

Studies on NA, MNA⁺, and NAD⁺ Compounds Influence on Human Lymphocytes Radio-Susceptibility and DNA Repair



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Introduction

Nicotinamide (NA), 1-methylnicotinamide (MNA⁺) and nicotinamide adenine dinucleotide (NAD⁺) participate in many biological processes, including the regulation of energy metabolism, signal transduction and DNA repair. Nicotinamide is one of the two primary forms of vitamin B₃ (niacin). This compound is a principal substrate for NAD⁺ synthesis. Third investigated compound, 1-methylnicotinamide (MNA⁺) is a primary metabolite of nicotinamide [1].

Aim of Study

The aim of study was to investigate the influence of the pre-treatment with nicotinamide (NA), 1-methylnicotinamide chloride (MNA⁺) or NAD⁺ on the levels of the radiation induced DNA damage as well as on the DNA repair efficiency.

Materials and Methods

Cells:

Human lymphocytes isolated from whole blood sample of a healthy young nonsmoking male

Chemical treatment

Thawed lymphocytes were incubated with fresh solutions of NA, MNA⁺ or NAD⁺ in final concentrations 80 mM. Compounds were obtained from and stored at room temperature in no water and light conditions.

X-rays irradiation

To evaluate influence of NA, MNA⁺ and NAD⁺ on the induction of the DNA damage lymphocytes were exposed to 2 Gy of X-rays (Rtg 250kV, (MCN 323). Dose rate: 1Gy/min) without or after the chemical pre-treatment.

Analiza uszkodzeń DNA metodą kometową

The initial and induced DNA damage level was measured with the alkaline version of the single cell gel electrophoresis (SCGE) assay (known also as Comet assay). The amount of DNA damage was analyzed with mikroskop epifluorescence and Komet 3.0 (Kinetic Img.) software. Degree of damage evaluated from:

TM- comet tail moment (% of DNA in a tail x comet length)

Results

➤ Our results show statistically significant decrease of DNA's radio-sensitivity of lymphocytes irradiated in the presence of all investigated compounds in comparison to the control cells irradiated without pre-treatment (Fig.1).

➤ In both types of cells: proliferating or G₀ pre-treated with MNA⁺ or NAD⁺, the percent of residual DNA damage was similar or higher than observed in controls.

➤ Presence of NA in G₀ cells medium resulted in a decrease of the residual DNA damage level ($RD_{TM} = 20.2 \pm 12$ for cells treated with NA and $RD_{TM} = 36.3 \pm 14$ for control) (Fig.3a).

➤ However, in proliferating cells presence of NA caused an increase of the unrepaired DNA damage in comparison to control ($RD_{TM} = 39.1 \pm 17$ for cells treated NA and $RD_{TM} = 15.8 \pm 13$ for control) (Fig.3b).

References:

1. J. Gębicki i wsp., Polish Journal of Pharmacology 2003, 55 (109-112).
2. A. Cebulska-Wasilewska. Mut. Res. 544 (2003) 289-297.

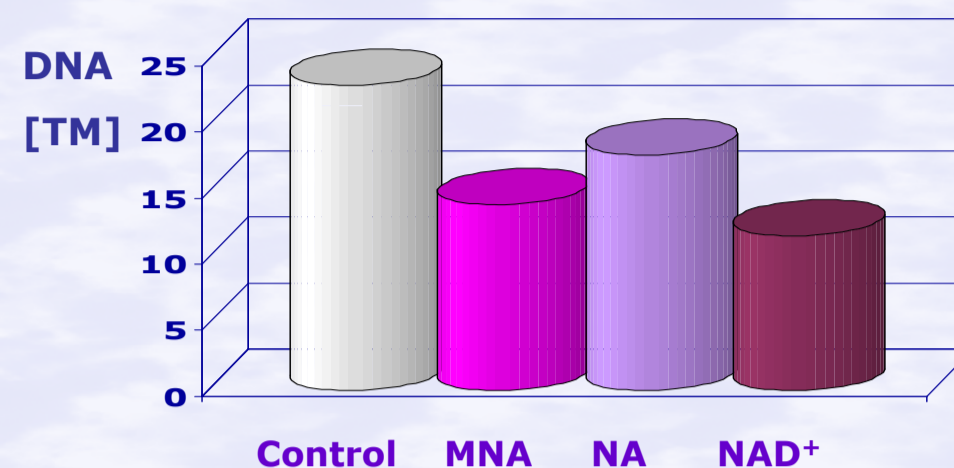


Fig.1 Radiosensitivity without and with 80μM (MNA, NA lub NAD⁺) pretreatment

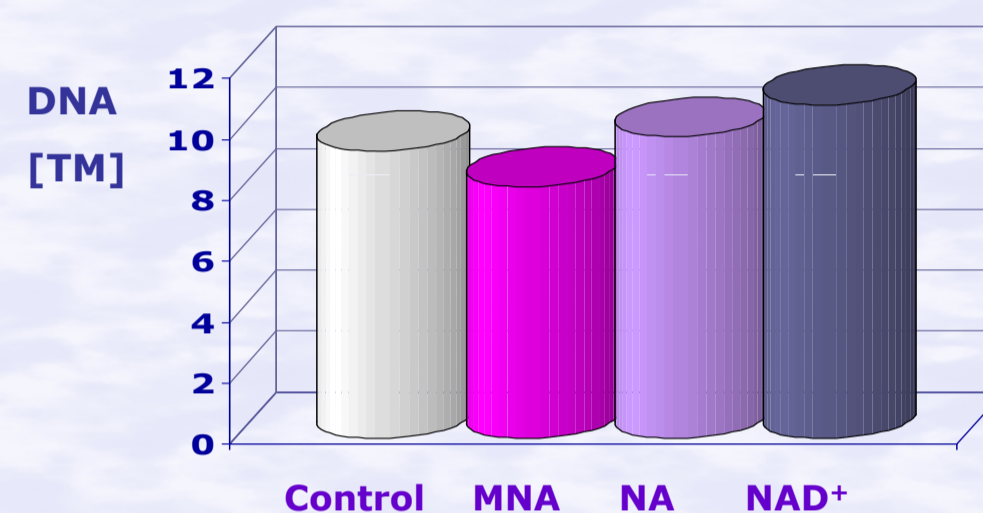
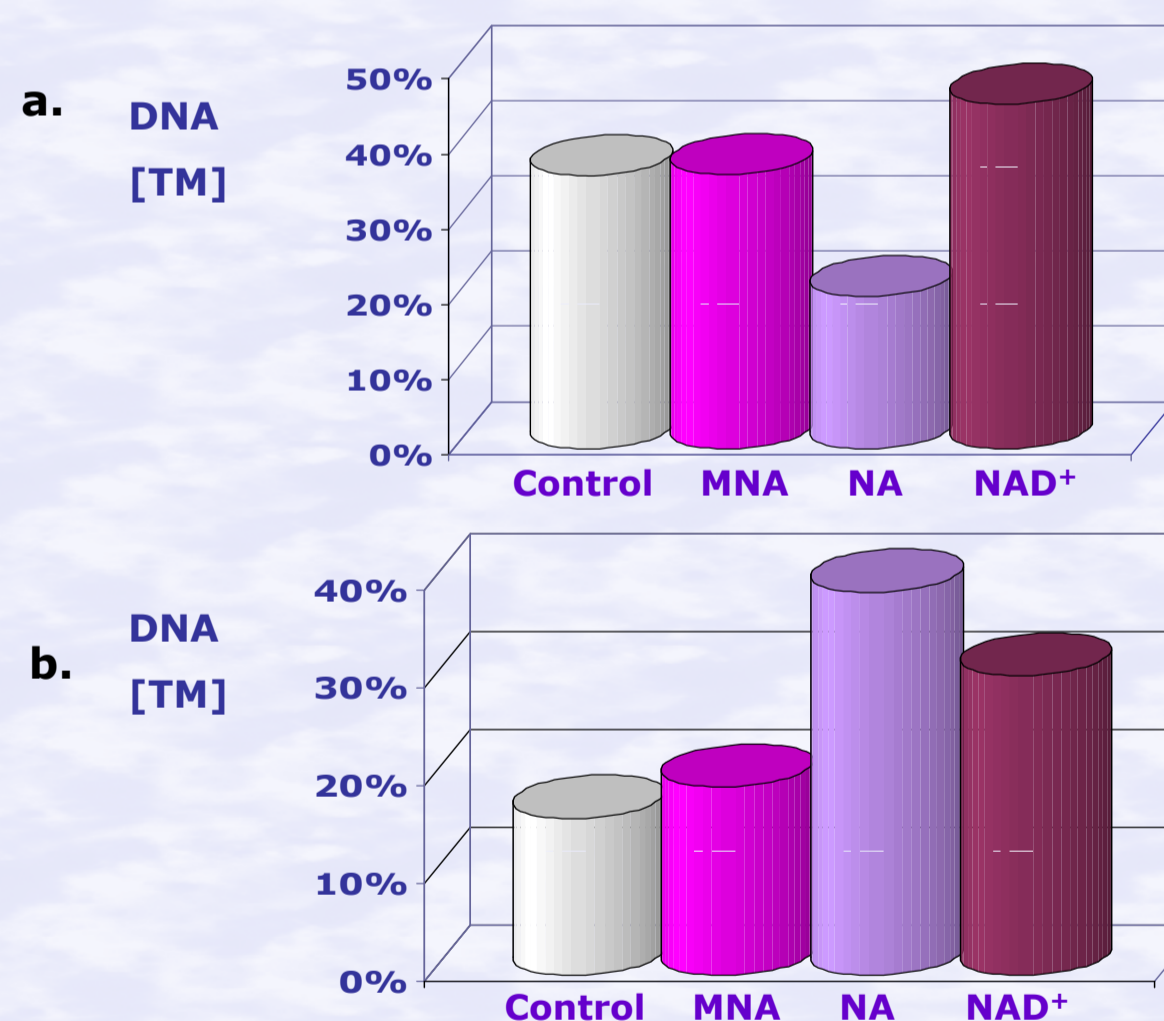


Fig.2 Influence of MNA, NA lub NAD. No irradiation



Figs.3 Percent of unrepaired DNA after post-irradiation incubation in resting G₀ (a) or proliferating (b) cells with or without MNA, NA, NAD⁺ pretreatment.

Conclusions

- ❑ Our results suggested that all investigated compounds protected DNA during genotoxic agent action.
- ❑ However, presence of these compounds during DNA repair might trigger a disturbance of this process resulting in decrease of the repaired DNA damage.