

A Pilot Study Using Reflectance Confocal Microscopy (RCM) in the Assessment of a Novel Formulation for the Treatment of Melasma

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ABSTRACT

Introduction: Melasma is a common pigmentary disorder caused by abnormal melanin deposits within the skin. Hydroquinone (HQ) is presently the most popular depigmenting agent, however the treatment of melasma remains unsatisfactory, resulting in a need to evaluate new depigmenting agents.

Objective: The objective of this study was to assess, using standard methods and a novel technique, in vivo Reflectance Confocal Microscopy (RCM), the efficacy and safety of a new non-HQ bleaching agent Dermamelan[®] (Mesoestetic, Barcelona, Spain) in the treatment of melasma.

Methods: Ten women with melasma were enrolled in an open-label trial lasting four months. Patients were of Fitzpatrick skin types II–IV. A non-HQ depigmenting agent (Dermamelan) was applied once-daily for three months. Melasma Area and Severity Indices (MASI) were measured. Standard and UV-light photographs were taken and in vivo RCM, which detects pigmentary changes at a cellular level, was done. Evaluations were performed before treatment, on the first, second and third month of treatment and one month after treatment. Upon cessation of the trial, patients completed a questionnaire regarding efficacy and tolerance.

Results: At baseline, RCM detected hyperpigmented keratinocytes in all patients, dendritic cells in 2/10 patients, and melanophages in 2/10 patients. Based on the MASI score, Dermamelan treatment improved melasma by 50 percent. This was confirmed by standard and UV-light photography. Maximum therapeutic effect was usually reached by one month of treatment and was maintained at one month following its completion. Interestingly Dermamelan treatment also induced a statistically significant decrease of pigmented epidermal keratinocytes as detected by RCM. Patients with melanophages on RCM at baseline had a poorer outcome, but not those with dendritic cells. Mild irritation was the only adverse event observed during treatment. The majority of patients were satisfied with the result.

Conclusion: This study suggests that Dermamelan produces significant rapid improvement of melasma at a clinical and cellular level and demonstrates the potential of RCM to monitor and possibly predict efficacy of a new depigmenting agent in the treatment of melasma.

J Drugs Dermatol. 2011;10(11):1260-1264.

INTRODUCTION

Melasma is a common and disabling acquired hypermelanosis, most commonly affecting women, which presents as irregular brown-grey macules of the face. Aetiological factors include genetic predisposition, ultraviolet light exposure, and sex hormones.¹ The pathogenesis of melasma is not yet fully understood. Two histological patterns of pigmentation are observed: an epidermal and a dermal pattern. In the epidermal type, melanin deposition is increased in basal and suprabasal keratinocytes. The dermal type is characterized by melanin deposition in perivascular melanophages. Both patterns may be seen in patients with mixed-type melasma.² The efficacy of HQ and non-HQ in the treatment of melasma, is frequently unsatisfactory, notwithstanding the potential side-effects

of hydroquinone.^{3,4} In vivo reflectance confocal microscopy (RCM) is a novel technique, which allows noninvasive imaging of the epidermis and the upper dermis at a near-histological resolution. Because melanin is the strongest endogenous contrast in human skin, pigmentary disorders are ideal for RCM examination.⁵ In melasma, it has been shown that RCM accurately detects melanin distribution and in addition identifies the type of melanin-containing cells.^{6,7} RCM could therefore be very useful to evaluate new depigmenting agents. We conducted a clinical trial to evaluate the efficacy and safety of a novel non-hydroquinone formulation Dermamelan[®] (Mesoestetic, Barcelona, Spain) for the treatment of melasma, using RCM to monitor pigment changes within the skin.

METHODS

Ten subjects with facial melasma, of Fitzpatrick skin type II–IV, were recruited for this open label study. Patients had received no treatment for melasma for at least two months before the study. They applied Dermamelan (azelaic acid, kojic acid, alpha arbutin, glycirrhiza glabra extract and ascorbic acid) once daily for 12 weeks. Patients were assessed at baseline, after the first, second, and third month of treatment, and one month after the end of treatment. Each visit consisted of a Wood's lamp examination, assessment of the MASI score, standardized normal and UV-light photography, and RCM examination of melasma lesions. Adverse events were noted at all visits. Irritation was graded on a four-point scale (0: none, 1: mild, 2: moderate, 3: severe). Compliance was also noted. At the end of trial, the patients filled in a self-evaluation questionnaire regarding efficacy and tolerance. Appropriate informed consent was obtained from all subjects.

Reflectance Confocal Microscopy

RCM images were acquired by means of a near-infrared reflectance confocal laser scanning microscope (Vivascope 1500, Lucid Inc, Rochester, NY, USA), which uses an 830 nm laser beam with a maximum power of 22 mW. Each image corresponds to a horizontal section at a selected depth with a 500x500 μm field of view, and a resolution of 1000x1000 pixels. Two types of images were collected, blocks, and stacks. A sequence of block images was acquired at the suprabasal layer, dermo-epidermal junction (DEJ), and dermis to explore a 4x4 mm field of view/lesion. Stack images were recorded at the center of the lesions and in areas of interest. In order to quantify the changes during treatment, each single image, (500x500 μm) at 40 \pm 10 μm depth for basal cell layer and 70 \pm 10 μm depth for dermis, was evaluated independently by two different observers, and scored as: 1, 0–25%; 2, 25–50%; 3, 50–75%; and 4, 75–100% for epidermal pigmentation. The mean value was used for statistical evaluation. The presence of epidermal dendritic cells or dermal melanophages was assessed qualitatively, e.g. as present (+) or not (-).

Statistical Analysis

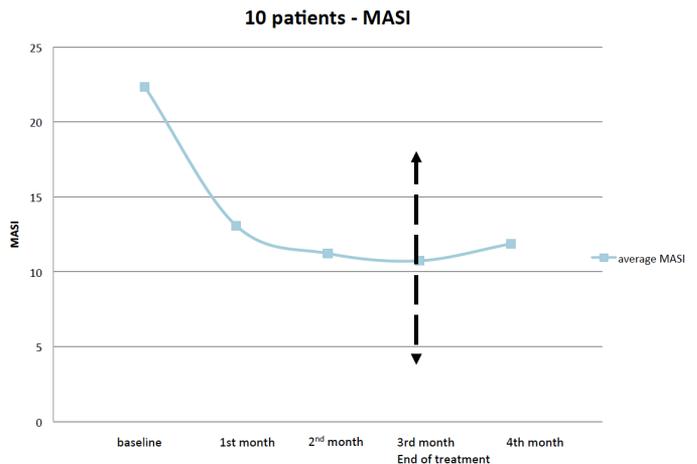
Evaluation of efficacy on MASI score and RCM measurements was analyzed using the Wilcoxon signed rank test.

RESULTS

At baseline, according to Wood's lamp examination, nine patients had epidermal melasma, and one had epidermal and dermal melasma. RCM examination detected hyperpigmented keratinocytes in all patients, along with epidermal dendritic cells in two patients and dermal melanophages in two (Table 1). After completion of treatment, the mean decrease of pigmentation, based on the MASI score was 50 percent (V0:22; V3:11, $P=0.008$) (Figure 1). Maximum therapeutic effect was usually reached after one month of treatment, was maintained throughout treatment and for one month following its completion. These results were confirmed by standard and UV-light

photography (Figure 2). In parallel to this clinical improvement, RCM detected a statistically significant decrease of hyper-refractile cobble-stoning cells, corresponding to pigmented keratinocytes, in the basal cell layer ($P=0.02$) (Table 1) (Figure 2). Interestingly, both patients with dermal melanophages on RCM at baseline had only a partial clinical response, with persistent melanophages evident on RCM follow-up (Table 1) (Figure 3). By contrast, the presence of dendritic cells did not correlate with response to treatment. Mild irritation was the only adverse event observed. The majority of patients were satisfied with efficacy and tolerance.

FIGURE 1. Compared to baseline (0), MASI score was significantly reduced during treatment (months one through three) and one month after the end of treatment (month four).



DISCUSSION

Dermamelan is a new, non-hydroquinone formulation, containing a combination of depigmenting agents. We performed a clinical trial to evaluate its efficacy and tolerance in the treatment of melasma, using RCM, a modality not previously employed for this purpose. In addition, we used established methods of assessment, such as the MASI score and standard and UV-light photography. Regarding the classification of melasma, RCM detected dermal melanophages in two out of ten cases (20%), while the Wood's lamp disclosed a dermal pattern in only one of these patients. This is in accordance with our previous finding that RCM is more accurate than the Wood's lamp in determining melanin depth.⁵ In two out of ten cases (20%), we found evidence of dendritic, highly refractile cellular structures in the epidermis. As reported previously, these cells probably correspond to activated melanocytes.⁶ We observed a rapid improvement in most patients with a significant mean reduction in the MASI score (50%). The reported efficacy of other melasma treatments ranges from a 32 percent to 75 percent reduction in the MASI over 12 weeks. The most efficacious agents reported to date are hydroquinone containing preparations.³ Our results favorably compare Der-

TABLE 1.**Results of RCM Examination**

	V0 Pigmented Keratinocytes	V3 Pigmented Keratinocytes	V0 Melanophages	V3 Melanophages	V0 Dendritic Cells	V3 Dendritic Cells
Patient 1	2	1	-	-	+	+
Patient 2	3	1	-	-	-	-
Patient 3	3	2	+	+	-	-
Patient 4	3	2	-	-	-	-
Patient 5	3	2	-	-	-	-
Patient 6	2	1	-	-	-	-
Patient 7	3	2	-	-	-	+
Patient 8	4	4	+	+	-	+
Patient 9	2	1	-	-	-	-
Patient 10	2	1	-	-	+	+

Epidermal pigmentation was scored on RCM images as: 1, 0–25%; 2, 25–50%; 3, 50–75%; and 4, 75–100%. RCM showed a statistically significant decrease of pigmented keratinocytes between V0 (m=2.7) and V3 (m=1.7) ($P=0.02$).

FIGURE 2. A representative case of epidermal melasma: **a-b)** standard light, **c-d)** UV light, and **e-f)** RCM. Appearance at day 0 (**a,c,e**) and day 90 (**b,d,f**) illustrate the complete clearance of melasma and pigmented keratinocytes (appearing as diffuse bright cells on the RCM image).

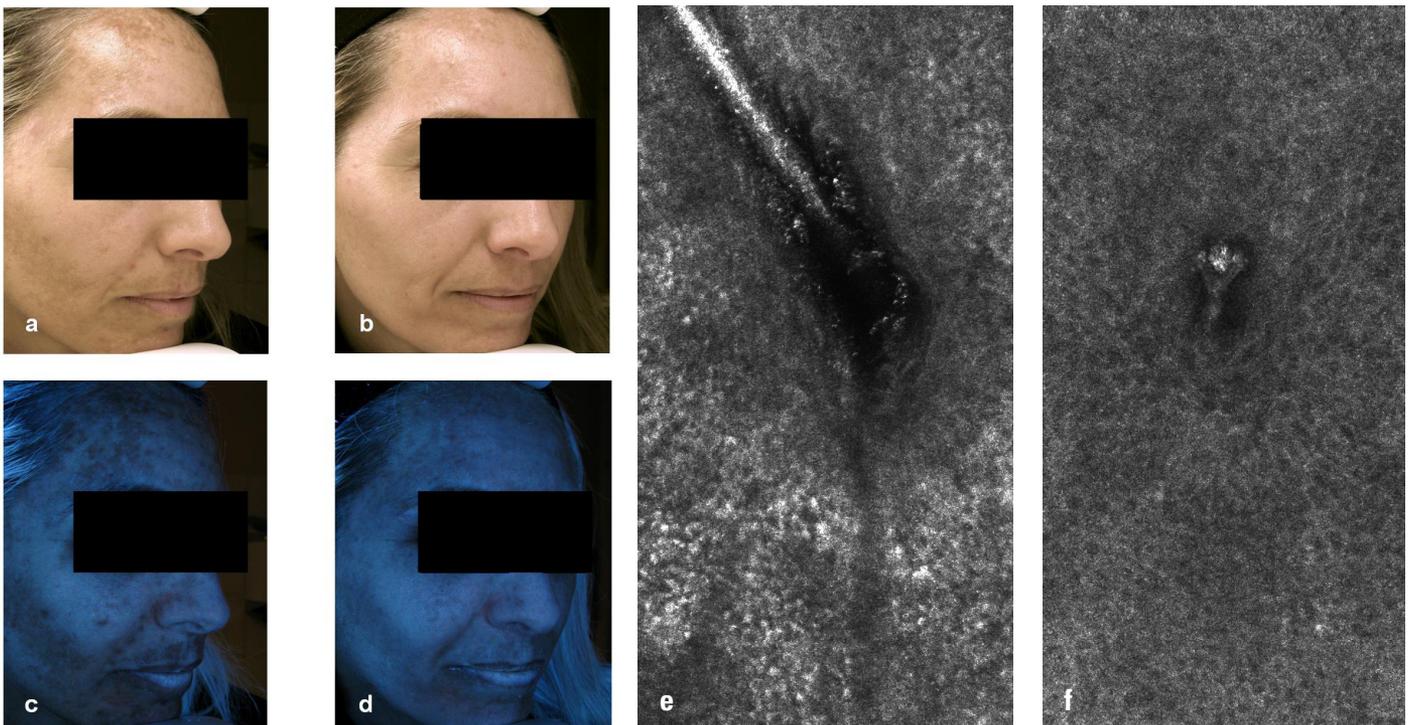
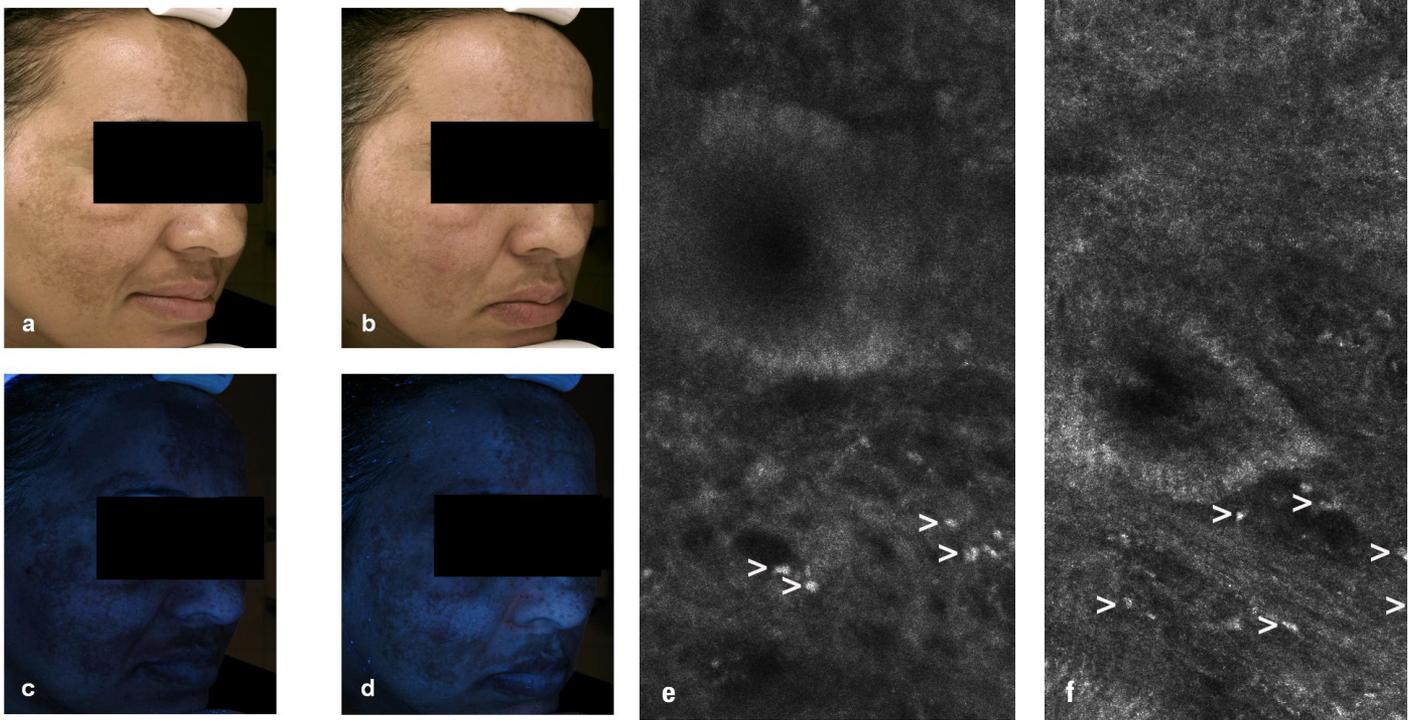


FIGURE 3. A representative case of epidermal-dermal melasma: **a-b)** standard light, **c-d)** UV light, and **e-f)** RCM. Appearance at day 0 (**a,c,e**) and day 90 (**b,d,f**) illustrate the persistence of melasma and melanophages (appearing as scattered bright cells with arrow-heads on RCM image).



This study suggests that Dermamelan produces a significant and rapid improvement in melasma at a clinical and cellular level and demonstrates the potential for RCM to monitor and even predict efficacy of a new depigmenting treatment in melasma.

mamelan with previously published treatments. Dermamelan is devoid of hydroquinone and has an excellent tolerance. A larger randomized control trial with a hydroquinone containing agent is needed to confirm these preliminary results.

In parallel to the clinical improvement, we noted a statistically significant decrease in pigmented keratinocytes on RCM images. Interestingly, we found that patients with exclusively epidermal hyperpigmentation on RCM, performed better than patients with dermal melanophages. This difference did not reach statistical significance, possibly because of the small size of our group. These findings raise the possibility that in contrast to the Wood's lamp,⁸ RCM might predict the response of melasma lesions to treatment and could therefore serve to select the most appropriate therapy for each patient.

CONCLUSION

This study suggests that Dermamelan produces a significant and rapid improvement in melasma at a clinical and cellular level and demonstrates the potential for RCM to monitor and even predict efficacy of a new depigmenting treatment in melasma.

ACKNOWLEDGEMENTS

The confocal scanning laser microscope used in this study was obtained through a grant from the Conseil General des Alpes-Maritimes.

DISCLOSURES

Jean Luc Levy MD is a consultant for Mesoestetic, and Philippe Bahadoran MD PhD has received speakers' honoraria from Mesoestetic. All other authors have no relevant conflicts of interest to disclose.

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