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Nicotinamide forestalls pathology and cognitive decline in Alzheimer mice: evidence for improved neuronal bioenergetics and autophagy procession

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ABSTRACT

Impaired brain energy metabolism and oxidative stress are implicated in cognitive decline and the pathologic accumulations of amyloid β-peptide (Aβ) and hyperphosphorylated tau in Alzheimer's disease (AD). To determine whether improving brain energy metabolism will forestall disease progress in AD, the impact of the β-nicotinamide adenine dinucleotide precursor nicotinamide on brain cell mitochondrial function and macroautophagy, bioenergetics-related signaling, and cognitive performance were studied in cultured neurons and in a mouse model of AD. Oxidative stress resulted in decreased mitochondrial mass, mitochondrial degeneration, and autophagosome accumulation in neurons. Nicotinamide preserved mitochondrial integrity and autophagy function, and reduced neuronal vulnerability to oxidative/metabolic insults and Aβ toxicity. β-Nicotinamide adenine dinucleotide biosynthesis, autophagy, and phosphatidylinositol-3-kinase signaling were required for the neuroprotective action of nicotinamide. Treatment of 3xTgAD mice with nicotinamide for 8 months resulted in improved cognitive performance, and reduced Aβ and hyperphosphorylated tau pathologies in hippocampus and cerebral cortex. Nicotinamide treatment preserved mitochondrial integrity, and improved autophagy-lysosome procession by enhancing lysosome/autolysosome acidification to reduce autophagosome accumulation. Treatment of 3xTgAD mice with nicotinamide resulted in elevated levels of activated neuroplasticity-related kinases (protein kinase B [Akt] and extracellular signal-regulated kinases) and the transcription factor cyclic adenosine monophosphate (AMP) response element-binding protein in the hippocampus and cerebral cortex. Thus, nicotinamide suppresses AD pathology and cognitive decline in a mouse model of AD by a mechanism involving improved brain bioenergetics with preserved functionality of mitochondria and the autophagy system.

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1. Introduction

Compromised brain energy metabolism and oxidative stress are implicated in the etiology of Alzheimer's disease (AD), contributing to the neurodegenerative process and associated cognitive deficits (Blass et al., 2002; Dumont and Beal, 2011; Kapogiannis and Mattson, 2011; Kennedy et al., 1995; Mattson, 2004; Nunomura et al., 2001). Age-related free radical production damages proteins, lipids, and nucleic acids, and impairs cellular organelle functions. The progressive accumulation of the 2 major AD pathologic hallmark proteins, amyloid β-peptide (Aβ) and tau, might exacerbate oxidative stress (De Felice et al., 2007; Mattson, 2004; Rottkamp et al., 2001). On the other hand, Aβ neurotoxicity is enhanced under conditions of mitochondrial dysfunction and cellular energy deficits (Arias et al., 2002).

Mitochondria are the major organelle for energy production in neurons, and are also a major source of free radicals and a target of oxidative damage. Growing evidence indicates that oxidative mitochondrial damage and dysfunction are critical factors in age-related neurodegenerative diseases including AD (Gibson et al., 2010; Hirai et al., 2001; Mattson, 2004; Moreira et al., 2006; Sultana and Butterfield, 2010). Mitochondria are capable of dividing and growing (biogenesis), and are also subject to degradation and removal when they become dysfunctional and damaged; mitochondria turnover every 2–4 weeks in neurons (Menzies and Gold, 1971). Accordingly, one approach for counteracting the adverse effects of aging and AD is to preserve mitochondrial quality and homeostatic dynamics, which would be particularly important for long-lived postmitotic cells such as neurons (Terman et al., 2010).

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In neurons, as in other cell types, the macroautophagy-lysosome system is the major degradation pathway responsible for turnover of defective organelles and aggregated proteins (Cherra et al., 2010; Simonsen et al., 2008). Autophagy is also a recycling process that is regulated by cellular energy state and oxidative stress signaling (Hsu et al., 2009; Scherz-Shouval and Elazar, 2007); it is activated by nutrient deprivation (Miwa et al., 2008; Mizushima et al., 2004; Scott et al., 2007) and is inhibited by the kinase mammalian target of rapamycin (mTOR) (Yu et al., 2010). When nutrients are lacking, mTOR repression shifts cellular metabolism toward autophagy. The links between bioenergetic state, oxidative stress, and autophagy in aging and age-related neurodegenerative diseases remain to be established.

Suppression of autophagy can result in neurodegeneration that involves the accumulation of aggregated proteins and damaged organelles (Batlevi and Spada, 2011; Cherra et al., 2010; Hara et al., 2006; Komatsu et al., 2006). However, in physiological and pathological settings, the accumulation of autophagosomes might represent either an adaptive response to stress or a defective autophagy-lysosomal process that triggers cell death (Banerjee et al., 2010; Scherz-Shouval and Elazar, 2007). Autophagy can be stimulated by a range of mild stressors including caloric restriction (Egan et al., 2011), oxidative stress (Karna et al., 2010; Scherz-Shouval and Elazar, 2007) and inhibition of mTOR (Ravikumar et al., 2004). Perturbations of autophagy occur during normal aging and might be exacerbated or dysregulated in neurodegenerative disorders (Rubinsztein et al., 2011). It was recently observed that autophagosomes are abundant in neurons in AD, where aberrations in macroautophagy might occur (Li et al., 2010; Moreira et al., 2010). In addition, both Aβ accumulation and tau neurofibrillary tangles have been observed to be associated with the autophagic pathway (Hung et al., 2009; Yu et al., 2005). Such observations have provoked controversial viewpoints, such as whether autophagosome accumulation indicates an increase in autophagic activity or rather is a consequence of impaired autophagic degradation, and whether autophagy promotes neuronal cell death or is neuroprotective (Banerjee et al., 2010).

It was recently reported that mitochondrial biogenesis and axonal transport of mitochondria are impaired in association with synaptic degeneration in a mouse model of AD, by a mechanism involving cytotoxic actions of Aβ (Calkins and Reddy, 2011; Calkins et al., 2011). In addition, Manczak et al. (2011) provided evidence for an abnormal interaction between dynamin-related protein 1 (Drp1) and Aβ related to mitochondrial abnormalities in neurons in the brains of AD patients. Drp1, which is also called dynamin-like protein 1 (DLP1), plays a critical role in mitochondrial biogenesis. Thus, there is reason to believe that impaired mitochondrial bioenergetics and accumulation of damaged, dysfunctional mitochondria occurs in vulnerable neurons in AD. Therapeutic interventions that sustain mitochondrial bioenergetics might therefore protect synapses and neurons against dysfunction and degeneration in AD.

In the present study we determined whether improving neuronal bioenergetics via provision of the nicotinamide (NAM) would enhance autophagy and ameliorate neuronal dysfunction and degeneration in experimental models of AD. NAM, an amide of vitamin B3, is the essential precursor of β-nicotinamide adenine dinucleotide (NAD^+) in mammalian cells (Belenky et al., 2006). NAM is converted to NAD^+ through the activity of nicotinamide phosphoribosyltransferase (NAMPT), a rate-limiting enzyme in NAD^+ biosynthesis. As an energy substrate and cofactor for electron transfer, NAD^+ is essential in multiple steps of aerobic energy metabolic reactions and diverse biological processes (Belenky et al., 2006). NAD^+ can serve as an adenosine donor and source of high energy phosphate for the synthesis of adenosine triphosphate (ATP). Depletion of NAD^+ impairs glycolysis, the tricarboxylic acid cycle, and mitochondrial oxidative phosphorylation (Sheline et al., 2000). Moreover, the reduced form of nicotinamide adenine dinucleotide (NADH):NAD^+ redox state influences mitochondrial free radical production and antioxidant capacity (Ghosh et al., 2012; Starkov and Fiskum, 2003). NAM modulates NAD^+ and NADH redox levels. In mitochondria, NAD^+ and NADH are in a dynamic state, because NADH generation in the tricarboxylic acid cycle requires NAD^+. NADH, the reduced form of NAD^+, is the substrate and electron donor in complex I of the mitochondrial electron transport chain, and is oxidized to NAD^+ during mitochondrial respiration (Liu et al., 2008).

Accumulating evidence reveals roles for NAD^+ in regulating bioenergetics, cell survival, and the involvement of perturbed NAD^+-dependent processes in age-related neurologic diseases and diabetes (Sauve, 2008). NAD^+-dependent signaling pathways are involved in the aging process and lifespan determination in model organisms (Houtkooper et al., 2010). For example, NAM modulates sirtuin activity, both as a precursor of the NAD^+-required several members of sirtuin family, and as an inhibitor of histone deacetylases. In addition, NAM prevents poly adenosine diphosphate ribose polymerase 1 (PARP1) activation and subsequent NAD^+ depletion induced by DNA strand breaks (Alano et al., 2010). NAM enhances glycolysis and might reduce the accumulation of abnormal protein aggregates and glycation end products associated with neurodegenerative disorders (Hipkiss, 2009). NAD^+ can also stimulate lysosomal acidification by modulating the proton gradient, which is essential for autophagosome-lysosome fusion and the optimum function of lysosomal proteases (Hsu et al., 2009).

NAM moves rapidly (within minutes) across the blood–brain barrier by facilitated diffusion and is converted to NAD^+ (Spector and Johanson, 2007). Green et al. (2008) found that treatment of 3xTgAD mice (an animal model of AD) with NAM results in amelioration of cognitive deficits, and provided evidence for a role for sirtuin 1 activation in this cognition-preserving action of NAM. We previously found that NAM preserves cellular NAD^+ levels and improves cell survival under conditions of metabolic and excitotoxic stress in neurons and in a rodent model of stroke (Liu et al., 2009). Here we report that oral administration of NAM ameliorates cognitive deficits, and Aβ and hyperphosphorylated tau (p-Tau) pathologies, in 3xTgAD mice. We show that the benefit of NAM in AD mice involves elevation of NAD^+ levels and increased resistance of mitochondria to oxidative stress, and enhancement of the autophagy-lysosome process. NAM treatment results in activation of signaling pathways critical for neuronal survival and synaptic plasticity including protein kinase B (Akt) and extracellular signal-regulated kinases (ERKs), and the transcription factor cyclic adenosine monophosphate (AMP) response element-binding protein (CREB).

2. Methods

2.1. Primary neuronal cell cultures

Methods for the preparation and maintenance of dissociated cultures of embryonic rat cerebral cortical cells have been described previously (Mattson et al., 1995). Dissociated neurons were seeded onto polyethylenimine-coated plastic culture dishes (for biochemical assays) in MEM medium with 15% fetal bovine serum for 4 hours, and the medium was then changed to serum-free Neurobasal Medium containing B-27 supplements (Invitrogen), 1 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), and 2 mM gentamycin (Sigma). The medium contained 0.8 mM Mg^{2+}. Cultures were maintained at 37 °C in a 6% CO_2/94% air atmosphere. All experiments were performed using 7- to 9-day-old cultures. All treatments were done in the same culture medium for a duration of 6 hours, unless stated otherwise.