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Physicochemical and Immunological Properties of RiVax Loaded in PLGA Nanoparticles

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Introduction

Ricin is a glycoprotein poison that consists of two subunits (RTA&RTB) which can be used as a bioterrorist weapon based on the CDC list. There are two RTA-based vaccine candidates, including RiVax and RVEc, against poisoning caused by this toxin. Although various studies have been conducted on the immunogenicity of RiVax alone and with the help of adjuvants, modern controlled release systems such as nanoparticles have not been used to improve the immunogenicity of this protein.

Material and Methods

The gene coding recombinant A chain (RiVax, containing 2-point mutation in A chain of ricin include ribosomal toxicity site and the motif responsible for causing vascular leak syndrome was inserted in pET21a (+). The RiVax recombinant protein was purified following transformation of recombinant plasmid to *E. coli* BL21 (DE3) and expression confirmation. For purification of Rivax, Ni-NTA affinity chromatography column was exploited. The collected fractions were analyzed on SDS-PAGE. The gels were stained by Coomassie Blue method. The collected fractions containing purified Rivax were pooled and concentrated to at least 1 mg/ml and stored at 4°C until use. The PLGA polymer (lactide: glycolide 75:25) was purchased from Sigma-Aldrich. Water-oil-water method (double emulsion with solvent evaporation) was used for NPs preparation. The size, scattering index and zeta potential of produced NPs were characterized by DLS instrument (Malvern, British). The measurements were performed at ambient. Scanning Electron

Microscopy was used for characterization of morphological properties of NPs. The Stability of recombinant RiVax during loading of NPs, evaluation of particle yield, loading efficiency and capacity, Stability of recombinant RiVax during loading on PLGA NPs, in vitro release of RiVax from PLGA NPs, and Immunization of mice were performed in this work.

Result and Discussion

The purpose of this study was the synthesis of PLGA nanoparticles (NPs) containing RiVax and evaluation of physicochemical parameters and immunization potential of this system as compared to naked RiVax. In this research, after purification of RiVax, Water-oil-water emulsion method (double emulsion with solvent evaporation) was used for loading of RiVax in PLGA NPs. water soluble peptides and proteins in an organic solution (Dichloromethane) of PLGA dispersed in this method. The hydrophobic specificity of the prepared NPs would be increased due to increase in surfactant concentration and therefor the cellular uptake of them would be reduced because the surface properties of NPs are effective in this process. Then this complex (water in oil) converted to a water/oil/water emulsion by adding polyvinyl alcohol as a surfactant. 2.5 % polyvinyl alcohol was used in order to either NPs preparation with smaller size or increase in hydrophilic of NPs. The average size of free and loaded NPs was 238 and 190 nm, respectively. Also, the Zeta potential for the PLGA NPs containing RiVax and free one was -21.6 and -14.7, respectively. In order to confirm the correct protein structure, prepared PLGA NPs containing RiVax were disturbed by complete release solution and the absorption rate of NPs was directly measured and recognized that the amount of entrapped protein was 64 % of total protein. The results indicated that the protein was stable during all stage of entrapment. The ratio of lactic acid to glycolic acid in PLGA composition used in this investigation was 75:25 with the slow release rate, due to the high amount of lactic acid. The NPs containing RiVax and naked protein was administrated into different groups of mice. 8 weeks after last immunization, the mice were analyzed. Results showed that the size of NPs was 190nm and the release pattern of protein from nanoparticle is slow (12 % of protein during 40 days). According to the results for the rate of release of RiVax from PLGA NPs and the immune responses, it is suggested to use a PLGA with more ratio of glycolic acid to increase the release rate as a function of time to acquire a considerable immunity in the animal model in the future works.

Conclusions

The purpose of this study is to make PLGA nanoparticles containing RiVax with a slow-release rate and to evaluate the physicochemical parameters and immunogenic potential of this system compared to RiVax alone. According to the above results, it was found that the nature of the release of PLGA nanoparticles depends on the ratio of lactic acid to glycolic acid and this factor has a significant effect on the amount of antigen release or on the immune response.

Keywords: PLGA nanoparticles (NPs), RiVax, Ricin, Immunization.

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Declaration of conflict of interest

The student and other researchers have no conflict of interest. The supervisors are members of the academic faculty of the university and the consultant professor is also a respected member of the academic faculty of the university.

Statement on ethics

This experimental study was carried out after the approval of the Ethics Committee of Imam Hossein University with code IR.IHU.REC.1396.1805.