International Journal of Pharmaceutics 447 (2013) 22–30

Contents lists available at SciVerse ScienceDirect

International Journal of Pharmaceutics

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

Enhanced transcutaneous immunization via dissolving microneedle array loaded with liposome encapsulated antigen and adjuvant

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ARTICLE INFO

Article history:
Received 2 November 2012
Received in revised form 6 January 2013
Accepted 3 February 2013
Available online 11 February 2013

Keywords:
Dissolving polyvinylpyrrolidone microneedles
Liposome encapsulated antigen and adjuvant
Transcutaneous immunization

ABSTRACT

Transcutaneous immunization (TCI) with dissolving microneedle arrays (DMAs) is a promising vaccine administration method. In this work, we developed a TCI device consisting of dissolving polyvinylpyrrolidone (PVP) microneedles array, where in the tips are loaded with antigen and adjuvant encapsulated in liposomes. The microneedles could effectively be inserted into the skin and completely dissolve within 3 min. As a test-case, we selected ovalbumin (OVA) as a model antigen, CpG OND as adjuvant and cationic liposome (Lip) as a microparticulate vehicle for co-deliver antigens and adjuvant. Mice were immunized transcutaneously with DMAs containing OVA, OVA-CpG OND, OVA encapsulated in Lip, OVA-CpG OND encapsulated in Lip and conventional intramuscular injection (IM) with OVA solution, respectively. The results show that the anti-OVA IgG antibody level in the group immunized with the DMA containing OVA-CpG OND encapsulated in Lip was significantly higher than that of the other groups. Furthermore, it significantly increased the level of IgG2a ($P < 0.05$) and achieved the shift of immune type from predominate Th2 type to a balance Th1/Th2 type. In conclusion, the DMA TCI device can effectively deliver the Lip encapsulating CpG OND-OVA into skin, enhancing the immune response and change the immune type.

1. Introduction

Vaccination has become one of the most powerful tools for preventing pathogen infection. Most of the conventional vaccines are delivered by intramuscular injection (IM), but this method have several inherent limitations: the requirement for trained nurses, the risk of the needle-related disease induced by reusing needles and syringes, the fear and stress of the children and parents, storage and transportation in cold chain (Matsuo et al., 2012) These disadvantages are especially serious in developing countries. So development of an easy-to-use vaccination method is an urgent task.

As an attractive vaccination strategy, transcutaneous immunization (TCI) can avoid these disadvantages (Bal et al., 2010). In the skin, the stratum spinosum has a lot of antigen-presenting cells (APCs)-Langerhans cells (LCs), a kind of dendritic cells (DCs), which can capture and present antigens (Ag) to the lymph to enhance the immune response. The underlying dermis layer is rich in mast cells, monocytes and dendritic cells, which also can encounter an antigen and migrate to the lymph (Kupper, 2004; Romani et al., 2010). But the epidermis with the high-density LCs is more ideal target site for vaccine delivery. However, the stratum corneum, on the top layer of the skin, acts as a physical barrier to prevent the penetration of vaccine (Naik et al., 2000).

To improve the transcutaneous penetration of vaccine, several microneedle systems have been developed (Kis et al., 2012). These microneedles can effectively penetrate the stratum corneum and make a large enough micro-hole as the transport pathway for the proteins and other large microparticulate (Prausnitz, 2004; Li et al., 2009, 2010). Henry et al. first reported the microneedle as a novel transcutaneous drug delivery system in 1998 (Henry et al., 1998). Since then, the microneedle system has become a promising TCI strategy to deliver various types of vaccine through the skin. Recently three groups have reviewed the work of microneedle-based transcutaneous drug delivery (Maaden et al., 2012; Kis et al., 2012, Kim et al., 2012). In recent years the dissolving microneedles made from biodegradable materials has attracted wide attention, because it can easily incorporate drugs and eventually disappear in the skin and thereby leave no biohazardous sharp waste. Polyvinylpyrrolidone (PVP) is a dissolving material and used as a binder in many pharmaceutical tablets. PVP has high water solubility, which facilitates rapid dissolution once inserted into the skin. Sullivan et al. has proved that the microneedle made from PVP could be inserted into the skin and easily dissolved in the skin (Sullivan

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http://dx.doi.org/10.1016/j.ijpharm.2013.02.006

et al., 2008, 2010) and Ke et al. reported that the PVP microneedles containing hollow PLGA microspheres also keep enough mechanically strong to insert into skin (Ke et al., 2012). So the PVP seems better candidate material for dissolving microneedles.

It is known that most of the subunit protein vaccines require an effective adjuvant (Aguilar and Rodriguez, 2007). Besides, transcutaneous immunization always induces a shift to Th2-type immune response compared with the IM (Quan et al., 2010; Koutsonanos et al., 2009). So Th1-type adjuvant to balance the Th1/Th2 immune response in the TCI process is necessary. It has been reported that CpG oligodeoxynucleotides (CpG ODN) is a potent adjuvant and could improve the Th1-type immune response (Chu et al., 1997; Weeratna et al., 2001).In addition, optimizing the formulation of subunit vaccines could help to improve the immunogenicity. Liposomes are elegant nanoparticulates that have been used successfully for the delivery of protein antigens (Kirby et al., 2008; Kersten and Crommelin, 2003; Perrie et al., 2008). It has become clear that cationic liposomes are one of the most effective liposomal delivery systems for antigens to antigen presenting cells (Nakanishi et al., 1999; Christensen et al., 2009).

Recently, Li et al. reported a flexible liposomes used in transcutaneous vaccines. The liposome vaccine encapsulating ovalbumin (OVA) and Toll-like receptor (TLR) ligands could elevate the OVA-special IgG, but needed 200 μg OVA in every dose and one hour to deliver the drug (Li et al., 2011). Slütter et al. reported transcutaneous immunization results of cationic liposomes loaded with OVA and CpG by microneedle array pre-treatment to enhance penetration of skin. But liposomes were found to decrease rather than improve the immunogenicity. They thought that the liposome is unsuitable for application via the transcutaneous, likely due to poor penetration of the microneedle pre-treated skin (Slütter et al., 2011). DeMuth et al. reported an approach for transcutaneous vaccines using PLGA microneedles coated with stabilized lipid nanocapsules ICMVs (interbilayer-cross-linked multilamellar lipid vesicles) loaded with the model vaccine OVA and the molecular adjuvant monophosphoryl lipid A. The results showed a more balanced Th1/Th2 response and higher IgG titer. The microneedles coated lipid nanocapsules for vaccine transcutaneous delivery has the potential for noninvasive delivery applications (DeMuth et al., 2012).

In this work, we developed a novel dissolving microneedle with PVP containing cationic liposome microparticulates for co-delivery of antigen and adjuvant by microneedle technology. The impact of the different dissolving microneedle formulations incorporating OVA antigen, CpG adjuvant and cationic liposome on the (antibody mediated) immune response was investigated in vivo. Furthermore the immune responses by transcutaneous and intramuscular injection were compared.

2. Materials and methods

2.1. Subjects and materials

Female Balb/c mice (6–8 weeks old) used in this study were purchased from Beijing Xinglong Experimental Animals Ltd. Co. (China). All mice were housed under a special pathogen-free condition. All research protocols followed the Guide for the Care and Use of Laboratory Animals (1996).

1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), cholesterol, dimethyldioctadecylammonium (DDA) and ovalbumin (OVA) were purchased from sigma (USA). Polydimethylsiloxane (PDMS, Sylgard 184) was purchased from Dow Corning (USA). Polyvinylpyrrolidone - K17 (PVP-K17) and PVP-K30 were purchased from boal NKY pharmaceuticals Ltd. (China). Horseradish peroxidase(HRP)-labeled goat anti-mouse IgG, IgG1, IgG2a were purchased from Santa Cruz Biotechnology(USA). FITC-OVA was purchased from Beijing Biosynthesis Biotechnology Co., Ltd (China). The CpG-ODN1826 (5′-TCC ATGACG TTC CTG ACG TT-3′) (Chu et al., 1997; Weeratna et al., 2001) was synthesized by Beijing Sunbiotech Co., Ltd (china).

2.2. Encapsulation of OVA and CpG ODN in the cationic liposome

The cationic liposomes containing OVA (Lip-OVA) were prepared as previously described (Jaafari et al., 2007). The lipid phase consisting of DPPC (10 mg/ml), cholesterol (3.4 mg/ml) and DDA (5.5 mg/ml) was dissolved in ethanol in a round bottom flask. The ethanol was removed by rotary evaporation and a thin lipid film on the flask's bottom remained. Then the lipid film was hydrated as follows: firstly, 2 ml deionized water was added, and mixed at 45 °C for 30 min; secondly, 2 ml 10 mM PBS (pH 7.4) was added drop by drop; finally, the 6 ml PBS containing OVA was added and mixed for 10 min. The resulting liposomes suspensions were passed through a high pressure homogenizer for three times at 300 ± 100 bar pressure. The liposomes formulations were extruded through two stacked 100 nm pore size polycarbonate filters 10 times to produce unilamellar vesicles with a uniform size (EmulsiFlex-C5, Avestin® Inc., Canada). The cationic liposomes containing OVA and CpG ODN (Lip-OVA-CpG ODN) were prepared following the same method.

2.3. Encapsulation efficiency and morphology, particle size

In order to determine the encapsulation efficiency, the unencapsulated OVA and CpG ODN in liposome formulation were separated using Amicon-Ultra-15 (100 k) centrifugal filter. The amount of OVA encapsulated in the liposomes was measured using the bicinchoninic acid (BCA) method (Morton and Evans, 1992). The amount of CpG OND was determined with UV absorption at 260 nm.

The sizes of all liposomes were determined by dynamic light scattering (DLS) (Zetasize; 3000 HS, Malvern, UK). The morphology of empty liposomes and the Lip-OVA-CpG OND were charactered by transmission electron microscope (JEM-2100, Japan) with an accelerating voltage of 80 kV.

2.4. Physical stability of liposomes in dissolving microneedles

The empty liposome or the Lip-OVA-CpG OND solution was mixed with PVP-K30 (1:1, w/w) and then kept at a temperature at 37 °C for 2 h to dry solution thoroughly. The samples were re-suspended and used to determine the encapsulation efficiency and analyze the morphology and size of liposomes as previously described (Li et al., 2012).

2.5. OVA stability in dissolving microneedles

For the stable of OVA, the integrity of OVA or OVA in liposome loaded in microneedle was identified through sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions. SDS-PAGE experiments were performed on 5% stacking gel and 15% separation (resolving) gel as previously described (Lavelle et al., 1999). The OVA samples were heated to 100 for 10 min prior to running. In the presence of the strong reducing agent sodium dodecyl sulfate (SDS) and heat, the proteins were dissociated before they were applied on the gel. Samples were loaded onto the vertical slab gel and subjected to electrophoresis at 160 mV, using a Bio-Rad mini protean II dual slab cell (Bio-Rad, Hemel Hempstead, UK). Gels were subsequently fixed and stained with Coomassie brilliant blue staining.