Preparation of polyacrylic acid and its activation on macrophages

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Abstract. With the rapid and efficient development of animal husbandry industry, intensive and large-scale has become the main body, and the corresponding use of vaccines and vaccine adjuvants has gradually increased. Polyacrylic acid has been widely used in vaccine adjuvants because of its safety and low toxicity. In this paper, polyacrylic acid was been prepared by aqueous solution polymerization, taking redox system as catalyst. The molecular structure of polymer was characterized by FT-IR. Viscosity average molecular weight was tested on a ubbelohde viscometer. Moreover, activation of polyacrylic acid on macrophages was analyzed by cell culture method. Results showed that the vaccine prepared by polyacrylic acid as adjuvant and chicken ovalbumin could stimulate macrophages to produce higher IL-1β.

1. Introduction

Animal vaccines are biological preparations made by bacteria, viruses and so on, which can make the body produce specific immunity. With the decrease of immunogenicity, adjuvants are needed to enhance the immune response. Adjuvant is a kind of non-specific immune enhancer [1-2]. When injected into the body together with antigen, it can enhance the immune response of the body to the antigen and improve the immunogenicity of the antigen. With the continuous progress of drug research and development, many kinds of new adjuvants have emerged at home and abroad in recent years [3]. As the most classical adjuvant, aluminum salt adjuvant has been used in clinical practice for 80 years, which can effectively induce humoral immune response, but has no effect on cellular immunity. The emulsion adjuvants include oil/water and oil/water adjuvants, such as FIA, Montanide 65 adjuvant, etc. Most of the commonly vaccines are made up of such adjuvants and antigens. But this kind of emulsion contains mineral oil, which can't be metabolized and remain in the injection site to cause some side effects, which has certain toxicity. However, polyanionic polymers have better performance in vaccine adjuvants, which has been confirmed by most researchers [4]. In 1988, L. A. TH. Hilgers esterified polyacrylic acid with weight average molecular weight of 45000 with butanol or octanol to obtain polyacrylate. It was found that the alkyl polycrylate showed a good secondary immune response, and the study showed that the relationship between the immune performance and esterification rate of the polymer [5-6]. In this paper, polyacrylic acid polymer with small molecular weight was synthesized using redox catalyst. The
The adjuvant prepared from this kind of polymer has low viscosity, easy injection and good safety performance. The activation of macrophages was also studied in macrophage culture in mice.

2. Experimental

2.1. Materials
The raw materials are as follows: Acrylic acid and sodium bisulfite, Tianjin Damao Chemical Reagent Factory; Ammonium persulfate, Tianjin Yongda Chemical Reagent Co., Ltd; Sodium hydroxide, Tianjin Feng Chuan chemical reagent Technology Co., Ltd; PBS and OVA were supplied by Institution of biology, Hebei Academy of Sciences. The water was distilled.

2.2. Characterizations
The structure of polyacrylic acid was tested on Fourier Transform Infrared Spectrometer (FT-IR); Viscosity average molecular weight was measured by Ubbelohde viscometer with inner diameter of 0.5-0.6mm. The viscosity of the product was measured at 30 ± 0.05 °C with NaOH of 2mol / L as solvent; the relationship between intrinsic viscosity and viscosity average molecular weight of polymer can be expressed by mark houdwink equation:

\[ \eta = 3.38 \times 10^{-3} \times M^{0.43} \]  

(1)

Where \( \eta \) represents viscosity (Pa.s) and M represents viscosity average molecular weight.

The activation of polyacrylic on macrophages was tested by cell culture. The macrophages of 1.0×10^6 cells/mL in a 24-well plate were cultured for 24 h and for experiment.

2.3. Preparation of Polyacrylic acid
Half mass of acrylic acid with concentration of 40% and ammonium persulfate/ sodium bisulfite was added into a flask equipped with a stirrer, a thermometer and a dropping funnel. The system temperature rises to 40 °C, and the remaining monomers and initiators with a drop funnel was slowly add. Polyacrylic acid polymer was obtained after 3 hours of reaction. The solid polyacrylic acid can be obtained by vacuum drying and dehydration. The experimental process is as follows.

\[ \text{Figure 1. Synthesis route of polyacrylic acid} \]

2.4. Preparation of vaccine
Dilute polyacrylic acid with PBS to a concentration of 125ug / ml, and neutralize it with sodium hydroxide solution to a pH value of 7.4. The concentration of OVA was 5ug/ml. Vaccine was prepared in the ratio of 1:1 with above polyacrylic acid solution and OVA. This sample was used for the experiment of activation of macrophages.
3. Results

3.1. FT-IR analysis of molecular structure of polyacrylic acid

![Figure 2. FT-IR spectra of polyacrylic acid](image)

The molecular structure of polyacrylic acid is shown in Fig.1. It can be seen that absorption peak of methylene and methylene in the molecular structure of polyacrylic acid was 2976cm⁻¹. 1692cm⁻¹ is the characteristic absorption peak of C = O bond. While No Characteristic absorption peak was appeared near 1640cm⁻¹, which proves the successful preparation of polyacrylic acid polymer.

3.2. Effect of polymerization temperature on molecular weight of polyacrylic acid

The polymerization temperature in redox system has a great influence on the molecular weight of the polymer. Too high temperature will lead to violent reaction of the system, which will reduce the molecular weight. The initiator decomposes rapidly and generates more free radicals at high temperature, leading to the increasing of the rate of continuous initiation and chain termination and decreasing of molecular. When the reaction temperature is too low, the initiator's initiation temperature cannot be reached and the polymerization reaction is slow [7]. Therefore, the oxidation-reduction reaction temperature selected in the bulk system is 40 °C.

3.3. Effect of initiator content on viscosity average molecular weight of polyacrylic acid

![Figure 3. Effect of initiator content on viscosity average molecular weight of polyacrylic acid](image)
With the increase of initiator dosage, the molecular weight of polymer decreased, which was in accordance with the rule that the length of kinetic chain was inversely proportional to the square root of initiator concentration. The higher the concentration of initiator, the higher the concentration of free radicals. Therefore, the more active centers of polymerization can be formed, resulted more polymer chains. Then the chain length of each molecular chain is shortened, which leads to the decrease of molecular weight.

3.4. Activation of macrophages by polyacrylic acid

![Figure 4](image)

Figure 4. The activation effect of polyacrylic acid on macrophages

Proinflammatory cytokines can mediate the activity of antigen-presenting cells, and the level of proinflammatory cytokines is one of the markers of the activation of antigen-presenting cells. IL-1β is an important pro-inflammatory cytokine. Macrophages can secrete IL-1β. The IL-1β secretion of macrophages stimulated by adjuvants can reflect the activation level of macrophages [8]. We added OVA-PAA to macrophage culture medium and taking PBS/OVA as blank control group. The results showed that the concentration of IL-1 β in macrophage culture medium added ova / PAA was as high as 132pg / ml, while that in blank control group was 21pg / ml. It is concluded that PAA can induce macrophages to secrete IL-1β and has a good activation effect on macrophages.

4. Conclusion

In this paper, polyacrylic acid polymer was successfully prepared by redox catalysis system, and its structure was characterized. The effect of initiator amount on viscosity average molecular weight was studied. In this paper, OVA was selected as antigen and polyacrylic acid as adjuvant to prepare vaccine. PAA has excellent ability to activate macrophages through the culture of macrophages in vitro. On the basis of this work, polyacrylic acid will have a broader application prospect in cellular immunology. The application of polyacrylic acid in vaccine needs to be explored deeply in the future.

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