Lipid Peroxidation in Nicotinamide-Deficient and Nicotinamide-Supplemented Rats

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Received for publication: April 4, 2000

Abstract: Supplementation or deficiency of nicotinamide in rats may interfere with the oxidative balance, with excess leading to greater lipid peroxidation, measured by TBARS, and deficiency causing a greater consumption of antioxidants such as vitamin E and glutathione. Urinary N-methylnicotinamide excretion was much more marked in the supplemented group, whereas the difference between deficient and control animals was non-significant.

Key words: Nicotinamide, lipid peroxidation, glutathione, vitamin E, rats

Introduction

Nicotinamide, a water-soluble vitamin of the B group, is a precursor of nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP), coenzymes involved in a wide variety of processes of energy transfer inside the cell [1]. Nicotinamide protects pancreatic cells against a variety of toxic and immunomediated aggressions such as those occurring in streptozotocin-induced diabetes [2]. The protective effect of nicotinamide may be due to its action as a free radical scavenger and to its effect on the inhibition of the enzyme poly ADP-ribose synthetase and on the repletion of NAD levels inside the cell [3].

Studies on the antioxidant effect of nicotinamide against the damage caused by free radicals may be of benefit in situations of toxicity. On the other hand, it is also necessary to determine the toxicity of nicotinamide supplementation.

Thus the objective of the present study was to assess the effect of different nicotinamide concentrations in the diet on the rate of lipoperoxidation and to determine the variations in the concentrations of antioxidant substances such as vitamin E and GSH in animal receiving diets containing different nicotinamide concentrations.

Materials and Methods

The study was conducted on newly weaned male Wistar rats weighing on average 47.71 ± 2.88 g from the Central animal House of the Faculty of Medicine of Ribeirão

Preto, University of São Paulo. The animals were divided at random into three groups of 24 rats each: CD group, rats receiving a nicotinamide-deficient diets CN group, rats receiving a normal diet; CS group, rats receiving a nicotinamide-supplemented diet.

The diets were prepared according to the 1993 recommendations of the American Institute of Nutrition [4]. Dietary nicotinamide supplementation corresponded to amounts 17-fold higher (500 mg/kg diet) than those recommended for a normal rat diet (30 mg/kg diet). the deficient diet did not contain nicotinamide in the composition of the vitamin mixture.

The rats were housed in individual stainless steel cages and once a week in metabolic cages in a room with natural ventilation and controlled artificial lighting (12 hours light/12 hours dark). The animals were allowed to adapt to the environment for three days and then started to receive the respective diets and water *ad libitum* for six weeks. At the end of the experiment the animals were sacrificed by cardiac pucture.

Blood was centrifuged and used to measure glycemia and α-tocopherol. Samples of liver were immediately placed in liquid nitrogen (–196°C) and stored at –70°C for later determination of thiobarbituric acid reactive substances (TBARS) and α-tocopherol.

Lipoperoxidation was quantified indirectly by TBARS determination in the liver according to the method of Uchiyama and Mihara [5]. Vitamin E concentrations in liver were determined by HPLC by the method of Arnauld *et al* [6]. Protein concentration in the liver was determined by the method of Lowry *et al* [7]. Hepatic reduced glutathione was determined by the method of Sedlack and Lindsay [8]. Plasma glucose was evaluated with a Labtest kit (cat. No. 28, Labtest Brazil). Urinary N'methylnicotinamide was determined by the method of Carpenter and Kodicek [9].

Data are reported as mean ± standard deviation. Analysis of variance was used to determine the differences between groups, with the level of significance set at $p < 0.05$.

Results and Discussion

The results (means ± SD) concerning blood glucose levels, hepatic TBARS, hepatic GSH, hepatic vitamin E and urinary N'methylnicotinamide are presented in Table I.

Nicotinamide deficiency or supplementation had no effect on glycemia. The levels of TBARS, an indirect indicator of lipid peroxidation, were significantly increased in the liver of animals receiving nicotinamide supplementation, with a consequent higher lipoperoxidation rate. Signs of toxicity, especially represented by the appearance of urinary metabolites derived from nicotinamide catabolism, may occur when the rats receive a diet containing 5% nicotinamide in its composition [10]. On the other hand, there was a significant decreased in hepatic concentrations of the antioxidants vitamin E and glutathione in the nicotinamide-deficient group, probably due to the lack of this vitamin in various reactions involving energy transfer or even as a free radical scavenger. Urinary N-methylnicotinamide excretion was much more marked in the supplemented group, whereas the difference between deficient and control animals was non-significant. We conclude that both the excess and the deficiency of nicotinamide may interfere with the oxidative balance, with excess leading to greater lipid peroxidation and deficiency causing a greater consumption of antioxidants such as vitamin E and glutathione.

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Table I: Biochemical parameters evlauated in rats receiving diets with different nicotinamide levels

	Glycemia (mg %)	TBARS (nmol/mg p)	GSH (nmol/mg p)	Vitamin E (µg&g t)	N'MN (µg/24hs)
Deficient	104.09 ± 13.88[a]	0.65 ± 0.07[ab]	14.60 ± 1.30[a]	27.32 ± 8.53[a]	30.86 ± 15.37[a]
Control	128.25 ± 14.61[a]	0.63 ± 0.03[a]	33.24 ± 3.66[b]	52.62 ± 13.57[b]	28.42 ± 15.03[a]
Supplemented	135.35 ± 30.07[a]	0.72 ± 0.06[b]	41.99 ± 9.27[b]	65.05 ± 14.02[b]	227.60 ± 90.86[b]

Different letters indicate differences between groups ($p < 0.05$).

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