 hrs in a shaker for adsorption. The microspheres were later separated by centrifugation and the supernatant containing the vaccine protein was estimated by limes flocculation method. [14-16]

4.3) Limes Flocculation

When the concentration of toxoid or toxin is kept constant and the concentration of antigen is varied in the mixtures of constant volume, the mixture flocculating first is that which contains the most nearly equivalent quantities of toxoid and antigen. [7, 17] 2µl of the supernatant containing the vaccine protein were added to each 6 flocculation tube. To this, different concentration of antigen (20, 40, 60, 80 and 100µl) was added. If the mixtures do not flocculate, the procedure is repeated with lower/ higher concentration of antigen on depending on the test result. The final volume was made up to 1ml with normal saline. The tube which flocculates first was noted.

4.4) In vitro Release

In vitro release of TT from chitosan microspheres was examined in phosphate buffer (pH 7.4, 0.1M) at 37°C. 40mg of TT loaded microspheres was taken on a small nylon bag dipped in 100ml conical flask containing 50ml of phosphate buffer. The flask was stirred using a magnetic stirrer at 90-95 revolution per minutes in an incubator maintained at 37°C. 5ml of sample was withdrawn at various time intervals (5 days) and centrifuged; the supernatant containing the antigenic TT was analyzed by limes flocculation method. The invitro studies were repeated for other batches of microspheres in the same manner. [18]

5) RESULT AND DISCUSSION

The tetanus toxoid was chosen as the candidate antigen to develop a vaccine for single administration as part of the children vaccine initiation programme in 1990 by the WHO. [19, 20] Prolong release of macro molecular drugs from polymeric matrices has received increasing attention in recent years in view of the fact that many of the future drugs are going to be recombinant DNA origin and will be macromolecules of very high molecular weights. Although still in its inception stages, sustained vaccine release is one area where there has been an considerable amount of research activity done, because of the need to immunize large populations particularly in the developing world. [12-26]

The Chitosan microspheres were smooth and spherical without aggregation as seen in SEM (Fig 1). All the microspheres were below 300µm in diameter and the particle size distribution
The volume and viscosity of the polymer have shown to have a direct influence on the particle size of the chitosan microspheres. The study revealed that the crosslinking of chitosan microspheres was found to be prolonging with the mixed batch prepared by mixing the four batches with varying viscosity and volume showed 56.10% release in 100 days. The smaller size in the mixed batch could release TT immediately and the bigger particle showed a delay in release which is essential in prolonging the action of the antigenic TT.

In order to be effective most vaccines require two or three primary immunizations followed by booster doses every few months or years. Patient compliance for such multiple shot immunization therapy is rather poor in the developing countries. It was seen that the crosslinking with glutaraldehyde effectively controls the drug diffusion from the chitosan matrix and can prove to be a carrier for prolonging the drug release.

6) CONCLUSION

The aim of this study was to stabilize TT adsorbed chitosan microspheres for the release of the antigen for a prolonged period. The encapsulation of TT antigen could denature the antigenic property of Tetanus Toxoid vaccine due to the harsh environment created by the organic solvent used. Here we have tried with the incorporation of TT antigen by adsorption method by swelling the chitosan microspheres. The antigenic TT loading on chitosan microspheres was estimated by limes flocculation method. The TT adsorbed chitosan microspheres were subjected to In vitro release. The release characteristics of TT antigen In vitro from low viscosity grade polymer was onset due to the small size particle and with microspheres prepared from higher viscosity polymer showed a delay in the release. The release of TT from chitosan microspheres was found to be prolonging with the mixed batch prepared by mixing the different volume and viscosity of chitosan polymer. The study revealed that the crosslinking of chitosan microspheres by glutaraldehyde could effectively control the drug diffusion from the chitosan matrix and can prolong the action of the therapeutic agents.

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7) REFERENCE