Photodynamic Therapy in the Dermatological Field and Enhanced Cutaneous Absorption of Photosensitizer

Makio Akimoto1, Kazuhisa Maeda2, Tokuya Omi3, Tomonori Nishimura4, and Michio Miyakawa5

1Kanto Gakuin University, 1-50-1, Mutsuurahigashi, Kanazawa-ku, Yokohama 236-8501, Japan
2Tokyo University of Technology, 1404-1, Katakura, Hachioji, Tokyo 192-0982, Japan
3Queen’s Square Medical Center, 2-3-5, Minatomirai, Yokohama 220-6208, Japan
4Atom Giken Co., Ltd., 92-2, Katase, Fujisawa, Kanagawa 251-0032, Japan
5Niigata University, 8050 Ikarashi-2, Niigata 950-2181, Japan

Abstract — The combination of light and chemicals to treat skin diseases is widely practiced in the field of dermatology, and has led to the concept of photodynamic therapy in recent years. In PDT for skin cancer, 5-aminolevulinic acid is applied topically to the affected area to be absorbed percutaneously through passive diffusion, and typically requires 4–6 h before performing PDT. In this study, we attempted to reduce the absorption period in PDT by ionizing ALA using direct current pulsed iontophoresis and Bowen’s disease. In all subjects, protoporphyrin IX production was confirmed after iontophoresis, and its production levels were comparable to the conventional occlusive dressing technique. Skin biopsies from the treated lesion showed the disappearance of tumour cells.

1. INTRODUCTION
In recent years, the application of optical technology to clinical medicine has been thriving. Many apparatus applying optical technology have been developed and are widely used in clinical settings; e.g., lasers have produced revolutionary results in surgery, most cases of chemical tests on specimens have been conducted using optical techniques, and automated assays have been developed, resulting in tremendous advances in patient diagnostics and medical examinations. Furthermore, a non-invasive treatment method has been recently developed, in which certain drugs given to the body are activated by light to exert their effects, and the therapeutic effects of this modality have drawn close attention. The modality is referred to as photodynamic therapy, and is based on the combined use of photosensitizers and photoradiation. That method administers a photosensitizer with affinity for the tumor cells, followed by light irradiation which results in the selective disruption of only the tumor cells in the body. Since photodynamic therapy (PDT) is less invasive than surgical therapy, it can be used in patients with serious complications and also in elderly patients, and it is being increasingly applied in many clinical fields, especially dermatology [1]. In the field of dermatology, it is widely recognized that PDT, in which an excitation light is applied to externally applied 5-aminolevulinic acid (5-ALA), a porphyrin precursor, is effective for the treatment of superficial malignant tumors of the skin. However, since the percutaneous absorption of photosensitizers is extremely low, treatment using these substances requires a long time, making it difficult to establish this method as a standard treatment modality and to apply it on an outpatient basis. In this study, PDT was optimized by introducing the photosensitizer into the skin within a short time by iontophoresis, which involves the application of a microelectric current to the skin to increase the percutaneous absorption of ionic drugs. The results of this study confirm that iontophoresis indeed enhances the percutaneous absorption of the photosensitizer within a short time, which dramatically reduces the time required for treatment. The findings of this study are reported herein. Since 5-ALA has an extremely low percutaneous absorbency, there are several methods to improve its absorption such as liposomal 5-ALA, and iontophoresis may dramatically enhance its absorption [2–4].

2. MECHANISMS OF PHOTODYNAMIC THERAPY
Photodynamic therapy is a new treatment for a wide variety of malignancies and pre-malignant dysplasias, as well as some non-cancer indications. Therapeutic response to PDT is achieved through the activation of a non-toxic photosensitizer located within the neoplastic tissue, using visible light
Table 1: Photosensitizer absorption spectra.

<table>
<thead>
<tr>
<th>Photosensitizer</th>
<th>Wavelength (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematoporphyrin derivative (HpD)</td>
<td>630</td>
</tr>
<tr>
<td>5-aminolevulinic acid (5-ALA)</td>
<td>410, 505, 540, 580, 635</td>
</tr>
<tr>
<td>Tin etiopurpurin (SnET&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>447, 660</td>
</tr>
<tr>
<td>N-asparyl-chlorin e6 (NPe6)</td>
<td>664</td>
</tr>
<tr>
<td>Chloraaluminium phthalocyanine (AℓPcS)</td>
<td>675</td>
</tr>
<tr>
<td>Benzoporphyrin derivative-monoacid ring A (BPD-MA)</td>
<td>456, 690</td>
</tr>
<tr>
<td>Lutetium texapyrin (Lu-Tex)</td>
<td>732</td>
</tr>
</tbody>
</table>

Figure 1: Mechanism of Photodynamic therapy.

The photosensitizer excited by light induces two types of reactions: A type-I reaction, which is the electron transport process and a type-II reaction which is the energy transport process. The free radicals produced by the type-I reaction react with dissolved oxygen in the tissues to produce various oxidized substances, which elicits a free-radical chain reaction. In the type-II reaction, the photosensitizer in the target cells is changed to the excited singlet state by the light energy. At that time, part of the substance returns to the ground state while emitting fluorescence, while the majority of the substance is changed to the triplet state. The triplet state returns partly to the ground state while emitting phosphorescence, however, the remaining converts energy to dissolved oxygen in the target tissues. The oxygen supplied with energy is excited and changes to singlet oxygen, which causes degenerative necrosis of the target cells. The therapeutic effect of PDT is mainly the result of the type-II reaction, but the effect varies with the type of photosensitizer used.

One of the most problematic aspects of PDT using HpD is that these agents exhibit a relatively slow rate of clearance from the skin. Consequently, patients receiving either drug systemically are rendered photosensitive and must remain out of sunlight or strong artificial light for 6 to 10 weeks after treatment to avoid severe sunburn. This problem seriously reduces the acceptability of this form of therapy, especially when other treatments are available and when the aim of the therapy is palliation rather than cure. This is achieved by the administration of 5-aminolevulinic acid (5-ALA), the first precursor of haem after the feedback control point. Because the resulting accumulation of PpIX is relatively short-lived, any skin photosensitivity which may occur is resolved by 24 hours point treatment. An attractive and proven PDT procedure for treating superficial is
the topical application of the haem precursor in the form of a cream emulsion 4–6 hours prior to light treatment. ALA applied topically passes rapidly through abnormal epidermis, or normal skin tripped of 15µm thick stratum corneum layer and, bypassing the feedback inhibition, is converted within the mitochondria to Protoporphyrin IX (PpIX). The fluorescent and photodynamically active substance Protoporphyrin IX is synthesized via the haem cycle in cells exposed to ALA. Once the sensitizer has been taken up by the cells in the target tissue, they are activated by a precise wavelength of non-thermal light. The sensitizer molecules become excited and in turn excite tissue oxygen to a highly toxic, but short lived singlet oxygen species. The singlet oxygen attacks the cellular membrane resulting in phototoxic damage and cell death. Toxicity occurs either as a result of direct cell kill or by attacking the endothelial layer of blood vessels thus causing vascular shutdown which starves all tissue downstream of nutrients. The body then naturally removes the dead tissue leaving the wound to heal uniquely via tissue regeneration as opposed to the normal fibrotic scarring.

3. IONTOPHORESIS MECHANISM AND DEVICES

Trans-dermal administration of drug is assuming an important place in modern drug therapy. It is used for non-ionized drugs required in a small dosage. Transdermal administration can be passive or facilitated. In passive administration, the non-ionized drug traverses the skin through the stratum corneum. The skin, being a semi-permeable membrane, allows only a small amount of any drug molecule to passively penetrate the skin. Ionized drugs do not easily penetrate this barrier and are not suitable for routine trans-dermal delivery unless an external source of energy is provided to drive the drug across the skin. Facilitated diffusion can utilize either phonophoresis or electrical (iontophoresis) energy. Iontophoresis increases the penetration of electrically charged drugs into surface tissues by the application of an electric current. Electrical energy assists the movement of ions across the stratum corneum according to the basic electrical principle of “like charges repel each other and opposite charges attract”. The drug is applied under an electrode of the same charge as the drug, and a return electrode opposite in charge to the drug is placed at a neutral site on the body surface. The operator then selects a current below the level of the patient’s pain threshold and allows it to flow for an appropriate length of time. The electrical current significantly increases the penetration of the drug into surface tissues by repulsion of like charges and attraction of opposite charges. The two classically considered prerequisites for iontophoretic treatment are that the drug must be charged (or modified to carry a charge) and that the disease process must be at or near a body surface. A typical iontophoresis device consists of direct current pulsed type delivery system and electrodes. Wires are then connected between the unit and the active and passive electrodes, and the unit set for current and time. In the iontophoresis process, the current, beginning at the device, is transferred from the electrode through the ionized drug solution as ionic flow. The drug ions are moved to the skin where the repulsion continues moving the drug through the trans-appendageal structures and stratum corneum interstices via the aqueous pores. The larger the electrode surface, the greater the current the device must supply to provide a current density for moving the drug. Iontophoresis enhances transdermal drug delivery by three mechanisms: (a) Ion-electric field interaction provides an additional force that drives ions through the skin, (b) the flow of electric current increases the permeability of the skin, and (c) electro-osmosis produces bulk motion of solvent that carries ions or neutral species with the solvent stream. Electroosmotic flow occurs in a variety of membranes and is in the same direction as the flow of counter-ions. It may assist or hinder drug transport. Since human skin is negatively charged above pH 4, counter ions are positive ions and electro-osmotic flow occurs from anode to cathode. Thus, anodic delivery is assisted by electro-osmosis but cathodic delivery is retarded. Because of the electro-osmotic flow, transdermal delivery of a large anion (negatively charged protein) from the anode compartment is more effective than that from the cathode compartment. The drug reservoir consists of a gauze/cloth or gel pad to which the solution is applied or the solution is injected through a port into the reservoir electrode combination. Wires are connected between the microprocessor unit and the active and passive electrodes.

4. SUBJECTS AND EXPERIMENTAL METHODS

Five patients who were diagnosed with actinic keratosis (two men and three women in ages ranging from 49 to 94 years, with an average of 79.6 years) and Bowen’s disease at the outpatient clinic of the Department of Dermatology, Aichi Medical University, attended this study. Informed consent was obtained from all patients following a full written and oral explanation.
Iontophoresis used in the present study (TransDermaionto System, Atom Giken Co., Ltd., Kanagawa, Japan) was a direct-current pulsed type [5]. For iontophoresis, 20% ALA was dissolved in distilled water, and then current was adjusted based on the area of the functional electrode between 0.25 and 0.50 mA/cm$^2$. In all subjects, the pulse wave was set at 50 kHz, and ALA was applied to lesion for 10 min. The principal of iontophoresis is shown in Fig. 2. After application, the affected area was washed using distilled water and was shielded from light. One hour after ALA application, a fluorescent spectrometer was used to measure protoporphyrin IX (PpIX) photosensitizer production. PDT was performed by excimer dye laser and emits 630 nm with pulsed light irradiation at 50 J/cm$^2$ per session. PDT was repeated three times, weekly (total dose: 150 J/cm$^2$). One week after the last PDT, a skin biopsy was performed in order to assess the therapeutic effects.

5. RESULTS AND DISCUSSION

In topical 5-ALA-PDT, sufficient PpIX accumulation in tumour cells by 5-ALA diffusion is necessary. With regard to the length of the topical ALA application tome, the British guidelines [6] for topical PDT recommend 4–6 hours, but it has been clarified that because of superior permeability, sufficient effects can be obtained in about 3 hour with methyl 5-ALA. To reduce these procedures, application of 5-ALA using iontophoresis has been studied. Figure 3 shows an example of the therapeutic effect. In this study, only 10 minutes of application of 5-ALA and a 1 hour follow-up showed the PpIX positive reaction within the target.

With iontophoresis, it took one hour for the entire treatment, ranging from the delivery of 5-ALA to the end of the treatment. Thus, the time needed for treatment can be drastically shortened with iontophoresis. Table 2 summarizes the clinical features of patients. When compared with previous studies, our direct-current pulsed iontophoresis had a higher charge because the concentration of ALA was higher at 20% solution. With the equipment used in the present study, the pulse mode makes it possible to avoid the functional electrode depolarization associated with direct current iontophoresis, and ALA can be efficiently applied. In our study, there were no marked differences in the average PpIX production between 1 h after iontophoresis and > 4 h after occlusive dressing technique. We think that ALA was apparently able to penetrate tumour cells faster with direct-current pulsed iontophoresis when compared with percutaneous absorption based on passive diffusion. Rapid ALA diffusion into cells also quickly depleted the rate-limiting enzyme, thus
Table 2: Summary of the comparison of iontophoretic PDT and external PDT.

<table>
<thead>
<tr>
<th></th>
<th>Iontophoretic PDT</th>
<th>External PDT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time from ALA to cure</td>
<td>1 hour</td>
<td>4 ~ 6 hours</td>
</tr>
<tr>
<td>Quantity PpIX AK</td>
<td>2.88 (n = 5)</td>
<td>5.54 (n = 39)</td>
</tr>
<tr>
<td>BD</td>
<td>15.34 (n = 2)</td>
<td>6.68 (n = 28)</td>
</tr>
</tbody>
</table>

accumulating PpIX inside tumour cells. The pain varies with the sites and the sizes of lesions and also the light source, but it is thought that the pain can mainly be attributed to the tissue damage caused by the thermal radiation accompanying the photoradiation and by the stimulation of nerve fibers by 5-ALA. Local anesthesia may need to be considered in some patients. From such a viewpoint, the delivery of 5-ALA within a short time by iontophoresis is useful, also in terms of shortening the treatment time.

6. CONCLUSION
Along with the increase in the number of elderly patients, the number of patients with superficial malignant tumors of the skin also seems to be on the increase. Under this circumstance, the need for the non-invasive modality of PDT is also increased. Since 5-ALA, a photosensitizer with high tumor affinity, is hardly absorbed percutaneously under normal circumstances, the effect of iontophoresis in enhancing the absorption within a short period of time is useful from the viewpoint of improving the quality of the PDT. In conclusion, our date suggested that when compared with conventional occlusive dressing technique, PDT could be performed much more rapidly with iontophoresis, resulting in lower a patient burden.

ACKNOWLEDGMENT
This research was supported by the Department of Dermatology, Aichi Medical University.

REFERENCES