Transdermal Delivery of Macromolecules Using Skin Electroporation

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Received September 27, 1999; accepted October 9, 1999

Purposes. (1) To evaluate the feasibility of transdermal delivery of macromolecules by skin electroporation. (2) To assess the influence of the molecular weight of the permeant on transport and examine whether there exists a "cut-off" value of molecular weight. (3) To localize the transport pathways of the macromolecules in the skin.
Methods. FITC-dextran (FD) of increasing molecular weight (4.4, 12 and 38 kDa) were used as model macromolecules to study the extent of transport across hairless rats skin *in vitro* and to localize their distribution in the skin by confocal scanning laser microscopy.
Results. Electroporation enhanced the transport of the macromolecules as compared to passive diffusion. The transdermal delivery by skin electroporation of FITC and FD 4.4 was equivalent whereas transport of higher molecular weight FD was lower but significant. FITC and FD 38 were observed in the epidermis both around and in the keratinocytes.
Conclusions. Transdermal and topical delivery of macromolecules of at least 40 kDa can be achieved by skin electroporation.

KEY WORDS: electroporation; transdermal drug delivery; macromolecules; FITC-dextrans, topical drug delivery.

INTRODUCTION

While biotechnology produced macromolecules such as proteins or nucleic acids show great therapeutic promise, their delivery can be a significant impediment. They generally have low oral bioavailability, and often have short biological half-times. For chronic diseases, new methods of delivery should be developed to address these problems.

Transdermal drug delivery is an useful alternative to conventional routes of administration such as oral or injectable routes. It avoids degradation in the gastrointestinal tract and first pass hepatic metabolism. Transdermal administration allows steady or time varying controlled delivery and improves patient compliance. However, very few drugs can be administered transdermally due to the low permeability of the skin. For charged or polar molecules and for macromolecules, delivery of therapeutic quantities across the skin is difficult. Chemical and physical approaches to increase transdermal transport have been explored to enhance skin permeability and expand the range of drugs which can be delivered transdermally (1–3).

Recently, the intermittent application of short (e.g., msec) high voltage (e.g., 100 to 1000 V) pulses, i.e., electroporation, has been shown to increase the transport of molecules across skin by several orders of magnitude (4–5). Although the molecular mechanism by which electric fields interact with lipid bilayers is still incompletely understood, it is generally accepted that the application of an electric pulse creates transient aqueous pathways within the lipid bilayers including the stratum corneum (4–6). Skin electroporation has been shown to be an effective method for delivering transdermally dyes (calcein, sulforhodamin), drugs (metoprolol, fentanyl, LHRH) and macromolecules (heparin, oligonucleotides) through the skin (4,5,7–11). Furthermore, electroporation also allows control on the transdermal flux (4,5,7,11).

Transdermal delivery of peptides (<6 kDa) can be enhanced by skin electroporation (8). Transdermal transport of heparin (12 kDa) was increased in vitro by skin electroporation to potential therapeutic rates (9). Transdermal and topical delivery of oligonucleotides (5 kDa) can also be enhanced by skin electroporation (10–12). Moreover, high voltage pulses are known to transport macromolecules across cell membrane. Electroporation (also called electropermeabilisation) is a very efficient method for DNA transfection. Besides its extensive use for DNA transfection in cells in culture, in vivo electroporation in the presence of DNA plasmid can induce gene expression in several organs (13).

If macromolecules delivery by high voltage pulses has been reported, the influence of the molecular weight on delivery by skin electroporation has never been systematically investigated. The aims of this study were: 1) to evaluate the feasibility of transdermal delivery of macromolecules by skin electroporation, 2) to assess the influence of the molecular weight of the permeant, and examine whether there exists a "cut-off" value of molecular weight for drug delivery by electroporation, 3) to localize the transport pathways of the macromolecules in the skin. A series of FITC-dextran of increasing molecular weight (4.4 kDa, 12 kDa and 38 kDa) (FD 4.4, FD 12 and FD 38) were used as model macromolecules because these fluorescent permeants allow both the quantification of transdermal transport by fluorescence spectroscopy and the localisation in the skin by fluorescence microscopy.

MATERIALS AND METHODS

Chemicals

Fluorescein isothiocyanate (FITC) (isomer I; MW 389,4) and Fluorescein isothiocyanate-dextran (FD) with average molecular weights of 4400 (FD 4.4), 12000 (FD 12) and 38260 (FD 38) respectively were bought from Sigma Chemical Company (Saint-Louis, MO, USA). The FITC labelling ranged from 0,011 to 0,05 mole FITC/mol glucose. The salts used to prepare the buffers (for analysis) were obtained from UCB (Drogenbos, Belgium). Glucose (analytical grade) was bought from Merck-Belgado (Overijse, Belgium). Ultrapure water was used to prepare all aqueous solutions.

In Vitro Model of Permeant Transport

The experimental protocol for transdermal delivery has been described previously. Vertical diffusion cells made of two compartments separated by a freshly excised abdominal skin of 2–3 month-old hairless male rat (mutant Iops rat hairless;

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0724-8741/00/0100-0032$18.00/0

Iffa Credo, St Germain les Arbreles, France) (3 cm^2) were used. The donor compartment (1,5 ml) contained the permeant solution (FITC (250 μM) or FD (250 μM)) in HEPES buffer (0,04 M) at pH 7. The receiver solution (7,5 ml) was a phosphate buffer pH 7,4 (0,024 M) made isotonic with glucose. The receiver was thermoregulated at 37°C and continuously stirred magnetically. Pt electrodes (1 cm^2) were used, the cathode being placed in the donor compartment and the anode in the receiver compartment. The interelectrode distance was 1 cm (5,7).

Electroporation

The electrodes were connected to Easyject Plus (Equibio Ltd., Kent, England), or PulseAgile Model PA-4000 (CytoPulse Sciences, Columbia, USA), equipments used for electroporating bacterial and other cell membranes.

Easyject Plus delivers exponentially decaying (ED) capacitive discharge pulses. The pulse time constant is the length of time between the beginning of the pulse (maximum voltage) and the time when the voltage reaches 37% of its maximal value. This pulse length is measured by Easyject Plus. It depends on the electrical circuit resistance (composed of the shunt resistance of Easyject Plus and of the apparent resistance of the diffusion chamber) and the capacity of the electroporation apparatus: pulse time = resistance × capacity. 10 pulses of 150 V were generated with a 2310 Ω resistance and a 450 μF capacity to get a pulse time constant of approx 150 msec. The pulses were separated by 30 sec (5,7,11).

Pulse Agile produces square wave (SW) pulses. To transfer the same amount of charges, 891 pulses of 150 V with a pulse time 1.68 msec separated by 0.125 sec were used (SW S). We have also used 10 pulses of 150 V, with a pulse time 150 msec separated by 30 sec (SW L). Pulse voltage, pulse time and current were controlled with an oscilloscope (Hewlett Packard 54602B, USA) placed in parallel. The amount of charges transfered and energy applied were calculated as previously described (7,11,14). The transdermal voltages were approximately 30% of the applied voltage.

Measurement of the Extent of Transport

After skin electroporation, samples (0,4 ml) were taken from the receiver compartment at regular intervals for 6 h and were replaced with an equal volume of the receiver solution (5,7). The fluorescence in each sample was measured by luminescence spectrometer (Perkin Elmer Ltd. LS50B, Beaconsfield, Buckinghamshire, England). The ratio of the cumulative quantities detected in the receptor compartment to the membrane area was plotted as a function of time. The flux was calculated by linear regression. Even though it was very low, the "quenching" phenomenon or the fluorescence extinction of the different molecules was taken in account during the 6 h of the experiment.

Localisation of FITC or FD in the Skin

Standard Fluorescence Microscopy

To determine the permeation of the macromolecules into the skin after electroporation, the skin samples were snap frozen in isopentane cooled by liquid nitrogen and kept at −80°C before sectioning. Cryostat sections, 20 μm thick, were performed perpendicular to the skin surface with a Jung Cryostat. Sections were mounted without fixation with glycerol:water (9:1) with 0.002% 33258 Hoechst (W/V) for cell staining. The cross sections were viewed and photographed with a Leitz Orthoplan microscope equipped with Orthomat for microphotography. Sections were photographed using two different filters (λ_{exc} = 450–490 nm or 340–380 nm), to visualize FITC or Hoechst 33258, respectively. All photographs were taken with 10× or 25× objectives and exposure time of 30–90 sec (12).

Confocal Laser Scanning Microscopy (CLSM)

CLSM was chosen to localize FITC and FD in the epidermal cells as this technique allows localisation of fluorescent molecules without shrinkage or distortion of the living tissue (15,16). The skin samples were directly placed in a sample holder (epidermis upwards) and covered with a coverslip glass. The confocal microscope used was a BioRad MRC 1024 confocal unit equipped with an argon-krypton laser and mounted on a Zeis Axiovert 13SM inverted microscope. The FITC and FD were detected using a BioRad filter block, which selects the 488 nm laser line to illuminate the specimen, and transmits emitted light with a wave length in the range 522–535 nm. Images were acquired with a Zeiss Plan-Neofluor 40× oil immersion lens. Optical sectioning was performed parallel to the skin surface (xy planar optical section), at different focal planes (Z = −2.5 to −55 μm), the z axis being perpendicular to the plane of the skin surface. The skin surface (Z = 0 μm) is defined by the user as the brightest fluorescence image with a morphology characteristic of the stratum corneum surface. All images were the average of 3–7 scans, and were obtained with the same optical aperture, filter block, lens, block level and scan speed. Different laser intensities were used as function of the emission intensity (12).

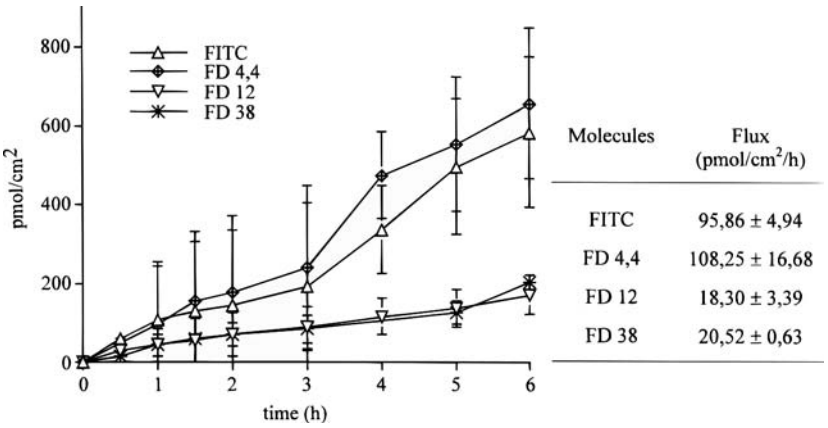
To investigate the autofluorescence properties of the skin, samples were first investigated with the confocal microscope in the absence of FITC. The autofluorescence of rat skin was found to be very low with the confocal settings used in this study (data not shown).

Stability of the Permeants

To determine whether electrolytic or metabolic degradation of the FITC labelled dextrans occurred, size exclusion gel chromatography with a Sephadex G25 column (Pharmacia, Brussels, Belgium) was used to analyse the FD solutions 1) before and after exposure to high voltage pulses, 2) after a 6 hours contact with the skin both in the donor and receptor compartment. 30 fraction of 0.4 ml were collected. The fluorescence in the collected fraction was measured as previously described.

Statistical Analysis

The results are expressed as mean ± the standard error of the mean (sem, n = 3 to 4). The cumulative quantities detected in the receiver compartment to the membrane area were compared by one way analysis of variance (Anova). The fluxes were compared by the student t-test (* $p < 0.05$ or ** $p < 0.01$).



Molecules	Flux (pmol/cm²/h)
FITC	95,86 ± 4,94
FD 4,4	108,25 ± 16,68
FD 12	18,30 ± 3,39
FD 38	20,52 ± 0,63

Fig. 1. Cumulative quantities and fluxes of FITC, FD 4,4, FD 12 and FD 38 detected in the receptor compartment as a function of time, after exponentially decaying electroporation (10 * 150V–150 msec). donor solution: 250 μM.

RESULTS

Influence of the Molecular Weight on Transport

To evaluate the influence of the molecular weight on the transdermal delivery by skin electroporation, the transport across skin of FITC and FITC-dextran of increasing molecular weight (FD 4.4; FD 12 and FD 38) was quantified.

The transdermal transport of FITC or FD by passive diffusion was below the limit of detection (10^{-12} M) whereas FITC and FD were detected in the receptor compartment after skin electroporation, confirming that electroporation enhances significantly transdermal delivery. As shown in Fig. 1, the cumulative amount of FITC and FD 4.4 delivered into the receptor solution were equivalent ($p > 0.05$) whereas the extent of transport of higher molecular weight FD was lower ($p < 0.01$). The fluxes were one order of magnitude lower for the FD 12 and FD 38 than FITC and FD 4.4. A "cut-off" limit was not reached since significant transport occurred. These data show that transdermal delivery of macromolecules of at least 40 kDa can be achieved by skin electroporation.

As previously reported, macromolecules such as heparin or dextran can enhance electroporation-assisted delivery (9,17,18). It has been hypothesized that these molecules could be entrapped in the aqueous pathways created by electroporation therefore inducing a sustained increase in skin permeability (18). Hence, as for heparin (9), elevated FD transport could occur after pulsing, explaining why a significant transport of FD 12 and FD 38 was observed. To check this hypothesis, we compared the transdermal transport of FITC in the presence or absence of dextran (MW 12 kDa). The data shown in Fig. 2 demonstrate that the macromolecule dextran enhances FITC transport.

Influence of the Pulse Form on the Extent of Transport

Both exponentially decaying pulses (ED) and square wave pulses (SW) have been used for transdermal drug delivery, DNA transfection or electrochemotherapy (4,5,13,18). Two main type of protocols have been used for transdermal drug delivery: intermittent short (1 ms) high voltage pulses and a few medium voltage pulses. Previous studies showed that long medium voltage ED pulses were more efficient than short high voltage ED pulses to enhance transdermal drug delivery (14). Other studies have compared exponentially decaying (ED) pulses and square wave (SW) pulses applied at equal energy (7,12). Transdermal delivery was lower for the SW pulses than for ED pulses. In order to check if the pulse waveform influences the extent of transport of macromolecules, transdermal delivery of FITC and FD by ED or SW pulses were compared. As electrophoresis is the main mechanism involved in transdermal permeation by skin electroporation, we compared SW and ED using the same amount of charges transferred by the electroporation protocols tested (14).

Long square wave pulses (SW L) (10 pulses of 150 V with a pulse time 150 msec separated by 30 sec), short square wave (SW S) (891 pulses of 150 V with a pulse time 1,68 msec separated by 0.125 sec) and exponentially decaying (ED) (10 pulses of 150 V with a pulse time ± 150 msec separated by 30 sec) pulses were applied. They transferred the same amount of charges (0.63 C) and had the same voltage (150 V).

ED and SW (SW S and SW L) pulses induced the same transport of FITC ($p > 0.05$) or FD 12 ($p > 0.05$) (Fig. 3). These results demonstrate that when the same amount of charges is transferred by high voltage pulses, the pulse waveform does not influence the delivery of macromolecules.

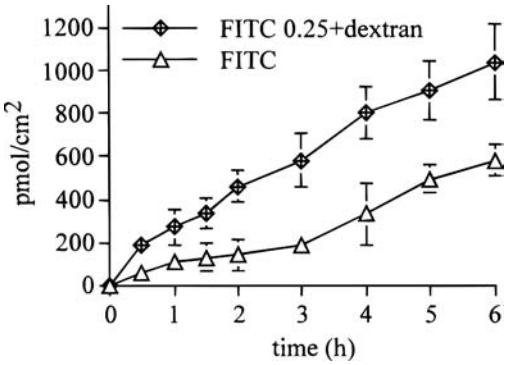


Fig. 2: Cumulative quantities of FITC detected in the receptor compartment as a function of time after exponentially decaying electroporation (10 * 150V–150 msec). donor solution: FITC (250 μM) or FITC (250 μM) + Dextran 12 kDa (250 μM).

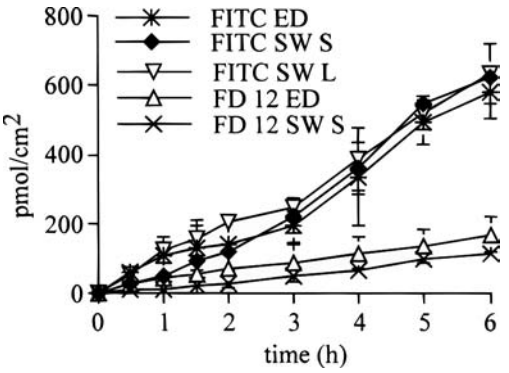


Fig. 3: Cumulative quantities of FITC and FD 12 after exponentially decaying (ED), square wave short (SW S) and square wave long (SW L) electroporation (ED = 10 * 150V–150 msec separated by 30 sec; SW S = 891 * 150 V–1.68 msec and separated by 0.125 sec; SW L = 10 * 150V–150 msec separated by 30 sec).

Macromolecules Localization in the Skin

To evaluate the depth of permeation of FITC and FD in the skin, cryostat sections stained with 33258 Hoechst were viewed at two wavelengths by standard fluorescence microscopy. FITC and FD 38 did not penetrate through the stratum corneum by passive diffusion. After skin electroporation, FITC was observed in the epidermis and in the dermis whereas for FD 38, the fluorescence was mainly recovered in the epidermis and less in the dermis (data not shown). As reported previously, the transport of FD 38 was localized in regions (LTRs) (12,20–22). The transport of FITC was more homogeneous: due to its hydrophobicity, FITC could bind to the stratum corneum.

These results confirm that the macromolecules administered by electroporation are less transported through the skin than FITC. However, as reported previously for oligonucleotides (MW 5kDa), the transport of the macromolecules in the skin is feasible.

CLSM was previously used to visualize the iontophoretic passage of fluorescent molecules (15) or to study the influence of molecular weight on iontophoretic transport (16), as well as to study the localized transport regions (LTRs) after skin electroporation (12,20–21). CLSM was used to evaluate the distribution of FITC and FD 38 at the cellular level in the viable epidermis immediately after electroporation.

FITC was only found at the level of the stratum corneum after passive diffusion (data not shown). Immediately after electroporation of intact skin, FITC and FD were detected at the level of the epidermis (large hexagonal cells) and for FITC, up to the basal cells (small cells). FITC and FD 38 were seen in the epidermis both around the cell membranes and in the cells (Figs. 4 and 5). In contrast to the oligonucleotides (12), the fluorescence was not found in the nucleus but remained in the cytosol of the keratinocytes (Figs. 4 and 5). These results demonstrate that high voltage pulses permeabilize both the stratum corneum and the keratinocytes and could be used to deliver topically macromolecules.

Stability of the Fluorescent Permeants

The electrolytic or metabolic degradation of the labelling of dextran by FITC was controlled with a Sephadex G25 exclusion column. For FITC, the fluorescence was found from the

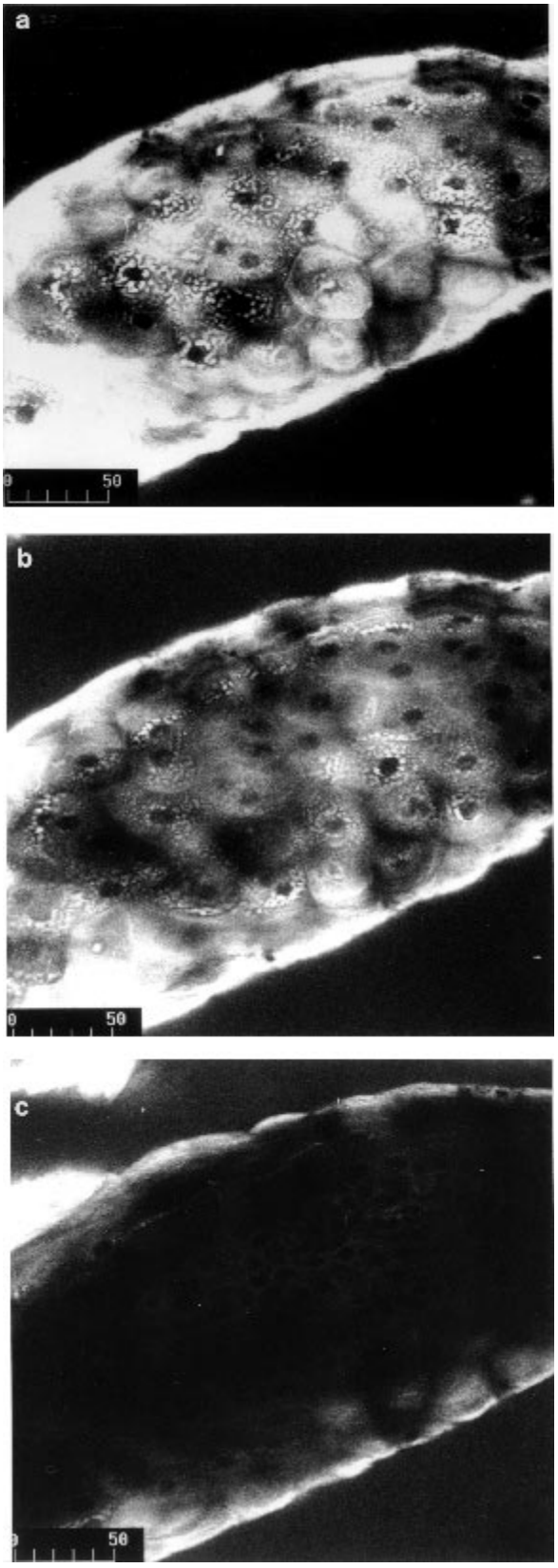


Fig. 4: xy-planar LSCM sections showing the distribution of FITC in intact skin immediately after electroporation (SW L). a: Z = 27 μm. Scale bar = 50 μm. b: Z = 36 μm. Scale bar = 50 μm. c: Z = 51 μm. Scale bar = 50 μm.

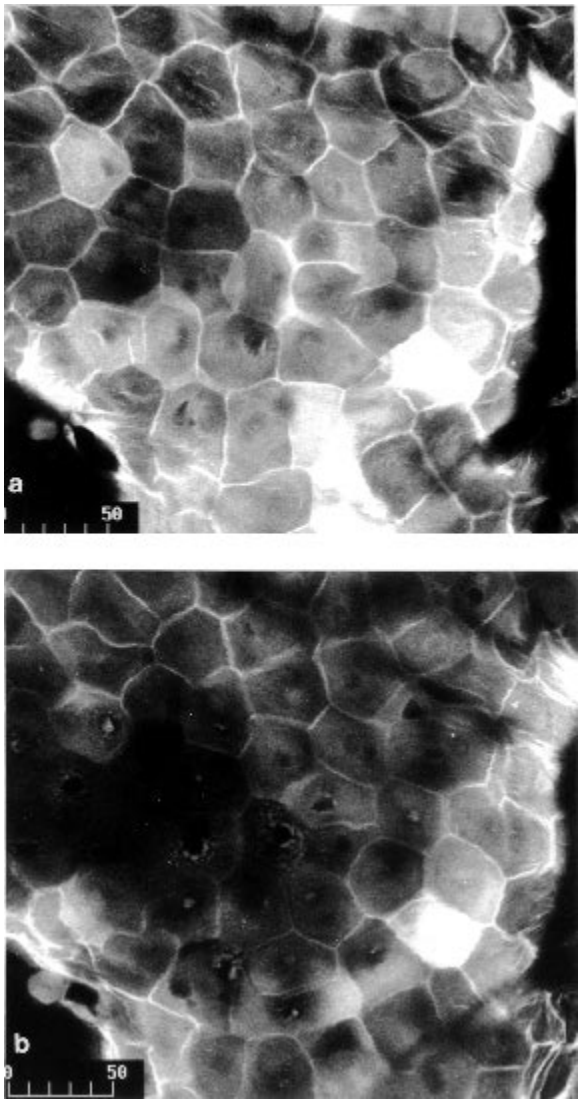


Fig. 5: xy-planar LSCM section showing the distribution of FD 38 in intact skin immediately after electroporation (ED). a: Z = 18 μm. Scale bar = 50 μm. b: Z = 24 μm. Scale bar = 50 μm.

9th fraction with a maximum of fluorescence at the 14th fraction. For the other solutions of FD, the fluorescence was detected from the 2^{d} fraction to the 4th fraction (data not shown). The different molecules of FD had the same elution profile before and after electroporation as well as after prolonged contact with the skin both in the donor compartment and in the receptor compartment after crossing the skin. No FITC was eluted demonstrating the resistance to degradation and the integrity of the covalent linkage of FITC to FD in the experimental conditions used.

DISCUSSION AND CONCLUSIONS

The first key issue addressed in this study was to assess the feasibility of transdermal delivery of macromolecules by skin electroporation. Due to the great barrier properties of the skin, delivery of high molecular weight compounds across the skin was not a realistic option until now (3). However, our data show that skin electroporation could be a very promising alternative for non invasive delivery of macromolecules that warrants close attention. Transdermal transport of macromolecules larger than those previously studied (> 12 kDa) (9–10) demonstrates that transdermal delivery of macromolecules by high voltage is feasible. The reported mechanisms of molecular transport (6) suggest that the efficiency of electroporation to enhance macromolecules transport results from its ability to increase skin permeability by the creation of new pathways and to drive molecules into the skin by electrophoresis.

Therapeutic administration of macromolecules by skin electroporation could be clinically useful. Because FD present linear conformation, their relevance to assess the general delivery of biotechnology-produced therapeutic macromolecules should be discussed. The molecular size and the conformation (e.g., globular vs linear) should be taken in account and extrapolation to proteins or nucleic acids should be, at least in part, individually evaluated. The efficacy of transport which depends on the drug physicochemical properties, the formulation and the electrical parameters (23), the potency of the drug and safety concerns (24–25) will be the critical issues to address. Whether electroporation will be more interesting than other techniques used to enhance macromolecules delivery (e.g., liposomes or ultrasound) remains to be investigated (3).

The second key issue addressed for the first time in this study was the influence of the molecular weight on the extend of transdermal transport by skin electroporation.

The effect of molecular weight on drug transport was never systematically investigated and distinguished from influence of charges and lipophilicity. Previous reports suggest that transport of macromolecules is lower than small molecules transport (26). However, difference in experimental conditions such as drug physicochemical properties such as charge (e.g., high charges of heparin (9) vs low charges of FD), log P, size, conformation (e.g., linear conformation of FD, oligonucleotides (10) or heparin (9) vs globular conformation of lactalbumin (R. Vanbever and J. C. Weaver; personnal communication) which is not efficiently transported) as well as drug concentration applied on skin (e.g., the elevated concentration of heparin (9) may partly explain the potentially therapeutic transdermal flux achieved vs the low concentration of cyclosporin A (27) which was not delivered at therapeutic rates) did not allow to draw clear-cut conclusions. Our results indicate that increasing the molecular weight decreased the permeant delivery. In contrast to iontophoresis (16), a molecular weight "cut off" could not be deduced from these data.

Hence, transdermal delivery of macromolecules of at least 40 kDa can be achieved by skin electroporation. With the development of new biotechnology produced proteins and nucleic acids, transdermal delivery could be a promising alternative to avoid parenteral administration. The main limiting factor for the pharmaceutical development of therapeutic macromolecules delivery by high voltage pulses will be the bioavailability of the molecules: too low bioavailability could exclude the use of this non invasive approach of macromolecules delivery, e.g., in our non-optimized standard conditions less than 1% of FD 38 reached the receptor compartment.

The localization of the fluorescent permeants showed that electroporation allows a penetration of FD 38 in the epidermis both around the keratinocytes and in their cytosol, confirming (12) that high voltage pulses permeabilize both the stratum corneum and the keratinocytes. Whereas FITC stained all the

surface of the skin, the transport of FD 38 was localized in "localized transport regions" as reported previously (21,22). The results show that in addition to transdermal delivery, topical delivery of macromolecules by electroporation could also be enhanced. Besides oligonucleotides (12) or FD 38, molecules as large as plasmids can be delivered in the keratinocytes opening new possibilities for the therapeutic delivery of proteins, antigens or nucleic acids in the skin.

In conclusion, this study is the first to assess systematically the influence of the molecular weight of the permeant on transdermal and topical transport by skin electroporation. Electroporation enhanced the transport of macromolecules:passively, the penetration of FD was negligible whereas a significant transport and an intracellular penetration of FD were detected after high voltage pulse application. Transport depended upon molecular weight: the higher the molecular weight, the lower the transport. The absence of a 40 kDa "cut-off" and the delivery of the macromolecule of at least 40 kDa suggest that electroporation could be useful for therapeutic delivery of large macromolecules.

ACKNOWLEDGMENTS

The authors thank Equibio and Cytopulse for the electroporation devices, FRSMM 3.4564.98 (Belgium) for the financial support and the CELL unit (Université catholique de Louvain) and FRSMM 9.4531.34F for the LSCM. We also thank Rita Vanbever for helpful discussions.

REFERENCES

1. J. Hadgraft and R. H. Guy. Transdermal drug delivery. Marcel Dekker (1989).
2. R. H. Guy. Current status and future prospects for transdermal drug delivery. *Pharm. Res.* **13**:1765–1769 (1996).
3. M. R. Prausnitz. Reversible skin permeabilisation for transdermal delivery of macromolecules. *Crit. Rev. Ther. Drug Car. Syst.* **14**:455–483 (1997).
4. M. R. Prausnitz, V. G. Bose, R. Langer, and J. C. Weaver. Electroporation of mammalian skin : a mechanism to enhance transdermal drug delivery. *Proc. Nat. Acad. Sci., USA* **90**:10504–10508 (1993).
5. R. Vanbever, N. Lecouturier, and V. Préat. Transdermal delivery of metoprolol by electroporation. *Pharm. Res.* **11**:1657–1662 (1994).
6. J. C. Weaver and Y. A. Chizmadzhev. Theory of electroporation: a review. *Bioelectrochem. Bioenerget.* **41**:135–160 (1996).
7. R. Vanbever, E. Le Boulangé, and V. Préat. Transdermal delivery of fentanyl by electroporation I. Influence of electrical factors. *Pharm. Res.* **13**:559–565 (1996).
8. J. E. Riviere, N. A. Monteiro-Riviere, R. A. Rogers, D. Bommannan, J. A. Tamada, and R. O. Potts. Pulsatile transdermal delivery of LHRH using electroporation: drug delivery and skin toxicology. *J. Contr. Rel.* **36**:229–233 (1995).
9. M. R. Prausnitz, E. R. Edelman, J. A. Gimm, R. Langer, and J. C. Weaver. Transdermal Delivery of Heparin by skin electroporation. *Bio-Technology* **13**:1205–1209 (1995).
10. T. E. Zewert, U. F. Pliquett, R. Langer, and J. C. Weaver. Transdermal transport of DNA antisense oligonucleotides by electroporation. *Biochem. Biophys. Res. Commun.* **212**:286–292 (1995).
11. V. Regnier, T. Le Doan, and V. Préat. Parameters controlling topical delivery of oligonucleotides by electroporation. *J. Drug Target* **5**:275–289 (1998).
12. V. Regnier and V. Préat. Localisation of a FITC-labeled phosphorothioate oligodeoxynucleotide in the skin after topical delivery by iontophoresis and electroporation. *Pharm. Res.* **15**:1596–1602 (1998).
13. M. J. Jaroszeski, R. Gilbert, C. Nicolau, and R. Heller. In vivo gene delivery by electroporation. *Adv. Drug Del. Rev.* **35**:131–137 (1999).
14. R. Vanbever, U. F. Pliquett, V. Préat, and J. C. Weaver. Comparison of the effects of short, high-voltage and long, medium-voltage pulses on skin electrical and transport properties. *J. Contr. Rel.* **60**:35–47 (1999).
15. C. Cullander and R. H. Guy. Visualization of iontophoretic pathways with confocal microscopy and the vibrating probe electrode. *Solid States Ion* **53**:197–206 (1992).
16. N. G. Turner, L. Ferry, M. Price, C. Cullander, and R. H. Guy. Iontophoresis of poly-L-lysines: The role of molecular weight? *Pharm. Res.* **14**:1322–1331 (1997).
17. R. Vanbever, M. R. Prausnitz, and V. Préat. Macromolecules as novel transdermal transport enhancer for skin electroporation. *Pharm.Res.* **14**:638–644 (1997).
18. J. C. Weaver, R. Vanbever, T. E. Vaughan, and M. R. Prausnitz. Heparin alters transdermal transport associated with electroporation. *Biochem. Biophys. Res. Commun.* **234**:637–640 (1997).
19. L. M. Mir and S. Orlowski. Mechanisms of electrochemotherapy. *Adv. Drug Del. Rev.* **35**:107–118 (1999).
20. M. R. Prausnitz, J. A. Gimm, R. H. Guy, R. Langer, J. C. Weaver, and C. Cullander. Imaging regions of transport across human stratum corneum during high-voltage and low-voltage exposures. *J. Pharm. Sci.* **85**:1363–1370 (1996).
21. U. F. Pliquett, T. E. Zewert, T. Chen, R. Langer, and J. C. Weaver. Imaging of fluorescent molecules and small ion transport through human stratum corneum during high voltage pulsing: localized transport regions are involved. *Biophys. Chem.* **58**:185–204 (1996).
22. U. F. Pliquett, R. Vanbever, V. Préat, and J. C. Weaver. Local transport regions (LTRs) in human stratum corneum due to long and short "high voltage" pulses. *Bioelectrochem. Bioenerget.* **47**:151–161 (1998).
23. V. Préat, R. Vanbever, A. Jadoul, and V. Regnier. Electrically enhanced transdermal drug delivery: iontophoresis vs electroporation. P. Couvreur, D. Duchêne, P. Green, and H. Junginger (eds.), in *Transdermal administration: A case study, Iontophoresis*. Edition de la Santé, Paris, 1997, pp. 58–67.
24. A. Jadoul, J. Bouwstra, and V. Préat. Effects of iontophoresis and electroporation on the stratum corneum—Review of the biophysical studies. *Adv. Drug Del. Rev.* **35**:89–105 (1999).
25. R. Vanbever, D. Fouchard, A. Jadoul, N. De Morre, V. Préat, and J.-P. Marty. In vivo non-invasive evaluation of hairless rat after high-voltage pulse exposure. *Skin Pharmacol. Appl. Skin Physiol.* **11**:23–34 (1998).
26. M. R. Prausnitz. A practical assessment of transdermal drug delivery by skin electroporation. *Adv. Drug Del. Rev.* **35**:61–76 (1999).
27. S. Wang, M. Kara, and T. R. Krishnan. Topical delivery of cyclosporin A coevaporate using electroporation technique. *Drug Dev. Ind. Pharm.* **23**:657–663 (1997).