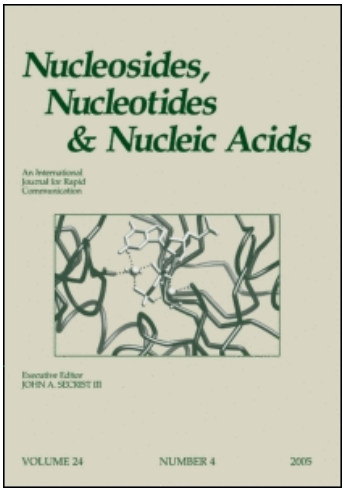

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CYTOPROTECTIVE EFFECTS OF NICOTINAMIDE DERIVATIVES IN ENDOTHELIAL CELLS

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□ *Following discovery of NAD^+-dependent reactions that control gene expression, cytoprotection, and longevity, there has been a renewed therapeutic interest in precursors, such as nicotinamide and its derivatives. We tested 20 analogues of nicotinamide for their ability to protect endothelial cells from peroxynitrite stress and their effect on poly (ADP-ribose) polymerase (PARP) activity. Several nicotinamide derivatives protected endothelial cells from peroxynitrite-induced depletion of cellular NAD^+ and ATP concentrations, but only some of these compounds inhibited PARP. We conclude that some nicotinamide derivatives provide protection of endothelial cells against peroxynitrite-induced injury independent of inhibition of PARP activity. Preservation of the NAD^+ pool was a common effect of these compounds.*

Keywords Nicotinamide; endothelium; cytoprotection; poly(ADP-ribose) polymerase (PARP); NAD; ATP

INTRODUCTION

There is renewed interest in nicotinamide (NA) and nicotinamide derivatives based on the discovery of non-vitamin-related cytoprotective properties.[1] Effects on the activities of NAD^+-dependent enzymes, such as histone deacetylase and poly(ADP-ribose) polymerase (PARP), could provide a basis for this effect of nicotinamide and derivatives,[2] as could effects on the availability of NAD^+. Clinical efficacy of high dose nicotinamide was proved in several clinical conditions such as diabetes and neurological disorders.[3,4] It is widely accepted that nicotinamide-mediated inhibition of PARP could contribute to some of these effects, but whether this mechanism

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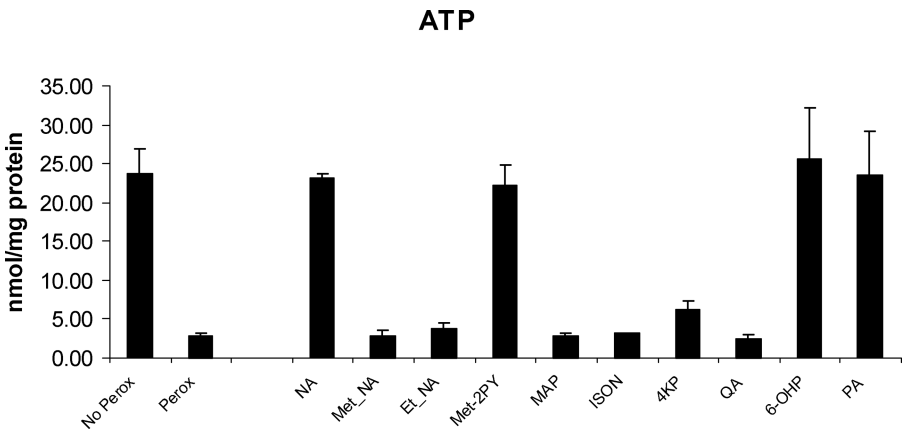

FIGURE 1 Effect of 30-minute pretreatment with nicotinamide analogues on ATP concentration in cultured endothelial cells exposed to 100 μM peroxynitrite for 60 minutes. Values represent means ±SEM, n = 3. Abbreviations are: Peroxynitrite (Perox), nicotinamide (NA), N-methylnicotinamide (Met_NA), ethylnicotinamide (Et_NA), N-methyl-2-pyridone-5-carboxamide (Met2PY), 1-methyl-3-acetylpyridine (MAP), isonicotinic acid (ISON), 4-ketopicolinic acid (4KP), quinolinic acid (QA), 6-hydroxypicolinic acid (6-OHP), picolinamide (PA).

can explain the effects of all related compounds has not been established. Therefore, we tested nicotinamide and a series of 20 nicotinamide analogues with regard to their cytoprotective effects in endothelial cells and their effects on inhibition of PARP.

METHODS

Cultured HMEC-1 cells were used in this study. Conditions for cell culture were described previously.[5] Confluent cultures were washed with Hanks Balanced Salt Solution (HBSS), and culture plate wells were filled with 1 ml of HBSS after the final wash. Nicotinamide analogues were then added at 1 mM concentration, and culture plates were incubated for 30 minutes. Compounds analysed included: nicotinamide (NA), N-methylnicotinamide (MetNA), ethylnicotinamide (EtNA), N-methyl-2-pyridone-5-carboxamide (Met2PY), N-methyl-4-pyridone-3-carboxamide (Met4PY), 4-pyridone-3-carboxamide (4PY), 2-pyridone-5-carboxamide (2PY), 4-pyridone-3-carboxamide-1-β-D-ribonucleoside (4PYR), isonicotinamide (ISONA), isonicotinic acid (ISON), 2-picolinic acid (2P), 3-hydroxypicolinic acid (3OHP), 4-ketopicolinic acid (4KP), 6-hydroxypicolinic acid (6OHP), 1-methyl-3-acetylpyridine (MAP), picolinamide (PA), 6-aminonicotinamide (6NH2NA), 6-hydroxynicotinic acid (6OHN), quinolinic acid (QA), and 6-aminonicotinic acid (6NH2N). After incubation with nicotinamide analogues, 100 μM peroxynitrite was added (except control) and incubation was continued for an additional 60 minutes. The incubation medium was then removed and frozen for further

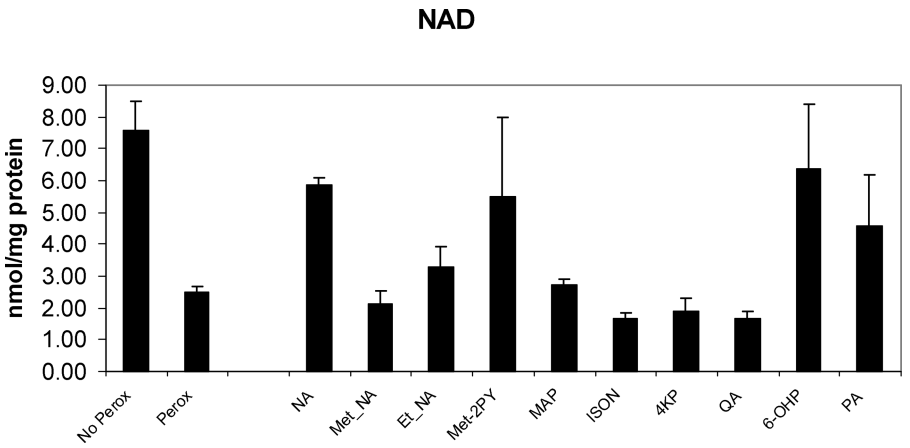

FIGURE 2 Effect of 30-minute pretreatment with nicotinamide analogues on NAD^+ concentration in cultured endothelial cells exposed to 100 μM peroxynitrite for 60 minutes. Values represent means ±SEM, n = 3. Abbreviations are as described in the legend to Figure 1.

analysis, and cells were extracted with 0.3 ml of 0.4 M perchloric acid. Analysis was performed by HPLC as described previously.[6] The protein precipitate was dissolved in 0.5 M sodium hydroxide and protein content was analysed by the Bradford method. The effect of nicotinamide derivatives on poly(ADP-ribosyl)-ation was evaluated with a commercially available PARP. enzyme preparation, incubated with different concentrations of nicotinamide analogues, and PARP activity was measured by evaluation of radioactivity incorporated from radiolabeled NAD^+ into histone proteins provided as substrate.[7]

RESULTS AND DISCUSSION

Among nicotinamide derivatives studied, some provided almost complete protection of ATP (Figure 1) and NAD^+ (Figure 2) pools in endothelial cells exposed to peroxynitrite. Steady-state ATP concentration decreased by 88% in control cells unprotected with NA but treated with peroxynitrite; levels of this metabolite were, however, maintained in cells pretreated with NA, Met2PY, 6OHP and PA (Figure 1). Other NA derivatives did not produce this effect. Changes in NAD^+ concentrations mirrored those in ATP concentrations (Figure 2), although the extent of NAD^+ depletion induced by peroxynitrite was less than that observed for ATP. Inhibition of poly(ADP-ribose) polymerase was demonstrated for NA and Met2PY. At 1 mM concentrations NA and Met2PY inhibited PARP 89% and 76%, respectively. However, no inhibition of PARP activity was observed with 6-OHP and PA even at 3 mM concentration.

We conclude that nicotinamide derivatives offer remarkable protection from metabolic injury caused by peroxynitrite. However, there is no single explanation available at present. For some nicotinamide derivatives, endothelial cell protection could be mediated by maintenance of the NAD^+ pool due to poly(ADP-ribose) polymerase inhibition while for others, the mechanism remains to be established. NAD^+ pool preservation seems to be a common element of these protective pathways. Since only PARP activity is sufficiently high in the cell to produce such a rapid decrease in the NAD^+ pool, compounds such as 6-OHP and PA that are not direct inhibitors of PARP could induce indirect inhibition of PARP activity.

REFERENCES

1. .Szabo,C.; Dawson,V.L. Role of poly(ADP-ribose) synthetase in inflammation and ischaemia-reperfusion. *Trends Pharmacol. Sci.* **1998**, 19, 287–298.
2. Hassa, P.O.; Haenni, S.S.; Elser, M.; Hottiger, M.O. Nuclear ADP-ribosylation reactions in mammalian cells: Where are we today and where are we going? *Microbiol. Mol. Biol. Rev.* **2006**,70, 789–829.
3. Maiese,K.; Chong, Z.Z. Nicotinamide: necessary nutrient emerges as a novel cytoprotectant for the brain. *Trends Pharmacol. Sci.* **2003**, 24, 228–232.
4. Cabrera-Rode,E.; Molina,G.; Arranz,C.; Vera,M.; et al. Effect of standard nicotinamide in the prevention of type 1 diabetes in first degree relatives of persons with type 1 diabetes. *Autoimmunity.* **2006**, 39, 333–340.
5. Smolenski, R.T.; Kochan, Z.; McDouall, R.; Page, C.; Seymour, A.L.; Yacoub, M.H. Endothelial nucleotide catabolism and adenosine production. *Cardiovasc. Res.* **1994**, 28, 100–104.
6. Smolenski, R.T.; Lachno, D.R.; Ledingham, S.J.; Yacoub, M.H. Determination of sixteen nucleotides, nucleosides and bases using high-performance liquid chromatography and its application to the study of purine metabolism in hearts for transplantation. *J. Chromatogr.* **1990**, 527, 414–420.
7. Bakondi, E.; Bai, P.; Szabo, E.E.; Hunyadi, J.; Gergely, P.; Szabó, C.; Virág, L. Detection of poly(ADP-ribose) polymerase activation in oxidatively stressed cells and tissues using biotinylated NAD substrate. *J. Histochem. Cytochem.* **2002**, 50, 91–98.