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Original Article

Effect of myristyl nicotinate on retinoic acid therapy for facial photodamage

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Abstract: Based on the hypothesis that skin barrier impairment is a contributor to side-effects associated with retinoic acid therapy, a double-blind, placebo-controlled pilot study examined the combined use of retinoic acid with myristyl nicotinate (MN), a lipophilic derivative of niacin that enhances skin barrier function, in female subjects with mild to moderate facial photodamage. The study involved a 1-month run-in period with placebo or MN prior to initiation of retinoic acid therapy for 3 months. Analysis of skin biopsies revealed that retinoic acid therapy resulted in stratum corneum thinning of approximately 25% ($P = 0.006$ versus baseline) that was ameliorated by MN use ($P < 0.005$). Therapy resulted in an increased rate of transepidermal water loss (TEWL) of approximately 45% ($P = 0.001$ versus baseline) and use of MN protected against the increase in TEWL with the strongest protection provided by prior use of MN ($P = 0.056$ versus placebo). MN use reduced the incidence of side-effects of the therapy and again prior use provided the greatest reduction of side-effects. Subjects showed statistically significant clinical improvement ($P < 0.05$ versus baseline) during the study. MN use did not interfere with any clinical improvement parameters and improved effects on temple laxity ($P = 0.01$ versus placebo). Analysis of changes in epidermal thickness, Ki67-positive cells and intensity of loricrin staining demonstrated that MN either improved or did not interfere with retinoic acid efficacy. These results show that prior and concurrent use of MN can mitigate barrier impairment and improve the tolerability of retinoic acid therapy for facial photodamage without interfering with efficacy.

Key words: facial photodamage – myristyl nicotinate – retinoic acid – skin barrier function

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Introduction

Retinoids, the natural metabolites of vitamin A and synthetic vitamin A analogues, are important regulators of skin function (1). All-*trans*-retinoic acid (vitamin A acid), the major naturally occurring biologically active retinoid, has been a focus of research into topical treatments for photodamaged skin for many years. Kligman et al. reported that retinoic acid could produce smoother, less wrinkled and less pigmented skin after a few months of treatment (2). Retinoids have prominent pharmacological effects in both epidermal and dermal compartments (3–5). In the epidermis of chronically photodamaged skin, long-term topical retinoid therapy results in dose-dependent increases in epidermal and granular layer thickness, stratum corneum compaction, decreased melanin content and improvement of epidermal atypia (1,5–7). Retinoids induce proliferation in keratinocytes, presumably mediated by epidermal growth factor receptor activation resulting in epidermal hyperplasia (8). Retinoic-acid-induced expression of keratins K6, K16 and K17, which are commonly expressed in the hyperproliferative epidermis, indicates that retinoids increase cell proliferation in the basal and/or lower spinous layers of the epidermis (9). Retinoids also can lighten hyperpigmented skin, reduce tyrosinase activity in cultured melanocytic cells (10,11), inhibit proliferation and lipid synthesis, and alter keratin expression in cultured human sebocytes (12). Dermal effects include increased fibroblast proliferation (4), increased collagen production (13) and reduced extracellular matrix degradation (1). Prolonged use of retinoic acid significantly increases collagen matrix deposition in dermal repair zones apparently responsible for the wrinkle reduction that accompanies retinoic acid treatment of photodamaged skin (5,11).

While retinoic acid provides multiple benefits to photodamaged skin (11), it is frequently accompanied by

Abbreviations: MN, myristyl nicotinate; TEWL, transepidermal water loss.

significant skin irritation that limits compliance with therapy (14). The most commonly reported retinoic acid treatment-related adverse effects are irritation, dryness, peeling, erythema and a sensation of burning on the skin (14). The mechanisms leading to retinoid side-effects are still incompletely understood, but retinoic acid therapy is known to impair barrier function as assessed by transepidermal water loss (TEWL) measurements (15). Barrier impairment has been attributed to retinoid-induced epidermal hyperplasia (16) and to alteration of the terminal differentiation programme (1). Erythema, which reflects the production of epidermal cytokines such as interleukin-1, may result from retinoid-stimulated keratinocyte proliferation directly or as a consequence of epidermal barrier impairment (17,18). Retinoid-induced stratum corneum thinning, often referred to as compaction (6,7), is likely related to barrier impairment as stratum corneum thickness is a major determinant of barrier function (19,20).

Myristyl nicotinate (MN), a niacin derivative developed for optimal topical delivery of nicotinic acid to skin (21,22), has been shown to enhance epidermal differentiation in photodamaged skin, resulting in increased stratum corneum and epidermal thickness and enhanced barrier function (22). Based on the hypothesis that barrier impairment contributes to the side-effects of retinoic acid therapy, we report here the results of a double-blinded study simultaneously using a placebo or MN-containing skin cream with retinoic acid therapy in subjects with mild to moderate facial photodamage. The results demonstrate that concomitant use of MN mitigates retinoic-acid-induced loss of barrier function and improves tolerability without interfering with the efficacy of retinoic acid. Finally, we report that 1-month use of MN prior to initiation of retinoic acid therapy provides additional barrier protection and tolerability of retinoic acid without interfering with, and in some cases, improving efficacy.

Methods

This study was a 12-week randomized, double-blinded, placebo-controlled evaluation of the effects of a 5% MN formulation on surrogate markers of barrier function, clinical and sensory irritation, and clinical efficacy associated with retinoic acid use. The study was conducted (July 2005 to December 2005) by a contract research organization, Thomas J. Stephens & Associates (Colorado Research Center, Colorado Springs, CO, USA). Healthy adult female subjects between the ages of 30 and 60, with a score of I to IV on the Fitzpatrick Skin Classification (23), mild to moderate photodamaged skin as defined by a modified Glogau Classification of I to II (24), and presence of dyschromia on the face as determined by a Woods light visual scan, were eligible for the study. The mean age of the study population was 43.4 ± 6.96 years and included 86.7% Caucasian, 8.3% Hispanic, 1.7% Asian and 3.3% other ethnicities. Subjects were randomly assigned to one of three groups of 20 subjects each [group 1 received placebo for 1 month prior to initiation of retinoic acid therapy (baseline), then placebo and retinoic acid (0.025%) from baseline to 12 weeks (P/P + RA); group 2 received placebo for 1 month, then MN (5%) and retinoic acid (0.025%) from baseline to 12 weeks (P/MN + RA); and group 3 received MN (5%) for 1 month, then MN (5%) and retinoic acid (0.025%) from baseline to 12 weeks (MN/MN + RA)]. The dose of retinoic acid was 0.1 g in all cases. This dose of retinoic acid was chosen for this study as the subjects had mild to moderate photodamage. Subjects also were provided with Cetaphil® liquid cleanser and Neutrogena Ultra Sheer SPF 30 sunscreen to use for facial cleansing and sun protection during the entire course of the study. Subjects applied the assigned test moisturizers at 2.5 mg/cm^2 [MN (5%) or a placebo that contained myristyl myristrate in place of MN] to the entire face twice a day after cleansing. During the usage phase of the study, subjects applied the retinoic acid (0.025%) to the face after test moisturizer application once a day in the evening.

The study was conducted in accordance with the applicable Good Clinical Practice regulations and guidelines and Institutional Review Board regulations. All subjects were required to read and sign an IRB-approved Informed Consent Form. The sample size was determined empirically. Each subject who qualified for enrollment was assigned a subject number that was used in all documentation. The numbers were unique and were assigned in ascending order using a computer-generated randomization schedule developed by the contract research organization. The numbers were concealed until after the intervention was assigned. The contract research organization enrolled and assigned subjects to groups. All participants, those administering the interventions, and those assessing the outcomes were blinded to group assignment. Analyses were not by 'intention-to-treat' but only on those who completed the study. Analyses were restricted to subjects with complete data sets; thus, the *n* for some measures differs slightly because of missing data points for a given individual.

Punch biopsies

A board-certified dermatologist collected a 2-mm punch biopsy from the right or left side of the face for 10 randomly selected subjects from each group at baseline and after 12 weeks of treatment. The punch biopsies were formalin-fixed, embedded in paraffin, cut into 5 μm cross-sections, mounted on slides and stained with haematoxylin–eosin (H&E). Tissue microarrays were generated using 1.5-mm cores from formalin-fixed, paraffin-embedded skin punch biopsies by the Translational Genomics Research Institute

Tissue Microarray Core Facility (TGEN, Phoenix, AZ, USA). Thirty 4-μm slices from each array were cut, mounted onto glass slides and immunohistochemistry was performed.

TEWL measurements

Subjects were required to equilibrate to ambient conditions for at least 20 min and were maintained between 66 and 72°F and a relative humidity between 15% and 55%. A Dermalab instrument was used to measure TEWL at two points above the skin surface, and the rate of water loss was calculated. Each TEWL measurement was averaged over a 1-min measurement period.

Image analyses

Histological images were taken of H&E-stained cross-sections with an Olympus inverted stage microscope using a 10× by 0.45 Apochromat objective and a Nikon digital CCD camera. ImageJ image analysis software (NIH) was used to examine the images and perform measurements. Suprapapillary epidermal thickness (as measured from the top of the dermal papilla to the top of the granular layer) and stratum corneum thickness (as measured from the top of the granular layer to the top of the stratum corneum) were measured. For each specimen, five different sites were measured and the average was calculated.

Immunohistochemistry analysis

Immunohistochemistry was performed using the Discovery XT autostainer by Ventana Medical Systems, Inc. (Tucson, AZ, USA). Diaminobenzidine detection solutions, the Ki67 antibody and the haematoxylin counter stain are proprietary reagents supplied by Ventana Medical Systems, Inc. for use on human tissues. Loricrin polyclonal antibody (PRB-145P; Covance Research Products, Berkeley, CA, USA) was used at a 1:100 dilution. Histological images were taken of Ki67 and loricrin-stained cross-sections with an Olympus inverted stage microscope using a 10× by 0.45 Apochromat objective and a Nikon digital CCD camera. After acquisition with a digital camera, the image files were analysed using Photoshop (Adobe, San Jose, CA, USA). For the Ki67 analyses, a line was drawn on the electronic images to demarcate the epidermis in two equal sections between the basal layer and the stratum corneum. Stained cells in the lower half of the epidermis were marked with a red dot while unstained cells were marked with a green dot. Proliferation index was calculated as the total number of stained cells divided by the total number of cells in the section. For quantitative analyses of loricrin staining, the method of Matkowskyj et al. was used (25). Briefly, the 'Magic Wand tool' of Photoshop was used to select the entire stained region of the histological image. A threshold tolerance value of 30 was used for this tool to capture all pixels falling within the threshold parameter selected, which was removed from the original image, placed in a new non-compressed TIFF file, and saved as the stained image. The original minus the stained region was saved as another non-compressed TIFF file as the unstained image. The amount of antibody staining was quantified using the TIFFalyzer program (University of Illinois Medical Center) outputting a RGB value between 0 (black) and 255 (white). Each deconstructed TIFF image, stained and unstained, was processed and an RGB value obtained. The final image energy was calculated by subtracting the stained image value from its unstained counterpart.

Clinical grading of tolerability and efficacy

Subjects were clinically graded on the right and/or left side of the face for efficacy/performance parameters and irritation/safety parameters at baseline and weeks 2, 4, 8 and 12. For tolerability assessment, parameters of scaling/peeling and degree of erythema were graded on a 3-point scoring system. The graders also questioned each subject at each scheduled visit and recorded the occurrence of tightness/dryness, stinging, burning and tingling side-effects. Clinical assessment of efficacy was assessed on a 5-point scale of five parameters associated with efficacy of retinoic acid therapy for facial photodamage that included dyschromia, fine lines, shallow wrinkles, tactile roughness and temple laxity.

Subject self-assessment of efficacy

Self-assessment questionnaires were administered at the completion of the study that requested study subjects to respond to questions with one of five choices (strongly agree, agree, neither agree nor disagree, disagree, strongly disagree) concerning their perception of a decrease in signs of ageing, disappearance of fine lines, increase in smoothness/softness, improvement in skin radiance and increase in healthy appearance of the skin.

Inclusion criteria

Eligible subjects were females between the ages of 30 and 60 who were in generally good health as determined by a health assessment questionnaire. Subjects were required to be willing to avoid direct sun exposure and the use of tanning beds for the duration of the study.

Exclusion criteria

Individuals were ineligible for this study if, in the opinion of the investigator, they had known allergies or sensitivities to products that may have influenced the study; exhibited any skin disorders on the test areas of the face that may have influenced the study; had known medical conditions, such as diabetes, that could affect wound healing; or were using medications that might have influenced the study (e.g. prescription strength steroids, prescription strength anti-inflammatory drugs or topical medications). Subjects

were ineligible if they were pregnant or nursing. Other exclusion criteria included hypertension or uncontrolled metabolic disease, atopic diseases such as asthma, atopic dermatitis of the face, arms or hands; known sensitivity to alpha- and beta-hydroxyacids; use of products containing hydroxyacids or retinoids such as Retin-A®, Renova®, microdermabrasion treatment or routinely used alpha-hydroxy-acids, beta-hydroxy-acid or poly-hydroxy-acid products within 1 month of the study start; routine use of skin lightening products within 1 month of study start; had ablative and non-ablative laser treatments as well as Thermage treatments on the face or arms; had a 'lunchtime' facial peel within the last year or currently enrolled on another facial usage study.

Results

Myristyl nicotinate prevents retinoic acid-associated stratum corneum thinning

Haematoxylin–eosin stained sections from periorbital biopsy samples obtained from study subjects at baseline and 12 weeks were used to determine stratum corneum thickness (Fig. 1). At baseline, the mean stratum corneum thickness of the P/MN + RA group was slightly higher than the P/P + RA group, although the difference was not statistically significant. The mean thickness of the MN/MN + RA group at baseline, which had been treated for 1 month with 5% MN, was approximately 11% higher than the other two groups, although the difference did not reach statistical significance at $P < 0.05$. However, previous studies have shown that treatment of photodamaged skin with 5% MN for 3 months results in an increase in stratum corneum thickness of more than 50% (22), and thus the trend towards a higher mean value in MN/MN + RA group compared with that of the other groups agrees with the known effects of MN on photodamaged skin. During the 12 weeks of retinoic acid therapy, the P/P + RA group experienced a reduction in stratum corneum thickness of approximately 24% ($P = 0.006$ versus baseline), but concurrent initiation of MN use with retinoic acid therapy did not result in stratum corneum thinning. The difference in stratum corneum thickness between the P/P + RA and P/MN + RA groups at 12 weeks of therapy was highly statistically significant ($P = 0.005$), even though the n in this pilot study was only 7. Stratum corneum thickness of the MN/MN + RA group decreased by approximately 2% (not statistically significant), but the difference in stratum corneum thickness between this group and the P/P + RA group at 12 weeks was highly statistically significant ($P = 0.003$). These data show that use of MN mitigates stratum corneum thinning associated with retinoic acid therapy.

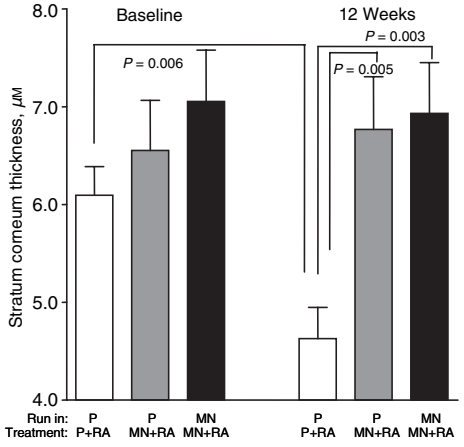

Figure 1. Effect of myristyl nicotinate (MN) on stratum corneum thickness during retinoic acid therapy. Biopsy samples from the periorbital regions were obtained at baseline and 12 weeks, haematoxylin–eosin (H&E)stained, and analysed for stratum corneum thickness ($n = 7$). Open bars represent the P/P group (P/P + RA), grey bars represent the P/MN group (P/MN + RA), and black bars represent the MN/MN group (MN/MN + RA). P = placebo. The letters in parenthesis before the slash describe the 30 day run in cream and those after the slash describe the 3 month treatment creams, placebo or MN+ retinoic acid, starting at base line. Error bars depict SEM. The *P*-values shown were derived from two-tailed Student's *t*-tests.

Myristyl nicotinate reduces retinoic acid-associated barrier impairment as assessed by TEWL measurements

Transepidermal water loss rates provide a non-invasive assessment of relative barrier function (20,26,27). Thus, TEWL measurements taken from the faces of study subjects were used as a surrogate marker of barrier function to compare placebo- and MN-treated groups. Figure 2 (panel a) shows the change in TEWL on the right cheek from baseline to 12 weeks for each group. The rates of TEWL increased in the P/P + RA group by approximately 45%, a value that was highly statistically significant ($P < 0.0001$) even though TEWL measurements made on the face have a documented high level of variability (19). The mean rates of TEWL also increased in the P/MN + RA and MN/MN + RA groups, although the changes from baseline for these groups were not statistically significant. The difference between the P/P + RA and MN/MN + RA groups at 12 weeks nearly reached statistical significance ($P = 0.056$). Figure 2 (panel b) shows the time course of changes from baseline for the three groups. The rates of TEWL for the P/P + RA and P/MN + RA groups were similar at 2 weeks of retinoic acid therapy, but the TEWL values for the P/MN + RA group were lower thereafter. In contrast, the TEWL values for the MN/MN + RA group were consistently lower than the other groups' at all time points following initiation of therapy. These results indicate that concurrent use of MN mitigates barrier impairment and that prior plus concurrent use provides greater barrier protection than concurrent use alone.

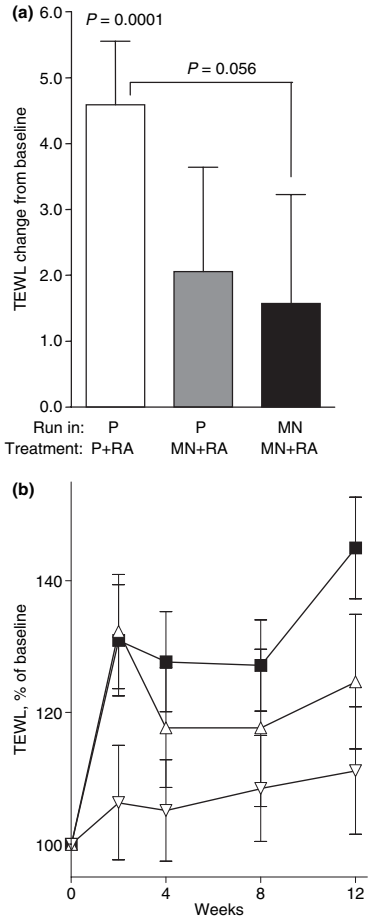

Figure 2. Effect of myristyl nicotinate (MN) on rates of transepidermal water loss (TEWL) during retinoic acid therapy. Rates of TEWL were determined on the right cheeks of study subjects as described in methods. The left panel shows the mean change in rates of TEWL from baseline to 12 weeks. Open bars represent the P/P group (P/P + RA) (n = 20), grey bars represent the P/MN group (P/MN + RA) (n = 20), and black bars represent the MN/MN group (MN/MN + RA) (n = 17). P=placebo. The letters in parenthesis before the slash describe the 30 day run in cream and those after the slash describe the 3 month treatment creams, placebo or MN+ retinoic acid, starting at base line. The *P*-values shown were derived from unpaired, two-tailed Student's *t*-tests. The right panel shows the per cent change in TEWL from baseline for the P/P + RA (squares), P/MN + RA (triangles), and MN/MN + RA (inverted triangles) groups. Error bars depict SEM.

Myristyl nicotinate improves the tolerability of retinoic acid therapy

Expert clinical grading assessed the tolerability of retinoic acid therapy. The parameters of tolerability such as scaling/peeling and degree of erythema were graded on a 3-point scoring system. The frequency of less severe parameters of tolerability typical of retinoic acid therapy that included tightness/dryness, stinging, burning and tingling also were recorded by the clinical graders. The degree of scaling/peeling was very low in all groups, remaining below 0.3 on the 3-point scale, and the degree of erythema also was relatively low as all scores were at 1.0 or below on the 3-point scale (Fig. 3, panel a), indicating an overall high degree of tolerance of the 0.025% concentration of retinoic acid when used with a moisturizer twice a day prior to and during therapy. There were no statistically significant differences between the placebo and MN groups in either parameter, although the grading of erythema was consistently slightly higher in the MN-treated subjects. Despite the low levels of scaling/peeling or erythema, a significant frequency of less severe but commonly encountered side-effects of retinoic acid was observed in this study (Fig. 3, panel b). For these tolerability parameters, a consistent pattern was observed as concurrent use of MN with retinoic acid decreased the frequency of tightness/dryness, stinging and burning, while prior and concurrent MN use further reduced the frequency of each of these parameters. Although the frequency of tingling reported was quite low (2%), the incidence of this side-effect was reduced to zero for the MN/MN + RA group. In addition to the clinical grading, study subjects completed self-assessment questionnaires that solicited information related to tolerability of the therapy. These self-assessments paralleled the clinical grading in all cases where the same parameter was assessed. Additionally, the study subjects reported a decreased frequency of comedones in the MN/MN + RA group. In total, the results show that use of MN improved the tolerability of retinoic acid therapy.

Myristyl nicotinate does not interfere with and in some cases improves the efficacy of retinoic acid therapy as assessed by clinical grading and patient self-assessment

Expert clinical grading and patient self-assessment were used to assess the effect of MN on the efficacy of retinoic acid therapy on visible clinical parameters. Clinical grading involved evaluation of dyschromia, fine lines, shallow wrinkles, tactile roughness and temple laxity as a function of treatment time (Fig. 4). Despite some differences in the degree of initial photodamage between the groups, similar rates of improvement for all three groups were observed for each of the parameters evaluated. The improvements were statistically significant relative to baseline ($P < 0.05$) at many of the time points. For tactile roughness, the MN/MN + RA group consistently showed greater improvement from weeks 4 to 12 compared with the P/P + RA group, although the difference did not reach statistical significance at $P < 0.05$. Grading of temple laxity showed a statistically significant greater improvement at 12 weeks ($P = 0.01$) in the MN/MN + RA group compared with the P/P + RA group and a trend for greater improvement in the P/MN + RA compared with P/P + RA was observed that did not reach statistical significance at $P < 0.05$.

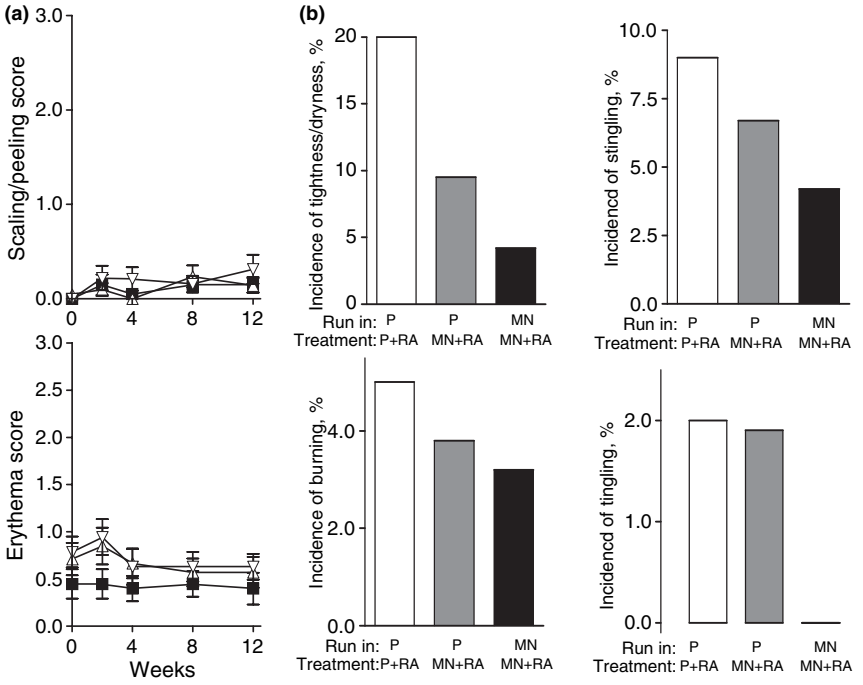

Figure 3. Effect of myristyl nicotinate (MN) on side-effects associated with retinoic acid therapy as assessed by clinical grading. (Panel a) Effect of myristyl nicotinate on the severity of scaling/peeling and erythema as assessed by clinical grading. The degree of severity was assessed on a 3-point clinical scale and mean values were determined for the placebo/placebo (squares) (n = 20), placebo/MN (triangles) (n = 21), and MN/MN (inverted triangles) (n = 19) groups. Error bars represent SEM. (Panel b) The incidence of tightness/dryness, stinging, burning and tingling side-effects. Open bars represent the P/P group (P/P + RA) (n = 20), grey bars represent the P/MN group (P/MN + RA) (n = 21), and black bars represent the MN/MN group (MN/MN + RA) (n = 19). P = placebo. The letters in parenthesis before the slash describe the 30 day run in cream and those after the slash describe the 3 month treatment creams, placebo or MN+ retinoic acid, starting at base line. Incidence rates were determined from clinical grading at 2, 4, 8 and 12 weeks.

Patient self-assessment of efficacy groups using MN rated efficacy higher than subjects in the P/P + RA group in all categories examined. These results indicate that concurrent or prior and concurrent use of MN does not interfere with retinoic acid efficacy and that MN use can result in improved efficacy in some cases.

Myristyl nicotinate does not interfere with efficacy of retinoic acid therapy as assessed by analysis of skin biopsies

Retinoid therapy is associated with an initial decrease in epidermal thickness and then after approximately 6 months of therapy an increase in epidermal thickness (1,6,7). Changes in epidermal thickness in each of the groups over the 12-week course of the retinoic acid therapy were assessed (Fig. 5). The mean values for the P/P + RA, P/MN + RA, and MN/MN + RA groups at the baseline were 37.9, 38.8 and 39.3 μm, respectively, which were not statistically significantly different even though the use of MN for 30 days showed a trend towards increased epidermal thickness, which is known to occur with an MN treatment over a longer period of time (22). The mean epidermal thickness of the group receiving retinoic acid and the placebo cream decreased by approximately 5% over the 12-week study, likely due to the limited duration of the study. The epidermal thickness of the group concurrently receiving MN increased by approximately 3%, and the group receiving MN prior to and concurrently with retinoic acid increased by approximately 10%. The difference between the P/P + RA and MN/MN + RA groups at 12 weeks was statistically significant (P = 0.0007), while the difference between P/P + RA and P/MN + RA groups showed a trend but did not reach statistical significance at $P < 0.05$. The difference between the P/MN + RA and MN/MN + RA groups at 12 weeks also reached statistical significance (P = 0.05). The results of epidermal thickness determinations support the possibility that MN use accelerates the efficacy of retinoic acid therapy.

The effects of MN on the frequency and localization of Ki67 as a marker of proliferation and on the intensity of loricrin staining as a marker of differentiation were assessed by immunohistochemistry. In all cases, Ki67-positive cells were located in the lower half of the epidermis and there was no effect of the 1-month run-in period with MN in comparison with the placebo on the percentage of Ki67-positive cells (data not shown). Retinoic acid therapy increased the frequency of Ki67-positive cells by 9–19%, consistent with a previous study (28), but there were no statistically significant differences between groups using placebo or MN formulations (data not shown). The 1-month use of MN resulted in a statistically significant (P = 0.01) increase in the intensity of loricrin (Fig. 6, panel a). In panels b, c, and d of Fig. 6 loricrin staining intensity of each of the three groups is compared at the initiation of retinoic acid therapy (baseline) and following 12 weeks. In each case, a statistically significant increase in staining was observed. These results indicate that both MN and retinoic acid stimulate increased loricrin expression and that MN does not interfere with the ability of retinoic acid to stimulate loricrin expression.

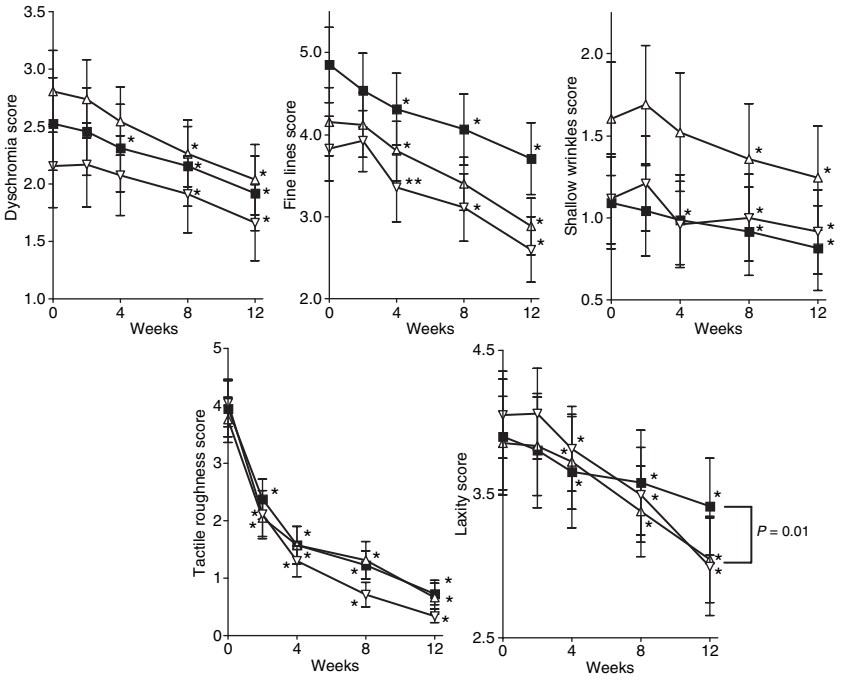

Figure 4. Effect of myristyl nicotinate (MN) on efficacy of retinoic acid therapy as assessed by clinical grading. Clinical assessment on a 5-point scale of five parameters associated with efficacy of retinoic acid therapy for facial photodamage over the course of the study was completed as described in 'Methods'. Mean values were determined for the P/P + RA (squares) (n = 20), P/MN + RA (triangles) (n = 21), and MN/MN + RA (inverted triangles) (n = 19) groups. The letters before the slash describes the 30 day run in cream and those after the slash represent the 3 month treatment period creams, placebo or MN+ retinoic acid, starting at base line. Error bars represent SEM; data points marked by * indicates $P < 0.05$ versus baseline using paired, two-tailed Student's t-tests. The P-value shown in the lower right panel was derived from an analysis of variance (ANOVA) with paired comparisons (Fisher's LSD) between the P/P + RA and MN/MN + RA groups.

Discussion

The correlation between stratum corneum thinning (Fig. 1) and increased rates of TEWL (Fig. 2) support the hypothesis that stratum corneum compaction is a factor in barrier impairment associated with retinoic acid therapy. In the study reported here, changes in stratum corneum thickness have been determined in paraffin-embedded samples subjected to the same conditions of preparation as the placebo samples. While prior studies have shown that the thickness of the stratum corneum is less in paraffin sections compared with frozen sections (29), paraffin sections have been used to assess stratum corneum morphology and changes in thickness in numerous studies (7,30–32). Previous studies of the effects of MN on photodamaged skin have shown that this agent stimulates increases in both epidermal and stratum corneum thickness and results in increased barrier function as evidenced by decreased rates of TEWL (22). Our results here show that concurrent and prior plus concurrent use of MN reduces stratum corneum thinning (Fig. 1) and reduces the increase in rates of TEWL in subjects on retinoic acid therapy (Fig. 2). A possible link between barrier impairment and the irritation potential of retinoic acid is supported by studies of other dermatology conditions that link barrier impairment with high irritation and/or inflammation potential (33–35). The results presented in this study support this link as the ability of MN to reduce stratum corneum thinning and reduce barrier impairment was coincident with a reduced frequency of tightness/dryness, stinging, burning and tingling side-effects (Fig. 3, left panel). It is interesting that 1-month pretreatment with MN showed a pattern of less side-effects than did use initiated concurrent with initiation of retinoic acid therapy (Fig. 3) and that the improved tolerability was coincident with positive effects of pretreatment on barrier impairment as assessed by TEWL measurements (Fig. 2). As previous studies have observed that MN-related increases in stratum corneum and epidermal thickness and decreases in rates of TEWL in photodamaged skin are progressive over a period of several months (22), additional studies on optimizing duration of pretreatment with MN on retinoid therapy for skin photodamage and possibly even acne are warranted. The feasibility of such studies is suggested by the excellent tolerability profile of formulations containing MN (22). It was also noted that a slightly higher grading of erythema in subjects using MN was observed. Prior studies of actinic skin damage have shown that MN treatment results in changes in skin tone (E.L. Jacobson, unpublished data), and whether this or other mechanisms led to the slightly higher erythema scoring also needs to be examined.

A key question addressed in this study was whether MN would negatively affect the efficacy of retinoic acid therapy. The results of clinical grading (Fig. 4) and of patient self-assessments argue that this is not the case. Similar rates of improvement were observed between placebo and MN groups, and for temple laxity at 12 weeks, a statistically significantly increased efficacy was observed with the use of MN. The epidermal thickness measurements reported here (Fig. 5) also indicate that MN use does not interfere with

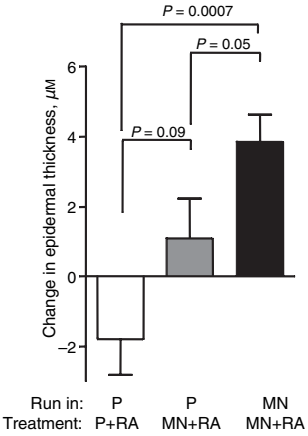

Figure 5. Effect of myristyl nicotinate (MN) on epidermal thickness during retinoic acid therapy. Biopsy samples from the periorbital regions were obtained at baseline and 12 weeks, H&E stained, and analysed for epidermal thickness. The number of biopsy samples analysed was 7 in each group. Open bars represent the (P/P + RA) group, grey bars represent the (P/MN + RA) group, and black bars represent the (MN/MN + RA) group. The letters in parenthesis before the slash describe the 30 day run in cream and those after the slash describe the 3 month treatment creams, placebo or MN+ retinoic acid, starting at base line. P = placebo; RA = retinoic acid. Error bars depict SEM. The *P*-values shown were derived from unpaired, two-tailed Student's *t*-tests.

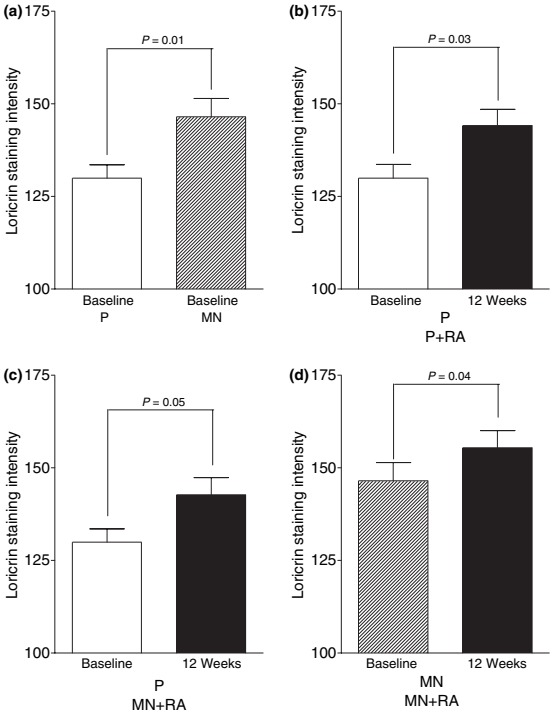

Figure 6. Effect of myristyl nicotinate (MN) on loricrin staining prior to and during retinoic acid therapy. The intensity of loricrin staining was determined as described in 'Methods'. (Panel a) Compares relative staining intensity following the 1-month run-in period with placebo (open bar, $n = 14$) or MN (stippled bar, $n = 9$) formulations. The relative staining intensity at baseline (open bar, $n = 14$) and 12 weeks (solid bar, $n = 7$) is shown for the (P/P + RA) group in panel b. (30 day run in cream/3 month treatment creams) P=placebo; RA=retinoic acid. (Panel c) Compares intensity at baseline (open bar, $n = 14$) and 12 weeks (solid bar, $n = 7$) for the (P/MN + RA) group. (Panel d) Compares intensity at baseline (stippled bar, $n = 9$) and 12 weeks (solid bar, $n = 9$) for the (MN/MN + RA) group. The *P*-values were determined using an unpaired Student's *t*-test for panel a and a paired two-sided Student's *t*-test for panels b, c, and d.

efficacy and are consistent with the possibility that MN accelerates the efficacy of retinoic acid, although it is also possible that the differences observed reflect the known property of MN to enhance epidermal thickness in photo-damaged skin (22). The intensity of loricrin staining was used as a marker of differentiation and the results show that MN alone stimulates loricrin expression (Fig. 5, panel a), that retinoic acid increases loricrin expression (Fig. 5, panel b), consistent with previous reports (32), and that MN does not interfere with increased loricrin expression during retinoic acid treatment (Fig. 5, panels c and d). In total, our results do not indicate any deleterious effect of MN on retinoic acid therapy.

The progression of actinic skin damage involves increased proliferation and de-differentiation of clones of damaged cells in the epidermis that can progress to actinic keratosis lesions and non-melanoma skin cancer (21). The ability of retinoic acid to reduce atypical cells in actinic skin damage suggests that it may provide skin cancer prevention benefit (11). The ability of oral retinoic acid to reduce skin cancer risk has been reported (36), providing additional support for a possible role in skin cancer prevention. The ability of MN to promote epidermal differentiation as evidenced by increases in epidermal and stratum corneum thickness (22) and loricrin expression (Fig. 6) raises the hypothesis that this agent also may limit the progression of early-stage actinic skin damage to actinic keratoses and non-melanoma skin cancer. Other studies also support the concept that optimal niacin status may limit carcinogenesis via enhancing genomic integrity (37,38). Indeed, the possibility that the combined use of retinoic acid and MN may effectively limit progression of actinic skin damage deserves consideration.

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