Effects of Fatty Acids and Iontophoresis on the Delivery of Midodrine Hydrochloride and the Structure of Human Skin

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Purpose. The purpose of this work was to investigate if fatty acids can increase the iontophoretic delivery of midodrine hydrochloride through human dermatomed skin and to observe the effects of iontophoresis and fatty acids on skin using SEM.

Methods. After prehydration for 1 h, human dermatomed skin was treated with 0–0.3 M fatty acids (oleic acid, linoleic acid, decanoic acid, and lauric acid) in propylene glycol (PG) for 1 h. Then the fatty acid solution was replaced by 1% midodrine hydrochloride aqueous solution, and 0.1 mA/cm^2 constant current was applied. Samples were taken over 24 h and analyzed by HPLC. After the treatments outlined above, the epidermis was separated, fixed with glutaraldehyde, and dehydrated for SEM.

Results. SEM studies revealed that only 1 h of treatment with fatty acids opened up the tightly compact stratum corneum cell layer, and the permeation study showed a significant increase of the permeability of skin to midodrine hydrochloride after fatty acid treatment.

Conclusions. Using 5% oleic acid pretreatment, with the electrical current offset at 0.1 mA/cm^2, the daily delivery of midodrine hydrochloride can provide an adequate clinical application. The enhancement of passive and iontophoretic delivery by fatty acids may be occurring through the same mechanism.

KEY WORDS: transdermal drug delivery; iontophoresis; midodrine; fatty acids.

INTRODUCTION

Skin is the largest organ of the human body, covering a surface area of approximately 2 m^2, and receives about one third of the total blood supply. Transdermal drug delivery (TDD) has been investigated for several decades, and several commercial products have been marketed successfully incorporating drugs such as clonidine, nicotine, and nitroglycerin. The benefits of TDD include the avoidance of the first-pass effect, often zero-order release of the drug, and higher patient compliance to the drug regimen. A major drawback, however, is that it is difficult to deliver many drugs in therapeutically effective quantities because of the rigid structure of the uppermost layer of the skin, the stratum corneum (SC).

Penetration enhancement including chemical and physical approaches is used to facilitate the penetration of drugs through SC to the underlying tissues. Fatty acids have been known to increase skin permeability since as early as 1961. Bettley (1) found that epidermis exposed to potassium oleate exhibited increased permeability to sodium salicylate and glucose. In recent years, much more work has been done to investigate the effects of fatty acids on skin (2–4). Several mechanisms of action explaining the enhancement effects of fatty acids have been proposed: (a) disruption of the barrier properties of SC lipids (2); (b) interaction of fatty acids with cellular proteins (5); (c) increased partitioning of the drug or solvent into the SC (6); and (d) formation of drug–fatty acid complex with a higher octanol/water partition coefficient than the drug has alone (7).

Unsaturated fatty acids with long carbon chains have been found to be more effective than the analogous saturated fatty acids. C_{12} and C_{14} fatty acids have an optimal balance of partition coefficient and affinity to lipids in the SC. Short-chain fatty acids have insufficient lipophilicity to penetrate the skin, and long-chain fatty acids have too much affinity to the lipids in SC and actually retard the penetration of drugs.

The permeation-enhancing effects of fatty acids are greatly influenced by the vehicle used. For example, compared with ethanol, polyethylene glycol (PEG) 400, and isopropanol, PG produces significantly greater enhancing effects. It is believed that PG can "drag" fatty acids into the skin (8).

Iontophoresis is a promising method for transdermal delivery of ionized drugs. The systemic effects of iontophoresis were first observed in 1879 by Munch. Fifteen minutes after he placed strychnine under the positive pole on shaved rabbit skin the rabbit died. As a result of advances in microelectronics and formulations in the past 5–10 years, iontophoresis has reemerged as an attractive approach for TDD.

The penetration of ionized drugs by iontophoresis can take place through either appendageal pathways or nonappendageal pathways. Although the appendageal route predominates according to the studies performed with electron microscopy (9), nonappendageal pathways also play a minor role (10). It must be borne in mind that generally, only small molecules with molecular weights less than 10,000 d can be delivered iontophoretically with no additional enhancement techniques.

A current density of less than 0.5 mA/cm^2 is normally regarded as tolerable and comfortable for patients. Birch Point Medical Inc. in Minnesota recently developed IontoPatch™, which has FDA clearance as an iontophoretic device, and it can be used to deliver high doses of drug for 12 to 24 h with an electric current of 0.1 mA/cm^2.

For breaking the "10,000 dalton rule," some other approaches (11), such as ultrasound (12), liposomal delivery (13), and chemical enhancers (14,15), have been used together with iontophoresis. Occasionally, the use of chemical enhancers was reported to result in reduced flux compared with iontophoresis alone (16,17). The combined effect of chemical enhancers and iontophoresis depends on the physicochemical properties of the penetrant and the enhancer and their behavior under the influence of an electric field.

Midodrine hydrochloride is the prodrug of desglymidodrine, which is an α_1-agonist and exerts its actions via activation of the α-adrenergic receptors of the arteriolar and venous vasculature, producing an increase in vascular tone and el-

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evation of blood pressure. Midrodrine is indicated for treating low blood pressure (hypotension). Its metabolism to desglymidodrine takes place in many tissues. Midodrine is available only as oral tablets in the United States, and dosing for an adult is 10 mg three times a day. Midodrine has the potential of causing supine hypertension, which can lead to blurred vision, headaches, and pounding in the ears, and administration should be stopped immediately if supine blood pressure increases excessively. With iontophoresis, the delivery of midodrine could be more precisely controlled and stopped at any time needed. The metabolic characteristics of midodrine suggest the dosing of transdermal delivery should be the same as that of oral delivery, i.e., 30 mg/day.

This paper discusses the effect of fatty acids (oleic acid, linoleic acid, decanoic acid, and lauric acid) and iontophoresis on the structure and permeability of human skin following application of midodrine hydrochloride.

MATERIALS AND METHODS

Materials

The stimulus isolator was purchased from World Precision Instruments, Inc. (Sarasota, FL). All chemicals and silver wire (0.5 mm) were from Sigma-Aldrich (Milwaukee, WI). Reagents were of analytic grade and used without further purification. Human skin from three donors including both sexes, dermatomed to 500 μm, was obtained from the National Disease Research Interchange (Philadelphia, PA). Franz diffusion cells with the cross section area of 0.64 cm^2 were purchased from PermeGear, Inc. (Bethlehem, PA). For the skin structure study, the epidermis was peeled off after heating of the skin to 60°C for 1 min. A Hitachi S-2500 scanning electron microscope was used for the skin structure studies.

Methods

Iontophoretic Delivery of Midodrine Chloride

All fatty acid solutions were made by dissolving the fatty acid in PG in concentrations of 0.065 M, 0.13 M, 0.26 M, or 0.31 M. Phosphate buffer (pH 7.4 at 37°C, made by dissolving PBS tablets provided by Sigma Aldrich. The prepared solution had 2.68 mM KCl, 1.47 mM KH_2PO_4, 136.89 mM NaCl, and 8.10 mM Na_2HPO_4) was used as the receptor solution in the Franz diffusion cells. Human skin was hydrated with the phosphate buffer for 1 h. After prehydration, fatty acid solution was added on the top of the skin for 1, 2, or 3 hours, followed by the removal of the fatty acid and washing of the skin with ethanol. Then 1% midodrine hydrochloride aqueous solution was added, and a 0.1 mA/cm^2 continuous direct current was applied for up to 24 h (n = 6 for all treatment groups). Skin from more than one donor was used in each treatment group.

Samples (300 μl) were taken at specified time points and replaced in the receptor of the diffusion cell by the same amount of phosphate buffer. The dilution of the receptor solution was accounted for in the permeability parameter calculations. All samples were frozen at –10°C before being analyzed by HPLC.

HPLC Method

HPLC analysis of samples was performed using a Hewlett Packard 1100 with a reverse-phase C_{18} column (Microsorb-MV™, 15 cm, 5 μm, Agilent Technologies) at a flow rate of 2 ml/min. Midodrine hydrochloride was detected at 210 nm with a mobile phase composition of acetonitrile:0.05 M monobasic potassium phosphate (30:70, pH 3.0) and injection volume of 20 μl. This method gave the linear range of 0.5 μg/ml to 500 μg/ml. The day-to-day RSD is less than 2%.

Data Analysis

The *in vitro* skin permeation data obtained was graphically plotted as the cumulative corrected amount of drug penetrated into the receptor as a function of time. The slope of the straight line portions of this plot (at steady state) yielded the values of flux (μg/cm^2 per h) and the cumulative corrected receptor concentrations at 24 h, Q_{24} (μg/cm^2).

The enhancement factor for flux was calculated using the following equation:

$$E = \frac{\text{Flux with enhancer or iontophoresis treated skin}}{\text{Flux with untreated skin (Control I)}}$$

Statistical analyses were performed using one-way analysis of variance (one-way ANOVA) followed by a least-significant-difference test (LSD) if the ANOVA indicated that a difference existed. The level of significance was 0.05 (18).

Table I. Iontophoretic Delivery of Midodrine Hydrochloride with Oleic Acid 0–12% (n = 6)

Enhancer	Enhancer treatment time (h)	Current applied (mA/cm^2)	Flux (μg/cm^2 h)	Q_{24} (μg/cm^2)	Enhancement factor
None (control I)	—	0	6.0 ± 1.3	160.1 ± 23.1	1
None (control II)	—	0.1	268.2 ± 20.4	6,274.4 ± 321.6	44.7
5% oleic acid	1	0	34.2 ± 2.5	767.4 ± 47.9	5.7
0% oleic acid (pretreatment with PG only)	—	0.1	233.2 ± 3.8	5,345.4 ± 163.3	38.9
2.5% oleic acid	1	0.1	300.6 ± 7.1	6740.3 ± 231.3	50.1
5% oleic acid	1	0.1	398.9 ± 11.5	8955.4 ± 223.9	66.5
5% oleic acid	2	0.1	525.2 ± 52.8	12,027.0 ± 1203.3	74.5
5% oleic acid	3	0.1	538.4 ± 46.5	12,552.2 ± 1558.8	87.5
10% oleic acid	1	0.1	493.6 ± 31.1	1,068,405 ± 765.5	82.3
12% oleic acid	1	0.1	586.6 ± 18.9	12,765.0 ± 471.2	97.8

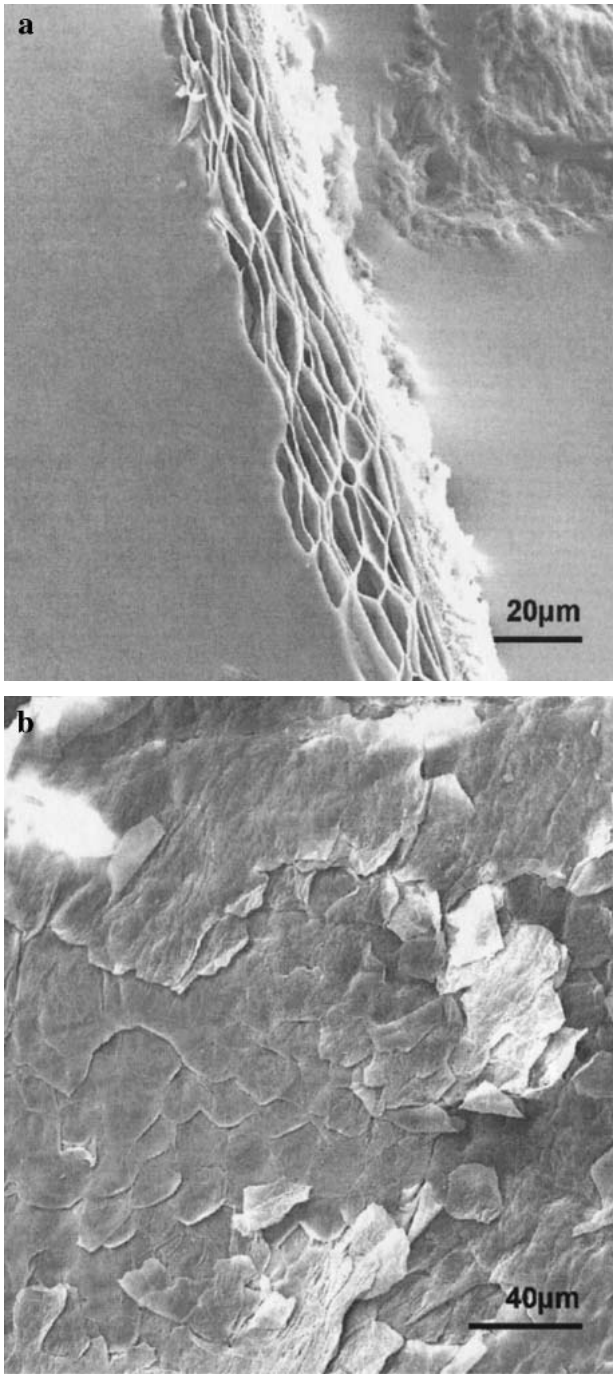

Fig. 1. a, Cross section of human epidermis after iontophoresis for 24 h. The thickness of the epidermis is approximately 20–30 μm. b, Surface of human epidermis after iontophoresis for 24 h. It resembles that of untreated skin.

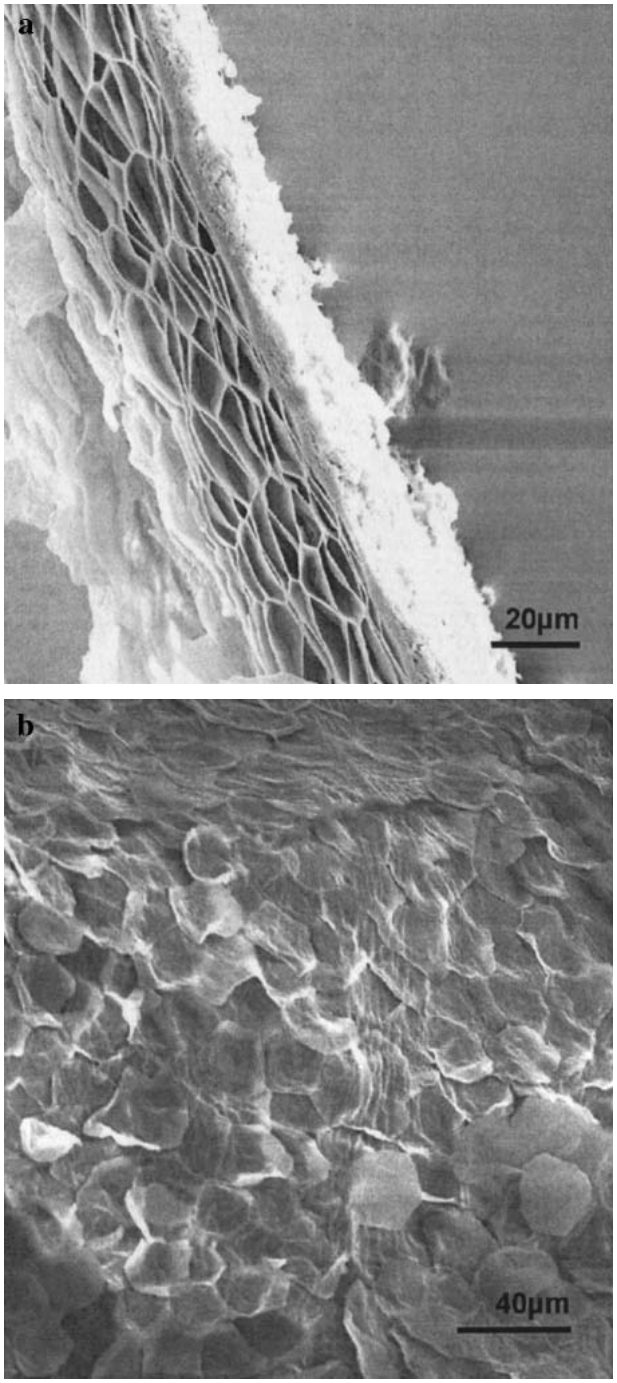

Fig. 2. a, Cross-section of human epidermis after treatment with 5% oleic acid for 1 h. The thickness of the epidermis is approximately 30–40 μm. b, Surface of human epidermis after treatment with 5% oleic acid for 1 h. It resembles that of untreated skin.

Preparation for SEM

Human epidermis before or after iontophoresis treatment was fixed with 5% glutaradehyde aqueous solution at 4°C for 1.5 h and then washed with water at 4°C overnight. The fixed skin was dehydrated with 30%, 50%, 75%, and 95% ethanol in water, for 2 h each, at room temperature and then with two changes of absolute ethanol for 2 h/change at room temperature. The dehydrated skin was dried with a critical-point drying machine and coated with gold.

RESULTS AND DISCUSSION

Effects of Iontophoresis and Oleic Acid Alone on the Delivery of Midodrine Hydrochloride

After prehydration, the skin was treated with 5% oleic acid, and then 0.1 mA/cm^2 electrical current was applied to determine the effects of iontophoresis and oleic acid. Control I had no oleic acid treatment and also no iontophoretic treatment, whereas control II had only iontophoretic treatment

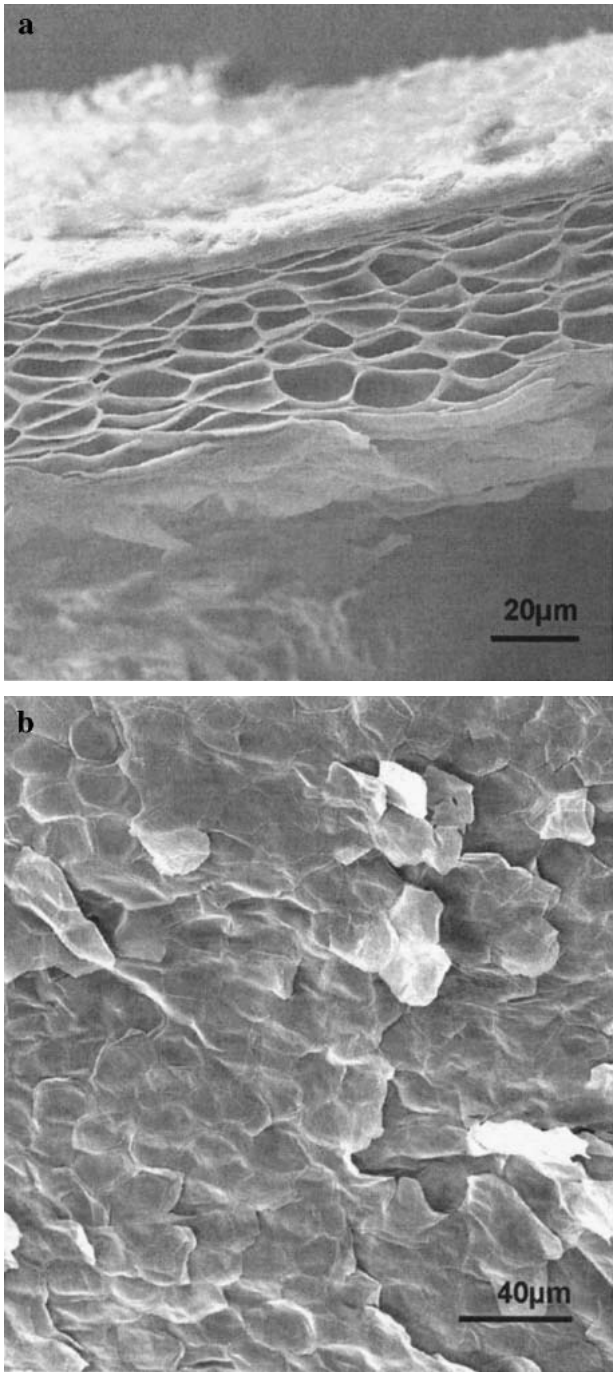

Fig. 3. a, Cross section of human epidermis after treatment with 2.5% oleic acid for 1 h and then iontophoresis for 24 h. The thickness of the epidermis is approximately 50 μm. b, Surface of human epidermis after treatment with 2.5% oleic acid for 1 h and then iontophoresis for 24 h. It resembles that of untreated skin.

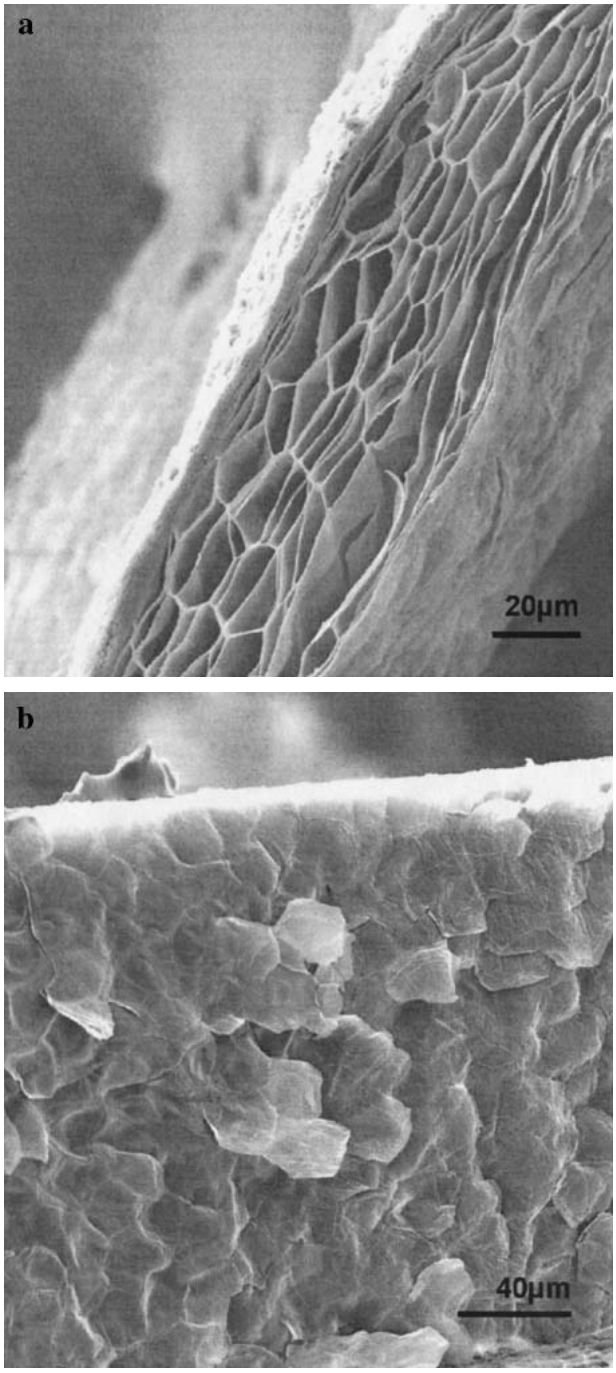

Fig. 4. a, Cross section of human epidermis after treatment with 5% oleic acid for 1 h and then iontophoresis for 24 h. The thickness of the epidermis is approximately 50–60 μm. b, Cross section of human epidermis after treatment with 5% oleic acid for 1 h and then iontophoresis for 24 h. It resembles normal skin without any treatment.

without oleic acid treatment. Control III had a treatment with the solvent PG only, no fatty acid added, and iontophoresis was applied thereafter. The permeation study results are shown in Table I. Pretreatment with 5% oleic acid in PG alone increased the flux of midodrine 5.7-fold, and iontophoresis increased the flux 44.7-fold compared with the relevant control I.

SEM micrographs of human epidermis with oleic acid or iontophoresis treatment alone are shown in Figs. 1 and 2. Iontophoresis did not show any significant effects on the structure of epidermis. The surface of the skin was normal, and there was no significant damage observed. Figure 2 shows how 5% oleic acid opened up SC cell layers significantly, and the whole epidermis was much more swollen. The surface of the skin did not have obvious damage following treatment with 5% oleic acid for 1 h.

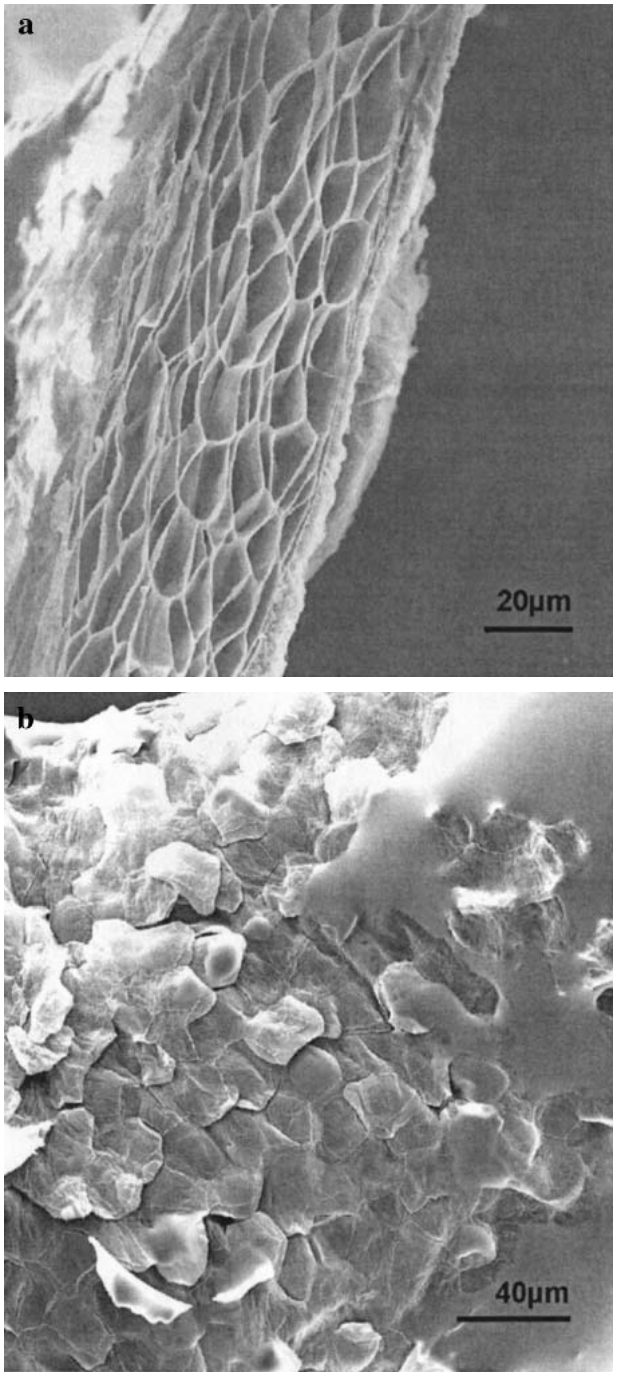

Fig. 5. a. Cross section of human epidermis after treatment with 5% oleic acid for 3 h and then iontophoresis for 24 h. The thickness of the epidermis is greater than 60 μm. b, Surface of human epidermis after treatment with 5% oleic acid for 3 h and then iontophoresis for 24 h. It is similar to normal skin without any treatment.

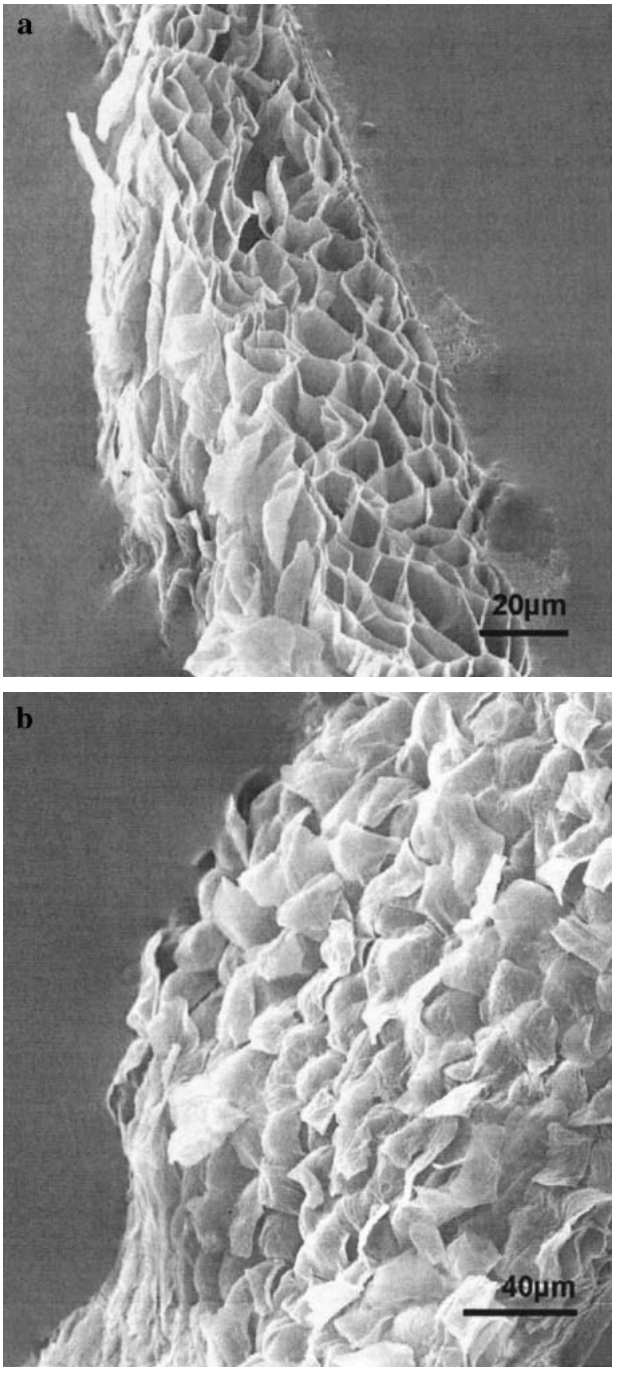

Fig. 6. a, Cross section of human epidermis after treatment with 10% oleic acid for 3 h and then iontophoresis for 24 h. The thickness of the epidermis is greater than 60 μm. b, Surface of human epidermis after treatment with 10% oleic acid for 3 h and then iontophoresis for 24 h. Detached corneocytes can be observed.

Transdermal Iontophoretic Delivery of Midodrine Hydrochloride with Various Concentrations of Oleic Acid as Enhancer

After prehydration, human epidermis was treated with 0%, 2.5% (0.065 M), 5% (0.13 M), 10% (0.26 M), and 12% (0.31 M) oleic acid in PG (v:v) for 1, 2, or 3 hours, and the midodrine hydrochloride was delivered iontophoretically for 24 h. The permeation study results are also presented in Table I. After treatment with pure PG (0% oleic acid), the skin had a lower permeability to midodrine delivered iontophoretically ($p < 0.05$). Treatment with 2.5%, 5%, 10%, and 12% oleic acid gave significantly higher permeation levels, up to 97.8-fold, compared with control I ($p < 0.05$). The combination effect of iontophoresis and treatment with 5% oleic acid for 1 h produced permeation higher than the sum of the effects of each technique alone, indicating the existence of synergism.

Table II. Iontophoretic Delivery of Midodrine Hydrochloride Using Fatty Acids as Enhancers (n = 6)

Enhancer	Enhancer treatment time (h)	Current applied (mA/cm^2)	Flux ($\mu g/cm^2 h$)	Q_{24} ($\mu g/cm^2$)	Enhancement factor
0.13 M (5%) Oleic acid	1	0.1	398.9 ± 11.5	8955.4 ± 223.9	66.5
0.13 M Linoleic acid	1	0.1	450.8 ± 37.6	9886.4 ± 811.8	75.1
0.13 M Decanoic acid	1	0.1	299.1 ± 54.7	6657.5 ± 1170.4	49.8
0.13 M Lauric acid	1	0.1	351.1 ± 9.3	7806.8 ± 359.9	58.5

SEM photomicrographs of the cross section and surface of epidermis treated with various concentrations of oleic acid and iontophoresis for 24 h are shown in Figs. 3–6. The cross sections reveal that the higher the concentration of oleic acid, the more the SC structure opened up. With the same concentration of oleic acid, 5%, 3 h of treatment opened up the SC more than the 1-h treatment. Treating with 5% oleic acid for up to 3 h did not change the surface structure of epidermis significantly. However, 10% oleic acid clearly damaged the surface of epidermis only after 1 h of treatment. One reported problem of fatty acids is their ability to cause irritation in the skin, so this result was expected (8).

The opening of cell layers in SC by oleic acid/PG could result from the oleic acid–induced breakdown and solubilization of macromolecular components, such as the filaggrin matrix protein of the stratum corneum, into smaller particles, resulting in a higher osmotic activity within the cells (3). The swelling of porcine skin was observed by Jiang *et al.* (19) and Bhatia *et al.* (3). Chemical and osmotic effects of oleic acid probably increased the diffusional volume within the SC, leading to a compromise of the SC barrier function. The increased diffusional volume could then be used by iontophoresis and increase the transport of the drug.

Oh *et al.* (20) investigated the electrical resistance change of hairless mouse skin after oleic acid treatment. They found that with treatment of the skin with 5% (w/w) oleic acid in PG, resistance dropped significantly, and the value of resistance did not then change with time.

Transdermal Iontophoretic Delivery of Midodrine Hydrochloride with Various Fatty Acids

Three other fatty acids with the same molar concentration (0.13 M) were used for enhancing iontophoretic delivery of midodrine hydrochloride. The treatment time for all fatty acids was 1 h. Results of the permeation study are presented in Table II.

All four fatty acids including oleic acid facilitated the iontophoretic delivery of midodrine ($p < 0.05$), and linoleic acid was the most effective, increasing the permeability by 75-fold compared with the control I.

CONCLUSIONS

Pretreatment with fatty acids in PG was found to cause the opening up of the tightly packed SC cell layers and thereby increased the permeability of the skin to midodrine hydrochloride delivered iontophoretically. The higher the concentration of oleic acid, the more the epidermis swelled, and the higher the resulting permeability of the skin. Pretreatment with 5% oleic acid for up to 3 h did not cause a significant change to the surface of human epidermis, whereas 10% oleic acid pretreatment for 1 h resulted in detachment and separation of the corneocytes on the surface of the skin.

Unsaturated long-chain fatty acids—oleic acid and linoleic acid—were more effective as enhancers of midodrine permeation than saturated fatty acids—lauric and decanoic acids. C_{12} lauric acid had a slightly greater enhancing effect than decanoic acid. The order of the enhancing effects of the fatty acids used in iontophoretic delivery are similar to that for passive diffusion (8), indicating that enhancement of passive and iontophoretic delivery by fatty acids may be occurring through the same mechanism.

From our data, using 5% oleic acid pretreatment, with the electrical current offset at 0.1 mA/cm^2, the daily delivery of midodrine hydrochloride of approximately 9 mg can be attained with the skin size of 0.64 cm^2. If one assumes a patch size of 2.1 cm^2, the therapeutic level of midodrine required (30 mg/day) can be reached by iontophoresis according to this study. For clinical use, iontophoretic delivery of midodrine is advantageous because it can be more precisely controlled and tailored to the patient's individual needs and terminated in order to avoid more severe drug side effects.

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