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Immunoevaluation of hydrophobic modified polyethyleneimine as cationic polymer adjuvant

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Abstract. Polyethyleneimine (PEI) is a cationic polymer that has excellent gene transfection effects as a gene transfection reagent. Its excellent proton sponge effect has attracted much attention in the field of vaccine adjuvants. However, its high positive charge density leads to high toxicity, which limits its application. In this paper, polyethyleneimine modified by stearyl chloride was used to reduce toxicity to obtain SA-PEI. The partical size of composite particles formed by SA-PEI adjuvant and ovalbumin (OVA) were smaller than 200 nm. In vivo experiments of Balb/c mice experiments showed that, compared with unmodified PEI, SA-PEI could significantly enhance the specific immune response to OVA and may promote the endocytosis of macrophages. This study clearly provides a promising cationic polymer adjuvant for the vaccine field.

Keywords: Polyethyleneimine, Adjuvant, Nanoparticle, Immunoevaluation.

1. Introduction

Vaccine is a biological product used to prevent disease and are an important part of medical science. Vaccination is one of the most effective methods for controlling viruses[1]. Vaccination enhances the body's immune response process, including guiding the host immune system to recognize and eliminate antigens in a more effective manner. Compared with the whole pathogen vaccine method, subunit vaccine has higher safety and precise targeting. However, these subunit vaccines are weakly immunogenic and require adjuvants to enhance the body's specific immune response to antigens[2, 3].

Commonly used adjuvants include aluminum salt adjuvant[4], oil emulsion adjuvant[5], liposome adjuvant[6], cytokine adjuvant [7] and polymer adjuvant[8, 9]. Among them, polymer adjuvant is a new type of adjuvant, and polyacrylates, polylactic acid and polypolysaccharides are often used as its polymer matrix.

Polyethyleneimine (PEI) is a kind of polymer, it is because of its unique structure, so that it has a high degree of cationic characteristics, but the amino density in the molecular chain is higher, and the cation forms a huge cationic cluster on the cell membrane, thereby inducing cell necrosis[10]. The primary amine and secondary amine in the PEI chain segment have good reactivity, so that we can better develop and customize its function in the application, therefore, polyethylene imide has been widely used in petroleum, water treatment, medicine, materials science and biomedicine and other fields [11, 12]. Among them, PEI is the most widely used nucleic acid transfection reagent. In recent years, more and more studies have shown that PEI has strong adjuvant activity and can enhance the anti-infection and anti-tumor effects of traditional vaccines[13].

Herein, we designed polyethylenimine modified with stearyl chloride (SA-PEI) as a cationic polymer adjuvant. SA-PEI can be combined with ovalbumin (OVA) modeled antigen to form composite particles. The long-chain alkyl hydrophobic segment in stearyl chloride can increase the lipid

solubility of the molecule and enhance the endocytosis of cells[14]. Therefore, using stearyl chloride to react the primary or secondary amine groups in the molecular structure of PEI can not only reduce cytotoxicity, but also enhance the targeting and endocytosis of macrophages. We found that SA-PEI adjuvant not only has small particle size, but also can effectively promote endocytosis.

2. Experiment

2.1 Materials

Polyethyleneimine (PEI, M_w=25 kDa) was obtained from BASF Co., Ltd. Stearyl chloride and triethylamine were obtained from Shanghai Aladdin Biochemical Technology Co., Ltd. Ovalbumin (OVA, chicken egg white) was purchased from Sigma-Aldrich Co., Ltd. Balb/c female mice was obtained from Hebei Medical University.

2.2 Synthesis of SA-PEI

During the reaction, the molar ratio of stearyl chloride: PEI primary amine group is 5:100. Take a certain amount of PEI according to the molar ratio and dissolve it in a three-mouth flask equipped with a mechanical stirring and thermometer, add an appropriate amount of trichloromethane and a certain amount of acid binding agent triethylamine (the same molar amount as stearyl chloride), stir, and ice bath under nitrogen atmosphere to 5℃. The quantitative stearoyl chloride was added to 10 g trichloromethane, slowly added to the flask, the drip time was controlled at about 30 min, and the reaction was mechanically stirred at 5℃ for 20 h. After the reaction, the reaction solution was collected, washed with isopropyl ether precipitation three times, centrifuged for 10 min (rotation speed 4000 rpm), and the sediment was vacuum-dried for 12 h to obtain an opaque white viscous substance, which is stearoyl chloride modified polyethyleneimine (SA-PEI).

2.3 Preparation of SA-PEI/OVA composite particles

A certain of OVA was added into purified water to prepare 5 mg/mL of OVA solution, and a certain amount of SA-PEI was added into purified water to prepare 1 mg/mL of SA-PEI solution (pH adjusted to 7.4). The quantitative OVA solution is added to the beaker, then the same volume of SA-PEI solution slowly drops into it, stirring for 20 min, after the completion of mixing to get SA-PEI/OVA composite particles. The preparation of PEI/OVA composite particles is the same as the above operation.

2.4 Structure of SA-PEI

The structure of SA-PEI was studied by fourier transform infrared spectrometer (FT-IR, IRL280301, USA), the scanning wavenumber from 4000 to 650 cm^{-1}.

2.5 Determination of particle size

The particle sizes of PEI/OVA composite particles and SA-PEI/OVA composite particles was measured on a PSS Z3000 (Particle Sizing Systems LLC, USA).

The test conditions were as follows: detection temperature 23℃, laser wavelength 633 nm, detection angle 90°, temperature equilibrium time in detection cavity 30 s before detection, and each sample was detected 3 times.

2.6 Immunoevaluation

Balb/c mice (4 to 6 weeks, 16 to 18 g, ♀) were selected as the animal model to evaluate the adjuvant performance of SA-PEI to OVA. The vaccine formulation is shown in Table 1.

Table 1. Immunization regimen

Sample	Adjuvant	Antigen	Solution
Blank	—	—	PBS(1×)
OVA	—	20 μg OVA	PBS(1×)
PEI	0.8 μg PEI	20 μg OVA	PBS(1×)
SA-PEI	0.8 μg SA-PEI	20 μg OVA	PBS(1×)

The method of immunization was thigh intramuscular injection, which was immunized twice, once at 0 and 14 days, respectively. After 28 days of primary immunization, serum was collected from orbital epicanthal blood, mouse plasma was placed in a drying oven at 37℃ for 30 min, moved to a refrigerator at 4℃ for 1 h, centrifuged and collected serum, and refrigerated at -20℃ for future antigen level detection. Subsequently, the IgG antigen levels in serum of mice were detected by ELISA double-anti-sandwich method.

3. Results and discussions

3.1 Structure of SA-PEI

The molecular structure of SA-PEI was characterized by FT-IR, and the results were compared with the infrared spectra of PEI, as shown in Figure 1.

The characteristic absorption peaks of -NH_2 anti stretching vibration in PEI and SA-PEI are 3357 cm^{-1} and 3353 cm^{-1}, respectively. Their symmetric stretching characteristic absorption peaks are 3279 cm^{-1} and 3274 cm^{-1}, respectively. The characteristic absorption intensity of -NH_2 in SA-PEI has decreased, indicating that some primary amino groups are involved in the reaction. The out of plane bending vibration peaks of -NH- in polyethyleneimine and stearoyl chloride modified polyethylene imine were 762 cm^{-1} and 746 cm^{-1}, respectively. The peak shapes after the reaction became sharper, indicating that the primary amine group of PEI participated in the reaction and generated more secondary amine groups. What's more, a characteristic absorption peak of C=O appeared at 1647 cm^{-1}. Based on the above analysis, it can be concluded that the alkyl hydrophobic segment has been successfully modified onto the PEI molecular structure.

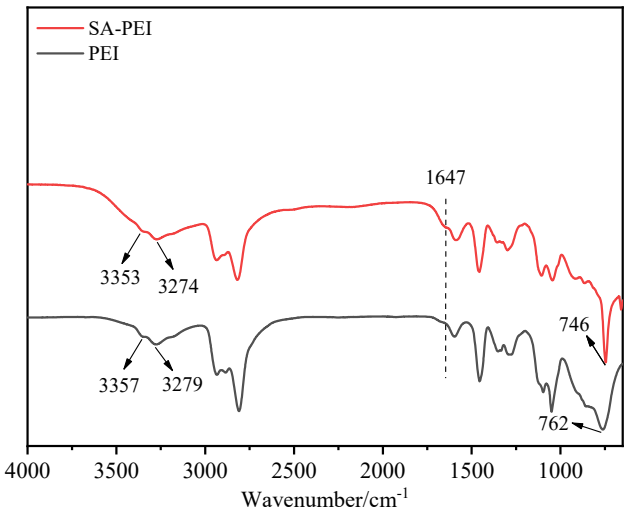

Figure 1. Infrared spectra of PEI and SA-PEI.

3.2 Analysis of particle size

The average particle size of SA-PEI was measured by dynamic light scattering particle size analyzer, and the results are shown in Figure 2.

As can be seen from Figure 2, the polymer/OVA mass ratio has shown a gradual increasing trend, in which SA-PEI/OVA composite particles have the smallest particle size when the mass ratio is 0.04, and the particle size does not exceed 200 nm, which is conducive to the function of antigen-presenting cells.

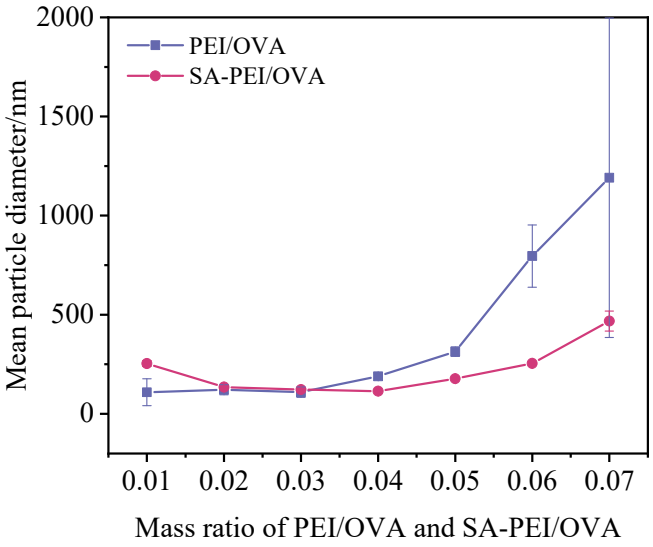

Figure 2. Particle size of PEI/OVA and SA-PEI/OVA composite particles.

3.3 Immunoevaluation

In order to evaluate the effect of SA-PEI on the specific immune response of model antigen OVA, the antigen specific IgG titer in serum of immunized mice was detected by Balb/c mice and compared with antigen control group and unmodified PEI.

As can be seen from Figure 3, the ability of SA-PEI immune group to induce OVA antigen specific IgG titer was significantly increased compared with PEI immune group. In addition, the antigen IgG titer of both groups was higher than that of pure antigen group, indicating that SA-PEI/OVA nanoparticles effectively enhanced antigen presentation. It may also be that the micro-hydrophobic zone effect of hydrophobic structure promotes endocytosis and deliver more antigens into cells. Hence, SA-PEI is expected to be used as a vaccine adjuvant.

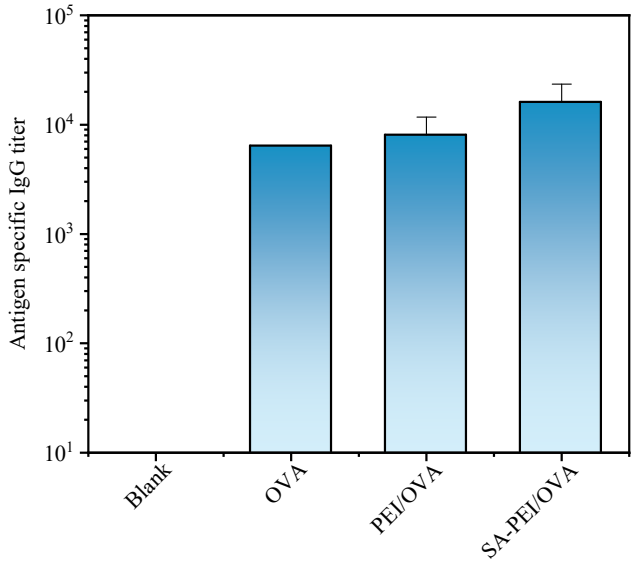

Figure 3. Antigen specific IgG titer of SA-PEI adjuvant to OVA.

4. Conclusion

In this paper, SA-PEI was prepared using PEI modified by stearyl chloride. The mass ratio of SA-PEI and model antigen OVA was 0.04 to form composite nanoparticles with average particle size less than 200 nm by electrostatic adsorption. According to in vivo immune experiment of Balb/c mice, SA-PEI significantly enhanced the antigen specific IgG titer response to OVA, which compared with PEI. Therefore, SA-PEI effectively promote the specific immune response of the body and provide an alternative vaccine adjuvant. Since then, our research group will still be committed to the research of this cationic polymer adjuvant.

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