The Utilization of Nanoemulsified Aminolevulinic Acid in Photodynamic Therapy for Actinic Keratosis

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Abstract

Actinic Keratosis (AK), the pre-malignant condition that precedes nonmelanoma skin malignancies, is still one of the most common dermatologic cases seen in the clinic. Despite the availability of numerous therapies, optimal treatment still needs to be explored. In recent years, photodynamic therapy has sparked interest in various diseases, including actinic keratosis. Hence, this review aims to highlight the role and efficacy of nanoemulsified aminolevulinic acid as a photodynamic therapy agent in the treatment of actinic keratosis. A systematic search was performed through MEDLINE, Web of Science, and EMBASE based on the specified PICO. The present study assesses primary outcomes based on lesion response, cosmetic results, clearance rate, patient satisfaction, and lesion reccurence. This paper found that the utilization of ALA-PDT has been shown to ameliorate AK manifestations by accelerating neocollagenesis, stimulating fibroblast synthesis, and eliciting death in target cells. As a result, we concluded that ALA-PDT is considered effective for treating AK lesions, notably due to its high clearance rate, low recurrence rate, and lack of complications due to its non-invasive approach. However, before administering ALA-PDT to AK patients, contraindications and precautions must be addressed.

Keywords: Actinic Keratosis; Photodynamic Therapy; Aminolevulinic Acid; Non-melanoma Skin Cancer; Phototherapy

1. Introduction

Skin cancer affects at least 20% of Americans by the age of 70, making it the most common cancer in both the United States and globally. Among all risk factors, persistent radiation of ultraviolet (UV) is tightly associated to 90% of non-melanoma skin cancers, proving its evidence as a human carcinogen [1]. Actinic keratosis (AK), one of its precancerous conditions, has emerged as one of the most prevalent dermatologic disorders encountered in practice in recent years. The AK itself is seen in 14% of all dermatologic visits in the clinic [2].

Actinic keratosis (AK) is a pre-malignant dermatologic condition characterized by atypical keratinocyte proliferation resulting in hyperkeratosis, erythematous papules, and plaques as a result of prolonged actinic damage. Its macroscopic manifestation is generally reddish, scaly, and rough lesions observed on chronically ultraviolet-exposed skin areas. Several risk factors of AK including light skin color phenotype, exposure to UV radiation, exposure to hydrocarbons, and several other occupational chemical exposures. If not treated, 14-15% of AK will transform into invasive squamous cell carcinoma (SCC), the most prevalent malignancy in the globe. Despite the relatively low number, the presence of multiple lesions in different areas of the body might increase the likelihood of their progression from invasive to metastatic SCC [3].

Various modalities have been used as therapy in NMSC and AK, including surgical excision, cryotherapy, curettage, electrodessication, radiation therapy, and topicals. However, these methods are still considered ineffective because they are invasive and risky, have a long healing time, frequent recurrences, and require unaffordable costs [4]. Therefore, a new, more efficient and modern method is needed in handling AK. Due to the high accessibility of the skin to external light, various studies have been carried out to investigate various modalities that can be used as therapy in dermatology, including photodynamic therapy. However, certain mechanisms of ALA-PDT and its effect to AK, as well as the comparison between ALA-PDT to other therapies still remains unknown. Hence, this review aims to highlight the utilization of ALA-PDT therapy in AK, from its mechanisms, benefits and challenges, previous studies conducted, to results and comparison with other modalities.

1.1. Photodynamic Therapy (PDT)

Photodynamic therapy (PDT) is the application of a photosensitive compound to the skin followed by the administration of a light source with various spectrums. Often referred similar to radiation, PDT using prodrug active substances as a photosensitizer agents to emit the radiation. In the process, light with certain waves activates the photosensitization and induces oxygen-rich molecular reactions, which kill pathogenic cells [5]. In AK and SCC, PDT is known to demonstrate a high clearance rate, a short duration of therapy, and a near-perfect cosmetic outcome. The PDT method can also destroy the blood vessels that nourish the tumor by modulating the immune system. Photodynamic therapy in the world of dermatology is even dubbed as a promising therapy due to its near-perfect results [6]. In its application, photodynamic therapy (PDT) requires several components, such as: low-intensity visible light as an activator, a non-toxic prodrug as a photosensitizer (PS), and target cells depending on the patient's disease. Depending on the photosensitizing agent used, PDT can produce reactive oxygen species or singlet oxygen that will damage the target cell structure that has been penetrated with PS agents [7, 8]. Furthermore, PDT will selectively eradicate tumors through several reaction cascades, both photochemically, immunologically, to physiologically. At the cellular level, the PDT mechanism acts in the mitochondria by inducing oxidative damage to target cells, resulting in an apoptotic cascade that kills diseased tissue or pre-malignant cells in AK [9].

1.2 Aminolevulinic Acid (ALA)

An agent that can be used as a photosensitizer in AK and SCC therapy is aminolevulinic acid (ALA). Although it can be used topically, its use through the PDT method has been shown to have higher efficacy in a shorter duration. Therefore, the PDT therapy method using ALA agents deserves further exploration as a modality in treating AK and SCC. In a previous studies, ALA has been widely administered as a photosensitizer in PDT therapy. This is because ALA is known as a "prodrug" which has the ability to penetrate through the stratum corneum to various skin tumors, including basal cell (BCC) and squamous cell carcinoma (SCC). Aminolevulinic acid (ALA) could transform into an endogenous photoactive porphyrin derivate, namely protoporphyrin IX (PpIX). Porphyrins will then absorb light energy and convert it into a therapeutic effect on cancer cells [7, 10]. However, one of the challenges often faced in the application of ALA-PDT is its bioavailability. Therefore, ALA is better emulsified with nanoliposome compounds to increase its availability and efficacy in AK therapy.

2. Methods

Systematic search was performed through three different electronic databases (MEDLINE, EMBASE, and Web of Science) by two independent authors. This study includes clinical trials with more than 10 patients from each study, including those with multiple AK lesions. Studies which assess the efficacy of ALA-PDT and/or compare it to other interventions for AK were included in this review. Keywords “aminolevulinic acid”, “photodynamic therapy”, “actinic keratosis”, and their synonyms were used to search relevant articles. Finally, four eligible studies were included to be analyzed further in this review.

3. Result

Table 1. Previous studies on utilizing ALA-PDT in AK treatment.

Author, Year	Patients (n)	Follow-up period	Comparison	Result
Scola *et al.,* (2012) [11]	20	Visits were scheduled from baseline, a day, 4 weeks, and 3 months after treatment for both groups.	Carbon dioxide laser ablation	Significantly higher reduction of AK lesion was seen after three monts of PDT treatment (P=0.0362) compared to carbon dioxide laser ablation. Both ALA-PDT and laser ablation demonstrated significant reduction of Ki-67 and p53 protein expression from baseline (Ki-67, median 49.5%; p53, median 64.8%) were seen 4 weeks following therapy in both procedures. Overall, patients favored ALA-PDT due to its superior clinical result.
Ulrich *et al.,* (2021) [12]	50	Maximum of 2 field-directed PDTs using BF-200 ALA.	Vehicle	BF-200 ALA significantly outperformed the vehicle in terms of overall lesion clearance rates (86.0% vs 32.9%; P.0001) and patient full clearance per patient's side (67.3% vs 12.2%, P.0001). The total lesion recurrence rate after one year follow-up was significantly lower after PDT treatment over vehicle (14.1% vs 27.4% P=.0068). The cosmetic effect of BF-200 ALA/PDT was more satisfactory to patients than the vehicle/PDT.
Berman *et al.,* (2014) [13]	24	Monitored at day 57 or 71.	Ingenol mebutate (IM)	Patients treated in ALA-PDT group had a significantly higher mean lesion reduction from the baseline compared to IM group alone and the combination of IM and ALA-PDT (97.5% $P<0.00001$ vs 91.7% vs 86.7%). The group treated by ALA-PDT had a significantly lower mean composite LSR score compared to IM and ALA-PDT plus IM.
Dirschka *et al.,*	630	Monitored at 6 and 12 months after the last PDT.	MAL	BF-200 ALA has a lower recurrence rate compared to MAL treatment. BF-200 ALA showed complete clearance and remained completely cleared for at least

(2012) [14]	12 months after PDT were higher in those treated with ALA-PDT (47% for BF-200 ALA vs 36% for MAL).

A previous study by Reinhold et al., [15] compared ALA-PDT therapy with placebo against AK. After 12 weeks post-therapy, two sessions of ALA-PDT resulted in significantly better AK multilesion clearance than placebo by a fantastic ratio of 94.3% vs. 22% ($P < 0.0001$). In addition, the cosmetic results of the lesions also showed significant improvement in the skin surface (repair of scaly, rough, scaling skin), hyper- and hypopigmentation, atrophy, to scarring in AK patients on ALA-PDT.

Another study by Ko et al., [16] also showed changes observed in patients with AK scale 3 (severe) before treatment and 12 months after 2 sessions of ALA-PDT therapy. It can be observed in Figure 3 that the severity of AK was drastically reduced and there were significant histopathological changes in the affected area. This study also compared the recurrence rates experienced by patients in a 12 month period between post-ALA-PDT and MAL-PDT therapy. The results showed that ALA-PDT had a significant difference compared to MAL-PDT, which only caused about 15.4% of recurrences compared to MAL-PDT (20.9%) ($P<0.05$).

One study by Scola et al., [11] also stated that ALA-PDT therapy could significantly reduce the expression of mutant-type p53 found in keratinocytes. The p53 mutations found in both AK and SCC suggest a central role in the transformation of malignancies from AK to SCC. In Figure 4, it can be observed that there was a decrease in p53 expression after ALA-PDT compared to baseline, indicting the process of slowing down the transformation of AK to SCC by ALA-PDT therapy.

4. Discussion

4.1. Biomechanisms

After topical adminstration of nanoemlusified ALA, ALA will penetrate target cells or transformed keratinocytes in the skin. Then, blue light as an ALA activator emitted by PDT will damage pretumor cells and those that have developed into tumors through several mechanisms: induction of apoptosis, blocking blood vessels of target cells, anti-inflammatory, immunosuppressive, anti-tumor, and modulation of the immune system. Following that, ALA-PDT will modulate the AK state by accelerating neocollagenesis, stimulating fibroblast production, and inducing apoptosis in target cells [7, 10, 17].

In the process of neocollagenesis, previous literature states that the application of ALA using the PDT method can accelerate skin remodeling through producing type I and type III pro-collagens. Pro-collagen will then increase skin collagen density and epidermal thickness, thereby repairing skin damaged by AK. In fact, previous in vivo studies suggest that elevations of type I and type III pro-collagen can be observed on biochemical and immunohistochemical analyzes that persist for at least 6 months after therapy [9, 18].

In addition, the induction of apoptosis in diseased tissue can occur not only due to the presence of ROS emitted by PDT, but also by blocking the vascularization pathway that nourishes the target cells. Both of these processes will then lead to the eradication of AK lesions and immune system modulation such as recruitment of dendritic cells, neutrophils, macrophages, and T cells. Moreover, ALA-PDT can produce a selective effect, where only diseased tissue undergoes apoptosis, without compromising healthy cells [8, 19].

In addition to these mechanisms, as described in the previous section, ALA can be transformed into protoporphyrin IX (PpIX). Porphyrins will then absorb light energy in the heme sequestration process which plays a role in anti-tumor activity in AK and SCC. In previous studies using mice, heme sequestration proteins (HeSPs) were found to decrease oxygen and ATP levels in tumor cells, thereby decreasing their ability to survive. In fact, HeSPs also decrease a key protein of tumor cell development, modulator of angiogenesis, and

decrease the density of blood vessels in tumor cells. This demonstrated that HeSPs induced by ALA-PDT can inhibit oxidative phosphorylation in tumor cell mitochondria to reduce tumor progression and progression [18].

4.2. Preparation Prior to Application

Prior to ALA-PDT therapy, the skin was pretreated by using stripping tape, microdermabrasion, and moderate acetone degreasing to reduce the thickness of the stratum corneum and enhance ALA absorption by the skin. The 5-ALA is approved by FDA for dermatologic treatment after a 14-18-hour incubation period and subsequent photoactivation with blue light. Longer incubation durations may enhance the adverse effects degree following ALA-PDT. However, shorter incubation hours (1-3 h) have shown comparable effectiveness in the clearance of AK lesion. Depending on the investigation, the wavelength of light employed ranges from 410 nm to 635 nm. However, in AK photorejuvenation, the blue light source was shown to be more successful in activating PpIX utilizing ALA-PDT [20].

4.3 Application

Nanoemulsified ALA cream with a dose of 10-20% was applied to patients' skin and treated for 4 hours using red or blue light radiation ranging from 570–670 nm. The ALA-PDT was radiated 20 cm from the target site using 76 J cm2 of total light dose for 20 minutes in each lesion. As a patient comfort measure, an air conditioner and a refrigerated conductive gel were used during the photoactivation process, and a cooling fan was used during exposure to blue light. In a longer incubation period, oral analgesics consumption, topical lidocaine, and ice packs can be used to increase patient comfort [12].

Following PDT treatment, applying an aloe vera-based gel to the treated skin area provides soothing effects for irritation and erythema. In order to avoid phototoxicity during the day, patients can be scheduled for treatment in the afternoon, so that they can leave the clinic at dusk. Patients are given protection according to the area being treated, such as the use of sunglasses when treating the facial area and used during the trip home to avoid the sun. Patients were instructed to avoid sunlight for 36 hours after treatment. The patient returned to the clinic at the interval of 1 week and 2 months after PDT for routine follow-up. Subsequent ALA-PDT sessions can be performed at 1-2 month intervals. In the treatment of AK, two sessions of ALA-PDT are required for optimal therapeutic results [3, 13].

5. Conclusion

Therapy using ALA-PDT in AK has a great potential to prevent its progression to SCC through the following mechanisms: accelerated neocollagenesis, stimulated fibroblast production, induces apoptosis in target cells, immunosuppression, anti-tumor, and modulation of the immune system. Its non-invasive approach at an affordable cost able to clear the manifestations of AK and is superior in terms of minimizing the recurrence incidence compared to other therapies such as MAL-PDT, vehicle/PDT, and ingenol mebutate. To enhance its bioavailability, nanoemulsified ALA can be applied in AK therapy.

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