A Randomized, Controlled, Double-Blind Study Evaluating Melanin-Encapsulated Liposomes as a Chromophore for Laser Hair Removal of Blond, White, and Gray Hair

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Introduction: Laser hair removal of blond and white hair is a complicated task with often unsatisfactory results as a result of a lack of laser-absorbing chromophore. In the present study, we investigated if repetitive external application of liposomal melanin (Lipoxome; Dalton Medicare B.V., Zevenbergschen Hoek, The Netherlands) enables removal of blond/white and gray hair with a diode laser.

Methods: Forty-two areas of blond, gray, or white facial and body hair of 16 patients were treated with a liposomal melanin spray (Lipoxome) and 3 cycles of 800 nm diode laser at intervals of 8 weeks (28–40 J/cm^2). A control group of 16 patients applied physiological saline spray before diode laser treatment. Hair regrowth was measured 8 weeks after each cycle and additionally 6 months after the last treatment by counting the number of terminal hairs compared with baseline pretreatment values. Complications and treatment outcomes were documented.

Results: Mean regrowth in the liposomal melanin group was 83% after 3 treatment cycles. Six months after therapy, average terminal hair count compared with baseline pretreatment showed 14% reduction. Although significant difference was seen compared with the control group showing a 10% reduction of hair growth after 6 months ($P < 0.05$), the clinical outcome was disappointing.

Conclusions: Melanin-encapsulated liposomal spray in combination with diode laser treatment showed significant higher efficacy in the treatment of white and blond hair compared with a control group. However, the clinically observed hair reduction was so weak that additional effort as well as higher costs argues against the application of the tested formulation.

Key Words: photothermolysis, liposomes, melanin, hair removal

(*Ann Plast Surg* 2007;58: 551–554)

Received August 1, 2006, and accepted for publication, after revision, August 23, 2006.

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ISSN: 0148-7043/07/5805-0551

DOI: 10.1097/01.sap.0000245129.53392.0e

Destroying hair follicles with lasers and other light sources has revolutionized the ability to eliminate unwanted hair. This technique was first used for the removal of hairs on flaps or grafts[1] and is today widely available for the treatment of unwanted hair. The primary principle of laser-assisted hair removal is damaging stem cells in the bulge area or replacing the hair follicle at the level of the dermis with connective tissue through thermal injury.[2] To selectively damage a hair follicle, laser energy has to be absorbed by a chromophore within a period equal to or less than the thermal relaxation time of the hair follicle. Melanin is the endogenous chromophore, which is present in the hair shaft, the outer root sheath of the infundibulum, and the matrix area.[3] Its concentration is dependent on the amount of pigment, which is responsible for hair color.[4] In the past, laser hair removal of blond and white hair was nearly impossible or with bad/disappointing results as a result of a lack of laser energy-absorbing chromophore in this type of hair.

The ability to color hair with melanin through liposomal targeting of melanin to hair follicles has been demonstrated in the past.[5] However, it has not been investigated if the combination of liposomal melanin (Lipoxome; Dalton Medicare, Zevenbergschen Hoek, The Netherlands) used to target melanin in the hair follicle by repetitive application and laser treatment has the ability to reduce regrowth and terminal hair count of blond, white, and gray hairs. In the present study, patients underwent laser therapy for previously treated hair. Clinical outcome was then measured by manual hair count.

MATERIALS AND METHODS

Study Population

Two groups of 16 healthy white volunteers (treatment group: 10 females and 6 males, median age: 54.7 years; control group: 11 females and 5 males, median age: 53.8 years) with white, gray, and light blond hair were enrolled in the study after having given informed consent. To ensure that the control population is representative of the test group, solely patients with skin type 2 (Fitzpatrick Classification) were enrolled in the study. All the subjects completed a physical examination at baseline to exclude any dermatologic or systemic condition (underlying endocrine disorders, intake of isotretinoin within the past month, herpes simplex infection, recent suntan). The volunteers were asked just to shave

TABLE 1. Treated Body Sites of the Liposomal Melanin and the Control Group

	Liposomal Melanin Group	Control Group
Chin	9	8
Upper lip	9	10
Legs	8	7
Backs	6	8
Shoulders	5	4
Underarms	3	4
Graft surface	2	1

and not to epilate their hair during the trial. The treated areas of light facial and body hair for both groups are compiled in Table 1. The study was performed in a university setting.

Treatment

After signing informed consent, all patients were photographed with digital photography with the same camera equipment (Fuji S2pro), lighting, and patient position before first, after each and last treatment. Patients were then randomized into 1 of the 2 groups. Additionally patients and investigators were blind to the study arms.

The first group was advised to spray liposomal melanin on the treatment areas 12 times a day (6 times in the morning and 6 in the evening) for a period of 8 weeks before each laser treatment.

Patients were advised to shave the areas 2 days before treatment and stop applying the liposomal melanin the night before laser treatment. The control group used physiological saline solution (0.9% NaCl) following the same mode of application. Because the application modus was inconvenient, we explained to the patients that it is pivotal to regularly use the spray according to our instructions to successfully treat the unwanted hair. Additionally, patients had to fill out a form that documented the time of application. Before each laser treatment, the numbers of blond, white, or gray terminal hairs were manually counted by 2 observers with a 6× magnifying apochromatic optical loupe within 3 cm^2 of a well-defined region of a treatment area. To be reproducible, the areas were marked and photographed before each treatment.

Hair reduction was defined as the average number of terminal hairs present before each treatment compared with the average number of terminal hairs before the first treatment. For both groups, treatment was performed with an 800-nm diode laser (Lumenis; Light Sheer, Dreieich, Germany) with a fluence range of 22 to 36 J/cm^2 and a pulse duration of 30 ms. Skin cooling was performed with a cold air system (SmartCool; Cynosure, Westford, MA) after applying a thin layer of ultrasound contact gel on the skin. To minimize perifollicular edema and erythema, ice was applied to the treatment sites immediately after treatment. Terminal hairs were manually counted before the first and 1 day before each treatment as well as 6 months after the last treatment to quantify photoepilatory efficiency. All complications and treatment outcomes were documented. Additionally, patients were asked to judge their overall satisfaction of treatment 6 months after the last treatment. Satisfaction survey consisted of a numbered scale (1 = completely satisfied, 2 = satisfied, 3 = moderate satisfaction, 4 = not satisfied, 5 = not satisfied at all).

Statistics

Explorative data analysis was performed using SPSS.12 for Microsoft (Microsoft, Redmond, WA). Null hypothesis was based on the assumption that there is no difference in efficacy (decrease of hair density) between the 2 groups used in the present study. The counted data followed the Poisson distribution. Comparisons between the terminal hair counts before the first and after the last treatment were performed using the 2-tailed t test for paired samples. Comparisons between the terminal hair counts of the treatment group and the control group were performed using the 2-tailed t test for unpaired samples. The rate of minor side effects was tested using the 2-sided Fisher exact test. Differences were considered significant when $P < 0.05$.

RESULTS

Patients using the liposomal melanin spray showed an average decrease of hair density of 17% 8 weeks after 3 treatment sessions (120 hairs ± 18.9 before therapy vs 100 hairs ± 18.7 after therapy; difference between means: 20; $P < 0.05$; 95% confidence interval [CI]: 16.37–23.88). Patients using the physiological saline solution had a decrease of hair density of 13% (115 hairs ± 16.3 before therapy vs 100 hairs ± 13.6 after therapy; difference between means: 14.6; $P < 0.05$; 95% CI: 12.9–16.35). Six months after therapy, terminal hair count of the liposomal group showed 14% reduction (120 hairs ± 18.9 before therapy versus 103 hairs ± 16.3 after therapy; difference between means: 17; $P < 0.05$; 95% CI: 15.00–19.25) compared with baseline pretreatment. The control group using physiological saline showed a 10% reduction of hair growth after 6 months (115 hairs ± 16.3 before therapy vs 104 hairs ± 14.9 after therapy; difference between means: 12; $P < 0.05$; 95% CI: 9.84–12.28). The differences of the results within each group and between the treatment and the control group were significant ($P < 0.05$) after 3 treatment sessions as well as after 6 months (Table 2). However, the patient and investigator noticed that hair reduction was very weak and clinical correlation was disappointing. No changes regarding hair thickness or color could be observed.

Side Effects

Rate of minor side effects was significantly higher in the liposomal melanin group (56% vs 12.5% in the control group; $P < 0.05$, Fisher exact test). Two patients (12.5%) developed small perifollicular urticae. Other side effects were mild erythema in 5 patients (31%) and folliculitis in 2 patients (12.5%). In the control group, 2 patients developed mild erythema (12.5%). There were no permanent side effects like hypo- or hyperpigmentation or scars seen in our study.

Patients Satisfaction

Mean satisfaction rate was 4.0 in the liposomal melanin group and 4.7 in the physiological saline group ($P > 0.05$).

TABLE 2. Mean Hair Count Before, 8 Weeks, and 6 Months After Last Treatment as Well as Reduction 8 Weeks and 6 Months After Last Treatment for Liposomal Melanin and Control Group ($P < 0.05$)

	Mean Hair Count Before Treatment (Hairs ± SD)	Mean Hair Count 8 Weeks After the Last Treatment (Hairs ± SD)	Mean Hair Count 6 Months After the Last Treatment (Hairs ± SD)	Reduction 8 Weeks After the Last Treatment (%)	Reduction 6 Months After the Last Treatment (%)
Liposomal melanin group	120 ± 18.9	100 ± 18.7	115 ± 16.3	17	14
Control group	115 ± 16.3	100 ± 13.6	104 ± 14.9	13	10

SD, standard deviation.

DISCUSSION

Removal of unwanted hair is performed for a variety of indications such as for aesthetic reasons, on hair-bearing flaps after reconstructive surgery, or in male-to-female transsexuals.[6–8] Different methods of hair removal such as shaving, plucking, and waxing are commercially available and used by the patients themselves, resulting in temporary hair reduction.[9]

To achieve permanent hair reduction, the physician can use several techniques. For classic electrolysis, a small, fine needle is inserted into the hair follicle followed by a pulse of electric current that damages and potentially destroys the hair follicle. Multiple treatment sessions are required to achieve a clinically significant result.[10]

The technique of laser-assisted hair removal has been described with the ruby laser (694 nm), alexandrite laser (755 nm), diode laser (800 nm), and neodymium:yttrium aluminum garnet (Nd:YAG) laser (1064 nm) for long-term hair reduction.[11–14] The mentioned lasers target melanin to achieve selective photothermolysis of the hair follicles. The same mechanism of action is used by intense pulsed light sources (IPL) that consist of a noncoherent filtered flash lamp that emits wavelengths ranging from 500 to 1200 nm.[15] It has also been successfully used for permanent hair reduction.[16] Both techniques, using either coherent (laser) or noncoherent (IPL) light, require a chromophore as a target.

The endogenous chromophore melanin, which is the target for laser hair removal, is not present in all types of hair. As a result, there is a lack of pigment in blond, gray, and white hair that led to the idea of external chromophore application. Nanni et al were the first to report on topically applied carbon particles suspended in mineral oil, which were massaged into the hair follicles in combination with a Q-switched Nd:YAG laser treatment.[17] Although permanent hair removal was not achieved, the concept of exogenous chromophores was followed further. Based on the idea that melanin could be a potential chromophore and act as a target for the diode laser (800 nm), topical melanin-encapsulated liposome spray was introduced to color the bulge region of the hair. A report of successful dyeing of the hair follicle with liposomal melanin can be found.[5] Although an observation by de Leeuw et al indicates that 62.5% of their patients experienced hair reduction of 95% after 8 treatments with the combination of Lipoxome and diode laser treatment, we were not able to confirm their observations.[18] We did observe a slightly higher rate of hair reduction in the liposomal melanin group of 4%. However, we performed only 3 laser treatments versus 8 treatments of Leeuw et al. We stopped laser treatment after 3 sessions because the patients did not respond as anticipated (Fig. 1A, B). Although patients had a very strong level of suffering from their unwanted hair and we additionally explained how important it was to regularly use their spray, a lack of compliance cannot be totally excluded. An accurate comparison with de Leeuw's data seems difficult because they are only published as a nonpeer-reviewed report on the Lipoxome manufacturer's web site without precise information about the study design.

The control group using physiological saline showed statistically less reduction of hair density compared with the liposomal melanin group (13% vs 17%, $P < 0.05$). Clinically, however, a similar reduction of hair density was observed. The reason for this might be that blond hair contains incompletely melanized melanosomes that could partially act as a chromophore. The same might be true for gray or white hair in which endogenous melanin in the hair follicles could be responsible for the observed hair reduction.

Considering the time-consuming application and the relatively high cost of the liposomal melanin spray, the authors propose that its use for laser hair removal is regarded skeptically. Additionally, we observed a higher rate of side effects. It is possible that part of the melanin is accumulating

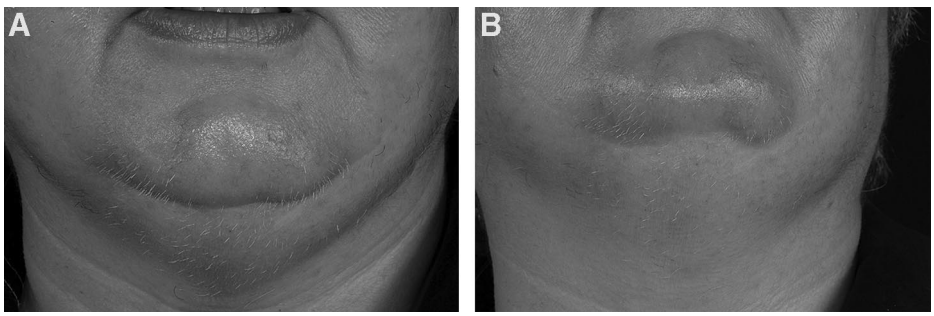

FIGURE 1. A, Chin and neck with multiple white hairs before therapy. B, The same patient 6 months after 3 cycles of liposomal melanin and laser treatment. Notice a very weak reduction of hair density.

in nonhair-bearing pores of the skin. Melanin deposits might cause a higher absorption of laser energy, which might be the reason for the higher rate of erythema after laser treatment compared with the control group.

In summary, the idea of selective dyeing of blond, white, and gray hair follicle may present the right approach for permanent laser hair removal; however, the method requires further development.

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