REVIEW ARTICLE

Enhancement techniques for improving 5-aminolevulinic acid delivery through the skin

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ABSTRACT

Photodynamic therapy (PDT) is a popular technique for skin cancer treatment. Protoporphyrin IX, which is a photosensitizing agent, converted enzymatically from the prodrug 5-aminolevulinic acid (ALA), is used as a photosensitizer in PDT for cancer. However, ALA penetrates with difficulty through intact skin; therefore, improving delivery systems for ALA in the skin will play an important role in ALA-PDT. Enhancement of ALA skin penetration can be achieved by physical methods, such as iontophoresis, laser, microneedles, ultrasound, and by adding chemical penetration enhancers, such as, dimethyl sulfoxide, oleic acid, and others, whereas some researches used lipophilic ALA derivatives and different vehicles to improve the transdermal delivery of ALA. This review introduces several enhancement techniques for increasing ALA permeation through the skin.

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INTRODUCTION

Skin cancer is the most common of all pathologies related to cancerous diseases.¹ Nearly two million new cases are diagnosed yearly, and that continues to increase worldwide. Photodynamic therapy (PDT) is a new modality for skin cancer treatment. In PDT, three components are combined to induce tumor destruction: a photosensitizer, light, and oxygen.² It is based on the accumulation of a porphyrin-related photosensitizer in tumor cells and its subsequent destruction when exposed to visible light. Singlet oxygen species are produced, which damage membranes and organelles, causing cell death and tumor ablation.³

The photosensitizer precursor: 5-aminolevulinic acid

The original photosensitizing mixture was termed the hematoporphyrin derivative (HPD). In 1975, Dougherty et al⁴ reported that HPD in combination with red light could completely eradicate mouse mammary tumor growth. Clinical trials were subsequently initiated with HPD to treat patients with bladder cancer and skin tumors.⁵ There are now increasing numbers of photosensitizers being used clinically, such as 5-aminolevulinic acid (ALA). ALA was approved by the U.S. Food and Drug Administration and is currently used in PDT. ALA, which is not a photosensitizer, is the precursor of protoporphyrin IX (PpIX), the derivatives of which are used as photosensitizers in PDT for cancer.⁶

ALA is synthesized in mitochondria under a negative feedback control mechanism by heme. In principle, the reaction initiated from the condensation of glycine and succinyl CoA, which is catalyzed by ALA-synthase, produces the first intermediate, which is further synthesized through a series of biochemical reactions. After those reactions, PpIX is converted into heme by ferrochelatase in the presence of iron (Figure 1).³

TRANSDERMAL DRUG DELIVERY SYSTEM

The skin is the largest organ of the human body. It is composed of three main histological layers: the epidermis, dermis, and subcutaneous tissues. The epidermis is further divided into two parts: the stratum corneum (SC) and viable epidermis, which includes other layers of the epidermis, such as the stratum granulosum, stratum spinosum, and stratum basale (Figure 2).⁷ The SC is the final product of epidermal cell differentiation, consists of 10–15 layers of corneocytes, and varies in thickness from approximately 10–15 μm.

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in the dry state to 40 μm when hydrated. It comprises a multilayered “brick and mortar” structure, consisting of various lipids (5–15%), including phospholipids, glycosphingolipids, cholesterol sulfate, and neutral lipids. The main protein (75–85%) in the SC is keratin. Intercellular lipids in the SC have a special role in regulating the SC barrier function.

Transdermal drug delivery systems (TDDSs) have been in clinical use for more than 30 years. TDDSs offer several advantages compared with the oral route and injections, such as the following:

- Reduction of first-pass metabolism in the liver;
- Improved patient compliance;
- Delivery topically and directly to the morbid area; and
- Reduced side effects of drugs.

However, TDDSs also have limitations, such as the skin state; properties of the drugs (e.g. molecular mass, dissociation coefficient \( pK_a \), and partition coefficient); types of vehicles; and the concentration of the drug. Because the SC has a barrier function, not all drugs are suitable for TDDSs; thus, several studies used permeability-enhancing techniques to increase the penetration of drugs.

**Use of ALA for topical PDT**

Application of ALA for PDT was first introduced by Kennedy et al. It was reported that ALA-PDT is useful for treating superficial skin cancers, actinic keratoses, psoriasis, cutaneous T-cells, basal cell carcinoma, and squamous cell carcinoma. An ideal photosensitizer shows a high tumor-to-normal-tissue ratio, exhibits
rapid accumulation in tumor tissues, and can be efficiently cleared from the body. In addition, ALA-PDT can be used as a long-term treatment without causing the accumulation of PpIX in normal skin. ALA is a hydrophilic molecule with a molecular weight of 167.6. According to the skin permeability theory, ALA has difficulty penetrating intact skin. The SC is the main barrier for percutaneous penetration of this exogenous substance. The permeability of hydrophilic ALA through intact skin is always low, making it difficult to achieve the desired therapeutic benefits. Numerous strategies were proposed to improve these penetration problems, such as modifying ALA’s structural properties, and changing the skin’s state by chemical and physical enhancement methods.

Chemical modification of the lead compound (ALA derivatives)

Despite the SC barrier, ALA might be able to penetrate through the skin and into tumors after topical application because of its small molecular weight. However, ALA is hydrophilic and zwitterionic, characteristics that make it difficult for ALA to penetrate the SC barrier. Therefore, lipophilic ALA ester derivatives have better potential for clinical use compared with ALA. The most frequently investigated ALA derivative is methyl (M)ALA. According to the determination of the partition coefficients of different alkyl esters, these compounds showed an increased affinity for the SC; hexyl-ester ALA and octyl-ester ALA showed the highest partition coefficients (Table 1) and increased affinities for the SC. The in vitro skin permeation test revealed that hexyl-ester ALA had a higher permeated amount than ALA (by about threefold) and other ester derivatives. Moreover, hexyl-ester ALA and octyl-ester ALA, but not ALA, were retained in the viable epidermis and dermis. However, Juzeniene et al. applied creams containing 0.2%, 2%, and 20% of ALA, MALA, and hexyl-ester ALA, respectively, to normal human skin of six volunteers to evaluate the topical application of these prodrugs. The amount and distribution of porphyrins formed in the skin were investigated noninvasively by means of fluorescence spectroscopy. The results showed that MALA induced comparably less PpIX in normal human skin than ALA and hexyl-ester ALA at low dosages (0.2% and 2%), whereas higher PpIX fluorescence was found in the ALA treatment group than that in the MALA and hexyl-ester ALA groups at a high dosage (20%).

Pretreatment with physical penetration enhancers

Recently, several innovative strategies were used to improve ALA penetration into skin, including iontophoresis, lasers, microneedles (MN), and ultrasound. Iontophoresis is a strategy that induces ionizable drugs through intact skin by the administration of a continuous, direct electrical current. It enhances drug transport across the SC without significant perturbation of the skin barrier. ALA is a zwitterion at physiological pH, indicating that the iontophoretic mechanism of electrotransport is primarily electro-osmosis, with little or no contribution from electromigration. In addition, one study found that ALA transport is more efficient from the anode than the cathode. Lopez et al. reported the effect of pH on iontophoretic delivery of ALA; they used anodal iontophoresis to evaluate the ALA transport efficiency at different pH values. The results showed that there was a significant increase, about 100 mM, at pH 7.4. Some researchers also estimated the optimized conditions for ALA electrotransport into and through the skin by adjusting the formulation composition and ionic strength. One group compared the anodal iontophoretic flux of ALA from a 10% solution with the drug’s passive flux from the same formulation to which 5% dimethyl sulfoxide (DMSO) had been added, and transport of ALA across the skin and the amount of produg delivered into the skin (SC and [epidermis–dermis]) were fourfold greater with iontophoresis compared with passive application of the DMSO formulation. Iontophoresis can also be used to control the delivery of ALA esters into the skin. Lopez et al. studied enhanced delivery of ALA esters by iontophoresis in vitro. They used anodal iontophoretic transport of ALA esters through porcine skin in an in vitro permeation test under a current of 0.5 mA/cm² for 2 hours. The results indicated that positively charged ALA esters with moderate lipophilicity showed an increasing iontophoretic flux through the skin. Greater than 50-fold enhancement compared with the zwitterionic parent ALA was observed for MALA. Iontophoresis of MALA and hexyl-ester ALA also increased the amount of produg delivered into the skin. The efficiency of ALA ester permeation by iontophoresis, compared with ALA itself, and the amount of produg that was transported into and across the skin were greatly enhanced.

Lasers are a very popular technology that is used for medical diagnoses and therapeutic purposes. They can effectively enhance drug delivery through the skin by ablating the SC with minimal residual thermal damage. Shen et al. evaluated the in vitro percutaneous absorption and in vivo PpIX accumulation in skin and tumors after topical ALA application combined with erbium yttrium–aluminum–garnet (Er:YAG) laser enhancement. ALA penetration after surface treatment with an Er:YAG laser also produced higher accumulations of PpIX within subcutaneous tumors of the superficial skin compared with those of the untreated group. The enhancement ratios of laser-treated skin ranged from 1.7-fold to 4.9-fold compared with those of the control group depending to the fluence used. Although it effectively enhanced ALA delivery, the skin needed 3–5 days to recover to a normal status after Er:YAG laser treatment. Lee et al. used a fractional Er:YAG laser to enhance skin permeation of ALA. The flux of ALA through laser-treated nude mouse skin and porcine skin showed higher levels than that through intact skin by 27–124-fold and 3–260-fold, respectively. The in vivo skin penetration depth also increased after laser treatment according to confocal scanning laser microscopic (CSLM) observations.

The first study using MNs as an enhancing technique to increase transdermal drug delivery was published in 1998. The overarching motivation for MNs is that they provide a minimally invasive means to drive molecules into the skin. Inserting drug molecules into the skin through MNs causes no pain or bleeding. Furthermore, MNs allow the delivery of drugs from dry dissolvable MNs rather than liquids. Donnelly et al. used silicon MN arrays to enhance the skin penetration of ALA in vitro and in vivo. Puncturing excised murine skin with 6 × 7 arrays of MNs 270 m in height, with a diameter of 240 µm at the base and an inter spacing of 750 µm, led to a significant increase in the transdermal delivery of ALA released from a biodegradable patch containing 19 mg/cm² ALA. MN puncture enhanced ALA delivery to the upper regions of excised porcine skin, but at a mean depth of 1.875 mm. In vivo experiments on nude mice showed that silicon MN puncture could reduce the application time and ALA dose required to induce high levels of the

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**Table 1** ALA and alkyl esters: general structure – HCl-NH-CH₃–CO-CH₂-CH₂CO-DMSO

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>Molecular mass (g/mol)</th>
<th>Log Kc ciné</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALA</td>
<td>H</td>
<td>167.6</td>
<td>1.3726</td>
<td>2.1233</td>
</tr>
<tr>
<td>Methylen-ester ALA</td>
<td>CH₃</td>
<td>181.6</td>
<td>0.2066</td>
<td>1.4345</td>
</tr>
<tr>
<td>Butyl-ester ALA</td>
<td>CH₃</td>
<td>223.8</td>
<td>0.2969</td>
<td>1.4647</td>
</tr>
<tr>
<td>Hexyl-ester ALA</td>
<td>CH₃</td>
<td>251.8</td>
<td>0.9122</td>
<td>1.4833</td>
</tr>
<tr>
<td>Octyl-ester ALA</td>
<td>CH₃</td>
<td>279.6</td>
<td>1.0221</td>
<td>1.5324</td>
</tr>
</tbody>
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- Log Kc ciné: decadic logarithm of partition coefficients of ALA and its n-alkyl esters between the stratum corneum and water (Kc ciné). ALA – 5-aminolevulinic acid.
photosensitizer PpIX in the skin. However, in spite of the success of ALA esters in increasing ALA cell permeation, the use of some of these compounds to treat skin cancer is still a matter of discussion, because of the fact that they appear to diffuse slowly across the skin. Among all ALA derivatives, MALA is used the most frequently. One study used the skin of 14 healthy volunteers to determine the erythema caused by ALA and MALA. The results showed that ultrasound could increase PpIX production both in tumors and skin of BALB/c nude mice bearing WiDr human colon adenocarcinomas. They also demonstrated that pulsed irradiated ultrasound (1 MHz) with an average intensity of 3 W/cm² for 10 minutes to the tumor area after administration of ALA (20% in an oil/water [O/W] emulsion applied topically to the surface of the tumor for 0.5–3 hours) increased the amount of PpIX in the tumors by 2.5-fold.

Ultrasound is cyclic sound pressure with a frequency greater than the upper limit of human hearing. In 1982, there was research that showed that ultrasound could increase blood circulation in tumors to improve the uptake of drugs. Ma et al demonstrated that ultrasound could increase PpIX production both in tumors and skin of BALB/c nude mice bearing WiDr human colon adenocarcinomas. They also demonstrated that pulsed irradiated ultrasound (1 MHz) with an average intensity of 3 W/cm² for 10 minutes to the tumor area after administration of ALA (20% in an oil/water [O/W] emulsion applied topically to the surface of the tumor for 0.5–3 hours) increased the amount of PpIX in the tumors by

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<th>Efficacy</th>
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<td>Different vehicles</td>
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<td>Lotion and cream</td>
<td>Casas et al</td>
<td>Male BALB/c mice 12 wk old</td>
<td>Maximal accumulation was found in the tumor 3 hr after ALA application with both cream and lotion preparations</td>
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<td>Cubic gel, HEC gel, and patch</td>
<td>Valenta et al</td>
<td>Porcine abdominal skin</td>
<td>ALA permeation through porcine skin after 48 hr was the highest from the patch (80.35%) followed by the cubic gel (66.4%)</td>
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<td>Liposomes</td>
<td>Pierre et al</td>
<td>Skin of male HRS/J hairless mice 4 wk old</td>
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<td>W/O, Bc, and O/W microemulsions</td>
<td>Araujo et al</td>
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<td>The developed microemulsion carried ALA to the deeper skin layers, increasing the PpIX of the skin</td>
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<td>Male BALB/c mice 12 wk old</td>
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<td>Different-pH ALA solution + liposomes with phloretin and 6-ketocholesterol</td>
<td>Auner et al</td>
<td>Porcine abdominal skin</td>
<td>In vitro permeation, pre-impregnation of porcine skin with liposome + phloretin or 6-ketocholesterol increased the ALA diffusion by about 1.7-fold at pH 7.0</td>
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<td>Physical enhancer</td>
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<td>Iontophoresis</td>
<td>Lopez et al</td>
<td>Pig ears (~700 mm)</td>
<td>In vitro: at pH 7.4, it was achieved by increasing the drug concentration in the anodal formulation to 100 nM</td>
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<td>Er:YAG laser irradiation of the skin increased the in vitro and in vivo efficacies of topical ALA delivery</td>
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<td>Fractional Er:YAG laser</td>
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<td>In vitro: 8-wk-old female nude mice skin and ear skin of 1-wk-old pigs</td>
<td>ALA permeation was effectively enhanced by ablating the SC with a low-fluence fractional laser</td>
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<td>MNs</td>
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<td>MN puncture reduced the application time and ALA dose</td>
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<tr>
<td>Ultrasound</td>
<td>Ma et al</td>
<td>Female BALB/c athymic nude mice 7–8 wk old bearing WiDr human colon adenocarcinomas</td>
<td>Ultrasound increased ALA delivery into the tumor and skin</td>
</tr>
</tbody>
</table>

**Table 2 Different types of enhancement methods, the model used, and efficacy in previous studies.**

DMSO – dimethyl sulfoxide; EDTA – ethylenediaminetetraacetic acid; PpIX – protoporphyrin IX; ALA – 5-aminolevulinic acid; HEC – hydroxyethyl cellulose; W/O – water in oil; Bc – bicontinuous; O/W – oil in water; SD – standard deviation; CP – cetylpyridinium chloride; MNs – microneedles; MALA – methyl 5-aminolevulinic acid.
approximately 45% within 1–2 hours. Furthermore, the amount of PpIX in the tumors after ultrasound treatment for 1 hour was similar to that of ALA applied alone for 3 hours.

**Pretreatment with chemical penetration enhancers**

Penetration enhancers may act by one or more of three main mechanisms: (1) disruption of the highly ordered structure of SC lipids; (2) interaction with intercellular proteins; and (3) improved partitioning of the drug, coenhancer, or solvent into the SC.

DMSO was one of the earliest and most widely studied penetration enhancers. DMSO may also extract lipids, making the horny layer more permeable by forming aqueous channels. Some studies showed it to be effective in promoting both hydrophilic and lipophilic permeants. Malik et al reported topical ALA application using three different creams (20% ALA alone; ALA with 2% DMSO; and ALA, DMSO, and 2% edetic acid disodium salt [ethylendiaminetetraacetic acid [EDTA]]) applied to mice bearing subcutaneously transplanted C26 colon carcinomas. PpIX production in the tumor and skin overlying the tumor was studied by laser-induced fluorescence and chemical extraction. The PpIX concentration after treatment was higher in the skin than that in the tumor. The efficiency of porphyrin production in the skin by the creams used was in the following order: ALA→DMSO→EDTA→ALA→DMSO→ALA. De Rosa et al used 20% DMSO in O/W emulsions to increase the in vitro permeation of ALA through hairless mouse skin. Other in vivo studies demonstrated a significant increase of about 2.5-fold in the amount of PpIX extracted from healthy hairless mouse skin after 3 hours of treatment with an O/W emulsion containing 10% ALA (w/w), 3% EDTA (w/w), and 20% DMSO (w/w). By CSLM imaging, it was concluded that the association of 10% ALA with 20% DMSO in O/W emulsions induced a significant increase in PpIX production and accumulation in hairless mouse skin. The effect of EDTA may be associated with the inhibition of ferrochelatase, an enzyme that converts PpIX into heme in the presence of iron. In that report, the presence of EDTA caused only a slight nonsignificant increase in the PpIX concentration in the skin. However, a subsequent report indicated that the combination of ALA, EDTA, and 20% DMSO may enhance the delivery of ALA to the skin in topical PDT.

Some reports using electron microscopy showed that a discreet lipid domain is induced within SC lipid bilayers on exposure to oleic acid, which enhanced the permeation of drugs across the skin. Therefore, Pierre et al reported oleic acid as an optimizer of the skin delivery of ALA in PDT. They measured the in vitro skin penetration and retention of ALA (1%) in the presence and absence of oleic acid (2.5%, 5.0%, and 10.0%) in propylene glycol using porcine ear skin as the membrane, and the in vivo accumulation of PpIX, 4 hours after application, was determined fluorometrically in healthy mice skin by chemical extraction of skin samples. The results showed that the flux and in vitro retention of ALA in viable epidermis increased in the presence of 10.0% oleic acid. The amounts of PpIX, evaluated both by chemical tissue extraction and in vivo measurements by an optical fiber probe, increased after applying ALA formulations containing 5.0% and 10.0% oleic acid. Moreover, in vivo kinetic studies showed an increase in skin PpIX accumulation when formulations containing 10% oleic acid were used; PpIX accumulation was also maintained for a longer period compared with the controls.

**Skin penetration of ALA with different vehicles**

The half-life of ALA in the body is quite short (about 45 minutes) because of its insufficient stability under physiological conditions. Vehicles may serve as a solubilization matrix, as a local depot for the sustained release of dermally active compounds, as a permeation enhancer, or as a rate-limiting membrane barrier to modulate systemic absorption of drugs through the skin.

In 1999, Casas et al reported the topical application of ALA delivered in different vehicles to the skin overlying a tumor and normal skin of mice. They used a lotion and cream, and the maximal accumulation was found in the tumor 3 hours after ALA application with both the cream and lotion preparations. The results showed that normal skin tissues and those overlying the tumor had different kinetic patterns, reflecting histological changes after the latter had been invaded by tumor cells. ALA lotion applied to the skin overlying the tumor induced higher accumulation of porphyrins in the tumor than the cream, and the lotion applied to normal skin appeared to be the most efficient for inducing total-body porphyrins. The following year, they also studied ALA in a saline lotion alone or with DMSO, cream, liposomes, and Vaseline, after topical application in a murine subcutaneous adenocarcinoma model. By measuring the uptake of 14C-labeled ALA, porphobilinogen accumulation, and some heme enzyme activities, they observed the effect of DMSO on porphyrin synthesis and ALA penetration through the skin. ALA in the saline lotion, without or with 10% DMSO, proved to be the most efficient vehicle for tumor porphyrin accumulation (mean ± standard deviation values are 1.75 ± 0.25 µg/g and 2.55 ± 0.39 µg/g, respectively), whereas the cream and liposomes induced lower levels and similar porphyrin accumulations of about 0.60 µg/g. Using the ALA + DMSO saline lotion, a higher porphyrin accumulation was found in skin overlying the tumor tissue and in the first 2 mm of the tumor, probably because of either increased ALA penetration, greater interconversion to porphyrin, or greater retention of ALA and/or porphyrins.

A new cubic gel and patch system containing additional carrageenan as the matrix was used as a vehicle for ALA. Valenta et al reported skin permeation and stability studies of ALA in the new gel and patch preparations. They used three vehicles: a cubic gel, a hydroxyethyl cellulose gel, and a patch. The in vitro ALA permeation through porcine skin after 48 hours was highest at 80.3% from the patch formulation, followed by the cubic gel at 66.4%. However, only about 40% of the ALA was chemically stable after 14 days of storage in the patch formulation, whereas no degradation of ALA was detected in the cubic gel over 90 days of observation.

Liposomes are one of the best drug delivery systems for low-molecular-weight drugs, imaging agents, peptides, proteins, and nucleic acids. Liposomes are microscopic vesicles consisting of one or more membrane-like phospholipid bilayers surrounding an aqueous medium. According to their particle size, liposomes are nanospheres or microspheres. Small unilamellar vesicles have dimensions of 20–100 nm, large unilamellar vesicles are larger than 100 nm, and multilamellar vesicles have dimensions exceeding 500 nm. Lipophilic agents are incorporated into the bilayers, whereas hydrophilic agents are found within the water phase inside the vesicles. Pierre et al worked on a delivery system for ALA based on liposomes with a lipid composition combined to obtain 10 mg of a mixture containing lipids at the following concentrations: ceramide (50%), cholesterol (28%), palmitic acid (17%), and cholesteryl sulfate (5%), in close approximation of the composition of mammalian stratum corneum lipid liposomes (SCLs) to optimize its skin delivery in PDT for skin cancers. SCLL encapsulated around 5.7% ALA, and the respective sizes were around 500 nm and 400 nm for SCLs and SCLLs containing ALA. The in vitro permeation profile was characterized using hairless mouse skin mounted in a modified Franz diffusion cell, and the results showed that SCLL preparations presented significantly higher skin retention in the epidermis without the SC + dermis, with decreased skin permeation compared with an aqueous solution. In contrast, Auner et al used different-pH ALA solutions (cetylpyridinium chloride and benzalkonium chloride at pH 7.0 and...
sodium-1-octanolsulfonic acid, sodium-1-heptanolsulfonic acid, and sodium-1-pentanolsulfonic acid monohydrate at pH 4.0) combined with liposomes containing phloretin and 6-ketocolesterol. Pre-impregnation of porcine skin with liposomes containing phloretin and 6-ketocolesterol as enhancers increased ALA diffusion by about 1.7-fold at pH 7.0. The transport enhancement of ALA and cetylpyridinium chloride combined with 6-ketocolesterol as the donor was 3.5-fold higher. Microemulsions are compositions of oil, water, surfactants, and cosurfactants. Compared with ordinary emulsions, microemulsions form on simple mixing of the components, and do not require high-shear conditions, are easily prepared, and have low viscosity. Microemulsions are of two basic types: O/W and water in oil (W/O). ALA was incorporated in W/O, bicontinuous (Bc), and O/W microemulsions produced by the titration of ethyl oleate and PEG-8 caprylic/capric glyceride (Labrasol): polyglyceryl-6 diuolate (3:1) mixtures with water. The in vitro and in vivo skin permeation of ALA was investigated using diffusion cells and CSLM, respectively. The O/W microemulsion decreased the ALA diffusion coefficient and retarded the drug release, but compared with other ALA carriers, it also significantly increased the in vitro drug skin permeation. In another study, 26 the in vivo permeation of an ALA microemulsion was observed by CSLM, and the red fluorescence of the skin homogeneously increased in the deeper skin layers, probably because of the formation of the photoactive PpIX.

Conclusions

PDT is a widely used technique to treat skin diseases, whereas ALA and MAL are the two most-often used drugs in PDT. Because there are some difficulties in ALA penetration through intact skin, many researches have used animal and human skins as in vitro models to evaluate whether the use of enhancers is beneficial for ALA-PDT efficiency. Combinations of these methods, including the design of carriers, prodrug modification, and the use of physical and chemical enhancers, are trends for PDT in the future.

References