



Radiofrequency-driven skin microchanneling as a new way for electrically assisted transdermal delivery of hydrophilic drugs

Amnon C. Sintov^{a,*}, Igor Krymberk^a, Dorit Daniel^b, Talli Hannan^b, Ze'ev Sohn^b, Galit Levin^b

^aThe Institutes for Applied Research, Ben Gurion University of the Negev, P.O. Box 653, Beer Sheva 84105, Israel

^bTransPharma Ltd., 3a Geron St., P.O. Box 222, Yehud, Israel

Received 27 November 2002; accepted 14 February 2003

Abstract

The aim of this study was to increase the skin penetration of two drugs, granisetron hydrochloride and diclofenac sodium, using a microelectronic device based on an ablation of outer layers of skin using radiofrequency high-voltage currents. These radiofrequency currents created an array of microchannels across the stratum corneum deep into the epidermis. The percutaneous penetration studies were first performed *in vitro* using excised full thickness porcine ear skin. An array of 100 microelectrodes/cm² was used in these studies. The skin permeability of both molecules was significantly enhanced after pretreatment with the radiofrequency microelectrodes, as compared to the delivery through the untreated control skin. Steady state fluxes of 41.6 µg/cm²/h ($r=0.997$) and 23.0 µg/cm²/h ($r=0.989$) were obtained for granisetron and diclofenac, respectively. The enhanced transdermal delivery was also demonstrated *in vivo* in rats. It was shown that diclofenac plasma levels in the pretreated rats reached plateau levels of 1.22±0.32 µg/ml after 3 h to 1.47±0.33 µg/ml after 6 h, as compared to 0.16±0.04 µg/ml levels obtained after 6 h in untreated rats. Similarly, application of granisetron patches (3% in crosslinked hydrogel) onto rats' abdominal skin pretreated with radiofrequency electrodes resulted in an averaged peak plasma level of 239.3±43.7 ng/ml after 12 h, which was about 30 times higher than the plasma levels obtained by 24-h passive diffusion of the applied drug. The results emphasize, therefore, that the new transdermal technology is suitable for therapeutic delivery of poorly penetrating molecules.

© 2003 Elsevier Science B.V. All rights reserved.

Keywords: Radiofrequency-microchannels; Radiofrequency ablation; Granisetron; Diclofenac; Transdermal delivery; Skin permeation

1. Introduction

The outmost dermal layer, the stratum corneum (SC), forms an effective barrier to the permeation of external chemicals; therefore, the transdermal ad-

ministration of drugs and other substances is remarkably restricted. Passive penetration of the SC is particularly difficult for hydrophilic and charged molecules. Consequently, transdermal delivery of drugs has been the subject of intensive research. In addition to the vehicle formulations and the chemical enhancers [1,2], physical methods such as microneedles [3,4], iontophoresis and electroosmosis [5–7], electroporation [8–13], and ultrasound [14] have

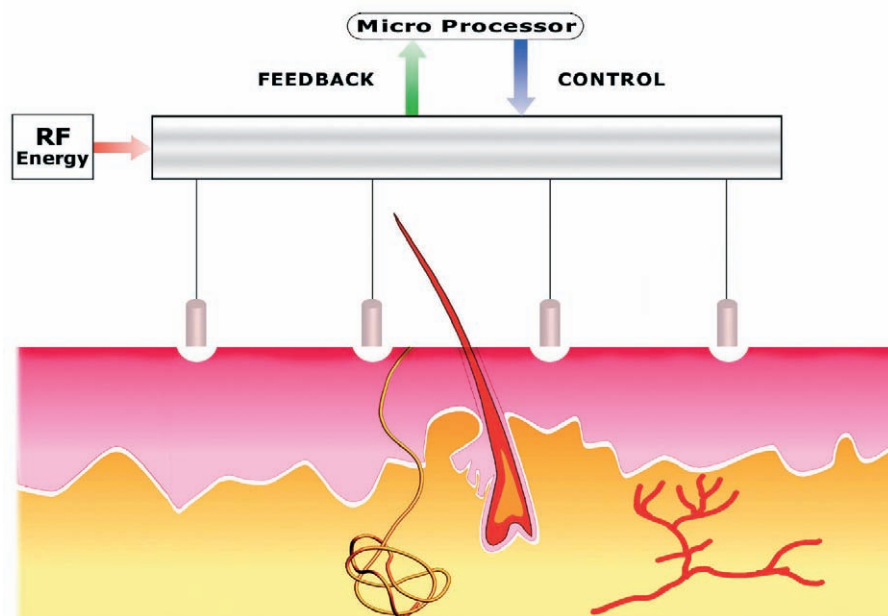
*Corresponding author. Tel.: +972-8-647-2709; fax: +972-8-647-2960.

E-mail address: asintov@bgumail.bgu.ac.il (A.C. Sintov).

been investigated. Synergistic interactions between chemical and physical enhancers as well as synergism between two physical means of drug enhancement were also studied [15–17].

Radiofrequency (RF) thermal ablation is a well-known and effective technology for electrosurgery and ablation of malignant tissues. The method involves placing of a thin, needle-like electrode directly into the tumor. During the application of RF energy, a frequency alternating current moves from the tip of the electrode into the tissue surrounding that electrode. As the ions within the tissue attempt to follow the change in the direction of the alternating current, their movements result in frictional heating of the tissue, producing coagulative necrosis and cell ablation. Operations using radiofrequencies are considered safe and convenient during the surgery, since cessation of neuromuscular stimulation occurs at approximately 100 kHz. That is, while the target tissue absorbs the heat energy released during the electrosurgery, the applied high-frequency current does not affect the proximal muscles [18–21]. This technology has been adapted as an optional physical enhancer of drug transport across the skin. Its potential in the creation of aqueous microchannels in the outer layer of the skin was studied.

The present paper is the first report describing this novel method in facilitating the transport of hydrophilic drugs, granisetron hydrochloride ($pK_a=9.4$, MW=348.9) and diclofenac sodium ($pK_a=4.0$, MW=318.1), through the SC barrier. We have chosen these drugs because the transdermal administration of charged and polar molecules is difficult due to the intrinsic lipophilicity of the SC. The method of using RF energy is based on creating an array of small microchannels across the SC into the viable epidermis by microablating skin cells. The high frequency electrical current conducted through the aqueous medium of the stratum corneum generates heat that brings about an instant removal of cells beneath the electrode. Due to the high velocity (1 ms per electrode), it is postulated that only heat conduction results in the creation of microchannels, and other mechanisms such as electrochemical reactions do not take place. Skin electroporation, which is operated by low duty cycle, high intensity electric-field pulsing, is also believed to create transient aqueous microchannels [8–13]. The creation of transient aqueous microchannels by RF energy has been evidenced for the first time in the present report. The operating principle of RF-microchanneling formation are shown in Scheme 1. As illustrated,



Scheme 1. Schematic presentation of RF-microchannels.

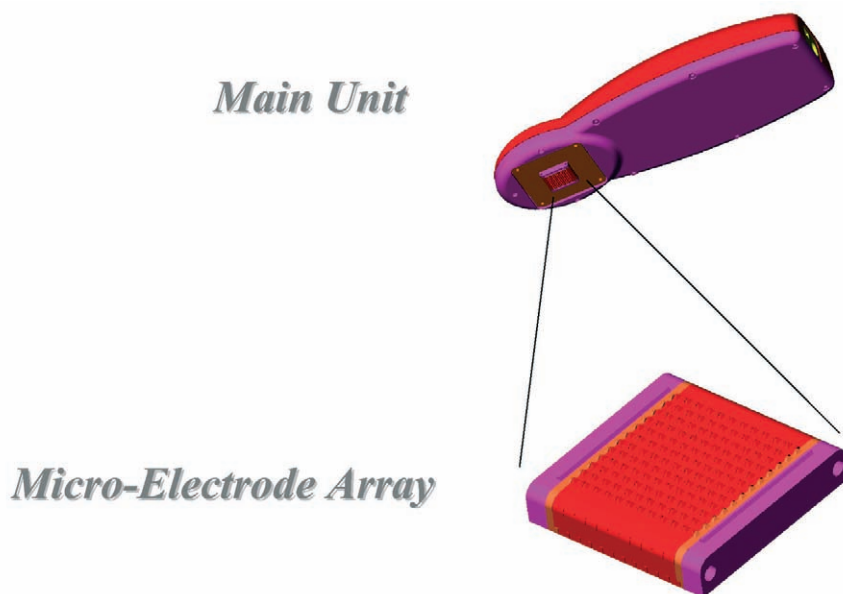
a closely spaced array of tiny electrodes is placed against the skin while an alternating current at radio frequency is applied to each of the microelectrodes. This forms RF-microchannels on the very outer layer of the skin through ablation of cells. The microchannels are designed to penetrate only the outer layers of the skin where there are no blood vessels or nerve endings, resulting in minimal skin trauma and neural sensation.

2. Materials and methods

2.1. Instruments and materials

The RF-microchannels generator (ViaDerm™, Transpharma Ltd, Israel) is illustrated in Scheme 2 [22]. ViaDerm™ generator is made of two primary components: a reusable electronic controller in a size similar to a phone handset, and a disposable array that can snap onto the end of the controller. The controller can communicate with a computer and be reprogrammed to change any of the critical parameters used. The controller is also able to measure and download electrical inputs during the ablation pro-

cess, such as a peak current, final current, voltage, time and energy. The array is made of a polycarbonate body and stainless steel electrodes of length 100 μm and diameter 40 μm . It has 140 electrodes spaced 1 mm from each other in a square matrix arrangement. In this prototype, each electrode is individually operated. The electrodes are designed to create micro-channels that are 40 μm wide and 70 μm deep. The operation of the RF-microchannel generator is simple and easy. The user holds the controller in his hand and presses the array against the test site on the skin. When a minimum pressure is placed on the skin the RF-generator is activated and the treatment begins automatically. Within seconds (typically less than a millisecond per burst per electrode) the array completes its work. The density of the microelectrode array used in these studies was 100 or 200 microelectrodes/ cm^2 . Each electrode received a multiple number of bursts of RF energy as programmed. The animal studies (rats) were done with the following conditions: applied voltage: 200 or 250 V; RF frequency: 100 kHz; burst length: 1 ms; number of bursts: 5; time between bursts: 15 ms. Excised porcine skin for in vitro testing and histological observation was treated with an applied



Scheme 2. The handset system and the microelectrodes array (ViaDerm™, Transpharma Ltd.) as designed to facilitate the transdermal drug delivery by transmission of radio frequency currents through the electrodes into the skin, and by the creation of microchannels.

voltage of 380 V and five bursts or with 330 V and two bursts, respectively.

Granisetron hydrochloride was obtained from Natco Pharma, Hyderabad, India. Diclofenac sodium was purchased from Sigma. The drug solutions at a concentration of 1% w/v were freshly prepared. Sodium diclofenac was dissolved in 1:4 ethanol–water, while granisetron HCl was dissolved in distilled water. For the pharmacokinetics experiments, granisetron in solution was soaked into a crosslinked polyethylene oxide wound dressing (Vigilon, Bard Inc.) to obtain a hydrogel patch containing 3% w/v of drug substance. This formulation was prepared and used in vivo instead of 1% w/v concentration utilized in the in vitro studies. All solvents were HPLC grade (Merck, Germany).

2.2. In vitro skin permeation study

The permeability of diclofenac sodium and granisetron hydrochloride through full thickness porcine ear skin was measured in vitro with a flow-through Franz diffusion cell system (Laboratory Glass Apparatus, Berkeley, CA). The diffusion area was 3.1 cm². Full-thickness porcine skin was excised from fresh ears of slaughtered white pigs (breeding of Landres and Large White, locally grown in Kibbutz Lahav, Israel). Transepidermal water loss measurements (TEWL, Dermalab[®] Cortex Technology, Hadsund, Denmark) were performed and only those pieces that the TEWL levels were less than 15 g/m²/h were mounted in the diffusion cells. Skin microchanneling was performed in cells defined as pretreatment group, then TEWL was measured again to control the operation (see Table 1). The skin pieces were placed on the receiver chambers with the stratum corneum facing upwards, and the donor chambers were clamped in place.

Drug solutions (1% w/v sodium diclofenac or

granisetron HCl) were pipetted into the donor chambers (0.5 or 1 ml of granisetron or diclofenac solutions, respectively). Phosphate buffered saline (PBS, pH 7.4) or ethyl alcohol–PBS (1:9) in granisetron or diclofenac experiments, respectively, was passed through the receiver cells at a flow rate of 2 ml/h. Samples from the receiver solutions were collected into tubes (using a fraction collector, Retriever IV, ISCO), at predetermined times for a 24-h period. The samples were kept at 4 °C until analyzed by HPLC.

2.3. HPLC analysis of samples from receiver solutions

2.3.1. Diclofenac

Aliquots of 20 ml from each sample were injected into a HPLC system, equipped with a prepacked C₁₈ column (Phenomenex LUNA[™], 5 mm, 150×4 mm). The HPLC system (ProStar modules, Varian Inc.) was equipped with an autosampler and a UV detector (Varian's ProStar model 310). The quantification of diclofenac was carried out at 280 nm. The samples were chromatographed using an isocratic mobile phase consisting of acetonitrile–sodium acetate buffer, pH 6.3 (35:65) at a flow rate of 1 ml/min.

2.3.2. Granisetron

Aliquots of 10 ml from each sample were injected into the HPLC system, equipped with a prepacked C₁₈ column (Phenomenex LUNA[™], 5 mm, 150×4 mm). The detection of granisetron was carried out at 305 nm. The samples were chromatographed using an isocratic mobile phase consisting of acetonitrile–sodium acetate buffer, pH 4.2 (40:60) at a flow rate of 0.75 ml/min.

Data were expressed as the cumulative drug permeation (Q_t) per unit of skin surface area, Q_t/S

Table 1

Summary of TEWL values obtained before and after treatment with RF currents in the overall experiments (values are expressed in g/m²/h)

	Drugs aimed to be tested	Control experiments (without RF treatment)	Tested skin before treatment	Tested skin after treatment
Excised porcine skin	Diclofenac	6.83±0.54	8.22±1.33	17.3±0.99
In vitro	Granisetron	8.38±0.93	10.67±1.2	23.4±2.06
Abdominal rat skin	Diclofenac	5.05±0.65	5.35±1.06	25.38±1.44
In vivo	Granisetron	6.16±0.98	5.7±0.98	23.73±4.33

($S=3.1 \text{ cm}^2$). The steady-state fluxes (J_{ss}) were calculated by linear regression interpolation of the experimental data.

2.4. Pharmacokinetic studies of transdermal drug in rats

Male Sprague–Dawley rats (400–500 g, Harlan Laboratories Ltd., Jerusalem, Israel) were anesthetized (5 mg/kg ketamine i.p.) and were placed on their back. Anesthesia was maintained with 0.1 ml ketamine (100 mg/ml) along the experiment. The procedure protocol related to animals was reviewed and approved by the Institutional Animal Care and Use Committee.

The abdominal skin hair was trimmed off and shaved carefully, and was cleaned with isopropyl alcohol. After 30 min, the transepidermal water loss was measured to check skin integrity. At this stage, RF-microchannels were generated on the shaved skin of a test group. After generation of RF-microchannels, TEWL was measured again and the obtained values were documented (see Table 1). It was obvious according to the TEWL data that the hair clipping and shaving did not cause apparent damage to the skin. Each test group of the diclofenac pharmacokinetic study consisted of three animals for the whole testing duration, while the test group of the granisetron study consisted of four animals for each time period ($n=4$; five sampling times; total of 20 rats). Each experiment was accompanied by a control group of animals that were not undergoing the pretreatment procedure ($n=6$ for diclofenac, $n=4$ with a total of 20 rats for granisetron application). Drug solutions (1% diclofenac sodium or 3% granisetron hydrochloride in a hydrogel sheet) were then applied on the skin surface. In the case of diclofenac solution, special containers glued to the skin by a silicon rubber were used to hold drug solution over the specified place. Skin surface areas of 1.4 cm^2 or 2.8 cm^2 ($1.4 \text{ cm}^2 \times 2$) were covered with granisetron patches and diclofenac solutions, respectively. Blood samples were taken under anesthesia from the tail vein (while monitoring diclofenac) or directly from the heart (while monitoring granisetron) into heparinized tubes. After centrifugation, plasma samples were kept at $-20 \text{ }^\circ\text{C}$ until analyzed for drug concentration.

2.5. HPLC analysis of plasma extracts

2.5.1. Diclofenac

Into 100 ml of plasma, 200 ml of methanol was added and mixed well. After centrifugation, aliquots of 20 ml from each vial were injected into the HPLC system, equipped with a prepacked C_{18} column (Phenomenex LUNA™, 5 mm, $150 \times 4 \text{ mm}$). The HPLC system (Shimadzu VP series) was equipped with an autosampler and a diode array detector. The quantification of diclofenac was carried out at 280 nm. The samples were chromatographed using an isocratic mobile phase consisting of acetonitrile–sodium acetate buffer, pH 6.3 (30:70) at a flow rate of 1.5 ml/min. Calibration curves (peak area vs. drug concentration) were linear over the range 1–20 mg/ml.

2.5.2. Granisetron

The procedure was basically performed according to Kudoh et al. [23]. Into 1 ml plasma, 500 ml of phosphate buffer (pH 7) was added and mixed well. The mixture was transferred on a 500-mg C-2 Bond Elute SPE cartridge pre-washed consecutively with methanol, water and phosphate buffer (pH 7). After plasma application, the SPE cartridge was washed with 2 ml water and 2 ml acetonitrile–water 40:60. The cartridge was dried under vacuum and granisetron was then eluted with 2 ml methanol followed by 2 ml methanol containing 1% trifluoroacetic acid. The combined eluent was dried at $40 \text{ }^\circ\text{C}$ under nitrogen and the residue was dissolved in 200 ml methanol–water 10:90. Aliquots of 30 ml from each sample were injected into the HPLC system, equipped with a prepacked C_8 column (Hypersil BDS C-8 $100 \times 3.0 \text{ mm}$, $3 \text{ }\mu\text{m}$). The HPLC system (1050 HP) was equipped with an autosampler, and a fluorescence detector (Model 1046A). The detection of granisetron was carried out at 305-nm excitation wavelength and 365-nm emission wavelength. The samples were chromatographed using an isocratic mobile phase consisting of acetonitrile–0.1 M acetate buffer (pH 4.7) containing 10 mM hexanesulfonate and 0.23 g/l EDTA (19:81) at a flow rate of 0.3 ml/min. Calibration curves (peak area vs. drug concentration) were linear over the range 2–100 ng/ml.

2.6. Histology

RF-microchannels were created at a density of 200 channels/cm² on the dorsal skin of a pig. Skin biopsies were taken immediately after RF-microchannels creation, by a biopsy punch, and preserved in 4% buffered formaldehyde solution. The samples were embedded in paraffin wax, cut to a thickness of 4–5 μm, stained with hematoxylin and eosin (HandE) and examined microscopically.

3. Results

The photomicrograph of Fig. 1 shows the microchannel produced by RF energy of 330 V applied voltage (100 kHz; two bursts). The tissue around the microchannel showed normal epidermal and dermal structure, with no pathological changes. The microchannel produced at these conditions measures about 30 μm in diameter and 70 μm in depth from the epidermal surface into the superficial dermis. The porcine epidermis is usually thinner than human

epidermis — 36.9 μm vs. 49.5 μm, respectively [26] — indicating that RF-microchannels would go into the human epidermis without perturbing the dermis.

The permeability of diclofenac sodium and granisetron hydrochloride through excised porcine ear skin was significantly enhanced after pretreatment with RF energy, as compared to the delivery through the untreated control skin (Figs. 2–3). After lag times of 3 and 9 h, pseudo steady state fluxes of 41.6 μg/cm²/h ($r=0.997$) and 23.0 μg/cm²/h ($r=0.989$) were obtained for granisetron and diclofenac, respectively. These results were compared to fluxes obtained after 24-h penetration through untreated intact skin (passive delivery) — 5.9 μg/cm²/h ($r=0.983$) and 6.0 μg/cm²/h ($r=0.988$) for granisetron and diclofenac, respectively. The concentration of the drugs in the donor was 10 mg/ml; therefore, the ‘apparent permeability coefficients’ (J_{ss} divided by donor concentration) after RF-microchanneling were 41.63×10^{-4} and 22.98×10^{-4} cm/h for granisetron and diclofenac respectively (Table 2). The values obtained for granisetron and diclofenac were 7.1 and 3.8 times, respectively, higher than the coefficients

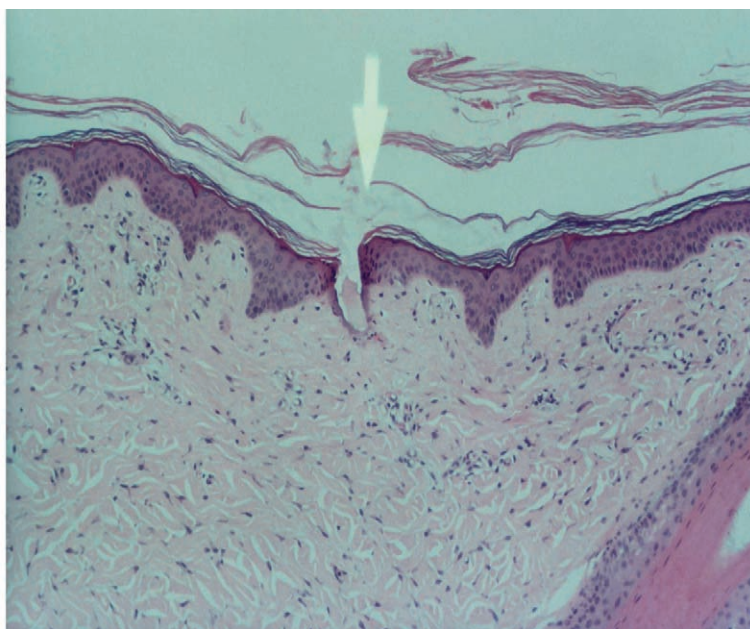


Fig. 1. Photomicrograph of cross-section of porcine skin treated with RF currents (density=200 electrodes/cm²) of 200 V applied voltage (100 kHz; five bursts) showing a localized microchannel intruding the epidermis into the superficial dermis (70 μm in length), hematoxylin–eosin staining, 400×.

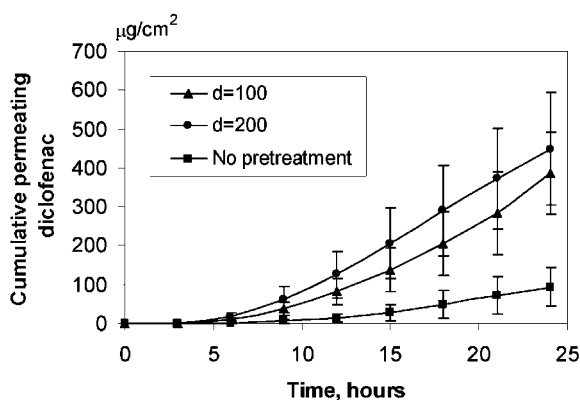


Fig. 2. In vitro percutaneous penetration of diclofenac sodium from 1% hydroalcoholic solution after skin was pretreated with RF currents of 200 V applied voltage (100 kHz; five bursts), using an array of 100 microelectrodes/ cm^2 (triangles) and 200 microelectrodes/ cm^2 (circles). Passive diffusion through untreated skin is also illustrated (squares). The in vitro testing was performed on porcine ear skin in Franz diffusion cells ($n=6$).

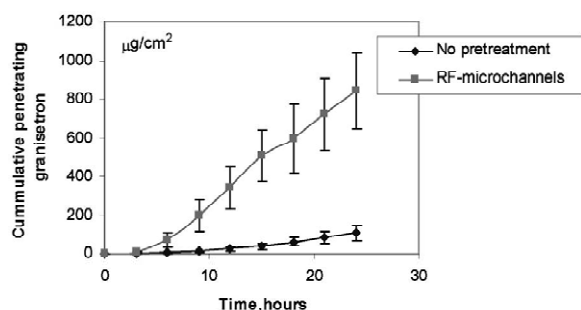


Fig. 3. In vitro percutaneous penetration of granisetron hydrochloride from 1% aqueous solution after skin was pretreated with RF currents of 200 V applied voltage (100 kHz; five bursts), using an array of 100 microelectrodes/ cm^2 (squares). Passive diffusion through untreated skin is also illustrated (diamonds). The in vitro testing was performed on porcine ear skin in Franz diffusion cells ($n=6$).

Table 2

Comparison of the apparent permeability coefficients obtained for granisetron and diclofenac transport through porcine skin

	Permeability coefficient (cm/h)	
	Granisetron	Diclofenac
Intact skin	5.86×10^{-4}	6.05×10^{-4}
RF ^a -treated skin	41.63×10^{-4}	22.98×10^{-4}

^a RF-microchannels were created using 100 electrodes/ cm^2 . Applied voltage: 200 or 250 V; RF frequency: 100 kHz; and number of bursts: 5.

obtained after a passive diffusion through the porcine skin. As shown in Fig. 2, by multiplying the density of the electrodes array from 100 to 200 electrodes/ cm^2 , the in vitro percutaneous penetration of diclofenac changed slightly (apparent permeability coefficient= 26.3×10^{-4} cm/h compared to 23.0×10^{-4} cm/h), although it was not statistically significant.

The enhanced transdermal delivery was also demonstrated in vivo in rats. It was shown that diclofenac plasma levels in the pre-treated rats reached plateau levels of 1.22 ± 0.32 $\mu\text{g}/\text{ml}$ after 3 h to 1.47 ± 0.33 $\mu\text{g}/\text{ml}$ after 6 h, as compared to 0.16 ± 0.04 $\mu\text{g}/\text{ml}$ levels obtained after 6 h in untreated rats (Fig. 4). This enhancement was achieved by using an array consisting of 100 electrode/ cm^2 and an applied voltage of 250 V. When a power of 200 V was applied, the drug plasma levels were significantly reduced ($P>0.05$), rising to only 0.93 $\mu\text{g}/\text{ml}$ after 6 h. Fig. 5 shows the pharmacokinetics profiles of granisetron in rats for 24 h after dermal application of patches containing 3% granisetron. A group of animals that were pretreated with an array of 100 electrodes/ cm^2 delivering an RF energy of 250 V applied voltage was compared with the untreated control group ($n=4$). The plasma levels of granisetron in the pretreated group reached a peak after 12 h with an averaged value of 239.3 ± 43.7 ng/ml. This enhanced con-

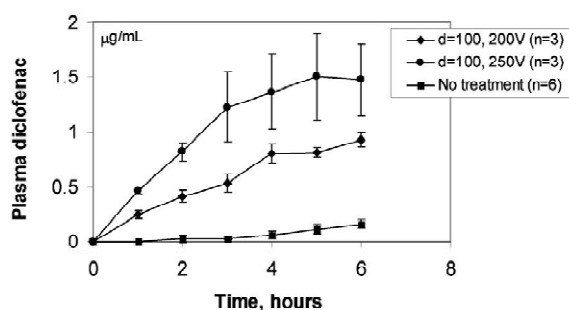


Fig. 4. Plasma levels ($\mu\text{g}/\text{ml}$) of diclofenac in rats after application of 1% hydroalcoholic solution on 2.8 cm^2 skin surface area. The diamond-shaped symbols represent skin pretreatment with RF currents of 250 V applied voltage (100 kHz; five bursts) ($n=3$), while the circle-shaped symbols represent skin pretreatment with RF currents of 200 V applied voltage ($n=3$). The pharmacokinetic profile of passive diffusion through untreated skin is represented by the square-shaped symbols ($n=6$).

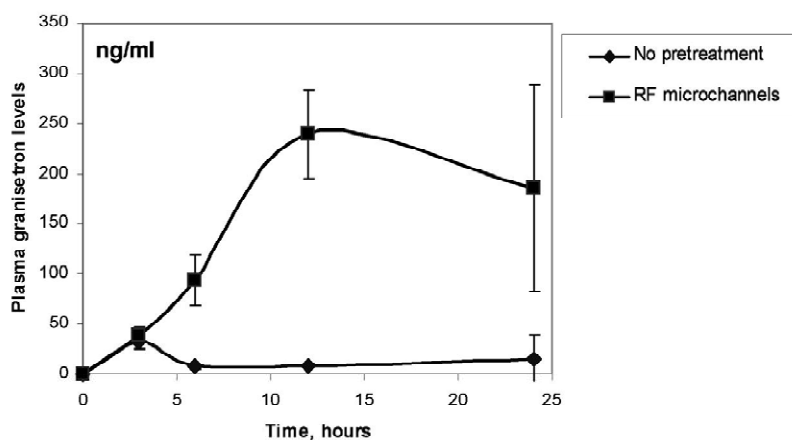


Fig. 5. Plasma levels (ng/ml) of granisetron in rats after application of 3% aqueous solution in Vigilon hydrogel patches on 1.4 cm² skin surface area. The square-shaped symbols represent skin pretreatment with RF currents of 200 V applied voltage (100 kHz; five bursts) ($n=4$). The pharmacokinetic profile of passive diffusion through untreated skin is represented by the diamond-shaped symbols.

centration was about 30 times higher than the plasma levels after dermal granisetron application, which passively absorbed to the blood circulation during 24 h, as demonstrated by the pharmacokinetics profile of the control group (Fig. 5).

4. Discussion

It has already been evidenced by many publications that high voltage electric-field pulses significantly increase the transdermal flux of drugs [8–13]. This phenomenon, which is called electroporation (or electropermeabilization), is believed to involve the creation of transient aqueous ‘pores’ in the lipid bilayers of the SC. While neither visual evidence of these pores has been reported nor the physical nature of the structural skin changes is known, the mechanism by which electroporation increases the transdermal flux is incomplete, thus being explained by theoretical models of transient aqueous pathways in planar lipid bilayer systems [24]. Our study presents for the first time a new method of creating visual pores or microchannels in the skin outer layers by radiofrequency currents. The mechanism by which these visual microchannels (Fig. 1) are created may be explained by the heat released during a fast flow of ions in the epidermis. Based on the known practice of needle ablation of

tumors and lesions by RF-electrodes [19], the RF power (<1 MHz) causes oscillatory movement of ions in the tissue with a velocity that is proportional to the electric field intensity. This ionic current results in a frictional energy loss, heating and coagulation necrosis. It has been postulated according to the fact that the immediate surroundings of the microchannel was not damaged that heat is created in the ablated cells themselves, rather than being conducted from an external source. Further mechanistic studies are needed to elucidate how RF currents work on skin tissue.

The fluxes and the permeability coefficients of diclofenac sodium and granisetron hydrochloride through porcine skin in vitro, as shown in Table 2 and Figs. 2–3, demonstrate that granisetron HCl is twice more permeable through RF-pretreated porcine skin than diclofenac sodium. The molecular weights of both drugs are similar (349 and 318 for granisetron and diclofenac salts, respectively), however, in the vehicle and in the physiological environment of the skin they are salts, which possess opposite charges. Since granisetron is a positively charged molecule and diclofenac is negatively charged polar molecule, it may imply that electrostatic attraction/repulsion ratios dictate the migration along the aqueous microchannels. This might also be an indication of a potential gradient that is created by RF microchanneling procedure in which, like ion-

tophoresis, monovalent cations cross the skin more easily than monovalent anions based on the net negative charge of the skin [25]. It is also reasonably postulated that the diffusion through the aqueous microchannels might be easier for the highly water-soluble granisetron hydrochloride (with an octanol/water partition coefficient of $K_{ow}=0.28$) than for the sparingly water-soluble diclofenac sodium ($K_{ow}=13.4$ at pH 7.4 and $K_{ow}=1545$ at pH 5.2).

Finally, RF microchanneling as performed in this study produced no skin damage in rats. There was no evidence of erythema and edema immediately after the pretreatment process, and 6 and 24 h after pretreatment when experiments were terminated. This may be explained by the fact that only a small portion of the total skin area is covered by microchannels. Since in the diameter of a microchannel is approximately 30 μm , its cross-section area is $7 \times 10^{-6} \text{ cm}^2$. By using a given density of 100 or 200 channels per centimeter square, it is found that less than 0.2% of the skin area is occupied by microchannels. Due to this small figure and the relatively low microchanneling depth, no apparent skin reaction was documented. Since toxicological research was beyond the scope of this paper, more studies including long-term follow-up and histopathological examinations should be conducted.

In conclusion, the results obtained in this study have shown that the permeability of polar hydrophilic molecules, which poorly penetrate the lipophilic SC barrier, was significantly enhanced through excised porcine ear skin and through rat skin in vivo by using radiofrequency microelectrodes. The mechanism by which they create microchannels in the outer layers of the skin still remains to be studied.

References

- [1] K.A. Walters, Penetration enhancers and their use in transdermal therapeutic systems, in: J. Hadgraft, R.H. Guy (Eds.), *Transdermal Drug Delivery, Developmental Issues and Research Initiatives*, Marcel Dekker, Inc., New York and Basel, 1989, pp. 197–246.
- [2] E.W. Smith, H.I. Maibach (Eds.), *Percutaneous Penetration Enhancers*, CRC Press, Boca Raton, FL, 1995.
- [3] S. Henry, D.V. McAllister, M.G. Allen, M.R. Prausnitz, Microfabricated microneedles: a novel approach to transdermal drug, *J. Pharm. Sci.* 87 (1998) 922–925.
- [4] D.V. McAllister, M.G. Allen, M.R. Prausnitz, Microfabricated microneedles for gene and drug delivery, *Ann. Rev. Biomed. Eng.* 2 (2000) 298–313.
- [5] P. Singh, P. Liu, S.M. Dinh, Facilitated transdermal delivery by iontophoresis, in: R.L. Bronaugh, H.I. Maibach (Eds.), *Percutaneous Absorption, Drugs–Cosmetics–Mechanisms–Methodology*, 3rd Edition, Marcel Dekker, Inc., New York and Basel, 1999, pp. 633–657.
- [6] M.J. Pikal, The role of electroosmotic flow in transdermal iontophoresis, *Adv. Drug Del. Rev.* 46 (2001) 281–305.
- [7] B.D. Bath, H.S. White, E.R. Scott, Visualization and analysis of electroosmotic flow in hairless mouse skin, *Pharm. Res.* 17 (2000) 471–475.
- [8] M.R. Prausnitz, V.G. Bose, R. Langer, J. Weaver, Electroporation of mammalian skin: a mechanism to enhance transdermal drug delivery, *Proc. Natl. Acad. Sci. USA* 90 (1993) 10504–10508.
- [9] R. Vanbever, N. Lecouturier, V. Preat, Transdermal delivery of metoprolol by electroporation, *Pharm. Res.* 11 (1994) 1657–1662.
- [10] J.E. Riviere, N.A. Monteiro-Riviere, R.A. Rogers, D. Bommannan, J.A. Tamada, R.O. Potts, Pulsatile transdermal delivery of LHRH using electroporation: drug delivery and skin toxicology, *J. Controlled Release* 36 (1995) 229–233.
- [11] R. Vanbever, E. Le Boulenger, V. Preat, Transdermal delivery of fentanyl by electroporation. I. Influence of electrical factors, *Pharm. Res.* 13 (1996) 559–565.
- [12] M.R. Prausnitz, A practical assessment of transdermal drug delivery by skin electroporation, *Adv. Drug Del. Rev.* 35 (1999) 61–76.
- [13] Q. Hu, W. Liang, J. Bao, Q. Ping, Enhanced transdermal delivery of tetracaine by electroporation, *Int. J. Pharm.* 202 (2000) 121–124.
- [14] J. Kost, S. Mitragotri, R. Langer, Phonophoresis, in: R.L. Bronaugh, H.I. Maibach (Eds.), *Percutaneous Absorption, Drugs–Cosmetics–Mechanisms–Methodology*, 3rd Edition, Marcel Dekker, Inc., New York and Basel, 1999, pp. 615–631.
- [15] S. Mitragotri, Synergistic effect of enhancers for transdermal drug delivery, *Pharm. Res.* 17 (2000) 1354–1359.
- [16] D. Bommannan, J. Tamada, L. Leung, R.O. Potts, Effect of electroporation on transdermal iontophoretic delivery of luteinizing hormone releasing hormone (LHRH) in vitro, *Pharm. Res.* 11 (1994) 1809–1814.
- [17] S-L. Chang, G.A. Hofmann, L. Zhang, L.J. Defetos, A.K. Banga, The effect of electroporation on iontophoretic transdermal delivery of calcium regulating hormones, *J. Controlled Release* 66 (2000) 127–133.
- [18] F. Izzo, C.C. Barnett, S.A. Curley, Radiofrequency ablation of primary and metastatic malignant liver tumors, *Adv. Surg.* 35 (2001) 225–250.
- [19] S. Nahum Goldberg, Radiofrequency tumor ablation: principles and techniques, *Eur. J. Ultrasound* 13 (2001) 129–147.
- [20] L. Solbiati, T. Ierace, M. Tonolini, V. Osti, L. Cova, Radiofrequency thermal ablation of hepatic metastases, *Eur. J. Ultrasound* 13 (2001) 149–158.
- [21] F.J. McGovern, B.J. Wood, S. Nahum Goldberg, P.R. Mueller, Radiofrequency ablation of renal cell carcinoma via

- image guided needle electrodes, *J. Urol.* 161 (1999) 599–600.
- [22] Z. Avrahami, Transdermal Drug Delivery and Analyte Extraction, US Patent No. 6,148,232, (2000).
- [23] S. Kudoh, T. Sato, H. Okada, H. Kumakura, H. Nakamura, Simultaneous determination of granisetron and 7-hydroxy-granisetron in human plasma by high-performance liquid chromatography with fluorescence detection, *J. Chromatogr. B* 660 (1994) 205–210.
- [24] J.C. Weaver, Y.A. Chizmadzhev, Theory of electroporation: a review, *Bioelectrochem. Bioenerg.* 41 (1996) 135–160.
- [25] R.R. Burnette, B. Ongpipattanakul, Characterization of the preselective properties of excised human skin during iontophoresis, *J. Pharm. Sci.* 76 (1987) 765–773.
- [26] R. Panchagnula, K. Stemmer, W.A. Ritschel, Animal models for transdermal drug delivery, *Meth. Find. Exp. Clin. Pharmacol.* 19 (1997) 335–341.