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The NAD+ precursor nicotinic acid improves genomic integrity in human peripheral blood mononuclear cells after X-irradiation

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ABSTRACT

NAD+ is an essential cofactor for enzymes catalyzing redox-reactions as well as an electron carrier in energy metabolism. Aside from this, NAD+ consuming enzymes like poly(ADP-ribose) polymerases and sirtuins are important regulators involved in chromatin-restructuring processes during repair and epigenetics/transcriptional adaption. In order to replenish cellular NAD+ levels after cleavage, synthesis starts from precursors such as nicotinamide, nicotinamide riboside or nicotinic acid to match the need for this essential molecule. In the present study, we investigated the impact of supplementation with nicotinic acid on resting and proliferating human mononuclear blood cells with a focus on DNA damage and repair processes.

We observed that nicotinic acid supplementation increased NAD+ levels as well as DNA repair efficiency and enhanced genomic stability evaluated by micronucleus test after x-ray treatment. Interestingly, resting cells displayed lower basal levels of DNA breaks compared to proliferating cells, but break-induction rates were identical. Despite similar levels of p53 protein upregulation after irradiation, higher NAD+ concentrations led to reduced acetylation of this protein, suggesting enhanced SIRT1 activity. Our data reveal that even in normal primary human cells cellular NAD+ levels may be limiting under conditions of genotoxic stress and that boosting the NAD+ system with nicotinic acid can improve genomic stability.

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1. Introduction

1.1. NAD+ supplementation

Several studies have reported that administration of NAD+ precursors such as nicotinamide, nicotinic acid (niacin, NA) or nicotinamide riboside can increase the intracellular NAD+ concentration in various tissues or cellular compartments *in vitro* and *in vivo* [1–3]. In the past, nicotinic acid has been in clinical use, at millimolar concentrations in the tissue, as a cholesterol lowering drug displaying atheroprotective effects [4,5]. However, this treatment was accompanied by unwanted side effects (reviewed in [5]), most prominently prostaglandin-dependent vasodilation in the skin (flushing) [6] and hepatotoxicity [7] (for review, see [8]), with the latter becoming negligible by applying niacin in a different formulation [9] (reviewed in [10]). Nevertheless, high niacin dosing can result in discomforting flushes [11], but niacin is still in use [12,13] and also marketed as a food supplement. Several studies showed protective effects of high NAD+ levels in model systems as well as in humans under certain pathological conditions or in the context of the aging process, possibly due to preserving energy production in mitochondria [14–20]. Apart from the important function of intracellular NAD+ in mitochondrial respiration or as coenzyme in a vast range of redox-reactions, NAD+ also participates in DNA repair and maintenance of genomic stability. J.B. Kirkland's group demonstrated the relevance of niacin status on genomic integrity, DNA repair and protection from carcinogenesis using animal models focusing on niacin deficiency [21–23]. The importance of NAD+ in this context was highlighted in reports showing that NA deficiency results in increased chromosomal instability [24] and higher cancer incidence [25]. Likewise, high concentrations of nicotinamide or NA delayed carcinogenesis [26], improved repair capacity after γ-irradiation above 40 Gy in mouse melanoma cells

Abbreviations: IR, X-ray irradiation; MN, micronuclei; NA, nicotinic acid; PARP, poly(ADP-ribose) polymerase; PAR, poly(ADP-ribose); PBMC, human peripheral blood mononuclear cells; PHA-L, leucoagglutinin.

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[27] and facilitated recovery of neuronal functions after hypoxia [28]. There is also increasing evidence that micronutrients have an important impact on the maintenance of gross genomic stability. In a study by Fenech et al. it was shown that low intake of nicotinic acid was associated with an increased micronucleus frequency [29], a widely accepted measure of genomic stability [30].

1.2. Consumers of NAD^+: poly(ADP-ribose) polymerases and sirtuins

Immediately after a genotoxic insult cells activate various responses that contribute to cell survival or death. One of the first reactions is poly(ADP-ribosyl)ation (PARylation) of proteins mediated by nuclear poly(ADP-ribose) polymerases (PARPs) [31,32]. The most active enzyme in this regard is PARP1 (and to a lesser extent PARP2 [33]), which covalently attaches units of ADP-ribose in a stepwise fashion to target proteins including itself, synthesizing a negatively charged polymer by using NAD^+ as substrate. Depending on the level of DNA damage and intracellular NAD^+ status, PARP1 and its product poly(ADP-ribose) (PAR) mediate the recruitment of DNA repair factors to sites of lesions, facilitate DNA repair and help maintain genomic integrity under conditions of moderate stress [34,35]. In this scenario a tolerable proportion of total cellular NAD^+ is used for polymer synthesis. In contrast, drastic and irreversible NAD^+ depletion [36] as a result of hyperactivation of PARP1 under severe genotoxic stress conditions can lead to cell death [37,38]. This paradigm is also apparent in inflammatory diseases and neurodegenerative disorders [39,40] (reviewed in [41]). One parameter determining the cellular response to stress is the level of available NAD^+, which is crucial not only for PAR synthesis [42], but also for the enzymatic action of sirtuins (SIRTs) [43,44]. We could show that supplementation of human peripheral blood mononuclear cells (PBMC) with NA not only raises NAD^+ levels, but enhances PAR formation after genotoxic stress and protects from damaged-induced necrotic cell death [45]. Conversely, NA deficiency results in impaired PARP1 functions in rats [46,47].

Sirtuins are homologues of the yeast enzyme Sir2 [43,48] and have important functions in regulating cellular responses to particular types of signals and stressors [49,50]. They use NAD^+ in order to de-acetylate proteins, forming 2′O-acetyl-ADP-ribose as product [51]. Both PARPs and SIRTs have been implicated in genome stabilization [49,52–55] and crosstalk between members of the two enzyme families has been published [56–58], as was proposed already ten years ago [55]. For example, abrogation of cellular PAR formation by *Parp1* gene deletion, silencing or PARP inhibitors is known to sensitize cells to many genotoxic agents and to increase genomic instability [34]. In contrast, PARP1 overexpression leads to the suppression of DNA damage-induced genomic instability [59]. It was demonstrated that SIRT1 and PARP1 share some important tasks, *i.e.* both are regulating chromatin structure [52,60–63] and repair [64,65], and both dampen the activity of the transcription factor and stress response protein p53, SIRT1 by de-acetylation [66] and PARP1 by covalent and non-covalent modification by PARylation [67–69], although the impact of this modification is less well understood. Interestingly, it has been reported that also SIRT6 and PARP1 cooperate in DNA repair [70].

1.3. Mononuclear blood cells as a model system

Human PBMC are primary cells proficient in DNA damage response cascades and repair pathways and therefore an ideal model for DNA repair studies and most relevant to the understanding of biochemical and molecular mechanisms in human physiology [71–73]. Furthermore, they are widely used in epidemiological studies to investigate the correlation of various parameters including DNA repair capacity and cancer risk [74]. As DNA repair might vary throughout the cell cycle, analyzing resting and proliferating PBMC might yield different results. But quiescent PBMC in G_0 phase can be easily stimulated for proliferation by treatment with phytohemagglutinin (PHA-L) [75]. In this way, cells from the same donor can be monitored for their response to diverse treatments in the resting and the proliferating state, thus excluding inter-individual variations. PBMC have been reported to utilize supplemented NAD^+ precursors [76,77] and express nicotinate phosphoribosyltransferase as well as nicotinamide phosphoribosyltransferase, making this cell type a suitable model for our approach.

1.4. Aim

In the present work, we wanted to study whether increased cellular levels of NAD^+ can influence cellular responses after genotoxic treatment in PBMC from healthy, non-niacin-deficient subjects, as there is a lack of human data addressing this issue. We focused on the biological consequences of elevated NAD^+ levels in human PBMC regarding PARP1/SIRT activities or downstream effects related with genomic integrity. To assess how modulated NAD^+ levels may contribute to physiological or pathophysiological outcomes we set out to investigate various end points including (i) DNA damage, (ii) DNA repair, (iii) the influence on genomic stability and (iv) sirtuin-1 (SIRT1) activity after treatment with ionizing radiation (IR) using varying doses. It was of particular interest to find out whether supplementation of nicotinic acid at pharmacologically relevant concentrations is able to improve the cellular status in the context of DNA damage in normal human cells from healthy donors.

2. Materials and methods

2.1. Chemicals and reagents

Biocoll separating solution and fetal calf serum (FCS) were purchased from Biochrom (Berlin, Germany). Antibiotics and RPMI 1640 culture medium were from Invitrogen (Darmstadt, Germany), standard chemicals from Roth (Karlsruhe, Germany), Sigma-Aldrich (Munich, Germany) or Merck (Darmstadt, Germany) if not stated otherwise.

2.2. Isolation of peripheral blood mononuclear cells [45]

Blood sampling was carried out in accordance with the Declaration of Helsinki and with approval of the University of Konstanz Ethics Committee, from healthy donors aged 24–45 years giving informed consent. Venous blood was obtained using the S-Citrate-Monovette blood collection system from Sarstedt (Nümbrecht, Germany). Cells were separated *via* Biocoll gradient centrifugation. Briefly, the freshly drawn blood was mixed with an equal volume of PBS (137 mM sodium chloride; 10 mM disodium hydrogen phosphate; 3 mM potassium dihydrogen phosphate; pH 7.4) and layered on 15 ml of Biocoll separating solution, followed by centrifugation at 800 × *g* for 15 min at room temperature. The PBMC layer was collected and washed twice with PBS. Isolated cells were incubated in standard culture medium (RPMI 1640 medium supplemented with 10% FCS, 100 U/ml penicillin and 100 μg/ml streptomycin) at 37 °C with 5% CO_2 in a humidified atmosphere. NA was added to the culture medium at a final concentration of 15 μM and cells were incubated 5 h before DNA damage induction.

2.3. Mitogen stimulation of cells

To investigate NAD^+ levels and DNA strand break repair at different cell cycle stages we challenged cells with X-irradiation, either

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