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Optimizing the energy status of skin cells during solar radiation

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Abstract

Ionizing- and ultraviolet-radiation cause cell damage or death by directly altering DNA and protein structures and by production of reactive oxygen species (ROS) and reactive carbonyl species (RCS). These processes disrupt cellular energy metabolism at multiple levels. The formation of DNA strand breaks activates signaling pathways that consume NAD, which can lead to the depletion of cellular ATP. Poly(ADP)-ribose polymerase (PARP-1) is the enzyme responsible for much of the NAD degradation following DNA damage, although numerous other PARPs have been discovered recently that await functional characterization. Studies on mouse epidermis in vivo and on human cells in culture have shown that UV-B radiation provokes the transient degradation of NAD and the synthesis of ADP-ribose polymers by PARP-1. This enzyme functions as a component of a DNA damage surveillance network in eukaryotic cells to determine the fate of cells following genotoxic stress. Additionally, the activation of PARP-1 results in the activation of a nuclear proteasome that degrades damaged nuclear proteins including histones. Identifying approaches to optimize these responses while maintaining the energy status of cells is likely to be very important in minimizing the deleterious effects of solar radiation on skin.
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1. Introduction

1.1. Early evidence that radiation disrupts energy metabolism

World War II precipitated considerable concern over the physiological consequences of ionizing radiation and on the mechanisms of cell death after exposure to this physical agent. In 1957, Maass and Rathgen [1] reported that when cultured tumor cells were exposed to non-lethal doses of X-rays (25 krad) the rate of glycolysis was dramatically reduced but returned to normal 1 h after exposure. This was not the consequence of the degradation of glucose by X-rays, since the addition of 0.1 mM glucose to the medium 40 min after irradiation did not restore a normal rate of glycolysis. Assessing intermediates in the Embden–Meyerhof pathway in irradiated cells revealed an accumulation of fructose-1,6-diphosphate and of dihydroxyacetone-3-phosphate as well as a decrease in pyruvate. These results indicated inhibition of glyceraldehyde-3-phosphate dehydrogenase, known at that time as triose phosphate dehydrogenase. This enzyme requires nicotinamide adenine dinucleotides (NAD) and it was found that the level of NAD in irradiated cells was 50% of that in control cells. The loss of NAD after irradiation was time-dependent and continued even after glucose had been added to the culture medium, indicating that the loss was not a result of the direct action of X-rays on NAD but of a slower, possibly enzymatic reaction. Maass and Schubert [2] and Holzer and Frank [3] reported similar findings. Later, Scaife [4] conducted similar experiments observing a dose-dependent decrease of NAD in laboratory rodents and in cultured thymocytes exposed to 0–800 rad of X- and γ-rays and suggested that the degradation of NAD was the consequence of an enzyme 'liberated' following the irradiation. Yamada et al. [5] analyzed thymocytes obtained from Wistar rats exposed to X-rays (400 rad) and placed in culture. They observed that the weight of the thymus in irradiated rats decreased 4 h

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after the irradiation because the number of thymocytes progressively decreased with time after exposure to X-rays. The modifications induced by X-rays on glycolysis in rat thymocytes were analogous to those reported by Maass and Rathgen, in particular for fructose-1,6-diphosphate, dihydroxyacetone-3-phosphate and glyceraldehydes-3-phosphate. In both cases, NAD dropped by 50% and the enzyme triosephosphate dehydrogenase was inhibited.

One hypothesis derived from these data is that cells die after exposure to X-rays because they lack sufficient ATP to perform the physiological functions required for survival. ATP synthesis may have been inhibited because of the activation of an enzyme able to degrade NAD. At that time it was still too early for enzymologists to ask about the 'induction' of enzymes, but that question was asked by biochemists in the years after, when it became possible to study the control of gene expression. The persisting questions were: what happens to NAD after exposure of cells to X-rays and what metabolic processes are responsible for this loss?

1.2. NAD is degraded by ADP-ribose polymer metabolism

In the late 1960s, three independent laboratories identified an activity in nuclei that converted radioactivity from exogenous NAD radiolabelled in the adenine ring but not in the nicotinamide ring to a bound form on proteins [6]. Ultimately, the labeled moieties on protein were identified as ADP-ribose polymers [7]. Characterization of the enzyme that catalyzed polymer formation demonstrated that NAD was cleaved such that the vitamin nicotinamide was released and ADP-ribose was transferred to the acceptor protein. The enzyme catalyzes the transfer of additional ADP-ribose residues forming a homopolymer of ADP-ribose that also displays multiple points of branching. The name for this enzyme evolved from poly(ADP-ribosyl) synthetase to poly(ADP-ribosyl) transferase to poly(ADP-ribose) polymerase, and is now commonly called PARP-1.

Early studies seeking the cellular functions of PARP-1 showed that the enzyme is activated by DNA strand breaks [8,9]. Investigators including Shall and co-workers [10,11] and others identified selective inhibitors of the enzyme resulting in evidence that PARP-1 played some role in base excision repair. Subsequently, molecular genetic approaches to deleting and restoring PARP-1 activity from cell and animal model systems led to our current understanding of the function of this enzyme. PARP-1 is now known to be a component of the DNA damage surveillance network of eukaryotic cells designed to respond to various genotoxic stresses. By sensing DNA strand breaks and modulating downstream events following a break, PARP-1 plays a very important role in cell death or survival outcomes due to effects on NAD and ATP metabolism (for reviews see Refs. [12,13] and discussion below).

1.3. Effects of solar radiation on skin cell NAD and ATP metabolism

The activation of poly(ADP-ribosyl)ation by solar radiation was shown, independently, by our teams in France (P.U.G.) and the USA (E.L.J. and M.K.J.). The Jacobsons exposed cultured human fibroblasts to 5 J/m^2 of UV-C radiation from a germicidal lamp. This wavelength of 254 nm is not found within the solar spectrum on earth, but was chosen to investigate the effects of DNA damage from UV light to skin cells. They observed the accumulation of ADP-ribose polymers not seen in control, non-irradiated cells. Polymer accumulation was greatly reduced in cells treated with a widely used inhibitor of PARP-1, 3-aminobenzamide, added immediately after exposure to UV-C [14]. Giacomoni and Balard performed kinetic analyses to measure NAD per mg protein in the epidermis of hairless mice as a function of time and dose after irradiation from a solar simulator (UV-B plus UV-A) equipped with a UV filter WG305 and an IR filter H325 [15] (see Fig. 1). For doses ranging from 1200 to 9600 J/m^2, the NAD content of the epidermis decreased after irradiation and reached a minimum 60 min after exposure, then recovered over the next hour, but decreased again and did not return to control values for at least 24 h. The level of NAD dropped to 15% of the control for the highest UV dose, and to 40% of the control for the lowest dose. This change in NAD after

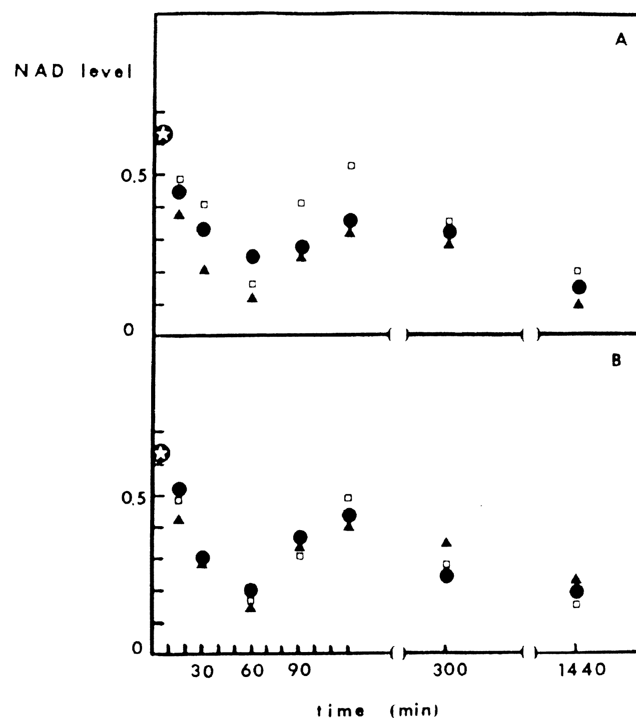

Fig. 1. NAD degradation after UV-A+UV-B from a solar simulator. NAD level in the lower (A) or in the upper (B) part of the back versus time after irradiation. □, 1200 J/m^2; ●, 4800 J/m^2; ▲, 9600 J/m^2. Redrawn from Ref. [15].

irradiation with a solar simulator is due to activation of PARP-1.

Other authors have explored the effect of UV or other kinds of DNA damage on the metabolism of NAD. As a historical note, these studies contributed significantly to understanding the role of PARP-1 in processes leading from an initial insult to eventual cell recovery or to cell death. It was recognized that nicks introduced into DNA were the common factor that led to the activation of PARP-1 [8,9]. PARP-1 is activated by processes as different as differentiation [16] or the treatment by any agent that directly or indirectly introduces nicks into DNA. Indeed, PARP-1 activation was observed during the course of the differentiation of myoblasts to myotubes (which leads to the elimination of nuclei and to the degradation of DNA), and in isolated nuclei treated with X-rays or DNase. The activation could not be achieved with supercoiled DNA or with single stranded DNA, and was independent of the sequence of the nicked DNA added [9]. It was therefore possible to surmise that an oxidative stress, such as the one provoked by UV or menadione, triggers the increase of intracellular calcium with the consequent activation of endogenous nucleases. This provokes the nicking of DNA, the activation of PARP-1, and NAD depletion. NAD depletion could be responsible for the drop of the membrane potential and the morphological modifications observed after UV irradiation in cultured cells. Other aspects of the cellular response to insult are now known to be the modulation of the expression of the bcl-2 gene, fragmentation of DNA, nuclear segmentation, and eventually cell death by apoptosis. A teleological yet successful model suggested that, when the original DNA damage is beyond the capacity of error-free repair and the probability of many mutations or transformation is high if the cell survives, NAD depletion blocks glycolysis and/or ATP synthesis leading to cellular suicide. This made a strong contribution to the concept of 'programmed cell death' [17,18].

2. Clinical effects of solar radiation

2.1. Erythema

Post-UV erythema is the consequence of a variety of concomitant phenomena triggered by UV on cells and tissues. Ley pointed out in 1985 that the stimulation of DNA repair in marsupials had an effect on the minimal erythemal dose [19]. *Monodelphis domestica* were exposed to increasing doses of UV-B, and subsequently untreated or exposed to a dose of UV-A. Since marsupials have photolyase, exposure to UV-A triggers photoreactivation, a DNA repair process that does not generate nicks. The results of the experiments were that the dose of UV-B necessary to provoke the appearance of erythema was larger when UV-B irradiation was followed by UV-A irradiation, and that the number of pyrimidine dimers was smaller in UV-B- and UV-A-irradiated animals than in animals irradiated only with UV-B. This was the first experiment to link DNA damage and erythema.

This observation has led to the following hypothesis. UV-B-generated DNA damage is repaired by endogenous DNA repair systems. In placental mammals, this system generates nicks (placental mammals such as humans and guinea pigs do not have photolyase) and nicks activate PARP-1. PARP-1 consumes NAD, and when the damage is significant, the cells secrete pro-inflammatory signals and trigger an inflammatory process, clinically characterized by an erythema. If this model holds, then the intensity of the erythema could be lessened by optimizing cellular NAD in skin after erythemal UV-B irradiation. Because NAD molecules carry two negative charges at physiological pH, NAD would not be expected to cross the cell membranes unless it is encapsulated in liposomes or analogous vehicles that facilitate crossing of membranes by charged molecules. NAD encapsulated in non-ionic liposomes or in liposomes, or dissolved in phosphate buffered saline (PBS) was applied to the back of hairless guinea pigs after exposure to 2 MED and the erythema was assessed 4 and 24 h after the irradiation. The results are reported in Table 1. The addition of NAD in non-ionic liposomes as well as in other vehicles dramatically reduces the intensity of erythema induced by a solar simulator (UV-B and UV-A) [20], similar to indomethacine. It should be noted that indomethacine is known to inhibit erythema by blocking the generation of pro-inflammatory signals. Further studies are needed to determine the precise mechanism by which optimizing NAD blocks UV-induced erythema, but a testable hypothesis is that maintenance of energy status and/or enhancement of DNA repair pathways facilitated by NAD protects cells and eliminates the need for pro-inflammatory signals.

2.2. UV-induced immune-suppression

It has been reported that topically applied nicotinamide behaves as an anti-tumor agent [21], and this might be in keeping with an effect on the immune response. A method to test the immune response after UV irradiation is to transfer spleen cells from UV-irradiated mice, nicotinamide-treated or control, into naïve mice, which are then injected with syngeneic, antigenic tumor cells, and follow the growth of the tumor in the recipient mice. The irradiation takes place for 10 weeks before the splenocytes are collected and injected into naïve mice. Gensler et al. using a similar protocol [22] observed that splenocytes from UV-irradiated donor mice treated with nicotinamide twice weekly, starting 2 weeks before the UV irradiation and throughout the experiment, prevented tumor formation in recipient mice. Indeed tumors in mice receiving splenocytes from UV-irradiated, nicotinamide-treated donors grew only to the same size as in control mice (40

Table 1
Erythema in hairless guinea pigs, treated with NAD after exposure to UV-B

N	Sample	Average erythema
12	Control	0.77
	versus	
	NAD in n.i.liposomes	0.29
12	Control	0.90
	versus	
	n.i.liposomes	0.69
12	Control	1.23
	versus	
	PBS+NAD	0.73
12	Control	1.15
	versus	
	PBS	1.46
12	Control	1.08
	versus	
	liposomes+NAD	0.79
12	Control	0.73
	versus	
	liposomes	0.85
12	Control	0.81
	versus	
	indomethacine	0.48
12	Control	0.60
	versus	
	indomethacine solvent	0.63
12	Control	1.63
	versus	
	control	1.50

Effect of topical application of NAD on UV-B-induced erythema. Groups of Hartley albino guinea pigs were epilated 72 h before irradiation and at time $t=0$ were exposed to a constant UV-B dose, corresponding to one minimal erythemal dose (MED). Thirty, 60 and 90 min after exposure, 0.1 ml of NAD was topically applied to a 7-cm^2 surface (NAD was 20 mM). At $t=4$ h, the erythema for every animal was read according to the following score (0, no erythema; 0.5, barely visible erythema; 1, erythema; 2, clear erythema; 3, marked erythema; 4, intense erythema) and averaged.

mm^2) whereas mice receiving splenocytes from UV-irradiated donors not treated with nicotinamide had tumors as large as 90 mm^2 and above. UV-B irradiation consisted of 5 weekly 30-min exposures to banks of six FS40 Westinghouse fluorescent lamps. Mice received ~602 kJ/m^2. The effect of nicotinamide was also studied on the induction of skin cancers by UV irradiation. Mice were exposed to UV (total dose 1090 kJ/m^2 in 17 weeks) and observed until week 25. Half of them were treated with 40 μmol of nicotinamide in 200 μl of acetone. Application of nicotinamide to UV-irradiated mice reduced skin tumor incidence from 75% to 42%, and the average count of tumors per mouse dropped from 0.95 to 0.50. This group working with that of Elaine Jacobson also showed that oral nicotinic acid employed in the same model also protected against photo-induced immunosuppression and tumor formation [23]. In the later study, the NAD content of the skin was elevated up to threefold and correlated with the protective effects observed. Since topical nicotinamide administered in acetone would be expected to rapidly partition into the vasculature from the skin, it is likely that the effects of both studies represent enhanced vascular distribution of the precursors of NAD to skin cells. It will be interesting to test approaches that focus on improved delivery of NAD precursors to the skin cells per se in this model.

2.3. Niacin, NAD, and energy status in humans

Severe niacin deficiency in humans was first identified as the cause of pellagra in 1937 [24]. Pellagra is distinguished as the disease of the 4-Ds presenting clinical symptoms of (i) dermatitis involving a mylar rash, severe sunlight sensitivity, and Casal's necklace; (ii) diarrhea; (iii) dementia; and finally (iv) death. While these clinical symptoms have not been directly linked to specific metabolic pathways, it is likely that the dermatitis and severe sunlight sensitivity can be explained by the role of the active form of niacin, NAD, in PARP-1-catalyzed reactions following exposure to UV radiation. Additionally, research in the last 20 years uncovered three different families of ADP-ribosyltransferase reactions, including those catalyzed by PARP-1, that consume NAD as a substrate. It is likely that other clinical symptoms of pellagra may relate to functions of niacin and NAD involving the family members of mono-ADP-ribosyltransferases or ADP-ribosyl cyclases [25]. Alternatively, the very recently discovered additional members of the PARP family [26] may have functions that are responsible for these symptoms.

Discovering the cause and cure of pellagra has limited but not eliminated niacin deficiency in humans. Furthermore, in contrast to the consequences of frank niacin deficiency presenting as pellagra, suboptimal niacin nutriture in humans may be a factor in chronic diseases of long-lived populations, such as cancer and aging. One of us (E.L.J.) has observed that the NAD content of normal skin in subjects diagnosed with squamous cell carcinoma is significantly lower than that of individuals showing precancerous lesions [27]. Optimal niacin status has not been determined in humans with intracellular NAD as an endpoint and the metabolic regulation of intracellular NAD levels is poorly understood. Only recently has a biochemical assessment method been developed that focuses on intracellular NAD as the relevant endpoint of niacin status for humans [28]. Applying this method, it was found that as much as 15% of people living in Western populations are niacin deficient [29]. These data are very interesting in light of the fact that blood cell NAD content in humans is ~20 to 25% of that in other species such as rodents [28]. Fu et al. [30] showed data from a metabolic-ward study that challenges the existing dogma that dietary tryptophan is readily converted to niacin under conditions of mild

dietary deficiency. Thus, the available niacin in the human diet may be less than we have previously thought. It is also becoming quite clear that supplying the skin with micronutrients by dietary approaches alone is leaving our aging population with many deficiencies. Taken together, these findings raise the interesting question as to whether niacin status (NAD content) in human skin is optimal for genotoxic stresses that result directly and indirectly from exposure to sunlight.

We have studied the consequences of treating cultured fibroblasts and keratinocytes with solar simulated light as a function of NAD content. The data shown in Fig. 2 indicate that human skin fibroblasts with a suboptimal NAD content have reduced survival following exposure to solar simulated light. Other biological consequences of suboptimal NAD include genomic instability as has been seen in experiments using a 'comet assay', a sensitive method for determining DNA integrity in single cells. Cells depleted of NAD by restricting its precursors in the culture medium form comets even in the absence of exposure to solar simulated light, while control cells maintain tight compact nuclei. These data support the hypothesis that the NAD content is important in maintaining genomic integrity of skin cells. It is likely that niacin and/or NAD is functioning to support ADP-ribose polymer metabolism activated by DNA strand breaks and to provide energy for apoptosis where levels of DNA damage preclude efficient repair. Independent of the mechanism, it is important to determine the optimal nutrient amounts and routes of delivery to skin cells for niacin to prevent and reverse skin damage from radiation.

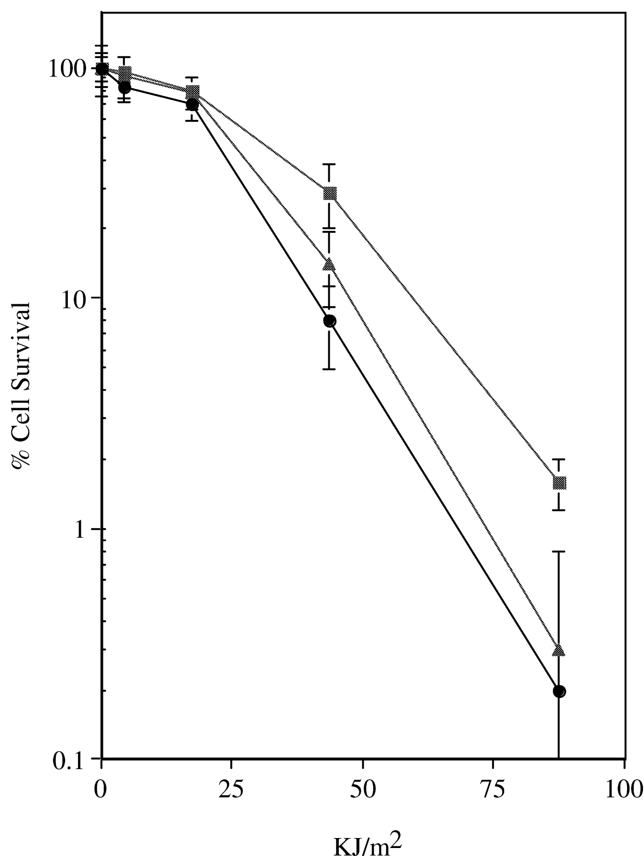

Fig. 2. The effect of cellular NAD in human skin fibroblasts on cell survival following solar simulated light treatment. Cells propagated in the absence of added nicotinamide (circles), 0.1 μM nicotinamide (triangles), or 33 μM nicotinamide (squares) were treated with various doses of solar simulated light and allowed to grow for 7 days. Cell survival was determined by the MTS assay.

Solar light results not only in DNA damage but in protein damage as well. The interactions between DNA damage and protein damage caused by solar radiation are complex and they also involve niacin, NAD and PARP-1 in at least three possible ways. Firstly, a metabolic consequence of ADP-ribose polymer synthesis following genotoxic stress is the formation of free ADP-ribose by the turnover of ADP-ribose polymers. Free ADP-ribose has been shown to be a potent protein glycating agent that leads to the formation of protein advanced glycation end-products (AGEs) in nuclear proteins including histones [31]. Protein-AGEs are known to cause damage to DNA and protein through formation of reactive carbonyl (RCS) and reactive oxygen species (ROS) [32–34]. Recent evidence shows that protein-AGE also are photosensitizers of the UV-A portion of solar light. Cells treated with solar simulated light in the presence of glycated proteins are killed by doses of radiation that are otherwise nontoxic as can be seen in Fig. 3. In this experiment, the proliferation potential of cultured human keratinocytes was examined following exposure to solar simulated light (UV) in the presence of bovine serum albumin (BSA) or glycated BSA (AGE–BSA). While the presence of BSA during UV exposure had no significant effect on proliferation potential, the presence of AGE–BSA enhanced the toxicity of UV. Follow-up experiments revealed that the AGE–BSA was enhancing the toxicity of the UV-A portion of the solar simulated light. Similar results have been presented by Masaki et al. [32]. These results suggest that

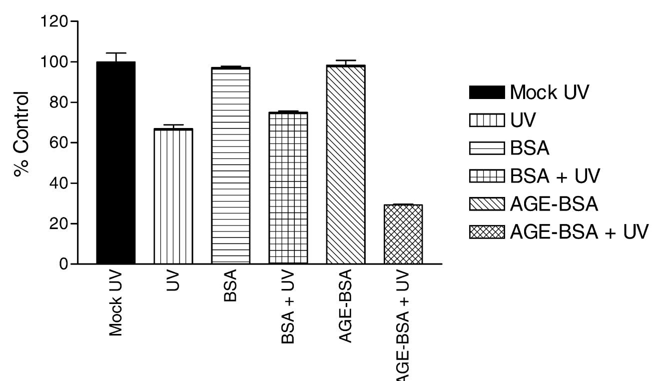

Fig. 3. The effect of protein-AGE and solar simulated light on human keratinocytes. Cells were treated with 300 s of solar simulated light yielding 1.2 kJ/m^2 of UV-B and 23 kJ/m^2 of UV-A and/or the indicated agent(s) and allowed to grow for 3 days. Cell numbers were determined and % of control growth was calculated.

agents that block protein-AGE photosensitization and/or protein-AGE formation may be very important in protecting skin from sun damage as AGE is known to accumulate over time in human skin. Secondly, recent evidence suggests that NAD(P)H may act as a direct antioxidant by scavenging peroxynitrite-derived free radicals as well as aliphatic peroxyl and alkoxyl radicals [35]. Therefore, raising intracellular NAD may be of great importance for direct protection against photooxidative stress caused by solar radiation. A third way in which NAD metabolism likely plays an important role in skin cell responses to protein damage is suggested by a recent study demonstrating that activation of PARP-1 stimulated the nuclear proteasome that detects and specifically degrades damaged histones [36].

Our understanding of the involvement of NAD in cellular responses to radiation has progressed greatly since the early questions about the fate of NAD following radiation exposure were raised many years ago. While we still have much to learn in this regard, it is clear that the enzyme PARP-1 plays an important role in cell responses involving NAD. The consumption of the bioactive form of niacin is thought to be involved in cell responses to the genotoxic stress that results from exposure to radiation, both solar and ionizing. It is clear that the activation of PARP-1 by DNA strand breaks leads to complex signaling pathways that can enhance cell survival, result in cell death by apoptosis, or cause energy loss that leads to necrosis. 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-kB pathway and by preventing ATP depletion and DNA repair through PARP-1 cleavage [12,13]. Finally, when the damage is very high, PARP-1 activation can lead to cellular necrosis through depletion of first NAD and then ATP with a resulting loss of all energy-dependent functions. Since the cells of the skin are exposed to many different doses of solar radiation ranging from no apparent change to erythema to severe sunburn, it seems likely that each of the pathways discussed above are relevant to humans. The dependence of these mechanisms on skin cell NAD content and energy status indicates that maintenance of an optimal niacin status in skin, especially skin exposed to solar radiation, is a desirable goal.

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