The role of Nicotinamide in photoprotection of Human Primary Keratinocytes from oxidative stress damages UV-induced

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Introduction

Non-melanoma skin cancers (NMSCs) are the most common malignancies in the Caucasian population and the incidence rate is increasing worldwide. The main risk factor is ultraviolet (UV) radiation exposure, which can directly damage the DNA, increase reactive oxygen species (ROS) production, activate local inflammation, and deplete cellular energy, leading to genomic instability and cell death. Nicotinamide, the amide form of vitamin B3, is a water-soluble molecule assumed by daily diet and is the precursor of NAD (nicotinamide adenine dinucleotide) a key coenzyme for different processes, such as cell energy production and DNA damage repair. NAM protects HaCat from UV-induced impairment restoring cell energy and enhancing DNA repair. However, little is known about its effects on human primary keratinocytes. This study aims to elucidate the role of NAM in photoprotection against UV-induced oxidative stress in human primary keratinocytes isolated from the cancerization field (CF-HPKs).

Material and Methods

CF-HPKs were isolated from the perilesional skin of NMSCs patients, aged 50-80 years, surgically treated at Dermatological Unit of AOU Maggiore della Carità, Novara, Italy. Then, cells were treated with three different concentrations of NAM (5, 25, and 50 μM) for 18, 24, and 48 hours before 400 mJ/cm^2 UVB exposure. We evaluated cell viability (MTT assay), DNA repair enzyme expression (OGG1), intracellular ROS (DCFDA assay), and Nitric Oxide (Measure-IT High sensitivity kit) production and protein (indirect immunofluorescence) and genomic (qRT-PCR) expression of oxidative stress markers (iNOS, SOD-1 and GPX-1) and inflammatory cytokines (IL-1β, IL-8 and TNF-α).

Results

Cell viability was not affected by either NAM or UVB treatment in all conditions. OGG1 gene expression was significantly enhanced by UVB exposure and NAM pretreatment protects cells from DNA damages, decreasing OGG1 expression, with major effects with 25 μM concentration given for 48h. After UVB exposure, NAM pretreatment at all concentrations decreased the NO release in comparison with respective UVB-exposed control cells, without any significant differences between concentrations. UVB significantly increased iNOS protein expression. NAM pretreatment with 25 μM concentration given for 24h significantly reduce its expression. UVB exposure significantly increased ROS production in HPKs in comparison with untreated cells. 5 and 25 μM concentrations given for 24 and 48h before UVB exposure were the most effective one. Then, we investigated the gene expression of two antioxidants: SOD-1 and GPX1. SOD-1 mRNA was significantly higher in UVB exposed cells in comparison with untreated groups and NAM pretreatment seemed to revert this trend, however without any significant difference. GPX-1 expression was weakly enhanced by UVB irradiation, even if the comparison with control cells (untreated) failed to demonstrate significant differences. The only concentration able to decrease GPX1 gene expression was the 50 μM given 48h before irradiation. UVB exposure weakly increased mRNA expression of pro-inflammatory cytokines (IL-1β, IL-8, TNF-α), however without any significant results. NAM pretreatment seemed to decreased cytokines expression, however only HPKs pretreated with 25 μM 24 and 48h before irradiation showed a significant reduction of IL-1β gene expression.

Discussion

Our results demonstrated that NAM pretreatment protects CF-HPKs from UVB impairment effects, enhancing DNA repair, decreasing ROS production and antioxidants expression, decreasing NO release, and blocking local inflammation with major effects pre-treating cells with 25 µM NAM 24h before irradiation.