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Original Article

A topical lipophilic niacin derivative increases NAD, epidermal differentiation and barrier function in photodamaged skin

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Abstract: The effects of myristyl nicotinate (MN), a nicotinic acid derivative designed to deliver nicotinic acid to skin without vasodilatation, on subjects with photodamaged skin have been studied. MN increased skin cell nicotinamide adenine dinucleotide (NAD) by 25% ($P = 0.001$) demonstrating effective delivery of nicotinic acid to skin. Relative to placebo, MN treatment of photodamaged facial skin increased stratum corneum thickness by approximately 70% ($P = 0.0001$) and increased epidermal thickness by approximately 20% ($P = 0.001$). In two separate studies, MN treatment increased rates of epidermal renewal by 6% ($P = 0.003$) to 11% ($P = 0.001$) and increased the minimal erythemal dose by 8.9 ($P = 0.07$) and 10% ($P = 0.05$) relative to placebo. MN treatment resulted in reductions in the rates of transepidermal water loss (TEWL) of approximately 20% relative to placebo on cheeks ($P = 0.012$) and arms ($P = 0.017$) of study subjects. Results of a tape stripping challenge before and after MN treatment demonstrated a significant correlation ($P = 0.03$) between increased skin NAD content and resistance to changes in TEWL for MN treated but not placebo subjects. Rates of TEWL changed more rapidly and to a greater extent in atopic subjects compared with normal subjects. The results indicate that MN enhances epidermal differentiation and barrier function in skin, suggesting that this method of nicotinic acid delivery may prove useful in limiting progression of actinic skin damage and possibly in treating other conditions involving skin barrier impairment.

Key words: atopic skin – epidermal differentiation – niacin/NAD – photodamage – skin barrier function

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Introduction

Chronic solar exposure to skin (photoaging) is associated with multiple alterations in structure and function (1–3). These changes include rearrangement of collagen and elastic fibres in the extracellular matrix of the dermis, irregularities in melanocyte and keratinocyte morphology in the epidermis (4), decreases in stratum corneum thickness, flattening of the dermal–epidermal junction (1, 5), and impairment of skin barrier function (6–8). Early stage photodamage can result in skin hyperpigmentation, loss of skin smoothness and wrinkling while later stage photodamage can result in development of actinic (solar) keratosis, a hyperproliferative lesion thought to be a continuum with non-melanoma skin cancer (9), and to melanoma skin cancer (10). While use of sunscreens represents a front line strategy for limiting actinic damage, their efficacy is still limited by inadequate use, incomplete spectral protection and potential dermal toxicities (11). Therefore, other approaches to limit the progression of skin damage could compliment the use of sunscreens (12, 13).

Preclinical studies suggest that nicotinic acid may provide benefit to photodamaged skin by mechanisms that involve both nutrient and drug effects of this agent. Regarding nutrient effects, nicotinic acid and the other B3

Abbreviations: H&E, haematoxylin–eosin; MED, minimal erythemal dose; MN, myristyl nicotinate; PARP, poly(ADP-ribose) polymerase; ROS, reactive oxygen species; TEWL, transepidermal water loss; UV, ultraviolet light.

vitamer, niacinamide, can serve as precursors to nicotinamide adenine dinucleotide (NAD), albeit by different metabolic pathways (14). The oxidized form of NAD is consumed as a substrate in poly(ADP-ribose) polymerase-1/2 (PARP-1/2) catalyzed reactions that promote genomic stability following DNA damage such as that caused by ultraviolet (UV) light (15, 16). UV exposure can lead to depletion of NAD in skin cells and intact skin, resulting in insufficient NAD available for PARP-1/2 activity (17, 18). Supplementation with nicotinic acid at levels that increase skin NAD content inhibits UV-induced carcinogenesis and photoimmune suppression in an animal model (19). NAD functions as a hydride ion acceptor and donor in biological redox reactions central to energy metabolism, and as a large organ with a high rate of turnover, the epidermal compartment of skin has a high-energy requirement (20). NAD is the precursor for NADPH (21), which is essential for synthesis of many lipids including ceramides important in skin barrier function (22), and serves as the source of reducing equivalents for cellular antioxidants needed to counter UV induced reactive oxygen species (23). Regarding drug effects, a G-protein coupled nicotinic acid receptor (24, 25), present in skin (26), is likely involved in release of the cytokine leptin (27). In skin, leptin-mediated signalling pathways show protective effects that include enhancing epidermal differentiation (28–30) and wound healing (31, 32).

We report here clinical evaluations of myristyl nicotinate (MN), a lipophilic derivative of nicotinic acid developed to deliver nicotinic acid to skin following topical application (33). Our results indicate that this approach to deliver nicotinic acid to skin offers a new tool for management of skin photodamage. Additionally, the ability to enhance skin barrier function suggests that MN may benefit other dermatology conditions that involve barrier impairment.

Materials and methods

Overview of clinical studies

Data obtained from three clinical studies are described in this communication. All studies were conducted in accordance with applicable Good Clinical Practice regulations and guidelines and Institutional Review Board (IRB) regulations. All subjects were required to read and sign an IRB-approved Informed Consent Form. Sample size was determined empirically. Each subject who qualified for enrollment was assigned a subject number that was used on all subject documentation. These unique numbers were assigned in ascending order using a computer-generated randomization schedule developed by the contract research organization (CRO) conducting each trial. Numbers were concealed until after the intervention was assigned. Each CRO 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. Two occurrences of allergic or adverse reactions to the formulations were noted in the 96 subjects who enrolled in these studies. Both of these were classified by clinical evaluators as a mild rash appearing within 24 h of the first application, possibly related to study product, which resolved in three and 9 days, respectively, and both subjects continued in the study. Analysis was restricted to subjects with complete datasets, thus the *n* for some measures differs slightly because of missing data points.

Study 1

Study 1 was an 18-week assessment (12/06/00–04/12/01) conducted by the Skin Study Center, Broomall, Pennsylvania, USA. It involved 16 healthy adult females between 35 and 45 years old with Fitzpatrick Skin Types I–IV. Subjects applied a simple, oil in water lotion containing 1% MN to one total forearm (complete coverage from wrist to elbow) and placebo lotion (myristyl myristate in the placebo replaced MN contained in the active) to the other total forearm in a double-blinded protocol. All applications were 0.5 ml once per day per site (1 mg/cm^2). Individuals were ineligible if, in the opinion of the investigator, they had known allergies or sensitivities to products; exhibited any skin disorders on the test areas of the face, upper inner arms or volar forearms; had known medical conditions, such as diabetes, that could affect wound healing or; were using medications which might have influenced the study (e.g. prescription strength steroids, anti-inflammatory drugs or topical medications). Subjects were ineligible if they were pregnant or nursing.

Study 2

Study 2 was a 12-week randomized, double-blind, placebo-controlled evaluation of the effects of a 5% MN formulation on human skin NAD and photoaging parameters. It was conducted (12/12/02–04/07/03) by Thomas J. Stephens & Associates, Colorado Springs, CO, USA. Female subjects ages 35–60, with a Fitzpatrick Skin Classification score of I–IV, moderate to advanced photodamaged skin defined by a Modified Glogau Classification of I–III (34), and significant dyschromia on the face determined by a score of ≥4 on the 10-cm visual acuity scale, participated in the study. The mean age was 48.2 ± 6.8 and 85% were Caucasian and 15% Hispanic. Subjects were randomly assigned to one of two groups of 30 (active or placebo where the formulations were identical except that myristyl myristate in the placebo replaced MN contained in the active). Test materials were applied to face and total forearms of subjects each morning and evening, 0.5 ml to the face (1 mg/cm^2) and 1 ml to

each arm (2 mg/cm^2). A separate group of atopic subjects, qualified for a substudy by having confirmed atopic criteria based on a questionnaire validated by the CRO, participated only in TEWL measurements on the volar forearms. Subjects were required to be willing to avoid direct sun exposure on the forearms and the use of tanning beds for the duration of the study. Individuals were ineligible 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, upper inner arms or volar forearms; had known medical conditions, such as diabetes, that could affect wound healing or; were using medications which might have influenced the study (e.g. prescription strength steroids, anti-inflammatory drugs, or topical medications). Subjects were ineligible if they were pregnant or nursing. Other exclusion criteria included; hypertension or uncontrolled metabolic disease, such as thyroid disease; known sensitivity to alpha- and beta-hydroxyacids or lactic acid; use of products containing hydroxy acids, or retinoids within 1 month of enrollment; participation in dansyl chloride patch-type studies within 24 months of enrollment; and/or concurrent participation in clinical studies involving the arms.

Study 3

Study 3 involved an 8-week double-blinded, placebo-controlled study to evaluate biophysical effects of formulations containing niacin derivatives on skin. The subjects were females in generally good health with Modified Glogau Classification, I–II; Fitzpatrick Classification, I–III, between the age of 30 and 60 years with a mean age of 44.1 ± 7.3 where 92% were Caucasian, 6% were Hispanic and 2% were Hispanic/Caucasian. The study was conducted (06/14/05–10/08/05) by Thomas J. Stephens & Associates, Colorado Springs, CO, USA. Twenty subjects were examined at baseline and weeks 2, 4, and 8 during use of a 5% MN formulation applied to one total forearm and a placebo formulation (formulations were identical to those in Study 2) applied to the other total forearm (arm assignment based on a predetermined randomization provided by the test facility) (2 mg/cm^2). Inclusion and exclusion criteria and doses were the same as described in Study 2.

Skin biopsy analyses

A board-certified dermatologist collected a 4-mm punch biopsy from the dorsal forearm of 20 randomly selected subjects (nine in placebo and 11 in the MN group) at baseline and after 12 weeks of treatment in Study 2. Samples were stored at −80°C and NAD was measured as reported previously (35). A 2-mm periocular punch biopsy was taken by a board-certified dermatologist at baseline (right side) and 12 weeks (left side) on randomly selected subjects in Study 2. The biopsies were formalin-fixed, embedded in paraffin, cut into 5-μm cross-sections, mounted on slides, and stained with haematoxylin–eosin (H&E). Images were taken of H&E-stained cross-sections with a Nikon Eclipse TE300 microscope using a 10× by 0.45 Apochromat objective and a Coolsnap Photometrics digital CCD camera. IMAGEJ software (NIH) was used to analyze the images. 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 at five different sites and the average was calculated.

Stratum corneum turnover determination

Application of test formulations to the right and left upper inner arms began following baseline assessments. A 5% dansyl chloride suspension in white petrolatum was applied under occlusion on each site after 4 weeks of treatment. Six hour later, a second application was made and the dressings renewed. After 24 h, the occlusive dressings were removed and the test sites were washed. After drying, each site was examined under a Wood's lamp and the acceptability of staining determined. Application of test formulations resumed on the evening the dye patches were removed. The degree of residual staining from the dansyl chloride was assessed three times per week by a clinician in blinded fashion for 4 weeks or until all sites were no longer fluorescent under Wood's lamp illumination.

Minimal erythemal dose (MED) determination

Minimal erythemal dose is defined here as the time of light exposure producing a minimally perceptible erythema reaction discernible 16–24 h after irradiation with a single port solar simulator (Solar Light Co., Philadelphia, PA, USA) equipped with a 150-watt xenon arc lamp (Model 16S, Solar UV Simulator, Solar Light Co.). A spectral output similar to that of the natural solar spectrum (emissions of UVA+B, 290–400 nm) was obtained by using a combination of the UG-5 or UG-11 and WG-320 filters (Schott Glass Technologies, Elmsford, NY, USA). The output of the solar simulator was monitored with a 3D-600 m (Solar Light Co.), and calibrations occurred just prior to use. After 18 weeks of test formulation use in Study 1 and 12 weeks of use in Study 2, MED was determined by exposing six squares of skin on the volar forearms to varying doses of UV. Each irradiated site received 25% more exposure than the previous site, starting at 0.64 of the estimated MED for that skin type (0.64, 0.8, 1.0, 1.25, 1.56 and 1.95 times the estimated MED) and 16–24 h later, the MED was determined by the site that exhibited the least perceptible erythema.

Transepidermal water loss (TEWL) measurement

Subjects were equilibrated to ambient conditions for at least 20 min and maintained between 66 and 72°F and rel-

ative humidity of 15–55%. A Dermalab instrument was used to measure TEWL at two points on the right volar forearm and left outer lower cheek, averaged over a 1-min measurement period.

Tape-stripping challenge

The right anterior volar forearm was compromised by applying acetone to the forearm for 30 s, then applying and removing 10 strips of 1'' Blenderm tape in a cross-wise pattern. TEWL measurements were made before and after tape stripping as described above.

Results

A topical MN formulation elevates NAD, a biomarker of niacin delivery, in human skin

Skin NAD content was used as a biomarker of nicotinic acid delivery following topical application of formulations containing MN. NAD provides a biomarker for all phases of delivery including partitioning from stratum corneum to living layers of the epidermis, conversion to nicotinic acid by skin esterases, and cellular uptake and bioconversion to NAD. Twenty subjects completed a phase of Study 2 where 4-mm punch biopsies of the forearm were obtained at baseline and after 12 weeks use of test formulations. These subjects were randomly assigned to placebo ($n = 9$) and 5% MN groups ($n = 11$). Biopsies were processed and analyzed for skin NAD content, expressed as pmol NAD per microgram protein (Fig. 1). At baseline, the MN group had slightly higher but not statistically significantly different NAD relative to the placebo group. After 12 weeks, no significant increase in NAD was observed in the placebo group, while the MN group increased skin NAD to an average of 125% of baseline ($P = 0.016$). The difference in NAD between placebo and MN groups at 12 weeks was highly statistically significant ($P = 0.008$). These data demonstrate that MN delivered nicotinic acid to skin in a manner that allowed conversion to nicotinic acid and cellular uptake and bioconversion to NAD.

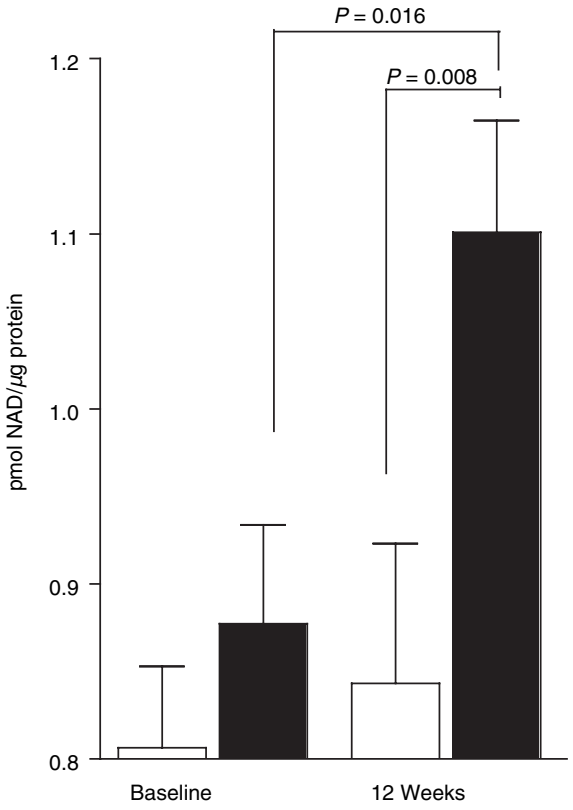

Figure 1. Effects of myristyl nicotinate on skin nicotinamide adenine dinucleotide (NAD) content. Skin punch biopsies obtained in Study 2 at baseline and 12-weeks post-treatment from the forearm were analyzed for NAD content, which is expressed relative to protein. Solid bars represent subjects randomized to myristyl nicotinate treatment ($n = 11$) and open bars represent subjects in the placebo group ($n = 9$). The values shown are group means ± SEM and the *P*-values were derived from a paired, two-tailed Student's *t*-test for within group comparisons and unpaired analyses for between group comparisons.

MN promotes epidermal differentiation in photodamaged skin without increasing photosensitivity

The effect of MN on epidermal histology in photodamaged skin was evaluated by comparison of cheek biopsy samples from placebo and MN-treated subjects from Study 2 at baseline and after 12 weeks of application. Increases of stratum corneum thickness are associated with increased epidermal differentiation and impaired differentiation resulting in decreased stratum corneum thickness occurs in atopic dermatitis (36). Visible increases in the stratum corneum thickness and in epidermal thickness as a result of increased cellularity were observed for subjects receiving MN. An example is shown in Fig. 2, where (a) shows a placebo subject and (b–d) show subjects that applied formulations containing 5% MN. Data summarizing changes in stratum corneum and epidermal thickness for all subjects (Fig. 2e and f) demonstrated a highly significant difference from baseline to 12 weeks between MN and placebo-treated groups for both parameters ($P = 0.0001$, both parameters, $n = 31$ for MN and 27 for placebo). The mean increase in stratum corneum thickness in the MN group was approximately 70% (6.35 μm) while the placebo group increased only slightly (0.89 μm). Epidermal thickness in the placebo group decreased by an average of 4.47 μm (approximately 8%) while the MN group increased in thickness by 7.75 μm (approximately 13%), a differential effect of approximately 20%.

Two studies examined the effect of MN on the rate of stratum corneum turnover following staining with dansyl chloride as a surrogate marker of epidermal renewal. Study 1 involved treatment with a 1% MN formulation once per day and Study 2 involved treatment with a 5% formulation

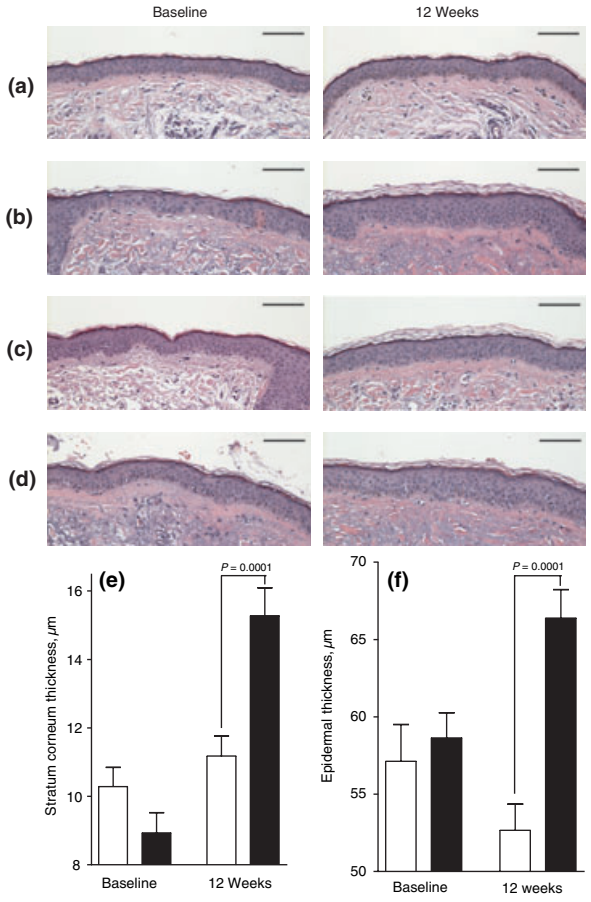

Figure 2. Effects of myristyl nicotinate (MN) on stratum corneum and epidermal thickness. Representative histological analyses of placebo and MN-treated skin biopsies: H&E slides were prepared from peri-ocular skin punch biopsies from subjects in Study 2. H&E from a representative placebo subject (a) at baseline and 12 weeks of treatment and from three representative subjects treated with MN (b–d) at baseline and 12 weeks of treatment. The bar in the upper right corner represents 100 microns. In (e), the mean stratum corneum thicknesses ± SEM for placebo ($n = 27$) and MN ($n = 31$) groups are shown at baseline and after 12 weeks of treatment. In (f), the mean epidermal thicknesses ± SEM for placebo ($n = 27$) and MN ($n = 31$) groups are shown at baseline and after 12 weeks. The *P*-values shown are derived from an unpaired, two-tailed *t*-test of change from baseline to 12 weeks between groups.

twice per day. In both studies, formulations were applied for 4 weeks prior to assessment of turnover time by evaluation of dansyl fluorescence extinction on alternate days for 4 weeks starting at day 6 after application to determine the $t_{1/2}$ for turnover. The presence of MN increased the rate of dansyl turnover relative to placebo by approximately 11.3% in Study 1 ($P = 0.001$) and 6.3% ($P = 0.003$) in Study 2 (Table 1), demonstrating enhanced epidermal renewal by MN.

As topical formulations that stimulate epidermal turnover have been shown to result in the occurrence of photosensitivity (37), the effect of MN on the minimum time of UV exposure to elicit erythema (MED) was measured in both Study 1 and 2. The results (Table 1) show that MN formulations increased the MED by approximately 9–10% compared with placebo. The results from Study 1 approached but did not reach statistical significance ($P = 0.07$, $n = 16$), while the data from Study 2 did reach statistical significance at $P = 0.05$ ($n = 24$). These studies demonstrate that use of MN does not result in development of photosensitivity but rather that its use can provide mild photoprotection.

MN enhances skin barrier function

The importance of the skin barrier in normal homeostasis and its impairment in a number of dermatology conditions are the areas of considerable current research in dermatology (38, 39). Stratum corneum thickness has been identified as an important determinant of skin barrier function (40) and the ability of MN to increase stratum corneum thickness in photodamaged skin (Fig. 2) along with providing photoprotection (Table 1) suggested that this compound may be enhancing skin barrier function. Accordingly, effects of MN on skin barrier function were examined by using three different approaches.

Changes in the rates of TEWL have long been used as a marker of changes in skin barrier function (38, 40–42). Accordingly, changes in rates of TEWL on both the face and arms of subjects in Study 2 were determined at baseline and after 12 weeks for both placebo and MN formulations (Fig. 3). Skin cream moisturizers in general have been shown to increase barrier function (43, 44) and the placebo formulation in this study applied to the face of study subjects resulted in decreased rates of TEWL of 1.0 g/m^2/h, which corresponded to a decrease of approximately 11%. However, formulations containing 5% MN decreased water loss by approximately 2.5 g/m^2/h or 250% relative to the placebo formulation ($P = 0.012$, $n = 29$ for placebo and 31 for MN groups). The rates of TEWL on the arms of placebo subjects increased over the course of the study while the MN group decreased, but again a highly statistically significant differential effect between placebo and MN groups ($P = 0.017$, $n = 28$ for placebo and 30 for MN groups) was observed. The increase in rates of TEWL on the arms of placebo subjects likely reflects seasonable changes in skin barrier function, which occurred between December and April in the study shown.

A second approach to examine effects of MN on skin barrier function involved a study of the effects of tape stripping on changes in the rates of TEWL. In this study, the rates of TEWL were determined both before and following a standard regimen of tape stripping at both baseline and after 12 weeks. Prior studies have shown that a strengthened skin barrier is more resistant to increases in rates of TEWL following a standard regimen of tape stripping (45). The changes in TEWL values (ΔTEWL) at 12 weeks minus the

Table 1. Effect of myristyl nicotinate (MN) on epidermal renewal and MED

MN	*n*	Placebo	*n*	MN-dependent change	*P*-value
Dansyl turnover time, $t_{1/2}$ (days)					
Study 1 (1%) 17.2 ± 0.44	16	19.4 ± 0.41	16	11.3%	0.001
Study 2 (5%) 13.4 ± 0.185	34	14.3 ± 0.226	34	6.44%	0.003
MED (s)					
Study 1 (1%) 29.4 ± 2.6	16	27.0 ± 1.8	16	8.9%	0.07
Study 2 (5%) 48.7 ± 1.8	24	44.3 ± 0.41	24	10.0%	0.05

The $t_{1/2}$ for epidermal renewal was measured as described in Materials and methods after 4-week use of formulations. MED was determined as described in Materials and methods.

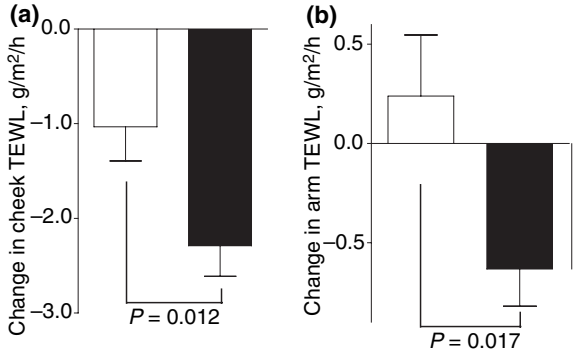

Figure 3. Effects of a 5% myristyl nicotinate (MN) formulation on skin barrier function assessed by changes in rates of transepidermal water loss (TEWL) on the face and volar forearms of subjects in Study 2. Panel (a) shows the change in TEWL rates on the cheek from baseline to 12 weeks while (b) shows the same measurements on the volar forearms. Open bars represent the placebo group and the black bars represent the MN-treated group. The values are mean ± SEM where $n = 29$ and 28 for the placebo group in A and B, respectively, and 31 and 30 for the MN-treated groups, respectively. The *P*-values are derived from unpaired, two-tailed Student's *t*-tests.

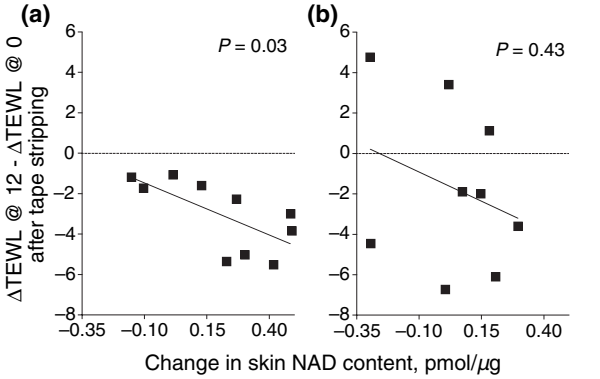

Figure 4. Correlation analysis of change in skin nicotinamide adenine dinucleotide (NAD) content and changes in TEWL before and after a tape stripping challenge from Study 2. The changes in transepidermal water loss (TEWL) values (ΔTEWL) at baseline minus the ΔTEWL values at 12 weeks ($\Delta TEWL_{12}-\Delta TEWL_0$) are plotted as a function of the change in skin NAD, which serves as a biomarker of delivery by MN. Panel (a) shows the data for MN-treated subjects and (b) shows the data for the placebo group where $n = 10$ and 9 subjects, respectively. The Pearson *r*-value for (a) = −0.68 and for (b) = −0.13. *P*-values were derived from the same analysis as for the Pearson *r*-value.

ΔTEWL values at baseline ($\Delta TEWL_{12}-\Delta TEWL_0$) were then plotted as a function of the change in skin NAD, which serves as a biomarker of delivery by MN (Fig. 4). In this plot, a value of zero represents no change in barrier function between baseline and 12 weeks, negative values indicate an improvement in barrier function and positive values indicate a decrease in barrier function. The results for the MN-treated group (panel a) show that all subjects improved in barrier function ($\Delta TEWL_{12}-\Delta TEWL_0 < 0$). Additionally, a strong correlation between the change in skin NAD and $\Delta TEWL_{12}-\Delta-TEWL_0$ values (Pearson $r = -0.68$, $P = 0.03$, $n = 10$) was observed for the MN group. Correlation analysis (panel b) did not show a significant correlation in the placebo group between change in NAD and $\Delta TEWL_{12}-\Delta TEWL_0$ (Pearson $r = -0.13$, $P = 0.43$, $n = 9$).

Studies have shown that rates of TEWL are greater in subjects with atopic skin (38, 46, 47). Accordingly, changes in the rates of TEWL in a group of atopic subjects were compared with a normal group (Fig. 5). While the effects of the placebo formulation were similar in the two populations,

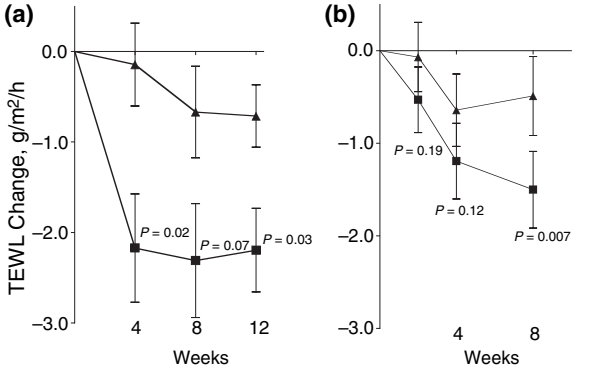

Figure 5. Comparison of effects of myristyl nicotinate (MN) on changes in the rates of transepidermal water loss (TEWL) in atopic and non-atopic subjects. The time course of TEWL changes on the forearms is shown in (a) for atopic subjects from Study 2 treated twice daily with placebo (triangles, $n = 6$) and 5% MN (squares, $n = 7$) and shown in (b) for non-atopic subjects in Study 3 treated twice daily with placebo (triangles, $n = 24$) and 5% MN (squares, $n = 24$). The *P*-values are derived from a paired two-tailed Student's *t*-test.

rates of TEWL decreased more rapidly and to a greater extent in atopic subjects than in non-atopic subjects. At 4 weeks, the first measurement made in atopic individuals, a highly significant decrease in TEWL was observed in treated subjects relative to the placebo group ($P = 0.02$, $n = 7$ and 6, respectively.) while statistically significant differences were not observed until 8 weeks in the normal subjects.

Discussion

A number of preclinical studies indicating that nicotinic acid could benefit photodamaged skin by several possible mechanisms provided the basis for the clinical studies described here. A major obstacle to the delivery of therapeutic amounts of nicotinic acid to any tissue is its ability to cause a peripheral vasodilatation that leads to a skin flushing response, an effect that is not harmful but is intensely disliked by most patients (48, 49). Delivery of nutrients taken orally to the epidermal compartment of skin is inherently inefficient and, combined with skin flushing side effects, oral delivery of nicotinic acid to skin with high patient compliance seems unlikely. Likewise, topical application of formulations containing nicotinic acid per se at levels above 0.05% is not feasible because they elicit intense vasodilatation at the site of application (33). The feasibility of topical delivery of nicotinic acid without causing vasodilatation was established by demonstrating in animal models that delivery of nicotinic acid by topical application of long chain ester derivatives such as MN can achieve delivery of relatively large amounts of nicotinic acid without skin flushing. The rate of diffusion of the ester derivative from the stratum corneum decreases as its lipophilicity increases, allowing the rate of delivery to be reduced such that the levels of free nicotinic acid released by skin esterases remain below the threshold for vasodilatation (33). Recent studies indicate that the skin flushing response is mediated by activation of receptors on epidermal Langerhans cells (50) or macrophages at concentrations above 0.1 mM (51). The topical application of formulations containing up to 5% MN did not induce skin flushing in the subjects involved in these trials and even higher concentrations do not cause this effect (data not shown). Using skin NAD as a biomarker of nicotinic acid delivery, MN was shown to deliver nicotinic acid to skin (Fig. 1) in human subjects effectively. As the method assesses intracellular NAD, the increases are likely derived primarily from the epidermis where the density of cells is much greater than in the dermis. However, determining which skin layer is most affected is difficult as the method for measurement requires intact cells where NADase activity has not been activated by disruption of the tissue.

Chronic sun exposure to skin often leads to thinning of the stratum corneum, sometimes referred to as compaction (1). Our studies (Fig. 2) show that MN treatment leads to an increase in stratum corneum thickness in photodamaged facial skin that averaged approximately 70% in the population studied. 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 (52), paraffin sections have been used to assess stratum corneum morphology and changes in thickness in numerous studies (53–57). The relative changes in stratum corneum thickness observed in this study strongly suggest that MN promotes epidermal differentiation in photodamaged skin. This conclusion also is supported by MN-stimulated increases in epidermal cellularity and thickness (Fig. 2) and rates of epidermal renewal (Table 1). The effect of MN on biomarkers of epidermal differentiation will be reported elsewhere (E. L. Jacobson et al. in preparation). The ability of MN to stimulate epidermal renewal without causing photosensitivity (Table 1) is in contrast to the effects of other agents that stimulate epidermal differentiation. An example is retinoic acid treatment where stimulation of epidermal renewal is accompanied by increased photosensitivity (37).

Recent studies have identified stratum corneum thickness as a key component of skin barrier function (40, 42), thus the ability of MN to increase stratum corneum thickness (Fig. 2) provided an indication that it may improve skin barrier function. TEWL measurements have been widely used in the non-invasive assessment of stratum corneum function and the utility of changes in TEWL as a reflection of changes in permeability barrier status has been validated (47). The absolute values of TEWL vary with the site of measurement, age of the individual and the presence of atopic skin (58, 59). In the present study, changes in rates of TEWL over time were assessed as a measure of change in barrier function. Consistent with other studies (43, 44), our results show that daily use of a skin moisturizer can improve the barrier function of photodamaged skin as evidenced by decreases in TEWL observed with placebo formulations (Fig. 3a). However, marked additional decreases in TEWL observed in subjects using MN indicate that this agent provides additional skin barrier enhancement, which together with the observed increases in stratum corneum thickness leads to the conclusion that MN improves barrier function in photodamaged skin. Additionally, the studies reported here indicate that MN also improves barrier function in relatively sun-protected skin as rates of TEWL were reduced on volar forearms of normal (Figs 3b–5b) and atopic subjects (Fig. 5a). The ability of skin to resist increases in rates of TEWL following a standardized regimen has been used in evaluation of changes of skin barrier function

(45) and this method of evaluation also supports the conclusion that MN improves barrier function (Fig. 4).

While our studies cannot distinguish between nutrient and drug effects of nicotinic acid in stimulation of epidermal differentiation and increased barrier function, prior studies suggest that both mechanisms likely are involved. DNA damage is central to skin photodamage and recent advances in the understanding of the role of niacin-dependent PARPs in DNA repair mechanisms (26) and the requirement for relatively high cellular concentrations of NAD for optimal PARP activity (16, 60) support a possible role of niacin as a protective skin micronutrient. Niacin deficiencies can mimic radiation- and chemical-induced DNA damage in terms of increased single- and double-strand breaks and/or oxidative lesions (61–63). Conversely, enhancing NAD in skin is associated with inhibition of UV-induced skin cancer and immunesuppression (19). Niacin-derived NAD has long been known to be essential in energy production and the constant renewal of the epidermis makes this compartment of skin vulnerable to micronutrient losses and UV exposure can cause further depletion (18). Niacin-derived NADPH is essential in the formation of several lipid components of the stratum corneum that are important determinants of epidermal barrier function (64). Thus, it seems reasonable to postulate that improved niacin status reflected in increased NAD and NADP content would benefit epidermal differentiation and maintenance of the epidermal barrier. Finally, the presence of a nicotinic acid receptor present in skin (26) and the stimulation of leptin release (27) support a drug effect of nicotinic acid as leptin has been shown to exert protective effects that include stimulation of epidermal differentiation, wound healing, and immune function (28–30, 65–67).

It is interesting that formulations containing the other B3 vitamer, nicotinamide, improve rosacea (68) and acne (69) and formulations containing 1-methylnicotinamide improve rosacea (70). Nicotinamide also inhibits melanosome transfer in hyperpigmentation (71) and anti-aging effects of formulations containing nicotinamide have been reported (72). The effects of nicotinamide likely involve mechanisms distinct from MN as topical application of formulations containing nicotinamide did not affect skin NAD content in a similar clinical trial where subjects applied a nicotinamide (4%) containing formulation for 8 weeks (E. L. Jacobson et al., manuscript in preparation). Further, nicotinamide does not bind to the nicotinic acid receptor (25).

The ability of MN to stimulate epidermal differentiation and barrier function in skin suggests that this agent may be useful in limiting the effects of skin photodamage either alone or in combination with other agents. The development of abnormal cell populations that can lead to actinic keratosis and non-melanoma skin cancers involves progressive cellular dedifferentiation. Agents that promote differentiation may limit progression of abnormal cell populations in these clinical conditions. Any prevention strategy requires long-term compliance with therapy, which in turn requires a strong record of safety and tolerability in addition to efficacy. The use of nicotinic acid for modification of blood lipids has established a strong record of safety for this agent (49) and the ability of MN to deliver therapeutic amounts of nicotinic acid to skin without the skin flushing side effects provides tolerability long lacking for this agent. Finally, the ability of MN to stimulate barrier function may benefit other conditions such as atopic dermatitis where skin barrier function is compromised (39, 73).

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