Oral Niacin Prevents Photocarcinogenesis and Photoimmunosuppression in Mice

Helen L. Gensler, Tedine Williams, Arnold C. Huang, and Elaine L. Jacobson

Abstract: Topical nicotinamide (niacinamide) has demonstrable preventive activity against photocarcinogenesis in mice. To better understand how this vitamin prevents ultraviolet (UV) carcinogenesis, we tested systemic administration of another form of the vitamin, niacin, and its capacity to elevate cutaneous nicotinamide-adenine dinucleotide (NAD) content as well as to decrease photoimmunosuppression and photocarcinogenesis. BALB/cAnNTacBR mice were fed the AIN-76A diet supplemented with 0%, 0.1%, 0.5%, or 1.0% niacin throughout the experiment. UV irradiation consisted of five 30-minute exposures per week to banks of six FS40 Westinghouse sunlamps for 22 weeks in the carcinogenesis experiments, yielding a total cumulative dose of approximately 1.41 x 10^8 J/m^2 of UV-B radiation. Dietary supplementation with 0.1%, 0.5%, or 1.0% niacin reduced the control incidence of skin cancer from 68% to 60%, 48%, and 28%, respectively, at 26.5 weeks after the first UV treatment. Two potential mechanisms by which niacin prevents tumor formation were identified. Photoimmunosuppression, critical for photocarcinogenesis, is measured by a passive transfer assay. Syngeneic, antigenic tumor challenges grew to an average of 91.6 ± 19.7, 79.8 ± 11.5, 41.9 ± 11.7, or 13.2 ± 4.1 mm^2 in naive recipients of splenocytes from UV-irradiated mice treated with 0%, 0.1%, 0.5%, or 1.0% niacin supplementation, respectively, demonstrating niacin prevention of immunosuppression. Niacin supplementation elevated skin NAD content, which is known to modulate the function of DNA strand scission surveillance proteins p53 and poly(ADP-ribose) polymerase (PARP) activity (13–15) but increases malignant transformation (reviewed in References 16 and 17). Both p53 and PARP are critical in cellular responses to UV-induced DNA damage.

Introduction

Nonmelanoma skin cancers are the most frequent of human cancers, approximating 62.5% of the expected incidence for all nonskin cancers combined (1). Nonmelanoma skin cancers have been increasing in incidence at an annual rate of 3–6% (2). An increase in lifetime exposure to ultraviolet (UV) radiation, due to longer life expectancy and ozone depletion, likely accounts for this increasing incidence (3). Nonmelanoma skin cancers represent considerable morbidity (4). Squamous cell carcinoma and basal cell carcinoma can be invasive and tend to present as multiple primary tumors on visible areas of the body (5–8). Multiple surgical excisions are costly and potentially disfiguring (4). The use of sunscreens alone may not be sufficient protection against skin cancer development (9). These observations indicate an increasing need for novel skin cancer prevention strategies.

Skin cancers result from premutagenic DNA damage and immunosuppression induced by UV radiation (10,11). Previous studies in our laboratory have demonstrated that topical niacinamide prevents immunosuppression and skin cancer induction in a mouse model of photocarcinogenesis (12). The hypothesis that orally administered niacin protects against skin carcinogenesis derives from these studies and other evidence that low niacin nutrure adversely affects DNA repair processes, including p53 expression and poly(ADP-ribose) polymerase (PARP) activity (13–15) but increases malignant transformation (reviewed in References 16 and 17). Both p53 and PARP are critical in cellular responses to UV-induced DNA damage.

Although oral niacin is used clinically to lower blood cholesterol and coronary atherosclerosis (18), its ability to influence skin cancer has not been well studied. The present investigation was designed to evaluate the capacity of dietary niacin (vitamin B3) supplementation to reduce photocarcinogenesis and photoinmunosuppression in mice. Because niacin is a precursor to nicotinamide-adenine dinucleotide (NAD) and because p53 function and PARP activity are highly sensitive to NAD concentration (15), we also investigated the possibility that niacin supplementation contributes to skin carcinogenesis prevention by mechanisms dependent on skin NAD content.

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Materials and Methods

Animals

Six-week-old specific-pathogen-free female BALB/cAnNTacBR (H-2k) mice were purchased from Taconic Laboratory Animals (Germantown, NY) and quarantined for two weeks before treatment. Animals were housed in microisolators under barrier conditions with a 12:12-hour light-dark cycle at 20 ± 2°C (SD). They were cared for according to the US Department of Health and Human Services Guidelines for Animal Care.

Administration of Niacin

Groups of 25 mice each received the basal diet, which consisted of the AIN-76A rodent diet containing 10% corn oil and 30 mg niacin/kg diet, supplemented with 0%, 0.1%, 0.5%, or 1.0% niacin ( Dyets, Bethlehem, PA). All diets were pelleted (Dyets) without heat or steam and stored under refrigeration for <10 weeks. Animals were fed ad libitum and given fresh diet three times per week for three weeks before UV treatments began and throughout the study. Niacin intake was calculated from the diet consumed. Animals were supplied with sterilized water ad libitum.

UV Irradiation

After three weeks of niacin administration, mice were exposed to UV radiation. The animals were shaved dorsally with animal clippers each week. The filter tops, food, and water bottles were removed from the microisolators while mice were exposed to UV radiation emitted by banks of six FS40 Westinghouse fluorescent sunlamps for 30 minutes per day, 5 days/wk. This UV regimen continued for 22 weeks in the carcino genesis experiments and for 11 weeks in the passive transfer assays. The cages were systematically rotated during the study to compensate for any differences in flux at different positions under the lamps. The lamps emit a continuous spectrum from 270 to 390 nm, with peak emission at 313 nm. Approximately 75% of the energy output is in the wavelength range of 280-340 nm, with 23% in the 340- to 390-nm range. The total UV-B radiation dose was approximately 1.41 × 10^6 J/m² in the carcinogenesis experiments and approximately 0.705 × 10^6 J/m² in the passive transfer experiments, as measured with a UVX Digital Radiometer with a UVX-31 sensor (Ultraviolet Products, San Gabriel, CA).

Passive Transfer Assay

The capacity of niacin supplementation to prevent photoimmunosuppression was measured in the current investigation. The reduced ability of UV-B-irradiated mice to reject syngeneic, antigenic tumor cells is mediated by immunosuppression. This response is measured by a passive transfer assay in which splenocytes from a UV-irradiated mouse are injected into a naive unirradiated mouse (19). The accelerated growth of antigenic tumor cells implanted in recipients of splenocytes from UV-irradiated donors compared with unirradiated donors is a measure of the transferred immunosuppression. This assay for immunosuppression is currently considered to result from the transfer of splenic T helper 2 cells (12,20,21). Spleens were excised under aseptic conditions from 15 mice/group after 11 weeks of UV-B treatment. Cells were isolated from spleens and trituration with RPMI 1640 medium without fetal bovine serum, washed, and resuspended in similar medium. Splenocytes were injected into the lateral tail veins of 15 naive mice per treatment group. Within 24 hours, recipients were challenged with 5 × 10^6 UVM12 (syngeneic, antigenic, UV-induced) tumor cells injected intradermally into the flanks. Individual tumor length and width were measured twice weekly with vernier calipers.

Tissue Extraction

Dorsal and ventral skin samples were excised from mice after 29.5 weeks of niacin supplementation and 4.5 weeks after UV treatments were terminated, at the time of final tumor measurements. Samples were immediately frozen in liquid nitrogen and stored at −80°C. For NAD and protein analyses, the tissue was ground in liquid nitrogen by mortar and pestle. The powder was then transferred to a 15-ml centrifuge tube containing 1 ml of 1 M NaOH. This mixture was rapidly neutralized with 0.27 ml of 2 M H₃PO₄, so that total time in alkali was under two minutes. Next, 0.13 ml of 2 M phenazine ethosulfate was added to convert NADH to the oxidized state (22). Finally, 1.4 ml of 1 M HClO₄ was added to precipitate the total protein. Total time from dissolving to perchloric acid addition was under five minutes. Samples were placed on ice for at least 10 minutes and centrifuged at 4°C for 10 minutes at 3,000 rpm. Each supernatant was collected and neutralized with 1 M KOH-0.33 M K₂HPO₄. The supernatants were assayed for NAD; the precipitates were dissolved in 1 M NaOH for protein measurement. All reagents for these assays were purchased from Sigma Chemical (St. Louis, MO).

NAD and Protein Assays

NAD was assessed as described previously (22,23). The NAD assay is based on the principle of enzymatic cycling between oxidized and reduced states, in which NAD is rate limiting for a series of amplification reactions. Control groups, which received no supplemental niacin, consisted of 6 mice each; supplemented groups consisted of 9 or 10 mice each. The Bradford method was employed to determine protein concentration (24).

Statistical Analysis

Differences in primary tumor incidence between the experimental groups were analyzed by the Wilcoxon rank sum test. The generalized estimating equation model was em-
ployed to compare tumor multiplicity between niacin-supplemented and unsupplemented groups of UV-irradiated mice. Analysis of variance was performed on the passive transfer results to test for a treatment effect on the tumor challenge growth rate. Dunnett’s test of multiple comparisons was then used to determine which treatment groups significantly differed in the rate of tumor growth. For the NAD assay, the Q test was first applied to the data to determine whether questionable values should be rejected. This test was conducted at the 95% confidence level. The data were then analyzed using the MIXED procedure in the statistical analysis program SAS 6.11. The MIXED procedure fits a variety of mixed linear models to data, and a mixed linear model is a generalization of the standard linear model, with the generalization being that the data are permitted to exhibit correlation and nonconstant variability. In this manner, we were able to account for differences and variability in duplicate or triplicate samples from the same mouse before we compared the differences between each mouse in each group, resulting in more precise group means than if each single value carried equal weight. Least-squares means for each group were calculated, and a multiple comparison between these means was made after application of a Tukey-Kramer adjustment for the P values.

Results

Reduction of Skin Tumor Development by Niacin Supplementation

UV-irradiated mice fed unsupplemented diets developed a 68% skin tumor incidence by 26.5 weeks after the first UV exposure (Figure 1A). Diet supplementation with 0.1%, 0.5%, or 1.0% niacin reduced this incidence to 60%, 48%, or 28%, respectively. This dose response yielded a statistically significant reduction in tumor incidence in mice fed the higher two doses of niacin (p = 0.0378 and p = 0.0257, Wilcoxon rank sum test). The results of the multiplicity of tumors are presented in Figure 1B. Supplementation of the basal diet with 0.1%, 0.5%, or 1.0% niacin reduced the number of tumors per mouse from the control level of 0.72 to 0.6, 0.48, or 0.4, respectively, at 26.5 weeks after the first UV treatment. Supplementation with 0.5% or 1.0% niacin significantly reduced tumor multiplicity (p = 0.0069 and p = 0.0143, respectively, generalized estimating equation model), whereas 0.1% niacin supplementation did not (p = 0.4719). The UV-induced tumors were predominantly squamous cell carcinoma, as previously reported for this protocol (25). No visible differences were observed between tumors from control and tumors from niacin-fed mice.

Niacin Ingestion and Body Weights of UV-Irradiated Mice

Niacin ingestion was calculated from diet consumption recorded on 50% of the animals of each group. Unirradiated mice fed the basal diet ingested an average of 0.99 ± 0.03 mg of niacin day per mouse throughout the experiment, and UV-irradiated mice had a similar intake. As shown in Table 1, UV-irradiated mice fed the 0.1%, 0.5%, or 1.0% niacin supplements averaged intakes of 2.54 ± 0.56, 13.93 ± 3.3, or 28.89 ± 6.0 mg of niacin per day per mouse, respectively, throughout the study. These results demonstrate an increased amount of niacin ingestion in mice fed higher concentrations of niacin in their food. The body weights of all the UV-irradiated mice were similar (Table 1), suggesting that tumor prevention was not influenced by nutritional factors other than niacin.

Influence of Niacin Supplementation on Photoimmunosuppression

The ability of a host to reject an antigenic tumor is dose dependent on the number of tumor cells injected. In this
Table 1. Niacin Consumption

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Food Consumed, g/mouse/day</th>
<th>Dietary Niacin, g/kg diet</th>
<th>Niacin Ingested, mg/mouse/day</th>
<th>Body Wt, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV</td>
<td>2.86 ± 0.55</td>
<td>0.03</td>
<td>0.09 ± 0.02</td>
<td>18.2 ± 0.54</td>
</tr>
<tr>
<td>UV + 0.1% niacin</td>
<td>2.47 ± 0.54</td>
<td>1.03</td>
<td>2.54 ± 0.56</td>
<td>18.2 ± 0.77</td>
</tr>
<tr>
<td>UV + 0.5% niacin</td>
<td>2.77 ± 0.66</td>
<td>5.03</td>
<td>13.93 ± 3.3</td>
<td>18.07 ± 1.37</td>
</tr>
<tr>
<td>UV + 1.0% niacin</td>
<td>2.88 ± 0.60</td>
<td>10.03</td>
<td>28.89 ± 6.0</td>
<td>18.55 ± 0.91</td>
</tr>
</tbody>
</table>

a: Values are means ± SD. UV, ultraviolet radiation.

experiment all tumor challenges had some growth, so the difference in size between the unirradiated and the UV-irradiated groups indicates the extent of enhanced growth due to photoimmunosuppression. Change in tumor challenge surface area, as a measure of size, is presented in Figure 2. The mean tumor surface area was 21.9 mm² in recipients of splenocytes from unirradiated control mice at 32 days after the tumor challenge. Recipients of splenocytes from UV-B-irradiated mice treated with 0%, 0.1%, 0.5%, or 1.0% niacin supplementation displayed mean tumor surface areas of 91.6, 79.8, 41.9, or 13.2 mm², respectively. Analysis of variance indicates a significant treatment effect on tumor challenge size over time (p = 0.0001). Dunnett’s test shows that tumor challenge size was significantly smaller in recipients of splenocytes from UV-irradiated mice treated with 0.5% or 1.0% niacin than in recipients of splenocytes from UV-irradiated mice treated with 0% or 0.1% niacin. These results demonstrate that supplemental niacin, within the range of 0.5–1.0% of diet, prevents UV-induced suppression of the ability to reject antigenic tumors in a dose-dependent manner.

**Discussion**

Topical nicotinamide has demonstrable preventive activity against photocarcinogenesis in mice (12). To better understand how this vitamin prevents UV carcinogenesis, we tested another form of the vitamin, a systemic route of administration, and its capacity to elevate skin NAD content as well as to decrease photoimmunosuppression and photocarcinogenesis. Tumor incidence and multiplicity were decreased in a dose-dependent manner by supplemental niacin within the range of 0.5–1% of the diet, without visible toxicity.

Photoimmunosuppression of the ability to reject syngeneic, antigenic tumors is critical for the development of most

**Influence of Niacin Supplementation on Skin NAD**

UV exposure was directed to the dorsal shaved skin of mice after three weeks of niacin supplementation. To assess the ability of ingested niacin to enhance NAD levels in UV-irradiated as well as in unirradiated skin, unirradiated ventral skin and dorsal UV-irradiated skin were collected. For each mouse, one, two, or three skin samples were collected at different sites from the dorsal and ventral surfaces at 4.5 weeks after cessation of UV treatments. The samples were extracted to measure total NAD, and these values were expressed relative to protein content to normalize the data.

Niacin supplementation elevates NAD, as shown in Figure 3. The relationship of niacin concentration in the diet to NAD in skin fits a logarithmic function, suggesting that NAD content approaches saturation at 0.5–1.0% niacin supplementation. UV-irradiated mice receiving 0.5% and 1.0% niacin supplementation had significantly higher skin NAD content than UV-irradiated mice receiving no additional niacin (p < 0.0001 for dorsal skin and p = 0.0114 for ventral skin in 0.5% niacin-supplemented mice; p = 0.0025 for dorsal skin and p = 0.0017 for ventral skin in 1.0% supplemented mice). Neither dorsal nor ventral skin NAD values in the 1% niacin group were significantly different from values in the 0.5% niacin group, suggestive of cutaneous NAD saturation. The

![Figure 2](image-url)  
*Figure 2. Mean surface area of UVM12 tumor challenges in naive recipients of splenocytes from UV-irradiated donors treated with niacin supplementation. Mice were fed supplemented diets for 3 wk before UV radiation treatments began. UV irradiation consisted of five 30-min exposures per week for 11 wk. Splenic leukocytes of UV-irradiated mice were injected intravenously into naive recipients. Within 24 h, 5 × 10⁵ UVM12 cells were injected intradermally into flanks of recipients. Values (means ± SD) represent surface areas from tumor challenges of each group. Rates of increase of tumor surface areas in UV-irradiated mice treated with 0.5–1.0% niacin were significantly lower than those in UV-irradiated control mice (p = 0.0001, Dunnett’s analysis).*
UV-induced murine skin tumors (26) and is effectively blocked by topical nicotinamide (12). The present results indicate that oral niacin also prevents UV-induced immunosuppression. Although the underlying basis for this immunomodulation is not yet known, NAD, the major metabolite of niacin, has recently been found to inhibit responses of T cells, including CD4 T helper cells, which are thought to mediate photoimmunosuppression (12,27). Niacin supplementation was more effective in preventing photoimmunosuppression than photocarcinogenesis, since 1% dietary niacin rendered UV-irradiated mice as resistant to a tumor challenge as unirradiated mice (Figure 2), whereas 1% niacin supplementation reduced, but did not totally prevent, tumor development in UV-irradiated mice (Figure 1A).

These results suggest that niacin or NAD can act as a potent immunoregulator and that inhibition of photoimmunosuppression is a mechanism by which niacin prevents UV-induced carcinogenesis.

Additional mechanisms by which niacin supplementation prevents nonmelanoma skin cancer likely include protection against oxidative DNA damage by acting as an antioxidant (28), as well as modulation of PARP, an enzyme known to affect base excision repair, and p53 expression. Expression of p53 is necessary for efficient nucleotide excision repair of cyclobutane pyrimidine dimers and 6-4 photoproducts induced in DNA by UV-C (200–280 nm) and UV-B (280–320 nm) wavelengths (29,30). Chinese hamster lung fibroblast cells with reduced NAD content display downregulation of p53 levels and inability to mount a p53 response to etoposide, a topoisomerase II inhibitor that customarily results in increased p53 expression (13). Functional PARP is required for base excision repair, the pathway for repair of oxidative DNA damage, such as that induced by the UV-A (320–400 nm) portion of UV radiation (31–33). NAD is a substrate for PARP, a nuclear enzyme that binds to and is activated by DNA strand scissions (34,35) and interacts with numerous proteins involved in DNA repair (36,37). PARP activity can be limited by NAD concentration (14,15). We demonstrate here that niacin ingestion can determine NAD concentration in the skin, as had been previously reported in a number of human tissues (38). Exposure of normal human fibroblasts to UV radiation results in activation of PARP and concomitant utilization of NAD (35). In the experiments reported here, no UV-induced loss of NAD was observed, since the mice were maintained on the niacin supplementation diets for 4.5 weeks after the UV treatments were terminated before tissue sampling. NAD biosynthesis throughout the experiment, including the interim after UV irradiation, should have prevented or reversed any direct effects of UV-induced lowering of NAD in the irradiated skin. The fact that no significant differences were observed between ventral and dorsal skin in this study supports this suggestion.

In summary, our results demonstrate that niacin supplementation inhibits photocarcinogenesis and photoimmunosuppression in mice. Furthermore, ingestion of niacin leads to enhanced levels of NAD in skin, even in skin that had been repeatedly exposed to UV radiation, an agent known to enhance NAD consumption. These results suggest that prevention of skin carcinogenesis by supplemental niacin results
from blockage of UV-induced immunosuppression and cutaneous NAD depletion. Maintenance of NAD levels in skin with consequent PARP enhancement of base excision repair of UV-induced oxidative DNA damage likely contributes to the skin cancer prevention capacity of supplemental niacin.

Acknowledgments and Notes

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References