Biomaterials 57 (2015) 50–58

Contents lists available at ScienceDirect

Biomaterials

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

Clinical study and stability assessment of a novel transcutaneous influenza vaccination using a dissolving microneedle patch

Sachiko Hirobe [a], Hiroaki Azukizawa [b], Takaaki Hanafusa [b], Kazuhiko Matsuo [a], Ying-Shu Quan [c], Fumio Kamiyama [c], Ichiro Katayama [b], Naoki Okada [a, **], Shinsaku Nakagawa [a, *]

[a] Laboratory of Biotechnology and Therapeutics, Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamadaoka, Suita, Osaka 565-0871, Japan
[b] Department of Dermatology, Course of Integrated Medicine, Graduate School of Medicine, Osaka University, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan
[c] CosMED Pharmaceutical Co. Ltd., 32 Higashikujokawanishi-cho, Minami-ku, Kyoto 601-8014, Japan

ARTICLE INFO

Article history:
Received 6 January 2015
Received in revised form
1 April 2015
Accepted 3 April 2015
Available online 24 April 2015

Keywords:
Drug delivery
Hyaluronic acid
Immune response
ELISA

ABSTRACT

Transcutaneous immunization (TCI) is an attractive vaccination method compared with conventional injectable vaccines because it is easier to administer without pain. We developed a dissolving microneedle patch (MicroHyala, MH) made of hyaluronic acid and showed that transcutaneous vaccination using MH induced a strong immune response against various antigens in mice. In the present study, we investigated the clinical safety and efficacy of a novel transcutaneous influenza vaccine using MH (flu-MH), which contains trivalent influenza hemagglutinins (15 μg each). Subjects of the TCI group were treated transcutaneously with flu-MH, and were compared with subjects who received subcutaneous injections of a solution containing 15 μg of each influenza antigen (SCI group). No severe local or systemic adverse events were detected in either group and immune responses against A/H1N1 and A/H3N2 strains were induced equally in the TCI and SCI groups. Moreover, the efficacy of the vaccine against the B strain in the TCI group was stronger than that in the SCI group. Influenza vaccination using MH is promising for practical use as an easy and effective method to replace conventional injections systems.

1. Introduction

Infectious diseases are the leading cause of death worldwide. Therefore, the development of safe and effective vaccines is of paramount importance. For example, the H1N1 swine influenza outbreak in Mexico in 2009 [1] and the reemergence of tuberculosis [2] warrant increasing emphasis on vaccine development and indicate the need for global mass vaccination to avoid the risk of pandemic. However, conventional vaccinations are administrated with subcutaneous or intramuscular injections, which require medical personnel with technical skills and is accompanied with the risk of needle-related diseases and injuries. Moreover, antigen solutions require cold chain storage and transportation systems. Therefore, the development of vaccines, which are easy-to-use in administration and superior in stability of formulations, is critically important.

We have developed a transcutaneous microneedle delivery device, which meets the requirements of “the only application” vaccination system. This technique is readily adaptable for widespread practical use, particularly in developing nations with inadequate public health resources. Many nonclinical studies describe the efficacy of a transcutaneous vaccination using microneedles fabricated with silicon or metal and soluble polymers [3,4], but few reports demonstrate the safety and efficacy of these vaccines in humans. Only one transcutaneous influenza vaccine using silicon microneedles induced immune responses in humans [5]. However, microneedles made of metal, stainless steel, or silicon have risks of fractures, leaving fragments in the skin. Therefore, we developed a

Abbreviations: APC, antigen-presenting cell; EMA, European Medicines Agency; FCS, fetal calf serum; GMT, geometric mean titer; HA, hemagglutinin; HI, hemagglutination inhibition; MH, MicroHyala; PBMC, peripheral blood mononuclear cell; PBS, phosphate-buffered saline; PPS, per protocol set; RBC, red blood cell; SCI, subcutaneous immunization; TBS-T, Tris–HCl-buffered saline containing 0.1% Tween-20; TCI, transcutaneous immunization.

* Corresponding author. Tel.: +81 6 6879 8175; fax: +81 6 6879 8179.
** Corresponding author. Tel.: +81 6 6879 8176; fax: +81 6 6879 8179.
E-mail addresses: okada@phs.osaka-u.ac.jp (N. Okada), nakagawa@phs.osaka-u.ac.jp (S. Nakagawa).

http://dx.doi.org/10.1016/j.biomaterials.2015.04.007
0142-9612/

dissolving microneedle patch (MicroHyala; MH) fabricated from hyaluronic acid which is a component of skin tissue [6]. Microneedles on MH penetrate the stratum corneum, which acts as a physical barrier on the outermost layer of the skin, and are dissolved by water in the skin. Subsequently, MH efficiently delivers various materials that can be loaded into the microneedles to abundant immunocompetent cells such as Langerhans cells, dermal dendritic cells, and keratinocytes in the epidermis and dermis below the stratum corneum [7]. Previously, we have reported that the delivery of a transcutaneous vaccine using MH induced an antigen-specific primary immune response against various antigens such as ovalbumin, tetanus–diphtheria toxoid, influenza HA antigens, and recombinant malaria SE36 antigen [8].

In order to lead the fundamental researches, which showed that microneedle formulations are safe and effective in animals, to the practical use, we conducted a clinical study of transcutaneous influenza vaccinations using MH and made compared them with conventional vaccine systems. Moreover, we performed the stability testing of formulation for the development of cold chain-free vaccine.

2. Material and methods

2.1. Preparation of flu-MH

MHs were fabricated using micromolding technologies with sodium hyaluronate as the base material in a clean room [6]. Sodium hyaluronate (JP grade, Kikkoman Biochemifa Company, Tokyo, Japan), dextran 70 (JP grade, Meito Sangyo, Nagoya, Aichi), and povidone (JPE grade, BASF Japan, Tokyo, Japan) were dissolved in distilled water at a weight ratio of 11:8:1 and were mixed with trivalent seasonal influenza hemagglutinin (HA) antigens [The Research Foundation for Microbial Diseases of Osaka University, Suita, Japan; 2011–2012 season strain: A/California/07/2009 (H1N1), A/Victoria/210/2009 (H3N2), and B/Brisbane/60/2008]. The aqueous solution was cast into micromolds and was dried in a desiccator at room temperature, and MHs containing influenza HA antigens (flu-MHs) were then separated from micromolds. Some samples of flu-MHs were subjected to microorganism test. The resulting flu-MH contained trivalent seasonal influenza HA antigen (15 μg each) in 200 microneedles, which are 800 μm long. The flu-MH system comprised of patches with an area of 0.8 cm^2 that were fixed onto 2.3 cm^2 adhesive films. Subsequently, flu-MHs were packed in aluminum laminated polyethylene terephthalate films and were stored at 4 °C until clinical use or at 4 °C, 25 °C, or 40 °C for 6 month for stability testing.

2.2. Clinical study

2.2.1. Vaccination

Forty healthy men (20–49 years of age) volunteered and were enrolled in the study. Written informed consent was received before enrollment. Subjects were randomly divided into two groups of 20 and were treated with transcutaneous immunization (TCI; TCI group) or subcutaneous immunization (SCI; SCI group). One subject (TCI-16) in the TCI group was eliminated from the study because of a vasovagal syncope when his blood was drawn during the first vaccination. Trivalent influenza HA antigens were administered twice and the second vaccination was administered 3 weeks after the first. The study protocol is summarized in Table 1. The TCI group received flu-MHs containing 15 μg of each influenza HA antigen and were applied to the skin of the left upper arm for 6 h using a handheld applicator. The SCI group received subcutaneous injections in each left upper arm of 0.5 mL of Influenza HA Vaccine "BIKEN" (The Research Foundation for Microbial Diseases of Osaka University, Suita, Japan), which contained >15 μg of each influenza antigen. All clinical procedures were approved by Institutional Review Board for Clinical Research at Osaka University Hospital.

Table 1
Clinical protocol for assessing the safety and efficacy of the flu-MH.

Day	0	2	7	21	23	28	42
Vaccination	1st			2nd			
	●			●			
Assessment of local reactions		●	●	●	●	●	●
Blood test	●	●	●	●	●	●	●
Antibody measurement	●		●	●		●	●
Cytokine production	●			●			●

2.2.2. Sample collection

Blood samples were collected before and at 2, 7, and 21 days after vaccination. Sera were obtained by centrifugation of blood samples at 5000 rpm for 15 min. Peripheral blood mononuclear cells (PBMCs) were separated from blood samples taken before and 21 days after vaccination using a BD Vacutainer® CPT™ Cell Preparation Tube with Sodium Citrate (BD, Franklin Lakes, USA). Nasal wash samples were obtained according to a modified protocol of Ainai et al. [9]. Subjects washed their nasal cavities using a nose irrigation solution and a device (Hananoa; Kobayashi Pharmaceutical, Osaka, Japan), and the liquid from nasal washes was collected. Dental cotton was immersed in nasal washes to remove crude materials, and the collected liquid and dental cotton were placed on 0.45 μm filter units (Nalgene, Thermo Fisher Scientific). Subsequently, filtrates were concentrated to 1.0 mg/mL total protein using Vivaspin centrifugal concentrators (Vivaspin 20, MWCO = 30,000; Sartorium Stedim Biotech, Aubagne, France).

2.2.3. Confirmation of microneedle skin insertion

Microneedles with flu-MH were observed after application using a stereo microscope (VHX-1000; KEYENCE, Osaka, Japan). Because microneedles dissolve after insertion into skin, the remaining microneedles were counted and the ratio of dissolved microneedles was calculated.

2.2.4. Assessment of local and systemic adverse reactions

The presence and diameter of epidermal erythematous lesions were evaluated, and the presence of purpura was observed by applying pressure to erythematous areas using a glass plate and observing temporary disappearance of erythema. Subsequently, pigmentation, induration, pressure-induced pain, fever, and the presence of water blisters were assessed, and standard peripheral blood tests and biochemical tests of liver and renal function were performed.

2.2.5. Hemagglutination inhibition (HI) test

HI tests of sera and nasal washes were performed by SRL, Inc. A/California/7/2009 (H1N1) and B/Brisbane/60/2008 strains were detected in HI tests using chicken red blood cells (RBCs), and the A/Victoria/210/2009 (H3N2) strain was detected in HI tests using turkey RBCs. In these experiments, sera and nasal washes were treated with receptor-destroying enzyme [RDE (II); Denka Seiken Co., Ltd., Tokyo, Japan] and RBCs to remove nonspecific hemagglutination inhibitors. Sera and nasal washes were then diluted 1:10 and 1:2, respectively, and samples were then serially diluted half-fold and incubated with each HA antigen for 1 h at 37 °C. One hour after adding 0.5% RBCs, HI titers were determined as the reciprocal of the highest serum dilution that inhibited hemagglutination.