International Journal of Biological Macromolecules 82 (2016) 264–272

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

The adjuvanticity of ophiopogon polysaccharide liposome against an inactivated porcine parvovirus vaccine in mice

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ARTICLE INFO

Article history:
Received 19 June 2015
Received in revised form 22 October 2015
Accepted 27 October 2015
Available online 31 October 2015

Keywords:
Ophiopogon polysaccharide liposome
Porcine parvovirus
Adjuvant activity

ABSTRACT

In this study, the adjuvant activity of ophiopogon polysaccharide liposome (OPL) was investigated. The effects of OPL on the splenic lymphocyte proliferation of mice were measured in vitro. The results showed that OPL could significantly promote lymphocyte proliferation singly or synergistically with PHA and LPS and that the effect was better than ophiopogon polysaccharide (OP) at most of concentrations. The adjuvant activities of OPL, OP and mineral oil were compared in BALB/c mice inoculated with inactivated PPV in vivo. The results showed that OPL could significantly enhance lymphocyte proliferation, increase the proportion of $CD4^+$ and $CD8^+$ T cells, improve the HI antibody titre and specific IgG response, and promote the production of cytokines, and the efficacy of OPL was significantly better than that of OP. In addition, OPL significantly improved the cellular immune response compared with oil adjuvant. These results suggested that OPL possess superior adjuvanticity and that a medium dose had the best efficacy. Therefore, OPL can be used as an effective immune adjuvant for an inactivated PPV vaccine.

1. Introduction

In recent years, with the continuous development of animal husbandry in China, the number of breeds and overall quantity of livestock and poultry has greatly increased. However, animal epidemics still occur frequently, such as those of Newcastle disease, avian flu, porcine parvovirus and porcine reproductive and respiratory syndrome, and impose a serious cost on livestock breeding and cultivation [1]. It is estimated that the direct loss caused by the disease and death of animals is nearly 40 billion yuan every year in China, which is equivalent to approximately 60% of the total output value in animal husbandry. Therefore, it is crucial to prevent and treat animal epidemics.

Porcine parvovirus (PPV) is classified in the family Parvoviridae and genus Parvovirus and is one of the most important pathogens. PPV prevents reproduction in sows. It results in sterility, abortion, stillbirth, foetal mummy and foetal malformation [2]. In addition, PPV often combines with other pathogens to create a mixed infection, directly leading to sow breeding obstacles [3]. Since its discovery, PPV has spread all over the world. At present, infections caused by PPV are very serious in China, and the antibody positive rate of swine is over 90%. PPV is mainly spread in the digestive tract and respiratory tract. The disease caused by PPV leads to significant economic losses to the pig breeding industry because of its widespread and severe harm [4].

For an important sow reproductive disturbance disease, vaccination is the best way to prevent and control the spread of PPV. Because PPV has the potential to generate enormous damage in sows and breeder boars, vaccination is required to prevent its outbreak in domestic large-scale pig farms. As a result, a PPV vaccine is currently in great demand in the marketplace. There are several types of PPV vaccines, including an attenuated vaccine, inactivated vaccine, subunit vaccine and DNA vaccine [5]. Although the attenuated vaccine has been used for several years in some countries, inoculation with the attenuated vaccine poses potential security problems, such as spreading the virus and the virus reverting to a pathogenic strain [6]. In addition, the attenuated vaccine is

Abbreviations: PPV, porcine parvovirus; OPL, ophiopogon polysaccharide liposome; OP, ophiopogon polysaccharide; HI, haemagglutinin inhibition; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PBS, phosphate buffered saline; PHA, phytohaemagglutinin; LPS, lipopolysaccharide; DMSO, dimethyl sulfoxide; CC, cell control; OA, oil adjuvant; BC, blank control.

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http://dx.doi.org/10.1016/j.ijbiomac.2015.10.084
0141-8130/

expensive and can cause other side effects. It has been shown that a subunit or DNA vaccine only generates moderate levels of protection. Therefore, the inactivated vaccine is the most effective type of PPV vaccine used in animals to prevent PPV infection today [7,8]. The inactivated vaccine possesses some advantages, such as high security, a long-acting antibody level, easy preservation, and so on. Although the inactivated vaccine is commonly used to prevent PPV, it has some specific deficiencies, such as cellular immune efficacy and failure to improve the immunogenicity of the antigen. In addition, it cannot effectively prevent and control PPV by humoral immunity. Therefore, adopting various efforts to enhance cellular immune responses to the inactivated PPV vaccine is the main direction of current research [9,10].

As a natural product, polysaccharide from medicinal plants can not only activate the immune cells, enhance the antibody titre, and promote the secretion of cytokines but can also exert important roles in cancer therapy and virus prevention [11]. Of the various biological activities of polysaccharides, its immunomodulatory efficacy is the most significant [12]. In addition, polysaccharide does not generate serious side effects when it enters the body and modulates the immune function. Polysaccharide is the perfect immunopotentiator for clinical use.

Ophiopogon japonicus (*Radix Ophiopogonis Japonici*), as a traditional Chinese herbal medicine, is recorded in Shen Nong's Materia Medica, and it is clinical application has been more than 2000 years. As the main active ingredient of ophiopogon japonicus, ophiopogon polysaccharide (OP) has strong immune-enhancing activity, such as enhancing cellular and humoral immunity, improving the activity of natural killer cells, enhancing the pinocytic and phagocytic function of macrophages, and so on [13]. However, because of its approximately 2-nm particle size and high hydrophilicity, when entered the body, OP is quickly excreted, which leads to a low distribution in the target tissue and instability in the organism. All of the unsatisfactory pharmacokinetic peculiarities above seriously limit the use of OP [14].

To improve bioavailability and exert better pharmacological function, OP was made into OP liposome (OPL), and its immune effect was investigated in our previous work. The results showed that OPL could enhance the non-specific and specific immune response in chickens and that their efficacy was significantly better than the efficacy of OP [15]. Thus, in the present study, the adjuvant activity of OPL to improve the inactivated PPV vaccine was investigated by determining its effect on lymphocyte proliferation, the relative proportion of T lymphocyte subpopulations ($CD4^+$ and $CD8^+$), haemagglutinin inhibition (HI) antibody titre, specific IgG titre and secretion of cytokines in immunized mice. The purpose of this study was to investigate whether OPL could improve the adjuvant activity of OP and enhance humoral and cellular immunity against the PPV vaccine. This study will provide theoretical support for the further development of a new type of adjuvant for PPV vaccines.

2. Materials and methods

2.1. Reagents

RPMI-1640 (GIBCO) supplemented with 100 IU mL^{-1} streptomycin, 100 IU mL^{-1} benzylpenicillin and 10% foetal bovine serum was used to culture cells. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, American Co.) was dissolved into 5 mg mL^{-1} with calcium and magnesium-free phosphate-buffered saline (PBS, pH 7.2). Phytohaemagglutinin (PHA, Sigma) and lipopolysaccharide (LPS, Sigma) as T-cell and B-cell mitogens were dissolved into 0.1 and 0.05 mg mL^{-1} with RPMI-1640, respectively. These reagents were sterilized by filtering through a 0.22-μm Millipore membrane filter. Dimethyl sulfoxide (DMSO) was purchased from Shanghai Lingfeng Chemical Co. Ltd.

2.2. Preparation of OPL

Based on the results of our previous experiment, OPL were prepared using the reverse-phase evaporation method [16]. The entrapment rate of OPL was 64.95%, and the average particle size was 245.3 nm. For the in vitro tests, OPL were dissolved into five working concentrations (125–7.813 μg mL^{-1}) in twofold serial dilutions with RPMI-1640. In vivo, it was diluted into three doses (4.0, 2.0 and 1.0 mg mL^{-1}) with physiological saline. They were sterilized and stored at 4 °C for the experiment.

2.3. Vaccine and virus

Inactivated PPV (SD-1 strain) was donated by the China Institute of Veterinary Drug Control. The inactivated PPV was made into an OPL adjuvant vaccine by mixing with OPL at three doses, an OP adjuvant vaccine by mixing with OP at 4.0 mg mL^{-1}, and an oil adjuvant (OA) vaccine by emulsifying with mineral oil according to the literature [17]. All of the vaccines contained the same amount of antigen.

2.4. Splenic lymphocyte proliferation assay in vitro

The method for preparing splenic lymphocytes was performed according to a previous report [18]. Briefly, a spleen was collected, placed in Hank's solution, minced and passed through a fine steel mesh to obtain a cell suspension. After centrifugation, the cells were washed and resuspended in RPMI-1640. Then, the cells were adjusted to 5.0×10^6 mL^{-1}. Finally, 100 μL of the splenic lymphocytes was seeded into a 96-well plate, and PHA, LPS (20 and 10 μg·mL^{-1}), or medium was then added to a final volume of 200 μL. The plates were incubated in a humid atmosphere with 5% CO_2 at 37 °C for 44 h. All tests were carried out in quadruplicate. Then, 20 μL of MTT was added into each well and incubated for an additional 4 h. Then, 100 μL of DMSO was added. The absorbance of the cells in each well was evaluated using a microplate reader (Bio-Rad, USA) at 570 nm (A_{570} value) as the index of splenic lymphocytes proliferation.

2.5. In vivo experimental design

2.5.1. Animals

Five-week-old BALB/c mice weighing 20–22 g were purchased from the Laboratory Animal Center of Fourth Military Medical University (Shannxi, China). They were housed in an environmentally controlled animal facility maintained at a temperature of 24 ± 1 °C, humidity of 50 ± 10%, and a 12/12 h light/dark cycle. All of the procedures were in strict accordance with the PR China legislation on the use and care of laboratory animals and the guidelines established by the Institute for Experimental Animals of Northwest A&F University and were approved by the university committee for animal experiments. Prior to experiment, the PPV antibody titres of all of the mice were confirmed to be negative using a haemagglutination inhibition (HI) test.

2.5.2. Adjuvant activity

Three hundred BALB/c mice were randomly divided into six groups and housed separately. The mice in the OPL_H (4.0 mg mL^{-1}), OPL_M (2.0 mg mL^{-1}) and OPL_L (1.0 mg mL^{-1}) groups were subcutaneously injected with 0.3 mL of the corresponding OPL-PPV vaccines, in the OP and OA groups with 0.3 mL of OP- (4.0 mg mL^{-1}) and OA-PPV vaccine, and in blank control (BC) group with 0.3 mL of physiological saline, repeated at 14 days of age. On D_7, D_{14}, D_{21},