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物理性促進作用對於ALA的經皮吸收效果評估以及在光動力治療上的應用

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Enhancement of topical 5-aminolaevulinic acid delivery by erbium:YAG laser and microdermabrasion: a comparison with iontophoresis and electroporation

Summary

Background: 5-aminolaevulinic acid (ALA) is used as a protoporphyrin IX-precursor for the photodynamic therapy of superficial skin cancer and cutaneous metastases of internal malignancies. However, the permeability of hydrophilic ALA across the skin is very low.

Objectivesandmethods: The objective of this study was to optimize and enhance the invitroskin permeation of ALA by two resurfacing techniques: erbium:yttrium-aluminium-garnet (Erb:YAG) laser and microdermabrasion. Light microscopic changes in pig skin caused by these techniques were also compared. The electrically assisted methods, iontophoresis and electroporation, were also used to facilitate ALA permeation across laser-or microdermabrasion-treated skin.

Results: Among the modalities tested in this study the Erb:YAG laser showed the greatest enhancement of ALA permeation. The laser fluence was found to play an important role in controlling the drug flux, producing enhancement ratios from 4-fold to 246-fold relative to the control. The skin permeation of ALA across microdermabrasion-treated skin was approximately 5–15-fold higher than that across intact skin. Both the ablated effect of the stratum corneum (SC) and ALA flux were proportional to the treatment duration of microdermabrasion. The application of iontophoresis or electroporation alone also increased the ALA permeation by approximately 15-fold and 2-fold, respectively. The incorporation of iontophoresis or electroporation with the resurfacing techniques caused a profound synergistic effect on ALA permeation.

Conclusions: This basic study has encouraged the further investigation of ALA permeation by laser or microdermabrasion.

Keywords: 5-aminolaevulinic acid, electroporation, erbium:YAG laser, iontophoresis, microdermabrasion, photodynamic therapy

Skin cancer is the most common of all cancers. Topical photodynamic therapy (PDT) of skin tumours.[1] ALA is application of 5-aminolaevulinic acid (ALA) is an a drug precursor, in that the photosensitizer, protoincreasingly popular method of photosensitization for porphyrin IX, is formed invivoafter the exogenous application of ALA. ALA-induced PDT has been used experimentally in a variety of superficial nonmelanoma skin cancers including basal cell carcinoma, squamous cell carcinoma, solar and actinic keratosis and Bowen's disease.[2] ALA is a hydrophilic molecule with a molecular weight of 167-7 and a stratum corneum (SC)/water partition coefficient of 0-1. As expected from skin permeability theory, ALA permeates poorly across intact skin. The commonly used dose of 20% for ALA for clinical use is so high that it may cause irritation of the skin.

The erbium:yttrium-aluminium-garnet (Erb:YAG) laser and microdermabrasion are both new tools which

can be used for ablation of the SC with relatively few sequelae. The Erb:YAG laser emits light with a 2940-nm wavelength which corresponds to the main peak of water absorption. This property enables this laser to ablate the SC with minimal residual thermal damage. Microdermabrasion is a process that uses aluminium oxide crystals and negative pressure superficially to peel off the outer surface of the skin.

The purpose of the present study was to evaluate the feasibility of the Erb:YAG laser and microdermabrasion for enhancing and controlling the topical delivery of ALA. Previous studies showed that iontophoresis, an electrical enhancement method, can increase the transport of ALA across the skin.[1,8] Electroporation is another method to enhance drug permeation through skin by electrical high-voltage pulses, which can create aqueous micropores within the skin. Our study also investigated whether iontophoresis or electroporation combined with resurfacing techniques can produce synergistic effects for enhancing ALA delivery. Our previous studies indicated that the Erb:YAG laser is safe and effective when used for ablation of the SC layers to enhance the skin absorption of drugs.[9,10] However, the animal model used in those studies was nude mouse skin, which is more permeable than human skin.[11] The most relevant animal model for human skin is the pig.[12] The present study utilized pig skin instead of nude mouse skin as the barrier to examine the topical delivery of ALA.

Materialsandmethods

Skin samples

Pigs (Landrace · Duroc hybrids, approximately 1-week old) were supplied from the laboratory of Dr Chi-Feng Hung, Fu Jen Catholic University, Taipei Hsien, Taiwan. Full-thickness skin was excised from the dorsal region. The hair of skin samples was carefully removed with curved surgical scissors. The subcutaneous adipose tissue was also dissected away.

Erbium:yttrium-aluminium-garnet laser assembly

The Erb:YAG laser (Continuum Biomedical, Santa Clara, CA, U.S.A.) has a wavelength of 2940 nm and a pulse duration of 250 ls. An articulated arm was used to deliver the laser beam onto the skin. Output energies of 0Æ45–1Æ35 J pulse$^{)1}$ with a beam spot size of 7 mm in diameter could achieve fluences of 1Æ2–3Æ5Jcm$^{)2}$. The energy of the laser pulse was monitored with an energy meter (Nova Display, Ophir, Israel) before and after treatment.

Microdermabrasion assembly

The microabrasor (Pepita-C; Mattioli Engineering, Florence, Italy) has a system of aspiration–compression within a flexible tube, which is connected to the machine and a handpiece. Aluminium oxide crystals are fired from the compression system via a nozzle at a vacuum pressure of 3 bar with the handpiece in contact with the skin for 3, 5, or 10 s.

Iontophoresis assembly

A pair of Ag/AgCl wires, having an effective length of 15 mm, was used as electrodes by immersing them in a diffusion cell for the invitropermeation experiments, with the anode in the donor compartment and the cathode in the receptor compartment. The electrodes were connected to a current power supply (Model 7651, Yokogawa, Japan). A current density of 0Æ5mAcm$^{)2}$ was applied for 6 h to stimulate the permeation of ALA.

Electroporation assembly

Electroporation was performed using an exponential decay pulse generator (Electro Cell Manipulator 630; Genetronics, San Diego, CA, U.S.A.). Platinum electrodes (0Æ5 · 1Æ5 cm) were used, each located 3 cm from the skin in the invitrodiffusion cell. The anode was positioned in the donor compartment, while the cathode was in the receptor compartment. The electroporation protocol consisted of one pulse per 30 s, applied for 10 min. The pulse voltage was 300 V, and pulse length was 200 ms. Voltages were expressed as applied values, not as transdermal values.

Histological examination of pig skin

Immediately after treatment, a specimen of the exposed area was taken for histological examination. The adjacent untreated skin was also assessed as a control. Each specimen was fixed in a 10% buffered formaldehyde solution at pH 7Æ4 for at least 48 h. Each section was dehydrated using ethanol, embedded in paraffin wax and stained with haematoxylin and eosin. For each skin sample, three different sites were examined and evaluated under light microscopy (Eclipse 4000, Nikon, Japan). Photomicrographs of the three randomly selected sites of each skin sample were taken with a digital camera (Coolpix 950, Nikon, Japan). The digital photomicrographs were then processed with Adobe PhotoDeluxe (Adobe Systems, San Jose, CA, U.S.A.), and the SC and epidermal thicknesses were calculated with ImagePro-plus 4.0 (Media Cybernetics, Silver Spring, MD, U.S.A.).

In vitro topicaldeliveryof5-aminolaevulinicacid

The diffusion cell used in this invitroexperiment was a horizontal diffusion assembly. A piece of excised pig skin was mounted with the SC side facing towards the donor compartment. After pretreatment with the Erb:YAG laser or with microdermabrasion, the skin surface was wiped with a cotton wool swab several times. The receptor compartment (8 mL) was filled with stable pH 5 citrate-phosphate buffer.[13] The donor compartment was filled with 8 mL of 0-5% (w/v) ALA in pH 5 buffer. The available area of the cell was 0-785 cm^2. The compartments were maintained at 37°C and stirred with a magnetic bar at 600 r.p.m. At appropriate intervals, 300-lL aliquots of receptor medium were withdrawn and immediately replaced with an equal volume of fresh receptor solution. Each experiment included four replicates.

High-performanceliquidchromatographyanalysisfor5-aminolaevulinicacid

The fluorescence derivation of ALA samples was based on a modification of the Hantzsch reaction.[14] The ALA content of the various samples was analysed with a high-performance liquid chromatography (HPLC) system consisting of a Hitachi L-7110 pump, a Hitachi L-7200 sample processor, and a Hitachi L-7480 fluorescence detector. A 25-cm long, 4-mm inner diameter stainless steel RP-18 column (Merck, Darmstadt, Germany) was used. The mobile phase, consisting of methanol–water– acetic acid (50 : 50 : 1) was used at a flow rate of 0Æ7 mL $min^{)1}$. The wavelength of the fluorescence detector was set to excitation at 378 nm and emission at 467 nm. The column oven was set to 40 C.

Data analysis

In the invitrotopical delivery of ALA, the total amount of drug permeability across a unit diffusion surface and into the receptor was calculated and plotted as a function of time. The flux was calculated from the slope of the linear portion of the cumulative amount–time plots for a zero-order model and expressed as the mass of drug passing across 1 cm^2 of skin over time. The present study showed that limited ablation-pretreated areas of diffusion of the skin was 49Æ02% for the 7-mm diameter handpiece of the Erb:YAG laser, and 96Æ05% for the 0Æ754-cm^2 oval-shaped handpiece of the microdermabrasion unit of the total permeated skin surface area. Data of the cumulative amount and flux of the treated area were extrapolated to an area of 100% ablation of the permeated area by the following equation:

$$Q_{100\%} = [Q_{treat} - (Q_{control} * (A_{100\%} - A_{treat} / A_{100\%}) * 100\%)] * A_{100\%} / A_{treat}$$

where $Q_{100\%}$ represents the permeation data across 100% treated skin (calibrated data), Q_{treat} represents the data across partially treated skin in the Franz cell, control represents the data across nontreated skin, 100% is the area (cm^2) of the total diffusion area, and treat is the area of the laser-or microdermabrasion-treated site. The ratio of the permeation data across 100% treated skin to the value across untreated skin (control group) was calculated as the enhancement ratio (ER). The statistical analysis of differences between different treatments was performed using unpaired Student's t-tests. A 0Æ05 level of probability was taken as the level of significance.

Results

Histological examination of pig skin

Light microscopy indicated no observable damage to whole skin in the untreated group (data not shown). The density of hair follicles of the pig skin seemed to be similar to that of human skin. The pig SC is approximately 10 lm thick as shown in Table 1, which is also similar to human SC (about 10 lm).[15] As shown in Figure 1a, the SC had loosened up as a result of laser treatment with 1Æ2Jcm$^{)2}$ (arrows). No significant difference (t-test, P>0Æ05) was observed in the SC or epidermal thickness between the control and 1Æ2Jcm$^{)2}$ -treated skin (Table 1). No gross changes in the viable epidermis or dermis were noted when compared with control.

Table 1. Stratum corneum and epidermis thickness of pig skin after ablation with an erbium:yttrium-aluminium-garnet (Erb:YAG) laser and microdermabrasion

Ablation type	Fluence (J cm^{-2})[a] or duration (s)[b]	SC thickness (μm)	Viable epidermis thickness (μm)
Control	0	9·36 ± 1·86	10·11 ± 1·39
Erb:YAG	1·2	7·10 ± 1·76	12·44 ± 2·28
Erb:YAG	1·4	5·80 ± 1·11	11·97 ± 1·46
Erb:YAG	2·1	5·29 ± 0·79	8·94 ± 1·25
Erb:YAG	2·6	5·05 ± 1·71	9·21 ± 1·64
Erb:YAG	1·2 × 2 pulses	3·95 ± 0·95	11·37 ± 1·47
Erb:YAG	1·4 × 2 pulses	3·31 ± 1·06	8·70 ± 2·22
Portable laser	3·5	2·13 ± 1·84	7·36 ± 3·30
Erb:YAG	3·5	4·66 ± 1·50	5·65 ± 1·18
Microdermabrasion	3	5·51 ± 1·01	10·71 ± 0·80
Microdermabrasion	5	4·93 ± 1·87	9·61 ± 1·35
Microdermabrasion	10	2·05 ± 1·70	10·21 ± 2·03

[a]Fluence is the unit for Erb:YAG laser. [b]Duration is the unit for microdermabrasion. Each value represents the mean ± SD ($n = 3$).

Light microscopy showed a slight thinning of the SC after treatment with 1-4Jcm^{-2} laser (Table 1). The ablation amount of the SC by 1-4Jcm^{-2} was more than that by 1-2Jcm^{-2} and in the control group (t-test, P<0.05). As shown in Table 1, there were no statistically significant differences in the SC or epidermal thickness with laser irradiation of from 1-2Jcm^{-2}to 2-6Jcm^{-2}. However, a thermal effect was observed in skin after treatment with 2Æ6Jcm$^{)2}$ laser irradiadiation, as shown in Figure 1b. Thermal necrosis was seen and the SC appeared condensed and homogeneously aggregated (arrows). The SC loosened up and was partially ablated by two passes of 1-2J cm$^{)2}$ fluence (arrows, Fig. 1c). SC removal with two passes at 1Æ2Jcm$^{)2}$ was deeper (t-test, P<0.05) than that with only one pass (Table 1). However, this phenomenon was not observed in the groups treated with 1-4Jcm^{-2} fluence as there was no significant difference (t-test, P>0.05) between the remaining SC thickness, using one or two pulses.

The disadvantages of the larger device size and high price of the laser may be resolved by using a novel product designed as a portable Erb:YAG laser (Laser-Assisted Anesthetic Delivery System, Norwood Abbey Ltd, Australia), which is cheaper and more convenient. This system emits 3-5Jcm^{-2} fluence for 300 lsbya 3-mm diameter handpiece. As shown in Figure 2a, the SC layers were lost after treatment with the portable laser (thick arrows). The epidermal thickness was, however, not affected by this portable laser (Table 1). The underlying epidermis showed homogenization and degeneration which may indicate prominent thermal

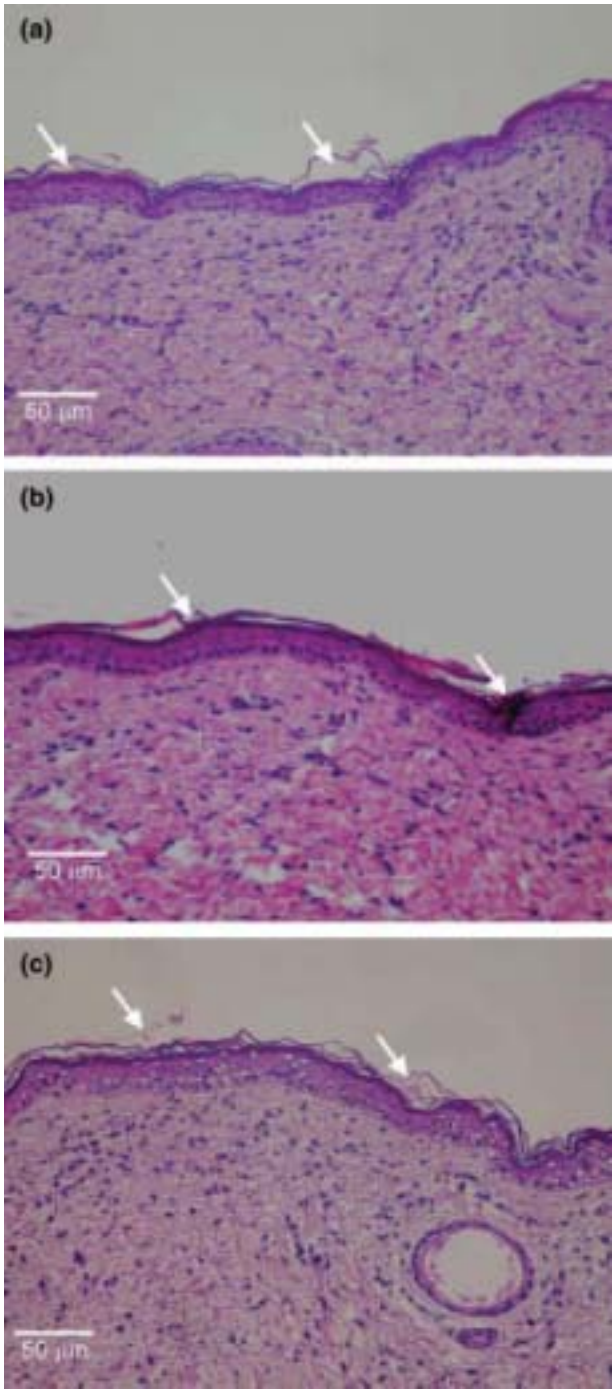

Figure1.Histological examination of pig back skin with (a) erbium:yttrium-aluminium-garnet (Erb:YAG) laser treatment at 1Æ2Jcm^{-2};
(b) Erb:YAG laser treatment at 2Æ6Jcm^{-2}; and
(c) Erb:YAG laser treatment at 1Æ2Jcm^{-2} with two pulses (original magnification · 200).

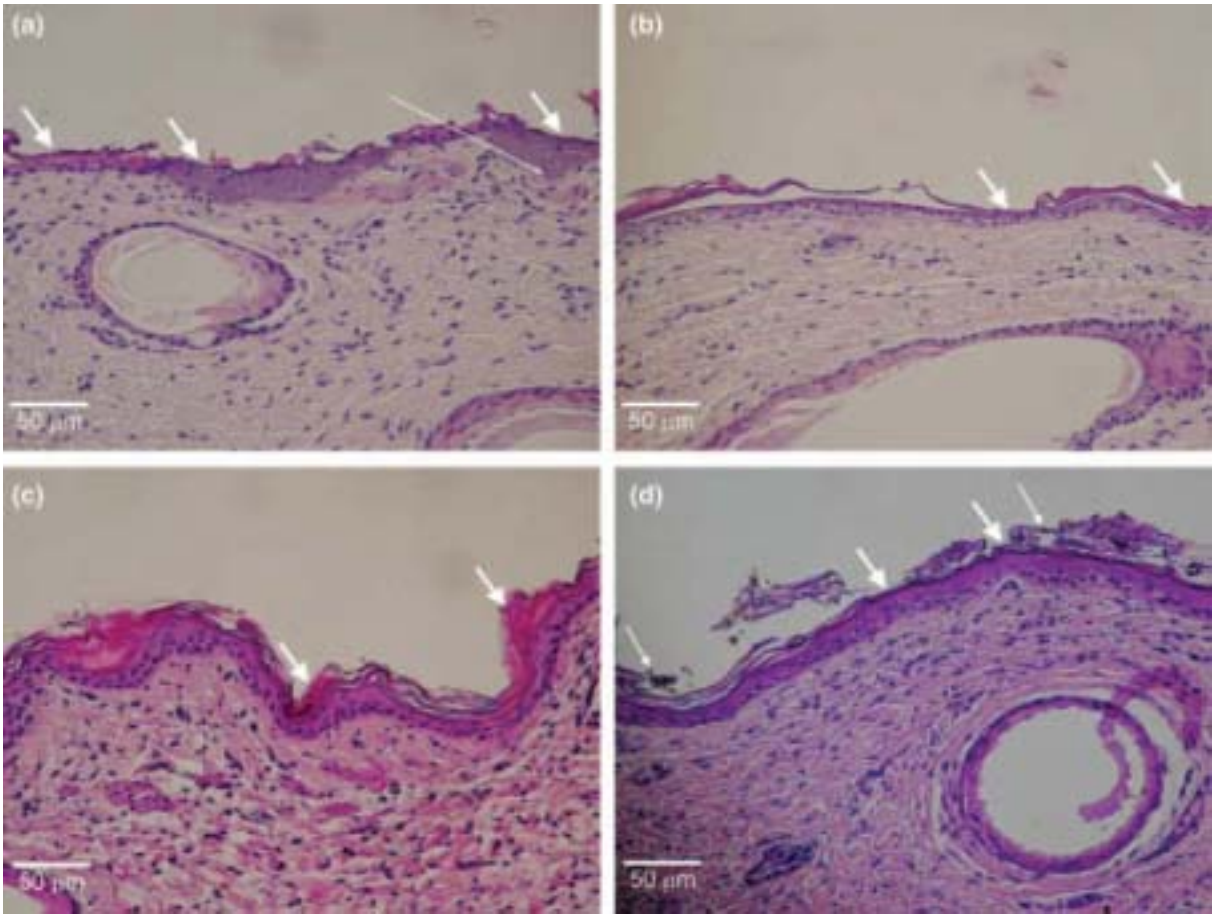

Figure2.Histological examination of pig back skin with (a) portable laser treatment at 3Æ5Jcm^{-2}; (b) erbium:yttrium-aluminium-garnet laser treatment at 3Æ5Jcm^{-2}; (c) microdermabrasion for 3 s; and (d) microdermabrasion for 10 s (original magnification · 200).

damage (thin arrows). The heating effect of the Erb:YAG laser is often observed with the higher fluences such as 5Jcm^{-2} The same 3-5Jcm^{-2} energy emitted by the traditional laser produced different effects on the skin from that of the portable laser. As shown in Figure 2b, a thin layer of the SC remained from the viable epidermis (arrows). The underlying epidermis was thinner than the control (Table 1). Moreover, the thermal effect was less for the traditional laser than for the portable laser. This might be due to the longer pulse duration of the portable laser (300 ls) compared with that of the Erb:YAG laser.

[16]

From histological observations of skin treated with microdermabrasion for 3 s (Fig. 2c), there was SC thinning with focal compaction and homogenization (arrows). Retention of aluminium oxide crystals was also observed on the surface of the SC. The level of SC thinning and homogenization was more prominent after 10 s of treatment with microdermabrasion (thick arrows, Fig. 2d and Table 1). There were also more remaining aluminium oxide crystals on the skin surface than were seen in the group treated for 3 s (thin arrows). No alteration was noted in the morphology and thickness of the viable epidermis with treatment

from 3 to 10 s when compared with the control. It was found that the viable epidermis thickness was almost not changed by all the formulations used except the Erb:YAG laser at the highest fluence (3-5Jcm^{-2}, Table 1). Hence epidermal change may not be related to the enhancement of ALA delivery by the ablative tools.

Topical delivery of 5-aminolaevulinic acid by theerbium:yttrium-aluminium-garnetlaser

Figure 3 shows the cumulative amount of ALA (阵g cm^{-2}) in the in vitro receptor as a function of time after laser irradiation at various fluences. Erb:YAG laser exposure of a limited area of the skin surface of about 49% of the permeated area increased ALA permeation via the skin for all fluences tested. Extrapolating the original flux data of the laser-irradiated area to an area of 100% exposure (normalized flux) resulted in permeation enhancement ratios (ERs) from 4Æ06 to 246Æ73 for fluences of from 1Æ2to 2Æ6Jcm$^{)2}$ (Table 2). It is clear that a single pulse at 1Æ2Jcm$^{)2}$ was sufficient to enhance significantly the transport of ALA across the skin. There was a linear relationship between the ALA flux and the laser fluences tested (correlation coefficient, r ¼ 0Æ99). However, the inclusion of higher fluences such as the two pulses mode and 3Æ5J cm$^{)2}$ would reduce this correlation.

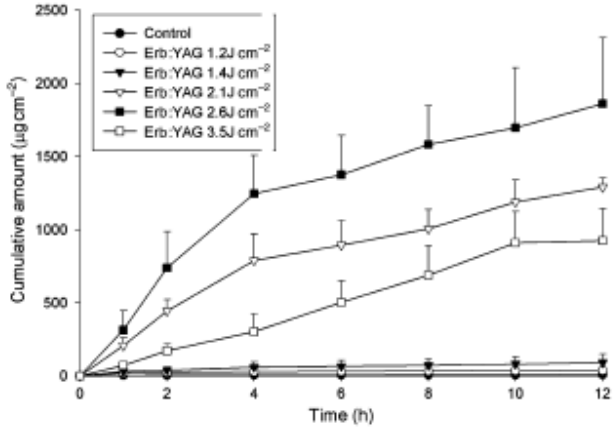

Figure 3. *In vitro* cumulative amount–time profiles of topical ALA permeation with erbium:yttrium-aluminium-garnet (Erb:YAG) laser treatment of skin by a 7-mm diameter handpiece at various fluences: ●, control group; ○, fluence of 1·2 J cm^{-2}; ▼, fluence of 1·4 J cm^{-2}; ▽, fluence of 2·1 J cm^{-2}; ■, fluence of 2·6 J cm^{-2}; □, fluence of 3·5 J cm^{-2}. Each value represents the mean ± SD ($n = 4$).

Topical delivery of 5-aminolaevulinicacid by microdermabrasion

Microdermabrasion for various treatment periods (3– 10 s) significantly enhanced (t-test, P<0.05) the skin permeation of ALA from 5-to 15-fold as depicted in Table 2. An excellent correlation (r=0.99) was found to exist between the normalized flux of ALA and the durations of exposure to microdermabrasion. Table 1 also demonstrates a linear relationship (r=0.99) between treatment duration and the etched thickness of the SC.

Topical delivery of 5-aminolaevulinic acid by theerbium:yttrium-aluminium-garnet laser and

microdermabrasion combined with electrically assisted methods

Figure 4a shows the permeated amount of ALA as a function of time with or without iontophoresis. As evident from the figure, the application of iontophoresis enhanced ALA permeation relative to the control by approximately 15-fold according to the permeated amount of ALA at the end of experiment. The permeated amount of ALA levelled off after cessation of the current density at 6 h. To clarify the mechanism of ALA permeation by iontophoresis, the release rate of drug across a cellulose membrane (Spectra-Por 5, molecular weight cut-off of 12 000–14 000) was studied as shown in Figure 4b. The presence of iontophoresis only caused a slight increase in ALA release compared with the control.

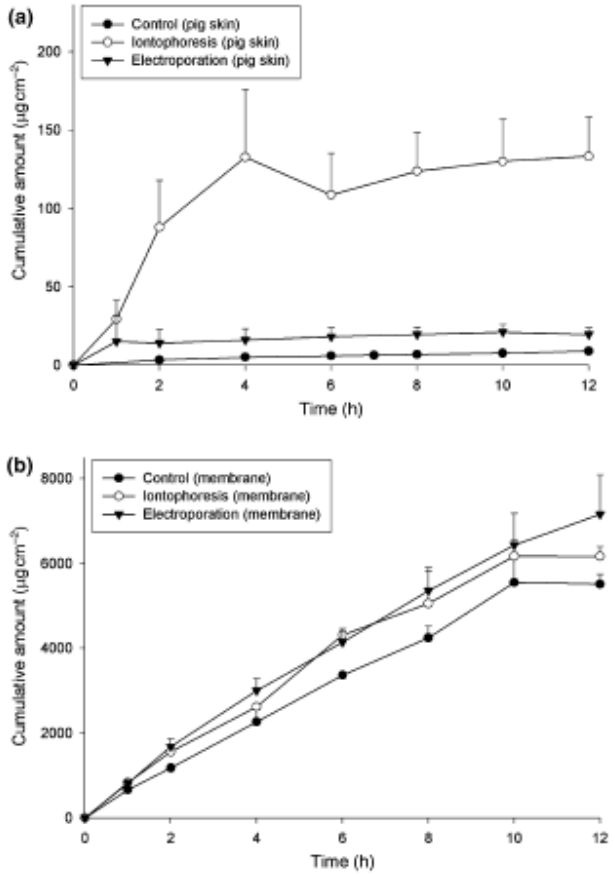

Figure 4. *In vitro* cumulative amount–time profiles of topical 5-aminolaevulinic acid permeation by iontophoresis or electroporation across (a) pig skin, and (b) a cellulose membrane: ●, control group; ○, iontophoresis at 0·5 mA cm^{-2} for 6-h application; ▼, electroporation at 300 V, 200 ms for 20 pulses. Each value represents the mean ± SD ($n = 4$).

As shown in Figure 4a, electroporation significantly enhanced ALA permeation compared with passive diffusion by a 2Æ2-fold increase. ALA permeation reached the highest level within the first hour. The release profiles of ALA by electroporation alone are shown in Figure 4b. As with iontophoresis, the release of ALA with electroporation was slightly higher than that without electroporation.

Figure 5a demonstrates the skin permeation of ALA after the combination of laser pretreatment and iontophoresis. A synergistic effect was observed with laser exposure of 1-4Jcm^{-2} oupled with iontophoresis.

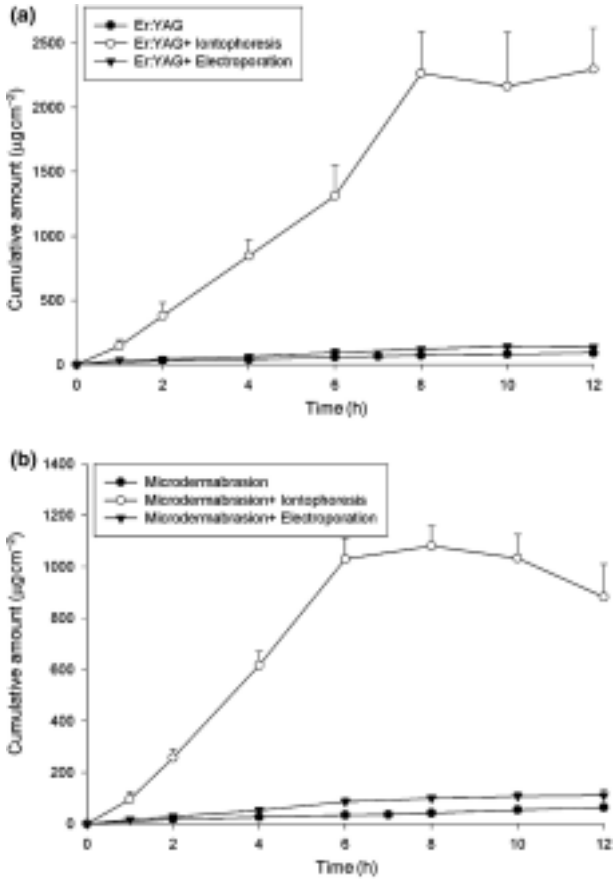

Figure5.Invitrocumulative amount–time profiles of topical 5aminolaevulinic acid permeation by iontophoresis or electroporation across (a) erbium:yttrium-aluminium-garnet (Er:YAG) laser treated-skin: d, fluence of 1Æ2Jcm$^{)2}$ alone; s, fluence of 1Æ2Jcm$^{)2}$ pretreatment following by iontophoresis at 0Æ5mAcm$^{)2}$ for 6-h application; ., fluence of 1Æ2Jcm$^{)2}$ pretreatment following by electroporation at 300 V, 200 ms for 20 pulses, and (b) micro-dermabrasion-treated skin: d, microdermabrasion for 5-s alone; s, microdermabrasion for 5-s pretreatment followed by iontophoresis at 0Æ5mAcm$^{)2}$ for 6-h application; ., microdermabrasion for 5-s pretreatment following by electroporation at 300 V, 200 ms for 20 pulses. Each value represents the mean ± SD (n ¼ 4).

Electroporation alone produced less enhancement of ALA permeation than iontophoresis alone, and combined use with an Erb:YAG laser also showed the same trend of synergistic effects (Fig. 5a). The same phenomenon was determined with the combination of micro-dermabrasion (5 s) and electrically assisted methods (Fig. 5b).

Discussion

The ablation amount of the SC by 1-4J cm^{-2} of energy of the Erb:YAG laser was more than that by 1-2J cm^{-2}[2] and the control group (t-test, $P<0.05$). This may indicate that the ablation threshold of the laser on pig skin was about 1-4J cm^{-2}. This threshold approximated the value for human skin (about 1-6J cm^{-2}).[17] ALA possessed very low permeation in the group that was without laser treatment (Fig. 3). The rate-limiting step for hydrophilic ALA molecule uptake into the skin lies at the level of the SC.[1] Part ablation of the SC by the laser reduced the inherent barrier properties of the skin to ALA and thus enhanced skin permeation. Table 2 shows that an increase in laser intensity led to further enhancement of ALA flux from 1Æ2to 2-6J cm^{-2}. The linear relationship between ALA flux and laser fluences tested suggested that ALA permeation can be precisely controlled by using an Erb:YAG laser with fluences of 1-2–2-6J cm^{-2}. When calculating the

relationship between ALA flux and the etched depth of the SC, however, only a correlation coefficient of 0-82 was obtained. This may indicate that forces other than SC ablation predominated in increasing ALA permeation after laser exposure. The histological observations of pig skin indicated that fluences of 2Æ1 and 2Æ6J cm$^{)2}$ made the remaining SC more condensed and homogenized than fluences of 1-2J and 1-4J cm^{-2}. Another explanation may be that the laser affected the remaining SC and the underlying epidermis through ultrastructural alterations.[9] This may have been due to the photomechanical acoustic effect generated by the laser. Local propagation of the acoustic shock wave may cause cracking of the epidermis.[18,19]

It was found that the higher fluences or energies such as 1-4J cm^{-2} for two pulses, 3-5J cm^{-2} for one pulse and the portable laser emission did not necessarily produce more enhancement than the lower fluences. ALA may first be partitioned into the SC, after which it passes across the skin by various pathways. It can be seen that the remaining SC detached from the underlying epidermis after treatment of 1-4J cm^{-2} with two pulses and 3-5J cm^{-2} (Figs 1d and 2a). Ablation of the SC layers reduced the inherent barrier properties of the skin and thus increased ALA permeation; however, the partitioning of ALA into the SC and subsequent diffusion into the epidermis may be retarded because of the effect of detachment.

The histological results showed that microdermabrasion could effectively remove a portion of the SC layers. A significant relationship existed between the treatment duration, the etched thickness of the SC, and the normalized flux of ALA. Hence the enhancement of ALA permeation by microdermabrasion may simply be generated by the ablation effect on the SC. It was perceived that the Erb:YAG laser and microdermabrasion ablated the SC layers to similar levels (Table 1). However, the results of ALA flux enhancement showed a great discrepancy between these two resurfacing tools. This may confirm the influence that factors other than skin ablation contributed to the enhancement of ALA permeation by the laser. The scanning electron microscope showed intervening spaces between the keratinocyte aggregates after irradiation with the Erb:YAG laser.[10]

Iontophoresis has been successfully used for promoting the skin permeation of ALA in recent studies.[1,8,20] With pK_a values of 3-90 and 8-05, it is clear that ALA at pH 5 in the donor compartment was protonated and thus was offered the opportunity to take advantage of the cation permselectivity of the skin as the isoelectric point of skin is pH 3–4 which possesses negative charge at pH 5.[21] Iontophoresis alone significantly enhanced ALA permeation. The permeated amount of ALA was not further increased after cutting off the current density. This result indicates that electrorepulsive movement under the electric field is important with ALA. The cellulose membrane was very permeable to ALA compared with the pig skin. This observation confirms that the SC indeed provides significant barrier characteristics towards ALA permeation. The presence of iontophoresis only caused a slight increase in ALA release. This suggests that the force of electrorepulsive drift is limited for ALA release. This may indicate that reduction of the SC barrier properties contributed to the main enhancement of ALA permeation by iontophoresis.

ALA permeation reached the highest level within the first hour of application of electroporation. The rapid initial transport which occurred during pulsing may be attributed to both electrophoresis as well as diffusion across highly permeabilized skin.[22] The result of ALA release by electroporation may demonstrate that the permeation-enhancing effects of electroporation were mainly attributed to the formation of micropore structures in the skin; the direct electromotive force on ALA was not as important. According to our data, ALA permeation comparing the four physical enhancement methods alone (laser, microdermabrasion, iontophoresis, and electroporation), the Erb:YAG laser was consistently the most potent method for ALA permeation via skin, followed by iontophoresis, microdermabrasion and electroporation. Hence direct abrasion of the SC rather than the modification of skin or increasing the diffusion of the drug itself may contribute to the greater ability to enhance ALA permeation.

The synergism of the laser and iontophoresis or electroporation on ALA permeation could provide the advantage of overcoming the difficulty of ALA permeation across intact skin. The synergistic effect was also shown by the combination of microdermabrasion for 5 s with iontophoresis or electroporation (Fig. 5b). As the superficial layers of the SC were removed, there was a gradual drop in the electrical resistance of the skin.[19] The combined use of electrically assisted methods with laser may thus further promote ALA

permeation.

Conclusions

The potential of some methods to promote ALA permeation into or across the skin was examined previously, including incorporation of permeation enhancers such as dimethyl sulphoxide, iontophoresis, increasing the skin temperature, and manufacturing self-adhesive thin films as vehicles.[1,4,8,20,23,24] However, these enhancing methods never achieved more than a 10-fold increase in ALA delivery. In our study the Erb:YAG laser greatly enhanced ALA permeation with 4–305-fold increases. The ablation effect, ultra-structural alterations, and ALA partitioning of the skin may contribute to the enhancing mechanism of the laser treatment. Microdermabrasion moderately increased skin permeation of ALA by simply ablating and homogenizing a portion of the SC layers. Both the peeling thickness of the SC and ALA flux showed good correlation with the treatment duration from 3 to 10 s. The combination of iontophoresis or electroporation with the peeling techniques could further promote ALA delivery to a great extent. The commonly used dose of 20% ALA in clinical practice almost always produces skin lesions. The methods examined in the present study can enhance ALA permeation, and thus reduce the ALA dose required. So the cost of PDT may also be reduced. Further studies on the feasibility of invivoand clinical uses of these techniques for topical ALA delivery are in progress.

References

1 Rhodes LE, Tsoukas MM, Anderson RR, Kollias N. Iontophoretic delivery of ALA provides a quantitative model for ALA pharmacokinetics and PpIX phototoxicity in human skin. JInvestDermatol1997; 108: 87–91. 2 Peng Q, Warloe T, Berg K etal.5-Aminolevulinic acid-based photodynamic therapy. Clinical research and future challenges. Cancer1997; 79: 2282–308. 3 Tsai JC, Chen IH, Wong TW, Lo YL. Invitro⁄invivocorrelations between transdermal delivery of 5-aminolevulinic acid and cutaneous protoporphyrin IX accumulation and effect of formulation. BrJDermatol2002; 146: 853–62.

4 De Rosa FS, Bentley MVLB. Photodynamic therapy of skin cancers: sensitizers, clinical studies and future directives. PharmRes2000; 17: 1447–55.

5 Manaloto RMP, Alster T. Erbium:YAG laser resurfacing for refractory melasma. DermatolSurg1999; 25: 121–3. 6 Lloyd JR. The use of microdermabrasion for acne: a pilot study. DermatolSurg2001; 27: 329–31.

7 Tsai RY, Wang CN, Chan HL. Aluminum oxide crystal micro-dermabrasion. A new technique for treating facial scarring. DermatolSurg1995; 21: 539–42.

8 Lopez RFV, Bentley MVLB, Delgado-Charro MB, Guy RH. Optimization of aminolevulinic acid delivery by iontophoresis. JControlRelease2003; 88: 65–70.

9 Lee WR, Shen SC, Lai HH etal.Transdermal drug delivery enhanced and controlled by erbium:YAG laser: a comparative study of lipophilic and hydrophilic drugs. JControlRelease2001; 75: 155–66.

10 Lee WR, Shen SC, Wang KH etal.The effect of laser treatment on skin to enhance and control transdermal delivery of 5-fluorour-acil. JPharmSci2002; 91: 1613–26.

11 Fang JY, Fang CL, Sung KC, Chen HY. Effect of low frequency ultrasound on the invitropercutaneous absorption of clobetasol 17-propionate. IntJPharm1999; 191: 33–42.

12 Moser K, Kriwet K, Naik A etal.Passive skin penetration enhancement and its quantification invitro. EurJPharmBiopharm2001; 52: 103–12.

13 Bunke A, Zerbe O, Schmid H etal.Degradation mechanism and stability of 5-aminolevulinic acid. JPharmSci2000; 89: 1335–41.

14 Oishi H, Nomiyama H, Nomiyama K, Tomokuni K. Fluorometric HPLC determination of 5-aminolevulinic acid in the plasma and urine of lead workers: biological indicators of lead exposure. JAnalToxicol1996; 20: 106–10.

15 Lee S, Kollias N, McAuliffe DJ etal.Topical drug delivery in humans with a single photomechanical wave. PharmRes1999; 11: 1717–21.

16 Grevelink JM, Ross EV, Anderson RR. Lasers in dermatology. In: Fitzpatrick'sDermatologyinGeneralMedicine(Freedberg IM, Eisen AZ, Wolff K, Austen KF, Goldsmith LA, Katz SI, Fitzpatrick TB, eds). New York: McGraw-Hill Health Professions Division, 1999.

17 Polnikorn N, Goldberg DJ, Suwanchinda A, Ng SW. Erbium:YAG laser resurfacing in Asians. DermatolSurg1998; 24: 1303–7.

18 Puliafito CA, Steinert RE. Short-pulsed Nd-YAG laser microsurgery of the eye. IEEEJQuantElect1984; 20: 1442–8.

19 Nelson JS, McCullough JL, Glenn TC etal.Mid-infrared laser ablation of stratum corneum enhances invitropercutaneous transport of drugs. JInvestDermatol1991; 97: 874–9.

20 Lopez RFV, Bentley MVLB, Delgado-Charro MB, Guy RH. Iontophoretic delivery of 5-aminolevulinic acid (ALA): effect of pH. PharmRes2001; 18: 311–15.

21 Elfsson B, Wallin I, Eksborg S etal.Stability of 5-aminolevulinic acid in aqueous solution. EurJPharmSci1998; 7: 87–91.
22 Vanbever R, De Morre N, Pre´at V. Transdermal delivery of fentanyl by electroporation. II. Mechanisms involved in drug transport. PharmRes1996; 13: 1360–6.
23 Moan J, Berg K, Gadmar O etal.The temperature dependence of protoporphyrin IX production in cells and tissues. PhotochemPhotobiol1999; 70: 669–73.
24 Lieb S, Szeimies R, Lee G. Self-adhesive thin films for topical delivery of 5-aminolevulinic acid. EurJPharmBiopharm2002; 53: 99–106.