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GENE DELIVERY

DNA electrotransfer into the skin using a combination of one high- and one low-voltage pulse

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Abstract

Electroporation is an effective alternative to viral methods to significantly improve DNA transfection after intradermal and topical delivery. The aim of the study was to check whether a combination of a short high-voltage pulse (HV) to permeabilize the skin cells and a long low-voltage pulse (LV) to transfer DNA by electrophoresis was more efficient to enhance DNA expression than conventional repeated HV or LV pulses alone after intradermal injection of DNA plasmid. GFP and luciferase expressions in the skin were enhanced by HV+LV protocol as compared to HV or LV pulses alone. The expression lasted for up to 10 days. Consistently, HV+LV protocol induced a higher Th2 immune response against ovalbumin than HV or LV pulses. Standard methods were used to assess the effect of electric pulses on skin: the application of a combination of HV and LV pulses on rat skin fold delivered by plate electrodes was well tolerated. These data demonstrate that a combination of one HV (700 to 1000 V/cm; 100 μs) followed by one LV (140 to 200 V/cm; 400 ms) is an efficient electroporation protocol to enhance DNA expression in the skin.

Keywords: Electropermeabilization; Electroporation; Electrotransfer; Skin; Non-viral gene therapy; DNA vaccination; DNA plasmid

1. Introduction

When an electric field is applied to a cell or cell system, a non-uniform transmembrane potential is induced in the exposed cells. If the induced transmembrane potential is above the threshold value, cell membrane becomes permeabilized and thus more conductive. That increases the uptake of some molecules into the cells, such as drugs or DNA. Reversible increase of the cell membrane permeability caused by the electric field is called electropermeabilization or electroporation [1]. Electroporation has been used for different applications, such as electrochemotherapy, transdermal drug delivery and gene transfection. Electrochemotherapy is a treatment of solid tumors

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which combines a cytotoxic non-permeant drug with locally delivered permeabilizing electric pulses. It is very successful in eliminating local tumors, e.g. subcutaneous tumors and is more efficient than the chemotherapy alone [2,3]. Transdermal drug delivery has many advantages over conventional routes of drug administration. However, the barrier properties of the skin limit transdermal drug transport. One of the methods to enhance it is electroporation which causes reversible permeabilization of the outer layer of the skin — the stratum corneum [4,5]. Since the first report of Neumann [6], electroporation has been widely used to introduce small molecules and macromolecules, including DNA, into prokaryotic and eukaryotic cells in vitro. Electroporation is currently one of the most efficient and simple non-viral method of gene transfer in vivo [7].

Skin is an attractive target tissue for gene therapy for a variety of reasons. Its accessibility facilitates in vivo gene delivery. Skin is also a very good target organ for DNA vaccination because of the large number of potent antigen presenting cells, critical to an effective immune response. If necessary, large areas of skin can be treated and can easily be monitored [8]. Beside viral methods that are controversial because of their safety issues, chemical and physical methods have been developed to enhance gene expression in the skin [9]. Electroporation seems particularly effective to improve DNA transfection after intradermal [9–14] and topical [15] delivery without any significant alteration of skin structure.

However, the effect of electrical parameters and electrode design on the efficacy of transfection in the skin and the mechanism of enhancement have not been studied systematically so far. It has been shown for muscle tissue that efficient cell electrotransfection can be achieved using combinations of high-voltage (HV) and low-voltage (LV) pulses. Luciferase-encoding DNA was injected in skeletal muscle and luciferase expression was studied after various pulse combinations. HV pulses alone resulted in a high level of muscle permeabilization (permeabilizing pulse), but very low DNA transfer. However, in combination with one or more LV pulses (electrophoretic pulse), a large increase in DNA transfer occurred [16–19].

We hypothesized that DNA electrotransfer into the skin is also a two-step process consisting in membrane permeabilization and DNA electrophoresis and that a combination of a high-voltage pulse to permeabilize the target cells, followed by a low-voltage pulse to electrophoretically transport the DNA would improve gene transfection in the skin too. Hence, the efficacy of the delivery of DNA in the skin was investigated using a combination of HV+LV pulses in comparison to protocols reported in literature [10–20]. The qualitative and quantitative measure of the expression of two reporter genes in the skin, the kinetics of this expression, intradermal DNA vaccination and skin tolerance using HV+LV pulses were investigated.

2. Materials and methods

2.1. Reporter genes and plasmid injection

The electrotransfer of gene into the skin was evaluated and optimized with two reporter genes, pCMVluc and pCMVGFP. We prepared the plasmids using a Qiagen kit for plasmid purification. The plasmids were injected intradermally (50 μg/25 μl PBS) 30 s before the application of the electric pulses, using a Hamilton syringe with a 27-gauge needle.

2.2. Animals

The animals used in all studies except the vaccination study, were male Wistar rats from Laboratoires Janvier, France, 8–10 weeks old. They were anaesthetized with 700 μl of a mixture of ketamine (100 mg/kg, Ketalar, Panpharma) and xylazine (40 mg/kg, Rompun, Bayer). The skin on the back was shaved 1–2 days prior to the experiments, first with an electric razor, then with a depilatory cream (Veet for sensitive skin) to thoroughly remove all the hair. Shaving allowed a better visualization of DNA injection and electroporated area. We placed 5 to 8 electroporation sites on the back of each rat.

For the vaccination study we used 6-week-old female Balbc mice (Janvier, France). They were anaesthetized with 15 μl of a mixture of ketamine and xylazine. The skin on the back was shaved with depilatory cream (Veet for sensitive skin) 1 day prior to immunization.

2.3. DNA electrotransfer

For the delivery of HV and/or LV pulses, we used a square-wave electropulsator Cliniporator (IGEA, Carpi, Italy). Different protocols, all consisting of one HV pulse (700 or 1000 V/cm 100 μs), followed by one LV pulse (80, 140 or 200 V/cm 400 ms), using no lag between them were tested. We compared these protocols with protocols reported in literature [10–20]. The electric pulses were delivered about 30 s after the intradermal injection of plasmid (50 μg/25 μl PBS), using two parallel, stainless-steel plate electrodes of 0.5 mm thickness and 4 mm distance between them (IGEA, Carpi, Italy). The area of the electrodes in contact with skin was about 1 cm × 1 cm. To assure good contact between the skin fold and the electrodes, a conductive gel (EKO-GEL, ultrasound transmission gel, Egna, Italy) was applied. The electrodes used for the vaccination study were 2.5 mm apart, due to the lower thickness of mouse skin.

2.4. GFP localization

Two days after the electroporation, the rats ($n = 3$ per group) were sacrificed and skin samples were taken. Both the epidermal and dermal sides of the skin were observed without fixation or freezing with a confocal microscope [15]. Two blinded observers evaluated the fluorescence intensity (2 skin samples per rat).

2.5. Luciferase assay

Two days after the electroporation (1, 2, 3, 4, 7, 10, 14, 21, 25 days for the kinetic study), the rats were sacrificed and the electroporated areas of the skin were taken. The skin samples were weighed to 200 mg, cut into pieces and homogenized in 1 ml cell culture lysis reagent solution (10 ml cell culture lysis reagent (Promega) diluted with 40 ml distilled water and supplemented with one tablet of protease inhibitor cocktail (Boeringher Mannheim)). After centrifugation at 12000 rpm for 10 min at 4 °C, we assessed the luciferase activity on 10 μl of the supernatant, using a luminometer, with delay time 3 s and integration time 15 s, starting after the addition of 50 μl of Luciferase Assay Substrate (Promega) to the skin lysate. The results from the luminometer were collected in relative light units (RLU). The final results were expressed as pg of luciferase per mg of tissue by calibration with purified firefly luciferase protein (Sigma).

2.6. Vaccination study

The immune response after delivery of a plasmid coding for an immunogenic model protein ovalbumin (pcDNA 3.1-OVA) was assessed. Mice were injected intradermally with 2 × 15 μl of this plasmid coding for ovalbumin at 2 mg/ml (groups 1–4), ovalbumin at 1 mg/ml (group 5), ovalbumin 1 mg/ml + adjuvant Alum (group 6) and PBS (negative control, group 7). Electric pulses (1 HV + 1 LV pulses in the first two groups and 6 HV pulses [11] in the third group) were applied 30 s after DNA injection. Two and four weeks after the priming, 2 boosts were applied. Blood samples were collected by retroorbital bleeding 2, 4 and 6 weeks after priming.

The humoral immune response i.e. titers of antibodies (IgG) to ovalbumin in the serum was measured by ELISA. Isotypes (IgG1, IgG2a or IgG2b) were determined using appropriate secondary antibodies as described previously [21]. The antibodies were first measured in the pools coming from individual mice in equivalent part. Individual mice responses were measured when a positive response was detected in the pooled sera.

2.7. Tolerance study

Side effects on the skin of one HV and one LV pulses (1000 V/cm 100 μs + 200 V/cm 400 ms) generated by the Cliniporator were investigated by standard methods [22–24]. Skin folds with or without gel with the electrodes applied for as long as needed to deliver pulses (30 s) were used as controls.

As Cliniporator measures the voltage and the current during pulsing, conductivity changes were estimated. Non-invasive bioengineering methods were used to evaluate in vivo if electroporation induced a trauma in the skin (transepidermal water loss TEWL, chromametry). Histology was used to investigate the effect on the skin structure. TEWL measurement is a non-invasive method for assessing the skin barrier function. The probe of the Tewameter TW 210 (Germany) was placed on the electroporation site and the measurements were taken

Table 1
The results of the immunization study

Group	Injection	Electroporation	Week 4		Week 6	
			IgG[a]	Responder[b]	IgG[a]	Responder[b]
1	DNA	700 V/cm 100 μs+200 V/cm 400 ms	3.41 ± 0.44	8/8	3.49 ± 0.40	5/5
2	DNA	700 V/cm 100 μs+200 V/cm 400 ms 4.2 s lag	3.24 ± 0.35	5/5	4.14 ± 0.13	5/5
3	DNA	6 × 1750 V/cm 100 μs (8 Hz)	2.61 ± 0.16	3/7	3.03 ± 0.29	7/7
4	DNA	/	2.80 ± 0.42	2/7	2.86 ± 0.45	8/8
5	OVA	/	3.39 ± 0.64	8/8	3.49 ± 0.38	8/8
6	OVA+Alum	/	4.0 ± 0.14	6/6	4.45 ± 0.2	5/5
7	PBS	/	/	/	/	/

The results are expressed in: a) Mean IgG titer (± S.D.) in responding mice determined by ELISA in individual mice, and b) number of mice showing IgG titers higher than the background values.

when TEWL values stabilized. TEWL values are expressed in g/m^2 h. Skin color and erythema were measured by Minolta Chromameter CR-200 (Minolta, Japan) calibrated using a white calibration tile. During measurements the apparatus was perpendicularly kept to the skin surface. The measurements were taken right before, right after the delivery of pulses, 30, 60, 120 min and 24 h after pulsing. We separately measured the anode and the cathode side of each electroporation site. For the histology study, the tissue was fixed in a 4% formalin solution for at least a week and embedded in paraffin wax. Sections 3 μm thick were cut perpendicularly to the surface of the skin and stained with hematoxylin–eosin.

2.8. Statistical analysis

For the statistical analysis of the results, we used Sigma Stat for Windows, version 2.0, Jandel Corporation. When normality test over the experimental groups failed, the data was represented with a median (horizontal line), 25th and 75th percentile (grey box) and 10th and 90th percentile (error bars). Black dots represent all the outliers. The ANOVA on ranks and Dunnet's test were used to compare different protocols.

3. Results

3.1. GFP expression in the skin

To localize the expression of a gene in the skin after intradermal injection of a plasmid followed by electroporation and to compare the efficacy of different pulsing protocols to enhance this gene expression, a plasmid coding for GFP was used as a reporter gene [15].

The control epidermis showed some autofluorescence of the hair follicles (see Fig. 1a, d, f) but no fluorescence in the dermis (data not shown). After intradermal injection of the plasmid without electroporation, a very slight and diffuse fluorescence was observed in some area of the dermis (Fig. 1b).

When only one HV pulse (1000 V/cm 100 μs) (Fig. 1c) or only one LV pulse (200 V/cm 400 ms) (Fig. 1d–e) was applied, the expression of GFP remained very low both in the epidermis (Fig. 1d) and to a lesser extent in the dermis (Fig. 1c, e).

The expression of GFP was enhanced by a combination of one HV pulse (1000 V/cm 100 μs) and one LV pulse (80, 140 or 200 V/cm 400 ms). The fluorescence was elevated in some part of the dermis (Fig. 1g, h). Expression in the epidermis was also observed (Fig. 1f). A semi-quantitative analysis indicates that 140 or 200 V/cm pulses were more efficient than 80 V/cm LV pulses and that the expression at the anodal side was slightly higher than expression at the cathodal side.

3.2. Luciferase expression in the skin

To confirm that the combination of one HV and one LV pulse is more efficient than HV or LV pulses alone, a quantitative study with another reporter gene coding for luciferase, was conducted. The efficiency of the HV and LV pulses to enhance gene transfer in rat skin was investigated in order to find the most efficient combination of the high- and low-voltage pulses delivered by the Cliniporator. As shown in Fig. 2, two HV+LV protocols (1: 1000 V/cm 100

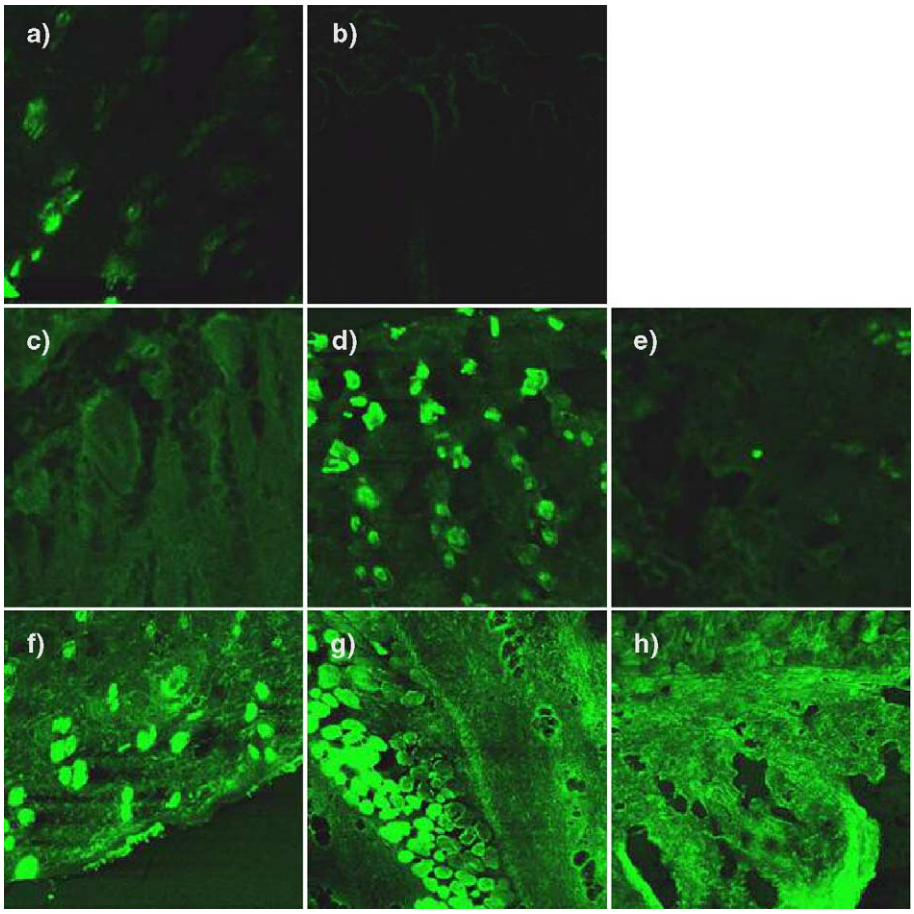

Fig. 1. Expression of GFP in the epidermis (a, d, f) or in the dermis (b, c, e, g, h) after intradermal injection of 50 μg of a plasmid coding for GFP; (a–b) no electroporation; (c) 1000 V/cm 100 μs pulse; (d, e) 200 V/cm 400 ms; (f, g) 1000 V/cm 100 μs+140 V/cm 400 ms; (h) 1000 V/cm 100 μs+200 V/cm 400 ms.

μs+140 V/cm 400 ms, or 2: 700 V/cm 100 μs+200 V/cm 400 ms) were compared with those reported in literature so far [10–20], consisting of 6 HV pulses (6×1750 V/cm 100 μs) (3) or 6 LV pulses (6×250 V/cm 20 ms) (4). Four control groups were included in the study. In the first control group the rats were only administered plasmid DNA intradermally without electroporation (5). In the second and third control groups the intradermal injection was followed by a single HV pulse (6) or by a single LV pulse (7). Basal luciferase activity of skin samples was also measured (8).

The application of electrical pulses increased luciferase expression: higher expressions were detected in protocols 1 to 4 as compared to the protocols 5–8 ($p<0.05$). HV+LV pulses (protocols 1 and 2) induced a statistically significant higher luciferase expression than repeated HV pulses or LV pulses (protocols 3 and 4).

3.3. Kinetics of the luciferase expression

To follow the kinetics of gene expression in the skin, the expression of the gene coding for luciferase was measured between day 1 and day 25 after application of HV+LV pulses (700 V/cm 100 μs+200 V/cm 400 ms). The control group was injected with plasmid DNA intradermally, without electroporation.

The kinetic study shows the highest expression on the first day after the electroporation and a rapid drop towards the 4th day. A low expression can be seen for up to about 10 days after. In the control group of

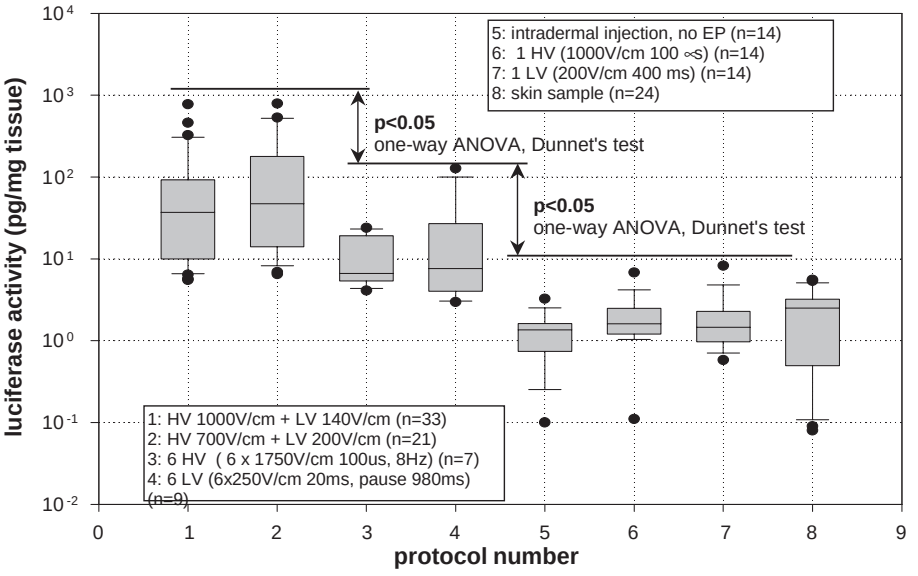

Fig. 2. Luciferase expression after different electroporation protocols: Group 1: HV+LV (1000 V/cm 100 μs+140 V/cm 400 ms); Group 2: HV+LV (700 V/cm 100 μs+200 V/cm 400 ms); Group 3: 6 HV (6×1750 V/cm 100 μs, 8 Hz); Group 4: 6 LV (6×250 V/cm 20 ms, pause 980 ms); Group 5: DNA injection, no electroporation; Group 6: 1 HV (1000 V/cm 100 μs); Group 7: 1 LV (200 V/cm 400 ms); Group 8: skin sample. All groups except group 8 were injected intradermally with 50 μg of pCMVluc. Results are represented on a log scale with pg of luciferase per mg of tissue. Normality test over the experimental groups failed, so the data is represented with a median (horizontal line), 25th and 75th percentile (grey box) and 10th and 90th percentile (error bars). Black dots represent all the outliers.

intradermal injection alone, without electroporation, some expression can be seen the first day after the injection, then the expression dropped close to zero already the second day (Fig. 3). A statistical difference ($p<0.05$) between electroporated rats and control rats was observed at days 2, 3, 4 and 7.

These data suggest that skin DNA electrotransfer would be more appropriate for short term treatment of the skin or for immunization.

3.4. Immunization study

As skin is also a very attractive target tissue for DNA vaccination and as gene expression in the skin is rather pulsed, an immunization study using a plasmid coding for a model antigen ovalbumin was performed [21]. HV+LV protocols were compared to electroporation protocols described in the literature [10–20]. Two and four weeks after the priming, two boosts were applied.

IgG responses were detected in the mice immunized with ovalbumin or the plasmid coding for ovalbumin whereas no response was observed in the control group 7. Application of electric pulses increased the immune response (groups 1, 2, 3 versus group 4, $p<0.05$). The groups receiving HV+LV protocols had a higher immune response (groups 1, 2) than the group treated with 6 HV pulses (group 3) ($p<0.05$). The IgG levels after DNA electrotransfer with HV+LV were equivalent to the IgG level in mice immunized with ovalbumin but lower than in mice immunized with ovalbumin and the standard adjuvant alum (Table 1).

No IgG2a and IgG2b response was detected (except for 2 mice in group 1), suggesting that only a Th2 response was induced.

3.5. Tolerance of the skin to HV+LV pulses

The trauma on the skin induced by HV+LV electroporation (1000 V/cm 100 μs+200 V/cm 400 ms) was investigated by non-invasive bioengineering methods to check if the pulses induce an erythema (chromametry) or impairment of barrier function (transepidermal water loss) and by histology to investigate the effect on the skin structure [22–24].

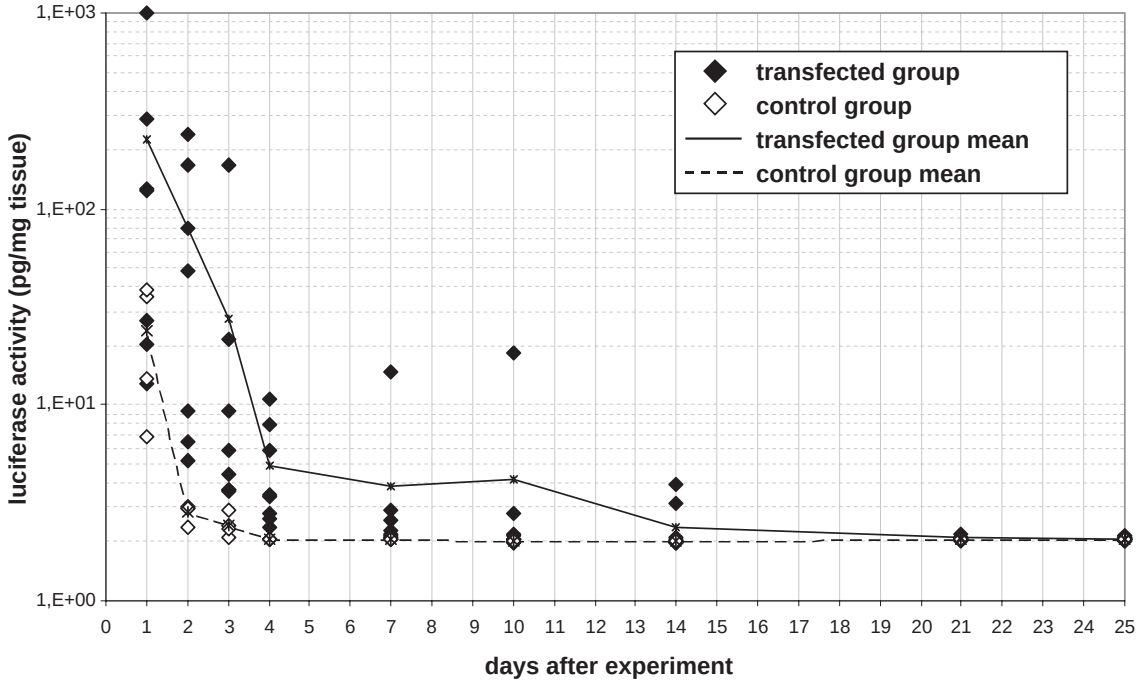

Fig. 3. Kinetics of luciferase expression in the skin after intradermal injection of 50 μg pCMVluc. (♦) Electroporation, 700 V/cm 100 μs+200 V/cm 400 ms ($n=8$); (◇) No electroporation ($n=4$). The data is represented with mean values.

A slight muscle contraction was observed after the HV pulse. Changes in conductivity were measured during pulsing. They varied from one animal to another and depended on the voltage amplitude used. The higher the voltage, the higher the conductivity change (or the lower the skin resistance). For exam-

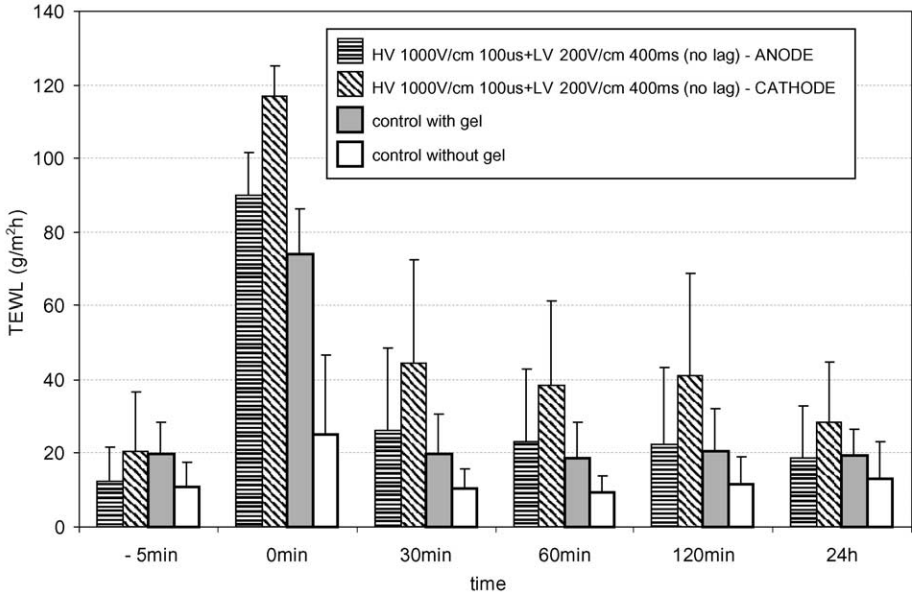

Fig. 4. Transepidermal water loss (TEWL) values in rat skin as a function of time after electroporation.

ple, the conductivity during a 100 μs pulse at 1000 V/cm increased up to 50%.

An increase in the TEWL values right after the delivery of the pulses can be observed (Fig. 4) but the controls where we applied conductive gel also had high TEWL values, suggesting that the high TEWL values resulted from evaporation of gel water from the skin surface. Although electroporation does disrupt the skin barrier as evidenced by the low skin resistance, the change in TEWL was small and short. TEWL values were down to normal within 30 min. TEWL values on the anode side of the electroporation side were consistently lower than the ones on the cathode side.

Values of parameter a (redness of skin) indicate that no erythema was induced by HV+LV pulses (data not shown). During the experiments we noticed some redness with the naked eye. The electroporation site turned red a few minutes after pulsing but it was a short-term change. There was a consistent difference between the anode and the cathode side of the electroporation sites. The reason why we did not record that with the chromameter right after pulsing might be the vascular lock in the minutes following the electroporation [25].

No damage in the histological structure of the skin was observed. Neither inflammation nor necrosis was detected 24 h after application of HV+LV pulses.

4. Discussion

The efficiency of combination of HV+LV pulse on DNA electrotransfer in skin was investigated. As already proposed for gene transfection in skeletal muscle [16–19], the rationale for this electroporation protocol was that the first, high-voltage pulse results in a high level of cell permeabilization (permeabilizing pulse), while the second, low-voltage pulse provides a driving force for transport of DNA into cells (electrophoretic pulse). So far the protocols reported in literature for DNA electrotransfer into the skin consisted of 6 to 8 HV pulses or of 6 to 16 LV pulses [10–15]. To check if the combination of 1 HV+1 LV pulses enhances gene expression in skin, both a localization of GFP expression and a quantitative measure of luciferase activity were evaluated. An enhanced expression of GFP in the skin was observed when a combination of HV+LV was used. One HV or one LV alone resulted in a very low transfection, comparable to the one of the control group where no pulse was applied whereas GFP expression in the epidermis and dermis was enhanced by the combination of HV+LV pulses. The quantification of luciferase activity further supports the hypothesis that HV+LV pulses are more efficient than several HV or LV pulses. Indeed, luciferase activity was enhanced by 2 orders of magnitude when a combination of HV+LV pulse was applied whereas a low expression was detected after application of 6 HV or 6 LV pulse only. These data confirm the hypothesis that as reported for the muscle [16–19], the association of a HV+LV is more efficient for DNA electrotransfer in the skin than repeated HV or LV. This also demonstrates that both the permeabilizing HV pulse and the electrophoretic LV pulses are required.

Luciferase expression in the skin varied over a factor of 100 after electrotransfer. This could partly be attributed to variable electrical properties of the skin or variable biological responses to DNA injection. Alternatively, the variation could be caused by experimental conditions e.g. DNA injection.

Kinetic study of the luciferase expression showed the highest expression on the first days after the experiment, then it dropped rapidly as reported previously for other delivery methods [8]. Hence, skin DNA electrotransfer would be more adapted to a short-term gene expression in the skin for immunization or skin treatment than for the secretion of a therapeutic protein in the blood.

The combination of HV+LV pulse was also tested for efficiency in skin immunization. This immunization experiment demonstrated that i) electroporation enhances the immune response induced after intradermal injection of a DNA plasmid coding for an antigen [11,14] ii) our protocols (1 HV+1 LV pulse) are more efficient than protocols (6 to 8 HV pulses) previously described.

The proposed protocols have proven to be efficient, so the safety aspect of such electric pulses was also studied. Side effects were investigated by standard methods. Some disruption of the skin barrier function due to electroporation was observed as evidenced by the low skin resistance, but the change in TEWL values was small and transient. A slight and transient erythema was observed visually but not by chromametry. The erythema and TEWL values on the anode

site of the electroporation site were consistently lower than the ones on the cathode side. The reasons for the difference are unknown but could be attributed to local changes in ion concentration or pH imbalance. These data confirm that the application of HV and/or LV pulses on rat skin forming a fold between two plate electrodes is well tolerated [22–24].

In conclusion, the combination of one high-voltage pulse to permeabilize the skin cells followed by one low-voltage pulse to transfer DNA enhances DNA transfection in the skin, compared to the protocols used so far. Such HV+LV pulses also have no major effects on skin for the voltage amplitudes used.

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References

[1] J.C. Weaver, Y.A. Chizmadzhev, Theory of electroporation: a review, Bioelectrochem. Bioenerg. 41 (1996) 135–160.
[2] L.M. Mir, S. Orlowski, Mechanisms of electrochemotherapy, Adv. Drug Deliv. Rev. 35 (1999) 107–118.
[3] R. Heller, R. Gilbert, M.J. Jaroszeski, Clinical applications of electrochemotherapy, Adv. Drug Deliv. Rev. 35 (1999) 119–129.
[4] A.-R. Denet, R. Vanbever, V. Préat, Skin electroporation for transdermal and topical delivery, Adv. Drug Deliv. Rev. 56 (2004) 659–674.
[5] M.R. Prausnitz, A practical assessment of transdermal drug delivery by skin electroporation, Adv. Drug Deliv. Rev. 35 (1999) 61–76.
[6] E. Neumann, M. Schaefer-Ridder, Y. Wang, P.H. Hofschneider, Gene transfer into mouse lyoma cells by electroporation in high electric fields, EMBO J. 1 (7) (1982) 841–845.
[7] S. Somiari, J. Glasspool-Malone, J.J. Drabick, R.A. Gilbert, R. Heller, M. Jaroszeski, R.W. Malone, Theory and in vivo application of electroporative gene delivery, Mol. Ther. 2 (3) (2000) 178–187.
[8] V. Préat, N. Dujardin, Topical delivery of nucleic acids in the skin, STP Pharma Sci. 11 (2001) 57–68.
[9] S. Mehier-Humbert, R.H. Guy, Physical methods for gene transfer: improving the kinetics of gene delivery into cells, Adv. Drug Deliv. Rev. 57 (5) (2005) 733–753.
[10] J. Glasspool-Malone, S. Somiari, J. Drabick, R.W. Malone, Efficient nonviral cutaneous transfection, Mol. Ther. 2 (2) (2000) 140–146.
[11] J.J. Drabick, J. Glasspool-Malone, A. King, R.W. Malone, Cutaneous transfection and immune responses to intradermal nucleic acid vaccination are significantly enhanced by in vivo electropermeabilization, Mol. Ther. 3 (2) (2001) 249–255.
[12] L. Zhang, E. Nolan, S. Kreitschitz, D.P. Rabussay, Enhanced delivery of naked DNA to the skin by non-invasive in vivo electroporation, Biochim. Biophys. Acta 1572 (2002) 1–9.
[13] M. Lee Lucas, L. Heller, D. Coppola, R. Heller, IL-12 plasmid delivery by in vivo electroporation for the successful treatment of established subcutaneous B16.F10 melanoma, Mol. Ther. 5 (6) (2002) 668–675.
[14] L. Zhang, G. Widera, D. Rabussay, Enhancement of the effectiveness of electroporation-augmented cutaneous DNA vaccination by a particulate adjuvant, Bioelectrochemistry 63 (1–2) (2004) 369–373.
[15] N. Dujardin, P. Van Der Smissen, V. Préat, Topical gene transfer into rat skin using electroporation, Pharm. Res. 18 (1) (2000) 61–66.
[16] L.M. Mir, M.F. Bureau, R. Rangara, B. Schwartz, D. Scherman, Long-term, high level in vivo gene expression after electric pulse-mediated gene transfer into skeletal muscle, Med. Sci. 321 (1998) 893–899.
[17] L.M. Mir, M.F. Bureau, J. Gehl, R. Rangara, D. Rouy, J.-M. Caillaud, P. Delaere, D. Branellec, B. Schwartz, D. Scherman, High-efficiency gene transfer into skeletal muscle mediated by electric pulses, Proc. Natl. Acad. Sci. U. S. A. 96 (8) (1999) 4262–4267.
[18] M.F. Bureau, J. Gehl, V. Deleuze, L.M. Mir, D. Scherman, Importance of association between permeabilization and electrophoretic forces for intramuscular DNA electrotransfer, Biochim. Biophys. Acta 1474 (2000) 353–359.
[19] S. Satkauskas, M.F. Bureau, M. Puc, A. Mahfoudi, D. Scherman, D. Miklavcic, L.M. Mir, Mechanisms of in vivo DNA electrotransfer: respective contributions of cell electropermeabilization and DNA electrophoresis, Mol. Ther. 5 (2) (2002) 133–140.
[20] R. Heller, M. Jaroszeski, A. Atkin, D. Moradpour, R. Gilbert, J. Wands, C. Nicolau, In vivo gene electroinjection and expression in rat liver, FEBS Lett. 389 (3) (1996) 225–228.
[21] C. Lombry, A. Marteleur, M. Arras, D. Lison, J. Louhahed, J.C. Renauld, V. Préat, Local and systemic immune responses to intratracheal instillation of antigen and DNA vaccines in mice, Pharm. Res. 21 (1) (2004) 127–135.
[22] R. Vanbever, D. Fouchard, A. Jadoul, N. De Morre, V. Préat, J.P. Marty, In vivo non-invasive evaluation of hairless rat skin after high-voltage pulse exposure, Skin Pharmacol. Appl. 11 (1998) 23–34.
[23] N. Dujardin, E. Staes, Y. Kalia, P. Clarys, R. Guy, V. Préat, In vivo assessment of skin electroporation using square wave pulses, J. Control. Release 79 (1–3) (2002) 219–227.
[24] R. Vanbever, V. Préat, In vivo efficacy and safety of skin electroporation, Adv. Drug Deliv. Rev. 35 (1999) 77–88.
[25] J. Gehl, T. Skovsgaard, L.M. Mir, Vascular reactions to in vivo electroporation: characterization and consequences for drug and gene delivery, Biochim. Biophys. Acta 1569 (2002) 51–58.