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Skin targeted DNA vaccine delivery using electroporation in rabbits
II. Safety

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Abstract

The Achilles heel of gene-based therapy is gene delivery into the target cells efficiently with minimal toxic effects. Viral vectors for gene/DNA vaccine delivery are limited by the safety and immunological problems. Recently, nonviral gene delivery mediated by electroporation has been shown to be efficient in different tissues including skin. There are no detailed reports about the effects of electroporation on skin tissue, when used for gene/DNA vaccine delivery. In a previous study we demonstrated the efficacy of skin targeted DNA vaccine delivery using electroporation in rabbits [Medi, B.M., Hoselton, S., Marepalli, B.R., Singh, J., 2005. Skin targeted DNA vaccine delivery using electroporation in rabbits. I. Efficacy. Int. J. Pharm. 294, 53–63]. In the present study, we investigated the safety aspects of the electroporation technique in vivo in rabbits. Different electroporation parameters (100–300 V) were tested for their effects on skin viability, macroscopic barrier property, irritation and microscopic changes in the skin. Skin viability was not affected by the electroporation protocols tested. The electroporation pulses induced skin barrier perturbation and irritation as indicated by elevated transepidermal water loss (TEWL) and erythema/edema, respectively. Microscopic studies revealed inflammatory responses in the epidermis following electroporation using 200 and 300 V pulses. However, these changes due to electroporation were reversible within a week. The results suggest that the electroporation does not induce any irreversible changes in the skin and can be a useful technique for skin targeted DNA vaccine delivery.

Keywords: DNA vaccine; Electroporation; Skin; Safety

1. Introduction

Cutaneous gene delivery is attractive, as skin is the most accessible somatic tissue (Khavari, 1997). Skin also represents a potential target for DNA vaccine delivery due to the presence of functional bone marrow derived epidermal Langerhan's cells and dermal dendritic cells, which are specialized for induction of immune responses (Tuting et al., 1998). The possibility of using viral vectors for gene/DNA vaccine delivery is limited by the safety and immunological problems associated with the use of viral vectors in humans (Verma and Somia, 1997; Abdallah et al., 1995). The viral vectors are antigenic by themselves and can cause severe inflammatory responses. An alternative approach to genetic immunization is the gene transfer using nonviral methods. Recent setbacks in gene therapy with viral vectors further accelerated the search for efficient nonviral gene delivery systems (Verma, 2000). The nonviral gene delivery methods have significant clinical potential. However, the efficiency of transfection using plasmid/naked DNA as such is low due to extracellular and intracellular barriers (Ma and Diamond, 2001; Herweijer and Wolff, 2003). Following the report of gene expression after direct plasmid DNA injection (Wolff et al., 1990), several studies examined the possibility of vaccination using plasmid DNA coding antigens (DNA vaccines) in vivo (Ulmer et al., 1993; Raz et al., 1994; Lagging et al., 1995). Although direct injections of DNA vaccine do induce immune response in smaller animals, the delivery of the DNA to target cells is not optimal, especially in higher animals (Whalen, 1996; Srivastava and Margaret, 2003). Several chemical and physical methods have been reported to enhance the immunogenicity of DNA vaccines, primarily by increasing the transfection efficiency and thereby the antigen expression (Herweijer and Wolff, 2003). Most of these methods

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are not suitable for routine use due to the inefficiency, in vivo clearance, toxicity and formulation/manufacturing complexities involved. Furthermore, large amount of genetic material is needed to induce the response with injections. Recently, gene delivery mediated by electroporation has been shown to be efficient (Aihara and Miyazaki, 1998; Glasspool-Malone et al., 2000; Medi and Singh, 2003; Zhang et al., 2002).

Electroporation involves application of controlled, short and high voltage electric pulses to permeabilize the target cell/tissue reversibly for macromolecules such as genes/proteins. Electroporation has been evaluated in animals and humans for the delivery of chemotherapeutic agents with high efficiency (Mir et al., 1998; Sersa et al., 2000). Furthermore, it has been employed in studies involving delivery of plasmid DNA in vivo to different types of tissues with improved transfection efficiency. Most of these studies involve insertion of electrode needles into the tissue after plasmid DNA injection, which may not be feasible for use in humans. To be therapeutically useful, the DNA vaccine must be delivered inside the cells before it can express antigen molecules (Doria-Rose and Haigwood, 2003). This requires efficient membrane permeabilization to allow the DNA vaccines to enter the cells. Cutaneous gene delivery using topical electroporation needs no specialized procedures as the pulses would be applied topically with tweezer type of electrodes, following the injection of plasmid DNA. In a previous study (part I), we demonstrated the efficacy of skin targeted DNA vaccine delivery using electroporation in rabbits (Medi et al., 2005). However, the major factor in the clinical acceptability of electroporation mediated gene/DNA delivery is its effect on the target tissue. The electropermeabilization may leave the target tissue damaged depending upon the electrical parameters associated with the electroporation (Lefesvre et al., 2002). The technique to be clinically acceptable for use in gene/DNA delivery, there should be no permanent damage to the skin. The detailed report on the effects of electroporation on skin safety is lacking. In the present study, we address the issues of skin safety from different electroporation parameters in vivo in New Zealand White (NZW) rabbits.

2. Materials and methods

2.1. Materials

MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] and dimethyl sulfoxide (DMSO) were obtained from Sigma Chemical Company (St. Louis, MO, USA). Dulbecco's modified eagle medium (DMEM) and fetal bovine serum (FBS) were purchased from Invitrogen Corporation (Carlsbad, CA, USA). Deionized water obtained with a Barnstead Nanopure Infinity® ultrapure water system (Barnstead, Boston, MA), having resistivity of $\geq$18 MΩ cm was used to prepare all solutions and buffers.

2.2. Animals

New Zealand White (NZW) rabbits (*Oryctolagus cuniculus*), 10 weeks old and about 2.0–3.0 kg body weight, were used in the study. The animals were housed in North Dakota State University (NDSU), Department of Veterinary Technology Animal Care Facilities and cared for in accordance with the "Guide for the Care and Use of Laboratory Animals" (National Academy Press, 1996). All the animal experiments were performed according to the protocols approved by Institutional Animal Care and Use Committee of NDSU.

2.3. Effect of electroporation on skin viability

The hair on the back of the rabbits was closely clipped using an electric clipper carefully without any damage to the skin, 24 h prior to the beginning of the study. At the beginning of the experiment, the rabbits were anesthetized using 30 mg/kg pentobarbital sodium (Nembutal®) given intraperitoneally. An area of the skin was swabbed with 70% (v/v) isopropyl alcohol. Electroporation site corresponding to cathode and anode was marked. The marked site was pinched and clamped with stainless steel tweezer electrode (10 mm × 5 mm, CUY 663B, NEPA Gene Co, Chiba, Japan) and electric pulses were applied using a square-wave electroporator (CUY21 EDIT Version, NEPA Gene Co, Chiba, Japan). The treatment conditions tested were control (no pulses), five pulses of 100 V and 10 ms, five pulses of 100 V and 30 ms, five pulses of 200 V and 10 ms, five pulses of 300 V and 10 ms. The interval between each pulse was 1 s. The electroporation treatment conditions tested were based upon the previous study on DNA vaccine delivery (Medi et al., 2005). The effect of electroporation pulses on the skin viability was investigated by taking skin samples from rabbits by punch biopsy immediately (0 h) and 24 h after electroporation along with control using MTT assay. The viability of the skin samples was assessed at both cathodal and anodal sites. The samples were weighed immediately and incubated with 2 mg/ml of MTT prepared in DMEM containing 5% FBS for 3 h at 37 °C under 5% CO_2 in a 24-well plate. The water-insoluble formazan was extracted using DMSO for 1 h under shaking and the absorbance of the samples was measured by spectrophotometer at 540 nm. The results were expressed as percent viability calculated using the following equation:

$$\%\ \text{skin viability} = \frac{\text{absorbance of the test sample} \times 100}{\text{absorbance of the control}}$$

2.4. Effect of electroporation on macroscopic skin barrier function and skin irritation

The electroporation pulses were applied to the skin as described in the previous section (*Effect of electroporation on skin viability*). The effect of electroporation on macroscopic skin barrier function was studied by measuring the transepidermal water loss (TEWL). TEWL can be considered a determinant indicative of the functional state of the cutaneous barrier (Maibach et al., 1984) and provides a method for assessing macroscopic changes in the barrier properties of the stratum corneum (SC) (Abrams et al., 1993). TEWL was measured quantitatively with a Tewameter™ (Courage and Khazaka, Cologne, FRG), before electroporation (baseline) and