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ORIGINAL ARTICLE

Melanocyte activation and skin barrier disruption induced in melasma patients after 1064 nm Nd:YAG laser treatment

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Abstract

Melasma is a frequently acquired hyperpigmentary skin disorder, for which several therapies are available. Among them, 1064 nm QS Nd:YAG laser therapy is an effective method, but the recurrence rate of laser treatment is still high. The aim of the present study was to elucidate the mechanism of the high relapse rate of melasma after 1064 nm Nd:YAG laser treatment. Twenty-five female melasma patients were treated with 1064 nm Nd:YAG laser for 10 times. The lesional skin and non-lesional skin were evaluated by means of a reflectance confocal laser scanning microscope before and after laser treatment. Melanin content and transepidermal water loss (TEWL) were measured by an MPA9 skin multifunction tester accordingly. The melanin index value was significantly decreased in the lesional skin after laser treatment, while the non-lesional skin had no difference. The dendritic cells were observed at the level of the dermal-epidermal junction (DEJ) in the lesions of 8 patients before laser treatment, while after laser treatment, the dendritic cells were observed in all 25 subjects. Moreover, there was significant difference between the TEWL value of the lesions before and after laser treatment. Furthermore, the TEWL value was higher in lesions of the 8 subjects which had dendritic cells compared with other 17 subjects which had no dendritic cells, no matter before or after laser treatment. The relapse patients of melasma had higher TEWL value compared with the non-relapse patients. Melanocyte activation and skin barrier disruption may be related to the high relapse rate of melasma after laser treatment.

Keywords Melasma · Melanocytes · Skin barrier · 1064 nm Nd:YAG laser

Introduction

Melasma is a melanogenesis dysfunction disease which results in localized, chronic acquired hyperpigmentation of the skin. It occurs symmetrically on human face, especially in woman. Due to the pathogenesis, mechanism of melasma is still unclear, the treatment for melasma is challenging, and the recurrence rate is high. The 1064 nm Q-switched Nd: YAG laser has been successfully used to treat melasma in recent years and get marked clinical improvement [1–3]. The melasma patients in this study were treated with low energy 1064 nm Q-switched (QS) Nd: YAG laser and were satisfied with the clinical therapy effects after 10 times treatment. But the relapse rate of these melasma patients was high 3–6 months after the endpoint of laser treatment and little is known about its mechanism.

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It has been verified that skin barrier recovery rate was significantly delayed and most of the lipid metabolism-associated genes were downregulated in the lesional skin of melasma [4]. This indicates that skin barrier function may be involved in the pathogenesis of melasma. Recent study showed that melanized keratinocytes displayed superior barrier function in comparison to lightly pigmented keratinocytes [5]. It means that melanin is involved in the formation of skin barrier function. In the process of laser treatment, melanin is gradually demolished from keratinocytes in the epidermis. Therefore, the suspection can be supported that laser treatments attenuate skin barrier function and it may be partly responsible for the relapse of melasma.

In the present study, we determined the changes of skin barrier function and melanin content after 1064 nm wavelength QS Nd:YAG laser treatment. We found that after Nd:YAG laser treatment, the trans-epidermal water loss (TEWL) was increased and more dendritic cells could be seen in the dermal-epidermal junction (DEJ) of lesional skin of melasma.

Material and methods

Subjects

Twenty-five female patients with melasma were enrolled in this study. The patients were recruited in the laser cosmetic center of the Third Hospital of Hangzhou, China. The mean age of the patients was 34 years (28–43 years) and their skin types were Fitzpatrick 3–4. It was ensured that bleaching agents, steroids, or any kind of laser treatment and systemic treatment were not used for the melasma patients within 6 months. Pregnancy or breast feeding was excluded from this study. The written informed consent was obtained from all patients before laser treatment.

Laser treatment

The patients were treated with a wavelength of 1064 nm QS Nd:YAG laser (MedLite C6, Hoya ConBio, USA). A fluence of 2.0–3.0 J/cm^2, spot size of 6 mm, and repeated frequency of 10 Hz was used for the treatment. The hand piece was moved slowly to treat the whole face for two or three times until a slight erythema was developed. The treatment was performed for 10 weeks at 1-week intervals and no other procedures were taken after laser treatment except moisture and sunscreen usage daily.

Reflectance confocal microscope (RCM)

The images of lesional skin and normal skin were acquired by means of a reflectance confocal laser scanning microscope (Vivascope 1500; Lucid Inc., USA). Each RCM image corresponded to a horizontal section with an effective 500 × 500 μm view field. The confocal features observed in the melasma lesions were the number of dendritic cells in the basal cell layer. To quantify the changes before and after laser treatment, each single image at 40 ± 10 μm depth for basal cell layer was evaluated by two observers independently.

Transepidermal water loss measurements

Melanin index and TEWL was measured with an MPA9 skin multifunction tester (Courage Khazaka, German). The test was performed at room temperature for 20–26 °C, relative humidity 40–60% of the room, with no ventilation and no direct sunlight. No topical medications or cosmetics were allowed to use on the measurement day. Each patient was measured at the lesional skin of cheek and non-lesional normal skin of neck. The measurements were repeated three times and the melanin index and TEWL (g/ m^2 / h) value were the average measured value.

Statistical analysis

The statistical analysis was carried out using SPSS statistical package 10.0 (SPSS Inc., Chicago, IL, USA). The data were normally distributed and were expressed as mean ± standard deviation. Differences between melanin index or TEWL values before and after laser treatment were evaluated by student's t test. All the statistical analyses were performed at a bilateral significance level of 0.05.

Results

Melanin content of melasma patients after laser treatment

The skin pigmentation significantly faded from the appearance of the lesional skin of melasma after 1064 nm Nd:YAG laser treatment for 10 times (Fig. 1a, b). Before laser treatment, a large amount of melanin can be observed at the basal layer of the lesional skin of melasma. After laser treatment for 10 times, the melanin index value was significantly decreased at the basal layer of the lesional skin of melasma. There was significant difference of the melanin index in lesional skin

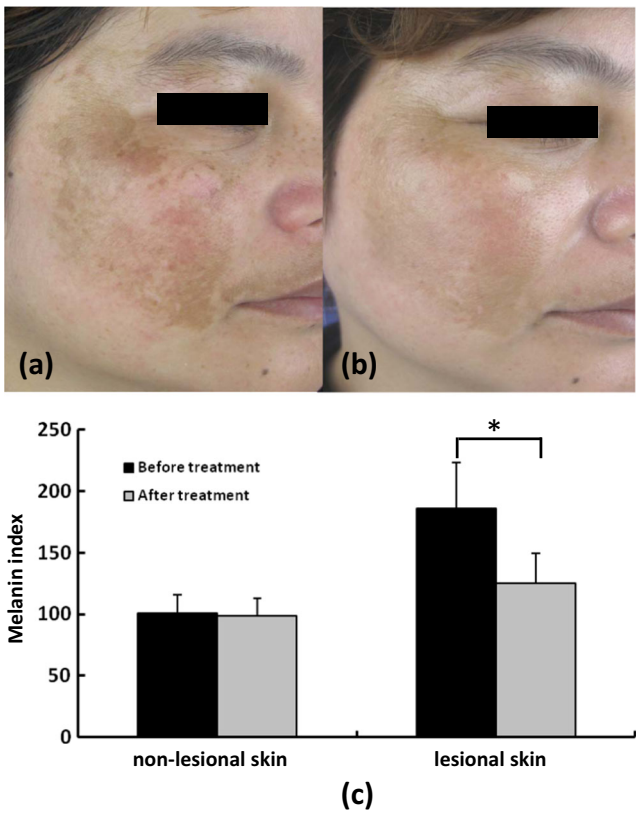

Fig. 1 Melanin content in melasma patients after laser treatment. **a** Lesional skin before laser treatment. **b** Lesional skin after 10 times laser treatment. **c** *$P < 0.05$, melanin index after 10 times laser treatment (gray) compared with the one before laser treatment (black)

before and after laser treatment (185.92 ± 37.47 vs. 124.95 ± 24.26). The melanin index of non-lesional skin was lower compared with the lesional skin. After laser treatment for 10 times, it had no significant change of melanin index of the non-lesional skin (100.90 ± 15.12 vs. 98.92 ± 14.36) (Fig. 1c).

Dendritic cells of melasma patients after laser treatment

In 8 of 25 melasma patients, RCM images showed bright dendritic cells at the level of the DEJ in the lesional skin before 1064 nm Nd:YAG laser treatment. The RCM images of lesional skin without dendritic cells were shown in Fig. 2c and the images of lesional skin with dendritic cells were shown in Fig. 2e. After laser treatment for 10 times, bright dendritic cells could be observed in the lesional skin of all the 25 melasma patients. And the lesional skin with dendritic cells showed more bright dendritic cells (Fig. 2f) compared with the ones without dendritic cells after laser treatment (Fig. 2d). There were no bright dendritic cells in the non-lesional skin of all 25 patients before and after laser treatment. (Fig. 2a, b).

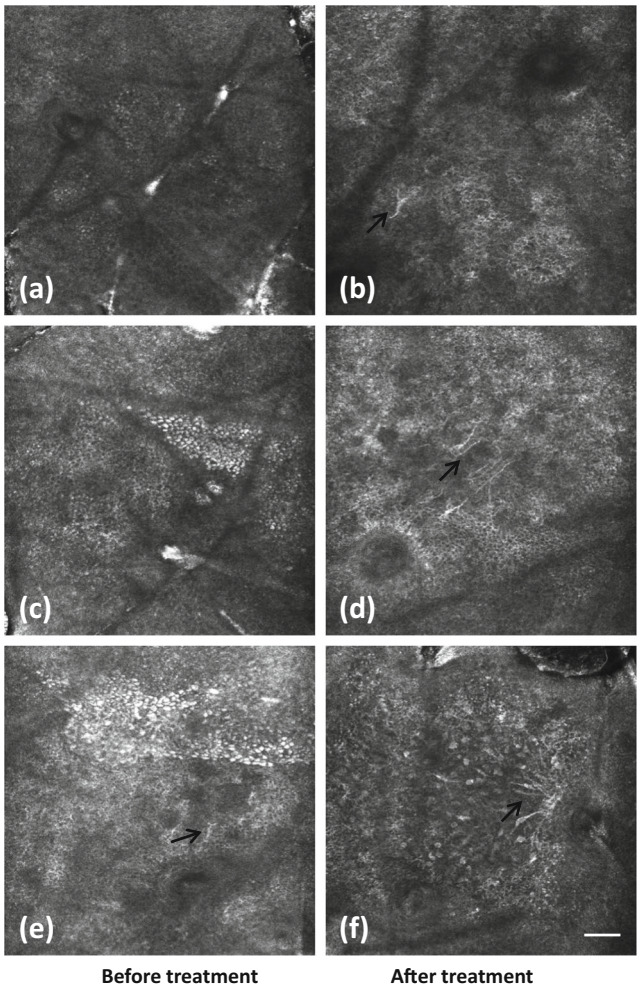

Fig. 2 Dendritic cells in melasma patients after laser treatment. **a** Non-lesional skin before laser treatment. **b** Non-lesional skin after laser treatment. **c** Lesional skin without DC before laser treatment. **d** Lesional skin without DC after laser treatment. **e** Lesional skin with DC before laser treatment. **f** Lesional skin with DC after laser treatment. The dendritic cells (black arrow) correspond to activated melanocytes. DC: dendritic cell. Scale bar 50 μm

TEWL value of melasma patients after laser treatment

There was no significant difference in the trans-epidermal water loss (TEWL) level between the lesional skin and peripheral non-lesional skin (18.18 ± 4.36vs.16.08 ± 4.62, g/m^2/h). After 10 times treatment with 1064 nm Nd:YAG laser, the TEWL value of the lesional skin was 25.34 ± 5.44 g/m^2/h and it was higher than the ones before laser treatment (18.18 ± 4.36 g/m^2/ h). There was significant difference between the two groups (Fig. 3a).

When compared the lesional skin with dendritic cells and without dendritic cells, the TEWL value was 21.83 ± 2.29 g/m^2/ h of the 8 ones with dendritic cells, and it was higher than the other 17 patients without dendritic cells (16.13 ± 3.90 g/m^2/ h). After laser treatment for 10 times, the TEWL value of the lesions with dendritic cells was 30.74 ± 3.31 g/m^2/h and it was

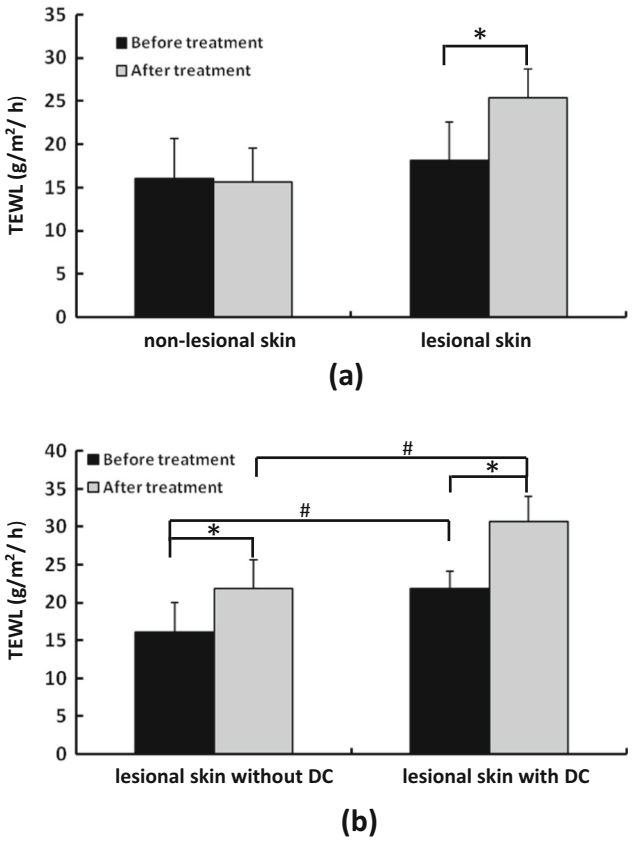

Fig. 3 TEWL value in melasma patients after laser treatment. **a** TEWL of non-lesional skin or lesional skin before and after 10 times laser treatment. **b** TEWl of lesional skin without or with DC before and after 10 times laser treatment. *$P < 0.05$, TEWL after treatment (gray) compared with TEWL before laser treatment (black). $^{\#}P < 0.05$, TEWL of lesional skin with DC compared with the one without DC

higher than the ones without dendritic cells (21.79 ± 3.89 g/m^2/h). There was significant difference between the two groups (Fig. 3b).

Relapse of melasma is related to high TEWL value after laser treatment

The recurrence of melasma was confirmed by the CLSM findings which showed the melanin level returned to previous baseline levels and the recurrence rate was verified to be as high as 68% in patients 3 months after the endpoint of laser treatment. The TEWL value after 10 times laser treatment was evaluated retrospectively in the 17 relapse patients compared with the 8 non-relapse patients. After the endpoint of laser treatment, the TEWL value of the relapse ones was 26.56 ± 2.91 g/m^2/h and it was higher than the 8 non-relapse ones (19.54 ± 3.43 g/m^2/ h). There was significant difference between the two groups (Fig. 4).

Discussion

Melasma is an acquired skin pigmentary disorder characterized by symmetrical hyperpigmented lesions on the exposed area, especially on the face. The treatment of melasma is challenging and it includes a variety of therapeutical options including topical formulations, chemical peels, lasers, and light source [6]. Recent studies have shown that 1064 nm QS Nd:YAG laser therapy is an effective method for the treatment of melasma. The 1064 nm QS Nd:YAG laser can precisely target melanin particles in melasma lesions and the melanin particles were significantly decreased after the laser treatment [2, 7]. The changes of melanin content can be observed by confocal laser scanning microscopy (CLSM). CLSM is a non-invasive technique and a promising clinical diagnostic tool for the evaluation of the skin up to the papillary dermis. CLSM facilitates the real-time in vivo examination of the skin at a level that permits the visualization of microscopic structures and individual cells. It has been widely used to detect pigmented lesions [8, 9]. In this study, the CLSM results showed that after 10 times 1064 nm Nd:YAG laser treatment, the concentration of melanin was significantly decreased in the epidermis, which coincided with the degree of clinical improvment. The fluence of the laser used in our study is 2.0–3.0 J/cm^2, spot size 6 mm, and frequency of 10 Hz. It indicates that 1064 nm QS Nd:YAG laser treatment is an effective method to treat melasma patients.

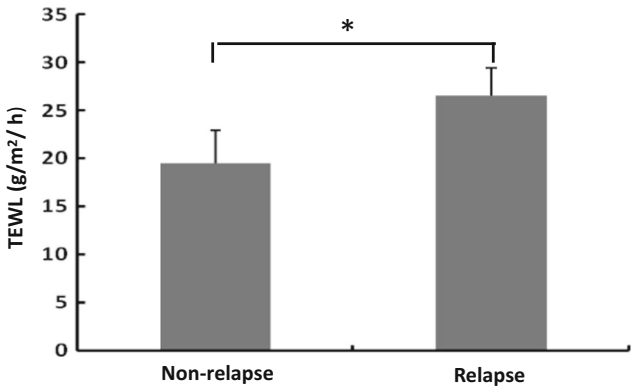

Fig. 4 Relapse of melasma is related to high TEWL value after laser treatment. TEWL of relapse melasma and non-relapse melasma after 10 times laser treatment. *$P < 0.05$, TEWL of relapse melasma compared with TEWL of non-relapse melasma

Although the present study showed marked improvement in melasma after 1064 nm QS Nd:YAG laser treatment, the high recurrence rate of melasma is still a challenge. With 3-month follow-up observation, the rate of the recurrence was as high as 64% in melasma patients treated with 1064 nm QS Nd:YAG laser [10]. Melanocytes in melasma lesions are thought to be highly active and easily stimulated. Some research thought the large-spot, low-energy mode combined with the longer wavelength of the QS Nd:YAG laser, was not easy to be absorbed by melanin and might enable the laser to suppress melanocyte function without stimulating them [11, 12]. But the presence of activated dendritic-shaped cells was still reported to be found in melasma after QS Nd:YAG laser treatment [10]. Our results showed there were more dendritic melanocytes after 1064 nm QS Nd:YAG laser treatment. The dendritic cells were observed in the lesions of 8 patients before laser treatment, while after laser treatment the dendritic cells were observed in all the 25 subjects. It means Nd:YAG laser treatment can induce the activation of epidermal melanocytes. Previous study showed that Q-switched alexandrite laser removed most melanin from the epidermis; however, numerous activated melanocytes were observed on day 7 and continued to be observed until day 28 in the subjects [13]. It has also been elucidated that the presence of bright dendritic cells in melasma after laser treatment had an early relapse of melasma and these cells correspond to activated melanocytes [10]. Therefore, it is confirmed that the activation of melanocytes is responsible for the relapse of melasma after laser treatment.

As for the mechanism of melanocytes activation in melasma after laser treatment, some signs indicate that it may be involved in skin barrier eruption. Previous studies showed that a delayed barrier recovery rate, impaired stratum corneum integrity, and the thinning of stratum corneum were observed in melasma lesions [14, 15]. The putative

melanosomes in dendrites of darkly pigmented human melanocytes were more acidic than comparable vesicular structures in lightly-pigmented subjects. The melanized keratinocytes displayed superior barrier function in comparison to lightly pigmented keratinocytes [5]. These results illustrated that pigmentation enhanced barrier function in human skin; thus, it can be suspected the clearance of the melanin by QS Nd:YAG laser might attenuate the skin barrier function. As a compensatory mechanism, melanocytes might be activated to produce more melanin to restore skin barrier function, which can be clinically manifested as recurrence of melasma. Our results showed TEWL value of melasma lesions was upregulated after 1064 nm QS Nd:YAG laser treatment, which indicated the eruption of skin barrier function. Even before laser treatment, the TEWL value of the lesions with dendritic melanocytes was higher than the lesions without dendritic cells, which indicated the already existed skin barrier disfunction in lesional skin of melasma. After QS Nd:YAG laser treatment, the worsened skin barrier function may partly be the cause of the recurrence of melasma. According to our findings in the present study, we propose that melanocytes activation and skin barrier disruption may be related to the high relapse of melasma after laser treatment.

Our observation also showed that 68% of recurrences were found in melasma patients 3 months after the endpoint of laser treatment. The TEWL values after laser treatment were higher in the relapse patients compared with the non-relapse ones. It confirmed the inference that the disruption of skin barrier was related to the recurrence of melasma. Therefore, targeting the recovery of skin barrier function is promising for melasma treatment. However, further studies are warranted to deeply elucidate the complicated mechanism of the relapse of melasma subjected to laser treatment.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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