Appendix S1

MATERIALS AND METHODS

Patients

Patients were enrolled at Shanghai Skin Diseases Hospital. For this open-label, randomized and controlled prospective study, women of 18 years or older with biopsy-proven active VLS who had the ability to sign written informed consent, willingness to comply with the study requirements, and no plan to conceive or breastfeed during the study were recruited. Exclusion criteria were: subjects who received systemic or local treatment within the past 6 months, those diagnosed with other vulvar dermatoses or carcinoma, and those hypersensitive to clobetasol propionate, ALA or any of the components of the ointments (S1). The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki. All eligible patients were randomized to either ALA-PDT or clobetasol propionate group using sequentially numbered envelopes. The random sequence in the envelopes was produced by computer programme. The sequentially numbered opaque envelopes were opened only after each patient agreed to participate.

Clinical evaluation

Photographs of VLS lesions were taken with a digital camera for measuring the lesion size using the AutoCAD software. Horizontal visual analogue method was used to evaluate disease extent (including lesion scale and signs) and symptoms. The lesion scale was graded as: 0 = none, 1 = less than 25%, 2 = 25–50%, 3 = 51–75%, 4 = more than 75% of the vulvar surface affected. The affected area mainly included the surface of the mons pubis, clitoris labia majora, labia minora and perineum. The severity of clinical signs of hyperkeratosis, atrophy, sclerosis, and depigmentation were each graded as: 0 = absent, 1 = mild, 2 = moderate, 3 = severe. The severity of symptoms (pruritus, burning and pain feeling) was also graded as: 0 = absent, 1 = mild, 2 = moderate, 3 = severe (15).

Therapeutic regimens

In this study, patients were randomized to either the ALA-PDT or the clobetasol propionate group. Other treatments were not allowed during treatment and follow-up.

ALA-PDT. Patients were asked to urinate before the procedure started. After routine cleaning, freshly prepared 10% 5-ALA cream (Shanghai Fudan-Zhangjiang Bio-Pharmaceutical Co. Ltd, Shanghai, China) was applied evenly to lesions plus a 1-cm margin and incubated under light protection for 3 h. The lesions were then irradiated with 100 J/cm^2 of red light generated by a diode laser (633 nm; Biolitec AG, Jena, Germany) at 100 mW/cm^2. The power density was determined using a power meter (SZG-100 W, Shanghai Yinle Instrument Co. Ltd, Shanghai, China). The diameter of light spot was 2 cm. The lesion was irradiated area by area if its diameter was larger than 2 cm. The same PDT procedure was repeated 3 times at 2-week intervals, i.e. PDT was performed on days 0, 14, 28 and 42. Two weeks after the fourth session of ALA-PDT, day 56 was defined as the end of ALA-PDT treatment.

Clobetasol propionate. After routine cleaning, a thin layer of 0.05% clobetasol propionate ointment (ShunFeng Pharmaceutical Co. Ltd, China) was applied by patient herself every night for 8 weeks. The ointment should cover the lesions plus a 5-mm margin, and the thickness of the ointment should be approximately 1 mm (17).

Examination of protoporphyrin IX (PpIX) fluorescence

To examine PpIX generation and distribution, 5-ALA-applied area was exposed to a 410-nm light-emitting diode (LED) light and fluorescence images were captured by a digital camera equipped with a 420-nm long-pass filter. The presence of PpIX was presented as brick-red colour (18).

To examine the kinetics of PpIX generation, the *in situ* measurement of PpIX fluorescence was carried out on 3 patients prior to light irradiation using fluorescence spectroscopy (Curalux; Munich, Germany, excitation wavelength 405 nm, emission wavelength 440–800 nm) (S2). PpIX fluorescence intensity in the lesional skin, perilesional skin (0.5 cm from lesions) and adjacent normal skin (2 cm from lesions, control without ALA) were measured after 1, 2, and 3 h of incubation in the 1st ALA-PDT. The PpIX fluorescence intensity in the lesional skin was measured after 1, 2 and 3 h of incubation in each time of ALA-PDT.

Evaluation of clinical outcomes

Evaluations were performed by the same examiners, who did not know which treatment was received by patients. The treatment responses, i.e. reduction in lesion size, were evaluated at 2, 4, 6 and 8 weeks after the start of the treatment. They were graded as: complete response = 100% lesion disappeared; partial response = > 60% lesion clearance; minimal response = 20–59% lesion clearance; and poor or no response = < 20% clearance. Patients from both groups were followed-up for 6 months. Changes in each horizontal visual analogue score of individual patients were analysed before treatment, at the end of treatment (week 8) and 6 months after the end of treatment. Recurrence was defined as lesion occurring, characterized as hyperkeratosis, atrophy, sclerosis, and depigmentation, after a complete response.

During each PDT session, the patients were also asked to report the severity and duration of treatment-related pain. The maximal painful sensation was evaluated with an 11-point pain intensity numeric rating scale (PI-NRS) (0 = no pain, 10 = worst possible pain) (S3). The patients with PI-NRS $\geq$ 7 were given a lidocaine injection (Harvest Pharmaceutical Co. Ltd., Shanghai). Post-treatment reactions (e.g. erythema, oedema and erosion) were recorded. For the clobetasol propionate group, local reactions, such as corticosteroid-dependent dermatitis and secondary infection, were recorded, if any.

Statistical analysis

Results were analysed using statistics software (PASW Statistics 19, IBM SPSS Statistics, Armork, NY, USA). The analysis was based on the per protocol (PP) population. Two-way analysis of variance (ANOVA) was used to compare the PpIX fluorescence intensity of 2 groups. The rate of complete response was compared between the 2 groups using χ^2 test. Mann–Whitney rank sum test was used to compare the objective and subjective scores of 2 groups. Wilcoxon rank-sum test was used to compare the objective and subjective scores changes before and after treatment. Independent *t*-test was used to compare lesion size between the 2 groups. Paired-sample *t*-test was used to compare the lesion size reduction after treatment. PI-NRS values were compared using 1-way ANOVA. *p*-value < 0.05 was considered statistically significant.

SUPPLEMENTARY REFERENCES

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