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Rabies vaccination in dogs using a dissolving microneedle patch

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ABSTRACT

Because humans get rabies primarily through dog bites, stray dog population control and mass or mandatory vaccination of domestic dogs and other animals has virtually eliminated human rabies in industrialized countries. However, thousands of people in developing countries die of rabies each year due to the inability to control dog populations and implement mass vaccination because of financial, logistical and other challenges. The availability of an easier-to-administer and more cost-effective vaccine may help to address some of these issues. Here, we propose the use of dissolving microneedle patches for simple and potentially cost-effective rabies vaccination, and assess the safety and immunogenicity of microneedle patch vaccination using a rabies DNA vaccine in dogs. The vaccine was stable upon formulation and storage for at least 3 weeks at 4 °C in a microneedle patch. For vaccination, the patches were applied to the inner ear by hand without an applicator. Microneedle patches were well tolerated in the skin, with mild erythema, minimal wheal formation and complete resolution of skin reactions within 7 days, and generated no systemic adverse events. Microneedle patches were at least as immunogenic as intramuscular injection at the same dose, as demonstrated by similar serum neutralizing antibody titers. A ten-fold lower vaccine dose administered by microneedle patch generated a weaker immune response compared to full-dose intramuscular vaccination. We conclude that dissolving microneedle patches may provide an innovative approach to mass vaccination of dogs.

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1. Introduction

Rabies is an acute, often fatal encephalitis caused by viruses in the Rhabdoviridae family [1]. The disease is zoonotic and human infection usually results from a bite or scratch from an infected animal or direct contact of skin wounds with virus containing saliva. Although all warm-blooded animals can be reservoirs of rabies, dogs account for 99% of human deaths due to rabies and pose a potential threat to >3.3 billion people [2].

Human rabies has been almost eliminated in industrialized countries by strict control of stray animal populations, widespread and often mandatory vaccination of dogs and other animals, as well as the availability of vaccines for humans [3]. Only specific groups of humans that have a recognized higher risk of exposure (e.g., veterinarians) are typically vaccinated against rabies for prevention, but post-exposure prophylactic vaccines and immunoglobulins are available to people who become exposed to the virus [4]. These measures have caused the number of deaths due to rabies in the United States to drop to just one to two per year [5]. However, globally, an estimated 26,000 to 61,000 deaths are caused by rabies each year, >95% of which occur in Africa and Asia due to dog bites [3]. Rabies occurs mainly in remote rural communities where children between the age of 5–14 years are the most frequent victims [6], and limited access to healthcare facilities with the high cost and complex schedule of post-exposure vaccines for humans often makes it difficult to provide medical care to people that become exposed to the virus [7].

In developing countries, more extensive vaccination of dogs and humans is often limited by the high cost of vaccination and a lack of trained personnel to administer the vaccines. As the main source of exposure is stray dogs, the vaccination of such a population poses very specific problems including capture, identification, and sheer numbers. There is some evidence that intradermal vaccination using one-fifth to one-tenth the dose of rabies vaccine can be effective in humans, thereby potentially enabling significant cost savings or at least making an extended use of available vaccine doses [2,8–10]. This dose sparing is believed to be due to targeting of the vaccine to resident dendritic cells in the skin, such as Langerhans and dermal dendritic cells, which are able to mount a more robust immune response [11–14]. However, intradermal injection requires specifically trained healthcare personnel and successful injection into the skin is unreliable [15,16]. Thus, low-cost intradermal post-exposure prophylaxis of humans is sometimes practiced in resource-poor settings. However, intradermal pre-exposure vaccination in dogs is generally not available. A simple and reliable method of intradermal rabies vaccination could therefore enable more widespread vaccination at lower cost.

Another method of cost savings is through DNA vaccination. Human DNA vaccines could be much less costly to manufacture compared to

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inactivated virus vaccines. This is because Human DNA vaccines can be produced in large quantities by bacterial fermentation processes [17]. For animal vaccines this could potentially also be the case. For veterinary rabies vaccination, live virus vaccines for oral use and inactivated cell culture vaccines for parenteral use have been used traditionally. Recombinant rabies DNA vaccines have been developed recently and are in trials for immunization of dogs in developing countries [18].

In this study, we propose that delivery of a rabies DNA vaccine using a microneedle patch could enable more widespread rabies vaccination of dogs and humans by enabling minimally trained personnel to carry out vaccination. Microneedles are <1 mm long and deliver vaccines to the skin's epidermis and dermis using a patch that is simply and painlessly applied to the skin by personnel with minimal training [19–25]. In a dissolving microneedle patch, an array of microneedles is attached to a backing such that it can be applied to the skin by hand like a bandage. After insertion into the skin, the microneedles dissolve in the skin within minutes, thereby delivering the vaccine contained in them and not generating sharps waste [26–36]. In a dissolving microneedle patch, the vaccine is encapsulated within the microneedles. Another design of microneedle patches is the coated microneedle patch that consists of insoluble microneedles that are coated with vaccine on the surface [37]. This study evaluated the use of dissolving microneedle patches.

Vaccination using a microneedle patch could simplify rabies vaccination of dogs, especially stray dogs in developing countries, since the microneedle patches could be easily applied by hand on a dog's ears by personnel with minimal training. While oral rabies vaccine exists, it has limited use when vaccinating dogs because the bait in which the vaccine is contained does not always lead to complete delivery of vaccine and the use of oral vaccines is usually limited to areas with minimal human activity so as to ensure safe distribution [3]. Microneedle patch vaccination could also be attractive in industrialized countries, where dogs and their owners may prefer a painless, less-invasive method of vaccination.

Microneedle patches have previously been studied for delivery of a number of vaccines for eventual human applications [34,38–45], but have not previously been studied for rabies vaccination or for veterinary vaccination applications. The goal of this project is to develop an easy-to-administer rabies vaccine suitable for use in dogs that enables cost savings, is safe and is at least as immunogenic as conventional intramuscular vaccination. We therefore developed and characterized dissolving microneedle patches for rabies vaccination and then assessed safety and immunogenicity in a small clinical study in beagle dogs.

2. Materials and methods

2.1. Fabrication of microneedle patch

Polydimethylsiloxane (PDMS) molds containing a 10 × 10 array of conical microneedles (base diameter 300 μm and height 650 μm) were used for microneedle patch fabrication by a two-step micromolding process. (i) Vaccine fill: The vaccine (i.e., proprietary DNA plasmid provided by Merial Inc. isolated from *E. coli* culture using the EndoFree Plasmid Giga Kit (Qiagen, Germantown, ND)) was mixed 1:1 with 15% w/v sucrose (Sigma-Aldrich, St. Louis, MO) and applied to the microneedle mold. Vacuum was then applied for 45 min. Excess vaccine was removed and the mold was allowed to dry for 90 min. (ii) Polymer matrix fill: The polymer matrix solution was composed of 18% (w/v) polyvinyl alcohol (EMD Millipore, Billerica, MA) and 18% (w/v) sucrose (Sigma-Aldrich) in sterile water. The solution was heated to 80 °C for 6 h before use to facilitate dissolution of the polyvinyl alcohol. The matrix solution was applied onto the mold and exposed to vacuum for 4 h. The mold was left in a chemical hood overnight to dry.

To remove the dried microneedle patches, a 2.3 cm-diameter disc of polymethylmethacrylate (McMaster-Carr, Atlanta, GA) was covered on one side with double-sided tape (MacTac, Stow, OH) and applied to the back of the mold. The resulting patch was gently peeled away from the mold and stored in a dark, sealed foil pouch with desiccant at 4 °C until use. The microneedles were 680 μm tall and 300 μm wide at the base.

As a quality control measure, a representative sample of patches from each batch was tested for DNA loading, supercoiling and sterility, as described below. Microneedle patches were imaged by brightfield microscopy (SZX12, Olympus, Center Valley, Pennsylvania). Microneedle patches were applied to the animals for vaccination three weeks after fabrication, as described below, after all testing had been completed.

2.2. Quantification of DNA loaded into microneedle patch

DNA concentrations were measured using the nucleic acid setting on Nanodrop 2000 (Thermo Fisher, Waltham, MA). The patch was dissolved in deionized autoclaved water to determine the dose contained in the patch. A placebo patch containing no vaccine was used as a negative control to subtract any interference from the microneedle matrix materials.

2.3. Quantification of DNA supercoiling

DNA supercoiling was measured using agarose gel electrophoresis. A 0.8% agarose gel was run with Tris-acetate buffer and the gel was stained with ethidium bromide. The gel was imaged using a Kodak 200 gel logic camera system (Kodak, Rochester, NY) and the relative intensities of the bands were used to calculate the percentage of supercoiled DNA.

2.4. In-vitro expression assay for DNA stability

An in-vitro expression assay was used to confirm the ability of the vaccine to express the rabies G protein in-vitro (i.e., the vaccine antigen). CHO-K1 cells (ATCC CCL-61, American Type Culture Collection, Manassas, VA) were transfected with rabies DNA obtained from reconstituted patches using Lipofectamine (Life Technologies, Carlsbad, CA) and stained with mouse anti-rabies glycoprotein monoclonal antibody clone 24-3F-10 (EMD Millipore, Billerica, MA) and FITC-conjugated rabbit anti-mouse IgG (Sigma, St. Louis, MO). For a sample to be declared satisfactory, cells needed to show easily visible and similar level of green fluorescence as compared to the control.

2.5. Insertion of microneedle patches into dog ears ex-vivo

Excised dog ears were obtained from animals euthanized as part of a separate study and the skin was carefully shaved with a razor to remove fur. Microneedle patches containing sulforhodamine dye (to simulate vaccine) were applied to the skin on the inner ear pinna by pressing down with the thumb, left on the skin for 15 min and then removed. The skin site and microneedle patches were imaged before and after insertion.

2.6. Safety and immunization study

The study was approved by the Institutional Animal Care and Use Committees (IACUC) at Merial and Georgia Tech. Male and female beagle dogs aged 5 to 11 months were used in the clinical study. The dogs were seronegative for rabies and were excluded from the study if they had eczema or inflammation at the injection sites at the time of the study. The dogs were vaccinated by intramuscular (IM) injection (50 μg DNA), microneedle patch (50 μg DNA) and microneedle patch (5 μg DNA) (n = 5 per group). A placebo (i.e., no vaccine) microneedle patch group was also included in the study (n = 2). Four weeks after the first dose, all dogs were given a booster using the same route and dose as the initial vaccination (Fig. 1).

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