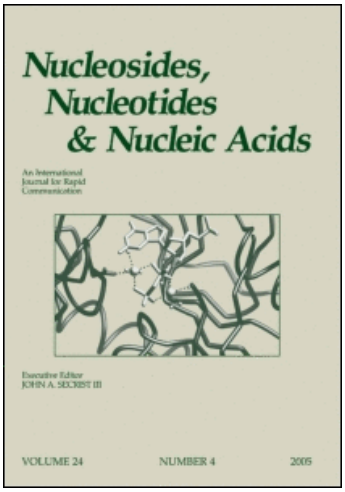

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The Effect of N-Methyl-2-Pyridone-5-Carboxamide—A Nicotinamide Catabolite on Poly ADP-Rybosylation and Oxidative Stress Injury in Endothelial Cells

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THE EFFECT OF N-METHYL-2-PYRIDONE-5-CARBOXAMIDE—A NICOTINAMIDE CATABOLITE ON POLY ADP-RYBOSYLATION AND OXIDATIVE STRESS INJURY IN ENDOTHELIAL CELLS

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▫ *This study evaluated the effect of nicotinamide (NA) and its endogenous metabolite 2PY (N-methyl-2-pyridone-5-carboxamide) on the activity of poly(ADP-ribose) polymerase (PARP) and on peroxynitrite-induced injury in endothelial cells. 2PY and NA inhibited isolated PARP with half-maximal constants of 0.53 mM and 0.025 mM, respectively. Exposure to peroxynitrite caused a decrease of the NAD pool in cultured endothelial cells to below 10% of initial level. Addition of 2PY or NA provided partial protection from peroxynitrite-induced NAD depletion, with NA being more effective. 2PY and NA also provide protection from ATP depletion. We conclude that NA as well as 2PY protect from oxidative stress injury in endothelial cells by inhibition of PARP and protection from NAD depletion. This, in turn, protects energetics, allowing maintaining cellular ATP.*

Keywords Nicorinamide, N-Methyl-2-Pyridone-5-Carboxamide, Poly(ADP-Ribose) Polymerase, Endothelium

INTRODUCTION

The poly(ADP-ribosyl)-ation is an essential process in DNA repair, but, if excessively stimulated, may impair cellular viability by depletion of the NAD pool

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and induction of apoptosis. One study has demonstrated deleterious effects of inhibitors of poly(ADP-ribose) polymerase (PARP),[1] another has shown that it may protect from ischemia reperfusion injury.[2] We studied whether 2PY (N-methyl-2-pyridone-5-carboxamide), a product of nicotinamide metabolism that excessively accumulates in chronic renal failure (CRF),[3] could inhibit PARP. Subsequently, we have analyzed the effect of 2PY on oxidative stress injury induced by peroxynitrite in endothelial cells.

MATERIALS AND METHODS

The effects of 2PY and nicotinamide (NA) on poly(ADP-ribosyl)-ation have been evaluated with commercially available isolated PARP and endothelial cell lysates. The enzyme preparation was incubated with different concentratioins of 2PY or NA and the activity was measured by evaluation of radioactivity incorporation from radiolabeled NAD into histone proteins provided as a substrate.[4] Cellular effects were studied with cultured human endothelial cell line (PIEC). Confluent cultures were exposed to 100 μM peroxynitrite for 60 min followed by measurement of cellular ATP and NAD by HPLC, as we have described previously.[5] 2PY or NA was added at 1, 3, or 10 mM concentration, and 10 mM imidazole was given to controls.

RESULTS AND DISCUSSION

Half-maximal concentrations for the inhibition of PARP by 2PY were 0.53 mM and at 0.75 mM with the isolated PARP and endothelial cell homogenate,

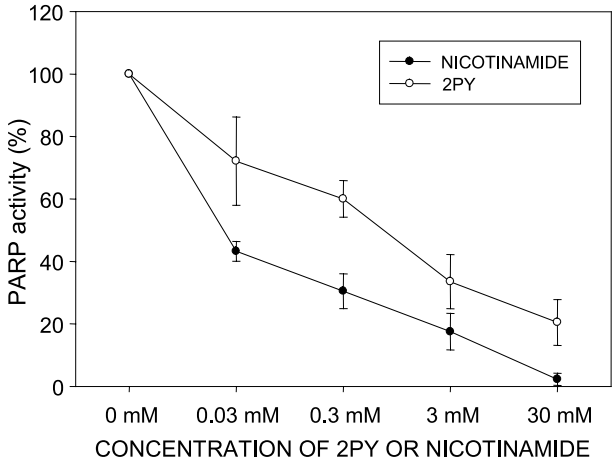

FIGURE 1 The effect of 2PY or nicotinamide on the activity of isolated poly(ADP-ribose) polymerase. Values are means ± SEM, $n = 3$.

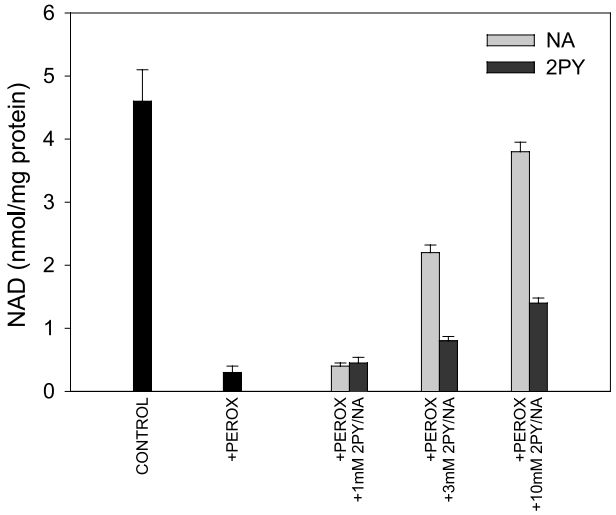

FIGURE 2 The effect of 2PY or nicotinamide (NA) on the concentration of NAD in human endothelial cells exposed to 100 μM peroxynitrite (Perox) for 60 min. Values are means ± SEM, $n = 6$.

respectively. NA was a more potent inhibitor of PARP with half-maximal inhibition at 0.025 mM. Comparison of the effect of 2PY with NA on the activity of the isolated enzyme is presented in Figure 1. In cultured endothelial cells, 2PY and NA attenuated the peroxynitrite-induced NAD pool decrease. NAD concentrations following exposure to peroxynitrite with and without NA or 2PY are presented on Figure 2. The endothelial cell ATP decreased from 16.1 ± 2.1 to 2.3 ± 0.6 nmol/mg cellular protein after peroxynitrite exposure in controls in these experiments. Addition of 2PY or NA protected from peroxynitrite-induced ATP decrease already at 1 mM concentration. Maximal effect was observed at 10 mM 2PY or NA, with ATP maintained at 10.2 ± 1.7 and 13.1 ± 1.5 in cells treated with 2PY and nicotinamide, respectively.

Our data indicate that 2PY, similar to NA, is an inhibitor of PARP, although its effect is weaker. At the normal physiological concentrations of 2PY, which is about 1 μM, inhibition of PARP by 2PY is probably not significant, unless 2PY preferentially accumulates in specific compartments.

However, 2PY concentration may increase up to 100 μM in plasma of patients with renal failure. In this situation the possibility of inhibition of PARP has to be taken into consideration. It is difficult to address further implications of this inhibition as it has been found deleterious[1] in some studies but protective in the other experiments.[2] The most likely possibility is that PARP inhibition is beneficial under specific pathological conditions such as brief ischemia with reperfusion but deleterious if this effect is extended in time. However, final assessment requires further studies.

ACKNOWLEDGMENT

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