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Mitigation of gamma-radiation induced abasic sites in genomic DNA by dietary nicotinamide supplementation: Metabolic up-regulation of NAD$^+$ biosynthesis

Vipen Batra*, Binita Kislay

Radiation Biology and Health Sciences Division, Bhabha Atomic Research Centre, Mumbai 400085, India

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ABSTRACT

The search for non-toxic radio-protective drugs has yielded many potential agents but most of these compounds have certain amount of toxicity. The objective of the present study was to investigate dietary nicotinamide enrichment dependent adaptive response to potential cytotoxic effect of ^{60}Co γ-radiation. To elucidate the possible underlying mechanism(s), male Swiss mice were maintained on control diet (CD) and nicotinamide supplemented diet (NSD). After 6 weeks of CD and NSD dietary regimen, we exposed the animals to γ-radiation (2, 4 and 6 Gy) and investigated the profile of downstream metabolites and activities of enzymes involved in NAD$^+$ biosynthesis. Increased activities of nicotinamide phosphoribosyltransferase (*NAMPT*) and nicotinamide mononucleotide adenylyltransferase (*NMNAT*) were observed up to 48 h post-irradiation in NSD fed irradiated mice. Concomitant with increase in liver *NAMPT* and *NMNAT* activities, NAD$^+$ levels were replenished in NSD fed and irradiated animals. However, NAMPT and *NMNAT*-mediated NAD$^+$ biosynthesis and ATP levels were severely compromised in liver of CD fed irradiated mice. Another major finding of these studies revealed that under γ-radiation stress, dietary nicotinamide supplementation might induce higher and long-lasting poly(ADP)-ribose polymerase 1 (PARP1) and poly(ADP-ribose) glycohydrolase (PARG) activities in NSD fed animals compared to CD fed animals. To investigate liver DNA damage, number of apurinic/apyrimidinic sites (AP sites) and level of 8-hydroxy-2′-deoxyguanosine (8-oxo-dG) residues were quantified. A significant increase in liver DNA AP sites and 8-oxo-dG levels with concomitant increase in caspase-3 was observed in CD fed and irradiated animals compared to NSD fed and irradiated mice. In conclusion present studies show that under γ-radiation stress conditions, dietary nicotinamide supplementation restores DNA excision repair activity via prolonged activation of PARP1 and PARG activities. Present results clearly indicated that hepatic NAD$^+$ replenishment might be a novel and potent approach to reduce radiation injury.

1. Introduction

Dietary modulation to combat various forms of stress, including that caused by gamma (γ)-radiation, has been demonstrated as effective strategy [1]. Among all the cellular targets of radiation, DNA is considered to be the most critical molecule. Nicotinamide, the amide form of vitamin B3 (niacin) functions as substrate for metabolic pathways linked to DNA repair reactions [2]. There is a limited amount of data documenting the effect of dietary nicotinamide (pyridine-3-carboxamide) supplementation on protection against γ-radiation induced DNA damage [3–5]. Mammals predominantly use nicotinamide as a precursor for nicotinamide adenine dinucleotide (NAD$^+$) biosynthesis [6]. Several studies suggest that nicotinamide, by functioning as precursor for NAD$^+$, might influence repair of radiation generated purine and pyrimidine free sites (AP sites/abasic sites) in DNA [7–9]. The base excision repair (BER) dependent repair of AP sites in mammalian cells introduces DNA single strand breaks (SSBs) as intermediates, which in turn activate the nuclear enzyme, poly(ADP)-ribose polymerase 1 (PARP1) [10]. PARP1 assists DNA repair events by utilizing NAD$^+$ for formation of poly-ADP-ribose (PAR) derivatives of DNA repair proteins [11]. Due to this underlying mechanism, cellular NAD$^+$ status has been increasingly demonstrated to alter the cell susceptibility to γ-radiation exposure, highlighting the possible role of dietary nicotinamide in DNA repair [12,13]. Nicotinamide is recycled to NAD$^+$ by two enzymes, nicotinamide phosphoribosyltransferase (*NAMPT*) and nicotinamide mononucleotide adenylyltransferase (*NMNAT*), which convert nicotinamide to NMN and NMN to NAD$^+$, respectively [14].

* Corresponding author at: Radiation Biology and Health Sciences Division, Modular Laboratories, Room No. 3-47-S, Bhabha Atomic Research Centre, Mumbai 400085, India. Tel.: +91 22 25590411; fax: +91 22 25560750/22 25505151.
E-mail address: batravipen@gmail.com (V. Batra).

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Existing data clearly demonstrates that immediately after radiation exposure, PARP1 binds to the AP site [15,16]. Earlier studies also suggest that PARP1 may be involved in coordination of AP sites repair process by attracting other BER proteins [17]. When activated by DNA strand breaks, PARP1 uses NAD^+ as a substrate to form ADP-ribose polymers on DNA repair proteins [18]. High levels of DNA strand breaks have been found to induce extensive polymer formation, with a concomitant lowering of cellular NAD^+ levels, which adversely affects BER dependent repair of AP sites [19,20]. The ensuing depletion of NAD^+ might inhibit glycolytic generation of ATP with consequent ATP depletion, eventuating in cell death. However, besides modifying several DNA repair proteins, PARP1 modifies itself by poly (ADP-ribosyl)ation, which results in a general inhibition of PARP1 activity [21,22]. The in vivo half-life of poly-ADP ribose (PAR) is less than a minute [23]. Poly(ADP-ribose) glycohydrolase (PARG) degrades PAR polymers synthesized by PARP1 [24]. PARG therefore maintains the active state of PARP1 by continuously removing inhibitory ADP-ribose residues from PARP1 [25]. The regulation of PARG activity may therefore influence PARP1-mediated AP sites repair [26].

γ-Radiation induced free radicals react with DNA and inflict damage to purine and pyrimidine bases [27,28]. In this study, we focused on 8-hydroxy-2′-deoxyguanosine (8-oxo-dG), a DNA metabolite, as a marker for radiosensitivity. Ogg1 is the primary enzyme responsible for the excision of 8-oxo-dG from DNA. Earlier studies [29] have indicated that Ogg1 binding to PARP-1 plays a functional role in the repair of oxidative DNA damage. Guanine is the highly susceptible target for γ-radiation mediated oxidative reactions because of its low redox potential [29]. Moreover, 8-oxo-dG, is potentially mutagenic because of its ability to form base pairs with both cytosine and adenine [30]. Thus, tissue 8-oxo-dG levels are one of the most mutagenic lesions, and the most abundant source of AP site in genomic DNA [31]. Delayed repair of AP sites can also result in replication induced double strand breaks (DSB) [32]. Therefore reducing AP sites in DNA may be an approach to decrease the adverse effects of γ-radiation.

Existing evidence suggests that nicotinamide deficiency may impair poly (ADP-ribosylation) of DNA repair proteins, leading to accumulation of AP sites in DNA [33]. We therefore asked whether dietary nicotinamide supplementation has the potential to mitigate radiation induced AP sites and delay cell death and, if so, whether this result is due to metabolic up-regulation of NAD^+ biosynthesis. Development of new therapeutic strategies using nicotinamide as radio-protective agent rests heavily upon the elucidation of metabolic pathways that link this nutrient to DNA repair reactions.

2. Materials and methods

2.1. Chemicals and reagents

ATP, bicin, casein, L-dithiothreitol (DTT), deoxycholic acid, EDTA, (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), Igepal CA-630, dimethyl thiazolyl diphenyl tetrazolium (MTT), nicotinamide, nicotinamide adenine mononucleotide (NMN), nuclear extraction kit, protease inhibitor cocktail, Tris–HCl, triton X-100, tween 20 and yeast alcohol dehydrogenase were purchased from Sigma–Aldrich Chemical Co (St. Louis, MO, USA). De-ionized water was purified with a Milli-Q water purification system (Millipore, Bedford, MA). All other chemicals/reagents were purchased from reputed manufacturers and were of analytical grade.

2.2. Animal maintenance and feeding

Male Swiss mice obtained from the departmental animal house facility of Bhabha Atomic Research Center (BARC) were maintained (for 6 weeks) on normal control diet (CD) and nicotinamide supplemented diet (NSD) based on AIN-93 M formula [34], which recommends a protein content of 14% (w/w) for rodents (Table 1). The mice were given free access to food and water throughout the study. Mice were housed three per cage in a room with a constant temperature of 23 ± 1 °C and a 12 h light–dark cycle. No significant change in the total food consumption and body weight was noticed, in the CD fed animals compared to animals maintained on NSD. The average initial body weight of the mice was 22.7 ± 1.4 g.

Table 1
Composition of normal control diet (NCD) and nicotinamide supplemented diet (NSD).

Component (g/kg)	NCD	NSD
Cornstarch	465.692	465.692
Casein	140.000	140.000
Dextrinized cornstarch	155.000	155.000
Sucrose	100.000	100.000
Soyabean oil	40.000	40.000
Cellulose fiber	50.000	50.000
Mineral mix (AIN-93M-MX)	35.000	35.000
Vitamin mix with nicotinamide[a]	10.000	10.000
L-Cysteine	1.800	1.800
Choline bitartarate	2.500	2.500
Ter-butylhydroquinone	0.008	0.008
Supplements		
Nicotinamide	–	2.000

Casein supplied by central Drug House (P) Ltd. (New Delhi, India), cornstarch and dextrinizrd cornstarch (feed grade) by Vijaya Enterprises (Mumbai, India), sucrose by Sisco research laboratory, (Mumbai, India), soyabean oil by Bharat Foods Cooperative Ltd. (Gandhidham, India), cellulose fiber by Maple Biotech (P) Ltd, (Pune, India), Mineral mix by MP biomedicals, USA, L-cysteine, choline bitaratarate and ter-butylhydroquinone by Sigma–Aldrich Company, (St. Louis, USA).

[a] Vitamin Mix AIN-93-VX (g/kg) Nicotinic acid 3.0, ca pantothenate 1.6, Pyridoxin-HCl 0.700, Thiamine-HCl 0.600, Riboflavin 0.600, Folic acid 0.200, D-Biotin, Vitamin B_{12} (0.1% IN Mannitol) 2.500, α-tocopherol powder (500 IU/g) 15.00, Vitamin A palmitate (250,000 U/g) 1.6, Vitamin D_3 (400,000 U/g) 0.25, Phylloquinone 0.08, Sucrose 959.7.

2.3. Experimental design

The mice were randomly distributed into fourteen different groups of five animals each under identical conditions and were grouped as follows:

Group 1	Served as CD fed control (CD-C) and was not irradiated.
Group 2	Served as NSD fed control (NSD-C) and was not irradiated.
Group 3, 4 and 5	CD fed animals received 2, 4, and 6 Gy of ^{60}Co γ-radiation, respectively and liver was removed after 24 h of irradiation.
Group 6, 7 and 8	CD fed animals received 2, 4, and 6 Gy of ^{60}Co γ-radiation, respectively and liver was removed after 48 h of irradiation.
Group 9, 10 and 11	NSD fed animals received 2, 4, and 6 Gy of ^{60}Co γ-radiation, respectively and liver was removed after 24 h of irradiation.
Group 12,13 and 14	NSD fed animals received 2, 4, and 6 Gy of ^{60}Co γ-radiation, respectively and liver was removed after 48 h of irradiation.

Animals were killed by cervical dislocation and liver was removed after different time intervals, as indicated above. The handling and sacrifice of the mice were done as per the guidelines issued by BARC (Bhabha Atomic Research Center) animal ethics committee. Animals (6 weeks old) were subjected to total body γ-radiation at a rate of 50 cGy/min by using a ^{60}Co Theratron Junior Teletherapy unit (Atomic Energy of Canada Ltd., Ottawa, Canada). Area of exposure was kept constant.

2.4. Sample preparation for NAMPT and NMNAT quantification

Liver tissue was rinsed at least twice with phosphate buffered saline. To 100 mg of incised tissue, 1 ml of cell lysis buffer (50 mM Tris–HCl; pH 7.5, 5 mM EDTA; 150 mM NaCl; 0.1% lauryl sulfate, sodium salt in deionized water (DW); 0.5% deoxycholic acid in DW; 1% Igepal CA-630 in DW; protease inhibitor cocktail (Sigma–Aldrich, St. Louis, USA) was added followed by incubation for 15 min. It was followed by transfer of sample, along with cell lysis buffer to pre-chilled micro-homogenizer where the tissue was homogenized. The lysed cells were centrifuged (12,000 × *g*, 10 min) and protein-containing supernatant was removed to a chilled test and kept on ice till used for NAMPT and NMNAT quantification.

2.5. NAMPT and NMNAT assay

NAMPT was measured by sandwich immunometric assay (Nampt (mouse/rat) Intracellular ELISA kit, Biovision research products, CA, USA). Briefly, it involved