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Note

The Effects of Niacin on DNA Repair after *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine Treatment in Normal Human Lymphocytes

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We have investigated the effects of niacin on NAD levels and on DNA repair in human lymphocytes. When lymphocytes were incubated in culture medium with various concentrations of niacin, incubation of lymphocytes with nicotinic acid at 5 μM or nicotinamide at 10 mM caused a 2–3 fold increase in NAD content. Under these conditions lymphocytes were treated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG). Interestingly, the rejoining of DNA strand breaks was promoted by nicotinic acid but nicotinamide inhibited the rejoining.

Key words: niacin; DNA repair; human lymphocytes; poly(ADP-ribose)polymerase; *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine

NAD is an important coenzyme in oxidation-reduction reactions, and is also a substrate for poly(ADP-ribose)polymerase (EC 2.4.2.30: PARP). Poly ADP-ribosylation is a post translational modification for nuclear proteins, including PARP itself, which is stimulated in the presence of DNA strand breaks. So, this reaction has been associated with many important cellular processes, such as cell proliferation, gene transcription, cell differentiation, alteration of chromatin structure, apoptosis, and DNA repair.[1,2] Many researches have been done to discover the biological function of poly ADP-ribosylation. Especially, an important role for poly ADP-ribosylation has been suggested in DNA repair, but no detailed mechanism of poly ADP-ribosylation in DNA repair is known.

Fujiwara *et al.* investigated the cause of hypersensitivity to ultraviolet in Cockayne's syndrome patients.[3] PARP activity was normal in fibroblasts of these patients, but intracellular level of NAD was low (60–70%) as compared with normal human fibroblasts. Further, this result was confirmed by Berger *et al.* in lymphocytes of Fanconi's anaemia patients.[4] These observations of the low level of NAD in the cells of patients with this highly carcinogenic and genetic syndrome suggest that there is a close relationship between the intracellular level of NAD and the ability of DNA repair. Consequently, in this research, we have paid attention to the effects of elevated NAD level on DNA repair in normal human lymphocytes incubated with the culture medium with various concentrations of niacin *in vitro*.

Normal human lymphocytes were isolated from heparinized peripheral blood from healthy human volunteers by density gradient centrifugation with Ficoll-Paque (Pharmacia Biotech. Co., Uppsala, Sweden). The cells were suspended in RPMI 1640 medium (Gibco BRL Co., Grand Island, N.Y., U.S.A.) with 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine, and 10% heat-inactivated (56°C, 30 min) fetal bovine serum (Gibco BRL Co., lot 38N9543) (complete medium) at density of 1.0×10^6 cells/ml, and were cultured with the media with various concentrations of nicotinic acid or nicotinamide at 37°C and 5% CO_2 for varying times to elevate NAD level in the cells.

To estimate NAD content, lymphocytes were treated with ice-cold 0.5 M perchloric acid for 15 min. After neutralization with KOH, NAD content was measured by the enzymatic cycling assay method of Jacobson *et al.*[5]

After lymphocytes were treated with various concentrations of MNNG for 1 h, cells were washed and cultured with complete medium. DNA strand breaks were measured by the fluorometric analysis of DNA unwinding developed by Birnboim and Jevcak.[6]

All results are presented as mean ± SD from triplicate independent experiments. The data were analyzed for statistical significance by Student's *t* test.

The effects of nicotinic acid or nicotinamide on NAD content in human lymphocytes are shown in Fig. 1. In the case of nicotinic acid, the increase in NAD content was observed at lower concentrations, and especially incubation with 5 μM nicotinic acid resulted in about a 3-fold increase as compared to the control level. But, at higher concentrations, the increase in NAD content was gradually becoming smaller. With nicotinamide, the increase in NAD content was almost proportional to the nicotinamide concentration. Incubation with 10 mM nicotinamide resulted in about a 2-fold increase of NAD concentration. It is suggested that the effects of nicotinic acid and nicotinamide on the increase in NAD level in the cells may due to the difference of regulation of intracellular level of NAD. Flechner *et al.* compared the efficiency of nicotinic acid and nicotinamide for a precursor of NAD biosynthesis in guinea pig leukocytes.[7] Nicotinic acid

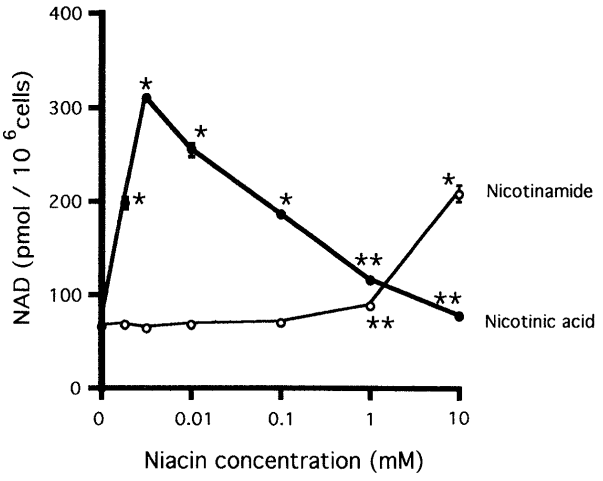

Fig. 1. The Effects of Niacin on NAD Level in Normal Human Lymphocytes.

Results are presented as mean ± SD from three independent experients. Student's *t* test, $*p < 0.0001$ and $**p < 0.01$ when compared to nonsupplemented cells at the same time of incubation; no symbols not significant when compared to the corresponding control cells. Parts of SDs were less than the size of the symbols.

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Abbreviations: MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; PARP, poly(ADP-ribose)polymerase.

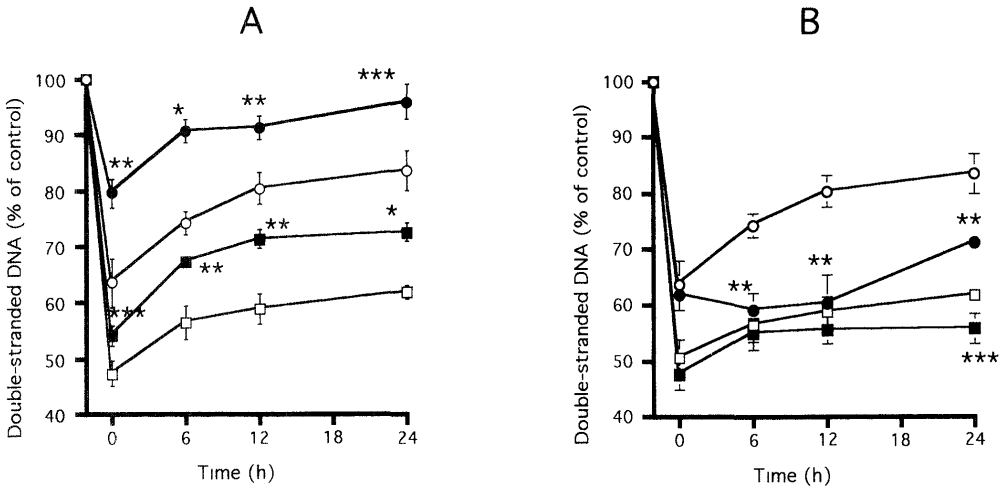

Fig. 2. Effects of Niacin on Formation of DNA Strand Breaks and Rejoining of Double-stranded DNA.

A, with 5 μM nicotinic acid; B, with 10 mM nicotinamide. The percentage of double-stranded DNA was calculated as described in ref. 6. "Control" of Y axis corresponds to the percentage of double-stranded DNA in non-MNNG treated cells. ○ ●, 0.5 μg/ml MNNG treatment; □ ■, 1.0 μg/ml MNNG treatment. Open symbols, control; closed symbols, niacin treatment. Results are presented as mean ± SD from three independent experiments. Student's t test, * $p<0.001$, ** $p<0.01$, and *** $p<0.05$ when compared to nonsupplemented cells at the same time of incubation; no symbols not significant when compared to the corresponding control cells. Parts of SDs were less than the size of the symbols.

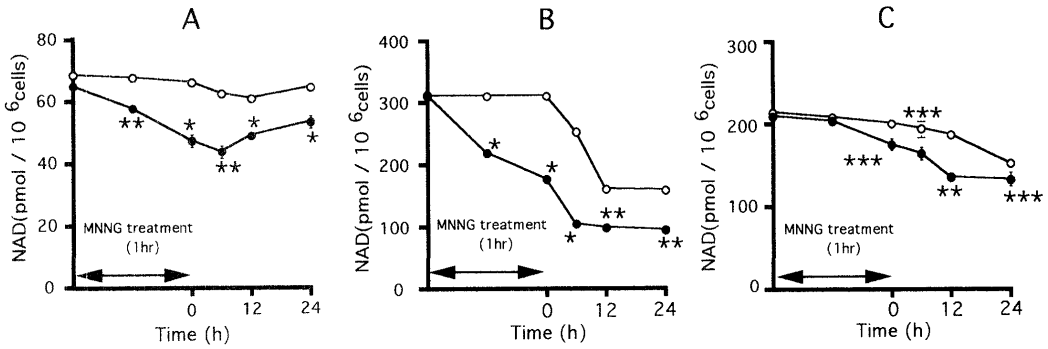

Fig. 3. Time-dependent Change of NAD Level with MNNG Treatment.

A, control; B, with 5 μM nicotinic acid; C, with 10 mM nicotinamide. ●, 0.5 μg/ml MNNG treatment, ○, no MNNG treatment. Results are presented as mean ± SD from three independent experiments. Student's t test, * $p<0.0001$, ** $p<0.001$, and *** $p<0.05$ when compared to nonsupplemented cells at the same time of incubation; no symbols not significant when compared to the corresponding control cells. Parts of SDs were less than the size of the symbols.

was superior as a precursor of NAD biosynthesis to nicotinamide. Moreover, nicotinamide is known as an inhibitor for NAD-degrading enzyme, *i.e.*, PARP and NAD glycohydrolase *in vitro*. Carson *et al.* investigated the effect of 3-aminobenzamide, a PARP inhibitor, on NAD degradation in resting human lymphocytes.[8] As the result, NAD degradation was prevented by 3-aminobenzamide in culture medium. This observation is very interesting, because it is suggested to relate poly ADP-ribosylation with regulation of the intracellular level of NAD content. Further, NAD is synthesized and degraded continuously *in vivo*, and the intracellular NAD level may be kept at certain level.[9] Therefore, synthesis of NAD may be promoted by nicotinic acid, and oppositely, degradation of NAD may be prevented by nicotinamide, and then the apparent NAD level is increased 2–3 fold. Moreover, we must also examine the uptake of niacin in human lymphocytes.

At an elevated NAD level, lymphocytes were treated with MNNG at the concentrations of 0.5 and 1.0 μg/ml. When the NAD level in the cells was increased with the addition of 5 μM nicotinic acid, the rejoining of DNA strand breaks was promoted as shown in Fig. 2. Although 10 mM nicotinamide addition also increased NAD levels in the cells, the rejoining of DNA strand breaks was inhibited.

To discover the reason of these results, we focused our attention on the NAD metabolism. First, we examined the time-dependent change of NAD content with MNNG treatment as shown in Fig. 3. Dramatic degradation of NAD was observed when lymphocytes were previously incubated in the medium containing 5 μM nicotinic acid and treated with 0.5 μg/ml MNNG. Oppositely, when cells were previously incubated in the medium containing 10 mM nicotinamide, the degradation of NAD was small. Further, in changes of NAD content after MNNG treatment, we guess that the intracellular level of NAD returned to previous levels after the change to the complete medium.

As stated above, at first, we have taken account of poly ADP-ribosylation. So, we have suggested that nicotinic acid may stimulate NAD synthesis, and then the level of NAD may be increased. Since NAD is a substrate for PARP, the phase related to poly ADP-ribosylation in the DNA repair reaction may be promoted. Nicotinamide may be not only a precursor of NAD biosynthesis, but also an inhibitor for NAD-degrading enzyme. Therefore, when poly ADP-ribosylation is inhibited, the NAD content is increased, and DNA repair may be suppressed. The DNA repair assay that we used in this experiment was developed by Birnboim *et al.*,[6] and is a very sensitive and excellent method. But, at present, we cannot tell the biological meaning of the promotion (maximum 15%) in the rejoining of DNA strand break observed in this study. So we must take account of the biological effect of niacin supplementation on DNA repair, and have to investigate the various effects on DNA repair, further.

After this, we want to explain a part of biological role of poly ADP-ribosylation in DNA repair, and especially direct our attention to NAD metabolism.

We have paid attention to previous papers which described the relationship between niacin and DNA repair. At first, Berger *et al.* investigated how unscheduled DNA synthesis was promoted by PARP inhibitor in human lymphocytes[10-12] and cultured hepatocytes of rats.[13] Weitberg investigated the effects of nicotinic acid on oxygen radical-induced DNA damage in lymphocytes from human subjects receiving niacin supplements.[14] This treatment increased the NAD content, and promoted the DNA repair reaction after exposure to oxygen radicals. Zhang *et al.*[15] and Kirkland *et al.*[16,17] reported the effects of niacin supplementation or deficiency on NAD content and poly ADP-ribosylation in various tissues from rats. As already described, the relationship between niacin and DNA repair has been suggested strongly. And, in most of the research, the authors directed their attention only to poly ADP-ribosylation. Wang *et al.* investigated a biological role of poly ADP-ribosylation with PARP gene knock-out mice.[18] DNA repair was not inhibited in primary fibroblasts from PARP knock-out mice. Therefore, they have considered that PARP is not always essential to DNA repair. So, we must also take account of participation of another factor besides poly ADP-ribosylation in our research. In this paper, we showed that the effect on DNA repair was opposite in nicotinic acid and nicotinamide, though the NAD content was increased by both. We have considered that the intracellular level of NAD and NAD metabolism (*i.e.*, NAD synthesis and degradation, mono-, poly-ADP-ribosylation, cyclic ADP-ribose synthesis *etc.*) is a key factor in controlling the cellular response, *e.g.* DNA repair, apoptosis, cell differentiation *etc.*

Moreover, the cause of cancer and aging may due to the accumulation of error in DNA. Therefore, we want to use our knowledge for promoting the ability of DNA repair with the aid of a vitamin, nicotinic acid.

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