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Original Research

# Ultrasound imaging demonstration of the improvement of non-ablative laser remodeling by concomitant daily topical application of 0.05% retinaldehyde

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*BACKGROUND*: Retinaldehyde has been proven to be effective in the reduction of facial wrinkles. It has also demonstrated its usefulness when used before and after laser skin resurfacing.
*OBJECTIVE*: A monocentric, comparative, randomized, double-blind study was performed to evaluate the efficacy of retinaldehyde versus excipient in combination with non-ablative laser remodeling treatment.
*METHODS*: A total of 16 female patients (mean age 45 years) were enrolled for neck line and forehead rhytid treatment. They were randomly assigned into two groups. The RAL group (eight patients) was treated with a non-ablative laser (1540 nm Er:glass, 10 J/cm$^2$ per pulse, three pulses, 2 Hz repetition rate, 4 mm spot, $+5^{\circ}$C cooling) and daily topical application of 0.05% retinaldehyde immediately after the first laser treatment and up to 3 months after the fifth treatment. The CTRL group (eight patients) was treated under similar conditions, except with a daily application of excipient. The thickness of the skin (forehead and neck) was measured by ultrasound imaging before the first treatment, 1 month after the third treatment, 1 month after the fifth treatment and 3 months after the fifth treatment.
*RESULTS*: An increase of dermal thickness was observed for all patients treated by laser (groups RAL and CTRL) on the forehead and neck. However, the increase was greater for the RAL group (retinaldehyde) when compared with the CTRL group (excipient). Three months after the fifth treatment, the increase in dermal thickness (%) was, respectively, 5.27 versus 1.13 for the forehead and 10.54 versus 3.57 for the neck. The difference between groups was statistically significant in favor of the retinaldehyde group for the forehead ($p<0.05$) and of limited significance for the neck ($p=0.08$).
*CONCLUSION*: When considering the reduced number of patients in each group, the statistical analysis demonstrates an evident advantage of using retinaldehyde versus excipient. This study demonstrates that irradiation with a 1540 nm Er:glass laser can be potentiated with concomitant daily topical application of 0.05% retinaldehyde. *J Cosmet Laser Ther* 2004; 6: 5–9

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# Introduction

Photodamaged skin is characterized by fine and coarse wrinkling, rough texture, sallow color, and uneven pigmentation.

Studies with topical all-trans-retinoic acid have documented the efficacy of this retinoid in treating photodamage.[1–3] Retinaldehyde (RAL), the immediate precursor of retinoic acid, has been shown to exert biological activity in the skin. It has been clinically demonstrated that RAL is efficient and well tolerated for the improvement of the signs of photoaging.[4,5]

In addition, non-destructive, non-ablative laser devices have gained immense popularity in the past 2 years in treating photodamage.[6] This is largely because they entail little to no healing time. These high-tech systems work by bypassing the surface of the skin or epidermis and treating the layers underneath. These new lasers also heat water to stimulate collagen and promote dermal remodeling.[7–9] Since they are less intense than ablative or vaporizing lasers, non-ablative procedures generally pose minimal downtime, few side effects and brief recovery periods. However, non-ablative treatments may require multiple sessions, and can take several weeks to months for the optimal results of treatment to be seen.[10,11]

This clinical study aimed to evaluate a possible synergetic effect of daily application of RAL in combination with non-ablative laser remodeling treatment.

# Materials and methods

## Laser

The Er:glass 1540 nm Aramis–Quantel laser is a laser devoted to dermatological treatment (patent 5.897.549). The emitted wavelength (1.54 $\mu$m) is of particular interest owing to high water absorption. This is a flashlamp pumped system. The wavelength is obtained from a specific co-doped Yb-Er:phosphate glass material, optimized for highly efficient pumping absorption. The laser head is optimized to reduce pump radiation absorption by water and it is based on high diffusion materials. The design of the laser cavity is simple, assuming high efficiency and good stability. It works in normal mode delivering up to 5 J in 3 ms and can work either in single shot mode or in a pulse train mode with a repetition rate up to 3 Hz. The beam is delivered by an optic fiber. An aiming beam is provided by a red laser diode. Internal cooling avoids water connection and only standard power outlet (10A) is required. The system is compact and monitored by a microprocessor assuming high reliability and compliant with all medical norms (FDA approved and CE marked).

For this study, the laser was tuned at 10 J/cm$^2$ per pulse. The treatment consisted of three pulses (30/cm$^2$) at 2 Hz repetition rate applied a with 4 mm spot handpiece connected to a cooling system.[11]

## Cooling system

The skin was cooled using the Constans Handpiece (Quantel Medical) This is a cryo-sapphire-tip handpiece which is in direct contact with the skin. The cooling was obtained thanks to purified tetrafluorothane cryogen circulating in the tip. This handpiece had an 8 mm diameter viewing area and included a real-time temperature monitor at the sapphire for immediate feedback. This handpiece was connected to an electronic unit allowing temperature stability within a degree during treatment. For this clinical study, the cooling temperature was set at $+5^{\circ}$C and contact was maintained for at least 2 s before firing the laser.[11]

## Retinaldehyde

Retinaldehyde is the natural precursor of tretinoin, and its metabolism in the skin has been extensively studied. Saurat et al showed that the application of RAL on human skin exerts a biological retinoid effect;[12] 0.05% retinaldehyde (RAL) has restorative effects on skin aging and photoaging. The beneficial effects of RAL are due to some recovery in the quality or density involved in skin suppleness and elasticity (e.g. collagen and elastic fiber network).[13]

## Ultrasound imaging

Ultrasound is a unique quantitative and qualitative tool for evaluating a cosmetic's effect on the skin. With this technology it is possible to calculate changes in skin thickness and relate this to product performance. Ultrasound uses high-frequency sound waves to create an image of the skin and its immediate substrate. A high frequency signal is sent out from the emitting source into the skin. When the sound wave strikes a tissue it sends out an 'echo' and for each tissue layer another echo is created. The size or amplitude of each of these echoes in conjunction with the difference in time it takes for them to return to the emitting source provide the information needed to produce a two-dimensional representation of the skin.

In this study, skin thickness was determined with a high-resolution B-mode real-time ultrasonic scanner: DermCup 2020 (MT, Toulouse, France). High resolution was obtained by means of a strongly focused, 20 MHz center frequency transducer, with a 25 MHz bandwidth at –6 dB. This system displays 10 frames per second. The scanning field is 6 mm (laterally) $\times$ 5 mm (axially). The resolution is 0.2 mm (laterally) and 0.08 mm (axially). Once the two-dimensional picture has been created it is possible to see the structure of the skin as well as measure the thickness of the epidermis, the dermis or subcutaneous fat. In measuring dermal thickness the computer calculates the distance between two points and provides a measurement of this distance with an accuracy of 0.01 mm.

Results of dermal thickness were expressed as means $\pm$SEM. Differences between the retinaldehyde and the control group were analyzed using the Wilcoxon test.

## Clinical protocol

For each patient, age, sex, and phototype were recorded. Phototype was evaluated using Fitzpatrick's classification (I–VI). All neck and forehead areas involved in this

protocol had never undergone any previous aesthetic treatments (lifting, filling injections, peelings, laser treatments).

Contraindications to the enrolment were the following: history of other laser procedures on the neck or the forehead collagen-related diseases, treatment by Accutane completed or arrested within 2 years, keloids, pregnancy, peeling, dermabrasion, fillings and anti-aging treatments (creams, tablets).

Only neck lines and forehead rhytids were considered in this procedure. On each patient, the treated area was traced on a sketch accompanying each patient file. This sketch was used for reproducible positioning of the probes used for the measurement of thickness and mechanical properties of the skin.

Unwanted effects were systematically noted before and after every treatment (1 = none, 2 = erythema, 3 = edema, 4 = blister, 5 = hyperpigmentation, 6 = hypopigmentation, 7 = bruising, 8 = skin whitening, 9 = scarring). Pain was evaluated by the patient on a scale of 1–4 and recorded (1 = none, 2 = minimal, 3 = bearable, 4 = unbearable).

Patients applied either 0.05% retinaldehyde (RAL) or excipient (CTRL) on their faces once daily. The application started immediately after the first treatment session and was stopped 3 months after the fifth laser session.

### *Procedure and follow-up*

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the local CCPPRB committee (# 01 CJLA 065 RV 1575D). All patients gave informed consent for treatments and photographs.

Sixteen female patients were enrolled for neck line and forehead rhytid treatment, with phototypes from I to IV. They were randomly assigned into two groups: group RAL (eight patients, mean age: 43.5 years) was treated with the non-ablative laser (1540 nm Er:glass, 10 J/cm$^2$ per pulse, three pulses, 2 Hz repetition rate, 4 mm spot, +5°C cooling) and daily topical application of 0.05% retinaldehyde immediately after the first laser treatment and up to 3 months after the fifth treatment. Group CTRL (eight patients, mean age: 45.2 years) was treated under similar conditions, except with a daily application of excipient.

Patients were treated every month, with a total of five treatments and a control visit 3 months after the last treatment; the duration of the study was 7 months.

For each session, digital pictures were taken before and after treatment, and pain and secondary effects were recorded. Measurement of the thickness and the mechanical properties of the skin were performed before the first treatment, 1 month after the third treatment, 1 month after the fifth treatment and 3 months after the fifth treatment in the Centre Jean Louis Alibert, Toulouse, France. Patients did not use sun protection before or after the treatment even during the summer because the melanin absorption at 1.54 $\mu$m is very low. No anesthesia was performed. The area was treated using juxtaposed 4 mm shots. One shot consisting of three pulses. Since there was no clinical endpoint visible with this technique, a slight overlapping was accepted.

## *Results*

The treatments were well tolerated by the patients and no anesthetic was used. For all the treatments, no side effects were reported. All the patients scored 1 for side effects. When using this laser with the parameters given above there was no immediate visible effect, no swelling, no erythema (except a very transient one for a few seconds due to the refreshment of the skin by the cooling device), and no bleaching. There was also no late visible side effect such as dyschromia (this wavelength is almost not absorbed by the melanin), scabbing, or blisters. The treatment was really imperceptible, with treated areas and untreated sites indistinguishable from each other. Therefore, the search for a visible clinical endpoint was not possible with this procedure.

### *Ultrasound imaging*

Measurements were performed before, 1 month after the third treatment, 1 month after the fifth treatment and 3 months after the fifth treatment.

On the neck (CTRL group), the dermal thickness was $1.44 \pm 0.12$ mm before starting treatment. It increased progressively to reach $1.61 \pm 0.11$ mm at 1 month after the third treatment and $1.61 \pm 0.12$ mm at 1 month after the fifth treatment. Three months after five treatments the dermal thickness decreased to $1.49 \pm 0.05$ mm. For the RAL group, the results were the following: $1.44 \pm 0.14$ mm before treatment, $1.56 \pm 0.21$ mm at 1 month after the third treatment, $1.65 \pm 0.17$ mm at 1 month after the fifth treatment and finally $1.59 \pm 0.14$ mm at 3 months after the fifth treatment. When comparing the values obtained at each point the groups were not significantly different. However, the limit of significance was reached ($p = 0.08$) at 3 months after the fifth treatment (Figure 1).

The percentage increase in dermal thickness is 3.57% (before versus 3 months after five treatments) for the CTRL group versus 10.54% for the RAL group.

The ratio of the RAL:CTRL percentage dermal thickness increase is equal to 2.95.

On the forehead (CTRL group), the dermal thickness was $1.78 \pm 0.11$ mm before treatment. It increased progressively to reach $1.85 \pm 0.10$ mm at 1 month after the third treatment and $1.95 \pm 0.14$ mm at 1 month after the fifth treatment. Three months after five treatments the dermis thickness decreased to $1.80 \pm 0.11$ mm. For the RAL group, the results were the following: $1.87 \pm 0.22$ mm before treatment, $2.02 \pm 0.24$ mm at 1 month after the third treatment, $1.99 \pm 0.25$ mm at 1 month after the fifth treatment and finally $1.97 \pm 0.21$ mm at 3 months after the fifth treatment. When comparing the values obtained at each point the groups were not significantly different, except when comparing the CTRL and RAL groups at 3 months

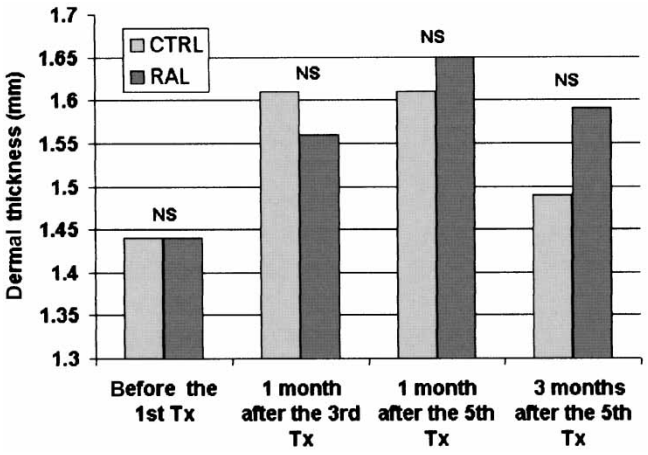


*Figure 1*
*Neck (eight patients): dermal thickness (mm) as a function of time. When comparing the values obtained at each delay, the groups are not significantly different. However, 3 months after the fifth treatment (Tx), the limit of significance is almost reached (p = 0.08): 1.49 ± 0.05 mm (CTRL group) versus 1.59 ± 0.14 mm at 3 months (RAL group).*

after the fifth treatment (Figure 2). The result is statistically significant in favor of the RAL group ($p = 0.03$).

The percentage increase in dermal thickness is 1.13% (before versus 3 months after five treatments) for the CTRL group versus 5.27% for the RAL group.

The ratio of the RAL:CTRL percentage dermal thickness increase is equal to 4.66.

## Discussion

This study presents a clinical evaluation of non-ablative remodeling of neck lines and forehead rhytids with concomitant daily topical application of 0.05% retinaldehyde (RAL) or excipient. A 1.54 $\mu$m Er:glass laser was used for this study since previous clinical studies on periorbital and perioral rhytids have demonstrated that irradiation with this laser can lead to new collagen formation, dermis thickening, reduction of anisotropy of the skin and clinical improvement without any adverse effect.[11,14–16] RAL was used since it has been demonstrated already that it was efficient and well tolerated for the improvement of the signs of photoaging.[4,5] The association of RAL after laser skin resurfacing has been proposed already. Sachsenberg-Studer et al have demonstrated that RAL could contribute to a more rapid decline of post-laser erythema usually observed after laser resurfacing.[17]

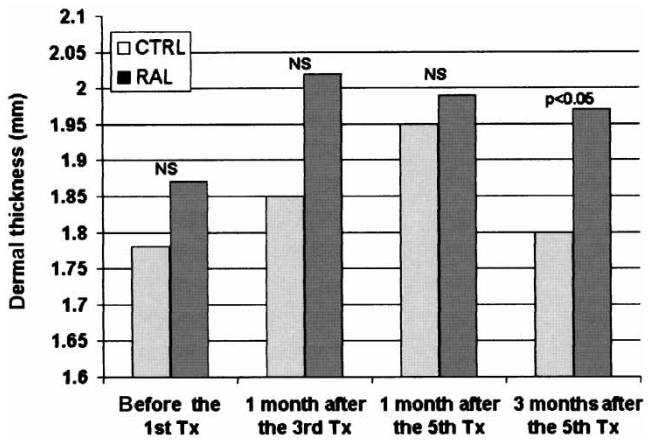


*Figure 2*
*Forehead (eight patients): dermal thickness (mm) as a function of time. When comparing the values obtained at each delay, the groups are not significantly different except when comparing the CTRL group and the RAL group at 3 months after the fifth treatment (Tx) (p < 0.05): 1.80 ± 0.11 mm (CTRL group) versus 1.97 ± 0.21 mm (RAL group).*

In order to compare the two groups, B-mode high-resolution ultrasound, providing indisputable data, was used. This technique is much more relevant than 'before' and 'after' photographs; it is a rapid and sensitive tool for measuring skin thickness. Its validity was confirmed by parallel measurement of skin thickness using the histological technique.[18,19]

The results of this clinical evaluation on two groups of eight female patients' neck lines and forehead rhytids after non-ablative laser remodeling show an increase of dermal thickness in both groups (RAL and excipient). This increase of dermal thickness was expected and is in accordance with the previous clinical studies using a 1.54 $\mu$m laser.[11,14] Similarly, the slight reduction observed 3 months after the final session, when compared with the value obtained 1 month after completion of the treatment, was expected. This slight reduction is due to a horizontal rearrangement of the new collagen fibers. This horizontal rearrangement (remodeling phase) is observed a few weeks after treatment.[20–22]

However, the increase is greater for the two RAL groups (neck and forehead) when compared with the two CTRL groups. The percentage increase of dermal thickness is, respectively, 5.27 versus 1.13 for the forehead and 10.54 versus 3.57 for the neck. The difference between groups is statistically significant in favor of the retinaldehyde group for the forehead ($p < 0.05$) and of limited significance for the neck ($p = 0.08$). The ratio of the RAL:CTRL percentage dermal thickness increase is equal to 4.66 (forehead) and 2.95 (neck).

## Conclusion

When considering the reduced number of patients in each group, the statistical analysis demonstrates the evident advantage of using 0.05% retinaldehyde versus excipient. This study demonstrates that irradiation with a 1540 nm Er:glass laser can be potentiated with concomitant daily topical application of 0.05% retinaldehyde.

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