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ORIGINAL PAPER

Multi-targeted Effect of Nicotinamide Mononucleotide on Brain Bioenergetic Metabolism

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Abstract

Dysfunctions in NAD^+ metabolism are associated with neurodegenerative diseases, acute brain injury, diabetes, and aging. Loss of NAD^+ levels results in impairment of mitochondria function, which leads to failure of essential metabolic processes. Strategies to replenish depleted NAD^+ pools can offer significant improvements of pathologic states. NAD^+ levels are maintained by two opposing enzymatic reactions, one is the consumption of NAD^+ while the other is the re-synthesis of NAD^+. Inhibition of NAD^+ degrading enzymes, poly-ADP-ribose polymerase 1 (PARP1) and ectoenzyme CD38, following brain ischemic insult can provide neuroprotection. Preservation of NAD^+ pools by administration of NAD^+ precursors, such as nicotinamide (Nam) or nicotinamide mononucleotide (NMN), also offers neuroprotection. However, NMN treatment demonstrates to be a promising candidate as a therapeutic approach due to its multi-targeted effect acting as PARP1 and CD38 inhibitor, sirtuins activator, mitochondrial fission inhibitor, and NAD^+ supplement. Many neurodegenerative diseases or acute brain injury activate several cellular death pathways requiring a treatment strategy that will target these mechanisms. Since NMN demonstrated the ability to exert its effect on several cellular metabolic pathways involved in brain pathophysiology it seems to be one of the most promising candidates to be used for successful neuroprotection.

Keywords Nicotinamide adenine dinucleotide · Nicotinamide mononucleotide · Mitochondria · Acetylation · Brain

Introduction

Nicotinamide adenine dinucleotide (NAD^+) is a ubiquitous and abundant molecule in biological organisms that is required as a cofactor or substrate for about 500 cellular reactions, therefore playing a key role in cellular metabolism. Originally NAD^+ was determined as a cofactor for redox reactions [1]. NAD^+, its reduced form NADH, and its phosphorylated forms (NADP and NADPH) are involved in glycolysis, the pentose phosphate pathway, oxidative phosphorylation, TCA cycle, as well ketone bodies, lipids, and amino acid metabolism. Apart from facilitating enzymatic reactions associated with transfer of electrons from one molecule to another, NAD^+ also serves as a substrate for several NAD^+ consuming enzymes associated with post-translational modifications or second messenger generation [1–6]. The pyridine nucleotide metabolism has a cyclic nature since NAD^+ degradation is the major biological source of nicotinamide (Nam) and at least part of Nam is used for conversion back to NAD^+ [7].

Recently, the interest in NAD^+ and its related metabolic pathways dramatically increased. This is because new findings revealed that dysfunctions in NAD^+ metabolism are associated with several diseases and pathologic states. Decreased NAD^+ levels were observed under conditions of neurodegenerative disease, following acute brain injury, in diabetes, or during aging. Loss of NAD^+ results in impaired mitochondrial function, thus failure in cellular bioenergetics metabolism and in perturbation of essential metabolic processes. Strategies that attempt to replenish NAD^+ pools therefore can offer significant improvements of tissue in pathologic state.

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Two opposing enzymatic processes determine cellular NAD^+ levels. One represents activity of enzymes that consume NAD^+ (degradation by NAD^+ glycohydrolases) and the other facilitates re-synthesis of NAD^+ pools. Consequently, there have been two main approaches to improve tissue NAD^+ levels. Since NAD^+ consumption is accelerated by over-activation of NAD^+ glycohydrolases under bioenergetic stress conditions, the main focus of neuroprotection was on inhibition of NAD^+ degrading enzymes [8–11]. In another approach, the preservation of NAD^+ pools was achieved by supplying the tissue with precursors for NAD^+ synthesis [12–20].

In this short review we will focus on the advantages of therapeutic approaches that use NAD^+ synthesis precursors to improve the pathologic conditions associated with neurodegeneration and acute brain injury.

Major Pathways of NAD^+ Catabolism

As mentioned above, there are several NAD^+ glycohydrolases that contribute to NAD^+ catabolism. A main NAD^+ consuming enzyme is poly-ADP-ribose polymerase 1 (PARP1), which is activated particularly following DNA damage [9, 21, 22]. PARP1, by poly-ADP-ribosylation (PAR) of histone and non-histone proteins, activates the repair of single stranded DNA breaks [23]. Significant or sustained DNA damage can lead to over-activation of PARP1, resulting in depletion of intracellular NAD^+ pools, decreased activity of NAD^+-dependent enzymes and accumulation of PAR polymers [24–26]. Both pharmacologic and genetic inhibition of PARP1 activity demonstrated significant protection against acute brain injury [8–11].

Poly-ADP-ribose polymerization is a post-translational modification that is removed by poly-ADP-ribose glycohydrolase (PARG) [27]. There are two major forms of PARG [27, 28]. The 110 kDa $PARG_{110}$ is localized predominantly in nucleus and the 60 kDa $PARG_{60}$ is found only in the cytoplasm [29, 30]. The original hypothesis of PARP1 over-activation induced NAD^+ depletion as a major death factor was challenged by data showing that the poly-ADP-ribose molecules *per se* are toxic to the cells [31]. Thus, overexpression of PARG was protective and animals with reduced levels of PARG showed significantly increased brain damage following ischemic insult [31]. However, there are several reports suggesting that inhibition of PARG activity is protective [32–34] and the selective knockdown of nuclear form of $PARG_{110}$ reduces genotoxic agent induced cell death [35]. However, focal ischemia in the same transgenic mouse model with selective knockdown of $PARG_{110}$ resulted in increased infarct volume when compared to wild type (WT) animals [30]. These conflicting data suggest that the mechanisms responsible for cellular death following PARP1 over-activation are still elusive and the significant contribution of the individual adverse factors might depend on the type of the insult or pathologic conditions. Furthermore, strong and prolonged inhibition of PARP1 activity will compromise the main biological role of this enzyme, damaged DNA repair, which can have adverse effects on cells survival after longer recovery periods.

Another enzyme that has a significant role in NAD^+ metabolism is the ectoenzyme CD38. CD38 generates Nam and second messengers, cyclic ADP-ribose or ADP-ribose from NAD^+ [36, 37]. In CD38 knockout (CD38KO) mice, brain NAD^+ pools were reported significantly higher when compared to WT animals [38], however, the changes in NAD^+ levels were dependent on the brain region [39]. It was demonstrated that CD38 expression levels increase with age, which leads to decline in cellular NAD^+ [40]. Interestingly, stereologic quantification of hippocampal neurons in adult CD38KO animals showed about 15% higher number of CA1 neurons when compared to WT mice [39]. Unexpectedly to previous findings, although these animals exhibit higher number of hippocampal neurons their PAR levels were 140% higher compared to WT mice due to lower expression of nuclear PARG isoform [39]. Furthermore, the most abundant immunoreactivity of PAR antibody was detected in the perinuclear region of neurons, and following ischemic insult there was an additional increase in PAR predominantly within astrocytes [39]. The experiments with CD38KO animals also revealed that CD38 significantly contributes to post-ischemic NAD^+ depletion during the late recovery period, after 4 h of reperfusion [39]. These data suggest that apart from PARP1, CD38 also contributes to increased post-ischemic NAD^+ consumption.

Recently, a research group exploring mechanism of axonal degeneration discovered an enzyme, SARM1 (sterile alpha and Toll/interleukin-1 receptor motif-containing 1), that is triggered by axonal damage and initiates a local destruction program involving rapid breakdown of NAD^+ after injury [41, 42]. SARM1 is a negative regulator of Toll-like receptor-activated transcription program [43] and it has intrinsic NADase activity, cleaving NAD^+ into ADP-ribose, cyclic ADPR, and Nam [42].

Another post-translational modification that is regulated by NAD^+ is protein acetylation. This modification is carried out by acetyltransferases that transfer the acetyl group from acetyl-CoA to the lysine residue of the target protein [44]. Enzymes that remove the acetyl group from the acetylated lysine are represented by several classes of deacetylases [44–46]. Sirtuins (SIRTs), NAD^+ depended class III deacetylases, remove Nam from NAD^+ and transfer the acetyl group from the lysine residue to the ADP-ribose moiety [47–49]. During this process NAD^+ is consumed and Nam plus *O*-acetyl-ADP-ribose is released. SIRTs deacetylate histone and non-histone proteins, thus they regulate gene

expression and also different metabolic pathways within the cell. Generally, increased sirtuin activity is considered beneficial for cells since sirtuin dependent deacetylation is associated with protective effects against neurodegeneration, acute brain injury, prolonged life span, suppression of adverse effects of diabetes, and beneficial impact of exercise [47–50]. So far it was not shown whether increased sirtuin activity can also significantly reduce cellular NAD^+ pools, however, low NAD^+ level leads to increased protein acetylation. There are several isoforms of sirtuins that are localized to specific intracellular compartments. Sirt1, Sirt6, and Sirt7 were predominantly found in the nucleus, Sirt2 is considered to be cytosolic enzyme, and mitochondria harbor several sirtuins (Sirt3-5), although only Sirt3 was shown to have robust deacetylase activity and to control the mitochondrial acetylome [51, 52].

NAD^+ Replenishment

To maintain tissue NAD^+ pools cells express enzymes that can generate and replace the consumed NAD^+. There are two major metabolic pathways that serve this purpose. NAD^+ can be synthesized de novo from tryptophan, however, the main source of NAD^+ is from the salvage pathway [2], where it is generated in two enzymatic reactions that use Nam, nicotinic acid (NA), or nicotinamide riboside (NR) as precursors, respectively. In mammals, Nam is considered to be the main precursor for NAD^+ synthesis [53]. In the first rate-limiting step of the salvage pathway, nicotinamide phosphoribosyltransferase (NAMPT) forms nicotinamide mononucleotide (NMN) from Nam and 5-phosphoribosyl-1-pyrophosphate (PRPP). In the next step, NMN is converted to NAD^+ by NMN adenylyl transferase (NMNAT) in the presence of ATP [5, 54]. Thus, the salvage pathway is part of the cyclic NAD^+ metabolism where the product of NAD^+ glycohydrolases, Nam, is recycled to generate new NAD^+ (Fig. 1).

Stimulating NAD^+ Synthesis as a Strategy for Therapeutic Intervention

As we mentioned above, pathologic conditions associated with neurodegenerative diseases or excessive bioenergetic and oxidative stress lead to depletion of cellular NAD^+ pools. Apart from inhibiting the NAD^+ degrading enzymes a simple approach to improve cellular metabolic conditions is to stimulate the NAD^+ generation by increasing the substrate

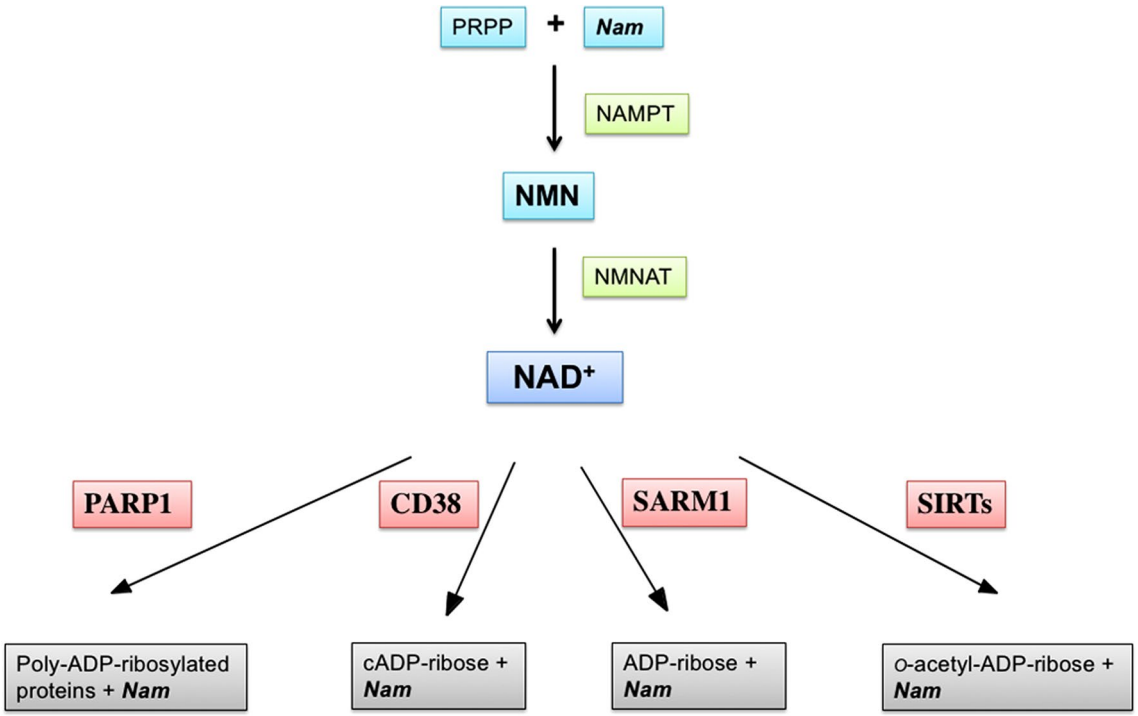

Fig. 1 Schematic diagram showing the major pathways of NAD^+ metabolism. NAD^+ can be degraded by poly-ADP-ribose polymerase (PARP1), CD38 ectoenzyme, sterile alpha and toll/interleukin-1 receptor motif-containing 1 enzyme (SARM1), and sirtuins (SIRTs). These enzymes remove nicotinamide (Nam) from NAD^+ and generate poly-, cyclic-, mono-, or *O*-acetyl-ADP-ribose. Nam is recycled into NAD^+ by the salvage pathway. NAMPT generates NMN from Nam and PRPP. In the next step NMN is used by nicotinamide mononucleotide adenylyltransferase (NMNAT) to generate NAD^+

supply for the salvage pathway. Therefore, Nam was one of the first metabolic compounds used to elevate NAD^+ levels [55, 56]. Since Nam is readily available and can pass the cellular membranes, it was the most logical candidate to use for treatment of conditions of brain tissue NAD^+ deficiency.

Administration of Nam improved bioenergetics following brain ischemia or oxidative stress and ameliorated ischemic and traumatic brain injury [13, 14, 57, 58]. The ability of Nam to pass the cellular membranes explains its rapid penetration of the blood–brain barrier (BBB) [59], however, it also suggests that the Nam can be readily removed from the tissue by blood flow [5]. Consequently, although Nam administration showed improved post-ischemic outcome at 125 mg/kg dose, the most neuroprotective dose of Nam was rather high at 500 mg/kg [57]. Several mechanisms were suggested to play a role in Nam neuroprotection following acute brain injury. These included prevention of ATP depletion [55, 57], inhibition of PARP1, CD38, and SIRTs [9, 60, 61], lipid peroxidation [62], anti-inflammatory activity [63], and prevention of apoptosis [55, 64].

Since Nam is a substrate for the Nampt enzyme its impact on the NAD^+ synthesis is limited by Nampt activity. Therefore, administration of Nam under conditions where the Nampt enzyme is inhibited or dysfunctional will not lead to increased NAD^+ synthesis by the salvage pathway.

NMN as a Promising Candidate for Treatment of NAD^+ Deficiency

To even more efficiently facilitate NAD^+ synthesis, by bypassing the rate-limiting step in the salvage pathway, one can administer NMN that will directly feed into the one-step enzymatic generation of NAD^+ via NMNAT. There are several reports showing that intraperitoneal administration of NMN can significantly increase brain tissue NAD^+ levels within 15 min post-injection ([65, 66], for review see [67]). This suggests that there is an active transport of NMN or its metabolites into the intracellular compartments, where it is converted to NAD^+. However, the mechanisms of NMN transport across the BBB or cellular and mitochondrial membranes need to be determined.

Recently published data suggest that NMN is metabolized extracellularly to NR by CD73 [68]. NR is then transported across the plasma membrane through dipyridamole-sensitive nucleoside transporters [69] and phosphorylated intracellularly by NR kinase 1 (NRK1) to NMN [2, 69, 70]. Finally, intracellular NMN is converted to NAD^+ by isoforms of NMNAT [2]. Experiments with NRK1 knockout mice demonstrated the requirement of NRK1 activity for generation of NAD^+ from NR and NMN [71]. Interestingly, although not all the enzymes required for generation of NAD^+ from NR or NMN are present in plasma, already 5 min after an intraperitoneal injection of NMN the plasma NAD^+ levels increased significantly. These data suggest that NMN is rapidly absorbed from the gut into blood, however, the NMN in vivo pharmacokinetics and its conversion to NAD^+ in cells or blood are so far poorly understood. Thus, NMN and NR demonstrated superior pharmacokinetics when compared to Nam, primarily due to involvement of active transport mechanisms that allow for better control of the translocation process of these compounds from plasma into intracellular compartments. As a result there is more effective intracellular accumulation of NMN and its conversion to NAD^+.

NMN administration improved several neuronal functions in the brain and showed significant neuroprotection against both neurodegenerative conditions or acute brain injury [15, 16, 19, 72–75]. There were several mechanisms identified as targets of NMN induced changes in cellular metabolism. These were linked to its direct inhibitory effect of PARP1 and CD38 or indirectly regulating gene expression and enzyme activity via modulating acetylation of histone and non-histone proteins due to increased NAD^+ levels (Fig. 2). Since the most effective dose for Nam administration was 500 mg/kg, the majority of studies with NMN treatment used similar 300–1000 mg/kg doses. However, high intracellular levels of NMN can be less effective or have adverse effects under particular pathologic conditions [15, 76]. Reports from cell culture studies of the Wallerian degeneration model support these findings by showing that accumulation of intracellular NMN due to inhibition of NMNAT promotes axonal degeneration [77]. While so far there are no reports of significant adverse effects of NMN on physiological parameters like temperature, blood pressure, cerebral blood flow [15], or other forms of toxicity, the studies that used different doses of NMN suggest that pharmacological titration of NMN administration should be applied to determine the most effective treatment protocol for particular disease conditions.

The beneficial outcomes of NMN administration were reported via downstream effects of SIRTs stimulation, particularly SIRT1 and SIRT3. The protective effect of SIRT1 activation against ischemic brain damage were showed after treatment of animals with SIRT1 activator, resveratrol [78–80]. Similarly, mitochondrial SIRT3 mediates neuroprotection following ischemic insult [81]. Therefore, it was not surprising that NMN administration, which leads to increased cellular NAD^+ levels, stimulated SIRT1, and ameliorated several pathologic conditions. For example, NMN-induced activation of SIRT1 prevented axonal degeneration [82], similarly overexpression of Nampt or NMN treatment reduced brain infarction following focal ischemia via the AMPK pathway which is SIRT1 dependent [74]. It was also reported that NMN can stimulate Nrf2 expression or neurogenesis via increased activity of SIRT1 [18, 75].

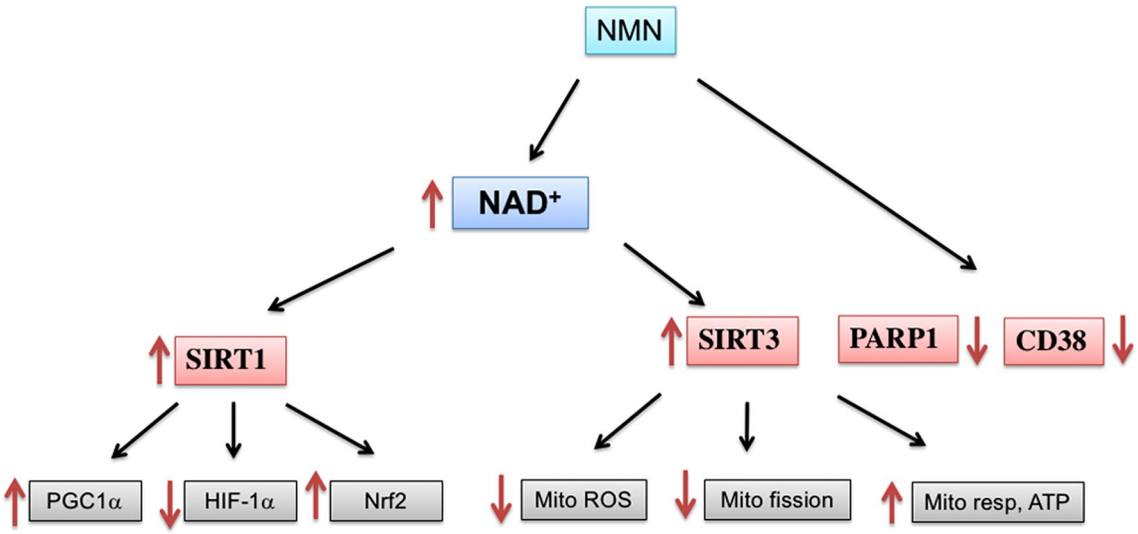

Fig. 2 Schematic diagram showing direct and indirect NMN targets. Administration of NMN leads to inhibition of PARP1 and CD38 activity. Cellular and mitochondrial NAD^+ levels are increased following NMN treatment causing increased activity of NAD^+- dependent deacetylases SIRT1 and SIRT3. As a result, PGC1α is deacetylated and its activity increases, SIRT1—dependent hypoxia inducing factor 1α (HIF-1α) accumulation is decreased, and nuclear factor (erythroid-derived 2)-like 2 (Nrf2) protein levels are increased. Higher SIRT3 activity deacetylates mitochondrial proteins leading to increased oxidative phosphorylation and ATP production (mito resp, ATP), more efficient mitochondrial reactive oxygen species (mito ROS) detoxification, and inhibition of mitochondrial fission (mito fission)

Furthermore there are reports of neuroprotective mechanisms by NMNAT (enzyme that uses NMN to generate NAD^+) due to its possible chaperon activity [83].

The beneficial effect of NMN administration was also showed in models of heart disease [20, 84–87]. Additionally, NMN was able to reverse pathologic alterations induced by aging via modulation of PGC1α , HIF-1α, and CD38 activity [17, 76, 88, 89] (for review see [90]), or diabetes via activation of SIRT1 [65, 91].

We have reported a dramatic neuroprotection of NMN against ischemic insult following a single, about 60 mg/kg, dose administration [15]. Additionally, in a mouse model of Alzheimer's disease repeated NMN administration every other day for 28 days improved mitochondrial functions, normalized brain SIRT1 and CD38 levels [16]. Surprisingly, we found that NMN administration can also affect mitochondrial dynamics after both chronic and single intraperitoneal treatment [16, 66]. After NMN administration the size distribution of brain mitochondria was shifted towards longer organelles [66]. The NMN treatment resulted in significant increase of hippocampal mitochondria NAD^+ levels leading to improved bioenergetics metabolism as reflected in higher brain tissue ATP [66]. Increase in intra-mitochondrial NAD^+ activated SIRT3 led to deacetylation of mitochondrial proteins including manganese superoxide dismutase [66]. This was accompanied by lower mitochondrial reactive oxygen species generation and reduction of Drp1 phosphorylation, ultimately leading to reduced fission [66].

Finally, we found that in addition to the dramatic neuroprotection, NMN also inhibits NAD^+ glycohydrolase activity of PARP1 and CD38 [15, 39].

Conclusion

Research from our and other laboratories demonstrates that NMN has multiple effects on cellular metabolism under both physiologic and pathologic conditions. Following NMN treatment there was a significant reduction of PAR, increase in brain and mitochondrial NAD^+ levels, and normalization of post-ischemic mitochondrial proteins acetylation and morphology. Thus, NMN has a multi-targeted effect and acts as a combination of several neuroprotective compounds (as PARP1 and CD38 inhibitors, sirtuin activators, mitochondrial fission inhibitors and NAD^+ supplements).

Another advantage of using NMN administration as a therapeutic approach is that it is an endogenous cellular metabolic compound. Thus, even at the highest dose used no toxicity or side effects after NMN injection was detected, nor were there significant changes in animal physiological parameters [15]. Since many neurodegenerative diseases and acute brain injury due to ischemic or traumatic attack activate several mechanisms that can lead to cell death, these diseases lead to complex and devastating neurologic conditions. Therefore, a successful treatment strategy needs to implement a multi-targeted approach that will affect several

mechanisms in multiple cell types. Since NMN demonstrated the ability to exert its effect on several cellular metabolic pathways involved in brain pathophysiology, it seems to be one of the most promising candidates to be used for successful neuroprotection.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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