Mapping the role of NAD metabolism in prevention and treatment of carcinogenesis

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Abstract

Studies presented here show that cellular NAD, which we hypothesize to be the relevant biomarker of niacin status, is significantly lower in humans than in the commonly studied animal models of carcinogenesis. We show that nicotinamide and the resulting cellular NAD concentration modulate expression of the tumor suppressor protein, p53, in human breast, skin, and lung cells. Studies to determine the optimal NAD concentrations for responding to DNA damage in breast epithelial cells reveal that DNA damage appears to stimulate NAD biosynthesis and that recovery from DNA damage occurs several hours earlier in the presence of higher NAD or in cells undergoing active NAD biosynthesis. Finally, analyses of normal human skin tissue from individuals diagnosed with actinic keratoses or squamous cell carcinomas show that NAD content of the skin is inversely correlated with the malignant phenotype. Since NAD is important in modulating ADP-ribose polymer metabolism, cyclic ADP-ribose synthesis, and stress response proteins, such as p53, following DNA damage, understanding how NAD metabolism is regulated in the human has important implications in developing both prevention and treatment strategies in carcinogenesis. (Mol Cell Biochem 193: 69–74, 1999)

Key words: skin, lung and breast cancer, p53 expression, cyclic ADP-ribose, poly(ADP-ribose) metabolism, niacin status

Introduction

Considerable evidence now indicates that the NAD content of cells influences cellular responses to genomic damage by multiple mechanisms. NAD is directly consumed for the synthesis of ADP-ribose polymers and cyclic ADP-ribose. The metabolism of ADP-ribose polymers appears to be involved with responses that can lead to normal cellular recovery, apoptosis, or necrosis [1, 2]. Cyclic ADP-ribose is a potent calcium releasing agent that also may mediate signalling pathways leading to apoptosis or necrosis [3, 4]. Finally, recent studies have shown that the NAD content of cells modulates the expression of stress proteins that play important roles in responses to genomic damage [5], including the tumor suppressor protein, p53 [6]. Consequently, NAD metabolism is a target for both prevention and treatment of cancer. The scheme shown in Fig. 1 outlines relationships in NAD metabolism that remain poorly defined. While it is known that dietary nicotinamide and nicotinic acid serve as precursors of NAD in many human tissues, much less is known about the conversion of tryptophan to NAD. The liver and perhaps the kidney are capable of the latter pathway. Since 60 mg of tryptophan consumed in protein is often assumed to be converted to 1 mg of niacin, tryptophan accounts for nearly one half of calculated niacin consumption in Western diets [7]. However, tryptophan does not appear to be a source of tissue NAD in humans under conditions of restricted niacin intake over a period of a few weeks [8]. Thus, it is likely that niacin intake may be significantly less than reported [7].

Measuring the relationship of dietary intake of NAD precursors and the circulating levels of various precursors that supply the tissues has been technically difficult. Recently, our laboratory has developed methods that allow measurement of nicotinic acid and nicotinamide in fasted serum samples [9]. Preliminary studies that control niacin in animal models suggest that a given concentration of precursor can produce vastly different effects on NAD in the various tissues [10–13]. The optimal intracellular NAD content for eliciting protective biochemical responses following DNA damage.

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has not been defined. Given the relationship between NAD content and biochemical pathways involved in carcinogenesis, we are attempting to determine the interrelationships shown in Fig. 1 to understand how niacin metabolism and tissue NAD impact the prevention and treatment of cancer.

Results and discussion

Species and tissue differences in requirements for NAD precursors

The first step in characterizing the relationships of NAD metabolism shown in Fig. 1 for humans is the establishment of reliable methods for assessing niacin status. Biochemical assessment of niacin status has traditionally involved measurement of urinary oxidation products of nicotinamide. However, discovery of reactions that use NAD as a substrate in ADP-ribose transfer reactions and the relationship between NAD content and expression of stress response proteins such as p53, have led us to hypothesize that intracellular NAD may be a more relevant measure of niacin status. A biochemical measure of niacin status where the ratio of NAD to NADP in blood cells reflects dietary intake has been developed from the metabolic ward studies of Fu et al. and our observations on the distribution of NAD in whole blood [8, 14, 15]. We refer to this ratio as niacin number.

Since studies of carcinogenesis frequently employ rodents as animal models, we have compared niacin status as measured by blood cell pyridine nucleotides in mice, rats, and humans as a function of niacin intake. The data of Table 1 are compiled from our laboratory and others as indicated. The values are shown as niacin number and as NAD per ml of blood, because many earlier studies have only measured the latter. We have compiled the data in both formats for means of comparison. The NAD content of human blood in an individual consuming the RDA for niacin is less than 50% of that in rats and less than 25% of that in mice consuming proportional dietary niacin. These data indicate that the niacin status of humans is considerably lower than that of rodents. Additionally, analyses from a large Western population within The Malmö Diet and Cancer Study reveal a large variability in the human population. The data in Table 1 represent the values for 95% of a population where n is equal to 1300. A range of NAD content of 3 fold is observed. Approximately 15–20% of individuals in this population have significant niacin deficiency [16]. Because pharmacological doses of niacin are frequently administered as therapy for hypercholesterolemia, we have been able to measure the effect of introducing niacin supplementation on human blood cell NAD. As shown in Table 1, a mean niacin number of 175 in this population increased nearly 4 fold to 663 after 2 months of niacin therapy. It is interesting that this therapy elevates human niacin status to that of mice. However, in contrast to