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**BASIC SCIENCE REVIEW**

# 5-Aminolevulinic Acid-Mediated Photodynamic Therapy on Wound Healing: A Systemic Review of Human Evidences

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**Backgrounds:** Skin wounds are a kind of refractory disease frequently encountered in clinic, which brings enormous burden to patients. Great efforts to address the dilemma of wound healing have yielded some encouraging results, but they are still unsatisfactory. 5-aminolevulinic acid (ALA)-mediated photodynamic therapy (PDT) has been developed as a promising noninvasive treatment for skin wounds. A systematic review was performed to determine the existing evidence base for the clinical use of ALA-PDT on refractory wounds.

**Methods:** We conducted a PubMed search for English literature related to the clinical treatment of human skin ulcers by ALA-PDT published from 2012 to 2022, and performed a systematic review.

**Results:** 25 studies were ultimately selected in the present review and summarized, including six studies of skin cancer, five studies of chronic leg ulcers, three studies of erosive pustular skin diseases, and two studies of erosive oral lichen planus symptoms. A total of 335 patients had

approximately 355 ulcers, of which 315 ulcers received photodynamic therapy and 276 improved with ALA-PDT. 20% ALA solution, wavelength between 600 nm and 670 nm, energy density of 120 J/cm$^2$, frequency of once per week and 3 sessions were the most selected therapeutic parameters. Generally, ALA-PDT for wounds was effective with main adverse events of mild to moderate pain, and follow-up was generally conducted within 1 year.

**Conclusions:** This systematic review summarized the commonly used therapeutic parameters for ALA-PDT in the clinical treatment of skin wounds. After ALA-PDT alone or ALA-PDT combined with curettage, antibacterial and surgical treatment, all wounds healed well, and the adverse reactions were mainly mild to moderate pain, which could be relieved by cooling. ALA-PDT had proven to be a promising wound treatment modality through evidence of safety and efficacy.

Skin wounds are considered to be a common refractory disease encountered by dermatologists, mainly caused by trauma, surgery and various diseases. The management of skin wounds is a major public health problem worldwide, especially chronic wounds, which impose a heavy physical and financial burden on patients and have a profound deleterious impact on their quality of life[1, 2]. The prevalence of chronic wounds in developed countries occupied approximately 1-2% of the total population[3]. Among the 8.2 million medicare beneficiaries in 2014, skin wound management costed $28.1 billion to $96.8 billion[4], which was the main consumption of medical resources and patient well-being.

Extensive efforts have been made to address the dilemma of wound healing, including debridement, antibiotics, dressings, offloading, surgical revascularization, autologous flap transplantations, hyperbaric oxygen, negative pressure, growth factors, gene therapy and stem

cell therapy, etc[5-10]. Although all of these wound care techniques are proved to have achieved encouraging performance in terms of enhancing cutaneous wound healing, it is worth noting that they possess high risk of high cost, scarring formation, abnormal pigmentation and are not consistently effective[10-12]. Therefore, it would be highly desirable to identify more efficient and economically therapeutic regimens for wound healing.

Photodynamic therapy (PDT) has emerged as a promising, non-invasive, minimally invasive modality. It was proposed by Oscar Rabb in 1900 and has developed rapidly in various medical specialties since the 1960s[13]. The mechanism of PDT involves a combination of non-toxic dyes called photosensitizers (PSs), molecular oxygen, and specific wavelengths of light to photochemically produce reactive oxygen species (ROS) to selectively cause cell death in target tissues or have immunoregulation[14]. PDT has shown successful outcomes in the clinical treatment of certain skin diseases, including acne vulgaris, rosacea, genital warts and superficial tumors[15]. The improvement of wound healing by PDT may be mainly attributed to its microbicidal effect. Literature support the antibacterial use of PDT in bacterial infections where conventional treatment has failed[16-19]. The potential of PDT antimicrobial therapy involves the management of superficial pathogens including fungi, bacteria and mycoplasma.

The success of PDT is closely related to the photophysical and photochemical properties of PSs[20]. PSs are dyes that absorb energy from a light source and transfer it to another molecule. One of the major PSs currently used in clinical trials is 5-aminolevulinic acid (ALA) approved by the Food and Drug Administration (FDA), which is widely used for acute and chronic wounds[21, 22]. Compared with other PSs, ALA is a natural intermediate in the heme biosynthesis pathway and can be converted into high levels of protoporphyrin IX (PpIX) in bacteria[23]. More

importantly, ALA has outstanding properties of high penetrability, selective accumulation in target tissues, and rapid metabolism[24, 25], which allow it to penetrate the matrix and selectively accumulate to compromise the target cells such as bacteria. We previously demonstrated that ALA-PDT had antimicrobial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) planktonic strain in vitro and and further confirmed its safety in the treatment of MRSA-infected mouse wounds[26, 27].

Recently, many studies have shown that ALA-PDT improves wound healing in humans. Human studies have suggested that ALA-PDT has an antimicrobial effect on bacteria isolated from chronic wounds in infected humans, improves ulcer healing and is well tolerated[28, 29]. Thus, ALA-PDT may be a suitable alternative approach for various types of wounds. It remains a great challenge to explore the optimal therapeutic parameters of ALA-PDT and fully explore the potential of ALA-PDT in the treatment of skin ulcers caused by various skin diseases in clinical practice. However, at present, no review of ALA-PDT application for skin ulcers has been ever conducted to systematically assess the clinical efficacy, regiments and tolerance of ALA-PDT therapy. Current studies on the promotion of ALA-PDT in human skin ulcers have focused on a few case reports and few clinical studies. Here, we performed an evidence-based review of the efficacy, therapeutic parameters and side effects of ALA-PDT on various human skin wounds, including bruising, burns, infections, tumors and other diseases. It is hoped that this review will promote the clinical application and development of ALA-PDT on skin ulcerative diseases.

## Evidence synthesis

### Study design and focused question

The systematic review is organized in accordance with the Preferred Reporting Items for Systematic Review and Meta-Analysis（PRISMA）guidelines illustrated in figure 1[30], which improves the reporting quality of the systematic review. The addressed PICO question was: Is ALA-PDT effective and safe in the management of cutaneous wounds?

**Search strategy**

As schematized in figure 1, a bibliographic search of the National Library of Medicine's PubMed database was accessed from 2012 to 2022, using Boolean operators with the following keywords in different combinations: "photodynamic" OR "photochemotherapy" OR "photochemistry" OR "photosensitizing agents" OR "photosensitizer" AND "wound" OR "ulcer" OR "burn" OR "excision" OR "injury" OR "abrasions" OR "pressure sores" OR "re-epithelialization" OR "regeneration" OR "erosive" OR "fistula" OR "tissue repair".

**Inclusion criteria**

Titles and abstracts that met inclusion criteria were screened and evaluated (Original studies; Clinical trials; Case report; Written in English; Published between 2012 and 2022), initially incorporating literature related to ALA-PDT and wound healing in human skin.

**Exclusion criteria**

Studies that did not fit the inclusion criteria and were correlated with other branches (other languages than English, guidelines, opinion articles, comments to the editor, letters to the editor and conference abstracts, review, interviews, updates, errata and unpublished studies) were excluded.

**Study selection and data extraction**

All extracted data were crosschecked by two individual authors (Jianhua Huang and Hongwei Wang), who independently conducted literature searches via database, assessed potentially relevant studies and performed data extraction based on eligibility criteria. Any discrepancy between reviewers involving study inclusion was resolved by discussion until consensus was reached. The data extracted from the accepted studies were tabulated on the following topics: title, previous treatment, study design, study groups, patients, wound, location, bacterial species, photosensitizer, administration route, incubation time, light source, wavelength, intensity, energy fluency, combined management, sessions, follow-up, outcome, assessment, safety evaluation, pain relief methods, author (year). Any data that was not available was stated in the form using NA.

**Quality assessment**

The systematic review went through several phases, beginning with reliance on an authoritative database to provide accuracy in publication searches. Special care was taken in determining key words, which were jointly decided by multiple researchers and had good sensitivity and specificity. Discussions were held to determine whether those selected publications should be retained in this review. Publication identification criteria are monitored and discussed to ensure robustness and rigor of research findings.

**Data Synthesis**

As explained in the results section, a meta-analysis could not be performed due to methodological heterogeneity of the included studies, such as variations in study groups, laser/photosensitizer parameters. Therefore, the pattern of the present study was mainly summarized information. For all included studies, data were narrated and tabulated.

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Fig. 1. Flow diagram of studies screening and selection process for the systematic review according to the PRISMA guidelines.

**Evidence synthesis**

**Results of literature search and selection**

Based on primary search on Pubmed, initially 170 potentially eligible records were identified from 2012 to 2022. Elimination of reviews and non-English written literature resulted in 154 articles. After screening titles and abstracts, a total of 125 articles that did not meet the inclusion criteria were excluded. Of the remaining 29 full-text articles that underwent assessment for eligibility, 4 papers were eliminated as irrelevant to the objective of the review. Therefore, 25 studies were ultimately selected in the present review and summarized in Table 1 (Fig. 1).

**Characteristics of the studies**

***Study design***

Study characteristics were summarized in Table 1. A total of 25 papers consisted of 9 case reports[16, 18, 19, 31-36], 5 pilot study[17, 29, 37-39], 3 case series[40-42], 5 randomized controlled trials[28, 43-46] and 3 prospective observational studies[47-49]. Across the 25 included articles, a total of 335 patients were enrolled in all studies, ranging in age from 15 to 93 years.

***Disease profile***

Data on this table showed that studies assessed the clinical application of ALA-PDT on different types of human skin wounds, including six studies of skin cancers[31, 33, 36, 37, 43, 44], five

studies of chronic leg ulcers[28, 29, 31, 39, 46], three studies of erosive pustular skin diseases[16, 38, 42], and two studies of bilateral erosive oral lichen planus symptoms[40, 41].

***Number distribution of patients***

All of the studies exclusively enrolled the patients only with skin wounds that were deemed "refractory" or had not responded well to one or more therapies. 335 patients had a total of approximately 355 ulcerative lesions, of which 315 ulcers underwent photodynamic therapy, and 276 improved after ALA-PDT treatment. A total of 164 men (56.6%) and 126 women (43.4%) were treated in studies reporting the sex of the subjects. The mean age of subjects was about 55.41 years. Demographic information in each study was presented below by literature type: Case reports: 1-3 patients, aged 15-80 years, 7 males and 4 females; Prospective studies: 10-33 patients, aged 18-65 years, 58 males and 34 females; Pilot studies: 5-19 patients, aged 22-91, 12 males and 5 females; Randomized controlled studies: 18-76 patients, 35-85 years old, 80 males and 60 females; Case series: 8-12 patients, aged 20-93, 7 males and 23 females.

In terms of population distribution of patients, skin cancers (105 patients)[31, 33, 36, 37, 43, 44], leg and foot ulcers (62 patients) [28, 29, 31, 39, 46] and anal fistula (59 patients)[48, 49] were the most common diseases in the population, while carbuncle[32], chromosomiasis[18], leishmaniasis[34], hidradenitis suppurativa[35], and ulcerative necrobiosis lipoidica[19] were only a small subset with only one case in each study. CO2 laser injury (33 patients)[47], erosive oral lichen planus (22 patients)[40, 41], Bowen's disease (BP) (18 patients)[45], erosive pustular dermatosis (14 patients)[16, 38, 42], furuncle and abscess (4 patients)[17], trauma (3 patients)[17] were moderate.

**Previous treatments**

The majority of patients receiving ALA-PDT had attempted 1 or more of the following previous treatments: dressings, topical steroids, topical dapsone, topical platelet-derived growth factor, antimony acid magnesium injection, oral corticosteroids, antibiotics antibacterial treatment, fungi, topical 0.1% tacrolimus, external use traditional Chinese medicine, cryotherapy, infrared light, narrow-band UVB phototherapy, radiosurgery, debridement, incision drainage, curettage, skin graft and fistula surgery[16-19, 29, 31, 34, 38, 42, 46, 48]. As reported in the studies, all of the previous treatment options covered above were switched to or combined with ALA-PDT in response to failure or poor response in the subjects. Interventions to remove scabs and scales, such as curettage and debridement, were essential pretreatments prior to ALA-PDT[18, 29, 31, 38, 42]. However, some selected studies did not provide the information regarding previous treatments[28, 32, 35-37, 39-41, 43-45, 47, 49]. One case report clearly stated that the patient had not received any treatment prior to PDT[33].

**Photosensitizer parameters**

There was a significant methodological heterogeneity in photosensitizer formulation, photosensitizer concentration, incubation time, interval, laser excitation wavelength, light dose, and mode of light delivery (Table 1).

***Photosensitizer concentration***

The concentration of PSs application is associated with the overall effect of PDT treatment. It is worth to note that higher concentrations of photosensitizer does not necessarily contribute to bacterial elimination or photodynamic efficacy correspondingly. To optimize efficiency of PDT, a range of PSs concentrations were investigated[26, 50]. We previously measured the fluorescence intensity after topical application by different ALA concentrations in a mouse

model of ulcer and preliminarily concluded the appropriate concentration of 10% to 30%[27]. Additionally, high concentrations also induce imperative cytotoxic effect of PSs. We reported dark toxicity of ALA at 5 and 50 mM after 12 h dark incubation[26]. A variety of ALA concentrations were included in this systematic review. 20% ALA was used in 14 studies[16-18, 28, 29, 32, 33, 38, 39, 42, 44-47], with other concentrations being 10%[19, 36, 37, 43], 5%[35, 41], 4%[40], and 2%[31, 48, 49]. This result also accorded with our animal experiment groping basically.

***Photosensitizer formulation***

The topical formulations of ALA exert great impact on the penetration of ALA and the amount of generated porphyrin. For example, saline or 10% DMSO proved to be the most efficient vehicle for porphyrin accumulation in tumor, superior to creams and liposomes[51]. The ALA forms used in this systematic review varied by solutions, emulsions, creams, gels and ointments. ALA solutions were used in the majority of studies (14 studies, 182 subjects)[16, 29, 31, 32, 37, 42-47, 49], followed by ALA gels (5 studies, 91 subjects)[33, 34, 38-41, 48], emulsions (2 studies, 17 subjects)[17, 28], creams (3 studies, 3 subjects)[18, 35, 36], and ointments (1 study, 1 subject)[19]. This might be due to the superior homogeneity and cutaneous permeability of the solution compared to creams or gels[52].

***Incubation time***

Most researchers chose 4 h under occlusion as an incubation time (9/25)[28, 29, 32, 33, 35, 36, 43, 44, 47], while 2 h was preferred in 6 studies (6/25)[16-18, 41, 48, 49]. The remaining incubation periods (1.5 h[46], 3 h[19], 5 h[45] and 6 h[31]) were relatively unpopular, while four studies[34, 37, 39, 40] did not describe the incubation time. The exposure time of the wound to PSs in dark is one of the determinants affecting treatment protocols. Practically, a sufficiently

long incubation time ensures adequate absorption and quantity of PSs in target cells. With the extension of incubation time (before saturation), PDT becomes more antibacterial[53]. Our previous in vivo studies verified that the production of PpIX increased significantly after incubation in ALA for 3 h and reached the highest level after 6 h[26, 27]. Therefore, 4 h is reasonable for ALA incubation in wounds as implied in this review.

***Administration route***

PSs can be administered intravenously, orally, or topically, depending not only on the photosensitizer type but also on the disease. Systemic PSs leads to degeneration of microcirculation, which is suitable for the treatment of tumors due to its avoidance of possible metastases[54]. Local application of PSs poses a minimal effect on blood vessels. Such local vascular preservation is essential for wound healing, and as a result, external application of PSs is believed to be the favorable route[55, 56]. Most studies in present review preferred topical delivery for ALA, with the exception of two studies[34, 39] that did not reveal the route of administration. For those patients with carbuncles[32], abscesses[17], laser holes[47] and anal fistulas[48, 49], gauze or cotton balls soaked in ALA solution were inserted into the cavity to achieve adequate administration.

**Photochemotherapy parameters**

The types, wavelengths, power densities, energy densities and treatment schemes of light delivery used in the included articles were detailed in Table 1.

***Light sources***

A variety of light sources have been used in conjunction with PSs, such as lasers and light-emitting diodes (LEDs)[57, 58]. The laser is a high-fluency monochromatic light

corresponding to the specific wavelength of maximum absorption of PSs, which works with precise focus without causing considerable damage to adjacent tissues[59]. LEDs are a light source composed of interconnected electronic components with strong reliability, high power and easy-to-use property. LEDs have advantage over lasers in that they can provide a wider range of radiation treatment over a larger area of the skin surface[60]. Overall, the included studies were carried out mainly with lasers (11/25)[17, 28, 32, 33, 40, 45, 47-49] and LEDs (9/25)[18, 19, 29, 35-37, 39, 41, 43, 46]as radiation sources, with the exception of three studies based on a blue light (BLU-U; DUSA Pharmaceuticals)[16, 38, 42] and one study that used a lamp[31]. The type of light source could alter the energy output mode of light during PDT process and consequently influence effect of PDT. For example, seven studies performed interstitial irradiation with optical fibers introduced deep into sinus tracts[17, 32, 33, 41, 47-49]. Light source details were not displayed in two studies[34, 44].

***Irradiance***

It is worth emphasizing that too high irradiance is prone to injury, pain and even deterioration of the condition, so the irradiation time of high irradiance is shorter, while that of low irradiance is longer. A considerable variation was observed in the irradiance parameters among the included studies. Seventeen studies[17-19, 29, 31-33, 35-37, 41, 43-45, 47-49] described irradiance with a range of 20 mW/cm$^2$[31] to 1 W/cm$^2$[49], among which a power density of 100 mW/cm$^2$[32, 37, 45, 47] was universally employed. Eight studies[16, 28, 29, 34, 38-40, 42, 46] did not report irradiance.

***Wavelength***

The light source with appropriate wavelength should be selected according to the absorption of PSs[61]. Red light penetrates deeper than the green and blue which penetrate tissue least

efficiently. Thus, most wavelengths for PDT are based on the visible red range (620-720 nm)[62]. Despite weak penetration of 410 nm, it is considered beneficial for disinfecting infected skin wounds or other infectious surfaces[60]. The wavelengths in this review ranged between 417 nm and 780 nm, with the majority between 600 nm and 670 nm (20/25)[17-19, 28, 29, 32, 33, 35-37, 39-41, 43-49]. Ultraviolet light (417 nm) was used in two experimental schemes[38, 42]. Another study[31] employed a wavelength of 560-780 nm and no access to wavelength information occurred in two studies[16, 34].

***Fluence***

In addition to wavelength, the effectiveness of PDT highly depends on the dosimetric parameters applied, which is related to irradiance and light exposure time. Our animal investigation showed that 25 J/cm$^2$ was more favorable than 75 J/cm$^2$ or 100 J/cm$^2$ for PDT in the treatment of skin ulcers, suggesting the avoidance of excessive injury caused by high energy density[27]. In this review, the delivered light doses varied from 10 J/cm$^2$ to 600 J/cm$^2$ among the studies, with the most common fluence falling around at 120 J/cm$^2$[29, 32, 36, 37, 43, 47]，followed by 80 J/cm$^2$[28, 39, 40, 46]. The study that did not directly show the energy density could be derived from time and irradiance. Since one study[16] only gave the illumination time (16 min and 40 s) without irradiance, the fluence could not be obtained. Another study[34] failed to show dosage of light.

***Sessions and frequency***

The ultimate efficacy of PDT is also owed to the proper number of session and frequency. It might be due to the difficulty of maintaining anti-microbial effects with a single application of ALA-PDT, and thus, multiple ALA-PDT sessions are generally required. In the present review, the sessions varied greatly: generally 1-6 times, and the vast majority are 3 times[18, 29, 32, 39, 45,

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47], followed by 10 times[28, 41], 9 times[35], 24 times[34]. ALA-PDT were delivered at 3-4 days to 1-2 months intervals. The treatment intervals in most studies were set at one week (9/25)[29, 32, 33, 37, 46, 47], 3-4 days[34, 39, 43, 44], while a few intervals were 2 weeks[19, 36], 10 days[17, 18], 10-15 days[35] or 1-2 months[31]. The unavailability of detailed description of treatment sessions and intervals was presented in other selected studies[16, 28, 38, 40-43, 45, 48, 49] (Table 1).

## Overall outcome

### *Assessment*

The primary endpoint evaluated as efficacy for PDT differed among the studies. Most articles were designated to evaluate wound areas (including wound surface conditions) before and after ALA-PDT. The results of treatment with ALA-PDT were evaluated by wound size（11/25）[17, 28, 29, 31, 37, 39, 41, 43-45, 49], morphological aspects（10/25）[19, 32, 33, 35-37, 42, 45, 47, 48], Visual Analogue Scale (VAS)（7/25）[19, 29, 37, 39, 40, 48, 49], histopathological examination（6/25）[36, 39, 42-45], recurrence（6/25）[29, 31, 32, 36, 45, 48], bacterial culture（5/25）[17, 28, 29, 31, 45], Numeric Rating Scales (NRS)（2/25）[28, 34], blood tests（2/25）[28, 45], cosmetic satisfaction（2/25）[44, 47], quality of life measures（1/25）[37], mycological detection（1/25）[18], REU (reticulation, erythema, ulceration) scoring system（1/25）[40], recurrence（2/25）[29, 31], hyperpigmentation（1/25）[43], urine tests（1/25）[45].

### *Wound healing*

All included studies variably evaluated clinical response to ALA-PDT (Table 1). Due to design heterogeneity, it was impossible to directly compare the efficacy of these studies. On the whole, almost all studies reported significant clinical regression of ALA-PDT on wound healing with a wide range of response among the studies. Some studies[17-19, 29, 31-34, 37] achieved cure

outcome by claiming “healed”, “healed completely”, “fully cure”, “clinical cure”, “basically healed” and “fully healed” without specific rates, and others demonstrated high healing rates with explicit response percentages (40%[28], 50%[31], 97%[47], 65.3%[48], 72.73%[45] and 80%[49], respectively). Healing outcomes in five studies[16, 35, 36, 38, 42] were reported by means of lesion clearance, reporting as “resolved”[16, 42], “cleared”[38], “disappeared”[36] and “resolution”[35]. Reduction in wound areas after ALA-PDT treatment were presented in three studies[17, 31, 39]. The remaining recovery endpoints received were reported as an increase in cosmetic satisfaction[44, 47], improvement in signs and symptoms[19, 32, 33, 35-37, 40, 42, 47].

***Randomized controlled trial***

In addition to case reports, there were few randomized controlled trials (RCT) of ALA-PDT on human skin wounds. Through our literature search, we found that in the past 10 years, there were only 5 RCT among 25 studies[28, 43-46]. RCT demonstrated that ALA-PDT groups were superior to the control group in treating skin ulcers caused by various diseases. One RCT study[44] compared a cohort receiving ALA-PDT supplementation after primary surgery with a cohort receiving secondary resection and found that the former was favorable in terms of financial burden, healing time, and cosmetic satisfaction at 8 months’ follow-up. Another RCT study[32] indicted a complete remission of 72.73% (8/11) with an overall clearance of 90.91% and a recurrence of 9% in the group of CO2 laser combined with ALA-PDT, while a healing rate of 63.63% (7/11) with a total clearance of 54.55% and a recurrence of 45.45% was detected in the CO2 laser alone group. The remaining three RCT respectively reported an antibacterial advantage of ALA-PDT over red light alone[46], a significant improvement in the ALA-PDT group over the octinidine salt treatment group exposed to placebo light[28], and improved survival and cosmetic satisfaction in patients with skin cancer when combined with surgery[43].

However, it is worth noting that the sample sizes of non-cancerous chronic ulcers were insufficient, with only 20 and 26 patients[28, 46], respectively, and further large-scale RCT are needed to demonstrate the outstanding efficacy of ALA-PDT.

***Histological analysis***

Histological examination was performed to better elucidate the response to ALA-PDT. Immunohistochemistry demonstrated the increased expression of BDCA2-positive plasmacytoid dendritic cells, MHC-II positive dendritic cells, TNF-α positive mast cells, TGF-β, CD4+/CD25+ T-Reg cells[39], and the inhibition of CyPA, CyPB and CD147 after ALA-PDT[43]. Furthermore, the clearance of tumor cells by ALA-PDT could be better exhibited histologically[36, 44]. Histological outcome of Bowen's disease revealed more uniform and normal skin architecture after treatment with ALA-PDT[45].

***Bacteria load***

Additionally, microbiological endpoint should be investigated to appreciate the outcome. Microbial infection in conjunction with wounds is one of the common complications that delay wound healing[63]. A large accumulation of microbial entities in the wound is succeeded by an infiltration of immune inflammatory cells. If not cleared, a persistent chronic inflammatory response may prevent tissue repair[64]. The common regiments for infected wounds, such as debridement, antibiotics, and dressings, have become increasingly less effective, because of the increasing emergence of multi-resistant bacterial species, alarming health care workers around the world of the need for better treatments. PDT represents a promising infection-fighting alternative that can overcome bacterial antibiotic resistance, and its indications have expanded to bacteria, fungi and viruses[65]. PDT mediated by singlet oxygen and free radicals interacts with multiple cellular structures and metabolic pathways in microbial cells, and this multi-target

effect significantly reduces the possibility of drug resistance[66]. In addition, PDT follows the principle of photoactivated non-toxic photosensitizers, and the preferential uptake of photosensitizers by rapidly proliferating cells means that PDT dose not harm host cells [67].

The most common culprits of infected wounds were Staphylococcus aureus[17, 28, 29, 31] and Pseudomonas aeruginosa[28, 29, 31, 46]. A number of studies[17, 18, 28, 29, 31, 46] that performed microbiological evaluations consistently demonstrated the inactivation action of ALA-PDT, both against fungi and bacteria. The photodynamic inactivation of ALA-PDT was carried out to treat wounds infected by the following microorganisms: Citrobacter freundii[29], Serratia marcescens[29], Enterobacteriaceae[28], Streptococcus pyogenes[28], E.aecalis[31], Morganella morganii[31], Mycobacterium abscessus[17], Acinetobacter baumannii[17], Finegoldia[17], Finegoldia magna[17], Mycobacterium ulcerans[17], Mycobacterium chelonae[17], fungus[18], Phlebotomine sandflies[34]. Microbial loads and species appraisal were conducted in six studies[17, 18, 28, 29, 31, 46] and were unavailable in the remaining 19 studies. Both fungi and bacteria were identified mainly by culture, and the culture in two studies[29, 46] were found to be accompanied by a VITEK-60 automated microbiology analyzer. Overall, the present review indicated that the use of ALA-PDT provided a considerable reduction in bacterial levels, which contributed greatly to the success of tissue repair.

**Follow-up**

The treatment responses were evaluated at various follow-up periods across studies, varying from one month to four years. In some studies[31, 38, 47], follow-up period of several months appeared to be insufficient when evaluating the wound healing results and a long-term follow up might have yielded better outcomes. Most patients were followed up within 1 year after ALA-PDT, for example, 4 months[16], 1 month[34, 46], 3 months[17, 18], 6 months[19, 45] and 8

months[28, 44]. The researchers preferred a one-year follow-up period, rather than longer or shorter time[33, 36, 37, 41, 43, 48], and only one study followed subjects for four years[40]. Follow-up information was not available in three other studies[32, 35, 39]. Recurrence was not observed in almost all follow-up periods.

**Combination therapy**

Unfortunately, bacterial or fungal infections associated with skin ulcers are often deeply localized and difficult to treat, resulting in low response to antibiotics alone and high recurrence rates. Some surgical and physical therapy methods have emerged in combination with antimicrobial agents, including surgery, laser therapy, hyperthermia and PDT[68, 69]. The antimicrobial efficacy of PDT alone for deep infection is considered unattainable. Therefore, photodynamic combined with antifungal agents was warranted to achieve better outcome, as seen in the included studies[70]. In present review, discrepancies of concurrent therapies with ALA-PDT were observed in 14 studies, including mild curettage (3 cases)[16, 38, 42], drainage (2 cases) [32, 44], antibiotics (4 cases) [17, 29, 48, 49] antifungal drugs (2 cases) [18, 34], CO2 laser (2 cases)[45, 47], surgery (1 case)[36] and corticosteroids (1 case) [36]. The remaining 11 studies[19, 28, 31, 33, 35, 37, 39-41, 43, 46] did not specify combined management.

**Adverse events**

Safety assessment of ALA-PDT was reported in 24 studies, except for one[44] that was not accessible. One study reported no adverse effects after ALA-PDT[38]. The most common adverse reaction was mild to moderate pain and basically tolerable, usually represented by a visual analog pain scale score, reported in 9 studies[19, 28, 29, 34, 37, 39-41, 49]. Except for one study involving a patient who dropped out of the study because of severe pain[37], no other studies were interrupted or stopped because of pain[18, 33, 36, 46]. Five studies stated that

pain was more severe[19, 28, 37, 40, 41], with the highest NRS at 10[28] and VRS at 9[37]. To mitigate pain during illumination process, some researches presented pain relief approaches[28, 29, 32, 34, 35, 37, 47-49]. Three studies adopted cooling approaches, namely, cooled fiber optic[28], dynamic cold air[47] and cold wet compress[35]. One study gave subjects oral nonsteroidal anti-inflammatory drugs (NSAIDs)[29, 48], whereas the other patients were relieved by use of anesthetics[34, 37, 49] and glucocorticoids[32]. It appeared that above strategies could play a vital role in reducing pain. Other local reactions were most commonly consisted of swelling[18, 28, 32, 35, 37, 43, 46, 47], erythema[16, 28, 32, 37, 43, 46, 47], burning[17, 18, 29, 40, 43, 45], pigmentation[17, 18, 40, 47], tingling[31, 45], exudation[18, 29], blisters[17, 32], erosion[17], fever[48] and phototoxicity[48], which generally subsided quickly.

**Differences in treatment**

Admittedly, the parameters and efficacy of photodynamic therapy certainly fluctuated between different diseases. As described above in the profiles of diseases, the types of literature included in this paper were mainly skin cancer and chronic skin ulcers, therefore, their photodynamic therapy differences were compared. The fluence required for skin cancer was relatively high. 120 J/cm$^2$ was reached in three literatures[36, 37, 43], while only one for chronic skin ulcer[29], which might be due to tumor cells requiring greater energy to be destroyed. Undoubtedly, the follow-up time after treatment was longer for neoplastic ulcers, with one year in four studies [31, 33, 36, 37, 43], while only two studies with one-year follow-up for chronic skin ulcers[29, 31]. Finally, the evaluation of cancerous ulcers emphasized pathology[36, 43, 44], whereas benign ulcers focused more on the reduction of wound bacteria[28, 29, 31, 46]. Therefore, photodynamic therapy for neoplastic ulcers was antibacterial and antitumor. Except for the slight differences in fluence, follow-up time and assessment method, no significant variations were found in other parameters, such as ALA concentration, incubation time and

sessions, which might be attributed to the insufficient number of diseases in this review, and a larger scale investigation was needed in the future.

**Limitations**

The main limitation of our present review was the heterogeneity (wavelength, power density, energy density, duration of exposure, frequency and number of sessions) among the regiments and the small number of studies included, not allowing a statistical analysis of the data in the form of meta-analysis. Regarding data search, we used PUBMED, one classic search engine, and other databases such as SCOPUS and EMBASE might contain several documents that meet the inclusion criteria. We made our effort to include literature on wounds, but it was inevitable to omit those could not be identified as “wound” from the name of the disease, especially those tumors and infectious diseases. Finally, the linguistic limitations of studies available in the English language might inevitably result in the unavailability of relevant publications in other languages.

**Conclusions**

Based on the results of the selected studies, this preliminary systematic review demonstrated beneficial role of ALA-PDT alone or in combination with lasers, antibiotics, antifungals, and antiparasitic agents in the management of wounds in clinic with few or no side effects. Further systematic reviews with large numbers of patients, more databases, and longer time spans are warranted.

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