Available online at www.sciencedirect.com
SCIENCE DIRECT
International Journal of Pharmaceutics 316 (2006) 58–67
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Facilitated skin penetration of lidocaine: Combination of a short-term iontophoresis and microemulsion formulation

Amnon C. Sintov *, Roy Brandys-Sitton

Department of Pharmacology and School of Pharmacy, E. D. Bergmann Campus, Ben Gurion University of the Negev, P.O. Box 653, Beer Sheva 84105, Israel

Received 11 October 2005; received in revised form 29 January 2006; accepted 17 February 2006
Available online 6 March 2006

Abstract

The objective of this study was to demonstrate the potential of the application of a short-term iontophoresis on the topical delivery of lidocaine hydrochloride from a microemulsion-based system. Five- and 10-min durations of anodal iontophoresis applied onto porcine skin were examined in combination with a microemulsion containing 2.5% lidocaine hydrochloride. A similar combination (10-min iontophoresis with microemulsion in the anodal electrode) was also examined in vivo in a rat model. It was shown in vitro that by combining microemulsion application with a 10-min iontophoresis of 1.13 mA/cm^2 electric current density, a significantly increased flux was obtained compared with a combination of aqueous drug solution with the same iontophoresis protocol. In vivo studies revealed that 57.71 ± 18.65 and 18.43 ± 9.17 μg cm^{-2} were reached in the epidermis and dermis, respectively, at $t = 30$ min of microemulsion application, when iontophoresis was applied for 10 min. In contrast, the application of aqueous solution-iontophoresis resulted in a relatively lower drug accumulation (21.44 ± 10.42 and 5.30 ± 2.25 μg cm^{-2} in the epidermis and dermis, respectively, at $t = 30$) with more rapid clearance of the drug from the skin. Ten-minute application of a low-current electric field on a new topical microemulsion appears to make significant changes in skin permeability. The potential advantages of this procedure include significantly increased flux, accumulation of a large skin drug depot, short lag times, reduced irritation (compared to long-term iontophoresis), simplicity and ease of compliance.

Keywords: Microemulsion; Iontophoresis; Percutaneous penetration; Topical delivery; Lidocaine

1. Introduction

The skin's outermost layer, the stratum corneum, has excellent barrier properties that limit drug delivery into and through the skin. Nevertheless, the outstanding advantages of the administration of drugs by the dermal route have motivated intensive research activity in this area. As a result of this activity, various methods and strategies have emerged to overcome the skin barrier and to improve drug transport into the skin and into the blood circulation (Barry, 2001; Davis et al., 2002). Some methods are based on chemical enhancers and various vehicle formulations (Walters, 1989; Smith and Maibach, 1995). Other methods rely on physical techniques, such as microneedle technologies (Henry et al., 1998; McAllister et al., 2000), iontophoresis (Singh et al., 1999; Marro et al., 2001; Guy et al., 2001), electroporation (Prausnitz et al., 1993; Vanbever et al., 1994, 1996; Riviere et al., 1995; Prausnitz, 1999; Hu et al., 2000) and ultrasound (Kost et al., 1999). In addition, various combinations of enhancing methods have been tested and some of them have indeed resulted in improved skin penetration (Bommannan et al., 1994; Mitragotri, 2000; Chang et al., 2000; Le et al., 2000; Bose et al., 2001; Denet et al., 2003; Fang et al., 2004). Many of these combinations involve two or three physical methods, such as iontophoresis and electroporation (Bommannan et al., 1994; Chang et al., 2000; Bose et al., 2001; Denet et al., 2003), iontophoresis, electroporation and laser treatment (Fang et al., 2004), or iontophoresis and ultrasound (Le et al., 2000). Other methods comprise combinations of an electrically assisted method with chemical enhancers (Mitragotri, 2000; Bhatia et al., 1997; Kalia and Guy, 1997; Zewert et al., 1999). In addition, the combined use of electrically assisted drug delivery methods with well-designed formulations, such as those based on liposomes, has also been reported (Fang et al., 1999; Conjeevaram et al., 2003; Essa et al., 2004). There are, however, only few studies on

* Corresponding author. Tel.: +972 8 647 2709; fax: +972 8 647 2960.
E-mail address: asintov@bgu.ac.il (A.C. Sintov).

0378-5173/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2006.02.034

the combination of an electrical method with drugs formulated in microemulsions (Kantaria et al., 1999; Boinpally et al., 2004).

Iontophoresis involves the application of a low-level electrical current that facilitates migration of drug ions or very polar drugs from the transdermal delivery system through the skin membranes (Green et al., 1993). Under the influence of the iontophoretic current, three mechanisms are involved in drug transport: (1) an existing passive skin permeation, (2) electrorepulsion, in which the ion is repelled from an electrode of the same charge, and (3) electroosmosis, which is the convective movement of a solvent through a charged "pore" in response to the preferential passage of counter-ions when an electric field is applied (Guy et al., 2000). Thus, in addition to electrorepulsion, an electroosmotic solvent flow occurs at the physiological pH in the anode-to-cathode direction, resulting in transport of cations into the negatively charged skin. An example of a cation used frequently in clinical practice for local anesthesia is lidocaine hydrochloride. Recently, new iontophoretic systems have become available for transdermal lidocaine delivery (e.g., Iontocaine, Iomed, Inc., Salt Lake City, UT, USA; Lidosite, Vyteris Inc., Fair Lawn, NJ, USA), in which lidocaine hydrochloride in delivered in an aqueous solution or in an aqueous gel (Naik et al., 2000; Kearns et al., 2003). Although effective, there is still a need to reduce the long delay between drug application and the local anesthetic effect.

Therefore, in the present study, we set out to demonstrate the potential advantages of iontophoretic delivery of lidocaine hydrochloride from a microemulsion system to the skin. We have previously demonstrated the potential of a microemulsion system based on commonly used nonionic surfactants and a new co-surfactant, tetraglycol (Sintov and Shapiro, 2004). In that study, a w/o microemulsion formulation containing 2.5% lidocaine (as base) was shown to passively deliver significant amounts of drug to the dermis in relatively short periods of time (30 min). Since more rapid onset of local anesthesia has always been preferable, we made a further attempt to shorten the time of treatment by combining a short-term iontophoresis (5 or 10 min) with the topical treatment. In this report, the same type of topical microemulsion loaded with lidocaine hydrochloride was further evaluated in vitro and in vivo in combination with short-term (maximum 10 min) anodal iontophoresis. The mechanism by which this microemulsion system contributes to the iontophoretic drug delivery is based on its lipophilic nature, which enables increased intercalation of aqueous nano-droplets into the stratum corneum. Charge transport in the microemulsion during iontophoresis is probably facilitated by the flexibility of the surfactant film, allowing hopping of drug ions within nano-droplet clusters.

2. Materials and methods

2.1. Materials

Lidocaine as a hydrochloride salt was obtained from Sigma Rehovot, Israel. PEG-40 stearate and glyceryl oleate were obtained from Uniqema, Bromborough Pool, Wirral, UK. Isopropyl palmitate and tetraglycol were purchased from Sigma, Rehovot, Israel. High-performance liquid chromatography (HPLC) grade solvents were obtained from J.T. Baker (Mallinckrodt Baker, Inc., Phillipsburg, NJ, USA).

2.2. Preparation of microemulsions

Microemulsions were prepared as previously described (Sintov and Shapiro, 2004) by dissolving lidocaine hydrochloride in a mixture of polyethylene glycol (PEG-40) stearate and glyceryl oleate (surfactants), tetraglycol (co-surfactant), isopropyl palmitate (oil) and 0.01 M sodium chloride solution (aqueous phase). The ratio of surfactants in the system was 3:2 glyceryl oleate–PEG-40 stearate, and the cosurfactant/surfactants ratio was 1.8. The final concentration of lidocaine HCl in the microemulsion was 2.5 wt%. The microemulsion formed spontaneously at room temperature as a clear monophasic liquid. The microemulsion was obtained with a mean droplet size of approximately 15 nm diameter as determined at 18.3 °C by dynamic light scattering (ALV instruments Type CGS-3, ALV/LSE-5003 digital correlator, ALV GmbH, Langen, Germany). The ternary phase diagram for this microemulsion system loaded with lidocaine HCl is shown in Fig. 1.

2.3. In vitro skin permeation study

The permeability of lidocaine through porcine skin was determined in vitro with a Franz diffusion cell system (Crown Bioscientific, Inc., Clinton, NJ, USA). The diffusion area was 1.767 cm^2 (15 mm diameter orifice), and the receptor compartment volumes varied from 11 to 12 ml. The solutions in the receiver side were stirred by externally driven, Teflon-coated magnetic bars. Each set of experiments was performed with at least five diffusion cells ($n \geq 5$). Full-thickness porcine skin

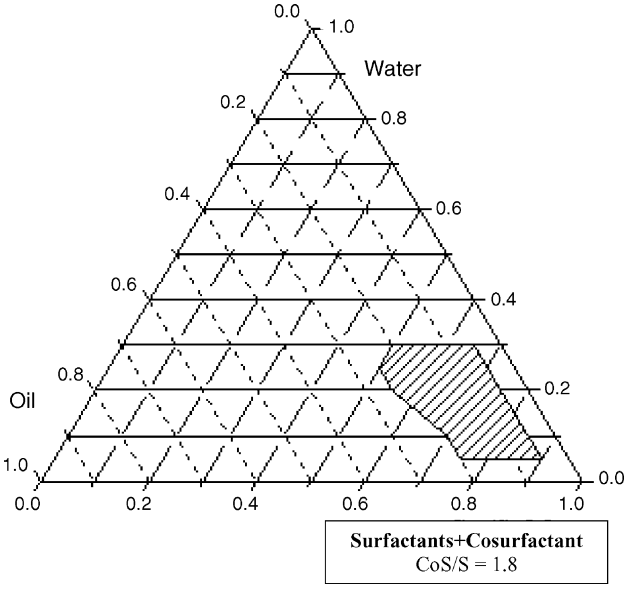

Fig. 1. Pseudo-ternary phase diagram of the new microemulsion system. 'Oil' is isopropyl palmitate and 'surfactants' are mixture of PEG-40 stearate and glyceryl oleate at a ratio of 2:3.

was excised from the ears of freshly slaughtered white pigs (breeding of Landres and Large White, locally grown in the Institute of Animal Research, Kibbutz Lahav, Israel). Skin sections (about 2 cm × 2 cm) were excised, and subcutaneous fat was removed with a scalpel. Transepidermal water loss (TEWL) measurements (Dermalab® Cortex Technology, Hadsund, Denmark) were performed on all the pieces, and only those for which TEWL levels fell within the specification (<15 g/m^2 h) were mounted in the diffusion cells, ready for testing. Although TEWL is less sensitive to small barrier impairments than low-frequency impedance measurements, it is a more practical and quite informative criterion when significant barrier disruption is apparent (Sekkat et al., 2002). The skin pieces were kept at −20 °C and were used for penetration studies within 2 weeks after slaughter of the animal. Each skin section was placed with the stratum corneum facing up on the receiver compartments, and the donor compartments were then clamped in place. The receiver compartment, defined as the side facing the dermis, was filled with phosphate buffer (4 mM, pH 7.4)–ethyl alcohol (analytical grade) (7:3). In experiments testing pH-dependant iontophoresis, the receiver compartment was filled with 0.1 M phosphate buffer (Na_2HPO_4 and NaH_2PO_4) adjusted to pH 4, 7.4 or 8.5. After 15 min of skin washing at 37 °C, the receiver medium was discarded, and the chambers were refilled with a fresh buffer-ethanol solution. Ethyl alcohol was used to ensure sink conditions throughout the experiment. One-milliliter aliquots of the microemulsion or sodium chloride (3 mM) solution, each containing 2.5% lidocaine HCl, were applied on the skin in the cells. Samples (2 ml) were withdrawn from the receiver solution at predetermined time intervals, and the cells were replenished up to their marked volumes with fresh buffer-ethanol solution each time. The samples were taken into 1.5-ml amber vials, and stored at −20 °C until analyzed by HPLC.

2.4. Skin partitioning of topical lidocaine applied to rats

The animal protocol was reviewed and approved by the institutional Animal Care & Use Committee, which complies with the Israeli Law of Human Care and Use of Laboratory Animals, 1994 (also approved by the Division of Compliance, OPRR, OD of the US-NIH as a foreign institution with compliance to the standards for Human Care and Use of Laboratory Animals, approval: #A5060-01); the institution has adopted policies regarding animal care and use as outlined in the guide for the Care and Use of Laboratory Animals of the National Academy of Sciences, USA. Each male Sprague–Dawley rat (400–500 g, Harlan Laboratories Ltd., Jerusalem, Israel) was anesthetized (75 mg/kg ketamine *i.p.* and 7 mg/kg xylazine) and was placed on its back. The hair on abdominal skin was trimmed off, and the TEWL was determined to verify skin integrity. The abdomen was then gently washed with distilled water. Anesthesia was maintained with 0.1 ml ketamine (100 mg/ml) throughout the experiment. Microemulsions or sodium chloride (3 mM) solutions containing 2.5% lidocaine hydrochloride (1 ml) were applied to the skin surface (over an area of 1.7 cm^2) via open containers glued to the skin on one end with a silicon rubber. After 5 or 10 min of anodal iontophoresis (see Section 2.6), the electrodes were disconnected from the power supply, and the tested microemulsion or the solution was left in the containers until the end of the experiment period. The rats were killed by ethyl ether aspiration after 30, 120 or 240-min of the in vivo study. The drug-exposed skin areas were swabbed three or four times with three layers of gauze pads, washed for 30 s with running water, wiped carefully, tape-stripped (×10 strips) and harvested from the animals.

2.5. Ag/AgCl electrodes

Silver wire (1.0 mm diameter, annealed, 99.9% Alfa Aesar®, Ward Hill, MA, USA) was used. For the in vivo studies, both electrodes were prepared by folding 15 cm of the silver wire in such a way as to create a round flat loop (14–15 mm diameter) with a of straight piece wire at the one end. For the in vitro studies, the anode electrode was prepared in the same way, but the receiver side electrode (cathode) was prepared by folding the 15 cm wire so as to form a 5-cm triple-stranded wire. The wires were coated by placing a silver electrode in a 1 M KCl solution with a Pt reference electrode and applying a constant current (1 A) between the electrodes to produce a uniform AgCl coating. The coated electrodes were checked for conductivity before use.

2.6. Anodal iontophoresis

The experiments were conducted in parallel to the passive diffusion studies (see Sections 2.3 and 2.4) using the same set-up. In the in vitro experiments, the coated Ag/AgCl anode (flat loop) was positioned in the donor compartment approximately 1 cm from the skin, and the cathode (straight wire) was inserted into the receiver compartment through the sampling port. In the in vivo experiments, the anode was dipped in the drug-containing solution or microemulsion approximately 1 cm above the skin, and the cathode (return electrode) was dipped in saline solution 4–5 cm away from the anode toward the animal's chest. The electrodes were connected to power supply leads from the iontophoretic device (Phoresor II Model PM 850, Iomed, Salt Lake City, UT, USA); the anode was connected to the positive output and the cathode, to the negative output of the power supply. A constant current of 2 mA (current densities of 1.13 and 1.17 mA/cm^2 in the in vitro and in vivo experiments, respectively) was passed for 5 or 10 min, and then the power supply was turned off and passive diffusion was continued. In the in vitro studies, the experiments were continued for 8 h from the time of drug application, while in the in vivo studies the application was terminated after 30, 120 and 240 min from the time of drug application.

2.7. Skin separation and extraction

This process has been described previously (Sintov and Shapiro, 2004). The dermis of each skin section was heat separated (60 °C for exactly 60 s) from the stratum corneum–epidermis layer. Each skin layer was then cut to small pieces and the pieces were inserted into 2-ml vials. The skin

pieces in each vial were extracted with 1 ml of ethyl alcohol for 1 h at 40 °C in a shaking water bath (150 rpm). The extracts were injected into the HPLC system. The total recovery of known quantities of lidocaine impregnated into skin pieces and processed as described above was 97.1 ± 3.93%.

2.8. HPLC analysis of samples from receiver solutions and skin extracts

Receiver solutions: Aliquots of 20 μl from each vial were injected onto the HPLC system, equipped with a prepacked CN column (Nucleosil 100-5 CN, 5 μm, 250 mm × 4 mm, Macherey-Nagel, Dueren, Germany). The HPLC system (Shimadzu VP series) consisted of an autosampler and a diode array detector. The quantitation of lidocaine was performed by integration of the peaks detected at 210 nm. The samples were chromatographed using an isocratic mobile phase consisting 0.02 M phosphate buffer pH 6.0–acetonitrile (4:6) at a flow rate of 0.8 ml min^{-1}. A calibration curve (peak area versus drug concentration) was constructed by running standard lidocaine solutions in a phosphate buffer/ethanol (7:3) for each series of chromatographed samples. Calibration curves were linear over the range of 0.5–10 μg ml^{-1}. Data were expressed as the cumulative lidocaine permeation per unit of skin surface area, Q_t/S ($S = 1.767\ cm^2$). The steady-state fluxes (J_{ss}) were calculated by linear regression interpolation of the experimental data at a steady state:

$$J_{ss} = \frac{\Delta Q_t}{\Delta T \cdot S}$$

2.9. Statistical analysis

All data such as cumulative drug penetration amounts, skin levels and penetration fluxes are expressed as mean ± standard deviation (S.D.). The statistical difference between the skin penetration profiles of the formulations was analyzed. The first analysis comprised a two-way unweighted means analysis of variance (ANOVA) test for the differences among group means was first run. However, this test required normal distribution of the data. If normality of data analyzed by the ANOVA procedure failed with the Kolmogorov–Smirnov test, the nonparametric Mann–Whitney rank sum test for unpaired data was applied at a significance level of 0.05.

3. Results and discussion

3.1. Formulation

For most currently available formulations for local anesthesia (e.g., EMLA cream, Astra, Sweden), there is a long delay between application and anesthetic effect even when an occlusive dressing is used. However, several studies have shown that the response time can be shortened by formulating the local anesthetics tetracaine (amethocaine) and pentacaine into microemulsions (Zabka and Benkova, 1995; Changez and Varshney, 2000; Arevalo et al., 2004). The findings of the latter studies are in keeping with a number of in vitro (Osborne et al., 1991; Trotta et al., 1996; Delgado-Charro et al., 1997; Parra et al., 1997; Dreher et al., 1997; Schmalfuss et al., 1997; Kreilgaard et al., 2000; Alvarez-Figueroa and Blanco-Mendez, 2001; Rhee et al., 2001; Lee et al., 2003) and in vivo studies (Kemken et al., 1992; Kreilgaard, 2001; Kreilgaard et al., 2001; Sintov and Shapiro, 2004) that have shown that microemulsion formulations provide improved transdermal and dermal drug delivery properties. Many microemulsion formulations are based on short- and medium-chain alcohols, while others are based on poloxamers or propylene glycol as co-surfactants in combination with polysorbates (Tween) 80 or 85. In this study we used a microemulsion system formulated with a combination of tetraglycol and PEG-40 stearate as the main constituents of the monophasic preparation (Fig. 1). It has previously been demonstrated that this composition plays a major role in the penetration enhancement of drugs through the stratum corneum (Sintov et al., 2003; Sintov and Shapiro, 2004). Furthermore, our preliminary results with iontophoresis (Table 1) showed that a formulation containing PEG-40 stearate is a better promoter of percutaneous lidocaine penetration than corresponding formulations containing polysorbate 80. By using polysorbate 80 and 10-min application of 2 mA current, fluxes of 9.92 ± 2.24 and 6.43 ± 0.35 μg cm^{-2} h^{-1} (after lag time of 1 h) were obtained for microemulsions containing 20 and 50% water, respectively. As can be seen in Table 1, these values are significantly lower than the initial flux (1–4 h after application) of 41.60 ± 16.16 μg cm^{-2} h^{-1} measured after the same iontophoretic treatment for a microemulsion formulation containing PEG-40 stearate. Further examination of the table shows that: (a) these values are significantly lower than the flux of 22.63 ± 6.12 μg cm^{-2} h^{-1} measured for a sodium

Table 1
Transdermal fluxes of lidocaine hydrochloride from sodium chloride solution and two microemulsion-based systems (containing polysorbate-80 or PEG-40 stearate) without and with 10 min of iontophoresis

Transdermal method	Transdermal flux (μg cm^{-2} h^{-1})				
	Solution	Polysorbate 80		PEG-40 stearate	
		20% water	50% water	Initial flux[a]	Late flux
Passive diffusion	2.43 ± 0.88	3.75 ± 0.76[b]	2.17 ± 1.04	–	1.58 ± 2.28
Iontophoresis (2 mA for 10 min)	22.63 ± 6.12	9.92 ± 2.24[b]	6.43 ± 0.35	41.60 ± 16.16[c]	13.26 ± 6.75

[a] A different penetration rate between 1 and 4 h was obtained only after PEG-40 stearate-containing microemulsion was applied with iontophoresis.
[b] Significant difference ($p < 0.05$) between the data obtained for 20% water and 50% water.
[c] Significant difference ($p < 0.05$) between the data obtained for PEG-40 stearate microemulsion and aqueous solution.

chloride solution after iontophoresis implying on a reduced diffusivity (see explanation below) and (b) the values for the polysorbate 80-containing formulations indicate that drug penetration flux through the iontophoresed skin can be increased by reducing the water content in the microemulsion (Table 1). The decrease in the water content probably serves to invert the water-continuous (o/w) preparation to oil-continuous microemulsion. These findings regarding microemulsions with polysorbate 80 as the surfactant may provide the basis for elucidating the mechanism by which combinations of certain microemulsions and iontophoresis may be effective. It has already been shown that interacting colloidal particles may give rise to percolation and phase separation (Paul and Moulik, 1997). In w/o microemulsions, percolation is manifested in a rapid increase in the electrical conductivity as the surfactant and water volume fraction is finely varied at a constant temperature (Paul and Moulik, 1997). In microemulsions, the mechanisms leading to percolation and the charge- or ion-transport process are currently the subject of controversy. The 'static percolation' approach attributes the phenomenon to the transfer of ions from one nano-droplet to another through open water channels in a bicontinuous structure, while the 'stirred percolation' approach attributes percolation to charge transport via hopping of ions within nano-droplet clusters transiently formed during attractive interactions and 'sticky' collisions between the water globules. A number of publications from the group of Eicke (Eicke and Meier, 1996; Eicke et al., 1996, 1999) have shown that minute modifications of the interfacial composition of the nano-droplets cause marked shifts of the percolation limit. It is evident, therefore, that factors like increases in temperature and amounts of water as well as changes in surfactant type and structure may significantly affect the percolation picture. It is most likely, therefore, that the substitution of PEG-40 stearate with polysorbate 80 obstructs percolation by rigidifying the interface between the water of the inner phase and the oil-continuous phase, resulting in a significant decrease in migration of lidocaine ions during iontophoresis and reduced diffusivity through the water channels within the percolation clusters. Interfacial rigidity may be formed by certain surfactants (e.g., polysorbates) due to strong hydrogen bonding interactions occurring between the bulky 'polar head' groups in the interface as well as between the polar groups and drug molecules.

3.2. In vitro percutaneous lidocaine penetration

Drug penetration through iontophoresed skin depends on the physiological pH of the skin. It has previously been shown (Merino et al., 1999; Lopez et al., 2001; Marro et al., 2001) that both the pH environment of the skin and the degree of ionization of the drug dictate the mechanism of the iontophoretic drug transport. As summarized in Table 2, a short process (maximum of 10 min) of anodal iontophoresis increased the penetration flux of lidocaine (in 3 mM NaCl solutions) by one order of magnitude, with no statistically significant differences between the three pH environments used in the receiver compartments. These findings may imply that: (a) the dominant mechanism of lidocaine transport is electrorepulsion, even at the isoelectric point of the skin (pI = pH 4) and not only when the skin is negatively charged (at pH 7.4 or 8.5) and (b) the electrorepulsive force overwhelms electroosmosis, even at pH 8.5, where only 20% of lidocaine in the skin is in the ionized state. Nevertheless, there was a trend indicating on some decrease of the iontophoretic penetration flux at pH 8.5, perhaps due to partial replacement of an electrorepulsive component with a relatively slower electroosmotic flow.

Table 2
pH dependency of the iontophoretic transport fluxes of lidocaine hydrochloride from a 3 mM sodium chloride solution through porcine skin in vitro

Transdermal method	Transdermal flux ($\mu g\,cm^{-2}\,h^{-1}$)		
	pH 4.0	pH 7.4	pH 8.5
Passive diffusion	2.12 ± 0.89	2.43 ± 0.88	1.57 ± 1.53
Iontophoresis (2 mA for 5 min)	15.04 ± 2.28	12.88 ± 3.47	11.19 ± 4.21
Iontophoresis (2 mA for 10 min)	24.17 ± 7.66	22.63 ± 6.12	16.64 ± 0.30

As an immediate consequence of preliminary skin irritation tests on rats and rabbits (unpublished data), we chose anodal iontophoresis process of a constant current of 2 mA applied for a period of 10 min (current density of 1.13 mA/cm^2). Although this electrical current density is considered to be relatively high (versus 0.5 mA/cm^2), skin irritation was not expected, since current application was continued only for 10 min rather than the 6-h period previously used (Marro et al., 2001). Indeed, it was found that this procedure resulted in only slight or almost no skin irritation compared with a higher current intensity (4 mA, 10 min, Fig. 2). It should be noted that a previous report by Kearns et al. (2003) described an anodal iontophoretic procedure with a current of 1.78 mA applied for 10 min. This procedure applied with commercial patches containing lidocaine/epinephrine was studied on pediatric patients demonstrating that the 92–100% of the subjects had no evidence of erythema or edema.

Fig. 3A shows the percutaneous penetration profiles of lidocaine obtained by 5 and 10-min of iontophoresis with a current of of 2 mA compared with the profiles obtained by passive diffusion. Fig. 3B and Table 3 show how the same experimental conditions affect the in vitro penetration of lidocaine from a microemulsion vehicle. In contrast to the linear profiles obtained from the lidocaine solution, the application of a microemulsion

Table 3
Transdermal fluxes of lidocaine hydrochloride from sodium chloride solution and from microemulsion without and with 5 and 10 min of iontophoresis

Transdermal method	Transdermal flux ($\mu g\,cm^{-2}\,h^{-1}$)		
	Solution	Microemulsion	
		Initial flux[a]	Late flux
Passive diffusion	2.43 ± 0.88	–	1.58 ± 2.28
Iontophoresis (2 mA for 5 min)	12.88 ± 3.47	15.02 ± 6.42	5.70 ± 3.11
Iontophoresis (2 mA for 10 min)	22.63 ± 6.12	41.60 ± 16.16[b]	13.26 ± 6.75

[a] Penetration rate from 1 to 4 h after microemulsion application.
[b] Significant difference ($p < 0.05$) between the data obtained for the microemulsion and those for aqueous solution.

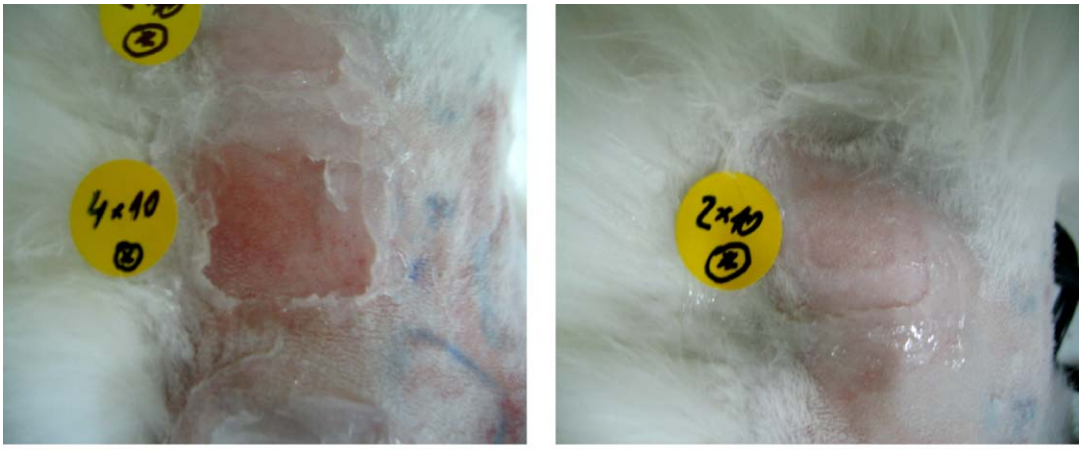

Fig. 2. Photographs of skin sites on a rabbit model after microemulsion application and 10-min iontophoresis at two different currents: (A) 4 mA and (B) 2 mA.

resulted in an initial burst of high-rate drug penetration through the skin, which lasted about 3 h, followed by a continuous low flux. It should be pointed out that for local anesthetics, the 'burst effect' is of prime importance since it speeds up the onset of the effect. As shown in Table 3, the microemulsion vehicle significantly increased the initial lidocaine flux obtained after 10 min of the iontophoresis treatment compared with solution application under the same treatment conditions ($p < 0.05$), but it did not make a significant difference after 5 min of iontophoresis. For the aqueous solution, the 5 and 10 min of short-term iontophoresis increased the lidocaine flux from 2.43 ± 0.88 (passive diffusion) to 12.88 ± 3.47 and $22.63 \pm 6.12\ \mu g\ cm^{-2}\ h^{-1}$, respectively. The value of the flux for the combination of microemulsion with 5 min of short-term iontophoresis ($15.02 \pm 6.42\ \mu g\ cm^{-2}\ h^{-1}$) was similar to that obtained for the combination of solution with 5 min of short-term iontophoresis. However, for the 10 min of short-term iontophoresis, the flux for the microemulsion ($41.60 \pm 16.16\ \mu g\ cm^{-2}\ h^{-1}$) was almost double that of the solution ($22.63 \pm 6.12\ \mu g\ cm^{-2}\ h^{-1}$) ($p < 0.05$). The late steady-state flux (i.e., between 3 and 8 h) was obviously similar in both microemulsion and solution combination treatments, indicating that the changes in skin properties can be reversed.

3.3. In vivo cutaneous lidocaine delivery

The in vitro penetration flux is usually a good indicator for the transdermal skin permeability of a drug, but it does not provide any insight into the intradermal drug distribution or its concentration gradient along the skin layers. Finding that the transdermal flux is relatively high may imply that the intradermal flux of the drug is also high, but they may not necessarily indicate whether a large depot of the drug has been created in the skin at steady state. This information can be derived only by monitoring the skin compartment in vitro or in vivo.

The penetration of lidocaine solution into the dermis and the epidermis after 10 min of iontophoresis as a function of time, as compared with the passive diffusion of the drug, is

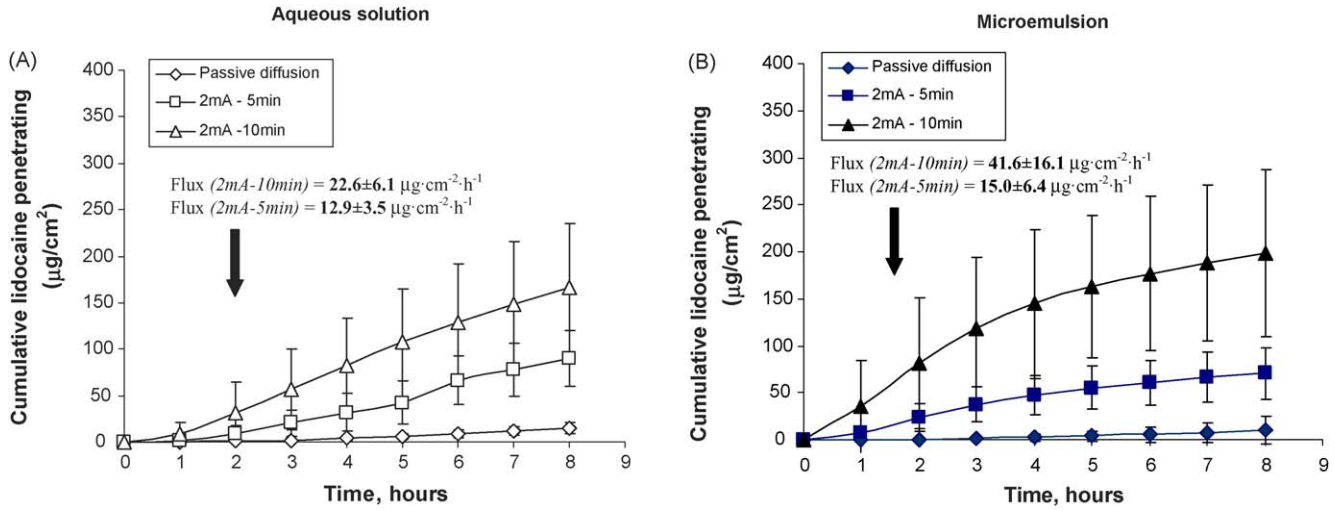

Fig. 3. Cumulative lidocaine penetration through porcine skin in vitro after short-term iontophoresis (2 mA or 1.13 mA/cm^2) for 5 or 10 min, as compared with passive drug permeability; (A) iontophoresis and aqueous solution of lidocaine hydrochloride and (B) iontophoresis and microemulsion. Note that the added effect of microemulsion to the iontophoretic drug delivery was obtained only after 10 min of application of the 2 mA electrical current.

Table 4
Concentrations of lidocaine in skin layers after application of lidocaine HCl-containing solution or microemulsion to rats in vivo

Time (min)	Lidocaine concentration ($\mu g\,cm^{-2}$)			
	Iontophoresis (2 mA for 10 min)		Passive diffusion	
	Epidermis	Dermis	Epidermis	Dermis
(A) Solution				
30	21.44 ± 10.42	5.30 ± 2.25	1.45 ± 0.87	0.26 ± 0.15
120	7.97 ± 4.11	2.23 ± 1.75	2.86 ± 1.65	1.43 ± 1.19
240	10.83 ± 8.30	2.32 ± 2.55	3.58 ± 2.81	1.65 ± 2.15
(B) Microemulsion				
30	57.71 ± 18.65**	18.43 ± 9.17**	1.51 ± 1.00	0.22 ± 0.18
120	51.95 ± 21.59**	17.51 ± 9.53**	6.78 ± 4.30	1.01 ± 0.78
240	35.68 ± 8.50**	19.60 ± 7.92**	16.18 ± 9.39**	2.17 ± 0.17

** Significantly different ($p \ll 0.05$) as compared with the corresponding values obtained for the aqueous solution.

shown in Fig. 4 and Table 4A. The passive diffusion of lidocaine from the aqueous drug solution was characterized by slowly increased levels of the drug in the skin layers up to 3.58 ± 2.81 and $1.65 \pm 2.15\ \mu g\,cm^{-2}$ in the epidermis and dermis, respectively, after 4 h. Twenty minutes after the 10-min iontophoresis (at $t = 30$ min), high drug levels of 21.44 ± 10.42 and $5.30 \pm 2.25\ \mu g\,cm^{-2}$ were found in the epidermis and dermis, respectively. After 2 and 4 h, respectively, the skin drug levels dropped rapidly to about half, i.e., to the same levels found in the dermis after passive diffusion. The results for the combi-

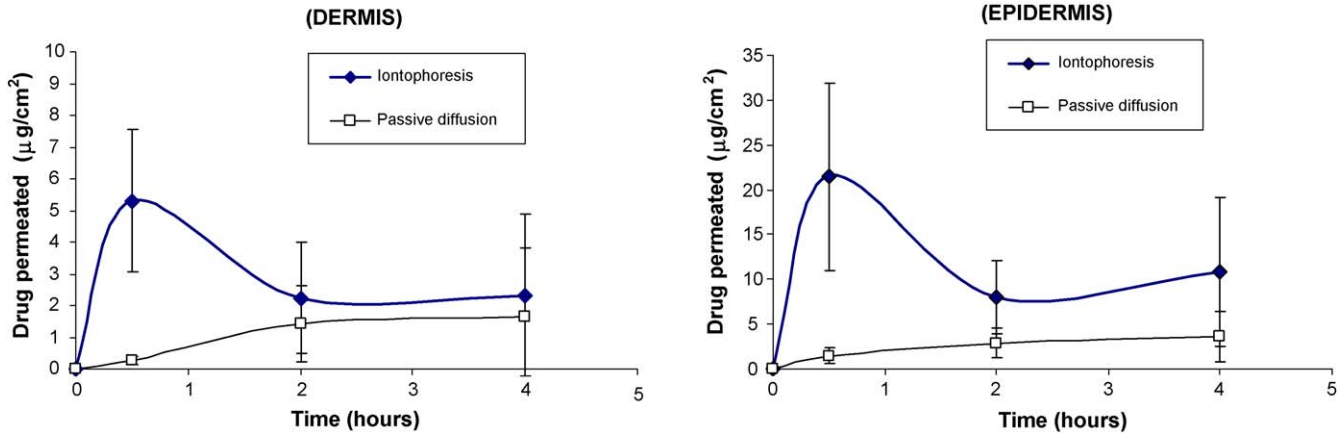

Fig. 4. Iontophoretic delivery of a 2.5% lidocaine HCl solution into the DERMIS and EPIDERMIS ($n = 7$) of rats. The solution was applied with the anode using a 10-min iontophoresis at current density of 1.17 mA/cm^2.

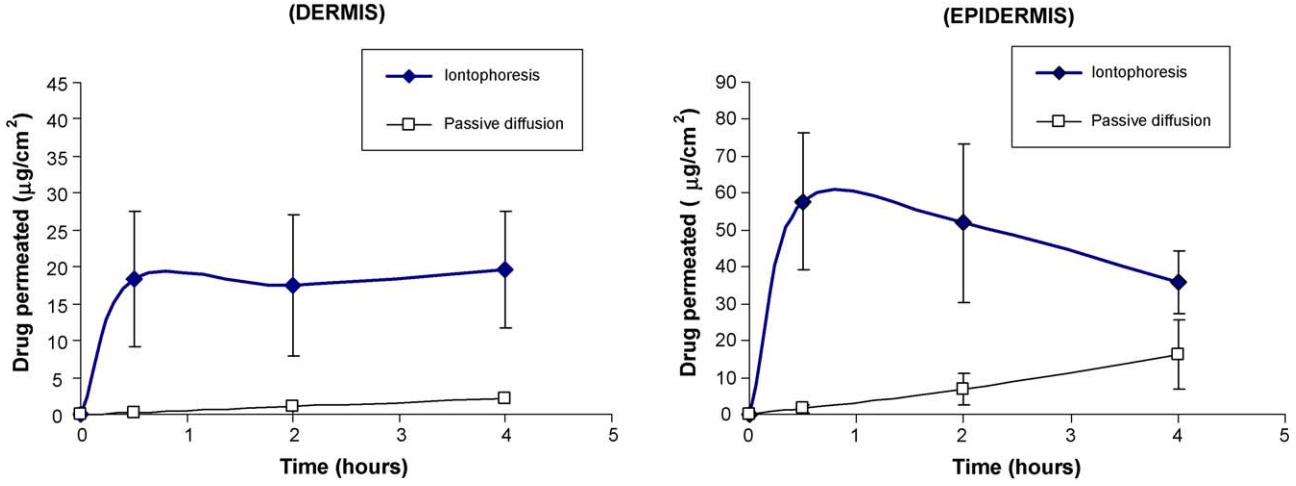

Fig. 5. Iontophoretic delivery of a 2.5% lidocaine HCl microemulsion into the DERMIS and EPIDERMIS ($n = 6$) of rats. The microemulsion was applied with the anode using a 10-min iontophoresis at current density of 1.17 mA/cm^2.

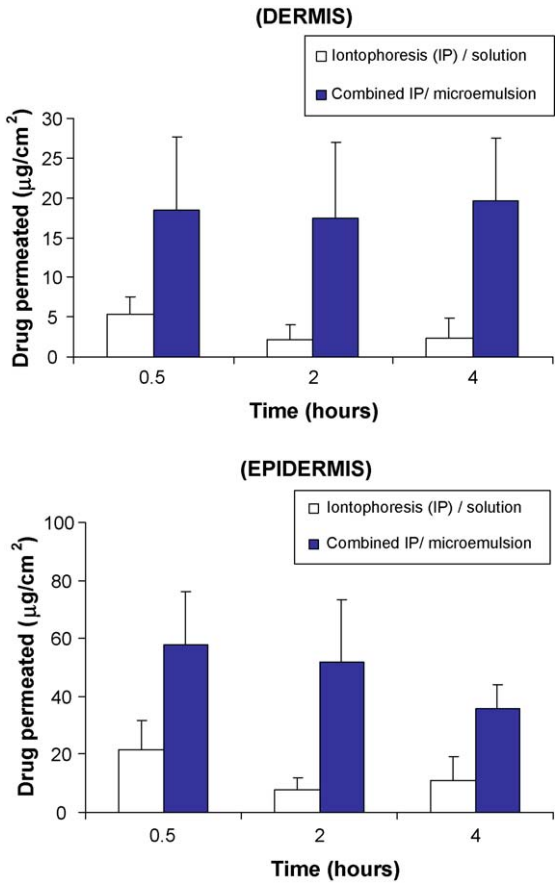

Fig. 6. Effect of microemulsion vehicle on iontophoretic delivery of lidocaine hydrochloride to rats. The drug preparations were applied with the anode using a 10-min iontophoresis at current density of 1.17 mA/cm^2.

nation of microemulsion with iontophoresis are shown in Fig. 5 and Table 4B. With this combination, lidocaine accumulation after 30 min was approximately three- and two-fold higher in the dermis and the epidermis, respectively, than the accumulation after applying the aqueous solution.

Although significantly higher levels of lidocaine were obtained after short-term iontophoresis (Table 4B) as compared with the aqueous solution, it should be noted that the passive diffusion of lidocaine from the microemulsion was also characterized by an increased drug accumulation in the skin layers, especially in the epidermis. After 4 h of passive diffusion from the microemulsion, 16.18 ± 9.39 and $2.17 \pm 0.17\ \mu g\ cm^{-2}$ were found in the epidermis and dermis, respectively. After 10 min of the iontophoresis, concentrations of 57.71 ± 18.65 and $18.43 \pm 9.17\ \mu g\ cm^{-2}$ were reached in the epidermis and dermis, respectively, at $t = 30$ min of microemulsion application. After the combined treatment, the drug depot was maintained in the dermis for as long as 4 h (Figs. 5 and 6), while the lidocaine concentration in the epidermis decreased slowly to $35.68 \pm 8.50\ \mu g\ cm^{-2}$ by that time. As presented in Fig. 6, the values obtained throughout the experiment for the microemulsion/iontophoresis combination were significantly higher than those obtained for the aqueous solution/iontophoresis combination ($p \ll 0.05$). The accumulation of a large skin drug depot for a prolonged period of time may indicate on the potential of the microemulsion/iontophoresis treatment to accelerate and amplify the anesthetic effect in the clinical practice.

4. Conclusions

Application of an electric field for 10 min appears to cause significant enhancement of lidocaine permeation through the stratum corneum. We found the combination of a microemulsion formulation of lidocaine with 10 min of iontophoresis (electric current density of 1.13 mA/cm^2) to be the most effective delivery protocol. Both the in vitro and the in vivo studies demonstrated that this combination increased the flux in comparison with that obtained with the aqueous lidocaine solution. The in vivo studies revealed that this combination gave relatively high drug concentrations in both the dermis and epidermis for up to 4 h. In contrast, application of the aqueous solution/iontophoresis combination resulted in relatively lower drug accumulation with more rapid clearance of the drug from the skin.

The advantages of the combined microemulsion/10-min iontophoresis procedure includes significantly increased fluxes, accumulation of a large skin drug depot, short lag times, possibility of reducing irritation (compared to long-term iontophoresis), simplicity and ease of compliance. The explanation for the phenomenon that the less electrically conducting system (relative to the aqueous solution) – the microemulsion – increased iontophoretic delivery of lidocaine ions may lie in the hopping of ions within nano-droplet clusters, but it still remains to be thoroughly investigated.

Acknowledgments

This work was performed in partial fulfillment of the requirements for the *M.Sc.* degree of R. Brandys-Sitton. The authors are grateful for the expert technical assistance of Mr. Igor Krymberk, Ms. Lillia Shapiro and Ms. Clara Behar-Canetti. We also gratefully acknowledge the help of Prof. Yoram Oren in preparation of the electrodes and the assistance of Ms. Inez Mureinuk in the preparation of the manuscript.

References

Alvarez-Figueroa, M.J., Blanco-Mendez, J., 2001. Transdermal delivery of methotrexate: iontophoretic delivery from hydrogels and passive delivery from microemulsions. Int. J. Pharm. 215, 57–65.

Arevalo, M.I., Escribano, E., Calpena, A., Domenech, J., Queralt, J., 2004. Rapid skin anesthesia using a new topical amethocaine formulation: a preclinical study. Anesth. Analg. 98, 1407–1412.

Barry, B.W., 2001. Novel mechanisms and devices to enable successful transdermal drug delivery. Eur. J. Pharm. Sci. 14, 101–114.

Bhatia, K.S., Gao, S., Freeman, T.P., Singh, J., 1997. Effect of penetration enhancers and iontophoresis on the ultrastructure and cholecystokinin-8 permeability through porcine skin. J. Pharm. Sci. 86, 1011–1015.

Boinpally, R.R., Zhou, S.L., Devraj, G., Anne, P.K., Poondru, S., Jasti, B.R., 2004. Iontophoresis of lecithin vesicles of cyclosporin A. Int. J. Pharmaceut. 274, 185–190.

Bommannan, D., Tamada, J., Leung, L., Potts, R.O., 1994. Effect of electroporation on transdermal iontophoretic delivery of luteinizing hormone releasing hormone (LHRH) in vitro. Pharm. Res. 11, 1809–1814.

Bose, S., Ravis, W.R., Lin, Y.J., Zhang, L., Hofmann, G.A., Banga, A.K., 2001. Electrically-assisted transdermal delivery of buprenorphine. J. Control. Release 73, 197–203.

Chang, S.-L., Hofmann, G.A., Zhang, L., Deftos, L.J., Banga, A.K., 2000. The effect of electroporation on iontophoretic transdermal delivery of calcium regulating hormones. J. Control. Release 66, 127–133.

Changez, M., Varshney, M., 2000. Aerosol-OT microemulsions as transdermal carriers of tetracaine hydrochloride. Drug Dev. Ind. Pharm. 26, 507–512.

Conjeevaram, R., Chaturvedula, A., Betageri, G.V., Sunkara, G., Banga, A.K., 2003. Iontophoretic in vivo transdermal delivery of beta-blockers in hairless rats and reduced skin irritation by liposomal formulation. Pharm. Res. 20, 1496–1501.

Davis, A.F., Gyurik, R.J., Hadgraft, J., Pellett, M.A., Walters, K.A., 2002. Formulation strategies for modulating skin penetration. In: Walters, K.A. (Ed.), Dermatological and Transdermal Formulations. Marcel Dekker, Inc., New York, pp. 271–317.

Delgado-Charro, M.B., Iglesias-Vilas, G., Blanco-Mendez, J., Lopez-Quintela, M.A., Marty, J.-P., Guy, R.H., 1997. Delivery of a hydrophilic solute through the skin from novel microemulsion systems. Eur. J. Pharm. Biopharm. 43, 37–42.

Denet, A.R., Ucakar, B., Preat, V., 2003. Transdermal delivery of timolol and atenolol using electroporation and iontophoresis in combination: a mechanistic approach. Pharm. Res. 20, 1946–1951.

Dreher, F., Walde, P., Walther, P., Wehrli, E., 1997. Interaction of a lecithin microemulsion gel with human stratum corneum and its effect on transdermal transport. J. Control. Release 45, 131–140.

Eicke, H.-F., Meier, W., 1996. Interfacial charge transport in water/oil microemulsions stabilized by ionic-nonionic surfactant mixtures. Biophys. Chem. 58, 29–37.

Eicke, H.-F., Meier, W., Hammerich, H., 1996. Water/oil microemulsions from binary ionic-nonionic surfactants. Colloids Surf. A 118, 141–148.

Eicke, H.-F., Meier, W., Hammerich, H., 1999. Charge transport as suitable tool to study near-percolation phenomena. Colloids Surf. A 156, 249–256.

Essa, E.A., Bonner, M.C., Barry, B.W., 2004. Electrically assisted skin delivery of liposomal estradiol; phospholipid as damage retardant. J. Control. Release 95, 535–546.

Fang, J.Y., Sung, K.C., Lin, H.H., Fang, C.L., 1999. Transdermal iontophoretic delivery of enoxacin from various liposome-encapsulated formulations. J. Control. Release 60, 1–10.

Fang, J.Y., Hung, C.F., Fang, Y.P., Chan, T.F., 2004. Transdermal iontophoresis of 5-fluorouracil combined with electroporation and laser treatment. Int. J. Pharm. 270, 241–249.

Green, P.G., Flanagan, M., Shroot, B., Guy, R.H., 1993. Iontophoretic drug delivery. In: Walters, K.A., Hadgraft, J. (Eds.), Pharmaceutical Skin Penetration Enhancement. Marcel Dekker, Inc., New York, pp. 311–333.

Guy, R.H., Kalia, Y.N., Delgado-Charro, M.B., Merino, V., Lopez, A., Marro, D., 2000. Iontophoresis: electrorepulsion and electroosmosis. J. Control. Release 64, 12–132.

Guy, R.H., Delgado-Charro, M.B., Kalia, Y.N., 2001. Iontophoretic transport across the skin. Skin Pharmacol. Appl. Skin Physiol. 14, 35–40.

Henry, S., McAllister, D.V., Allen, M.G., Prausnitz, M.R., 1998. Microfabricated microneedles: a novel approach to transdermal drug. J. Pharm. Sci. 87, 922–925.

Hu, Q., Liang, W., Bao, J., Ping, Q., 2000. Enhanced transdermal delivery of tetracaine by electroporation. Int. J. Pharmaceut. 202, 121–124.

Kalia, Y.N., Guy, R.H., 1997. Interaction between penetration enhancers and iontophoresis: effect on human skin impedance in vivo. J. Control. Release 44, 33–42.

Kantaria, S., Rees, G.D., Lawrence, M.J., 1999. Gelatin-stabilised microemulsion-based organogels: rheology and application in iontophoretic transdermal drug delivery. J. Control. Release 60, 355–365.

Kearns, G.L., Heacook, J., Daly, S.J., Singh, H., Alander, S.W., Qu, S., 2003. Percutaneous lidocaine administration via a new iontophoresis system in children: tolerability and absence of systemic bioavailability. Pediatrics 112, 578–582.

Kemken, J., Ziegler, A., Mueller, B.W., 1992. Influence of supersaturation on the pharmacodynamic effect of bupranolol after dermal administration using microemulsions as vehicle. Pharm. Res. 9, 554–558.

Kost, J., Mitragotri, S., Langer, R., 1999. Phonophoresis. In: Bronaugh, R.L., Maibach, H.I. (Eds.), Percutaneous Absorption, Drugs-Cosmetics-Mechanisms-Methodology, third ed. Marcel Dekker, Inc., New York, pp. 615–631.

Kreilgaard, M., Pedersen, E.J., Jaroszewski, J.W., 2000. NMR characterization and transdermal drug delivery potential of microemulsion systems. J. Control. Release 69, 421–433.

Kreilgaard, M., 2001. Dermal pharmacokinetics of microemulsion formulations determined by in vitro microdialysis. Pharm. Res. 18, 367–373.

Kreilgaard, M., Kemme, M.J.B., Burggraaf, J., Schoemaker, R.C., Cohen, A.F., 2001. Influence of a microemulsion vehicle on cutaneous bioequivalence of a lipophilic model drug assessed by microdialysis and pharmacodynamics. Pharm. Res. 18, 593–599.

Le, L., Kost, J., Mitragotri, S., 2000. Combined effect of low frequency ultrasound and iontophoresis: application for transdermal heparin delivery. Pharm. Res. 17, 1151–1154.

Lee, P.J., Langer, R., Shastri, V.P., 2003. Novel microemulsion enhancer formulation for simultaneous transdermal delivery of hydrophilic and hydrophobic drugs. Pharm. Res. 20, 264–269.

Lopez, R.F.V., Bentley, M.V.L.B., Delgado-Charro, M.B., Guy, R.H., 2001. Iontophoretic delivery of 5-aminolevulinic acid (ALA): effect of pH. Pharm. Res. 18, 311–315.

Marro, D., Guy, R.H., Delgado-Charro, M.B., 2001. Characterization of the iontophoretic permselectivity properties of human and pig skin. J. Control. Release 70, 213–217.

McAllister, D.V., Allen, M.G., Prausnitz, M.R., 2000. Microfabricated microneedles for gene and drug delivery. Ann. Rev. Biomed. Eng. 2, 298–313.

Merino, V., Lopez, A., Kalia, Y.N., Guy, R.H., 1999. Electrorepulsion versus electroosmosis: effect of pH on the iontophoretic flux of 5-fluorouracil. Pharm. Res. 16, 758–761.

Mitragotri, S., 2000. Synergistic effect of enhancers for transdermal drug delivery. Pharm. Res. 17, 1354–1359.

Naik, A., Kalia, Y.N., Guy, R.H., 2000. Transdermal drug delivery: overcoming the skin's barrier function. Pharm. Sci. Technol. Today 3, 318–326.

Osborne, D.W., Ward, A.J., O'Neill, K.J., 1991. Microemulsions as topical drug delivery vehicles: in vitro transdermal studies of a model hydrophilic drug. J. Pharm. Pharmacol. 43, 450–454.

Parra, J.L., Coderch, L., Yuste, I., de la Maza, A., 1997. Incorporation of nonsteroidal anti-inflammatory drugs into specific monophasic formulations. Colloids Surf. A: Physicochem. Eng. Aspects 123–124, 115–123.

Paul, B.K., Moulik, S.P., 1997. Microemulsions: an overview. J. Dispersion Sci. Technol. 18, 301–367.

Prausnitz, M.R., Bose, V.G., Langer, R., Weaver, J., 1993. Electroporation of mammalian skin: a mechanism to enhance transdermal drug delivery. Proc. Natl. Acad. Sci. U.S.A. 90, 10504–10508.

Prausnitz, M.R., 1999. A practical assessment of transdermal drug delivery by skin electroporation. Adv. Drug Deliv. Rev. 35, 61–76.

Rhee, Y.-S., Choi, J.-G., Park, E.-S., Chi, S.-C., 2001. Transdermal delivery of ketoprofen using microemulsions. Int. J. Pharmaceut. 228, 161–170.

Riviere, J.E., Monteiro-Riviere, N.A., Rogers, R.A., Bommannan, D., Tamada, J.A., Potts, R.O., 1995. Pulsatile transdermal delivery of LHRH using electroporation: drug delivery and skin toxicology. J. Control. Release 36, 229–233.

Schmalfuss, U., Neubert, R., Wohlrab, W., 1997. Modification of drug penetration into human skin using microemulsions. J. Control. Release 46, 279–285.

Sekkat, N., Kalia, Y.N., Guy, R.H., 2002. Biophysical study of porcine ear skin in vitro and its comparison to human skin in vivo. J. Pharm. Sci. 91, 2376–2381.

Singh, P., Liu, P., Dinh, S.M., 1999. Facilitated transdermal delivery by iontophoresis. In: Bronaugh, R.L., Maibach, H.I. (Eds.), Percutaneous Absorption, Drugs-Cosmetics-Mechanisms-Methodology, third ed. Marcel Dekker, Inc., New York, pp. 633–657.

Sintov, A.C., Krymberk, I., Gavrilov, V., Gorodischer, R., 2003. Transdermal delivery of acetaminophen for pediatric use: effects of vehicle formulations on the percutaneous penetration. J. Pharm. Pharmacol. 55, 911–919.

Sintov, A.C., Shapiro, L., 2004. New microemulsion vehicle facilitates percutaneous penetration in vitro and cutaneous drug bioavailability in vivo. J. Control. Release 95, 173–183.

Smith, E.W., Maibach, H.I., 1995. Percutaneous Penetration Enhancers. CRC Press, Boca Raton, FL.

Trotta, M., Pattarino, F., Gasco, M.R., 1996. Influence of counter ions on the skin permeation of methotrexate from water–oil microemulsions. Pharm. Acta Helv. 71, 135–140.

Vanbever, R., Lecouturier, N., Preat, V., 1994. Transdermal delivery of metoprolol by electroporation. Pharm. Res. 11, 1657–1662.

Vanbever, R., Le Boulenge, E., Preat, V., 1996. Transdermal delivery of fentanyl by electroporation. I. Influence of electrical factors. Pharm. Res. 13, 559–565.

Walters, K.A., 1989. Penetration enhancers and their use in transdermal therapeutic systems. In: Hadgraft, J., Guy, R.H. (Eds.), Transdermal Drug Delivery, Developmental Issues and Research Initiatives. Marcel Dekker, Inc., New York, pp. 197–246.

Zabka, M., Benkova, M., 1995. Microemulsions containing local anesthetics. Part 6. Influence of microemulsion vehicle on in vivo effect of pentacaine. Pharmazie 50, 703–704.

Zewert, T.E., Pliquett, U.F., Vanbever, R., Langer, R., Weaver, J.C., 1999. Creation of transdermal pathways for macromolecule transport by skin electroporation and a low toxicity, pathway-enlarging molecule. Bioelectrochem. Bioenerg. 49, 11–20.