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Photodynamic therapy with riboflavin-tryptophan gel in facial skin rejuvenation: a pilot study

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Introduction

Photodynamic therapy (PDT) has been used as the treatment for non-melanoma skin cancer such as actinic keratosis, squamous cell carcinoma in situ and basal cell carcinoma particularly in co-morbid and immunocompromised patients¹. Nowadays, there are many studies demonstrate PDT for cosmetic dermatology uses as photorejuvenation. It provides an excellent efficacy with high tolerability²⁻⁵.

Many clinical trials described rejuvenating effect of PDT on photo-damaged skin^{2, 6}. Anne Le Pillouer-Prost et al.⁷ reviewed that topical PDT improves lentigos, skin tone, skin roughness, texture and fine wrinkles. Side effects were only mild to moderate, non-scarring and can recover in short period of time. PDT could also improve dyspigmentation and reduce actinic elastosis. At present, there are two available photosensitizers (PS) referring to 5- α -aminolevulinic acid (ALA) and methyl-ester of ALA (MAL). In 2011, Palm, MD et al.⁸ performed a comparison research of blue versus red light sources for photodynamic therapy using methyl aminolevulinate in photodamaged skin. He concluded that blue and red light have similar efficacy as the light source for methyl aminolevulinate(MAL)-PDT when combined with other light sources. Side effects following MAL-PDT with red versus blue light were similar and mild in severity. Different formulation of photosensitizers and light sources were adjusted for skin rejuvenation as shown in many published papers. Although, treatment protocols for skin cancers are strongly established, treatment parameters for photo-aging are not yet standardized and deviate among different studies⁹.

Riboflavin (vitamin B2) and Tryptophan has been introduced as a new photosensitizer for photodynamic therapy recently without side effects. Riboflavin is a promising alternative photosensitizing molecule. In this study, we use photodynamic therapy with riboflavin tryptophan to evaluate the efficacy in facial skin rejuvenation.

Methods

Subjects

Fourteen subjects with age ranged between 30–50 years old were recruited from the dermatology out-patient clinic, Thailand Tobacco Monopoly Hospital. Inclusion criteria were subjects displaying with Fitzpatrick photo skin types I–IV.

Exclusion criteria were subjects who had undergone laser treatment or any other ablative/nonablative cosmetic intervention within the last six months, subjects with any history of laser treatment or trauma to the test site, Fitzpatrick scale skin type V–VI, patients with diseases associated with Koebner phenomena or light sensitive skin diseases, pregnant or lactating women, and patients who had used topical retinoids, alpha-hydroxy acids, or topical vitamin C within 3 months prior to the study.

Materials

- Riboflavin-tryptophan gel (RT gel): BR PDT Gel[®] in which the ingredients are Riboflavin, Tryptophan, Glycolic Acid, Niacinamide (BR PHARM Co.Ltd., Gangwon-do, Korea).

- Light source: Omnilux PDT[™] LED System (GlobalMed Technologies Co., CA, USA)

Treatment

All patients were carefully examined for any clinical suspicion of pre-cancerous or skin malignancies. This study will be conducted at the dermatology out-patient clinic, Thailand Tobacco Monopoly Hospital between March and July 2017.

Patients underwent 4 sessions PDT treatment on the face (avoiding the eye area, nose, and lips) with RT gel. The photosensitizers were applied and covered under plastic film occlusion for 30 minutes. The remaining products will be cleaned off using normal saline after the incubation. Then, the face was illuminated with blue light 415 nm, 48 J/cm² for 20 min. The light source was maintained at 5 cm from the nose tip. A wet dressing of gauze soaked in cooled normal saline was provided for 10 minutes after the irradiation. Subjects will be treated with PDT- RT gel at 1 week intervals for 4 sessions, totally.

Assessment

Baseline digital photography was performed on all subjects. Lighting and ambient conditions for photography were standardized throughout the trial. Image analysis and photo-aging assessment were conducted by the principal investigator. We used Antera 3D™ (Miravex Ltd., Dublin, Ireland) to measure wrinkles and texture of facial skin in every evaluation sessions of the study which were 1 week interval during (day 0, 7, 14 and 21) treatment and 2 weeks interval post-treatment (day 35 and 49).

Statistical methods

Data were analyzed using software IBM SPSS for Windows version 22 (IBM Corp., Armonk, NY, USA). Statistical significance was defined as a p-value less than 0.05. Non-Parametric analysis of covariance, Wilcoxon signed ranks was used to assess changes from baseline for each parameter.

Results

A total of 14 subjects with facial photo-aging signs referring to skin roughness and wrinkles volunteered to participate in the study. The mean age of subjects in this group (30–50) was 36.3 years old. Antera 3D was used to obtain macroscopic facial skin images for pretreatment baseline, 1 week, 2 weeks, 3 weeks, 5 weeks and 7 weeks after the treatment. An example of general appearance of the same particular part of the cheek was shown in figure 1. Noninvasive skin profilometry by Antera 3D provided a three-dimensional digital reconstruction of the skin. Skin texture were analyzed and demonstrated as roughness index (Figure 2). Median of the score were presented in table 1. The skin texture improved throughout the evaluation time line as the median value of roughness index declined (figure 4). At the fourth evaluation or 3 weeks after PDT with RT gel, skin texture was statistically significant improved with p-value 0.035 ($p < 0.05$). At the last follow-up, the median of roughness index decreased from 19.13 to 16.02. However, the results comparing day 0 and day49 of the treatment was not statistically significant. Wrinkle was also measured by Antera 3D at the same specific point on the cheek demonstrated by indentation index (Figure 3). Figure 5 illustrated the diminished tendency of wrinkle from baseline to each evaluation period. The significant reduction of wrinkle was observed at the fourth evaluation or 3 weeks after the treatment with p-value 0.030 ($p < 0.05$), corresponding with skin texture. The median of indentation index declined from 17.36 to 15.12. Nevertheless, the difference between pre and posttreatment was not statistically significant.

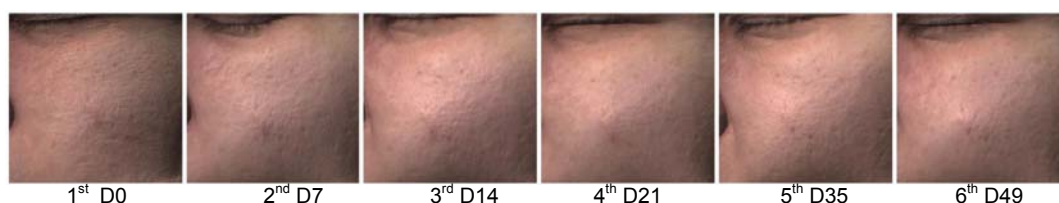


Figure 1. An example of macroscopic facial skin images of the same particular part of the cheek by Antera 3D in every evaluation time.

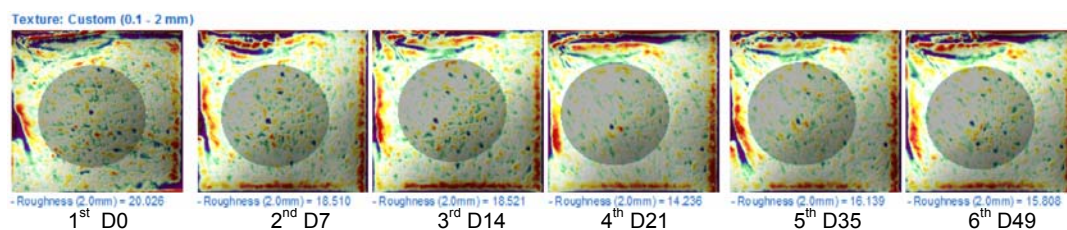


Figure 2. An example of roughness index of the same particular part of the cheek by Antera 3D in every evaluation time.

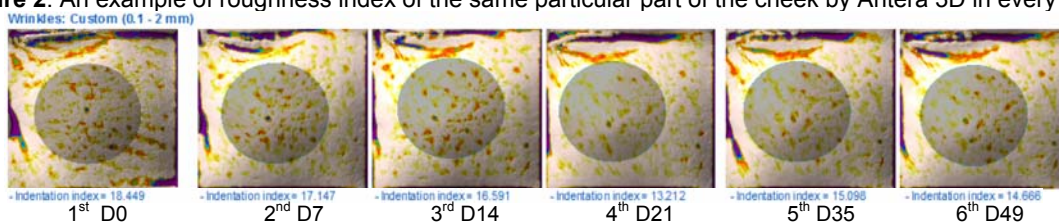
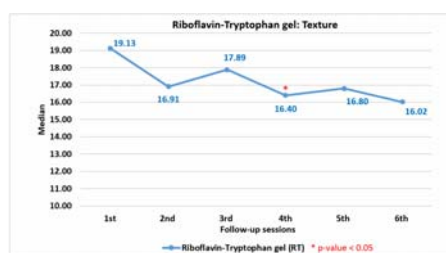


Figure 3. An example of indentation index of the same particular part of the cheek by Antera 3D in every evaluation time.

Table 1. All median of roughness index and indentation index measured by Antera 3D.

Evaluation	Texture	Wrinkles
(n=14)	Median [Min ,Max]	Median [Min ,Max]
1 st	19.13 [9.61 ,32.9]	17.36 [9.96 ,30.2]
2 nd	16.91 [9.54 ,32.33]	16.3 [9.84 ,29.65]
3 rd	17.89 [10.32 ,34.34]	16.75 [10.48 ,31.33]
4 th	16.4 [9.32 ,30.51]	15.32 [9.14 ,28.61]
5 th	16.8 [10.91 ,34.1]	15.73 [10.71 ,31.31]
6 th	16.02 [9.99 ,35.04]	15.12 [10.12 ,32.94]
	p-value	p-value
1 st vs. 2 nd	0.331	0.331
1 st vs. 3 rd	0.363	0.397
1 st vs. 4 th	0.035*	0.030*
1 st vs. 5 th	0.331	0.300
1 st vs. 6 th	0.096	0.124

Values presented as median (min, max). P-value corresponds to Wilcoxon Signed Ranks test.

**Figure 4.** Linear graph illustrated median value of roughness index**Figure 5** Linear graph illustrated median value of indentation index

Discussion

Photodynamic therapy (PDT) with photosensitizer could activate photochemical biomolecule electron transfer leading to reactive oxygen species (ROS) or free radicals production. These activation of ROS or free radicals cause tissue damage including cell apoptosis or necrosis, membrane, mitochondrial damage and many signaling molecules activation. In 2011, Rossi et al.¹ stated about the molecular mechanism of rejuvenation which could cause by TGF- β and FGF production accelerating fibroblast activation and collagen type I-III synthesis. Besides, PDT diminished collagen-degrading enzymes including matrix metalloproteinases-1, 3, and 12. Nevertheless, the exact molecular mechanism of rejuvenation on net dermal collagen production from PDT was still not fully understood. The experiment by Park et al.¹⁰ concluded that net MMP increased and type I Collagen mRNA production decreased which contradicted with previous studies.

Topical application of ALA or MAL before PDT with light sources including blue or red light or IPL, were considered as a new non-invasive, safe and effective procedure for the treatment of photodamaged skin^{11, 12}. Goldman et al.¹³ performed photorejuvenation research using the blue light source to illuminate the face after 1-hour topical application of ALA. He documented that ALA-PDT with blue light improved facial skin elasticity and reduced skin thickening in patients with photodamaged skin. There were significant changes in photodamage signs at 1 and 2 month after each treatment without scars or pigmentary changes at the site of ALA-PDT. In 2003, Touma¹⁴ studied the effectiveness of ALA and blue light for the treatment of actinic keratosis and diffuse photodamage. After 1 and 5month of the treatment, there was a significant declined in actinic keratosis and significant improvement in photo damaged parameters, such as skin quality, fine wrinkling, and sallowness with good to excellent satisfaction rated by 80% of the patients. In our study, we found that PDT with RT gel also had good efficacy for facial rejuvenation with texture improvement and wrinkle reduction corresponding with the previous studies. Besides, only minimal side effects were recorded such as mild erythema and burning sensation which could be reversible within hours without scar. However, PDT with RT gel provided significant improvement since the third week after treatment which was earlier than PDT with ALA in previous studies. So, we might assume that PDT with RT gel provided faster improvement in skin rejuvenation than PDT with ALA.

Riboflavin with blue light or IPL, activates tryptophan and forms radicals which react with water molecules and generates ROS. In this study, we performed photodynamic therapy with RT gel using blue light by Omnilux PDT™ LED System as the light source. The statistical significant improvement of both skin texture and wrinkle comparing between three weeks post-therapy and baseline indicating the benefit of photodynamic

therapy with riboflavin tryptophan in photo-ageing skin. Both skin roughness and wrinkle also decreased throughout the evaluation period. However, we assumed that the reason for non-significant differences between baseline and post-therapy was the small sample size and too short interval of follow-up time. Further randomized-controlled trials split-face of photodynamic therapy with riboflavin tryptophan gel are recommended in the future. Moreover, more studies are required to standardize the optimal parameters for the treatment of skin rejuvenation regarding to different photosensitizing agents, light sources and delivery techniques and incubation times.

Conclusion

Photodynamic therapy has become one of the most often chosen treatment for photorejuvenation in cosmetic dermatology due to excellent efficacy with high tolerability. At present, the treatment protocols of PDT for rejuvenation are not yet standardized and the precise mechanism of action of PDT on dermal collagen production in the skin is still uncertain. Skin texture and wrinkle improvement in this study demonstrated the usefulness of photodynamic therapy with riboflavin tryptophan for facial skin rejuvenation. PDT with riboflavin tryptophan gel was non-invasive and well-tolerated by the patients. Nonetheless, further randomized-controlled trials with more sample size and longer follow-up period of time are needed.

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