Vaccine 31 (2013) 3403–3409

Contents lists available at SciVerse ScienceDirect

Vaccine

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

Measles vaccination using a microneedle patch☆

Chris Edens[a], Marcus L. Collins[b], Jessica Ayers[d], Paul A. Rota[b,**], Mark R. Prausnitz[a,c,*]

[a] Coulter Department of Biomedical Engineering at Georgia Tech and Emory University, Georgia Institute of Technology, Atlanta, GA 30332, United States
[b] Division of Viral Diseases, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention, Atlanta, GA 30333, United States
[c] School of Chemical and Biomolecular Engineering, Georgia Institute of Technology, Atlanta, GA 30332, United States
[d] Animal Research Branch, Division of Scientific Resources, Centers for Disease Control and Prevention, Atlanta, GA 30333, United States

ARTICLE INFO

Article history:
Received 22 June 2012
Received in revised form 22 August 2012
Accepted 24 September 2012
Available online 5 October 2012

Keywords:
Measles vaccination
Microneedle patch
Vaccine stability
Cotton rat

ABSTRACT

Measles vaccination programs would benefit from delivery methods that decrease cost, simplify logistics, and increase safety. Conventional subcutaneous injection is limited by the need for skilled healthcare professionals to reconstitute and administer injections, and by the need for safe needle handling and disposal to reduce the risk of disease transmission through needle re-use and needlestick injury. Microneedles are micron-scale, solid needles coated with a dry formulation of vaccine that dissolves in the skin within minutes after patch application. By avoiding the use of hypodermic needles, vaccination using a microneedle patch could be carried out by minimally trained personnel with reduced risk of blood-borne disease transmission. The goal of this study was to evaluate measles vaccination using a microneedle patch to address some of the limitations of subcutaneous injection. Viability of vaccine virus dried onto a microneedle patch was stabilized by incorporation of the sugar, trehalose, and loss of viral titer was less than 1 $\log_{10}(TCID_{50})$ after storage for at least 30 days at room temperature. Microneedle patches were then used to immunize cotton rats with the Edmonston-Zagreb measles vaccine strain. Vaccination using microneedles at doses equaling the standard human dose or one-fifth the human dose generated neutralizing antibody levels equivalent to those of a subcutaneous immunization at the same dose. These results show that measles vaccine can be stabilized on microneedles and that vaccine efficiently reconstitutes in vivo to generate a neutralizing antibody response equivalent to that generated by subcutaneous injection.

1. Introduction

Despite the widespread availability of an inexpensive and effective vaccine, measles virus is one of the leading causes of vaccine-preventable morbidity and mortality among children worldwide [1]. High levels of coverage are necessary for interruption of measles transmission. Measles vaccination programs have dramatically reduced the incidence of disease in both developed and developing countries [2,3]. More than 4.5 million measles deaths have been prevented as of 2008 through implementation of the vaccination strategies developed by WHO and UNICEF. Global mortality has declined by 74% from an estimated 733,000 deaths in 2000 to 139,300 in 2010 [4]. Measles elimination, defined as the absence of endemic transmission of virus, has been achieved and sustained in the WHO Region of the Americas since 2002, and four of the five other WHO Regions, European, Eastern Mediterranean and Western Pacific, have targeted measles for elimination by 2020 or earlier [5].

The measles vaccine is currently delivered by subcutaneous injection using a needle and syringe. This delivery method creates the requirement for specifically trained healthcare personnel to administer each vaccine dose, typically at centralized locations. In contrast, the global campaign to eradicate polio has been possible, in part, because of the simplicity of delivering the oral polio vaccine, which can be administered by minimally trained personnel. Decreasing the logistical challenges associated with delivery of measles vaccine could increase vaccination coverage and reduce vaccination campaign costs.

Hypodermic injections create hazardous medical waste which must be safely destroyed. Preventing needle theft and reuse through responsible disposal methods adds significant costs to vaccination campaigns. For example, a relatively small measles vaccination campaign in the Philippines generated over 130,000 kg of sharps waste [6]. Another logistical challenge with the standard vaccination scheme is the requirement of a cold chain for vaccine

☆ The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention, US Department of Health and Human Services.

* Corresponding author at: School of Chemical and Biomolecular Engineering, Georgia Institute of Technology, Atlanta, GA 30332, United States. Tel.: +1 404 894 5135.
** Corresponding author. Tel.: +1 404 639 4181.
E-mail addresses: par1@cdc.gov (P.A. Rota), prausnitz@gatech.edu (M.R. Prausnitz).

0264-410X/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.vaccine.2012.09.062

storage and transport. After reconstitution, multi-dose vials must be used within 2 h or discarded [7]. This leads to vaccine wastage and increased program costs. A delivery system that eliminates the need for reconstitution and reduces or eliminates the need for cold storage and transport could enable more efficient use of measles vaccine and decrease the cost per delivered dose.

Measles vaccination using a microneedle patch may be able to address some of the limitations of conventional hypodermic injection and thereby facilitate measles mortality reduction and elimination programs. Microneedles are micron-sized needles made of metal or polymer that are designed to achieve the efficacy of hypodermic injection with the simplicity of a patch [8,9]. Microneedles offer the possibility of eliminating or mitigating many of the logistical challenges associated with the current vaccination strategy, including reduced cost, simplified transport and storage, and increased safety. The microneedles used in this study remain on the patch after it is removed and could present a small risk for disease transmission as a sharps hazard. However, microneedles can also be fabricated from dissolving polymers in which case no potentially infectious, sharps waste would be generated [10,11]. Microneedles require a small amount of force to penetrate the skin, and once the barrier layer has been penetrated, the vaccine is rapidly released into the skin. The microscopic wound created by the patch is superficial and heals quickly [12]. With the correct excipient conditions other vaccines have been stabilized onto a microneedle patch [13,14]. If this high level of temperature stability could be extended to the live-attenuated measles vaccine, the cost and logistical issues associated with vaccine transport and storage could be decreased significantly. Finally, the small size of the microneedle patch would limit sharps waste following large-scale vaccination campaigns. This would decrease transport costs while also minimizing the potential for reuse.

Measles vaccine has been previously delivered to the skin using a variety of methods including the Mantoux method [15] and jet injection [16,17]. While some studies have shown improvements after intradermal delivery [18], others found lower neutralizing antibody titers when compared with traditional delivery routes [19,20]. The inferior serologic response to intradermal vaccination seen in these studies could result from the low dose of measles vaccine delivered (as low as 5% of the standard dose). Neither study investigated the response to a standard subcutaneous dose (at least 10^3 $TCID_{50}$) delivered intradermally.

Stabilization of the measles vaccine in a dry state has also been previously examined. Viral infectivity loss after drying has been mitigated through both excipient selection and drying process optimization [21,22]. Some of these dry powder vaccines were shown to be efficacious after delivery to the respiratory tract of non human primates [23–26]. However, these stabilization methods used drying processes such as spray drying and lyophilization, which are not easily compatible with microneedle fabrication and coating.

Microneedles have been used successfully as an experimental delivery system for a number of different vaccines including live virus and bacteria, inactivated virus, virus-like particles, protein sub-unit, DNA and live viral vaccines against influenza and a number of other diseases [8,10,27–39]. However, measles vaccine has never been studied before using microneedles. In this study, we first examined the ability of excipients to stabilize the live-attenuated measles vaccine during fabrication and storage. We then compared the immune response to vaccination using a microneedle patch to conventional subcutaneous injection in the cotton rat model.

2. Materials and methods

2.1. Preparation of live-attenuated measles vaccine

The measles vaccine strain, Edmonston-Zagreb, was obtained from the collection at the Centers for Disease Control and Prevention and this strain is used in many WHO pre-qualified measles vaccines. To achieve the high titers need for coating of the microneedles, the vaccine virus was propagated in Vero cells maintained in Dulbecco's Modified Eagle Medium (DMEM) (Gibco, Grand Island, NY) and 2% fetal bovine serum (FBS, Gibco). Infected cells were harvested when the cytopathic effect was maximal; the cell suspension was freeze-thawed once before low-speed centrifugation to remove cellular debris [40]. The viral titer (50% tissue culture infective dose, $TCID_{50}$) was measured by end-point titration. The virus was then aliquoted and stored at −70 °C until use. For the end-point dilution assay, 10-fold dilutions of the viral stock were prepared in DMEM with 2% fetal bovine serum and used to infect multiple wells of Vero cell monolayers in 24-well tissue culture plates. Plates were incubated for 7 days and scored visually for the presence or absence of viral cytopathic effect. The $TCID_{50}$ was then calculated using the Karber method [41].

2.2. Vaccine stability studies

Live measles vaccine virus with an initial viral titer of 10^6 $TCID_{50}$/mL was mixed with excipients at specific concentrations. Excipients used in this study included carboxymethylcellulose (CMC, CarboMer, San Diego, CA), trehalose (Sigma–Aldrich, St. Louis, MO), fish gelatin (Sigma–Aldrich), myo-inositol (Sigma–Aldrich), and Lutrol F68 (BASF, Mt. Olive, NJ). A 2 μL drop of each resulting solution was applied to a sterile chip of stainless steel measuring 3 mm × 4 mm to simulate the surface of a stainless steel microneedle. We used this simple method of coating to screen formulations since coating actual microneedles is more time consuming. Each 2 μL drop contained a mixture of 50% measles virus solution and 50% excipient solution. The chips were allowed to dry at room temperature (22 °C) in a Class II biosafety cabinet or an incubator (37 °C). In some cases, the air was de-humidified during storage by placing the stainless steel chip inside of a 50 mL plastic tube containing desiccant (Drierite, Sigma–Aldrich) and wrapped in Parafilm (Sigma–Aldrich). After specified storage times, the vaccine coated onto the chips was reconstituted in 1 mL DMEM and viral titers were measured in Vero cells as described above.

2.3. Microneedle fabrication and coating

Stainless steel microneedles were fabricated by first defining the microneedle shape lithographically and then etching the microneedles in a chemical bath. This produced patches each containing a single row of five microneedles that were 750 μm long and measured 200 μm × 50 μm at the base (Fig. 1). We chose this microneedle patch design because the measles vaccine dose is sufficiently small that a full dose can be coated onto just five microneedles and because coated microneedles of similar design have been successfully used for vaccination and drug delivery in a number of published studies [9]. We chose a microneedle length of 750 μm because it matches the thickness of rat dorsal skin, which is generally reported in the range of 700–1000 μm [42,43]. Thus, we believe vaccine coated on the microneedles was deposited along the needle track in the epidermis and dermis; it is possible that a small fraction of the vaccine was delivered to the subcutis in the case of thin skin.