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SCIENCE DIRECT
Vaccine 24 (2006) 4667–4670
Vaccine
www.elsevier.com/locate/vaccine

A novel electroporation device for gene delivery in large animals and humans

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Available online 1 September 2005

Abstract

Intramuscular injection of plasmid DNA followed by electrical stimulation (electroporation) is an efficient method for achieving therapeutic levels of encoded proteins or eliciting efficient immune responses in smaller animals such as mice and rats. Electroporation in larger animals and humans poses new technical challenges, the main difficulty being to maintain efficacy while limiting invasiveness and pain.

Here we present data using a new device for combined injection and electroporation in large animals and humans. The device injects DNA through two needles during insertion into the muscle and thus distributes the injection volume along the needles which also serve as electrodes. Since the electrical field is strongest close to the needle-electrode, a near perfect match between the DNA and the electric field is achieved. We show that using moderate amounts of DNA: (1) muscle tissue is transfected along the entire length of the needle path, (2) the efficacy is higher compared to when the DNA is injected between the electrodes, (3) level of protein expression can be tightly controlled by the number of treatments, and (4) efficient immunization is achieved.

Keywords: DNA vaccination; Electroporation; Sheep

1. Introduction

Gene based vaccines or therapeutics can be delivered via viral or non-viral vectors. While avoiding complication and safety issues often associated with viral vectors, non-viral gene delivery has in general suffered from low efficiency of transfer. Application of short electrical pulses (electroporation) can be used to enhance gene delivery [1–3] DNA vaccination in large animals have resulted in improved cellular and humoral immune responses when DNA injection is combined with electroporation [4–7]. As the method is entering into clinical trials, there is an urgent need for equipment and procedures that reduces invasiveness, pain and the per patient cost.

One of the main challenges for efficient electroporation in larger animals is to assure correct match between the electrical field and the injected DNA. Current designs for electroporation in large animals and humans typically consist of an array of 3–6 custom-made needle electrodes that are inserted 1–2 cm into the muscle. DNA is injected with a standard syringe into the area covered by the electrode array where after electrical pulses are applied (Fig. 1, upper panel). A relatively large volume of DNA is needed to ensure overlap with the electrical field applied to the muscle. Hence, additional diffusion time after injection and before the application of electrical pulses, therefore improve the transfection [8].

The Inovio electroporation device makes use of two standard disposable syringes with needles, which is used for both injection and application of electrical pulses (Fig 1, lower panel). DNA is injected during insertion of the needles and, therefore, distributed along the entire path of the needle-electrode so that a larger portion of muscle cells are covered by both DNA and the electrical field needed for transfection.

Studies in human volunteers on electroporation of skin [9] and muscle (unpublished results) have shown that above perception threshold increased current flow will lead to augmented sensation of pain. Lowering the electrical field will

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doi:10.1016/j.vaccine.2005.08.068

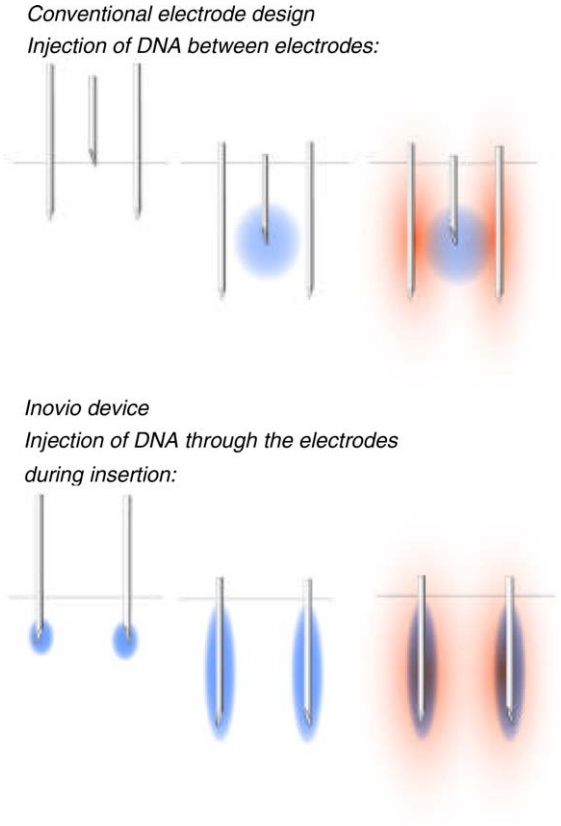

Fig. 1. Principles of DNA injection and electroporation. DNA (blue) can be injected between (upper panel) or through (lower panel) the electrodes. The electrical field (red) is applied after injection.

therefore be important for reducing electroporation-induced pain. As the current density will be highest close to the electrodes efficient electroporation should be achievable using milder electrical conditions when the DNA is located in this area. Furthermore, since distribution of DNA outside the electrical field is avoided efficient transfection can be achieved using moderate amounts of DNA.

2. Materials and methods

Sheep and rabbits were injected with DNA encoding β-galactosidase (β-gal) or mouse IgG2b anti-NIP (mIgG-NIP). Electroporation was performed using 5 pulses of 20 ms at 250 mA (Inovio pulse generator). Serum samples were analyzed by ELISA as described in [10].

3. Results and discussion

3.1. Design of the electroporation device

An injection/electroporation device was designed in collaboration with Automasjonsutvikling (Branbu, Norway).

Fig. 2. The novel injector/electrode device consists of an outer "house" (A) with an inner "wagon" (B) carrying two syringes with needles that slides within the house. The assembled version is shown in (C).

The device consists of an outer "house" (Fig. 2A) with an inner "wagon" (Fig. 2B) carrying two standard 1 ml syringes with needles, 8 mm apart. A gearing system presses the piston of the syringes when the wagon slides forward in the house in order to inject DNA during the insertion. The needles subsequently serve as electrodes. The use of needles as electrodes has been described previously [11]. The main advantage achieved with Inovio's device is the guarantied distribution of DNA around the full needle length.

3.2. Muscle fibers along the needle path are transfected with β-gal and GFP after DNA injection and electroporation

Twenty-five micrograms of DNA encoding β-gal in 50 μl saline was injected into *M. glutaeobiceps* and/or *M. semitendinosus* of anesthetized sheep through each needle during insertion. Subsequently, electroporation was performed. Three days later the sheep was terminated and tissue samples from the transfected area were removed, sliced in 1–2 mm thick sections and stained for β-gal activity (Fig. 3). Note the two barrel-shaped transfected areas along the trajectory of the electrodes and fibers transfected both above and below the tendon which could probably not have been achieved without injection during insertion (Fig. 3a). Fig. 3b shows results from a similar experiment comparing injection of DNA with (+EP) or without electroporation (−EP).

Continuous injection during insertion limits the risk of depositing the whole injection volume in tissue pockets void of muscle cells, i.e. adipose tissue or between muscle bundles, which is a risk using conventional intramuscular injection. Furthermore the influence of natural borders such as tendons or muscle fascia is reduced allowing a larger area of tissue to be transfected.

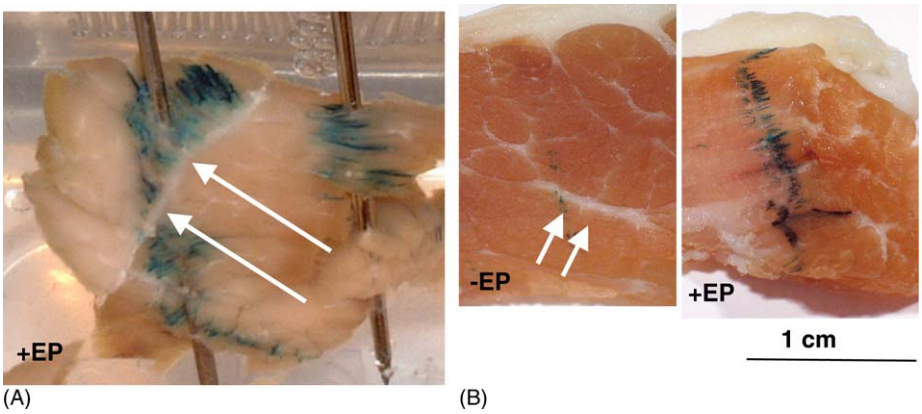

Fig. 3. β-Galactosidase in sheep muscle. Syringes placed in the Inovio device was inserted 13 mm into the muscle tissue while 25 μg/50 (l DNA encoding β-galactosidase was injected. Five pulses of 20 ms length and of 250 mA was then applied (+EP) while one area only received the DNA (−EP). Three days later, the muscle was dissected and stained for β-galactosidase activity. The arrows indicate a tendon (in A) and a pocket of adipose tissue (in B). A and B are results from two different experiments.

3.3. High and reproducible transfection level is achieved by electroporation using the Inovio device

Transfection efficiency using the Inovio device was compared with a conventional system where DNA was injected between two needle electrodes, 8 mm apart (Fig. 1, upper panel). DNA encoding mIgG2-NIP, 50 μg/100 μl, was injected followed by electroporation. Treatments were performed twice in each sheep ($n = 5$). Seven days later blood samples were collected and analyzed for mIgG-NIP by ELISA. As shown in Fig. 4, electroporation using the Inovio device was more efficient than using the device injecting DNA between the electrodes. These results suggest that there is a good match of injected DNA and the electric field allowing low volumes to be used. It should be noted, however, that increasing the number of electrodes and/or injection volume, could enhance the transfection efficiency of the conventional electroporation devices.

To determine if it is possible to control dosing of the encoded protein, electroporation mediated delivery of 50 μg/100 μl DNA encoding mIgG-NIP was performed either two or four times in each animal ($n = 5$). The results show that doubling the number of treatments lead to a twofold increase in mIgG-NIP 7 days after transfection (Fig. 4). This demonstrates the ease of manipulating the level of gene expression using the Inovio device.

3.4. Electroporation induced humoral response

To investigate the usefulness of the Inovio device for induction of immune responses, sheep were injected with 100 μg DNA encoding mIgG-NIP followed by electroporation. Sera were assayed for sheep anti-mouse IgG2b 14, 28 and 48 days after treatment. The results show that significant

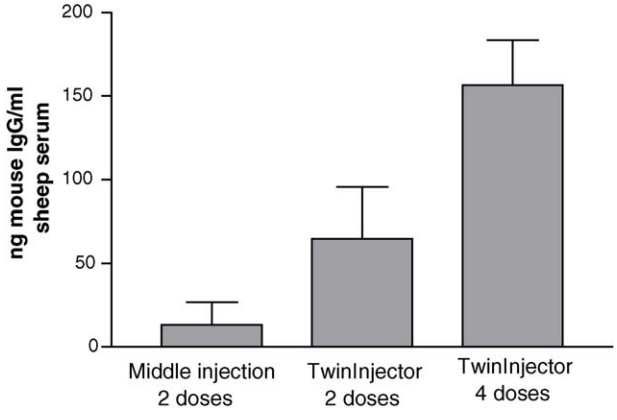

Fig. 4. Expression using two different injection systems. Electroporation was performed following injection of DNA encoding mouse IgG2b anti-NIP in sheep muscle using a conventional electrode design (Middle injection) or trough the electrodes during insertion (Inovio device). The procedure was performed two (two doses) or four (four doses) times injecting a total DNA of 100 or 200 μg. Seven days later sera were assayed for mouse IgG. Error bars represents the standard deviation.

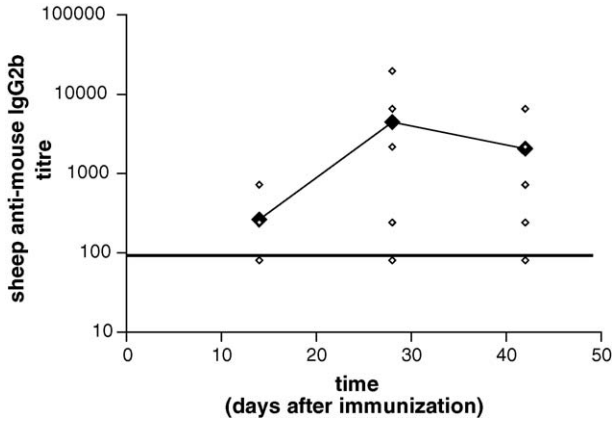

Fig. 5. Humoral response in sheep. The Inovio device was used to deliver a single dose of DNA (100 μg) encoding mouse IgG2b anti-NIP in sheep muscle followed by electroporation. At indicated time points sera were assayed for mouse IgG. Results are presented as mean (black diamonds) as well as single (open diamonds) endpoint titres ($n = 9$). The horizontal line represents the cut-off value.

antibody titres can be achieved in sheep after a single immunization with small amounts of DNA using the Inovio device for genetic immunization (Fig. 5). It should be noted that all sheep had positive antibody titres at some stage during the time course of the experiment.

4. Conclusions

We have here shown that electroporation using a novel injection/electroporation device which injects DNA during insertion of the needles, results in efficient transfection and immune responses in larger animals using mild electrical condition and moderate amounts of DNA. We are now in the process of optimizing parameters such as injection volume and electrical conditions for clinical use.

Acknowledgements

We thank Birgit Wanvik and Liiban Ali Warsame for excellent technical assistance and Agnes Klouman and Sigbjørn Moen for help with handling the sheep.

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