Introduction

The Asian patient’s expectations for cosmetic dermatological treatment of pigmented lesions are high, and

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the effectiveness of laser and light treatments for pigmented lesions with many types of devices, such as
KTP-532, ruby, alexandrite and Nd-YAG lasers and intense pulsed light (IPL) systems, has been reported.
1,2 The effectiveness of these treatments for senile lentigo, postinflammatory hyperpigmentation and
melasma has also been reported. On the other hand, many reports have described that the treatment of post inflammatory hyperpigmentation (PIH) and melasma should be avoided at all costs, particularly in the Asian skin phenotype due to its propensity for PIH formation.

There are many patients with melasma in Asian populations. This disease is often found especially in middle-aged females and beyond, and many patients with melasma visit our hospital for treatment. However, the effects of topical preparations and chemical peeling are limited, and conventional wisdom advises that laser and light treatments are basically forbidden in the treatment of melasma in this skin type. In particular, a high frequency of recurrence some time after treatment with lasers, such as the ruby laser, is problematic.

Recently, many studies have reported the clinical effectiveness of laser toning with the Q-switched Nd-YAG laser for the treatment of melasma. In this study, the clinical effects of laser toning on melasma were investigated and the results were compared with those of a Q-switched ruby laser from a histopathological point of view.

**Subjects and Methods**

**Subjects**

Healthy adult Japanese volunteers (n = 8, aged 41 to 57 years [mean 52.5], all female, Fitzpatrick skin type III) were recruited. All subjects had melasma, with fairly homogeneous bilateral involvement as is typical of this condition. Subjects with chronic illness, atopic dermatitis, contact dermatitis, photosensitivity, a history of scarring or poor wound healing, vascular disease, or cutaneous disease were excluded. Written informed consent was obtained from each volunteer and the study was approved by the Ethics Committee of the Queen’s Square Medical Center.

**Nd:YAG Laser Toning**

For laser toning, a Q-switched Nd-YAG laser, MedLite C6 (HOYA ConBio, Fremont, CA, USA) was used. Only one side of each patient’s face was irradiated, randomly assigned (QS:YAG group). The laser toning parameters for the present study were set as follows: wavelength of 1064 nm, spot size of 6 mm, pulse width of 5-7 ns, fluence of 3.0 J/cm² and a repetition rate of 10 Hz. The end point of irradiation was the appearance of a mild general erythema in the irradiated area, after from 5-7 passes with an approximate 20% overlap. Treatment was performed once a week for 4 consecutive weeks. After irradiation, only cooling was given for approximately 10 minutes and no topical treatment was applied.

**Q-Switched Ruby Laser**

A Q-switched ruby laser was used on the contralateral side to the Nd:YAG laser toning for comparison (QS:Ruby group). The Q-switched ruby laser (The Ruby Z1, JMEC, Tokyo, Japan) was applied at the wavelength of 694.3 nm with a spot size of 4 mm in diameter, pulse width of 20 ns, a fluence of 4.0 J/cm², and a repetition rate of 0.5 Hz. A single pass was given, with approximately 20% overlapping. The macroscopic endpoint was a white frost-like change to the epidermis for each shot.

**Histopathological observation**

Three millimeter punch biopsies were taken from the QS:YAG group immediately after 4 irrigations and from the QS:Ruby group immediately after a single irradiation, and prepared for histopathological assessment with light- and transmission electron microscopy.

Briefly, each specimen was fixed in glutaraldehyde (2.5%) and later with osmium tetroxide (1%). After dehydration with graded ethanol, specimens were embedded in Epok 812 (Oken Shoji Co., Ltd, Tokyo). All samples were observed with a light microscope after toluidine blue staining. Ultrathin sections were obtained with an Ultracut N ultramicrotome (Reichert-Nissei) and diamond knife. Sections were stained with oolong tea extract for connective tissue, uranyl acetate, and lead citrate for transmission electron microscopic examination (75 kV, Hitachi H-7500, Hitachi, Tokyo, Japan).

**Results**

**Clinical observation**

Clinically, laser toning was performed 4 times, and a macroscopic comparison was made before and after the 4th treatment session. In the case of ruby laser treatment, only 1 treatment was given and a macroscopic comparison was made before and after this single session. In both the QS:YAG and QS:Ruby groups a decrease in melasma was found in all cases, but recurrence was soon noted in all the QS:Ruby sides, in agreement with previous reports. Figure 1 shows a typical example of Nd:YAG laser toning results at baseline (Fig. 1A) and after the 4th session (Fig. 1B), with Figure 2 showing the result in another patient after...
only 2 laser toning sessions compared with baseline (Fig. 2B and 2A respectively), with maintenance of the treatment 3 weeks after the final session (Fig. 2C) and at an extended follow-up of 2 years (Fig. 2D).

Ultrastructural observation, QS:YAG group

Figure 3 shows photomicrographs of a typical QS:YAG specimen. In the nuclei (N) of epidermal cells typically appeared after 4 Nd:YAG laser toning sessions. However, a marked decrease in the number of melanin granules was found in the cytoplasm (Fig. 3A). In addition, in the dermis just below the dermoepidermal junction, fibroblasts showed an almost normal appearance and incorporated melanin granules (arrows) into their cytoplasm (Fig. 3B).

Ultrastructural observation, QS:Ruby group

Immediately after irradiation with the ruby laser, a high concentration of heterochromatin in the nucleus (N) was noted, and the necrotic appearance of the cytoplasm with large and small vacuoles (V) was observed in epidermal cells (Fig. 4A). Just below the dermoepidermal junction, the degenerative and necrotic appearance of fibroblasts with large vacuoles (V) was observed in the dermis just below the dermoepidermal junction. Capillary vessels (Cap), however, appeared normal (Fig. 4B).

Fig. 1: Melasma in a 50-year-old female. a) Findings at baseline. b) Findings after 4 Nd:YAG laser toning sessions, with clear improvement in pigment.

Fig. 2: Melasma in a 44-year-old female. a) Findings at baseline, b) after only 2 laser toning sessions, c) the improvement is maintained 3 weeks after the 4th treatment session, and d) is still maintained in the long-term at 2 years post-treatment.
Fig. 3: Electron micrographs of tissue in a typical QS:YAG-treated specimen. A) The nuclei (N) of epidermal cells showed a normal appearance after 4 ND:YAG laser toning treatment sessions, but at the same time a marked decrease in the number of melanin granules (arrows) was found in the cytoplasm. B) Additionally, in the dermis just below the dermoepidermal junction, fibroblasts showed an almost normal appearance and had incorporated melanin granules (arrows) into their cytoplasm. (Scale bars are as shown)

Fig. 4: Electron micrographs of tissue in a typical QS:YAG-treated specimen. A) Immediately after irradiation with the ruby laser, a high concentration of heterochromatin was noted in the nucleus (N) and the necrotic appearance of the cytoplasm with large and small vacuoles (V) was observed in epidermal cells (arrows: melanin granules). B) In the superficial dermis, the degeneration and necrosis of fibroblasts with large vacuoles (V) were observed. Capillary vessels (Cap) appeared normal (arrows: melanin granules). (Scale bars are as shown)
Discussion

Topically-applied preparations, such as hydroquinone and tretinoin, and chemical peeling, and laser and light treatments with ruby and alexandrite lasers and IPL have been reported for the treatment of melasma. However, topical preparations have problems in that the patients sometimes feel some uncomfortable aftereffects in their skin and are not necessarily fully satisfied. Chemical peeling has a problem in that patients with darker skin types are sometimes at risk for hyperpigmentation and hypopigmentation.

There have been quite a few reports showing the effectiveness of IPL. However, hyperpigmentation can be a problematic side effect with this treatment in the Asian skin phenotype, and combined therapy with hydroquinone and other drugs is recommended.

Laser treatment of pigmented lesions has produced very good results and is particularly effective for solar lentigines and freckles (ephilides). However, this treatment is considered to be contraindicated for melasma, because it is ineffective or recurrence occurs even if it works in many cases. For example, Tse et al. have reported that both Q-switched Nd:YAG and Q-switched ruby lasers showed no effect in the investigation of 20 patients. The Q-switched ruby laser has a wavelength of 694.3 nm, which has melanin as a chromophore. However, poor results or recurrences in the early stages after treatment have been reported in most studies.

On the other hand, it has recently been reported that laser toning, which irradiates over multiple passes with a low-fluence Q-switched Nd:YAG laser, is effective for melasma. One reason for this efficacy must lie with the comparative absorption rates between 694.3 nm and 1064 nm, with the latter being much lower on the absorption curve than the former. Even with comparative fluences, therefore, higher melanin-specific absorption could be expected for the ruby wavelength, resulting in the creation of a stronger photothermal effect.

From the histochemical viewpoint, Kim et al. have reported on Nd:YAG laser toning for melasma, and demonstrated that Fontana-Masson and Melan-A stains revealed a marked decrease in melanin granules and a decrease in melanocytes in the basal layer, findings similar to those in punctate leukoderma, and have speculated that the decrease in pigment following Nd:YAG laser toning is phototoxicity-induced damage to melanocytes, which is unlikely to easily lead to repigmentation. Mun et al. have reported that, as a result of laser toning for melasma in 16 patients, a decrease in pigment was clinically found and three-dimensional scanning electron microscopy (3D SEM) revealed a dramatic decrease in the size and number of melanocytic dendrites, which deliver melanin to the daughter keratinocytes, although the melanocytes themselves survived.

In the present study, a decrease in melasma was found in all patients who underwent laser toning with the low-fluence Nd:YAG laser over 4 sessions, and no increase in pigment was observed in patients for whom follow-up was available after treatment. Furthermore, in the histopathological investigations, after 4 laser toning sessions with the Nd:YAG laser, a marked decrease in the number of melanin granules was found in epidermal cells and incorporation of melanin granules into fibroblasts was observed in the dermis just below the dermo-epidermal junction. The decrease in melanin granules with sparing of the cellular architecture following Q-switched Nd:YAG treatment was consistent with the reports by Kim and colleagues in the zebrafish and Mun et al. in human subjects, based on the concept of subcellular selective photothermolysis.

On the other hand, changes in the basal layer of the epidermis in the present study seemed to be somewhat greater than those reported by Mun et al. This is considered to be caused by the somewhat higher output (5 to 7 passes at a fluence of 3.0 J/cm²) in this study than in the study by Mun et al. (2 passes with a fluence of 1.6-2.0 J/cm²), in addition to the differences due to the Q-switched laser systems used. In the present study, a decrease in pigment was observed in all cases following Nd:YAG laser toning, no adverse effects were observed and incorporation of melanin granules was histologically found in fibroblasts after irradiation. It is considered from these observations that the degradation of melanin granules was more promoted following the Nd:YAG treatment in the present study, suggesting that these conditions lead to a greater improvement in melasma lesions. The fact that cells previously containing the melanin granules remained alive, as was shown by the selective destruction of melanosomes in melanocytes in the Mun study referenced above, would also account for the longer period before repigmentation, as the melanocytes seemed to have undergone a dendrectomy: in addition, it has been reported that levels an essential substance mediating the transfer of melanin from melanocyte to daughter keratinocyte, PAR-2, decreased following laser toning sessions, likewise for tyrosinase-related proteins TRP-1 and -2, part of the...
intramelanocytic melanin oxidization process. The lesser degree of both epidermal and dermal cellular damage seen in the photomicrographs in the present study would also be related to a lower inflammatory response, thus mediating PIH induction.

As for the ruby laser, immediately after irradiation condensed nuclei and the appearance of cytoplasmic vacuoles were observed and an overall necrotic appearance was observed in epidermal cells. The degenerative appearance of melanin granules was also seen, so it could be said that the Q-switched ruby laser had met the criterion for producing selective photothermolysis of the melanin, but at the expense of damage to the cells containing the melanin. In the dermis just below the dermoepidermal junction, the degenerative and necrotic appearance of fibroblasts with large vacuoles was observed. This could possibly lead to a greater inflammatory reaction, and potential PIH formation. Furthermore, although the basal layer keratinocytes may also have been fatally damaged, replacement keratinocytes are known to differentiate and migrate from stem cells in the dermis, and melanin production would thus probably resume faster than in the case of the Q-switched Nd:YAG laser treatment. These observations indicate that both epidermal and dermal skin damage is much greater following treatment with one Q-switched ruby laser session than with 4 Nd:YAG laser toning sessions.

In the actual gross clinical findings, the ruby laser-irradiated area showed a change with a crust-like whitening phenomenon immediately after irradiation in all patients. However, laser toning caused only an even erythema without such a change in the appearance of the epidermal morphology.

Following ruby laser treatment in the present study, a decrease in pigment was observed after the treatment of melasma and degenerated melanin was also histologically observed immediately after irradiation, consistent with the decrease in pigment. However, as reported in other articles in the literature referenced above, a re-increase in pigment was also found in almost all cases. The authors regret that clinical photography from only the Qs:YAG treated sides was kept and shown in the present study, and this is a limitation to the study as far as the macroscopic aspect is concerned. However, the main focus of the study was on the histopathological findings, rather than the macroscopic aspect. Furthermore, recurrence of melasma after Qs:Ruby treatment is well-documented compared with post Qs:YAG laser toning (see Figure 2C, D above), and this is something that the authors will be sure to address in any future study.

Generally, clinical and histological pigmentation is often observed after inflammation. After the extensive degenerative appearance of the epidermis as observed in the present study following Q-switched ruby laser treatment, repair of such tissue through fibroblast activation is considered to occur extensively, and this is known to be linked with melanogenesis, leading to an increase in pigment some time after treatment during the remodeling process.

On the other hand, in Nd:YAG laser toning, a decrease in melanin granules was found, but the degenerative appearance of the epidermis was scarcely observed even after 4 treatment sessions, and the dermis appeared more or less normal. The minor damage to the skin was another factor most probably related to the absence of a re-increase in pigment in the clinical follow-up, in addition to the speculated delayed melanocytic melanogenesis given above.

Conclusions

Although Q-switched Nd:YAG laser toning and Q-switched ruby laser treatment achieved good melasma pigment removal in the Japanese skin immediately after treatment, the former gave better short-term and longer-term results for melasma in the Asian skin type III female than treatment with the latter, both histopathologically and macroscopically, with less epidermal and dermal damage seen for the Nd:YAG laser toning.

References