

Reprint from:
Alberts, David S.; Hess, Lisa M. (Eds.)
Fundamentals of Cancer Prevention
pp 139–160
© Springer-Verlag Berlin Heidelberg 2005

Developing Topical Prodrugs for Skin Cancer Prevention

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The skin plays multiple roles in protection from environmental insults yet skin damage, particularly that derived from sunlight, constitutes a major public health problem. End stage skin damage in the form of non-melanoma skin cancers (NMSC) are the most frequent malignancies in the United States with more than 1,000,000 cases diagnosed annually (Karagas, Greenberg et al. 1999). Melanoma skin cancer is the most rapidly increasing cancer. Actinic keratosis (AK), skin lesions that can progress to NMSC are far more prevalent than skin cancers. The occurrence of DNA damage and cellular responses to DNA damage are major determinants of skin damage including skin cancer (Ames 2001; Ullrich 2002). A compelling body of evidence now indicates that there are multiple targets for reducing skin damage and that several key micronutrients are candidates for skin damage prevention. However, a major challenge for the development of prevention strategies for skin damage is the difficulty of delivering micronutrients to skin. Delivery to skin via the blood circulation of nutrients taken orally is inherently inefficient since this delivery is distal to other organs, particularly the liver, which removes many agents by first pass metabolism. In addition the major cell targets for prevention of skin cancer are located in the epidermis which is non-vascular.

Described here are strategies to limit skin damage and thus skin cancer by targeting multiple mechanisms that include preventing DNA damage, enhancing DNA repair, preventing immune suppression, and preventing migration of transformed cells from epidermis to dermis. Further, an approach for delivery of key protective agents to skin cells using prodrugs specifically tailored for topical delivery is described. Finally, this approach is illustrated using niacin as a model micronutrient demonstrating that topical delivery of this polar compound to skin cells via prodrugs is feasible and that targeted delivery provides prevention benefit for skin.

Strategies for Intervention

Genotoxic stress is known to be a major factor in skin damage. While the mechanisms that cause skin damage are complex and incompletely understood, genotoxic stress in the form of DNA damage is a major factor. Figure 1 shows the primary sources of genotoxic stress in both the dermis and epidermis of skin and the consequences of this stress. Three interrelated sources of genotoxic stress in skin are reactive oxygen species (ROS), reactive carbonyl species (RCS) and sunlight. Sunlight is the major source of skin damage as it leads to DNA damage directly via formation of pyrimidine

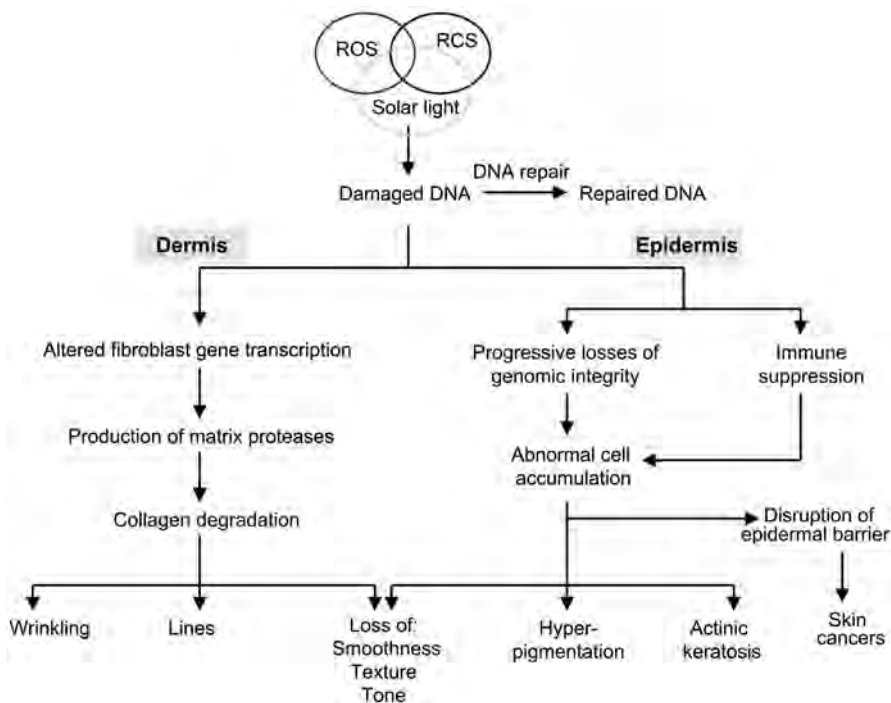


Fig. 1. Central role of DNA damage in skin damage

dimers and other photoproducts (Ullrich 2002) and indirectly via generation of ROS and RCS by photooxidation and photosensitization reactions (Wondrak, Cervantes-Laurean et al. 2002; Wondrak, Roberts et al. 2002; Roberts, Wondrak et al. 2003). Indeed, sunlight has been documented as a complete carcinogen. While the UVB region of sunlight, the region responsible for most of the direct DNA damage by sunlight, is the most effective at initiation of squamous cell carcinoma (SCC), recent studies have shown that solar simulated light containing more predominantly UVA rays that induce ROS also cause SCC formation (Pentland, Schoggins et al. 1999; Agar, Halliday et al. 2004). The involvement of ROS in the promotion and progression phases of skin cancer is well established (Perchellet and Perchellet 1989). ROS include superoxide, hydroxyl radical, hydrogen peroxide, singlet oxygen, nitric oxide, peroxyxynitrite, and hypochlorite. All cells are exposed to ROS during the normal course of energy metabolism and/or immune surveillance in addition to sunlight exposure. While ROS are involved in normal cell signaling pathways, increased ROS formation during oxidative stress disrupts signaling pathways causing negative consequences for normal cell function. In addition to DNA, proteins also are targets for damage by ROS in skin. Carbonyl stress, mediated by RCS from metabolic sources, lipid peroxidation, and glycoxidation targets skin cell DNA and extracellular matrix proteins with accumulation of protein advanced glycation end products (AGEs) during chronological and actinic aging of skin (Wondrak, Cervantes-Laurean et al. 2002; Wondrak, Jacobson et al. 2002; Wondrak, Roberts et al. 2002; Roberts, Wondrak et al. 2003). Recently AGEs have been

identified as potent UVA sensitizers of photooxidative stress in human skin, establishing a vicious cycle of RCS and ROS formation in sunlight induced genotoxic stress.

Figure 1 also overviews two major consequences of genotoxic stress in skin. First, chronic DNA damage results in progressive losses of genomic integrity that result in and are required for end stage skin damage in the form of skin cancer. These progressive losses of genomic integrity lead to altered growth properties of damaged keratinocytes such as unresponsiveness to terminal differentiation signals leading to epidermal hyperplasia and progressively to detectable skin lesions diagnosed as actinic keratosis (Jeffes and Tang 2000; Lober, Lober et al. 2000). Cell populations present in actinic keratosis lesions can progress to transformed cell populations that represent epidermal carcinoma in situ (Horowitz and Monger 1995; Guenther, Hurwitz et al. 1999). Subsequent cellular changes occur including induction of matrix proteases that facilitate disruption of the integrity of the epidermal barrier leading to invasion of the dermis, the point at which the damage process is diagnosed as SCC. A second major consequence of DNA damage in skin is the suppression of immune responses that would normally detect and remove damaged cells. While mechanisms of immune suppression extend well beyond DNA damage, the latter represents a major factor in immune sup-

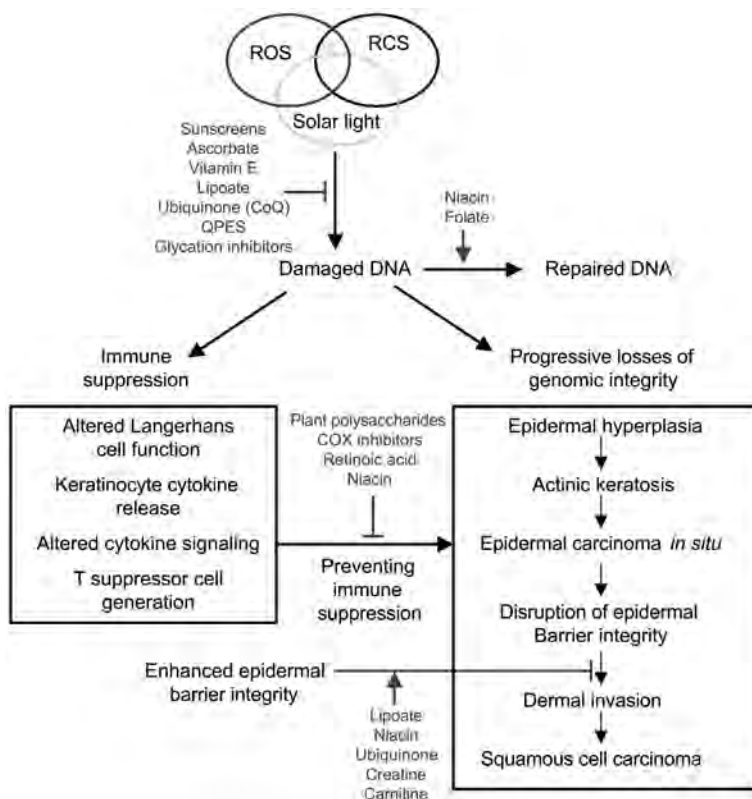


Fig. 2. Opportunities for treatment and prevention of skin damage

pression. The consequences of genotoxic stress include altered migration and antigen presentation by Langerhans cells, stimulation of cytokine release by keratinocytes that likely alters cytokine signaling required for normal immune surveillance including generation of T suppressor cells. Given the complexity of damage pathways and the down stream consequences, it seems likely that a combination strategy to prevent skin damage will be essential.

Figure 2 identifies opportunities for epidermal intervention by various agents where substantial evidence suggests the possibility of modulating the consequences of genotoxic stress. These opportunities include preventing DNA damage, enhancing DNA repair, preventing immune suppression both by preventing DNA damage and by mechanisms downstream from DNA damage, and strengthening the integrity of the epidermal barrier to prevent migration of transformed cells from the epidermis.

Strategy 1: Preventing DNA damage

Sunscreens. The value of sunscreens in preventing DNA damage has clearly been demonstrated in animal models in which sunscreens applied prior to solar simulated light (SSL) exposure prevented p53 mutations and dramatically reduced skin cancer incidence (Ananthaswamy, Ullrich et al. 1999). Further, sunscreens prevent photoimmune suppression in mice (Reeve, Bosnic et al. 1998) and man (Fourtanier, Gueniche et al. 2000). Despite growing use of sunscreens, their inability to protect across a broad spectrum of solar radiation combined with poor public knowledge of appropriate selection and use have not led to decreases in skin cancer incidence. While the use of sunscreens needs to be an integral part of an overall strategy to reduce skin damage, their inability as a single agent to reduce skin damage illustrates the need for a combination of prevention agents.

Ascorbate (Vitamin C). Vitamin C has been used widely in recent years as a skin protective agent. It is known to function as an antioxidant and likely serves multiple roles in collagen synthesis (Geesin, Darr et al. 1988). Further, this vitamin is important in recycling of reducing power in cells by exchange of electrons with vitamin E (Beyer 1994). A study in mice has shown inhibition of phorbol ester induced skin tumor promotion using a lipophilic ester of vitamin C which, in its free form, is unstable and has poor penetration through the stratum corneum (Smart and Crawford 1991). Further studies of stable, deliverable forms of vitamin C designed for optimal uptake by cells may prove beneficial in limiting DNA damage and improve skin cancer prevention. It is important to consider, however, that this compound can serve both as an antioxidant and a pro-oxidant.

Tocopherol (Vitamin E). Topical application of tocopherol has been shown to decrease the incidence of ultraviolet-induced skin cancer in mice (Berton, Conti et al. 1998; Burke, Clive et al. 2000). Vitamin E provides protection against UV-induced skin photodamage through a combination of antioxidant and UV absorptive properties (McVean and Liebler 1997). Topical application of alpha-tocopherol on mouse skin inhibits the formation of cyclobutane pyrimidine photoproducts (Chen, Barthelman et al. 1997). However, topically applied alpha-tocopherol is rapidly depleted by UVB radiation in a dose-dependent manner (Liebler and Burr 2000) as vitamin E in skin can

absorb UV light and generate the tocopheryl radical (Kagan, Witt et al. 1992). Hence, vitamin E in skin may act in two conflicting manners, as a radical scavenger and possibly as a photosensitizer. Indeed, tocopherol has been shown to exacerbate UVA induced DNA damage in vitro (Nocentini, Guggiari et al. 2001). However, reductive antioxidants (ascorbate, thiols, ubiquinols, etc.) can reduce tocopherol radicals back to tocopherol (Kagan, Serbinova et al. 1990). Unlike the soluble vitamins that are too hydrophilic for optimal delivery through the stratum corneum, vitamin E is more lipophilic than is optimal for delivery into skin. Thus, when applied topically as the parent compound, residence time on the surface of skin is prolonged making the agent susceptible to UVB light absorption and possible conversion to a tocopheryl radical, which is a potential photosensitizer. On the other hand, when Vitamin E is stabilized by derivatization to a prodrug and effectively delivered into skin possessing an environment of reductive antioxidants (ascorbate, thiols, ubiquinols, etc.) or formulated with antioxidants, tocopherol radical formation can be eliminated by three mechanisms: (1) decreased exposure to UVB on the surface of skin due to rate of delivery, (2) stability of vitamin E due to derivatization to prodrug, and (3) rapid conversion back to tocopherol of any tocopheryl radicals formed due to the presence of reductive antioxidants in skin cells and/or in the delivery vehicle. While preclinical data demonstrate that tocopherol has photoprotective properties, clinical data do not yet convincingly show that dietary supplementation is of significant therapeutic value in protection from acute or chronic photodamage. Further, use of ester derivatives of vitamin E to date have stabilized the molecule, but have increased the lipophilicity of the compound thereby decreasing its delivery to skin. This illustrates the general lack of understanding of delivery mechanisms for micronutrient benefits in skin. Thus, it seems likely that cutaneous bioavailability of dietary and existing preparations of topical tocopherol may be insufficient to combat photodamage in skin.

Lipoate. While a clear role for direct ROS scavenging by lipoate has not been firmly established, lipoate has a redox potential of -0.32 V, allowing it to reduce oxidized glutathione and ascorbate nonenzymatically in the skin antioxidative network (Guo and Packer 2000). In keratinocytes, lipoate is reduced to its active form, dihydrolipoate, which results in significant inhibition of the consumption of tocopherol and ubiquinone following UVA irradiation (Guo and Packer 2000). This protection presumably occurs via the role of lipoate along with ascorbate and tocopherol in the maintenance of redox balance. Lipoate topically applied to hairless mouse skin shows penetration and conversion to dihydrolipoate demonstrating cellular delivery although the efficacy of delivery was not examined in detail (Podda, Rallis et al. 1996). Lipoate *per se* is much too hydrophilic for effective topical delivery.

Ubiquinone (Coenzyme Q10). Ubiquinone offers the potential to reduce DNA damage directly as an ROS scavenger and by supporting redox cycles that resist oxidative stress (Tomasetti, Littarru et al. 1999). Ubiquinone and tocopherol are the major lipophilic antioxidants in skin (Shindo, Witt et al. 1994). The content of ubiquinone is 9 times higher in the epidermis than in the dermis and a strong role in protection against skin damage is suggested by the observation that skin ubiquinone content decreases rapidly following solar irradiation (Shindo, Witt et al. 1994). A recent study has demonstrated that in vivo supplementation with ubiquinone enhances the recovery

of human lymphocytes from oxidative DNA damage, supporting the hypothesis that this micronutrient can limit DNA damage in vivo (Tomasetti, Littarru et al. 1999). In addition to its role as a direct ROS scavenger, ubiquinone has been postulated to function as an integral part of antioxidant defense pathways that also include tocopherol, ascorbate, glutathione and NADPH (Podda and Grundmann-Kollmann 2001). Beneficial effects of topical ubiquinone on prevention and reversal of skin photoaging also have been reported although the same study reported that the extremely lipophilic nature of the molecule strongly limited bioavailability following topical application (Hoppe, Bergemann et al. 1999), again illustrating the desirability of an effective strategy for topical delivery of this micronutrient. As with the case of tocopherol, ubiquinone *per se* also is much too lipophilic for effective topical delivery.

Quenchers of Photoexcited States (QPES). We have coined the term QPES to refer to agents that physically quench or dissipate the energy transferred from sunlight to skin molecules, thereby inactivating photoexcited states that would ultimately interact with oxygen or other molecules to produce RCS and ROS, hydrogen peroxide and singlet oxygen in particular. UV and near visible chromophores in skin extracellular proteins (keratin, collagen, and elastin) are endogenous photosensitizers that mediate photo-damage in human skin (Wondrak, Roberts et al. 2002; Wondrak, Roberts et al. 2003). Small molecule quenchers of this novel class of sun damage targets are predicted to serve a chemopreventive role in suppressing photooxidative pathways of photocarcinogenesis and photoaging by direct physical quenching of photoexcited states that occurs without chemical depletion or need for metabolic regeneration of the active compound. This represents intervention at a very early step in the production of these UV induced deleterious species upstream of ROS formation. We have identified small polar compounds that accomplish these goals and envision that derivatives suitable for topical delivery will be required for optimal protective benefit to limit skin damage.

Glycation Inhibitors. Among the chromophores in skin that serve as photosensitizers of UV light are the nonenzymatically formed AGE chromophores generated from chemical reactions between reducing sugars and other RCS with protein amino groups followed by rearrangements and oxidation reactions. These structures form during intrinsic aging and accumulate at accelerated rates in photoaged skin. Interaction of AGEs with UV light readily generates ROS and more RCS forming a vicious cycle of skin damage (Wondrak, Cervantes-Laurean et al. 2002; Wondrak, Jacobson et al. 2002; Wondrak, Roberts et al. 2002; Roberts, Wondrak et al. 2003). Identifying agents to inhibit the formation of AGEs and optimize delivery of such agents could be very beneficial in limiting DNA damage that contributes to skin cancer.

Strategy 2: Enhancing DNA repair

Niacin. A major target for niacin (nicotinic acid) with regard to enhancing DNA repair is poly(ADP-ribose) polymerase-1 (PARP-1) and downstream signaling pathways, whose activity is enhanced by niacin due to increased availability of NAD, the bioactive form of niacin and a substrate for PARP-1. The involvement of PARP-1 as a target for cancer prevention by niacin is based on studies that have demonstrated the involvement of PARP-1 in the maintenance of genomic integrity following genotoxic stress (Jacobson

and Jacobson 1999; Rolli, Armin et al. 2000). PARP-1 functions in the synthesis of chromatin-associated polymers of ADP-ribose that function in cellular recovery from DNA damage and maintenance of genomic stability. The activation of PARP-1 by DNA strand breaks leads to complex signaling pathways that can enhance cell survival or result in cell death by apoptosis as shown in Figure 3. In cases where the amount of damage is relatively small, PARP-1 activation enhances cellular recovery by interaction with other proteins such as p53 and the nuclear proteasome to stimulate both DNA repair and histone degradation such that the cell can fully recover from the genotoxic stress. When the damage is relatively higher, PARP-1 plays a key role in effecting cell death by apoptosis through its transcriptional activation role involving the NF- κ B pathway and by preventing ATP depletion and DNA repair through PARP-1 cleavage (Jacobson and Jacobson 1999). Of direct relevance to skin, PARP-1 has been shown to be required for Fas, FasL mediated apoptosis critical to removal of badly damaged and potentially carcinogenic 'sunburn' cells that arise following sunlight exposures that lead to erythema (Hill, Ouhitt et al. 1999). Validation of niacin as a chemoprevention agent has been obtained in a mouse model where high dose oral niacin intake resulted in dose-dependent (1) increased skin NAD content, (2) decreased skin tumor incidence 70%, and (3) reduced immune suppression 86% (Gensler, Williams et al. 1999).

Folate. A major prevention target for folate relates to its role in providing precursors for DNA repair synthesis. It may also promote genomic integrity through its role in the generation of methyl groups needed for control of gene expression. Its cancer protection potential has been demonstrated by large-scale epidemiological and nutritional studies indicating that decreased folate status increases the risk of developing stomach (Fang, Xiao et al. 1997), colorectal and breast cancer (Prinz-Langenohl, Fohr et al. 2001). Consistent with a role in DNA repair, chromosome breaks and centrosome abnormalities have been observed in patients deficient in folate (Heath 1966; Chen, Reidy et al. 1989). In vitro, DNA strand breakage and uracil misincorporation increased in a time and concentration dependent manner after human lymphocytes were cultured with decreasing amounts of folate (Duthie and Hawdon 1998). DNA breaks are associated with an increased risk of cancer in humans. Moreover, folate deficiency impairs DNA excision repair in rat colonic

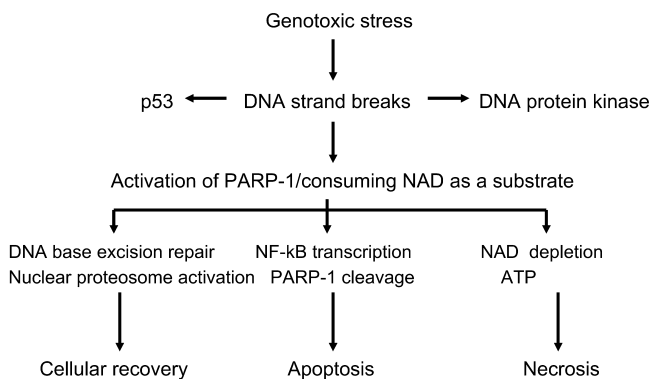


Fig. 3. Interrelationship of niacin metabolism and DNA damage and repair pathways

mucosa (Choi, Kim et al. 1998). These data indicate that folic acid deficiency affects the stability of cellular DNA at the chromosomal and molecular levels (Choi and Mason 2000). While folate deficiency has been extensively documented by analysis of human plasma, folate status within skin has not been widely investigated. Nevertheless, the inefficiency of delivery of nutrients to skin argues that documented folate deficiencies will extend to skin. Additionally, photolysis of folate appears likely to deplete this nutrient in sun-exposed skin (Jablonski and Chaplin 2000). It has been reported that fair-skinned patients undergoing photochemotherapy for dermatological conditions have low serum folate concentrations, suggesting that folate depletion may occur *in vivo* (Branda and Eaton 1978). With regard to delivery, folate *per se* is too hydrophilic for effective topical skin delivery.

Strategy 3: Preventing photoimmune suppression

While the mechanisms leading to photoimmune suppression are still poorly understood, DNA damage is a major factor leading to reduced immune surveillance (Ullrich 2002). Two major cell targets are Langerhans cells where DNA damage leads to suppression of cell migration and antigen presentation functions and keratinocytes where DNA damage results in altered cytokine signaling and reduced immune function potentially involved in generation of T suppressor cells (Elmets, Bergstresser et al. 1983; Cruz, Tigelaar et al. 1990). In addition to DNA, two other targets have been identified for immune suppression. The photoconversion of urocanic acid to cis-urocanic acid has been implicated in immune suppression, although the molecular mechanisms are still poorly defined (Moodycliffe, Norval et al. 1993). Also, ROS generation including membrane lipid peroxidation has been implicated in immune suppression (Ullrich 2002), possibly by altering signaling pathways at the membrane level, although DNA as the ultimate target is still a possibility. In view of the strong link between DNA damage and immune suppression, it is not surprising that agents that prevent DNA damage or enhance DNA repair reduce suppression (Gensler, Williams et al. 1999; Ullrich 2002). Additionally, several other agents offer promise for prevention of immune suppression at stages following DNA damage.

Plant Polysaccharides. A number of plant polysaccharides, such as those found in *Aloe barbadensis*, appear to prevent immune suppression by mechanisms distinct from those that do so by preventing DNA damage (Strickland 2001).

COX Inhibitors. Drugs that block production of PGE₂ by cyclooxygenase activity have been shown to reduce photoimmune suppression, suggesting a role of overproduction of prostaglandins in immune suppression (Shreedhar, Giese et al. 1998).

Retinoic Acid. Defective dendritic cell function caused by abnormal differentiation of these cells is an important mechanism of tumor escape from immune system control. All-trans-retinoic acid has been shown to induce maturation of these cells in cancer patients and this may suggest a role in modulating immune suppression (Almand, Clark et al. 2001).

Niacin. As described above, niacin has been shown to prevent photoimmune suppression (Gensler, Williams et al. 1999). While the ability of niacin to prevent immune

suppression may be due to its ability to limit DNA damage by enhancing DNA repair, it should be noted that niacin has recently been discovered to stimulate the release of leptin (Kim 2002). Leptin is emerging as a hormone that modulates numerous protective effects in skin including immune modulation. Thus, it is possible that niacin prevents immune suppression via effects on leptin secretion.

Strategy 4: Enhancing the Epidermal Barrier

The epidermis of skin is a constantly renewing tissue. This renewal involves a complex series of events that involves proliferation of keratinocytes in the basal layer followed by terminal differentiation that ultimately leads to an epidermal barrier whose integrity is crucial to the protection of the organism from environmental insults. Several points need to be considered with regard to micronutrients in epidermal turnover. First, there is a growing body of evidence indicating that a significant percentage of the American population is deficient in a number of micronutrients and the constant turnover of the epidermis makes this tissue particularly vulnerable to micronutrient depletion. While there is limited data on micronutrient content of skin, studies have demonstrated that micronutrient deficiencies observed in plasma also are observed in skin (Peng, Peng et al. 1993), a wide range of tissue NAD content has been observed in human skin (Jacobson, Shieh et al. 1999), and solar exposure has been demonstrated to deplete micronutrients (Jablonski and Chaplin 2000; Liebler and Burr 2000). Thus, skin is a likely site of micronutrient deficiencies with potentially adverse consequences leading to skin damage. Second, the constant renewal of the epidermal compartment places an important energy requirement on the organism. Thus, the nutritional status of micronutrients whose bioactive forms play important roles in cellular energy generation is important to the integrity of the epidermal barrier (Jacobson, Giacomoni et al. 2001). Micronutrients in this category include lipoate, niacin, ubiquinone, creatine, and carnitine. Third, the non-vascular nature of the epidermal compartment makes micronutrient delivery to this compartment inherently inefficient. The above considerations have led to the proposal that optimal energy metabolism will strengthen integrity of the epidermal barrier which in turn can lead to a decrease in skin cancer. Studies have shown that cell populations with altered growth properties within actinic keratosis lesions can be recognized by immune surveillance and removed. Alternatively, cell populations within such lesions can progress to cell populations (carcinoma in situ) that secrete proteases and other factors that allow escape from the epidermis. Thus, the status of the epidermal barrier integrity can be a deciding factor between the ultimate fates of removal or escape of abnormal cell populations from the epidermal compartment.

Innovative Agents for Skin Cancer Prevention are Needed

Several approaches have been taken to reduce the rate of skin damage and photodamage. Reducing the amount of damage that reaches critical biomolecules in the skin is the objective of sunscreens, antioxidants, and quenchers of photoexcited states (Figure 2). Sunscreens aim to directly absorb sunlight photons and thus lessen the amount of damage that reaches the skin, and quenchers are designed to deactivate excited state molecules in skin prior to interaction with oxygen to limit the amount of ROS formed.

Glycation inhibitors also are designed to limit ROS generation from solar irradiation of AGE-pigments in skin. Alternatively, antioxidants, which include vitamins C and E as active ingredients, are designed to intercept damage to the skin by capture of ROS generated by sunlight exposure. While the approaches designed to reduce damage to the skin are beneficial, they have inherent limitations, as neither sunscreens nor antioxidants can effectively eliminate oxidative stress. Sunscreens absorb only a portion of the rays of sunlight that cause damage, many are not photostable for more than a few minutes in sunlight, and the feasible levels of antioxidants in skin creams can only partially reduce oxidative stress. Another approach to treating skin deterioration involves accelerating the removal of the upper layers of damaged skin to allow replacement with undamaged skin. The application of retinoic acid (tretinoin, Renova and Retin A) results in an increased rate of cell turnover allowing new cells to mature and replace damaged cells, but a side effect is a weakened skin barrier and increased photosensitivity. Chemical peels using agents such as alpha-hydroxy acids or beta-hydroxy acids cause a chemical exfoliation of the top layers of skin, again allowing new cells to replace the damaged cells that have been removed. Topical formulations of 5-fluorouracil represent an aggressive therapy for removal of skin lesions and a topical formulation of the cyclooxygenase (COX) inhibitor diclofenac has been approved for treatment of actinic keratosis. Surgical or laser procedures also can remove damaged skin. While these approaches play important roles in the treatment of skin damage, the high irritation potential, increased sunlight sensitivity, and long downtime for patient recovery from facial disfigurement of most current treatments combined with the enormity of the problem indicates that new approaches to treat and prevent skin damage still are needed.

Lipophilic ester prodrugs have been used to increase the permeability of polar compounds for transdermal systemic drug delivery. With regard to micronutrients, some derivatives of vitamins C and E have been prepared and used in skin care products, but a systematic, scientific base for rational development of compounds and their evaluation demonstrating optimal delivery of protective agents to the cellular components of skin has not been reported. For example, the realization that vitamin E is more stable as an ester derivative resulted in the design of a compound that was less efficacious than the parent compound, but to our knowledge the flux rates into skin of vitamin E esters have not been studied (Alberts, Goldman et al. 1996). We predict that a more polar derivative of vitamin E also would stabilize the compound and improve delivery. This approach to optimize skin micronutrients and/or chemoprevent genotoxic stress in skin with multiple agents is complementary to and integrative with existing approaches and new developments such as designing more effective sunscreens to limit skin damage.

Topical Delivery: the Cornerstone of a Skin Damage Prevention Strategy

We have reviewed above evidence indicating that several agents are therapeutic candidates for skin damage prevention. However, a major challenge for the development of prevention strategies for skin damage relates to the difficulty of delivering small molecules to skin. Delivery to skin via the blood circulation of nutrients taken orally is inherently inefficient as delivery is distal to other organs and numerous cell targets for skin cancer prevention are located in the epidermis which is non vascular. The challenging

in delivering many micronutrients topically is that they are small molecules that do not have optimal properties to insure prolonged skin residence time required for efficacy. For example, niacin, ascorbate, lipoate, creatine, carnitine, and folate are too polar for effective delivery while vitamin E, vitamin E acetate, and ubiquinone are too lipophilic. To resolve this problem, we have studied niacin (nicotinic acid) as a model nutrient to determine the feasibility of optimizing topical delivery to skin cells. Briefly, the strategy is as follows. Prodrugs designed for optimal delivery are synthesized as esters or thioesters of the parent micronutrient or drug. Once delivered to the epidermis, the abundant and nonspecific esterases present there rapidly cleave the prodrugs back to the parent compound. The delivery properties are designed to provide a slow, continuous supply of micronutrient to skin cells to allow increased uptake by the cells. This strategy takes into consideration two distinct barriers that influence the delivery of small molecules to skin, lipophilicity of the stratum corneum and skin metabolic activity. Our formulation strategy controls the rate of partitioning of the prodrug in and out of the stratum corneum by designing derivatives with an optimal lipophilicity for such partitioning. Figure 4 shows a multiple compartment model that serves as the framework for the development of this delivery strategy. Briefly described below are features of the topical delivery strategy that have emerged from our research.

A Pronutrient Must Effectively Partition from the Topical Formulation Into the Stratum Corneum. The highly lipophilic nature of the stratum corneum dictates that a pronutrient be sufficiently lipophilic to effectively partition into the stratum corneum from the donor compartment, which can be a skin cream or lotion (arrow #1 in Figure 4). As described in more detail below, the required lipophilicity needed for diffusion from the stratum corneum into the epidermis predicts that an efficacious pronutrient should be sufficiently lipophilic to rapidly partition from the cream or lotion into the stratum corneum. We have synthesized esters of nicotinic acid that are lipophilic derivatives that allow rapid diffusion from the topical formulation into the stratum corneum.

The Pronutrient Must be Stable in the Topical Formulation. The lipophilicity of a prodrug should allow it to be formulated in a skin cream or lotion and the linkage of the nutrient derivative must be very stable in these formulations. We have shown that the prodrug lipophilicity optimal for delivery is such that the prodrug is easy to formulate in a cream or lotion. Also, in the case of niacin prodrugs, our developmental research examined and identified compounds that were stable to chemical hydrolysis when formulated in a cream or lotion.

The Pronutrient must Partition from the Stratum Corneum into the Epidermis at an Optimal Rate to Achieve Effective Delivery to the Cellular Components of Skin (#2 in Figure 4). Studies of drug structure-penetration relationships have provided useful information concerning partitioning from the stratum corneum to the epidermis (Tsai, Tayar et al. 1992; Potts and Guy 1993; Webber, Meyer-Trumpener et al. 1994). This rate of flux is controlled by a diffusion constant and for small uncharged molecules lipophilicity is the major factor that determines the diffusion constant. A correlation between skin permeability (P_B) and the physicochemical properties of the drug, such as octanol/water partition coefficient ($P_{oct/w}$) have proven to be of great value in predicting drug transport across skin. Figure 5 illustrates the relationship be-

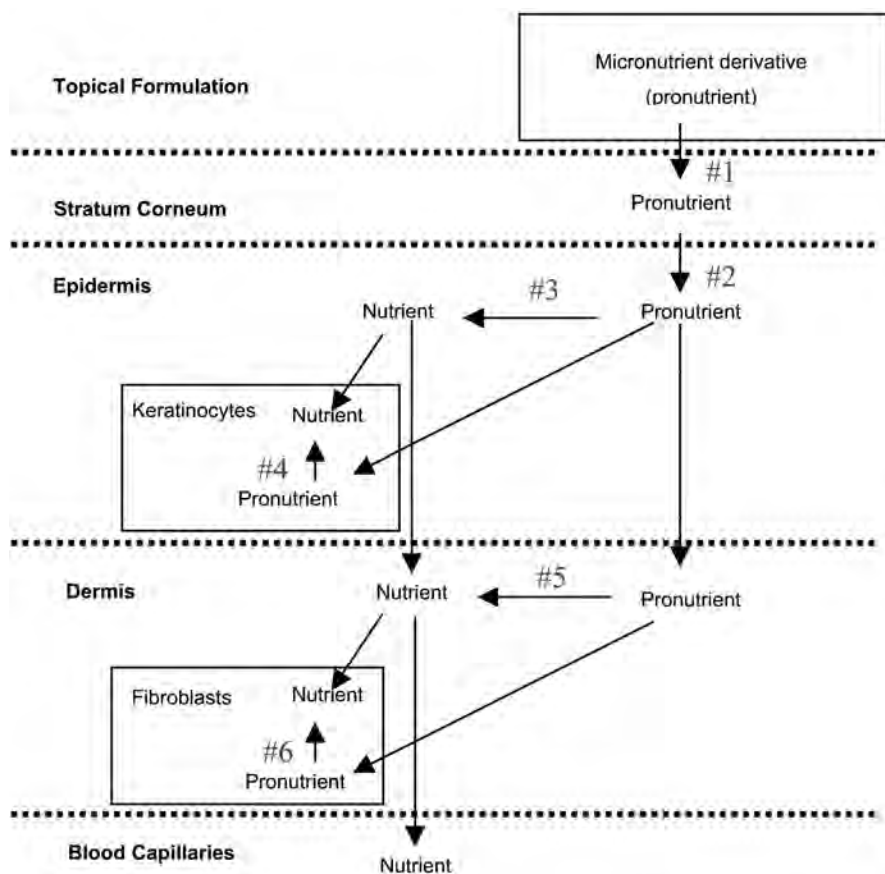


Fig. 4. Multiple compartment model for topical delivery

tween compound lipophilicity, rate of flux from stratum corneum, and skin residence time (Roberts, Anderson et al. 1978; Anderson, Higuchi et al. 1988).

A series of niacin esters were synthesized and their $\log P_{\text{oct/w}}$ values were determined. The values demonstrated a linear relationship between alkyl chain length of the niacin ester and the logarithm of the octanol/water partition coefficient. These data allowed us to relate prodrug lipophilicity to niacin delivery and thus allowed identification of the lipophilicity range that provides an optimal rate of prodrug and thus drug delivery.

The Pronutrient must be Efficiently Bioconverted to Active Nutrient in Skin. The delivery approach that we designed for niacin involved the bioconversion of the pronutrient to niacin by the action of skin esterases. Studies on the esterase distribution of skin have shown that the stratum corneum has little or no esterase activity, the epidermis has the highest activity and the dermis has reduced activity relative to the epidermis (Sugibayashi, Hayashi et al. 1999). Delivery should be possible whether the

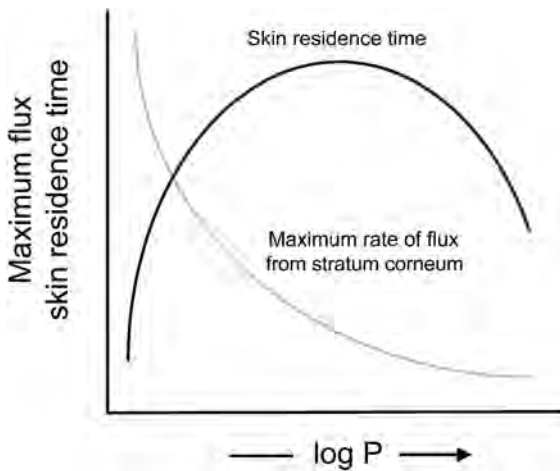


Fig. 5. Relationship between compound lipophilicity, rate of flux from stratum corneum, and skin residence time

bioconversion is extracellular (#3 and 5, Figure 4) or following uptake by the target cells (#4 and 6, Figure 4) since cells contain specific transport systems for niacin and the lipophilicity of the prodrug should make it readily bioavailable through passive diffusion also. Skin cells also contain esterases. Bioconversion was confirmed by experiments that determined the effect of niacin prodrugs on the content of the bioactive form of niacin, NAD, in skin cells. Thus, we have measured bioconversion of the prodrug to nutrient and then bioconversion of the nutrient to the active form of the vitamin in this case. In this manner, we were able to relate the major factor determining the rate of partitioning from stratum corneum to epidermis (prodrug lipophilicity) to the effectiveness of cellular delivery (skin cell NAD content).

Developing a Niacin Prodrug as a Potential Skin Cancer Prevention Agent Rationale. Nicotinic acid was selected for development as the first topical agent in a series of micronutrients based on its known effects in preclinical studies. The diagram in Figure 6 outlines the three predominant mechanisms of action of this compound. First, it has long been appreciated that nicotinic acid, as well as nicotinamide, can serve as the vitamin precursor of NAD and recent studies have demonstrated that NAD deficiency can occur in Western populations (Jacobson, Dame et al. 1995) and while the degree of deficiency may not reach that which elicits symptoms of pellagra, it may be relevant in the development of chronic disease states such as cancer. NAD is essential in energy metabolism and may be a very important factor in the continual epidermal renewal of skin, which is known to turnover approximately every 28–30 days. In addition, numerous dermal functions are energy demanding. Since skin is the largest organ of the body, maintenance of optimal NAD for energy in skin by dietary means could be challenging, particularly during aging and following photodamage. Secondly, NAD serves as a substrate for the enzyme PARP-1, which plays an essential role in maintenance of genomic integrity and NAD is rapidly consumed during genomic stresses such as UV radiation and environmental insults to skin (Jacobson, Antol et al. 1983). This pathway may also be important in immune function, which is criti-

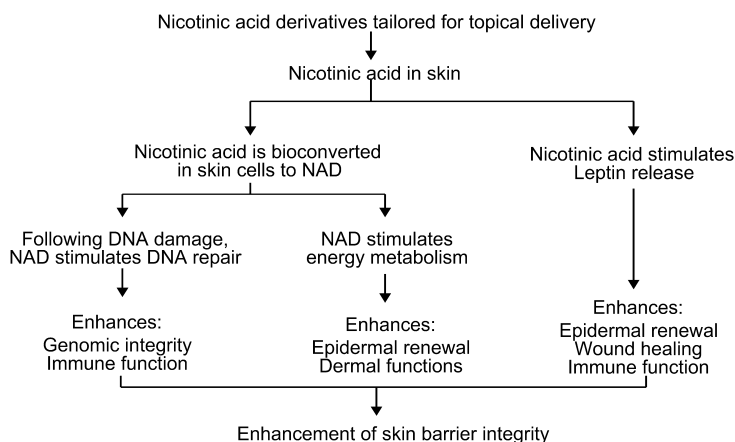


Fig. 6. Known effects of nicotinic acid in skin

cal following UV exposure. Thirdly, we have shown that nicotinic acid stimulates the release of the cytokine, leptin (Kim, Jacobson et al. 2002), which has been shown to function in epidermal renewal, wound healing, immune function, and inhibition of tumor promotion. Based on these findings, a development program for a topical prodrug of nicotinic acid was initiated.

Synthesis and Characterization of Niacin Prodrugs. The feasibility of developing a topical delivery system for skin protective agents was established by demonstrating that delivery of a niacin prodrug is controlled primarily by the rate of diffusion (lipophilicity). We synthesized, purified, and characterized niacin derivatives using alcohols varying in alkyl chain lengths from 8 to 18 carbon atoms to construct niacin derivatives.

To assess targeted delivery to skin, niacin derivatives were formulated in a compatible lotion and administered to the backs of hairless mice, once daily for three days. Skin samples from the site of application were evaluated for intracellular NAD content. This measurement assesses the net effect of diffusion of the prodrug through the stratum corneum to the cellular layers of skin, bioconversion to niacin, uptake by cells, and subsequent conversion to NAD. From Figure 7, it can be seen that derivatives having log P values ranging from around 6 to 10 were effective at targeting delivery to skin cells with tetradecyl nicotinate (TN or Nia-114) (Log $P_{oct/w}$ of 7.5) most effectively elevating NAD in mouse skin at the site of application. Also, it can be seen in Figure 7 that the free forms of the vitamin, nicotinamide and nicotinic acid did not effectively deliver to skin cells to increase skin cell NAD content.

The proposed topical delivery system was designed to target small molecules to skin with minimal systemic exposure. To evaluate this effect, skin samples taken from the abdominal area (distal to the application site) were compared to that taken from the back (site of application). Minimal changes in NAD occurred in abdominal samples while significant increases were observed in the samples from the back as shown in Figure 7. These data provide evidence for preferential delivery to the targeted tis-

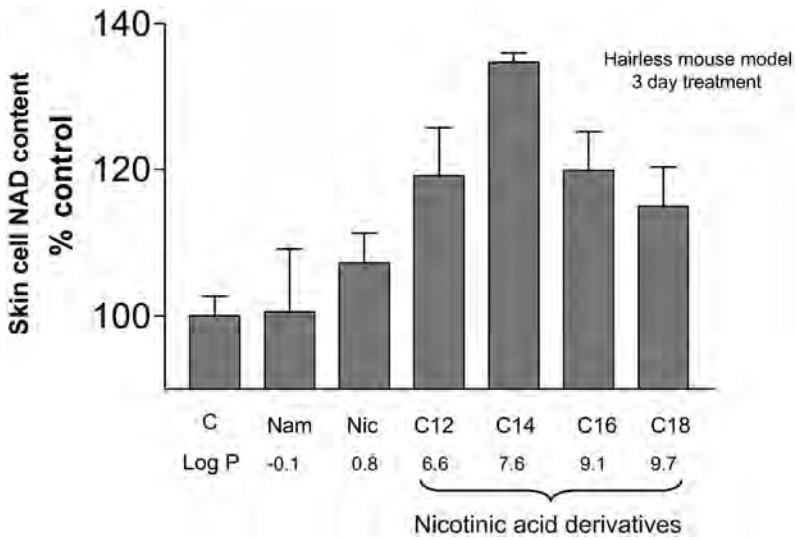


Fig. 7. Effect of niacin derivatives applied topically on skin cell NAD

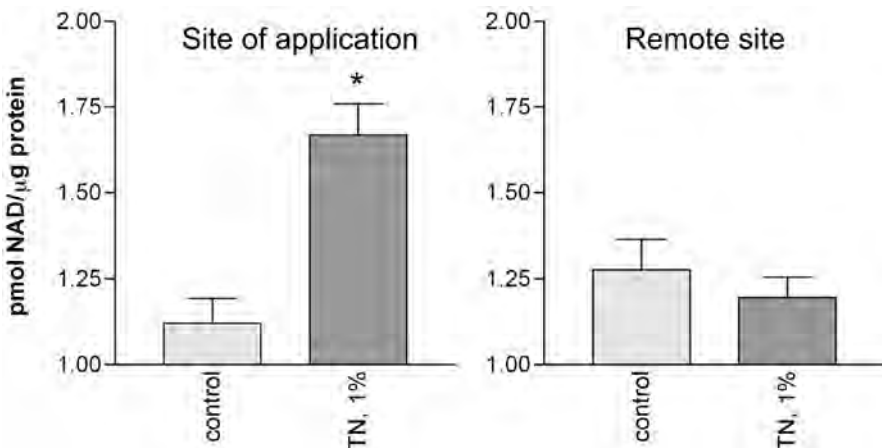


Fig. 8. Preferential dermal delivery by tetradecyl nicotinate in mouse skin

sue. These data show that tetradecyl nicotinate delivers niacin at a slow sustained rate at the site of delivery on the stratum corneum, allowing hydrolysis of the prodrug at a rate suitable for efficient uptake and bioconversion by skin cells. In contrast, lauryl nicotinate increased skin NAD content at both the site of application and at a distal site (data not shown), indicating tissue saturation at remote sites due to transdermal delivery. Using this lead candidate, tetradecyl nicotinate, dose- and time-response studies were carried out to determine the dosing concentration and schedule for optimal delivery to skin cells (Figure 9). Using 7 days as an end point, concentrations of tetradecyl

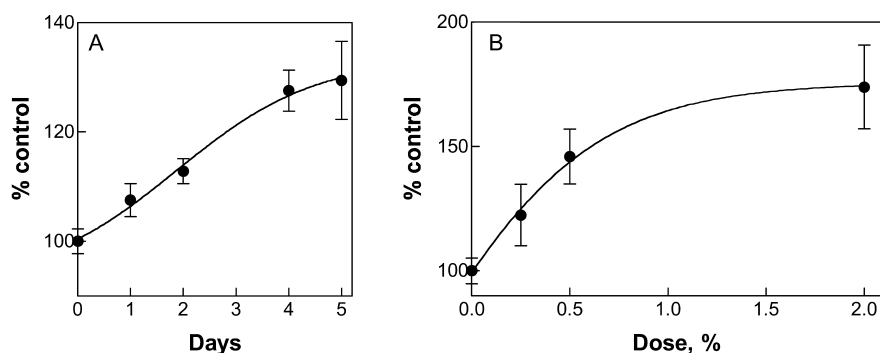


Fig. 9. Dose (A) and time (B) effects of topical tetradecyl nicotinate treatment on NAD content of hairless mouse skin

cyl nicotinate up to 1.0% increased NAD in skin. The time course of skin NAD content is shown for 1% tetradecyl nicotinate. A plateau was observed at about 5 days.

Tetradecyl nicotinate was found to be stable at room temperature and at elevated temperatures for extended periods of time appropriate for further development. In addition, bioconversion studies showed that it was readily converted to nicotinate and the free alcohol, demonstrating that tetradecyl nicotinate functions effectively as a prodrug of niacin for topical delivery.

Clinical Development of Tetradecyl Nicotinate (Nia-114)

Clinical Evaluation of Nia-114. This compound has undergone extensive safety evaluation *in vitro* and *in vivo* to determine the repetitive epidermal contact potential of a test material to induce primary or cumulative irritation and/or contact sensitization. The test material at 5% showed no potential for dermal irritation or allergic contact sensitization. Skin creams containing this compound are extremely well tolerated with daily use. No irritation was reported by study subjects or detected by study physicians. Measurements designed to detect even minimal irritation as skin redness showed a trend away from redness for Nia-114 treated skin. These data demonstrate that the controlled delivery of niacin using the prodrug strategy eliminates the vasodilation effects that occur when niacin is applied topically or taken orally. The prodrug strategy was designed to provide slow continuous delivery where the concentration of niacin reaching the circulation would be below the threshold to induce vasodilation. With these safety and tolerability evaluations completed, the clinical effects on skin of Nia-114 were then evaluated in multiple studies that have used a double blinded, placebo-controlled study design. Results of these studies are summarized below.

Nia-114 Simultaneously Increases Skin Cell Turnover and Skin Barrier Integrity. The effects of Nia-114 on stratum corneum turnover was measured by disappearance of Dansyl staining as a surrogate measure of the rate of skin cell turnover. Treatment with Nia-114 resulted in a highly statistically significant stimulation of skin cell turnover in the range of 7 to 11% compared to placebo. Thus, it is similar to other treatments for photo-damage in its property to increase skin cell turnover; however, the magnitude of the ef-

fect is less than that observed for other treatments. The advantage of this approach to stimulate skin cell turnover is that the turnover occurs in a manner that strengthens skin barrier integrity as described below while other treatments that stimulate turnover do so to such a degree that skin barrier integrity is seriously compromised.

The effect of Nia-114 on the integrity of the skin barrier has been determined in a number of different ways that include determination of the rates of transepidermal water loss (TEWL), TEWL following a standard regimen of stratum corneum removal by tape stripping to assess effects on the upper epidermis, and by stratum corneum conductance determinations. Each of these methods has shown that Nia-114 strengthens the integrity of the skin barrier. The relationship between rates of TEWL and skin barrier integrity has been validated showing that decreased rates clearly reflect a more intact skin barrier (Reeve, Bosnic et al. 1998). Nia-114 treatment resulted in a highly statistically significant ($p=0.006$ versus placebo) decrease in the rate of TEWL of nearly 20% above the effect of the placebo alone. The effect of Nia-114 on skin barrier integrity has been assessed following removal of the stratum corneum layer of skin using a standardized protocol of tape stripping. Nia-114 treated arms showed a 20% decrease in the rate of TEWL at 18 weeks of treatment ($p=0.07$ versus placebo). Stratum corneum conductance measurements showed a highly statistically significant progressive increase in skin barrier integrity for Nia-114 treated skin compared to placebo of 10% at weeks 12 ($p=0.05$) and 18 ($p=0.01$). The unique ability of Nia-114 to simultaneously stimulate skin cell turnover and strengthen skin barrier integrity is consistent with the known roles of nicotinic acid (see Figure 6). It will be interesting to determine whether strengthening the integrity of the epidermal barrier will limit progression of in situ cancers to metastatic cancers. Further studies will be needed to verify this hypothesis.

Nia-114 Dramatically Increases Skin Barrier Integrity in Compromised Skin. Figure 10 shows the results of skin barrier assessment with placebo and Nia-114 treatment at 4 and 8 weeks in a group of atopic study subjects measuring rates of transepidermal

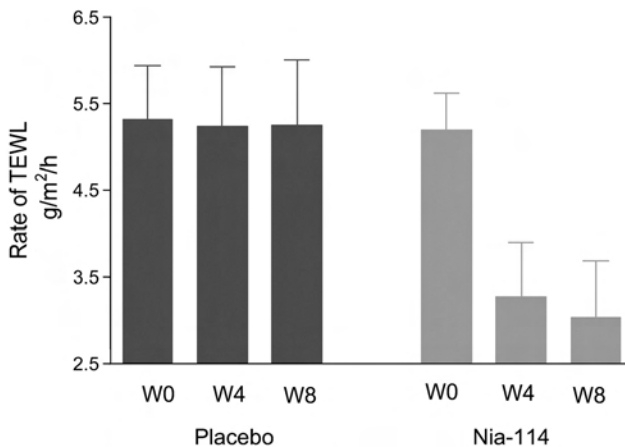


Fig. 10. Decrease in rate of transepidermal water loss during treatment with Nia-114 in individuals with atopic skin

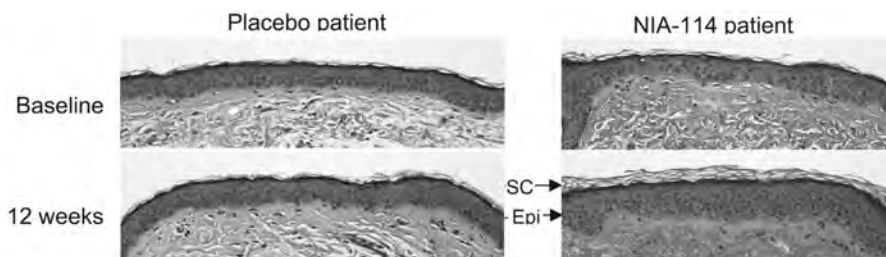


Fig. 11. Histological analyses of Nia-114 effects on skin

water loss (TEWL) to assess barrier integrity. Research has shown that rates of TEWL are strongly correlated with severity of clinical symptoms in atopic subjects (Chamlin, Kao et al. 2002). The results show that the placebo had no significant effect on barrier function while the Nia-114 treatment resulted in an approximately 35% and 45% increase in barrier function at 4 and 8 weeks. This effect of Nia-114 on skin barrier integrity in atopic subjects has exciting implications for clinical evaluation of benefits of this compound (Chamlin, Kao et al. 2002). Furthermore, it has been reported recently that low levels of leptin are strongly correlated with the risk of atopic skin, which is of interest with regard to the leptin releasing property of nicotinic acid (Jacobson, Kim et al. 2002) and the role of leptin release in preventing skin tumor promotion (Thuillier, Anchiraico et al. 2000). The data of Figure 11 show examples of histological analyses of skin punch biopsies from a clinical trial evaluating the effects of Nia-114 on skin. The increase in layers of corneocytes of the stratum corneum, responsible for barrier function, observed over 12 weeks of treatment as compared to the placebo is remarkable and correlates with effects measured by physical methods.

Nia-114 Confers Photoprotection. The effect of Nia-114 treatment on the minimum time of UV exposure to cause erythema also was determined in two separate sets of experiments. Nia-114 treatment results in a photoprotective effect of approximately 9% relative to control (data not shown). Erythema following UV exposure results from DNA damage, and the increased skin resilience following treatment is consistent with the known effects of nicotinic acid on enhancement of DNA repair and strengthening skin barrier integrity. The photoprotective effect of Nia-114 treatment contrasts sharply with other treatments for photodamage where photosensitivity is often observed.

Development of Nia-114 as a Skin Cancer Prevention Agent. Preclinical data has generated a body of evidence that has led to a RAPID Award from National Cancer Institute of the National Institutes of Health for development of Nia-114 as a skin cancer prevention agent. The RAPID program supports preclinical toxicology and pharmacology studies, filing an Investigational New Drug Application (IND) with the US Food and Drug Administration, and the planning and execution of Phase I clinical evaluation.

Conclusion

The complexity of processes that lead to skin damage are such that successful skin prevention strategies almost certainly will require a combination of agents that can provide prevention benefit by impacting different aspects of skin damage. Effective topical delivery of protective agents provides a solution to the difficulty of delivering to skin compounds taken orally. Topical delivery allows prevention to be targeted to the sites of damage, namely sun exposed skin, while minimizing systemic exposure. An increasing body of evidence indicates that key micronutrients can combat skin damage by multiple mechanisms including the reduction of genotoxic stress that is clearly a major factor in accumulated skin damage. A critical factor in any prevention strategy must be the safety of the prevention agent since long-term human exposure will be required. The tolerance and safety of micronutrients makes them excellent prevention candidates. The known protective effects of nicotinic acid summarized in Figure 6 have led us to develop a topical prodrug of this micronutrient and to begin development of this agent for skin cancer prevention. The data presented in this chapter indicate that the approach we have initiated allows effective delivery and provides benefit to skin with the potential to serve as a component of skin cancer prevention strategies. The approach is applicable to numerous other micronutrients and small molecule agents that have potential for skin cancer prevention.

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