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Nicotinamide downregulates gene expression of interleukin-6, interleukin-10, monocyte chemoattractant protein-1, and tumour necrosis factor-α gene expression in HaCaT keratinocytes after ultraviolet B irradiation

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Summary

Ultraviolet (UV) radiation has profound effects on human skin, causing sunburn, inflammation, cellular-tissue injury, cell death, and skin cancer. Most of these effects are mediated by a number of cytokines produced by keratinocytes. In this study we investigated whether nicotinamide (NCT), the amide form of vitamin B3, might have a protective function in reducing the expression of interleukin (IL)-1β, IL-6, IL-8, IL-10, monocyte chemoattractant protein (MCP)-1 and tumour necrosis factor (TNF)-α in UV-irradiated keratinocytes. HaCaT cells were treated with UVB in the presence or absence of NCT, and cytokine mRNA levels were examined by quantitative real-time PCR. NCT significantly downregulated IL-6, IL-10, MCP-1 and TNF-α mRNA expression, whereas it did not exert any significant effect on IL-1β or IL-8 expression. Because of its ability to decrease these cytokine mediators after UV exposure, NCT is a possible therapy to improve or prevent conditions induced or aggravated by UV light.

Short ultraviolet (UV)B (290–320 nm) is able to increase levels of interleukin (IL)-1β, IL-6, IL-8, IL-10, monocyte chemoattractant protein (MCP)-1 and tumour necrosis factor (TNF)-α gene expression in keratinocytes. Nicotinamide (NCT), also known as niacinamide, is the pyridine-3-carboxylic acid amine form of niacin, a component of the vitamin B complex. NCT is the precursor for nicotinamide adenine dinucleotide (NAD^+), and acts as an inhibitor of poly(ADP-ribose) polymerase (PARP)-1.[1] This enzyme, potentially activated by UV radiation, decreases cellular energy production through glycolytic blockade, resulting in ATP depletion. Moreover, by enhancing transcription of nuclear factor κB-mediated transcription, PARP-1 plays a pivotal role in the expression of inflammatory mediators such as cytokines, chemokines and adhesion molecules.[1,2] As well as its antioxidant and immunomodulating effects, NCT has also been shown to have anti-inflammatory properties after stimulation with bacterial lipopolysaccharide[3] or with *Propionibacterium acnes*.[4] In addition, *in vivo* studies have shown that NCT is able to provide protection against UV-induced immunosuppression in humans.[5,6]

The aim of this study was to investigate whether NCT could modulate UVB-induced expression of IL-1β, IL-6, IL-8, IL-10, MCP-1 and TNF-α in spontaneously immortalized HaCaT keratinocytes.

Report

HaCaT cells were treated with UVB in the presence or absence of NCT, and mRNA levels of IL-1β, IL-6, IL-8,

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IL-10, MCP-1 and TNF-α were measured by quantitative real-time (qRT) PCR.

To calculate the optimal UVB irradiation dose, HaCaT cell viability was determined at 6 and 24 h after treatment with 5, 25, 50, 100 and 150 mJ/cm^2 UVB (TL12 lamp; Philips, Eindhoven, the Netherlands), using Trypan blue. UVB irradiation at 5 and 25 mJ/cm^2 did not significantly affect cell viability, whereas 50, 100 and 150 mJ/cm^2 induced viability loss of 70%, 40% and 28%, respectively (Fig. 1a). Hence, UVB 100 mJ/cm^2 was chosen as the stimulation dose for further experiments because it corresponds to the mean UVB minimal erythema dose for the Italian population, and should therefore reproduce a reliable *in vivo* condition.

To identify the optimal dose of NCT, cell viability was assessed 24 h after treatment with different doses (50 and 100 μM, and 1, 5 and 10 mM) of NCT (Sigma-Aldrich St. Louis, MO, USA). We found that none of the tested concentrations of NCT affected cell viability (Fig. 1b), therefore two intermediate concentrations were chosen: 100 μM and 5 mM.

All statistical analyses were performed using GraphPad Prism (version 4.0; GraphPad Software Inc, La Jolla, CA, USA). Student *t*-test was used to calculate differences between groups, and $P < 0.05$ was considered significant.

We assessed the viability of cells pretreated for 30 min with NCT and subsequently irradiated with UVB. NCT 5 mM resulted in a decrease in the cellular mortality induced by UVB irradiation, increasing the cell viability from 40% to 56%, whereas NCT 100 μM did not significantly improve cell viability (40% vs. 44%) (Fig. 1c). Previous studies performed on similar cell viability (~40%), and our finding that NCT (5 mM) was even able to increase it (~60%), enabled us to rely on such viability values for cytokine gene expression evaluation.[7] Thereafter, peaks of cytokine expression were detected in UVB-treated cells by monitoring production of specific mRNAs at 6, 12 and 24 h post irradiation.

Similarly, cytokine gene expression was assessed in cells treated with NCT 100 μM and 5 mM for 6 and 24 h. HaCaT cells were then pretreated with NCT 100 μM and 5 mM for 30 min, irradiated with UVB at 100 mJ/cm^2, and cultured for 6 or 24 h to evaluate the effect of NCT on UVB-induced cytokine gene expression. Total mRNA was isolated (RNeasy Mini Kit; Qiagen, Doncaster, Australia), cDNA was prepared (Transcriptor High Fidelity cDNA Synthesis Kit; Roche, Indianapolis, IN, USA) and gene expression was analysed using qRT-PCR in a thermal cycler (LightCycler; Roche) according to the manufacturer's instructions.

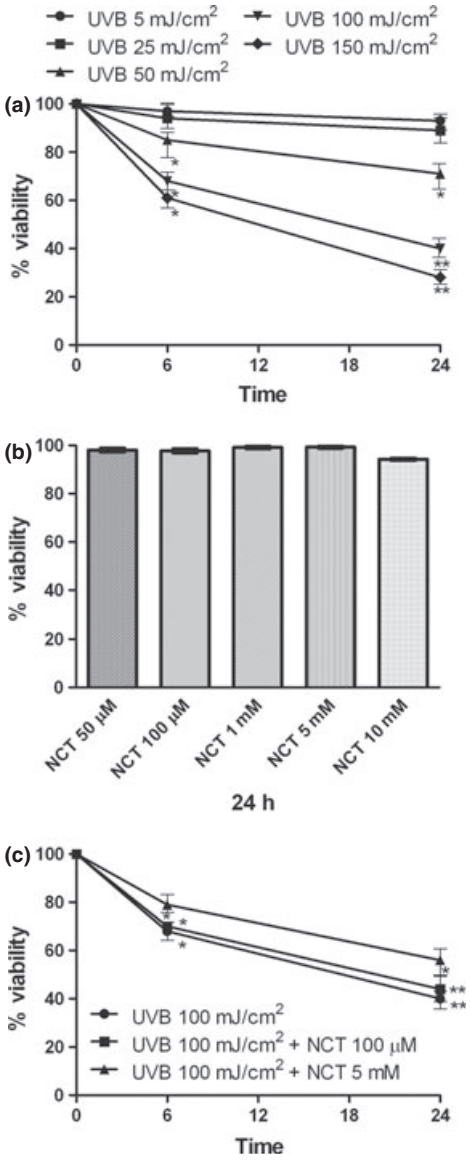

Figure 1 The viability of HaCaT cells after ultraviolet (UV)B exposure and nicotinamide (NCT) treatment. Cell viability was investigated after (a) exposure to different UVB doses (5, 25, 50, 100 and 150 mJ/cm^2) at 6 and 24 h; (b) after incubation with NCT (50 μM, 100 μM, 1 mM, 5 mM and 10 mM) for 24 h (not statistically significant); (c) after 30 min pretreatment with NCT (100 μM or 5 mM) and subsequent irradiation (UVB 100 mJ/cm^2) at 6 and 24 h. Cellular viability rate and statistical significance were determined with respect to the 100% viability of untreated control cells. Data are expressed as means ± SD of three independent experiments, each performed in duplicate. Student *t*-test:.*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

The time-course analysis of the investigated cytokines showed the highest mRNA expression occurred at 6 h after UVB irradiation for IL-1β, IL-6 and IL-8, whereas IL-10, MCP-1 and TNF-α mRNA peaks were recorded at 24 h after UVB exposure (Fig. 2a). No significant alteration was found after treatment of nonirradiated cells with NCT for 6 and 24 (data not shown). Conversely, NCT pretreatment of UVB-irradiated HaCaT cells was able to modulate cytokine gene expression. This was more effective with the higher tested concentration of 5 mM, which significantly reduced IL-6, IL-10, MCP-1 and TNF-α gene expression but was ineffective for IL-1β and IL-8. NCT 100 μM produced a significant decrease in IL-6, IL-10 and TNF-α, but was ineffective for IL-1β, IL-8 and MCP-1 (Fig. 2b).

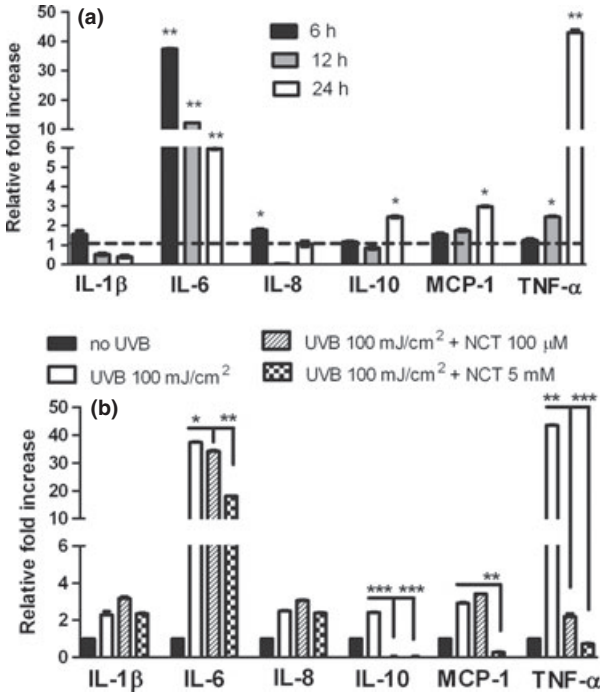

Figure 2 (a) Assessment of cytokine gene expression in HaCaT cells after ultraviolet (UV)B irradiation (100 mJ/cm^2). Dotted line indicates the reference value, represented by unirradiated HaCaT cells. mRNA expression of interleukin (IL)-1β, IL-6 and IL-8 reached its maximum at 6 h after UVB irradiation, whereas that of IL-10, monocyte chemoattractant protein (MCP)-1 and tumour necrosis factor (TNF)-α peaked at 24 h after UVB exposure. (b) Cytokine gene expression was measured in HaCaT cells after nicotinamide (NCT) pretreatment and subsequent UVB irradiation (100mJ/cm^2) vs. unirradiated HaCaT cells (no UVB). mRNA extraction was performed at 6 h post irradiation to evaluate IL-1β, IL-6 and IL-8, and 24 h post irradiation to assess IL-10, MCP-1 and TNF-α gene expression. NCT 5 mM significantly downregulated IL-6, IL-10, MCP-1 and TNF-α mRNA expression, but it did not exert any significant effect on IL-1β and IL-8 expression (b). Relative mRNA levels were determined by the comparative threshold cycle method and their expression was normalized to 18S mRNA expression. Student's *t*-test was used to calculate significant differences. Data are expressed as means ± SD of three independent experiments, each performed in duplicate. Student *t*-test:.*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

In this study, NCT pretreatment of HaCaT cells downregulated gene expression of the UVB-induced cytokines IL-6, IL-10, MCP-1 and TNF-α. NCT was more effective at the higher tested concentration, showing a dose-related trend. Moreover, NCT, at both tested concentrations was safe, with no decrease in cell viability observed.

Our results for IL-6 are in line with those of Ungerstedt[3] *et al.* and Ahn[8] *et al.*, who reported a decrease in IL-6 induced by NCT after exposure to UV and bacterial endotoxin, respectively. Our findings about the effect of NCT on TNF-α also agree with those of Ungerstedt *et al.*[3] who found that endotoxin-induced TNF-α increments were reduced by NCT. Our results for MCP-1 gene expression are in accordance with previous studies showing the ability of NCT to reduce MCP-1 secretion from multiple cell types exposed to oxidative stress or viral infection.[9] However, our data for IL-1β and IL-8 contrast with those of Grange *et al.*, who found that a high level of efficacy for NCT in reducing these cytokines after *P. acnes* stimulation.[4] This might be due to the fact that in our study IL-1β and IL-8 were only slightly enhanced by UVB, whereas there was a much more robust increase after the bacterial stimulation.[4]

NCT downregulated the UVB-induced overexpression of IL-10. This effect could modulate the UV-induced immunosuppression attributed to IL-10 that is thought to be able to shift the immune response towards a T-helper 2 pathway and to inhibit Langerhans cell function. Prevention of UV-induced immunosuppression has been shown after NCT topical application in men before or after UV exposure.[5,6] This study extends these findings, as the effect of NCT on IL-10 supports its anti-immunosuppressive potential and confirms the the recently proposed NCT preventive treatment for skin cancer.[10]

In conclusion, given that regulation of immunomodulatory cytokines is considered an essential part of treatment in multiple inflammatory diseases and that we have shown the ability of NCT to decrease these mediators after UV exposure, NCT may be an interesting option to improve or prevent UV-induced/aggravated clinical conditions.

Learning points

- NCT has antioxidant, immunomodulating and anti-inflammatory properties.
- NCT is able to modulate UVB-induced expression of IL-1β, IL-6, IL-8, IL-10, MCP-1 and TNF-α in HaCaT keratinocytes.
- NCT downregulation of UVB-induced IL-10 overexpression may represent an additional explanation of its anti-immunosuppressive mechanism, supporting the recently proposed use of NCT as a preventive treatment for skin cancer.
- Because regulation of immunomodulatory cytokines is considered an essential part of treatment in many inflammatory diseases, NCT may be an interesting option to improve or prevent clinical conditions induced or aggravated by UV irradiation.

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