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Effects of 1-methylnicotinamide and its metabolite *N*-methyl-2-pyridone-5-carboxamide on streptozotocin-induced toxicity in murine insulinoma MIN6 cell line

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1-Methylnicotinamide (MNA) is a primary metabolite of nicotinamide. In recent years several activities of MNA have been described, such as anti-inflammatory activity in skin diseases, induction of prostacyclin synthesis *via* COX-2, aortal endothelium protection in diabetes and hypertriglyceridaemia and increased survival rate of diabetic rats. 1-Methylnicotinamide was also suggested to protect pancreatic cells from streptozotocin *in vivo*. Streptozotocin toxicity is known to be mediated by poly-ADP-ribose polymerase. Nicotinamide and its derivatives have been shown to ameliorate poly-ADP-ribose polymerase-dependent nucleotide pool reduction. We aimed to verify if 1-methylnicotinamide and its metabolite, *N*-methyl-2-pyridone-5-carboxamide, can protect insulinoma cells from streptozotocin-induced toxicity. We found that *N*-methyl-2-pyridone-5-carboxamide, but not 1-methylnicotinamide, restores the pool of ATP and NAD^+ in streptozotocin-treated cells, but neither compound improved the cell viability. We conclude that inhibition of poly-ADP-ribose polymerase-dependent nucleotide pool reduction may not be sufficient to protect cells from streptozotocin toxicity.

Keywords: 1-methylnicotinamide, *N*-methyl-2-pyridone-5-carboxamide, streptozotocine, nucleotide pool

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INTRODUCTION

Depletion of the nucleotide pool, due to poly-ADP-ribose polymerase (PARP) activation in response to DNA damaging agents is believed to be one of the mechanisms leading to cell death. Nicotinamide and its derivatives receive a growing attention as potential PARP inhibitors.

1-Methylnicotinamide (MNA) is a primary metabolite of nicotinamide. In recent years several activities of MNA have been described, such as anti-inflammatory activity in skin diseases (Gebicki *et al.*, 2003; Wozniacka *et al.*, 2005), induction of prostacyclin synthesis *via* COX-2 (Chlopicki *et al.*, 2007), aortal endothelium protection in diabetes and hypertriglyceridemia (Bartus *et al.*, 2008) and increased survival rate of diabetic rats (Watala *et al.*, 2009; Przygodzki *et al.*, 2010) Occasional observations indicated that MNA can protect rats from streptozotocin (STZ)-induced diabetes (Watala *et al.*, 2009). It remained fairly in line with previous observations that nicotinamide treatment decreases streptozotocin toxicity and can protect against STZ-induced diabetes (Hoorens & Pipeleers, 1999; Hassan & Janjua, 2001). The STZ action on pancreatic cells has been shown to be dependent on PARP activity (Burkart *et al.*, 1999; Masutani *et al.*, 1999; Pieper *et al.*, 1999). Thus, it could be suggested that MNA or one of its metabolites protect pancreatic cells against STZ toxicity. One of the MNA metabolites found in circulation of rats fed MNA is *N*-methyl-2-pyridone-5-carboxamide (Met2PY) (Bartus *et al.*, 2008). It has been recently shown that Met2PY is an effective PARP inhibitor (Slominska *et al.*, 2008) Our present study was aimed at verifying whether MNA or Met2PY can be protective against STZ-induced toxicity *in vitro*. Using murine insulinoma cells (MIN6) as a model, we found that, despite restoring the pool of ATP and NAD^+ in STZ-treated cells, Met2PY has no effect on cell survival.

MATERIALS AND METHODS

Cell culture. MIN6 cells were maintained in DMEM containing 15% fetal bovine serum, 100 U/mL penicillin, 100 μg/mL streptomycin, and 25 mmol/L glucose at 37 °C in an atmosphere of 95% O_2/5% CO_2. Incubations with the tested substances for MTT assays were held in culture medium, those for for ATP and NAD^+ assays in Hank's buffered salt solution. For all the assays streptozotocin solution was freshly prepared in 0.1 M citrate buffer, pH 4.5, prior to its addition to the cells. Control cells were treated with an equal volume of citrate buffer.

ATP and NAD^+ quantitation. For nucleotide assay cells were grown on 24-well plates. The cells were incubated with STZ (5 mM) in the presence of MNA chloride or Met2PY (1 mM) for 1 h. The incubation was then continued for 1 h in fresh medium containing MNA or Met2PY, but free of STZ.

After the incubation the cells were washed twice with cold PBS (phosphate-buffered saline). To each well 300 ml of cold 0.4 M $HClO_4$ was added and frozen at –70 °C for further analyses.

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Abbreviations: MNA, 1-methylnicotinamide; Met2PY, *N*-methyl-2-pyridone-5-carboxamide; STZ, streptozotocin; MIN6, mouse pancreatic β cell line; PARP, poly-ADP-ribose polymerase

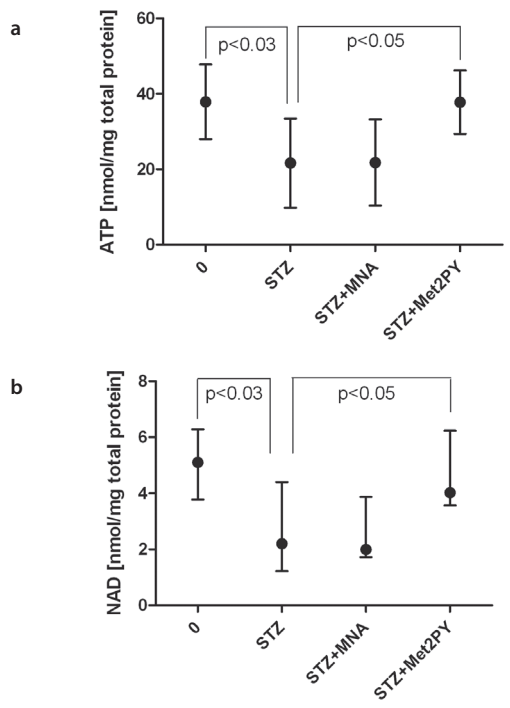

Figure 1. Effects of 1-methylnicotinamide (MNA) and *N*-methyl-2-pyridone-5-carboxamide (Met2PY) on ATP and NAD⁺ pools in streptozotocin-treated MIN6 cells
The cells were treated with 5 mM streptozotocin. MNA and Met-2PY were at 1 mM concentration. **(a)** ATP levels **(b)** NAD⁺ levels. Data shown as mean ±95% confidence interval **(a)**, or as median and interquartile range **(b)** due to lack of normal distribution.

After thawing the supernatants were collected and centrifuged (14000 r.p.m., 5 min, 4 °C). The pellets were dissolved in 0.5 M NaOH and pooled with the cell remnants from respective wells for protein assay. $HClO_4$ cell extracts were titrated with K_3PO_4 to reach pH between 5 and 6, centrifuged (14000 r.p.m., 5 min, 4 °C) and the supernatants were frozen at –70 °C for HPLC nucleotide quantitation (Smolenski *et al.*, 1990) Nucleotide concentration was expressed as nmol nucleotide/mg total protein.

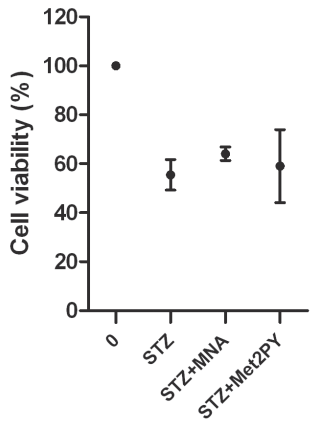

Figure 2. Effect of 1-methylnicotinamide (MNA) and *N*-methyl-2-pyridone-5-carboxamide (Met2PY) on viability of the 5 mM streptozotocin-treated MIN6 cells
The cells were treated with 5 mM streptozotocin. MNA and Met-2PY were at 1 mM concentration. Data shown as percentage of viability of control cells (mean ±95% confidence interval).

MTT assay. For MTT assay cells were grown on 96-well plates. The cells were incubated for 24 h with STZ (5 mM) in the presence of MNA chloride or Met2PY (1 mM). The incubation was then continued for 24 h in fresh medium containing MNA chloride or Met2PY but free of STZ.

The medium was removed and the cells were incubated with MTT (0.25 mg/ml, 3 h, 37 °C) The solution was removed and the formazan crystals formed were dissolved in DMSO (dimethysulfoxide). The absorbance was measured at 580 nm. The results are expressed as an absorbance averaged for 3 readings.

Statistics. Normality of data distribution and equality of variances were checked using Shapiro-Wilk's test and Levene's test, respectively. Homoscedatic data non-departing from normality were analysed with ANOVA, followed by Dunnett's test for multiple comparisons with control group. For data departing from normality reciprocal transformation was used and the data were analysed as above.

RESULTS AND DISCUSSION

STZ lowered the pools of ATP and NAD⁺ to a similar degree, to 66% and 60% of control values, respectively. Met2PY, but not MNA, partially restored the pool of both nucleotides (Fig. 1a, b). STZ decreased the viability of MIN6 cells to 50% and neither MNA or Met2PY were able to reverse this STZ-induced effect (Fig. 2). It has been shown previously that STZ lowers the nucleotide pool in murine pancreatic islet cells. This effect was PARP-dependent since it was not observed in cells isolated from PARP deficient-mice (Burkart *et al.*, 1999). Our results, indicating a sparing effect of Met2PY on the nucleotide pool and a lack of such an effect in the case of MNA, are consistent with previously published data on these compounds (Slominska *et al.*, 2008). The fact that Met2PY appeared of the same time ineffective in improving STZ-treated cell survival needs to be addressed. It should be noted that the nucleotide pool was assayed only 1 h after cells had been exposed to STZ for 1 h, whereas the cell viability was assayed 24 h after a 24 h exposure. We have deliberately chosen such an experimental setup in studying cell viability for reasons given below. When the nucleotide pools were assayed long time after the exposure to STZ (24 h), no differences were found between control and STZ-treated cells (not shown). This may be explained by the fact that the cells that survived a 24 h exposure to STZ had already restored their nucleotide pools. Likewise, a 2 h exposure to STZ does not result in lowered cell survival. This suggests that while short-term STZ action results in rather short term effects on cell metabolism related to a rapid decrease in ATP and NAD⁺ pools, the long-term effects of STZ inevitably result in cell death. On the basis of previously published experiments on the STZ mode of action, PARP activation and consequent nucleotide pool depletion seems to be the major contributor to the cell death (Burkart *et al.*, 1999; Masutani *et al.*, 1999; Pieper *et al.*, 1999). It has been shown that PARP is activated on DNA strand breaks resulting from the alkylating activity of STZ (Uchigata *et al.*, 1982). It can be then suggested that if the DNA damage exceeds a particular level, the restoration of nucleotide pools is not sufficient to protect the cell from death. It is also

important to notice that the ability of a particular factor to restore the nucleotide pools need not be a predictor of its anti-cytotoxic activity. Also, our present *in vitro* data do not support the notion that MNA or its metabolite Met2PY can protect pancreatic β-cells against STZ-induced toxicity in MNA-treated animals.

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