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Advanced Drug Delivery Reviews 35 (1999) 77–88

advanced drug delivery reviews

In vivo efficacy and safety of skin electroporation

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Abstract

This article reviews the studies on skin electroporation carried out in vivo in animals and emphasizes its potential therapeutic applications for transdermal and topical drug delivery. In agreement with in vitro studies, transport across skin due to high-voltage pulses in vivo was shown to increase by orders of magnitude on a timescale of minutes. Increased transdermal transport was measured by systemic blood uptake and/or pharmacological response, and demonstrated for calcein, a fluorescent tracer, fentanyl, a potent analgesic and flurbiprofen, an antiinflammatory drug. Combined electroporation with iontophoresis was shown to provide rapidly responsive transdermal transport of luteinizing hormone releasing hormone ex vivo as well. These data underline the potential of skin electroporation for improving the delivery profile of existing conventional transdermal patches, but also for replacing the injectable route.

High-voltage pulses can increase drug permeation within and across skin but are also an efficient tool to permeabilize the membrane of cells of the cutaneous or subcutaneous tissue. This was shown beneficial for targeting cutaneous cells with oligonucleotides or genes and might open new opportunities for gene therapy and DNA vaccination.

The safety of the application of high-voltage pulses on skin was assessed in vivo, using histological and visual scores, and bioengineering methods. While changes in skin barrier and function were observed, the irritation was mild and short-lived. Further optimization of the electrode configuration for improved targeting of the stratum corneum should still improve tolerance and levels of sensation.

Keywords: Transdermal drug delivery; Topical drug delivery; Skin; Electroporation; Iontophoresis

Contents

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0169-409X/99/$ – see front matter
PII: S0169-409X(98)00064-7

1. Introduction

Transdermal drug delivery has attracted considerable attention in recent years and the potential advantages of this mode of administration have been well documented. Transdermal delivery (1) avoids gastrointestinal degradation and the hepatic first-pass effect, (2) has potential for controlled and sustained delivery, and (3) is a user-friendly method and therefore improves patient compliance [1,2]. The major difficulty is that the skin is an excellent barrier to molecular transport. Conventional transdermal therapy is only applicable to small, potent and lipophilic solutes. Moreover, transport of most drugs across skin is very slow and lag-times to reach steady-state fluxes range in hours. When considering topical delivery to the skin, the same limitations apply. Achievement of a therapeutically effective level is therefore difficult without some form of facilitation, and chemical, electrical, mechanical, and ultrasonic approaches to increasing transdermal transport have been explored [1,2].

Electroporation (or electropermeabilization) involves the creation of transient aqueous pathways across lipid bilayer membranes by applying a short, high-voltage pulse [3–7]. This phenomenon occurs in the lipid bilayers of nonliving systems, such as liposomes or red blood cell ghosts, as well as the plasma membranes of living cells, either isolated or part of a tissue. Electrical exposures typically involve electric field pulses that generate transmembrane potentials of 0.5–1.0 V and last for 10 μs to 10 ms [4,5]. Both dramatic electrical behavior (reversible electrical breakdown) and significant molecular transport occur because of structural rearrangement of the cell membrane. Significant progress has been made by adopting the hypothesis that some of these rearrangements consist of temporary aqueous pathways, with the electric field playing the dual role of causing pore formation and providing a local driving force for ionic and molecular transport. Tremendously increased molecular transport is probably the most important result of electroporation for biological research. Gene transfer into cells or electrotransfection is a common application [4,5].

The biological composition and structure of the stratum corneum, the outermost layer of the skin, make it particularly attractive for electroporation. The stratum corneum is made of corneocytes embedded in a continuous extracellular matrix of lipids highly ordered in bilayers. Because the stratum corneum is the main barrier to transdermal transport, it has been suggested that electroporation of its intercellular lipid bilayers might enhance percutaneous exchange. Electroporation of the stratum corneum was first demonstrated in 1993 [8]. Upon application of high-voltage pulses, large increases in transdermal transport of molecules were observed in vitro with human skin and in vivo in the hairless rat. The concept of skin electroporation and the supporting preliminary data have motivated a number of subsequent studies, mainly in vitro but also a few in vivo in animals [9–13].

Electroporation experiments with skin generally used exponential-decay electric field pulses of 100 V to 1500 V applied voltage, 30–100 V transdermal voltages and 1 ms to hundreds of ms pulse duration [9–11]. Because the stratum corneum contains approximately 100 bilayer membranes in series, these transdermal voltages well correspond to the range of voltages used for electroporation in cells (i.e., 0.3–1.0 V per bilayer). Skin electrical resistance drops by several orders of magnitude on a timescale of microseconds during a pulse, so that peak electrical currents are in the range of 0.1–3.0 A. Electroporation of skin was shown to enhance and expedite transport across and/or into skin for many different compounds. Within a few minutes of high-voltage pulsing, molecular transport across skin increased by several orders of magnitude, mainly due to electrophoretic movement and diffusion through newly created aqueous pathways [14].

Iontophoresis differs from skin electroporation by the electrical protocol applied but also by its impact

on molecular transport and skin (see Sections 2.3 and 3.3) [1,2,15]. Iontophoresis can be defined as the movement of ions through the skin due to the application of a low intensity electrical current as a driving force. Current densities generally do not exceed 0.5 mA/cm^2 (transdermal voltage below 5 V) and the application may last minutes to hours. Unlike electroporation which acts directly on the skin making transient changes in tissue permeability, iontophoresis acts primarily on the drug, involving skin structural changes as a secondary effect.

In this article, in vivo studies on transdermal and topical drug delivery using skin electroporation are reviewed with emphasis on potential therapeutic applications. Reference to iontophoresis as a basis of comparison for skin electroporation is given whenever appropriate. Safety issues are discussed.

2. Skin electroporation for systemic drug delivery

2.1. Potential therapeutic applications

Extensive work on molecular transport across skin due to electroporation has been performed in vitro. Essential features of transport include (1) large flux increases for many different compounds, (2) rapidly responsive molecular transport and (3) control on transport magnitude achieved by controlling the electrical parameters of the high-voltage pulses and the physico–chemical properties of the drug and solution. This section provides a brief overview of these data as a basis for understanding potential therapeutic applications of skin electroporation for systemic drug delivery and the in vivo data developed afterwards. For an extended review on the information yielded in vitro, the reader is referred to another article appearing in this issue [14].

Electroporation of skin can increase transport across and/or into skin by up to four orders of magnitude for compounds ranging in size from small ions (e.g., Na^+, Cl^-) to moderate-sized molecules (e.g., metoprolol, calcein), to macromolecules (e.g., luteinizing hormone releasing hormone, LHRH; heparin), to latex microspheres, as well as for compounds presenting different solubilities (e.g., lipophilic as domperidone, or hydrophilic as LHRH) and different electrical charges (e.g., neutral as mannitol, or highly-charged as calcein) [8–11,14,16–23].

Molecular transport due to high-voltage pulses is rapidly responsive, with onset times for transport and lag-times to reach steady-state fluxes ranging in minutes [11,24,25]. Quickly responsive transport is illustrated in Fig. 1, where significant transport is observed after application of only a few pulses and steady state flux reached within half an hour of pulsing. Other experiments have showed how rapidly responsive behavior of transdermal transport by electroporation can be used to achieve complex delivery profiles [25]. Control on transport was achieved by controlling the electrical parameters of the pulses (i.e., pulse voltage, length, number and spacing) and by choosing the concentration of the drug applied on skin (Fig. 1) [8–11,14,16–18,20–23].

The characteristics of transport and the flexibility in the physico–chemical properties of the molecule being transported suggest several potentials of skin electroporation for systemic delivery of drugs. High-voltage pulses can potentially (1) improve the delivery profile of existing conventional transdermal patches by increasing transdermal transport and by shortening the lag-time for onset of action, (2) broaden the applications of transdermal delivery to drugs of variable physico–chemical properties, (3)

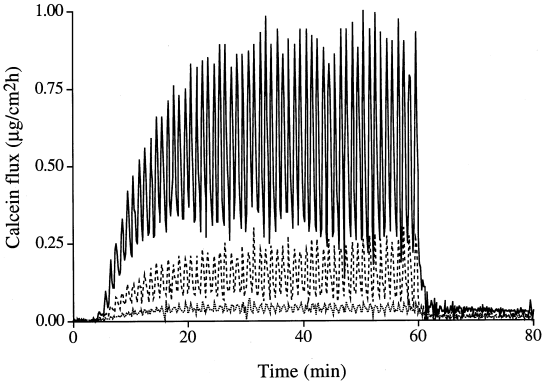

Fig. 1. Rapid temporal control on transdermal transport using skin electroporation. The time profiles of calcein transdermal flux due to electroporation at different transdermal voltages are shown. 1 ms pulses were applied to human epidermis in vitro at 1 pulse per minute for 1 h, at 270 V (solid line), 135 V (dashed line) or 115 V (dotted line). From Ref. [25], with permission.

replace the injectable route, both in the cases of the bolus injection (e.g., hormonal treatment or diagnosis) and continuous infusion as well as in the case of pulsed or programable administration (e.g., pain treatment).

2.2. In vivo efficacy

The in vitro features of transport have been shown to translate in vivo, in support of the potential therapeutic applications. In vivo data have especially underlined the enhanced transport and the rapid onset for transport. Transdermal fluxes of calcein, a moderate-sized, highly polar fluorescent molecule (623 Da, −4 charge) which does not normally cross skin in detectable quantities, were measured following application of low-duty cycle electric field pulses in vivo in hairless rats [8,26]. While calcein plasma concentrations of the controls (no electric field pulses) were below the detection limit, calcein plasma concentrations under pulsed conditions were two orders of magnitude higher. The fluxes did not increase with increasing voltage, which suggested that a rate-limiting step other than transport across the stratum corneum existed for calcein.

A subsequent more comprehensive study with fentanyl demonstrated, in addition to the enhanced transport, the fast onset of therapeutic action and the control achieved on the dose delivered transdermally using high-voltage pulses [12]. Fentanyl is currently administered by injections, passive transport across skin, or via the oral mucosa. Transdermal fentanyl delivery has been developed for the management of moderate to severe chronic pain. After placement of a fentanyl transdermal system, serum concentrations increase during the first 14 h and then reach a plateau [27]. Pain treatment can however require rapid and pulsed administration of drugs. Fentanyl delivered across skin by high-voltage pulsing in vivo in hairless rats exhibited an onset time of action reduced to a few minutes [12]. High-voltage pulsing was applied for 5 min. Immediately after pulsing, fentanyl plasma levels already reached a third of the maximal plasma concentrations attained approximately 1 h after the electroporation (Fig. 2). Elevated plasma concentrations were associated with strong pharmacological effects: deep analgesia was

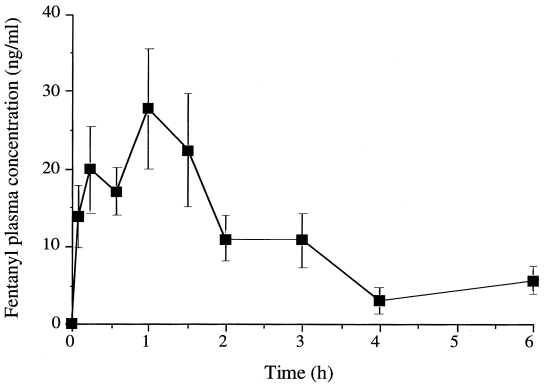

Fig. 2. Fentanyl plasma concentrations as a function of time after transdermal delivery using electroporation. Electroporation was carried out using 15 pulses of 250 V (voltage applied at the electrodes) and 200 ms, applied from time 0 to 5 min in hairless rat skin in vivo. Foams at the cathode and anode were soaked with a solution of fentanyl (400 μg/ml in citrate buffer 0.01 M at pH 5). From Ref. [12], with permission.

achieved and lasted for 1 hour. Without application of pulses, no antinociception effects were measured (Fig. 3a). Supraspinal side-effects of fentanyl, i.e., total blockade of the pinna and cornea reflexes and strong increases in skeletal muscle tone, were also observed immediately after the electroporation. When comparing electroporation to a subcutaneous injection, the onset time to achieve a pharmacological response was as fast. When comparing electroporation to an iontophoresis of 0.5 mA/cm^2 (i.e., maximal current clinically tolerated) applied for 5 min as the electroporation protocol, the onset time to achieve pharmacological response was faster and the response stronger and longer due to high-voltage pulsing (Fig. 3b). This study with fentanyl also demonstrated the control on transport obtained by controlling the electrical parameters of the pulses. As seen in Fig. 3c, electrical protocols transporting more electrical charges through skin induced stronger and longer antinociception effects of fentanyl.

Flurbiprofen, a potent nonsteroid antiinflammatory drug, has been used as a model drug to study transdermal transport due to high-voltage pulses in vivo in hairless rats as well. The peak in plasma concentration of flurbiprofen was attained very rapidly (within 15 min) after electroporation, in agreement

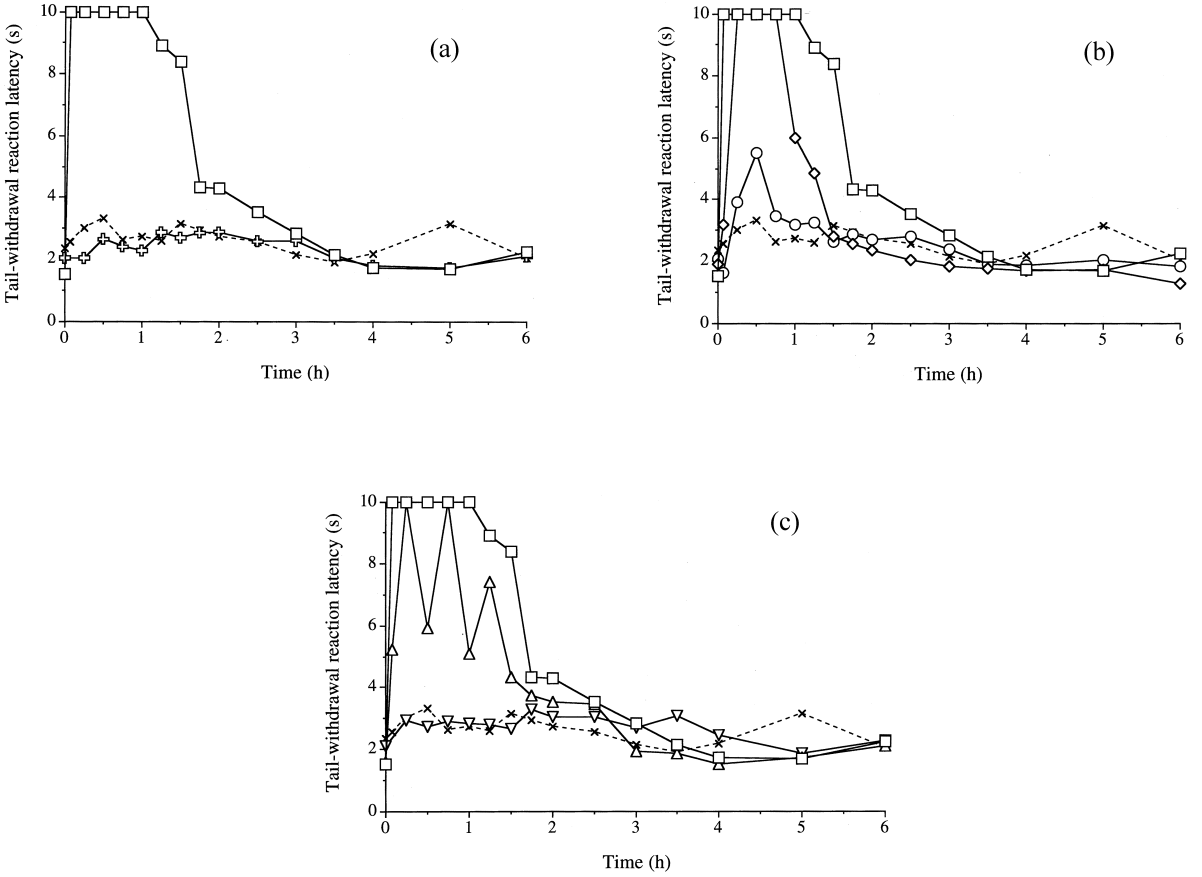

Fig. 3. Fast onset of analgesia of transdermal fentanyl using skin electroporation. (a) Tail-withdrawal reaction latency in vivo in the rat as a function of time after 15 pulses of 250 V (voltage applied at the electrodes) and 200 ms (□), after passive diffusion of fentanyl (control diffusion, +) or without any treatment applied (control, ×). (b) Tail-withdrawal reaction latency as a function of time after 15 pulses of 250 V and 200 ms (□), a subcutaneous injection of fentanyl (4 μg/100 g, ◇), an iontophoresis applied at 0.5 mA/cm^2 for 5 min (○), or without any treatment applied (control, ×). (c) Tail-withdrawal reaction latency as a function of time after 15 pulses of 250 V and 200 ms (□), 15 pulses of 100 V and 500 ms (△), 60 pulses of 500 V and 1.3 ms (▽), or without any treatment applied (control, ×). Pulse application started at time 0 and lasted for 5 min (250 and 100 V) or 15 min (500 V). Foams at the cathode and anode were soaked with a solution of fentanyl (400 μg/ml in citrate buffer 0.01 M at pH 5). From Ref. [12], with permission.

with the results with fentanyl (Fig. 4). This study with flurbiprofen is further discussed in Section 2.3 [28].

Finally, in vivo transdermal delivery of vesicles encapsulating drug using high-voltage pulses (i.e., ''electro-incorporation'') has been reported in hairless mice [29]. Although penetration of microspheres of a size up to 45 μm in the dermis was observed by microscopy, blood uptake of the encapsulated compound was not measured in the study.

2.3. Comparison with iontophoresis

Although, enhancement in molecular transport induced by high-voltage pulses or iontophoresis can achieve similar levels, the features of transdermal transport due to these electrical methods exhibit differences, which suggest that high-voltage pulsing may provide advantages over conventional iontophoresis. Flurbiprofen transport across skin in vivo in hairless rats was greater and more rapid, following

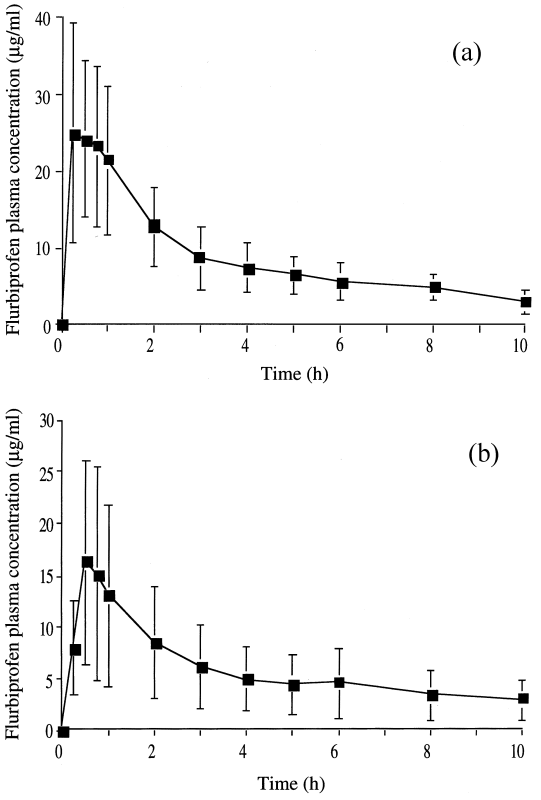

Fig. 4. Electroporation vs. iontophoresis-mediated transdermal transport of flurbiprofen. Plasma concentrations of flurbiprofen in the hairless rat in vivo are shown as a function of time after transdermal delivery using electroporation (a) or iontophoresis (b). Electroporation was carried out using 15 pulses of 150 V (voltage applied at the electrodes) and 150 ms, applied at a rate of 1 pulse per minute. Iontophoresis involved a 15 min exposure to 0.5 mA/cm^2 direct current, which corresponded to an amount of electrical charges transported equivalent to the electroporation protocol. Foams at the cathode and anode were soaked with a solution of flurbiprofen (10 mg/ml in 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid buffer 0.04 M at pH 7.4–ethanol, 90:10 v/v). From Ref. [28].

electroporation than following iontophoresis of the same amount of transported charges [28]. Plasma concentrations and the area under the curve were almost twice as high and the peak in plasma concentrations was reached earlier following electroporation (Fig. 4). Similar results were previously obtained with fentanyl [12].

The greater efficiency of electroporation vs. iontophoresis-mediated transdermal transport can be explained by results yielded in vitro. The efficiency of electric current in transporting a solute can be evaluated by calculating its transport number and by considering the surface area of the skin available to transport during electrical exposure [11,17,30]. Transport numbers, which are the fraction of total current carried by a given ionic species, were equivalent or greater during high-voltage pulsing vs. during iontophoresis. This suggests that pathways created at high-voltage present less steric and/or electrical hindrance to transport than those undertaken during iontophoresis.

The surface area of the skin available to transport was estimated as one to two orders of magnitude greater and eight orders of magnitude more rapidly accessible during high-voltage pulses than during iontophoresis [30,31]. This calculation was based on the measurement of skin electrical resistance during high-voltage pulsing or iontophoresis. By measuring skin resistance and assuming that ion transport pathways are filled with saline, the area fraction of skin made up of these pathways can be estimated [30]. After minutes to hours of iontophoresis, human skin resistance can drop from 100 000 $\Omega \cdot cm^2$ to between 1000 and 10 000 $\Omega \cdot cm^2$ over a characteristic time of minutes. The decrease in electrical resistance during iontophoresis corresponds to an increase in skin surface area occupied by ion transport pathways from 0.0001 to 0.001–0.01%. During high-voltage pulses, skin resistance drops even further, to 100 $\Omega \cdot cm^2$, on a timescale of microseconds, which corresponds to a still greater skin surface area available for transport, i.e., 0.1%. The larger surface area available for transport during electroporation may correspond to a shift from iontophoretic transport largely through shunt routes (hair follicles and sweat glands) to transport predominantly through electropores within the bulk of the stratum corneum [32,33]. The current is therefore more evenly distributed across the skin during electroporation than during iontophoresis, which is beneficial in terms of skin tolerance to the electric current but also means a skin surface area more efficiently utilized during electroporation than during iontophoresis.

Together, the data demonstrate that electric current delivered in a pulsed and high voltage/intensity manner (i.e., electroporation) transports more rapidly (e.g., earlier peak in plasma concentrations) and more efficiently (e.g., higher transport numbers and greater

skin surface area involved in the transport) the drug across skin than when delivered in a continuous low voltage/intensity manner (i.e., iontophoresis).

2.4. Combination with other enhancers

The combination of electroporation with other methods might still open new perspectives. In vitro studies demonstrated synergistic effects of skin electroporation with iontophoresis, ultrasound or macromolecules used as chemical enhancers (for review, see Ref. [14]). Combined electroporation to iontophoresis has also been tested ex vivo; being relevant in the context of in vivo studies, this method is further discussed below.

Enhanced iontophoretic transport of LHRH by the prior application of an electroporative high-voltage pulse (five- to ten-times the corresponding iontophoretic flux) was first demonstrated in vitro with human skin [10]. Riviere et al. [34] confirmed those data ex vivo with the isolated perfused porcine skin flap model. In these experiments, negligible LHRH was delivered transdermally by passive diffusion. Following the application of an electroporative pulse and thereafter an iontophoretic current for 30 min, LHRH flux dramatically increased in the venous effluent. When the electrical treatment ceased, LHRH flux dropped. The application of a subsequent pulse–iontophoresis episode resulted in a second dramatic increase in LHRH flux (Fig. 5). These data demonstrated the ability of the combination electroporation–iontophoresis to rapidly, repeatedly and reversibly enhance LHRH transport. It should be noted that in these experiments, a tight control on molecular transport was not attained with iontophoresis alone: LHRH transport across skin increased due to a first application of iontophoresis alone, but did not respond to a second episode of current (Fig. 5).

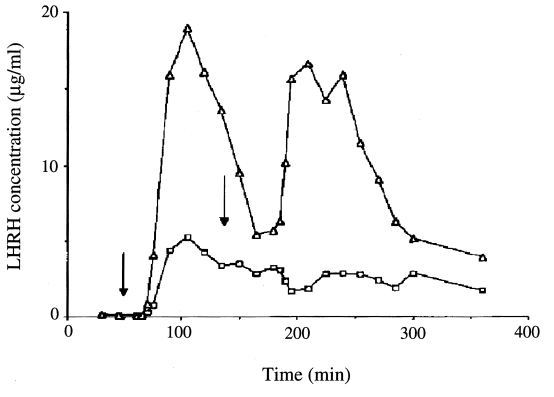

Fig. 5. Pulsatile transdermal delivery of LHRH using electroporation. LHRH was delivered in two separate episodes consisting of either one 500 V (voltage applied at the electrodes) pulse followed by 30 min of iontophoresis (△) or 30 min of iontophoresis alone (□). The arrows indicate the beginning of each treatment period. From Ref. [34], with permission.

3. Skin electroporation for topical drug delivery

3.1. Potential therapeutic applications

Skin electroporation could be particularly appropriate for topical delivery for the following reasons. (1) Skin electroporation temporarily permeabilizes the stratum corneum, the barrier to drug permeation and therefore could broaden topical delivery to drugs not suitable for delivery by passive diffusion, i.e., hydrophilic, charged and/or large-molecular-mass drugs [8–11,14,22,31,35]. (2) The application of high-voltage pulses can also enhance the permeability of viable cells underlying the stratum corneum as demonstrated in vivo by electrochemotherapy (i.e., the electropermeabilization of tumors to bleomycin using high-voltage pulses) or DNA transfection [36–42].

3.2. In vivo efficacy

Skin electroporation has been shown to increase transport of peptides and gene based drugs into skin in vitro. The overall concentration of cyclosporin A, a hydrophobic peptide, within skin was increased by an order of magnitude following high-voltage pulsing [43]. Potentially therapeutic concentrations ($=1$ μM) of antisense oligonucleotides could be achieved in the viable skin of hairless rats; the oligonucleotides were shown to reach the nucleus of the keratinocytes within minutes after the electroporation [23,44].

Electroporation-mediated transfection of cells of the cutaneous tissue in vivo has been reported as well [40–42]. Electroporation is commonly used for in vitro gene transfer of mammalian and other cells; studies also showed that electroporation is efficient

for the transfection of cells being part of a tissue, e.g., the skin [4,5]. Titomirov et al. [40] demonstrated that after intradermal injection of a plasmid in newborn mice, skin electroporation enhanced the transfection efficiency in fibroblasts. Zhang and coworkers [41,42] reported that the combination of electroporation to subsequent pressure increased the transport of a *lacZ* plasmid into the skin in vivo in hairless mice. Three days after the application of high voltage pulses, expression of the *lacZ* gene was observed in the dermis. These data suggest therefore that skin electroporation could be useful for topical delivery of peptides and antisense oligonucleotides as well as for topical delivery of DNA for gene therapy or vaccination.

3.3. Comparison with iontophoresis

Topical delivery of drugs by iontophoresis has been used for many years for diagnosis (e.g., cystic fibrosis) and therapeutic applications (e.g., local anesthetics) [15]. However, the use of iontophoresis has mainly been restricted to low-molecular-mass hydrophilic drugs. Both iontophoresis and electroporation were shown to enhance the topical delivery of oligonucleotides in vitro [23,44]. Oligonucleotide concentration in the skin was in the same range after application of the same amount of transferred charges. However, confocal laser scanning microscopy demonstrated that only electroporation led to a rapid nuclear uptake of the oligonucleotides in the keratinocytes. These data suggest that electroporation might be more efficient for delivering compounds inside cutaneous cells than iontophoresis. They confirm the hypothesis that the enhancement of both stratum corneum and keratinocyte permeability contributes to the intracellular delivery of drugs.

4. In vivo safety of skin electroporation

4.1. Skin integrity

A major factor in the clinical acceptability of electrically-enhanced transdermal or topical delivery is its effect on the skin. The alterations in skin induced by high-voltage pulsing have been investigated both in vitro and in vivo. In vitro investigation has given insights into the changes in stratum corneum structure. Briefly, skin resistance has been measured and shown to drop by up to three orders of magnitude during pulses and to recover either partially or fully to prepulse values after pulsing [11,31]. Recent studies of human stratum corneum using differential thermal analysis, X-ray scattering studies and freeze fracture electron microscopy have suggested that high-voltage pulses could induce a general perturbation of the stratum corneum lipid ultrastructure. These effects were similar to the disorganization observed after iontophoresis [45–47].

Relevant information on the alterations of skin induced by electroporation and their significance in terms of safety has also been yielded in vivo. Toxicology of an electroporative pulse followed by an iontophoretic current has been partially evaluated in vivo on pig skin, using histological scores and by scaling the degree of erythema, edema and the presence of petechia [34]. Erythema, edema and petechiae all increased significantly with increasing current in the absence of a pulse. The application of an electroporative pulse did not increase the iontophoretic-induced irritation at any current tested. The only skin alterations seen with electroporation alone were mild intraepidermal vacuolization and a transient erythema. However, all changes tended to decrease within 4 h after treatment.

The alterations of skin functions following the application of high-voltage pulses have also been evaluated in vivo in hairless rat using noninvasive bioengineering methods [13]. Chromametry, transepidermal water loss, laser Doppler flowmetry and corneometry were jointly used as noninvasive sensing of skin biophysical parameters. Mild erythema appeared within a few minutes after pulsing but decreased to control values within half an hour; some skin redness appeared however again a few hours later (Fig. 6). The effects were shown to be dependent on the electrical parameters of the pulses. Transepidermal water loss increased within a few minutes after electroporation as well, but recovered control values within an hour or several hours for larger pulses (Fig. 7). Changes in skin capacitance were overall not significant. No edema, nor burn were observed. High-voltage pulses did not induce stronger alterations of skin functions than an ion-

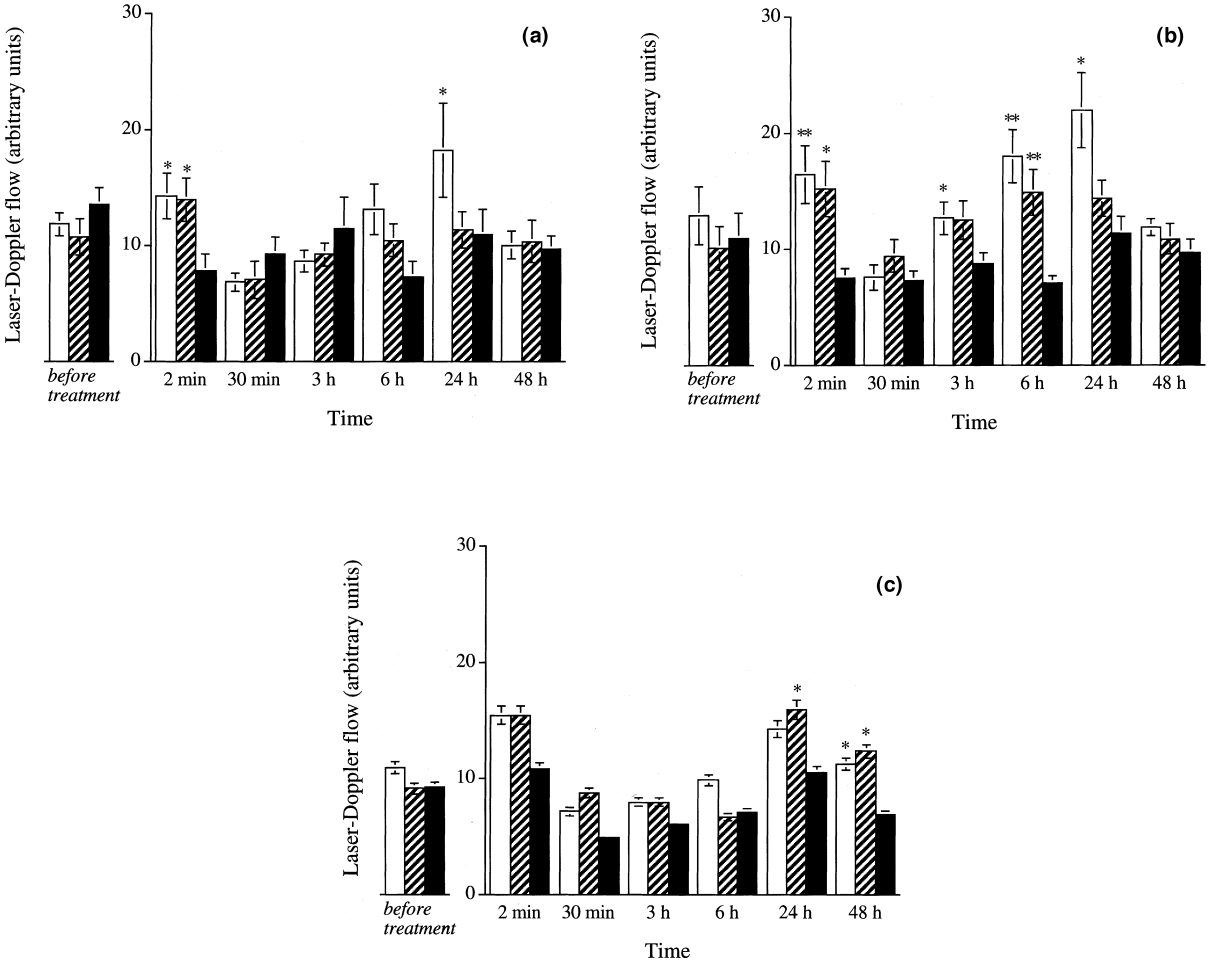

Fig. 6. Laser Doppler flowmetry measurement of skin blood flow following electroporation (a and b) or iontophoresis (c). Electroporation was carried out using 15 pulses of 100 V (voltage applied at the electrodes) and 500 ms (a), or 15 pulses of 250 V and 200 ms (b), applied at a rate of 1 pulse per minute in hairless rat skin in vivo. Iontophoresis involved a 1 h exposure to 0.5 mA/cm^2 direct current. White bars are measurements at the cathode, stripped bars at the anode and black bars at the control sites. Statistical significance: * $P < 0.05$ vs. control; ** $P < 0.01$ vs. control. From Ref. [13], with permission.

tophoresis of 0.5 mA/cm^2 applied for 1 h, which is considered to be safe (Figs. 6 and 7) [49]. The study on transdermal delivery of fentanyl reported earlier used the same electrical pulses as the present study on skin integrity [12]. Interestingly, molecular transport and alterations of skin did not fully correlate, where different pulsing conditions induced different changes in skin functions but transported similar quantities of fentanyl across skin.

Overall, the studies of skin exposed to high-voltage pulses in vivo have shown changes in skin barrier and function. However, the irritation was mild, short-lived, dependent on the electrical parameters of the pulses, and similar to that following conventional iontophoresis. This supports the safety of skin electroporation as a method to enhance transdermal and topical drug delivery.

4.2. Sensation and muscle stimulation

A strong clinical precedent of the application of high-voltage pulses on skin is the case of electro-

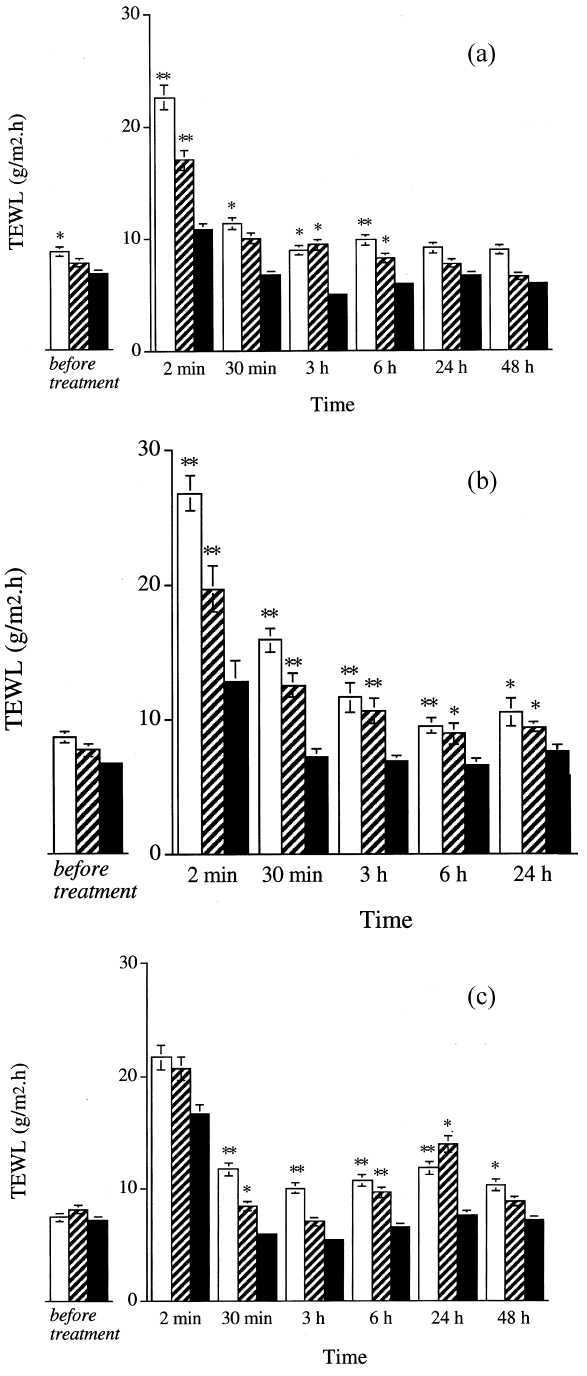

Fig. 7. Transepidermal water loss (TEWL) following electroporation (a and b) or iontophoresis (c). See Fig. 6 caption for experimental information. From Ref. [13], with permission.

chemotherapy [36–39]. Typically, eight pulses of 1.3 kV/cm and 100 μs each were applied directly on the patient tumors 5–15 min after an infusion of bleomycin. Minimal adverse side-effects of the electrochemotherapy treatment were observed. Instantaneous painless contractions of the underlying muscles during pulse delivery were reported.

Sensation due to current applied to skin is caused primarily by direct electrical excitation of nerves [49,50]. Effects range from sensation of localized heat or cold, through tingling and itching, slight pricking, muscle contraction, to outright pain. Whereas transdermal transport is impeded by the stratum corneum, the uppermost thin layer of the epidermis, nerves are located lower in the skin, in the dermis. By concentrating the electric field in the stratum corneum and by selecting the parameters of the high-voltage pulses, side-effects on sensation could therefore be minimized.

In vivo skin electroporation experiments with animals as well as electrochemotherapy have used parallel-plate electrodes attached to calipers across a skin fold [8,12,13,26,29,36–42]. Although this electrode configuration can effectively electroporate the stratum corneum, it also exposes underlying tissues to the high-voltage electric field, and it is not the most convenient method for application in humans. Using this design, rats exhibited muscle twitches in response to each pulse; when pulses were applied to the lower back, rats' hind legs kicked to each pulse. Sensation due to high-voltage pulses was also expressed through vocalization and spontaneous movements of the rats when they were not (or not fully) anesthetized [8,12,26]. A different electrode configuration which is more suited to implementation in a patch, has also been mentioned [29]. It consists of an array of interweaving electrode fingers called ''meander electrodes''. This configuration was theoretically expected to provide equipotential lines parallel to the electrode and to concentrate the electric field in the upper layers of the skin. However, no supportive experimental data were provided in this study. A microfabricated electrode array, another plane and closely-spaced electrodes system, is presently under development at the Massachusetts Institute of Technology [51].

Thresholds for sensation caused by pulses are nonlinear functions of pulse current, pulse length and

contact area. In general however, increased current/charge, pulse rate and pulse length all increase levels of sensation. Because sensation decreases dramatically for pulses shorter than about 1 ms, short pulses at high-voltage might provide significant increase in transdermal transport without sensation or pain. However, decreasing pulse length strongly decreases transdermal transport, so that more perspectives for eliminating sensation might be offered by developing an appropriate electrode configuration [50].

5. Conclusion

The effects of skin electroporation on molecular transport and skin properties have been extensively studied in vitro. The in vivo data have confirmed the enhancement and rapidity in transdermal transport due to high-voltage pulses and have underlined the potential of skin electroporation for improving the delivery profile of existing conventional transdermal patches and for replacing the injectable route. High-voltage pulses were also demonstrated as beneficial for enhancing transfection of cells of the cutaneous tissue in vivo, which might open opportunities for gene therapy and DNA vaccination. When considering the safety of the technique, high-voltage pulses were demonstrated as well-tolerated and as safe as iontophoresis. However, an optimized electrode configuration might still improve tolerance and levels of sensation.

Acknowledgements

We thank Samir Mitragotri and Mark R. Prausnitz for helpful discussion, and Jim E. Riviere for assistance in the preparation of this manuscript.

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