Effect of Ethanol on Iontophoretic Transdermal Delivery of 5-Aminolevulinic Acid through Yucatan Micropig Full-thickness Skin

KAZUSA ASAI, A. OCHIAI[1], N. KAWASHIMA[1] AND Y. TOKUOKA[1]*

Graduate School of Engineering, [1]Faculty of Biomedical Engineering, Toin University of Yokohama, 1614 Kurogane-cho, Aoba-ku, Yokohama 225-8503, Japan

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The effect of ethanol was investigated on iontophoretic transdermal delivery of 5-aminolevulinic acid through Yucatan micropig full-thickness skin. When 20 % ethanol was added into an aqueous 5-aminolevulinic acid solution, the iontophoretic permeation of 5-aminolevulinic acid through Yucatan micropig full-thickness skin was enhanced. The addition of ethanol also enlarged the partition of 5-aminolevulinic acid into Yucatan micropig full-thickness skin, which gave rise to enhancement of 5-aminolevulinic acid permeation. This result suggested that the combination system of iontophoretic delivery and 20 % ethanol could be a promising approach for the promotion of 5-aminolevulinic acid skin permeation.

Key words: Iontophoresis, 5-aminolevulinic acid, ethanol, Yucatan micropig full-thickness skin, permeability coefficient, diffusion coefficient, partition coefficient, lag time

5-Aminolevulinic acid-based photodynamic therapy (ALA-PDT) is one of the most effective treatments of skin diseases such as cutaneous carcinoma and intractable acne[1-4]. In ALA-PDT, ALA is administered topically, and an endogenous photosensitizer, protoporphyrin IX (PpIX), is accumulated in skin diseases. When PpIX is photoexcited by light with a specific wavelength ranged from 600 to 700 nm, it transfers the energy to molecular oxygen and forms cytotoxic singlet oxygen, leading to photochemical and photobiological processes that cause irreversible photodamage to the skin disease[5-7].

Skin has an outermost thin layer, *stratum corneum* and underlying viable epidermis and dermis. Since the *stratum corneum* is highly hydrophobic, dry, and a percutaneous barrier, hydrophilic compounds are inferior to hydrophobic compounds in permeating through the *stratum corneum*[8]. When ALA is topically applied to skin diseases in ALA-PDT, it is hard for ALA to permeate into the *stratum corneum* because of its hydrophilicity. In fact, in the clinical treatment of solar keratosis lesion and Bowen's disease by ALA-PDT, ALA is passively administered by percutaneous absorption for 4-6 h before light irradiation[9]. The long-time administration makes it difficult to establish ALA-PDT as a standard treatment modality and to apply to outpatients. Therefore, skin permeation of ALA needs to be improved.

Iontophoresis is a process to enhance skin permeation of ionized and charged compounds by application of a weak electrical current. So far, many researchers have studied iontophoresis for improving skin permeation of various types of drugs, insulin[10,11], methotrexate[12], metoprolol[13], indomethacin[14], luteinizing hormone releasing hormone[15], and so on. Besides, iontophoretic delivery can be applied to skin permeation of ALA[16-21]. As a result, iontophoresis can significantly enhance permeation of ALA across skin when compared to passive transdermal delivery. For example, the amount of ALA that passively diffuses across human *stratum corneum* in several hours can be delivered by the iontophoresis in 10 min or less[18]; iontophoresis could enhance human epidermal permeation of ALA by 15-fold[21].

Recently, other techniques such as ultrasound, electroporation, and chemical enhancers have been

*Address for correspondence
E-mail: tokuoka@toin.ac.jp

combined with iontophoresis[22]. The combination system seems to be more effective for transdermal delivery compared with the iontophoresis system alone. Moreover, ethanol is well known as a topical penetration enhancer and is often used in transdermal delivery systems[23]. Ethanol extracts lipids and proteins exerting a percutaneous barrier function on *stratum corneum*; thereby accelerating skin permeation of drugs.

In the present investigation, in order to enhance percutaneous ALA absorption within a shorter time, we determined *in vitro* iontophoretic transdermal delivery of ALA through Yucatan micropig (YMP) full-thickness skin with and without ethanol, evaluating the effect of the combination of iontophoresis and ethanol on ALA skin permeation.

YMP full-thickness skin employed for *in vitro* iontophoretic permeation experiments was purchased from Charles River Laboratories Japan, Inc. ALA (98 %) was obtained as ALA hydrochloride from Cosmo Bio Co., Ltd. Ethanol (99.5 %), acetylacetone (99.5 %), formaldehyde (36.0-38.0 %), sodium chloride (99.5 %), and agar (powder) were purchased from Kanto Chemical Co., Inc. A 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer solution was prepared with 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid and an aqueous sodium hydroxide solution (0.1 mol/l), which were purchased from Nacalai Tesque, Inc. All of the materials were used without further purification.

In vitro iontophoretic permeation experiment of ALA with and without ethanol through YMP full-thickness skin was carried out with the iontophoretic device described in fig. 1. YMP full-thickness skin was settled on the receiver compartment having an inner volume of 150 cm^3. The anode cell (donor compartment) was filled with 0.6 mol/l (100 mg/l) ALA solution prepared using distilled water with and without ethanol. The cathode cell and the receiver compartment were filled with the HEPES buffer solution (pH=7.4) containing 133 mmol/l sodium chloride. The platinum electrodes fixed in the anode and cathode cells by using agar gel containing 0.9 % sodium chloride were connected to a constant current source (HA-3001A, Hokuto Denko Corporation), and a current density of 0.132 mA/cm^2 was applied at 37° for 6 h. The effective diffusion area on the skin was 3.8 cm^2. A 200 µl of HEPES buffer solution from the receiver compartment was collected at specified time intervals. The concentration of ALA

permeated through the YMP skin was measured, and the cumulative amount of permeated ALA per unit skin area was calculated.

The concentration of ALA permeated through the YMP full-thickness skin into the buffered solution in the receiver compartment was measured by the fluorometric method described in the previous paper[24]. Fifty microliters of the buffered solution with ALA permeated was treated with a mixture of 3.5 ml of acetylacetone, ethanol, and water (15:10:75 in volume ratio) containing 4 g of sodium chloride per liter, followed by the addition of 450 µl of 85 ml/l aqueous formaldehyde solution. The treated solution was heated in boiling water for 30 min, cooled in cold water for 5 min. These reactions eventually gave us the fluorescent ALA derivative, 2-methyldeneamin-3,5-diacetyl-
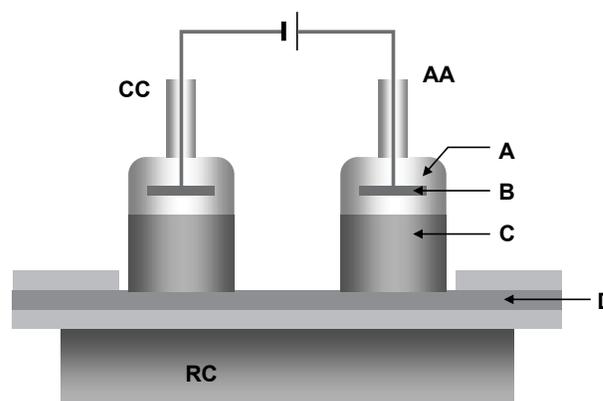

Fig. 1: Schematic representation of iontophoretic device used
RC: Receiver compartment; CC: cathode cell; AA: anode cell (donor compartment); A: agar gel, B: platinum electrode, C: electrolyte, D: YMP skin
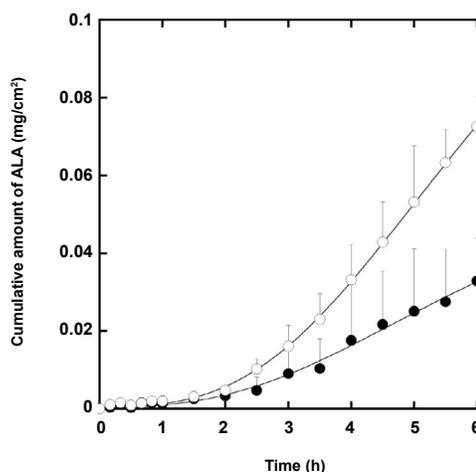

Fig. 2: ALA permeation through YMP full-thickness skin by iontophoresis with and without ethanol
Changes in cumulative amounts of ALA permeated through YMP full-thickness skin with permeation time by iontophoresis with and without ethanol. Values are mean±SD (n=3). The ALA solution with 20 % ethanol (—○—, EW=20:80) and without ethanol (—●—, EW=0:100) was employed as a sample

4,6-dimethylphenylpropionic acid. The fluorescence intensity of the ALA derivative was measured with a fluorescence spectrophotometer (FP-6500, Jasco Co.). The excitation and emission wavelengths were 363 and 473 nm, respectively.

Fig. 2 shows the change in cumulative amount of iontophoretically permeated ALA per unit YMP full-thickness skin area with the permeation time. The ALA solution with 20 % ethanol by weight (EW=20:80) and without ethanol (EW=0:100) was employed as a sample. The concentration of ALA was 0.6 mol/l in both the samples. In these iontophoretic deliveries, the cumulative amount of ALA increases with increasing permeation time. In comparison at the same permeation time, moreover, the cumulative amount of ALA in the iontophoretic delivery with 20 % ethanol is greater than that without ethanol. Since the dielectric constant of ethanol (24.6 at 20°) is lower than that of water (78.6 at 20°), the addition of ethanol probably inhibits ionization of ALA molecules. We then expected that ethanol suppressed ALA permeation in iontophoretic delivery. Contrary to our expectation, however, ethanol enhances the iontophoretic permeation of ALA through YMP skin.

Next, the permeation parameters were evaluated and estimated from the permeation profile shown in fig. 2. The flux, J, of ALA permeated through YMP skin was calculated from the slope of the steady-state portion of the permeation profile between the cumulative amount of permeated ALA per unit skin area and the permeation time[25]. The lag time, T_{lag}, which is the time at the beginning of permeation, was also estimated from the linear extrapolation of the steady-state portion to the X-axis of the permeation profile. Furthermore, calculated the permeability coefficient, K_p, diffusion coefficient, D, and partition coefficient, K, of ALA using the following Eqn. 1. $K_p = J/C_D = KD/L$ and Eqn. 2: $T_{lag} = L^2/6D$, where C_D is the initial concentration of ALA in the donor compartment and L is the diffusion path length, that is, the thickness of YMP skin. According to the previous paper, 0.2 cm to L was assigned in this study[26].

The permeation parameters calculated are summarized in Table 1. As a result, the addition of 20 wt. % ethanol enlarges the K_p of ALA in the iontophoretic delivery by a factor of approximately 2.5 (p<0.01 by Student's t-test). The T_{lag} of ALA in the iontophoretic delivery with 20 wt. % ethanol was almost equal to that without ethanol. Furthermore, the D was almost constant in the iontophoretic delivery with and without ethanol, but the K was much larger in the iontophoretic delivery with ethanol than without ethanol (p<0.01).

Compared to the K_p in the passively transdermal delivery with 40 % ethanol, 2.40×10^{-5} cm/h, which was estimated in previous reports[27], the K_p of ALA in the iontophoresis, shown in Table 1, becomes larger, regardless of ethanol. An aqueous ALA solution is acidic; for instance, pH of 0.6 mol/l ALA solution is 1.9. Since pKa of ALA in water at 25° is 4.1 and 8.9[28], moreover, ALA molecules are likely to be converted into the positively charged ions. Hence, the charged ALA ion is repelled by the similarly charged anodal electrode and is absorbed readily through the skin by the iontophoresis, which enhances the ALA skin permeation.

Along with fig. 2, the K_p of ALA in Table 1 indicates that the ALA permeation was accelerated by ethanol. From Eqns. 1 and 2, the transdermal delivery of a drug was governed by D and K of the drug. As shown in Table 1, the K of ALA in the iontophoretic delivery was significantly enlarged by ethanol, but the D was almost constant. It is considered, therefore, that ethanol enhances the partition of ALA into YMP full-thickness skin, leading to acceleration of permeation of ALA in iontophoretic delivery.

The K_p of ALA in the passively transdermal delivery with 20 % ethanol was not estimated in this study, but is probably the same as or less than that with 40 % ethanol, 2.40×10^{-5} cm/h, mentioned above. Also, the K_p of ALA in the iontophoretic delivery without ethanol was 8.08×10^{-5} cm/h. The summation of K_p of ALA in each delivery, 10.48×10^{-5} cm/h, was then less than the K_p in the iontophoretic delivery with 20 % ethanol, 19.28×10^{-5} cm/h. That is to say, the combination system of iontophoretic delivery and ethanol has a synergistic effect on the ALA skin permeation.

Anodal iontophoresis brings about not only flux of cationic ions, but also flux of a solvent owning to

TABLE 1: PERMEABILITY COEFFICIENT, LAG TIME, DIFFUSION COEFFICIENT, AND PARTITION COEFFICIENT OF ALA IN IONTOPHORESIS WITH AND WITHOUT ETHANOL

EW	K_p ($\times 10^{-5}$ cm/h)	T_{lag} (h)	D ($\times 10^{-3}$ cm²/h)	K ($\times 10^{-3}$)
0:100	8.08±2.16	2.08±0.88	3.75±1.98	4.73±1.50
20:80	19.28±1.16 *	2.26±0.32	2.99±0.45	13.00±1.16*

The ALA solution with 20 wt. % ethanol (EW=20:80) and without ethanol (EW=0:100) was employed as a sample. *P<0.01 by Student's t-test. Permeability coefficient (K_p), lag time (T_{lag}), diffusion coefficient (D), and partition coefficient (K)

electroosmosis[29]. Electroosmosis can also deliver neutral compounds such as urea, mannitol, and sucrose[30]. In this study, the electroosmotic flux of ethanol may occur in the mixed solvent and readily penetrates it into the YMP full-thickness skin, which gives rise to the synergistic effect of the combination system of iontophoresis and ethanol on the ALA skin permeation.

It is concluded that the addition of 20 % ethanol into an aqueous ALA solution enhances the iontophoretic delivery of ALA through YMP full-thickness skin. However, it is suspected that the addition of excess amount of ethanol reduces the iontophoretic effect on the ALA permeation, because of its low dielectric constant. Further studies are needed to confirm the optimum concentration of ethanol for iontophoretic delivery of ALA.

Conflicts of interest:

The authors declare that there is no conflict of interests in this paper.

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