

## Original Article

# Efficacy analysis of the aminolevulinic acid photodynamic therapy combined with recombinant human interferon $\alpha$ -2b ointment in the treatment of precancerous skin lesions and superficial basal cell carcinoma in elderly patients

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**Abstract:** Objective: To explore the efficacy of the aminolevulinic acid (ALA) photodynamic therapy (PDT) combined with recombinant human interferon (rhIFN)  $\alpha$ -2b ointment for the treatment of precancerous skin lesions and superficial basal cell carcinoma (sBCC) in elderly patients. Methods: A total of 101 elderly patients with precancerous skin lesions and sBCC, who were diagnosed and treated at Shanxian Central Hospital between January 2015 and January 2016 were randomly divided into 2 groups using a random number table method. A total of 51 patients were placed in the combined treatment group and 50 patients were placed in the control group. Patients in the control group were treated with conventional surgical methods for tumor resection, whereas the postoperative local skin was treated with rhIFN  $\alpha$ -2b ointment. Patients in the combined treatment group were treated with ALA PDT combined with rhIFN  $\alpha$ -2b ointment. The efficacy, surgical wound area, lesion area, cosmetic effect, and recurrence rate within 1 year were compared between two groups, and the safety was evaluated. Results: The complete remission rate in the combined group was significantly higher compared to those in the control group ( $P < 0.05$ ). However, no significant difference was observed in total effective rate between the two groups ( $P > 0.05$ ). The recurrence rate in the combined treatment group was significantly lower than that in the control group ( $P < 0.05$ ), and the average wound area and skin lesion area after treatment in the combined treatment group was significantly smaller compared to those in the control group (both  $P < 0.05$ ). The excellent and good rate of cosmetic effects in the combined treatment group were significantly higher compared to that in the control group ( $P < 0.05$ ). No severe postoperative reactions were observed in both groups. Conclusion: Compared with the control group, ALA PDT combined with rhIFN therapy had a better efficacy, a lower recurrence rate, and caused a smaller wound, indicating that it was a more effective method for the treatment of sBCC.

**Keywords:** Aminolevulinic acid, photodynamic therapy, superficial basal cell, recombinant human interferon  $\alpha$ -2b

## Introduction

In recent years, increasing environmental pollution and accelerated aging of the population have led to an increase in the incidence of skin tumors and precancerous skin lesions [1, 2]. In particular, middle-aged and elderly populations of over 60 years of age are among the most susceptible [3]. At present, treatment of this type of disease is mostly performed by surgical resection, laser, cryotherapy, topical external medicine, etc. Surgical resection is the most commonly used treatment method, through

which cancerous tissue can be completely removed. However, to ensure complete resection of the tumor, a part of normal tissue needed to be removed during the operation, resulting in an area of the wound that was larger after treatment. Skin tissue may be injured and a scar may be obvious and affect appearance. Moreover, the surgical approach is time-consuming and costly. Several patients had infections, scars, and injury to the adjacent line of defense, which limited clinical applications. Cryotherapy mainly kills cancer cells with low-temperature gas such as liquid nitrogen. During

treatment, the diseased area needs to be repeatedly frozen and thawed. Although pain is tolerable, the thawing process could cause adverse reactions, such as pain, swelling, and exudation, and the depth of treatment is limited. Although topical external medicine is directly applied to the local lesion, there is also an issue regarding treatment depth, which results in incomplete treatment and recurrence commonly [4]. Previous studies have shown that injection of recombinant human interferon (rhIFN) at the base of skin cancer effectively inhibited tumor growth and prevent its recurrence [5]. However, several patients were prone to fever, headaches, and other adverse reactions. rhIFN  $\alpha$ -2b ointment was an external preparation of rhIFN with good antiviral and immunomodulatory properties. External ointment application reduced the occurrence of adverse reactions caused by injection of rhIFN at the base. However, the depth of treatment was limited, and relapse was common.

With a gradual increase of the medical field, 5-aminolevulinic acid (ALA) photodynamic therapy (PDT) was born, which had good selectivity on lesion site. The damage to patients during surgery was limited, and there was no scar formation. However, clinical reports were not uniform about the exact therapeutic effect of this type of therapy in clinical practice [6, 7]. To evaluate the efficacy and safety of ALA PDT in the treatment of skin cancer, in this study, we investigated the effects of ALA PDT combined with rhIFN  $\alpha$ -2b ointment on precancerous skin lesions and superficial basal cell carcinoma (sBCC) in elderly patients.

### Materials and methods

#### General materials

A total of 101 elderly patients with precancerous skin lesions or sBCC who were diagnosed and treated at Shanxian Central Hospital between January 2015 and January 2016 were selected as study subjects and included 63 males and 38 females, with an age of 60-85 years (average  $71.5 \pm 13.3$  years old), the duration of the disease was 4-71 months (average  $27.6 \pm 11.4$  months). There were 80 lesions in the head and face, 9 in the auricle, 7 in the fingers, and 5 in the shoulder. The area of the lesions was 0.9-9.5 cm<sup>2</sup>, and the thickness of the lesions was less than 6 mm.

Inclusion criteria: Patients were with precancerous skin lesions or sBCC and diagnosed by clinical symptoms, signs, and histopathology, and all lesions were single skin lesions. Patients were treated for the first time or had not been treated with radiotherapy or chemotherapy prior to coming to the hospital. No lymph node involvement and distant metastasis characteristics was required and a tumor diameter  $\leq 20$  mm was needed [8, 9].

Exclusion criteria: Combination of melanoma, and seborrheic keratosis; patients with severe kidney, heart, liver, lung, or other diseases; patients who used immunomodulating agents and hormones during the last month; patients who had trouble in communicating with others; patients with a history of photosensitizer allergy or photosensitive disease; pregnant and lactating women.

The 101 patients were randomly divided into two groups using random number table method. Among them, 51 patients were placed in the combined group, and 50 patients were placed in the control group. This study was approved by the Ethics Committee of Shanxian Central Hospital and all patients signed informed consent.

#### Therapeutic methods

Combined group: Prior to surgery, a medical cotton swab with physiological saline was used to clean the diseased area and the disease-free skin surrounding this area. Then, pre-equipped ALA cream (Sigma Chemical Company, Saint Louis MO, USA) was applied to the tumor and to disease-free skin nearby (around 80 mm), then it was sealed with aseptic plastic film, and coated with black film to protect it from illumination. After 240 minutes, the sealed film was removed and the skin was treated with a PDT device (Ominlux, USA), at a wavelength of 635 nm, an output power of 80 mw/cm<sup>2</sup>, and an energy density of 100 J/cm<sup>2</sup>. The treatment area was evaluated based on the size of the tumor, and the treatment time was estimated to be about half an hour per week according to the size of the spot. The interval of treatment was over 7 days, and treatment was performed 3 to 5 times [10]. After that, the recombinant human interferon  $\alpha$ -2b ointment was wiped at the damaged part of the skin. After 8 hours, the saline was applied

**Table 1.** Comparison of two groups of clinical data

Groups	Age (year)	Gender (male/female)	Course of disease (d)	Lesion area (cm <sup>2</sup> )
Combined group (n=51)	70.8±13.5	35/16	26.5±10.7	3.35±1.03
Control group (n=50)	71.9±14.1	28/22	27.9±11.3	3.27±1.31
t/x <sup>2</sup>	0.401	1.715	0.639	0.342
P	0.609	0.190	0.524	0.733

to clean out the smear area once a day for three weeks. The drug was administered before going to bed and covered with local gauze to prevent the ointment from falling off.

Control group: Conventional surgical procedures for surgical resection were performed. After surgery, the damaged surface was treated by direct suture or flap transplantation according to the patient's diseased position and the size of the tension [11]. After surgery, rhulFN  $\alpha$ -2b ointment was applied to the local skin, and the method used was similar to that of the combined treatment group.

#### Observation indexes

The main observation indexes were as follows. (1) The clinical efficacy was evaluated according to literature standards: complete remission, the tumor was completely eliminated, with only pigmentation or pigment fading, and without recurrence within one year; partial remission, the tumor reduced by more than 50% in size; invalid, the tumor reduced less than 50% or with no therapeutic effect [12]. Total clinical effective rate = (total number of cases of complete remission + partial remission)/the total number of cases \* 100%. (2) Recurrence: After treatment, the patient was followed up for 1 year on a monthly basis. If new skin lesions were generated in and around the original regressed area, then it was considered as a recurrence. The recurrence rate between the two groups was compared and calculated as: recurrence rate = recurrence cases/total cases \* 100%. (3) Wound area and lesion area: The scar area caused by surgery and other treatments, and lesion area before and after operation were compared between the two groups.

Secondary observation indexes were as follows. (1) The cosmetic effect of the appearance of lesion was evaluated according to literature standards [13]. If no nodules and sclerosis were present in skin lesions and no or slight pigmentation was present, the skin was considered excellent. It was considered good that although no nodules and sclerosis

appeared, moderate skin pigmentation appeared. Moderate or mild nodules and sclerosis in the skin lesions were considered medium. Extensive nodular sclerosis was considered poor. (2) Safety evaluation was performed according to observation of adverse reactions during treatment.

#### Statistical methods

Data were analyzed by SPSS 17.0 software (Chicago, IL, USA), and measurement data are expressed as the mean  $\pm$  standard deviation ( $\bar{x} \pm sd$ ). Two independent sample t tests were used to compare measurement data that showed a normal distribution between groups. Paired t test was used compare the mean before and after intervention in the same group, and was expressed as t. The ratios were compared using X<sup>2</sup> test and Fisher's exact probability test, which was expressed as X<sup>2</sup>. The rank variables are evaluated by rank test, which was expressed as H. P<0.05 is considered statistically significant.

#### Results

##### Clinical data comparison

No significant differences were observed in age, gender, course of disease, lesion site, and lesion area between the two groups (all P>0.05) as shown in **Table 1**.

##### Clinical efficacy and recurrence

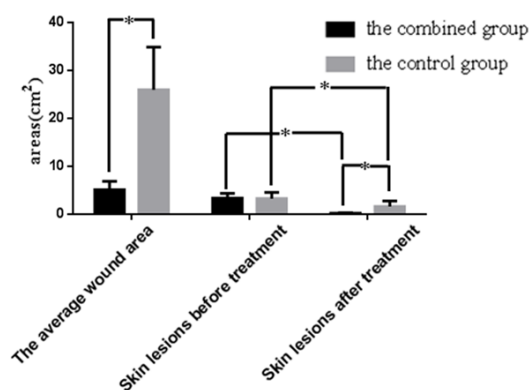
The complete remission rate in the combined treatment group was significantly higher compared to that in the control group (P<0.05). However, no significant difference was found in the total effective rate between the two groups (P>0.05). During the one-year follow-up, there were 4 cases of recurrence in the combined treatment group, with a recurrence rate of 7.84%. Twelve cases of recurrence were found in the control group, with a recurrence rate of 24.00%. Within one year, the recurrence rate in the combined treatment group was significantly lower compared to that in the control group (P<0.05) as shown in **Table 2**.

**Table 2.** Comparison of clinical efficacy and recurrence between the two groups of patients (n, %)

Groups	Complete remission	Partial remission	Inefficiency	Total efficiency	Recurrence rate
Combined group (n=51)	41 (80.39)	6 (11.77)	4 (7.84)	47 (92.16)	4 (7.84)
Control group (n=50)	29 (58.00)	16 (32.00)	5 (10.00)	45 (90.00)	12 (24.00)
$\chi^2/H$	5.951	6.068	0.122	0.122	4.944
P	0.015	0.014	0.727	0.727	0.026

**Table 3.** Comparison of wound healing between the two groups of patients (cm<sup>2</sup>)

Group	Combined group (n=51)	Control group (n=50)	t	P
Wound average area	5.12±1.73	25.98±8.91	16.408	<0.001
Skin lesion area				
Before treatment	3.35±1.03	3.27±1.31	0.342	0.733
After treatment	0.23±0.07	1.63±1.12	8.910	<0.001



**Figure 1.** Comparison of wound healing between two groups, \*P<0.05.

#### Comparison of wound healing

The average area of wound in the combined treatment group was significantly smaller than that in the control group, and the difference was statistically significant (P<0.05). Before treatment, there was no significant difference skin lesion area between the two groups (P>0.05). However, after treatment, the area of skin lesions in the combined treatment group was significantly smaller than that in the control group, and the difference was statistically significant (P<0.05) as shown in **Table 3** and **Figure 1**.

#### Cosmetic effect of the appearance of lesion

During the one-year follow-up, the excellent and good rate of cosmetic effect in the combined

treatment group was significantly higher compared to that in the control group, and the difference was statistically significant (P<0.05) as shown in **Table 4**.

#### Evaluation of safety

After treatment with PDT, no serious postoperative adverse reactions were observed. However, after surgery, patients showed different levels of skin redness, tingling, itching, and other adverse reactions, which were gradually relieved within 12 to 72 hours after strict light avoidance and symptomatic treatment. Skin photosensitivity reaction occurred in 15 patients in the combined treatment group but was relieved after anti-allergy therapy. In the control group, 4 cases had poor local skin flap healing, which resulted in skin scars.

#### Discussion

5-ALA is an important photosensitizer in the human body. It can be synthesized from glycine, caprylic acid, and coenzyme A in the processes of hemoglobin synthesis [14]. Only a small amount of ALA is contained in normal human cells, and the photosensitivity is low. However, due to the exuberant proliferation and metabolism of tumor cells, tumor cells can selectively absorb and accumulate exogenous 5-ALA and convert it into hemoglobin. If 600-800 nm red lights were applied, a photodynamic reaction would occur. Protoporphyrin could be activated, resulting in the production of singlet oxygen and free radicals, thereby having cytotoxic effects on proteins, nucleic acids, and lipid macromolecules, which lead to necrosis and apoptosis of tumor cells [15]. In recent years, ALA PDT had been gradually used in the treatment of skin tumors and precancerous lesions, which had definite curative effects and good cosmetic results, causing little damage to the patient. Only selected tumor cells

**Table 4.** Comparison of cosmetic effect of the appearance of lesion between the two groups of patients

Group	Excellence	Good	Medium	Poor	Excellent rate
Combined group (n=51)	41	7	3	0	48 (94.12)
Control group (n=50)	31	9	7	3	40 (80.00)
$\chi^2$					4.487
P					0.034

were killed and there was little influence on the surrounded tissues [16, 17]. However, due to the shorter duration for performing ALA PDT, its standard therapeutic dose and possible adverse reactions needed further investigation. Interferon is a common adjuvant drug for clinical anti-tumor therapy, which inhibits the synthesis of tumor cell genes, and induces necrosis and apoptosis of tumor cells. Moreover, it activates immune cells such as NK cells and mononuclear macrophages, increases the expression of immune cell surface antigens and antibodies, and enhances the body's immune defense function, thereby promoting apoptosis and necrosis of tumor cells [18]. Previously, drugs were mostly administrated through intratumoral or intramuscular injection, and the systemic flu-like symptoms were severe. It has been difficult for some patients to tolerate and thus the clinical application was limited [19]. rhuIFN  $\alpha$ -2b ointment as a topical external preparation may have a good local anti-tumor effect by external application on skin tumors [20].

This study was the first to apply ALA PDT combined with rhuIFN  $\alpha$ -2b ointment to elderly patients with precancerous skin lesions and sBCC. The results indicate that the complete remission rate was significantly higher in the combined treatment group when compared to the control group. The recurrence rate within 1 year was significantly lower compared to that in the control group, suggesting that the therapy had a good clinical effect on patients with precancerous lesions and sBCC. In addition, it could effectively alleviate the patient's degree of disease, improve the cure rate, and control the recurrence of the disease. According to Ramirez et al., the complete remission rate of PDT in patients with basal cell carcinoma was 76.5%, which was slightly lower compared to the findings of this study [21]. This was possibly related to the fact that subjects in this study were diagnosed with skin precancerous

lesions and sBCC. Therefore, the degree of affected tissue was limited and the disease condition was mild. Simultaneously, the average wound area and skin lesion area in the combination treatment group after treatment were significantly smaller compar-

ed to that in the control group. It was suggested that PDT was less traumatic to patients than conventional surgery, which could minimize the area of lesions and reduce the creation of scars during regenerative repair [22, 23]. During the 1-year follow-up, the excellent and good rate for cosmetic results in the combined group was significantly higher than that in the control group. The data indicate that after treatment with PDT combined with rhuIFN  $\alpha$ -2b ointment, patients demonstrate less trauma, show better recovery, and receive good cosmetic results.

In addition, patients in the combined treatment group had different degrees of skin redness, tingling, itching, and other adverse reactions after PDT treatment [24, 25]. This gradually remitted 12-72 hours after strict light avoidance and symptomatic treatment. In 15 patients, photosensitivity reactions occurred in the skin, which were relieved after anti-allergy therapy [26]. In none of the patients, serious adverse reactions were observed, suggesting that the therapy was well tolerated by the patients and had no significant toxic or side effects.

This study is a preliminary study, therefore, the subjects selected had precancerous skin lesions or superficial tumors with a tumor thickness of <6 mm. Previous studies have shown that PDT could be used as a pretreatment method to improve therapeutic efficacy for tumor lesions with a skin lesion that was protruding or had infiltrates of >6 mm in size. In this study, further research and discussion were not conducted and discussed, but would be further studied in future studies.

In summary, PDT has the following advantages. First, PDT for the treatment of precancerous lesions of the skin and sBCC has good efficacy, is less traumatic to patients with a better recovery, and meets the patient's cos-

metic requirements. Second, PDT can be applied to elderly patients who are unable to tolerate surgical resection or who need surgery on sites that will seriously impair organ function. Moreover, although after surgery patients may have different degrees of skin redness, tingling, itching, and other adverse reactions, patients mostly tolerate the procedure. Damage to the surrounding normal tissues is less and a relatively higher safety level is achieved. Finally, the approach is simple and convenient.

#### Disclosure of conflict of interest

None.

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## ALA PDT with rhuIFN $\alpha$ -2b ointment in treating precancerous skin lesions and sBCC

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