A clinical study of photodynamic therapy for chronic skin ulcers in lower limbs infected with Pseudomonas aeruginosa

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Received: 26 May 2014 / Revised: 21 September 2014 / Accepted: 27 October 2014 / Published online: 20 November 2014
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**Abstract** The objective of this study is to evaluate the antimicrobial activity and healing-promoting effect of topical photodynamic therapy (ALA-PDT) on chronic skin ulcers infected with *Pseudomonas aeruginosa* (PA). A total of 26 patients with chronic skin ulcers in lower limbs infected with PA were enrolled. The surface areas of the ulcers were treated with either δ-aminolevulinic acid (ALA)-mediated PDT (20 % ALA solution, 1.5 h incubation, 630 nm red light, 80 J/cm²) or red light alone, both once a week for two weeks. Before treatment, the wound areas and the bacteria levels in these two groups were comparable (*p* > 0.05). Results indicated that the bacteria levels in the skin ulcers of the light only group of 24 h post-treatment (3.4 × 10⁷ ± 7.1 × 10⁷ CFU/cm²) and pre-treatment (5.5 × 10⁷ ± 1.6 × 10⁸ CFU/cm²) were not significantly different. In contrast, the bacteria levels on the surfaces of the ulcers in the PDT group of 24 h post-treatment (6.3 × 10⁵ ± 1.7 × 10⁶ CFU/cm²) and pre-treatment (8.9 × 10⁷ ± 1.7 × 10⁸ CFU/cm²) were significantly different (*p* < 0.01). At seven days post treatment, the mean ulcer area in the red light group was reduced from 11.85 ± 6.83 to 7.8 ± 4.9 cm² (*p* < 0.01), that of PDT group from 12.72 ± 8.58 to 3.4 ± 3.4 cm² (*p* < 0.01). Better healing was seen in PDT group (*p* < 0.01). In conclusion, ALA-PDT is a potential modality to control PA infection and promote healing of chronic skin ulcers in lower limbs.

**Keywords** Photodynamic therapy · Skin ulcers · *Pseudomonas aeruginosa*

**Introduction**

Skin ulcer is a common disease. In most cases, these ulcers will affect the quality of life and are associated with higher medical expenses. In some cases, severe skin ulcers may lead to death [11]. Several factors result in the prolonged course of ulcers and difficulty in healing, such as nutrient metabolism, blood circulation, cell senescence, bacterial infections, and bacterial biofilm formation [22]. Bacterial infections are one of the most important causes of skin ulcers. The significant increases in the rates of infection with antibiotic- and multi-drug-resistant bacteria in recent years have hindered clinical treatment [13].

*Pseudomonas aeruginosa* (PA) can be associated with refractory skin ulcer [3, 31]. A study demonstrated that PA could be detected in >50 % cases of chronic refractory wounds [8]. The difficulty in treating PA in wound infection was primarily associated with the drug-resistant strains and bacterial biofilm formation [9, 19, 24]. Increasing antibiotics dose will not facilitate healing in this type of ulcer, therefore, new therapeutic methods that may locally eradicate PA and remove its biofilms are needed.
Photodynamic therapy (PDT) is considered to be a promising new treatment method for the eradication of microbes. PDT has been clinically approved for the treatment of several infectious skin diseases, such as acne and viral warts [7]. In addition, several studies have confirmed the efficacy of PDT in the treatment of bacterial infections and their biofilms [2, 12]. δ-Aminolevulinic acid (ALA) is a prodrug that is commonly used in dermatology. Its mode of action involves being absorbed by actively proliferating cells/bacteria and subsequently being converted into protoporphyrin IX (PpIX). Upon exposing to light of appropriate wavelength, PpIX will undergo photodynamic reactions to produce singlet oxygen to exert cytotoxicity effects on microbes and pathological cells [30]. Lee et al. [16] observed the effects of ALA-PDT on PA and its biofilm and found that ALA-PDT effectively inactivated PA and destroyed its biofilm. The efficacy of ALA-PDT was positively correlated with the laser dosage. After two treatments, all of the bacteria in the biofilm were eradicated. However, the specific mechanism for this action requires further elucidation. Additionally, Street et al. [27] demonstrated that methylene blue-based PDT at a dose >15.5 J/cm² could completely eradicate planktonic PA and inhibit biofilms at a rate of 99%. However, singlet oxygen quenchers could suppress this effect, which indicates that the effects of photodynamic disinfection and biofilm destruction are closely associated with the effect of singlet oxygen. In addition, in vivo studies of PDT treatment of PA infection have been reported. Hamblin et al. [10] used poly-L-lysine chlorin(e6) conjugate (pL-ce6)photodynamic antimicrobial chemotherapy (PACT) to treat wounds in mice infected with PA and found that the speed of wound healing in these mice was significantly faster than that in the silver nitrate control group. The eradication rates of PDT in planktonic bacteria, such as Staphylococcus aureus (including methicillin-resistant Staphylococcus aureus [MRSA]) [26], Streptococcus mutans, Streptococcus sobrinus, and Streptococcus sanguinis, can exceed 95%. However, different degrees of clearance have been observed in the biofilms of these bacteria when treated with PDT.

In this clinical study, ALA-PDT was applied for the treatment of chronic skin ulcers in lower limbs infected with PA. The antimicrobial and healing-promoting effects of ALA-PDT on chronic skin ulcers were compared with red light.

Materials and methods

Study design

This clinical study was a randomized controlled experiment. Consecutive patients with chronic skin ulcers in the lower limbs were enrolled and randomly divided into ALA-PDT group (the experimental group) and red light only group (the control group). Changes in the bacterial levels and wound areas and the side effects (including pain, redness, and swelling) in these two groups were observed and compared. This study was approved by the Ethics Committee of Daping Hospital of the Third Military Medical University. Informed consent was obtained from all of the patients.

Patient inclusion and exclusion criteria

The diagnosis of chronic skin ulcers was based on following criteria: chronic skin wounds that involved the dermis and did not heal after 3 months or did not exhibit a tendency toward healing [15, 21]. The presence of these ulcers indicated that the anatomical and functional integrity of the skin cannot be recovered through normal, orderly, and timely repair processes.

Inclusion criteria: Adult male and female patients (>25 years old) had a chronic skin ulcer in the lower limbs. The course of the skin ulcer was more than 3 months. The maximum diameter of a single ulcer area was smaller than 6 cm, and the area was smaller than 36 cm². PA infection was confirmed by bacterial culture. Blood glucose levels were normal (fasting blood-glucose ≤6.1). Patients agreed to participate in the study and signed informed consent forms.

Exclusion criteria: patients who were allergies to photosensitizers and their solvents; patients with serious medical illnesses, such as severe heart disease and severe liver and kidney dysfunction; patients with systemic infections that required timely treatment; patients with more than two bacterial or fungal infections on the skin ulcer surface; the causes of the ulcers included cancer, cutaneous vasculitis, fungus and tuberculosis and other special infections; pregnant and breast-feeding women; and patients with other conditions that warranted exclusion according to the researchers.

Measurement indicators and methods

Bacterial identification

Specimens were obtained from the wound surfaces of the ulcers using sterile cotton swabbing according to specimen collection guidelines [6]. The specimens were placed into sterile tubes, sealed, and sent to the laboratory for examination. Pathogen isolation was performed according to the “National Guide to Clinical Laboratory Procedures”. Bacterial identification was performed using a VITEK-60 automated microbiology analyzer (bioMérieux, Inc., France) and the corresponding identification cards in this
system. The VITEK-60 automated microbiology analyzer is an intelligent system for microbial identification and drug sensitivity assessment. The system uses biochemical reactions to identify bacteria. Based on the coloring conditions after culture, bacterial identification results could be accurately obtained in a short time period. Patients who had bacterial culture results that were positive for PA after 3 days and who met the inclusion criteria were included in the study [27].

**Bacterial count**

A sterile absorbent gauze square with a side length of 2 cm was attached to the skin ulcer surface, left for 2 min, and then directly placed into a flask that was filled with 10 ml of sterile broth medium. After doubling the dilution, 0.1 ml of diluted solution at different concentrations was evenly inoculated onto blood agar plates. The bacterial levels in the unit area (cm²) of the skin ulcer surface were calculated based on the number of colonies on the plates [29]. The calculation formula was as follows: bacterial count/cm² = number of colonies × dilution fold × 10/4. When the area was smaller than 4 cm², the actual area was used for the calculation.

**Measurement of the wound area**

The wound area was measured using plethysmography with transparent film and a scale ruler [4]. Tracings were made by placing sterilized transparency film over the wound and tracing the wound’s perimeter on the film. The counting procedure entailed tracing the outline of the tracing on metric graph paper and counting the number of square millimeters within the tracing.

**Treatment method**

In the ALA-PDT group, the skin that surrounded the ulcers was covered with adhesive tape. ALA solution (20 %) was freshly prepared by injecting 0.5 ml of water into a vial that contained 118 mg of ALA power (Shanghai Fudan-Zhangjiang Bio-Pharmaceutical Co., Ltd., Shanghai, China). The ALA solution was evenly coated on the ulcer surface and extended 5 mm around the ulcer. After incubation for 1.5 h in the dark, the surface was exposed to 630 nm light (Omnilux Red and Blue Light Therapy System) at a dose level of 80 J/cm² once a week for 2 weeks.

The control group was treated once a week for 2 weeks with the same dose of red light after saline was applied to the ulcers.

During the treatment, a standard wet dressing was used: a wet dressing with 3 % boric acid solution was used for

<table>
<thead>
<tr>
<th>Table 1 Characteristics of the patients and ulcer surfaces</th>
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<tbody>
<tr>
<td>Control group (red light only)</td>
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<tr>
<td>Experimental group (ALA-PDT)</td>
</tr>
<tr>
<td>Number of subjects</td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>Gender (M/F)</td>
</tr>
<tr>
<td>Causes of ulcer (venous/trauma)</td>
</tr>
<tr>
<td>Ulcer duration (month)</td>
</tr>
<tr>
<td>Median duration</td>
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<tr>
<td>Ulcer area (cm²)</td>
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<tr>
<td>Bacterial count (CFU/cm²)</td>
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<tr>
<td>Median duration</td>
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<tr>
<td>Biofilms</td>
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<tr>
<td>Pre-treatment method</td>
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</tbody>
</table>

12 h each day with the gauze changed every 2 h and a routine nursing dressing was used for the remaining time.

**Evaluation of outcomes**

The general conditions of the patients before treatment, 1 day post-treatment, and 1 week post-treatment were recorded. The wound surface conditions, including the presence of redness, swelling, pain, and exudates, and other side effects were observed. Routine blood and urine tests were performed before treatment and 1-week post-treatment to determine whether there were significant changes, and any side effects were recorded. The bacterial levels on the ulcer surfaces before treatment, 1-day post-treatment, and 1-week post-treatment were examined. Follow-up was conducted by telephone after 1-month.

**Statistical methods**

The statistical analysis was performed using SPSS13.0 software. Normality was examined using the one-sample
Kolmogorov–Smirnov (K–S) test as the non-parametric test (Table 1). A comparison of the means was made using the independent samples \( t \) test (age and disease duration), the paired samples \( t \) test (ulcer area), and the Wilcoxon \( W \) and \( Z \) tests (bacterial count) respectively (Tables 2, 3).

### Results

#### Patient characteristics and ulcer surface conditions

From June 2011 to June 2013, patients with chronic skin ulcers at our hospital were recruited for this study. The pathogen that infected the ulcer surface was confirmed as PA. The experimental and control groups each included 13 patients who would undergo treatment.

Table 1 describes the patient characteristics and the ulcer surface conditions. The K–S test indicated that the ages, male-to-female ratio, ulcer duration, bacterial counts, and ulcer areas in these two groups were comparable. The majority of the patients had traumatic ulcers. The average age was approximately 60 years, and the patients were mainly males.

#### Antimicrobial effects

The clinical presentations in the experimental group after 1–3 days of ALA-PDT revealed that the skin ulcer surfaces had healed: secretions were significantly reduced, and the skin ulcer surfaces were significantly cleaner (Fig. 1a–e). In contrast, the ulcer surfaces in the control group did not exhibit any significant changes.

### Table 2 Bacterial counts before and after treatment in two groups \((n = 13)\) \((\text{CFU/cm}^2)\)

<table>
<thead>
<tr>
<th></th>
<th>Pre-PDT</th>
<th>Post-PDT(24 h)</th>
<th>(P) value</th>
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</thead>
<tbody>
<tr>
<td>Control group</td>
<td>(5.5 \times 10^7 \pm 1.6 \times 10^8)</td>
<td>(3.4 \times 10^7 \pm 7.1 \times 10^7)</td>
<td>0.588</td>
</tr>
<tr>
<td></td>
<td>Median 2.9 (\times 10^6)</td>
<td>Median 7.2 (\times 10^4)</td>
<td></td>
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<tr>
<td>Experimental group</td>
<td>(8.9 \times 10^7 \pm 1.7 \times 10^8)</td>
<td>(6.3 \times 10^5 \pm 1.7 \times 10^6)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Median 5.8 (\times 10^6)</td>
<td>Median 0</td>
<td></td>
</tr>
<tr>
<td>(P) value</td>
<td>0.682</td>
<td>0.010</td>
<td></td>
</tr>
</tbody>
</table>

Bacterial measurements indicated that the bacterial levels in the control and experimental groups before treatment were comparable and were not significantly different \((p = 0.682 > 0.05)\). The bacterial levels in the control group before treatment and 24 h after the second treatment were \(5.5 \times 10^7 \pm 1.6 \times 10^8\) and \(3.4 \times 10^7 \pm 7.1 \times 10^7\) CFU/cm\(^2\), respectively. No significant differences were observed \((p > 0.05)\). The bacterial levels in the experimental group before treatment and 24 h after the second treatment were \(8.9 \times 10^7 \pm 1.7 \times 10^8\) and \(6.3 \times 10^5 \pm 1.7 \times 10^6\) CFU/cm\(^2\), respectively. This difference was statistically significant \((p < 0.01)\) (Table 2).

The results indicated that ALA-PDT significantly reduced the bacterial levels on the skin ulcer surfaces and had a bactericidal effect, whereas light alone did not have a significant bactericidal effect.

#### Reduction of the skin ulcer area

The skin ulcer areas in the control and experimental groups before treatment were comparable and were not significantly different \((p = 0.779 > 0.05)\). Seven days after the second treatment (14 days after the start of the study), the ulcer areas in the control group were reduced from 11.85 \(\pm\) 6.83 to 7.8 \(\pm\) 4.9 cm\(^2\), a difference that was statistically significant \((p \leq 0.01)\) (Table 3). Comparison of the ulcer areas in each lesion before and after treatment in the control and experimental groups revealed that the reduction in the ulcer area in the experimental group was more significant than that in the control group.

The results indicated that these two groups both benefited from PDT, which promoted healing of the skin ulcer surface. However, the outcome in the experimental group was more significant than that in the control group.

#### Adverse reactions

All of the patients in the experimental group experienced different degrees of pain during PDT, however, the pain...
was tolerable. After PDT treatment, significant redness and swelling was observed on and around the skin ulcer surface. The redness, swelling, and pain continued for 1–3 days. None of the patients in the control group felt uncomfortable before or after light exposure. In addition, none of the patients had any obvious systemic discomfort. The routine blood and urine test results before and after treatment did not reveal any obvious abnormalities. Follow-up was performed by telephone 1-month post-treatment, and none of the patients in these two groups had any obvious adverse reactions.

Discussion

This clinical study evaluated the effectiveness of red light alone and ALA-PDT on chronic skin ulcers in the lower limbs. Topical PDT showed significant bactericidal activity on PA \((p < 0.01)\) and significant healing-promoting effects on skin ulcers in the lower limbs that were infected with PA \((p < 0.01)\).

Few clinical reports on the application of PDT for infectious skin ulcers are available in the literature. Clayton et al. [5] reported on a 72-year-old female patient with chronic recalcitrant venous ulceration of the right lower leg for more than 1-year. The ulcerated area was 19.6 cm². Neoplasia was excluded, and bacterial culture indicated MRSA infection. The lesion was refractory to topical antiseptics (potassium permanganate, silver nitrate, and different bacteriostatic dressings). The skin ulceration significantly improved after ALA-PDT with red light of 630 nm. The bacterial culture was negative, and no adverse reactions were observed. This case report demonstrated that ALA-PDT was a viable option for treating infectious skin ulcers. Recently, Morley et al. [20] performed a phase II randomized placebo-controlled clinical study of 32 patients that included 16 patients with chronic leg ulcers and 16 patients with diabetic foot ulcers (each group had 8 active treatment recipients/8 placebo recipients). The photosensitizer was PPA904 [3,7-bis(N,N-dibutylamino) phenothiazin-5-ium bromide] or light dose 50 J/cm² of red light exposure. The results indicated PPA904-PDT immediately exhibited strong bactericidal effects: the median bacterial levels were 7.05 log_{10} before treatment and 5.83 log_{10} after treatment. Significant differences were detected between these two groups \((p < 0.01)\). After 3 months, only 12% of the patients in the control group exhibited complete ulcer healing, whereas 50% of the patients in the experimental group experienced complete healing. These clinical studies further confirmed that PPA904-PDT had significant bactericidal effects and healing-promoting effects on infectious skin ulcers.

Based on the results in this and previous studies, we consider PDT to be a promising method for treating chronic skin ulcers infected with bacteria. Analysis of its mechanism of action indicated that it might be associated with the following aspects. First, PDT may be associated with the bactericidal effect of photodynamic action and the reduction in the bacterial load on the ulcer surface, which results in the promotion of healing. It is well-known that bacterial infection plays an important role in the transition of skin ulcers from acute to chronic refractory [14]. Infection and slow healing have a reciprocal relationship in skin ulcer formation [3, 21]. Second, the anti-bacterial pathway of PDT is different from traditional antibiotic pathways; therefore, this therapy may have eradication effects on drug-resistant or multi-drug-resistant strains. PDT does not induce new drug resistance, has a destructive effect on bacterial biofilms, and may be used for repeated treatment [17, 28]. Third, red light has photo-regulatory,
anti-inflammatory, and healing-promoting effects [1]. Fourth, several novel studies have demonstrated that PDT has positive effects on histological aspects of wound healing [18, 23, 25]. Mills [18] proved MAL-PDT increases MMP-1, MMP-9 and TGF-b3 production during matrix remodelling, ultimately producing scars with improved dermal matrix architecture. Sahu’s study [25] showed that photodynamic treatment of wounds with pl–cp6 not only kills the bacteria but also reduces hyper-inflammatory response of P. aeruginosa-infected wounds in mice, leading to acceleration of wound healing. Currently, however, no basic research has fully elucidated related mechanisms, which still await further in-depth studies.

Our study further confirmed that ALA-photodynamic treatment could reduce the bacterial load of chronic skin ulcers in lower limbs infected with PA. It also promoted the healing of ulcer surfaces. However, the specific mechanism underlying this treatment still requires in-depth studies. This treatment also requires further validation using large sample size in clinical trials.

Acknowledgments This work was funded by the National Natural Science Foundation of China (NSFC, grant number 81271760 and 81471703).

Conflict of interest No conflicts of interest to declare.

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