REVIEW ARTICLE

5-Aminolevulinic acid-mediated photodynamic therapy for oral cancers and precancers

Hsin-Ming Chen a,b,c,d, Chuan-Hang Yu e,f, Hung-Pin Lin g,h, Shih-Jung Cheng b,c,d, Chun-Pin Chiang a,b,c,d*

a Graduate Institute of Oral Biology, School of Dentistry, National Taiwan University, Taipei, Taiwan
b Graduate Institute of Clinical Dentistry, School of Dentistry, National Taiwan University, Taipei, Taiwan
c School of Dentistry, National Taiwan University, Taipei, Taiwan
d Department of Dentistry, National Taiwan University Hospital, College of Medicine, National Taiwan University, Taipei, Taiwan
e School of Dentistry, College of Oral Medicine, Chung Shan Medical University, Taichung, Taiwan
f Department of Dentistry, Oral Medicine Center, Chung Shan Medical University Hospital, Chung Shan Medical University, Taichung, Taiwan
g Department of Dentistry, China Medical University Hospital, College of Medicine, China Medical University, Taichung, Taiwan
h School of Dentistry, College of Medicine, China Medical University, Taichung, Taiwan

Final revision received 22 August 2012; accepted 25 August 2012
Available online 21 December 2012

KEYWORDS
5-aminolevulinic acid; oral erythroleukoplakia; oral leukoplakia; oral verrucous hyperplasia; topical photodynamic therapy

Abstract Previous studies have used both systemic and topical 5-aminolevulinic acid (ALA)-mediated photodynamic therapy (PDT) to treat oral precancers including oral leukoplakia (OL), oral erythroleukoplakia (OEL), and oral verrucous hyperplasia (OVH) as well as oral cancers including oral verrucous carcinoma (OVC) and oral squamous cell carcinoma (OSCC). Systemic ALA-PDT has been used to treat oral dysplastic lesions and oral cancers with promising clinical outcomes. The efficacy of a regular topical ALA-PDT (fluence rate, 100 mW/cm²; light dose, 100 J/cm²) was tested on an extensive buccal OVC and an enhanced topical ALA-PDT (fluence rate, 200 mW/cm²; light dose, 200 J/cm²) on an early-invasive OSCC; complete regression of the carcinomas was demonstrated after 28 and 18 PDT treatments, respectively. Several previous studies showed relatively good outcomes for OL lesions treated with topical ALA-PDT. However, it was found that the regular topical ALA-PDT is very effective for OVH and OEL lesions but less so for OL lesions. Better PDT outcomes are significantly associated with OVH and OEL lesions with smaller size, pink to red color, epithelial dysplasia, or thicker surface keratin layer. Moreover, the thicker surface keratin layer on the OL lesions is responsible for the relatively poorer PDT outcomes for OL lesions. In addition, both light
emitting diode light- and laser light-mediated topical ALA-PDTs are comparative treatment modalities for O VH and OEL lesions. Methotrexate- or vitamin D3-preconditioned prostate or skin carcinoma cells can accumulate more intracellular protoporphyrin IX, resulting in an increased killing of these preconditioned cells by subsequent ALA-PDT. Because chemotherapy can help destroy carcinoma cells and tumor-associated vasculatures and cryotherapy pretreatment may help the diffusion of ALA into lesional epithelial cells, the chemotherapy or cryotherapy-combined topical ALA-PDT may be a new effective PDT alternative for treatment of oral precancers and cancers. It is concluded that topical ALA-PDT using either light emitting diode or laser light is very effective for OVH and OEL lesions but is relatively less effective for OL lesions. If OVC or early-invasive OSCC has no concurrent regional or distant metastasis, regular or enhanced topical ALA-PDT may have a high potential to treat these two particular kinds of oral cancer. A large-scale human clinical trial is needed to evaluate the efficacies of drug-preconditioned topical ALA-PDT and chemotherapy or cryotherapy-combined topical ALA-PDT on oral precancers, OVC and early-invasive OSCC in the near future.

Copyright © 2012, Association for Dental Sciences of the Republic of China. Published by Elsevier Taiwan LLC. All rights reserved.

Introduction

Oral squamous cell carcinoma (OSCC) is the most frequent malignant tumor of the oral cavity. In Taiwan, oral cancers rank as the sixth most prevalent cancer in both genders and account for the fourth most common cancers in males. Although there have been great advances in diagnosis and treatment of OSCC in recent years, the 5-year survival rate for advanced oral cancer patients has remained approximately 20%. Oral precancerous lesions are even more prevalent than oral cancers. Without further interventions, a part of these oral precancerous lesions finally transform into oral cancers. Therefore, one of the best strategies to prevent the occurrence of oral cancer is to treat the oral cancer at its precancerous stage to prevent its further malignant transformation.

Oral leukoplakia (OL), oral erythroleukoplakia (OEL), and oral verrucous hyperplasia (OVH) are three common oral precancerous lesions. Histologically, approximately 90% of OL lesions show hyperparakeratosis or hyperorthokeratosis and/or epithelial hyperplasia, whereas nearly all OEL lesions reveal some degree of epithelial dysplasia, carcinoma in situ or superficially invasive carcinoma. In addition, OEL lesions have higher mitotic and apoptotic indices than homogeneous and nodular OL lesions. Immunohistochemical study also demonstrates a higher Ki67 or p53 expression in OEL than in homogeneous and nodular OL lesions. Thus, OEL has a higher malignant transformation rate than OL. In Taiwan, both betel quid chewing and cigarette smoking are involved in multistate progression of oral precancers including OL and OEL lesions. A cohort study found an average dwelling period of 24 years for OL and of 7 years for OEL. Furthermore, the risks of developing oral cancer after 20 years of follow-up are 42.2% for OL and 95.0% for OEL. These findings suggest that OEL lesions have higher malignant transformation potential than OL lesions. A retrospective clinical study showed a malignant transformation rate of 3.1% and a mean malignant transformation duration of 54.6 months for 324 OVH lesions of Taiwanese patients. Wang et al also demonstrated a 5-year malignant transformation rate of 3% for 30 plaque-typed and of 17% for 30 mass-typed OVH lesions. The high malignant transformation rates of OEL and OVH lesions highlight the importance of early detection and treatment of these two types of oral precancerous lesions.

Surgical treatment of OL or other oral precancerous lesions may be performed either through conventional surgery, electrocauterization, laser ablation, or cryosurgery. Recurrence of OL lesions after surgical treatment has been reported in 10–35% of cases. Large oral precancerous lesions treated by total surgical excision frequently result in scar formation. Photodynamic therapy (PDT) is another effective treatment option for human oral precancerous lesions because it can be used repeatedly without cumulative side effects, results in little or no scar formation, has lower invasiveness, has the ability to treat multifocal lesions, and can be applied to patients who refuse surgery or have pacemakers and bleeding tendency.

PDT needs two individually nontoxic components, light and a photosensitizer, that work together to induce cellular and tissue destruction in an oxygen-dependent manner. The technique is based on the administration of an exogenous photosensitizer to render tumor tissue sensitive to light of a specific wavelength. The photosensitizers are normally inert and have a selective affinity to premalignant and malignant tissues. When a photosensitizer in tissues is activated by a light of specific wavelength, it transfers energy from light to molecular oxygen, resulting in generation of reactive oxygen species such as free radicals and singlet oxygen. There are three main mechanisms by which PDT mediates tumor destruction. Firstly, the free radicals and singlet oxygen can kill tumor cells directly. Secondly, PDT can damage the tumor-associated vasculature, leading to thrombus formation and subsequent tumor infarction. Thirdly, PDT-destroyed tumor tissue-released tumor-specific antigens can also activate an immune response against the residual tumor cells.

5-Aminolevulinic acid (ALA) itself is not a photosensitizer but serves as the biological precursor of the photosensitizer, protoporphyrin IX (PpIX), in the heme biosynthesis pathway. When ALA is topically applied on the oral lesions or systemically administered by the patients, it will be...
absorbed by the lesional epithelial cells and results in accumulation of relatively high concentration of PpIX in these cells.\textsuperscript{13,14} ALA is superior to other photosensitizers because it can be rapidly cleared from the tissues and the body within 48 hours and patients after ALA-mediated PDT (ALA-PDT) treatment have no problem of prolonged skin photosensitivity.\textsuperscript{12–14}

In this article, we review the previous studies evaluating the efficacies of PDT with systemically administered ALA (systemic ALA-PDT) or PDT with topically applied ALA (topical ALA-PDT) on oral precancers including OL, OEL, and OVH, and oral cancers such as oral verrucous carcinoma and OSCC.\textsuperscript{15–33} Previous works found that the methotrexate- or vitamin D3-preconditioned prostate or skin carcinoma cells can accumulate more intracellular PpIX due to the marked augmentation of coproporphyrinogen oxidase expression and the slight reduction of ferrochelatase expression, resulting in an increased killing of these preconditioned cells by subsequent ALA-PDT.\textsuperscript{34–41} Therefore, we also review a few studies assessing the effects of methotrexate- or vitamin D3-preconditioned ALA-PDT on killing of prostate or skin carcinoma cells \textit{in vitro} and \textit{in vivo}.\textsuperscript{42–49} Because chemotherapy can help destroy carcinoma cells or tumor-associated vasculatures and cryotherapy pretreatment may help the diffusion of ALA into lesional epithelial cells, we review several studies evaluating the efficacies of combination treatments of chemotherapy or cryotherapy plus ALA-PDT on cancer cell lines \textit{in vitro} or solid tumors implanted \textit{in vivo}.\textsuperscript{42–49} Finally, the effects of topical ALA-PDT or topical photosan-PDT on hamster buccal pouch precancers are also reviewed.\textsuperscript{50,51}

**Systemic ALA-PDT for oral cancers and precancers**

Systemic ALA-PDT has been used for treatment of oral cancerous and precancerous lesions with relatively good clinical outcomes.\textsuperscript{15,16} Grant et al\textsuperscript{15} first used PDT with orally administered ALA to treat four patients with advanced oral cancer. They found tumor necrosis in three of four patients treated with PDT. Fan et al\textsuperscript{16} used systemic ALA-PDT to treat six oral cancer and 12 oral dysplastic lesions. They observed a reduction in the size of the cancer in five of six patients after PDT, but only two showed a complete regression of the cancer. In addition, all 12 dysplastic lesions showed a significant regression to normal or less dysplastic after PDT.

**Topical ALA-PDT for oral cancers**

An extensive buccal verrucous carcinoma which was successfully eradicated by 28 treatments with a regular topical ALA-PDT protocol (fluence rate, 100 mW/cm\textsuperscript{2}; light dose, 100 J/cm\textsuperscript{2}) has been reported.\textsuperscript{17} Moreover, an enhanced topical ALA-PDT protocol (fluence rate, 200 mW/cm\textsuperscript{2}; light dose, 200 J/cm\textsuperscript{2}) was also used successfully to treat an early invasive OSCC that showed complete regression after 18 PDT treatment.\textsuperscript{18} Although only two oral cancer cases are treated with these two different topical ALA-PDT protocols, it is suggested that if there is no concurrent regional or distant metastasis for oral verrucous carcinoma or early invasive OSCC that is limited to the superficial \textit{lamina propria} of the oral mucosa, regular or enhanced topical ALA-PDT may be a treatment of choice for these two particular kinds of oral cancer.

**Topical ALA-PDT for oral or laryngeal leukoplakia**

Kubler et al\textsuperscript{19} treated 12 OL lesions with PDT after local application of 20% ALA cream. Of these lesions, complete response (CR) was found in five, partial response (PR) in four, and no response (NR) in three. Sieron et al\textsuperscript{20,21} treated 17 OL lesions with PDT after topical application of 10% ALA ointment or emulsion in two separate studies. CR was observed in 14 out of 17 OL lesions. Franco\textsuperscript{22} treated 12 recalcitrant laryngeal leukoplakia lesions with PDT using 585-nm pulsed dye laser after topical application of 20% ALA cream. He found CR in nine out of 12 laryngeal leukoplakia lesions.\textsuperscript{22} In addition, Shafirstein et al\textsuperscript{23} treated 17 OL lesions with topical ALA-PDT using high-power 585-nm pulsed dye laser after placing a gauze saturated with 20% ALA solution against the OL lesions for 1.5 hours. They demonstrated a >75% regression (significant response) in seven (41%) OL lesions and a >25% regression (PR) in nine (53%) OL lesions, resulting in an overall response rate of 94% at 90 days. In addition, three of the six OL lesions treated with PDT after intralesional injection of 20% ALA solution showed significant response. They found no significant difference in PDT outcome for OL lesions treated with ALA-PDT after either topical application or intralesional injection of 20% ALA solution.\textsuperscript{24} Jerjes et al\textsuperscript{25} used PDT with either 5-ALA or m-tetrahydroxyphenylchlorin (mTHPC) as the photosensitizer to treat a total of 147 consecutive patients with oral potentially malignant disorders. The 147 patients include 55 with homogenous leukoplakia, 73 with nonhomogenous leukoplakia, and 19 with erythroplakia. Histopathologically, moderate dysplasia is identified in 33 patients, severe dysplasias in 63 patients; and carcinoma \textit{in situ} in 32 patients. ALA cream is applied topically 3–4 hours prior to tissue illumination for patients with thin-mild/moderate dysplasia, and m-THPC is administered at a dose of 0.1 mg/kg of body weight intravenously into the midcubital vein 96 hours before light treatment for patients with thick mild-moderate, severe dysplasia and carcinoma \textit{in situ}. The final outcomes of the cohort show CR in 119 (81%) patients, PR in 12 (8.2%) patients, stable disease in five (3.4%) patients, and progressive disease in 11 (7.5%) patients. They concluded that topical ALA-PDT and systemic mTHPC-PDT offer an effective alternative treatment for oral potentially malignant disorders.\textsuperscript{24} Kawczyk-Krupka et al\textsuperscript{25} used topical ALA-PDT to treat 48 OL lesions. Of the 48 OL lesions, 30 were treated with PDT using the 630-nm Diomed laser light and a topically applied 20% ALA emulsion and 18 with PDT using the 635-nm argon-pumped dye laser light and a topically applied 10% ALA emulsion. They found CR in 20 (67%) of the former 30 OL lesions and in 15 (83%) of the latter 18 OL lesions. Moreover, recurrence was observed in 11 (37%) of the former 30 OL lesions and in two (11%) of the latter 18 OL lesions over a 6-month period.\textsuperscript{25}
A regular topical ALA-PDT with a 635-nm light-emitting diode (LED) light was used to treat 65 OL lesions once a week and another 32 OL lesions twice a week. Lesion response was characterized as follows: CR, lack of detectable lesion confirmed by clinical evaluation; PR, reduction of lesion by >20% in diameter; and NR, reduction of lesion by ≤20% in diameter. It was found that the 65 OL lesions treated with topical ALA-PDT once a week showed CR in five, PR in 33, and NR in 27. The 32 OL lesions treated with the same topical ALA-PDT twice a week demonstrate CR in 11 and PR in 21. The 32 OL lesions treated twice a week had a significantly better clinical outcome than the 65 OL lesions treated once a week. Furthermore, recurrence was found in one (20%) of the five CR OL lesions treated with PDT once a week and in two (18%) of the 11 CR OL lesions treated with PDT twice a week. Although this does not demonstrate a promising clinical outcome for OL lesions treated with regular topical ALA-PDT, the results of other studies do show that topical ALA-PDT is a relatively effective treatment modality for OL lesions.

**Topical ALA-PDT for oral verrucous hyperplasia**

A regular topical ALA-PDT with the 635-nm LED light was used to treat 36 OVH lesions once a week. It was found that all the 36 OVH lesions show CR after an average of 3.8 (range, 1–6) treatments of topical ALA-PDT. OVH lesions with a clinical appearance of a mass (2.9 ± 1.3 treatments), with greatest diameter <1.5 cm (3.0 ± 1.3 treatments), with the pink color (3.2 ± 1.4 treatments), with epithelial dysplasia (3.1 ± 1.5 treatments), or with surface keratin layer ≤40 μm (3.4 ± 1.4 treatments) need significantly fewer mean treatment numbers of PDT to achieve a CR than OVH lesions with a clinical appearance of a plaque or a combination type (4.7 ± 1.1 treatments), with the greatest diameter >1.5 cm (4.3 ± 1.4 treatments), with white color (4.8 ± 1.1 treatments), without epithelial dysplasia (4.2 ± 1.3 treatments), or with the surface keratin layer >40 μm (4.8 ± 0.7 treatments), respectively. Multivariate analyses showed that the clinical appearance of OVH lesions is the only independent factor for prediction of the PDT treatment number. No recurrence of the 36 OVH lesions was found after a follow-up period of 6–56 (mean, 26) months. It is concluded that complete regression of OVH lesions with a mean diameter of 1.7 (range, 0.5–3.1) cm can be achieved by less than seven treatments of topical ALA-PDT once a week. The PDT treatment outcome for OVH depends on the clinical appearance, size, color, epithelial dysplasia, and surface keratin thickness of the lesion. In addition, an enhanced topical ALA-PDT protocol was used successfully to treat an extensive buccal OVH lesion that showed complete regression after 18 PDT treatments.

For comparison, 40 OVH lesions were treated with topical ALA-PDT using the 635-nm laser light once a week. We found that all the 40 OVH lesions exhibit CR after an average of 3.6 (range, 1–6) PDT treatments. Moreover, the mean number of PDT treatments to achieve a CR for OVH lesions with the clinical appearance of a mass (2.8 ± 1.1 treatments), with greatest diameter <1.5 cm (2.2 ± 0.7 treatments), with pink color (3.0 ± 1.2 treatments), with epithelial dysplasia (2.5 ± 1.1 treatments), or with surface keratin layer ≤40 μm (3.0 ± 1.2 treatments) was significantly fewer than for OVH lesions with the clinical appearance of a plaque or a combination type (4.4 ± 0.8 treatments), with greatest diameter >1.5 cm (4.3 ± 0.8 treatments), with white color (4.4 ± 0.8 treatments), without epithelial dysplasia (4.2 ± 0.8 treatments), or with surface keratin layer >40 μm (4.4 ± 0.8 treatments), respectively. Multivariate logistic regression analyses showed that the size of OVH lesions was the only independent factor influencing the PDT treatment number. No recurrence of the 40 OVH lesions was found after a follow-up period of 8–37 (mean, 20) months. In addition, although the mean treatment number of PDT to achieve a CR for the 40 laser light-treated OVH lesions (3.6 ± 1.2 treatments) was lower than that for the 36 LED light-treated OVH lesions (3.8 ± 1.5 treatments), no significant difference was found.

**Topical ALA-PDT for oral erythroleukoplakia**

A regular topical ALA-PDT with the 635-nm LED light has also been used to treat 20 OEL lesions once a week. It was found that the 20 OEL lesions treated with topical ALA-PDT once a week show CR in 17 and PR in three. In addition, the 17 CR OEL lesions needed an average of 3.7 (range, 2–7) treatments of ALA-PDT to achieve CR of the lesions. Correlation analyses revealed that the mean number of PDT treatments needed to achieve a CR for OEL lesions with greatest diameter ≥1.5 cm (3.0 ± 0.9 treatments) or with surface keratin layer ≤30 μm (2.9 ± 0.9 treatments) was significantly fewer than for OEL lesions with greatest diameter ≥1.5 cm (4.5 ± 1.5 treatments) or with surface keratin layer >30 μm (4.6 ± 1.3 treatments), respectively. After a follow-up period of 16–76 (mean, 32) months, five (29%) of the 17 CR OEL lesions recurred. These five lesions recur 8–14 (mean, 11) months after the last PDT treatment. The five recurrent lesions were treated by the same PDT protocol as before and showed complete regression after 2–4 (mean, 2.8) treatments.

For comparison, 40 OEL lesions were treated with topical ALA-PDT using the 635-nm laser light once a week. Of the 40 OEL lesions, 38 showed CR after an average of 3.4 (range, 2–6) PDT treatments and 2 showed PR. It was found that the mean number of PDT treatments needed to achieve a CR for OEL lesions with greatest diameter <1.5 cm (2.7 ± 1.0 treatments) or with surface keratin layer ≤30 μm (2.7 ± 0.9 treatments) was significantly fewer than for OEL lesions with greatest diameter ≥1.5 cm (4.4 ± 1.3 treatments) or with surface keratin layer >30 μm (4.5 ± 1.2 treatments), respectively. After a follow-up period of 6–30 (mean, 18) months, eight (21%) of the 38 CR OEL lesions recurred. These eight OEL lesions recurred 6–14 (mean, 9) months after the last PDT treatment. The eight OEL recurrent lesions were treated by the same PDT protocol as before and showed complete regression after 1–3 (mean, 2) treatments. Although the mean number of PDT treatments to achieve a CR for the 38 laser light-treated OEL lesions (3.4 ± 1.4) was lower than that for the 17 LED light-treated OEL lesions (3.7 ± 1.4), no significant difference was found.
Why is topical ALA-PDT very effective for OVH and OEL lesions but less effective for OL lesions?

Previous studies have found that topical ALA-PDT is very effective for OVH and OEL lesions but less so for OL lesions. We suggest that the successful clinical outcomes for OVH and OEL lesions treated by either the LED or laser light-mediated topical ALA-PDT may be due to the ALA preparation, the topical ALA-PDT protocol used, and the characteristic morphological, histologic, and biological features of the OVH and OEL lesions themselves. In brief, the 20% ALA preparation is a gel form, which is adhesive to the oral mucosa, is partially resistant to the dilution of the saliva, and in turn helps the absorption of ALA from the mucosal surface. Moreover, the studies used a fractionated protocol to deliver light treatment (repeated cycles of 3-minute light illumination followed by 3-minute rest); the lesional epithelial cells might regenerate new PpIX and obtain new oxygen during multiple 3-minute resting periods, finally resulting in a more successful clinical outcome for our OVH and OEL lesions.

The verrucous appearance of the OVH lesion provides a large area for good retention and absorption of ALA on the surface. In general, OVH and OEL lesions with smaller size, pink to red color, epithelial dysplasia, and thinner surface keratin layer have better PDT outcomes than those corresponding lesions, respectively. Pink to red and dysplastic oral OVH and OEL Lesions usually have thinner surface keratin layer, leading to diffusion of more ALA into the lesional epithelial cells. Furthermore, dysplastic OVH and OEL lesions usually have a more permeable epithelium (due to wide intercellular spaces of the dysplastic epithelium); this also results in diffusion of more ALA into the lesional epithelial cells. In addition, dysplastic epithelium may retain more ALA than hyperplastic epithelium, and the thinner surface keratin layer may only have a minimal effect on the reduction of the light intensity. In addition, there are more epithelial cells in the cell division cycle in dysplastic OVH and OEL lesions than in nondysplastic OVH lesions. Dysplastic epithelial cells in the cell division cycle are more susceptible to destruction by PDT-generated singlet oxygen molecules and free radicals than those epithelial cells not in the cell division cycle. Sufficient photosensitizers and light dose finally result in a better clinical outcome for those OVH and OEL lesions with pink to red color, epithelial dysplasia, and thinner surface keratin layer.

Furthermore, mass-typed OVH lesions are highly protuberant and are usually situated on a smaller base area than plaque-typed or combination-typed OVH lesions. In our experience, the purely mass-typed lesions or the central masses of combination-typed lesions usually show complete regression after one or two treatments of PDT. In contrast, the purely plaque-typed lesions or the residual peripheral plaques of combination-typed lesions may need more PDT treatments to achieve a CR. Because PDT can damage the tumor-associated vasculature and mass-typed OVH lesions with a smaller base area usually have limited blood supply, these mass-typed OVH lesions are easily eradicated by PDT, probably through a combined mechanism of reactive oxygen species-mediated tumor cell killing and endothelial cell destruction followed by thrombus formation and tumor infarction.

LED versus laser light sources for ALA-PDT

To the best of our knowledge, most of the previous ALA-PDT studies used laser light to treat a variety of premalignant and malignant human lesions; we first tested the efficacy of an LED light-mediated ALA-PDT on oral premalignant lesions such as OVH, OEL, and OL. Laser machines can provide light with specific mono-wavelength; the laser system is well designed, more stable, and power adjustable. It can be used for a long period of several years without the need of changing the laser light source. However, it is heavier, more bulky, and more expensive. An LED light device can only provide light with a range of wavelengths but has the advantages of being a simpler, smaller, lighter, less expensive, and more portable light source than the laser machine. However, the LED chips are very sensitive to the heat generated during the PDT treatment and may deteriorate rather quickly after repeated use. This results in the need of frequent changes of the LED chips every 6–12 months. Therefore, the choice of using either the LED or laser light source depends on the budget of the institute. Our previous studies showed that the LED light-mediated topical ALA-PDT is as effective as the laser light-mediated topical ALA-PDT for treatment of OVH and OEL lesions. This finding suggests that the total light dose rather than the light source is more important for a successful PDT outcome.

Systemic ALA-PDT versus topical ALA-PDT

Although both topical and systemic ALA-PDT remedies are used for treatment of oral precancerous lesions, topical ALA-PDT is even superior to systemic ALA-PDT because the former uses a small amount of ALA (20–200 mg) per treatment and has no skin photosensitivity even within the initial 48 hours after PDT. Systemic administration of ALA to the patients has the side effects of nausea, vomiting, and elevation of blood levels of bilirubin and liver enzymes (e.g. aspartate transaminase). In contrast, direct topical application of ALA onto oral lesions does not have any systemic side effects. Therefore, we suggest the use of topical ALA-PDT as the first-line treatment of choice for human oral precancerous lesions, especially for both OVH and OEL lesions, in the near future.

Drug-preconditioned ALA-PDT

The efficacy of ALA-PDT depends on the amount of PpIX accumulated in the lesional epithelial or cancer cells. PpIX is the seventh product in the heme biosynthesis pathway. The increase in the expression of coproporphyrinogen oxidase (the enzyme that is responsible for the sixth step in heme production, converting coproporphyrinogen III into protoporphyrinogen IX) and the decrease in the expression...
of ferrochelatase (the enzyme that catalyses the terminal or eighth step in the biosynthesis of heme, converting PpIX into heme) are responsible for the increased accumulation of PpIX in lesional epithelial or cancer cells. Previous studies have shown that agents capable of inducing cellular differentiation can also cause an elevation in the level of PpIX in prostate cancer cells and skin epithelial cells. Because methotrexate can promote differentiation and PpIX accumulation in carcinoma cells, methotrexate may serve as a drug to augment the efficacy of ALA-PDT for tumor cell killing. Sinha et al. studied methotrexate-pretreated ALA-PDT for killing of prostate cancer cells. They found that prostate cancer cells pretreated by methotrexate and followed by ALA incubation can result in a threefold increase in intracellular PpIX level, which is due to the threefold augmentation in expression of coproporphyrinogen oxidase by methotrexate. Transfection of prostate cancer cells with a coproporphyrinogen oxidase-expressing vector also stimulates the accumulation of PpIX in cells. In addition, after exposure to 512-nm light, killing is significantly enhanced in methotrexate-pretreated cells. These findings suggest that methotrexate, when used to modulate intracellular production of endogenous PpIX, may provide a new combination PDT approach for certain cancers. Recent studies also demonstrated that low-dose methotrexate can enhance ALA-PDT in skin carcinoma cells in vitro and in vivo. Methotrexate preconditioning of monolayer cultures can preferentially augment intracellular PpIX levels two- to fourfold in carcinoma cells versus normal keratinocytes. Photodynamic killing is synergistically enhanced by the methotrexate-combined therapy compared to PDT alone. Methotrexate enhancement of PpIX levels is achieved over a broad methotrexate concentration range (0.0003–1.0 mg/L; 0.6 nM–2 mM). PpIX enhancement correlates with a fourfold increase in expression of coproporphyrinogen oxidase and no change or a slight decrease in expression of ferrochelatase. In vivo relevance is also established by demonstrating that methotrexate-preconditioning enhances PpIX accumulation in three models: organotypic cultures of immortalized keratinocytes, chemically induced skin tumors in mice, and human A431 squamous cell tumors implanted subcutaneously in mice. Therefore, combination therapy using short-term exposure to low-dose methotrexate followed by ALA-PDT should be further investigated as a new combination treatment modality to increase the efficacy of ALA-PDT on epithelial carcinomas.

Methotrexate is chosen as an attractive candidate agent because it is already used widely in the clinical arena, has relatively low toxicity, and exerts its own antitumor effects by inhibiting proliferation, promoting apoptosis, and promoting terminal differentiation. The ability to promote differentiation, in particular, suggests the idea of using methotrexate in combination with ALA-PDT. Androgens and vitamin D are also known to promote cellular differentiation and at the same time to enhance PpIX accumulation in prostate cancer cells. Sato et al. evaluated the vitamin D-enhanced PpIX production and photodynamic cell death in 3D organotypic cultures of keratinocytes. They found that PpIX levels, at 4 hours after addition of ALA (1 mM), are significantly increased in vitamin D-preconditioned cultures. Maximal PpIX induction is seen at the vitamin D concentration of 10⁻¹²–10⁻¹⁰ M. Phototoxic cell killing after exposure to 635-nm light is significantly increased in vitamin D-preconditioned cultures. Therefore, vitamin D may also be useful as a biological enhancer of ALA-PDT. Recently, Anand et al. showed that calcitriol, delivered topically or intraperitoneally, can raise tumoral accumulation of the PpIX up to 10-fold, mainly due to the augmentation of coproporphyrinogen oxidase expression and the reduction of ferrochelatase expression. Calcitriol-pretreated tumors undergo enhanced apoptotic cell death after ALA-PDT. Mechanistic studies have shown the activation of the extrinsic apoptotic pathway, with specific cleavage of caspase-8 and increased production of tumor necrosis factor-α in tumors treated with the calcitriol-preconditioned ALA-PDT. Very low doses of calcitriol (0.1–1 μg/kg body weight) are sufficient to elicit tumor-selective enhancement to ALA-PDT efficacy. The aforementioned findings suggest that methotrexate or calcitriol is a simple, nontoxic, and highly effective preconditioning agent to enhance the response of epithelial tumors to ALA-PDT. To the best of our knowledge, to date there has been no human clinical trial testing the methotrexate- or calcitriol-preconditioned topical ALA-PDT for oral precancers or cancers.

**Combination therapy of chemotherapy plus ALA-PDT**

Because chemotherapy can help destroy carcinoma cells and tumor-associated vasculatures, previous studies also tested whether the combination therapy of chemotherapy plus ALA-PDT are more effective than the ALA-PDT alone. It was found that low doses of doxorubicin and vincristine can increase the anti-cancer effect of ALA-PDT in murine leukemic cell lines. Decrease in cell survival is higher when the combination therapy of vincristine plus ALA-PDT is used compared to that of doxorubicin plus ALA-PDT. In addition, doxorubicin and vincristine chemoresistant LBR-D160 and LBR-V160 cell lines are also found to be sensitive to ALA-PDT alone, although the combination therapy does not augment its efficacy on chemoresistant cell lines.

Vadimezan, a tumor vascular-disrupting agent, has shown to have anti-vascular effects in both murine and human tumors via increasing vascular permeability and hemorrhagic necrosis. The local synthesis of cytokine TNF-α in murine tumors is found in part to cause the tumor destruction. Previous studies have shown that addition of intraperitoneal vadimezan to PDT using the systemically administered photosensitizers porfimer sodium (Photofrin™) and 2-(1 -hexyloxyethyl)-2-devinyl pyropheophorbide-a (HPPH; Photochlor™) dramatically enhances the destruction of solid tumors implanted in mice. In addition, Marrero et al. treated Colon26 tumors implanted in mice with either combination therapy of topical ALA-PDT (80 J/cm² delivered at 75 mW/cm²) plus vadimezan or topical ALA-PDT alone. For the combination therapy, 20% ALA solution and vadimezan solution (1 mg vadimezan dissolved in 40 μL of dimethyl sulfoxide) are topically applied onto the tumor
3 hours and 1 hour prior to light treatment, respectively. It was found that the combination therapy causes a substantial decrease in tumor blood flow up to 2 hours following light delivery and a significant decrease in tumor weight at the endpoint (tumor growth of controls to 400 µL). The combination therapy of chemotherapy plus ALA-PDT has not been tested for human oral cancers and precancers.

Combination therapy of cryotherapy plus topical ALA-PDT

Mi et al used the combination therapy of cryotherapy plus topical ALA-PDT and cryotherapy alone to treat 80 patients with multiple condylomata acuminata. For the combination therapy, the condylomata acuminata lesions were pretreated with cryotherapy and then a 20% ALA is applied to the condylomata acuminata lesions 3 hours before illumination with the 635-nm red light (100 mW/cm² and 100 J/cm²). The lesions were treated once a week. They found that after two treatments, the CR rates in the combination therapy group (cryotherapy plus topical ALA-PDT) and cryotherapy alone group were 32.4% (36/111) and 32.6% (43/132) in the anal area, 100% (32/32) and 54.5% (18/33) in the urethral meatus, and 94.2% (129/137) and 50.5% (56/111) in the external genitals, respectively. The recurrence rates in the combination therapy group and cryotherapy alone group were 24.3% (27/111) and 31.1% (41/132) in the anal area, 9.4% (3/32) and 39.4% (13/33) in the urethral meatus, and 3.6% (5/137) and 31.5% (35/111) in the external genitals, respectively. They concluded that the combination therapy of cryotherapy plus topical ALA-PDT is a more effective regimen for the treatment of multiple condylomata acuminata compared to cryotherapy alone. A previous study has shown that topical ALA-PDT and cryotherapy are comparable methods for treatment of OL lesions. However, so far, there has been no human clinical trial evaluating the efficacy of the combination therapy of cryotherapy plus topical ALA-PDT on oral precancers or cancers.

Topical ALA-PDT for hamster buccal pouch precancers

Hsu et al used 7,12-dimethylbenz(a)anthracene (DMBA)-induced hamster buccal pouch carcinogenesis model to produce 20 precancerous lesions, which were treated by topical ALA-PDT with either a light dose of 75 J/cm² (n = 10) or 100 J/cm² (n = 10) using the 640-nm LED light. They found that the 10 precancerous lesions treated by 75-J topical ALA-PDT showed CR in eight after an average of 3.4 (range, 2–6) treatments and PR in two. The 10 precancerous lesions treated by 100-J topical ALA-PDT demonstrated CR in seven after an average of 4.4 (range, 3–6) treatments and PR in three. Fisher’s exact test showed no significant difference in clinical outcome between these two treatment modalities. These findings indicate that both the 75-J and 100-J topical ALA-PDT treatment modalities are very effective for DMBA-induced hamster buccal pouch precancerous lesions and no significant difference in PDT outcome is found between these two treatment modalities. Photosan, a mixture of porphyrin oligomers, is intracellularly converted into the active photosensitizer, PpIX, in epithelial cells. For comparison, topical photosan-mediated PDT (topical photosan-PDT) was used to treat 14 DMBA-induced hamster buccal pouch precancerous lesions using the 640-nm LED light twice a week. It was found that all the 14 precancerous lesions showed a CR after an average of 3.8 (range, 3–5) PDT treatments. Our findings indicate that topical photosan-PDT is also a very effective treatment modality for DMBA-induced hamster buccal pouch precancerous lesions compared to topical ALA-PDT.

Topical ALA-PDT using either the LED or laser light is very effective for OVH and OEL lesions but is relatively less effective for OL lesions. Better PDT outcomes are significantly associated with OVH and OEL lesions with smaller size, pink to red color, epithelial dysplasia, or thinner surface keratin layer. Moreover, the thicker surface keratin layer on the OL lesions is responsible for the relatively poorer PDT outcomes for OL lesions. Therefore, topical ALA-PDT using either the LED or laser light may serve as the first-line treatment of choice for OVH and OEL lesions. Furthermore, if oral verrucous carcinoma or early-invasive OSCC has no concurrent regional or distant metastasis, a regular topical ALA-PDT (fluence rate, 100 mW/cm²; light dose, 100 J/cm²) or an enhanced topical ALA-PDT (fluence rate, 200 mW/cm²; light dose, 200 J/cm²) may have a high potential to treat these two particular kinds of oral cancers. A large-scale human clinical trial is needed to evaluate the efficacies of drug-preconditioned topical ALA-PDT and chemotherapy or cryotherapy-combined topical ALA-PDT on oral precancers, oral verrucous carcinoma, and early-invasive OSCC in the near future.

References


